Department of Chemistry MPhil Thesis

2010

Synthesis of Chalcone Epoxides and Studies of their Antimicrobial Activities

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University of Rajshahi

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Synthesis of chalcone epoxides and Studies of their antimicrobial activities

M.Phil Thesis

A Dissertation
Submitted to the Department of Chemistry, University of Rajshahi, in
partial fulfillments of the requirements for the degree of Master of
philosophy in Chemistry

SUBMITTED
BY
Md. Mamun-Ar-Rashid
Roll No. : 06201
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Session : 2006-2007

Second Science Building
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December, 2010

Organic Research Laboratory
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Dedicated
To
My Beloved
Parents

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Date:.................

Declaration Certificate

I here by certify that all the experimental and other works incorporated in the thesis entitled "Synthesis of chalcone epoxides and Studies of their antimicrobial activities" and submitted herein, were carried out by the candidate Mr. Md. Mamun-Ar-Rashid under my supervision. The work has been done in organic research laboratory of the Department of Chemistry, University of Rajshahi, Rajshahi, Bangladesh. Antibacterial and antifungal activities were carried out in Mycology and Plant Pathology Laboratory, Department of Botany, University of Rajshahi. The candidate has fulfilled all terms and conditions of M.Phil thesis course including presentation of the result of his study in seminar at the Department of Chemistry, University of Rajshahi, Rajshahi, Bangladesh.

I have gone through the final draft of the thesis and recommend its submission for the degree of Master of Philosophy (M.Phil).

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DECLARATION

I am Md. Mamun-Ar-Rashid, M.Phil Researcher, Roll No. 06201 Registration No. 1341, Session: 2006-2007, Department of Chemistry, University of Rajshahi, Rajshahi, Bangladesh, declaring that the thesis entitled "Synthesis of chalcone epoxides and Studies of their antimicrobial activities" is an original study and no part of the thesis has been submitted to any other University or institute for any degree or diploma. The thesis contains no materials previously published or written by any other person except reference is made in text of the thesis.

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I am also grateful to Dr. Jahan Ara Khanam, Professor, Department of Biochemistry and Molecular Biology, University of Rajshahi, for providing UV spectra.

I express my heartiest felling of gratitude to my wife, Mst. Mahfuza Akter, Ph.D. Fellow Department of Chemistry, University of Rajshahi for her unselfishness, stimulating influence, cooperation and help during the research work.

Finally I express my thanks to Mr. Zohurual Islam Babu and Mr. Faizul Islam Faruk, Computer Land, Rajshahi University for composing and printing this thesis paper.

Rajshahi University
Date:

(Md. Mamun-Ar-Rashid)

NOTATIONS

UV	Ultraviole
IR	Infrared
FTIR	Fourier Transform Infrared
^{1}H NMR	^{1}H Nuclear Magnetic Resonance
^{13}C NMR	^{13}C Nuclear Magnetic Resonance
s	Singlet
d	Doublet
dd	Doublet of a doublet
t	Triplet
dt	Doublet of a triplet
m	Multiplet
J	Coupling constant
TLC	Thin Layer Chromatography
R_f	Retardation Factor
b.p.	Boiling point
m.p.	Melting point
Hz	Hertz
MHz	Mega-hertz
δ	Chemical shift
TMS	Tetramethyl Silane
$CDCl_3$-d_6	Deuterated Chloroform
ppm	Parts per million
KBr	Potassium bromide
DMSO	Dimethyl sulphoxide
$CDCl_3$	Deuterated Chloroform
NA	Nutrient Agar
PDA	Potato Dextrose Agar
MIC	Minimum inhibitory concentration

CONTENTS

PART-I

Synthesis of some chalcone epoxides

PART-II

Abstract

ABSTRACT

Flavonoid compounds constitute one of the major classes of naturally occurring products. These types of compounds show antimicrobial (antibacterial and antifungal) activities and are used widely as drugs for the treatments of different types of diseases. Chalcone epoxides and their derivatives have attracted considerable attention due to their significant biocidal, pharmaceutical and antioxidant activities. Chalcone and its derivatives are used as inhibitors angiogenesis. Chalcone and its epoxides have been used as antitumor or anticancer agents and to treat a number of conditions or disease states in which angiogenesis is a factor, including angiongenic skin disease such as psoriasis, acne, rosacea, wartz, eczema, hemangiomas, lymphangiogenesis, among numerous others, as well as chronic inflammatory disease such as arthritis. They also control and reduce hypertension. In the light of these results we have chosen eight chalcones and their corresponding chalcone expoxides. We synthesized them and studied their biological activities. They were 2′-hydroxy-2,4,5-trimethoxychalcone epoxide; 2′-benzyloxy-2,4,5-trimethoxychalcone epoxide; 4′-methyl-2-chlorochalcone epoxide; 4′-methyl-4-chlorochalcone epoxide; 2′-hydroxy-4-nitrochalcone epoxide; 2′-benzyloxy-4-methoxychaclone epoxide; 2′-hydroxy-3,4-methylenedioxy chalcone epoxide; 2′-benzyloxy-3,4-methylene-dioxychalcone epoxide. The above chalcone epoxides were synthesized from their corresponding chalcones using $NaOH/H_2O_2$ as an oxidizing agent.

The biocidal activity of these chalcone epoxides along with their corresponding chalcones are determined against some selected bacterial and fungal strain. Both the chalcones and their corresponding chalcone

epoxides were screened *in vitro* for their antibacterial and antifungal activities against four human pathogenic bacteria viz. *Streptococcus-β-haemolyticus* (G^{+}), *Bacillus megatrium* (G^{+}), *Klebsiella* sp. (G^{-}) *Escherichia coli* (G^{-}) and four plants as well as molds fungi viz. *Rhizoctonia solani, Sclerotium rolfsii, Aspergillus niger* and *Aspergillus fumigatus*.

Some of the compounds showed moderate activity towards both the Gram-positive and Gram-negative bacteria. It has been found that the inhibition zones of the chalcone epoxides were more effective than their corresponding chalcones. Chalcones (**111** and **115**) did not show any inhibition activity against some selected bacteria. But their corresponding chalcone epoxides (**112** and **116**) show high inhibition activity against some selected bacteria. Others were very reactive. It has been also found that most of the compounds show fungicidal activity.

So our aim of the present study was to investigate the antimcrobial properties of the above chalcone epoxides and chalcones with the hope of adding new and potent chemotherapeutic agents to arsenal of weapons used against resistance organisms as well as other most lethal infectious diseases.

PART-I

Synthesis of chalcone epoxides

CHAPTER
I

General Introduction

CHAPTER-I

General Introduction

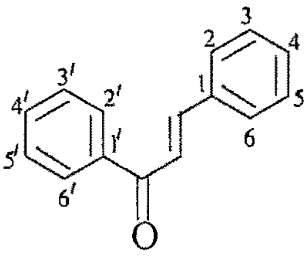
Synthesis of some chalcone epoxides

1.1 Discussion:

The world of nature abounds in organic compounds of every conceivable structural classes. The cells of living organisms, plants, fungi, bacteria, insects, other animals are the sites of intricate and complex biosyntheses that result in the fragmentation of many varieties of organic compounds, many of them of great practical importance to mankind. The structures of these naturally occurring compounds are often extremely complex and elucidation of their structures has been and continues to be a major challenge to organic chemists and biochemists alike. Structural and synthetic study of natural products constitutes one of the most fascinating and fruitful fields of study open to organic chemists.

1.2 Chalcones:

Benzylideneactophenones constitute a class of naturally occurring pigments, which are often referred to as 'Chalcones'. The term was first coined by kostanecki[1]. An interesting feature of chalcones (polyhydroxylated) is that they serve as the starting materials for the synthesis of another class of naturally occurring and widely distributed pigments called 'flavones'.

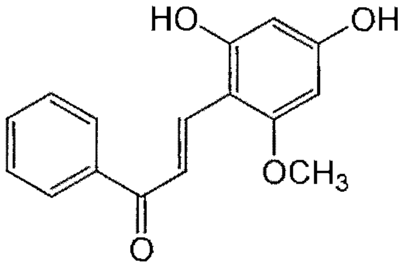
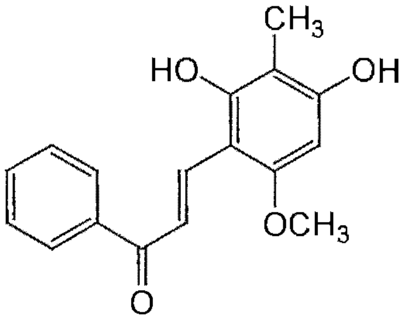
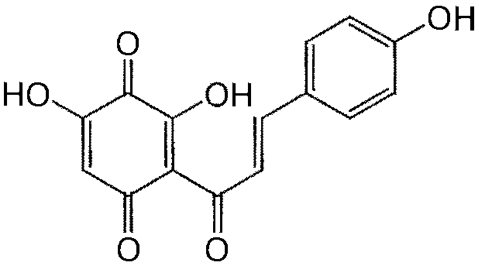
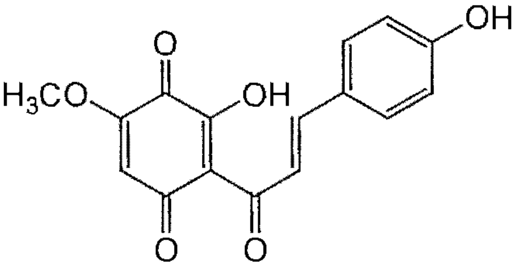


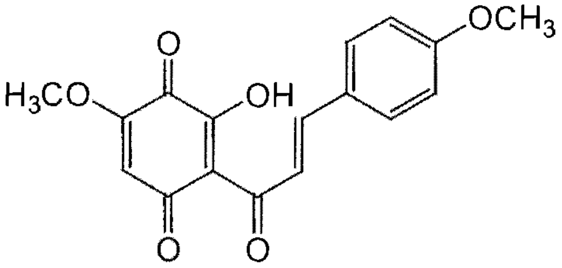
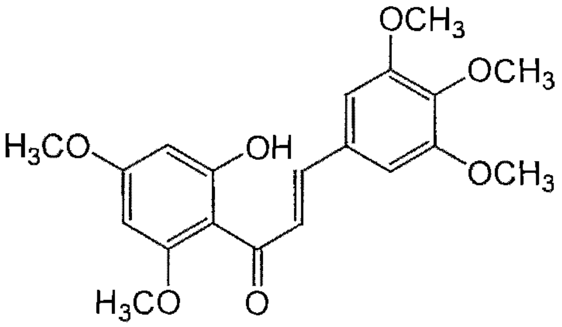
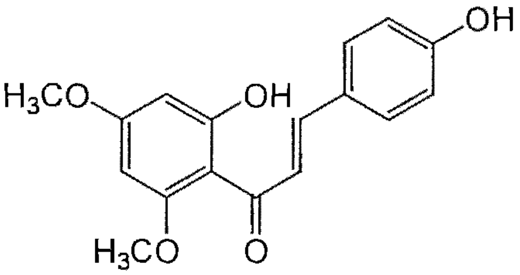
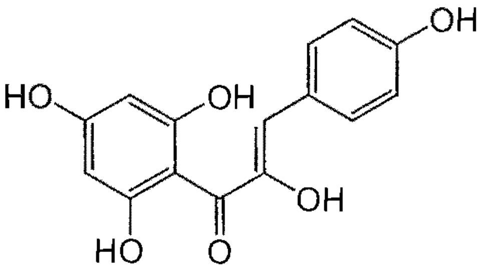
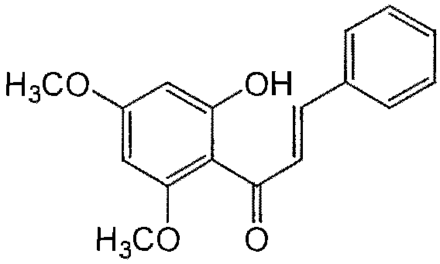
1

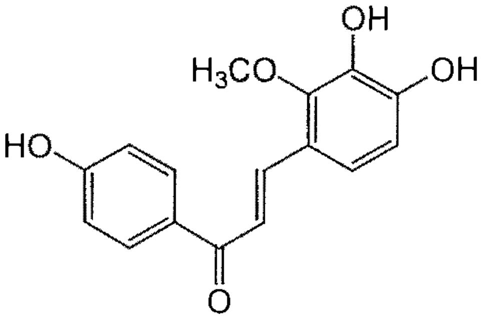
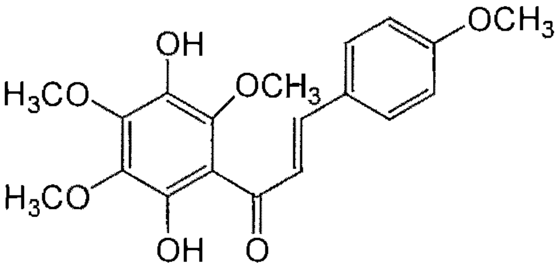
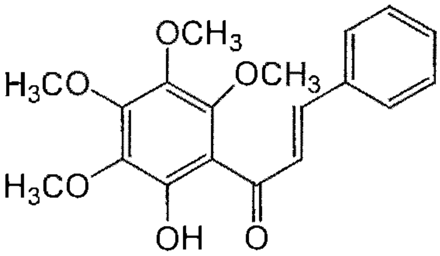
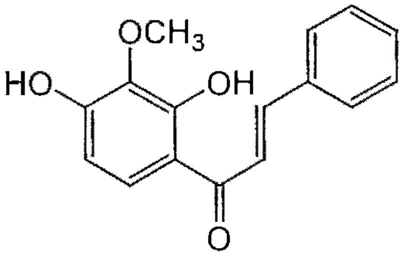
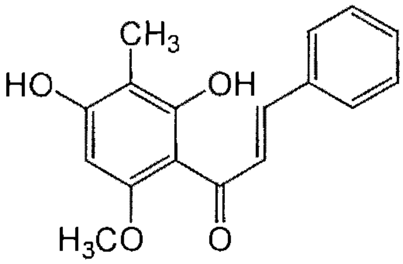
CHALCONE

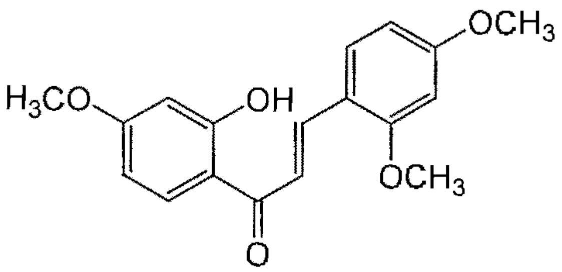
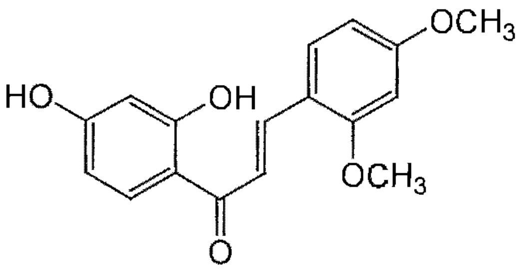
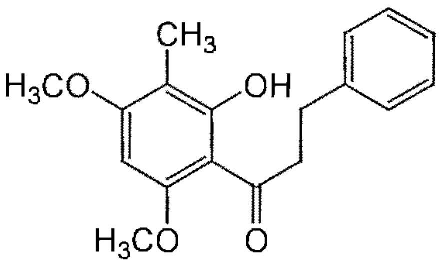
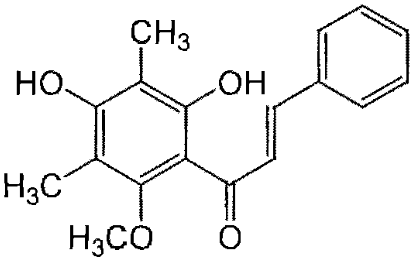
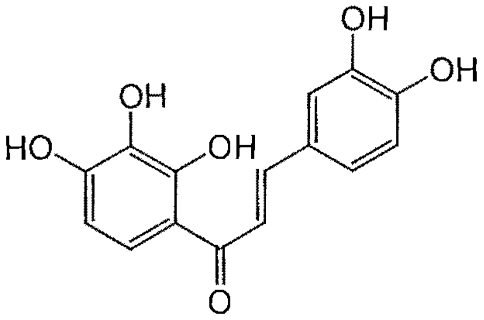
Chalcones form an important group of plant pigments because they are the precursors in the biosynthesis of flavonoids and are known to play an ecological role in nature in relation to colors of the leaves and flowers of plants. These bright yellow colored compounds are found most conspicuously in flowers. Naturally occurring chalcones are all hydroxylated to a greater or lesser extent. The list of naturally occurring chalcones given by Mabry *et al.*[2] can be supplemented with new chalcones which have been isolated recently as tabulated below.

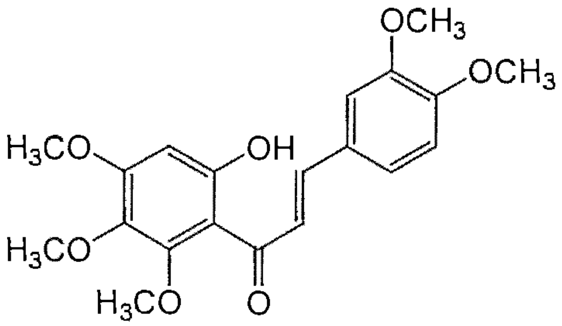
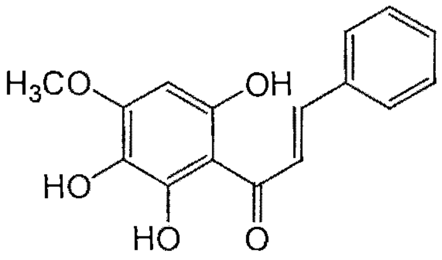
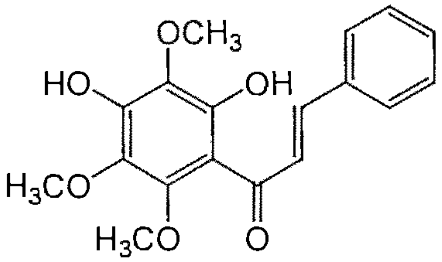
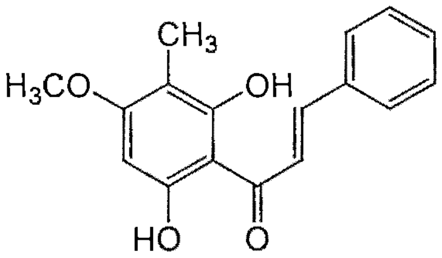
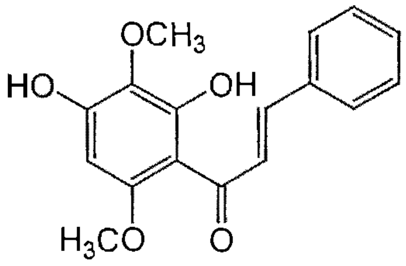
1.3 Naturally occurring chalcones:

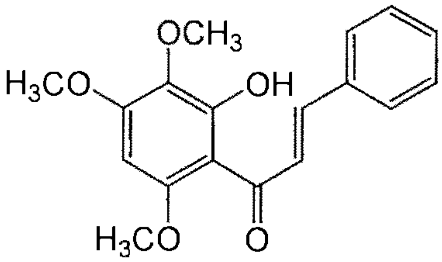
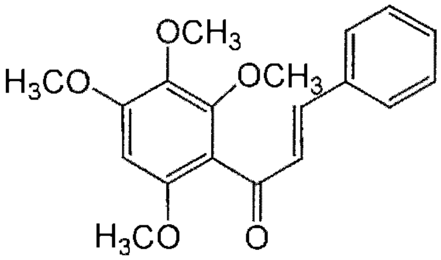
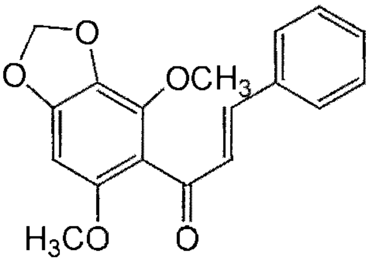
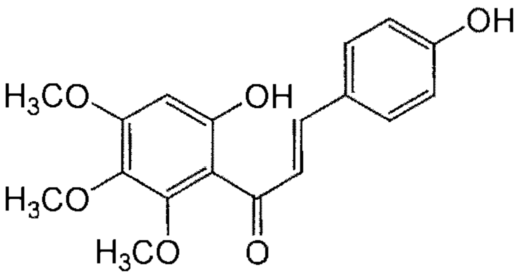
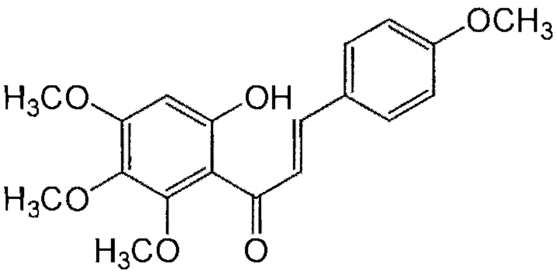
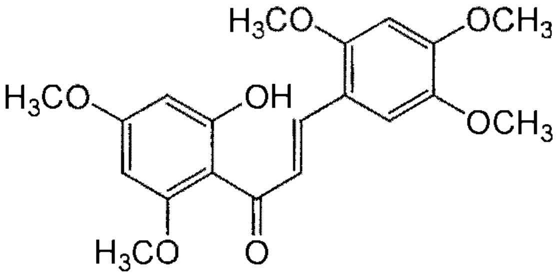
Name & Sl. No.	Structure	Source	Reference
2. 2,4-Dihydroxy-6-methoxychalcone.		Dragon's blood Resin.	Cardillo, *et al.*, (1971), *J. Chem. Soc.*, **C**, 3967.
3. 2,4-Dihydroxy-3-methyl-6-methoxychalcone.		*-do-*	-do-
4. Quinochalcone-A		*Carthamus tinctarius*	Obara, *et al.*, (1971), *J. Chem. Soc.*, 1357.
5.Quinochalcone-B		*-do-*	-do-

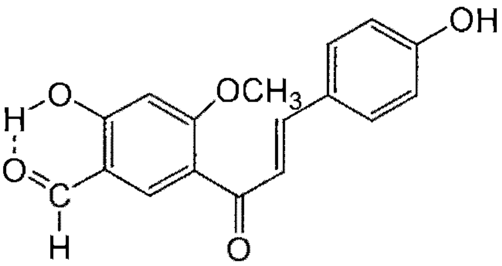
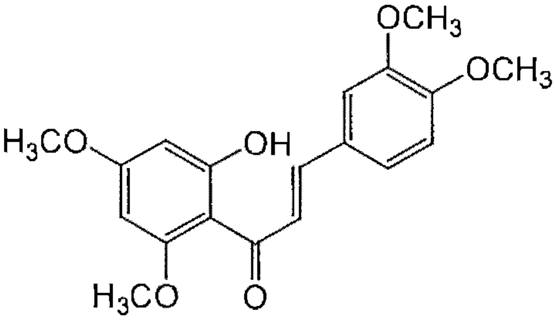
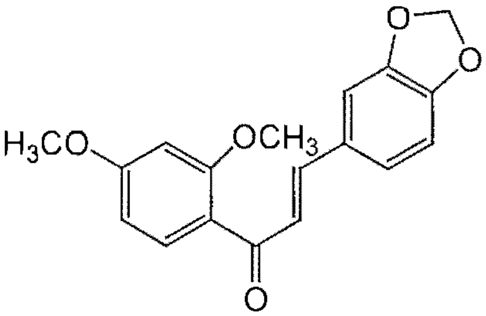
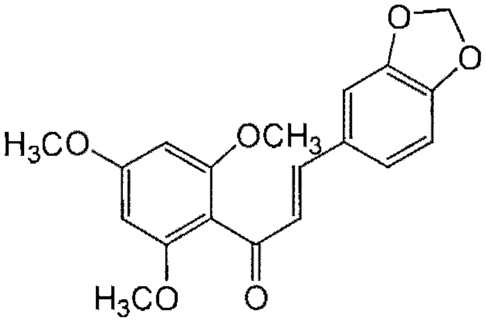
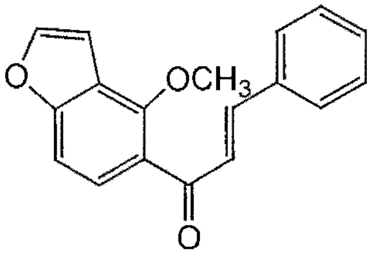
Name & Sl. No.	Structure	Source	Reference
6.Quinochalcone –C		*Carthamus tinctarius*	Obara, *et al.*, (1971), *J. Chem. Soc.*, 1357.
7. 2′-Hydroxy-3, 4,5,4′,6′-Pentamethoxychalcone.		*Merrillea caloxylon.*	Fraser, *et al.*, (1972), *Phytochemistry,* **11**, 868.
8.Flavokawain-C		*Piper methylsticum.*	Dutta, *et al.*, (1973), *Indian J.chem.* **11**,509
9. 2′,4′,6′,4,α-Pentahydroxy-chalcone.		*Berchemia zeyheri.*	Volsteedt, *et al.*, (1973), *Tet. Lett.*, 1001.
10. Pashanone		*Didymocarpus pedicellatus.*	Agarwal, *et al.* (1973), *Indian J.Chem.* **11**, 9.

Name & Sl. No.	Structure	Source	Reference
11. Licochalcone-B		*Glycyrrhiza glabra*	Saitoh, S.,*et al.*, (1975), *Tet. Lett.*,**50**,4461.
12.3′6′-Dihydroxy-2′,4′,5′,4-tetramethoxychalcone.		*Flemingia strobilifera.*	Bhatt, (1975), *Indian J. Chem.* **13**, 1105.
13. Kamakugiol		*Lindera erythrocarpa*	Liu, *et al.*, (1975), *J. Pharm. Soc.* (Japan*)*, **19**, 1114.
14. Lassein.		*Lassea nitida.*	Pederiva, *et al.*, (1975), *An. Assoc. Quim. Argent*; **63**,85.
15. Aurentiacin		*Didymocarpus pedicellatus.*	Aditya Chaudhury, *et al.*, (1976), *Phytochemistry* , **15**, 224.

Name & Sl. No.	Structure	Source	Reference
16. Cerasidin.		*Prunus cerasus.*	Parmer and Nagaranjan, (1977), *Phytochemistry,* **16** 1317.
17. Cerasin		-do-	-do-
18.2′-Hydroxy-4′.6′-dimethoxy-3′-methyldihydrochalcone		*Myrica gale*	Malterud, K. E., *et al.*, (1977), *Phytochemistry,* **16**, 1805.
19. 2′,4′-Dihydroxy-6′-methoxy-3′,5′-dimethylchalcone		*-do-*	-do-
20. 2′,3′,4′,3,4-Pentahydroxychalcone		*Albilizia adianthfolia*	Candy, *et al.*, (1978), *Phytochemistry,* **17**, 1807.

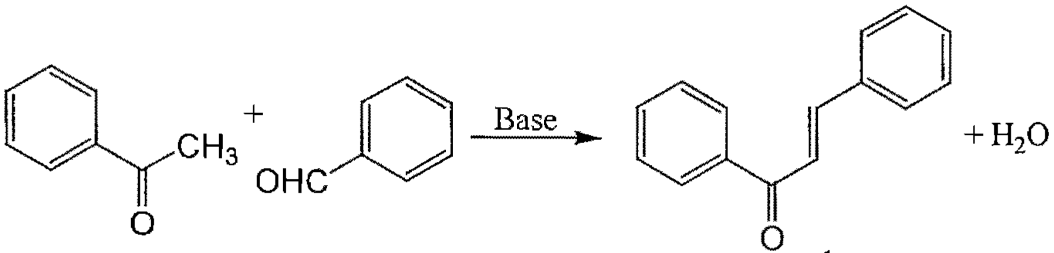
Name & Sl. No.	Structure	Source	Reference
21.2′-Hydroxy-4′,5′,6′,3,4-pentamethoxychalcone.		*Chromolaena odorata.*	Barua, *et al.*, (1978), *Phytochemistry,* 17, 1807.
22. Helilandin-B		*Helichrysum sutherlandii.*	Bohlmann, F., *et al.*, (1978), *Phytochemistry,* 17, 1935.
23. Isodidymocarpin		*Didymocarpus pedicellata.*	Bose and Aditya Chaudhury, (1978), *J. Indian Chem. Soc.*, 55, 1198.
24. Triangularin		*Pityrogramma triangularis.*	Mabry, *et al.*, (1978), *Phytochemistry,* 17, 586.
25.2′,4′-Dihydroxy-3′,6′-dimethoxychalcone		*Polygonum senegalense.*	Maradufa and Ouma, (1978), *Phytochemsitry*, 17, 580.

Name & Sl. No.	Structure	Source	Reference
26.2′-Hydroxy-3′,4′,6′-trimethoxychalcone		*Popowia cauliflora.*	Panichpol and Watermann, (1978),*Phytochemistry,* **17**, 1363.
27. 2′,3′,4′,6′-Tetramethoxychalcone		*-do-*	-do-
28. Helilandin-A		*Helichrysum sutherlandii*	Bohlmann, F., *et al.*, (1978), Phytochemsitry , **17**, 1935.
29. 2′,4-Dihydroxy-4′,5′,6′-trimethoxychalcone.		*Chromolaena*	Barua, *et al*, (1978), *Phytochemistry,* **17**, 1807.
30. 2′-Hydroxy-4,4′,5′,6′-tetramethoxychalcone.		*-do-*	-do- .
31.2′-Hydroxy-2,4,5,4′,6′-Pentamethoxychalcone.		*Derris robusta*	Chibber, S.S., *et al.*, (1979), *Phytochemsitry*, **18**, 2056.

Name & Sl. No.	Structure	Source	Reference
32. Isonebarachalcone.		*Psoralea corylifolia.*	Gupta, *et al.*, (1980), *Phytochemsitry,* 19(9), 2304.
33.2′-Hydroxy-4′,6′,3,4,-tetramethoxychalcone		*Pongamia pinata*	Tanaka, T. *et al.*, (1992), Phytochemsitry, **21** (3), 998.
34. 2′,4′-Dimethoxy-3, 4-methylenedioxychalcone		*-do-*	-do-
35. 2′,4′,6′-Trimethoxy-3,4-methylenedioxychalcone		*-do-*	-do-
36.2′-Methoxyfurano-[2″,3″: 4′,3′]-chalcone		*-do-*	-do-

1.4 General methods for the synthesis of chalcones:

The synthesis of chalcone, the parent member of the series, has been accomplished in a variety of ways, but perhaps the simplest method is the one involving the Claisen-Schmidt reaction. This is the reaction of acetophenone with benzaldehyde in the presence of aqueous alkali or sodium ethylate, resulting in the formation of α, β- unsaturated ketone[3] (**1**).

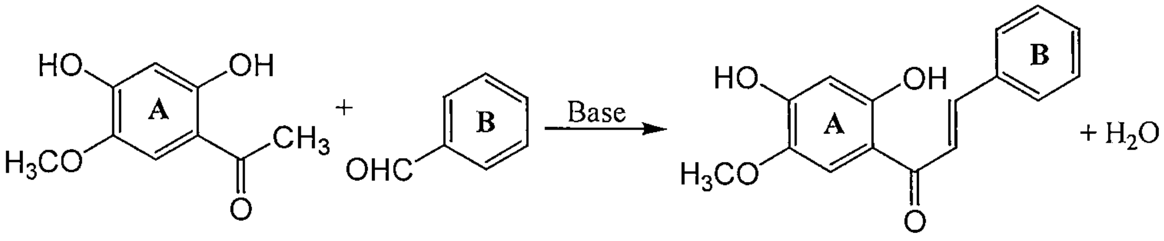


1

The substituted benzylideneacetophenones have likewise been obtained by condensing the appropriately substituted acetophenones with substituted benzaldehydes in the presence of alkali.

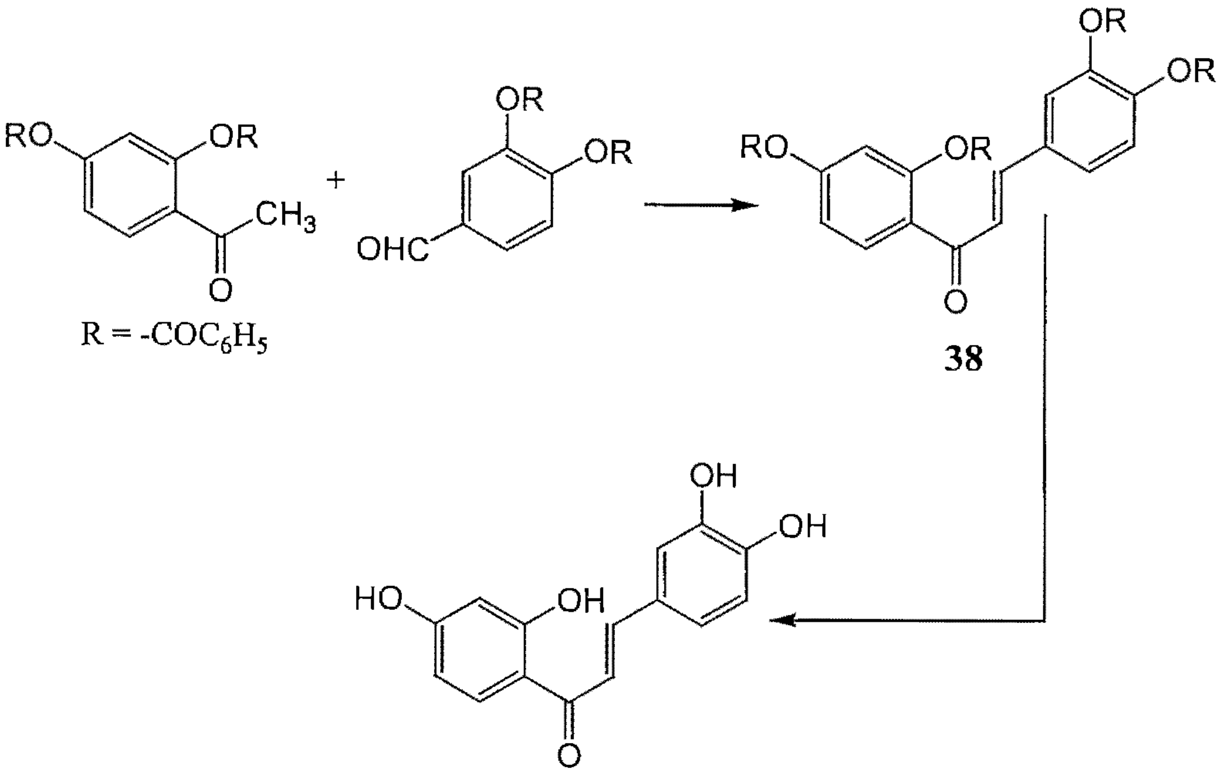
o-hydroxy acetophenones on acid[4] or base[5] catalyzed condensation with aldehydes yield either a chalcone or a flavanones or a mixture of both.

In Kostanecki's method[1], the starting materials are suitably substituted o-hydroxyacetophenone (furnishing A-ring) and benzaldehyde (furnishing B-ring) which are condensed in the presence of alcoholic costic potash to give chalcones. If only 2′-hydroxyl group of the chalcone is free, the condensation proceeds smoothly in 10% alcoholic costic potash in as high yield as 85% but in the case of compounds with a large number of free hydroxyl groups, higher concentration of alcoholic costic potash (50%-60%) is required. The yields are highest when alcoholic costic potash (50%-60%) is used at 0-20°C for 15-72 hours and when starting materials are methoxylated or benzylated. But for polyhydroxyacetophenones, good yields are obtained only when the condensation is performed at 0°C. Aditya Chaudhury *et al.*[6] synthesised flemichapparin **(37)** by the base catalyzed condensation of 2, 4-dihydroxy-5-methoxyacetophenone with benzaldehyde at 0-5°C for 8 days.



37

A modification of the kostanecki's method[1] was worked out by Russel *et al.*[7]. It involves the condensation of benzoylated derivatives of o-hyroxya- cetophenones with benzoylated derivative of *o*-hydroxy-benzaldhyde in the presence of dry hydrogenchloride at 0°C followed by hydrolysis with alkali to the free chalcone. Thus 2, 4-dibenzoyloxy-acetophenone condenses with dibenzoyloxyprotocatechuic aldehyde to give the tetrabenzoyloxycha- lcone **(38)** which on hydrolysis yields 2′,4′,3,4-tetrahydroxychalcone **(39)**.

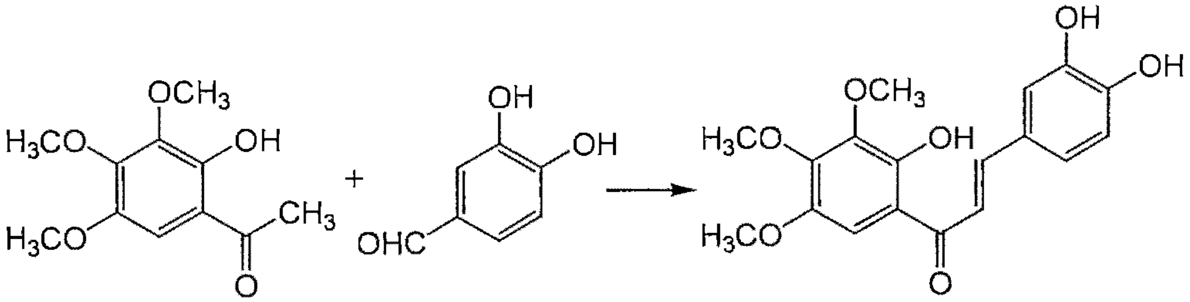


$R = -COC_6H_5$

38

39

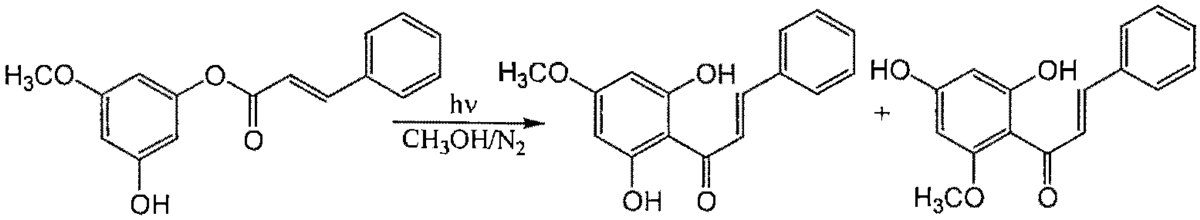
The synthesis of some chalcones has been accomplished by using sodium hydride as the base for the condensation. This method gives good yield even if the hydroxyl groups are not protected. Thus 2′,3,4-trihydroxy-3′,4′,5′-trimethoxychalcone(**40**) has been synthesized by Stout *et al.*[8] by using sodium hydride as the base.



40

Chalcones have also been synthesized by irradiating a solution of phenyl- cinnamate in methanol, with UV light (253 nm) for 1.5 hours under nitro- gen atmosphere. Synthesis of two isomeric chalcones **41** and **42** were reported by this method[9].

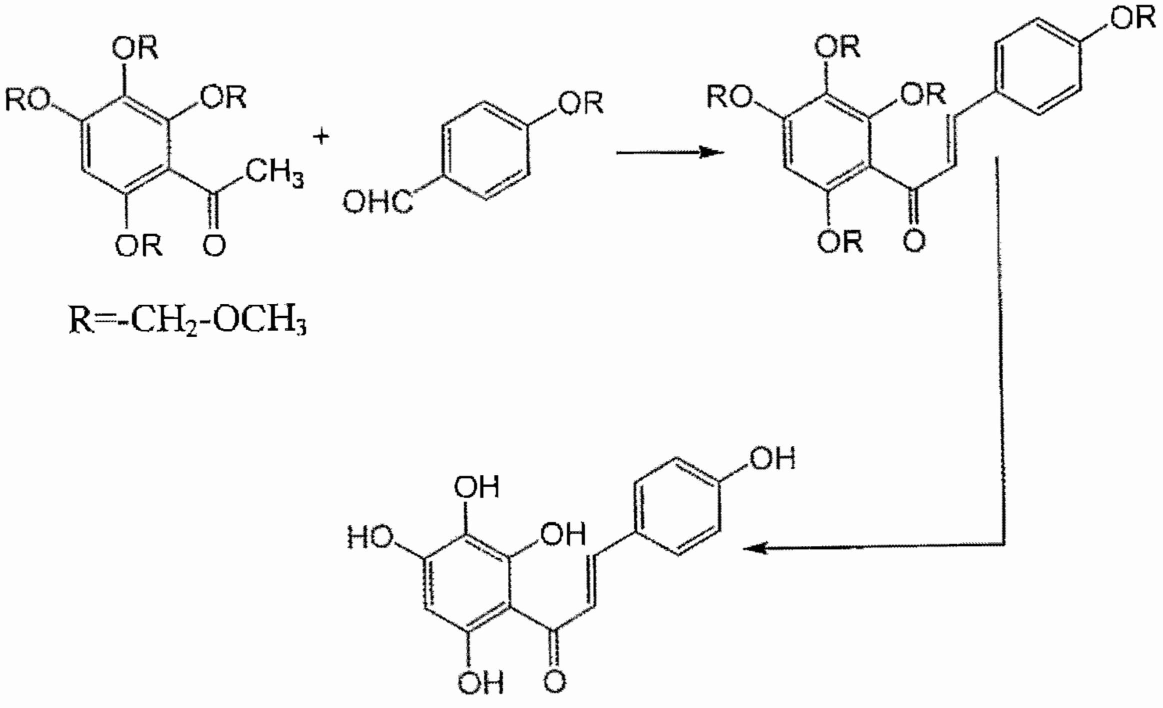
The synthesis of chalcones using organo phosphorus activated reagent[10] has been reported.



41 **42**

Lupi and co-workers[11] while synthesising various prenylated and chromeno chalcones under alkaline conditions have noticed the yield variation by changing the temperature, solvent and using different bases for condensation. Sodium hydroxide, sodium ethoxide and piperidine were used as bases in solvents like absolute alcohol, dry DMSO, anhydrous dioxane at a temperature varying between 25-100°C for a period ranging from 1 to 30 hours. The best yield was obtained when piperidine was used as a base in absolute alcohol for a period of 6 hours at a temperature between 60-70°C using a little excess of aldehyde than the stoichiometric amount.

Obara *et al.*[12] have reported the synthesis of 2′,3′,4′,4,6′-pentahydroxychalcone **(43)** in very good yield by the base catalyzed condensation of 2, 3, 4, 6-tetram- ethoxyacetophenone and methoxymethoxybenzaldehyde followed by demeth- oxymethylation using methanolic hydrochloric acid. Of the many methods available for protection of hydroxyl groups alkylation with methoxy methylchloride appeared to be attractive since excellent yields were obtained and the free hydroxychalcone could readily be obtained by heating in acid medium.

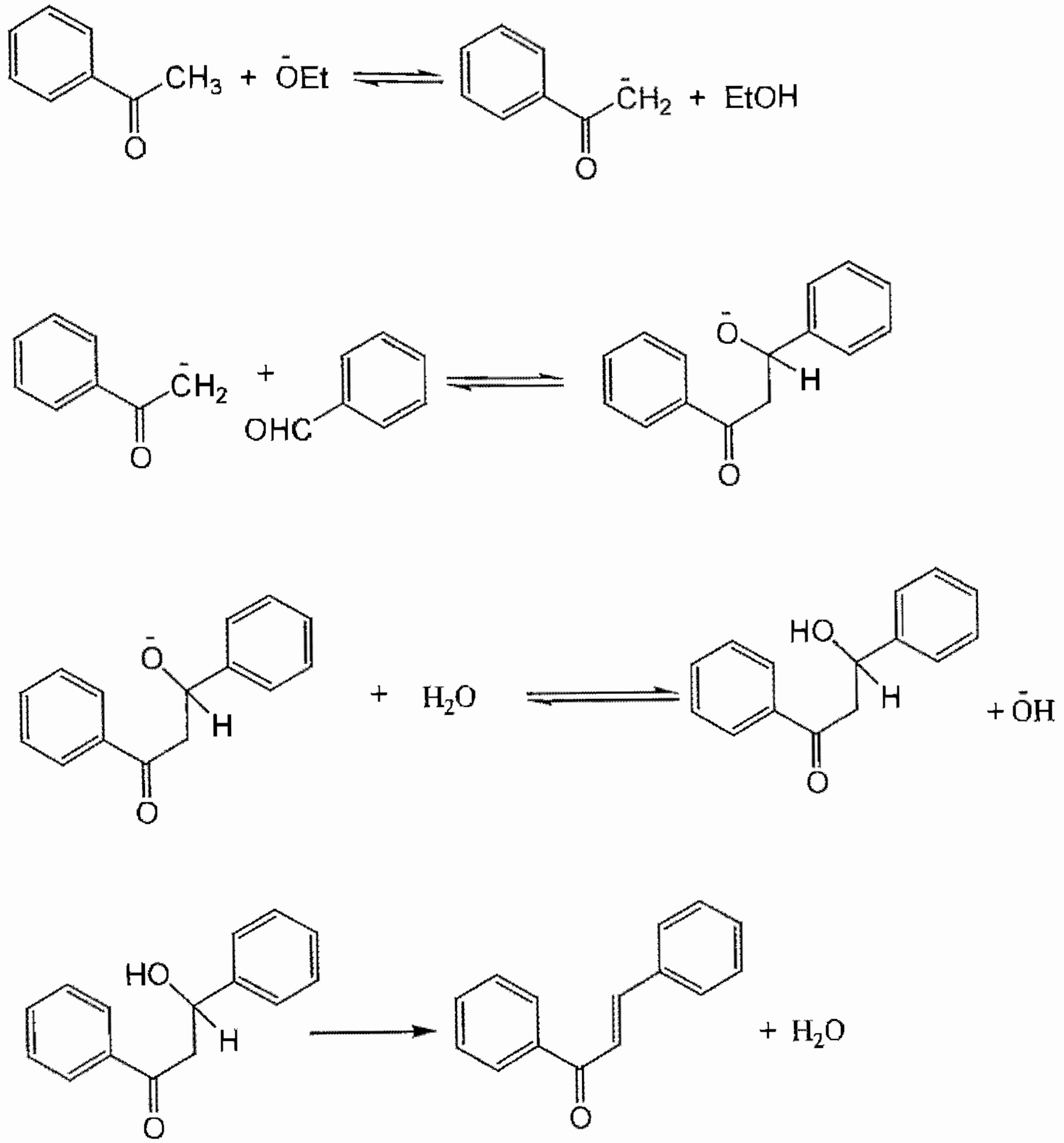


43

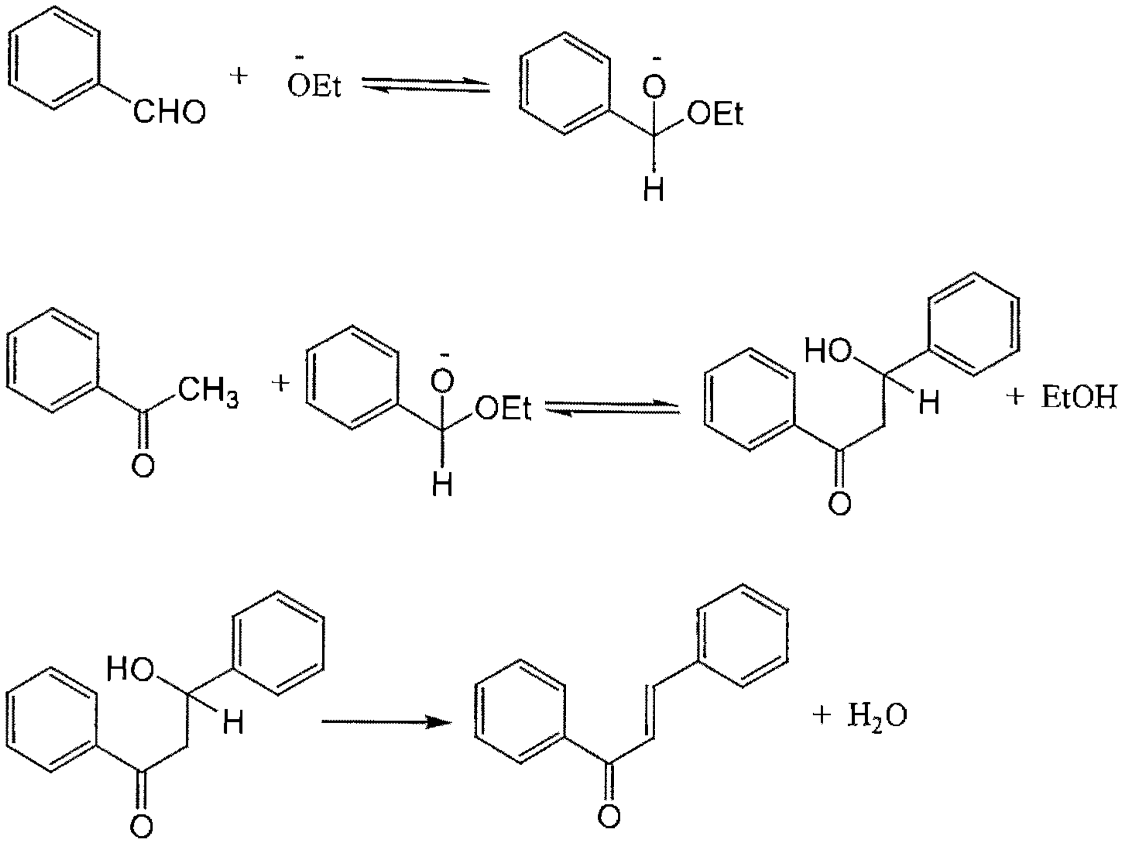
1.5 Mechanism of chalcone formation:

Kinetic studies have been reported for the base catalyzed formation of chalcone[13,14] and its derivatives[14,15]. Two alternative mechanisms have been advanced[16] for the reaction of benzaldehyde with acetophenone in the presence of basic catalyst.

Mechanism-I

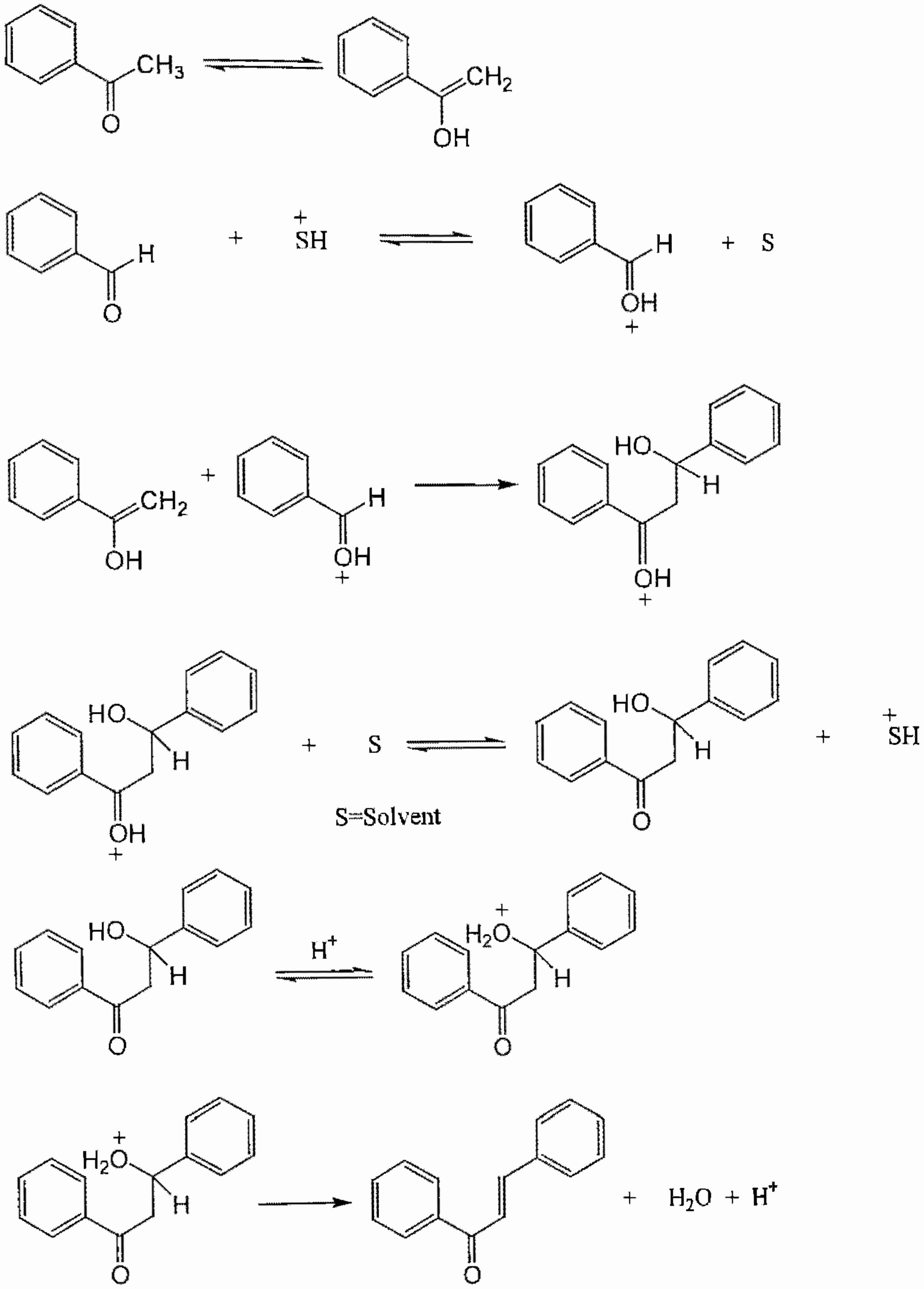


Mechanism-II



The formation of chalcone by the acid catalyzed condensation of acetophenone and benzaldhyde has been studied[17,18]. The rate of reaction is reported to depend on the first power of the concentration of acetophenone, the first power of the concentration of benzaldehyde, and the Hammett acidity function[17,18]. Also the concentration step has been shown to be the rate-determining step in this reaction. The following mechanism seems to be operable.

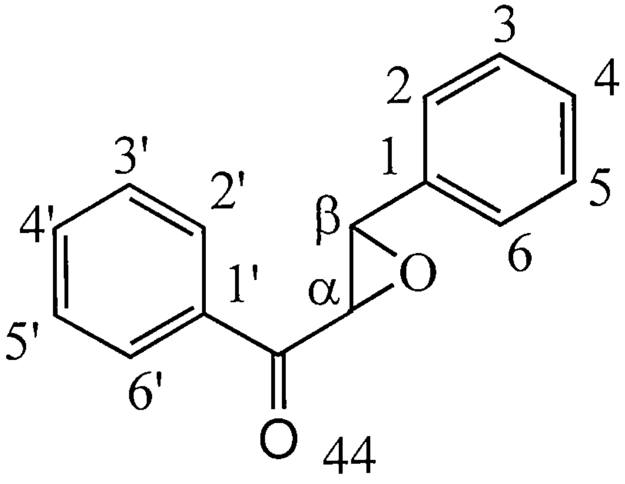
Mechanism :



1.6 Chalcone epoxides:

The chalcone epoxides which are also known as the anthoxanthins are white pigments which occur in the plant kingdom. Chalcone epoxides occur naturally in the free state or as glycoside or associated with tannins. Chemically the chalcone epoxides are very closely related to the anthocyanins, the chalcone epoxides are oxigenative derivatives of chalcones which may be partially alkylated. In almost all casses the positions 2, 3, 4, 5 and 6 are hydrodxylated and frequently one or more of the positions 3′,4′and 5′. The positions 2, 3, 5, 6 and 2′ are generally unmethylated but 3′ and 4′ are often methylated.

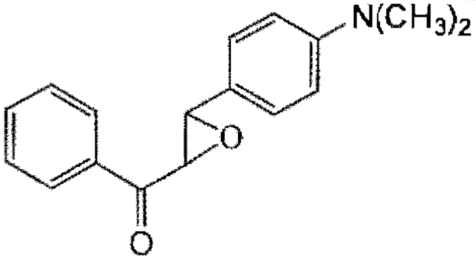
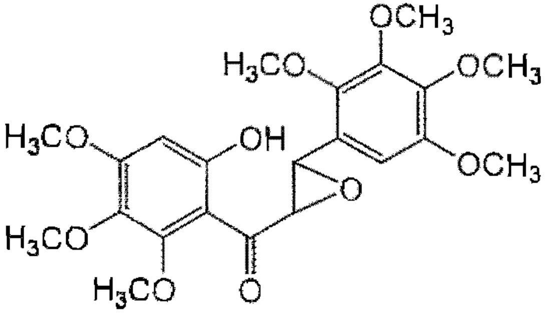
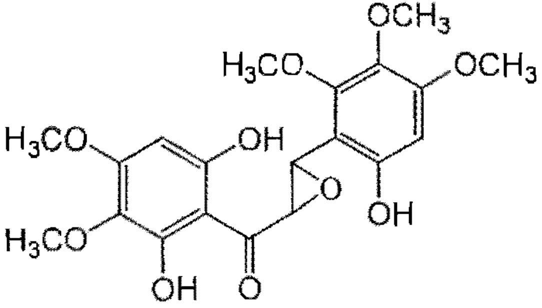
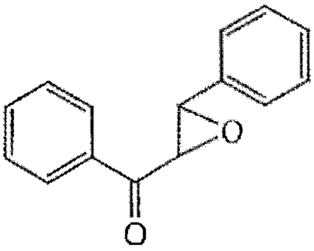
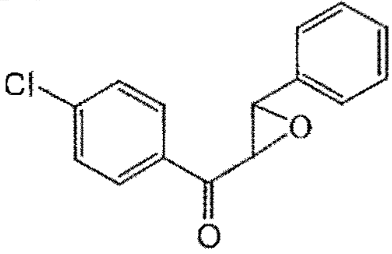
Since this section deals with the synthesis of chalcone epoxides, a brief list of naturally occurring and synthesized chalcone epoxides has been given.

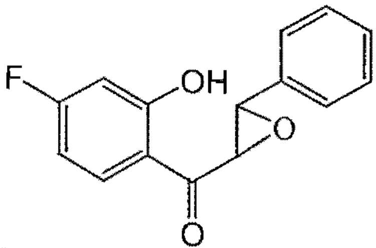
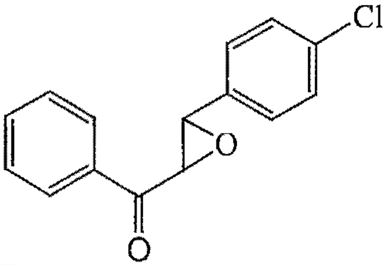
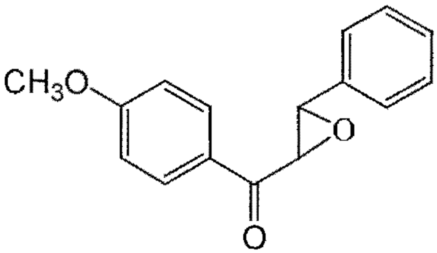
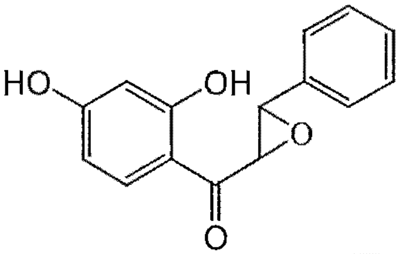
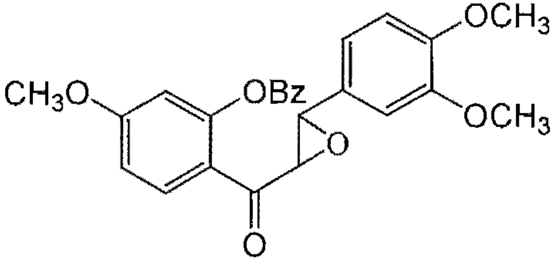
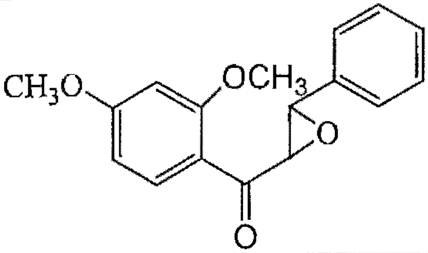


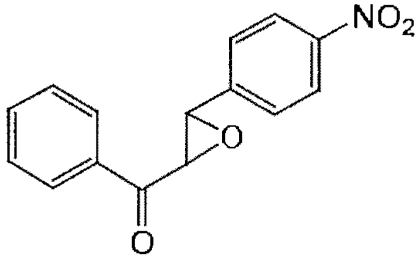
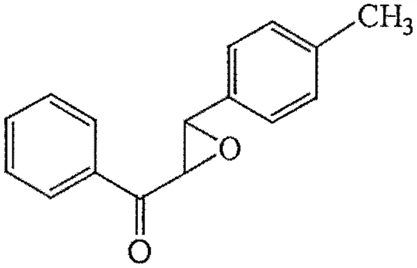
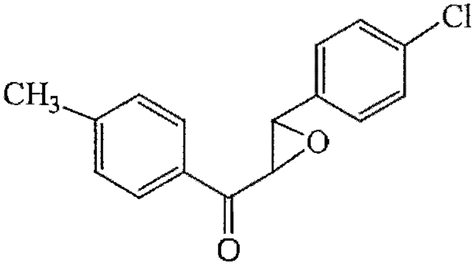
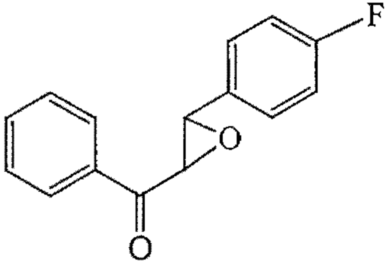
44

Chalcone epoxide

1.7 Naturally occurring and synthesized chalcone epoxides:

Name & Sl. No.	Structure	Reference
45. 4-Dimethylamino-chalcone epoxide		-do-
46. 2′-Hydroxy-4′,5′,6′,2,3,4,5,6-Heptamethoxychalcone epoxide		-do-
47. 2′,4′,6-trihydroxy-4′,5′,2,3,4-Penta-methoxychalcone epoxide		Malhotra, S., Sharma, V. K, (Late) Gupta, S. R. and Parmar, V. S., (1987), *Ind. J. Chem.*, 26B., 60.
48. Chalcone epoxide		-do-
49. 2′-Chloro chalcone epoxide		-do-

Name & Sl. No.	Structure	Reference
50. 2′-Hydroxy-4′-floro chalcone epoxide		Lin *et al.*, (1958), *J. Chinese Chem. Soc.*, (Taiwan), **5**, 60.
51. 4-chlorochalcone epoxide		-do-
52. 4′-Methoxychalcone epoxide		-do-
53. 2′,4′-Dihydroxychalcone epoxide		-do-
54. 2′-benzyloxy-3,4′,4-Trimethoxychalcone epoxide		-do-
55. 2′,4′-Dimethoxychalcone epoxide		-do-

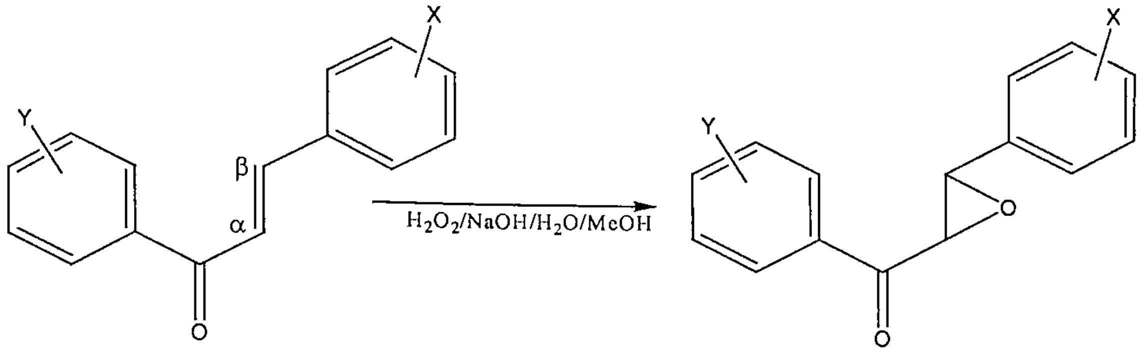
Name & Sl. No.	Structure	Reference
56. 4-Nitrochalcone epoxide		-do-
57. 4-methylchalcone epoxide		-do-
58. 4-Cloro-4′-methylchalcone epoxide		-do-
59. 4-Florochalcone epoxide		-do-

1.8 General methods for the synthesis of chalcone epoxides:

a) The above chalcone (101, 0.5g) was dissolved in methanol (40mL) and treated with aq. sodium hydroxide (1mL, 8%) and hydrogen peroxide (30%). It was shaken for 1 hr. and occasionally brought to its boiling point. It was kept aside overnight at room temperature. Water (50mL) was added and the solid that precipitated was filtered, washed and dried. When crystallized from methanol it separated as white needles.

b) 2.0 millimoles of the α,β–unsaturated ketone (chalcone) is dissolved in 2.0mL of methanol in a 10mL round bottom flask equipped with a magnetic stirring bar. 0.60mL of 30% H_2O_2 is added with stirring and the flask is cooled to 15-20°C in an ice-water bath[19]. 0.25mL of 4M NaOH is added dropwise with stirring over a period of 5 - 10 minutes. The reaction flask is removed from the cooling bath and stirred for another 15 minutes by which period a precipitate was formed. The reaction mixture is filtered and the filtrate washed with small amounts of cold water. The epoxy ketone (chalcone epoxide) is recrystallized from ethanol or ethanol/water.

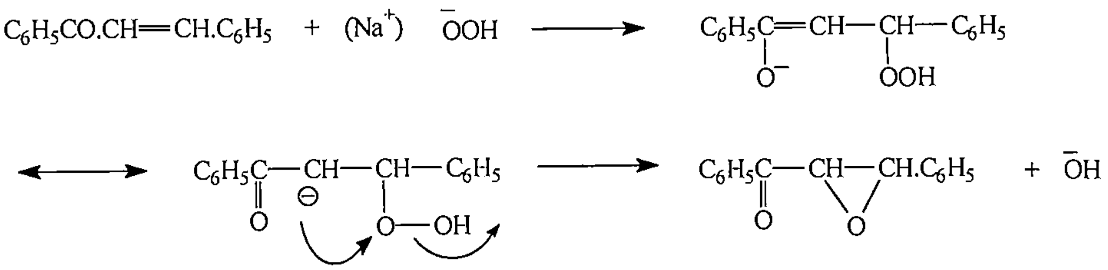
c) Place 3 g of benzalacetophenone (chalcone), 35mL of ethanol and 2.8mL of 30% hydrogen peroxide in an 100mL Erlenmeyer flask are mixed. The mixture is swirled and 6mL of 5% aqueous sodium hydroxide solution is added dropwise within 20 min. The reaction mixture is cooled so that the temperature does not rise above 30°C. After the addition of the alkali is completed, the mixture is allowed to stand for 10 min. Crystalline ppt (white) of chalcone epoxide is filtered, washed once with cold water and crystallized the crude product from a small volume of ethanol.



trans Chalcone epoxide :

In this experiment, the conversion of an α,β-unsaturated ketone (chalcone) to an epoxyketone using alkaline hydrogen peroxide is demonstrated. Note that an isolated olefinic bond undergoes epoxidation with peracids while the conjugated olefinic compounds (enones) form epoxides by sodium hydroperoxide (an anionic agent). The reaction is an example of 1,4-addition.

Mechanism of chalcones epoxides formation:



$$C_6H_5CO.CH{=}CH.C_6H_5 + (Na^+)\ \bar{O}OH \longrightarrow \underset{O^-}{C_6H_5C}{=}CH{-}\underset{OOH}{CH}{-}C_6H_5$$

$$\longleftrightarrow \underset{O}{\overset{}{C_6H_5C}}{-}\underset{\ominus}{CH}{-}\underset{O-OH}{CH}{-}C_6H_5 \longrightarrow \underset{O}{C_6H_5C}{-}CH{-}CH.C_6H_5 + \bar{O}H$$

CHAPTER
II

Present Work

CHAPTER-II

Present Work

2.1 Synthesis of some chalcone epoxides:

During the last fifty years synthetic as well as isolation works on the flavonoids are being done throughout the world. During the last thirty years synthetic works on the flavonoids are in progress in our laboratory. We have already published a good number of papers on the synthesis of these types of compounds.

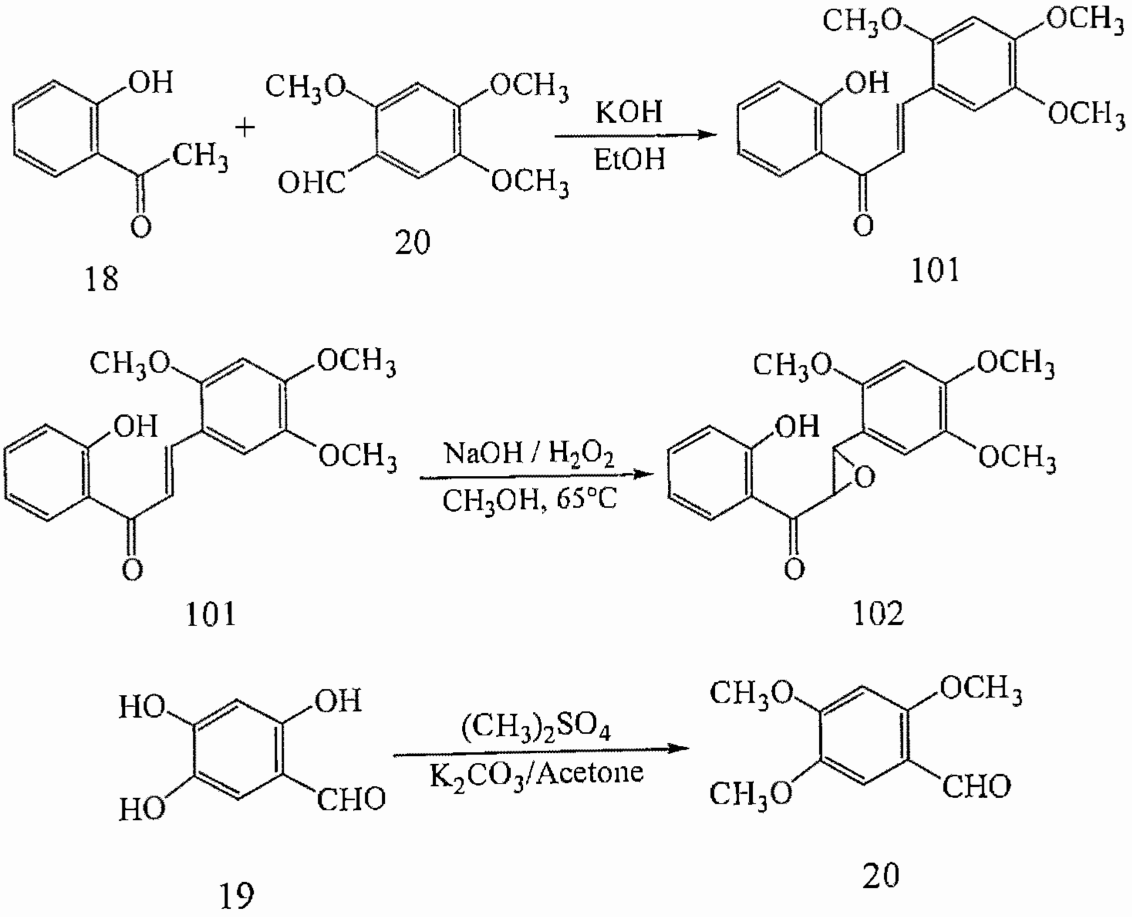
A large number of natural products including chalcone and chalcone epoxides are being reported in the literature every year and their structures need to be confirmed by synthesis. In the present chapter we describe the synthesis of 2′-hydroxy-2,4,5-trimethoxychalcone epoxide (**102**); 2′-benzyloxy-2,4,5-trimethoxychalcone epoxide (**104**); 4′-methyl-2-chlorochalcone epoxide (**106**); 4′-methyl-4-chlorochalcone epoxide (**108**); 2′-hydroxy-4-nitrochalcone epoxide (**110**); 2′-benzyloxy-4-methoxychaclone epoxide (**112**); 2′-hydroxy-3,4-methylenedioxy chalcone epoxide (**114**); 2′-benzyloxy-3,4-methylenedioxychalcone epoxide (**116**). The above chalcone epoxides were synthesized form their corresponding chalcones (**101, 103, 105, 107, 109, 111, 113 and 115**) using NaOH/H_2O_2 as an oxidizing agent.

The structures of the above compounds were assigned on the basis of spectral data together with their elemental analyses. The above chalcone epoxides were synthesized and tested for antimicrobial activities towards more human pathogenic bacteria and some plant pathogenic fungi.

2.1.1 Synthesis of 2′-hydroxy-2,4,5-trimethoxychalcone epoxide (102) by using NaOH / H_2O_2:

Alkaline condensation (Scheme-1) of 2-hydroxyacetophenone **(18)** and 2,4,5-trimethoxybenzaldehyde **(20)** gave the corresponding 2′-hydroxy-2,4,5-trimethoxychalcone **(101)**. Oxidation of this chalcone **(101)** with NaOH / H_2O_2 reagent furnished the corresponding 2′-hydroxy-2,4,5-trimethoxychalcone epoxide **(102)**. The constituent of the 2′-hydroxy-2,4,5-trimethoxychalcone epoxide was deduced on the basis of spectral data and elemental analysis. 2,4,5-trydroxybenzaldehyde **(19)** on methylation with dimethyl sulphate yielded 2,4,5-trimethoxybenzaldehyde **(20)**

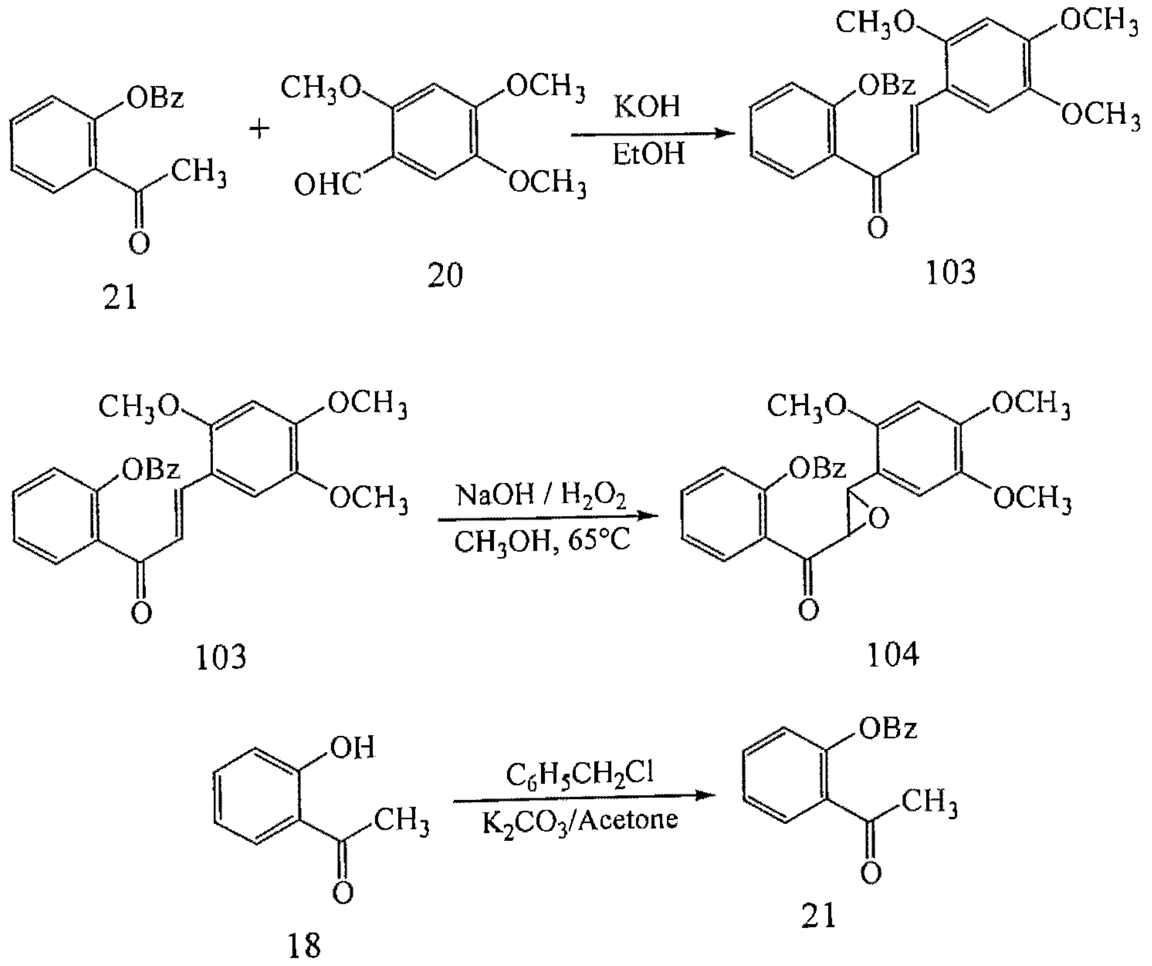
Scheme-1



2.1.2 Synthesis of 2′-benzyloxy-2,4,5-trimethoxychalcone epoxide (102) by using NaOH / H_2O_2:

Alkaline condensation of 2-benzyloxyacetophenone **(21)** and 2,4,5-trimethoxybenzaldehyde **(20)** gave the corresponding 2′-benzyloxy-2,4,5-trimethoxychalcone **(103)**. Oxidation of this chalcone **(103)** with NaOH / H_2O_2 reagent furnished the corresponding 2′-benzyloxy-2,4,5-trimethoxychalcone epoxide **(104)**. The constituent of the 2′-benzyloxy-2,4,5-trimethoxychlocone epoxide **(104)** was deduced on the basis of spectral data and elemental analysis. 2-hydroxyacetophenone **(18)** on treatment with benzyl chloride gave one product (Scheme-2) viz; 2-benzyloxyacetophenone **(21)**.

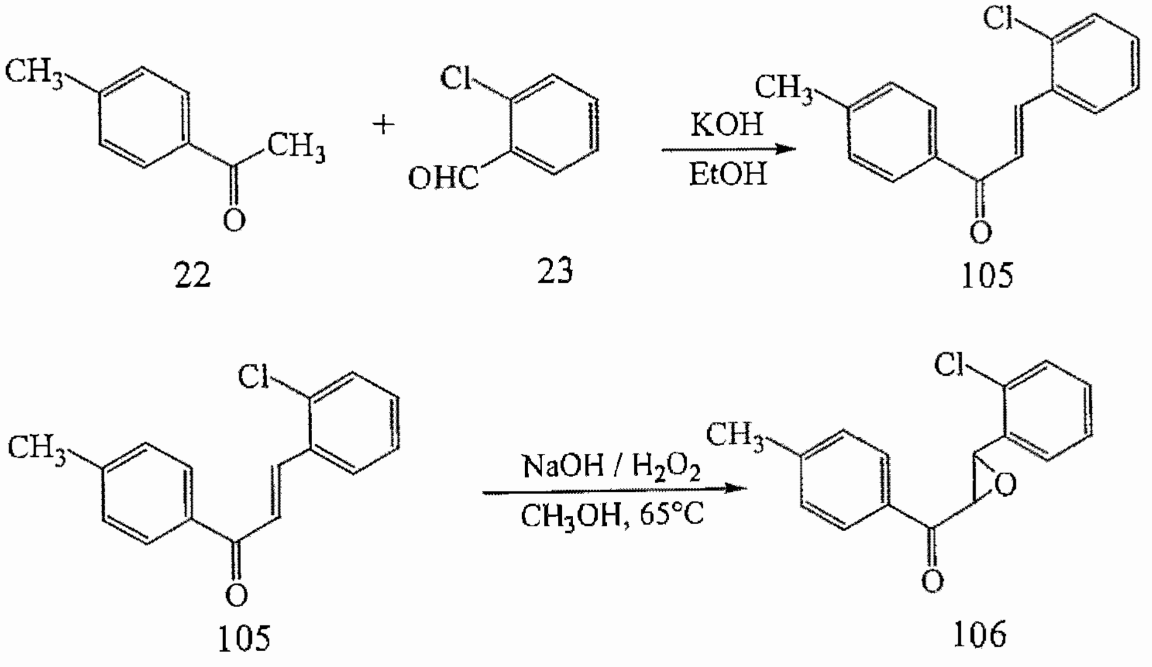
Scheme-2



2.1.3 Synthesis of 4′-methyl-2-chlorochalcone epoxide (106) by using NaOH / H_2O_2:

Cross aldol condensation (Scheme-3) of 4-methylacetophenone (**22**) and 2-chlorobenzaldehyde (**23**) under alkaline condition produced the corresponding 4′-methyl-2-chlorochalcone (**105**). Oxidation of this chalcone (**105**) using NaOH / H_2O_2 as an oxidizing agent furnished the corresponding 4′-methyl-2-chlorochalcone epoxide (**106**). The constituent of 4′-methyl-2-chlorochalcone epoxide (**106**) was deduced on the bases of spectral data i.e. UV, IR, ^{1}H-NMR and ^{13}C-NMR and elemental analysis.

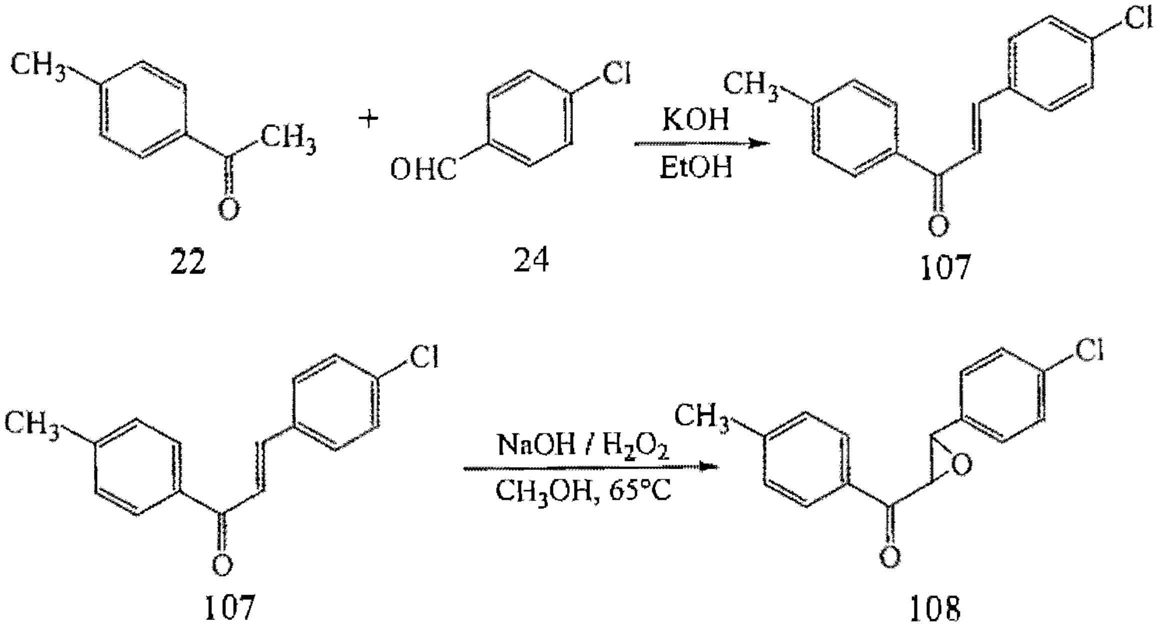
Scheme-3



2.1.4 Synthesis of 4′-methyl-4-chlorochalcone epoxide (108) by using NaOH / H_2O_2:

Cross aldol condensation (Scheme-4) of 4-methylacetophenone (**22**) and 4-chlorobenzaldehyde (**24**) under alkaline condition produced the corresponding 4′-methyl-4-chlorochalcone (**107**). Oxidation of this chalcone (**107**) using NaOH / H_2O_2 as an oxidizing agent furnished the corresponding 4′-methyl-4-chlorochalcone epoxide (**108**). The constituent of 4′-methyl-4-chlorochalcone epoxide (**108**) was deduced on the basis of spectral data i.e. UV, IR, ^{1}H-NMR and ^{13}C-NMR and elemental analysis.

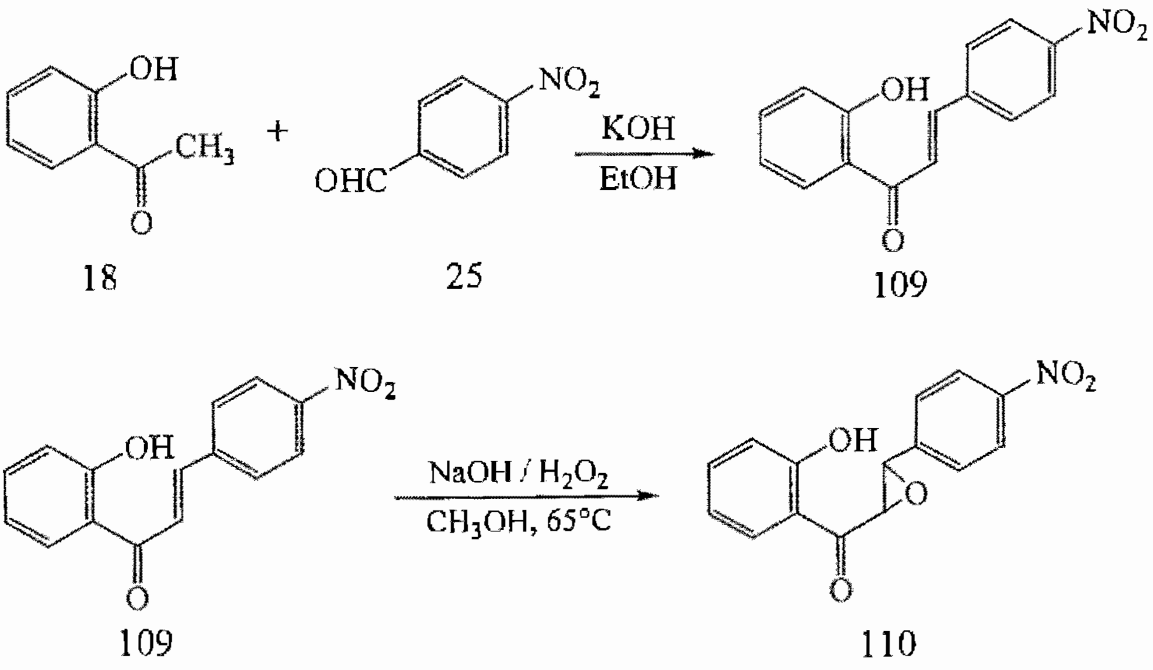
Scheme-4



2.1.5 Synthesis of 2′-Hydroxy-4-nitrochalcone epoxide (110) by using NaOH / H_2O_2:

Cross aldol condensation (Scheme-5) of 2-hydroxyacetophenone (**18**) and 4-nitrobenzaldehyde (**25**) under alkaline condition produced the corresponding 2′-hydroxy-4-nitrochalcone (**109**). Oxidation of this chalcone (**109**) using NaOH / H_2O_2 as an oxidizing agent furnished the corresponding 2′-hydroxy-4-nitrochalcone epoxide (**110**). The constituent of 2′-hydroxy-4-nitrochalcone epoxide (**110**) was deduced on the basis of spectral data i.e. UV, IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis.

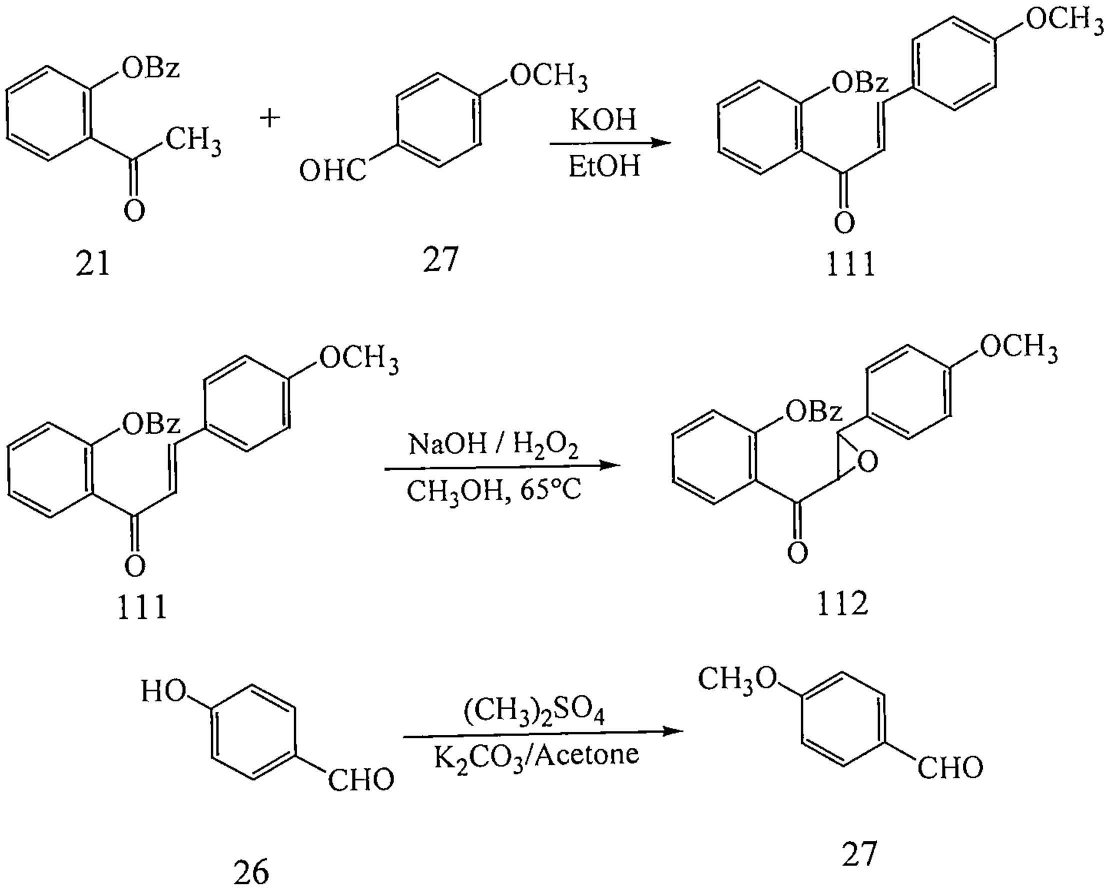
Scheme-5



2.1.6 Synthesis of 2′-benzyloxy-4-methoxychalcone epoxide (112) by using NaOH / H_2O_2:

Alkaline condensation of 2-benzyloxyacetophenone **(21)** and, 4-methoxybenzaldehyde **(27)** gave the corresponding 2′-benzyloxy-4-methoxychalcone **(111)**. Oxidation of this chalcone **(111)** with NaOH / H_2O_2 reagent furnished the corresponding 2′-benzyloxy-4-methoxy chalcone epoxide **(112)**. The constituent of the 2′-benzyloxy-4-methoxychalcone epoxide **(112)** was deduced on the basis of spectral data i.e. UV, IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis. 4-hydroxybenzaldehyde **(26)** on methylation with dimethyl sulphate

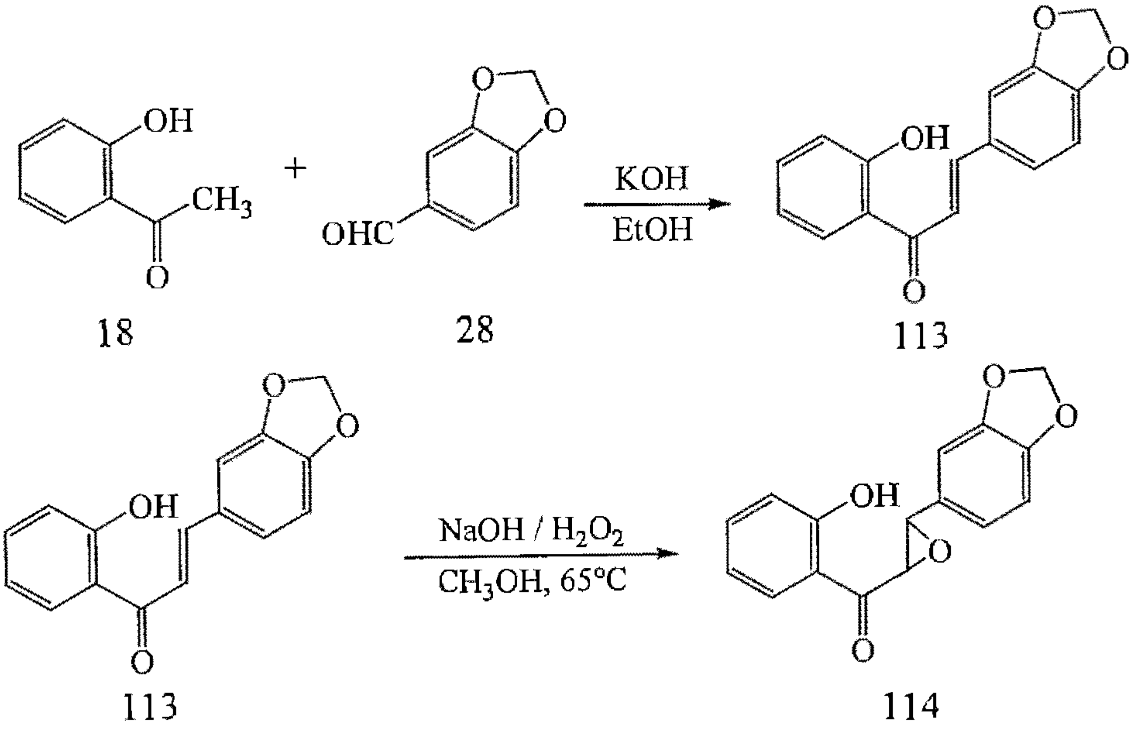
Scheme-6



2.1.7 Synthesis of 2′-hydroxy-3,4-methylenedioxychalcone epoxide (114) by using NaOH /H_2O_2:

Cross aldol condensation of 2-hydroxyacetophenone (**18**) and 3,4-methylenedioxybenzaldehyde (**28**) under alkaline condition produced the corresponding 2′-hydroxy-3,4-methylenedioxychalcone (**113**). Oxidation of this chalcone (**113**) using NaOH / H_2O_2 as an oxidizind agent furnished the corresponding 2′-hydroxy-3,4-methylenedioxychalcone epoxide (**114**). The constituent of 2′-hyroxy-3,4-methylenedioxychalcone epoxide (**114**) was deduced on the basis of spectral data i.e. UV, IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis.

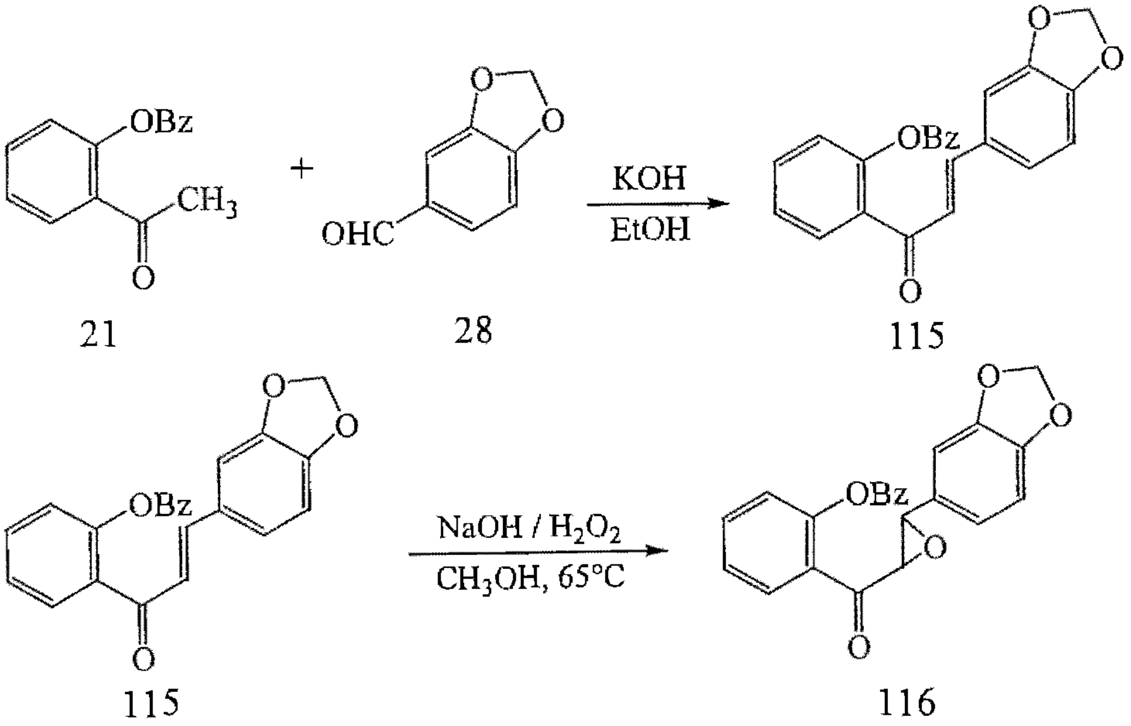
Scheme-7



2.1.8 Synthesis of 2′-benzyloxy-3,4-methylenedioxy chalcone epoxide (116) by using NaOH / H_2O_2:

Alkaline condensation of 2-benzyloxyacetophenone **(21)** and 3,4-methylenedioxybenaldehyde **(28)** gave the corresponding 2′-benzyloxy-3,4-methylenedioxychalcone **(115)**. Oxidation of this chalcone **(115)** with NaOH / H_2O_2 reagent furnished corresponding 2′-benzyloxy-3,4-methylenedioxychalcone epoxide **(116)**. The constituent of the 2′-benzyloxy-3,4-methylenedioxychalcone epoxide **(116)** was deduced on the basis of spectral data i.e. UV, IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis.

Scheme-8



CHAPTER
III

Experimental

CHAPTER-III

Experimental

3.1 Section-A (General Methods):

3.1.1 Melting point:

The melting points of the compounds were recorded on an electrothermal melting point apparatus (Gallenkamp). Care was taken to ensure that the heating was done at a steady rate. The melting points recorded and incorporated in this thesis are uncorrected.

3.1.2 Spectroscopic measurements:

Infrared spectra:

Infrared spectra were recorded on a DR-8400; SHIMADZU using KBr pellets for solids and neat for liquids in the College of Pharmacy, Yeungnum University, Gyeongsan 712749, South Korea and the characteristic peaks are expressed in cm^{-1}.

UV spectra:

UV spectra (λ_{max} in nm) were record on a UV-180, SMIMADZU, Double beam Spectrophotometer in the Department of Biochemistry and Molecular Biology, Rajshahi University, Rajshahi, Bangladesh.

^{1}H NMR spectra:

^{1}H NMR spectra were recorded on 'BRUKER 250 MHz SPECTRO-METER' College of Pharmacy, Yeungnum University, Gyeongsan 712749, South Korea. The solvents used were deuterated chloroform ($CDCl_3$-d_6) and Chemical Shifts were quoted on the δ scale relative to tetramethyl silane (TMS, $\delta = 0$) as an internal standard.

3.1.3 Thin Layer Chromatography (TLC):

The technique of thin layer chromatography was extensively used to monitor the progress of the reaction. Thin layer chromatographic plates (0.25mm thickness) were prepared by spreading a layer of silica gel (60 GF_{254}, E. MERCK) on clean and thoroughly dried glass plates (2g gel was mixed with 4 mL of distilled water for each plate of size 5 cm × 20 cm). These were activated by drying at 110°C before use.

3.1.4 Development of thin layer chromatography:

i) Using iodine tank

The chromatograms were developed in iodine vapor by placing the plate in the iodine tank.

ii) Using UV light

The presence of the compound as a single spot or a mixture can be identified by UV light.

iii) Using Spraying Agent

The spots were appeared if the plate was spread with the following spraying reagents. 2% Ferric chloride solution in water is used as a spraying reagent. 50% H_2SO_4 is also used as a spraying reagent and heating the plate.

3.1.5 Preparative Thin Layer Chromatography (PTLC):

The preparative glass plates (20 cm × 20 cm) were cleaned and dried. The plates were coated with silica gel slurry (silica gel 60 GF_{254}, E. MERCK, 16g mixed with 32mL of distilled water) with the help of spreader to yield a coating of 0.5 mm thickness. The plates were left at room temperature until their surface become completely dry the plates were then heated at 120°C in an electrical oven before use to increase activation. The solutions of compounds to be purified were applied with special type of thin glass capillaries at about 3cm from bottom of the plates. After drying, the plates were then placed vertically with the spotted end placed downward in chromatographic tanks so that the spotted mark of the compounds remained above the solvent. The plates were removed from the tanks when the solvent front reached almost to the upper edge of the plates (1 cm far from the upper edge). The plates were usually developed in appropriate solvent and was subsequently dried in the air. The plates were then viewed under UV light or the sides of the plates were exposed to iodine vapour to locate the position of the compounds. The zones bearing the compounds were scratched off from the plates and subsequently extracted with suitable solvent.

3.1.6 Column chromatography:

Column chromatography is a very useful technique for the separation of pure compounds from its mixture. For column chromatography silica gel (Kisel gel-66, 70-230 mesh, ASTM MERCK) as absorbent and solvent like n-hexane, petroleum ether, benzene, acetone, ethyl acetate etc. at different proportions was used as eluent. The column was prepared by slurry method silica gel being the stationary phase. The column was thorough cleaned and rinsed with acetone and then dried. It was then clamped properly and rinsed with solvent used in the preparation of silica gel slurry and cotton plug was fitted at the bottom. The column was half filled with appropriate solvent and the slurry was then poured into it, so that the packing was compact and uniform. Air bubble was avoided by packing the column as quickly as possible. The column was allowed to settle for an hour.

The mixture of the compound was completely dried and then was dissolved in eluting solvent and was carefully placed on the surface of the column, and eluted with desired solvent system. In case of polar compounds, the mixture was dissolved in suitable polar solvents and absorbed in small amount of silica gel in a flask. The solvent was removed completely under vacuum by rotary evaporator from silica gel and this silica gel with absorbed material was put on the surface of the silica gel column. The compounds were then collected as distinct bands. The purity of each band was further checked by TLC examination.

3.1.7 Purification of the reagents and solvent:

The reagents and solvents were purified and dried before use where necessary, and the rest were used as such from the bottle. All solutions in water, immiscible solvents which had been contact with water were dried over anhydrous sodium sulphate and magnesium sulphate, prior to evaporation. Solvent was usually removed with the help of a vacuum rotary evaporator. Dry hydrogen chloride gas (HCl) was produced by pouring conc. H_2SO_4 over a mixture o sodium chloride and ammonium chloride (5:1) moistended with conc. HCl gas thus produced were dried by passing them through the troughs containing good quality of conc. H_2SO_4. Pet. ether, diethyl ether and benzene were dried by using sodium wire and / or phosphorus pentaoxide (P_2O_5). Acetone and dioxane were dried by keeping over anhydrous K_2CO_3 and distilling the solvents. The liquid reagents were sometimes distilled by following standard method before use.

Acetic Acid:

About 100mL of acetic acid was taken in a round bottomed flask and 50g of $CaCl_2$ (anhydrous) was added to it. After 6 hour then acetic acid was decanted and it was distilled and collected at reduced pressure by maintaining its b.p.

Acetone:

To 500mL of acetone anhydrous K_2CO_3 (150g) was added and it was kept overnight. Then acetone was decanted off from K_2CO_3 and distilled to get pure acetone and preserved in a air tight container.

Methanol:

To 500mL of methanol CaO (50g) was a added and it was kept overnight. Then methanol was decanted off from CaO and refluxed it for 5-6 hours using $CaCl_2$ guard tube. After reflux it was distilled and collected to a round-bottomed flask. A white cake of Mg-turnings was formed in an another round bottomed flask and the whole mass of methanol as added to it. Again it was refluxed for 4 hours distilled out and collected it into an airtight container.

Ethanol:

To 500mL of ethanol CaO (50g) was added and it was kept overnight. Then ethanol was decanted off from CaO and refluxed it 5-6 hours using $CaCl_2$ guard tube. After reflux it was distilled and collected to a round bottomed flask. A white cake of Mg-turnings was formed in an another round bottomed flask and then whole mass of ethanol was added to it. Again it was refluxed for 4 hours distilled out and collected it into an airtight container.

Petroleum ether:

To 500mL of petroleum ether anhydrous $CaCl_2$ (20g) was added and it was kept overnight. Then petroleum ether was decanted off from $CaCl_2$ and distilled and collected at 40-60°C in to an air tight container.

Chloroform ($CHCl_3$):

At first chloroform was shacked with concentrated sulphuric acid after this it was again shacked with distilled water to remove acid. The solvent was then kept in calcium chloride for two hours then it was distilled (b.p. 61°C).

Diethyl ether (Et_2O):

Metallic sodium wire was added in diethyl ether and the mixture was kept overnight and was distilled over P_2O_5 at 35°C.

3.1.8 Cleaning and drying of glassware:

All glassware was cleaned and for most purposes, dried before employed in preparative work in the laboratory. The glassware was washed with a commercial household washing powder, which does not scratch glass, e.g. "Vim, Trix etc." The washing powder was either introduced directly into the apparatus or moistened with a little water, or it was applied to the dirty surface with a wet test-tube brush, which has been dipped into the powder. The operation was repeated if necessary. Finally, the apparatus was thoroughly rinsed with distilled water.

The most widely used cleansing agent was the 'chromic acid' cleaning mixture. It was a mixture of chromic acid and concentrated sulphuric acid. It was prepared by the following way '5 g of potassium dichromate were dissolved in 5mL of water in a 250mL beaker 100 m of concentrated sulphuric acid were then added slowly with constant stirring. The temperature will rise to 70-80°C. The mixture was allowed to cool to about 40°C and then transferred to a dry glass stoppered bottle.' Before using chromic acid mixture for cleaning the vessel was rinsed with water to remove organic matter. Sometime chloroform, acetone, rectified spirit was used as a cleansing agent.

Small and bulky both types of glass apparatus were dried by leaving it in an electrically heated oven maintained at 100-120°C for one to two hours. Sometimes the glassware was also dried with a commercial hair drier.

3.2 Section-B:

3.2.1 Synthesis of some chalcone epoxides and determination of their structures:

Methylation of 2,4,5-trihydroxybenzaldehyde (19):

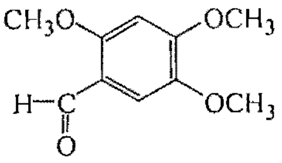
A mixture of 2,4,5-trihydroxybenzaldehyde (**19**, 3g), dimethylsulphate (2.5mL) and anhydrous potassium Carbonate (22.5g) in acetone (125mL) was refluxed for 3 hours. Acetone was removed by distillation and water (100mL) was added to the residue. The solution was acidified with dil HCl when a light yellow colored solid was precipitated out. It was collected and subjected to column Chromatography over silica gel. The elution was done with petroleum spirit-benzene (7:3). It crystallized from ethyl acetate-petroleum spirit as colorless needles (2.25 g), yield 75%, m.p. 92-95°C, R_f- 0.60 (benzene-acetone, 4:1).

Anal Found: C, 61.21; H, 6.16 Calc. for $C_{10}H_{12}O_4$; C, 61.61; H, 6.5%.

UV $\lambda_{max}^{CH_3OH}$ **:** 230 and 255 nm.

IR (KBr) ν_{max}**:** 3048, 2970, 2860, 1675, 1570, 1480, 1350, 1310, 1070, 1030, 880, 875, 760, 730, 690, 660, 630 cm^{-1}.

^{1}HNMR($CDCl_3$-d_6): δ 3.73 (s, 9H, C_2-OC$\underline{H}_3$, C_4-OC$\underline{H}_3$ and C_5-OC$\underline{H}_3$), 6.36 (s, 1H, C_3-$\underline{H}$), 7.10 (s, 1H, C_6-H), 10.24 (s, 1H, C_1-C$\underline{H}$O).



20

^{13}CNMR ($CDCl_3$-d_6): δ 56.0 (C_2-O$\underline{C}H_3$), 56.3 (C_4-O$\underline{C}H_3$ and C_2-O$\underline{C}H_3$), 101.2 (C_3), 115.6 (C_1), 117.3 (C_6), 140.4 (C_5), 154.4 (C_4), 156.5 (C_2), 190.0 (> C = O).

It was identified as 2,4,5-trimethoxybenzaldehyde (**20**)

Synthesis of 2′-hydroxy-2,4,5-trimethoxychalcone epoxide (102):

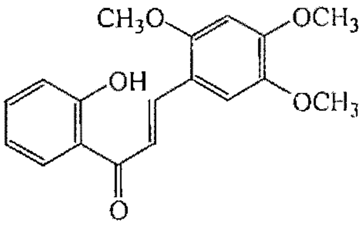
Synthesis of 2′-hydroxy-2,4,5-trimethoxychalcone (101):

A mixture of 2-hydroxyacetophenone (**18,** 10mmol, 1.36g) and 2,4,5-trimethoxybenzaldehyde (**20,** 1.1 eqv, 2.15g) in ethanolic solution of KOH (5% 15mL) was kept at room temperature for about 80 hours. The reaction mixture was diluted with ice cold water, acidified with cold dil. HCl and extracted with ether. The ether layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The reaction mixture was subjected to column chromatography over silica gel. The elution was done with acetone-n-hexane (1:3) and crystallized from petroleum spirit as yellow crystals (1.79g) yield 57% m.p. 120-122°C, R_f = 0.84 (benzene : acetone; 5:1).

Anal Found: C, 68.90; H, 5.52; Calc. for $C_{18}H_{18}O_5$; C, 68.78; H, 5.73%.

UV $\lambda_{max}^{CH_3OH}$: 235, 260 and 365 nm

IR (KBr) ν_{max}: 3450, 2938, 2842, 1677, 1609, 1549, 1474, 1413, 1339, 1283, 1187, 1135, 1062, 1004, 974, 871, 834, 819, 763, 737, 645 cm^{-1}.



101

^{1}HNMR ($CDCl_3$-d_6): δ 3.73 (s, 9H, C_2-$OC\underline{H}_3$, C_4-$OC\underline{H}_3$ and C_5-$OC\underline{H}_3$), 6.12 (s, 1H, C_3-H), 6.59 (s, 1H, C_6-H), 6.92 (d, 1H, J = 2.6 Hz, $C_{3'}$-H), 7.01 (m, 1H, $C_{5'}$-H), 7.37 (m, 1H $C_{4'}$-H), 7.39 (d, 1H, J = 16Hz, C_α-H), 7.64 (d, 1H, J = 8.6 Hz, $C_{6'}$-H), 8.17 (d, 1H, J = 16 Hz C_β-H), 13.08 (s, 1H, $C_{2'}$-$O\underline{H}$).

^{13}CNMR ($CDCl_3$-d_6): δ 56.3 (C_4-$O\underline{C}H_3$ and C_5-$O\underline{C}H_3$), 56.4 (C_2-$O\underline{C}H_3$), 100.6 (C_3), 113.8 (C_1 and C_6), 116.2 ($C_{3'}$), 121.6 ($C_{5'}$), 123.3 (C_α), 123.9 ($C_{1'}$), 131.1 ($C_{6'}$), 135.7 ($C_{4'}$), 139.8 (C_5), 142.8 (C_β), 147.8 (C_4), 153 (C_2), 158.5 ($C_{2'}$), 187 (>C=O).

It was identified as 2′-hydroxy-2,4,5-trimethoxychalcone (**101**).

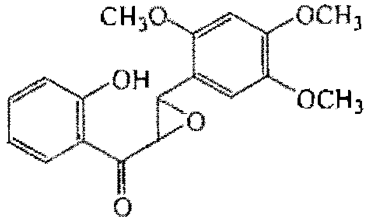
Synthesis of 2′-hydroxy-2,4,5-trimethoxy chalcone epoxide (102):

The above chalcone (**101**) 0.5g was dissolved in methanol (40mL) and treated with aq. sodium hydroxide (1mL, 8%) and hydrogen peroxide (30%). It was shaken for 1 hr. and occasionally brought to its boiling point. It was kept aside overnight at room temperature. Water (50mL) was added and the solid that precipitated was filtered, washed and dried. When crystallized from methanol it separated as white needles (376mg), yield 71.55% m.p. 133-135°C R_f 0.81 (benzene : acetone; 2:1)

Anal Found: C, 65.65; H, 5.20; Calc. for $C_{18}H_{18}O_6$; C, 65.45; H, 5.45%.

UV $\lambda_{max}^{CH_3OH}$ **:** 228, 255 and 361 nm.

IR (KBr) ν_{max}**:** 3433, 2882, 1704, 1579, 1512, 1489, 1424, 1361, 1304, 1271, 1193, 1134, 1033, 974, 908, 882, 811, 793, 761, 714, 639 cm^{-1}



102

^{1}HNMR ($CDCl_3$-d_6): 3.73 (s, 9H, C_2-OC$\underline{H}_3$, C_4-OC$\underline{H}_3$ and C_5-OC$\underline{H}_3$), 4.36 (d, 1H, J = 2 Hz, C_β-H), 4.43 (d, 1H, J = 2 Hz, C_α-H), 6.10 (s, 1H, C_3-H), 6.48 (s, 1H, C_6-H), 6.81 (d, 1H, J = 2.6 Hz, $C_{3'}$-H) 6.90 (m, 1H $C_{5'}$-H), 7.27 (m, 1H, $C_{4'}$-H), 7.72 (d, 1H, J = 2.6 Hz $C_{6'}$-H) 11.85 (s, 1H, $C_{2'}$-O$\underline{H}$).

^{13}CNMR ($CDCl_3$-d_6): 48.5 (C_β), 56.3 (C_2-O$\underline{C}H_3$, C_4-O$\underline{C}H_3$ and C_5-O$\underline{C}H_3$), 70 (C_α), 100.6 (C_3), 113 (C_6), 115.6 ($C_{3'}$), 116.6 (C_1), 121 ($C_{5'}$), 124.6 ($C_{1'}$), 130 ($C_{6'}$), 134.3 ($C_{4'}$), 139.8 (C_5), 148.1 (C_4), 152.2 (C_2), 157.4 ($C_{2'}$) 197.6 (>C=O).

It was identified as 2′-hydroxy-2,4,5-trimethoxychalcone epoxide (**102**).

Synthesis of 2′-benzyloxy-2,4,5-trimethoxychalcone epoxide (104)

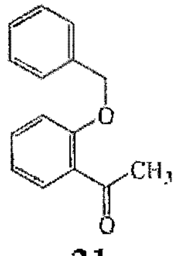
Benzylation of 2-hydroxyacetophenone (18)

A mixture of 2-hydroxyacetophenone (18, 10g), Benzylchloride (7.4mL) anhydrous potassium carbonaate (22g) and potassium iodide in acetone (250mL) was refluxed for 8 hr. Potassium salts were filtered off and the filtrate evaporated to dryness. The reaction mixture was subjected to column chromatography over silica gel. The elution was done with petroleum spirit-benzene (7:3), Petroleum spirit-benzene (1:4), benzene-ethyl acetate (9:1), when the following fractions labeled as I-III were obtained. 2-benzyloxyacetophenone (**21**) from fraction II as a pale yellow needles (4.9g) were obtained. Yield 50%, m.p. 112-114°C, R_f = 0.79 (benzene : acetone 9:1). There are no color with a alcoholic ferric chloride solution.

UV $\lambda_{max}^{CH_3OH}$: 242 and 293 nm.

IR (KBr) ν_{max}: 3043, 2970, 1647, 1560, 1458, 1360, 1320, 1254, 1164, 988, 830, 814, 745, 655, 613 cm^{-1}.

^{1}HNMR($CDCl_3$-d_6): δ 2.55 (s, 3H C_1-CO-C$\underline{H}_3$), 5.20 (s, 2H, C_2-OC$\underline{H}_2$-C_6H_5), 6.88 (d, 1H, C_3-H), 6.93 (m, 1H, C_5-H), 7.19 (s, 5H, C_2-OCH_2-$C_6\underline{H}_5$), 7.34 (m, 1H, C_4-H), 7.75 (d, 1H, C_6-H)



21

^{13}CNMR ($CDCl_3$-d_6): δ 23.1 (C_1-CO-C$\underline{H}_3$), 77.8 (C_2-OC$\underline{H}_2$-C_6H_5), 114 (C_3), 120.7 (C_5), 123 (C_1), 127.3 ($C_{2'}$ and $C_{6'}$), 127.4 ($C_{4'}$), 128.7 ($C_{3'}$ and $C_{5'}$) 129.6 (C_6) 133.9 (C_4) 140.9 ($C_{1'}$), 162.1 (C_2), 196.5 ($C_1\underline{C}OCH_{3'}$).

It was identified as 2-benzyloxyacetophenone (**21**)

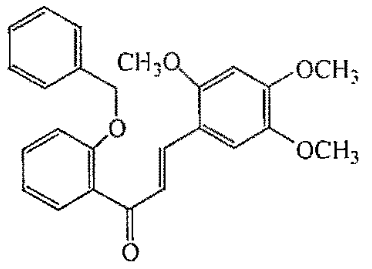
Synthesis of 2′-benzyloxy-2,4,5-trimethoxychalcone (103):

A mixture of 2-benzyloxyaetophenone (**21,** 10 mmol, 2.26g) and 2,4,5-trimethoxybenzaldehyde (**20**, 1.1 eqv, 2.15g) in ethanolic solution of KOH (5% 15mL) was kept at room temperature for about 80 hours. The reaction mixture was diluted with ice cold water, acidified with cold dil. HCl and extracted with ether. The ether layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The reaction mixture was subjected to column chromatography over silica gel. The elution was done with acetone-n-hexane (1:3) and crystallized from petroleum spirit as yellow crystals (2.38g), yield 56% m.p. 95°C. R_f = 0.92 (benzene-acetone; 4:1)

Anal Found: C, 74.05; H, 6.15 Calc. for $C_{25}H_{24}O_5$; C, 74.25; H, 5.94%

UV $\lambda_{max}^{CH_3OH}$ **:** 245, 290 and 391 nm

IR (KBr) ν_{max}**:** 3032 1666, 1606, 1548, 1472, 1415, 1337, 1281, 1189, 1132, 1064, 1002, 972, 873, 831, 817, 766, 739, 648 cm^{-1}.



103

^{1}HNMR($CDCl_3$-d_6): δ 3.74 (s, 9H, C_2-$OC\underline{H}_3$, C_4-$OC\underline{H}_3$ and C_5-$OC\underline{H}_3$), 5.21 (s, 2H, $C_{2'}$-$OC\underline{H}_2$-C_6H_5), 6.12 (s, 1H, C_3-H), 6.58 (s, 1H, C_6-H), 6.96 (d, 1H, $C_{3'}$-H), 7.01 (m, 1H, J=8.6 Hz, $C_{5'}$-H), 7.19 (s, 5H, $C_{2'}$-OCH_2-$C_6\underline{H}_5$), 7.39 (d, 1H, J = 16 Hz, C_α-H), 7.43 (m, 1H, $C_{4'}$-H), 7.70 (d, 1H, J = 8.6 Hz, $C_{6'}$-H), 8.17 (d, 1H, J = 16 Hz, C_β-H).

^{13}CNMR ($CDCl_3$-d_6): 56.3 (C_4-$O\underline{C}H_3$ and C_5-$O\underline{C}H_3$), 56.4 (C_2-$O\underline{C}H_3$), 77.8 ($C_{2'}$-$O\underline{C}H_2$-C_6H_5), 100.6 (C_3), 113.8 (C_1 and C_6), 114.6 ($C_{3'}$), 121.3 ($C_{5'}$), 122.3 ($C_{1'}$), 123.3 (C_α), 127.37 ($C_{2''}$, $C_{4''}$ and $C_{6''}$), 128.7 ($C_{3''}$ and $C_{5''}$), 130.7 ($C_{6'}$), 135.3 ($C_{4'}$), 139.8 (C_5), 140.9 ($C_{1''}$), 142.8 (C_α), 147.8 (C_4), 153.0 (C_2), 163.2 ($C_{2'}$) 187.0 (>C=O).

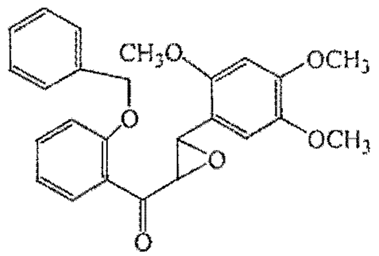
It was identified as 2′-benzyloxy-2,4,5-trimethoxychalcone **(103)**

Synthesis of 2′-benzyloxy-2,4,5-trimethoxychlcone epoxide (104):

The above chalcone (**103**) 0.5g was dissolved in methanol (40mL) and treated with aq. sodium hydroxide (1mL 8%) and hydrogen peroxide (30%). It was shaken for 1 hour and occasionally brought to its boiling point. It was kept aside overnight at room temperature. Water (50mL) was added and the solid that precipitated was filtered, washed and dried. When crystallized from methanol it separated as white needles (380mg), yield 73.46%, m.p. 105-107°C, $R_f = 0.70$ (benzene : acetone; 2:1).

Anal Found: C, 71.20; H, 5.93; Calc. for $C_{25}H_{24}O_6$; C, 71.42; H, 5.71%.

UV $\lambda_{max}^{CH_3OH}$: 235, 270 and 365.



104

IR (KBr) ν_{max}: 3240, 1701, 1577, 1510, 1484, 1426, 1367, 1302, 1278, 1199, 1131, 1035, 975, 906, 885, 812, 795, 762, 712, 636 cm^{-1}.

^{1}HNMR ($CDCl_3$-d_6): δ 3.73 (s, 9H C_2-OC$\underline{H}_3$, C_4-OC$\underline{H}_3$ and C_5-OC$\underline{H}_3$) 4.35(d, 1H, J = 2 Hz C_β-H), 4.41 (d, 1H, J = 2 Hz C_α-H), 5.22 (s, 2H, $C_{2'}$-OC$\underline{H}_2$-C_6H_5), 6.10 (s, 1H, C_3-H), 6.48 (s, 1H, C_6-H) 6.85 (d, 1H, $C_{3'}$-H), 6.90 (m, 1H, $C_{5'}$-H), 7.19 (s, 5H, $C_{2'}$-OCH_2-$\underline{C_6H_5}$), 7.33 (m, 1H, $C_{4'}$-H) 7.78 (d, 1H, J = 2.6 Hz, $C_{6'}$-H).

^{13}CNMR ($CDCl_3$-d_6): δ 48.5 (C_β), 56.3 (C_2-O$\underline{C}H_3$, C_4-O$\underline{C}H_3$ and C_5-O$\underline{C}H_3$), 70.0 (C_α), 77.8 ($C_{2'}$-O$\underline{C}H_2$-C_6H_5), 100.6 (C_3), 113 (C_6), 114 ($C_{3'}$), 116.6 (C_1), 120.7 ($C_{5'}$), 123.0 ($C_{1'}$), 127.39 ($C_{2''}$, $C_{4''}$, and $C_{6''}$), 128.7 ($C_{3''}$ and $C_{5''}$), 129.6 ($C_{6'}$), 133.9 ($C_{4'}$), 139.8 (C_5), 140.9 ($C_{1''}$), 148 (C_4), 152.2 (C_2), 162.1 ($C_{2'}$), 197.6 (>C=O).

It was identified as 2′-benzyloxy-2,4,5-trimethoxychalcone epoxide (**104**).

Synthesis of 4′-methyl-2-chlorochaclone epoxide (106):

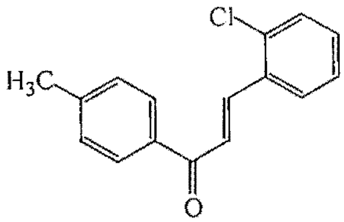
Synthesis of 4′-methyl-2-chlorochalcone (105):

A mixture of 4-methylacetophenone (**22,** 10 mmol 1.34g) and 2-chlorobenzaldehyde (**23,** 1.1 eqv, 1.54g) in ethanolic solution of KOH (5% 15mL) was kept at room temperature for about 80 hours. The reaction mixture as diluted with ice cold water, acidified with cold dil. HCl and extracted with ether. The layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The reaction mixture was subjected to column chromatography over silica gel. The elution was done with acetone-n-hexane (1:3) and crystallized from petroleum spirit as yellow crystals (1.59g), yield 83.24%, m.p. 116-118°C, $R_f = 0.81$ (benzene-acetone; 5:1).

Anal Found: C, 74.65; H, 5.35 Calc. for $C_{16}H_{13}OCl$; C, 74.85; H, 5.06%.

UV $\lambda_{max}^{CH_3OH}$: 242, 275 and 390 nm.

IR (KBr) ν_{max}: 3035, 2961, 1672, 1583, 1485, 1440, 1340, 1256, 1205, 1155, 1016, 974, 823, 752, 637 cm^{-1}.



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^{1}HNMR ($CDCl_3$-d_6): δ 2.35 (s, 3H, $C_{4'}$-$C\underline{H}_3$); 7.08 (m, 1H, C_4-H); 7.09 (m, 1H C_5-H); 7.22 (d, 1H, J = 2.6 Hz, C_3-H); 7.24 (d, 1H J = 2.6 Hz C_6-H); 7.25 (d, 2H, J = 8.6 Hz $C_{3'}$-H & $C_{5'}$-H); 7.39 (d, 1H, J = 16 Hz, C_α-H); 7.69 (d, 2H, J = 8.6 Hz $C_{2'}$-H and C_6'-H); 8.17 (d, 1H, J = 16 Hz, C_β-H).

^{13}CNMR ($CDCl_3$-d_6): δ 20.9 ($C_{4'}$-$\underline{C}H_3$), 123.3 (C_α), 126.5 (C_5), 127.6 (C_6), 129.1 (C_4), 129.6 ($C_{2'}$ & $C_{6'}$), 129.7 ($C_{3'}$ and $C_{5'}$) 131.5 (C_2), 133.7 ($C_{1'}$), 135.3 (C_1), 142.8 (C_β) 143.5 ($C_{4'}$), 187 (> C = O).

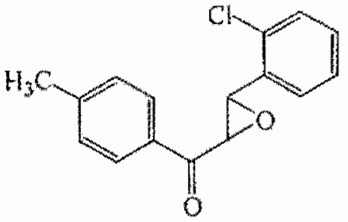
It was identified as 4′-methyl-2-chlorochaclone (**105**).

Synthesis of 4′-methyl-2-chlorochalcone epoxide (106):

The above chalcone (**105**) 0.5g was dissolved in methanol (40mL) and treated with aq. sodium hydroxide (1mL, 8%) and hydrogen peroxide (30%). It was shaken for 1 hour and occasionally brought its boiling point. It was kept aside overnight at room temperature. Water (50 mL) was added and the solid that precipitated was filtered, washed and dried. When crystallized from methanol it separated as white needles (374g), yield 70.40%, m.p. 100-102°C, $R_f = 0.81$ (benzene : acetone; 2:1).

Anal Found: C, 70.62; H, 4.80 Calc. for $C_{16}H_{13}O_2Cl$;C, 70.46; H, 4.77%.

UV $\lambda_{max}^{CH_3OH}$: 245 and 280 nm.



106

IR (KBr) ν_{max}: 3039, 2970, 1703, 1597, 1439, 1282, 1248, 1167, 1006, 860, 812, 749 cm^{-1}.

^{1}HNMR ($CDCl_3$-d_6): δ 2.33 (s, 3H, $C_{4'}$-C$\underline{H}_3$), 4.34 (d, 1H, C_β-H); 4.42 (d, 1H, C_α-H); 7.07 (m, 1H, C_5-H); 7.13 (m, 2H, C_4-H & C_6-H); 7.14 (d, 2H, $C_{3'}$-H and $C_{5'}$-H); 7.20 (d, 1H, C_3-H), 7.77 (d, 2H, $C_{2'}$-H and $C_{6'}$-H).

^{13}CNMR ($CDCl_3$-d_6): δ 20.9 ($C_{4'}$-$\underline{C}H_3$), 49 (C_β), 68.9 (C_α), 126.5 (C_5), 126.8 (C_6), 128.5 ($C_{2'}$ & $C_{6'}$), 128.8 (C_3), 129.1 ($C_{3'}$ & $C_{5'}$), 129.4 (C_4), 130.7 (C_2), 134.4 ($C_{1'}$), 138.1 (C_1), 142.1 ($C_{4'}$), 197.6 (> C = O).

It was identified as 4′-methyl-2-chlorochalcone epoxide (**106**).

Synthesis of 4′-methyl-4-chlorochalcone epoxide (108):

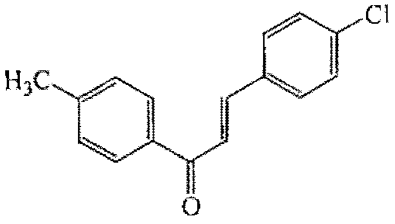
Synthesis of 4′-methyl-4-chlorochalcone (107):

A mixture of 4-methylacetophenone (**22**, 10 mmol 1.34g) and 4-chlorobenzaldehyde (**24**, 1.1 eqv. 1.54g) in ethanolic solution of KOH (5% 15mL) was kept at room temperature for about 80 hours. The reaction mixture was diluted with ice cold water, acidified with cold dil. HCl and extracted with ether. The ether layer was washed with water; dried over anhydrous Na_2SO_4 and evaporated to dryness. The reaction mixture was subjected to column chromatography over silica gel. The elution was done with acetone-n-hexane (1:3) and crystallized from petroleum spirit as yellow crystals (1.81g), yield 70.56%, m.p. 135-137°C R_f=0.83 (benzene : acetone; 5:1)

Anal Found: C, 74.61; H, 5.30 Calc. for $C_{16}H_{13}OCl$; C, 74.85; H, 5.06%.

UV $\lambda_{max}^{CH_3OH}$: 243, 265 and 385 nm.

IR (KBr) ν_{max}: 3038, 2950, 1683, 1564, 1487, 1440, 1367, 1340, 1319, 1304, 1263, 1234, 1205, 1174, 1159, 1130, 1089, 1022, 983, 943, 862, 819, 783, 760, 682, 644 cm^{-1}.



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^{1}**HNMR($CDCl_3$-d_6):** δ 2.36 (s, 3H, $C_{4'}$-$C\underline{H}_3$); 7.22 (d, 2H, J = 8.6 Hz, C_3-H & C_5-H), 7.24 (d, 2H, J = 8.6 Hz, C_2-H and C_6-H), 7.25 (d, 2H, J = 8.6 Hz $C_{3'}$-H & $C_{5'}$-H), 7.56 (d, 2H, J = 16 Hz, C_α-H), 7.69 (d, 2H, J = 8.6 Hz, $C_{2'}$-H & $C_{6'}$-H), 7.90 (d, 1H, J = 16 Hz, C_β-H).

^{13}**CNMR ($CDCl_3$-d_6):** δ 20.9 ($C_{4'}$-$\underline{C}H_3$), 123.3 (C_α), 127.6 (C_2 and C_6), 128.8 (C_3 & C_5), 129.6 ($C_{2'}$ and $C_{6'}$), 129.7 ($C_{3'}$ and $C_{5'}$) 133 (C_1 and C_4), 133.7 ($C_{1'}$), 142.8 (C_β), 143.5 ($C_{4'}$), 187 (> $\underline{C}$ = O).

It was identified as 4′-methyl-4-chlorochalcone (**107**)

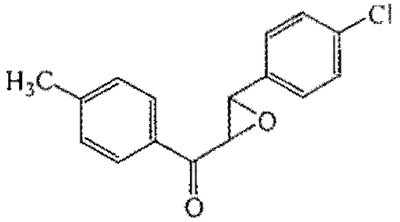
Synthesis of 4′-methyl-4-chlorochalcone epoxide (108):

The above chalcone (**107**) 0.5g was dissolved in methanol (40mL) and treated with aq. sodium hydroxide (1mL, 8%) and hydrogen peroxide (30%). It was shaken for 1 hr. and occasionally brought to its boiling point. It was kept aside overnight at room temperature. Water (50mL) was added and the solid that precipitated was filtered, washed and dried. When crystallized from methanol it separated as white needles (372 mg), yield 70.03%, m.p. 125-127°C, $R_f = 0.92$ (benzene : acetone 2:1).

Anal Found: C, 70.60; H, 4.81 Calc. for $C_{16}H_{13}O_2Cl$; C, 70.46; H, 4.77%.

UV $\lambda_{max}^{CH_3OH}$ **:** 238, 260 and 380 nm.

IR (KBr) ν_{max}**:** 3040, 2955, 1705, 1630, 1562, 1485, 1440, 1402, 1150, 1127, 1079, 1009, 980, 934, 862, 789, 779, 752 cm^{-1}.



108

^{1}HNMR ($CDCl_3$-d_6): δ 2.34 (s, 3H, C_4′-C$\underline{H}_3$); 4.35 (d, 1H, J = 16 Hz C_β-H); 4.42 (d, 1H, J = 16 Hz, C_α-$\underline{H}$); 7.13 (d, 2H J = 8.6 Hz, C_2-H and C_6-H); 7.14 (d, 2H, J = 8.6 Hz, $C_{3'}$-H and $C_{5'}$-H); 7.20 (d, 2H, J = 8.6 Hz, C_3-H and C_5-H); 7.77 (d, 2H, J = 8.6Hz $C_{2'}$-H and $C_{6'}$-H).

^{13}CNMR ($CDCl_3$-d_6): δ 20.9 ($C_{4'}$-$\underline{C}H_3$), 58.1 (C_β), 69.4 (C_α), 126.8 (C_2 and C_6), 128.5 ($C_{2'}$ and $C_{6'}$), 128.8 (C_3 and C_5), 129.1 ($C_{3'}$ and $C_{5'}$), 133.3 (C_4), 134.4 ($C_{1'}$), 135.8 (C_1), 142.1 ($C_{4'}$), 197.6 (>$\underline{C}$=O).

It was identified as 4′-methyl-4-chlorochaclone epoxide (**108**).

Synthesis of 2′-hydroxy-4-nitrochaclone epoxide (110):

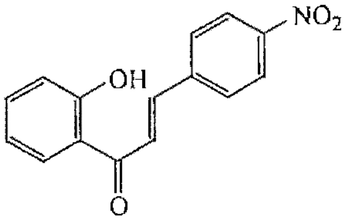
Synthesis of 2′-hydroxy-4-nitrochaclone (109):

A mixture of 2-hydroxyacetophenone (**18,** 10mmol, 1.36g) and 4-Nitrobenzaldehyde (**25,** 1.1 eqv. 1.66g) in ethanolic solution of KOH (5% 15mL) was kept at room temperature for about 80 hours. The reaction mixture was diluted with ice cold water, acidified with cold dil. HCl and extracted with ether. The ether layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The reaction mixture was subjected to column chromatography over silica gel. The elution was done with acetone-n-hexane (1:3) and crystallized from petroleum spirit as yellow crystals (1.98g), yield 73.60%, m.p. 105-106°C, $R_f = 0.83$ benzene : acetone; (5:1).

Anal Found: C, 67.10; H, 3.90 Calc. for $C_{15}H_{11}N$; C, 66.91; H, 4.08%.

UV $\lambda_{max}^{CH_3OH}$: 243, 265 and 385 nm.

IR (KBr) ν_{max}: 3455, 3035, 1681, 1568, 1487, 1440, 1406, 1367, 1340, 1319, 1304, 1263, 1234, 1205, 1174, 1159, 1130, 1089, 1022, 984, 945, 866, 760, 685, 649 cm^{-1}.



109

^{1}HNMR (CDCl-d$_6$): δ 6.92 (d, 1H, J = 2.6 Hz, $C_{3'}$-H); 7.01 (m, 1H $C_{5'}$-H); 7.37 (m, 1H, $C_{4'}$-H); 7.56 (d, 2H, C_2-H and C_6-H); 7.64 (d, 1H, $C_{6'}$ - H); 7.85 (d, 1H, J = 16 Hz, C_α-H); 8.04 (d, 1H, J = 16 Hz, C_β-H); 8.14 (d, 2H, C_3-H and C_5-H); 12.76 (s, 1H, $C_{2'}$-O$\underline{H}$).

^{13}CNMR (CDCl-d$_6$): δ 116.2 ($C_{3'}$), 121.6 ($C_{5'}$) 123.3 (C_α), 123.5 (C_3 and C_5), 123.9 ($C_{1'}$), 127.1 (C_2 and C_6), 131.1 ($C_{6'}$), 135.7 ($C_{4'}$), 141(C_1), 142.8 (C_β), 147.6 (C_4) 158.5 ($C_{2'}$) 187 (> $\underline{C}$ = O).

It was identified as 2′-hydroxy-4-nitrochalcone (**109**).

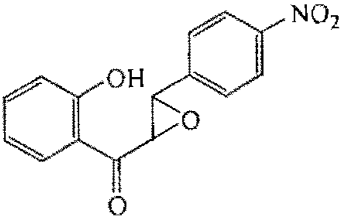
Synthesis of 2′-hydroxy-4-nitrochalcone epoxide (110):

The above chalcone (**109**) 0.5g was dissolved in methanol (40mL) and treated with aq. sodium hydroxide (1mL 8%) and hydrogen peroxide 30%. It was shaken for 1 hour and occasionally brought to its boiling point. It was kept aside overnight at room temperature. Water (50mL) was added and the solid that precipitated was filtered, washed and dried. When crystallized from methanol it separated as white needles (365 mg), yield 68.90%, m.p. 95-97°C, $R_f = 0.94$ (benzene : acetone; 2:1)

Anal Found: C, 61.40; H, 3.75 Calc. for $C_{15}H_{11}O_5N$; C, 61.15; H, 3.86%.

UV $\lambda_{max}^{CH_3OH}$: 245, 260 and 380 nm.

IR (KBr) ν_{max}: 3443, 3042, 1709, 1630, 1562, 1485, 1442, 1402, 1151, 1129, 1081, 1007, 978, 931, 862, 772, 752 cm^{-1}.



110

^{1}**HNMR (CDCl$_3$-d$_6$):** δ 4.35 (d, 1H, J = 2 Hz C_β-H); 4.42 (d, 1H, J = 2 Hz, C_α-H), 6.81 (d, 1H, $C_{3'}$-H), 6.90 (m, 1H, $C_{5'}$-H) 7.27 (m, 1H, $C_{4'}$-H), 7.45 (d, 2H, C_2-H and C_6-H), 7.72 (d, 1H, $C_{6'}$-H), 8.12 (d, 2H, C_3-H and C_5-H), 11.40 (s, 1H, $C_{2'}$-O$\underline{H}$)

^{13}**CNMR (CDCl$_3$-d$_6$):** δ 58.1(C_β), 69.7 (C_α), 115.6 ($C_{3'}$), 121 ($C_{5'}$), 123.5 (C_3 and C_5), 124.6 ($C_{1'}$), 126.3 (C_2 & C_6), 130 ($C_{6'}$), 134.3 ($C_{4'}$), 143.8 (C_1), 147.9 (C_4), 157.4 ($C_{2'}$), 197.6 ($> \underline{C} = O$).

It was identified as 2′-hydroxy-4-nitrochalcone epoxide (**110**).

Synthesis of 2′-benzyloxy-4-methoxychalcone epoxide (112):

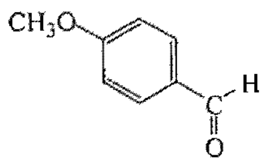
Methylation of 4-hydroxy benzaldehyde (26):

A mixture of 4-hydroxybenzaldehyde (**26**, 3 g), dimethylsulphate (2.5mL) and anhydrous potassium carbonate (6 g) in acetone (125mL) was refluxed for 3 hours. Acetone was removed by distillation and water (100mL) was added to the residue. The solution was acidified with dil. HCl when a light yellow colored solid was precipitated out. It was collected and subjected to column chromatography over silica gel. The elution was done with petroleum spirit-benzene (7:3). It crystallized from ethyl acetate-petroleum spirit as colorless needles (2.25g), yield 75%, m.p. 89-90°C, R_f = 0.60 (benzene:acetone 4:1).

Anal. Found: C, 70.34; H, 5.67; Calc. for $C_8H_8O_2$; C, 70.57; H, 5.92%.

UV $\lambda_{max}^{CH_3OH}$: 250 and 290 nm.

IR (KBr) ν_{max}: 3078, 2962, 1635, 1560, 1480, 1470, 1400, 1360, 1280, 1220, 1160, 1120, 825, 600 cm^{-1}.



27

^{1}**H NMR (CDCl$_3$-d$_6$):** δ 3.73 (s, 3H, C_4-OC$\underline{H}_3$), 6.96 (dd, 2H, J = 2.24 Hz and J = 7.6 Hz, C_3-$\underline{H}$ and C_5-H), 7.70 (dd, 2H, J = 2.24 Hz and 7.6 Hz, C_2-$\underline{H}$ and C_6-$\underline{H}$), 9.87 (s, 1H, C_1-C$\underline{H}$O).

^{13}C NMR (CDCl$_6$-d$_6$): δ 56.0 (C_4-O$\underline{C}H_3$), 114.6 (C_3 and C_5), 129.0 (C_1), 130.7 (C_2 and C_6), 167.8 (C_4) 190.0 (C_1-$\underline{C}$HO).

It was identified as 4-methoxybenzaldehyde (**27**).

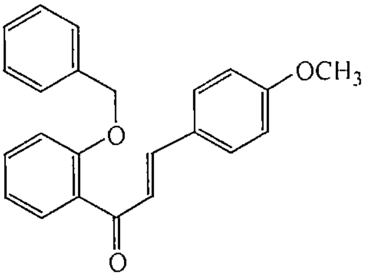
Synthesis of 2′-benzyloxy-4-methoxy Chalcone (111):

A mixture of 2-benzyloxy acetophenone (**21,** 10 mmole, 2.26g) and 4-methoxy benzaldehyde (**27,** 1.1 eqv, 1.5g) in ethanolic solution of KOH (5% 15mL) was kept at room temperature for about 80 hours. The reaction mixture was diluted with ice cold water, acidified with cold dil HCl and extracted with ether. The ether layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The reaction mixture was subjected to column chromatography over silica gel. The elution was done with acetone-n-hexane (1:4) and crystallized from petroleum spirit as yellow crystals (2.09g), yield 61%, m.p. 65-67°C, R_f 0.71 (benzene:acetone 5:1).

Anal Found: C, 80.11; H, 5.92; Calc. for $C_{23}H_{20}O_3$ C, 80.23; H 5.81%.

UV $\lambda_{max}^{CH_3OH}$: 216 and 308 nm.

IR (KBr) ν_{max}: 3042, 1679, 1557, 1508, 1358, 1257, 1213, 1169, 1131, 1003, 838, 739, 696, 647, 539 cm^{-1}



111

^{1}HNMR (CDCl$_3$): δ 3.73(s, 3H, C_4-OC$\underline{H}_3$), 5.20 (s, 2H, $C_{2'}$-OC$\underline{H}_2C_6H_5$), 6.72 (d, 2H, J = 2.6 Hz, C_3-H and C_5-H), 6.96 (d, 1H, $C_{3'}$-H), 7.01 (m, 1H, $C_{5'}$-H), 7.19 (s, 5H, $C_{2'}$-OCH$_2$-C$_6\underline{H}_5$), 7.19 (d, 2H, J = 2.6 Hz, C_2-H and C_6-H), 7.43 (m, 1H, $C_{4'}$-H), 7.56 (d, 1H, J = 16 Hz, C_α-H), 7.70 (d, 1H, $C_{6'}$-H), 7.90 (d, 1H, J = 16 Hz, C_β-H).

^{13}CNMR (CDCl$_3$): δ 56 (C_4-O$\underline{C}H_3$), 77.8 ($C_{2'}$-O-$\underline{C}H_2$-C_6H_5), 114 (C_3 and C_5), 114.6 ($C_{3'}$), 121.3 ($C_{5'}$), 122.3 ($C_{1'}$), 123.3 (C_α), 127.2 (C_1, C_2 and C_6), 127.3 ($C_{2''}$ and $C_{6''}$), 127.4 ($C_{4''}$), 128.7 ($C_{3''}$ and $C_{5''}$) 130.7 ($C_{6'}$), 135.3 ($C_{4'}$), 140.9 ($C_{1''}$), 142.8 (C_β), 161.2 (C_4), 163.2 ($C_{2'}$), 187 (> $\underline{C}$ = O).

It was identified as 2′-benzyloxy-4-methoxychalcone (**111**).

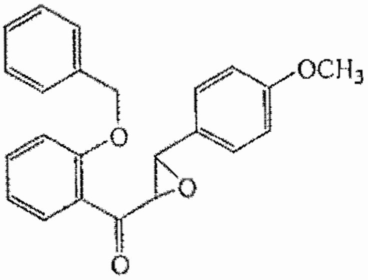
Synthesis of 2′-benzyloxy-4-methoxychalcone epoxide (112):

The above chalcone (**111**) 0.5g was dissolved in methanol (40mL) and treated with aq sodium hydroxide (1mL, 8%) and hydrogen peroxide (30%). It was shaken for 1 hour and occasionally brought to its boiling point, it was kept aside overnight at room temperature, water (50mL) was added and the solid that precipitated was filtered wash and dried. When crystallized from methanol it separated as white needles (421 mg) yield 80.15%, m.p. 75-76°C, R_f 0.71 (benzene : acetone; 4 : 1).

Anal Found: C, 76.78; H, 5.41; Calc. for $C_{23}H_{20}O_4$ C, 76.67; H 5.56%

UV $\lambda_{max}^{CH_3OH}$: 209 and 295 nm.

IR (KBr) ν_{max}: 3036, 1707, 1506, 1456, 1259, 1094, 779, 735, 697, 460 cm^{-1}.



112

^{1}HNMR ($CDCl_3$): δ 3.73 (s, 3H, C_4-OC$\underline{H}_3$), 4.37 (d, 1H, J = 2 Hz, C_β-H) 4.43 (d, 1H, J = 2 Hz, C_α-H), 5.19 (s, 2H, $C_{2'}$-OC$\underline{H}_2$-C_6H_5), 6.70 (d, 2H, C_3-H and C_5-H) 6.85 (d, 1H, $C_{3'}$-H), 6.90 (m, 1H, $C_{5'}$-H), 7.08 (d, 2H, C_2-H and C_6-H), 7.19 (s, 5H, $C_{2'}$-OCH_2-$C_6\underline{H}_5$) 7.33 (m, 1H, $C_{4'}$-H), 7.78 (d, 1H, $C_{6'}$-H).

^{13}CNMR ($CDCl_3$): δ 56 (C_4-O$\underline{C}H_3$), 58.1 (C_β) 69.7 (C_α), 77.8 ($C_{2'}$-O$\underline{C}H_2$-C_6H_5) 114 (C_3, C_5 and $C_{3'}$), 120.7 ($C_{5'}$), 123 ($C_{1'}$) 126.4, (C_2 and C_6), 127.3 ($C_{2''}$ and $C_{6''}$) 127.4 ($C_{4''}$) 128.7 ($C_{3''}$ and $C_{5''}$) 129.6 ($C_{6'}$), 130 (C_1) 133.9 ($C_{4'}$) 140.9 ($C_{1''}$) 161.5 (C_4), 162.1($C_{2'}$) 197.6 (> $\underline{C}$ = O).

It was identified as 2′-benzyloxy-4-methoxychalcone epoxide (**112**).

Synthesis of 2′-hydroxy-3,4-methylenedioxychalcone epoxide (114):

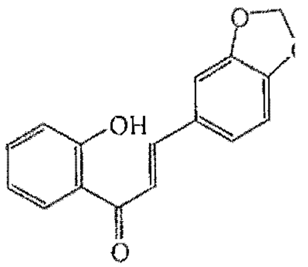
Synthesis of 2′-hydroxy-3,4-methylenedioxychalcone (113):

A mixture of 2-hydroxyacetophenone (**18,** 10 mmole, 1.36g) and 3,4-methylenedioxybenzaldehyde (**28,** 1.1 eqv, 1.65g) in ethanolic solution of KOH (5% 15mL) was kept at room temperature for about 80 hours. The reaction mixture was diluted with ice cold water, acidified with cold dil HCl and extracted with ether. The ether layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The reaction mixture was subjected to column chromatography over silica gel. The elution was done with acetone-n-hexane (1:4) and crystallized from petroleum spirit as yellow crystals (2.1g), yield 78.36%, m.p. 125-127°C, R_f 0.91 (benzene:acetone 4:1).

Anal Found: C, 71.93; H, 4.91; Calc. for $C_{16}H_{12}O_4$ C; 71.63; H 4.51%

UV $\lambda_{max}^{CH_3OH}$: 242, 280 and 355 nm.

IR (KBr) ν_{max}: 3458, 2908, 1641, 1620, 1577, 1504, 1493, 1440, 1373, 1354, 1309, 1242, 1203, 1161, 1130, 1097, 1037, 976, 933, 868, 846, 827, 804, 760, 748, 665, 625 cm^{-1}.



113

^{1}HNMR ($CDCl_3$): δ 5.90 (s, 2H, C_3-OC$\underline{H}_2$-C_4), 6.61 (d, 1H, J = 8.6 Hz, C_5-H), 6.70 (s, 1H, J = 8.6 Hz, C_2-H), 6.75 (d, 1H, J = 8.6 Hz C_6-H), 6.92 (d, 1H, J = 8.6 Hz, $C_{3'}$-H), 7.01 (m, 1H, $C_{5'}$-H), 7.37 (m, 1H, $C_{4'}$-H), 7.56 (d, 1H, J = 16 Hz C_α-H) 7.64 (d, 1H, J = 8.6 Hz, $C_{6'}$-H), 7.90 (d, 1H, J = 16 Hz C_β-H), 12.89 (s, 1H, $C_{2'}$ - OH).

^{13}CNMR ($CDCl_3$): δ 91.3 (C_3-O$\underline{C}H_2$O-C_4) 112.8 (C_2), 115.0 (C_5), 116.2 ($C_{3'}$), 119.5 (C_6), 121.6 ($C_{5'}$), 123.3 (C_α), 123.9 ($C_{1'}$), 128.2 (C_1), 131.1 ($C_{6'}$), 135.7 ($C_{4'}$), 142.8 (C_β) 146.8 (C_4), 147.5 (C_3), 158.5 ($C_{2'}$), 187.0 (>C = 0).

It was identified as 2′-hydroxy-3,4-dimethylenedioxy chalcone (**113**).

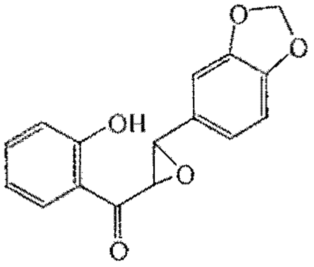
Synthesis 2′-benzyloxy-3,4-methenedioxychalcone epoxide (114):

The above chalcone (**113**) 0.5g was dissolved in methanol (40mL) and treated with aq sodium hydroxide (1mL, 8%) and hydrogen peroxide (30%). It was shaken for 1 hour and occasionally brought to its boiling point. It was kept aside overnight at room temperature. Water 50mL was added and the solid that precipitated was filtered, washed and dried. When crystallized from methanol it separated as white needles (379 mg) yield 84.31%, m.p. 215-217°C, R_f 0.57 (benzene : acetone; 4 : 1).

Anal Found: C, 67.72; H, 4.91; Calc. for $C_{16}H_{12}O_5$ C, 71.63; H 4.51%

UV $\lambda_{max}^{CH_3OH}$: 245, 282 and 375 nm.

IR (KBr) ν_{max}: 3464, 1697, 1504, 1454, 1415, 1367, 1300, 1261, 1167, 1114, 1074, 1037, 931, 914, 868, 831, 812, 763, 671 cm^{-1}.



114

^{1}**HNMR (CDCl$_3$):** δ 4.36 (d, 1H, J = 2 Hz C_β-H), 4.44 (d, 1H, J = 2 Hz, C_α-H), 5.91 (s, 2H, C_3-OC$\underline{H}_2$O-C_4), 6.59 (d, 1H, J = 2.6 Hz, C_5-H), 6.59 (s, 1H, C_2-H), 6.64 (d, 1H, C_6-H), 6.81 (d, 1H, $C_{3'}$-H), 6.90 (m, 1H, $C_{5'}$-H), 7.27 (m, 1H, $C_{4'}$-H), 7.72 (d, 1H, $C_{6'}$-H), 11.85 (s, 1H, $C_{2'}$-O$\underline{H}$)

^{13}**CNMR (CDCl$_3$):** δ 58.4 (C_β), 69.7 (C_α), 91.3 (C_3-O$\underline{C}H_2$O-C_4), 112.0 (C_2), 115.0 (C_5), 115.6 ($C_{3'}$), 118.7 (C_6), 121.0 ($C_{5'}$), 124.6($C_{1'}$), 130.0 ($C_{6'}$), 131.0 (C_1), 134.3 ($C_{4'}$), 147.1 (C_4), 147.5 (C_3), 157.4 ($C_{2'}$), 197.6 (> C = O).

It was identified as 2′-hydroxy-3,4-methylenedixy chalcone epoxide (**114**).

Synthesis of 2′-benzyloxy-3,4-methylenedioxychalcone epoxide (116):

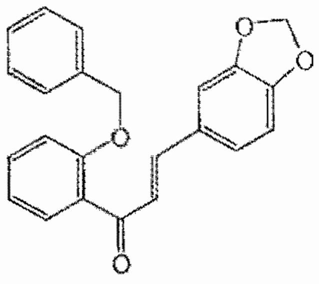
Synthesis of 2′-benzyloxy-3,4-methylenedioxychalcone (115):

A mixture of 2-benzyloxyacetophenone (**21**, 10 mmole, 2.26g) and 3,4-methylenedioxybenzaldehyde (**28**, 1.1 eqv, 1.65g) in ethanolic solution of KOH (5% 15mL) was kept at room temperature for about 85 hours. The reaction mixture was diluted with ice cold water, acidified with cold dil HCl and extracted with ether. The ether layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The reaction mixture was subjected to column chromatography over silica gel. The elution was done with acetone-n-hexane (1:4) and crystallized from petroleum spirit as yellow crystals (2.25g), yield 62.84%, m.p. 80-82°C, R_f 0.82 (benzene : acetone 5:1).

Anal Found: C, 77.21; H, 4.96; Calc. for $C_{23}H_{18}O_4$ C, 77.09; H 5.02%

UV $\lambda_{max}^{CH_3OH}$: 280 and 395 nm.

IR (KBr) ν_{max}: 3037, 1682, 1606, 1581, 1500, 1444, 1361, 1309, 1246, 1180, 1118, 1099, 1037, 1014, 989, 929, 862, 812, 752, 736, 684 cm^{-1}.



115

^{1}HNMR ($CDCl_3$): δ 5.22(s, 2H, $C_{2'}$-OC$\underline{H_2}$-C_6H_5), 5.94 (s, 2H, C_3-OC$\underline{H_2}$O-C_4), 6.61 (d, 1H, J = 8.6 Hz, C_5-H), 6.70 (s, 1H, J = 2.4 Hz, C_2-H), 6.75 (d, 1H, J = 8.6 Hz, C_6-H), 6.96 (d, 1H, $C_{3'}$-H) 7.01 (m, 1H, $C_{5'}$-H), 7.19 (s, 5H, $C_{2'}$-OCH$_2$-C_6-$\underline{H_5}$), 7.43 (m, 1H, $C_{4'}$-H), 7.56 (d, 1H, J = 16 Hz, C_α-H), 7.70 (d, 1H, $C_{6'}$-H), 7.90 (d, 1H, J = 16 Hz, C_β-H)

^{13}CNMR ($CDCl_3$): δ 77.8 ($C_{2'}$-O$\underline{C}$H$_2$-C_6H_5), 91.3 (C_3-O-$\underline{C}$H$_2$-O-C_4), 112.8 (C_2) 114.6 ($C_{3'}$), 115 (C_5), 119.5 (C_6), 121.3 ($C_{5'}$), 122.3 ($C_{1'}$), 123.3 (C_α), 127.3 ($C_{2''}$ and $C_{6''}$), 127.4 ($C_{4''}$), 128.2 (C_1) 128.7 ($C_{3''}$ and $C_{5''}$), 130.7 ($C_{6'}$), 135.3 ($C_{4'}$), 140.9 ($C_{1''}$), 142.8 (C_β) 146.8 (C_4), 147.5 (C_3), 163.2 ($C_{2'}$), 187 (>C=O).

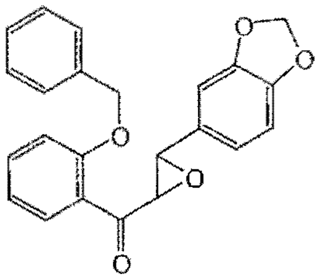
It was identified as 2′-benzyloxy-3,4-methylenedioxy chalcone (**115**).

Synthesis 2′-benzyloxy-3,4-methenedioxychalcone epoxide (116):

The above chalcone **(115)** 0.5g was dissolved in methanol (40 mL) and treated with aq sodium hydroxide (1mL, 8%) and hydrogen peroxide (30%). It was shaken for 1 hour and occasionally brought to its boiling point. It was kept aside overnight at room temperature, water 50mL was added and the solid that precipitated was filtered, washed and dried. When crystallized from methanol it separated as white needles (379 mg) yield 72.6%, m.p. 110-112°C, R$_f$ 0.78 (benzene : acetone; 6 : 1).

Anal Found: C, 73.67; H, 4.93; Calc. for $C_{23}H_{18}O_5$ C, 73.79; H 4.81%

UV $\lambda_{max}^{CH_3OH}$: 285 and 390 nm.



116

IR (KBr) ν_{max}: 3041, 1699, 1555, 1446, 1259, 1182, 983, 767 cm^{1}.

^{1}HNMR (CDCl$_3$): δ 4.37 (d, 1H, J = 2Hz, C$_\beta$-H), 4.43 (d, 1H J = 2Hz, C$_\alpha$-H), 5.21 (s, 2H, C$_{2'}$-OC$\underline{H}_2$-C$_6$H$_5$), 5.92, (s, 2H, C$_3$-OC$\underline{H}_2$O-C$_4$), 6.59 (s, 1H, C$_2$-H), 6.59 (d, 1H, J = 2.6 Hz, C$_5$-H), 6.64 (d, 1H, C$_6$-H), 6.85 (d, 1H, C$_{3'}$-H), 6.90, (m, 1H, C$_{5'}$-H), 7.19 (s, 5H, C$_{2'}$-OCH$_2$-C$_6$-$\underline{H}_5$), 7.33 (m, 1H, C$_{4'}$-H), 7.78 (d, 1H, C$_{6'}$-H).

^{13}CNMR (CDCl$_3$): δ 58.4 (C$_\beta$), 69.7 (C$_\alpha$), 77.8 (C$_{2'}$-O$\underline{C}$H$_2$-C$_6$H$_5$), 91.3 (C$_3$-O$\underline{C}$H$_2$, C$_4$) 112 (C$_2$), 114 (C$_{3'}$), 115 (C$_5$), 118.7 (C$_6$), 120.7 (C$_{5'}$), 123 (C$_{1'}$), 127.3 (C$_{2''}$ and C$_{6''}$), 127.4 (C$_{4''}$), 128.7 (C$_{3''}$ and C$_{5''}$), 129.6 (C$_{6'}$), 131 (C$_1$) 133.9 (C$_{4'}$), 140.9 (C$_{1''}$), 147.1 (C$_4$), 147.5 (C$_3$), 162.1 (C$_{2'}$), 197.6 (> C = O).

It was identified as 2′-benzyloxy-3,4-methylenedioxychalcone epoxide (**116**).

CHAPTER IV

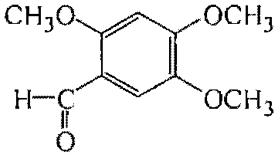
Results and Discussion

CHAPTER-IV

Results And Discussion

Part-1 of this dissertation is concerned with the synthesis and structural elucidation of some chalcone epoxides e.g. 2′-hydroxy-2,4,5-trimethoxychalcone epoxide **(102)**, 2′-benzyloxy-2,4,5-trimethoxychalcone epoxide **(104)**, 4′-methyl-2-chlorochalcone epoxide **(106)**, 4′-methyl-4-chlorochalcone epoxide **(108)**, 2′-hydroxy-4-nitrochalcone eppxide **(110)**, 2′-benzyloxy-4-methoxychalcone epoxide **(112)**, 2′-hydroxy-3,4-methylene-dioxychalcone epoxide **(114)**, 2′-benzyloxy-3,4-methylenedioxychalcone epoxide **(116)**.

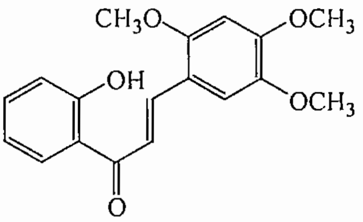
2,4,5-trihydroxybenzaldehyde **(19)** on methylation with dimethylsulphate yielded 2,4,5-trimethoxybenzaldehyde **(20)**. Compound **20** was obtained as a colorless needles m.p. 92-95°C. The UV spectrum of **20** showed absorption peaks in methanol at λ_{max} 230 and 255 nm. It gave no color with $FeCl_3$ solution and showed IR absorption band at ν 1675 cm^{-1} indicating the presence of a carbonyl group (>C=O).



20

The ^{1}H-NMR spectrum of compound **20** showed the presence of three methoxy group by one singlet at δ 3.73 (C_2-OC$\underline{H}_3$, C_4-OC$\underline{H}_3$ and C_5-OC$\underline{H}_3$) integrating for nine protons. The two aromatic protons which appeared as two singlets at δ 6.36 and 7.10 assigned to C_3-$\underline{H}$ and C_6-$\underline{H}$, integrating for one proton each respectively. A singlet at δ 10.24 was indicated the presence of an aldehydic proton integrating for one proton. The compound 20 was identified as 2,4,5-trimethoxybenzaldehyde.

Alkaline condensation of 2-hydroxy acetophenone (18) and 2,4,5-trimethoxybenzaldehyde (**20**) produced 2′-hydroxy-2,4,5-trimethoxy-chalcone (**101**). It was obtained as yellow crystals, m.p. 120-122°C. The structure of this chalcone **101** has been confirmed by spectral data and elemental analysis. The UV absorption band of **101** in methanol at 235, 260 and 365 nm suggested the presence of a chalcone skeleton[51,52].

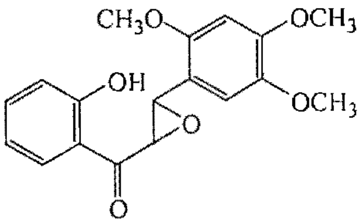


101

The IR absorption frequency at ν 3450 cm^{-1} indicated the presence of a hydroxyl group. It gave light brown color with alcoholic ferric chloride solution confirming that compound **101** has free hydroxyl group.

The absorption frequency at ν 1677 cm^{-1} showed the presence of a conjugated carbonyl group (>C=O). The ^{1}H-NMR spectrum explained the presence of three methoxy group in the B ring at δ 3.73 (C_2-OC$\underline{H}_3$; C_4-OCH$_3$; C_5-OC$\underline{H}_3$) as a singlet integrating for nine protons respectively. The two aromatic protons of B ring appeared as two singlets at δ 6.59 (C_6-H) and 6.12 (C_3-H) integrating for one proton each respectively. The other four aromatic protons of A ring appeared as two doublates and two multiplets at δ 6.92 (J = 2.6 Hz $C_{3'}$-H), 7.64 (J = 8.6 Hz $C_{6'}$-H) and δ 7.01 (M, $C_{5'}$-H), 7.37 (M, $C_{4'}$-H). The C_α-$\underline{H}$ and C_β-$\underline{H}$ protons of **101** appeared as two doublets at δ 7.39 (J = 16 Hz C_α-$\underline{H}$) and 8.17 (J = 16 Hz C_β-$\underline{H}$) integrating for one proton each respectively. One hydroxyl proton appeared as a singlet at δ 13.08 ($C_{2'}$-O$\underline{H}$). The yield of the chalcone (**101**) was 57%. The structure of the compound **101** was established by the above spectral data (UV, IR, ^{1}H-NMR and ^{13}C-NMR) and elemental analysis.

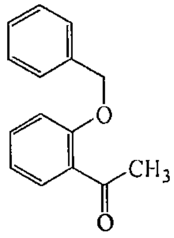
Oxidation of chalcone **101** into the corresponding 2′-hydroxy-2,4,5-trimethoxychalcone epoxide **(102)** using, NaOH/H_2O_2 as oxidizing agent was carried out. The yield of this epoxide **102** was 71.55% and it crystallized as white needles, m.p. 133-135°C. The structure of the epoxide **102** has been confirmed by spectral analysis. The UV absorption of **102** in methanol at λ_{max} 228, 255 and 361 nm suggested the presence of a epoxide skeleton. The IR absorption frequency at ν 1704 cm^{-1} showed the presence of a carbonyl group (>C=O). The C_α-$\underline{H}$ and C_β-$\underline{H}$ protons of **102** appeared as two doublets at δ 4.43 (J = 2Hz C_α-$\underline{H}$) and 4.36 (J = 2 Hz C_β-H) integrating for one proton each respectively.



102

The ^{1}H-NMR at δ 4.43 (C_α-$\underline{H}$) and δ 4.36 (C_β-$\underline{H}$) of the compound **102** indicated the epoxide ring comparing its corresponding chalcone **(101)**. Singlet at δ 11.85 (1H, $C_{2'}$-OH) indicated the presence of hydroxyl group. The other four aromatic protons of A ring which appeared as two doublets and two multiplets at δ 6.81 (1H J = 2.6 Hz $C_{3'}$-H), 7.72 (1H, J = 2.6 Hz $C_{6'}$-H) and δ 6.90 (1H, $C_{5'}$-H), 7.27 (1H, $C_{4'}$-H). The ^{1}H-NMR spectrum explained the presence of three methoxy group in the B ring at δ 3.73 (C_2-$OC\underline{H}_3$, C_4-OCH_3 and C_5-$OC\underline{H}_3$) as a singlet integrating for nine protons respectively. The two aromatic protons of B ring which appeared as two singlets at 6.10 and δ 6.48 assigned to C_3-$\underline{H}$ and C_6-$\underline{H}$ integrating for one protons each respectively.

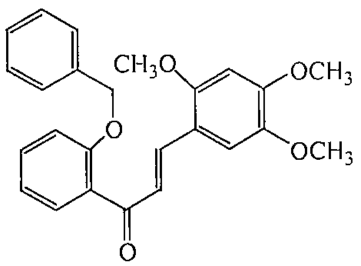
Benzylation of 2-hydroxyacetophenone (**18**) using benzyl chloride gave one products e.g. 2-benzyloxyacetophenone **(21)** 2-benzyloxyacetophenone **(21)** was obtained as brown oily liquid, b.p 112-114°C and its yield was 50%. The UV absorption band of **21** was appeared at λ_{max} 242 and 293 nm. It showed IR absorption frequency of 1647 cm^{-1} indicating the presence of a carbonyl group (>C=O) respectively. The ^{1}H-NMR spectrum of **21** showed presence of a benzyl group by two singlet at δ 5.20 (s, 2H, -$OC\underline{H}_2$-C_6H_5) and δ 7.19 (s, 5H, -OCH_2-$C_6\underline{H}_5$) integrating for two and five protons respectively. The three methyl protons adjacent to the carbonyl group (-$OCOC\underline{H}_3$) appeared at δ 2.55 integrating for three protons.



21

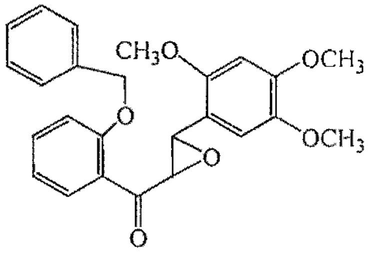
The aromatic protons were shown by two doublets at δ 6.88 (d, 1H, C_3-H) and δ 7.75 (d, 1H, C_6-H) and two multiplets at δ 6.93 (m, 1H, C_5-H) and δ 7.34 (m, 1H, C_4-H) integrating for one proton each respectively.

Alkaline condensation of 2-benzyloxyacetophenone (**21**) and 2,4,5-trimethoxybenzaldehyde (**20**) furnished 2′-benzyloxy-2,4,5-trimethoxy-chalcone (**103**). It was obtained as yellow crystals, m.p. 95°C. The structure of this chalcone **103** has been confirmed by spectral data and elemental analysis. The UV absorption band of **103** in methanol at λ_{max} 245, 290 and 391 nm suggested the presence of a chalcone skeleton[62]. The IR absorption frequency at ν 1666 cm^{-1} showed the presence of a conjugated carbonyl group (>C=O). The ^1H-NMR spectrum explained the presence of three methoxy group in the B ring at δ 3.74 (C_2-$OC\underline{H}_3$; C_4-$OC\underline{H}_3$ and C_5-$OC\underline{H}_3$), as a singlets integrating for nine protons, respectively. The two aromatic protons of B ring which appeared as two singlets at δ 6.58 and 6.12 assigned to C_6-$\underline{H}$ and C_3-$\underline{H}$ integrating for one proton each respectively. Two singlets at δ 5.20 (2H, -O-$C\underline{H}_2$-C_6H_5) and 7.19 (5H -O-CH_2-$C_6\underline{H}_5$) indicated the presence of benzyloxy group. The other four aromatic protons of A ring appeared as an ABC system at δ 6.96 (d, 1H, $C_{3'}$-H) and 7.01 (m, 1H, J = 8.6 Hz, $C_{5'}$-H) and 7.43 (m, 1H, $C_{4'}$-H) and 7.70 (d, 1H, $C_{6'}$-H). The C_α-H and C_β-H protons of **103** appeared as two doublets at 7.39 (J = 16 Hz) and 8.17 (J = 16 Hz) integrating for one proton each respectively. The yield of this chalcone was 56%. The structure of the compound **103** was established by the above spectral data (UV, IR, ^1H-NMR and ^{13}C-NMR) and elemental analysis.



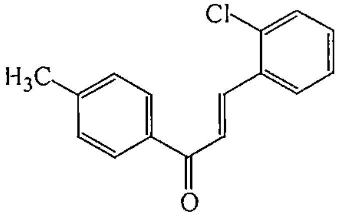
103

Oxidation of chalcone **103** into the corresponding 2′-benzyloxy-2,4,5-trimethoxychalcone epoxide (**104**) using NaOH / H_2O_2 as an oxidizing agent was carried out. The yield of this epoxide **104** was 73.46% and it crystallized as white needles, m.p. 105-107°C. The structure of this epoxide **104** has been confirmed by spectral data (UV, IR; ^{1}H-NMR and ^{13}C-NMR) and elemental analysis. The UV absorption band of **104** in methanol at λ_{max} 235, 270 and 365 nm suggested the presence of a epoxide skeleton. The IR absorption frequency at ν 1701 cm^{-1} showed the presence of a carbonyl group (>C=O). The C_α-$\underline{H}$ and C_β-$\underline{H}$ protons of **104** appeared as two doublets at δ 4.41 (J=2Hz) and 4.35 (J = 2Hz) integrating for one proton each respectively. The IR absorption frequency (>C=O, ν 1701 cm^{-1}) and the ^{1}H-NMR (C_α-H, δ 4.41 and C_β-H, δ 4.35) of the compound **104** indicated the epoxide ring comparing its corresponding chalcone (**103**). Two singlets at δ 5.20 (2H, C_2-O-C$\underline{H}_2$-C_6H_5) and 7.19 (5H, C_2-O-CH_2-$C_6\underline{H}_5$) indicated the presence of benzyloxy group. The other four aromatic protons of A ring which appeared as an ABC system at δ 6.85 (d, 1H, $C_{3'}$-H) and 6.90 (m, 1H, $C_{5'}$-H) and 7.78 (d, 1H, J = 2.6 Hz, $C_{6'}$-H) and 7.33 (m, 1H, $C_{4'}$-H). The ^{1}H-NMR spectrum explained the presence of three methoxy group in the B ring at δ 3.73 (C_2-OC$\underline{H}_3$, C_4-OC$\underline{H}_3$, C_5-OC$\underline{H}_3$ as one singlet integrating for nine protons respectively. The two aromatic protons of B ring which appeared as two singlets at δ 6.48 and 6.10 assigned to C_6-$\underline{H}$ and C_3-H integrating for one proton each respectively.



104

Alkaline condensation of 4-methylacetophenone **(22)** and 2-chlorobenzaldehyde **(23)** gave 4′-methyl-2-chlorochalcone **(105)**. It was obtained as yellow crystals, m.p. 116-118°C. The structure of the chalcone **105** has been confirmed by spectral data and elemental analysis.

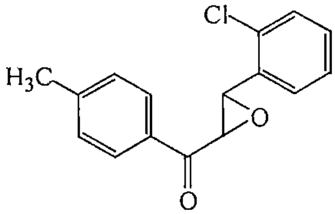


105

The UV absorption band of **105** in methanol at λ_{max} 242, 275 and 390 nm suggested the presence of a chalcone skeleton. The IR absorption frequency at ν 1672 cm^{-1} showed the presence of a conjugated carbonyl group (>C = O).

The ^{1}H-NMR spectrum explained the presence of methyl group in A ring at δ 2.35 (s, 3H, $C_{4'}$-C$\underline{H}_3$). The four aromatic protons of B ring appeared as two multiplets and two doublets at δ 7.09 (m, 1H, C_5-H); 7.08 (m, 1H, C_4-H) and 7.24 (d, 1H, J = 2.6 Hz C_6-H); 7.22 (d, 1H, J = 2.6 Hz C_3-H) integrating for one protons each respectively. The other four aromatic protons of A ring appeared as two doublets at δ 7.25 (d, 2H, J = 8.6 Hz, $C_{3'}$-H and $C_{5'}$-H) and 7.69 (d, 2H, J = 8.6 Hz, $C_{2'}$-H and $C_{6'}$-H). The C_α-H and C_β-H protons of **105** appeared as two doublets at δ 7.39 (J = 16 Hz, C_α-H) and 8.17 (J = 16 Hz, C_β-H) integrating for one proton each respectively. The yield of the chalcone was 83.24%. The structure of the compound **105** was established by the above spectral data (UV, IR, ^{1}H-NMR, ^{13}C-NMR) and elemental analysis.

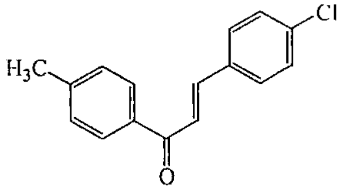
Oxidation of chalcone **105** into the corresponding 4′-methyl-2-chlorochalcone epoxide **(106)** using NaOH/ H_2O_2 as an oxidizing agent was carried out. The yield of the epoxide **106** was 70.40% and it crystallized as white needles, m.p. 100-102°C. The structure of the epoxide **106** has been confirmed by spectral data (UV, IR, ^{1}H-NMR and ^{13}C-NMR) and elemental analysis. The UV absorption band of **106** in methanol at λ_{max} 245 and 280 nm suggested the presence of a epoxide skeleton. The IR absorption frequency at ν 1703 cm^{-1} showed the presence of a carbonyl group (>C = O).



106

The C_α-H and C_β-H protons of **106** appeared as two doublets at δ 4.42 (1H, J = 2 Hz) and 4.34 (1H, J = 2 hz) integrating for one proton each respectively. The ^{1}H-NMR at 4.42 (C_α-H) and δ 4.34 (C_β-H) of the compound **106** indicated the epoxide ring comparing its corresponding chalcone (**105**). The ^{1}H-NMR spectrum explained the presence of methyl group in A ring at δ 2.33 (s, 3H, $C_{4'}$-$C\underline{H}_3$). The four aromatic proton of A ring appeared as two doublets at δ 7.14 (d, 2H, J = 8.6 Hz, $C_{3'}$-H and $C_{5'}$-H) and 7.77 (d, 2H, J = 8.6 Hz, $C_{2'}$-H and $C_{6'}$-H). The other four aromatic protons of B ring appeared as two doublets and two multiplets at δ 7.13 (d, 1H, J = 2 Hz, C_6-H); 7.20 (d, 1H, J = 2.6 Hz C_3-H) and 7.07 (m, 1H, C_5-H); 7.13 (m, 1H, C_4-H) integrating for one protons each respectively. The structure of the compound **106** was deduced by the above spectral data (UV, IR, ^{1}H-NMR, ^{13}C-NMR) and elemental analysis.

Alkaline condensation of 4-methylacetophenone (**22**) and 4-chlorobenzaldehyde (**24**) produce 4′-methyl-4-chlorochalcone (**107**). It was obtained as yellow crystals, m.p. 135-137°C. The structure of the chalcone **107** has been confirmed by spectral data and elemental analysis.

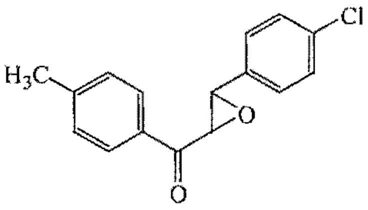


107

The UV absorption band of **107** in methanol at λ_{nax} 243, 265 and 385 nm suggested the presence of a chalcone skeleton. The IR absorption frequency at ν 1683 cm^{-1} showed the presence of a conjugated carbonyl group (> C=O).

The ^{1}H-NMR spectrum explained the presence of methyl group in the A ring at δ 2.36 (s, 3H, $C_{4'}$-C$\underline{H}_3$). The four aromatic protons of A ring appeared as two doublets at δ 7.25 (2H, J = 8.6 Hz, $C_{3'}$-H and $C_{5'}$-H) and δ 7.69 (2H, J = 8.6 Hz $C_{2'}$-H and $C_{6'}$-H) integrating for two protons each respectively. The other four aromatic protons of B ring appeared as two doublets at δ 7.24 (2H J = 8.6 Hz C_2-H and C_6-H) and δ 7.22 (2H, J = 8.6 Hz C_3-H and C_5-H) integrating for two protons each receptively. C_α-H and C_β-H protons of **107** appeared as two doublets at δ 7.56 (J = 16 Hz, C_α-H) and δ 7.90 (J = 16 Hz, C_β-H), integrating for one proton each respectively. The yield of the chalcone **107** was 70.56%. The structure of the compound **107** was established by the above spectral data (UV, IR, ^{1}H-NMR and ^{13}C-NMR) and elemental analysis.

Oxidation of chalcone **107** into the corresponding 4′-methyl-4-chlorochalcone epoxide (**108**) using NaOH/ H_2O_2 as an oxidizing agent was carried out. The yield of the epoxide **108** was 70.03% and it crystallized as white needles, m.p. 125-127°C. The structure of the epoxide **108** has been confirmed by spectral data (UV, IR, ^{1}H-NMR and ^{13}C-NMR) and elemental analysis. The UV absorption band of **108** in methanol at λ_{max} 238, 260 and 380 nm suggested the presence of an epoxide skeleton.

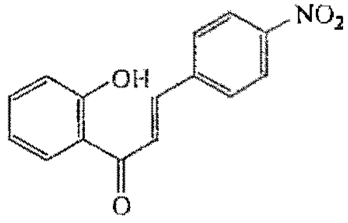


108

The IR absorption frequency at ν 1705 cm^{-1} showed the presence of a carbonyl group (>C=O). The C_α-H and C_β-H protons of **108** appeared as two doublets at δ 4.42 (1H, J = 2 Hz) and 4.35 (1H J = 2 Hz) integrating for one proton each respectively.

The ^{1}H-NMR at δ 4.42 (C_α-H) and δ 4.35 (C_β-H) of the compound **108** indicated the epoxide ring comparing its corresponding chalcone (**107**). The four aromatic protons of A ring appeared as two doublets at δ 7.14 (d, 2H, J = 8.6 Hz $C_{3'}$-H and $C_{5'}$-H) and 7.77 (d, 2H, J = 8.6 Hz $C_{2'}$-H and $C_{6'}$-H). The ^{1}H-NMR spectrum explained the presence of methyl group in A ring at δ 2.34 (s, 3H, $C_{4'}$-$C\underline{H}_3$). The other four aromatic protons of B ring appeared as two doublets at δ 7.13 (d, 2H, J = 8.6 Hz C_2-H and C_6-H) and 7.20 (d, 2H, J = 8.6 Hz C_3-H and C_5-H) integrating for two protons each respectively. The structure of the compound **108** was established by the above spectral data (UV, IR, ^{1}H-NMR and ^{13}C-NMR and elemental analysis).

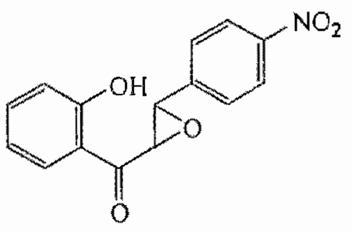
Alkaline condensation of 2′-hydroxyacetophenone (**18**) and 4-nitrobenzaldehyde (**25**) produced 2′-hydroxy-4-nitrochalcone (**109**). It was obtained as yellow crystals, m.p. 105-106°C. The structure of the chalcone **109** has been confirmed by spectral data and elemental analysis.



109

The UV absorption band of **109** in methanol at λ_{max} 243, 265 and 385 nm. suggested the presence of a chalcone skeleton. The IR absorption frequency at ν 3455 cm^{-1} indicated the presence of hydroxyl group and it gave light brown color with alcoholic ferric chloride solution confirmed that compound **109** has free hydroxyl group. The absorption frequency at ν 1681 cm^{-1} showed the presence of a conjugated carbonyl group (>C=O). The four aromatic protons B ring appeared as two doublets at δ 7.56 (d, 2H, J = 8.6 Hz, C_2-H and C_6-H) and 8.14 (d, 2H, J = 8.6 Hz C_3-H and C_5-H) integrating for two protons each respectively. The other four aromatic protons of A ring appeared as two doublets and two multiplets at δ 6.92 (d, 1H, J = 2.6 Hz, $C_{3'}$-H); 7.64 (d, 1H, J = 2.6 Hz $C_{6'}$-H) and δ 7.01 (m, 1H, $C_{5'}$-H); 7.37 (m, 1H, $C_{4'}$-H). The C_α-H and C_β-H protons of **109** appeared as two doublets at δ 7.85 (d, 1H = J = 16 Hz C_α-H); 8.04 (d, 1H, J = 16 Hz, C_β-H) integrating for one proton each respectively. One hydroxyl proton appeared as a singlet at δ 12.76 (s, 1H, $C_{2'}$-O<u>H</u>). The yield of the chalcone was 73.60%. The structure of the compound **109** was established by the above spectral data (UV, IR, ^{1}H-NMR and ^{13}C-NMR) and elemental analysis.

Oxidation of chalcone **109** into the corresponding 2′-hydroxy-4-nitrochalcone epoxide (**110**) using NaOH/ H_2O_2 as an oxidizing agent was carried out. The yield of the epoxide **110** was 68.90% and it crystallized as white needles. m.p. 95-97°C.

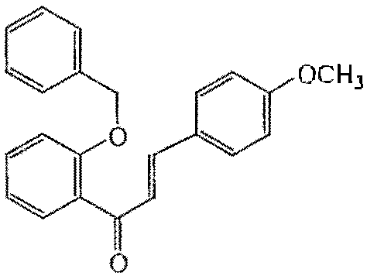


110

The structure of this epoxide **110** has been confirmed by spectral data and elemental analysis. The UV absorption band of **110** in methanol at λ_{max} 245, 260 and 380 nm suggested the presence of epoxide skeleton.

The IR absorption frequency at ν 1709 cm^{-1} showed the presence of a carbonyl group (>C=O). The C_α-H and C_β-H protons of **110** appeared as two doublets at δ 4.42 (1H, J = 2 Hz C_α-H) and 4.35 (1H, J = 2 Hz C_β-H) integrating one proton each respectively. The ^{1}H-NMR at δ 4.42 (C_α-H) and δ 4.35 (C_β-H) of the compound **110** indicated the epoxide ring comparing its corresponding chalcone (**109**). The four aromatic proton of A ring appeared as two doublets and two multiplets at δ 6.81 (d, 1H, J = 2.6 Hz $C_{3'}$-H); 7.72 (d, 1H, J = 2.6 Hz $C_{6'}$-H) and δ 6.90 (m, 1H, $C_{5'}$-H); 7.27 (m, 1H, $C_{4'}$-H). The other four aromatic protons of B ring appeared as two doublets at δ 7.45 (d, 2H J = 8.6 Hz C_2-H and C_6-H) and δ 8.12 (d, 2H J = 8.6 Hz, C_3-H and C_5-H) integrating for two protons each respectively. One hydroxyl proton appeared as a singlet at δ 11.40 ($C_{2'}$-OH). The structure of the compound **110** was established by the above spectral data (UV, IR, ^{1}H-NMR, ^{13}C-NMR) and elemental analysis.

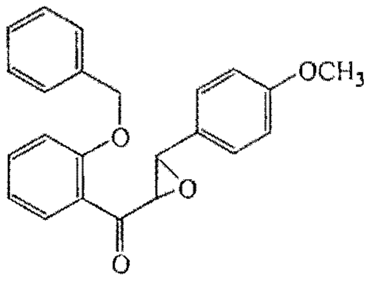
Alkaline condensation of 2-benzyloxyacetophenone (**21**) and 4-methoxybenzaldehye (**27**) produced 2′-benzyloxy-4-methoxychalcone (**111**). It was obtained as yellow crystals, m.p. 65-67°C. The structure of the chalcone (**111**) has been confirmed by spectral data and elemental analysis. The UV absorption band of **111** in methanol at λ_{max} 216 and 308 nm. suggested the presence of a chalcone skeleton. The IR absorption frequency at ν 1679 cm^{-1} showed the presence of a conjugated carbonyl group (>C=O). The ^{1}H-NMR spectrum explained the presence of one methoxy group in the B ring at δ 3.73 (s, 3H, C_4-OC$\underline{H}_3$).



111

The four aromatic protons of B ring appeared as two doublets at δ 6.72 (d, 2H, J = 2.6 Hz C_3-H and C_5-H) and 7.19 (d, 2H, J = 2.6 Hz, C_2-H and C_6-H) integrating for two protons each respectively. The other four aromatic protons of A ring appeared as two doublets and two multiplets at δ 6.96 (d, 1H, $C_{3'}$-H); 7.70 (d, 1H, $C_{6'}$-H) and 7.01 (m, 1H, $C_{5'}$-H); 7.43 (m, 1H, $C_{4'}$-H) integrating for one proton each respectively. Two singlets at δ 5.20 (s, 2H, $C_{2'}$-OC$\underline{H}_2$-C_6-H_5) and 7.19 (s, 5H, $C_{2'}$-OC$H_2$$C_6$$\underline{H}_5$) indicated the presence of benzyloxy group. The C_α-H and C_β-H protons of **111** appeared as two doublets at δ 7.56 (d, 1H, J = 16 Hz, C_α-H) and 7.90 (d, 1H, J = 16 Hz C_β-H) integrating for one proton each respectively. The yield of the chalcoen was 61%. The structure of the compound **111** was established by the above spectral data (UV, IR, ^{1}H-NMR and ^{13}C-NMR) and elemental analysis.

Oxdation of the chalcone **111** into the corresponding 2′-benzyloxy-4-methoxychalcone epoxide (**112**) using NaOH/ H_2O_2 as an oxidizing agent was carried out. The yield of the epoxide **112** was 80.15% and it crystallized as white needles, m.p. 75-76°C. The UV absorption band of **112** in methanol at λ_{max} 209 and 295 nm suggested the presence of a epoxide skeleton.



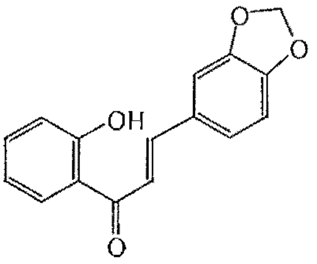
112

The IR absorption frequency at ν 1707 cm^{-1} showed the presence of a carbonyl group (>C=O). The C_α-H and C_β-H protons of **112** appeared as two doublets at δ 4.43 (1H J = 2 Hz C_α-H) and 4.37 (1H, J = 2 Hz C_β-H) integrating for one proton each respectively.

The ^{1}H-NMR at δ 4.43 (C_α-H) and δ 4.37 (C_β-H) of the compound **112** indicated the epoxide ring and compared with its corresponding chalcone (**111**). The ^{1}H-NMR spectrum explained the presence of one methoxy group in B ring a δ 3.73 (s, 3H C_4-$OC\underline{H}_3$). The four aromatic protons of A ring appeared as two doublets and two multiplets at δ 6.85 (d, 1H, $C_{3'}$-H); 7.78 (d, 1H, J = 8.6 Hz, $C_{6'}$-H) and δ 6.90 (m, 1H, J = 8.6 Hz $C_{5'}$-H); 7.33 (m, 1H, $C_{4'}$-H). The other four aromatic protons of B ring as two doublets at δ 6.70 (d, 2H, J = 2.6 Hz, C_3-H and C_5-H) and 7.08 (d, 2H, J = 2.6 Hz, C_2-H and C_6-H) integrating for two protons each respectively. Two singlets at δ 5.19 (s, 2H, $C_{2'}$-$OC\underline{H}_2$-C_6H_5) and δ 7.19 (s, 5H, $C_{2'}$-OCH_2-$C_6\underline{H}_5$) indicated the presence of benzyloxy group. The structure of the compound **112** was established by the above spectral data (UV, IR, ^{1}H-NMR, ^{13}C-NMR) and elemental analysis.

Alkalin condensation of 2-hydroxyacetophenone (**18**) and 3,4-methylenedioxybenzaldehyde (**28**) produced 2′-hydroxy-3,4-methylene-dioxychalcone (**113**). It was obtained as yellow crystals, m.p. 125-127°C. The structure of the chalcone **113** has been confirmed by spectral data and elemental analysis. The UV absorption band of **113** in methanol at λ_{max} 242, 280 and 355 nm suggested the presence of a chalcone skeleton.

The IR absorption frequency at ν 3458 cm^{-1} indicated the presence of a hydroxyl group and it gave light brown color with alcoholic ferric chloride solution confirmed that compound **113** has free hydroxyl group.

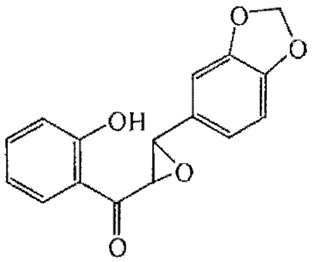


113

The absorption frequency at ν 1641 cm^{-1} showed the presence of a conjugated carbonyl group (>C=O). The ^{1}H-NMR spectrum explained the presence of methylenedioxy group in B ring at δ 5.90 (s, 2H, C_3-OCH_2O-C_4). The three aromatic protons of B ring appeared as one singlet at δ 6.70 (s, 1H, J = 8.4 Hz, C_2-H) and two doublets at δ 6.61 (d, 1H, J = 8.6 Hz, C_5-H) and 6.75 (d, 1H, J = 8.6 Hz, C_6-H) integrating for one proton each respectively. The other four aromatic protons of A ring as two doublets at δ 6.92 (d, 1H, J = 8.6 Hz, $C_{3'}$-H) and 7.64 (d, 1H, J = 8.6 Hz $C_{6'}$-H) and two multiplets at δ 7.01 (m, 1H, $C_{5'}$-H) and 7.37 (m, 1H, $C_{4'}$-H) integrating for one proton each respectively. The C_α-H and C_β-H protons of **113** appeared as two doublets at δ 7.56 (d, 1H, J = 16 Hz, C_α-H) and 7.90 (d, 1H, J = 16 Hz C_β-H) integrating for one proton each respectively. One hydroxyl proton appeared as a singlet at δ 12.89 (s, 1H, $C_{2'}$-OH). The yield of the chalcone (**113**) was 78.36%. The structure of the compound **113** was established by the above spectral data (UV, IR, ^{1}H-NMR and ^{13}C-NMR) and elemental analysis.

Oxidation of the chalcone **113** into the corresponding 2′-hydroxy-3, 4-methylenedioxychalcone epoxide **(114)** using NaOH/ H_2O_2 as an oxidizing agent was carried out. The yield of the epoxide **114** was 84.31% and it crystallized as white needles m.p. 215-217°C. The structure of the epoxide **114** has been confirmed by spectral data and elemental analysis.

The UV absorption band of **114** in methanol at λ_{max} 245, 282 and 375 nm suggested the presence of an epoxide skeleton. The IR absorption frequency at ν 3464 cm^{-1} indicated the presence of a hydroxyl group. The absorption frequency at ν 1697 cm^{-1} showed the presence of a carbonyl group (>C=O).

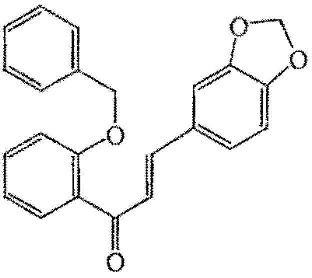


114

The C_α-H and C_β-H protons of **114** appeared as two doublets at δ 4.44 (1H, J = 2 Hz, C_α-H) and 4.36 (1H, J = 2 Hz C_β-H) integrating for one proton each respectively. The ^{1}H-NMR at δ 4.44 (C_α-H) and 4.36 (C_β-H) of the compound **114** indicated the epoxide ring and compared with its corresponding chalcone **(113)**. A singlet at δ 5.91 (s, 2H, C_3-OC$\underline{H_2}$O-C_4) indicated the presence of methylenedioxy group. The three aromatic protons of B ring appeared as one singlets at δ 6.59 (s, 1H, C_2-H) and two doublets δ 6.59 (d, 1H J = 2.6 Hz C_5-H) and 6.64 (d, 1H, C_6-H). The other four aromatic protons of A ring which appeared as two doublets at δ 6.81 (d, 1H, J = 8.6 Hz $C_{3'}$-H) and 7.72 (d, 1H, J = 8.6 Hz, $C_{6'}$-H) and two multiplets at δ 6.90 (m, 1H, $C_{5'}$-H) and 7.27 (m, 1H, $C_{4'}$-H) integrating for one proton each respectively. One hydroxyl proton appeared as a singlet at δ 11.85 (s, 1H, $C_{2'}$-OH). The structure of the compound **114** was established by the above spectral data (UV, IR, ^{1}H-NMR and ^{13}C-NMR) and elemental analysis.

Alkaline condensation of 2-beznyloxyacetophenone **(21)** and 3,4-methylenedioxybenzaldehyde **(28)** furnished 2′-benzyloxy-3,4-methylenedioxchalcone **(115)**. It was obtained as yellow crystals, m.p. 80-82°C. The structure of the chalcone **115** has been confirmed by spectral data and elemental analysis. The UV absorption band of **115** in methanol at λ_{max} 280 and 395 nm suggested the presence of chalcone skeleton. The absorption frequency at ν 1682 cm^{-1} showed the presence of a conjugated carbonyl group (>C=O). The ^{1}H-NMR spectrum explained the presence methylenedioxy group in the B ring at δ 5.94.

The four aromatic protones of A ring appeared as two doublets at δ 6.96 and 7.70 assigned to $C_{3'}$-H and $C_{6'}$-H and two multiplets at δ 7.01 and 7.43 assigned to $C_{5'}$-H and $C_{4'}$-H integrating for one proton each respectively.

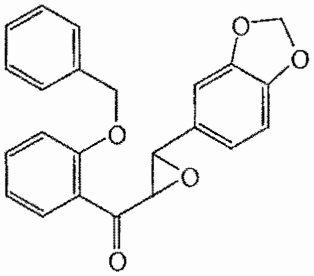


115

The other three aromatic protons of B ring appeared as one singlet at δ 6.70 (s, 1H, J = 2.4 Hz C_2-H) and two doublets at δ 6.61(d, 1H, J = 8.6 Hz C_5-H) and 6.75 (d, 1H, J = 8.6 Hz, C_6-H). Two singlets at δ 5.22 (s, 2H, $C_{2'}$-$OC\underline{H}_2$-C_6H_5) and δ 7.19 (s, 5H, $C_{2'}$-OCH_2-$C_6\underline{H}_5$) indicated the presence of benzyloxy group. The C_α-H and C_β-H protons of **115** appeared as two doublets at δ 7.56 (d, 1H, J = 16 Hz, C_α-H) and 7.90 (d, 1H, J = 16 Hz, C_β-H) integrating for one proton each respectively. The yield of the chalcone 62.84%. The structure of the compound **115** was established by the above spectral data (UV, IR, ^{1}H-NMR, ^{13}C-NMR) and elemental analysis.

Oxidation of chalcone **115** into the corresponding 2′-benzyloxy-3, 4-methylenedioxy chalcone epoxide (**116**) using NaOH/ H_2O_2 as an oxidizing agent was carried out. The yield of the epoxide **116** was 72.6% and it crystallized as white needles, m.p. 110-112°C. The structure of the epoxide **116** has been confirmed by spectral data and elemental analysis.

The UV absorption band of **116** in methanol at λ_{max} 285 and 390 nm suggested the presence of an epoxide skeleton. The IR absorption frequency at ν 1699 cm^{-1} showed the presence of a carbonyl group (>C=O).



116

The C_α-H and C_β-H protons of **116** appeared as two doublets at δ 4.43 (d, 1H, J = 2 Hz, C_α-H) and 4.37 (d, 1H, J = 2 Hz C_β-H) integrating for one proton each respectively. The ^{1}H-NMR at δ 4.43 (C_α-H) and δ 4.37 (C_β-H) of the compound **116** indicated the epoxide ring comparing its corresponding chalcone (**115**). A singlet at δ 5.92 (s, 2H, C_3-OC$\underline{H}_2$O-C_4) indicated the presence of methylenedioxy group. The three aromatic protons of B ring appeared as one singlet at δ 6.59 (s, 1H, C_2-H) and two doublets 6.59 (d, 1H, J = 2.6 Hz C_5-H) and 6.64 (d, 1H, C_6-H). The other four aromatic protons of A ring which appeared as two doublets at δ 6.85 (d, 1H, J = 8.6 Hz $C_{3'}$-H) and 7.78 (d, 1H, J = 8.6 Hz $C_{6'}$-H) and two multiplets at δ 6.90 (m, 1H, $C_{5'}$-H) and 7.33 (m, 1H, $C_{4'}$-H) integrating for one proton each respectively. Two singlets at δ 5.21 (s, 2H, $C_{2'}$-OC$\underline{H}_2$-C_6H_5) and 7.19 (s, 5H, $C_{2'}$-OCH$_2$-C_6-$\underline{H}_5$) indicated the presence of benzyloxy group. The structure of the compound **116** was established by the above spectral data (UV, IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis.

PART-II

Studies of antimicrobial activity of chalcone epoxides and their corresponding chalcones

CHAPTER
V

Introduction

CHAPTER-V

Introduction

Studies of antmicrobial activity of chalcone epoxides and their corresponding chalcones

5.1 General Discussion:

Chalcones and flavones are widely occurring in medicinal plants in their chemical constitutions[22,23] and these medicinal plants have been used for the treatment of various kind of diseases, such as intestinal parasites and colic in children, dysentery, diarroea, fever, skin diseases, bronchitis, whooping cough, leprous sores, painful rheumatic joints and other coetaneous diseases. Medicinal plants constitute an important natural wealth of a country. They play a significant role in primary health care service to rural people. The plant kingdom served as the best natural source of drugs and medicines from the start of human civilization. Various plat materials have been and still being processed and utilized as raw materials for pharmaceutical preparation such as antibiotic. Most of these plant constituents particularly the secondary metabolites such as flavonoids (chalcones, chalcones epoxides, flavones and flavanones), alkaloids, glycosides, essential oils, tannins, resins, saponins, waxes, cardinolides, butadinolides etc. have pronounced pharmacological action on animal and human physiological system. It is also capable of mitigating and curing human sufferings, health wounds, cuts, burns and other antimicrobial activities.

Chalcones form an important group of plant pigments. They are the precursors in the biosynthesis of flavonoids and are known to play an ecological role in nature in relation to colors of the leaves and flowers of plants. Chalcones and flavones show insecticidal, piscicidal, antibacterial, fungicidal, anti-oxidant, anti-cancerous and herbicidal activities. Therapeutical applications of flavonoid compounds are enormous. Some flavonoids like 'citrin' can decrease the permeability and increase the resistance of the capillary wall of the artery and nerves.

Bangladesh is predominantly an agricultural country depending mainly on crop plants, agricultural, medicinal and forest products for its economic development. Although crops play a vital role in the economy of the country and agroecological conditions are favorable for the production of various crops, the yields of crops is often poor. Among the various factors responsible for poor yield of crops, plant disease caused by various microorganisms play a significant role. Gradually men gathered sufficient knowledge of chemistry to inhibit or to kill the microorganism i. e. only inhibit the microbial growth are called 'statis'. But the chemicals which have ability to kill microorganisms are called 'cidal'. Various pesticides are classified as fungicides, viricides, bactericides etc.

It is universal truth that disease, decay and death have always been coexist with human life. Many fatal diseases caused by microorganisms viz. bacterial, fungal and viral attack are known. The treatment of diseases due to bacterial, viral and fungal invasion by chemical compounds were studied and used successfully without affecting the tissues of the host and any other side effects. Many compounds e.g. formaldehyde, iodine, phenol etc. are also active in destroying bacteria.

Antibiotics are the chemical substances obtained from certain non-pathogenic microorganism (Bacteria and Fungi) and are used for either killing or inhibiting the growth of pathogenic microorganism without affecting the host tissue.

In order to detect the antimicrobial activity of a new compound for the development as potential new antibiotic or chemotherapeutic agent *in vitro* antimicrobial screening is a useful technique. In general, antimicrobial screening is undertaken in two phases: a primary qualitative assay to detect the presence or absence of activity and secondary assay which quantifies the relative potency expressed as minimum inhibitory concentration (MIC) value of a pure active compound.

The words bactericide and fungicide have originated from Latin words: bacteria, fungus and caedo. The word caedo means 'to kill'. Thus literally speaking a bactericide and fungicide would be any agencies which have the ability to kill bacteria or fungus. By common usage the word is restricted to chemicals. Hence the words bactericide and fungicide mean a chemical capable of killing bacteria and fungus respectively.

It is not enough that a chemical has high bactericidal and fungicidal activity. Such as chemicals may have no utility unless it stands out in the tests and gives proof of significant control of diseases under varied field conditions. There are several factors which influence the performance of a bactericide and fungicide under different field conditions. They may be either physical or chemical in nature.

A good fungicide should be toxic to the parasite or inhibit the germination of its spores without causing phytotoxicity. It should be reasonably easy to prepare and not so expensive. It should be capable of even distribution from spraying or dusting machines on the surface to be covered should remain on the surface without running off (initial retention) and should stick to the surface after drying (tenacity). A good fungicide should be least toxic to human beings and cattle. This will eliminate dangers of accidental poisoning and make it safer for an operator to work.

The number of chemicals available that shows antibacterial activity runs into hundreds although all are not equally safe, effective and popular. Also different types of organic, aromatic, inorganic and heterocyclic compounds are employed as antibacterial agents. Salts of toxic metals and organic acids, organic compounds of mercury and sulfur, quinines, flavones and heterocyclic nitrogen, oxygen compounds are the major fungicides in use today. The flavonoid compounds are highly effective and most popular fungicides in use these days.

Many flavonoid compounds have significant antimicrobial activity and have been developed into antibiotics and fungicides. Some of these are in commercial use.

It is well known that a large number of biologically active compounds possess aromatic and heteroaromatic nucleus and flavonoid compounds in their chemical structures contain aromatic and heteroaromatic nucleus. It is also known that if an active nucleus is linked to another nucleus the resulting molecule may possess greater potential for biological activity[24]. The benzene and substituted benzene nuclei play an important role as common denominator for various biological activities. It was observed that many times the combination of two or more nuclei enhances biological profile many fold than its parent nuclei.

Danno G.I. *et al.*[25] performed antibacterial activities of a series of flavonoid compounds. He used two human pathogenic bacteria such as *Bacillus cereus* ATCC11778 and *Salmonella enteritidis*. The antibacterial activities of flavonoids were found to be enhanced by combining or mixing them. The combination of flavonoids were much more active than flavonoid alone.

Propolis (Bee glue) a resinous material derived by bees from plant juices was known for its therapeutic properties. Ethanolic Extract of Crude Propolis (EECP), Ethanolic Extract of Defatted Propolis (EEDP), Non Volatile fraction of Etheric Defatted Propolis (NVFEDP) and Aqueous Phase of Etheric Defatted Propolis (APEDP) were investigated for its antibacterial effect against a range of commonly encountered gram positive bacteria e.g. *Staphylococcus aureus, Staphylococcus epidermidis* and gram negative bacteria e.g. *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Citrobacter freundii* by Rhajaoui, M. *et al*[26]. Propolis has been used for thousand years in folk medicine for its biological activities such as anti-inflammatory[27,28,29], antiviral[30], antifungal[31], antiparasital[32], antitumoral[29], tissue and dental pulp regenerative[33,34] and antibacterial activity[29,30,35,36]. Rhajaoui *et al.*[26] reported that propolis activity is not due to the presence of one of particular substance but is a resultant of the complex action of various aromatic structures and of flavonoid compounds. They suggested that the antibacterial effect of propolis is the result of their flavonoid contents, like myricitine ‘3,7,4′,5′-tetramethylether’, quercetine ‘3,7,3-trimethyal ether’[37], pinobanksin ‘3,5,7-trihydroxyflavonone’, chrysin ‘5,7-dihydroxyflavone’[38], pinocembrin ‘5,7-dihydroxy flavone’[32,37,38] and their various esters of caffeic acid[29,39]. Actually several research teams support the basis of flavonoids implication in the antibacterial activity.

The flavonoids are a group of natural products founds in fruits, vegetables, nuts, seeds and flowers as well as in teas and wines are important constituent of human diet. They have been demonstrated to possess many biological and pharmacological activities such as anti bacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antimutagenic and antiallergic activities and inhibitory activities on several enzymes[40,41].

Thirty seven naturally occurring flavonoids were investigated for their inhibitory activities on the mouse brain monoamino oxidase (MAO) *in vitro* by Lee S.J. *et al.*[42]. Three flavonoids apigenin (IC_{50}, 1μ mol), Liquiritigenin (IC_{50}, 4μ mol) and genistein (5μ mol) were judged to be very active for flavonoids, quercetin, leuteolin and eupatilin were moderately active with IC_{50} values of 10-20 μ mol.

Carrvalho J.C. *et al.*[43] isolated a flavone named titonine (7,4′-dimethoxy-3′-hydroxyflavone) from the leaves of *Virola michelli* Heckel (Myristicaceae). Titonine was further subjected to methylation and acylation reaction yielding a 7,3′,4′-trimethoxyflavone and 7,4′-dimethoxy-3′-acetylflavone receptively. These compounds were evaluated for both anti-inflammatory and analgesic activity. The anti-inflammatory activity was evaluated in rats using the paw edema test with carragenin while the analgesic activity was determined in mouse using the writhing test method.

Aitken R.A. *et al.*[44] synthesized a new derivative of flavone-8-aceticacid (FAA) and evaluated anti-tumor activity of this flavone derivative.

Flavone ring system is of considerable interest due to several biological activities including antibacterial. Following these observations TunCbilek M. *et al.*[45] have designed and synthesized some o-substituted oxime derivatives on flavone B-ring and tested antibacterial activity against *S. aureus*, *E. coli* and *C. albicns*.

Artoindonesianin P a new prenylated flavone have been isolated from *Artocarpus Lanceifolius* 668 and evaluated cytotoxic activity by Hakim E.H. *et al.*[46].

Miroslawa K.B. *et al.*[47] have been isolated some biflavones from *Taxus baccata* and *Ginkgo biloba* and studied antifungal activity towards the fungi *Alternaria alternata, Fusarium culmorum* and *Cladosporium oxysporum.*

Jha H. *et al.*[48] have been tested antifungal effect of ten defferent flavonoids against *Alternaria tenuissima, Cladosporium cladosporioides, Spicellum roseum* and *Trichoderma hamatum* in concentration 2×10^{-4} (a) and 8×10^{-4} mol (b) on agar. They found that unsubstituted flavone was the most active substance in both the concentrations (a) and (b). They also reported that 3,5,7-trihydroxyflavone showed higher fungicidal activity than the 4′,5,7-trihydroxyflavone.

From the above discussion it is clear that the flavonoid compounds show antimicrobial activity and widely used as drugs. So we deliberately synthesized a number of chalcone epoxides e.g. 2′-hydroxy-2,4,5-trimethoxychalcone epoxide (**102**); 2′-benzyloxy-2,4,5-trimethoxy chalcone epoxide (**104**); 4′-methyl-2-chlorochalcone epoxide (**106**); 4′-methyl-4-chlorochalcone epoxide (**108**); 2′-hydroxy-4-nitrochalcone epoxide (**110**); 2′-benzyloxy-4-methoxychaclone epoxide (**112**); 2′-hydroxy-3,4-methylenedioxy chalcone epoxide (**114**); 2′-benzyloxy-3,4-methylenedioxy chalcone epoxide (**116**) and their corresponding chalcones (**101, 103, 105, 107, 109 111, 113** and **115**) finally antibacterial activities and antifungal activities of these chalcone epoxides and their corresponding chalcones were determined. The antibacterial and antifungal activities of their chalcone precursors were also determined. These chalcone epoxides and their corresponding chalcones were used (which were listed in table-1) for evaluation of antimicrobial activities and synthesis of these compounds are already discussed in the first part of this dissertation. The result obtained by *in vitro* antibacterial activity against both Gram-positive and Gram-negative organisms and also antifungal activities of these compounds (Table-1) are discussed in this part of this dissertation.

The capability of microorganism to antimicrobial agents can be determined *in vitro* by a number of methods. The disc diffusion technique[49,50] is widely accepted for preliminary investigations of materials which are suspected to possesses antimicrobial properties. Diffusion procedure as normally used in susceptible intermediate of resistant categories.

Diffusion assays are based on the ability of antibiotics to diffuse from a confined source through a PDA gel and create a concentration gradient. If the agar is seeded or streaked with sensitive organism a zone of inhibition will result where the antibiotic concentration exceeds the minimum inhibitory concentration (MIC) for that particular organism.

In the disc diffusion technique dried filter paper discs containing known amount of test material are placed on agar plates seeded with test organisms. These plates are kept at low temperature (4°C) for 24 hours. Initially the dried discs absorb water from the surrounding test medium and the drug is dissolved. The drug migrates through the adjacent test medium by concentration gradient of the drug according to physical law that govern diffusion of molecules through an agar gel[51]. As a result there is a gradual change of drug concentration in the agar surrounding each discs. Then the plates are incubated in an incubator at 37.5°C for 24 hours.

As the antibiotic diffusion progresses microbial multiplication also proceeds. At that point of time fungal multiplication proceeds more rapidly than the drug can diffuse and fungal cell which are not inhibited by the antimicrobial agents will continue to multiply until a lawn of growth can be visualizd. No growth will appear in the area where drug is present in inhibitory concentrations.

Generally more susceptible the test organism the larger is the zone of inhibition. Antimicrobial activities of the test compounds are expressed by measuring the zone of inhibition observed around the area. The diameter of the inhibition zone is usually measured to understand the extent of inhibition in different concentration.

The size of the inhibitory zones depends principally in the following factors:

(a) Intrinsic antimicrobial sensitivity of the test compounds.

(b) Growth rate of the test microorganisms.

(c) Diffusion rate of the drug, which is related to its water solubility.

(d) Number of concentration of the inoculated test organisms.

(e) Concentration / amount of the test sample.

(f) Thickness of the test medium in the petridishes.

5.2 Antibacterial Activity Testing:

Materials and Methods:

Evaluation of chemicals against bacteria:

In order to detect the antimicorbial activity of a new compound for the development as potential new antibiotic or chemotherapeutic agent *in vitro* antimicrobial screening is a useful technique. In general antimicrobial screening is undertaken in two phases: a primary qualitative assay to detect the presence or absence of activity and secondary assay which quantifies the relative potency expressed as minimum inhibitory concentration (MIC) value of a pure active compound.

In vitro antibacterial activities of the test chemicals were studied against four human pathogenic bacteria. The primary assay can be performed *in vitro* by a number of methods one of which is disc diffusion technique[49,50] by this method we could classify the organism as susceptible as well as resistance towards particular compounds.

The secondary assay is the serial broth dilution assay[52,53] which quantifies the antimicrobial activity of pure compound by providing the MIC value of the compound for specific susceptible organism. This is an important consideration for the screening of a new antibiotic substance.

Agar (PDA) was used as basal medium for test fungi. Dimethyl sulphoxide (DMSO) was used as a solvent to prepare desired solution (10 mg / mL) of the compounds initially. Proper control was maintained with dimethyl sulphoxide (DMSO). The materials and methods of the present investigation are described in details bellow.

Method:

Diffusion assay is based on the ability of antibiotics to diffuse from a confined source through the nutrient agar gel and create a concentration gradient. If the agar is seeded with a sensitive organism a zone of inhibition will be result where the concentration exceeds the minimum inhibitory concentration (MIC) for that particular organism.

The disc diffusion technique[49,50] involves utilization of paper disc on which a known amount of drug has been applied and dried. The discs are then placed on the agar plates seeded with test organisms and kept at low temperature (4°C) for 24 hours. A number of simultaneous events have been occurred during this time.

1. At first the dried disc absorbs water form the surrounding test medium and the drug dissolved in it.
2. Then the drug migrates through the adjacent test medium due to the concentration gradient.
3. It produces a concentration gradient surrounding the each disc.

The petridishes are then incubated in an incubator at 37.5°C for 8 hours. After incubation the microbial multiplication proceeds at the same time diffusion of compound also progresses. Logarithmic growth phase is initiated after the lag phase and at that time bacterial multiplication proceeds more rapidly than the drug can diffuse. The bacterial cells which are not inhibited by the antibacterial agent will continue to multiply until visualized. The activity of antibiotic is evidenced by the presence of a clear zone of inhibition surrounding the disc. Disc diffusion method is highly effective for rapidly growing bacteria and the activity of the test drugs are expressed by measuring the diameter of the zone of inhibition. Generally the zone of inhibition is proportional to the susceptibility of the organism.

Apparatus and reagents:

1. Filter paper disc
2. Nutrient agar (DIFCO)
3. Petridishes
4. Dimethyl sulphoxide (DMSO)
5. Alcohol-95%
6. Methanol
7. Inoculating loop
8. Sterile cotton
9. Sterile Forceps
10. Spirit lamp
11. Aseptic hood / Laminar air Flow horizontal with HEPA filter
12. Micropipette
13. Autoclave
14. Incubator
15. Refrigerator
16. Test tubes

Chemicals used:

Eight chalcone epoxides and their corresponding chalcones were used as the test chemicals. The chemicals were synthesized, isolated and characterized in the Organic Research Laboratory, Department of Chemistry, University of Rajshahi. The names of the tested compounds are listed in Table-1

Table-1: List of compounds used for antibacterial activities:

Compound No.	*Name of the test compounds*	**Molecular formula**
101	2′-hydroxy-2,4,5-trimethoxychalcone	$C_{18}H_{18}O_5$
102	2′-hydroxy-2,4,5-trimethoxychalcone epoxide	$C_{18}H_{18}O_6$
103	2′-benzyloxy-2,4,5-trimethoxychalcone	$C_{25}H_{24}O_5$
104	2′-benzyloxy-2,4,5-trimethoxychalcone epoxide	$C_{25}H_{24}O_6$
105	4′-methyl-2′-chlorochalcone	$C_{16}H_{13}OCl$
106	4′-methyl-2′-chlorochalcone epoxide	$C_{16}H_{13}O_2Cl$
107	4′-methyl-4-chlorochalcone	$C_{16}H_{13}OCl$
108	4′-methyl-4-chlorochalcone epoxide	$C_{16}H_{13}O_2Cl$
109	2′-hydroxy-4-nitrochalcone	$C_{15}H_{11}O_4N$
110	2′-hydroxy-4-nitrochalcone epoxide	$C_{15}H_{11}O_5N$
111	2′-hydroxy-4-methyoxychalcone	$C_{23}H_{20}O_3$
112	2′-hydroxy-4-methyoxychalcone epoxide	$C_{23}H_{20}O_4$
113	2′-hydroxy-3,4-methylenedioxychalcone	$C_{16}H_{12}O_4$
114	2′-hydroxy-3,4-methylenedioxychalcone epoxide	$C_{16}H_{12}O_5$
115	2′-benzyloxy-3,4-methylenedioxychalcone	$C_{23}H_{18}O_4$
116	2′-benzyloxy-3,4-methylenedioxychalcone epoxide	$C_{23}H_{18}O_5$

Test organism:

In the present study to screen the antibacterial activities of different chemicals and a number of bacterial strains were used as test organisms. Among these human pathogens two were Gram-positive and two were Gram-negative.

Collection of organisms:

The test tube cultures of the bacterial pathogens were collected from the Mycology and plant pathology laboratory, Department of Botany, University of Rajshahi, Rajshahi, Bangladesh.

Following type of test organisms have been studied:

Table-2: List of the test organisms.

1	*Streptococcus-β-haemolyticus*	Gram-positive (G^+)
2	*Bacillus megaterium*	Gram-positive (G^+)
3	*Klebsiella sp.*	Gram-negative (G^-)
4	*Escherichia coli*	Gram-negative (G^-)

Test materials used for the study:

The synthetic compounds at a concentration of 100, 200, 300 and 400 μg/disc were used for the investigation of antibacterial activity. These compounds were dissolved in dimethyl sulphoxide for screening of the antibacterial activity. *Kanamycine-50 (*K-50, 50μg/disc) was used as standard.

Preparation of medium:

The instant Nutrient Agar (NA) media is weighed and then reconstituted with distilled water in a conical flask according to specification (2.8% w/v). It was then heated in a water bath to dissolve the agar until a transparent solution was obtained and it was then autoclaved at 121°C for 15 minutes at 15 Ibs / sq. inch pressure.

Preparation of fresh culture:

The media prepared in the above section was dispensed in 5mL amount of clear test tubes to prepare slants. The test tubes were plugged with cotton and sterilized in an autoclave at 121°C for 15 minutes at 15 Ibs / sq. inch pressure. After sterilization the test tubes were kept in an inclined position for solidification. Finally the slants streaked with pure culture of the test organisms under laminar air flow and incubated for 24h.

Preparation of test plates:

1. 15 mL of Nutrient Agar medium which prepared the previous section was poured in clean test tubes and then plugged with cotton.
2. The test tubes were sterilized and allowed to cool at about 45°C to 50°C.
3. The medium in the test tubes were inoculated with fresh culture of the test bacteria by means of a sterile loop and agitated to ensure uniform dispersion of bacteria in the medium.
4. Finally, the medium was poured into sterile petridishes and agitated and clockwise anticlockwise right to left and left to right. Thus plates were ready for sensitivity test.

Preparation of discs containing sample:

A. Sample discs:

1. Solution of the synthetic compounds was prepared in dimethyl sulphoxide (DMSO) so that 10μL contained 100μg of the compounds.
2. Filter paper discs were taken in a petridish and sterilized by oven at 110°C for 1 hour.

3. 10μL and 20μL of the solution of each compound was placed in a particular disc with the help of a micropipette.
4. These discs were then air dried.

B. Standard discs:

Commercially available *Kanamycin-50 containing 50 μg/disc of this antibiotic was used as standard disc.

Placement of the discs and incubation:

1. The sample impregnated discs and standard antibiotic discs were placed gently on the solidified agar plates with the help of a sterile forceps to ensure contact to the medium.
2. The plates were then kept in a refrigerator at 4°C for overnight so that the materials that absorbed the discs could get sufficient time to diffuse into the medium.
3. Finally the plates were incubated at 37.5°C for 24 hours.

Determination of antibacterial activity of the test agents by measuring the zone inhibition:

After 24 hours incubation the antibacterial activity was carried out by measuring the zone of inhibition in millimeter (mm) by a transparent scale. The zones made by the samples were compared with that of the standard disc.

5.3 Antifungal and Activity Testing

Materials and Methods:

Evaluation of chemicals against fungi:

The antifungal activities of eight chalcones and eight chalcone epoxides were studied towards five plant pathogenic and molds fungi. The antifungal activity was assessed by poisoned food technique[54] in some modified condition[55].

Potato Dextrose Agar (PDA) was used as basal medium for test fungi. Dimethyl sulphoxide (DMSO) was used as a solvent to prepare desired solution (10 mg/mL) of the compounds initially. Proper control was maintained with Dimethyl sulphoxide (DMSO). The materials and methods of the present investigation are described in details bellow.

Apparatus and reagents:

1. Filter paper disc.
2. Nutrient agar (DIFCO)
3. Petridishes
4. Dimethyl sulphoxide (DMSO)
5. Alcohol-95%
6. Methanol
7. Inoculating loop
8. Sterile cotton
9. Sterile forceps
10. Spirit lamp
11. Aseptic hood / Laminar air flow horizontal with HEPA filter
12. Micropipette
13. Autoclave
14. Incubator
15. Refrigerator
16. Test tubes

Collection of fungal cultures:

As the test organisms are pathogenic all steps of the work were done with high precaution and aseptic condition which is mentioned bellow. The test tube cultures of the fungal pathogens were collected from the Mycology and plant pathology laboratory, Department of Botany, University of Rajshahi, Rajshahi, Bangladesh.

The names of the collected pathogens are listed bellow:

Table-3: List of the test pathogens:

1. *Rhizoctonia solani*	Molds
2. *Sclerotium rolfsii*	Molds
3. *Aspergillus niger*	Molds
4. *Aspergillus fumigatus*	Molds

Chemicals used:

The synthetic chalcones along with their corresponding chalcone epoxides were used as test chemicals. The chemicals were synthesized, isolated and characterized in the Organic Research Laboratory, Department of Chemistry, University of Rajshahi. The names of the tested compounds are listed in Table-4.

Table-4: List of compounds used for antifungal activities:

Compound No.	*Name of the test compounds*	**Molecular formula**
101	2′-hydroxy-2,4,5-trimethoxychalcone	$C_{18}H_{18}O_5$
102	2′-hydroxy-2,4,5-trimethoxychalcone epoxide	$C_{18}H_{18}O_6$
103	2′-benzyloxy-2,4,5-trimethoxychalcone	$C_{25}H_{24}O_5$
104	2′-benzyloxy-2,4,5-trimethoxychalcone epoxide	$C_{25}H_{24}O_6$
105	4′-methyl-2′-chlorochalcone	$C_{16}H_{13}OCl$
106	4′-methyl-2′-chlorochalcone epoxide	$C_{16}H_{13}O_2Cl$
107	4′-methyl-4-chlorochalcone	$C_{16}H_{13}OCl$
108	4′-methyl-4-chlorochalcone epoxide	$C_{16}H_{13}O_2Cl$
109	2′-hydroxy-4-nitrochalcone	$C_{15}H_{11}O_4N$
110	2′-hydroxy-4-nitrochalcone epoxide	$C_{15}H_{11}O_5N$
111	2′-hydroxy-4-methyoxychalcone	$C_{23}H_{20}O_3$
112	2′-hydroxy-4-methyoxychalcone epoxide	$C_{23}H_{20}O_4$
113	2′-hydroxy-3,4-methylenedioxychalcone	$C_{16}H_{12}O_4$
114	2′-hydroxy-3,4-methylenedioxychalcone epoxide	$C_{16}H_{12}O_5$
115	2′-benzyloxy-3,4-methylenedioxychalcone	$C_{23}H_{18}O_4$
116	2′-benzyloxy-3,4-methylenedioxychalcone epoxide	$C_{23}H_{18}O_5$

Medium used:

Standard PDA (Potato Dextrose Agar) medium was used throughout the work. The composition and preparative procedure of PDA medium described bellow:

Composition of PDA (for fungi):

1. Potato (Piece of cutting)	: 200g
2. D-glucose	: 20g
3. Agar	: 20g
4. Distilled water	: 1000mL

Procedure:

To prepare PDA medium potatoes are cut into small pieces and weighing about 200 g and boiled in 100mL of distilled water for an hour filtered and volume was made up to 1000mL by adding of more distilled water. Glucose and Agar were then added and the solution were mixed thoroughly with a glass rod with proper heating. After dissolving of agar the medium was transferred to a 500mL conical flask. Before autoclaving the conical flask was closed with the cotton plug and rapping with aluminum foil. The medium as then sterilized at 121°C under pressure for 15 minutes at 15 Ib psi. After autoclaving the medium was used for fungal culture.

Maintenance of culture:

Test tube slants of PDA medium were prepared for the maintenance of cultures. Small portions of mycelia of the test organisms were transferred to the test tubes separately from old cultures with the help of sterilized needles. A number of test tubes were freshly prepared for each fungal pathogen. The inoculated slants were incubated at room temperature under laboratory condition and 3 to 5 days old culture were used for antifungal screening.

Preparation of cultures:

Glass petridishes were sterilized and sterilized melted PDA medium (~ 45°C) was poured at the rate of 15mL in each petridish (90 mm). After solidification of the medium the small portions of mycelium of each fungus were separated carefully over the center of each PDA plant with the help of sterilized needles. Thus each fungus was transferred to a number of PDA plates. The PDA plates were then incubated at (25±2) °C and after five days of incubation they were ready for use.

Preparation of discs:

Sterilized discs were taken and the test material of known concentration was applied on the discs with the help of a micropipette. The solvents from the discs were evaporated by hot air blower. Control discs containing only the solvents were prepared.

Placement of the discs and incubation:

The prepared disc of samples was placed gently on the solidified agar plates freshly seeded with the test organisms with sterile forceps. Control disc was also placed on the test plates to compare the effect of the test samples and to nullify the effect of solvents respectively. The plates were then kept in a refrigerator at 4°C for 24 hours in order that the materials had sufficient time to diffuse to a considerable area of the plates. After this the plates were incubated at 37.5°C for 72 hours.

Calculation of the zone of inhibition:

After incubation the diameter of the zone of inhibition were observed and measured in mm by a transparent scale. Results obtained from these test are listed in tables 5 and 12.

CHAPTER VI

Results and Discussion

CHAPTER-VI

Results And Discussion

6.1 Studies of antimicrobial activity of some chalcones and Chalcone epoxides:

In the present study eight chalcones epoxides and their corresponding chalcones were screened *in vitro* for their antibacterial and antifungal activity against four human pathogenic bacteria viz. *streptococcus-β-haemolyticus* (G^+), *Bacillus megaterium* (G^+), *Klebsiella* sp (G^-) and *Escherichia coli* (G^-) and four plants as well as molds fungi viz. *Rhizoctonia solani, Sclerotium rolfsii, Aspergillus niger* and *Aspergillus fumigatus*. The bactericidal activities of the compounds are described bellow:

6.1.1 Antibacterial Screening:

The result of the inhibition zone against the selected bacteria due to the effect of compounds are mentioned in Table 5-8.

Table-5: Results of the antibacterial activity of the compounds against *Bacillus megaterium* (G^+)

Compound No.	Molecular formula	Diameter of the zone of inhibition (mm)				
		100 µg $disc^{-1}$	200 µg $disc^{-1}$	300 µg $disc^{-1}$	400 µg $disc^{-1}$	*K-50 50 µg $disc^{-1}$
101	$C_{18}H_{18}O_5$	9.0	14.1	20.4	26.5	22.3
102	$C_{18}H_{18}O_6$	9.5	15.2	21.5	28.1	
103	$C_{25}H_{24}O_5$	8.8	13.9	19.9	25.5	
104	$C_{25}H_{24}O_6$	9.1	14.2	20.1	26.3	
105	$C_{16}H_{13}OCl$	7.5	12.8	18.1	23.9	
108	$C_{16}H_{13}O_2Cl$	7.9	13.5	19.0	24.5	
107	$C_{16}H_{13}OCl$	7.8	13.2	19.5	24.9	
108	$C_{16}H_{13}O_2Cl$	8.1	13.9	20.1	25.4	
109	$C_{15}H_{11}O_4N$	8.1	14.3	18.3	23.4	
110	$C_{15}H_{11}O_5N$	8.5	15.1	18.9	24.5	
111	$C_{23}H_{20}O_3$	-	-	-	-	
112	$C_{23}H_{20}O_4$	6.6	11.1	15.2	19.1	
113	$C_{16}H_{12}O_4$	9.2	15.2	20.2	26.2	
114	$C_{16}H_{12}O_5$	9.8	16.3	21.1	27.5	
115	$C_{23}H_{18}O_4$	-	-	-	-	
116	$C_{23}H_{18}O_5$	5.8	9.9	12.6	15.8	

*K-50 Kanamycin standard disc 50 µg $disc^{-1}$.

Table-6: Results of the antibacterial activity of the compounds against *Streptococcus–β–haemolyticus* (G^+):

Compound No.	Molecular formula	Diameter of the zone of inhibition (mm)				
		100 µg $disc^{-1}$	200 µg $disc^{-1}$	300 µg $disc^{-1}$	400 µg $disc^{-1}$	*K-50 50 µg $disc^{-1}$
101	$C_{18}H_{18}O_5$	10.2	18.1	25.4	30.6	23
102	$C_{18}H_{18}O_6$	10.4	18.4	25.7	30.9	
103	$C_{25}H_{24}O_5$	8.1	14.5	18.4	21.7	
104	$C_{25}H_{24}O_6$	8.3	14.8	18.7	21.9	
105	$C_{16}H_{13}OCl$	7.8	13.7	18.0	22.2	
106	$C_{16}H_{13}O_2Cl$	9.1	13.9	18.2	22.5	
107	$C_{16}H_{13}OCl$	7.9	13.8	18.1	22.4	
108	$C_{16}H_{13}O_2Cl$	8.2	14.1	18.3	22.6	
109	$C_{15}H_{11}O_4N$	7.7	13.6	17.9	21.1	
110	$C_{15}H_{11}O_5N$	7.9	13.9	18.1	21.3	
111	$C_{23}H_{20}O_3$	-	-	-	-	
112	$C_{23}H_{20}O_4$	6.1	11.3	15.4	18.2	
113	$C_{16}H_{12}O_4$	10.1	17.9	24.1	28.8	
114	$C_{16}H_{12}O_5$	10.4	18.1	24.3	29.0	
115	$C_{23}H_{18}O_4$	-	-	-	-	
116	$C_{23}H_{18}O_5$	7.0	12.1	16.3	19.2	

*K-50 Kanamycin standard disc 50 µg $disc^{-1}$.

Table-7: Results of the antibacterial activity of the compounds against *Klebsiella sp.* (G$^-$):

Compound No.	Molecular formula	Diameter of the zone of inhibition (mm)				
		100 µg disc^{-1}	200 µg disc^{-1}	300 µg disc^{-1}	400 µg disc^{-1}	*K-50 50 µg disc^{-1}
101	$C_{18}H_{18}O_5$	7.8	13.9	19.1	23.2	22
102	$C_{18}H_{18}O_6$	8.1	14.3	19.5	24.0	
103	$C_{25}H_{24}O_5$	8.0	14.1	19.3	23.2	
104	$C_{25}H_{24}O_6$	8.1	14.4	19.7	22.8	
105	$C_{16}H_{13}OCl$	7.2	13.0	18.1	23.4	
106	$C_{16}H_{13}O_2Cl$	7.4	13.8	18.7	24.1	
107	$C_{16}H_{13}OCl$	7.1	12.9	18.3	23.9	
108	$C_{16}H_{13}O_2Cl$	7.3	13.3	18.7	24.2	
109	$C_{15}H_{11}O_4N$	7.6	12.8	18.9	23.8	
110	$C_{15}H_{11}O_5N$	7.8	13.2	19.3	24.4	
111	$C_{23}H_{20}O_3$	-	-	-	-	
112	$C_{23}H_{20}O_4$	5.5	9.9	14.1	18.3	
113	$C_{16}H_{12}O_4$	9.1	16.2	22.1	27.2	
114	$C_{16}H_{12}O_5$	9.6	16.6	22.5	27.6	
115	$C_{23}H_{18}O_4$	-	-	-	-	
116	$C_{23}H_{18}O_5$	6.9	12.1	17.9	22.8	

*K-50 Kanamycin standard disc 50 µg disc^{-1}.

Table-8: Results of the antibacterial activity of the compounds against *Escherichia coli* (G⁻):

Compound No.	Molecular formula	Diameter of the zone of inhibition (mm)				
		100 µg $disc^{-1}$	200 µg $disc^{-1}$	300 µg $disc^{-1}$	400 µg $disc^{-1}$	*K-50 50 µg $disc^{-1}$
101	$C_{18}H_{18}O_5$	8.7	13.9	19.00	23.6	22
102	$C_{18}H_{18}O_6$	9.2	14.1	19.8	25.1	
103	$C_{25}H_{24}O_5$	8.9	13.8	18.9	23.5	
104	$C_{25}H_{24}O_6$	9.5	14.4	19.1	24.2	
105	$C_{16}H_{13}OCl$	9.1	14.2	19.0	24.1	
106	$C_{16}H_{13}O_2Cl$	9.8	14.8	19.5	25.6	
107	$C_{16}H_{13}OCl$	9.0	14.1	18.8	23.9	
108	$C_{16}H_{13}O_2Cl$	9.6	15.2	19.2	25.4	
109	$C_{15}H_{11}O_4N$	8.0	13.1	18.2	23.5	
110	$C_{15}H_{11}O_5N$	8.7	13.9	18.8	24.6	
111	$C_{23}H_{20}O_3$	-	-	-	-	
112	$C_{23}H_{20}O_4$	5.5	9.1	12.7	15.3	
113	$C_{16}H_{12}O_4$	9.1	15.2	19.8	24.5	
114	$C_{16}H_{12}O_5$	9.9	16.4	20.2	26.2	
115	$C_{23}H_{18}O_4$	-	-	-	-	
116	$C_{23}H_{18}O_5$	5.2	8.8	12.3	15.2	

*K-50 Kanamycin standard disc 50 µg $disc^{-1}$.

a. *Bacillus megaterium* (G$^+$):

The inhibition zones of *Bacillus megaterium* due to the treatment of different compounds at the different concentrations are presented in table-5. The compounds, **101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 112, 113, 114** and **116** shown inhibition zones at concentration 100, 200, 300 and 400 μg disc^{-1}. Only compound **111** and **115** did not show any inhibition zone at the same concentration. It was found that the inhibition zone of compound **101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 113** and **114** were more effective than that of the other compounds such as **112** and **116**.

b. *Streptococcus-β-haemolyticus* (G$^+$):

The *in vitro* growth inhibitions of *streptococcus-β-haemolyticus* (G$^+$) due to the treatment of different compounds are shown in table-6. The screening data suggest that compounds **101, 102, 113** and **114** showed very good antibacterial activity. Compound **111** and **115** did not show antibacterial activity. Again compound **103, 104, 105, 106, 107, 108, 109, 110, 112** and **116** showed less inhibitions zones than that of other compounds such as **101, 102, 113** and **114** at the concentration. 100 μg disc^{-1}, 200 μg disc^{-1}, 300 μg disc^{-1} and 400 μg disc^{-1}.

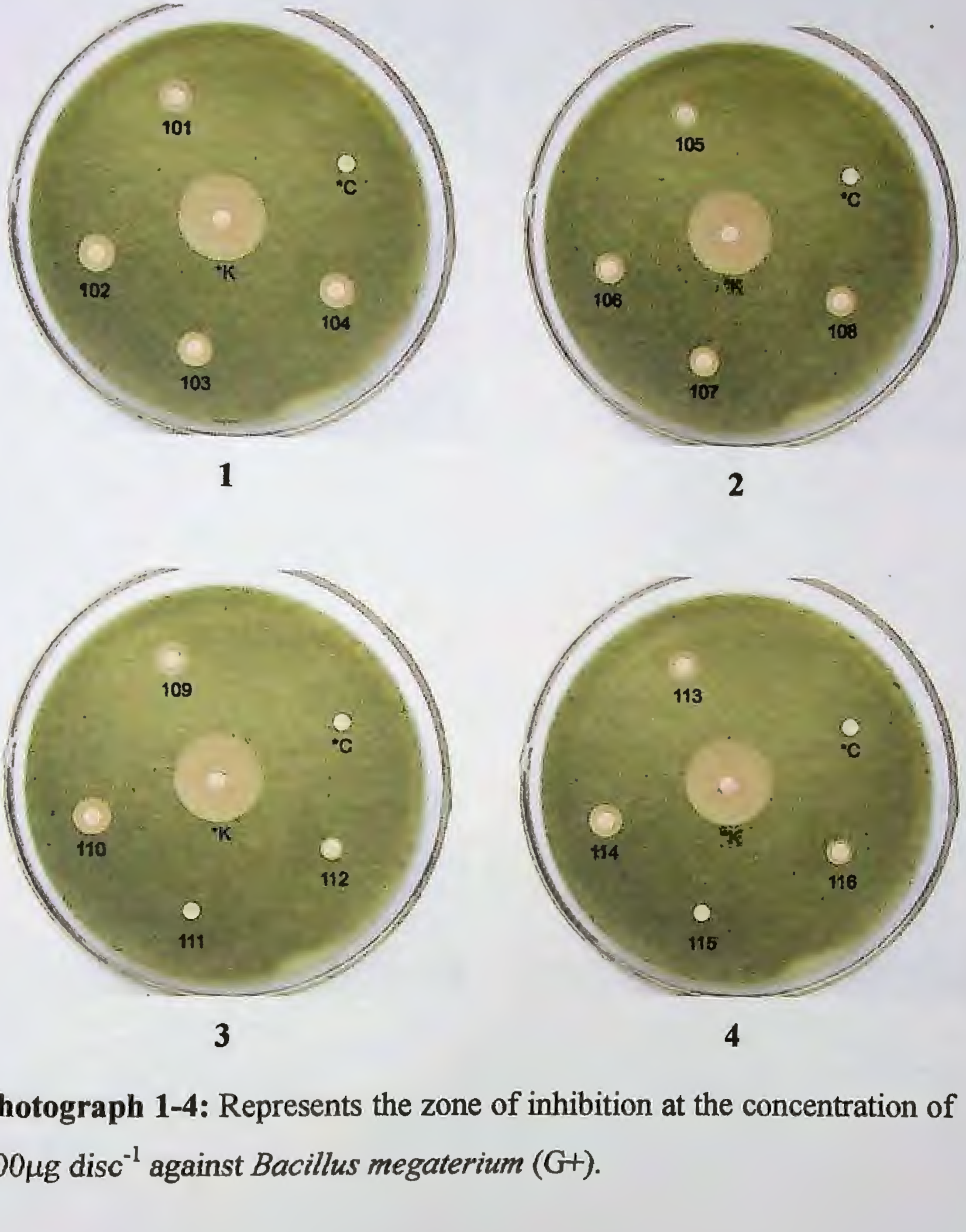
c. *Klebsiella* sp. (G$^-$):

The inhibition of this Gram negative bacteria for different compounds treatment are mentioned in table-7. The experimental data suggest that the compound, **101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 113** and **114** showed good antibacterial activity against the selected bacteria. The screening data suggest that the compounds **111** and **115** did not show any inhibition against this bacteria at the concentration 100, 200, 300 and 400 μg disc^{-1}. Compound **112** and **116** showed less inhibition zones than that of other compounds such as **101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 113** and **114**.

d. *Escherichia coli* (G⁻):

The screening data presented in Table-8 suggest that the tested compounds **101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 112, 113, 114** and **116** showed effective antibacterial activity at the concentration 100, 200, 300 and 400 μg disc^{-1}. But the compound **111** and **115** did not show antibacterial activity at the same concentration. It was found that the inhibition zones of compounds **101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 113** and **114** were more effective than that of the other compounds such as **112** and **116**.

From the inhibition zone diameter data analysis it was found that chalcone epoxide **102, 104, 106, 108, 110** and **114** and there precursor chalcones **101, 103, 105, 107, 109** and **113** were identified as the more active compounds in comparison with the chalcone epoxide **112** and **116** against *Bacillus megaterium* (G$^+$), *streptococcus-β-haemolyticus* (G$^+$), *Klebsiclla* sp. (G$^-$) and *Escherichia coli* (G$^-$). Chalcone **111** and **115** did not show any antibacterial activity towards both Gram positive and Gram Negative bacteria.



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Photograph 1-4: Represents the zone of inhibition at the concentration of 100μg $disc^{-1}$ against *Bacillus megaterium* (G+).

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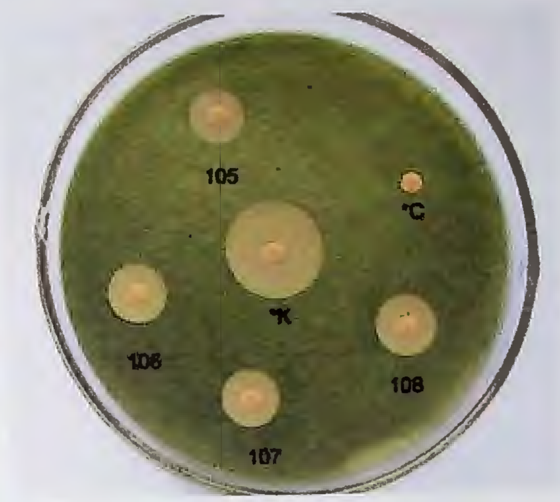

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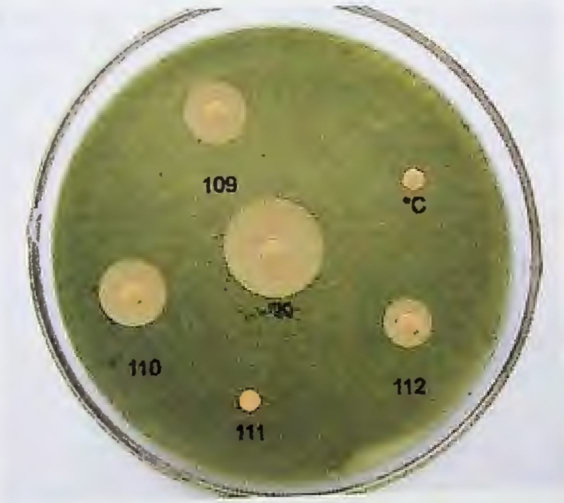

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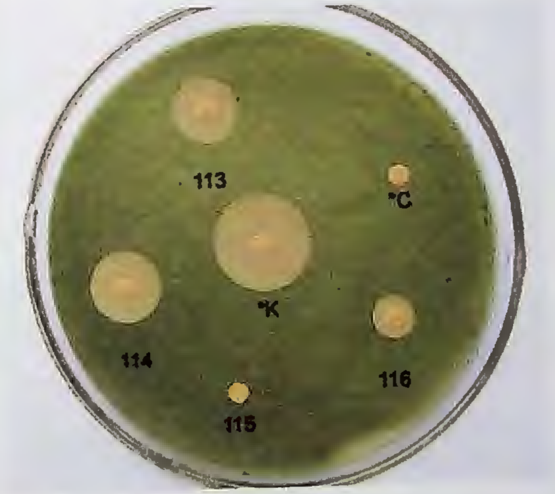

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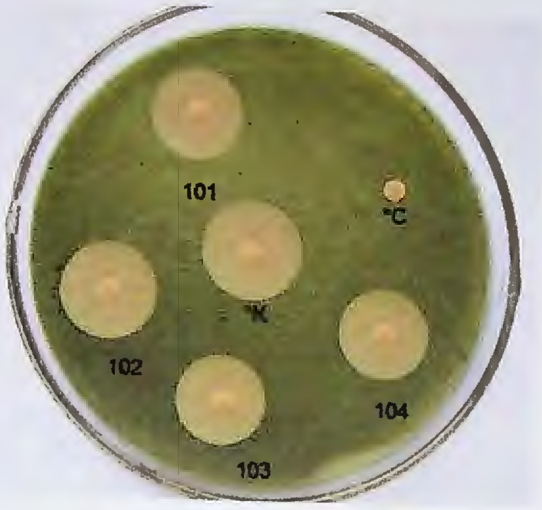
Photograph 5-8: Represents the zone of inhibition at the concentration of 200µg disc^{-1} against *Bacillus megaterium* (G+).



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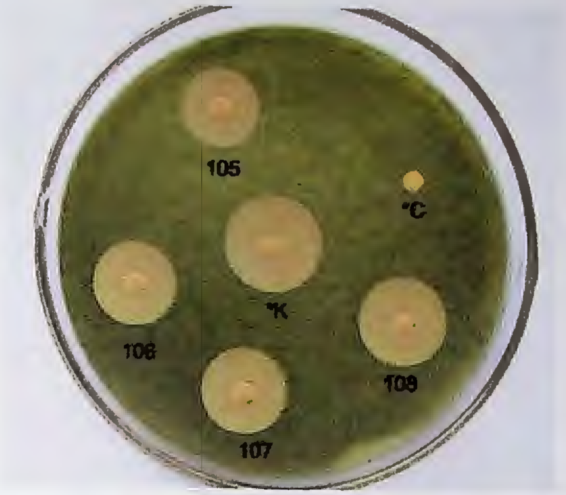

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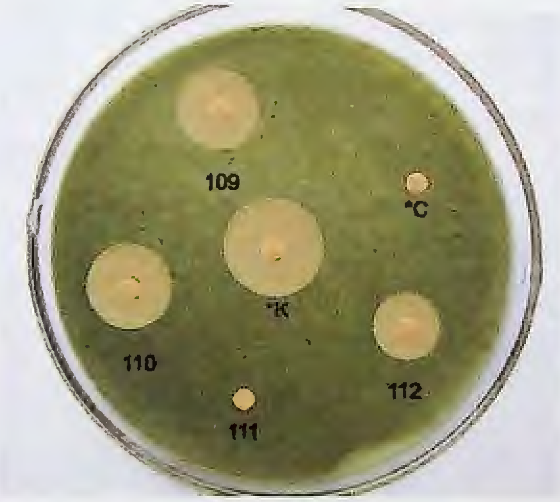

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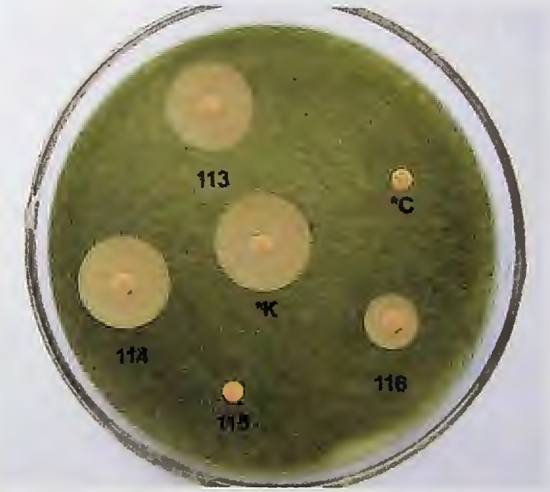

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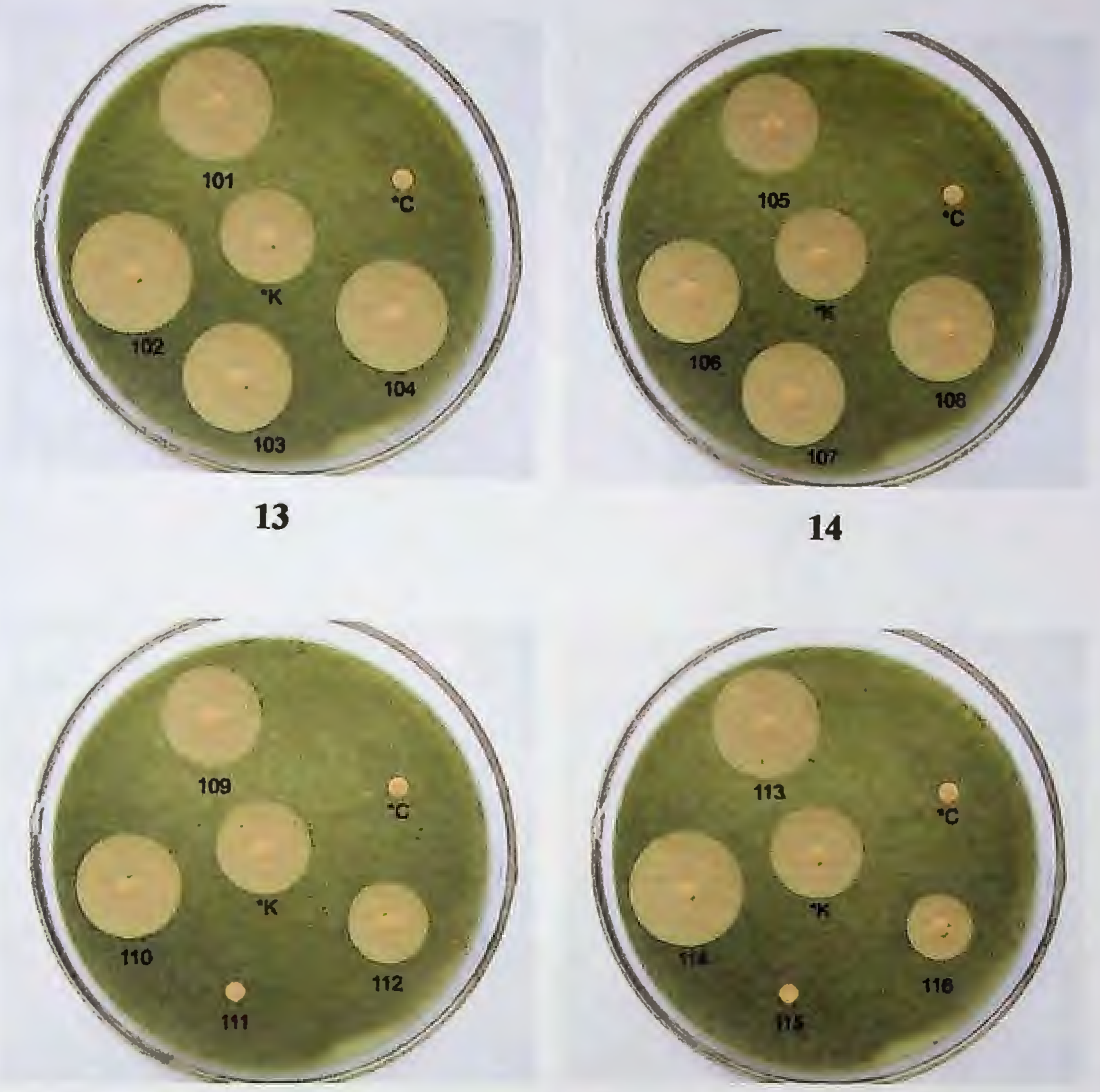
Photograph 9-12: Represents the zone of inhibition at the concentration of 300μg disc^{-1} against *Bacillus megaterium* (G+).



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Photograph 13-16: Represents the zone of inhibition at the concentration of 400μg $disc^{-1}$ against *Bacillus megaterium* (G+).

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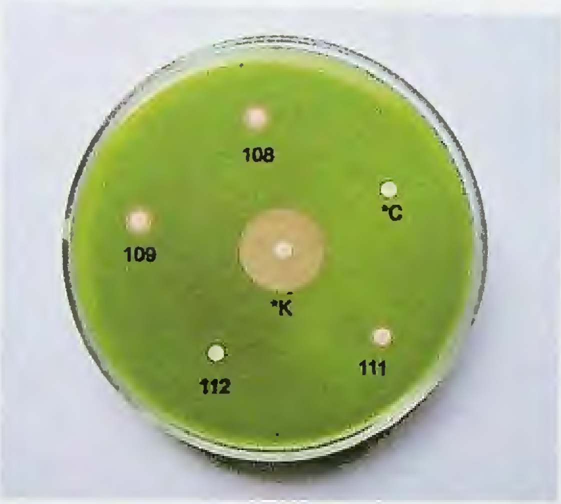

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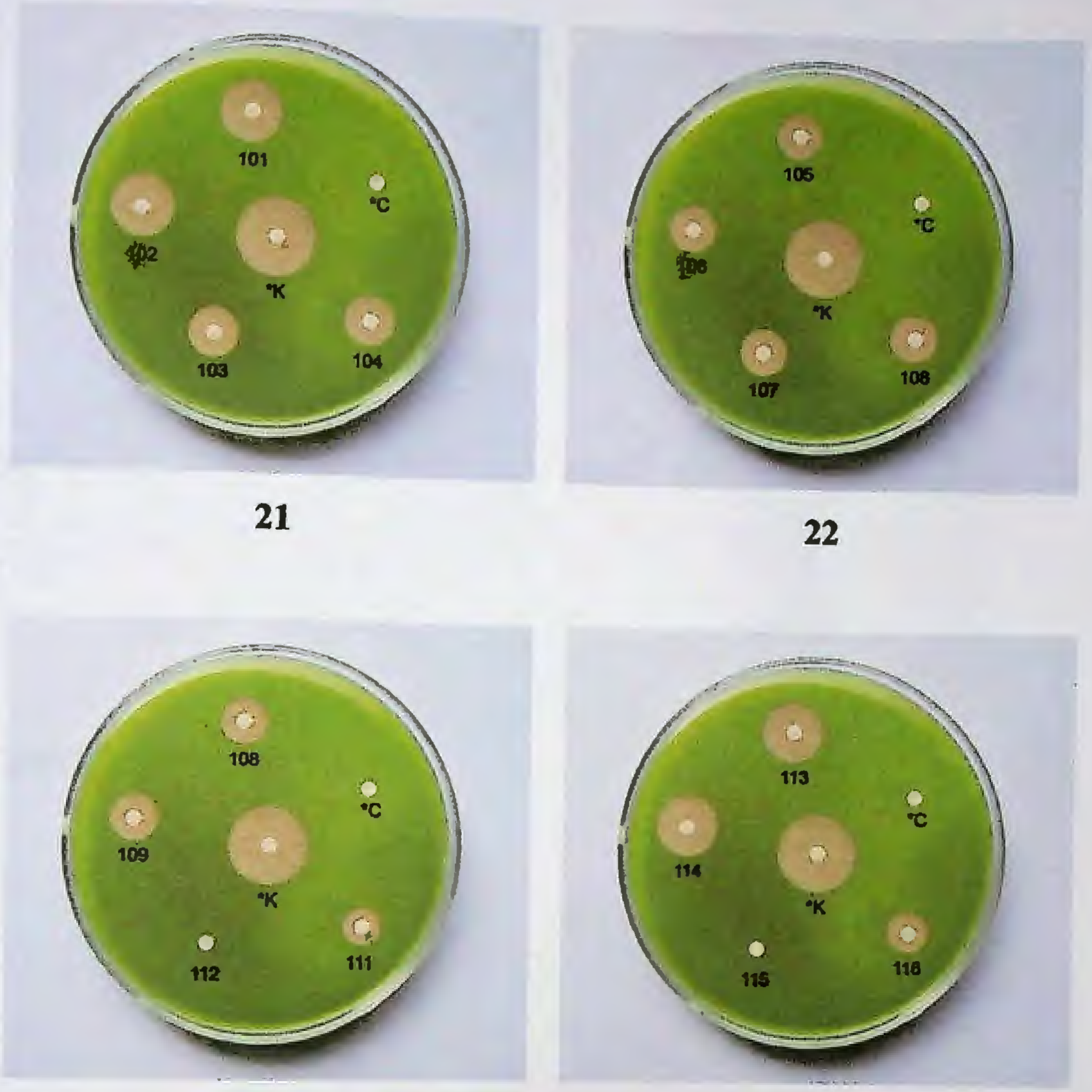
Photograph 17-20: Represents the zone of inhibition at the concentration of 100μg disc^{-1} against *Streptococcus–β–haemolyticus* (G+).



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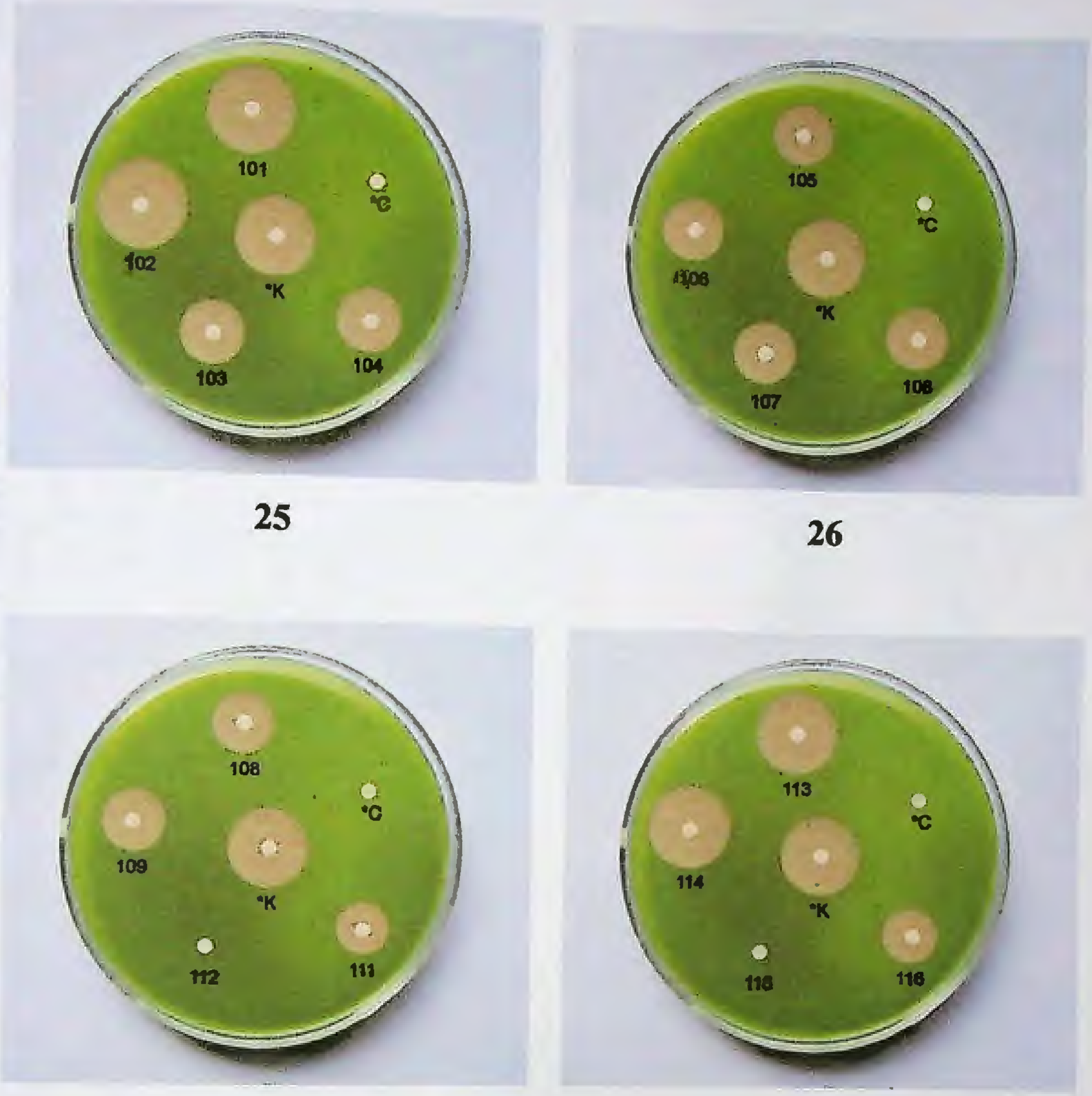
Photograph 21-24: Represents the zone of inhibition at the concentration of 200μg $disc^{-1}$ against *Streptococcus–β–haemolyticus* (G+).



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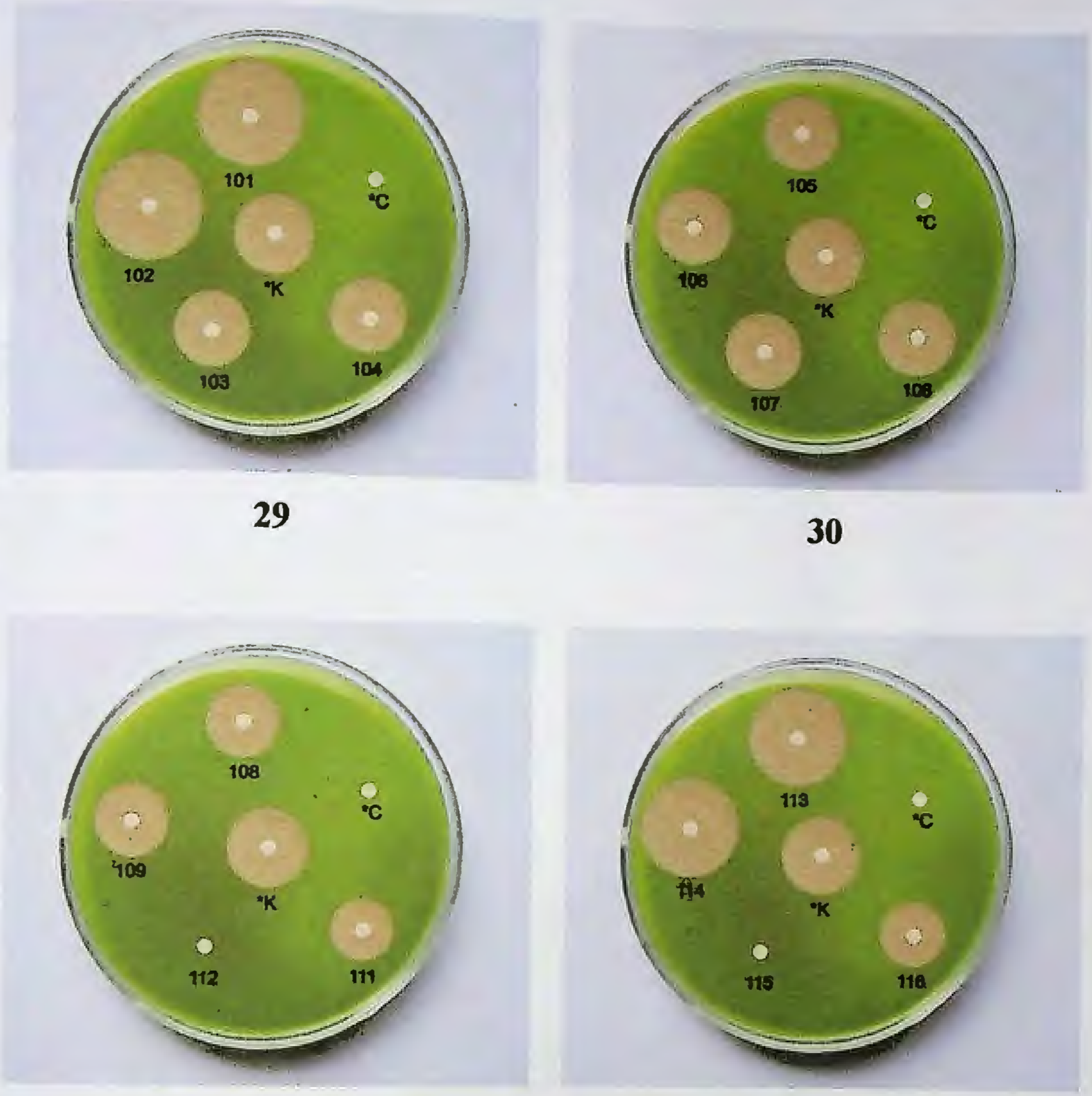
Photograph 25-28: Represents the zone of inhibition at the concentration of 300µg disc^{-1} against *Streptococcus–β–haemolyticus* (G+).



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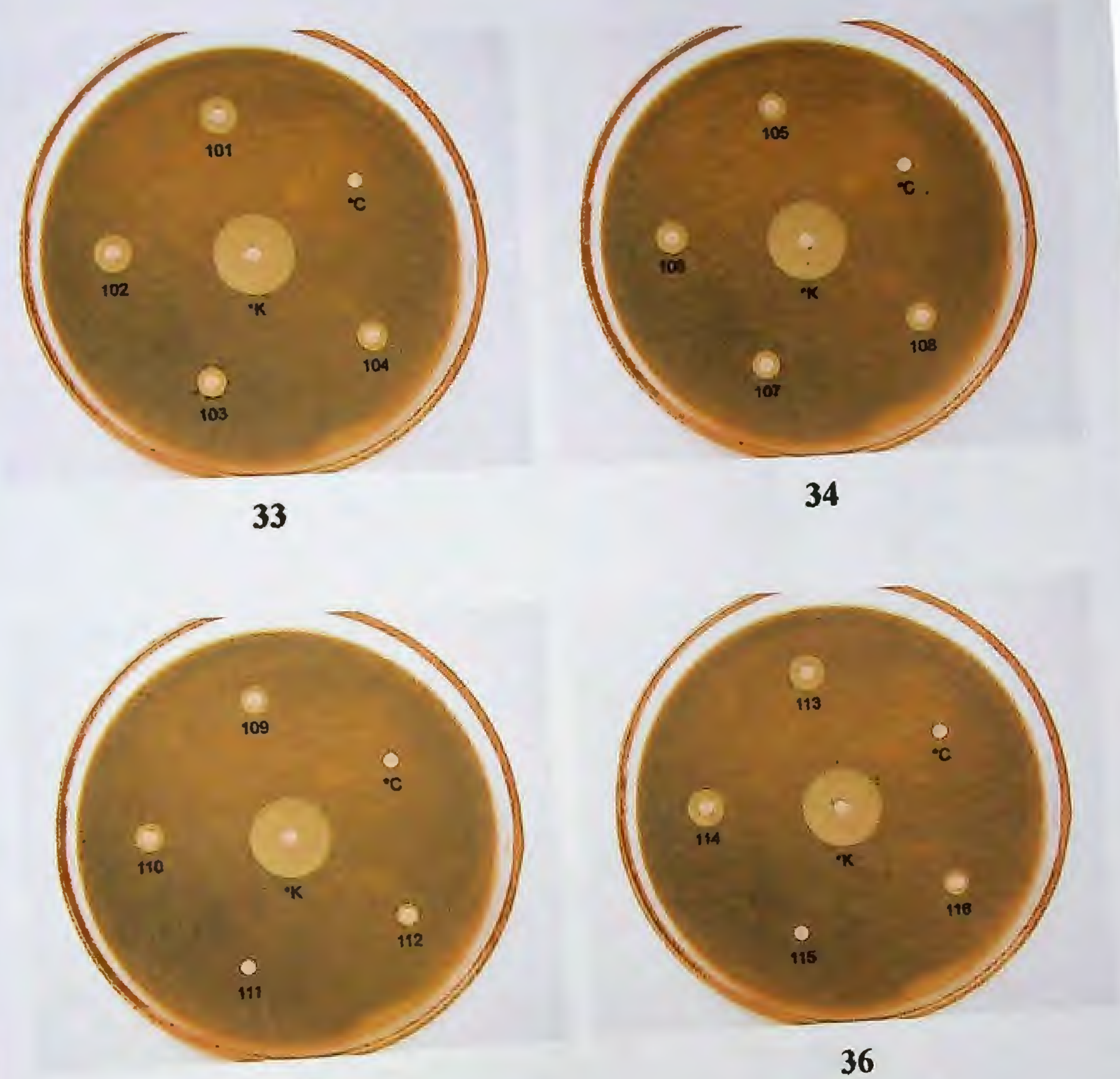
Photograph 29-32: Represents the zone of inhibition at the concentration of 400μg disc^{-1} against *Streptococcus–β–haemolyticus* (G+).



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Photograph 33-36: Represents the zone of inhibition at the concentration of 100µg disc^{-1} against *Klebsiella sp.* (G^{-}).

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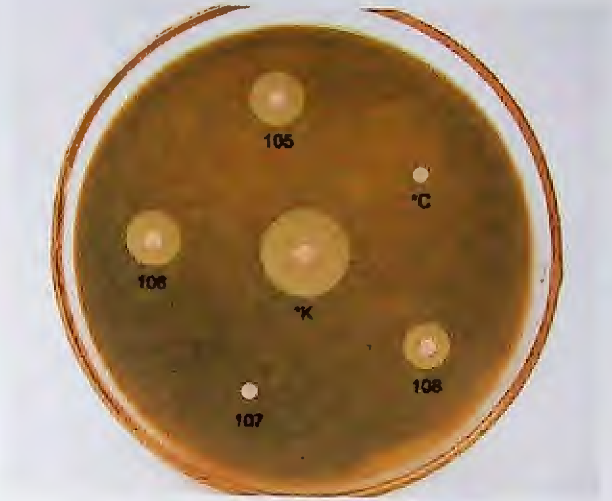

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Photograph 37-40: Represents the zone of inhibition at the concentration of 200μg disc^{-1} against *Klebsiella sp.* (G^{-}).

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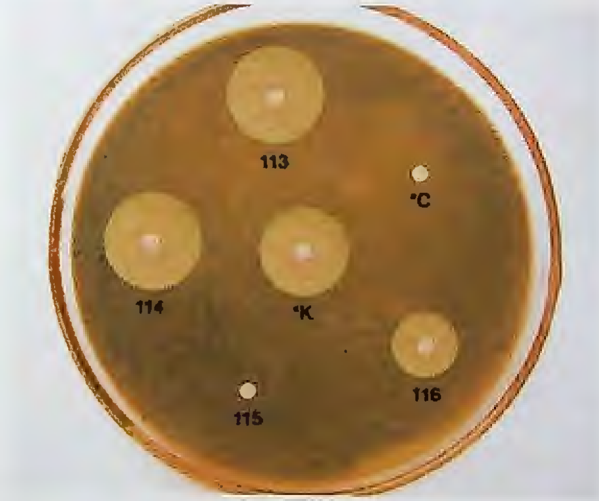

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Photograph 41-44: Represents the zone of inhibition at the concentration of 300μg $disc^{-1}$ against *Klebsiella sp.* (G^{-}).

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Photograph 45-48: Represents the zone of inhibition at the concentration of 400μg disc^{-1} against *Klebsiella sp.* (G^{-}).

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Photograph 49-52: Represents the zone of inhibition at the concentration of 100μg $disc^{-1}$ against *Escherichia coli* (G^{-}).

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Photograph 53-56: Represents the zone of inhibition at the concentration of 200µg $disc^{-1}$ against *Escherichia coli* (G^{-}).

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Photograph 57-60: Represents the zone of inhibition at the concentration of 300μg disc^{-1} against *Escherichia coli* (G^{-}).

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Photograph 61-64: Represents the zone of inhibition at the concentration of 400μg $disc^{-1}$ against *Escherichia coli* (G^{-}).

6.1.2 Antifungal Screening Effect of test Compounds on Mycelial growth:

The result of the inhibition zone of the four plants pathogenic as well as molds fungi due to the effect of compounds are presented in Table 9-12. *Rhizoctonic solani* (Molds), *Sclerotium rolfsii* (molds), *Aspergillus niger* (molds) and *Aspergillus fumigatus* (molds) were selected for mycelial growth test. The efficacy of the test compounds against the selected fungi are mentioned bellow:

Table-9: Results of the antifungal activity of the compounds against *Rhizoctonia solani*:

Compound No.	Molecular formula	Diameter of the zone of inhibition (mm)				
		100 µg disc^{-1}	200 µg disc^{-1}	300 µg disc^{-1}	400 µg disc^{-1}	*N-50 µg disc^{-1}
101	$C_{18}H_{18}O_5$	8.1	14.2	19.1	23.0	21
102	$C_{18}H_{18}O_6$	8.4	14.3	19.3	23.2	
103	$C_{25}H_{24}O_5$	7.9	13.7	18.5	22.6	
104	$C_{25}H_{24}O_6$	8.2	14.1	18.7	22.8	
105	$C_{16}H_{13}OCl$	8.6	14.4	19.3	22.2	
106	$C_{16}H_{13}O_2Cl$	9	14.7	19.5	22.5	
107	$C_{16}H_{13}OCl$	7.8	13.4	18.3	22.2	
108	$C_{16}H_{13}O_2Cl$	8.3	13.9	18.7	22.4	
109	$C_{15}H_{11}O_4N$	7.8	13.5	18.4	22.4	
110	$C_{15}H_{11}O_5N$	8.2	13.8	18.6	24.5	
111	$C_{23}H_{20}O_3$	-	-	-	-	
112	$C_{23}H_{20}O_4$	5.7	10.1	14.2	17.1	
113	$C_{16}H_{12}O_4$	9.8	16.9	21.7	25.9	
114	$C_{16}H_{12}O_5$	10.1	17.1	21.9	26.1	
115	$C_{23}H_{18}O_4$	-	-	-	-	
116	$C_{23}H_{18}O_5$	6.1	11.1	15.3	19.2	

*N-50; Nystatin standard disc (50µg disc^{-1}).

Table-10: Results of the antifungal activity of the compounds against *Sclerotium rolfsii*:

Compound No.	Molecular formula	Diameter of the zone of inhibition (mm)				
		100 μg disc^{-1}	200 μg disc^{-1}	300 μg disc^{-1}	400 μg disc^{-1}	*N-50 μg disc^{-1}
101	$C_{18}H_{18}O_5$	8.3	13.8	18.5	22.4	21.2
102	$C_{18}H_{18}O_6$	8.9	14.2	18.8	22.7	
103	$C_{25}H_{24}O_5$	7.5	13.4	18.1	22.2	
104	$C_{25}H_{24}O_6$	7.8	13.6	18.3	22.5	
105	$C_{16}H_{13}OCl$	7.8	13.3	18.1	22.2	
106	$C_{16}H_{13}O_2Cl$	8.1	13.6	18.2	22.5	
107	$C_{16}H_{13}OCl$	7.9	13.5	18.2	22.3	
108	$C_{16}H_{13}O_2Cl$	8.2	13.7	18.4	22.5	
109	$C_{15}H_{11}O_4N$	8.1	14.0	18.5	23.0	
110	$C_{15}H_{11}O_5N$	8.3	14.2	18.7	23.1	
111	$C_{23}H_{20}O_3$	-	-	-	-	
112	$C_{23}H_{20}O_4$	6.2	11.1	15.2	19.1	
113	$C_{16}H_{12}O_4$	9.9	16.8	22.3	27.5	
114	$C_{16}H_{12}O_5$	10.1	17.1	22.6	28.1	
115	$C_{23}H_{18}O_4$	-	-	-	-	
116	$C_{23}H_{18}O_5$	6.4	11.3	15.4	19.2	

*N-50; Naystatin standard disc 50 μg disc^{-1}.

Table-11: Results of the antifungal activity of the compounds against *Aspergillus niger*:

Compound No.	Molecular formula	Diameter of the zone of inhibition (mm)				
		100 µg disc^{-1}	200 µg disc^{-1}	300 µg disc^{-1}	400 µg disc^{-1}	*N-50 µg disc^{-1}
101	$C_{18}H_{18}O_5$	9.1	16.5	22.6	28.2	20.7
102	$C_{18}H_{18}O_6$	9.6	17.0	23.0	28.6	
103	$C_{25}H_{24}O_5$	8.6	15.1	21.2	27.1	
104	$C_{25}H_{24}O_6$	9.0	15.4	21.5	27.4	
105	$C_{16}H_{13}OCl$	9.1	16.4	22.5	28.1	
106	$C_{16}H_{13}O_2Cl$	9.5	16.8	22.9	28.4	
107	$C_{16}H_{13}OCl$	9.2	16.6	22.7	28.2	
108	$C_{16}H_{13}O_2Cl$	9.7	17.1	23.1	28.5	
109	$C_{15}H_{11}O_4N$	8.4	14.9	20.7	26.5	
110	$C_{15}H_{11}O_5N$	8.8	15.3	23.1	26.9	
111	$C_{23}H_{20}O_3$	-	-	-	-	
112	$C_{23}H_{20}O_4$	4.3	7.4	9.9	12.0	
113	$C_{16}H_{12}O_4$	10.4	19.1	26.6	31.4	
114	$C_{16}H_{12}O_5$	10.8	19.5	27.0	32.1	
115	$C_{23}H_{18}O_4$	-	-	-	-	
116	$C_{23}H_{18}O_5$	4.5	7.6	10.2	12.4	

*N-50; Naystatin standard disc 50 µg disc^{-1}.

Table-12: Results of the antifungal activity of the compounds against *Aspergillus fumigatus.*

Compound No.	Molecular formula	Diameter of the zone of inhibition (mm)				
		100 μg disc^{-1}	200 μg disc^{-1}	300 μg disc^{-1}	400 μg disc^{-1}	*N-50 μg disc^{-1}
101	$C_{18}H_{18}O_5$	8.5	15.1	21.3	26.8	20.9
102	$C_{18}H_{18}O_6$	8.9	15.4	21.7	27.1	
103	$C_{25}H_{24}O_5$	7.5	14.6	20.4	25.6	
104	$C_{25}H_{24}O_6$	7.8	14.9	20.7	25.9	
105	$C_{16}H_{13}OCl$	8.5	15.3	21.7	26.8	
106	$C_{16}H_{13}O_2Cl$	9.0	15.7	22.1	27.2	
107	$C_{16}H_{13}OCl$	8.3	15.3	21.1	26.2	
108	$C_{16}H_{13}O_2Cl$	8.7	15.6	21.8	26.8	
109	$C_{15}H_{11}O_4N$	8.0	14.9	21.8	26.9	
110	$C_{15}H_{11}O_5N$	8.5	15.4	22.2	27.2	
111	$C_{23}H_{20}O_3$	-	-	-	-	
112	$C_{23}H_{20}O_4$	4.4	7.5	10.1	12.2	
113	$C_{16}H_{12}O_4$	10.1	18.9	26.4	31.8	
114	$C_{16}H_{12}O_5$	10.5	19.3	26.7	32.1	
115	$C_{23}H_{18}O_4$	-	-	-	-	
116	$C_{23}H_{18}O_5$	4.6	7.7	10.3	12.5	

*N-50; Naystatin standard disc 50 μg disc^{-1}.

a. *Rhizoctonia Solani*:

The mycelial growth inhibition of *Rhizoctonia solani* due to the treatment of different compounds are given in table-9. Here the compounds, **101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 112, 113, 114** and **116** exhibited antifungal activities to this tested fungi at the concentration 100, 200, 300 and 400 μg disc^{-1}. It can be noted that compounds **101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 113** and **114** showed the greatest inhibitory effect against this fungi compared to the compound **112** and **116**. Chalcones **111** and **115** did not show any activity but their corresponding chalcone epoxides showed very good activity agaisnt Rhizoctonia solani.

b. *Sclerotium rolfsii*:

The inhibition Zones of *Sclerotium rolfsii* due to the treatment of different compounds are given in table-10. Here the compounds **101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 112, 113, 114** and **116** showed minimum inhibition against this organism at the concentration 100, 200, 300 and 400 μg disc^{-1}. From the result we can see that the compound **101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 113** and **114** showed the greatest inhibitory effect against this fungi compared to the compounds **112** and **116**. Chalcones **111** and **115** did not show any activity against this organism.

c. *Aspergillus niger*:

The inhibition zones of *Aspergillus niger* due to the treatment of different compounds are given in table-11. Here the compounds **101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 112, 113, 114** and **116** showed minimum inhibition against this organism of the concentration 100, 200, 300 and 400 μg disc^{-1}. For the result we can see that the compounds **101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 113** and **114** showed the greatest inhibitory effect against this test organism compared to the compounds **112** and **116**.

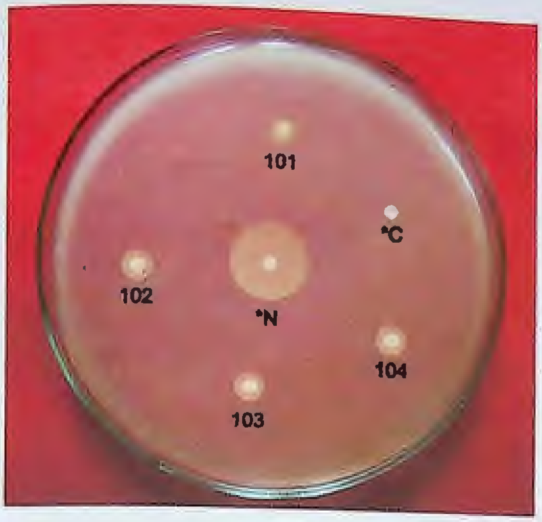
Chaclone epoxides **102, 104, 106, 108, 110** and **114** showed higher fungicidal effect compared to the other chalcone epoxides **112** and **116**. Chalcone **111** and **115** did not show any activity against this organism.

d. *Aspergillus fumigatus*:

The inhibition zones of *Aspergillus fumigatus* due to the treatment of different compounds are given in Table-12. Here the compounds **101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 112, 113, 114** and **116** showed minimum inhibition against this organism of the concentration 100, 200, 300 and 400 μg disc^{-1}. From the result we can see that the compounds **101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 113** and **114** showed greatest inhibitory effect against this test organism to the compound **112** and **116**. Chalcone epoxides **102, 104, 106, 108, 110** and **114** showed higher fungicidal effect compared to the other chalcone epoxides **112** and **116**. Chalcone **111** and **115** did not showed any activity against this organism.

The overall results of the present investigation showed that chalcone epoxides are somewhat effective that their corresponding chalcones towards the organism (Fungi). The effective inhibitor or fungicidial effect of all the tested compounds. are compared to the standard antibiotic Nystatin-50. From the above result it can be concluded that the epoxide ring system and presence of methoxy group ($-OCH_3$), hydroxy group (OH), nitro group (NO_2) and halogen group (Cl^-) are responsible for the antifungal effects.

Thus a comparative study on antimicrobial activities of the above compounds has been carried out successfully. The selected compounds have not been tested against the selected pathogens before. This is the first report regarding the effectiveness of the selected compounds against the selected pathogens.



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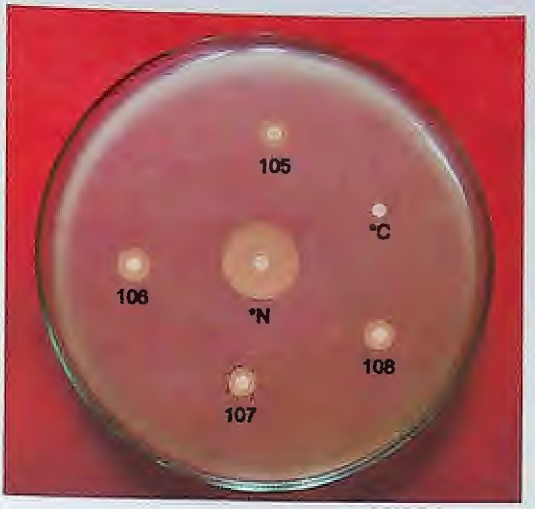

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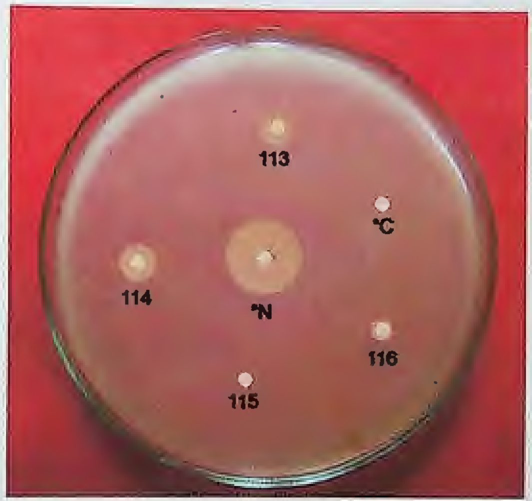
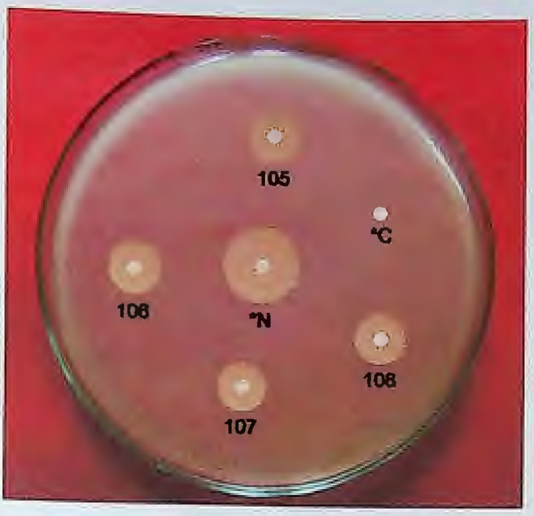


68

Photograph 65-68: Represents the zone of inhibition at the concentration of 100μg disc^{-1} against *Rhizoctonia solani.*

69



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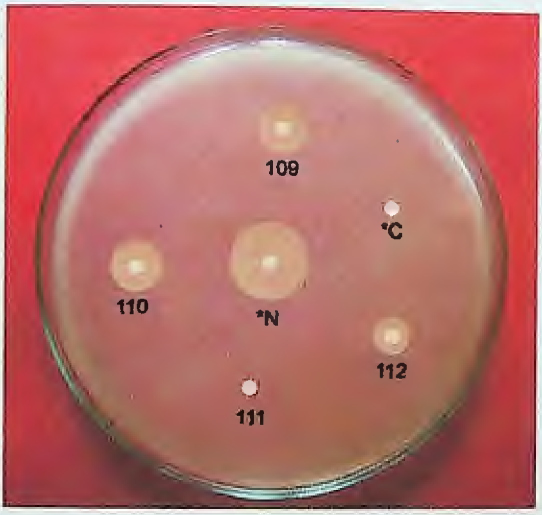

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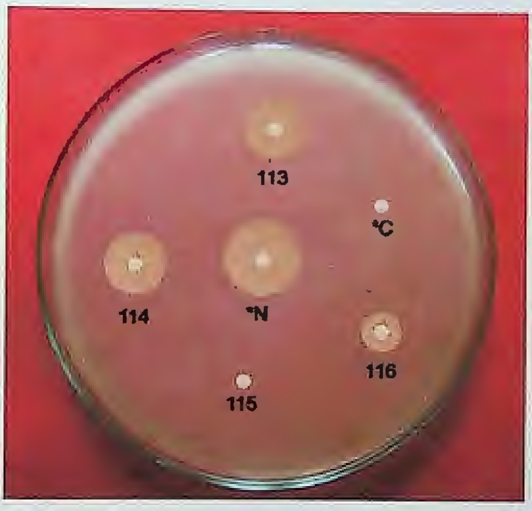

72

Photograph 69-72: Represents the zone of inhibition at the concentration of 200μg $disc^{-1}$ against *Rhizoctonia solani.*

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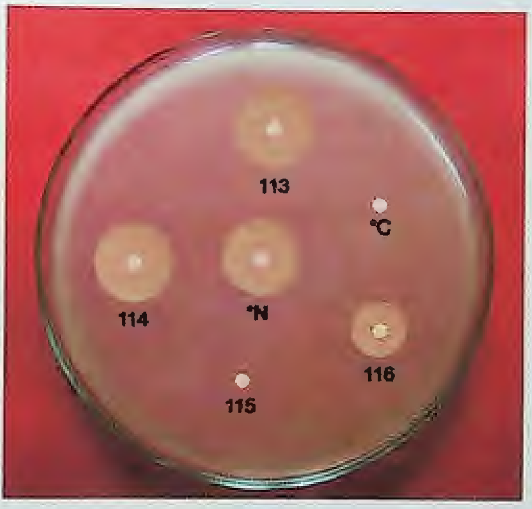

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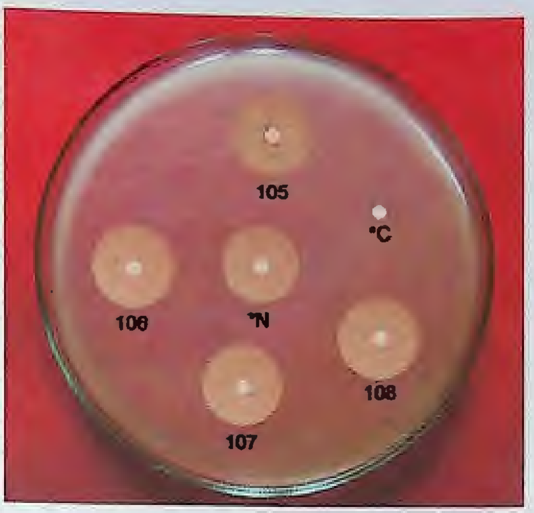
Photograph 73-76: Represents the zone of inhibition at the concentration of 300μg disc^{-1} against *Rhizoctonia solani.*

77



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Photograph 77-80: Represents the zone of inhibition at the concentration of 400µg disc^{-1} against *Rhizoctonia solani.*

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Photograph 81-84: Represents the zone of inhibition at the concentration of 100µg $disc^{-1}$ against *Sclerotium rolfsii*.

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Photograph 85-88: Represents the zone of inhibition at the concentration of 200μg disc^{-1} against *Sclerotium rolfsii.*

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Photograph 89-92: Represents the zone of inhibition at the concentration of 300μg disc^{-1} against *Sclerotium rolfsii.*

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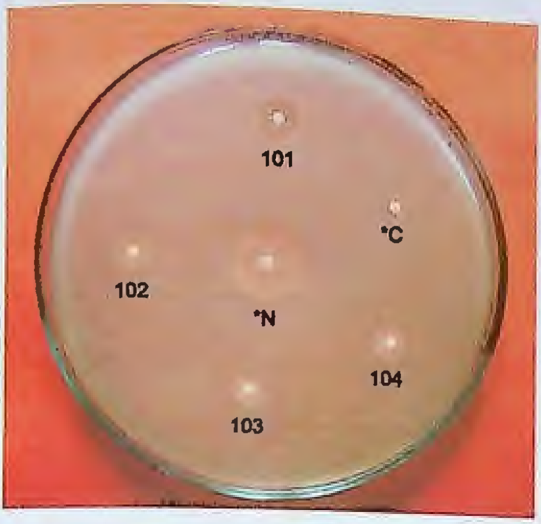
Photograph 93-96: Represents the zone of inhibition at the concentration of 400µg disc^{-1} against *Sclerotium rolfsii*.



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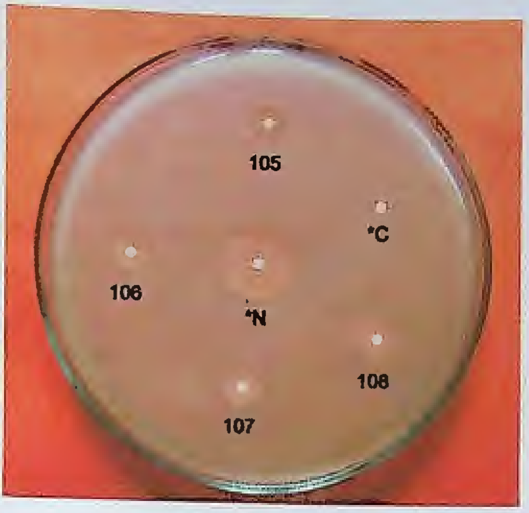

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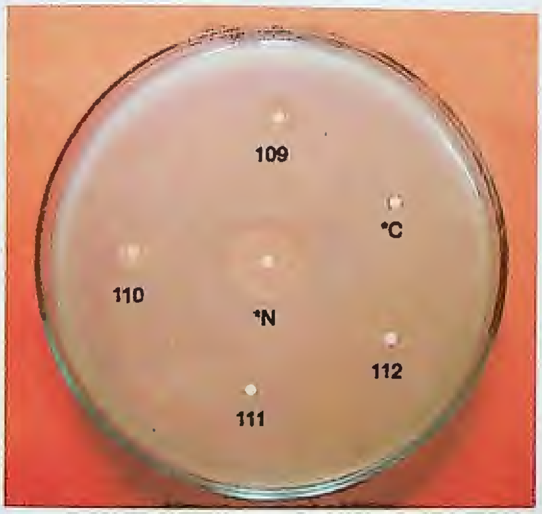

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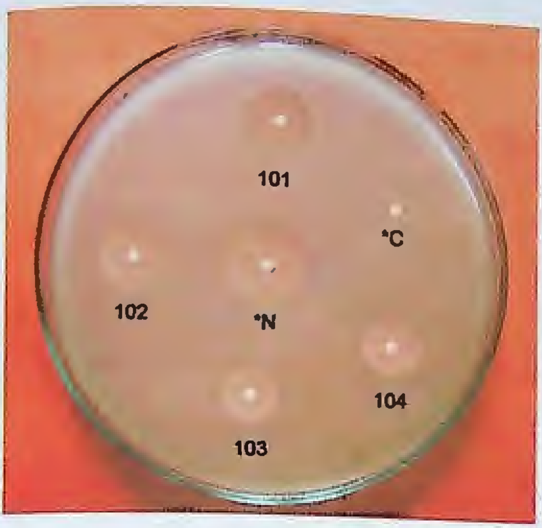
100

Photograph 97-100: Represents the zone of inhibition at the concentration of 100μg disc^{-1} against *Aspergillus niger*.



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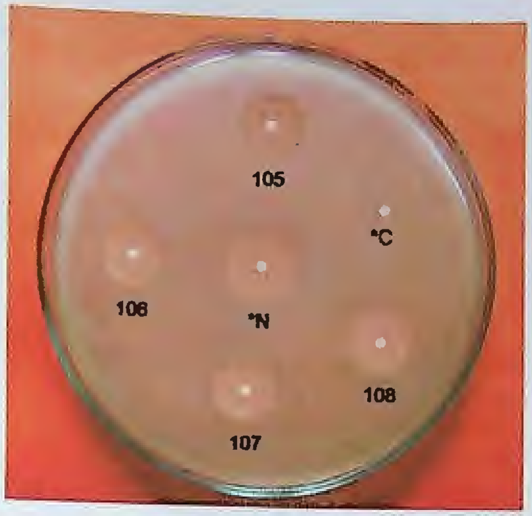

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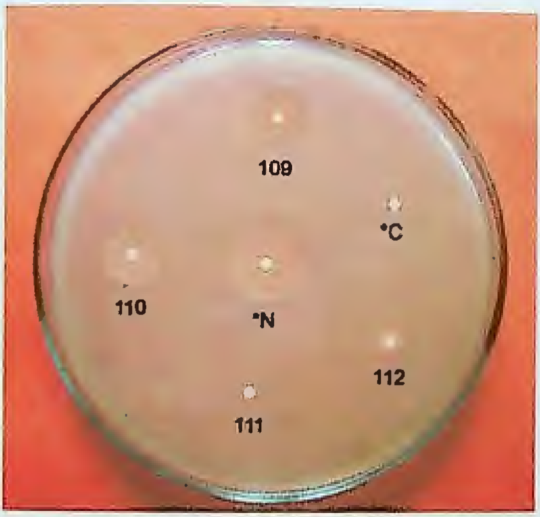

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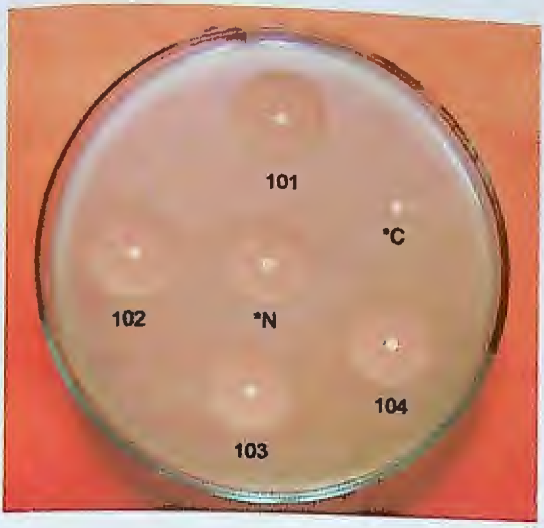
104

Photograph 101-104: Represents the zone of inhibition at the concentration of 200µg disc^{-1} against *Aspergillus niger*.



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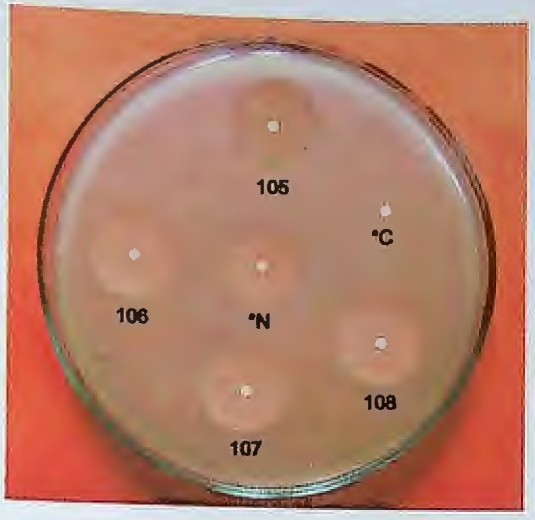

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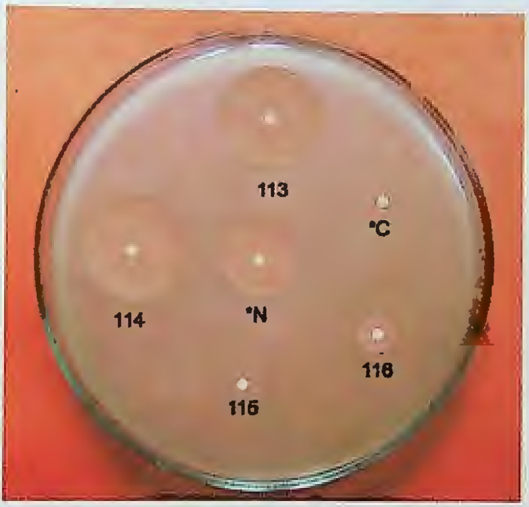

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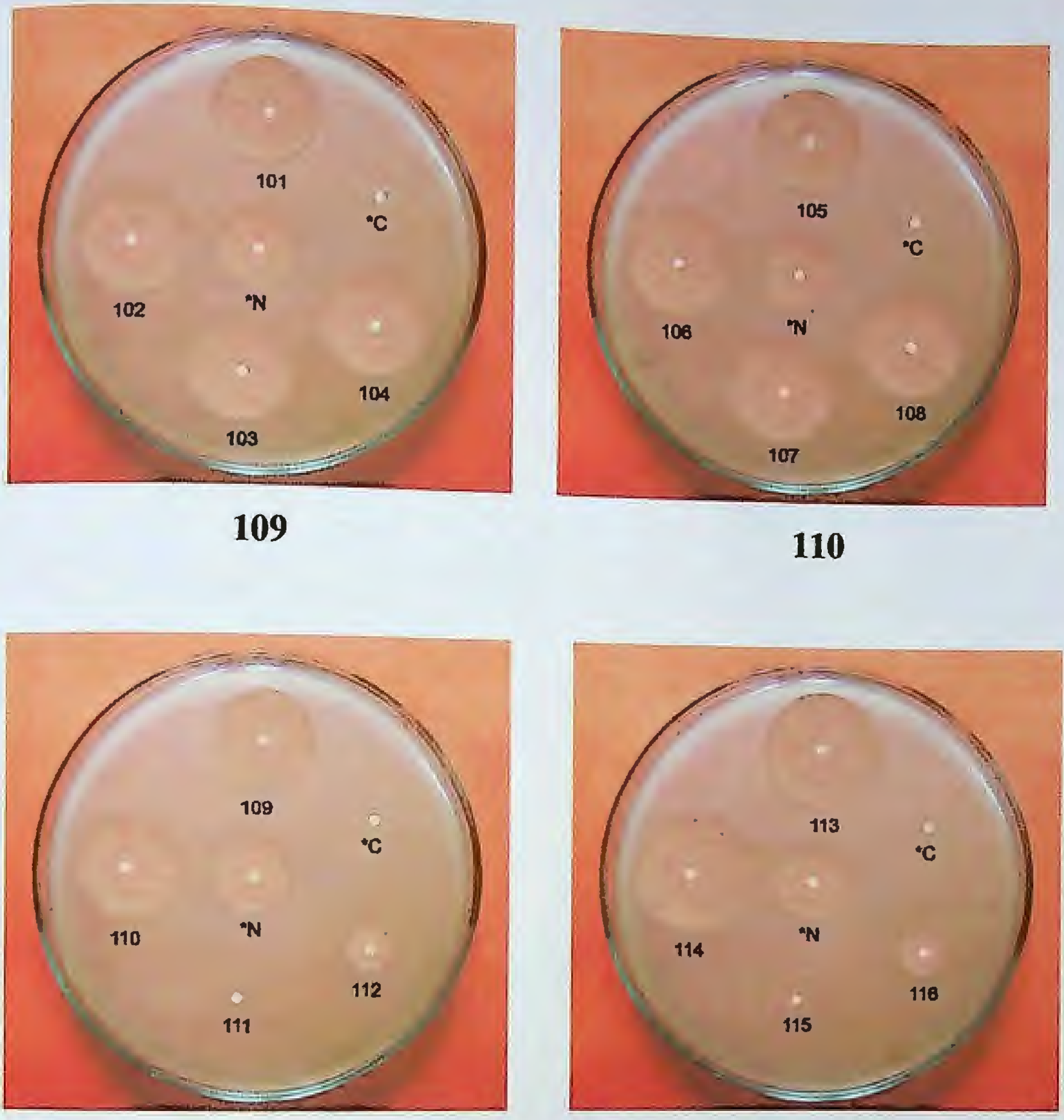
Photograph 105-108: Represents the zone of inhibition at the concentration of 300μg disc^{-1} against *Aspergillus niger*.



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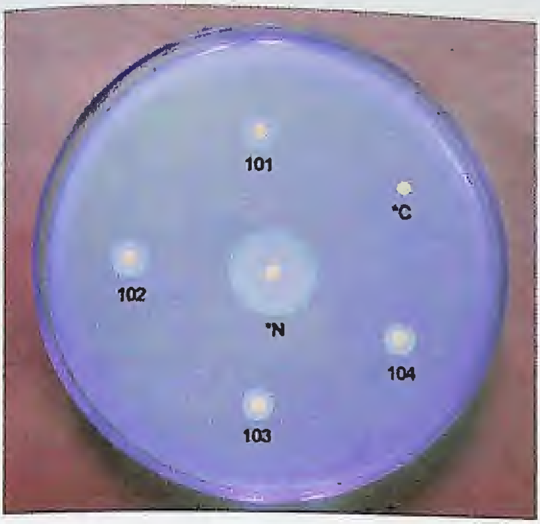
Photograph 109-112: Represents the zone of inhibition at the concentration of 400µg $disc^{-1}$ against *Aspergillus niger*.



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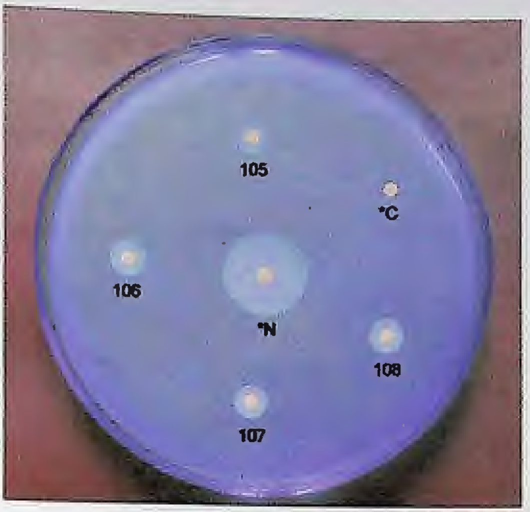

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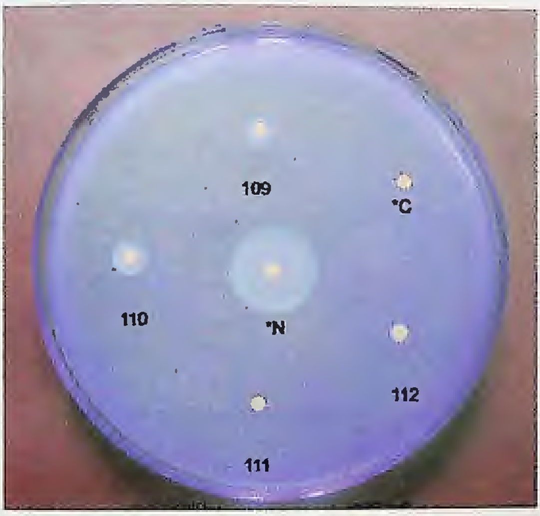

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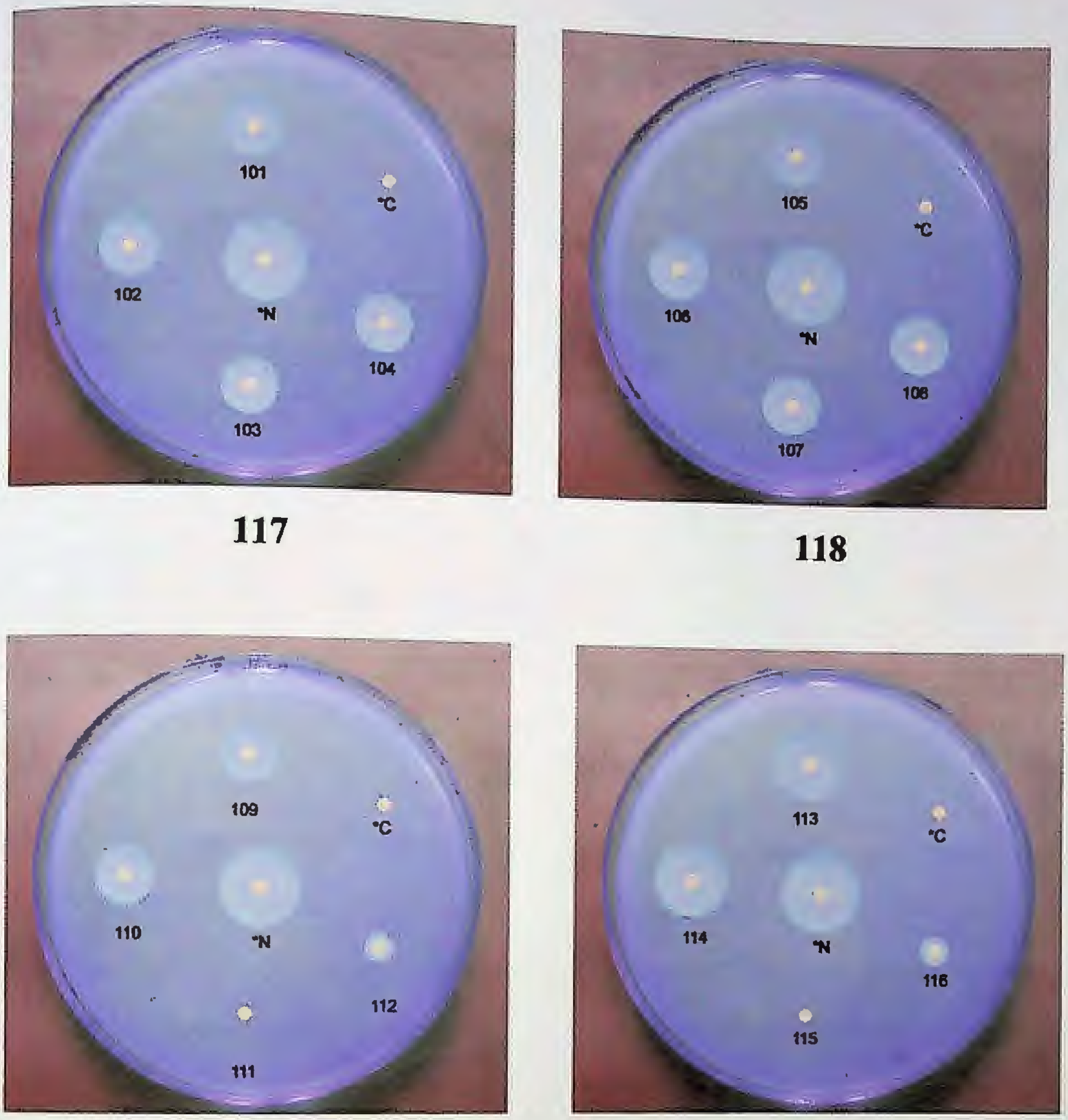
Photograph 113-116: Represents the zone of inhibition at the concentration of 100µg disc^{-1} against *Aspergillus fumigatus*.



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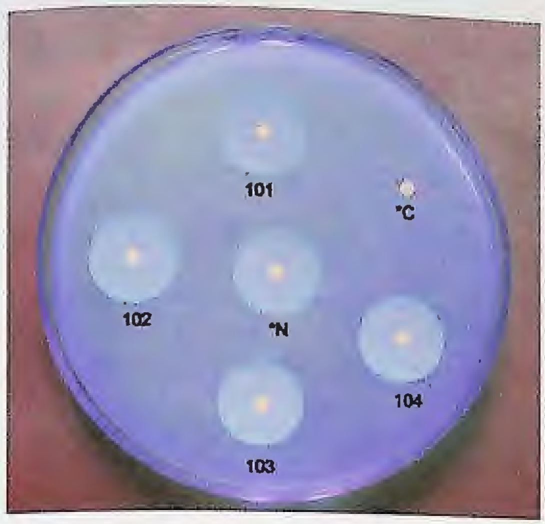
Photograph 117-120: Represents the zone of inhibition at the concentration of 200μg disc^{-1} against *Aspergillus fumigatus*.



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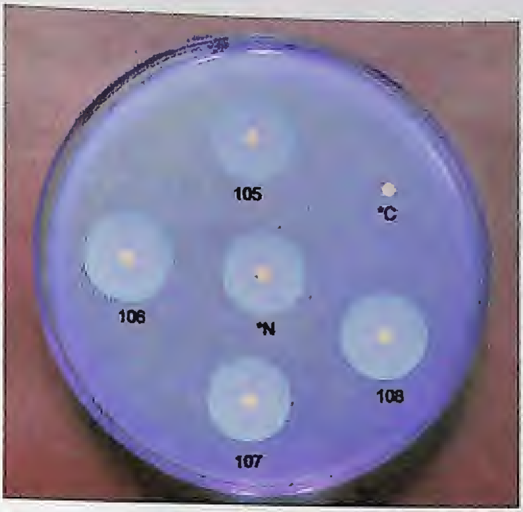

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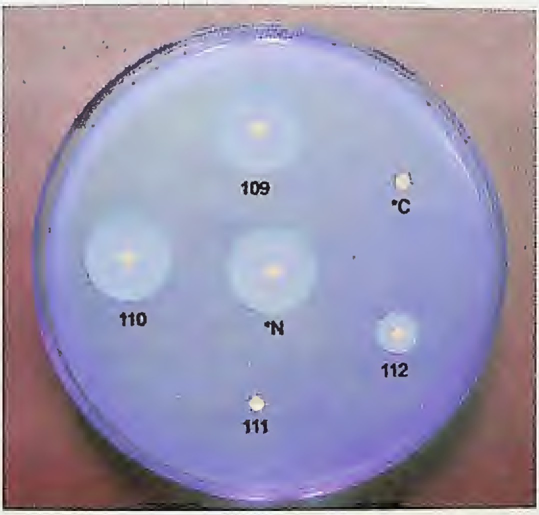
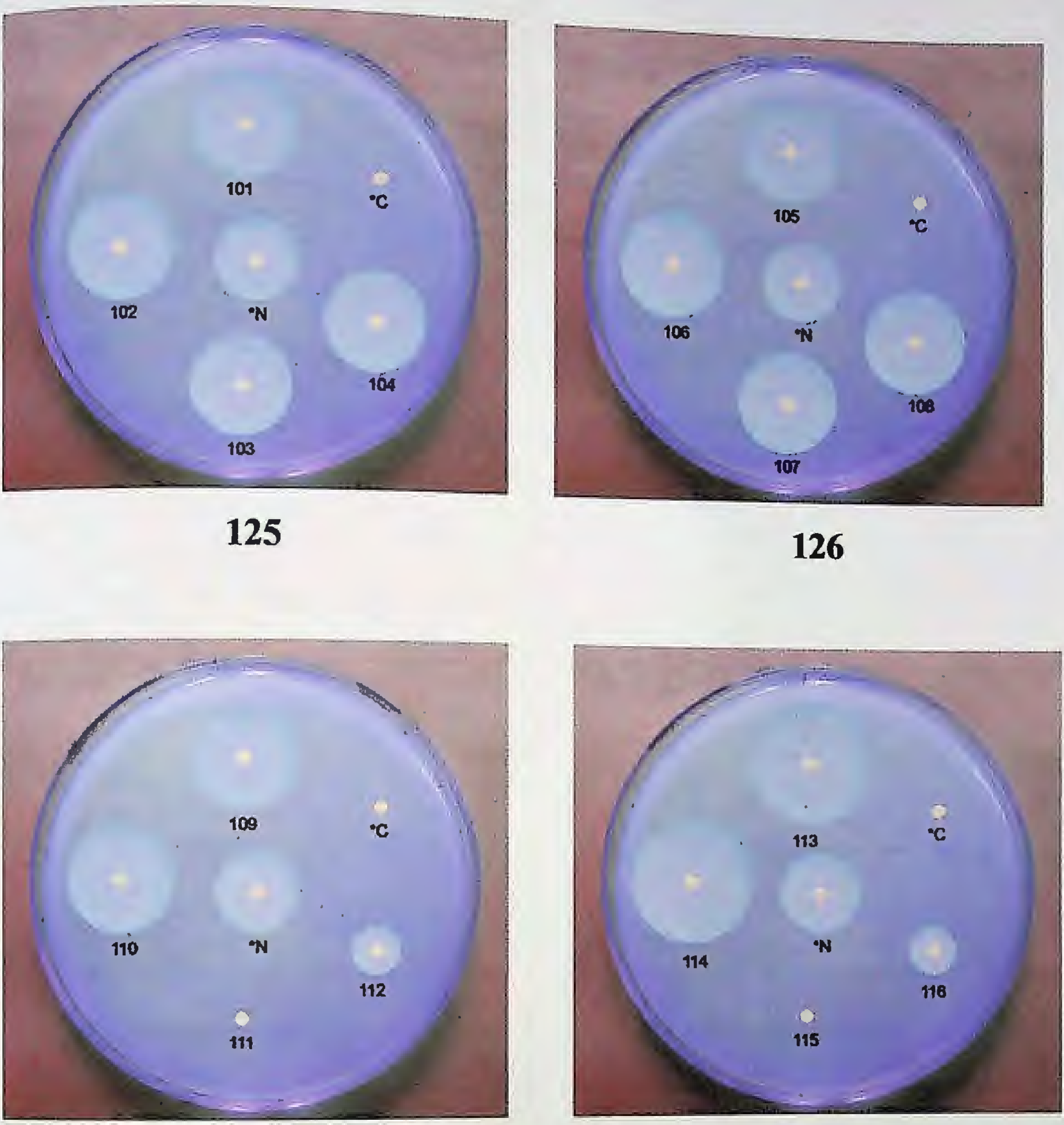


123

124

Photograph 121-124: Represents the zone of inhibition at the concentration of 300µg disc^{-1} against *Aspergillus fumigatus*.



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Photograph 125-128: Represents the zone of inhibition at the concentration of 400µg disc^{-1} against *Aspergillus fumigatus.*

CHAPTER VII

Summary

CHAPTER VII
SUMMARY

This Chapter gives an overview about the synthesis and characterization of chalcone epoxides as well as studies of their antibacterial and antifungal activity.

A large number of natural products including chalcone and chalcone epoxides that are being reported in the literature every year and their structures need to be confirmed by synthesis. In part-I, we described the synthesis of 2′-hydroxy-2,4,5-trimethoxychalcone epoxide; 2′-benzyloxy-2,4,5-trimethoxychalcone epoxide; 4′-methyl-2-chlorochalcone epoxide; 4′-methyl-4-chlorochalcone epoxide; 2′-hydroxy-4-nitrochalcone epoxide; 2′-benzyloxy-4-methoxychaclone epoxide; 2′-hydroxy-3,4-methylenedioxychalcone epoxide. 2′-benzyloxy-3,4-methylenedioxychalcone epoxide; The structures of the above compounds were assigned on the basis of their spectral data together with their elemental analysis. The chalcone epoxides were synthesized and tested for antimicrobial activities towards some human pathogenic bacteria and some plant pathogenic fungi.

Methylation of 2,4,5-trihydroxybenzaldehyde with dimethylsulphate and anhydrous potassium carbonate in acetone gave the corresponding 2,4,5-trimethoxybenzaldehyde. Alkaline condensation of 2-hydroxyacetophenone and 2,4,5-trimethoxybenzaldehyde gave the corresponding 2′-hydroxy-2,4,5-trimethoxychalcone. Oxidation of this chalcone with $NaOH/H_2O_2$ reagent furnished the corresponding 2′-hydroxy-2,4,5-trimethoxychaclone epoxides. The constituent of 2′-hydroxy-2,4,5-trimethoxychalcone epoxide was deduced on the basis of spectral data and elemental analysis.

2′-benzyloxy-2,4,5-trimethoxychalcone epoxide was synthesized by the following way. 2-hydroxyacetophenone on treatment with benzyl chloride gave 2-benzyloxyacetophenone. Alkaline condensation of 2-benzyloxyacetophenone and 2,4,5-trimethyoxybenzaldehyde gave the corresponding 2′-benzyloxy-2,4,5-trimethoxychalcone. Oxidation of this chalcone with NaOH/H_2O_2 reagent furnished the corresponding 2′-benzyloxy-2,4,5-trimethoxychalcone epoxide. The constituent of the 2′-bezyloxy-2,4,5-trimethoxychalcone epoxide was deduced on the basis of spectral data and elemental analysis.

In a program to synthesis of 4′-methyl-2-chlorochalcone epoxide and 4′-methyl-4-chlorochalcone epoxide the following procedure was adopted. Cross aldol condensation of 4-methylacetophenone and 2-chlorobenzaldehyde under alkaline condition produced the corresponding 4′-methyl-2-chlorochalcone. oxidation of this chalcone using NaOH/H_2O_2 as an oxidizing agent furnished the corresponding 4′-methyl-2-chlorochalcone epoxide. Again 4-methylacetophenone and 4-chlorobenzaldehyde maintaining the same procedure as above and produced the corresponding 4′-methyl-4-chlorochalcone epoxide. The constituent of both the chalcone epxoides were deduced on the basis of spectral data and elemental analysis.

In order to synthesis 2′-hydroxy-4-nitrochalcone epxoide, the following procedure has been under taken. Cross aldol condensation of 2-hydroxyacetophenone and 4-nitrobenzaldehyde under alkaline condition produced the corresponding 2′-hydroxy-4-nitrochalcone. Oxidation of this chalcone using NaOH/H_2O_2 as an oxidizing agent furnished the corresponding 2′-hydroxy-4-nitrochalcone epoxide. The constituent of 2′-hydroxy-4-nitrochalcone epoxide was deduced on the basis of spectral data and elemental analysis.

4-hydroxybenzaldehyde on methylation with dimethylsulphate yielded 4-methyoxybenzaldehye. Alkaline condensation of 2-benzyloxyacetophenone and 4-methoxybenzaldehyde gave the corresponding 2′-benzyloxy-4-methoxychalcone. Oxidation of this chalcone with $NaOH/H_2O_2$ reagent furnished the corresponding 2′-benzyloxy-4-methoxychalcone epoxide. The constituent of the 2′-benzyloxy-4-methoxychalcone epoxide was deduced on the basis of spectral data and elemental analysis.

In a progrm to synthesis 2′-hydroxy-3,4-methylenedioxychalcone epoxide and 2′-benzyloxy-3,4-methylenedioxychalcone epoxide the following procedure was adopted. Cross aldol condensation of 2-hydroxyacetophenone and 3,4-methylenedioxybenzaldehyde under alkaline condition gave the corresponding 2′-hydroxy-3,4-methylenedioxy chalcone. Oxidation of this chalcone using $NaOH/H_2O_2$ as an oxidizing agent furnished the corresponding 2′–hydroxy-3,4-methylenedioxychalcone epoxide. Again 2-benzyloxyacetophenone and 3,4-methylenedioxybenzaldehyde maintaining the same procedure as above and produced the corresponding 2′-benzyloxy 3,4-methylenedioxychalcone epoxide. The constituent of both chalcone epoxides were deduced on the basis of spectral data and elemental analysis.

The antibacterial and antifugnal activities of the above chalcone epoxides along with their corresponding chalcones were determined *in vitro*. The capability of microorganism to antimicrobial agents can be determined *in vitro* by a number of methods. The disc diffusion technique is widely accepted for preliminary investigations of materials, which are suspected to possesses antimicrobial properties. Diffusion procedure as normally used in susceptible, intermediate of resistant categories.

Diffusion assays are based on the ability of antibiotics to diffuse from a confined source through a PDA gel or NA and create a concentration gradient. If the agar is seeded or streaked with sensitive organism, a zone of inhibition will result were the antibiotic concentration exceeds the minimum inhibitory concentration (MIC) for that particular organism.

Eight chalcone epoxides and their corresponding chalcones were screened *in vitro* for their antibacterial and antifungal activity against four human pathogenic bacteria viz. *Streptococcus-β-haemolyticus* (G^+), *Bacillus megatrium* (G^+), *Klebsiella* sp. (G^-) *Escherichia coli* (G^-) and four plant as well as molds fungi viz. *Rhizoctonia solani, Sclerotium rolfsii, Aspergillus niger* and *Aspergillus fumigatus* in different concentrations. Some of the compounds show moderate activity towards both the Gram-positive and Gram-negative bacteria. It has been found that the inhibition zones of the chalcone epoxides were more effective than their corresponding chalcones. Some of the compounds did not show any inhibition against bacteria as well as fungi. It has also been found that most of the compounds shows fungicide activity.

CHAPTER VIII

References

CHAPTER-VIII

References

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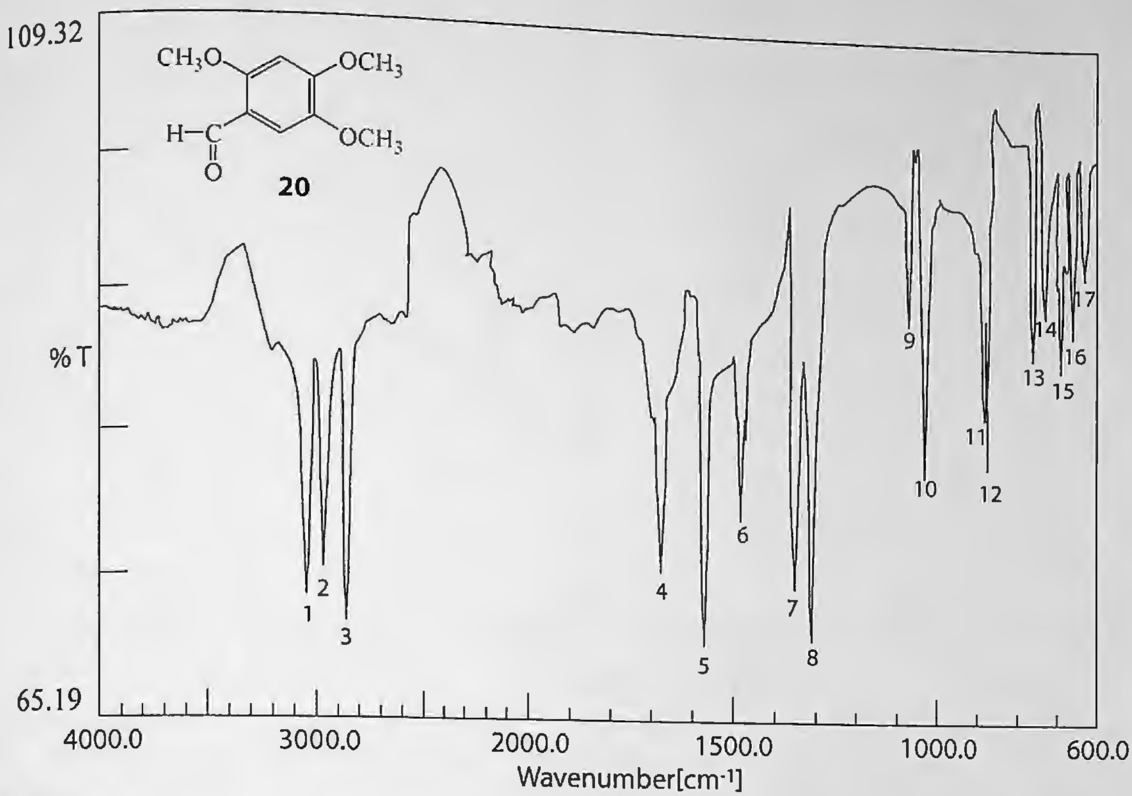
Appendix

Appendix



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Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

Comment
Copyright
Operator
Peak information

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2 2970.02
3 2860.13
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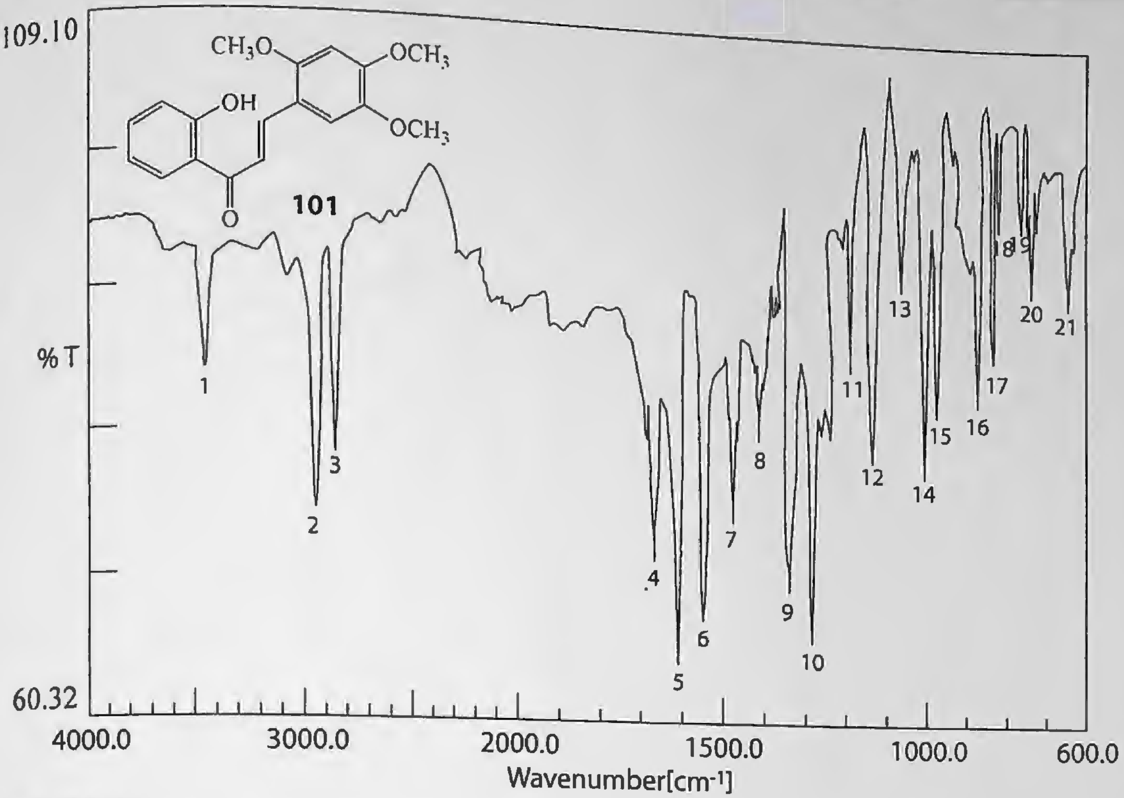
17 630.19



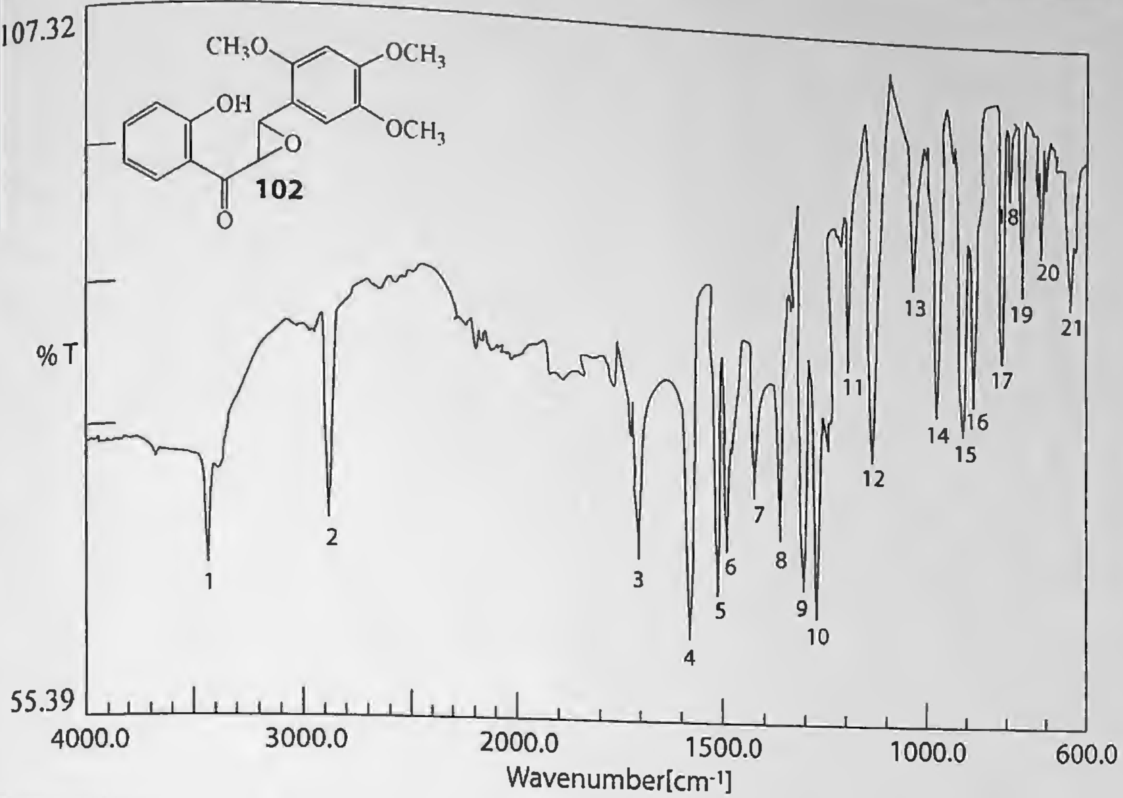
Parameter

Accumulation	40.15 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 8:11오후
Sample Name			

Gain	4	File Name	AIMR101.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

Comment
Copyright
Operator
Peak information

1	3450.32	8	1413.18	15	974.21
2	2938.21	9	1339.23	16	871.41
3	2842.10	10	1283.11	17	834.30
4	1677.09	11	1187.31	18	819.21
5	1609.12	12	1135.24	19	763.35
6	1549.36	13	1062.14	20	737.22
7	1474.13	14	1004.33	21	645.09



Parameter

Accumulation	50 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 10:31오후
Sample Name			

Gain	4	File Name	AIMR102.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

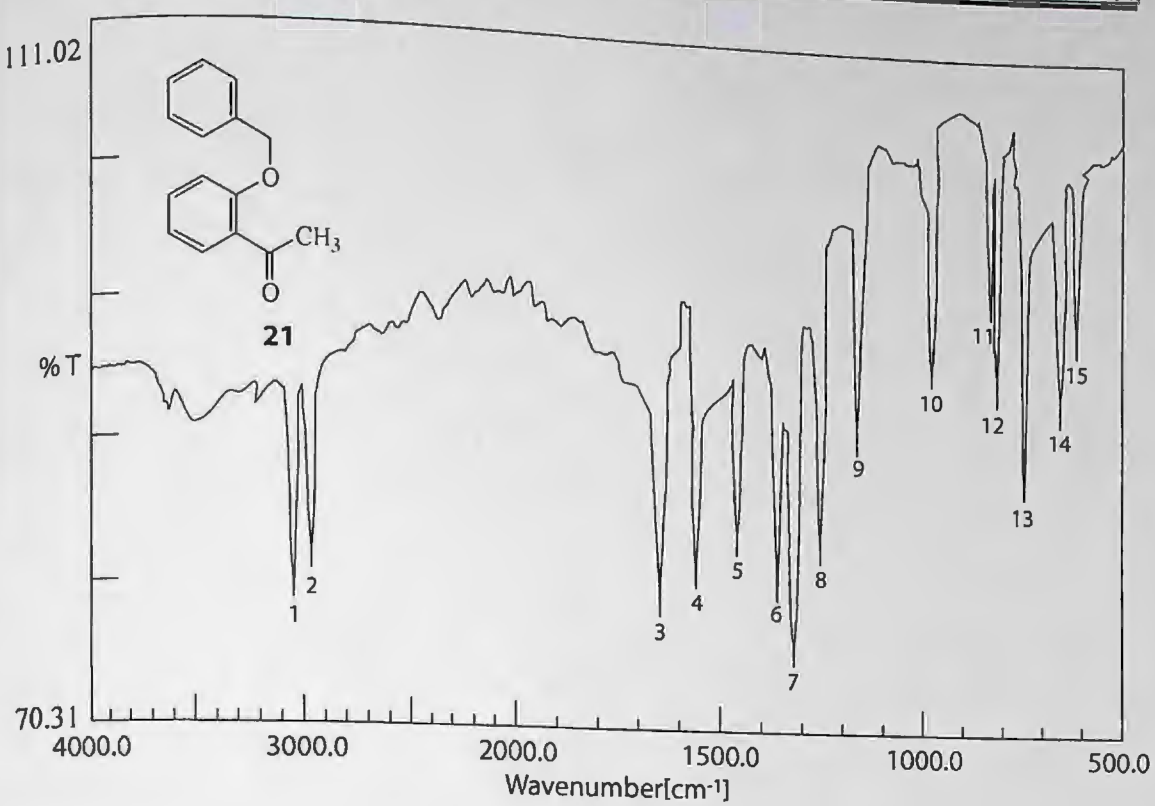
Comment

Copyright

Operator

Peak information

1	3433.21	8	1361.32	15	908.20
2	2882.23	9	1304.21	16	882.14
3	1704.41	10	1271.12	17	811.15
4	1579.11	11	1193.33	18	793.31
5	1512.32	12	1134.10	19	761.30
6	1489.12	13	1033.09	20	714.16
7	1424.22	14	974.23	21	639.11



Parameter			
Accumulation	45 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 11:31오후
Sample Name			

Gain	4	File Name	AIMR21.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

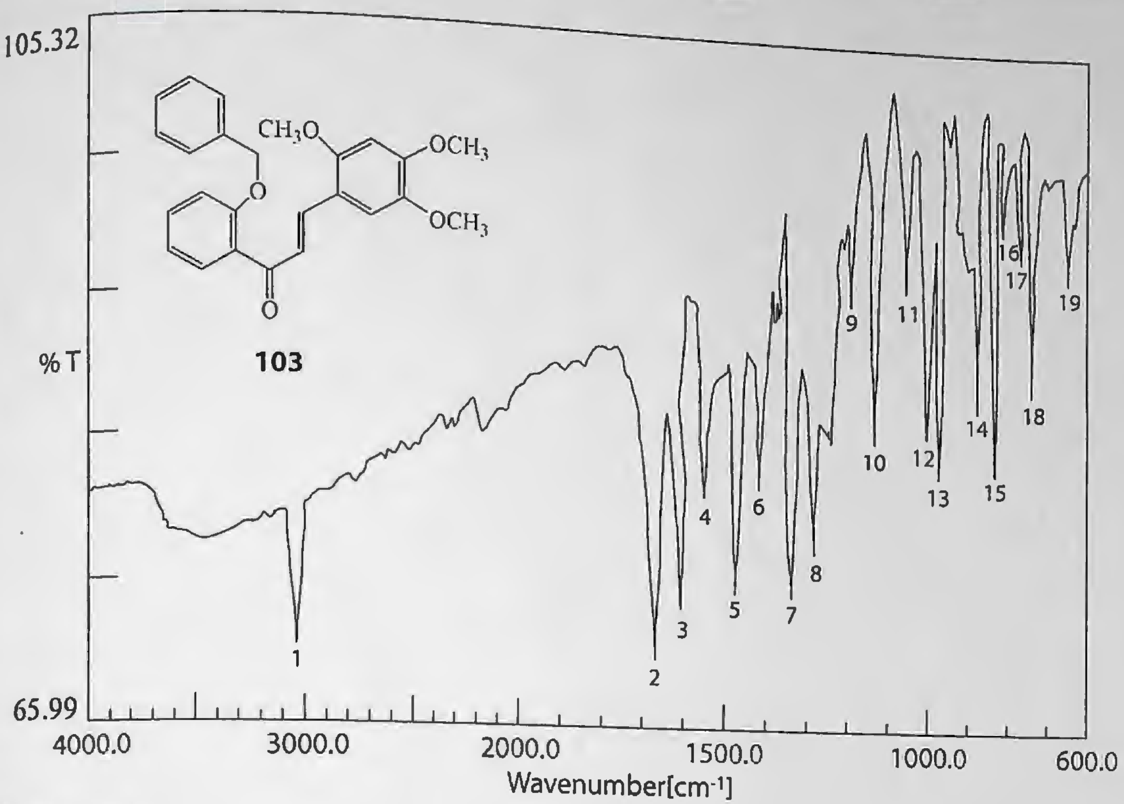
Comment

Copyright

Operator

Peak information

1	3043.16	6	1360.25	11	830.21
2	2970.21	7	1320.32	12	814.04
3	1647.32	8	1254.21	13	745.10
4	1560.06	9	1164.19	14	655.32
5	1458.14	10	988.11	15	613.36



Parameter

Accumulation	49 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 11:00 오후
Sample Name			

Gain	4	File Name	AIMR103.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

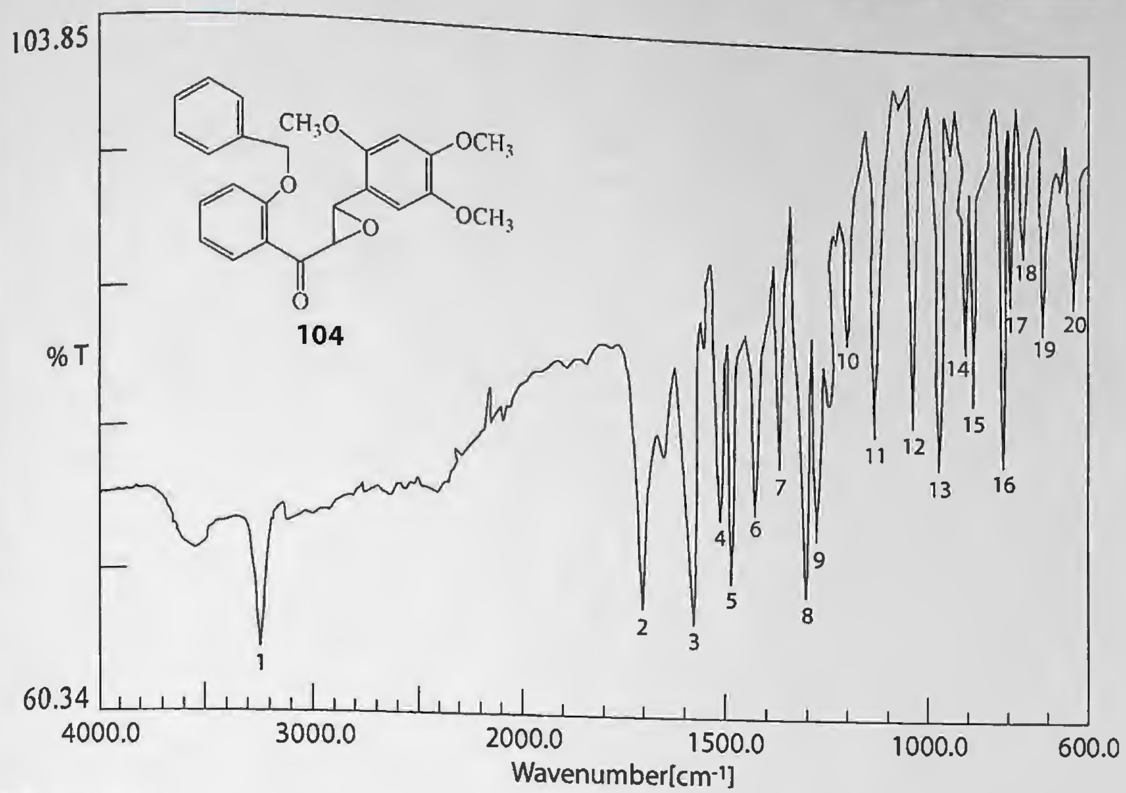
Comment

Copyright

Operator

Peak information

1	3032.21	8	1281.11	15	831.21
2	1666.10	9	1189.31	16	817.12
3	1606.23	10	1132.09	17	766.36
4	1548.15	11	1064.22	18	739.20
5	1472.32	12	1002.33	19	648.14
6	1415.36	13	972.15		
7	1337.21	14	873.32		



Parameter

Accumulation	51.25 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 9:10 오후
Sample Name			

Gain	4	File Name	AIMR104.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

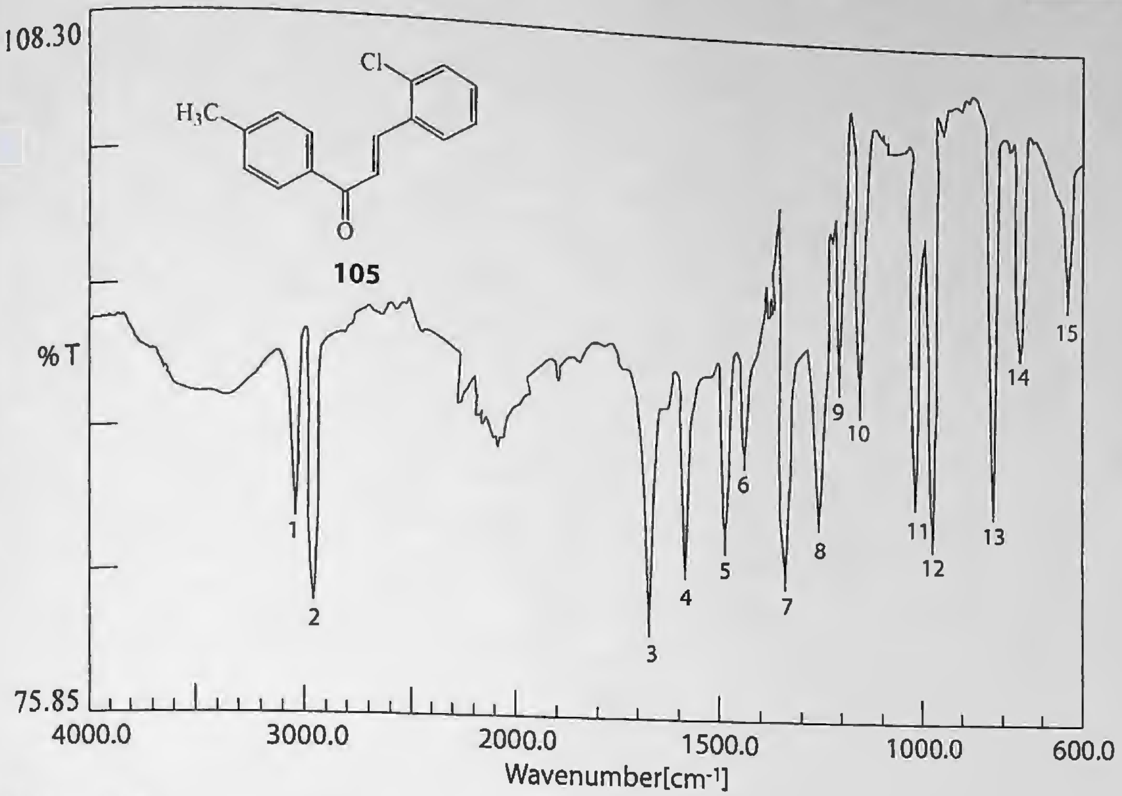
Comment

Copyright

Operator

Peak information

1	3240.21	8	1302.25	15	906.10
2	1701.10	9	1278.19	16	885.23
3	1577.21	10	1199.09	17	812.25
4	1510.32	11	1131.13	18	795.12
5	1484.36	12	1035.32	19	762.36
6	1426.21	13	975.24	20	636.20
7	1367.14	14	906.26		



Parameter

Accumulation	50.31 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 9:50 오후
Sample Name			

Gain	4	File Name	AIMR105.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

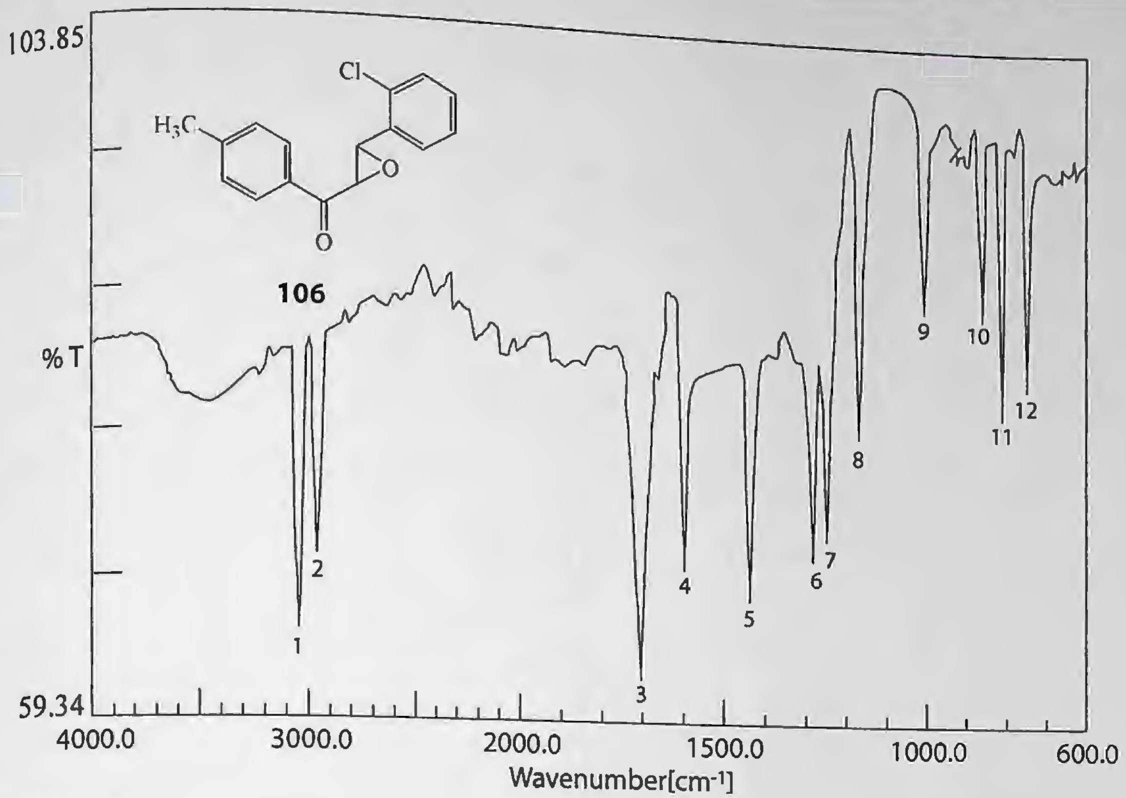
Comment

Copyright

Operator

Peak information

1	3035.21	6	1440.21	11	1016.32
2	2961.12	7	1340.14	12	974.14
3	1672.24	8	1256.19	13	823.21
4	1583.12	9	1205.20	14	752.14
5	1485.32	10	1155.09	15	637.30



Parameter

Accumulation	51.24 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 7:50 오후
Sample Name			

Gain	4	File Name	AIMR106.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

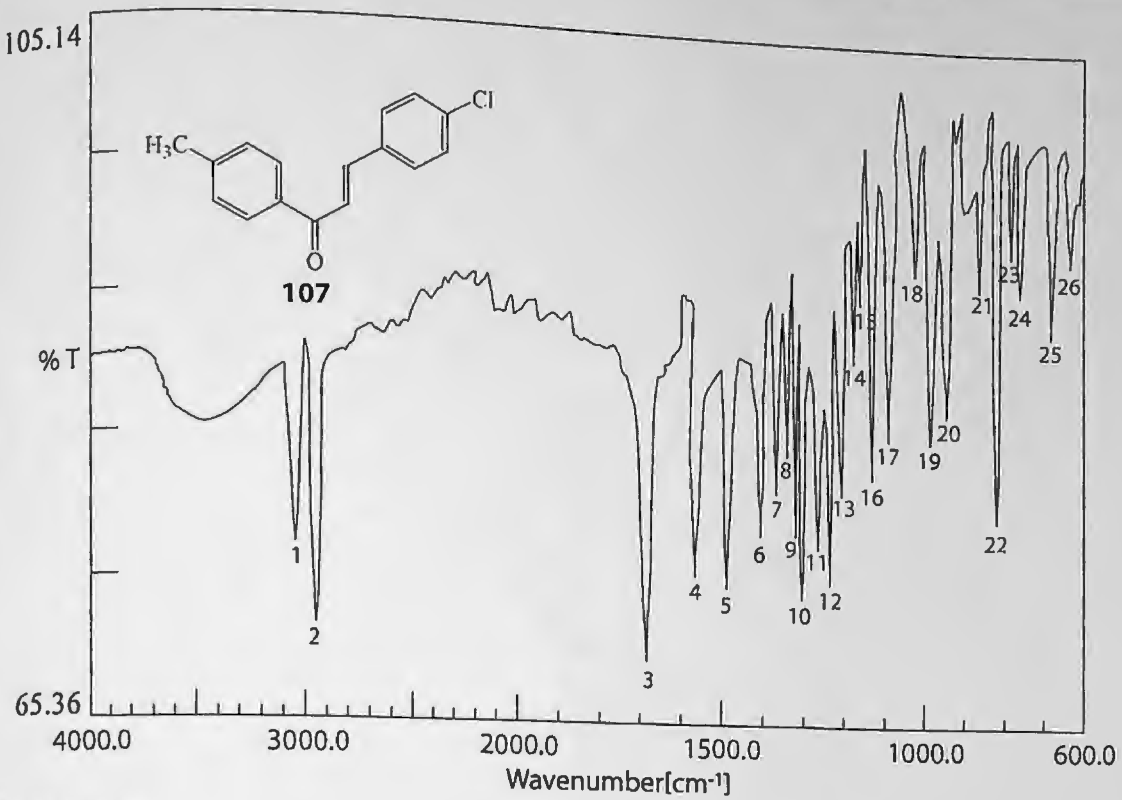
Comment

Copyright

Operator

Peak information

1	3039.21	5	1439.23	9	1006.31
2	2970.09	6	1282.21	10	860.12
3	1703.11	7	1248.10	11	812.24
4	1597.32	8	1167.25	12	749.09



Parameter

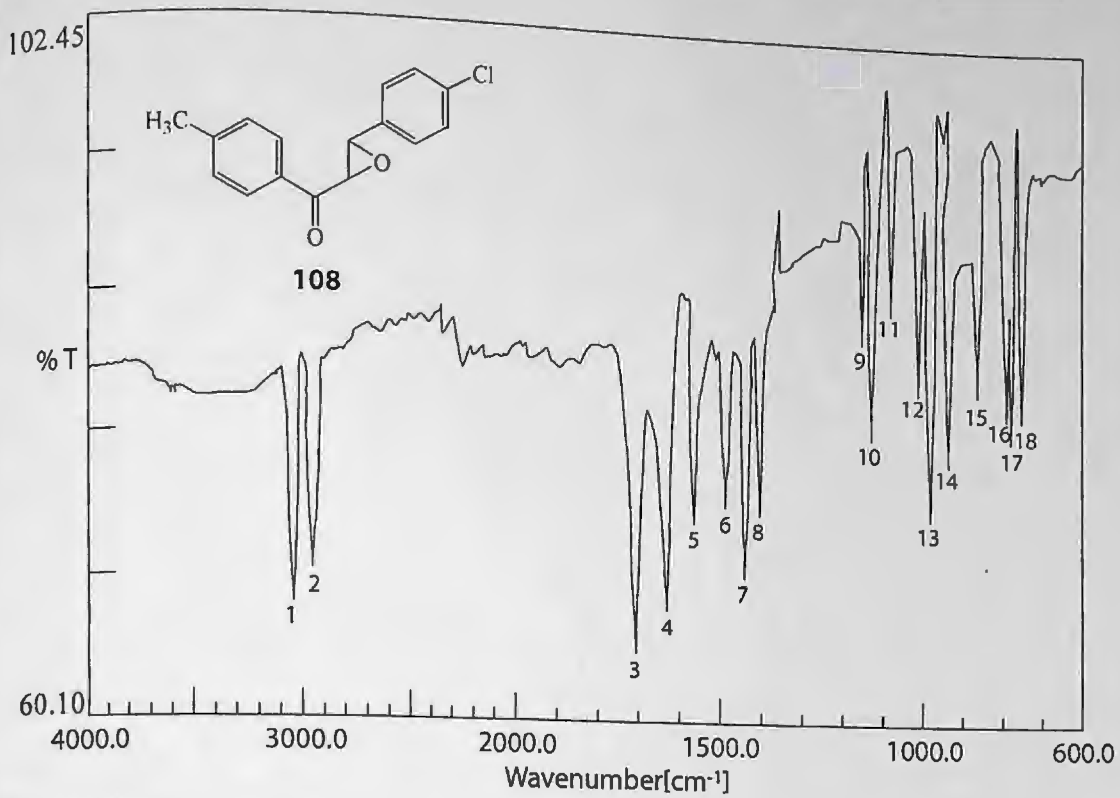
Accumulation	51.10 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 8:46 오후
Sample Name			

Gain	4	File Name	AIMR107.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

Comment
Copyright
Operator

Peak information

1	3038.23	10	1304.09	19	983.24
2	2950.25	11	1263.14	20	943.21
3	1683.10	12	1234.32	21	862.14
4	1564.23	13	1205.21	22	819.23
5	1487.24	14	1174.19	23	783.19
6	1440.36	15	1159.23	24	760.10
7	1367.20	16	1130.10	25	682.14
8	1340.15	17	1089.36	26	644.16
9	1319.18	18	1022.23		



Parameter

Accumulation	52 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 9:26 오후
Sample Name			

Gain	4	File Name	AIMR108.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

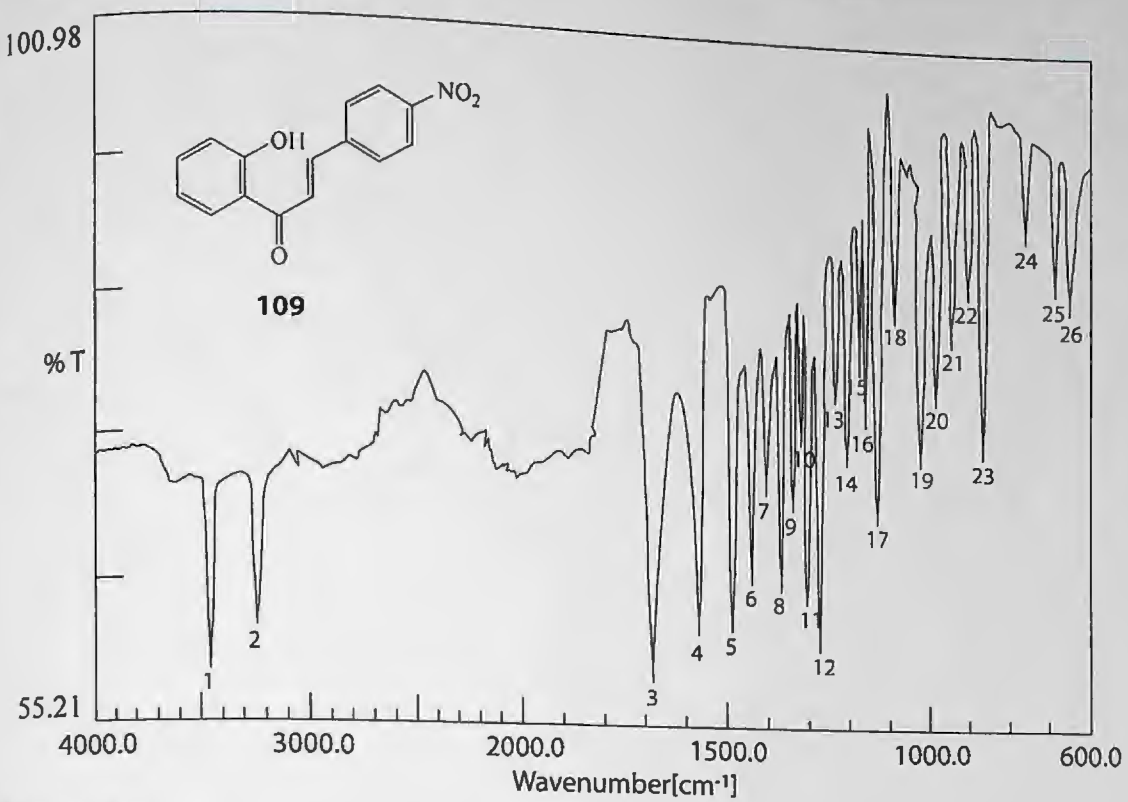
Comment

Copyright

Operator

Peak information

1	3040.23	7	1440.09	13	980.08
2	2955.21	8	1402.21	14	934.12
3	1705.12	9	1150.22	15	862.14
4	1630.10	10	1127.32	16	789.19
5	1562.19	11	1079.12	17	779.21
6	1485.20	12	1009.33	18	752.35



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Parameter

Accumulation	52.24 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 9:00 오후
Sample Name			

Gain	4	File Name	AIMR109.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

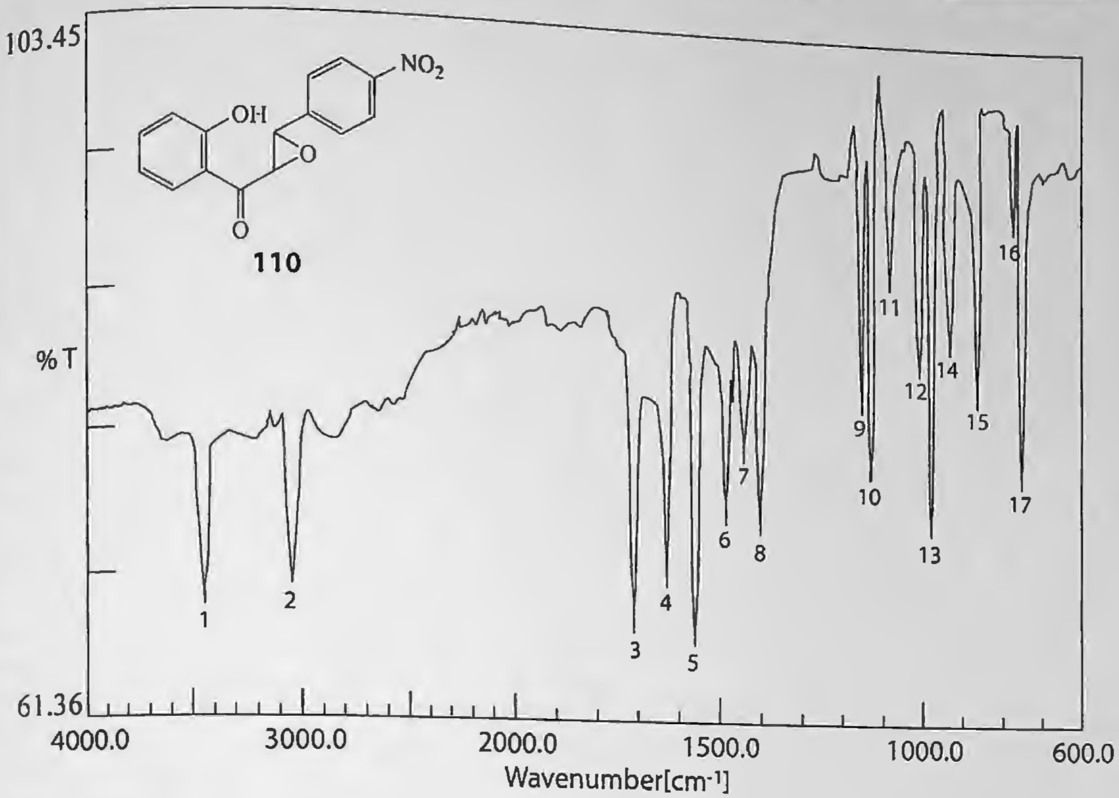
Comment

Copyright

Operator

Peak information

1	3455.21
2	3035.10
3	1681.19
4	1568.09
5	1487.23
6	1440.33
7	1406.12
8	1367.24
9	1340.21
10	1319.32
11	1304.22
12	1263.14
13	1234.19
14	1205.20
15	1174.14
16	1159.13
17	1130.18
18	1089.26
19	1022.32
20	984.10
21	945.24
22	866.18
23	760.17
24	685.10
25	649.07



Parameter

Accumulation	47 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 9:15 오후
Sample Name			

Gain	4	File Name	AIMR110.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

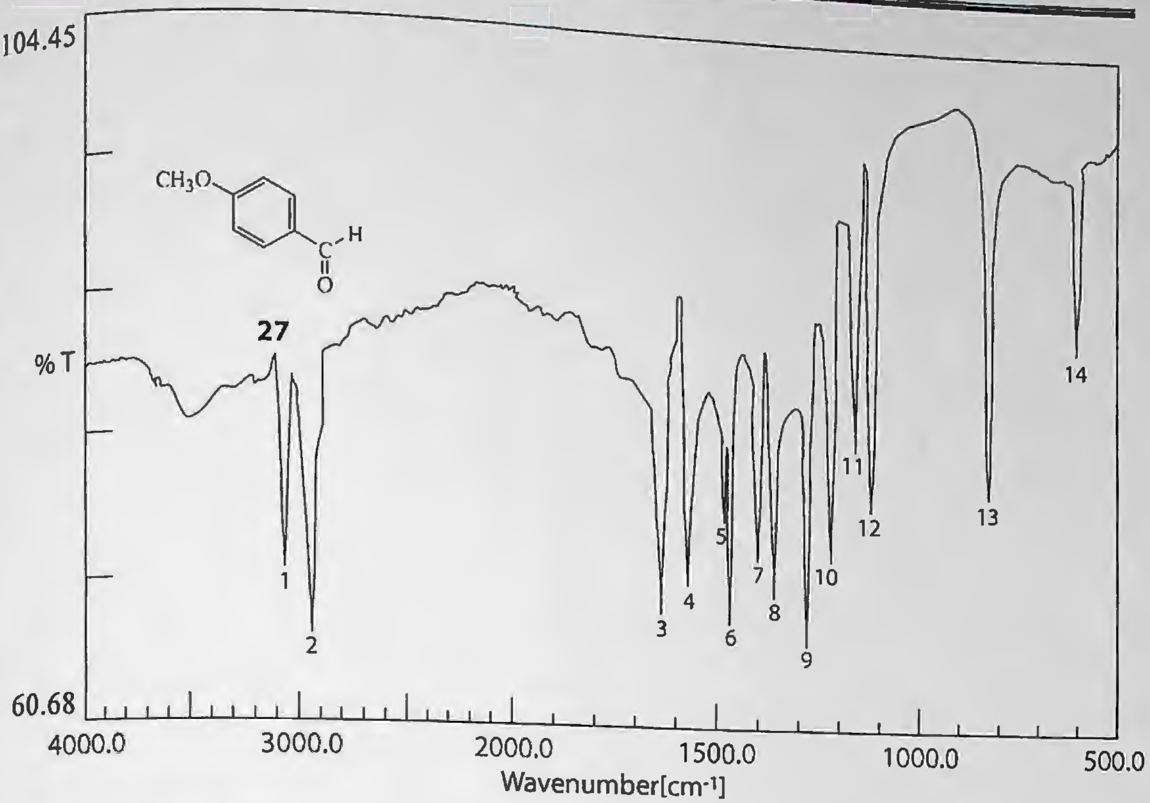
Comment

Copyright

Operator

Peak information

1	3443.15
2	3042.10
3	1709.19
4	1630.22
5	1562.23
6	1485.31
7	1442.21
8	1402.24
9	1151.10
10	1129.09
11	1081.14
12	1007.19
13	978.33
14	931.21
15	862.26
16	772.16
17	752.21



Parameter

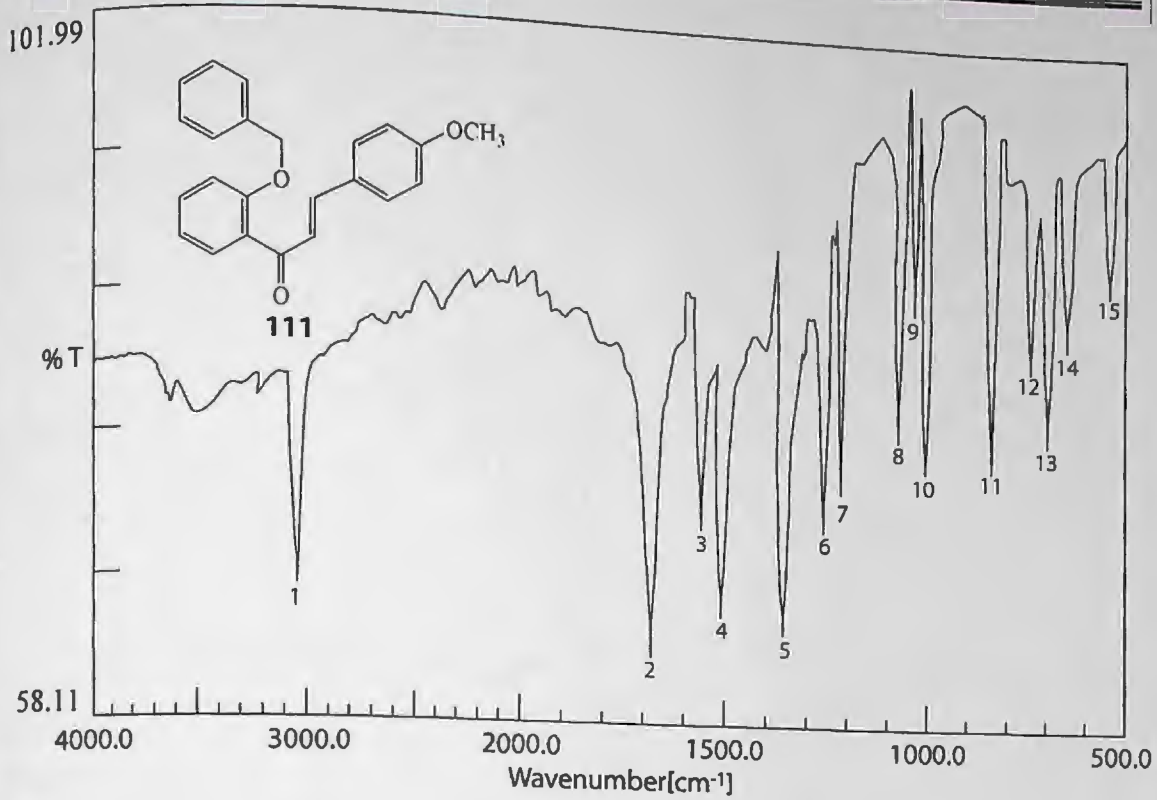
Accumulation	48.25 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 10:10오후
Sample Name			

Gain	4	File Name	AIMR27.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

Comment
Copyright
Operator

Peak information

1 3078.12
2 2962.09
3 1635.21
4 1560.24
5 1480.26
6 1470.19
7 1400.17
8 1360.26
9 1280.14
10 1220.20
11 1160.33
12 1120.21
13 825.33
14 600.30



Parameter

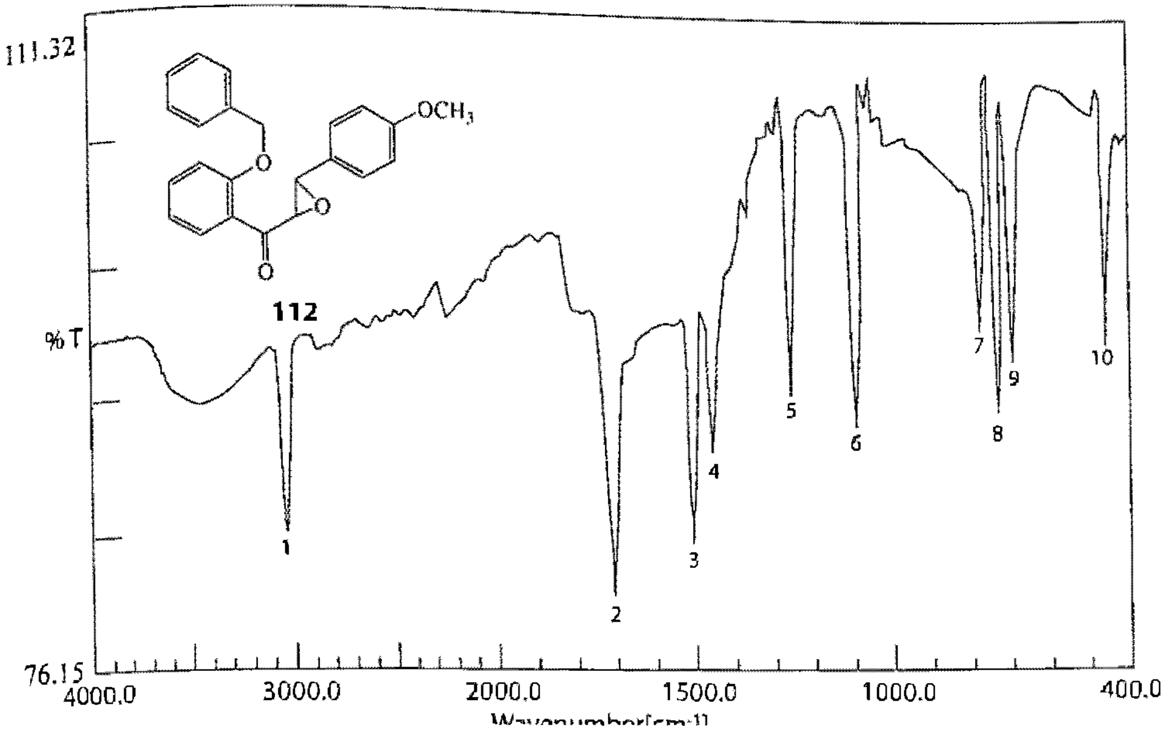
Accumulation	48 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 10:29 오후
Sample Name			

Gain	4	File Name	AIMR111.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

Comment
Copyright
Operator

Peak information

1	3042.04	6	1257.11	11	838.19
2	1679.12	7	1213.34	12	739.13
3	1557.14	8	1169.10	13	696.17
4	1508.34	9	1131.21	14	647.21
5	1358.21	10	1003.25	15	539.09



Parameter

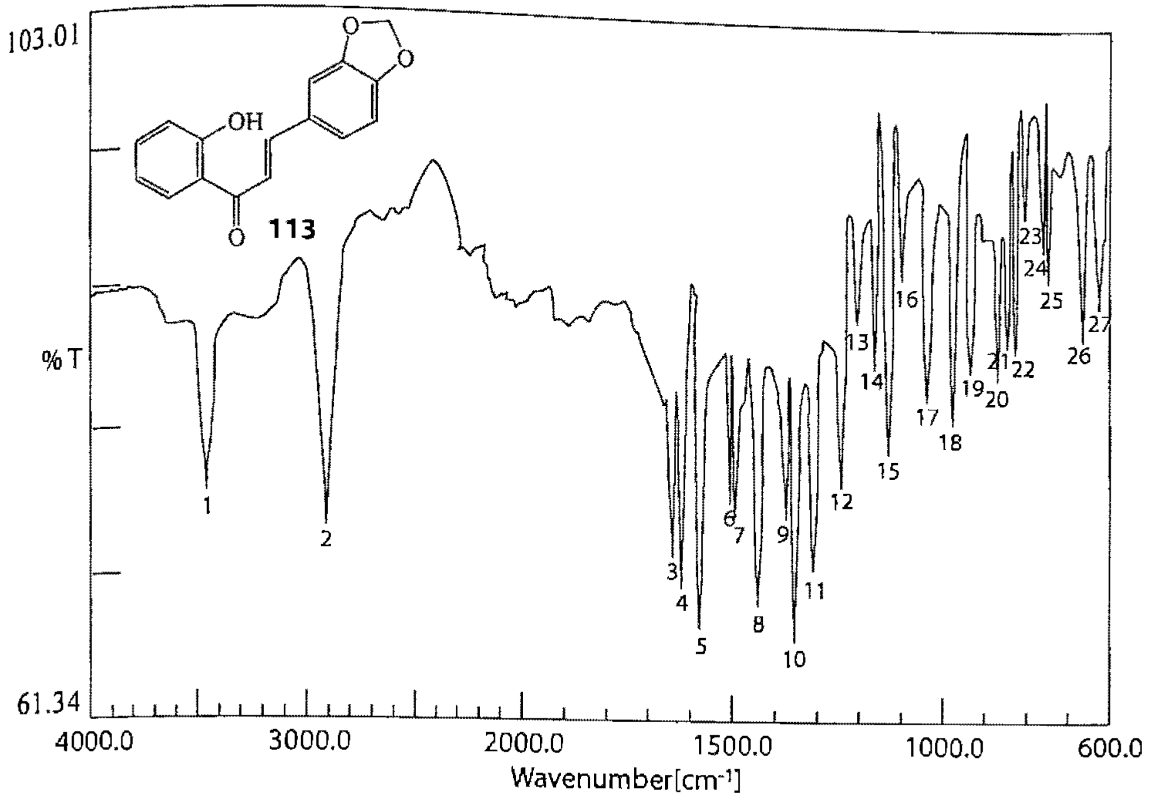
Accumulation	49.32 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 8:29 오후
Sample Name			

Gain	4	File Name	AIMR112.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

Comment
Copyright
Operator

Peak information

1 3036.09
2 1707.32
3 1506.12
4 1456.24
5 1259.24
6 1094.19
7 779.17
8 735.13
9 697.33
10 460.14



Parameter

Accumulation	49.54 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 10:39 오후
Sample Name			

Gain	4	File Name	AIMR113.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

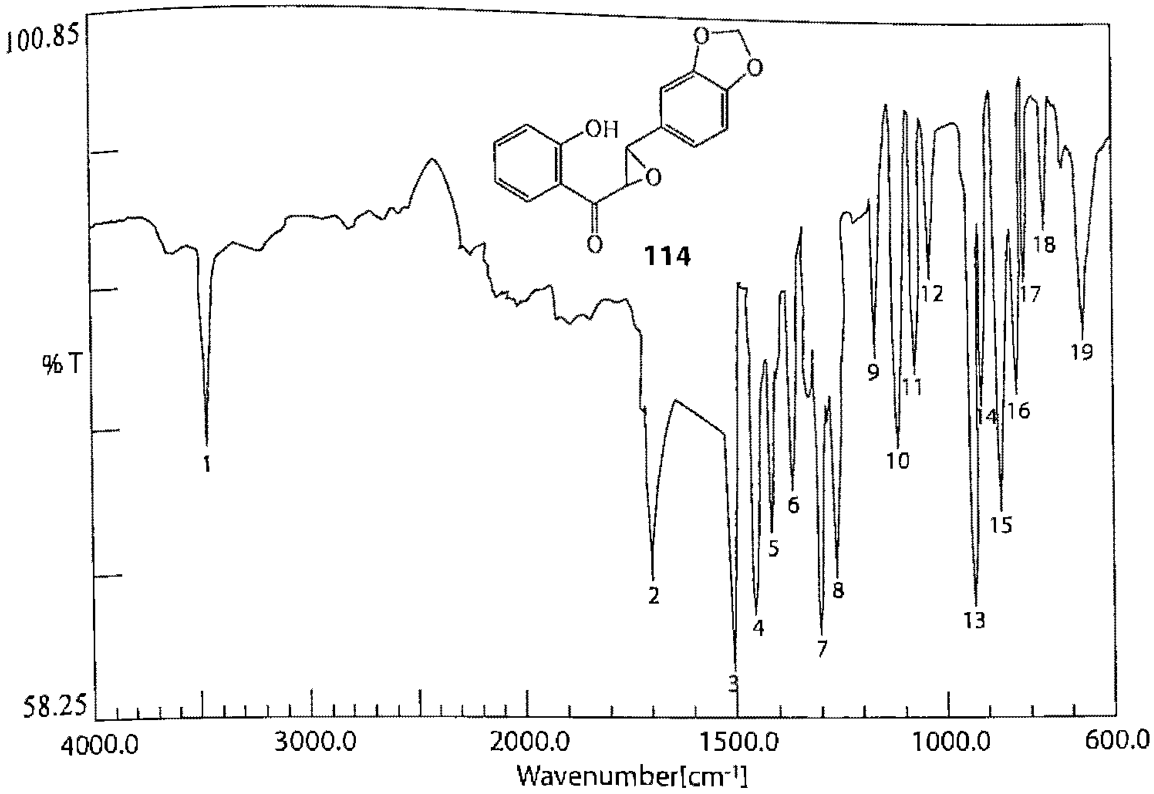
Comment

Copyright

Operator

Peak information

1	3458.21	10	1354.32	19	933.11
2	2908.14	11	1309.10	20	868.09
3	1641.12	12	1242.24	21	846.32
4	1620.32	13	1203.14	22	827.15
5	1577.19	14	1161.10	23	804.11
6	1504.21	15	1130.32	24	760.16
7	1493.25	16	1097.25	25	748.24
8	1440.14	17	1037.16	26	665.26
9	1373.13	18	976.19	27	625.13



Parameter

Accumulation	49.15 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 11:19 오후
Sample Name			

Gain	4	File Name	AIMR114.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

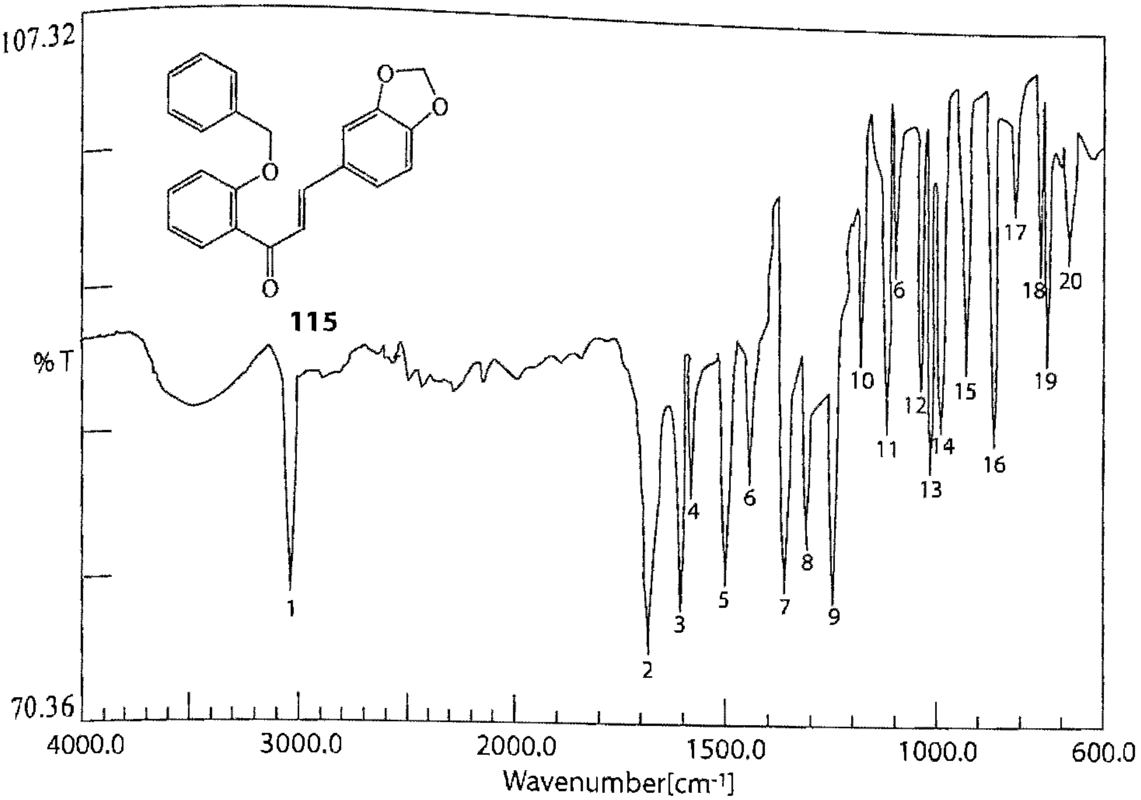
Comment

Copyright

Operator

Peak information

1	3464.01	8	1261.32	15	868.09
2	1697.35	9	1167.21	16	831.13
3	1504.21	10	1114.14	17	812.31
4	1454.10	11	1074.19	18	763.20
5	1415.24	12	1037.22	19	671.14
6	1367.26	13	931.26		
7	1300.14	14	914.18		



Parameter

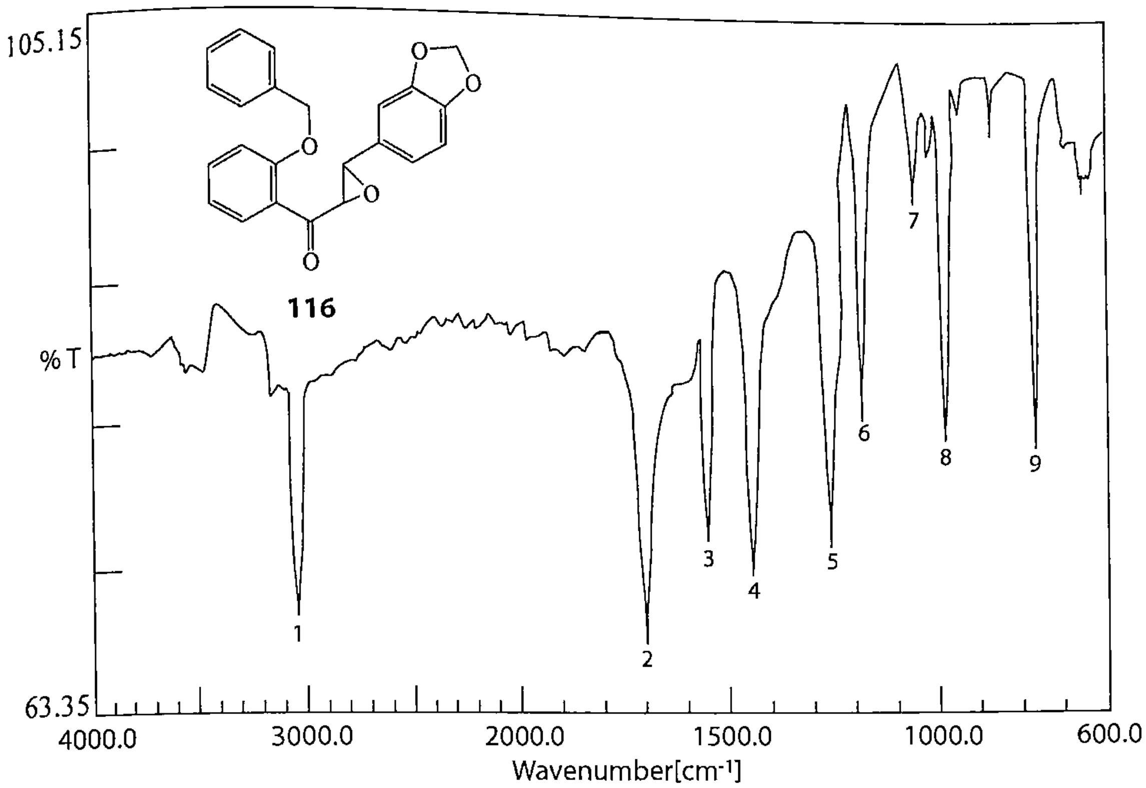
Accumulation	52.30 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 11:30 오후
Sample Name			

Gain	4	File Name	AIMR115.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	x 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

Comment
Copyright
Operator

Peak information

1	3037.20	8	1309.32	15	989.32
2	1682.19	9	1246.28	16	929.12
3	1606.32	10	1180.14	17	862.24
4	1581.14	11	1118.32	18	812.01
5	1500.10	12	1099.12	19	752.19
6	1444.19	13	1037.24	20	736.14
7	1361.36	14	1014.13	21	684.09



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Parameter

Accumulation	53 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 11:58 오후
Sample Name			

Gain	4	File Name	AIMR116.J1D
Aperture	5.0 mm	Light Source	Standard
Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

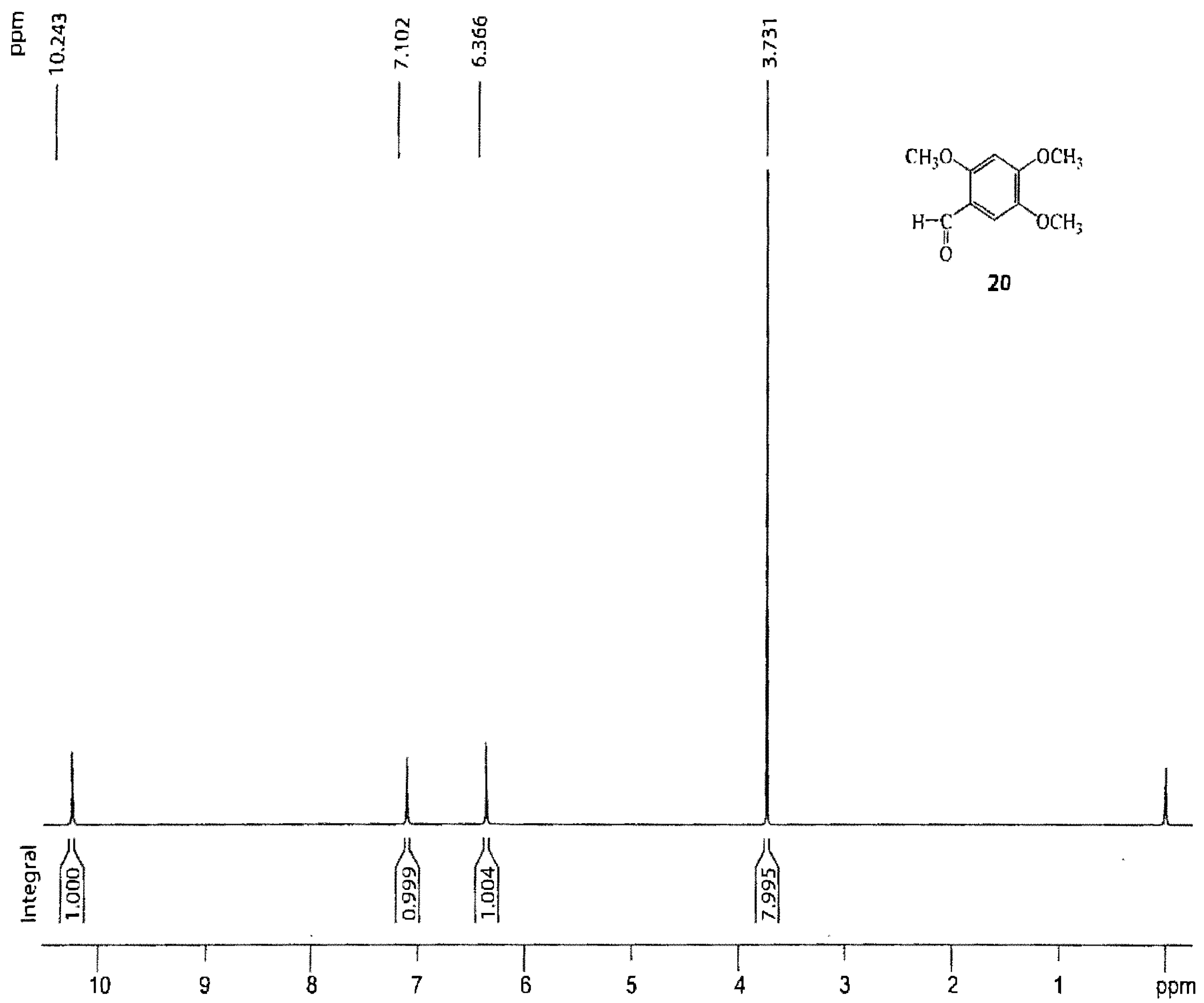
Comment

Copyright

Operator

Peak information

1	3041.10	4	1446.33	7	983.25
2	1699.21	5	1259.41	8	767.09
3	1555.23	6	1182.29		



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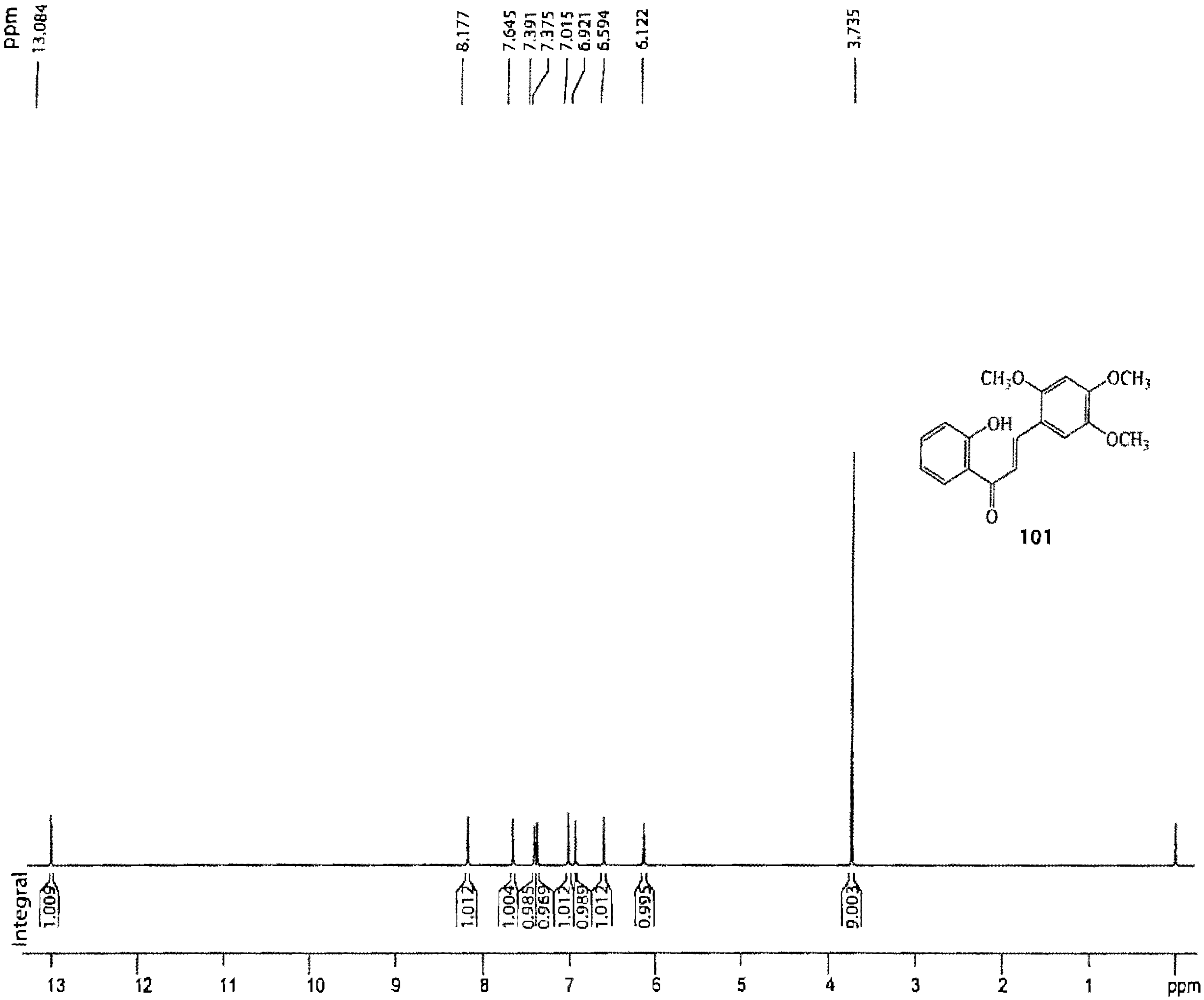
```
Current Data Parameters
NAME              MRAI-20
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20080902
Time                17.36
INSTRUM             spect
PROBHD   5 mm Dual 13C/
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                      6
DS                      0
SWH              5175.983 Hz
FIDRES           0.078979 Hz
AQ              6.3308277 sec
RG                  574.7
DW                 96.600 usec
DE                   6.00 usec
TE                  291.2 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  10.50 usec
PL1                  0.00 dB
SFO1          250.1315447 MHz

F2 - Processing parameters
SI                  32768
SF            250.1300059 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

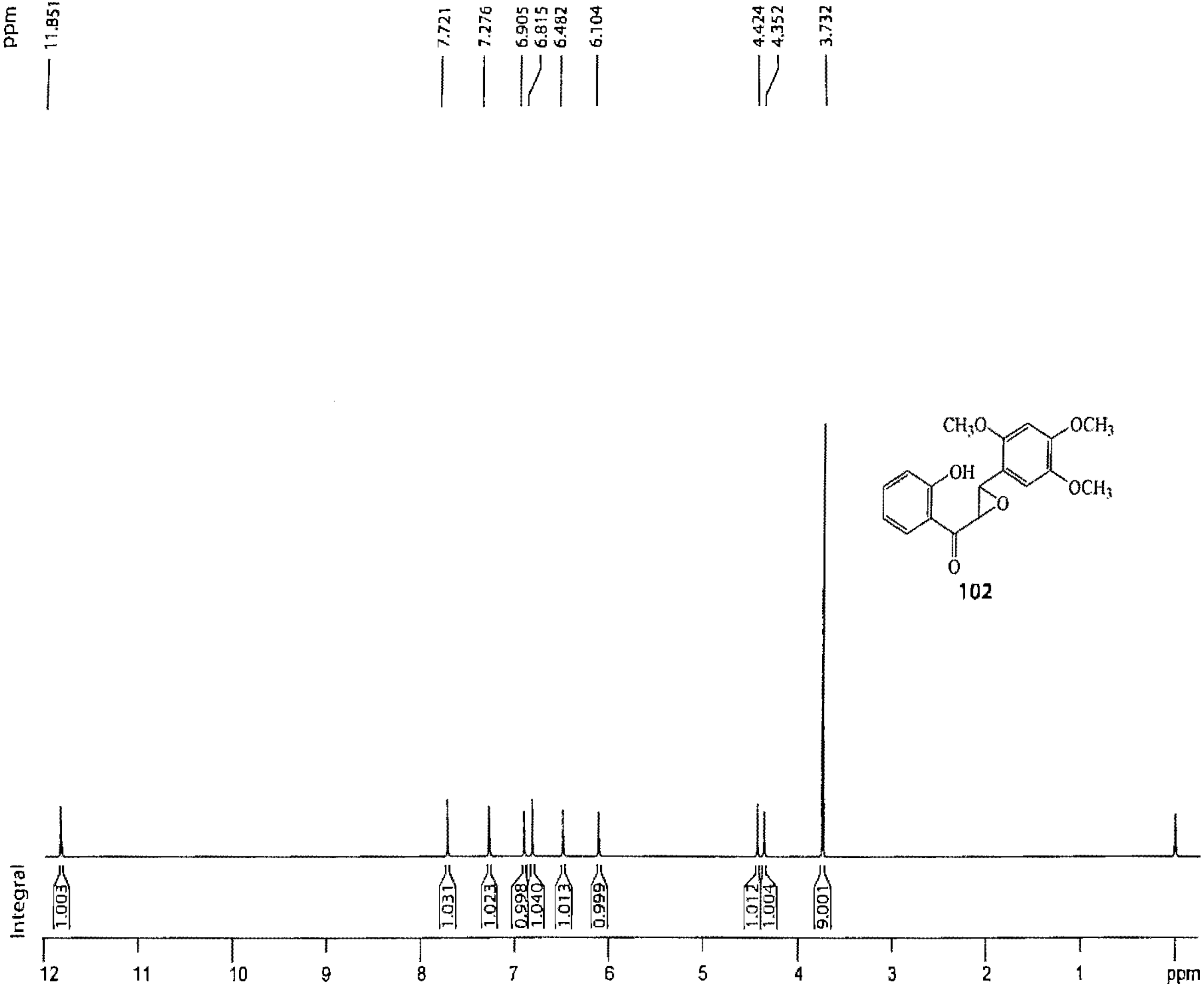


```
Current Data Parameters
NAME          MRAI-101
EXPNO                1
PROCNO               1

F2 - Acquisition Parameters
Date_           20080902
Time               17.36
INSTRUM            spect
PROBHD   5 mm Dual 13C/
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                     6
DS                     0
SWH          5175.983 Hz
FIDRES       0.078979 Hz
AQ          6.3308277 sec
RG                 574.7
DW            96.600 usec
DE              6.00 usec
TE               291.2 K
D1        1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1             10.50 usec
PL1              0.00 dB
SFO1     250.1315447 MHz

F2 - Processing parameters
SI                 32768
SF       250.1300059 MHz
WDW                   EM
SSB                    0
LB               0.30 Hz
GB                     0
PC                  1.00
```



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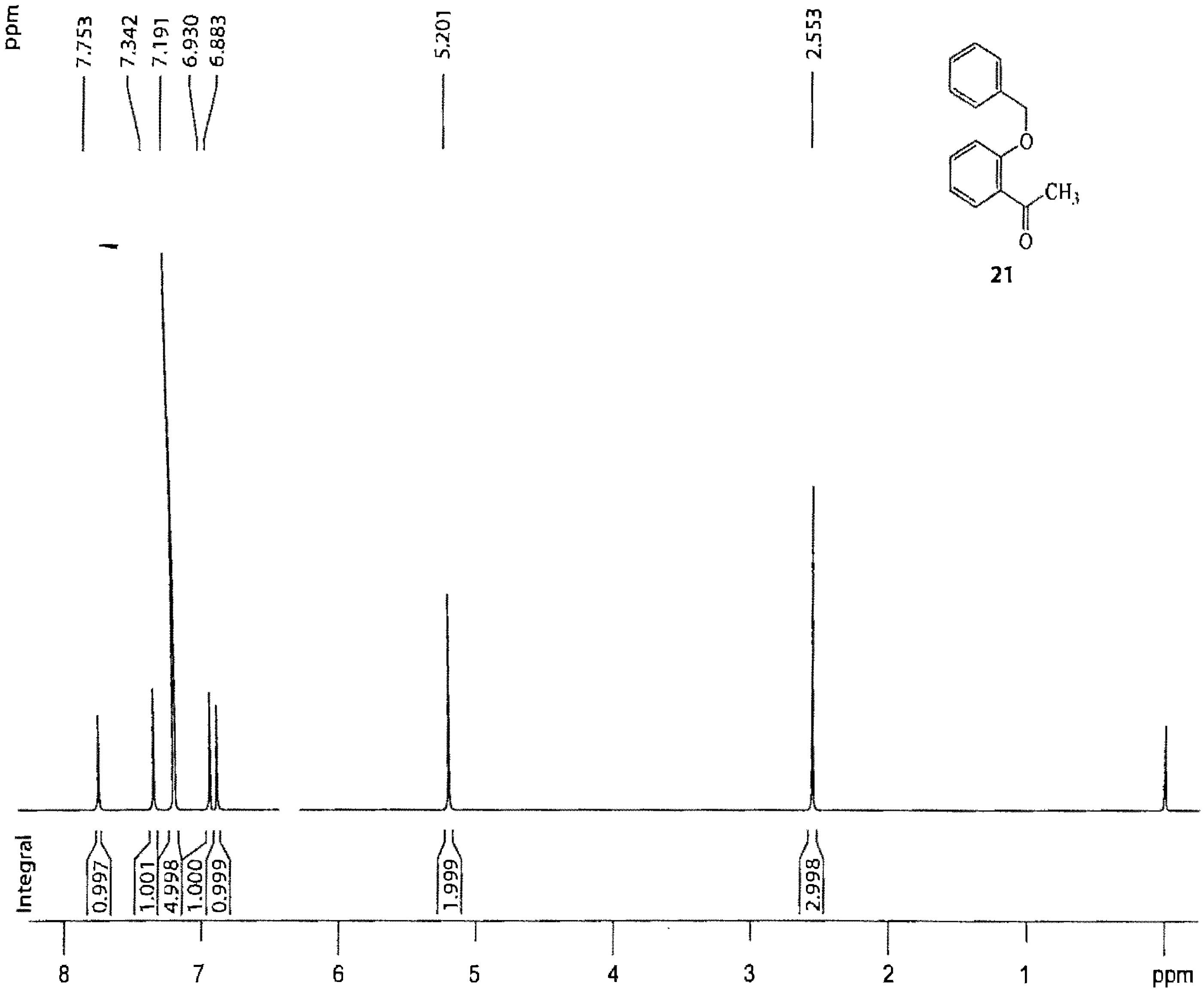
```
Current Data Parameters
NAME             MRAI-102
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20080902
Time                17.50
INSTRUM             spect
PROBHD   5 mm Dual 13C/
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                      9
DS                      0
SWH              5175.983 Hz
FIDRES           0.078979 Hz
AQ              6.3308277 sec
RG                  161.3
DW                 96.600 usec
DE                   6.00 usec
TE                  291.2 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  10.50 usec
PL1                  0.00 dB
SFO1          250.1315447 MHz

F2 - Processing parameters
SI                  32768
SF            250.1300068 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```



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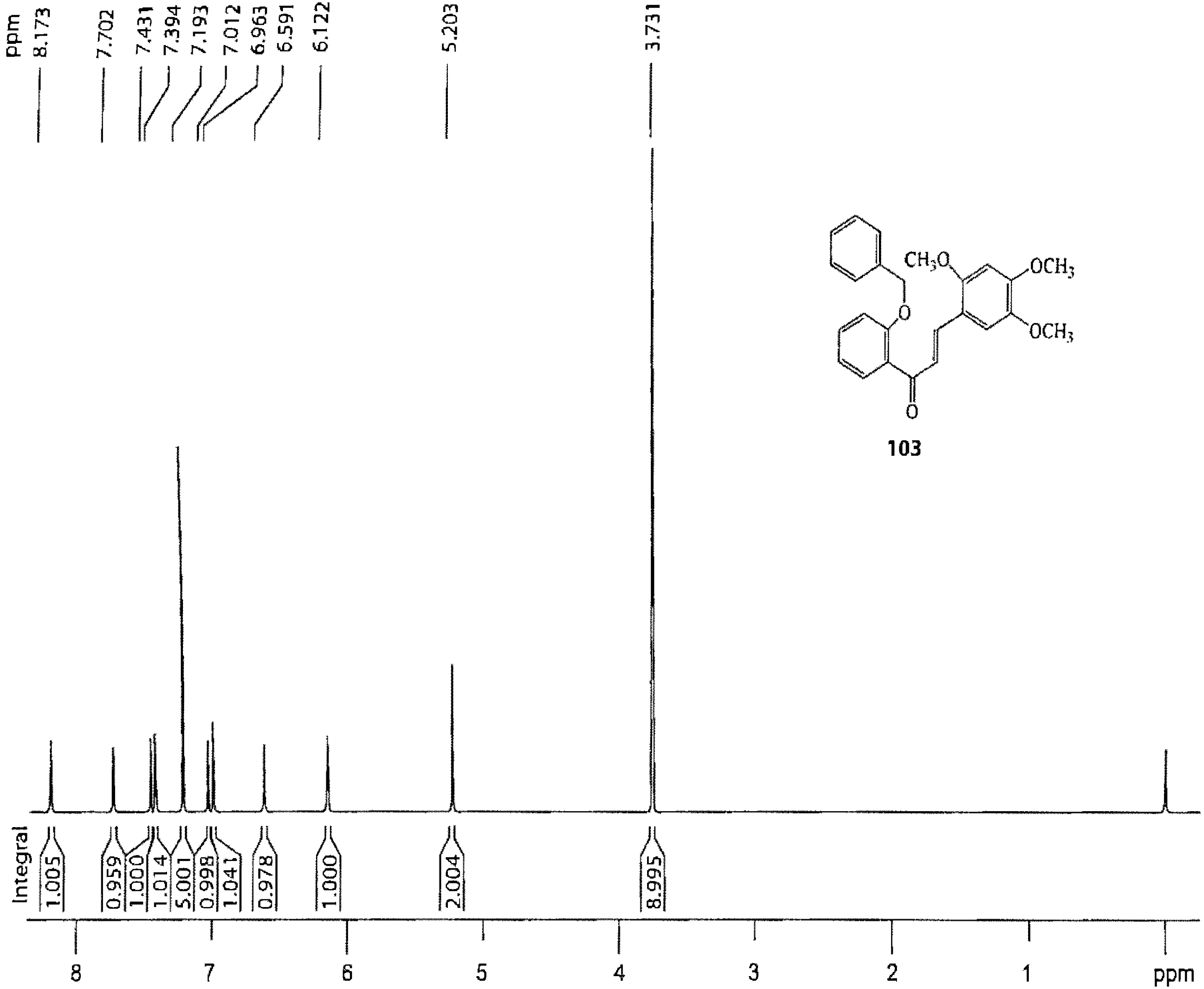
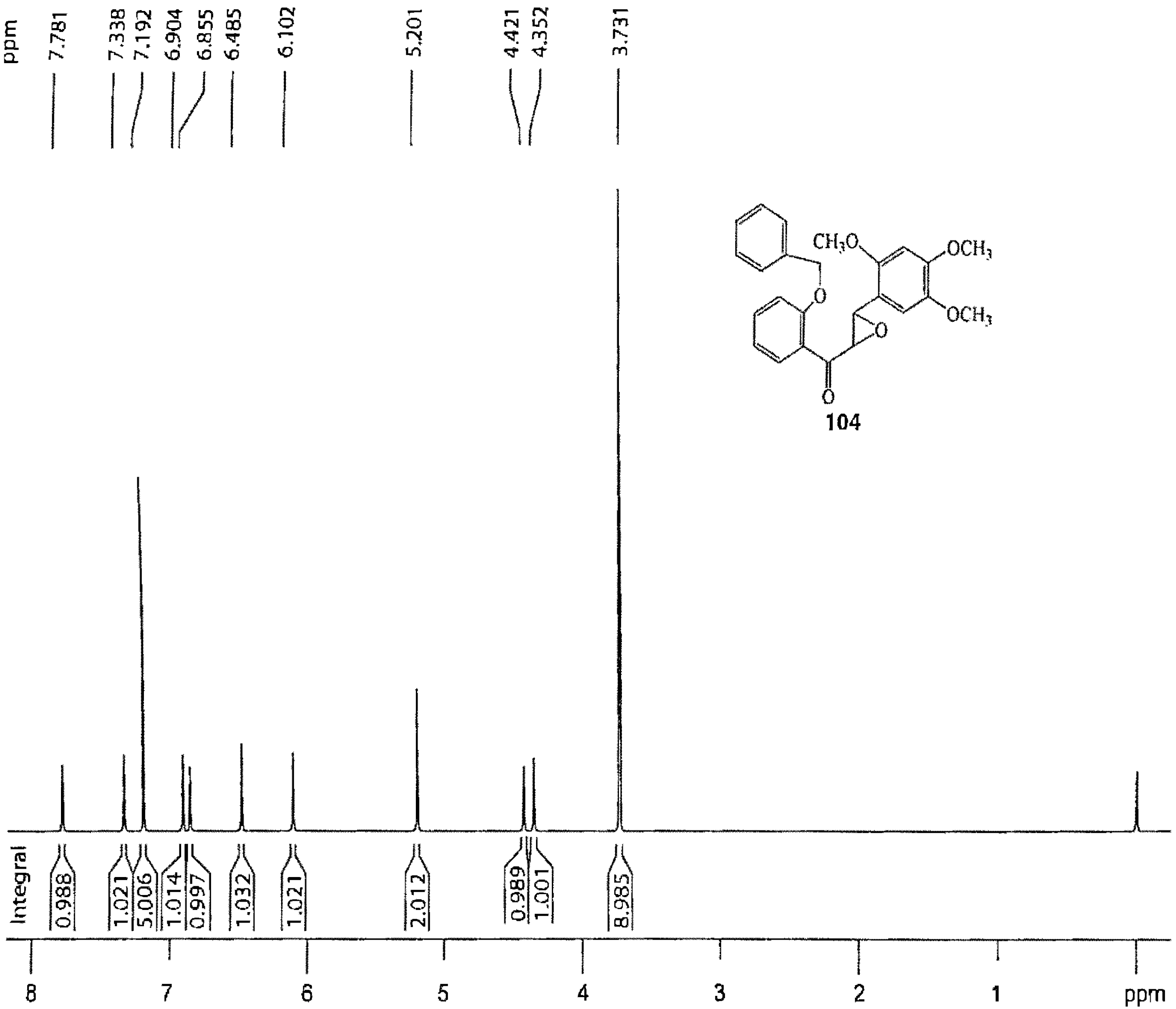
```
Current Data Parameters
NAME             MRAI-21
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20080902
Time               17.36
INSTRUM            spect
PROBHD   5 mm Dual 13C/
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                     6
DS                     0
SWH             5175.983 Hz
FIDRES          0.078979 Hz
AQ             6.3308277 sec
RG                 574.7
DW                96.600 usec
DE                  6.00 usec
TE                 291.2 K
D1            1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 10.50 usec
PL1                 0.00 dB
SFO1         250.1315447 MHz

F2 - Processing parameters
SI                 32768
SF           250.1300059 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



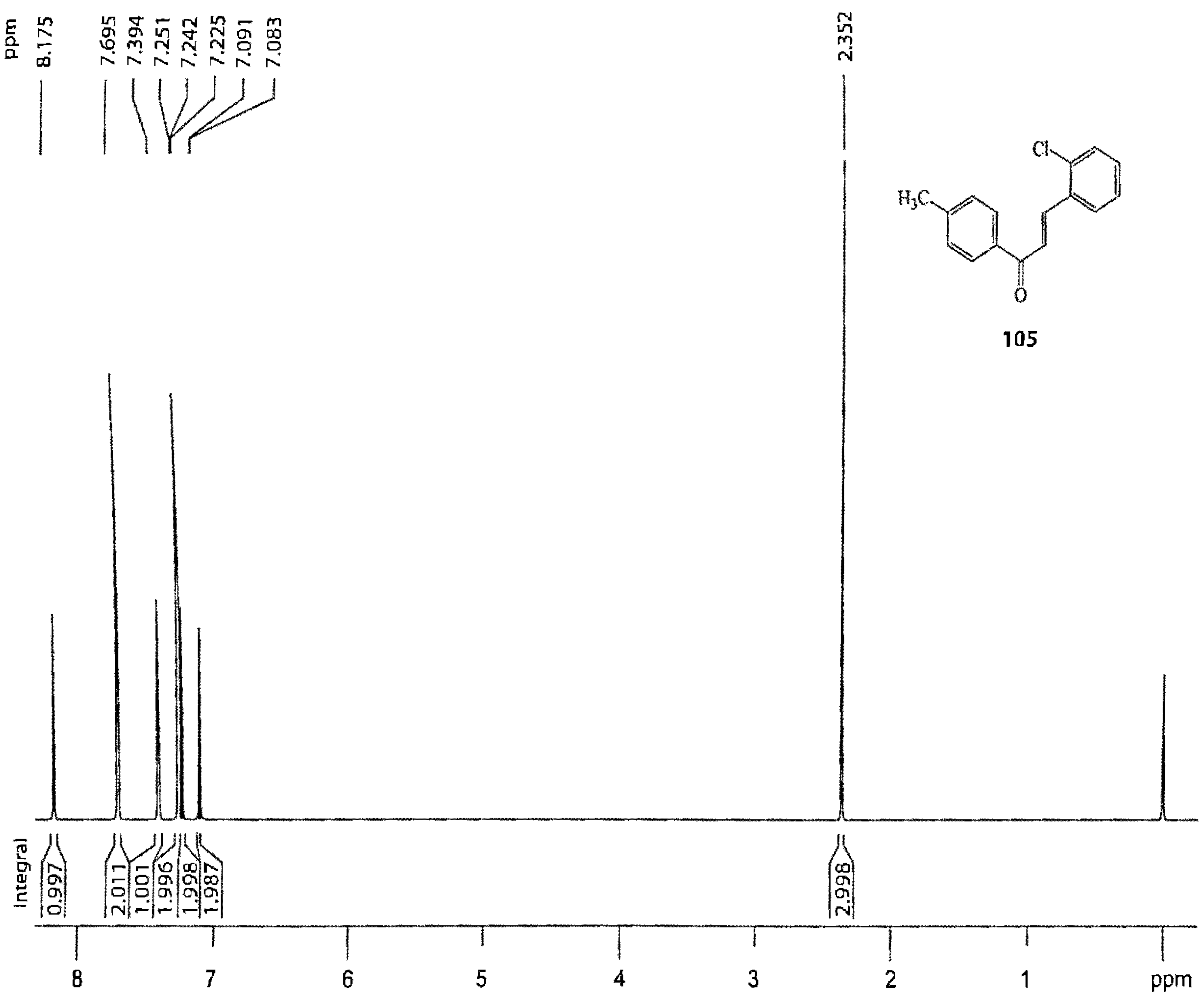
103

```
Current Data Parameters
NAME              MRAI-103
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20080902
Time                 17.18
INSTRUM              spect
PROBHD   5 mm Dual 13C/
PULPROG               zg30
TD                   65536
SOLVENT              CDCl3
NS                      16
DS                       0
SWH            5175.983 Hz
FIDRES         0.078979 Hz
AQ            6.3308277 sec
RG                     181
DW              96.600 usec
DE                6.00 usec
TE                 291.2 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1               10.50 usec
PL1                0.00 dB
SFO1        250.1315447 MHz

F2 - Processing parameters
SI                   32768
SF          250.1300044 MHz
WDW                     EM
SSB                      0
LB                 0.30 Hz
GB                       0
PC                    1.00
```



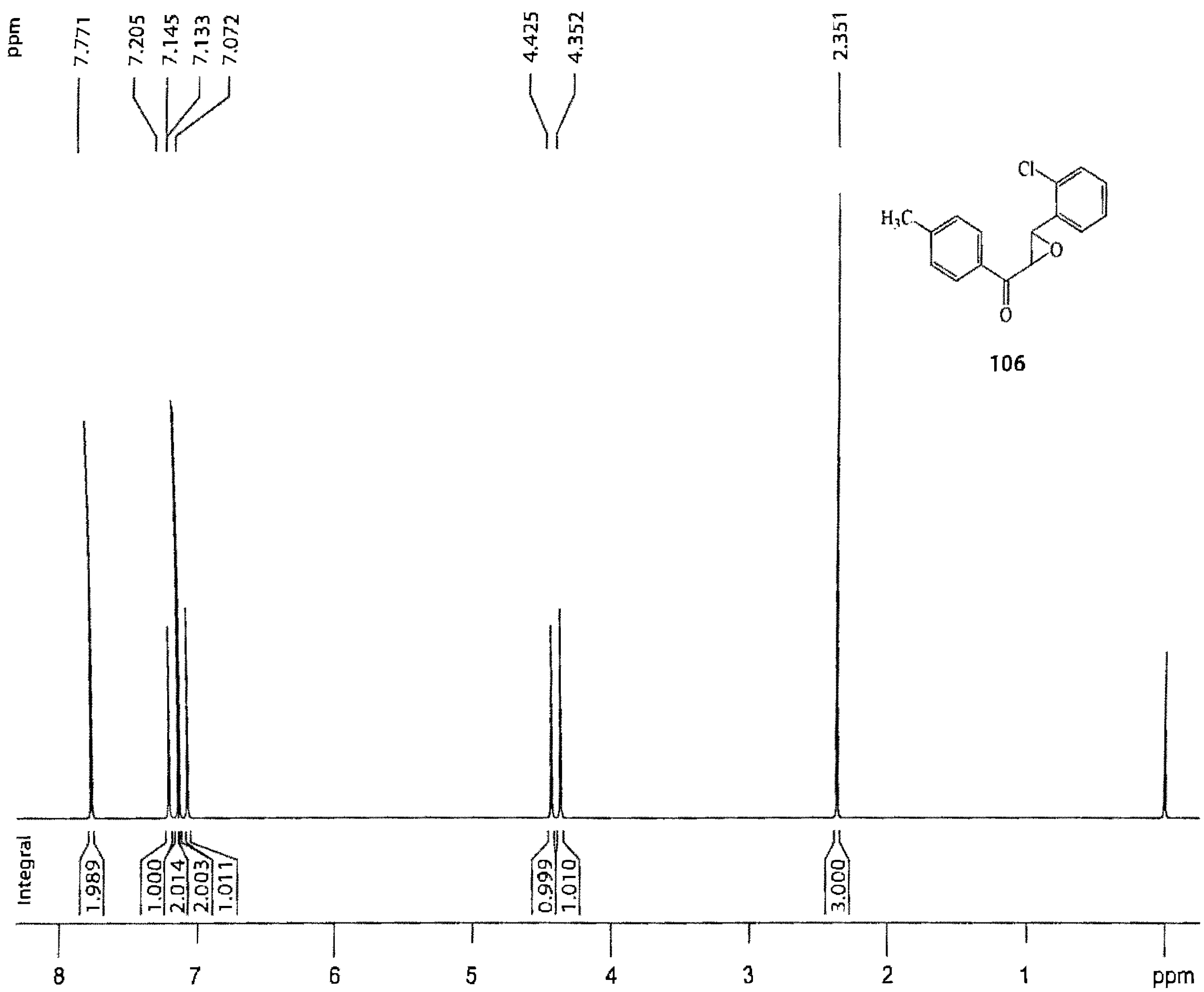
104

```
Current Data Parameters
NAME             MRAI-104
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20080902
Time                17.43
INSTRUM             spect
PROBHD   5 mm Dual 13C/
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                      9
DS                      0
SWH              5175.983 Hz
FIDRES           0.078979 Hz
AQ              6.3308277 sec
RG                  406.4
DW                 96.600 usec
DE                   6.00 usec
TE                  291.2 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  10.50 usec
PL1                  0.00 dB
SFO1          250.1315447 MHz

F2 - Processing parameters
SI                  32768
SF            250.1300060 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```



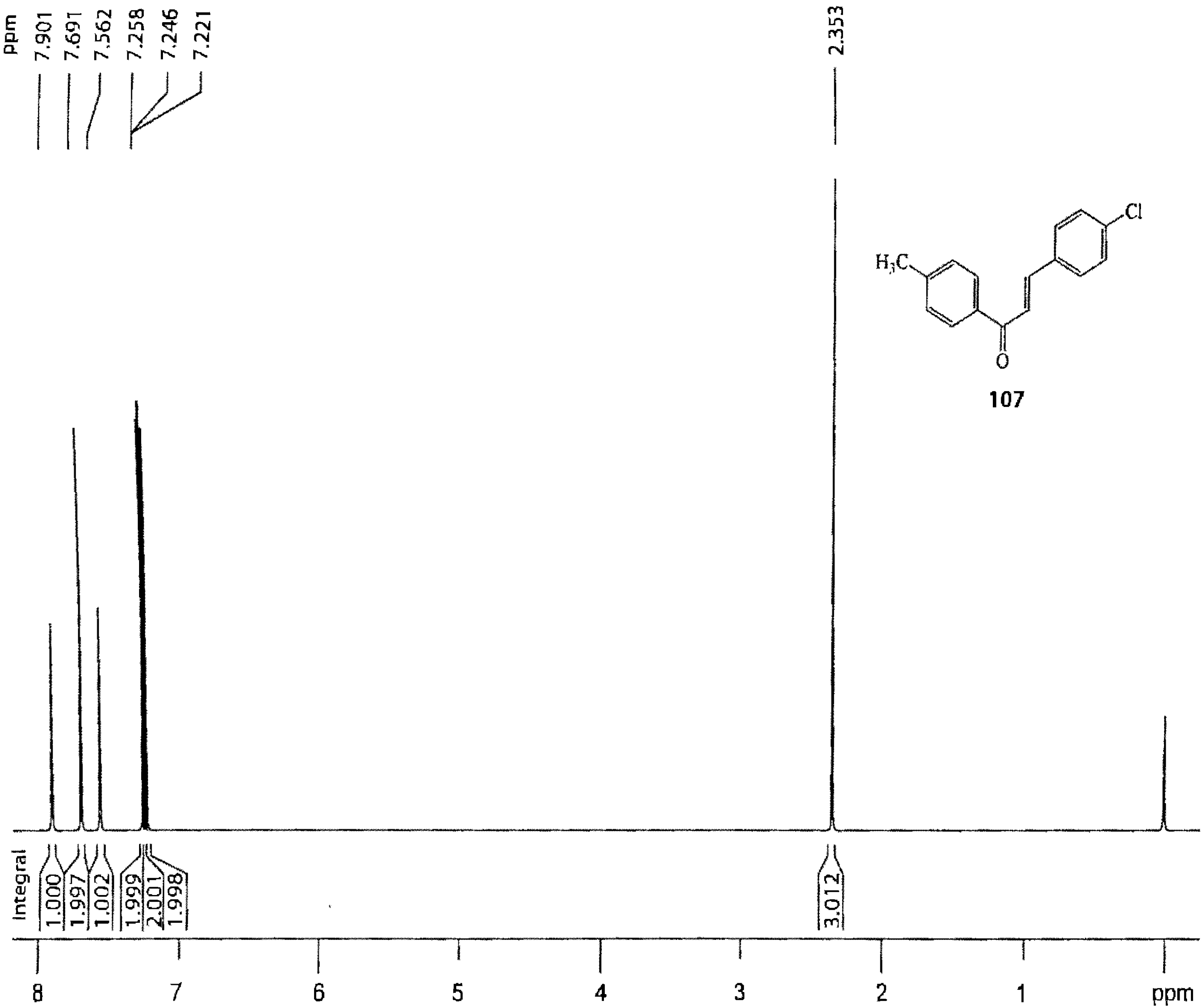
105

```
Current Data Parameters
NAME               MRAI-105
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20080902
Time                  17.36
INSTRUM               spect
PROBHD   5 mm Dual 13C/
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                        6
DS                        0
SWH                5175.983 Hz
FIDRES             0.078979 Hz
AQ                6.3308277 sec
RG                    574.7
DW                   96.600 usec
DE                     6.00 usec
TE                    291.2 K
D1               1.00000000 sec

======== CHANNEL f1 ========
NUC1                     1H
P1                    10.50 usec
PL1                    0.00 dB
SFO1            250.1315447 MHz

F2 - Processing parameters
SI                    32768
SF              250.1300059 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00
```

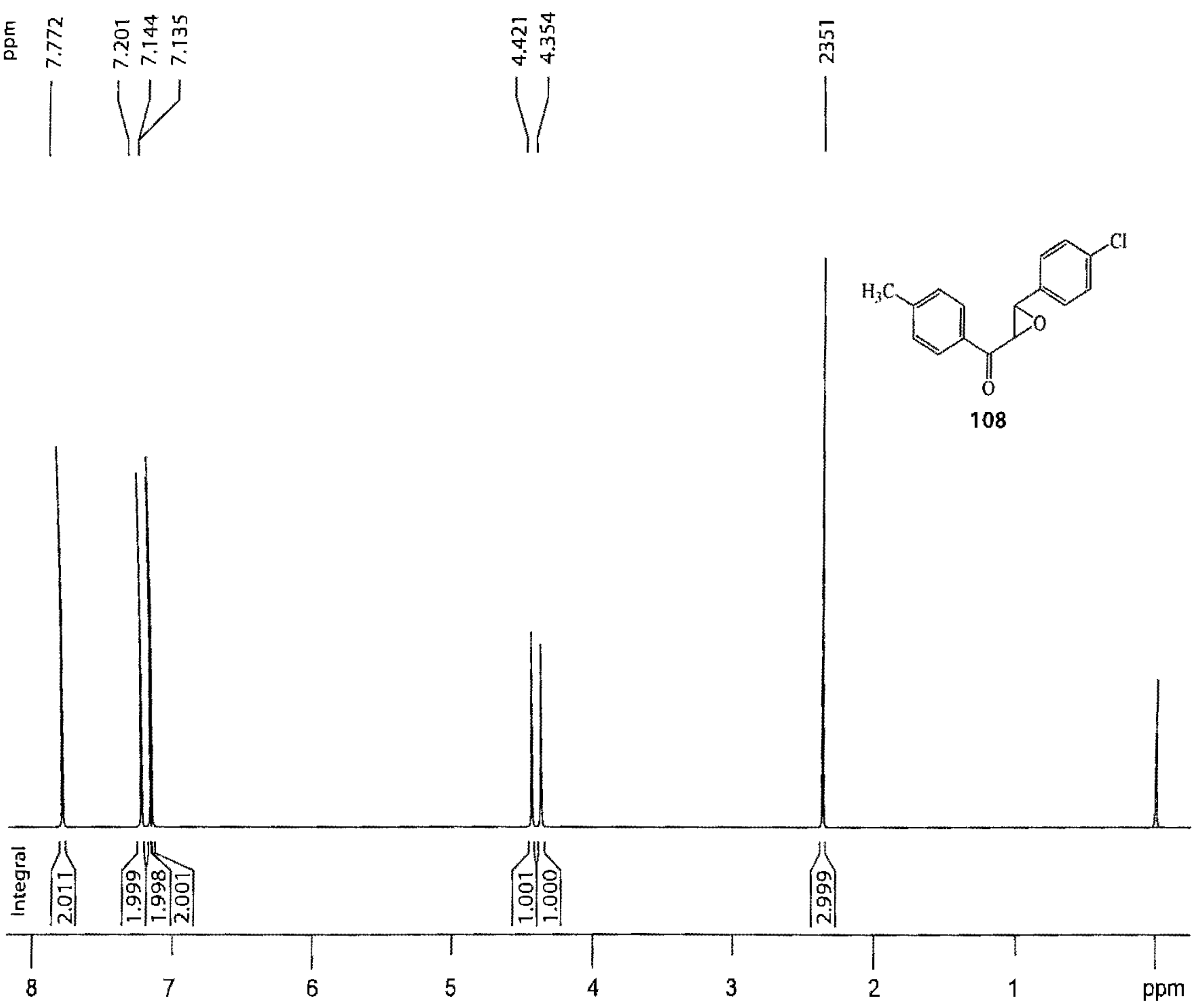


106

```
Current Data Parameters
NAME             MRAI-106
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20080902
Time                17.26
INSTRUM             spect
PROBHD   5 mm Dual 13C/
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     12
DS                      0
SWH            5175.983 Hz
FIDRES         0.078979 Hz
AQ            6.3308277 sec
RG                  228.1
DW                 96.600 usec
DE                   6.00 usec
TE                  291.2 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  10.50 usec
PL1                  0.00 dB
SFO1        250.1315447 MHz

F2 - Processing parameters
SI                  32768
SF          250.1300289 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

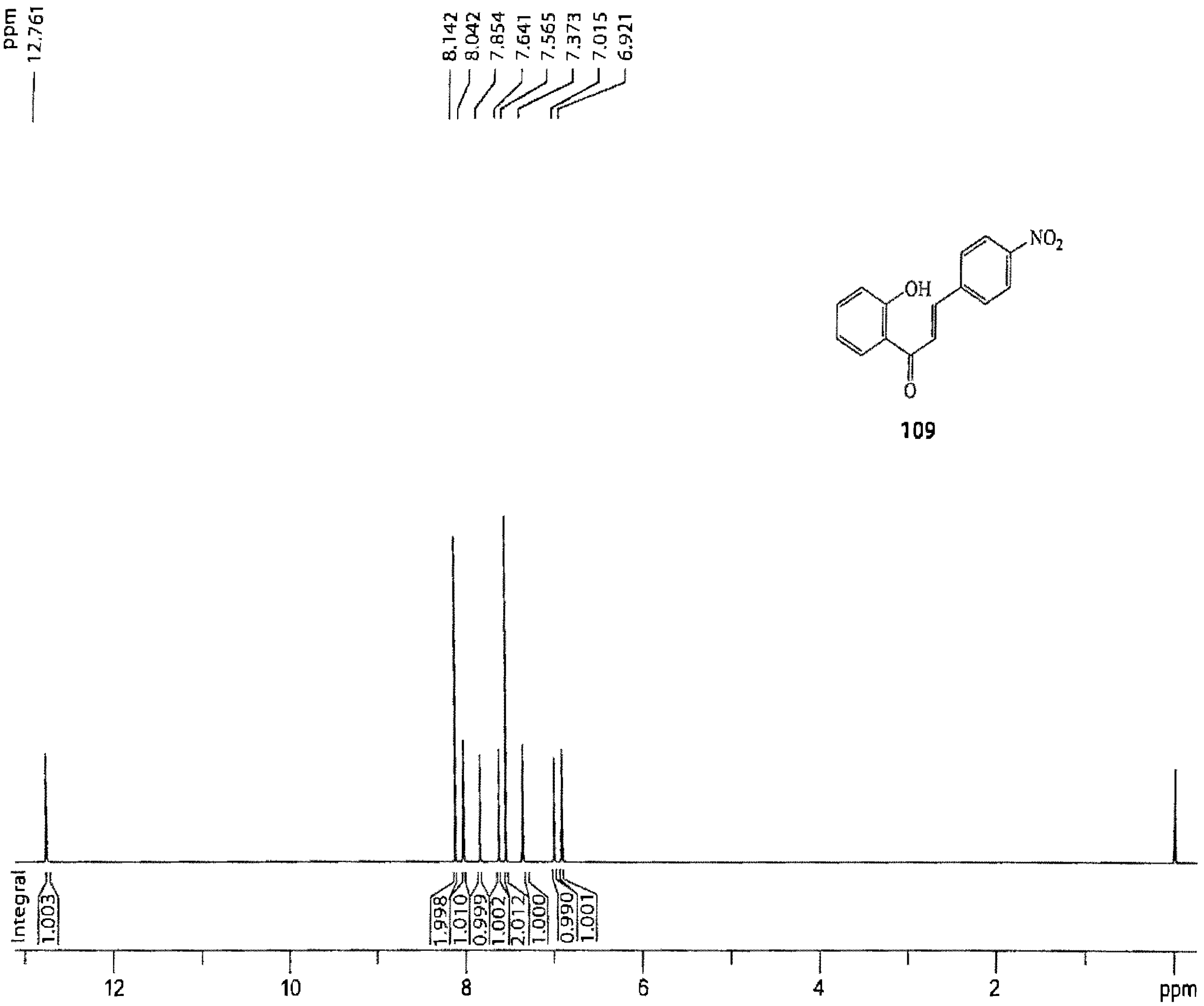


```
Current Data Parameters
NAME              MRAI-107
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20080902
Time                 17.26
INSTRUM              spect
PROBHD    5 mm Dual 13C/
PULPROG               zg30
TD                   65536
SOLVENT              CDCl3
NS                      12
DS                       0
SWH             5175.983 Hz
FIDRES          0.078979 Hz
AQ             6.3308277 sec
RG                   228.1
DW                  96.600 usec
DE                    6.00 usec
TE                   291.2 K
D1            1.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   10.50 usec
PL1                   0.00 dB
SFO1        250.1315447 MHz

F2 - Processing parameters
SI                   32768
SF          250.1300289 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```



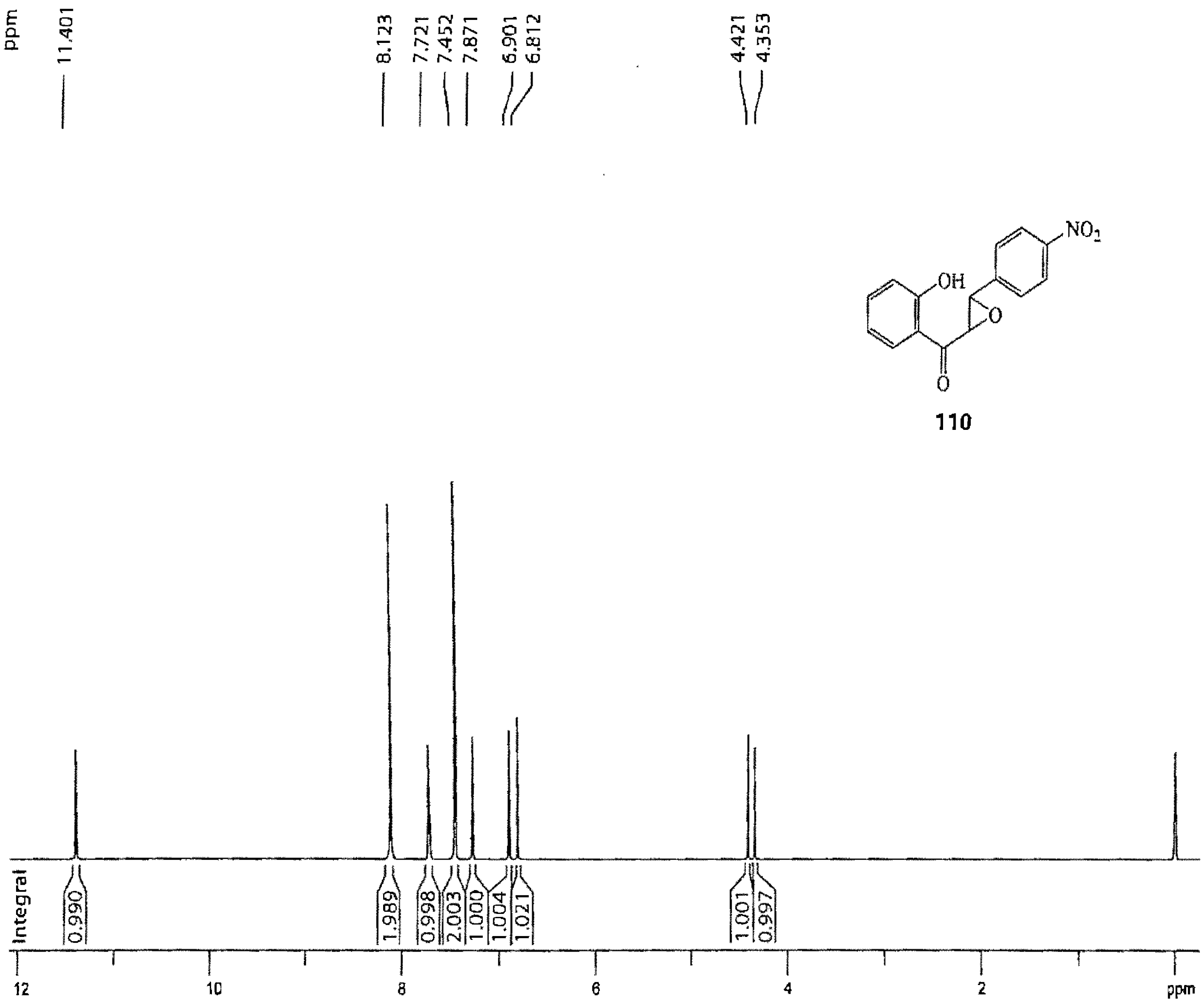
108

```
Current Data Parameters
NAME              KRAI-108
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20080902
Time                 17.50
INSTRUM              spect
PROBHD   5 mm Dual 13C/
PULPROG               zg30
TD                   65536
SOLVENT              CDCl3
NS                       9
DS                       0
SWH               5175.983 Hz
FIDRES            0.078979 Hz
AQ               6.3308277 sec
RG                   161.3
DW                  96.600 usec
DE                    6.00 usec
TE                   291.2 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   10.50 usec
PL1                   0.00 dB
SFO1           250.1318447 MHz

F2 - Processing parameters
SI                   32768
SF             250.1300038 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```



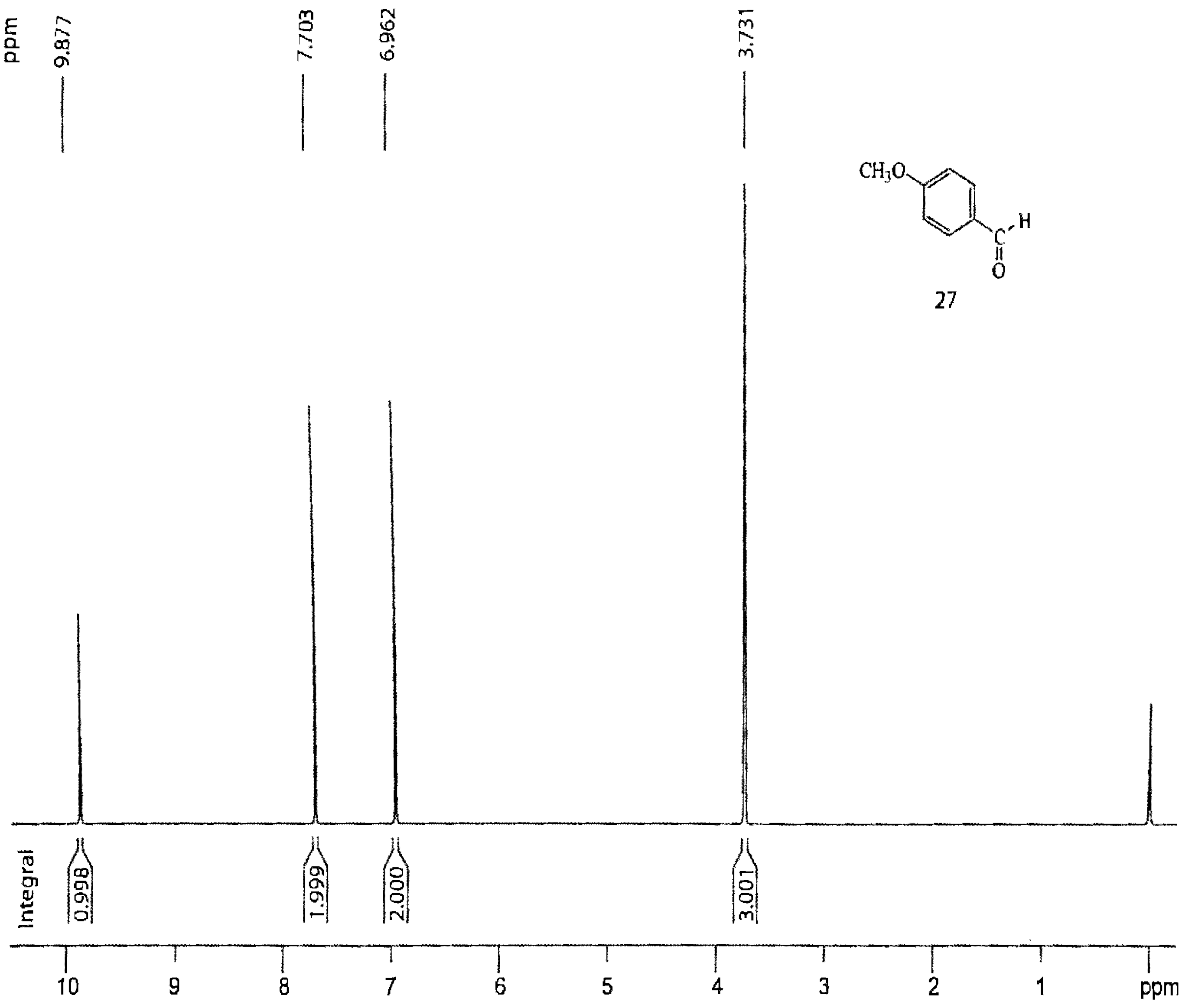
109

```
Current Data Parameters
NAME             MRAI-109
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20080902
Time                17.18
INSTRUM             spect
PROBHD   5 mm Dual 13C/
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      0
SWH              5175.983 Hz
FIDRES           0.078979 Hz
AQ              6.3308277 sec
RG                    161
DW                 96.600 usec
DE                   6.00 usec
TE                  291.2 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  10.50 usec
PL1                  0.00 dB
SFO1          250.1315447 MHz

F2 - Processing parameters
SI                  32768
SF            250.1300044 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```



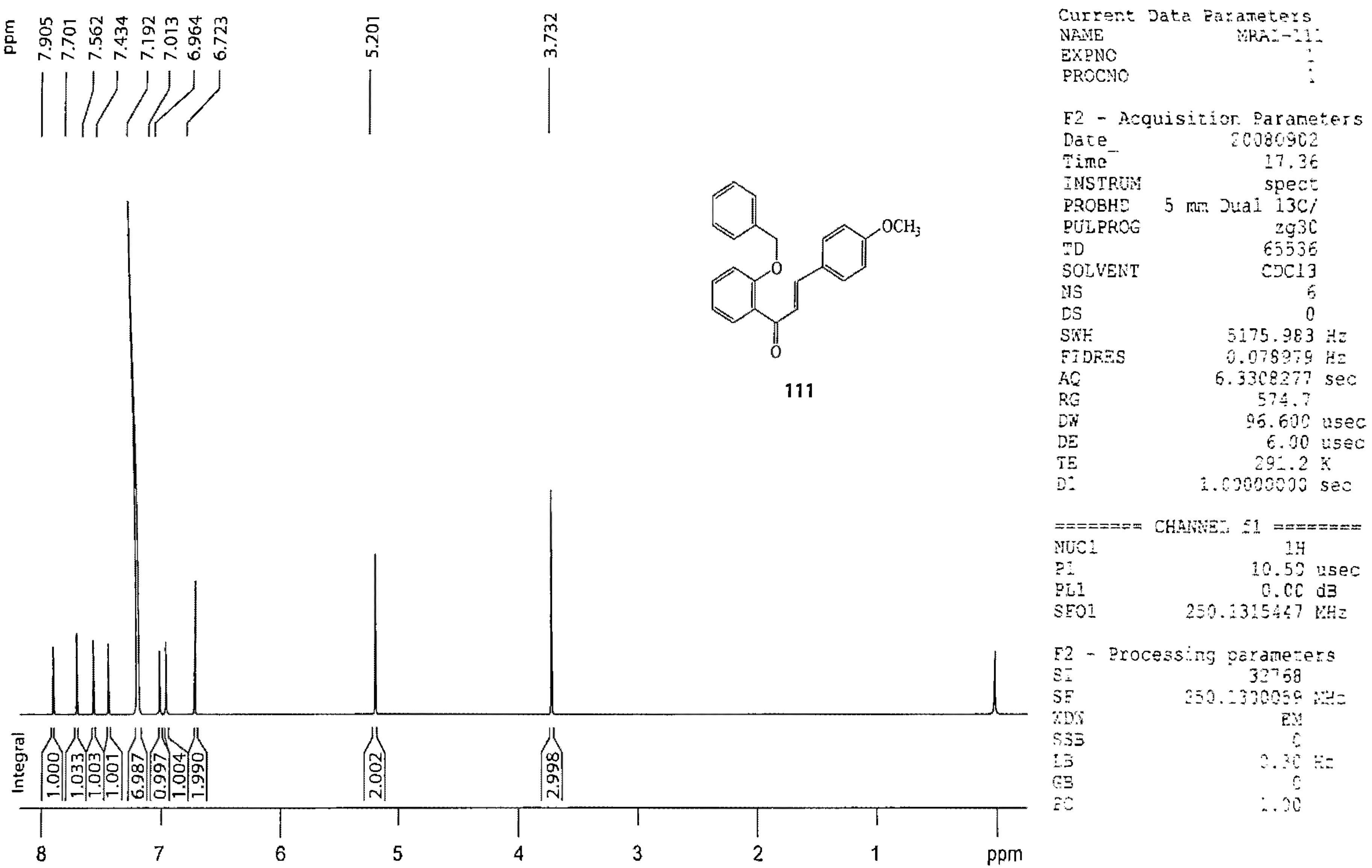
110

```
Current Data Parameters
NAME             MRAI-110
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20080902
Time                17.43
INSTRUM             spect
PROBHD   5 mm Dual 13C/
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                      9
DS                      0
SWH              5175.983 Hz
FIDRES           0.078979 Hz
AQ              6.3308277 sec
RG                  406.4
DW                 96.600 usec
DE                   6.00 usec
TE                  291.2 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  10.50 usec
PL1                  0.00 dB
SFO1          250.1315447 MHz

F2 - Processing parameters
SI                  32768
SF            250.1300060 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

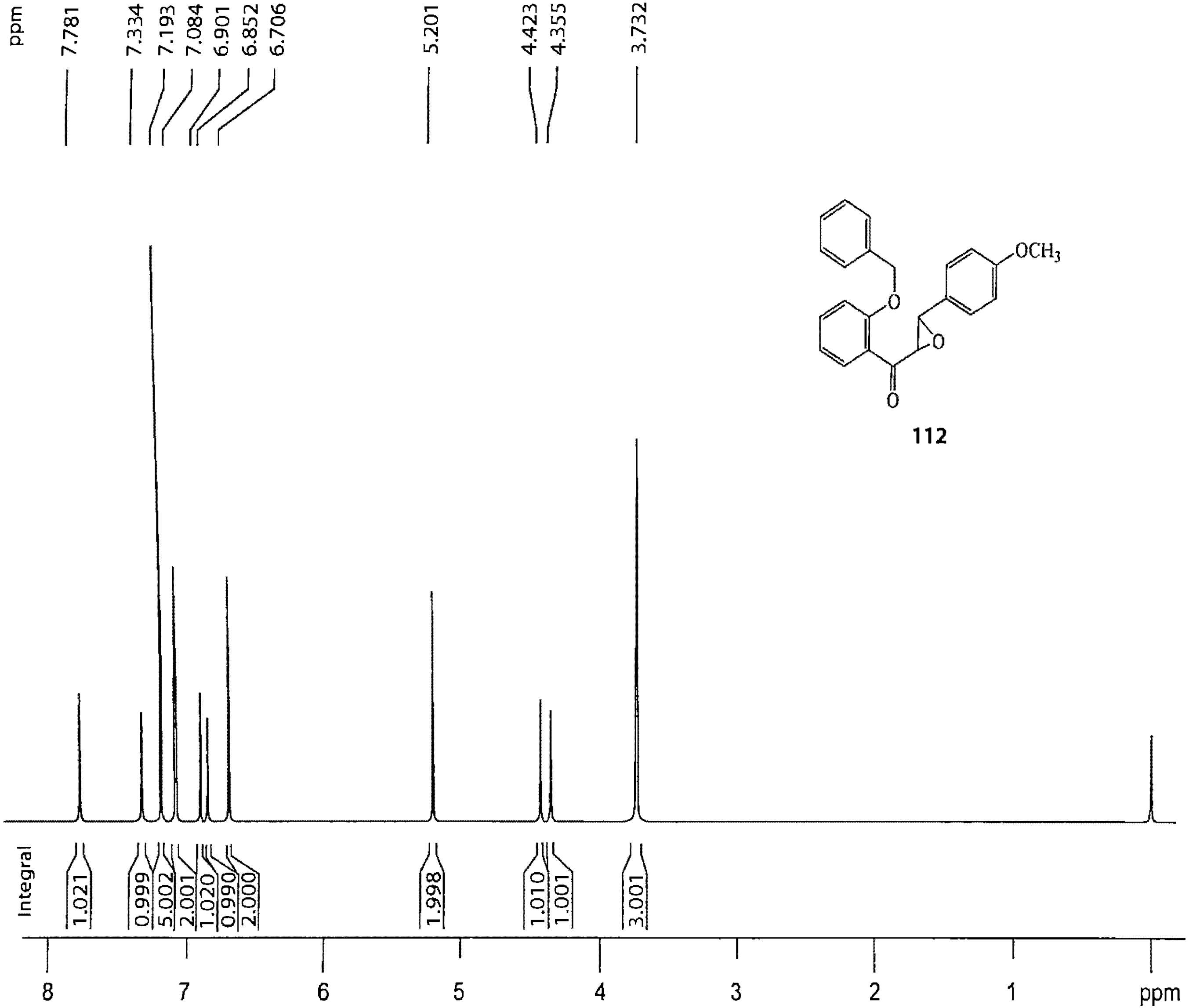


27

```
Current Data Parameters
NAME              MRAI-27
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20080902
Time                17.17
INSTRUM             spect
PROBHD    5 mm Dual 13C/
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                      6
DS                      0
SWH            5175.983 Hz
FIDRES         0.078979 Hz
AQ            6.3308277 sec
RG                  574.7
DW                 96.600 usec
DE                   6.00 usec
TE                  291.2 K
D1            1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  10.50 usec
PL1                  0.00 dB
SFO1          250.1315447 MHz

F2 - Processing parameters
SI                  32768
SF            250.1300059 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

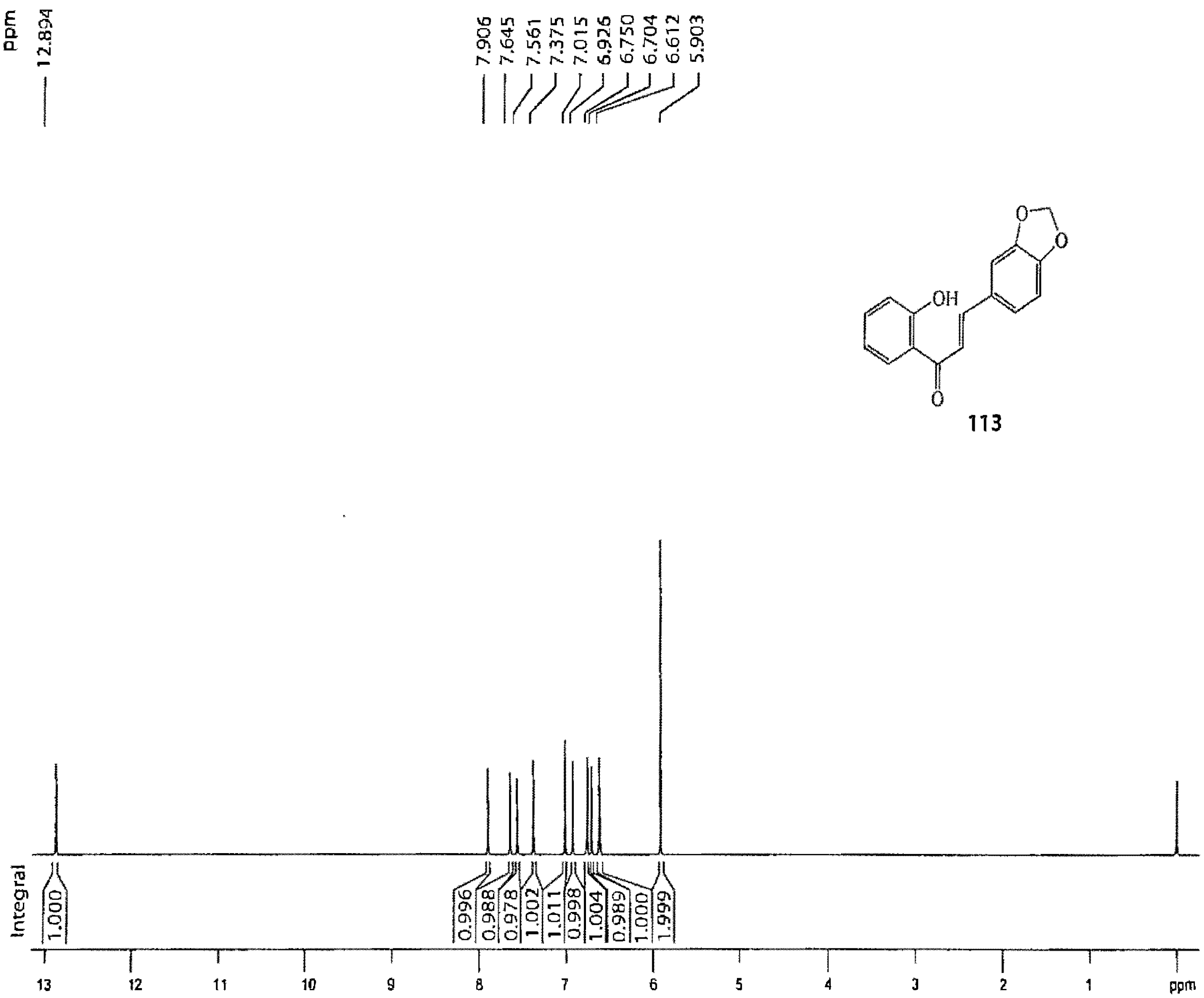


```
Current Data Parameters
NAME           MRAL-111
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20080902
Time              17.36
INSTRUM           spect
PROBHD   5 mm Dual 13C/
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                    6
DS                    0
SWH            5175.983 Hz
FIDRES         0.078979 Hz
AQ            6.3308277 sec
RG                574.7
DW               96.600 usec
DE                 6.00 usec
TE                291.2 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                 1H
P1                10.50 usec
PL1                0.00 dB
SFO1        250.1315447 MHz

F2 - Processing parameters
SI                32768
SF          250.1300059 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
```



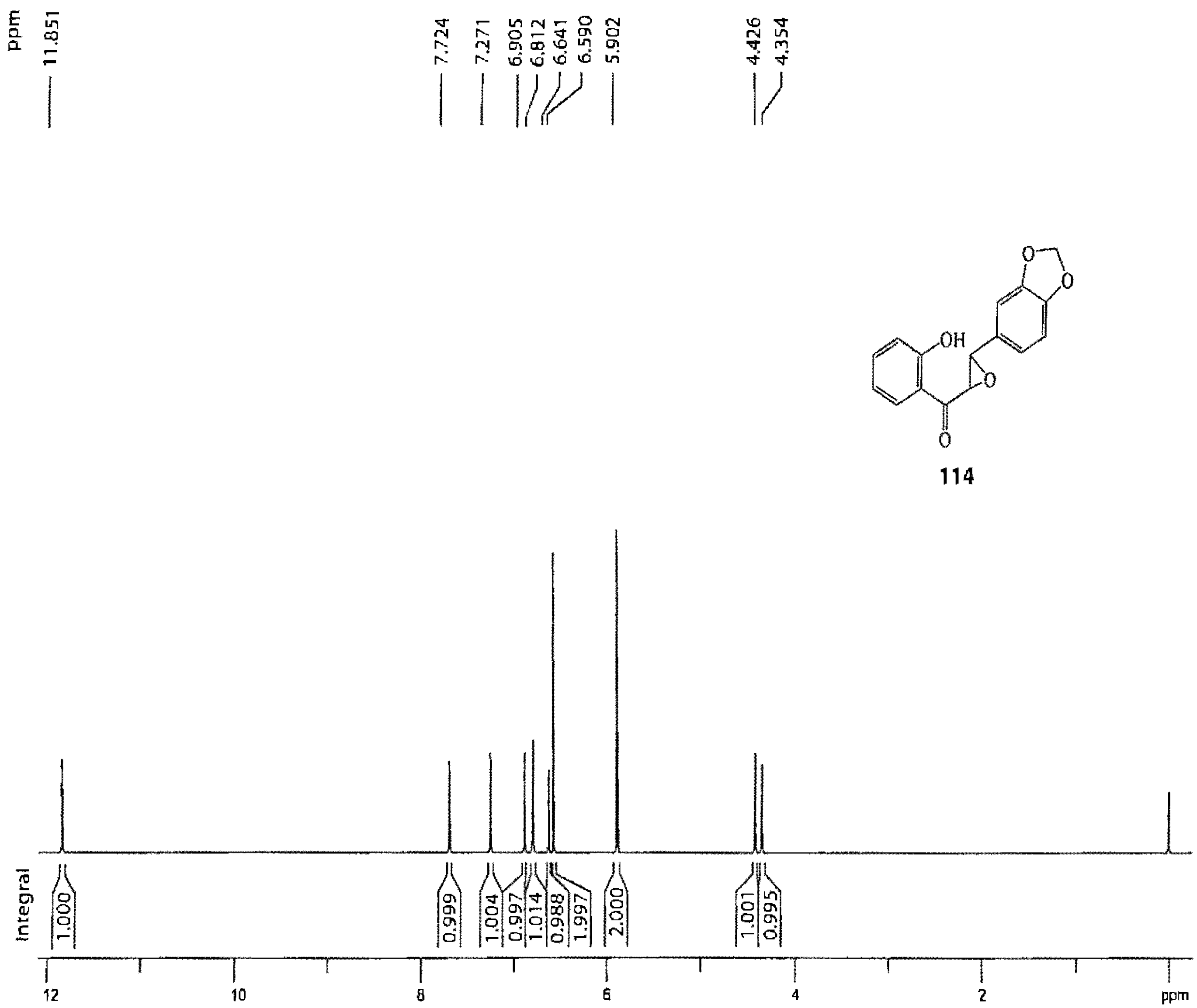
112

```
Current Data Parameters
NAME              MRAI-112
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20080902
Time                 17.18
INSTRUM              spect
PROBHD    5 mm Dual 13C/
PULPROG               zg30
TD                   65536
SOLVENT              CDCl3
NS                      16
DS                       0
SWH             5175.983 Hz
FIDRES          0.078979 Hz
AQ             6.3308277 sec
RG                     181
DW                96.600 usec
DE                  6.00 usec
TE                 291.2 K
D1            1.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                 10.50 usec
PL1                 0.00 dB
SFO1         250.1315447 MHz

F2 - Processing parameters
SI                   32768
SF           250.1300044 MHz
WDW                     EM
SSB                      0
LB                  0.30 Hz
GB                       0
PC                    1.00
```



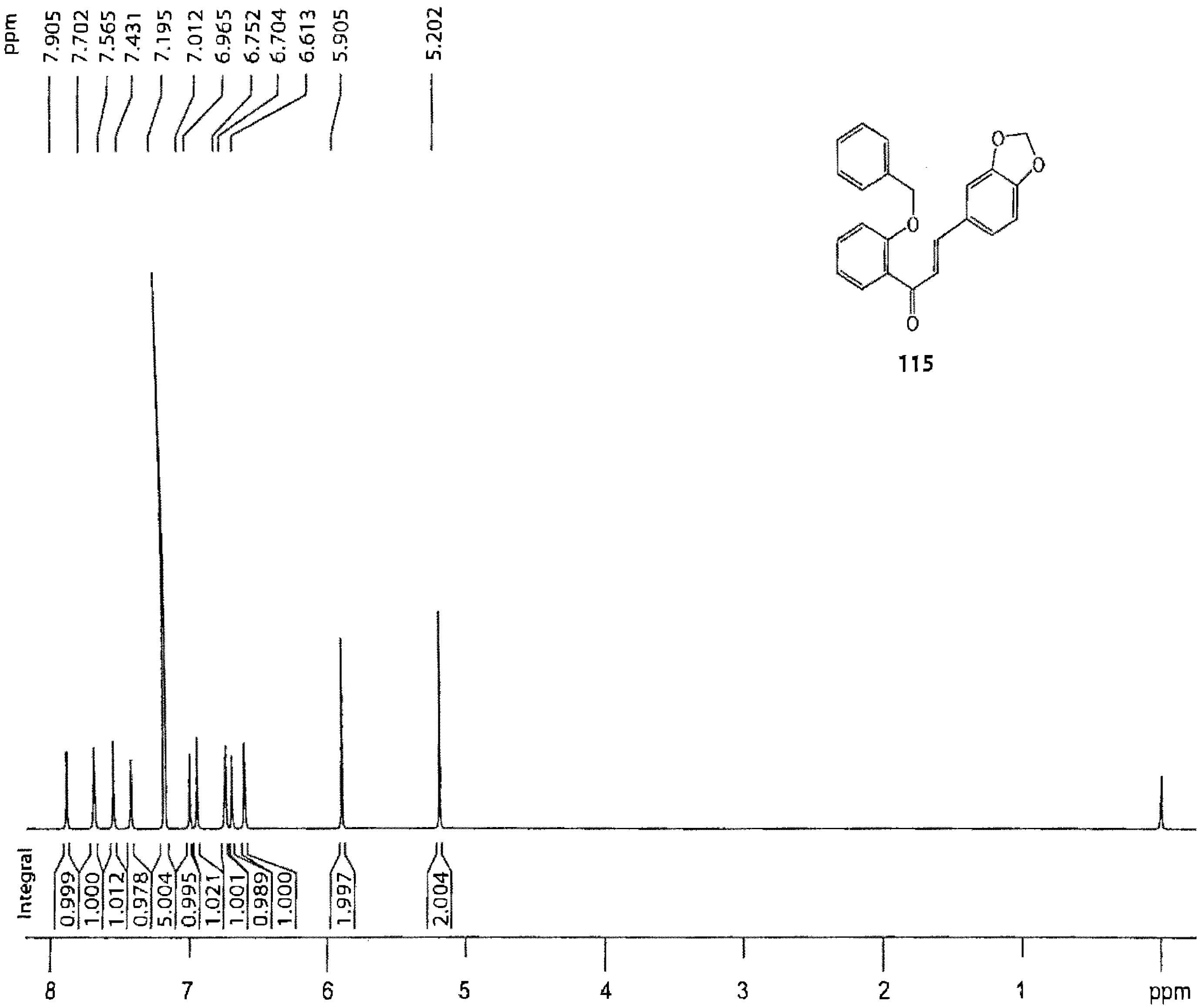
113

```
Current Data Parameters
NAME              MRAI-113
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20080902
Time                 17.18
INSTRUM              spect
PROBHD   5 mm Dual 13C/
PULPROG               zg30
TD                   65536
SOLVENT              CDCl3
NS                      16
DS                       0
SWH              5175.983 Hz
FIDRES           0.078979 Hz
AQ              6.3308277 sec
RG                     181
DW                  96.600 usec
DE                    6.00 usec
TE                   291.2 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   10.50 usec
PL1                   0.00 dB
SFO1           250.1315447 MHz

F2 - Processing parameters
SI                   32768
SF             250.1300044 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```



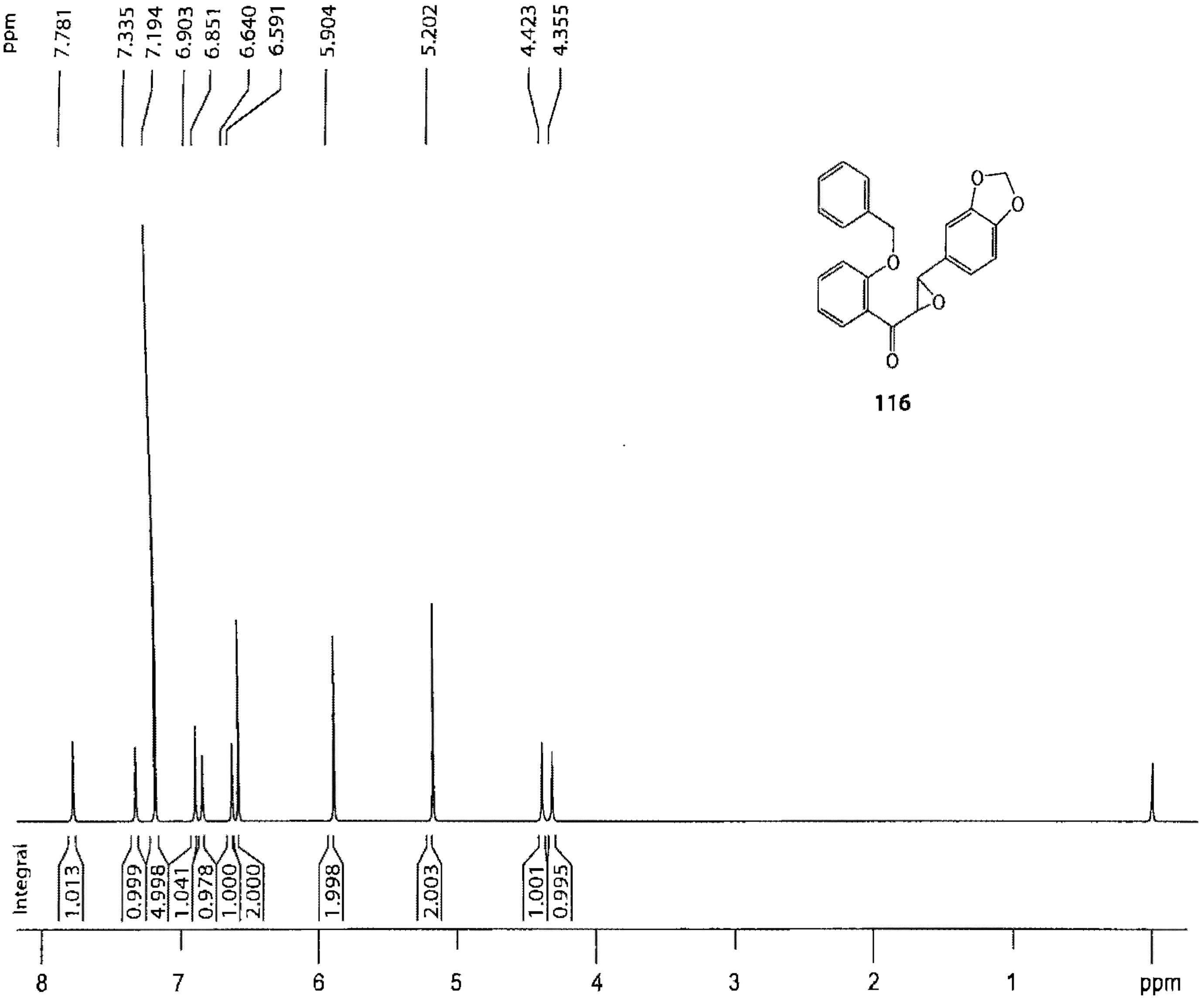
114

```
Current Data Parameters
NAME             MRAI-114
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20080902
Time                17.43
INSTRUM             spect
PROBHD   5 mm Dual 13C/
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                      9
DS                      0
SWH           5175.983 Hz
FIDRES        0.078979 Hz
AQ          6.3308277 sec
RG                  406.4
DW             96.600 usec
DE               6.00 usec
TE                291.2 K
D1         1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1              10.50 usec
PL1               0.00 dB
SFO1       250.1315447 MHz

F2 - Processing parameters
SI                  32768
SF         250.1300060 MHz
WDW                    EM
SSB                     0
LB                0.30 Hz
GB                      0
PC                   1.00
```

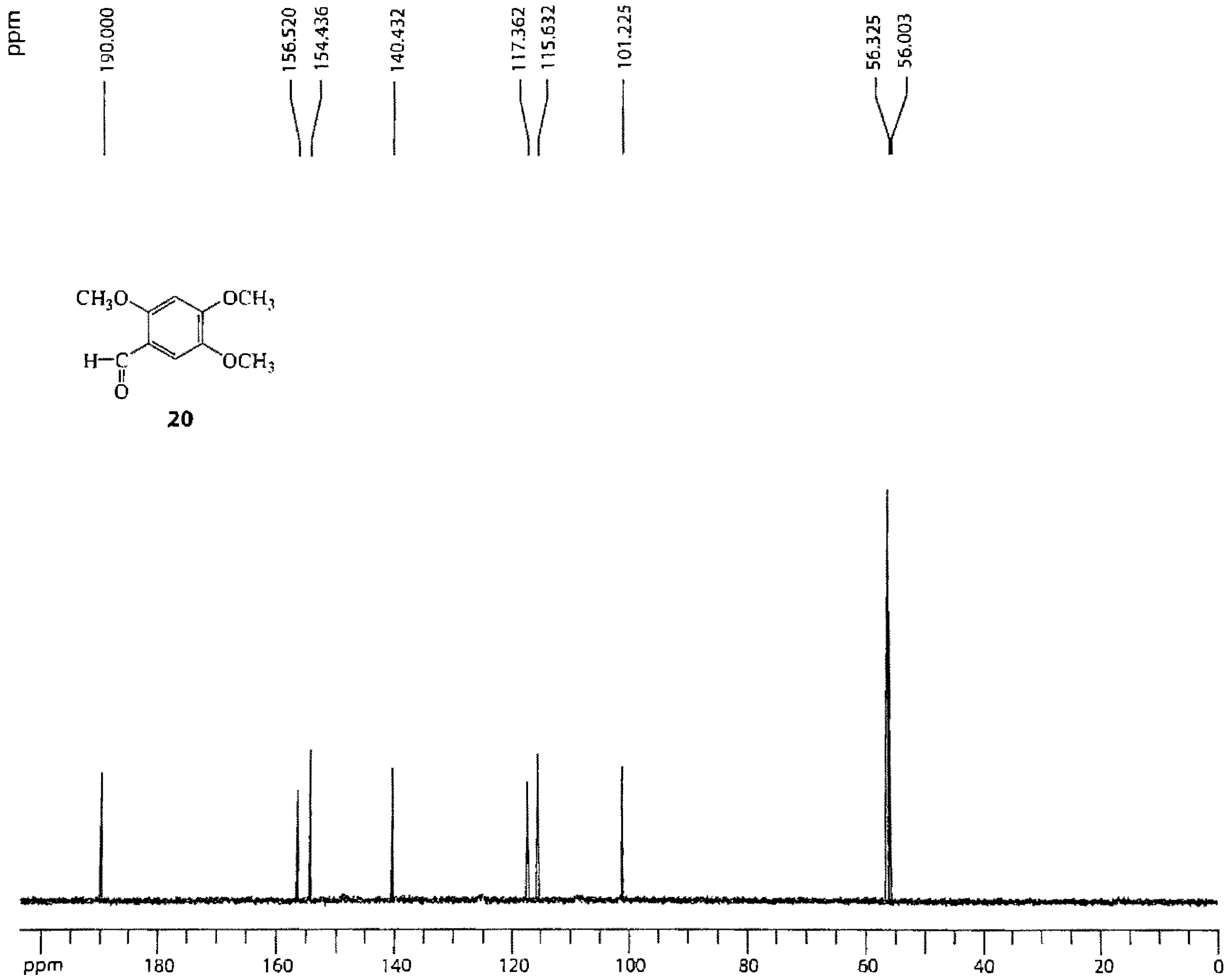


115

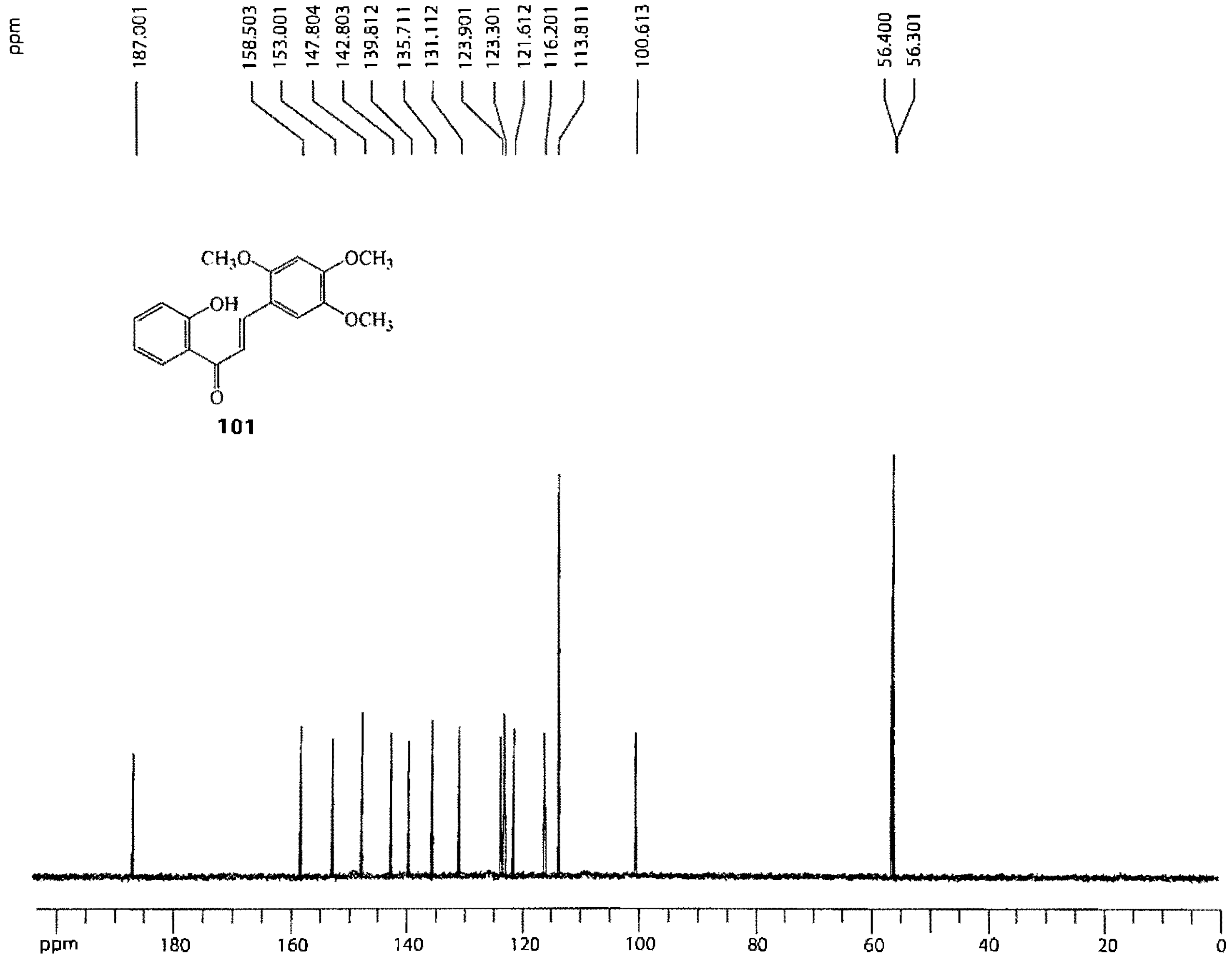
```
Current Data Parameters
NAME               MRAI-115
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20080902
Time                  17.36
INSTRUM               spect
PROBHD   5 mm Dual 13C/
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                        6
DS                        0
SWH                5175.983 Hz
FIDRES             0.078979 Hz
AQ                6.3308277 sec
RG                    574.7
DW                   96.600 usec
DE                     6.00 usec
TE                    291.2 K
D1               1.00000000 sec

======== CHANNEL f1 ========
NUC1                     1H
P1                    10.50 usec
PL1                    0.00 dB
SFO1            250.1315447 MHz

F2 - Processing parameters
SI                    32768
SF              250.1300059 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00
```



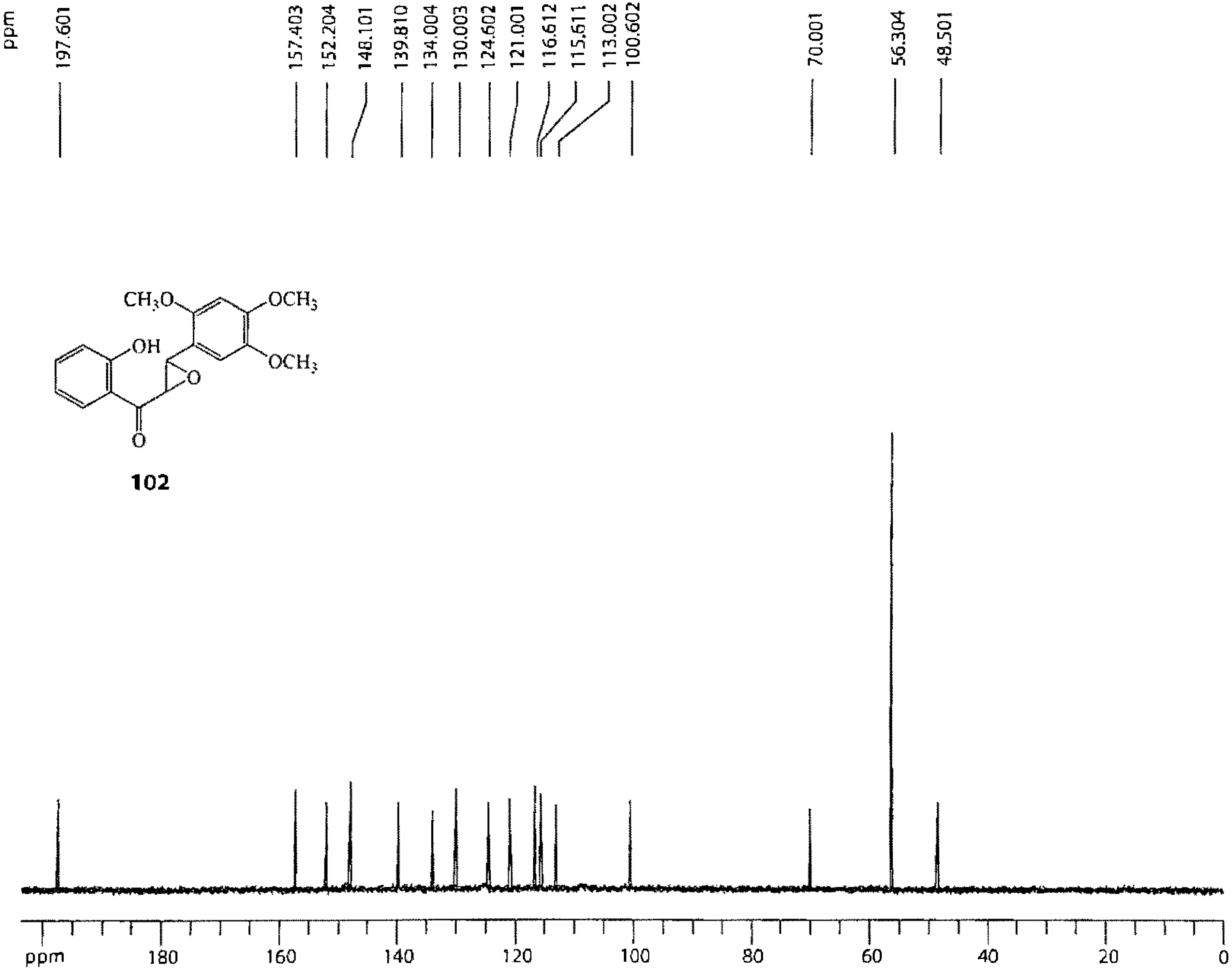
```
Current Data Parameters
NAME              XPAI-116
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20080902
Time                 17.50
INSTRUM              spect
PROBHD   5 mm Dual 13C/
PULPROG               zg30
TD                   65536
SOLVENT              CDCl3
NS                       9
DS                       0
SWH               5175.983 Hz
FIDRES            0.078979 Hz
AQ               6.3308277 sec
RG                   161.3
DW                  96.600 usec
DE                    6.00 usec
TE                   291.2 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   10.50 usec
PL1                   0.00 dB
SFO1           250.1315447 MHz

F2 - Processing parameters
SI                   32768
SF             250.1300058 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```



```
Current Data Parameters
NAME          MRAI-20
EXPNO               2
PROCNO              1

F2 - Acquisition Parameters
Date_        20090903
Time            12.44
INSTRUM         spect
PROBHD   5 mm Dual 13
PULPROG       zgpg30
TD              65536
SOLVENT          DMSO
NS                300
DS                  0
SWH         19841.270 Hz
FIDRES       0.302754 Hz
AQ          1.6515572 sec
RG              16384
DW             25.200 usec
DE               6.00 usec
TE              300.0 K
D12        0.00002000 sec
PL13            22.00 dB
D1         2.00000000 sec
CPDPRG2       waltz16
PCPD2           90.00 usec
SFO2      250.1312345 MHz
NUC2               1H
PL2              0.00 dB
PL12            22.00 dB
P1              10.20 usec
SFO1       62.9031573 MHz
NUC1              13C
PL1              0.00 dB
D11        0.03000000 sec

F2 - Processing parameters
SI              32768
SF         62.8952429 MHz
WDW                EM
SSB                 0
LB               1.00 Hz
GB                  0
PC               1.40

1D NMR plot parameters
CX              20.00 cm
F1P           200.701 ppm
F1           12623.13 Hz
F2P             5.825 ppm
F2             362.11 Hz
PPMCM         9.74389 ppm/cm
HZCM        612.85060 Hz/cm
```

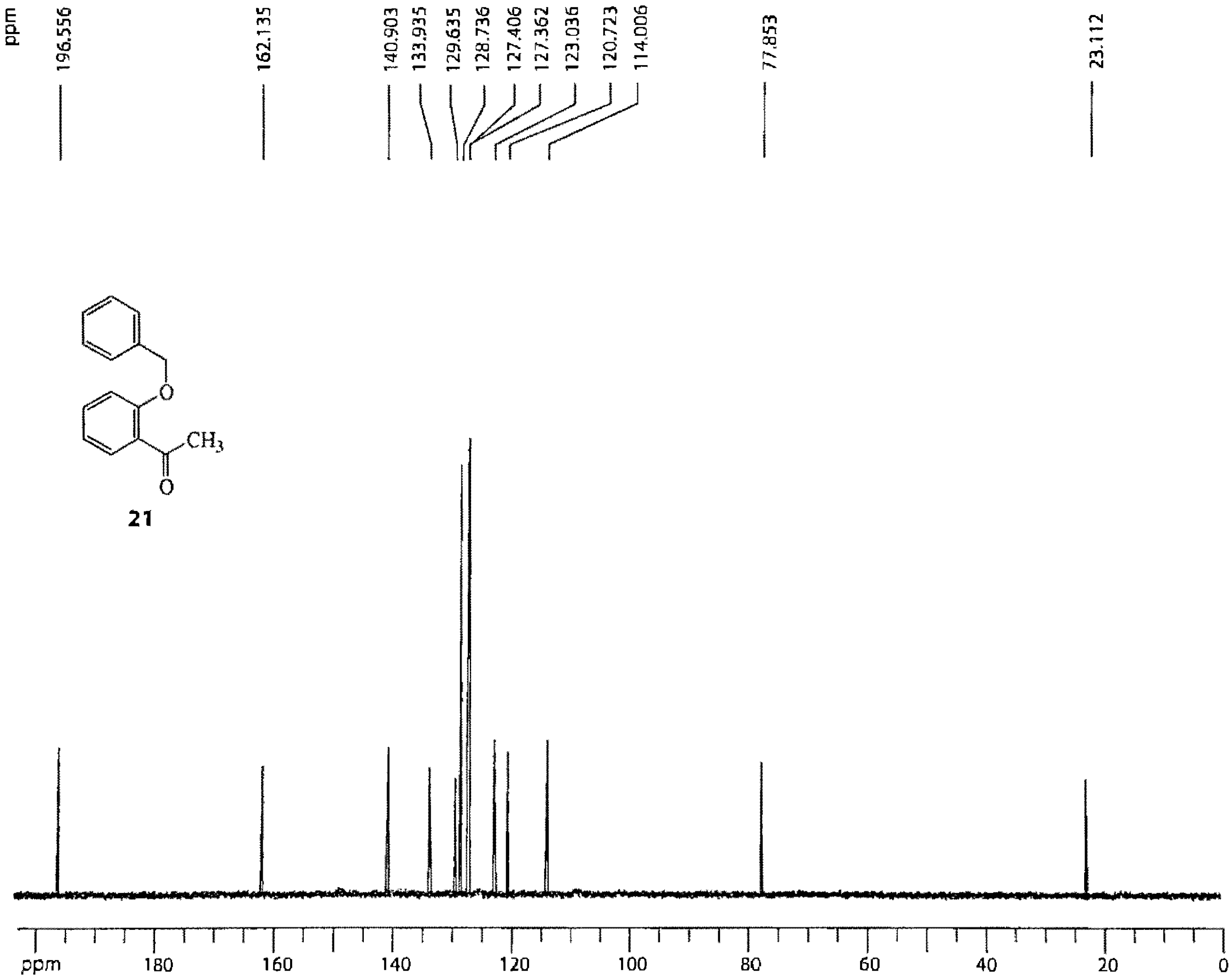


```
Current Data Parameters
NAME              MRAI-101
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_             20090305
Time                 12.44
INSTRUM              spect
PROBHD       5 mm Dual 13
PULPROG            zgpg30
TD                   65536
SOLVENT               DMSO
NS                     330
DS                       0
SWH           19841.270 Hz
FIDRES         0.302754 Hz
AQ           1.6515572 sec
RG                   16384
DW              25.200 usec
DE                6.00 usec
TE                 300.0 K
D12         0.00002000 sec
PL13              22.00 dB
D1          2.00000000 sec
CPDPRG2            waltz16
PCPD2            90.00 usec
SFO2        250.1312345 MHz
NUC2                    1H
PL2                0.00 dB
PL12              22.00 dB
P1               10.20 usec
SFO1         62.9031575 MHz
NUC1                   13C
PL1                0.00 dB
D11         0.03000000 sec

F2 - Processing parameters
SI                   32768
SF           62.8952429 MHz
WDW                     EM
SSB                      0
LB                 1.00 Hz
GB                       0
PC                    1.40

1D NMR plot parameters
CX                20.00 cm
F1P            200.701 ppm
F1            12623.13 Hz
F2P              5.802 ppm
F2              365.11 Hz
PPMCM        9.74399 ppm/cm
HZCM       612.65083 Hz/cm
```



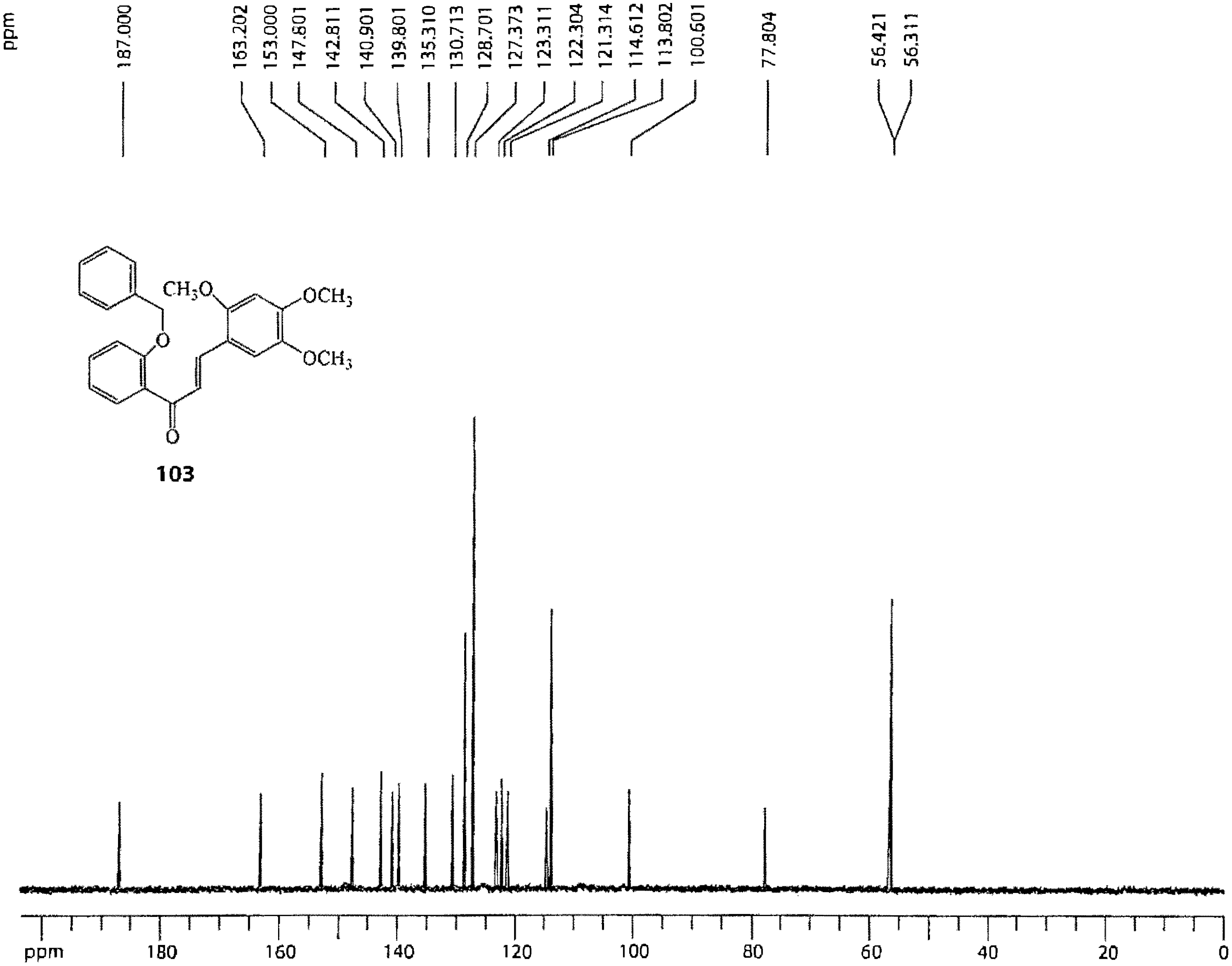
102

```
Current Data Parameters
NAME            MRAI-102
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20080005
Time               12.44
INSTRUM            spect
PROBHD     5 mm Dual 13
PULPROG          zgpg30
TD                 65536
SOLVENT             DMSO
NS                   330
DS                     0
SWH          19841.270 Hz
FIDRES        0.302754 Hz
AQ           1.6515572 sec
RG                 16384
DW              25.200 usec
DE                6.00 usec
TE               300.0 K
D12         0.00002000 sec
PL13             22.00 dB
D1          2.00000000 sec
CPDPRG2          waltz16
PCPD2            90.00 usec
SFO2       250.1312345 MHz
NUC2                  1H
PL2               0.00 dB
PL12             22.00 dB
P1               10.20 usec
SFO1        62.9031573 MHz
NUC1                 13C
PL1               0.00 dB
D11         0.03000000 sec

F2 - Processing parameters
SI                 32768
SF          62.8952429 MHz
WDW                   EM
SSB                    0
LB                1.00 Hz
GB                     0
PC                  1.40

1D NMR plot parameters
CX               20.00 cm
F1P            200.701 ppm
F1            12623.13 Hz
F2P              5.821 ppm
F2              366.11 Hz
PPMCM          9.74400 ppm/cm
HZCM         612.85060 Hz/cm
```



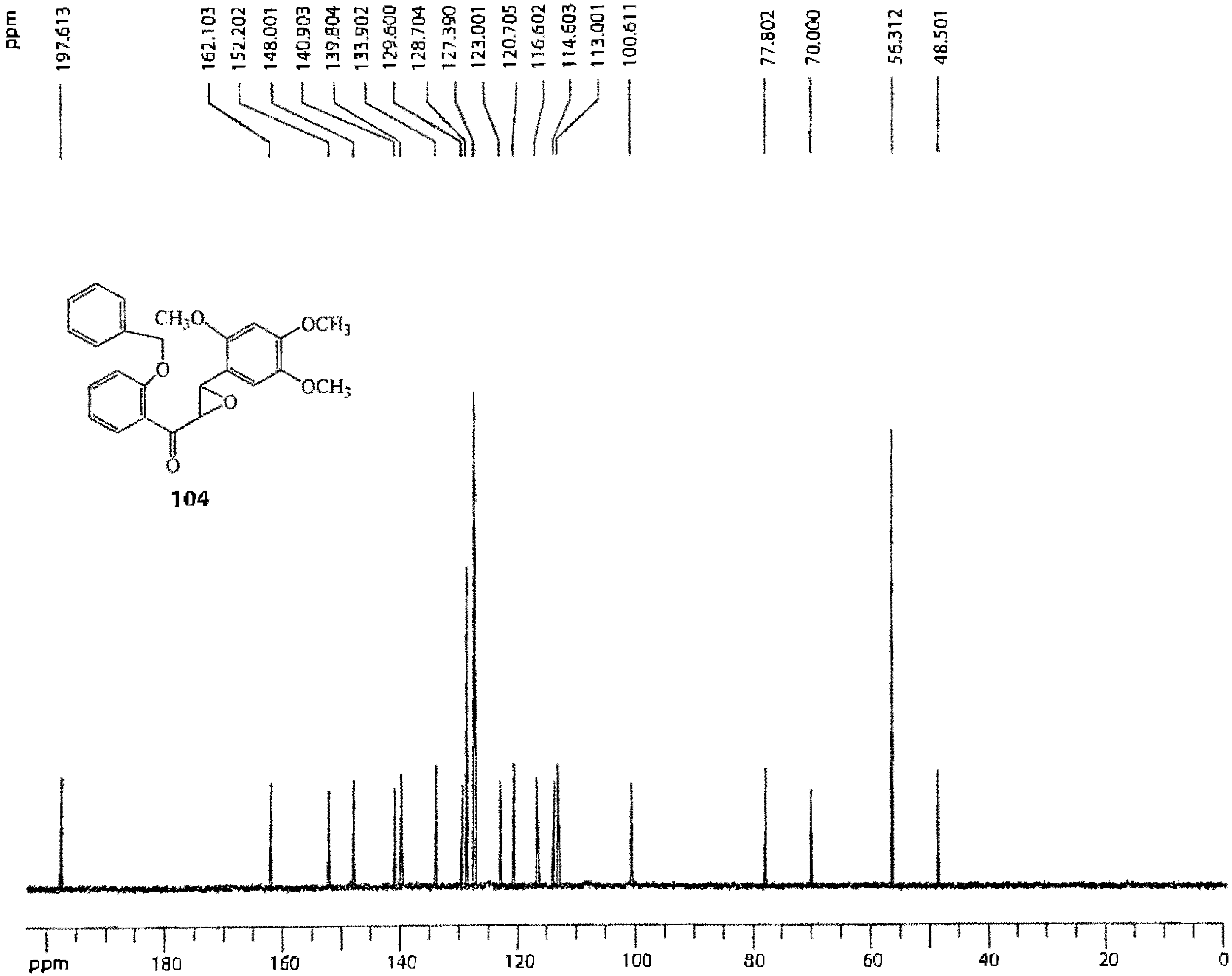
21

```
Current Data Parameters
NAME              HRAI-21
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_            20090303
Time                12.44
INSTRUM             spect
PROBHD     5 mm Dual 13
PULPROG           zgpg30
TD                  65536
SOLVENT              DMSO
NS                    300
DS                      0
SWH          19841.270 Hz
FIDRES        0.302754 Hz
AQ          1.6515572 sec
RG                  16384
DW             25.200 usec
DE               6.00 usec
TE                300.0 K
D12        0.00002000 sec
PL13             22.00 dB
D1         2.00000000 sec
CPDPRG2            waltz16
PCPD2           90.00 usec
SFO2       250.1312349 MHz
NUC2                    1H
PL2               0.00 dB
PL12             22.00 dB
P1              10.20 usec
SFO1        62.9031573 MHz
NUC1                   13C
PL1               0.00 dB
D11        0.03000000 sec

F2 - Processing parameters
SI                  32768
SF          62.8952420 MHz
WDW                    EM
SSB                     0
LB                1.00 Hz
GB                      0
PC                   1.40

1D NMR plot parameters
CX               20.00 cm
F1P             200.701 ppm
F1             12623.13 Hz
F2P               5.821 ppm
F2               366.11 Hz
PPMCM          9.74399 ppm/cm
HZCM         612.85365 Hz/cm
```

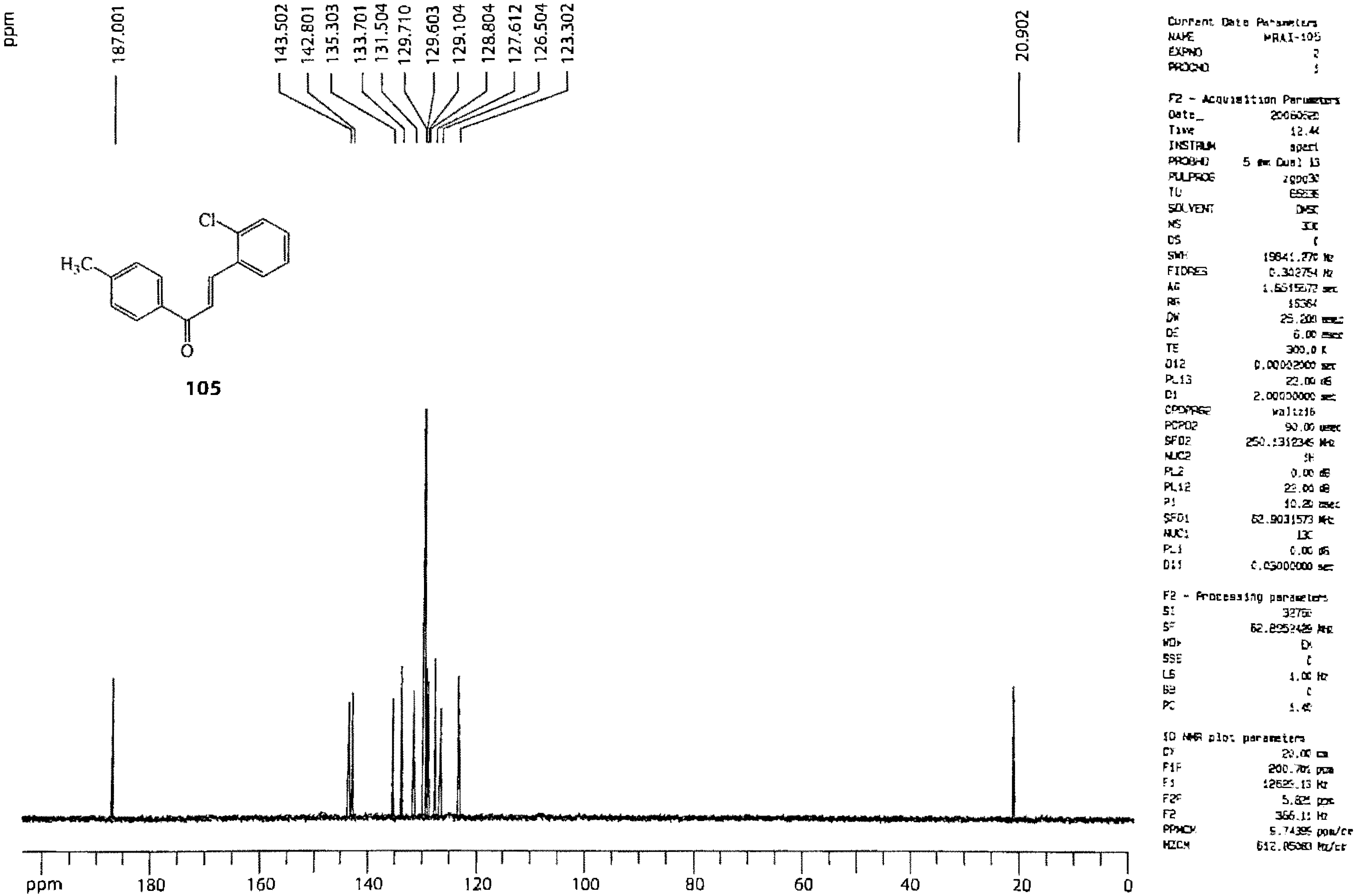


103

```
Current Data Parameters
NAME              MRAI-103
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_             20060520
Time                 12.44
INSTRUM              spect
PROBHD      5 mm Dual 13
PULPROG            zgpg30
TD                   65536
SOLVENT              CDCl3
NS                     300
DS                       0
SWH           19841.270 Hz
FIDRES         0.302754 Hz
AQ           1.6515572 sec
RG                   16384
DW              25.200 usec
DE                6.00 usec
TE                 300.0 K
D12         0.00002000 sec
PL13              22.00 dB
D1          2.00000000 sec
CPDPRG2            waltz16
PCPD2            90.00 usec
SFO2       250.1312346 MHz
NUC2                    1H
PL2                0.00 dB
PL12              22.00 dB
P1               10.20 usec
SFO1        62.9031573 MHz
NUC1                   13C
PL1                0.00 dB
D11         0.03000000 sec

F2 - Processing parameters
SI                   32768
SF          62.8952429 MHz
WDW                     EM
SSB                      0
LB                 1.00 Hz
GB                       0
PC                    1.40

1D NMR plot parameters
CX                20.00 cm
F1P              200.701 ppm
F1             12623.13 Hz
F2P               5.822 ppm
F2               366.11 Hz
PPMCM          9.74399 ppm/cm
HZCM         612.85083 Hz/cm
```

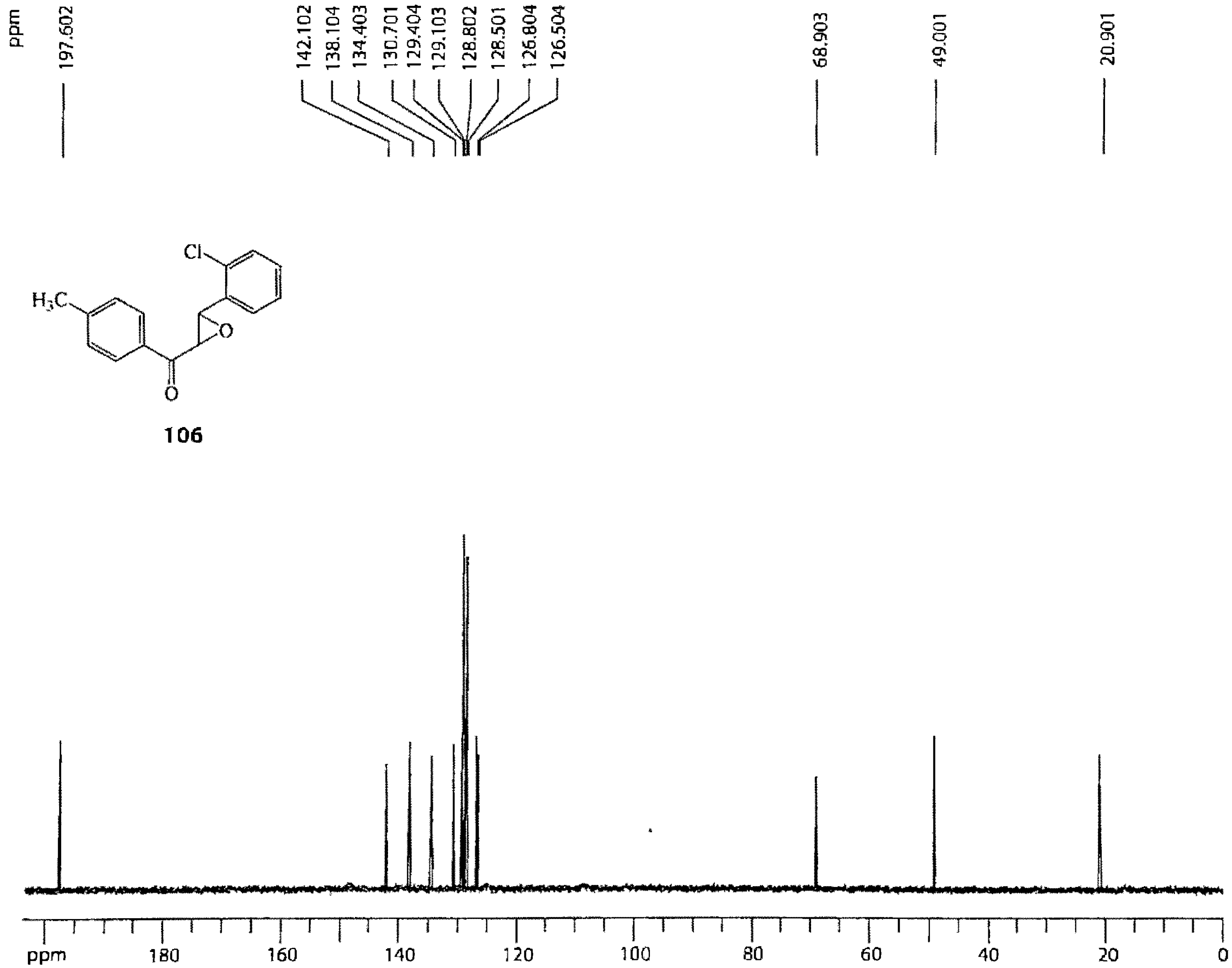


```
Current Data Parameters
NAME              MRAI-104
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_             20060614
Time                 16.10
INSTRUM              spect
PROBHD       5 mm Dual 13
PULPROG            zgpg30
TD                   65536
SOLVENT              CDCl3
NS                      86
DS                       0
SWH             19841.270 Hz
FIDRES           0.302754 Hz
AQ              1.6515572 sec
RG                   16384
DW                  25.200 usec
DE                    6.00 usec
TE                   300.0 K
D12             0.00002000 sec
PL13                 22.00 dB
D1              2.00000000 sec
CPDPRG2            waltz16
PCPD2                90.00 usec
SFO2           250.1315445 MHz
NUC2                    1H
PL2                   0.00 dB
PL12                 22.00 dB
P1                   10.20 usec
SFO1            62.9031573 MHz
NUC1                   13C
PL1                   0.00 dB
D11             0.03000000 sec

F2 - Processing parameters
SI                   32768
SF              62.8952481 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40

1D NMR plot parameters
CX                   20.00 cm
F1P                210.252 ppm
F1                13226.40 Hz
F2P                 -0.491 ppm
F2                  -30.88 Hz
PPMCM             10.53815 ppm/cm
HZCM             662.83258 Hz/cm
```



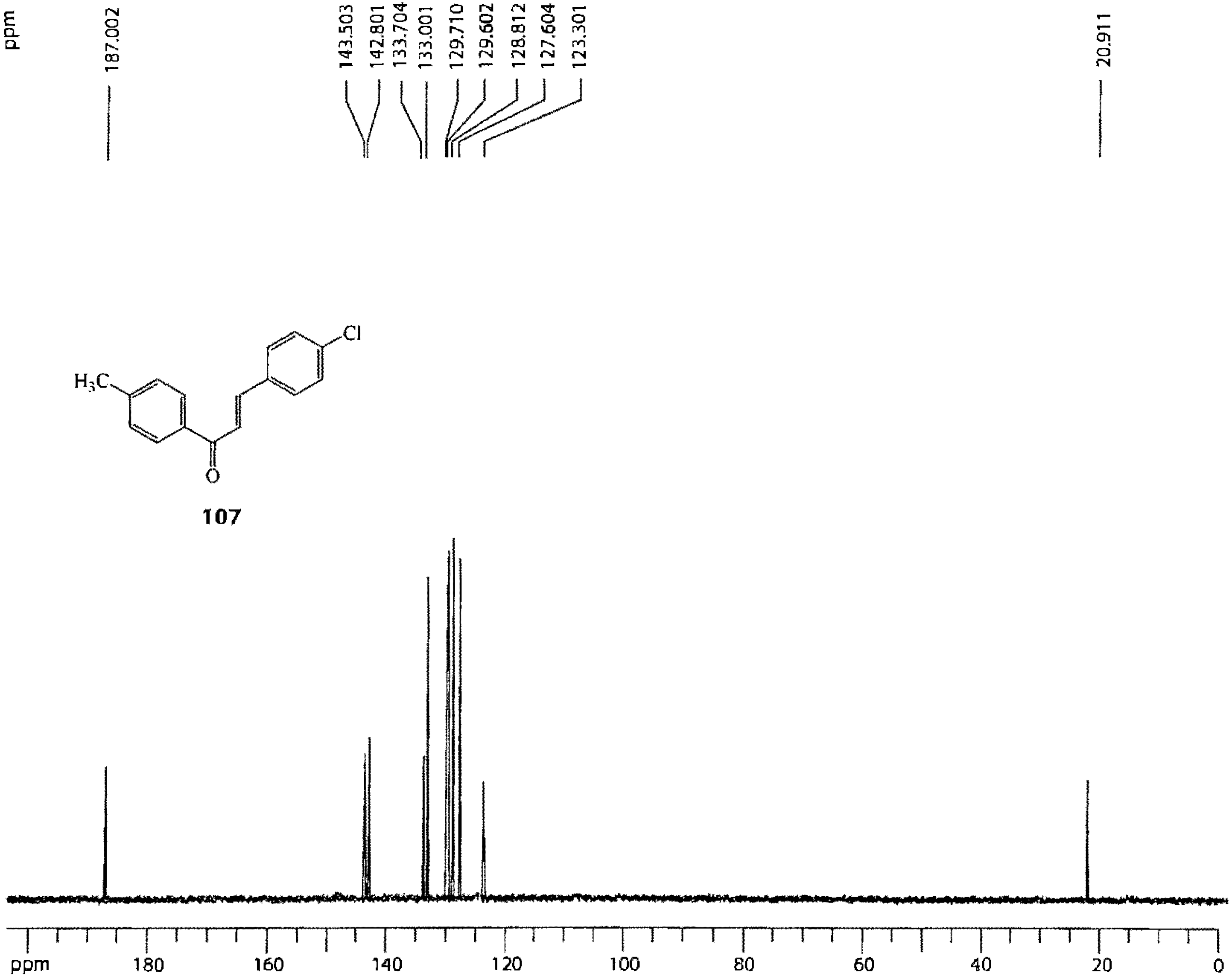
105

```
Current Data Parameters
NAME            MRAI-105
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20060522
Time               12.44
INSTRUM            spect
PROBHD     5 mm Dual 13
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                  300
DS                    0
SWH          19841.270 Hz
FIDRES        0.302754 Hz
AQ           1.6515572 sec
RG                16384
DW              25.200 usec
DE                6.00 usec
TE               300.0 K
D12         0.00002000 sec
PL13             22.00 dB
D1          2.00000000 sec
CPDPRG2         waltz16
PCPD2            90.00 usec
SFO2       250.1312345 MHz
NUC2                 1H
PL2               0.00 dB
PL12             22.00 dB
P1               10.20 usec
SFO1        62.9031573 MHz
NUC1                13C
PL1               0.00 dB
D11         0.03000000 sec

F2 - Processing parameters
SI                32768
SF          62.8952426 MHz
WDW                  EM
SSB                   0
LB                1.00 Hz
GB                    0
PC                 1.40

1D NMR plot parameters
CX               22.00 cm
F1P             200.704 ppm
F1             12623.13 Hz
F2P               5.825 ppm
F2               366.11 Hz
PPMCM           5.74395 ppm/cm
HZCM          612.85380 Hz/cm
```



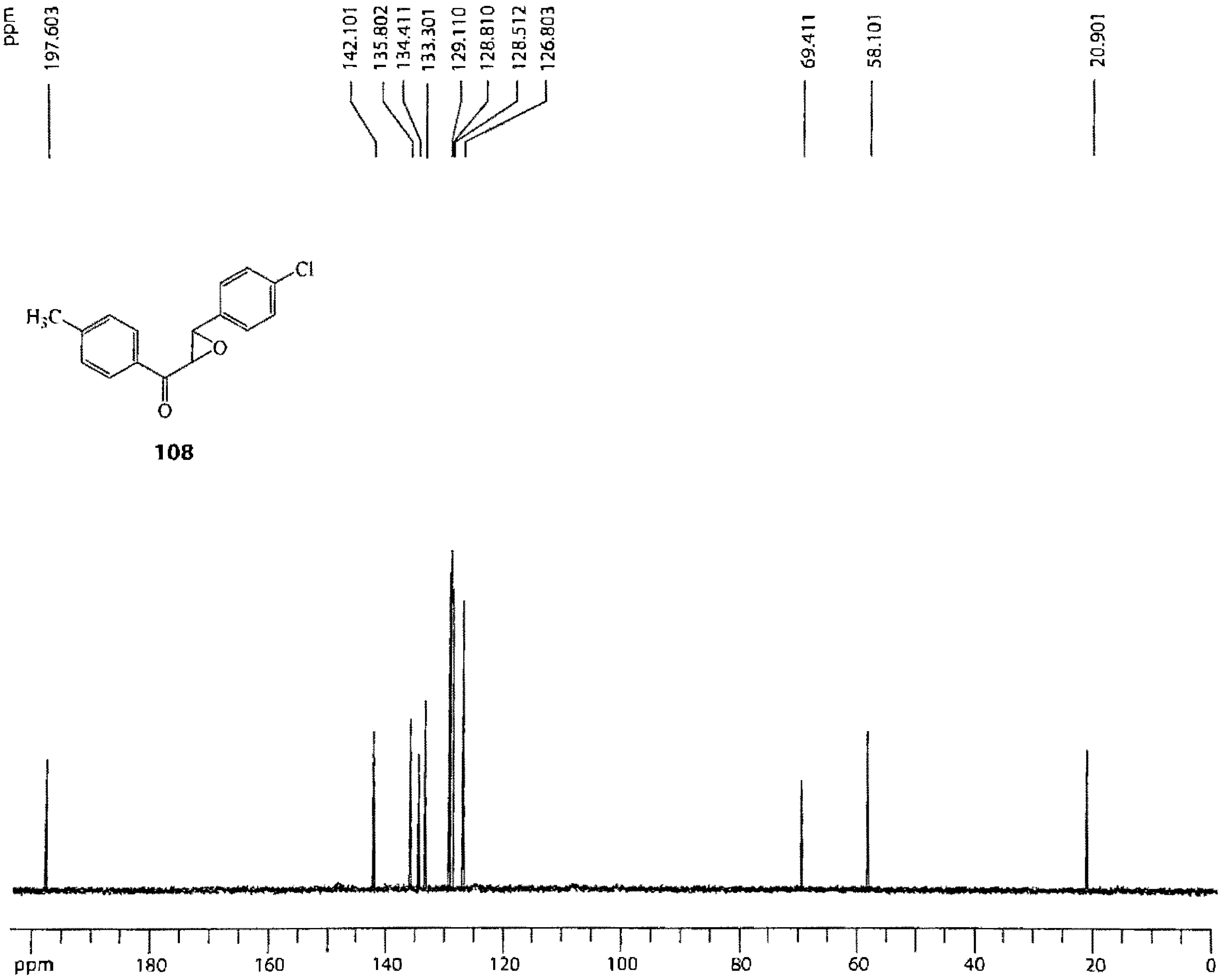
106

```
Current Data Parameters
NAME             MRAI-106
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_            20060614
Time                16.10
INSTRUM             spect
PROBHD      5 mm Dual 13
PULPROG            zgpg30
TD                  65536
SOLVENT             CDCl3
NS                     86
DS                      0
SWH             19841.270 Hz
FIDRES           0.302754 Hz
AQ              1.6515572 sec
RG                  16384
DW                 25.200 usec
DE                   6.00 usec
TE                  300.0 K
D12            0.00002000 sec
PL13                22.00 dB
D1             2.00000000 sec
CPDPRG2           waltz16
PCPD2               90.00 usec
SFO2          250.1315446 MHz
NUC2                   1H
PL2                  0.00 dB
PL12                22.00 dB
P1                  10.20 usec
SFO1           62.9031573 MHz
NUC1                  13C
PL1                  0.00 dB
D11            0.03000000 sec

F2 - Processing parameters
SI                  32768
SF             62.8952481 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40

1D NMR plot parameters
CX                  20.00 cm
F1P               210.292 ppm
F1               13226.40 Hz
F2P                -0.491 ppm
F2                 -30.85 Hz
PPMCM            10.53915 ppm/cm
HZCM            662.86255 Hz/cm
```



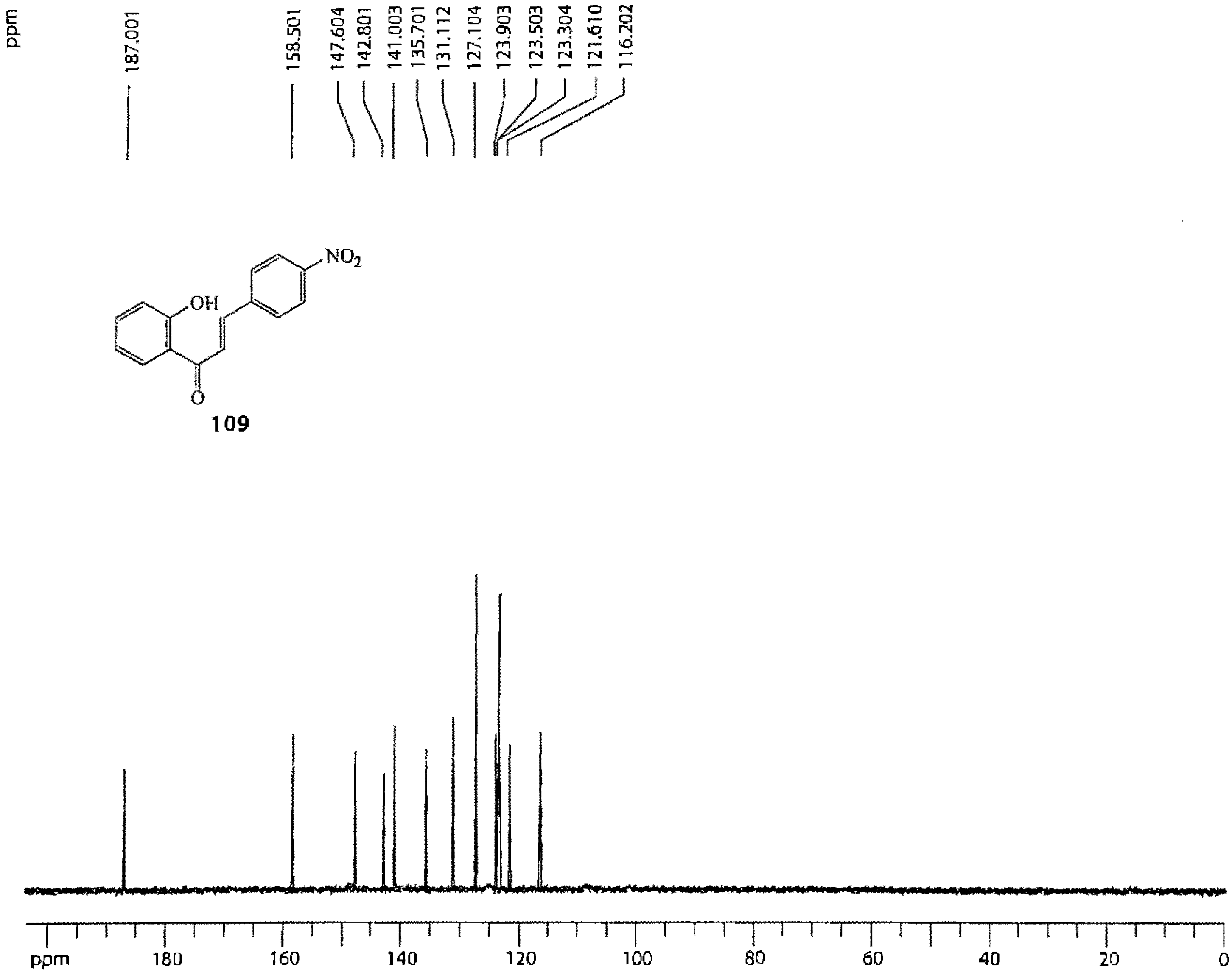
107

```
Current Data Parameters
NAME            MRAI-107
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20050620
Time               12.44
INSTRUM            spect
PROBHD      5 mm Dual 13
PULPROG          zgpg30
TD                 65536
SOLVENT             DMSO
NS                   330
DS                     0
SWH          19841.270 Hz
FIDRES        0.302754 Hz
AQ           1.6515572 sec
RG                 16384
DW                25.200 usec
DE                  6.00 usec
TE                 300.0 K
D12          0.00002000 sec
PL13               22.00 dB
D1           2.00000000 sec
CPDPRG2           waltz16
PCPD2              90.00 usec
SFO2        250.1312345 MHz
NUC2                  1H
PL2                 0.00 dB
PL12               22.00 dB
P1                 10.20 usec
SFO1         62.9031573 MHz
NUC1                 13C
PL1                 0.00 dB
D11          0.03000000 sec

F2 - Processing parameters
SI                 32768
SF           62.8952425 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40

1D NMR plot parameters
CX                 20.00 cm
F1P              200.701 ppm
F1              12623.13 Hz
F2P                5.821 ppm
F2                366.11 Hz
PPMCM            9.74399 ppm/cm
HZCM           612.85063 Hz/cm
```



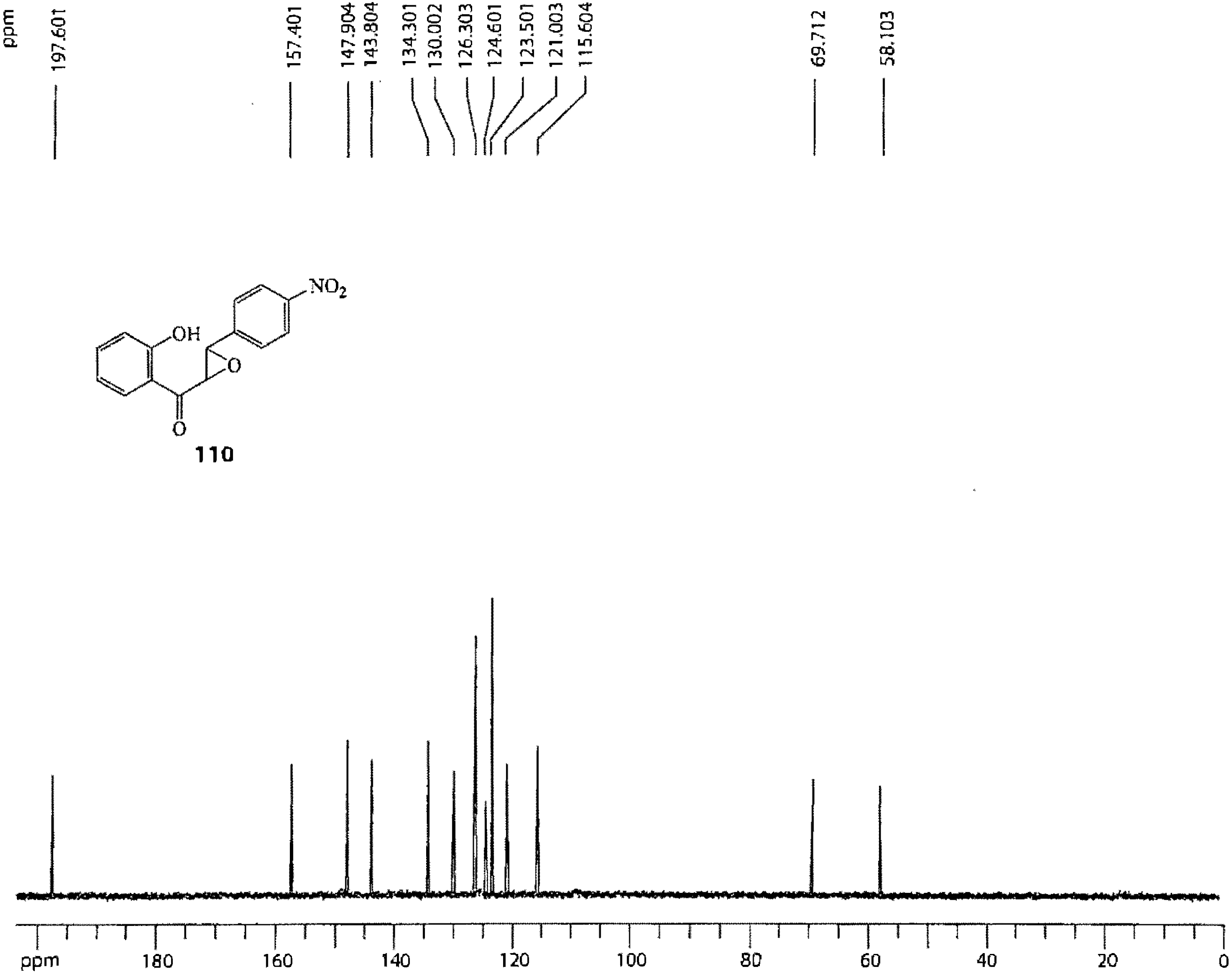
108

```
Current Data Parameters
NAME              MRAI-108
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_             20060614
Time                 16.10
INSTRUM              spect
PROBHD      5 mm Dual 13
PULPROG            zgpg30
TD                  65536
SOLVENT             CDCl3
NS                     82
DS                      0
SWH             19841.270 Hz
FIDRES           0.302754 Hz
AQ              1.6515572 sec
RG                  16384
DW                 25.200 usec
DE                   6.00 usec
TE                  300.0 K
D12            0.00002000 sec
PL13                22.00 dB
D1             2.00000000 sec
CPDPRG2           waltz16
PCPD2               90.00 usec
SFO2         250.1315445 MHz
NUC2                   1H
PL2                  0.00 dB
PL12                22.00 dB
P1                  10.20 usec
SFO1          62.9031575 MHz
NUC1                  13C
PL1                  0.00 dB
D11            0.03000000 sec

F2 - Processing parameters
SI                  32768
SF             62.8952481 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40

1D NMR plot parameters
CX                  20.00 cm
F1P               210.292 ppm
F1               13225.40 Hz
F2P                -0.491 ppm
F2                 -30.85 Hz
PPMCM            10.53915 ppm/cm
HZCM            662.86256 Hz/cm
```

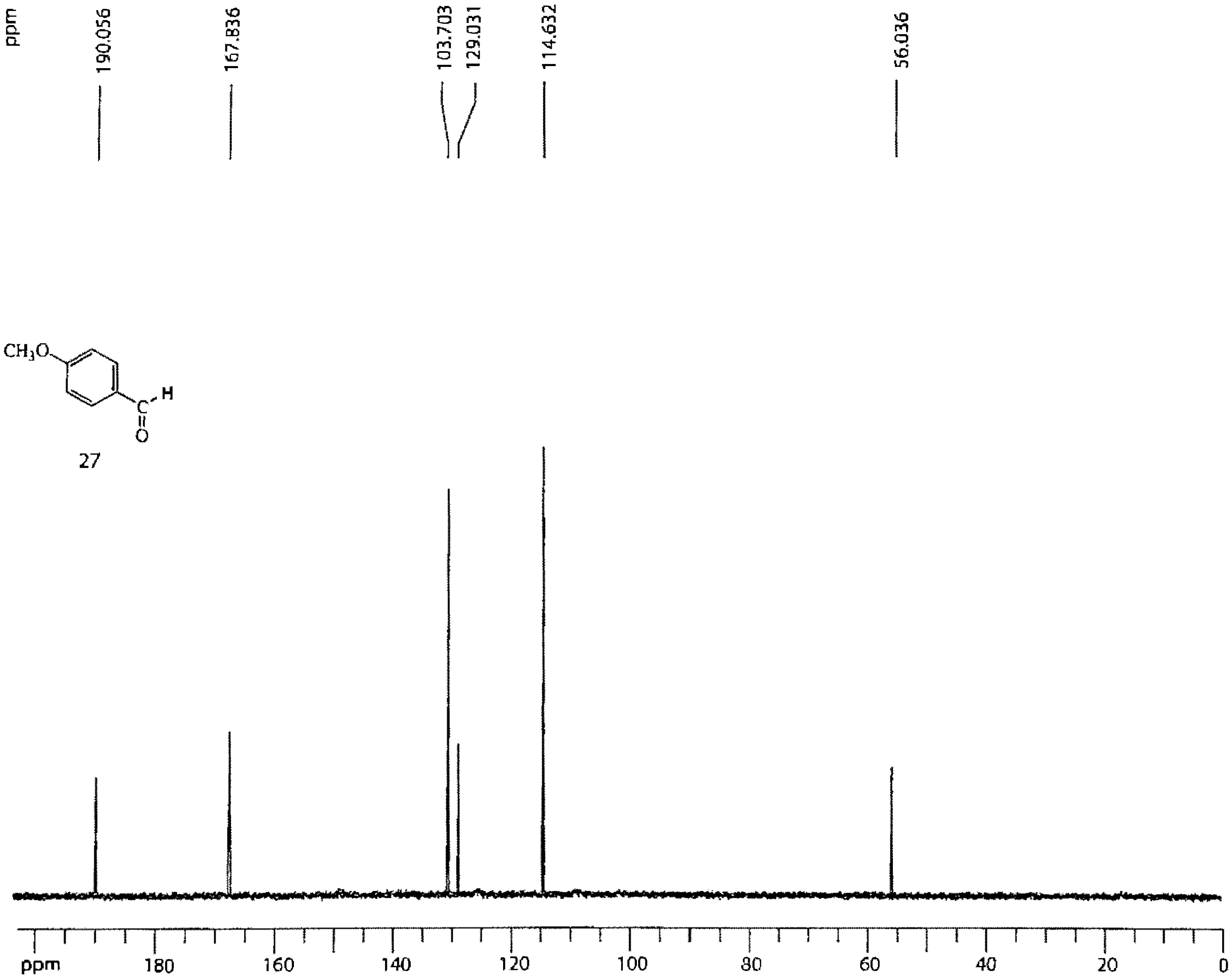


109

```
Current Data Parameters
NAME              MBA1-109
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_             20060620
Time                 12.44
INSTRUM              spect
PROBHD        5 mm Dual 13
PULPROG            zgpg30
TD                   65536
SOLVENT               DMSO
NS                     300
DS                       0
SWH           19841.270 Hz
FIDRES         0.302754 Hz
AQ           1.6515572 sec
RG                   16384
DW              25.200 usec
DE                6.00 usec
TE                 300.0 K
D12         0.00002000 sec
PL13              22.00 dB
D1          2.00000000 sec
CPDPRG2            waltz16
PCPD2            90.00 usec
SFO2        250.1312346 MHz
NUC2                    1H
PL2                0.00 dB
PL12              22.00 dB
P1               10.20 usec
SFO1        62.9031573 MHz
NUC1                   13C
PL1                0.00 dB
D11         0.03000000 sec

F2 - Processing parameters
SI                   32768
SF          62.8952429 MHz
WDW                     EM
SSB                      0
LB                 1.00 Hz
GB                       0
PC                    1.40

1D NMR plot parameters
CX                20.00 cm
F1P            200.701 ppm
F1             12623.13 Hz
F2P              5.821 ppm
F2               366.11 Hz
PPMCM        9.74398 ppm/cm
HZCM       612.85083 Hz/cm
```

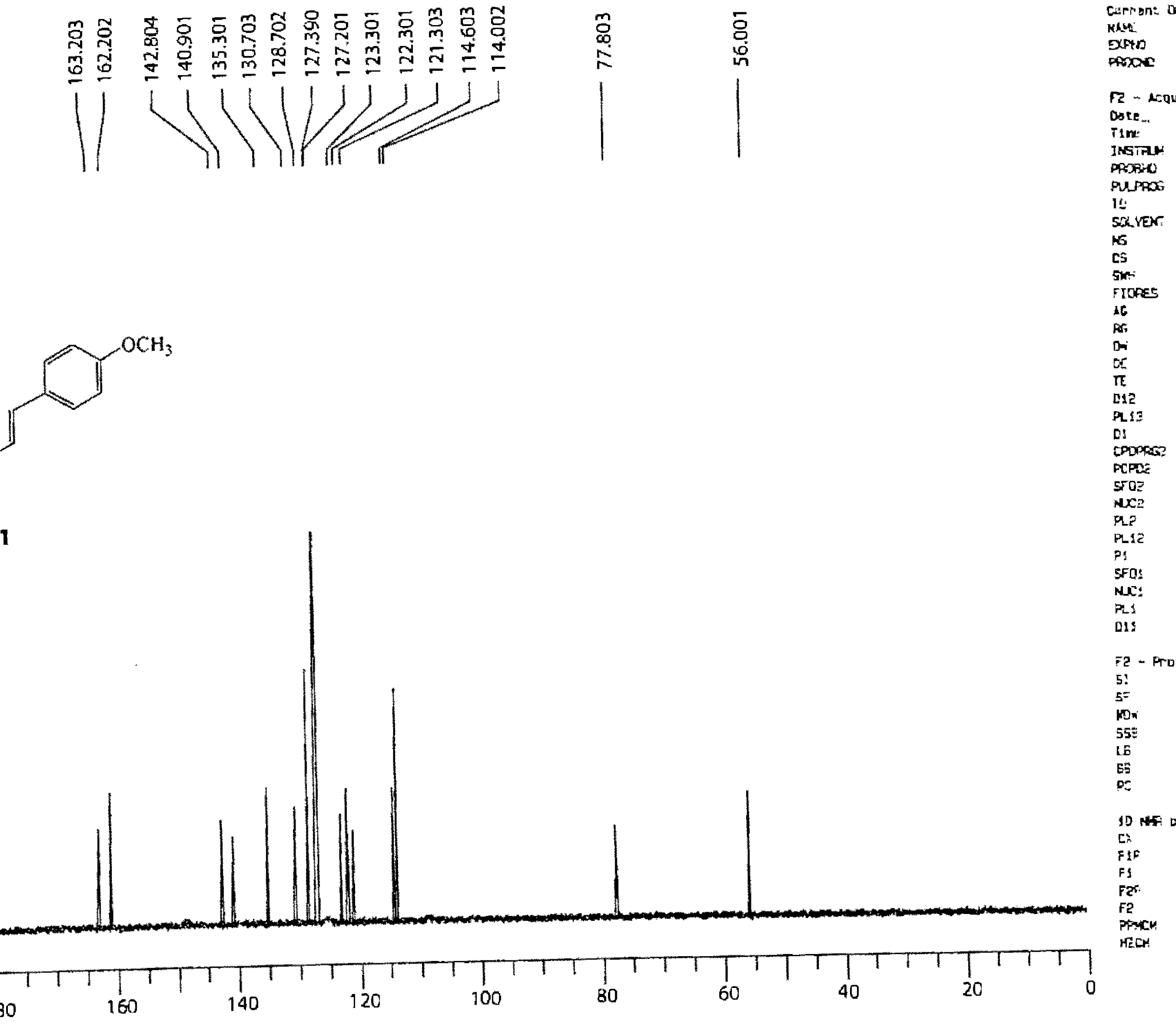


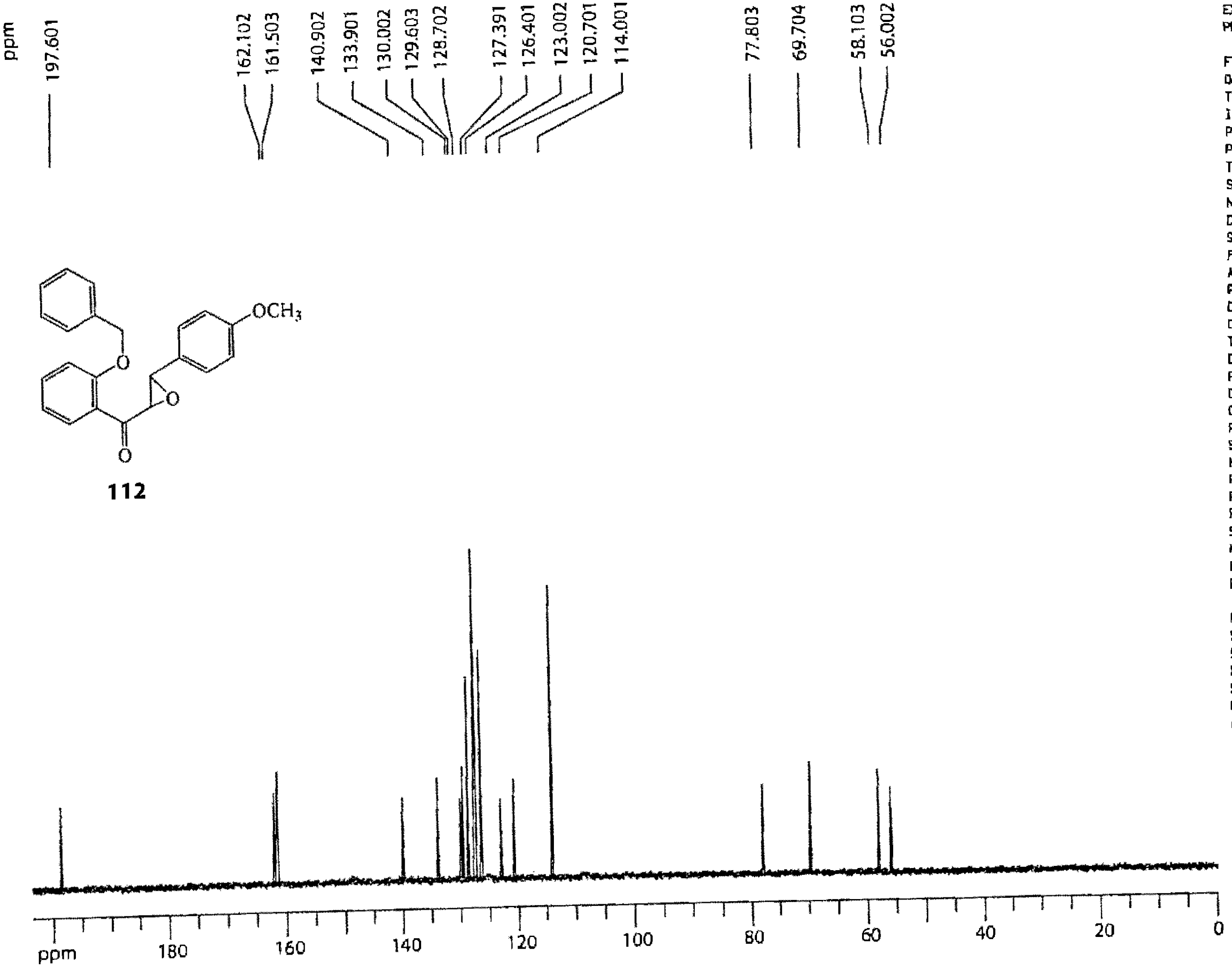
```
Current Data Parameters
NAME          MRAI-110
EXPNO                2
PROCNO               1

F2 - Acquisition Parameters
Date_         20060614
Time             16.10
INSTRUM          spect
PROBHD   5 mm Dual 13
PULPROG        zgpg30
TD               65536
SOLVENT          CDCl3
NS                  86
DS                   0
SWH          19841.270 Hz
FIDRES        0.302754 Hz
AQ           1.6515572 sec
RG               16384
DW              25.200 usec
DE                6.00 usec
TE               300.0 K
D12         0.00002000 sec
PL13             22.00 dB
D1          2.00000000 sec
CPDPRG2         waltz16
PCPD2            90.00 usec
SFO2       250.1315445 MHz
NUC2                1H
PL2               0.00 dB
PL12             22.00 dB
P1               10.20 usec
SFO1        62.9031573 MHz
NUC1               13C
PL1               0.00 dB
D11         0.03000000 sec

F2 - Processing parameters
SI               32768
SF          62.8952481 MHz
WDW                 EM
SSB                  0
LB                1.00 Hz
GB                   0
PC                1.40

1D NMR plot parameters
CX               20.00 cm
F1P            210.292 ppm
F1            13226.40 Hz
F2P             -0.491 ppm
F2              -30.88 Hz
PPMCM         10.53915 ppm/cm
HZCM         662.86252 Hz/cm
```



27

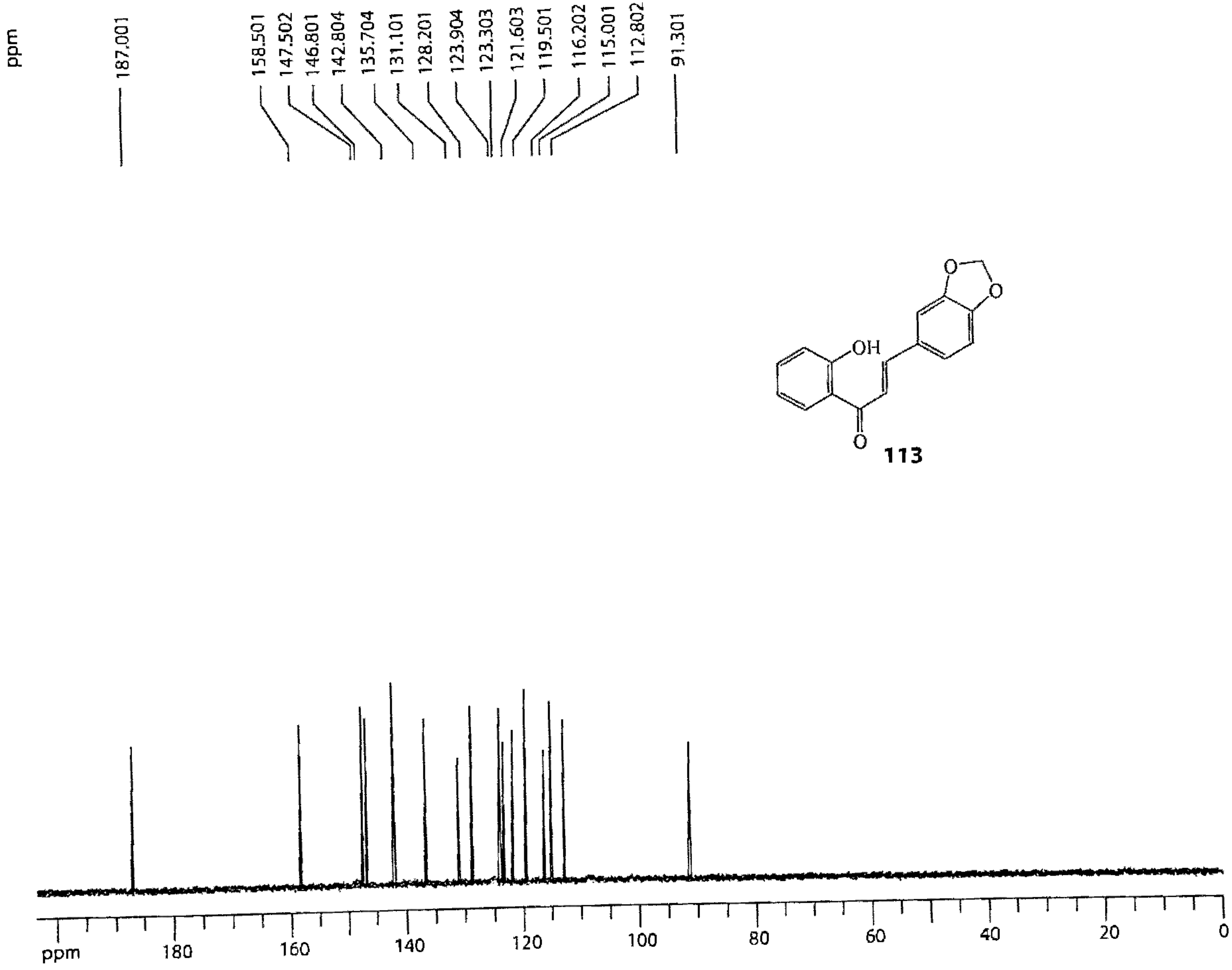
```
Current Data Parameters
NAME            MRAI-27
EXPNO                 2
PROCNO                1

F2 - Acquisition Parameters
Date_          20080003
Time              12.44
INSTRUM           spect
PROBHD    5 mm Dual 13
PULPROG         zgpg30
TD                65536
SOLVENT            DMSO
NS                  300
DS                    0
SWH           19841.270 Hz
FIDRES         0.302754 Hz
AQ            1.6515572 sec
RG                16384
DW               25.200 usec
DE                 6.00 usec
TE                300.0 K
D12          0.00002000 sec
PL13              22.00 dB
D1           2.00000000 sec
CPDPRG2         waltz16
PCPD2             90.00 usec
SFO2        250.1312349 MHz
NUC2                 1H
PL2                0.00 dB
PL12              22.00 dB
P1                10.20 usec
SFO1         62.9031573 MHz
NUC1                13C
PL1                0.00 dB
D11          0.03000000 sec

F2 - Processing parameters
SI                32768
SF           62.8952425 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40

1D NMR plot parameters
CX                20.00 cm
F1P             200.701 ppm
F1             12623.13 Hz
F2P               5.821 ppm
F2               366.11 Hz
PPMCM           9.74396 ppm/cm
HZCM          612.85083 Hz/cm
```


163.203
162.202
142.804
140.901
135.301
130.703
128.702
127.390
127.201
123.301
122.301
121.303
114.603
114.002
77.803
56.001
OCH$_3$
1
Current D
NAME
EXPNO
PROCNO
F2 - Acqu
Date_
Time
INSTRUM
PROBHD
PULPROG
TD
SOLVENT
NS
DS
SWH
FIDRES
AQ
RG
DW
DE
TE
D12
PL13
D1
CPDPRG2
PCPD2
SFO2
NUC2
PL2
PL12
P1
SFO1
NUC1
PL1
D11
F2 - Pro
SI
SF
WDW
SSB
LB
GB
PC
1D NMR p
CX
F1P
F1
F2P
F2
PPMCM
HZCM
80
160
140
120
100
80
60
40
20
0



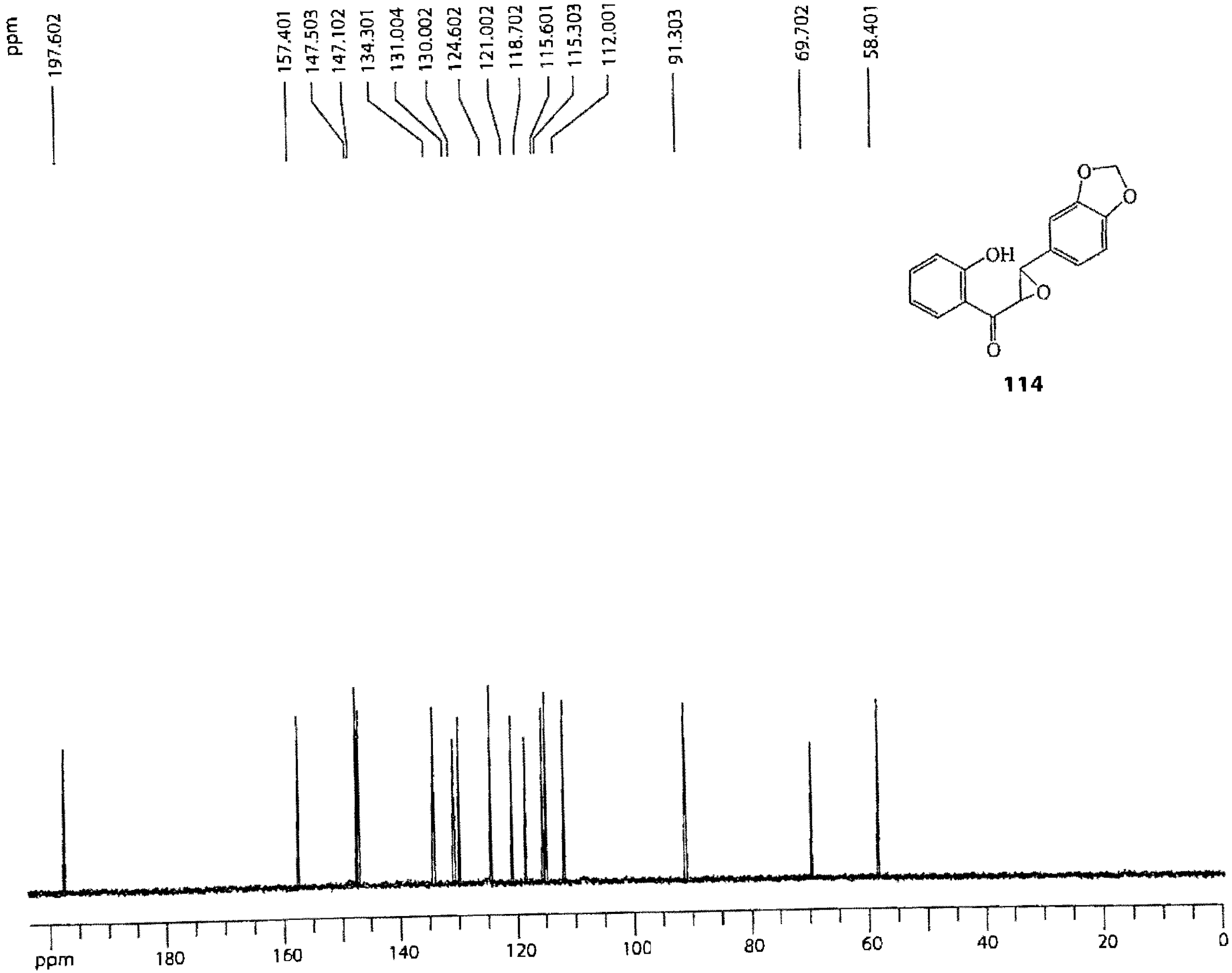
112

```
Current Data Parameters
NAME              KRAI-112
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_             20060514
Time                 16.10
INSTRUM              spect
PROBHD      5 mm Dual 13
PULPROG            zgpg30
TD                   65536
SOLVENT              CDCl3
NS                      66
DS                       0
SWH             19841.270 Hz
FIDRES           0.302754 Hz
AQ              1.6515572 sec
RG                   16384
DW                  25.200 usec
DE                    6.00 usec
TE                   300.0 K
D12            0.00002000 sec
PL13                 22.00 dB
D1             2.00000000 sec
CPDPRG2            waltz16
PCPD2                90.00 usec
SFO2           250.1315446 MHz
NUC2                    1H
PL2                   0.00 dB
PL12                 22.00 dB
P1                   10.20 usec
SFO1            62.9031573 MHz
NUC1                   13C
PL1                   0.00 dB
D11          · 0.03000000 sec

F2 - Processing parameters
SI                   32768
SF              62.8952481 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40

1D NMR plot parameters
CX                   20.00 cm
F1P                210.292 ppm
F1                13226.40 Hz
F2P                 -0.491 ppm
F2                  -30.88 Hz
PPMCM             10.53915 ppm/cm
HZCM             662.85255 Hz/cm
```



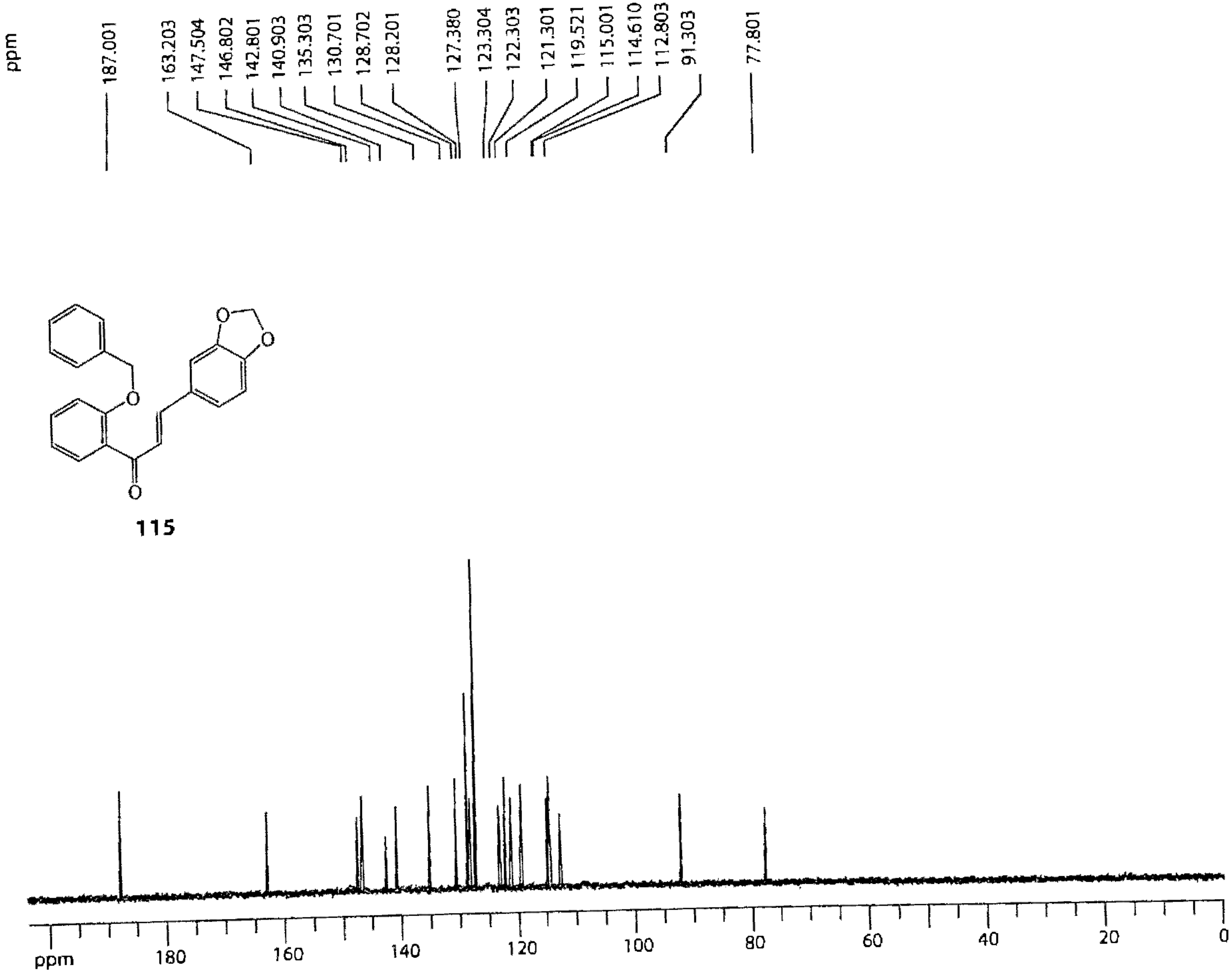
113

Current Data Parameters	
NAME	MRAI-113
EXPNO	2
PROCNO	1
F2 - Acquisition Parameters	
Date_	20060620
Time	12.44
INSTRUM	spect
PROBHD	5 mm Dual 13
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	300
DS	0
SWH	19841.270 Hz
FIDRES	0.302754 Hz
AQ	1.6515572 sec
RG	16384
DW	25.200 usec
DE	6.00 usec
TE	300.0 K
D12	0.00002000 sec
PL13	22.00 dB
D1	2.00000000 sec
CPDPRG2	waltz16
PCPD2	90.00 usec
SFO2	250.1312349 MHz
NUC2	1H
PL2	0.00 dB
PL12	22.00 dB
P1	10.20 usec
SFO1	62.9031573 MHz
NUC1	13C
PL1	0.00 dB
D11	0.03000000 sec
F2 - Processing parameters	
SI	32768
SF	62.8952429 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40
1D NMR plot parameters	
CX	20.00 cm
F1P	200.701 ppm
F1	12623.13 Hz
F2P	5.822 ppm
F2	366.11 Hz
PPMCM	9.74395 ppm/cm
HZCM	612.85083 Hz/cm

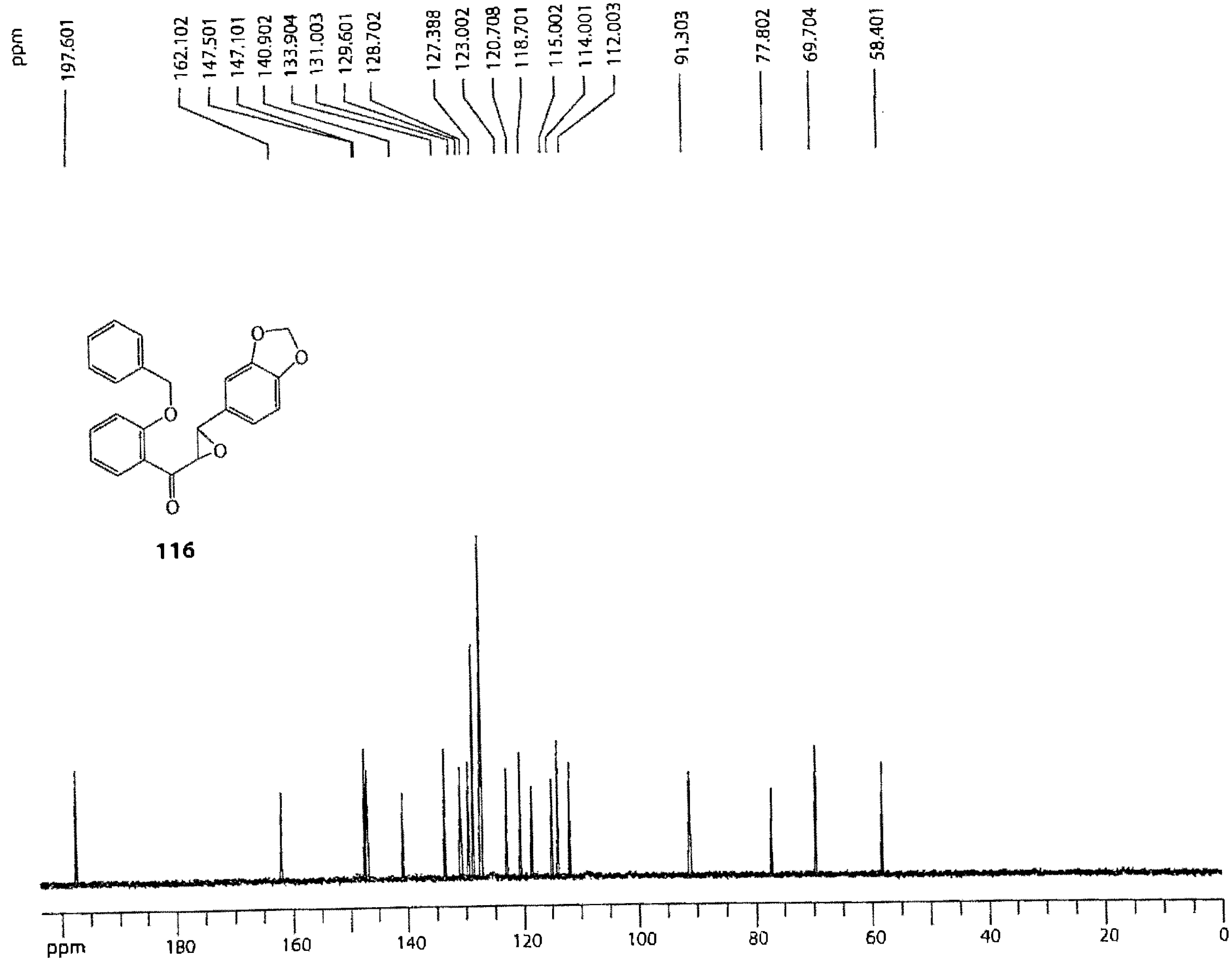


114

```
Current Data Parameters
NAME            MRA1-114
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20060514
Time               16.10
INSTRUM            spect
PROBHD     5 mm Dual 13
PULPROG          zgpg30
TD                 65536
SOLVENT            CDCl3
NS                    86
DS                     0
SWH            19841.270 Hz
FIDRES          0.302754 Hz
AQ             1.6515572 sec
RG                 16384
DW                25.200 usec
DE                  6.00 usec
TE                 300.0 K
D12           0.00002000 sec
PL13               22.00 dB
D1            2.00000000 sec
CPDPRG2          waltz16
PCPD2              90.00 usec
SFO2         250.1315445 MHz
NUC2                  1H
PL2                 0.00 dB
PL12               22.00 dB
P1                 10.20 usec
SFO1          62.9031575 MHz
NUC1                 13C
PL1                 0.00 dB
D11           0.03000000 sec

F2 - Processing parameters
SI                 32768
SF            62.8952481 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40

1D NMR plot parameters
CX                 20.00 cm
F1P              210.252 ppm
F1              13226.40 Hz
F2P               -0.491 ppm
F2                -30.85 Hz
PPMCM           10.53916 ppm/cm
HZCM           662.86255 Hz/cm
```



```
Current Data Parameters
NAME            MRAI-115
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20060521
Time               12.44
INSTRUM            spect
PROBHD      5 mm Dual 13
PULPROG          zgpg30
TD                 65536
SOLVENT             DMSO
NS                   330
DS                     0
SWH           19841.270 Hz
FIDRES         0.302754 Hz
AQ            1.6515572 sec
RG                 15384
DW                25.200 usec
DE                  6.00 usec
TE                 300.0 K
D12           0.00002000 sec
PL13               22.00 dB
D1            2.00000000 sec
CPDPRG2           waltz16
PCPD2              90.00 usec
SFO2         250.1312345 MHz
NUC2                  1H
PL2                 0.00 dB
PL12               22.00 dB
P1                 10.20 usec
SFO1          62.9031573 MHz
NUC1                 13C
PL1                 0.00 dB
D11           0.03000000 sec

F2 - Processing parameters
SI                 32768
SF            62.8952428 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40

1D NMR plot parameters
CX                 20.00 cm
F1P              200.701 ppm
F1              12623.13 Hz
F2P                5.822 ppm
F2                366.11 Hz
PPMCM            9.74395 ppm/cm
HZCM           612.85083 Hz/cm
```



116

```
Current Data Parameters
NAME               MRAI-116
EXPNO                     2
PROCNO                    1

F2 - Acquisition Parameters
Date_              20060614
Time                  15.10
INSTRUM               spect
PROBHD       5 mm Dual 13
PULPROG             zgpg30
TD                    65536
SOLVENT               CDCl3
NS                       66
DS                        0
SWH              19841.270 Hz
FIDRES            0.302754 Hz
AQ               1.6515572 sec
RG                    16384
DW                   25.200 usec
DE                     6.00 usec
TE                    300.0 K
D12             0.00002000 sec
PL13                  22.00 dB
D1              2.00000000 sec
CPDPRG2             waltz16
PCPD2                 90.00 usec
SFO2         250.1315446 MHz
NUC2                     1H
PL2                    0.00 dB
PL12                  22.00 dB
P1                    10.20 usec
SFO1          62.9031573 MHz
NUC1                    13C
PL1                    0.00 dB
D11             0.03000000 sec

F2 - Processing parameters
SI                    32768
SF            62.8952481 MHz
WDW                      EM
SSB                       0
LB                     1.00 Hz
GB                        0
PC                     1.40

1D NMR plot parameters
CX                    20.00 cm
F1P                 210.292 ppm
F1                 13226.40 Hz
F2P                  -0.491 ppm
F2                   -30.88 Hz
PPMCM              10.53915 ppm/cm
HZCM              662.86255 Hz/cm
```