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Synthesis of Chalcone Epoxides and Studies of their Antimicrobial Activities

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University of Rajshahi

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
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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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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).

Supervisor

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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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ACKNOWLEDGEMENT

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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

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
1

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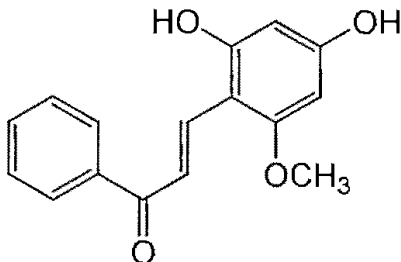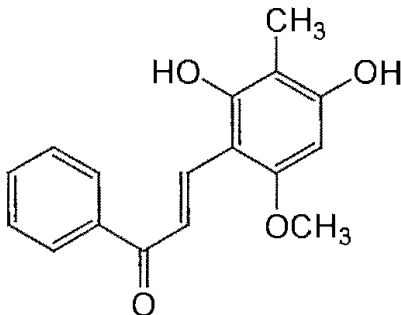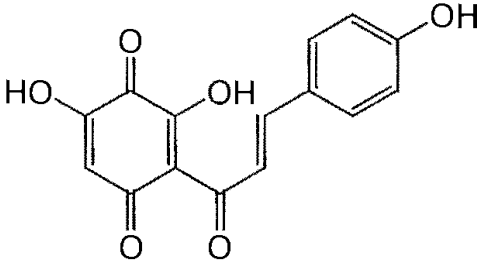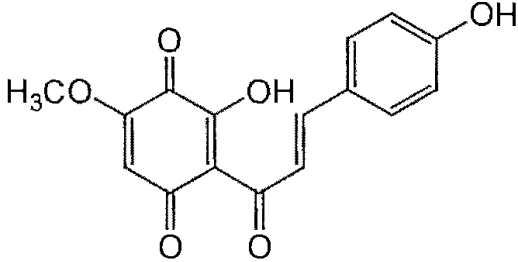
Benzylideneactophenones constitute a class of naturally occurring pigments, which are often referred to as 'Chalcones'. The term was first coined by kostanecki[1]. An interesting feature of chalcones (polyhydroxylated) is that they serve as the starting materials for the synthesis of another class of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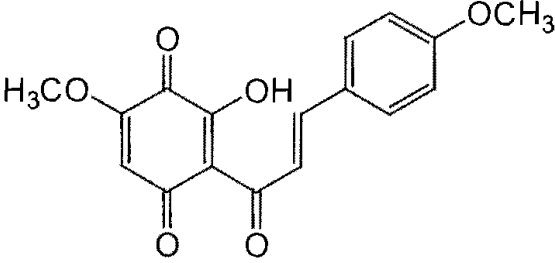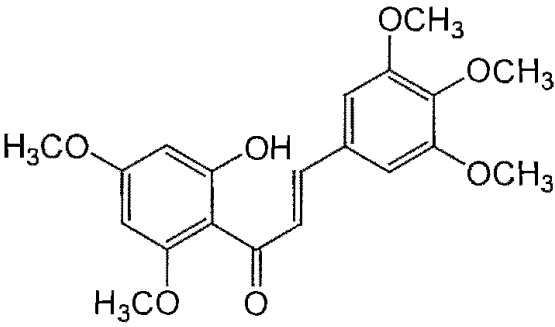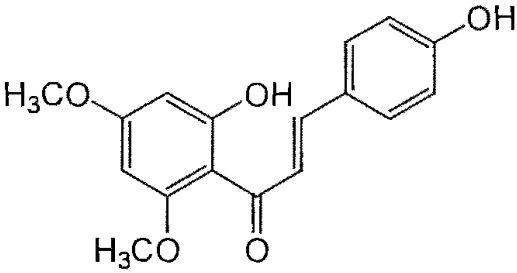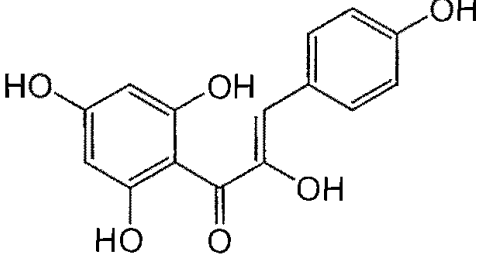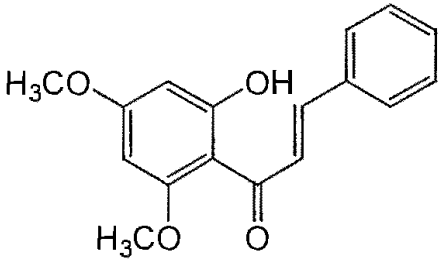
Chalcones form an important group of plant pigments because they are the precursors in the biosynthesis of flavonoids and are known to

1
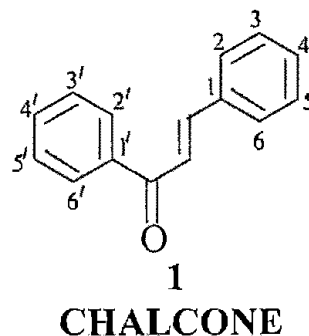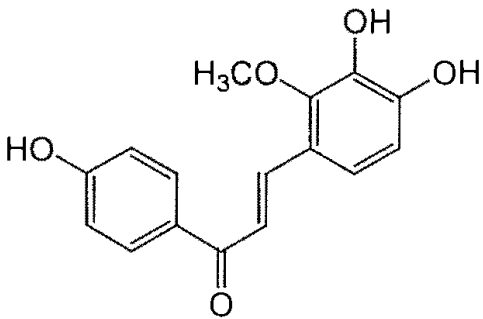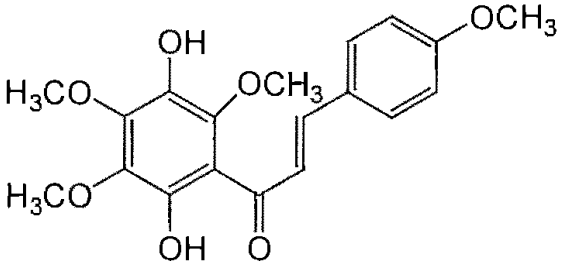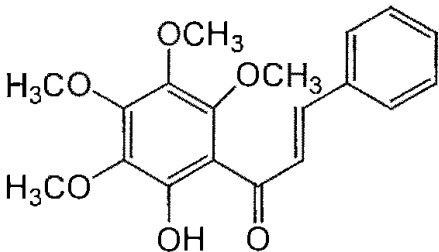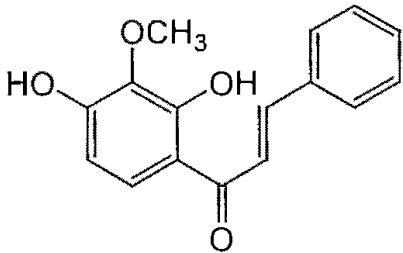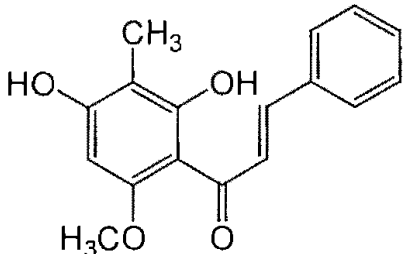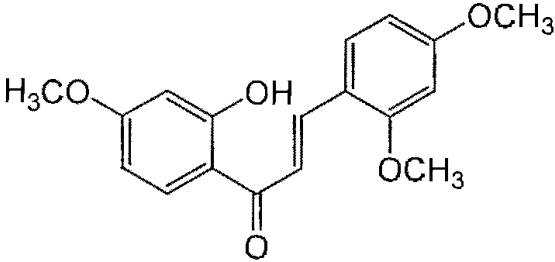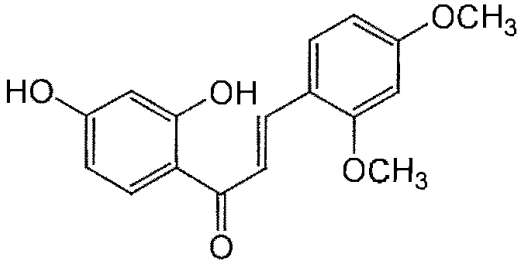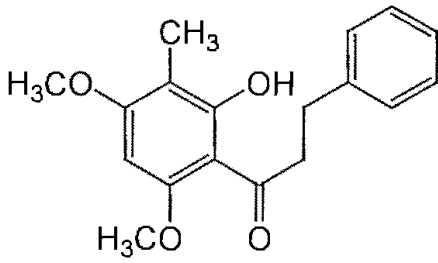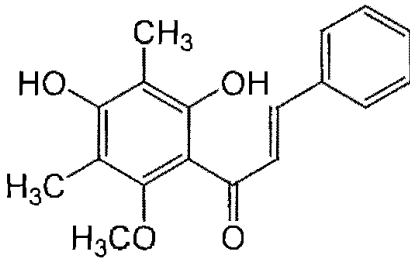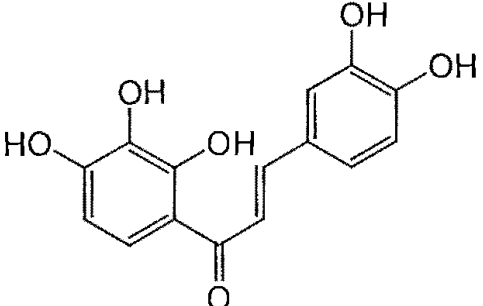
CHALCONE

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.
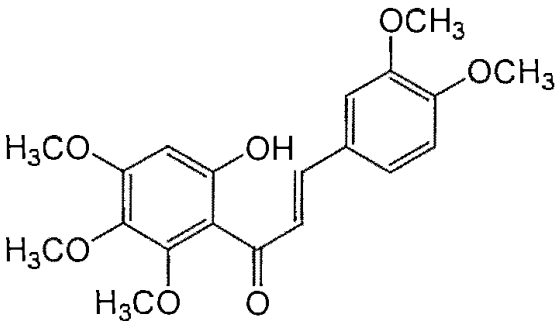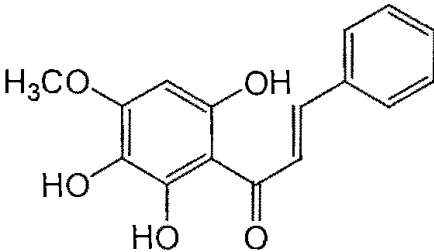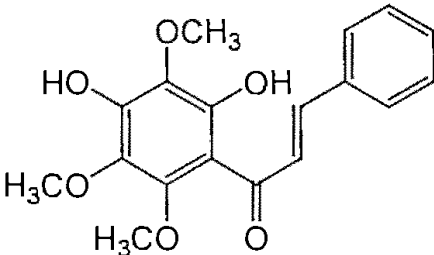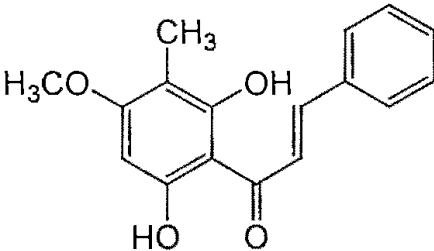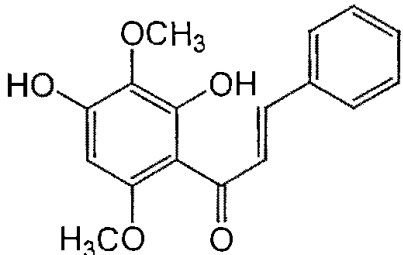
1.3   Naturally occurring chalcones:
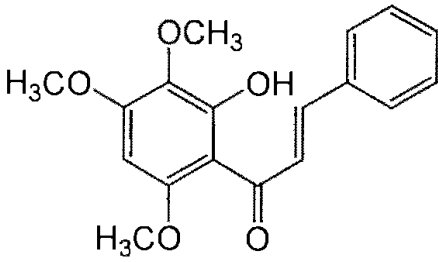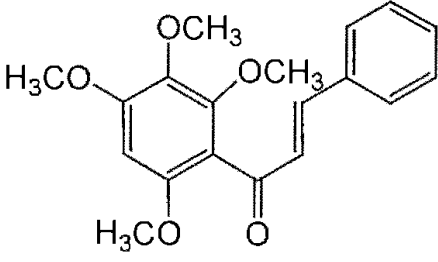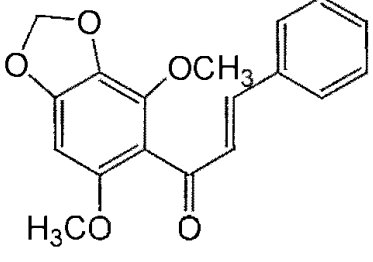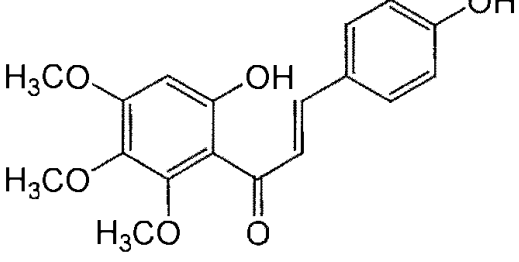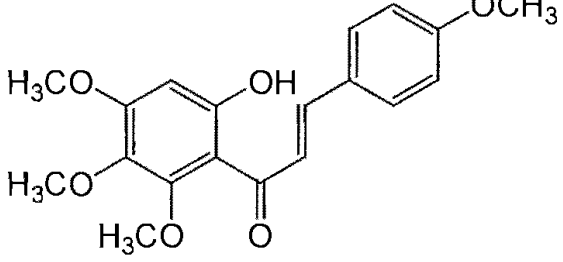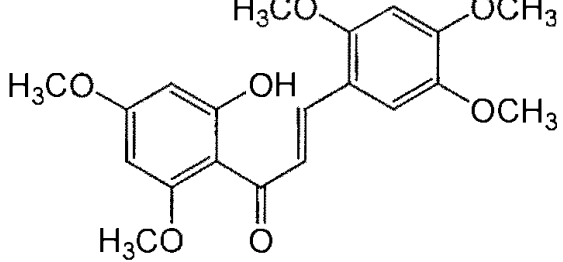
Name & Sl. No.	Structure	Source	Reference
2. 2,4-Dihydroxy-6-methoxychalcone.		Dragon's blood Resin.	Cardillo, *et al.*, (1971), *J. Chem. Soc.*, **C**, 3967.
3. 2,4-Dihydroxy-3-methyl-6-methoxychalcone.		-*do*-	-do-
4. Quinochalcone-A		*Carthamus tinctarius*	Obara, *et al.*, (1971), *J. Chem. Soc.*, 1357.
5. Quinochalcone-B		-*do*-	-do-
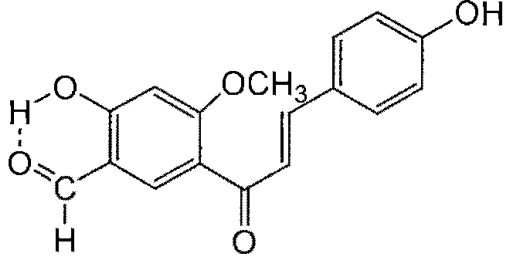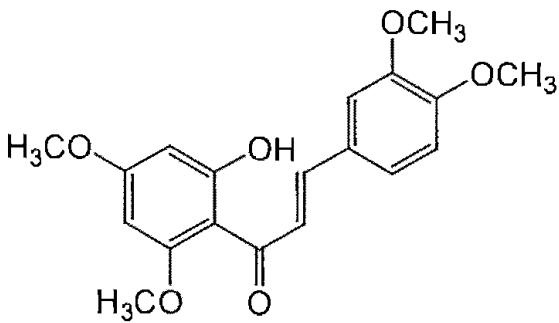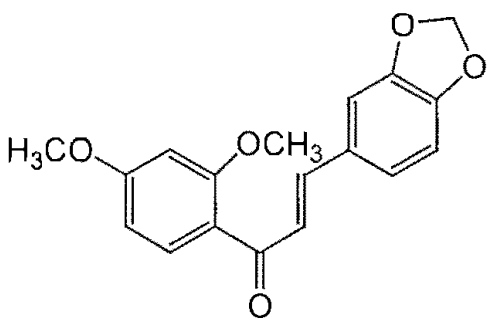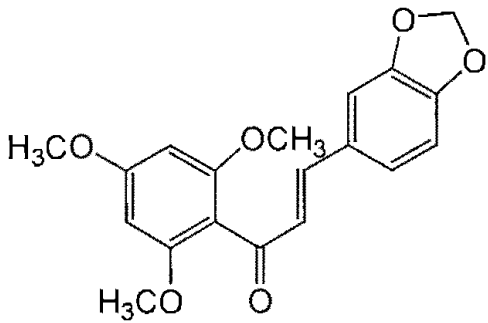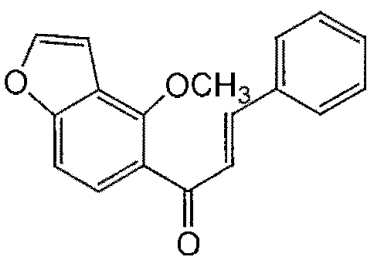
Name & Sl. No.	Structure	Source	Reference
6.Quinochalcone –C		*Carthamus tinctarius*	Obara, *et al.*, (1971), *J. Chem. Soc.*, 1357.
7. 2′-Hydroxy-3, 4,5,4′,6′-Penta-methoxychalco-ne.		*Merrillea caloxylon.*	Fraser, *et al.*, (1972), *Phyto-chemistry*, **11**, 868.
8.Flavokawain-C		*Piper methylsticum.*	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), *Phyto-chemistry,* **16** 1317.
17. Cerasin		-do-	-do-
18. 2'-Hydroxy-4',6'-dimethox-y-3'-methyldih-ydrochalcone		*Myrica gale*	Malterud, K. E., *et al.,* (1977), *Phytochemistry,* **16,** 1805.
19. 2',4'-Dihyd-roxy-6'-metho-xy-3',5'-dimet-hylchalcone		*-do-*	-do-
20. 2',3',4',3,4-Pentahydroxy-chalcone		*Albilizia adianthfolia*	Candy, *et al.,* (1978), *Phyt-ochemistry,* **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′-trimeth-oxychalcone		*Popowia cauliflora.*	Panichpol and Watermann, (1978),*Phytochemistry*, **17**, 1363.
27. 2′,3′,4′,6′-Tetramethoxy-chalcone		*-do-*	-do-
28. Helilandin-A		*Helichrysum sutherlandii*	Bohlmann, F., *et al.*, (1978), Phytochemsitry , **17**, 1935.
29. 2′,4-Dihydr-oxy-4′,5′,6′-tri-methoxychalco-ne.		*Chromolaena*	Barua, *et al*, (1978), *Phytochemistry*, **17**, 1807.
30. 2′-Hydroxy-4,4′,5′,6′-tetra-methoxychalco-ne.		*-do-*	-do- .
31. 2′-Hydroxy-2,4,5,4′,6′-Pentam-ethoxychalcone.		*Derris robusta*	Chibber, S.S., *et al.*, (1979), *Phytochemsitry*, **18**, 2056.

Name & Sl. No.	Structure	Source	Reference
32. Isonebarach-alcone.		*Psoralea corylifolia.*	Gupta, *et al.*, (1980), *Phytochemsitry,* 19(9), 2304.
33. 2'-Hydroxy-4',6',3,4,-tetra-methoxychalcone		*Pongamia pinata*	Tanaka, T. *et al.*, (1992), Phytochemsitry , **21** (3), 998.
34. 2',4'-Dimethoxy-3, 4-methylenedioxychalcone		*-do-*	-do-
35. 2',4',6'-Tri-methoxy-3,4-methylenedioxychalcone		*-do-*	-do-
36. 2'-Methoxyf-urano-[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).
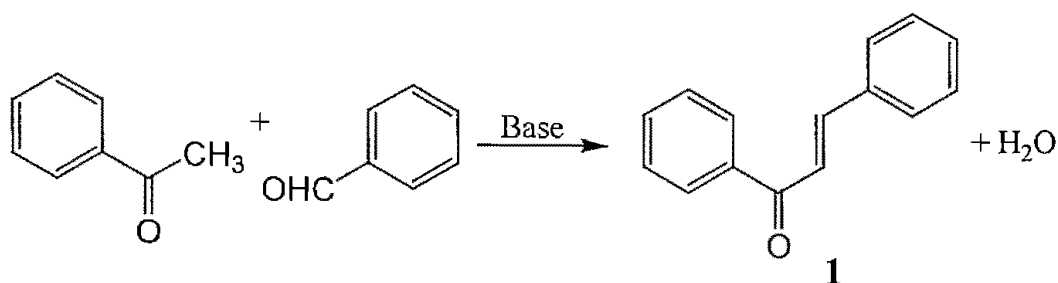
The substituted benzylideneacetophenones have likewise been obtained by condensing the appropriately substituted acetophenones with substituted benzaldehydes in the presence of alkali.

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
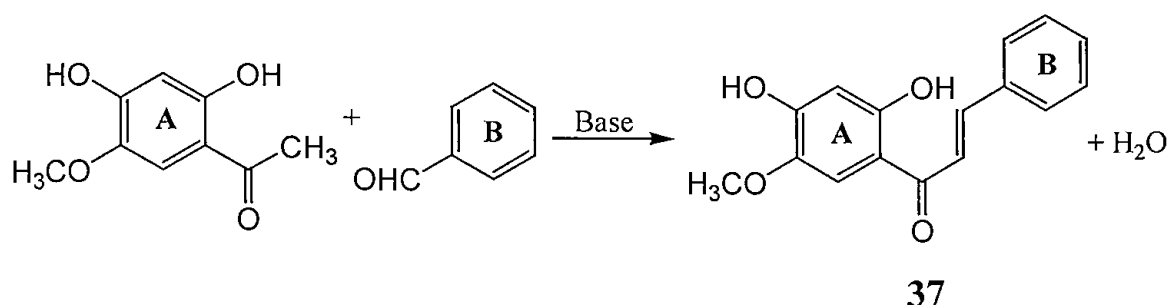
A modification of the kostanecki's method[1] was worked out by Russel *et al.*[7]. It involves the condensation of benzoylated derivatives of o-hyroxya- cetophenones with benzoylated derivative of *o*-hydroxy-benzaldhyde in the presence of dry hydrogenchloride at 0°C followed by hydrolysis with alkali 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$_6$H$_5$

38

39
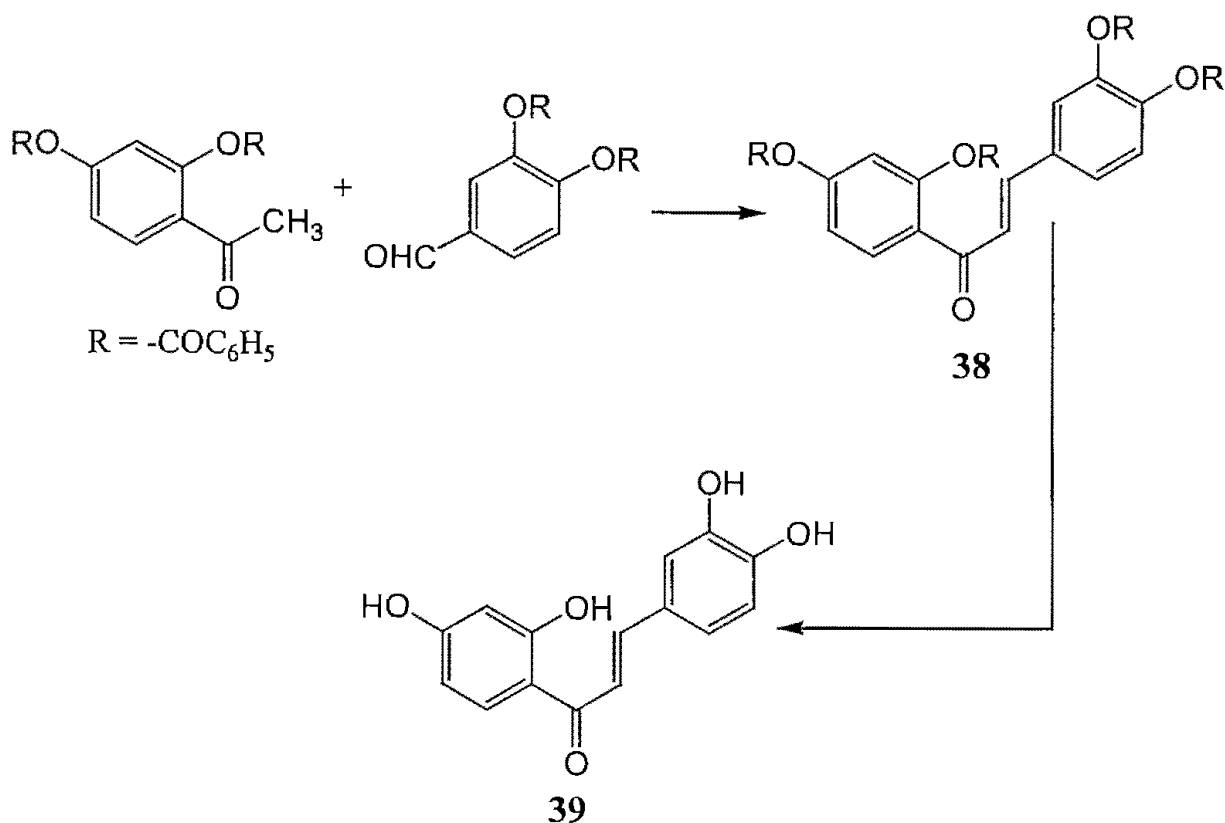
The synthesis of some chalcones has been accomplished by using sodium hydride as the base for the condensation. This method gives good yield even if the hydroxyl groups are not protected. Thus 2′,3,4-trihydroxy-3′,4′,5′-trimethoxychalcone(**40**) has been synthesized by Stout *et al.*[8] by using sodium hydride as the base.

40
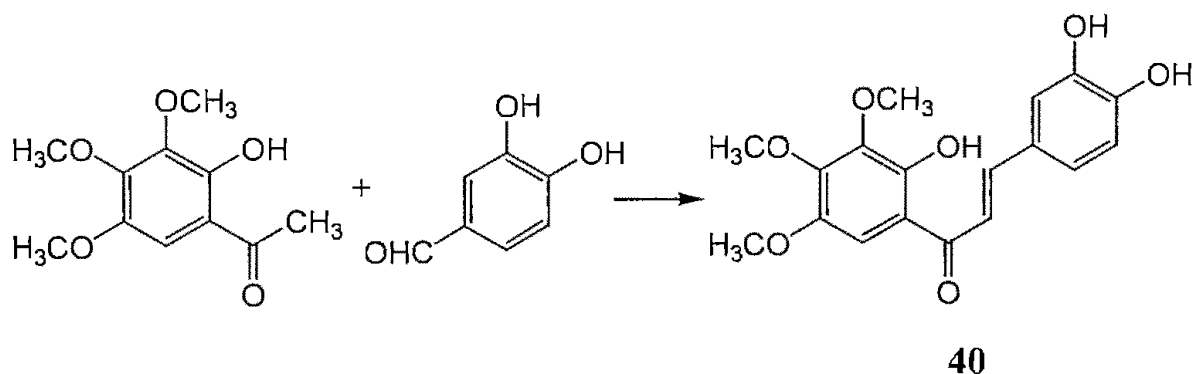
Chalcones have also been synthesized by irradiating a solution of phenyl- cinnamate in methanol, with UV light (253 nm) for 1.5 hours under nitro- gen atmosphere. Synthesis of two isomeric chalcones **41** and **42** were reported by this method[9].

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

41   **42**
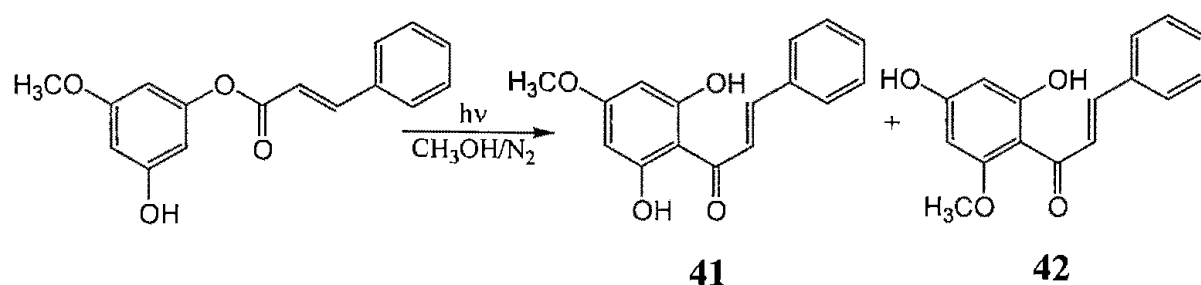
Lupi and co-workers[11] while synthesising various prenylated and chromeno chalcones under alkaline conditions have noticed the yield variation by changing the temperature, solvent and using different bases for condensation. Sodium hydroxide, sodium ethoxide and piperidine were used as bases in solvents 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.

$R= -CH_2-OCH_3$

43
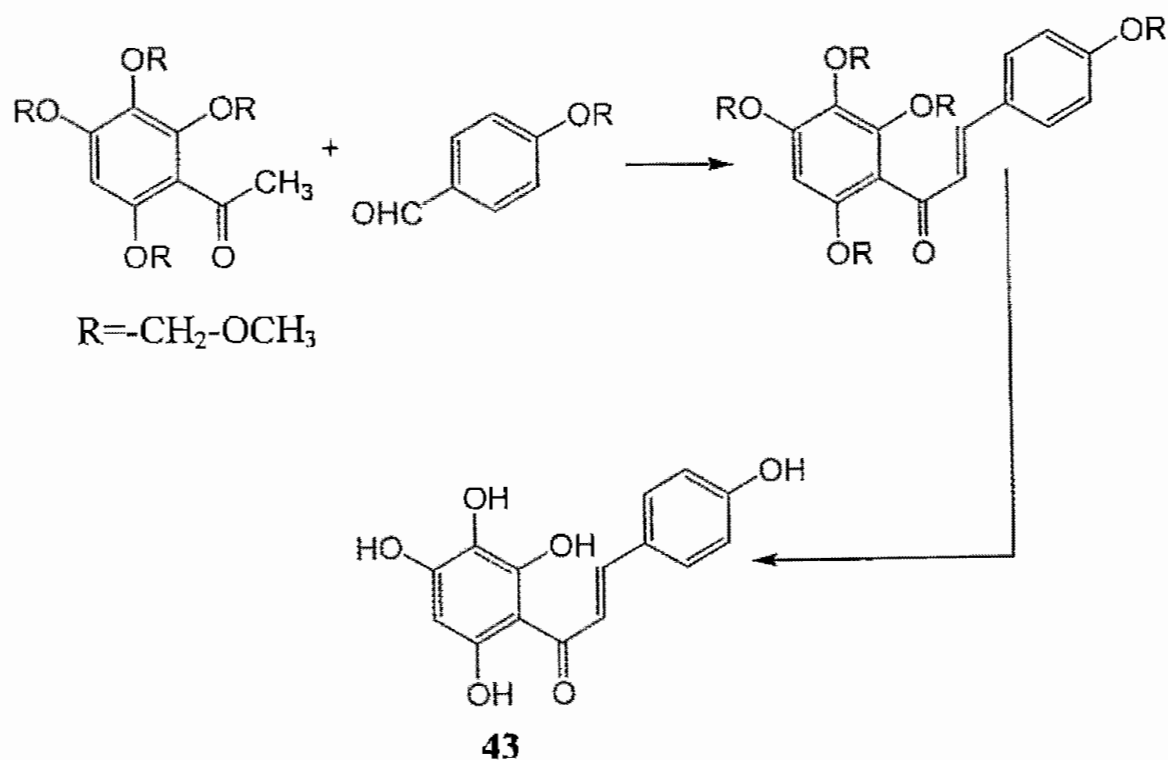
1.5   Mechanism of chalcone formation:

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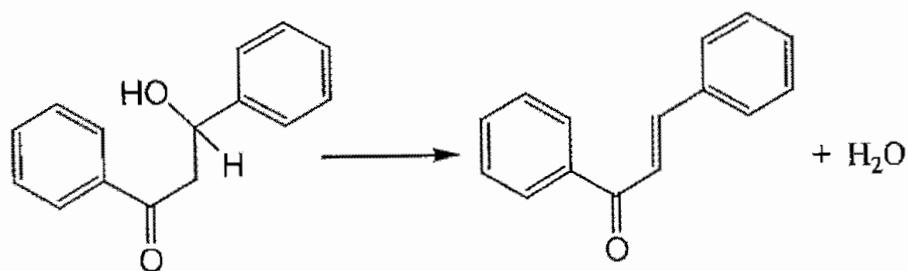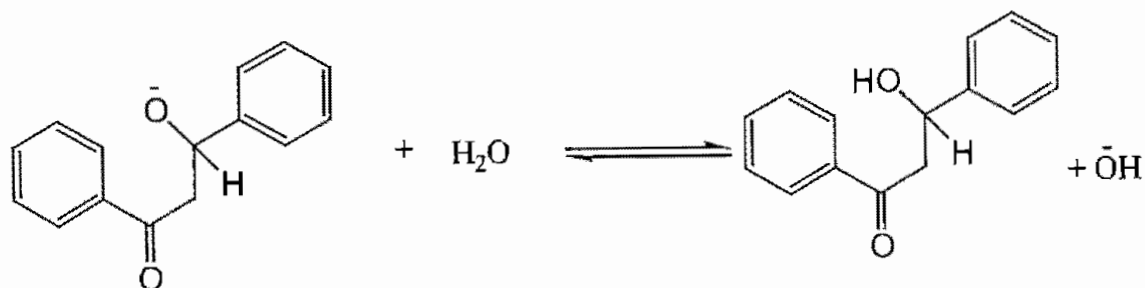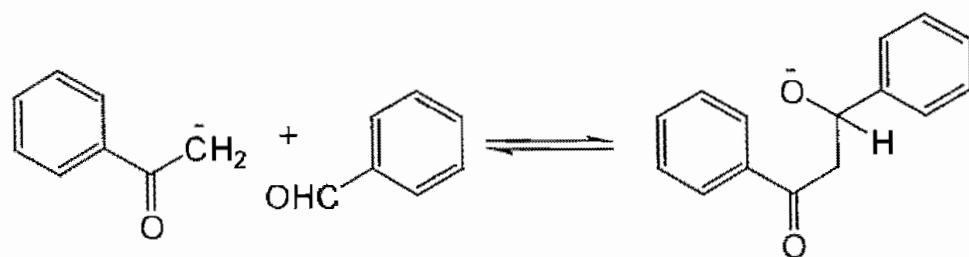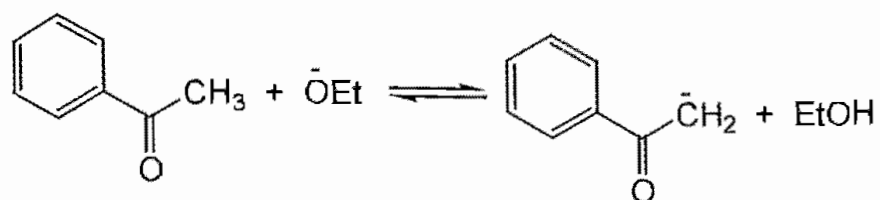
Mechanism-II
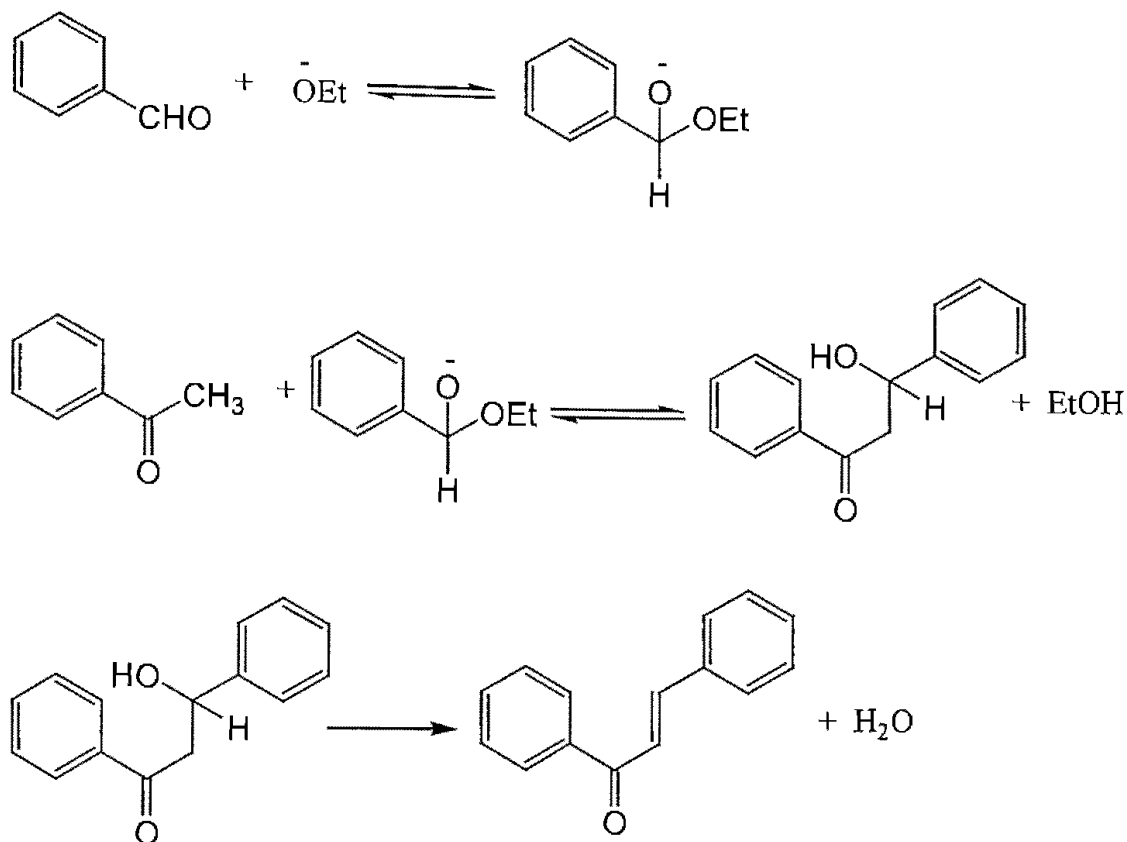
The formation of chalcone by the acid catalyzed condensation of acetophenone and benzaldhyde has been studied[17,18]. The rate of reaction is reported to depend on the first power of the concentration of acetophenone, the first power of the concentration of benzaldehyde, and the Hammett acidity function[17,18].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

Mechanism :
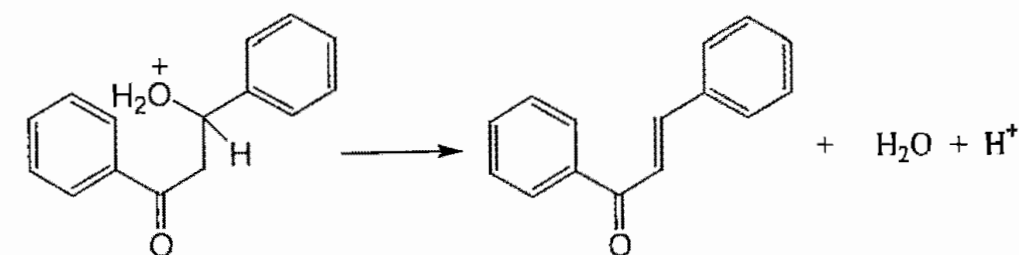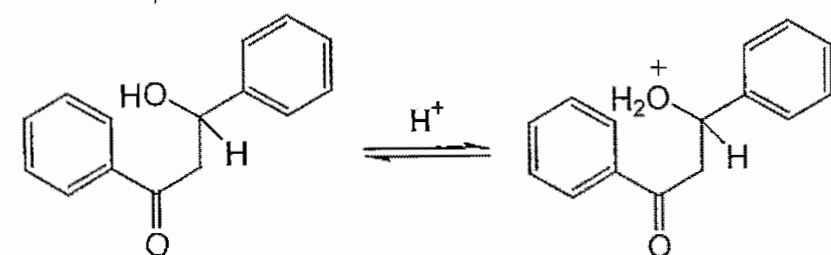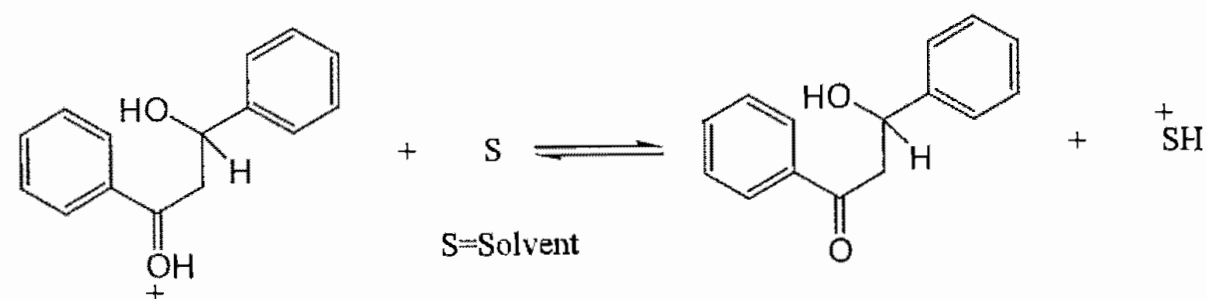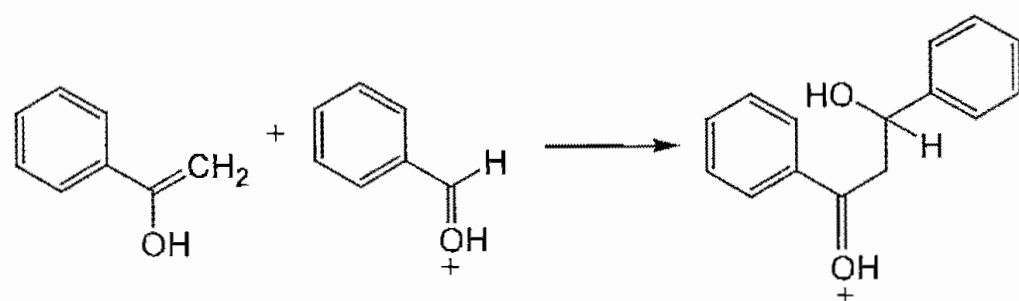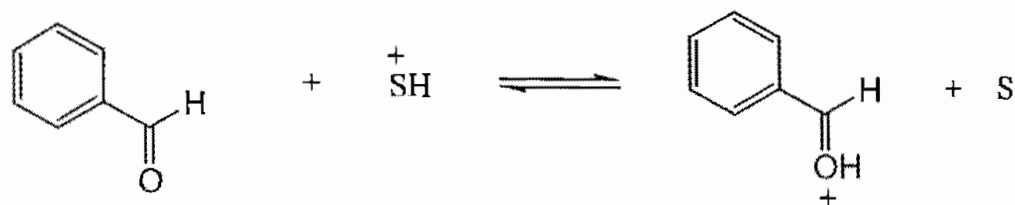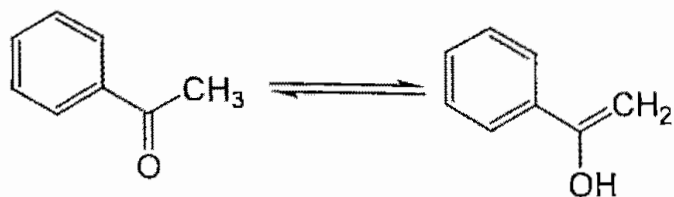
1.6   Chalcone epoxides:
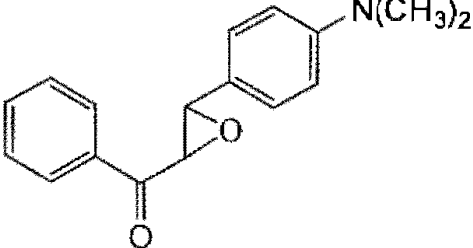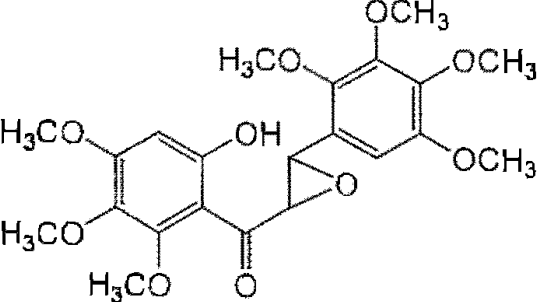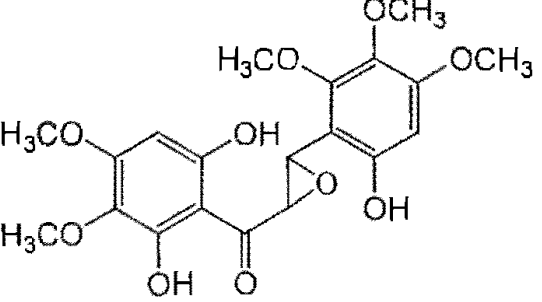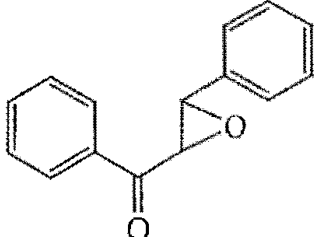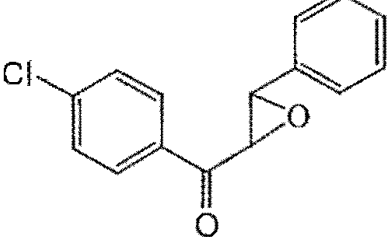
The chalcone epoxides which are also known as the anthoxanthins are white pigments which occur in the plant kingdom. Chalcone epoxides occur naturally in the free state or as glycoside or associated with tannins. Chemically the chalcone epoxides are very closely related to the anthocyanins, the chalcone 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.
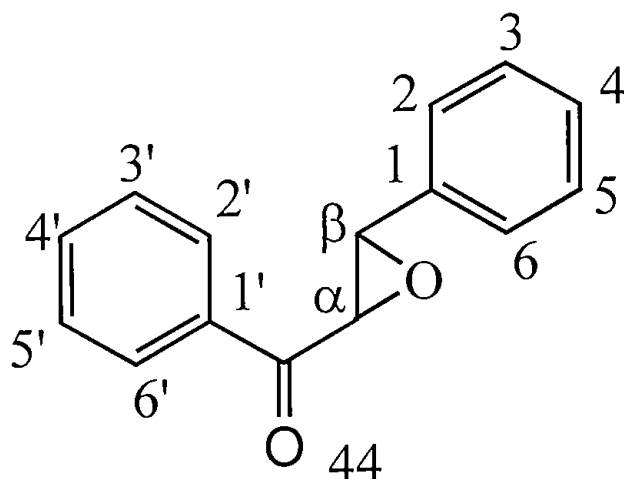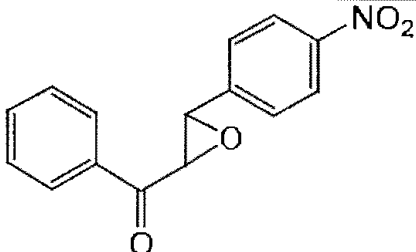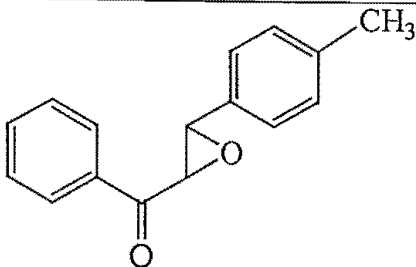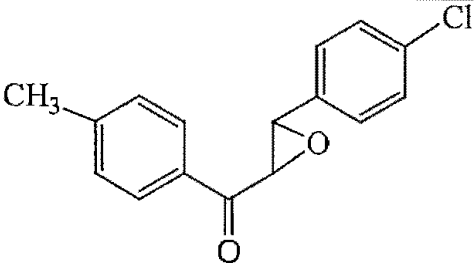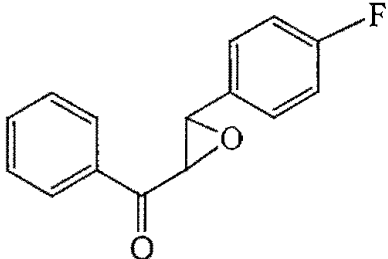
Chalcone epoxide

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.
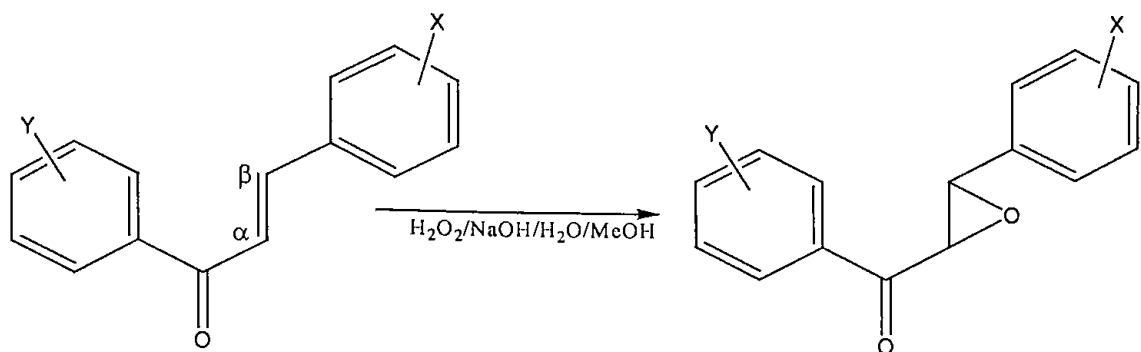
trans Chalcone epoxide :

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:
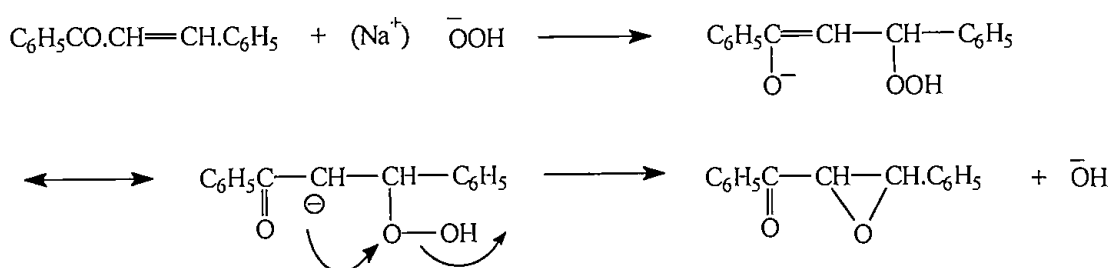
CHAPTER
II

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$_2$O$_2$ 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
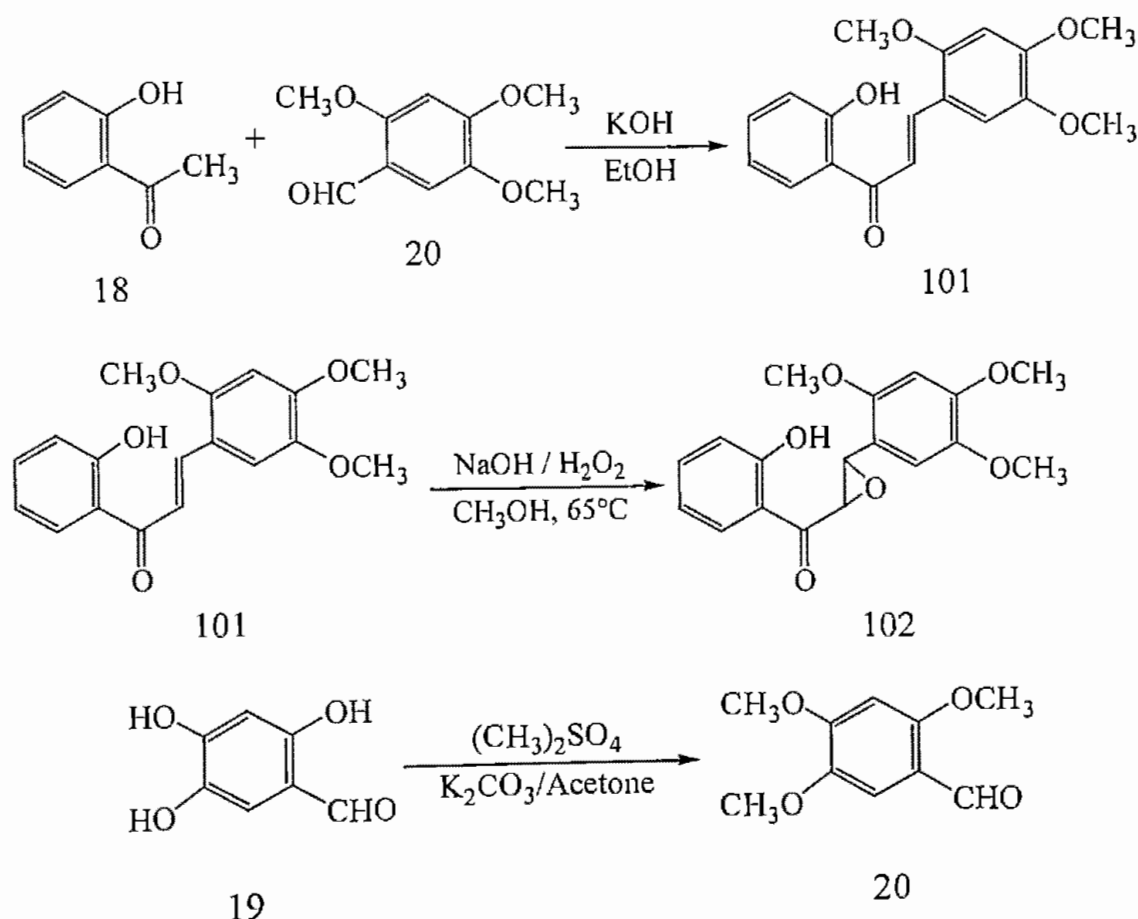
2.1.2 Synthesis of 2'-benzyloxy-2,4,5-trimethoxychalcone epoxide (102) by using NaOH / H₂O₂:

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 / H₂O₂ 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
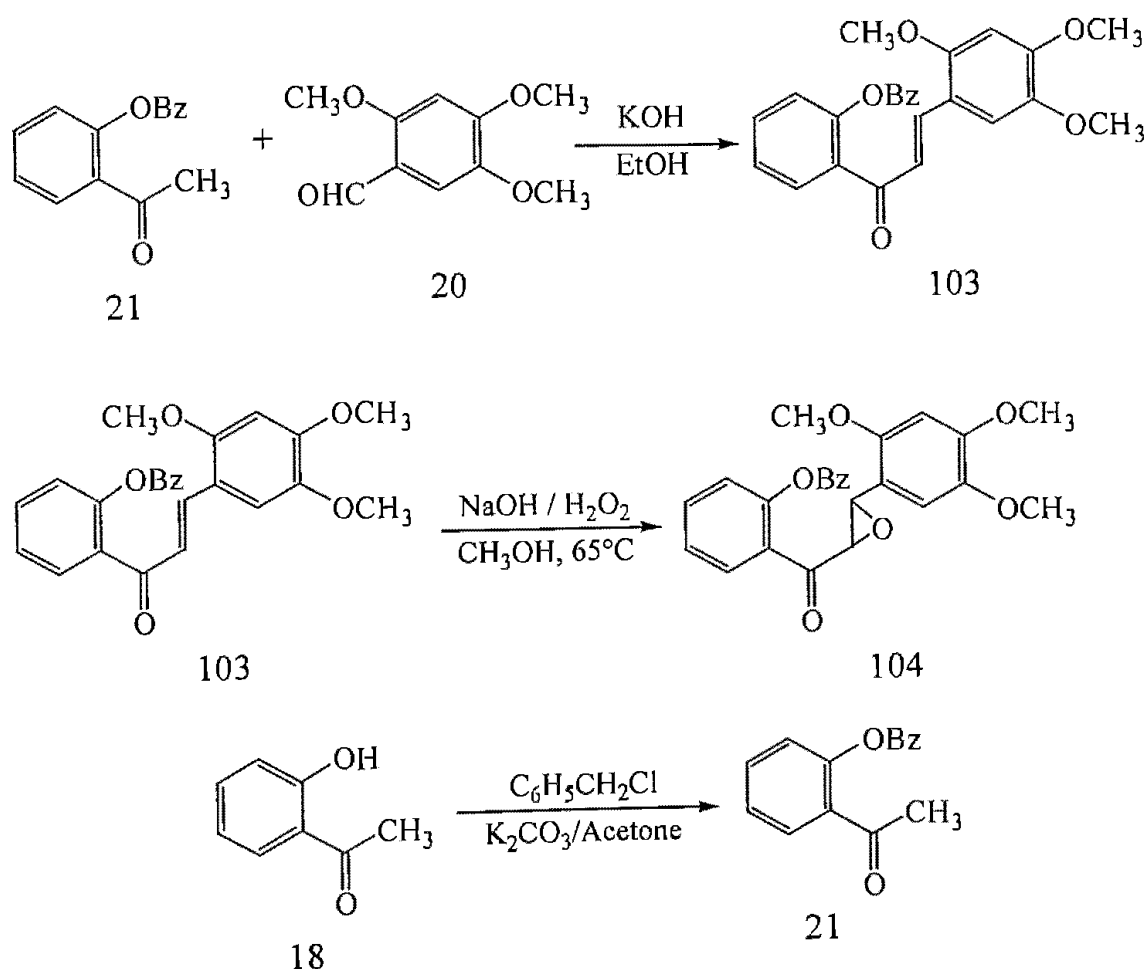
2.1.3 Synthesis of 4′-methyl-2-chlorochalcone epoxide (106) by using NaOH / H₂O₂:

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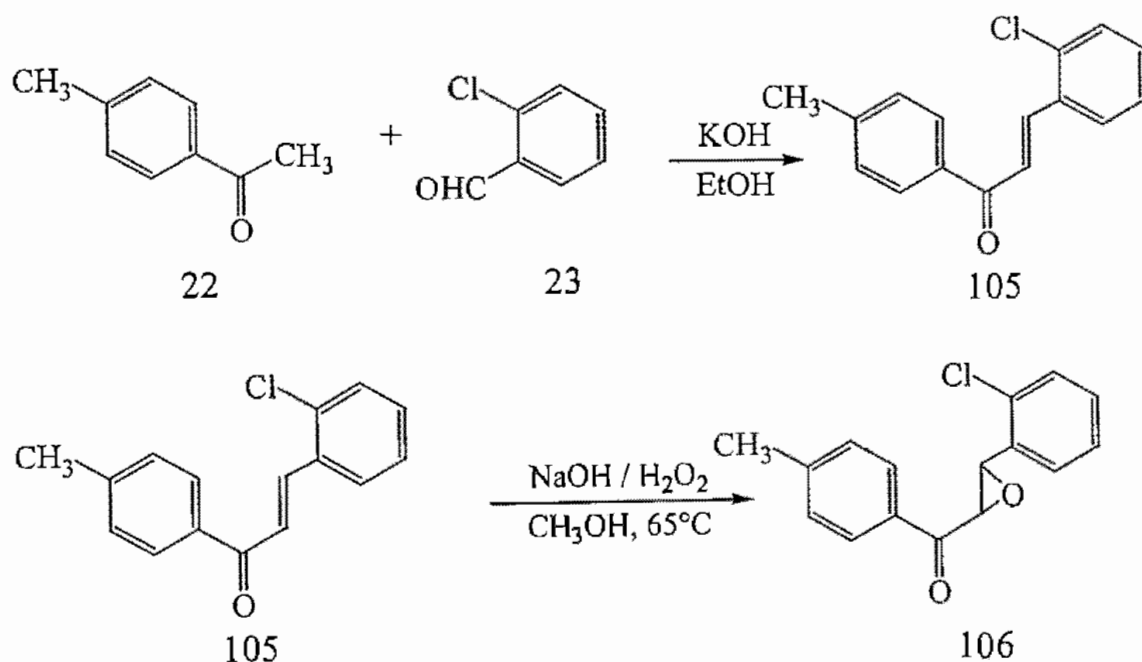
2.1.4 Synthesis of 4'-methyl-4-chlorochalcone epoxide (108) by using NaOH / H_2O_2:

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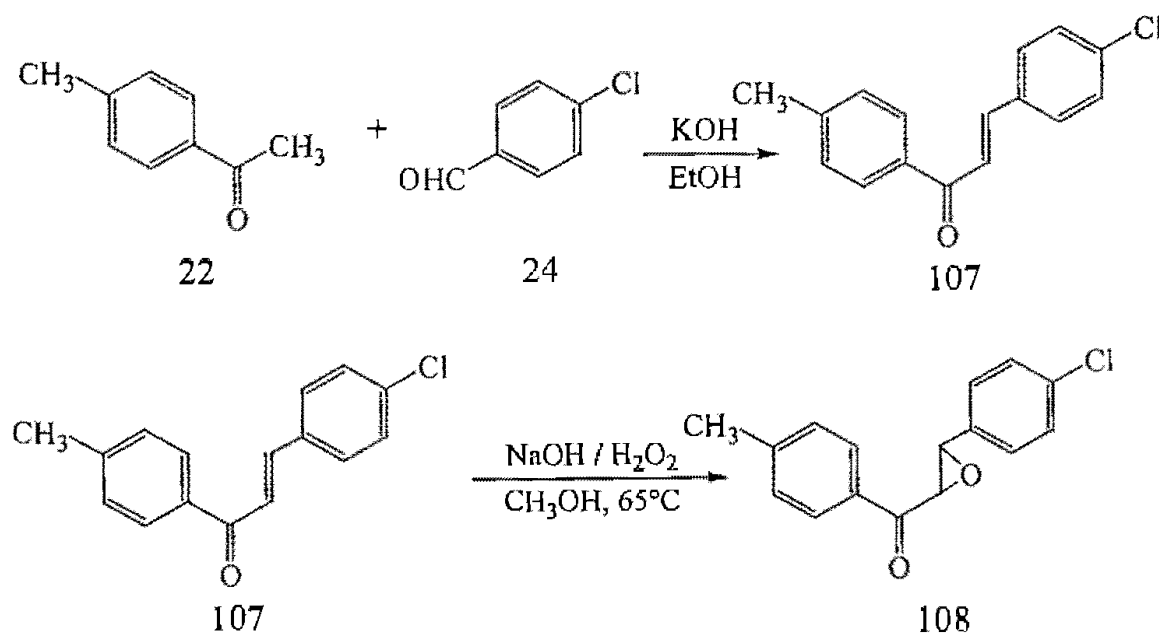
2.1.5 Synthesis of 2'-Hydroxy-4-nitrochalcone epoxide (110) by using NaOH / H_2O_2:

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
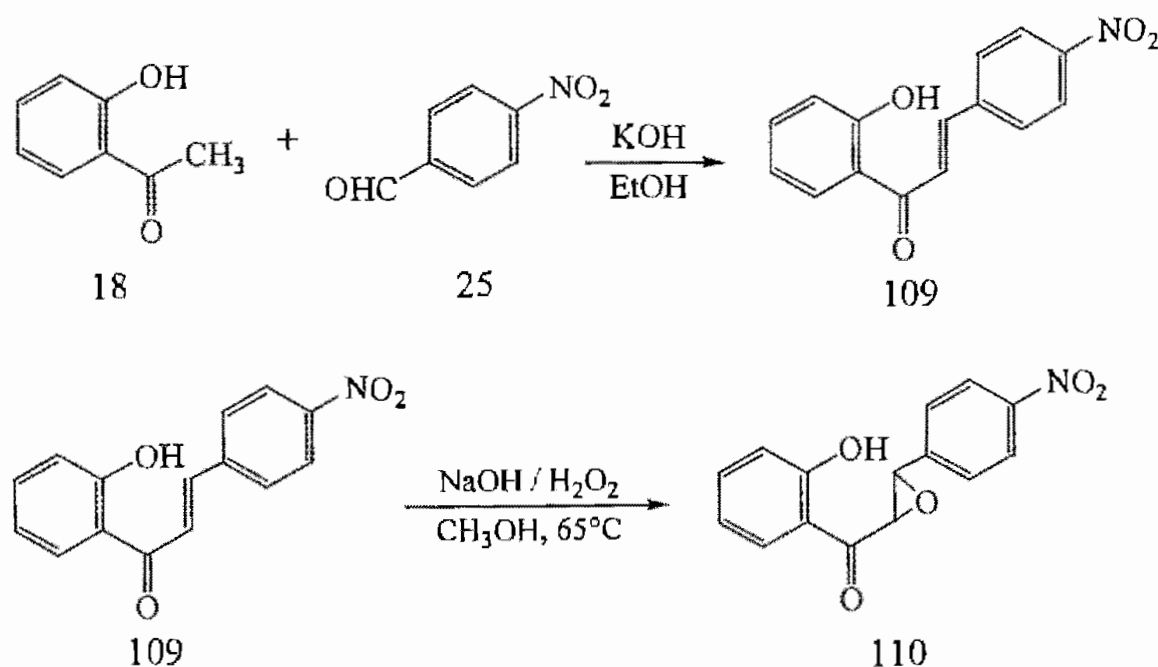
2.1.6 Synthesis of 2'-benzyloxy-4-methoxychalcone epoxide (112) by using NaOH / H₂O₂:

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 / H₂O₂ 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-hydroxybenz-aldehyde **(26)** on methylation with dimethyl sulphate

Scheme-6
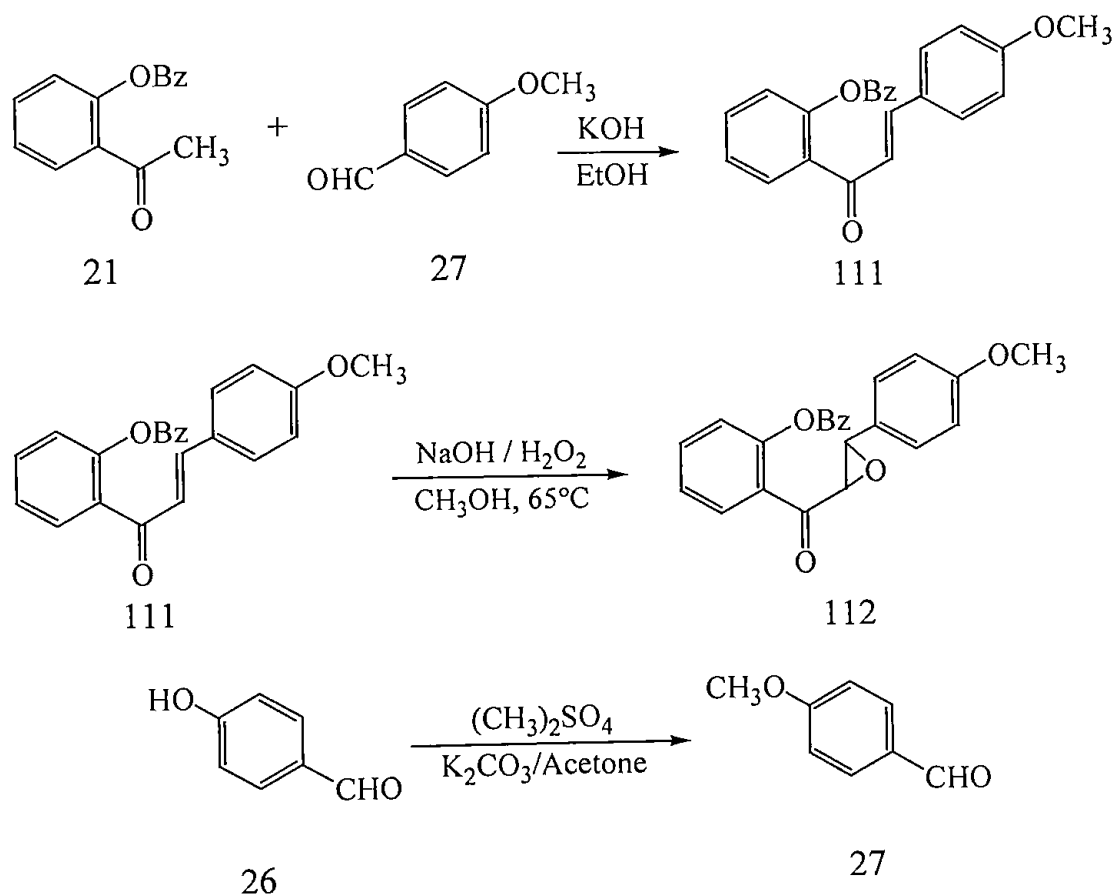
2.1.7 Synthesis of 2′-hydroxy-3,4-methylenedioxychalcone epoxide (114) by using NaOH /H$_2$O$_2$:

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$_2$O$_2$ 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
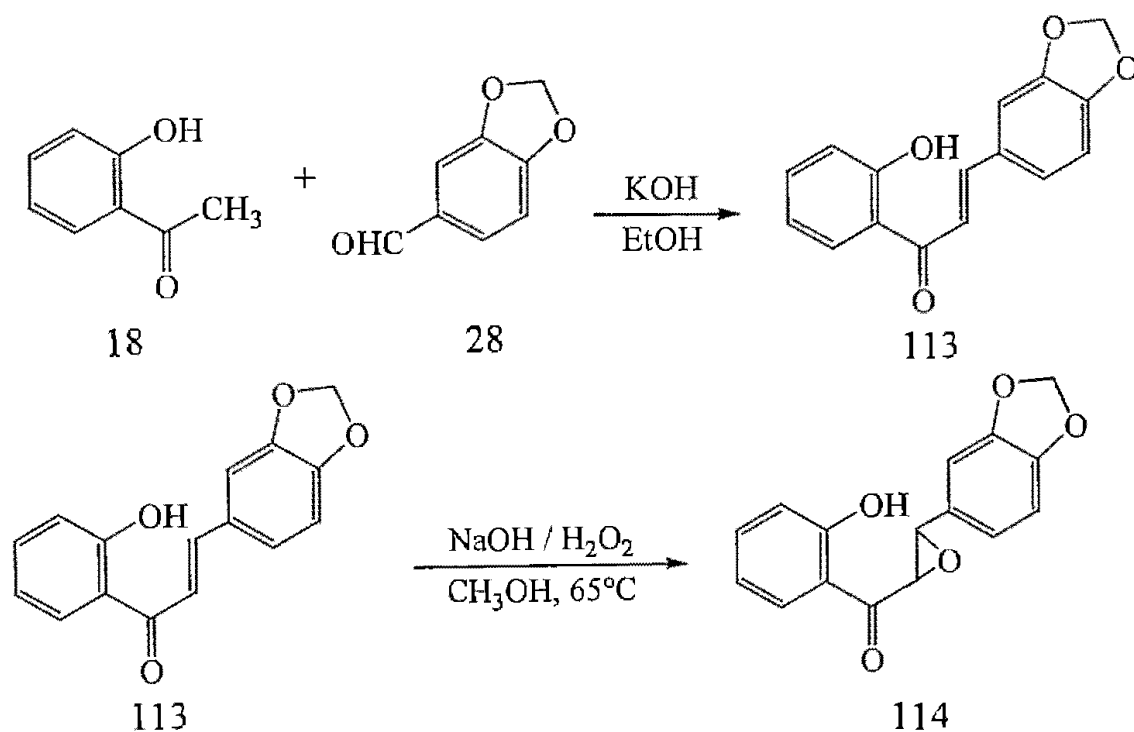
2.1.8 Synthesis of 2'-benzyloxy-3,4-methylenedioxy chalcone epoxide (116) by using NaOH / H_2O_2:

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
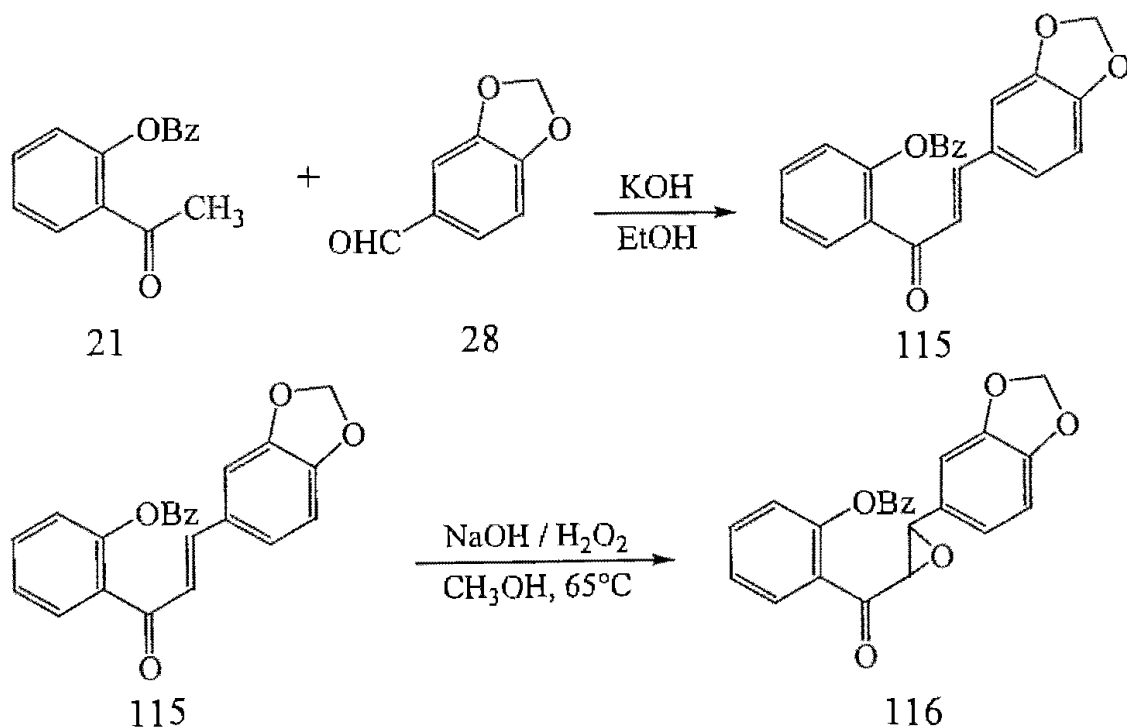
CHAPTER III

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 (CHCl₃):

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$_2$O):

Metallic sodium wire was added in diethyl ether and the mixture was kept overnight and was distilled over P$_2$O$_5$ 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) v_{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
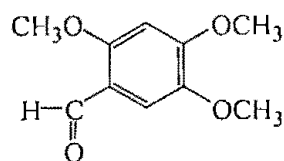
^{13}CNMR (CDCl$_3$-d$_6$): δ 56.0 (C$_2$-O$\underline{C}$H$_3$), 56.3 (C$_4$-O$\underline{C}$H$_3$ and C$_2$-O$\underline{C}$H$_3$), 101.2 (C$_3$), 115.6 (C$_1$), 117.3 (C$_6$), 140.4 (C$_5$), 154.4 (C$_4$), 156.5 (C$_2$), 190.0 (> C = O).

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) v_{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

¹HNMR (CDCl₃-d₆): 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$\underline{H}$).

¹³CNMR (CDCl₃-d₆): δ 56.3 (C_4-OC$\underline{H}_3$ and C_5-OC$\underline{H}_3$), 56.4 (C_2-OC$\underline{H}_3$), 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) v_{max}: 3433, 2882, 1704, 1579, 1512, 1489, 1424, 1361, 1304, 1271, 1193, 1134, 1033, 974, 908, 882, 811, 793, 761, 714, 639 cm^{-1}
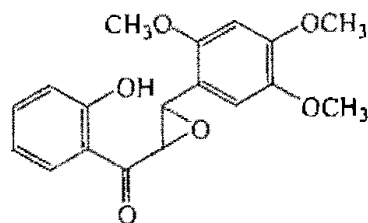
102

¹HNMR (CDCl₃-d₆): 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$\underline{H}$).

¹³CNMR (CDCl₃-d₆): 48.5 (C_β), 56.3 (C_2-O$\underline{C}$H₃, C_4-O$\underline{C}$H₃ and C_5-O$\underline{C}$H₃), 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$_6$H$_5$), 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-$\underline{C}$H$_3$), 77.8 (C$_2$-O$\underline{C}$H$_2$-C$_6$H$_5$), 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) v_{max}: 3032 1666, 1606, 1548, 1472, 1415, 1337, 1281, 1189, 1132, 1064, 1002, 972, 873, 831, 817, 766, 739, 648 cm⁻¹.

103

¹HNMR(CDCl₃-d₆): 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, $C_{2'}$-OCH₂-$C_6\underline{H}_5$), 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).

¹³CNMR (CDCl₃-d₆): 56.3 (C_4-OC$\underline{H}_3$ and C_5-OC$\underline{H}_3$), 56.4 (C_2-OC$\underline{H}_3$), 77.8 ($C_{2'}$-OC$\underline{H}_2$-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**)

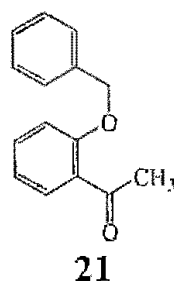## Synthesis of 2′-benzyloxy-2,4,5-trimethoxychlcone epoxide (104):

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.

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

104
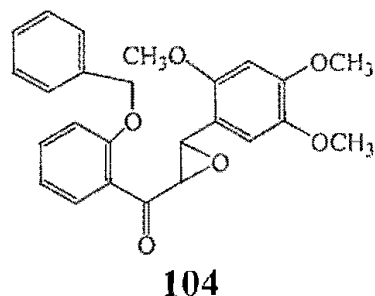
^{1}HNMR (CDCl$_3$-d$_6$): δ 3.73 (s, 9H C$_2$-OC$\underline{H}_3$, C$_4$-OC$\underline{H}_3$ and C$_5$-OC$\underline{H}_3$) 4.35(d, 1H, J = 2 Hz C$_\beta$-H), 4.41 (d, 1H, J = 2 Hz C$_\alpha$-H), 5.22 (s, 2H, C$_{2'}$-OC$\underline{H}_2$-C$_6$H$_5$), 6.10 (s, 1H, C$_3$-H), 6.48 (s, 1H, C$_6$-H) 6.85 (d, 1H, C$_{3'}$-H), 6.90 (m, 1H, C$_{5'}$-H), 7.19 (s, 5H, C$_{2'}$-OCH$_2$-C$_6\underline{H}_5$), 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$_\beta$), 56.3 (C$_2$-OC$\underline{H}_3$, C$_4$-OC$\underline{H}_3$ and C$_5$-OC$\underline{H}_3$), 70.0 (C$_\alpha$), 77.8 (C$_{2'}$-OC$\underline{H}_2$-C$_6$H$_5$), 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):
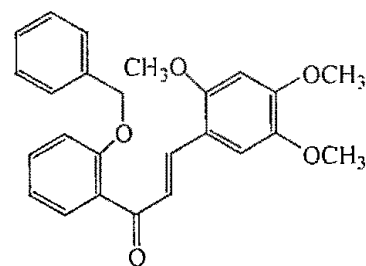
A mixture of 4-methylacetophenone (**22**, 10 mmol 1.34g) and 2-chlorobenzaldehyde (**23**, 1.1 eqv, 1.54g) in ethanolic solution of KOH (5% 15mL) was kept at room temperature for about 80 hours. The reaction mixture as diluted with ice cold water, acidified with cold dil. HCl and extracted with ether. 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}.
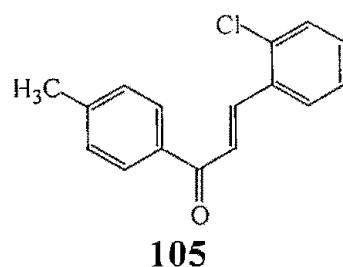
^{1}HNMR (CDCl$_3$-d$_6$): δ 2.35 (s, 3H, C$_{4'}$-C$\underline{H}_3$); 7.08 (m, 1H, C$_4$-H); 7.09 (m, 1H C$_5$-H); 7.22 (d, 1H, J = 2.6 Hz, C$_3$-H); 7.24 (d, 1H J = 2.6 Hz C$_6$-H); 7.25 (d, 2H, J = 8.6 Hz C$_{3'}$-H & C$_{5'}$-H); 7.39 (d, 1H, J = 16 Hz, C$_\alpha$-H); 7.69 (d, 2H, J = 8.6 Hz C$_{2'}$-H and C$_{6'}$-H); 8.17 (d, 1H, J = 16 Hz, C$_\beta$-H).

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^{13}CNMR (CDCl$_3$-d$_6$): δ 20.9 (C$_{4'}$-C$\underline{H}_3$), 123.3 (C$_\alpha$), 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$_\beta$) 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.

IR (KBr) v_{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$_\beta$-H); 4.42 (d, 1H, C$_\alpha$-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).

106

^{13}**CNMR (CDCl$_3$-d$_6$):** δ 20.9 (C$_{4'}$-$\underline{C}$H$_3$), 49 (C$_\beta$), 68.9 (C$_\alpha$), 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).
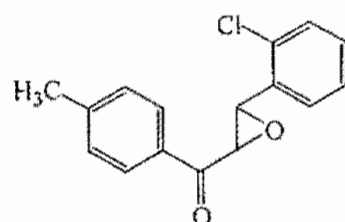
It was identified as 4′-methyl-2-chlorochalcone epoxide (106).

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}.

107
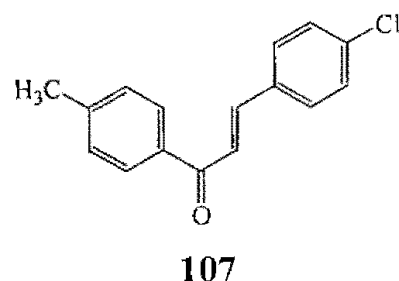
^{1}HNMR(CDCl$_3$-d$_6$): δ 2.36 (s, 3H, $C_{4'}$-C$\underline{H}_3$); 7.22 (d, 2H, J = 8.6 Hz, C_3-H & C_5-H), 7.24 (d, 2H, J = 8.6 Hz, C_2-H and C_6-H), 7.25 (d, 2H, J = 8.6 Hz $C_{3'}$-H & $C_{5'}$-H), 7.56 (d, 2H, J = 16 Hz, C_α-H), 7.69 (d, 2H, J = 8.6 Hz, $C_{2'}$-H & $C_{6'}$-H), 7.90 (d, 1H, 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) v_{max}: 3040, 2955, 1705, 1630, 1562, 1485, 1440, 1402, 1150, 1127, 1079, 1009, 980, 934, 862, 789, 779, 752 cm^{-1}.

108
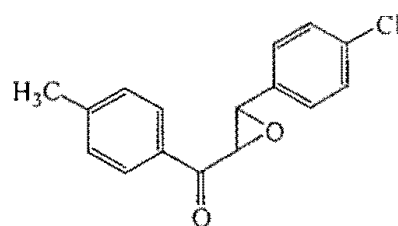
^{1}HNMR (CDCl$_3$-d$_6$): δ 2.34 (s, 3H, C$_4$'-CH$_3$); 4.35 (d, 1H, J = 16 Hz C$_β$-H); 4.42 (d, 1H, J = 16 Hz, C$_α$-H); 7.13 (d, 2H J = 8.6 Hz, C$_2$-H and C$_6$-H); 7.14 (d, 2H, J = 8.6 Hz, C$_3$'-H and C$_5$'-H); 7.20 (d, 2H, J = 8.6 Hz, C$_3$-H and C$_5$-H); 7.77 (d, 2H, J = 8.6Hz C$_2$'-H and C$_6$'-H).

^{13}CNMR (CDCl$_3$-d$_6$): δ 20.9 (C$_4$'-CH$_3$), 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 (>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) v_{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
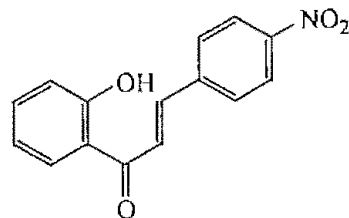
^{1}HNMR (CDCl-d$_6$): δ 6.92 (d, 1H, J = 2.6 Hz, $C_{3'}$-H); 7.01 (m, 1H $C_{5'}$-H); 7.37 (m, 1H, $C_{4'}$-H); 7.56 (d, 2H, C_2-H and C_6-H); 7.64 (d, 1H, $C_{6'}$ - H); 7.85 (d, 1H, J = 16 Hz, C_α-H); 8.04 (d, 1H, J = 16 Hz, C_β-H); 8.14 (d, 2H, C_3-H and C_5-H); 12.76 (s, 1H, $C_{2'}$-O$\underline{H}$).

^{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
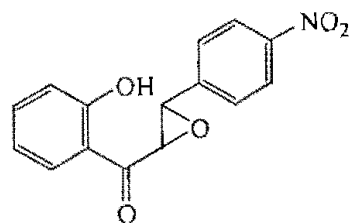
^{1}HNMR (CDCl$_3$-d$_6$): δ 4.35 (d, 1H, J = 2 Hz C$_\beta$-H); 4.42 (d, 1H, J = 2 Hz, C$_\alpha$-H), 6.81 (d, 1H, C$_{3'}$-H), 6.90 (m, 1H, C$_{5'}$-H) 7.27 (m, 1H, C$_{4'}$-H), 7.45 (d, 2H, C$_2$-H and C$_6$-H), 7.72 (d, 1H, C$_{6'}$-H), 8.12 (d, 2H, C$_3$-H and C$_5$-H), 11.40 (s, 1H, C$_{2'}$-OH)

^{13}CNMR (CDCl$_3$-d$_6$): δ 58.1(C$_\beta$), 69.7 (C$_\alpha$), 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) v_{max}: 3078, 2962, 1635, 1560, 1480, 1470, 1400, 1360, 1280, 1220, 1160, 1120, 825, 600 cm^{-1}.

27
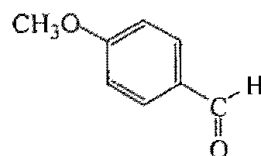
^{1}H NMR (CDCl$_3$-d$_6$): δ 3.73 (s, 3H, C$_4$-OC$\underline{H}_3$), 6.96 (dd, 2H, J = 2.24 Hz and J = 7.6 Hz, C$_3$-$\underline{H}$ and C$_5$-H), 7.70 (dd, 2H, J = 2.24 Hz and 7.6 Hz, C$_2$-$\underline{H}$ and C$_6$-$\underline{H}$), 9.87 (s, 1H, C$_1$-C$\underline{H}$O).

^{13}C NMR (CDCl$_6$-d$_6$): δ 56.0 (C$_4$-OC$\underline{H}_3$), 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}

^{1}HNMR (CDCl$_3$): δ 3.73(s, 3H, C$_4$-OC$\underline{H}_3$), 5.20 (s, 2H, C$_{2'}$-OC$\underline{H}_2$C$_6$H$_5$), 6.72 (d, 2H, J = 2.6 Hz, C$_3$-H and C$_5$-H), 6.96 (d, 1H, C$_{3'}$-H),
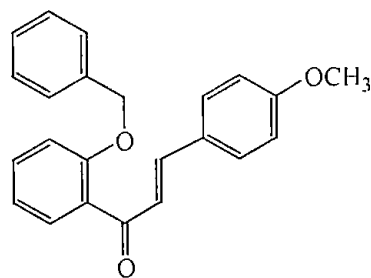
111

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$_\alpha$-H), 7.70 (d, 1H, C$_{6'}$-H), 7.90 (d, 1H, J = 16 Hz, C$_\beta$-H).

^{13}CNMR (CDCl$_3$): δ 56 (C$_4$-OC$\underline{H}_3$), 77.8 (C$_{2'}$-O-C$\underline{H}_2$-C$_6$H$_5$), 114 (C$_3$ and C$_5$), 114.6 (C$_{3'}$), 121.3 (C$_{5'}$), 122.3 (C$_{1'}$), 123.3 (C$_\alpha$), 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$_\beta$), 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) v_{max}: 3036, 1707, 1506, 1456, 1259, 1094, 779, 735, 697, 460 cm^{-1}.

112
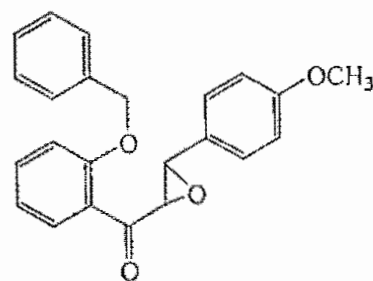
^{1}HNMR (CDCl$_3$): δ 3.73 (s, 3H, C_4-OC$\underline{H}_3$), 4.37 (d, 1H, J = 2 Hz, C_β-H) 4.43 (d, 1H, J = 2 Hz, C_α-H), 5.19 (s, 2H, $C_{2'}$-OC$\underline{H}_2$-C_6H_5), 6.70 (d, 2H, C_3-H and C_5-H) 6.85 (d, 1H, $C_{3'}$-H), 6.90 (m, 1H, $C_{5'}$-H), 7.08 (d, 2H, C_2-H and C_6-H), 7.19 (s, 5H, $C_{2'}$-OCH$_2$-$C_6\underline{H}_5$) 7.33 (m, 1H, $C_{4'}$-H), 7.78 (d, 1H, $C_{6'}$-H).

^{13}CNMR (CDCl$_3$): δ 56 (C_4-OC$\underline{H}_3$), 58.1 (C_β) 69.7 (C_α), 77.8 ($C_{2'}$-OC$\underline{H}_2$-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
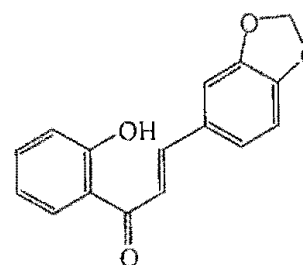
^{1}HNMR (CDCl$_3$): δ 5.90 (s, 2H, C$_3$-OC$\underline{H}_2$-C$_4$), 6.61 (d, 1H, J = 8.6 Hz, C$_5$-H), 6.70 (s, 1H, J = 8.6 Hz, C$_2$-H), 6.75 (d, 1H, J = 8.6 Hz C$_6$-H), 6.92 (d, 1H, J = 8.6 Hz, C$_{3'}$-H), 7.01 (m, 1H, C$_{5'}$-H), 7.37 (m, 1H, C$_{4'}$-H), 7.56 (d, 1H, J = 16 Hz C$_\alpha$-H) 7.64 (d, 1H, J = 8.6 Hz, C$_{6'}$-H), 7.90 (d, 1H, J = 16 Hz C$_\beta$-H), 12.89 (s, 1H, C$_{2'}$ - OH).

^{13}CNMR (CDCl$_3$): δ 91.3 (C$_3$-OC$\underline{H}_2$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$_\alpha$), 123.9 (C$_{1'}$), 128.2 (C$_1$), 131.1 (C$_{6'}$), 135.7 (C$_{4'}$), 142.8 (C$_\beta$) 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) v_{max}: 3464, 1697, 1504, 1454, 1415, 1367, 1300, 1261, 1167, 1114, 1074, 1037, 931, 914, 868, 831, 812, 763, 671 cm^{-1}.

114
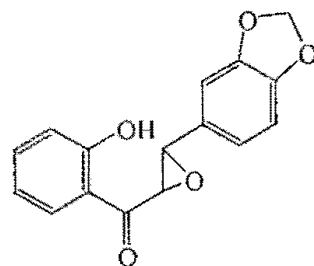
¹HNMR (CDCl₃): δ 4.36 (d, 1H, J = 2 Hz C_β-H), 4.44 (d, 1H, J = 2 Hz, C_α-H), 5.91 (s, 2H, C_3-OC$\underline{H}_2$O-C_4), 6.59 (d, 1H, J = 2.6 Hz, C_5-H), 6.59 (s, 1H, C_2-H), 6.64 (d, 1H, C_6-H), 6.81 (d, 1H, $C_{3'}$-H), 6.90 (m, 1H, $C_{5'}$-H), 7.27 (m, 1H, $C_{4'}$-H), 7.72 (d, 1H, $C_{6'}$-H), 11.85 (s, 1H, $C_{2'}$-O$\underline{H}$)

¹³CNMR (CDCl₃): δ 58.4 (C_β), 69.7 (C_α), 91.3 (C_3-OC$\underline{H}_2$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) v_{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 ($C_{2'}$-OCH$_2$-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).
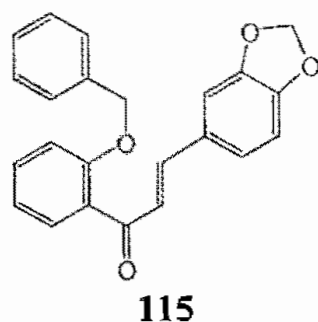
It was identified as 2'-benzyloxy-3,4-methylenedioxy chalcone (**115**).

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.

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

1HNMR (CDCl₃): δ 4.37 (d, 1H, J = 2Hz, C_β-H), 4.43 (d, 1H J = 2Hz, C_α-H), 5.21 (s, 2H, $C_{2'}$-OC$\underline{H}_2$-C_6H_5), 5.92, (s, 2H, C_3-OC$\underline{H}_2$O-C_4),
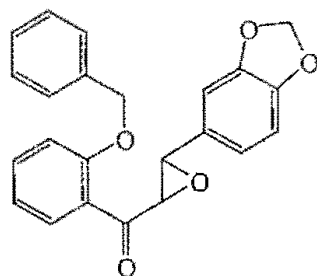
116

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**).

13CNMR (CDCl₃): δ 58.4 (C_β), 69.7 (C_α), 77.8 ($C_{2'}$-O$\underline{C}$H$_2$-C_6H_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 v 1675 cm^{-1} indicating the presence of a carbonyl group (>C=O).

The ^{1}H-NMR spectrum of compound 20 showed the presence of three methoxy group by one singlet at δ 3.73 (C$_2$-OCH$_3$, C$_4$-OCH$_3$ and C$_5$-OCH$_3$) integrating for nine protons. The two aromatic protons which appeared as two singlets at δ 6.36 and 7.10 assigned to C$_3$-H and C$_6$-H,
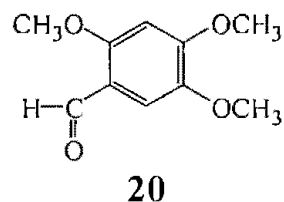
integrating for one proton each respectively. A singlet at δ 10.24 was indicated the presence of an aldehydic proton integrating for one proton. The compound 20 was identified as 2,4,5-trimethoxybenzaldehyde.

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].

The IR absorption frequency at v 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.
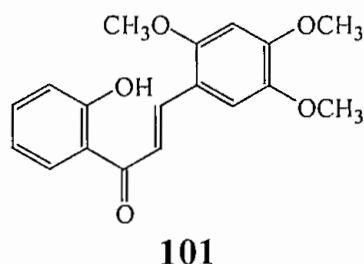
The absorption frequency at v 1677 cm^{-1} showed the presence of a conjugated carbonyl group (>C=O). The ^{1}H-NMR spectrum explained the presence of three methoxy group in the B ring at δ 3.73 (C_2-OC$\underline{H}_3$; C_4-OCH_3; C_5-OC$\underline{H}_3$) as a singlet integrating for nine protons respectively. 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 v 1704 cm^{-1} showed the presence of a carbonyl group (>C=O). The C_α-H and C_β-H protons of **102** appeared as two doublets at δ 4.43 (J = 2Hz C_α-H) and 4.36 (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 4.36 (C_β-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
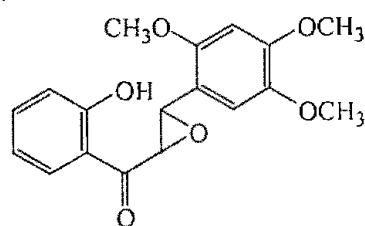
102

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-OCH$_3$, C_4-OCH$_3$ and C_5-OCH$_3$) 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-H and C_6-H integrating for one protons each respectively.

Benzylation of 2-hydroxyacetophenone (**18**) using benzyl chloride gave one products e.g. 2-benzyloxyacetophenone (**21**) 2-benzyloxyaceto-phenone (**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$_6$H$_5$) 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.

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 ^{1}H-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$_6$H$_5$) and 7.19 (5H -O-CH$_2$-C$_6$$\underline{H}_5$) indicated the presence of benzyloxy group. The
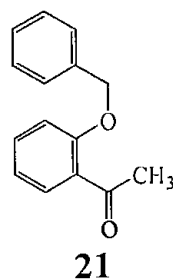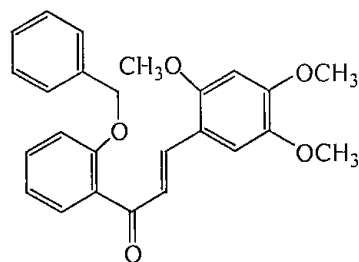
103

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$_\alpha$-H and C$_\beta$-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, ^{1}H-NMR and ^{13}C-NMR) and elemental analysis.

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

104
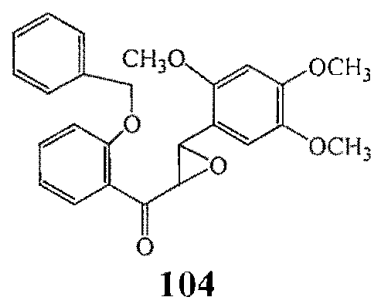
one proton each respectively. The IR absorption frequency (>C=O, ν 1701 cm^{-1}) and the ^{1}H-NMR (C_α-H, δ 4.41 and C_β-H, δ 4.35) of the compound **104** indicated the epoxide ring comparing its corresponding chalcone (**103**). Two singlets at δ 5.20 (2H, C_2-O-C$\underline{H}_2$-C_6H_5) and 7.19 (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.

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.

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 ($>C = O$).

105
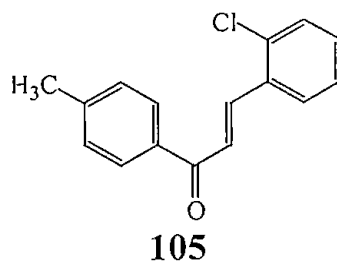
The ^{1}H-NMR spectrum explained the presence of methyl group in A ring at δ 2.35 (s, 3H, C$_{4'}$-C$\underline{H}_3$). The four aromatic protons of B ring appeared as two multiplets and two doublets at δ 7.09 (m, 1H, C$_5$-H); 7.08 (m, 1H, C$_4$-H) and 7.24 (d, 1H, J = 2.6 Hz C$_6$-H); 7.22 (d, 1H, 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$_\alpha$-H and C$_\beta$-H protons of **105** appeared as two doublets at δ 7.39 (J = 16 Hz, C$_\alpha$-H) and 8.17 (J = 16 Hz, C$_\beta$-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 $\lambda_{max}$ 245 and 280 nm suggested the presence of a epoxide skeleton. The IR absorption frequency at v 1703 cm^{-1} showed the presence of a carbonyl group ($>C = O$).

106
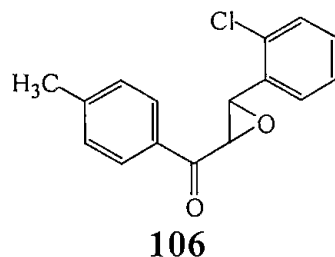
The C_α-H and C_β-H protons of **106** appeared as two doublets at δ 4.42 (1H, J = 2 Hz) and 4.34 (1H, J = 2 hz) integrating for one proton each respectively. The ^{1}H-NMR at 4.42 (C_α-H) and δ 4.34 (C_β-H) of the compound **106** indicated the epoxide ring comparing its corresponding chalcone (**105**). 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.

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 v 1683 cm^{-1} showed the presence of a conjugated carbonyl group (> C=O).

107
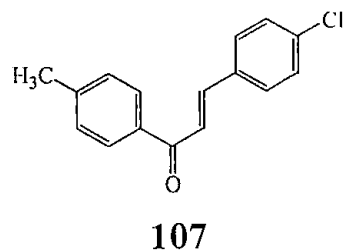
The ^{1}H-NMR spectrum explained the presence of methyl group in the A ring at δ 2.36 (s, 3H, $C_{4'}$-C$\underline{H}_3$). The four aromatic protons of A ring appeared as two doublets at δ 7.25 (2H, J = 8.6 Hz, $C_{3'}$-H and $C_{5'}$-H) and δ 7.69 (2H, J = 8.6 Hz $C_{2'}$-H and $C_{6'}$-H) integrating 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.

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.

108
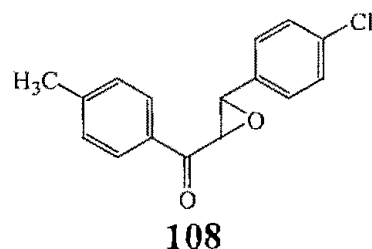
The ^{1}H-NMR at δ 4.42 (C_α-H) and δ 4.35 (C_β-H) of the compound **108** indicated the epoxide ring comparing its corresponding chalcone (**107**). The four aromatic protons of A ring appeared as two doublets at δ 7.14 (d, 2H, J = 8.6 Hz $C_{3'}$-H and $C_{5'}$-H) and 7.77 (d, 2H, J = 8.6 Hz $C_{2'}$-H 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.

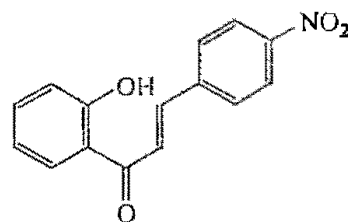The UV absorption band of **109** in methanol at λ_{max} 243, 265 and 385 nm. suggested the presence of a chalcone skeleton. The IR absorption frequency at v 3455 cm^{-1} indicated the presence of hydroxyl group and it gave light brown color with alcoholic ferric chloride solution confirmed that compound **109** has free hydroxyl group. The absorption frequency at v 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, $C_{2'}$-OH). 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.

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.
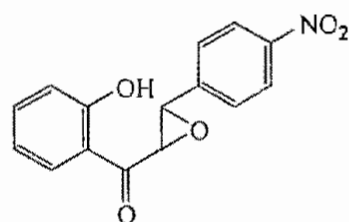
110

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.
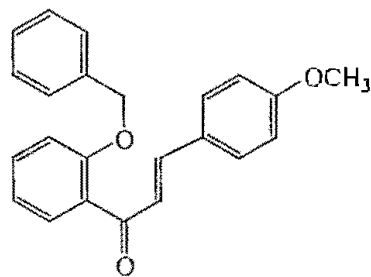
suggested the presence of a chalcone skeleton. The IR absorption frequency at v 1679 cm^{-1} showed the presence of a conjugated carbonyl group (>C=O). The ^{1}H-NMR spectrum explained the presence of one methoxy group in the B ring at δ 3.73 (s, 3H, C$_4$-OC$\underline{H}_3$).

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$_2'$-OCH$_2$C$_6\underline{H}_5$) indicated the presence of benzyloxy group. The C$_\alpha$-H and C$_\beta$-H protons of 111 appeared as two doublets at δ 7.56 (d, 1H, J = 16 Hz, C$_\alpha$-H) and 7.90 (d, 1H, J = 16 Hz C$_\beta$-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.

The IR absorption frequency at v 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.

112
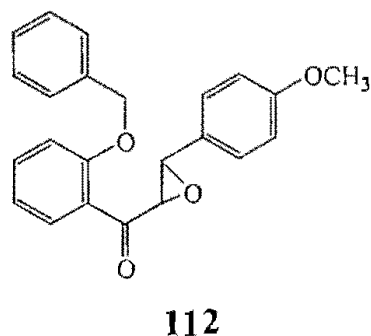
The ^{1}H-NMR at δ 4.43 (C_α-H) and δ 4.37 (C_β-H) of the compound **112** indicated the epoxide ring and compared with its corresponding chalcone **(111)**. The ^{1}H-NMR spectrum explained the presence of one methoxy group in B ring a δ 3.73 (s, 3H C_4-OC$\underline{H}_3$). The four aromatic protons 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
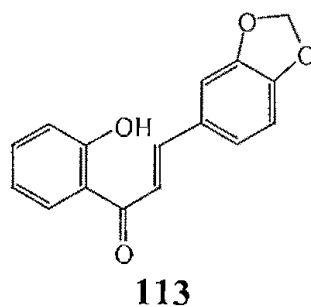
The absorption frequency at ν 1641 cm^{-1} showed the presence of a conjugated carbonyl group (>C=O). The ^{1}H-NMR spectrum explained the presence of methylenedioxy group in B ring at δ 5.90 (s, 2H, C_3-OCH$_2$O-C_4). The three aromatic protons of B ring appeared as one singlet at δ 6.70 (s, 1H, J = 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 v 3464 cm^{-1} indicated the presence of a hydroxyl group. The absorption frequency at v 1697 cm^{-1} showed the presence of a carbonyl group (>C=O).

114
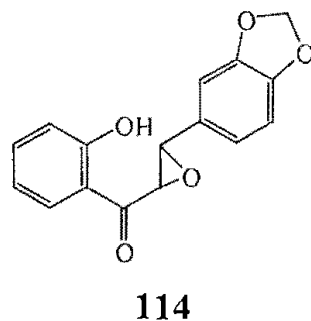
The C_α-H and C_β-H protons of **114** appeared as two doublets at δ 4.44 (1H, J = 2 Hz, C_α-H) and 4.36 (1H, J = 2 Hz C_β-H) integrating for one proton each respectively. The ^{1}H-NMR at δ 4.44 (C_α-H) and 4.36 (C_β-H) of the compound **114** indicated the epoxide ring and compared with its corresponding 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 v 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
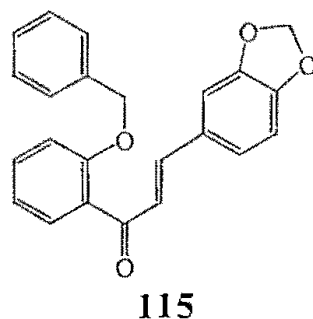
The other three aromatic protons of B ring appeared as one singlet at δ 6.70 (s, 1H, J = 2.4 Hz C_2-H) and two doublets at δ 6.61(d, 1H, J = 8.6 Hz C_5-H) and 6.75 (d, 1H, J = 8.6 Hz, C_6-H). Two singlets at δ 5.22 (s, 2H, $C_{2'}$-OC$\underline{H}_2$-C$_6$H$_5$) and δ 7.19 (s, 5H, $C_{2'}$-OCH$_2$-C$_6$$\underline{H}_5$) 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 v 1699 cm^{-1} showed the presence of a carbonyl group (>C=O).
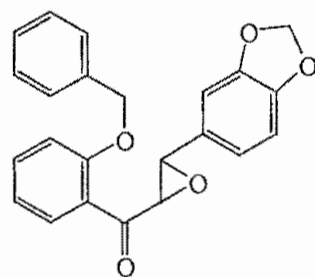
116

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 lbs / 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	
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	22.3
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	
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	23
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	
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	22
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	
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	22
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.
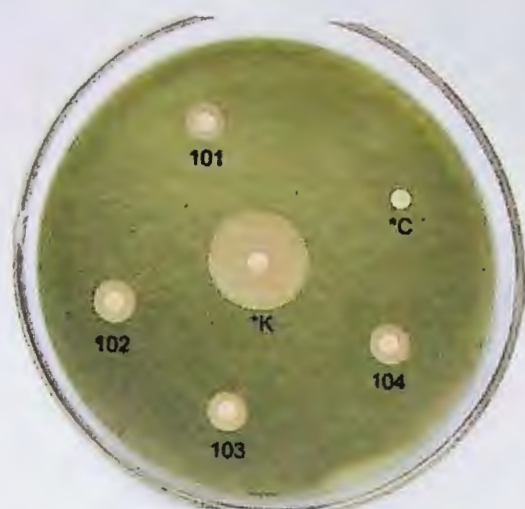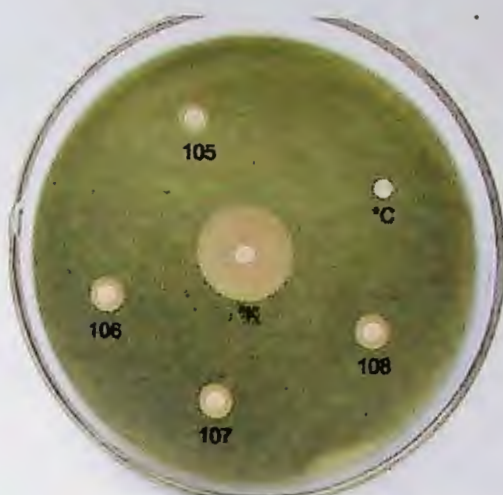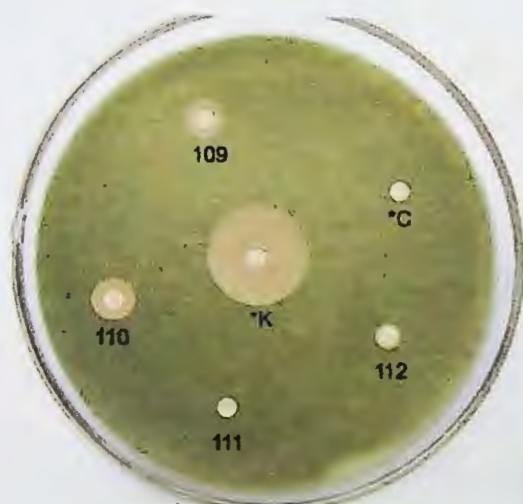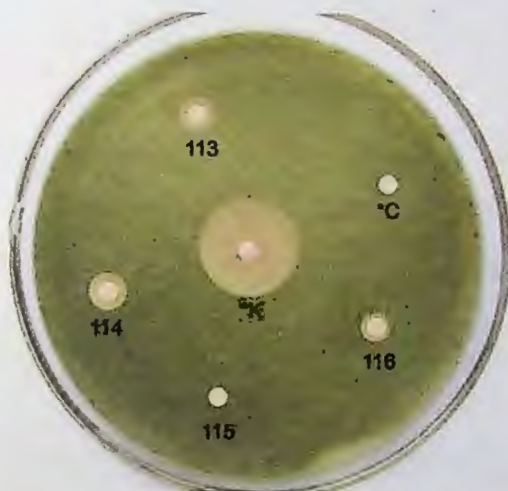
Photograph 1-4: Represents the zone of inhibition at the concentration of 100µg disc^{-1} against *Bacillus megaterium* (G+).
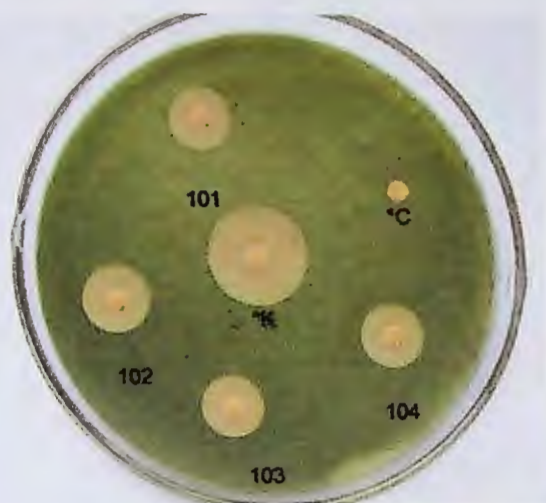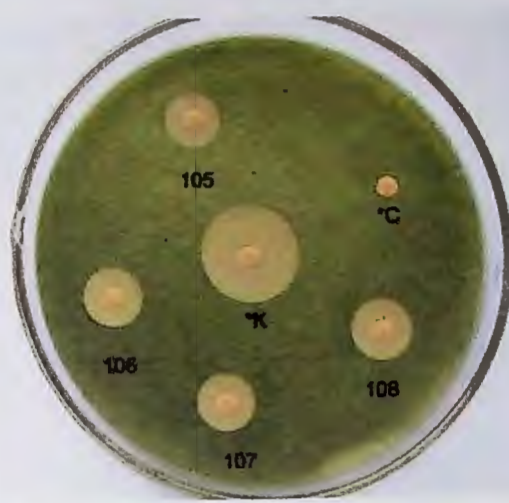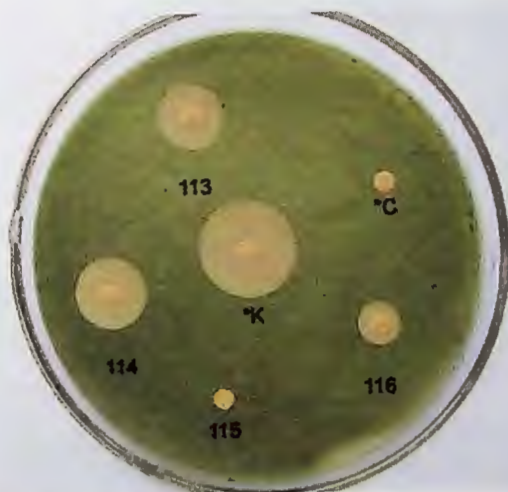
Photograph 5-8: Represents the zone of inhibition at the concentration of 200µg disc⁻¹ against *Bacillus megaterium* (G+).
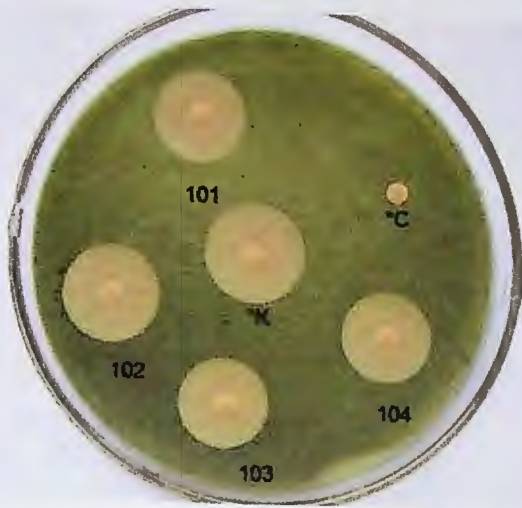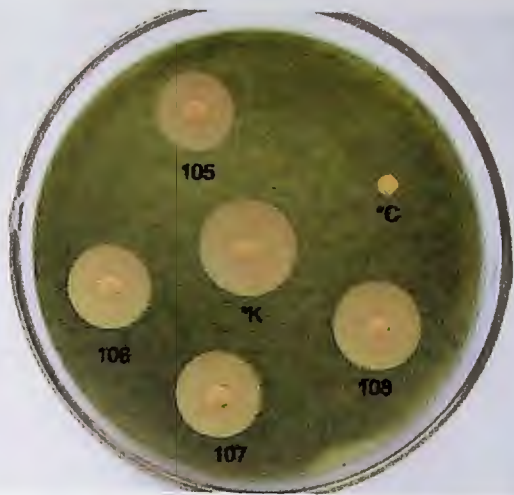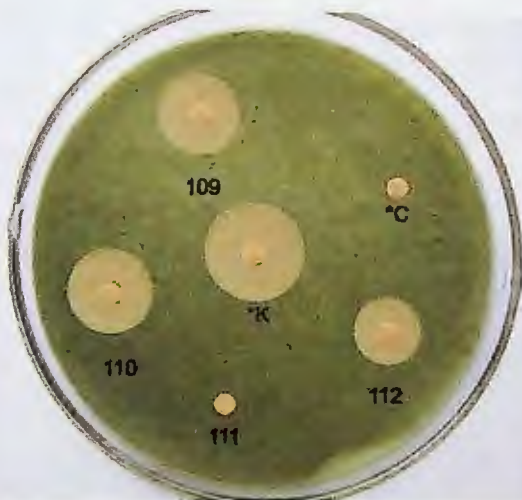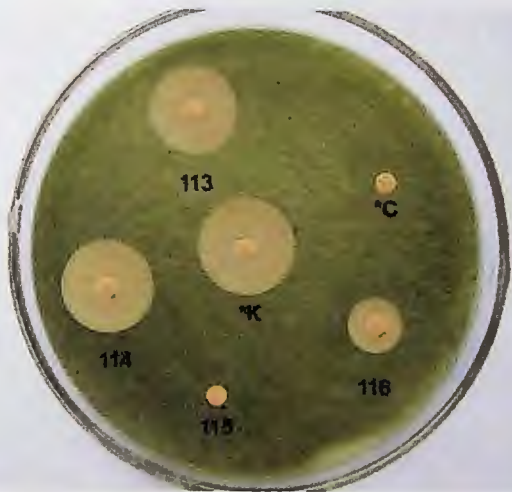
Photograph 9-12: Represents the zone of inhibition at the concentration of 300μg disc⁻¹ against *Bacillus megaterium* (G+).
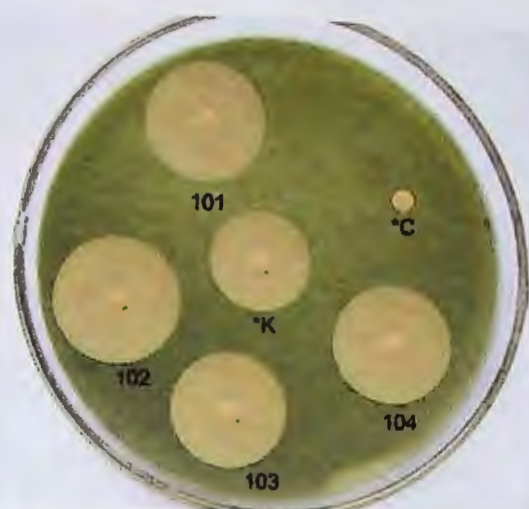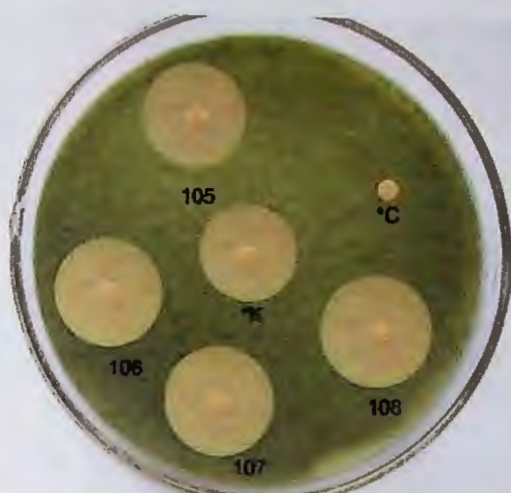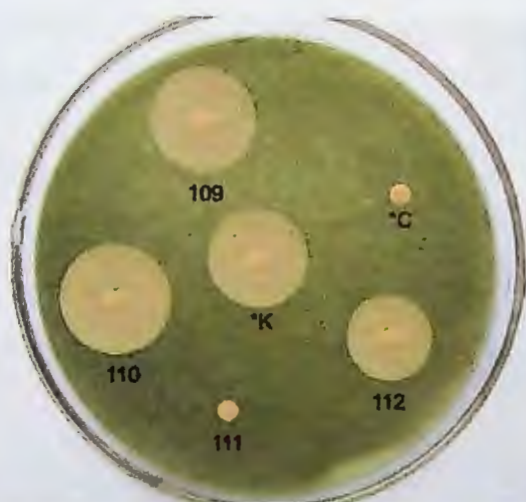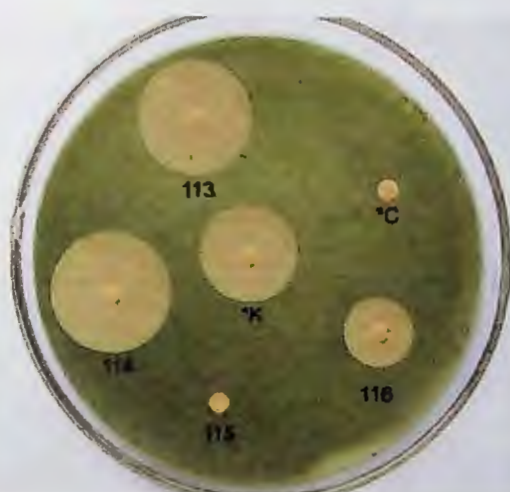
Photograph 13-16: Represents the zone of inhibition at the concentration of 400µg disc^{-1} against *Bacillus megaterium* (G+).
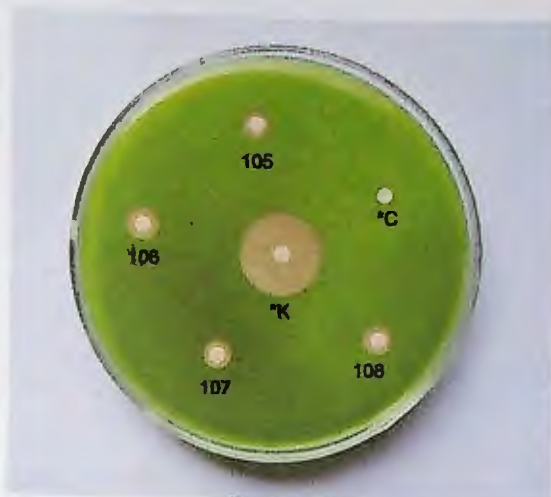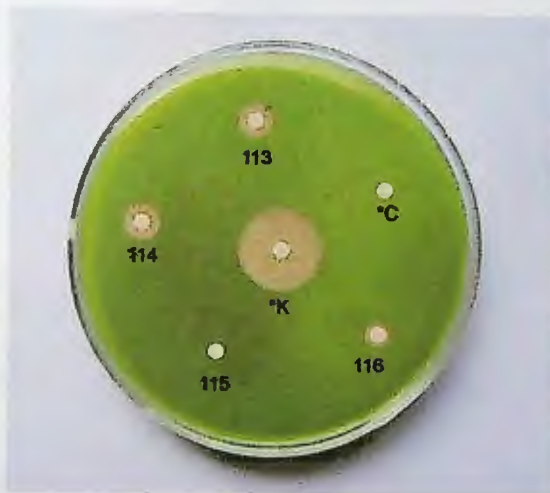
Photograph 17-20: Represents the zone of inhibition at the concentration of 100µg disc^{-1} against *Streptococcus–β–haemolyticus* (G+).

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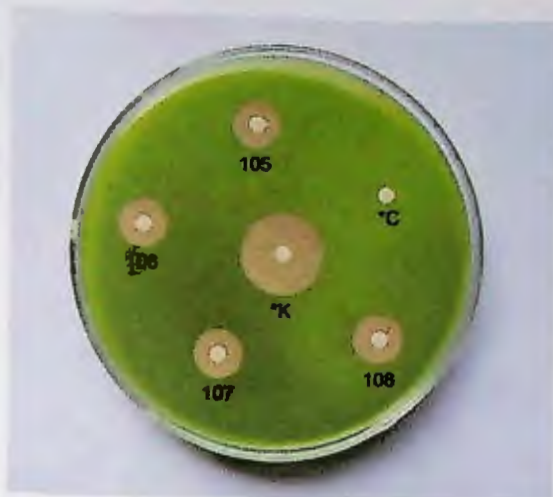
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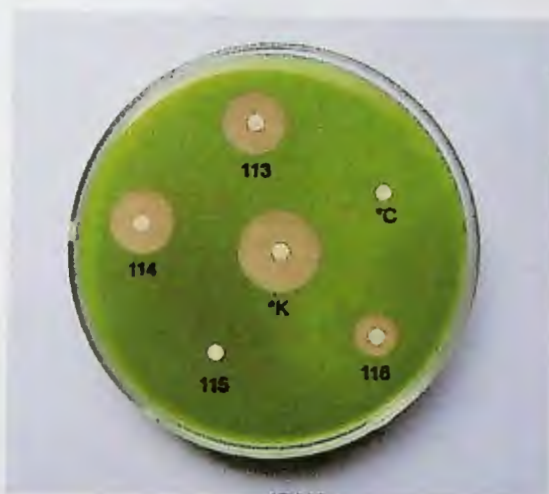
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Photograph 21-24: Represents the zone of inhibition at the concentration of 200μg disc^{-1} against *Streptococcus–β–haemolyticus* (G+).

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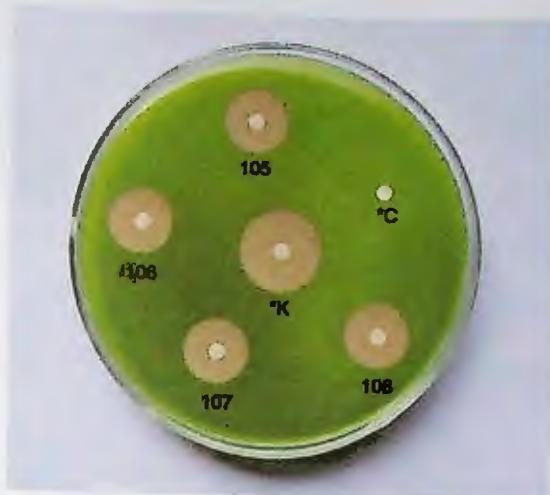
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Photograph 25-28: Represents the zone of inhibition at the concentration of 300µg disc^{-1} against *Streptococcus–β–haemolyticus* (G+).

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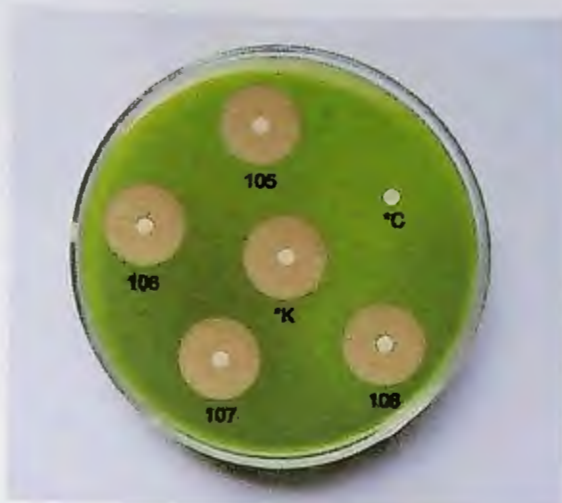
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Photograph 29-32: Represents the zone of inhibition at the concentration of 400µg disc^{-1} against *Streptococcus–β–haemolyticus* (G+).
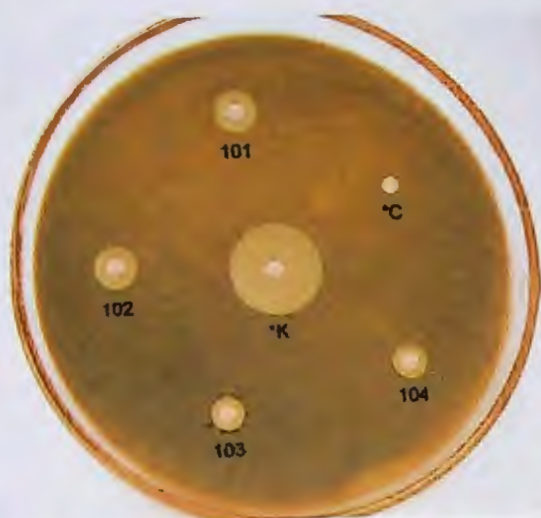
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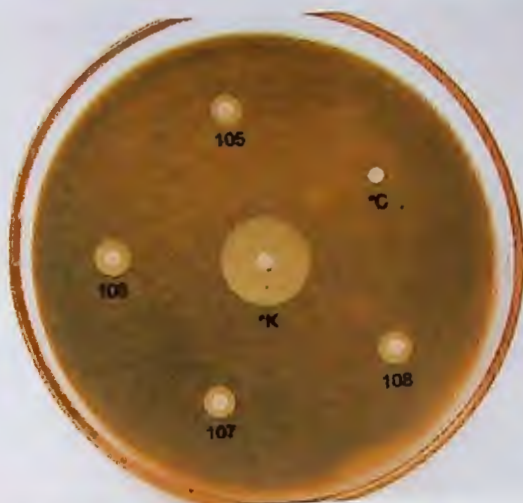
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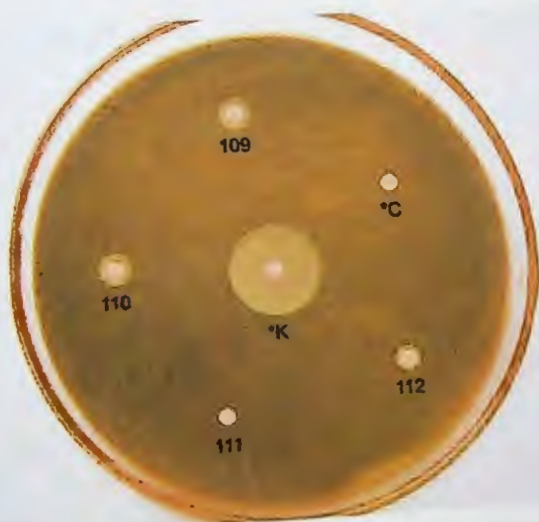
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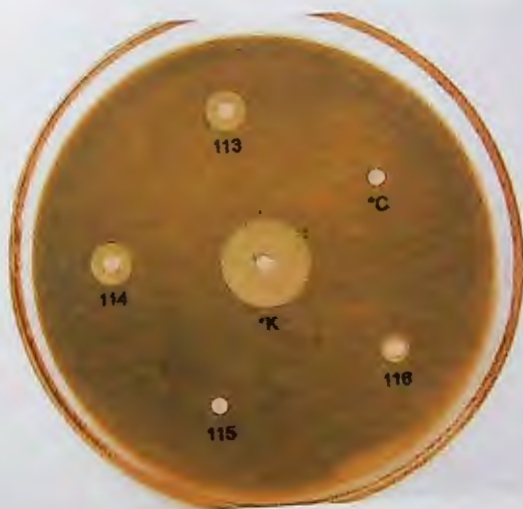
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Photograph 33-36: Represents the zone of inhibition at the concentration of 100µg disc^{-1} against *Klebsiella sp.* (G$^-$).
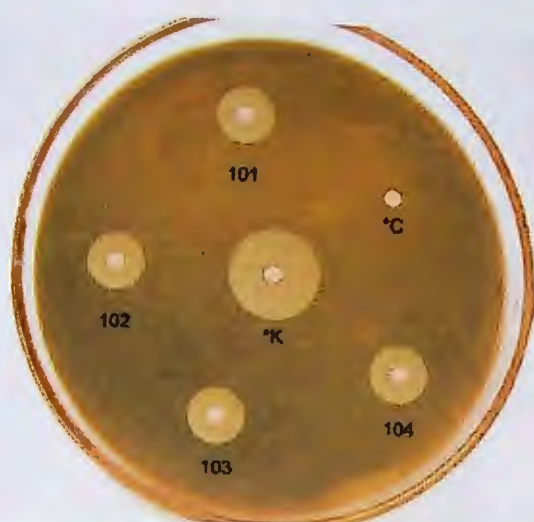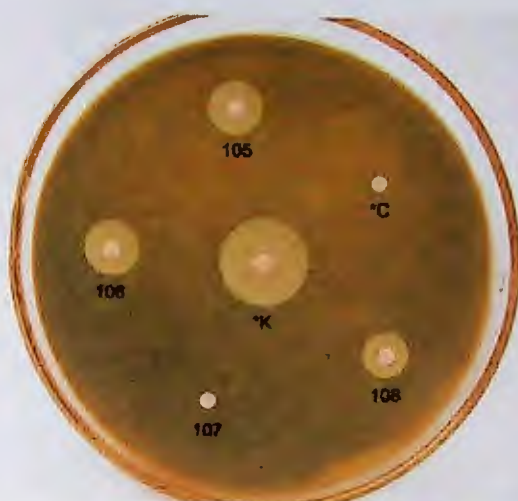
Photograph 37-40: Represents the zone of inhibition at the concentration of 200µg disc^{-1} against *Klebsiella sp.* (G$^-$).
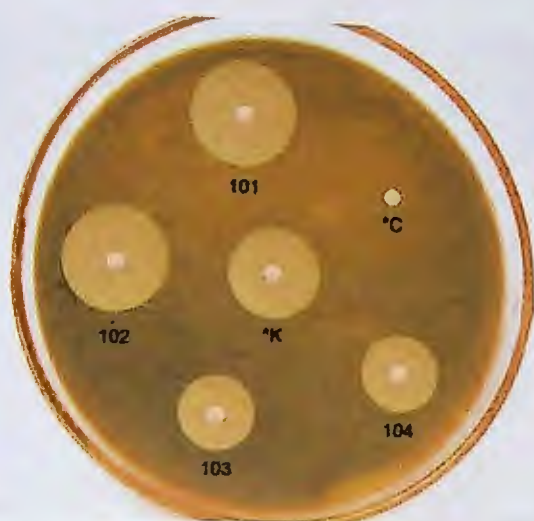
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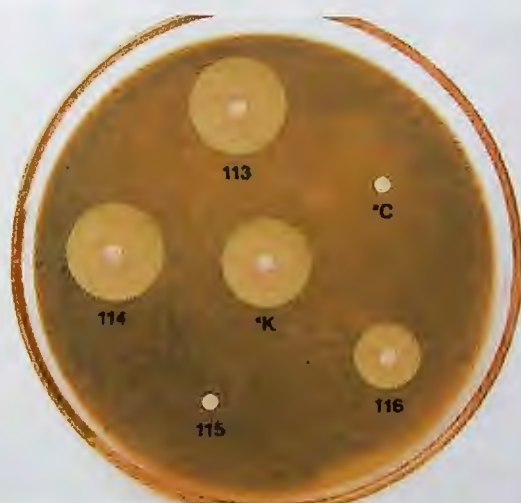
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Photograph 41-44: Represents the zone of inhibition at the concentration of 300µg disc^{-1} against *Klebsiella sp.* (G$^-$).

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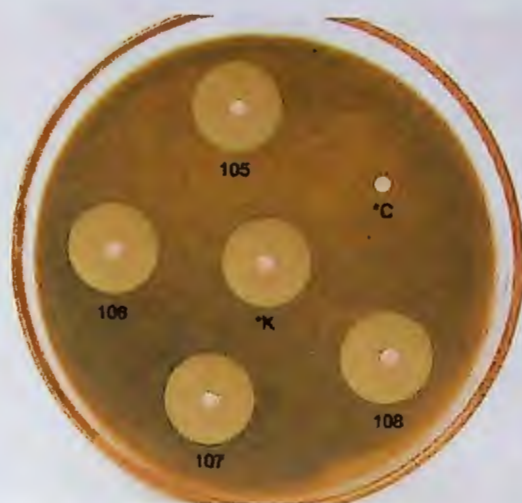
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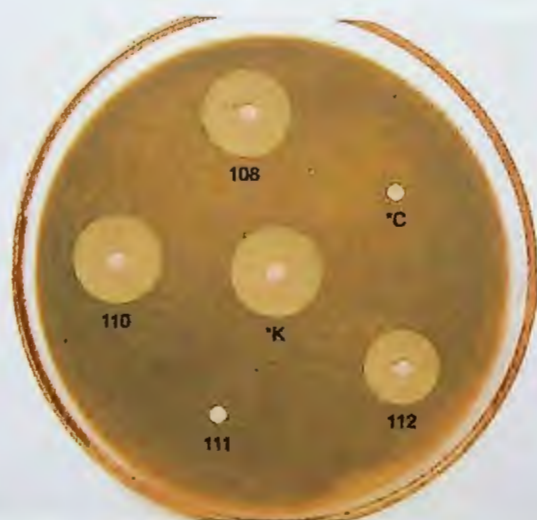
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Photograph 45-48: Represents the zone of inhibition at the concentration of 400µg disc^{-1} against *Klebsiella sp.* (G$^-$).

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

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	
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	21
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	
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	21.2
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)				
		$100\ \mu g$ $disc^{-1}$	$200\ \mu g$ $disc^{-1}$	$300\ \mu g$ $disc^{-1}$	$400\ \mu g$ $disc^{-1}$	*N-50 $\mu g\ disc^{-1}$
101	$C_{18}H_{18}O_5$	9.1	16.5	22.6	28.2	
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	20.7
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 $\mu g\ disc^{-1}$.

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	
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	20.9
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.
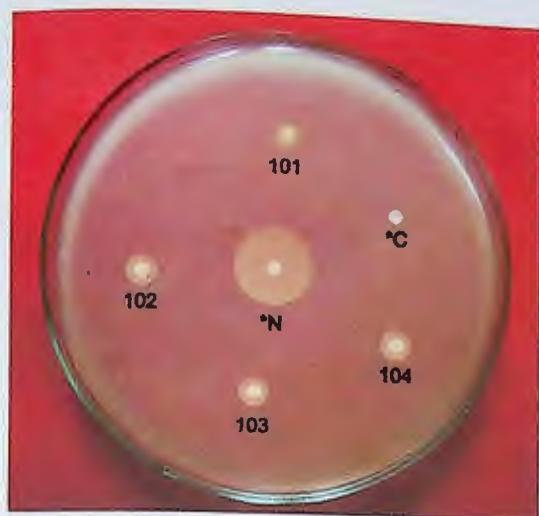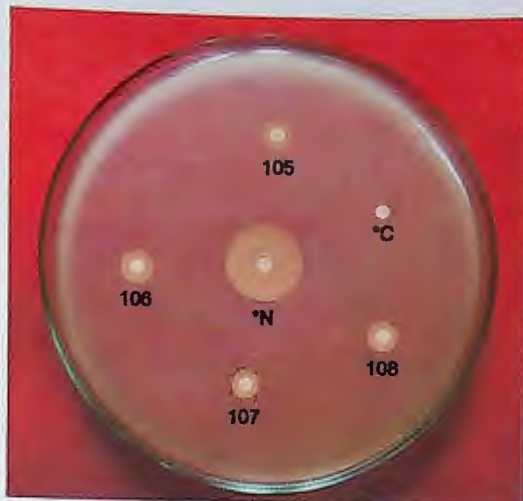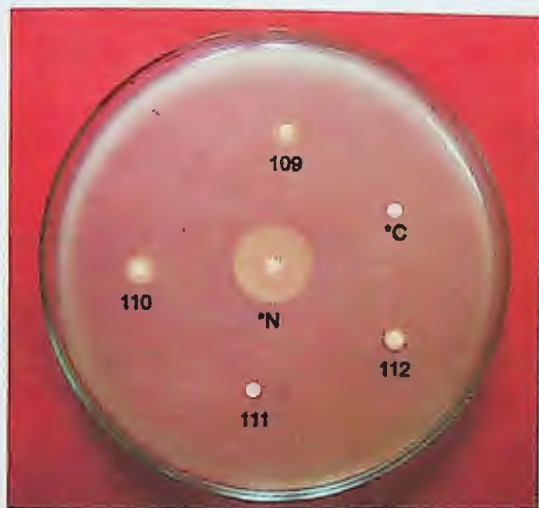
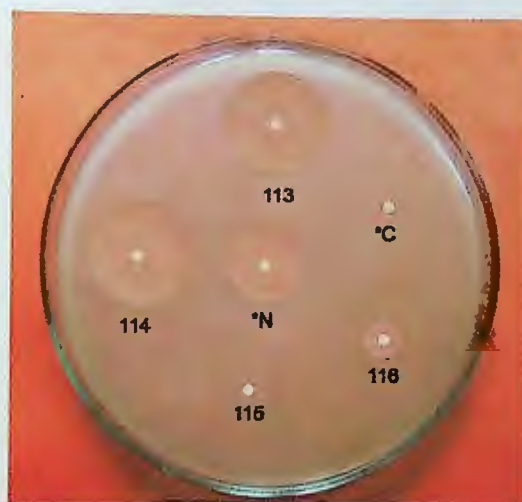**Photograph 65-68:** Represents the zone of inhibition at the concentration of 100μg disc^{-1} against *Rhizoctonia solani*.
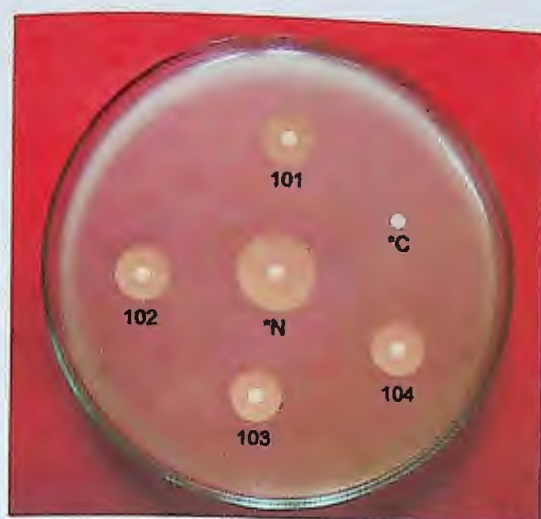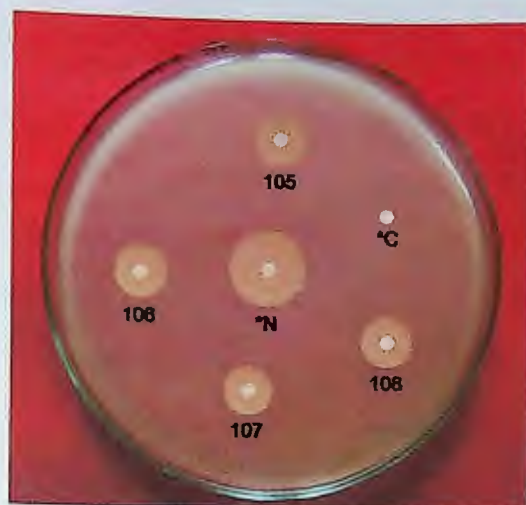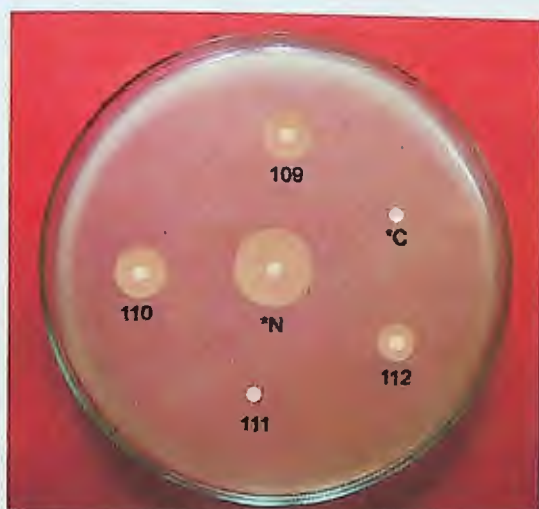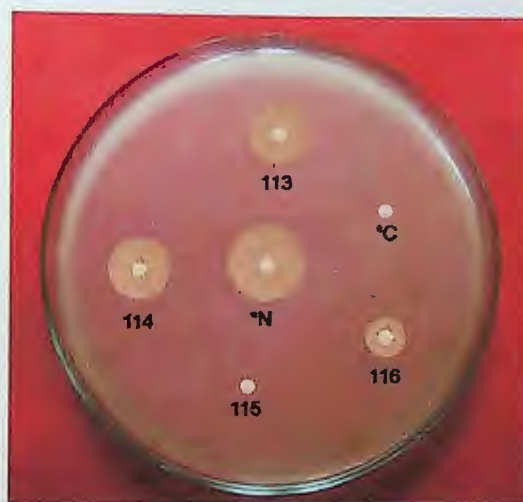
Photograph 69-72: Represents the zone of inhibition at the concentration of 200µg disc^{-1} against *Rhizoctonia solani*.
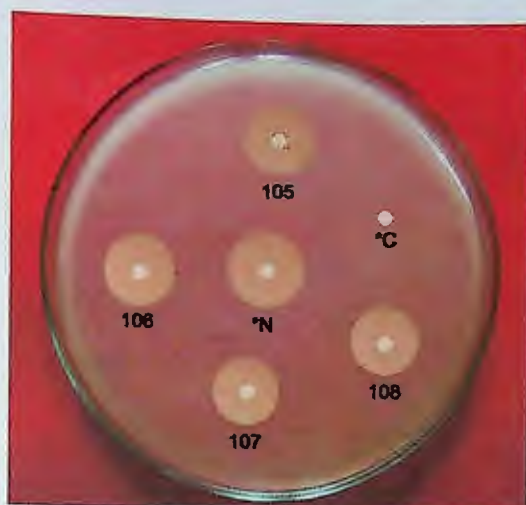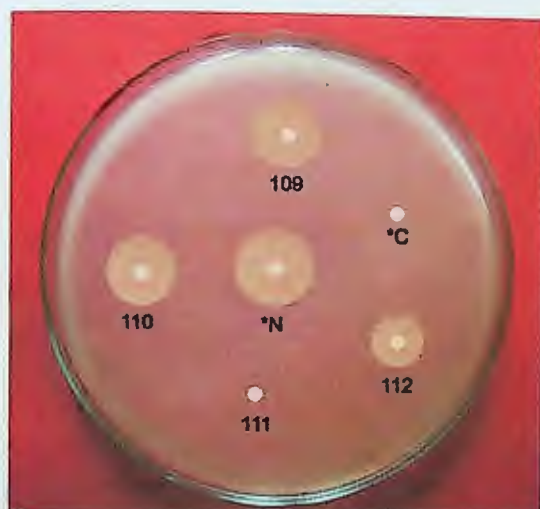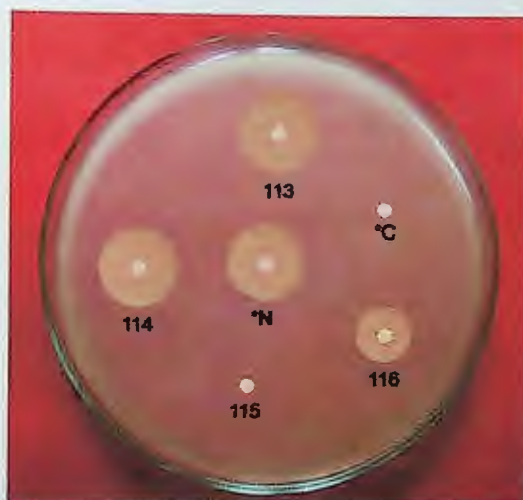
Photograph 73-76: Represents the zone of inhibition at the concentration of 300µg disc^{-1} against *Rhizoctonia solani*.
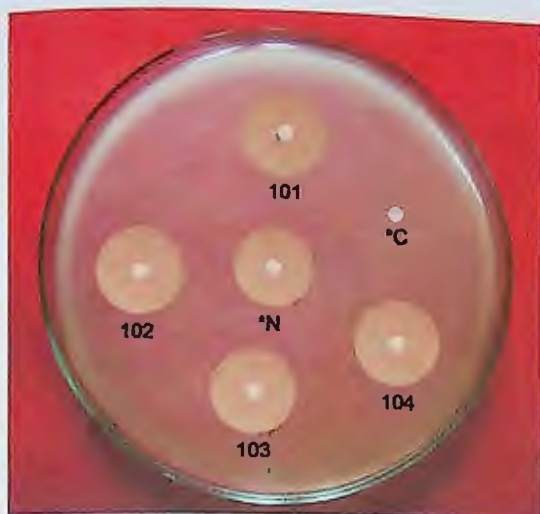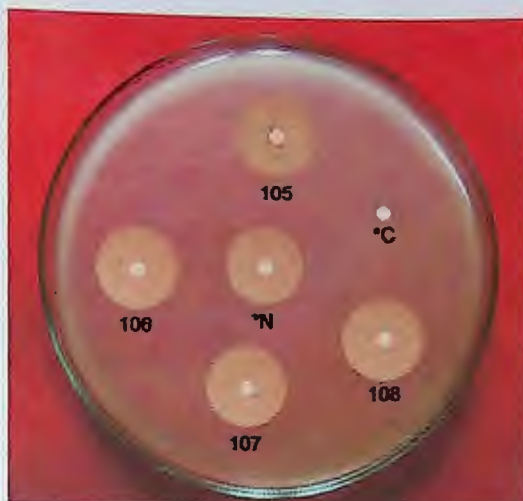
Photograph 77-80: Represents the zone of inhibition at the concentration of 400µg disc^{-1} against *Rhizoctonia solani*.

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

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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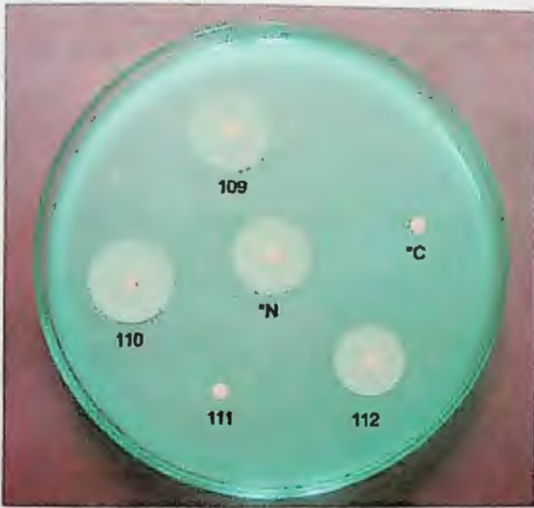
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Photograph 93-96: Represents the zone of inhibition at the concentration of 400µg disc^{-1} against *Sclerotium rolfsii*.
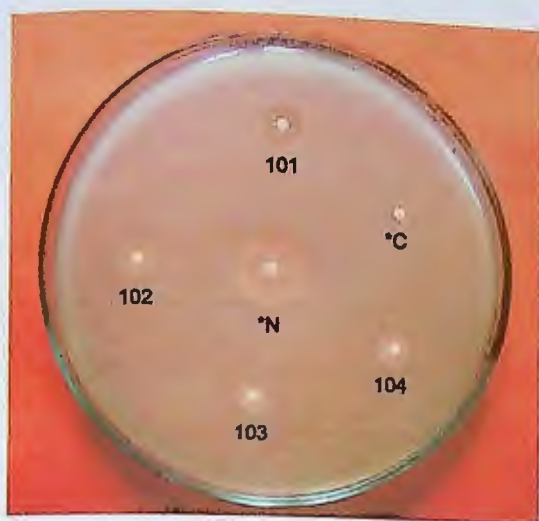
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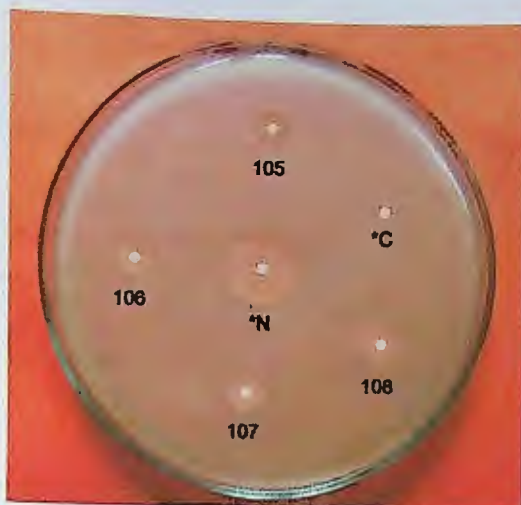
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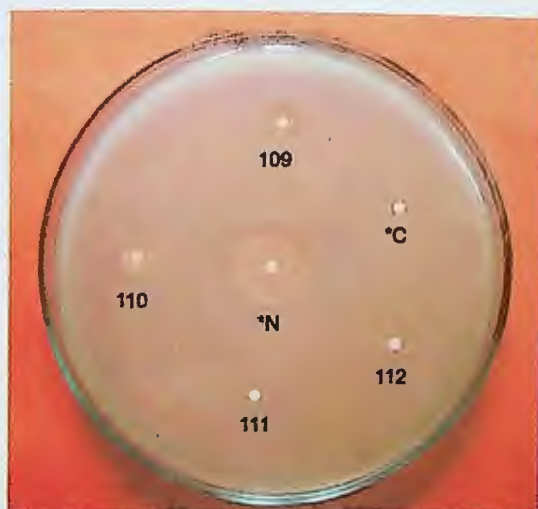
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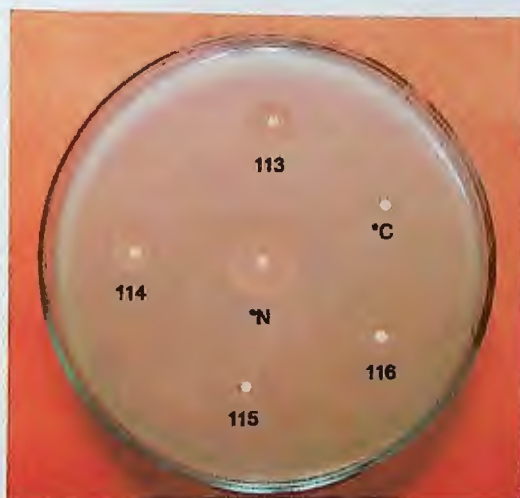
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Photograph 97-100: Represents the zone of inhibition at the concentration of 100μg disc^{-1} against *Aspergillus niger*.
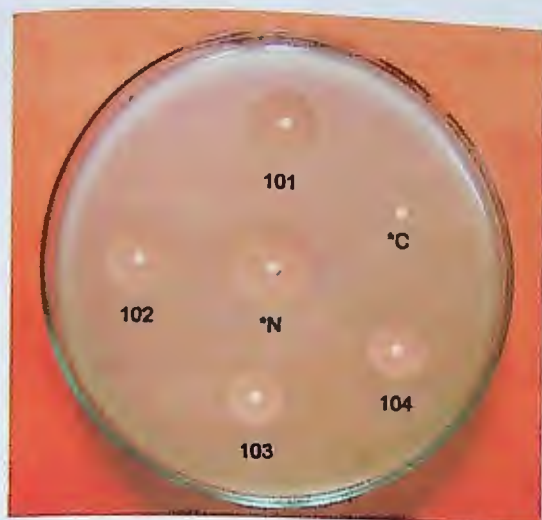
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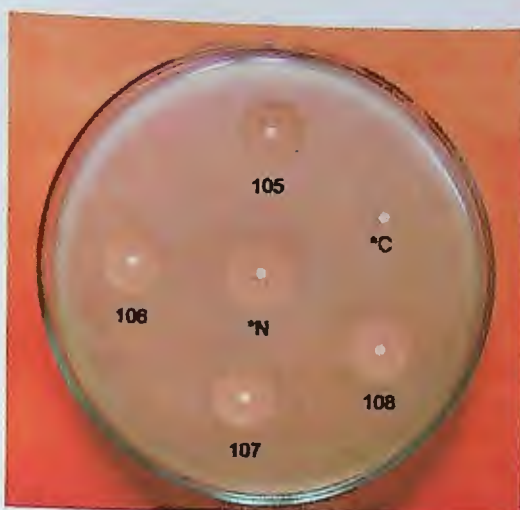
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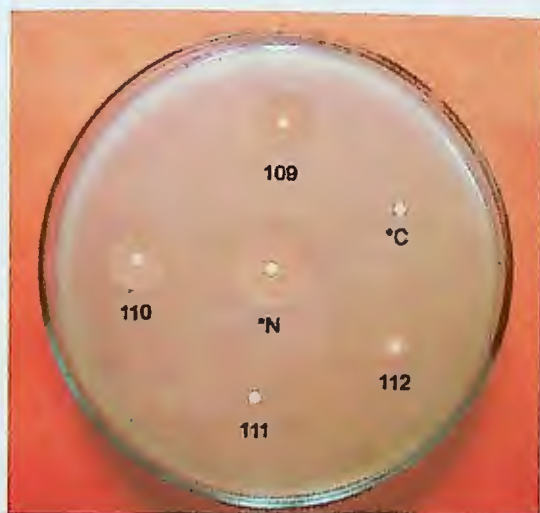
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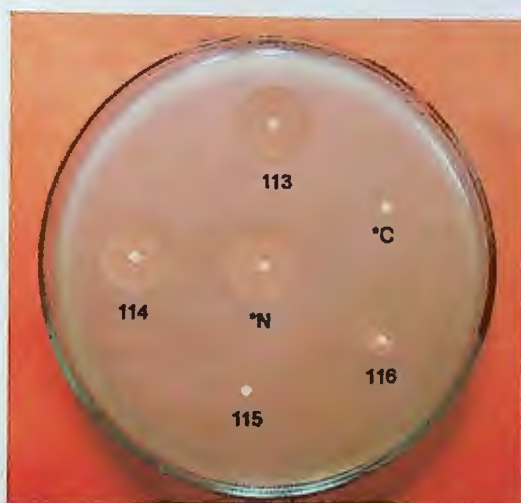
104

Photograph 101-104: Represents the zone of inhibition at the concentration of 200μg disc^{-1} against *Aspergillus niger*.
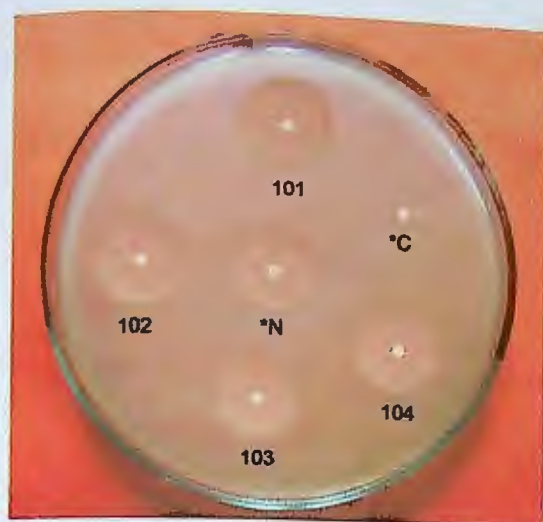
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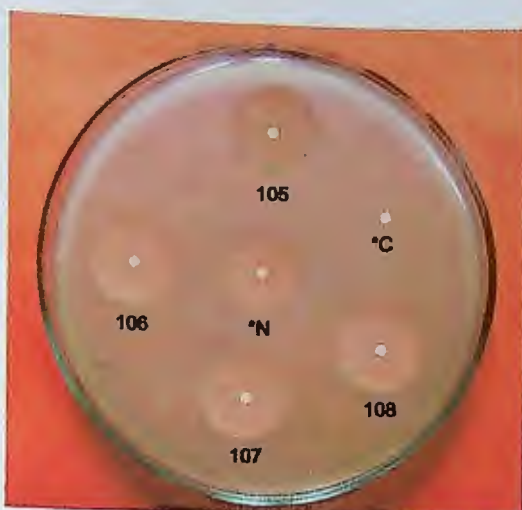
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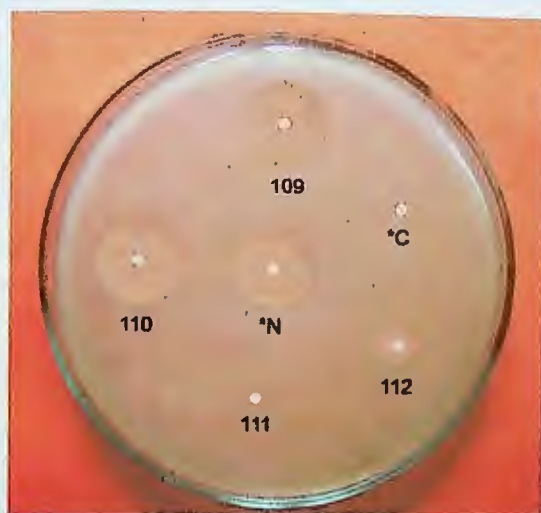
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108

Photograph 105-108: Represents the zone of inhibition at the concentration of 300μg disc^{-1} against *Aspergillus niger*.
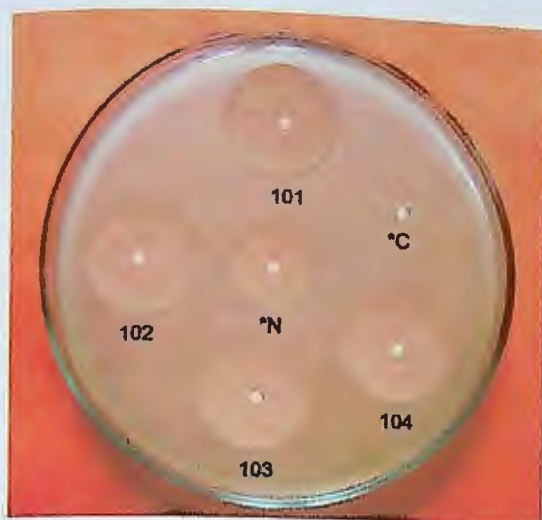
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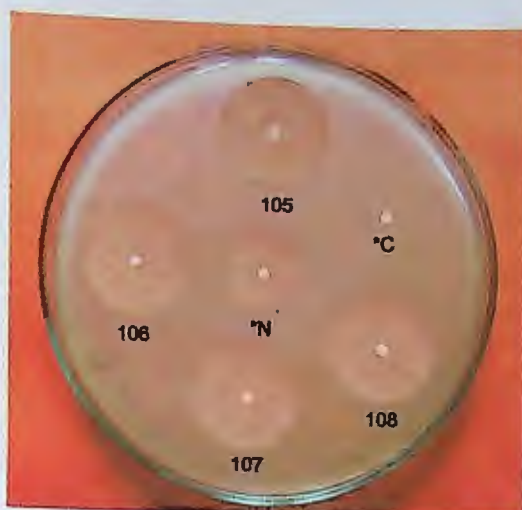
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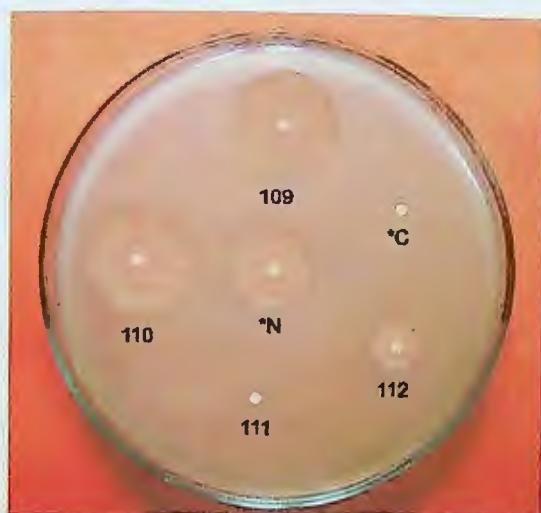
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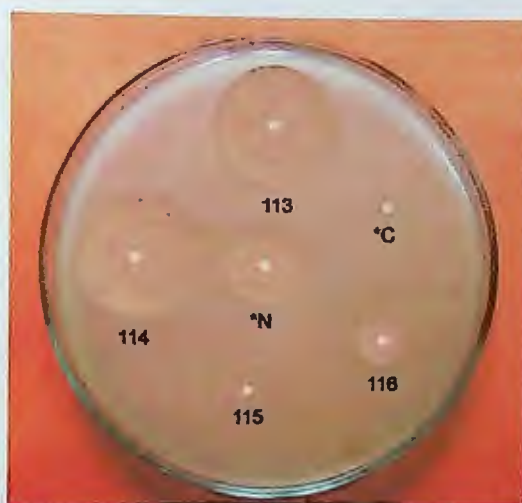
112

Photograph 109-112: Represents the zone of inhibition at the concentration of 400µg disc^{-1} against *Aspergillus niger*.
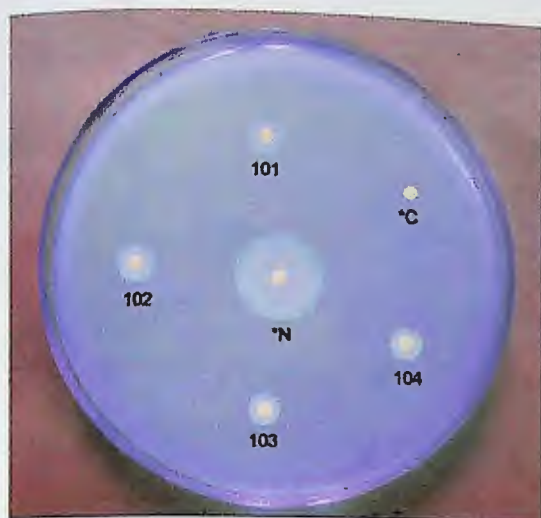
113
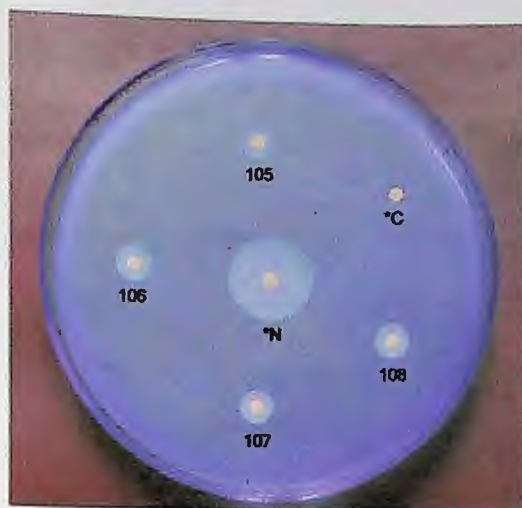
114

115

116

Photograph 113-116: Represents the zone of inhibition at the concentration of 100μg disc^{-1} against *Aspergillus fumigatus*.
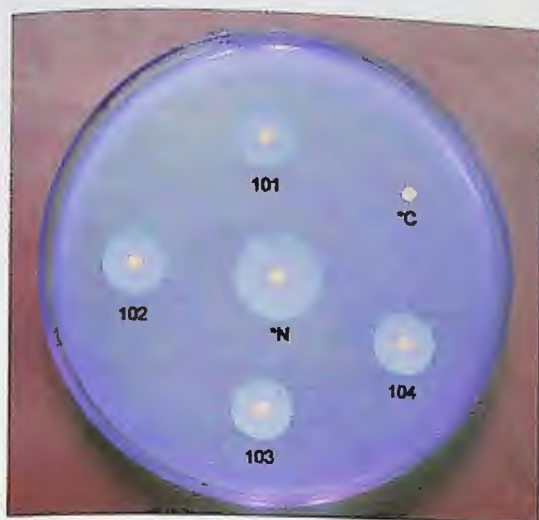
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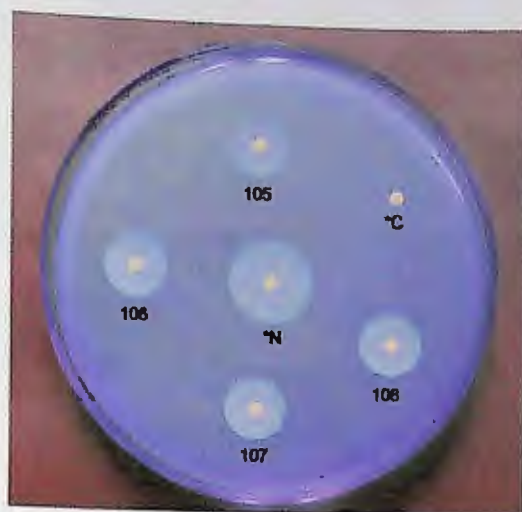
118

119

120

Photograph 117-120: Represents the zone of inhibition at the concentration of 200µg disc^{-1} against *Aspergillus fumigatus*.
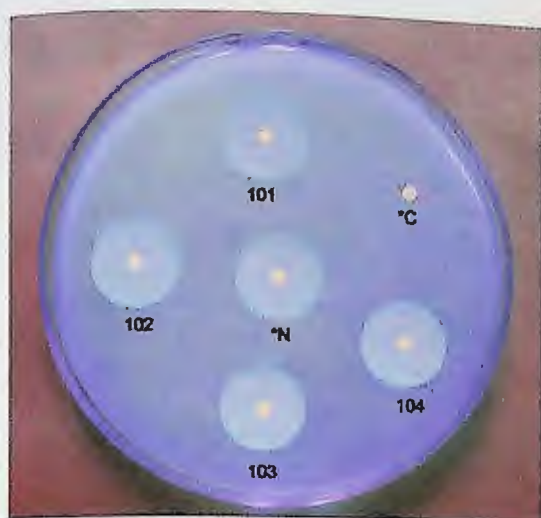
121

122

123

124

Photograph 121-124: Represents the zone of inhibition at the concentration of 300µg disc^{-1} against *Aspergillus fumigatus*.
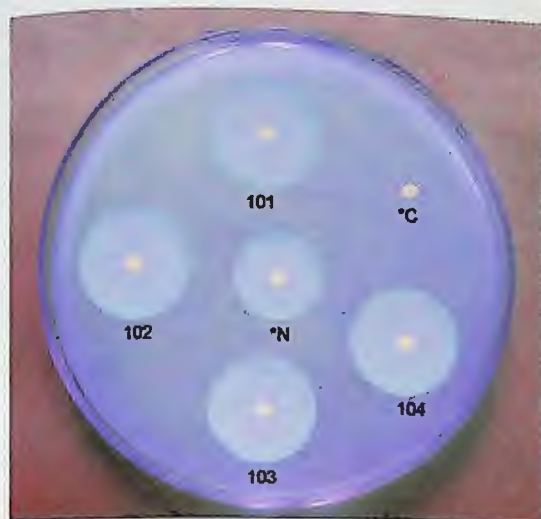
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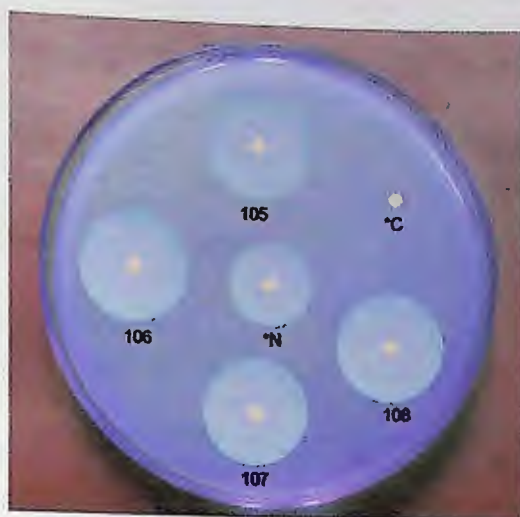
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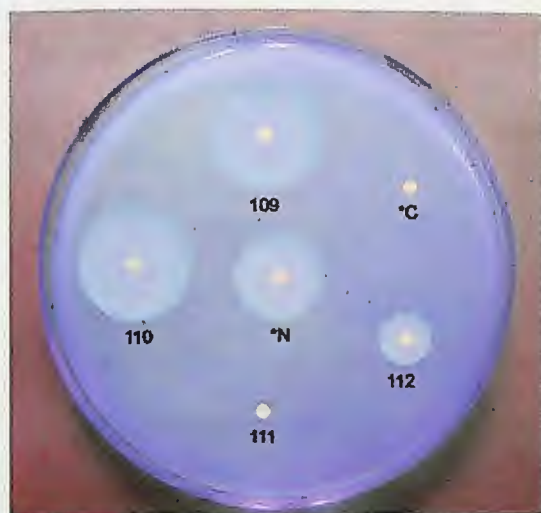
127

128

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$_2$O$_2$ 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$_2$O$_2$ 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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65.19		

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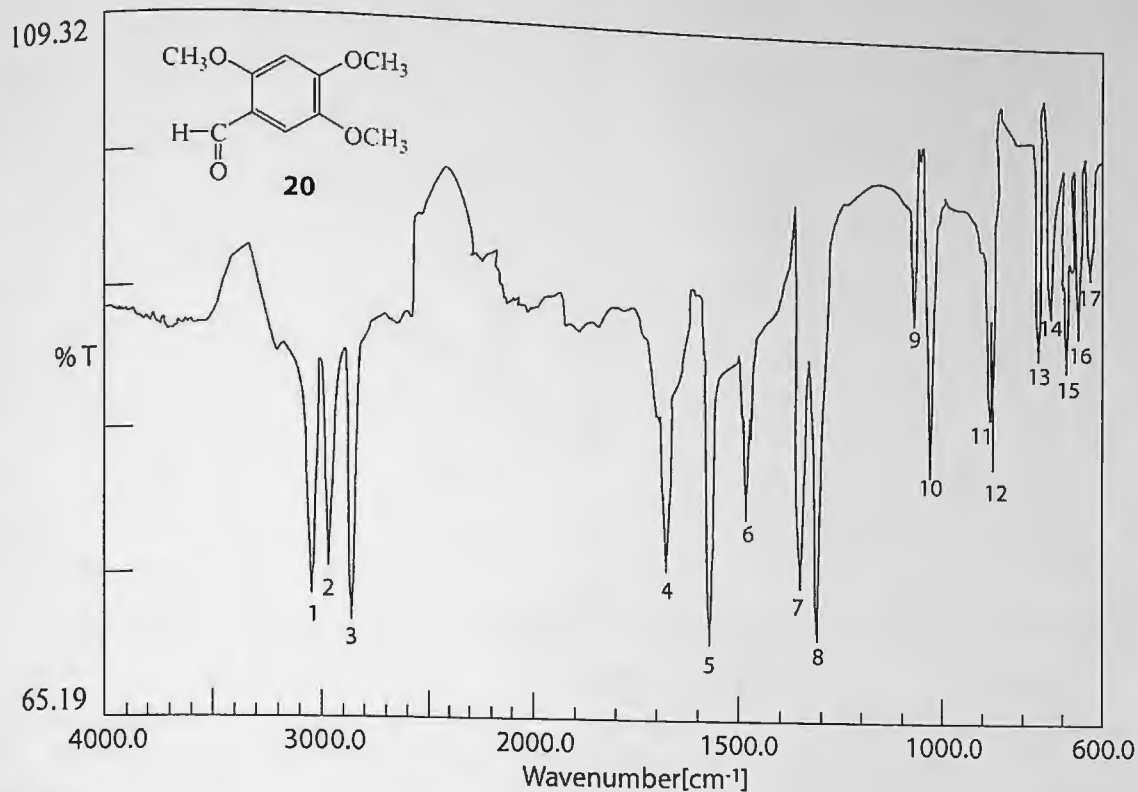
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Zero Filling	× 2	Detector	1
Speed	4.0 mm/sec	Beam splitter	KBr
Delay Time	0 sec		

Comment
Copyright
Operator
Peak information

1 3048.32	7 1350.03	13 760.08
2 2970.02	8 1310.21	14 730.36
3 2860.13	9 1070.12	15 690.21
4 1675.31	10 1030.31	16 660.29
5 1570.10	11 880.09	17 630.19
6 1480.41	12 875.35	

109.10

CH₃O ─ OCH₃

OH ─ OCH₃

O **101**

% T

1
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3
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11
12
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14
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16
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20
21

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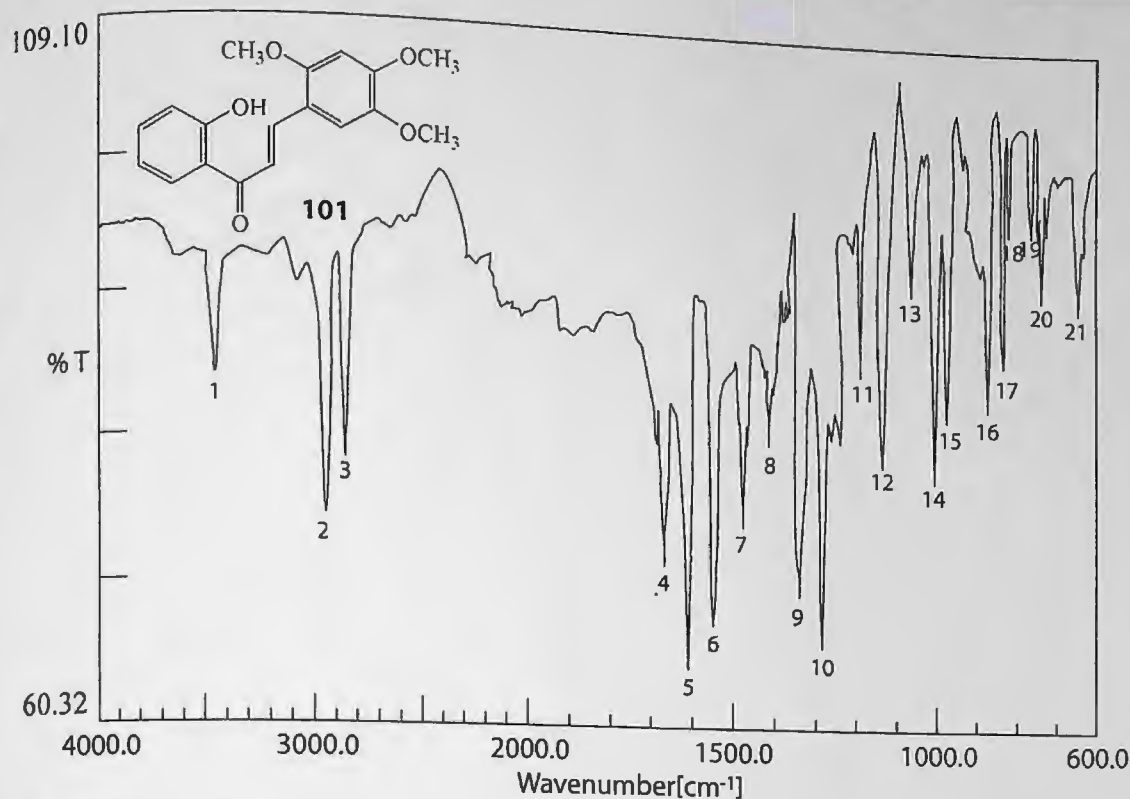
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Comment
Copyright
Operator
Peak information

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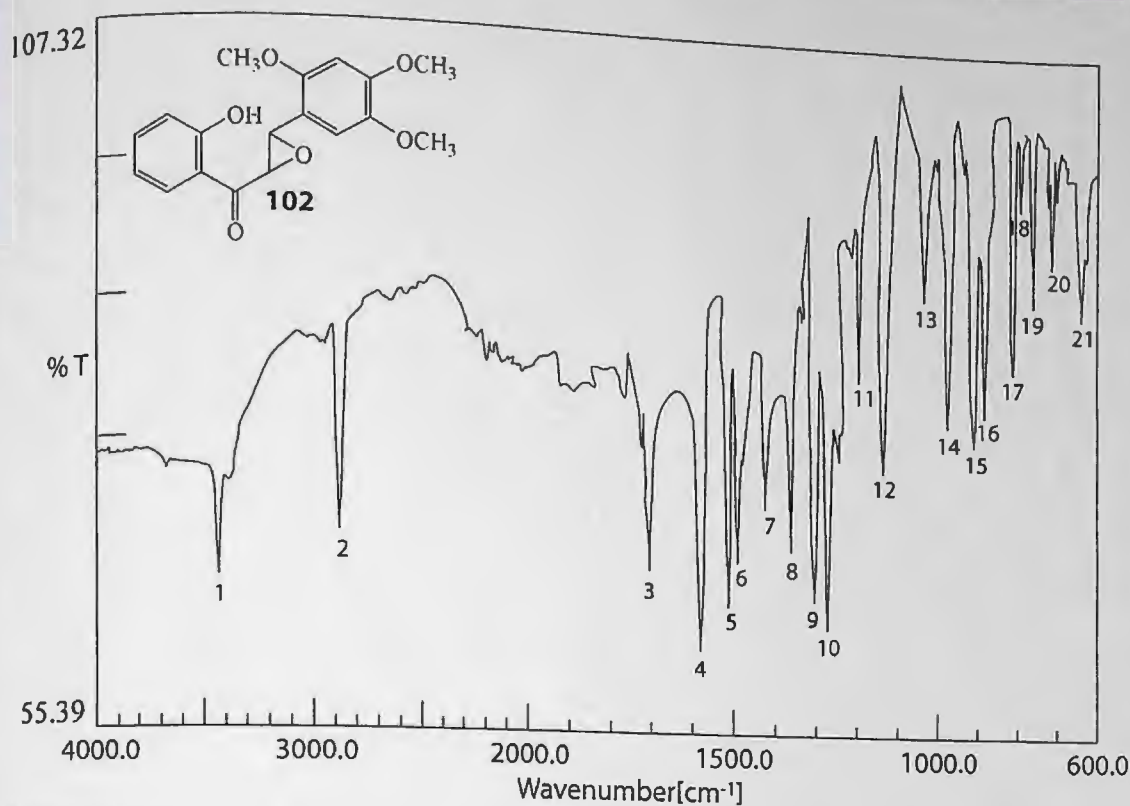
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Comment
Copyright
Operator
Peak information

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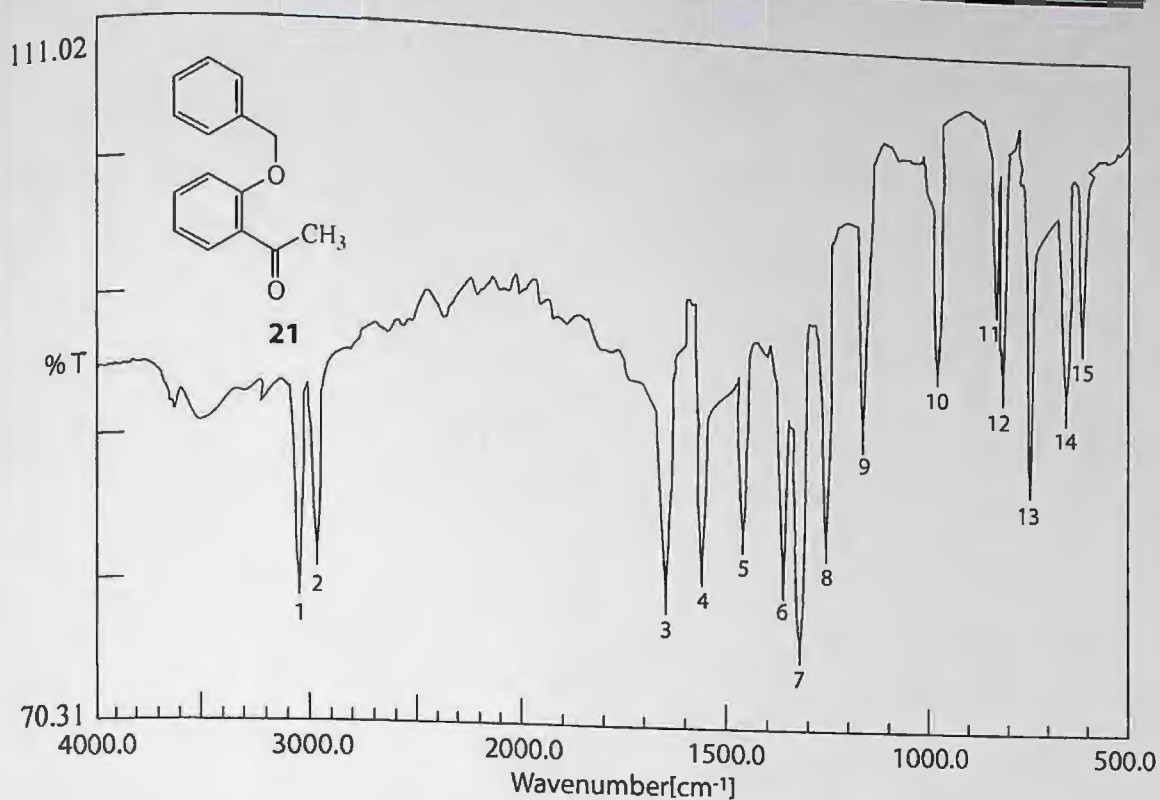
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Comment
Copyright
Operator
Peak information

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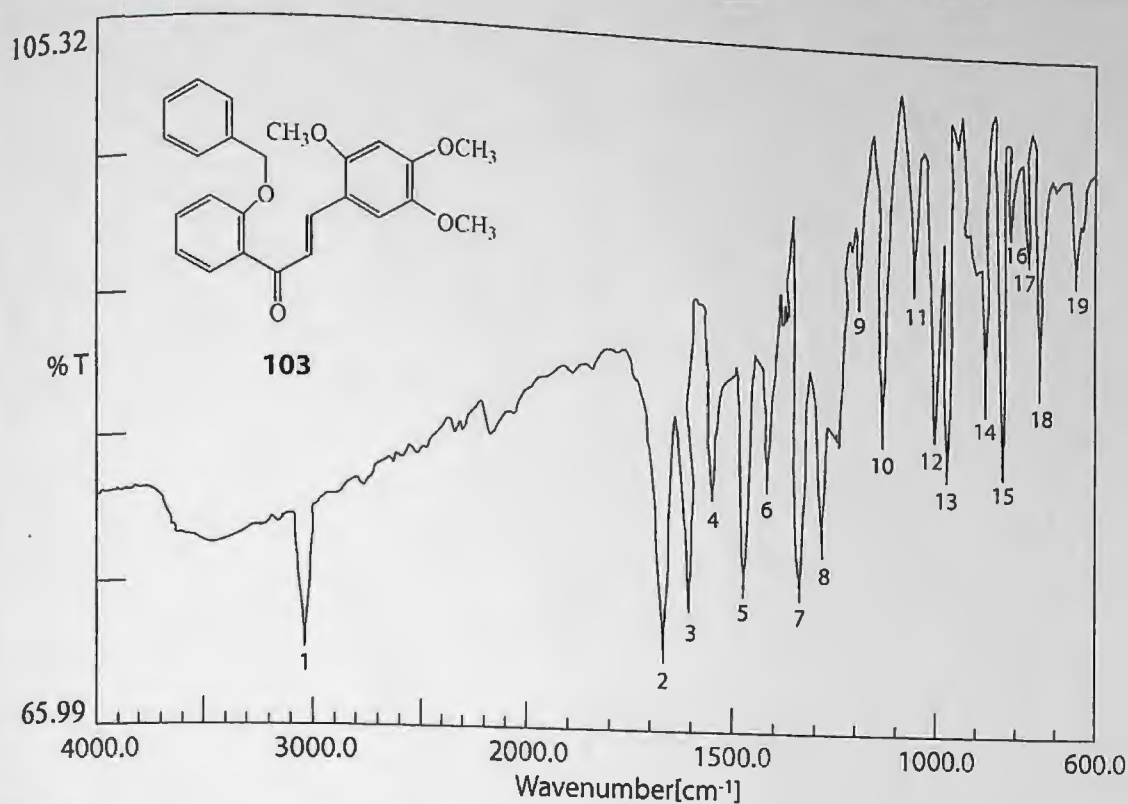
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Copyright
Operator
Peak information

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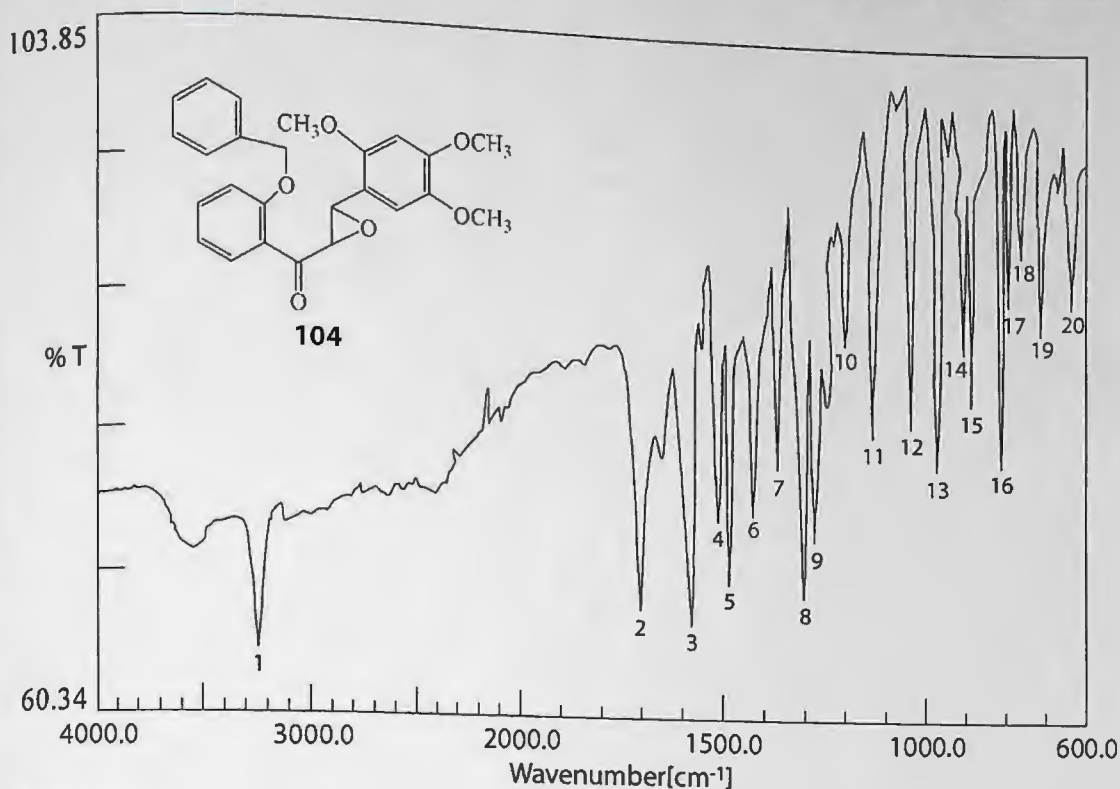
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Delay Time	0 sec		

Comment
Copyright
Operator

Peak information

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4 1510.32	11 1131.13	18 795.12
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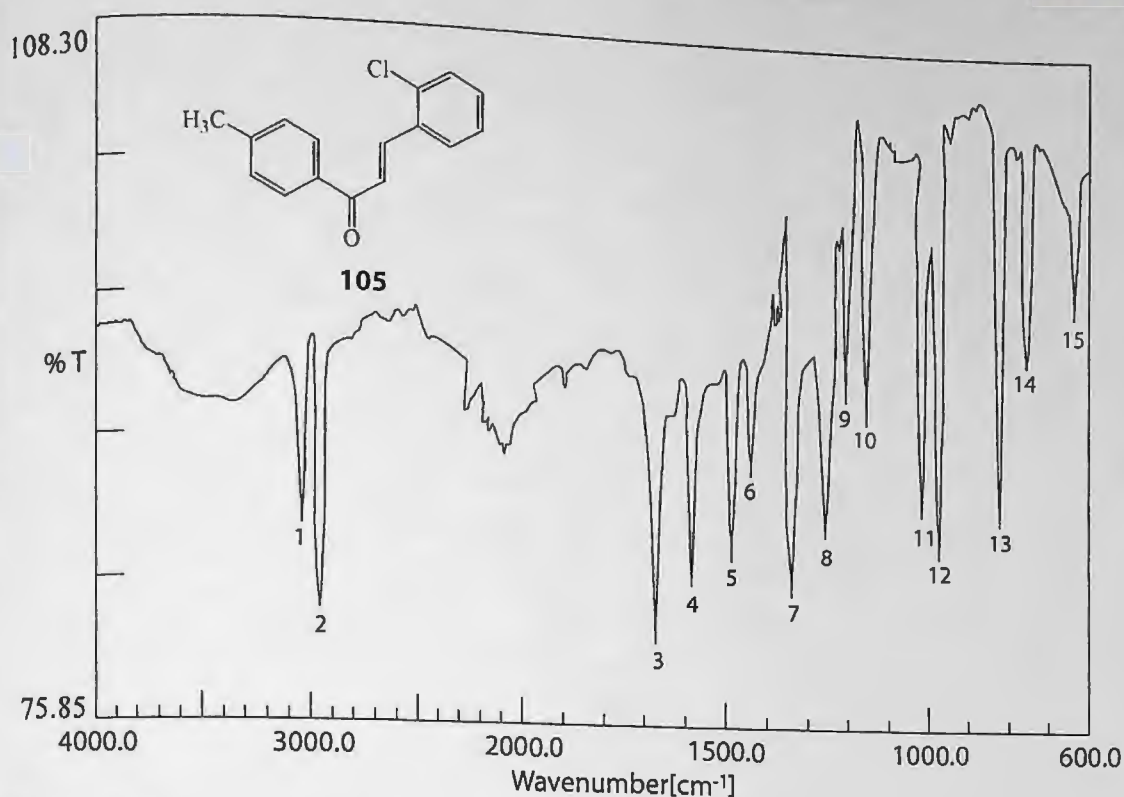
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Delay Time	0 sec		

Comment
Copyright
Operator

Peak information

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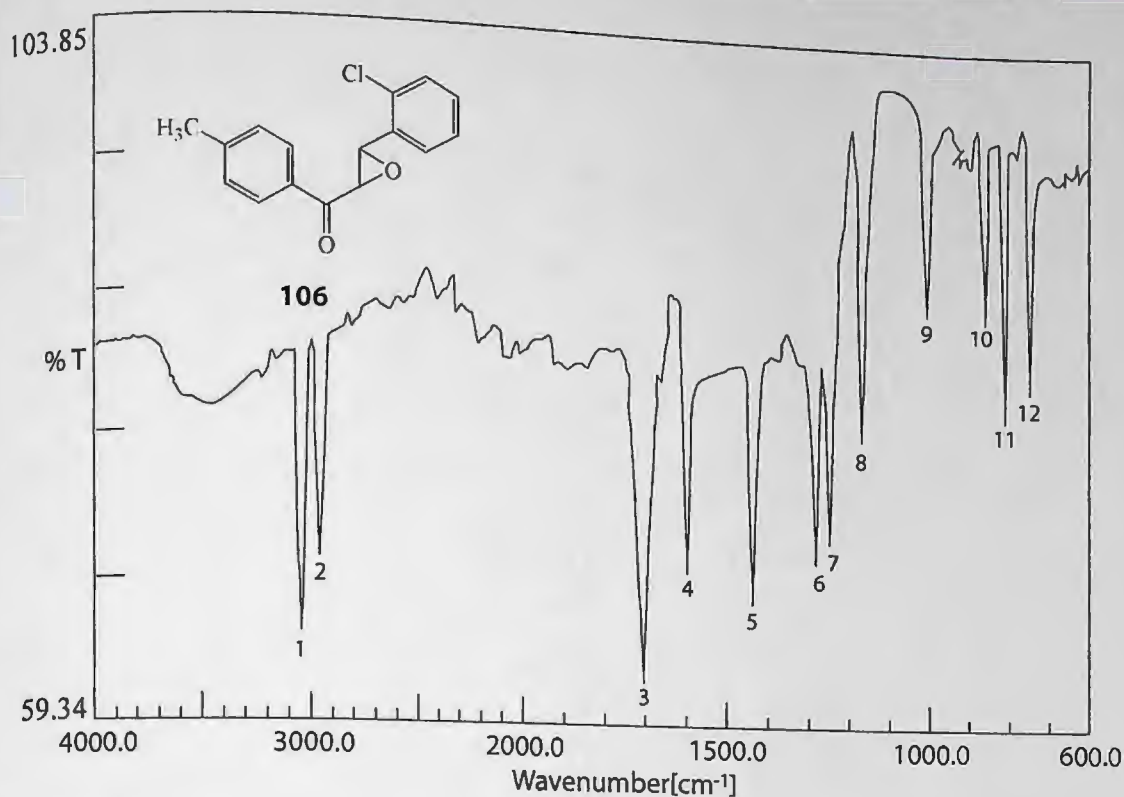
Parameter

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

105.14

%T

65.36

| 4000.0 | 3000.0 | 2000.0 | 1500.0 | 1000.0 | 600.0 |

Wavenumber[cm⁻¹]
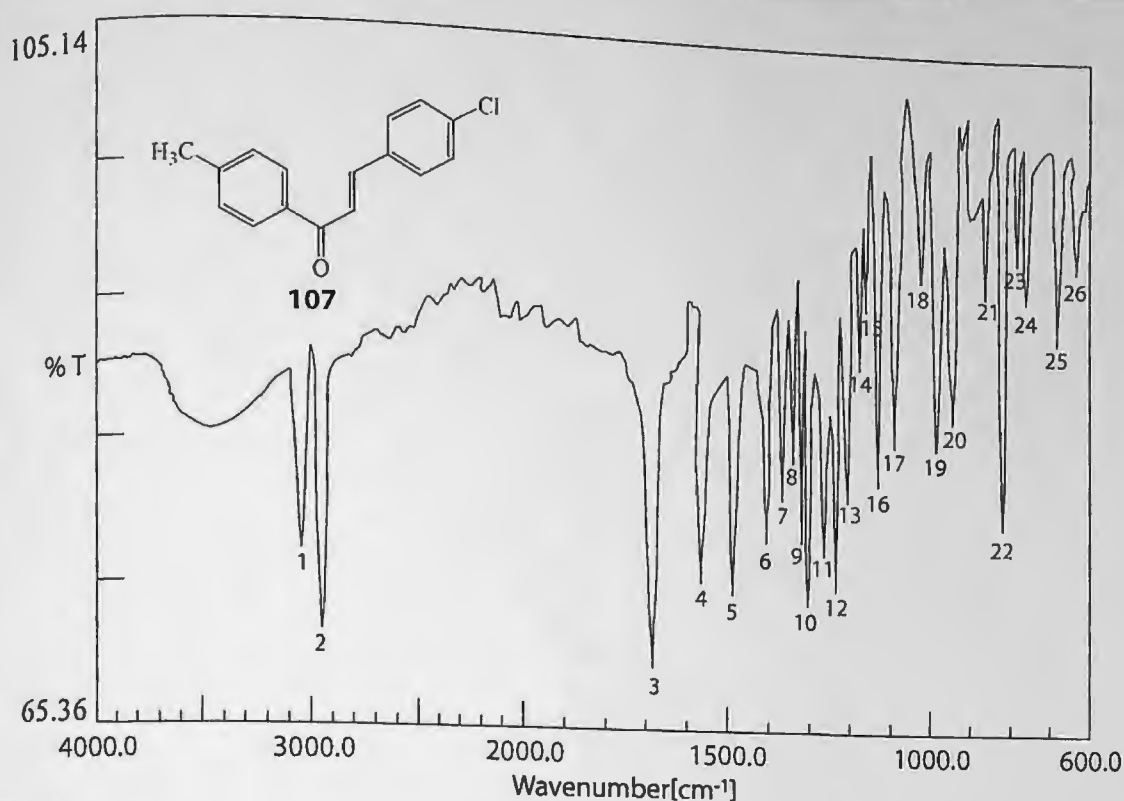
Parameter

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	
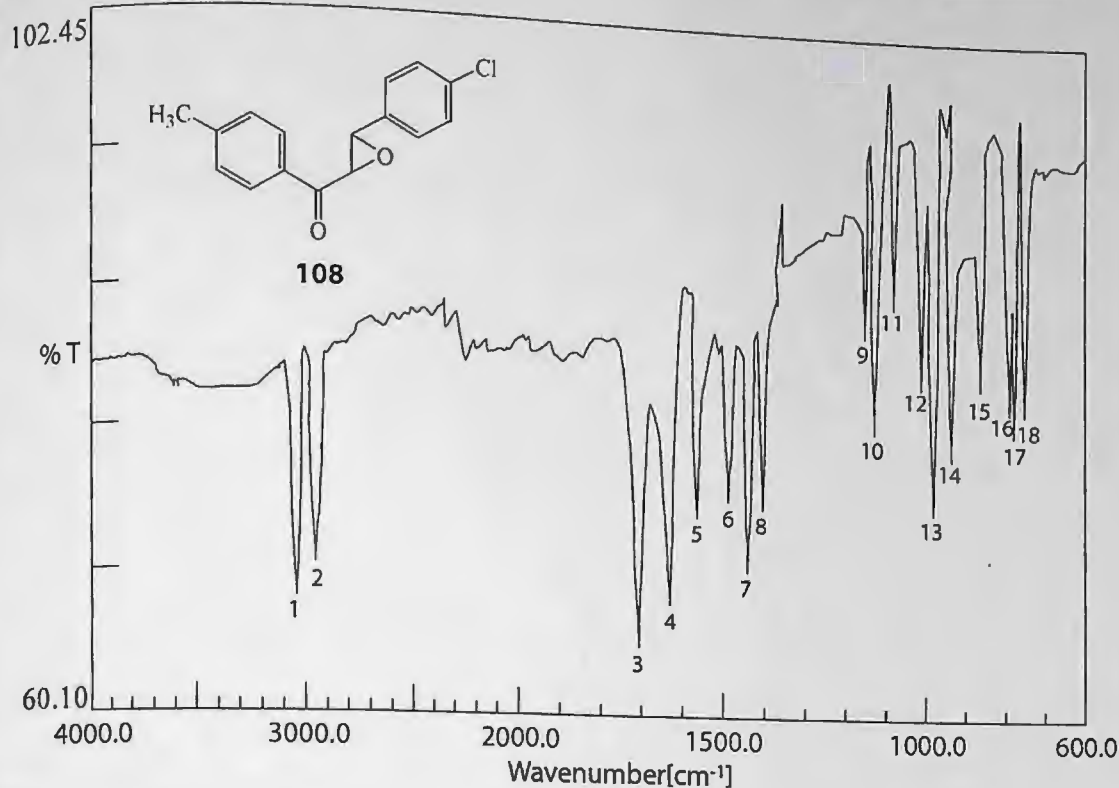
Parameter

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
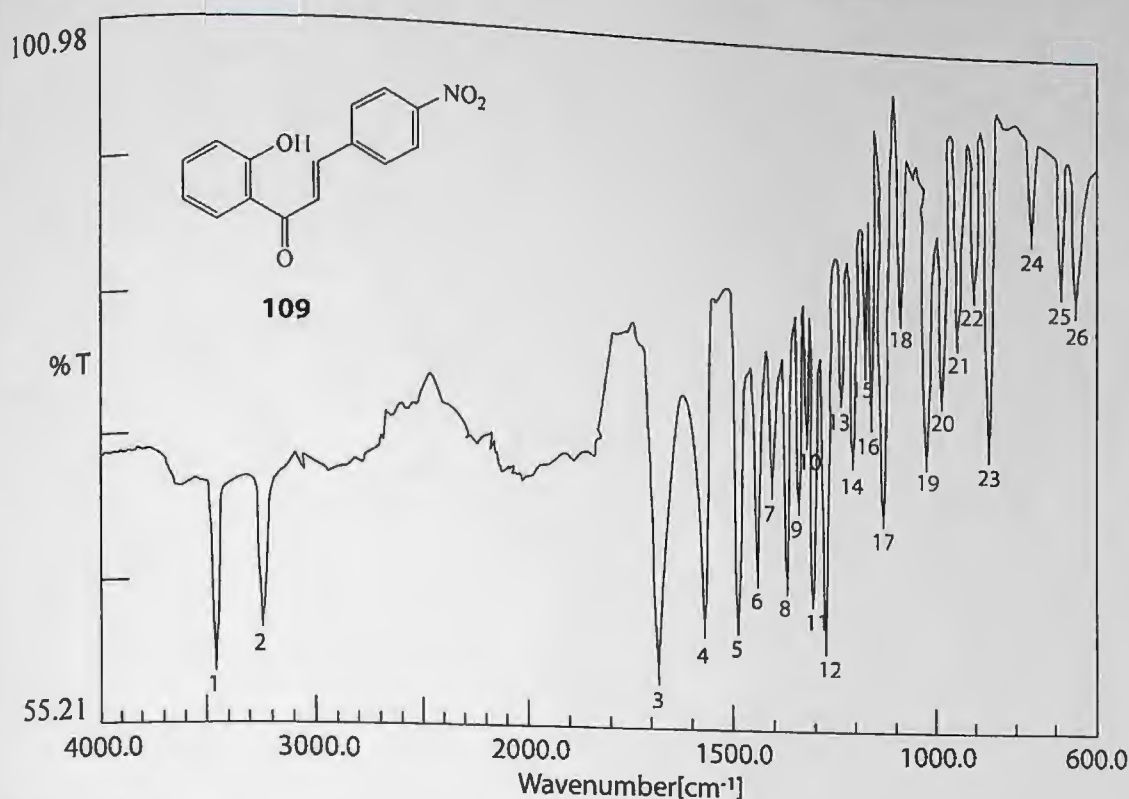
Parameter

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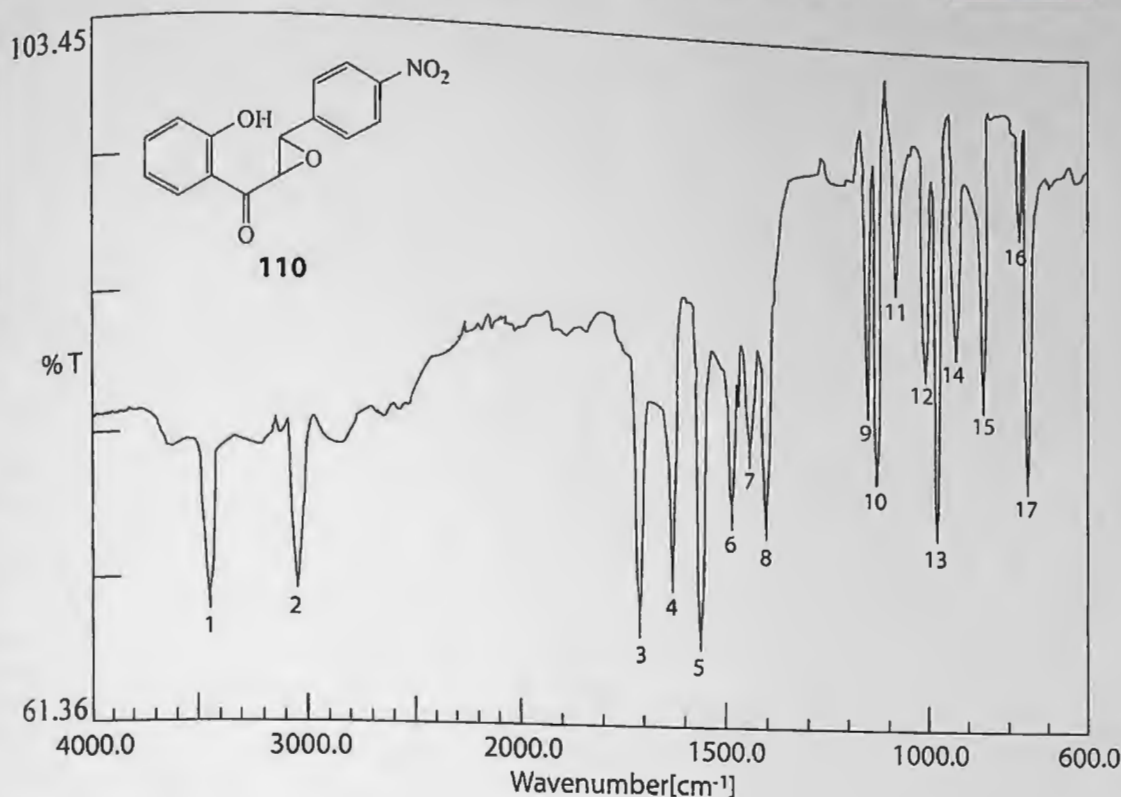
Parameter

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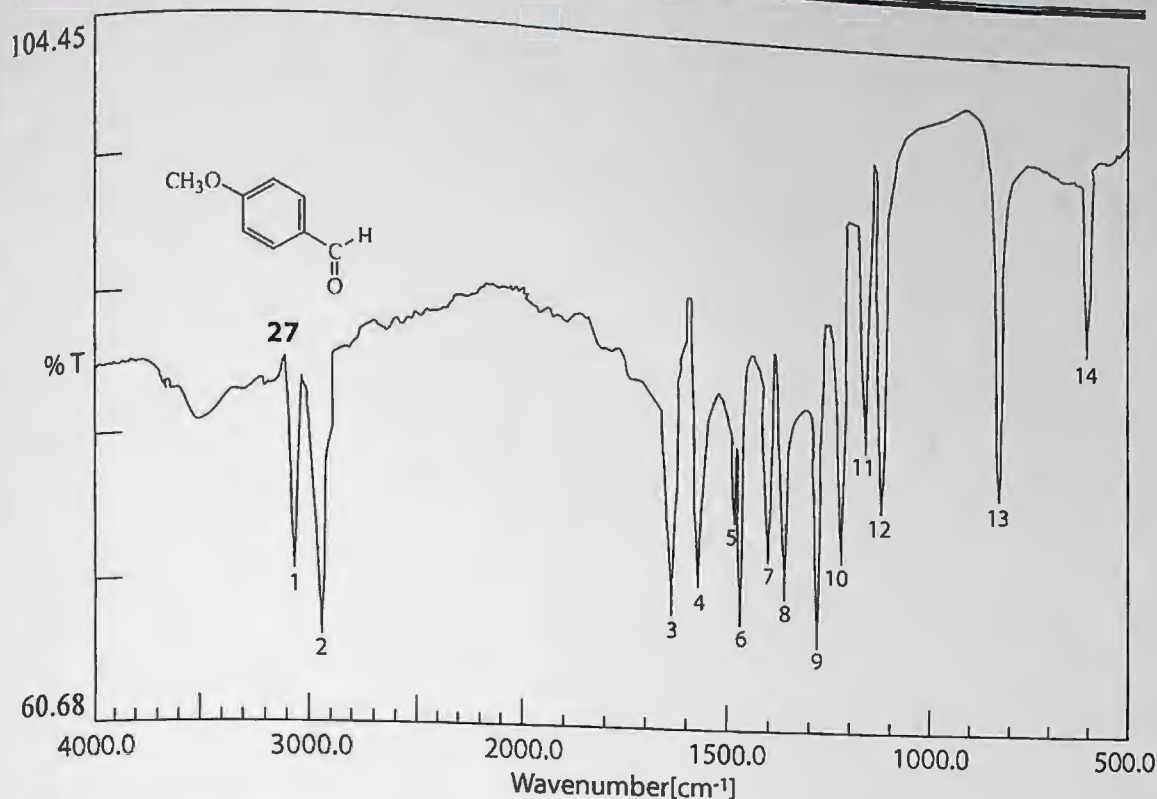
Parameter

Accumulation	48.25 Times	Apodization	Cosine
Resolution	4 cm-1	Date/Time	106-01-18 10:10 오후
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	6	1470.19	11	1160.33
2	2962.09	7	1400.17	12	1120.21
3	1635.21	8	1360.26	13	825.33
4	1560.24	9	1280.14	14	600.30
5	1480.26	10	1220.20		
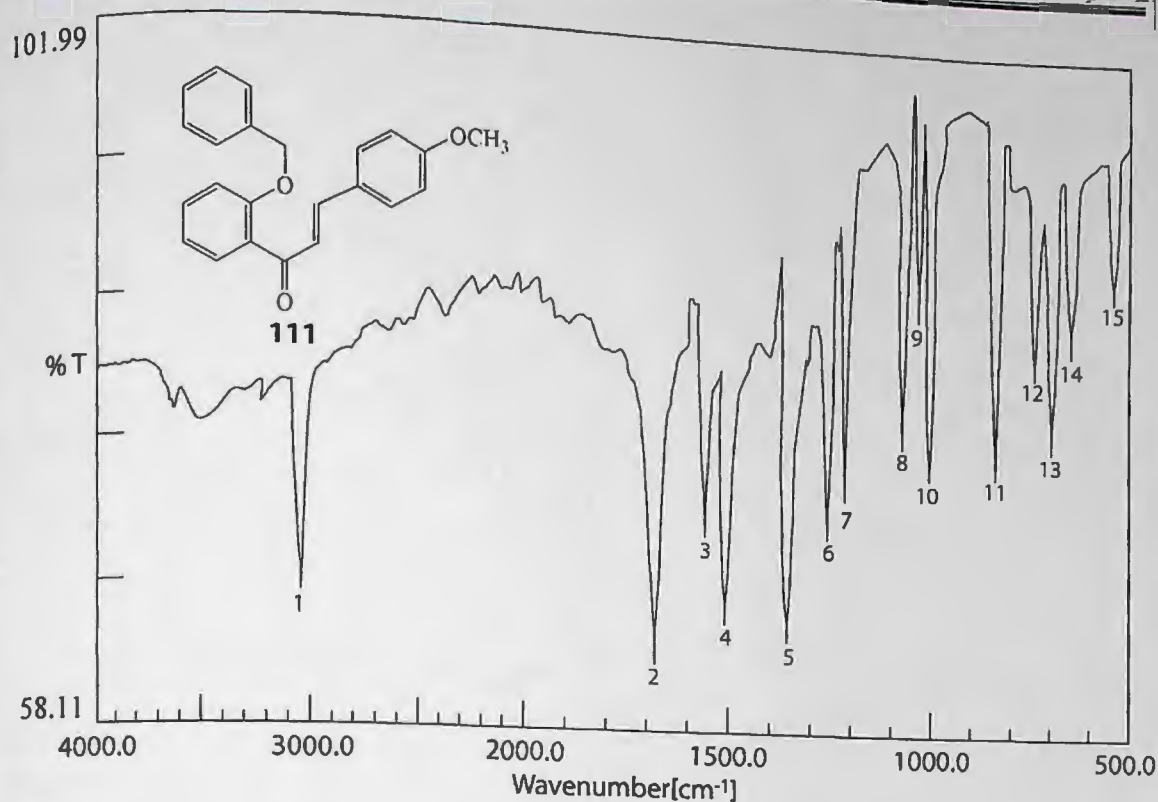
Parameter

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
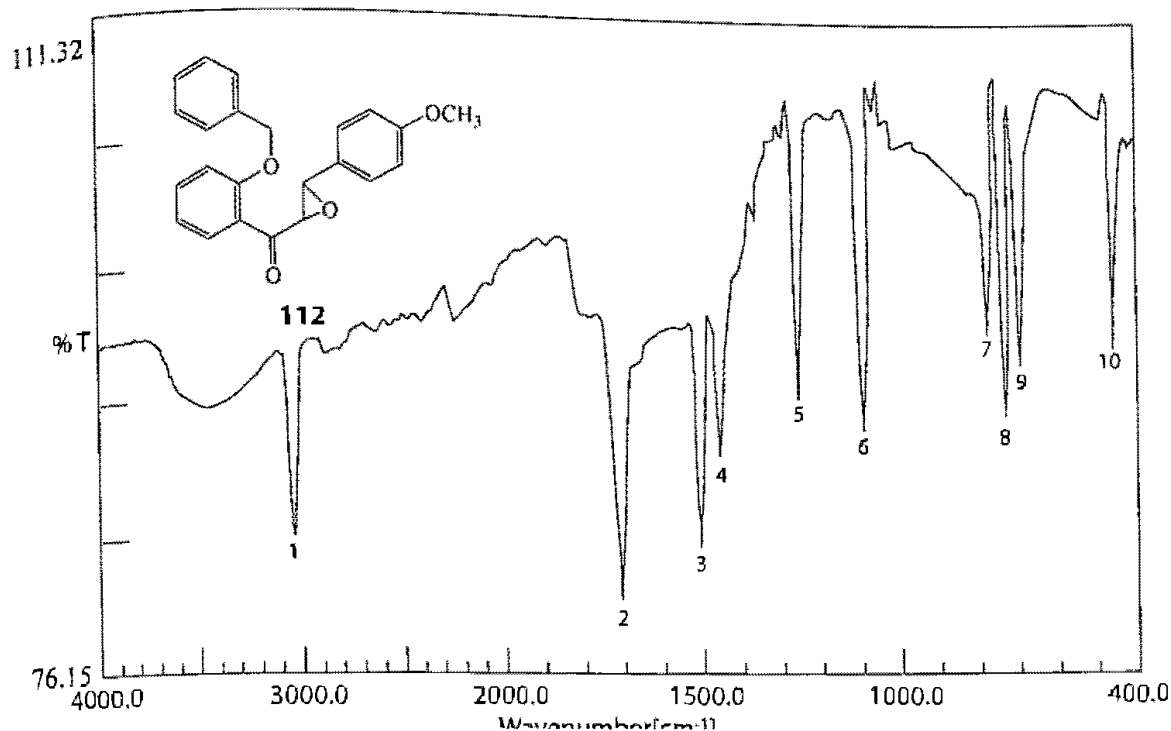
Parameter

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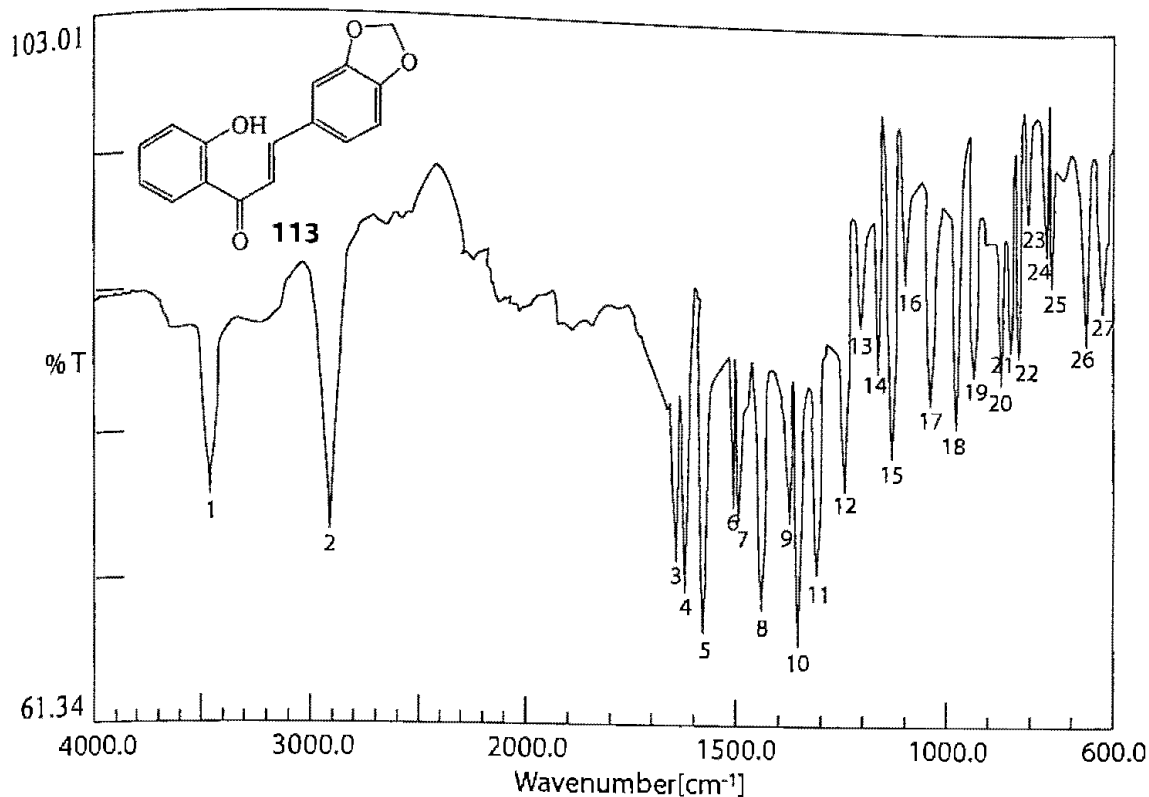
Parameter

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
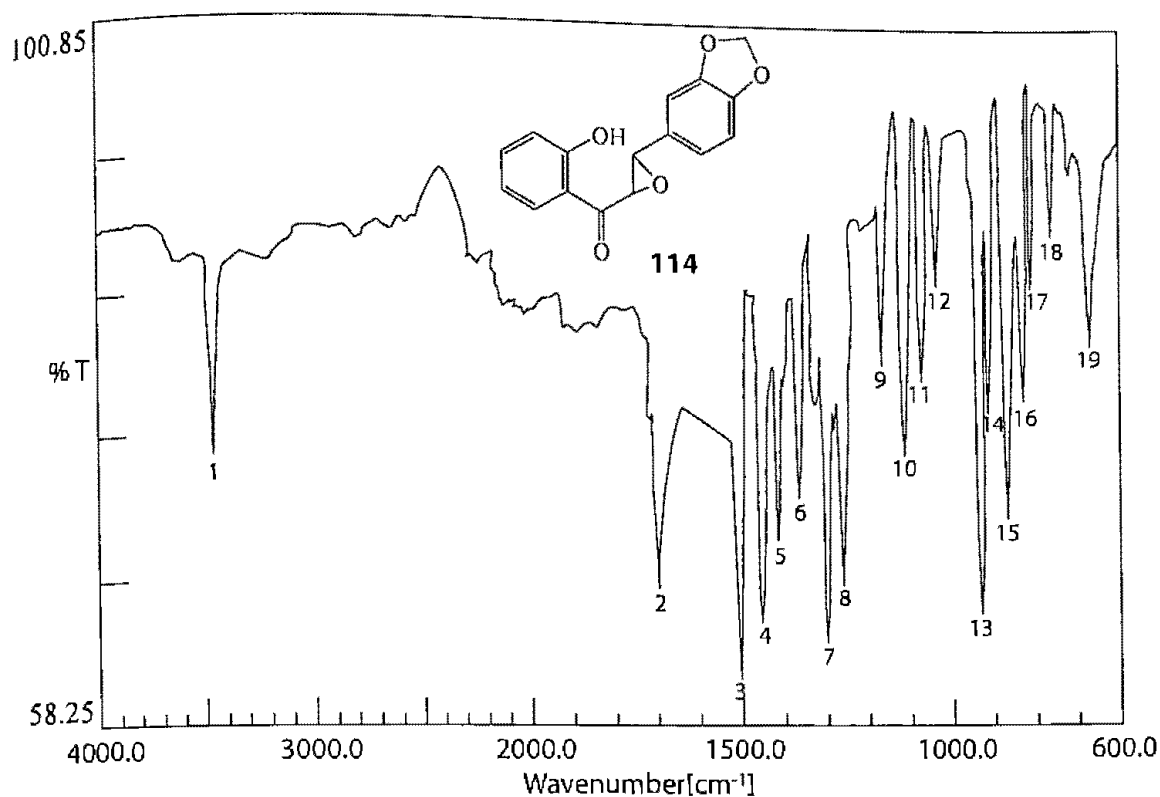
Parameter

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	
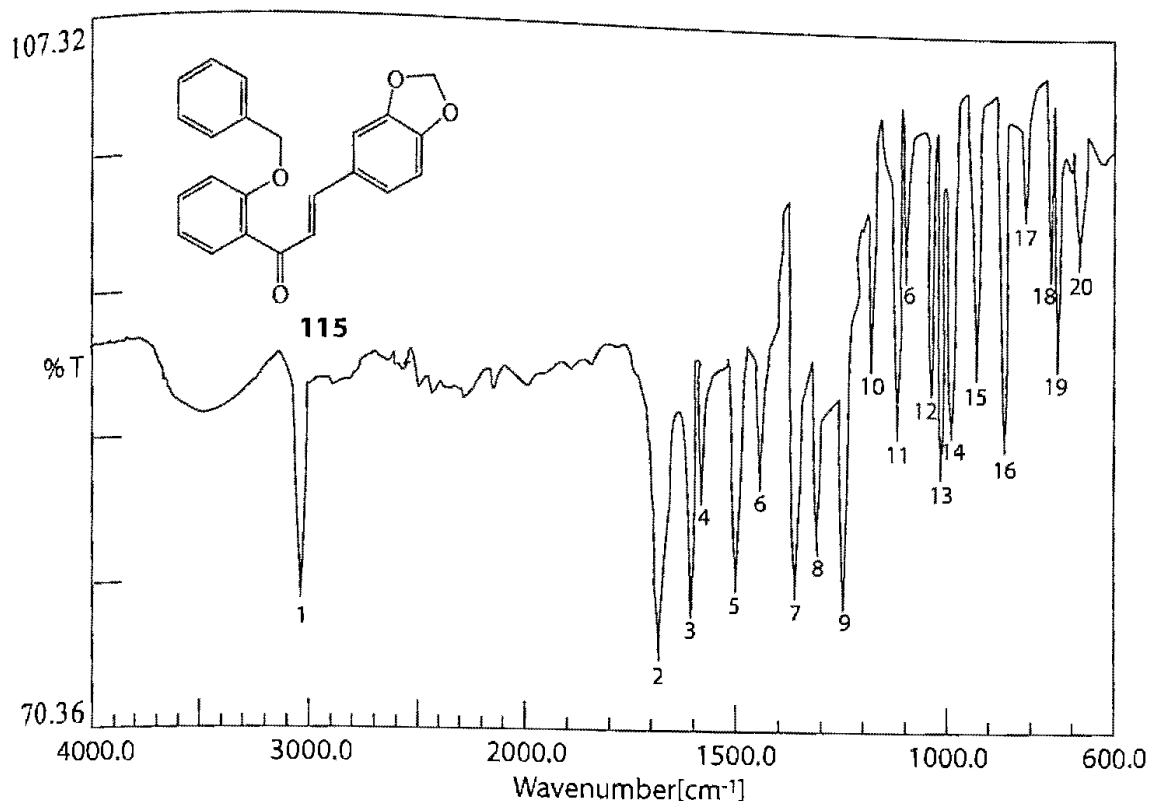
Parameter

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	× 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
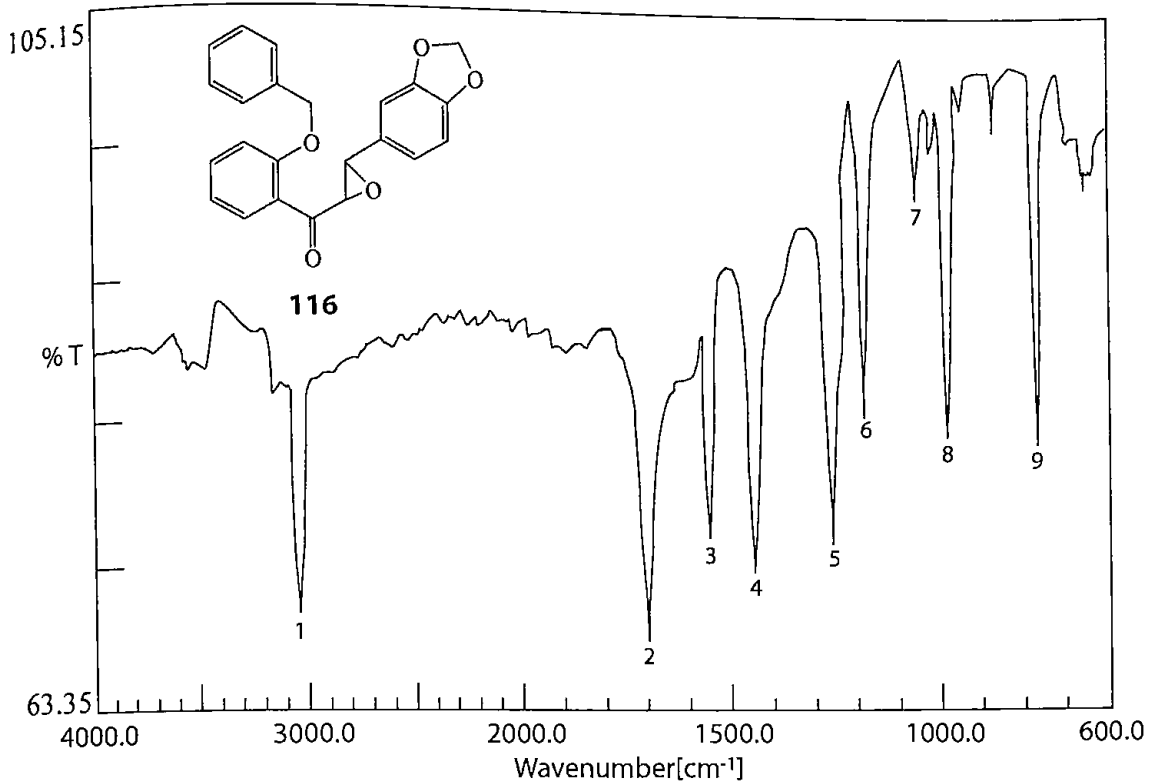
Parameter

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	
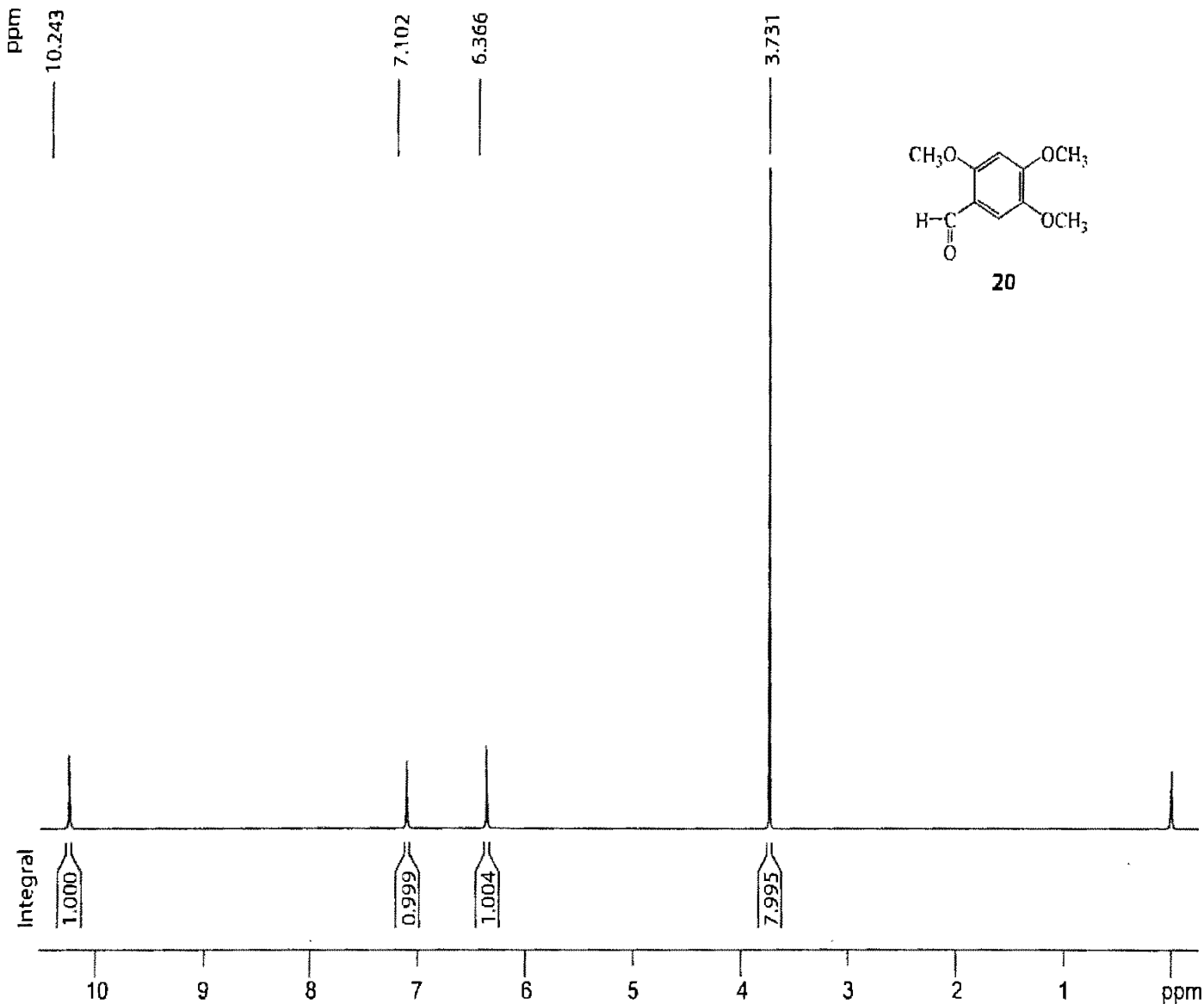
ppm

10.243

7.102

6.366

3.731

20

Integral

1.000   0.999   1.004   7.995

10   9   8   7   6   5   4   3   2   1   ppm

8.177

7.645
7.391
7.375
7.015
6.921
6.594

6.122

3.735
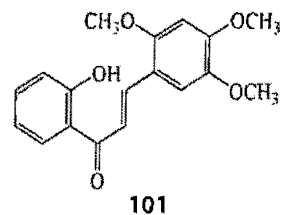
101

Integral

1.009

1.012
1.004
0.985
0.969
1.012
0.989
1.011

0.995

2.003

13 12 11 10 9 8 7 6 5 4 3 2 1 ppm

ppm
7.753
7.342
7.191
6.930
6.883

5.201

2.553

21

Integral
0.7697
1.001
4.998
1.000
0.999

1.999

2.998

8 7 6 5 4 3 2 1 ppm

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   1.30000000 sec

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

F2 - Processing parameters
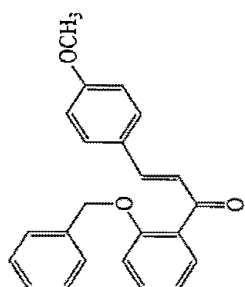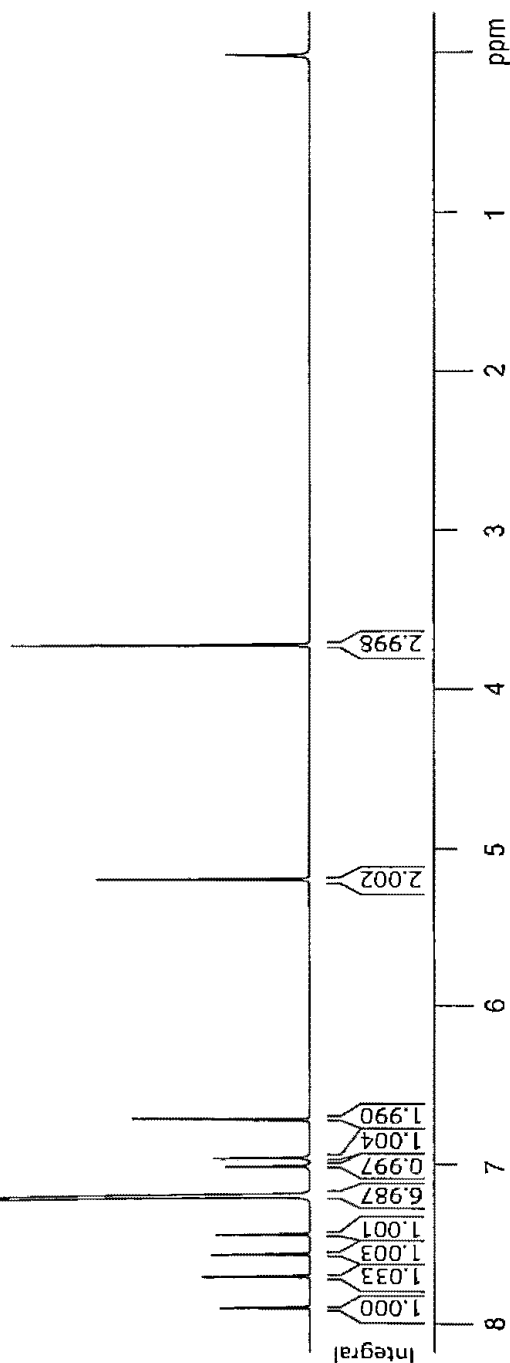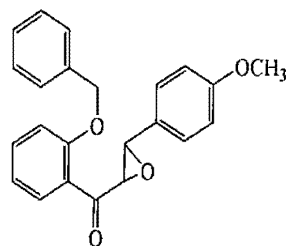SI   32768
SF   250.1300069 MHz
WDW   EM
SSB   0
LB   0.30 Hz
GB   0
PC   1.00

ppm
8.173

7.702
7.431
7.394
7.193
7.012
6.963
6.591

6.122

5.203

3.731

CH₃O

OCH₃

OCH₃

103

Integral

1.005

0.959
1.000
1.014
5.001
0.998
1.041

0.978

1.000

2.004

8.995

8   7   6   5   4   3   2   1   ppm

7.781

7.338
7.192
6.904
6.855
6.485

6.102

5.201

4.421
4.352

3.731

104

Integral

0.886

1.021
5.006
1.014
0.997

1.032

1.021

2.012

0.989
1.001

8.985

8   7   6   5   4   3   2   1   ppm

ppm
8.175

7.695
7.394
7.251
7.242
7.225
7.091
7.083

2.352

105

Integral

0.997

2.011
1.001
1.966
1.998
1.987

2.998

8   7   6   5   4   3   2   1   ppm

ppm
7.771
7.205
7.145
7.133
7.072
4.425
4.352
2.351

106

Integral
1.989
1.000
2.014
2.003
1.011
0.999
1.010
3.000

8 7 6 5 4 3 2 1 ppm

ppm 7.901 7.691 7.562 7.258 7.246 7.221

2.353

107

Integral

1.000 1.997 1.002 1.999 2.001 1.998

3.012

8   7   6   5   4   3   2   1   ppm

Integral

ppm
7.772
7.201
7.144
7.135
4.421
4.354
2351

2.011

1.999
1.998
2.001

1.001
1.000

2.999

108

H₃C

O

Cl

Current Data Parameters
NAME   HRM-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   252.2 K
D1   1.00000000 sec

======== CHANNEL f1 ========
NUC1   1H
P1   10.50 usec
PL1   0.00 dB
SFO1   250.1315447 MHz
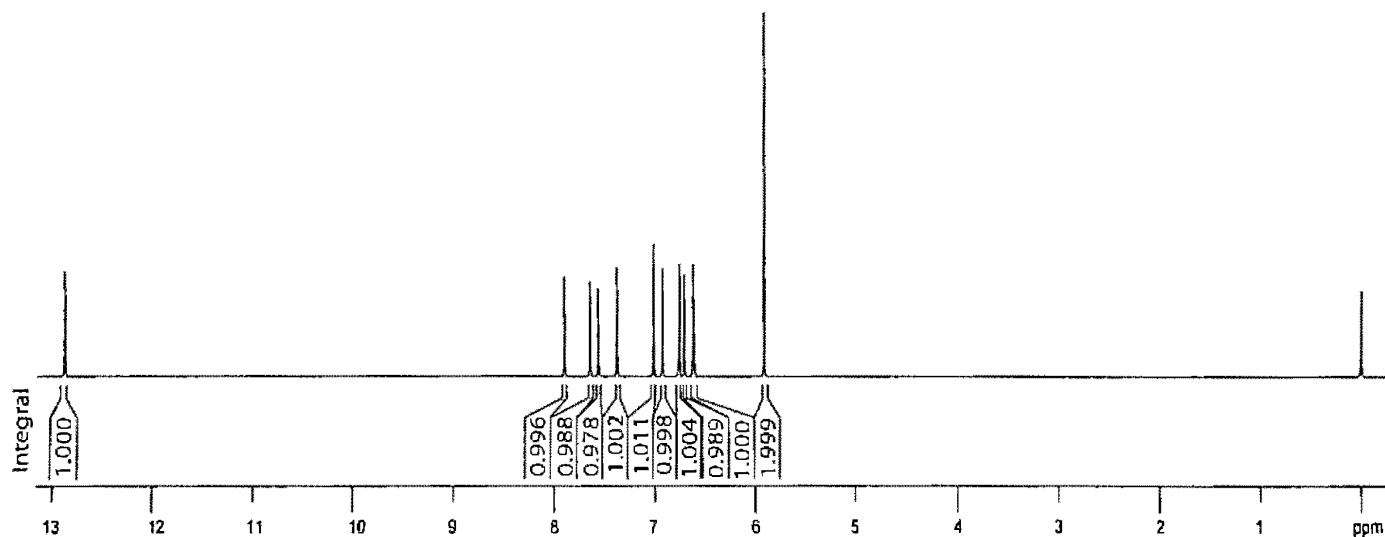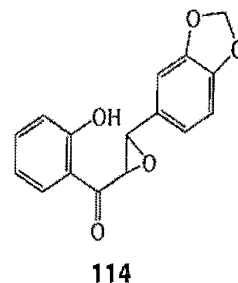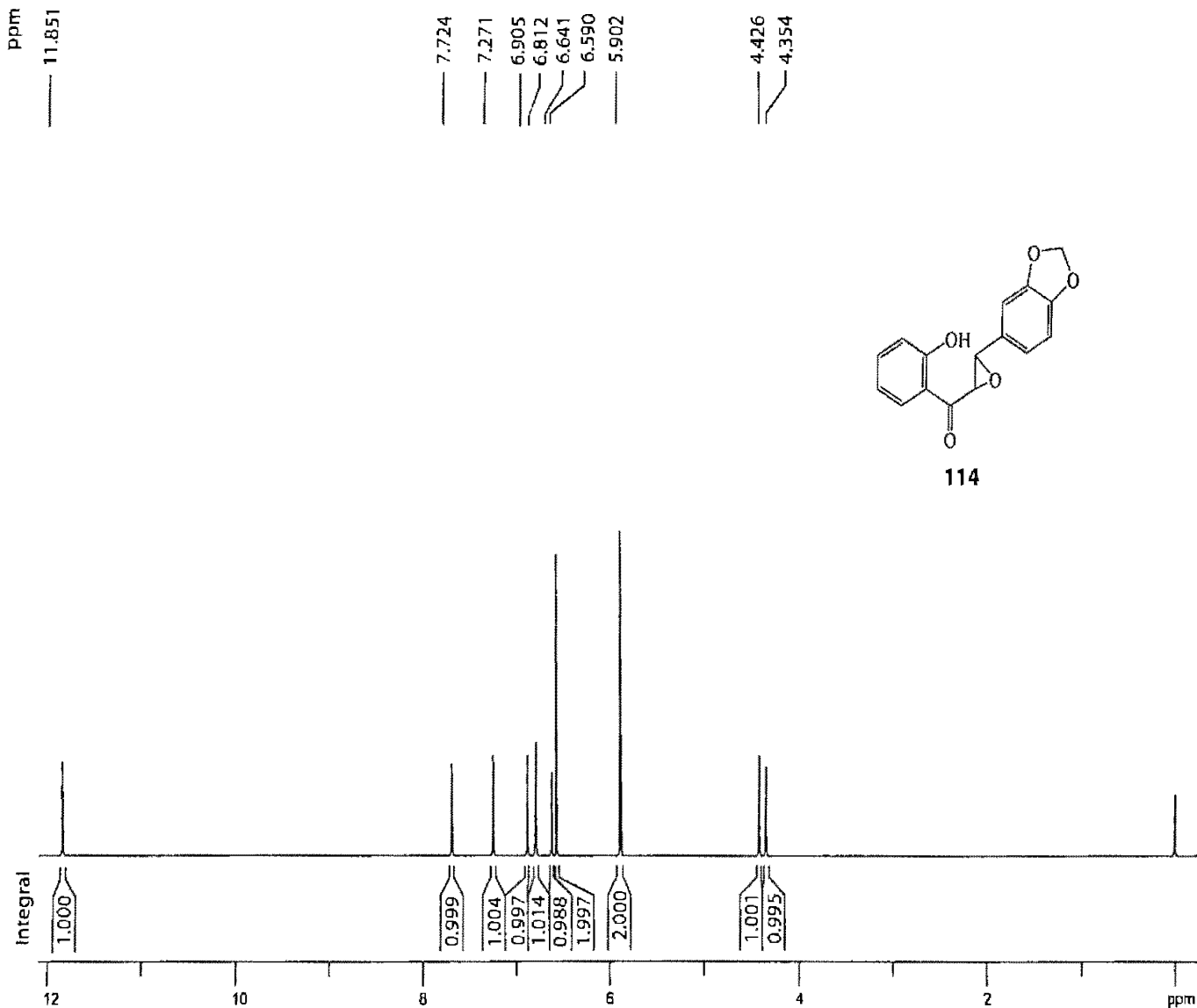
F2 - Processing parameters
SI   32768
SF   250.1300008 MHz
WDW   EM
SSB   0
LB   0.30 Hz
GB   0
PC   1.00

8.142
8.042
7.854
7.641
7.565
7.373
7.015
6.921
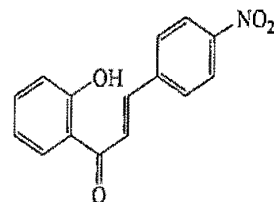
109
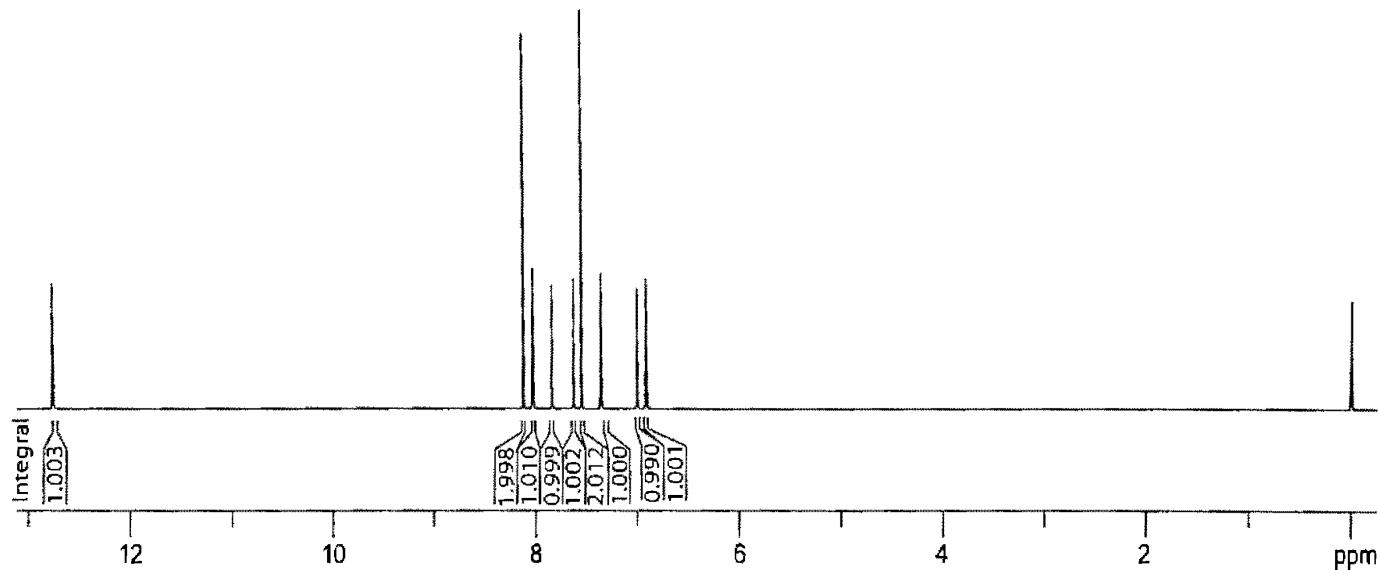
Integral
1.003

1.998
1.010
0.999
1.002
2.012
1.000
0.990
1.001

12   10   8   6   4   2   ppm

ppm — 11.401

8.123
7.721
7.452
7.871
6.901
6.812

4.421
4.353

110

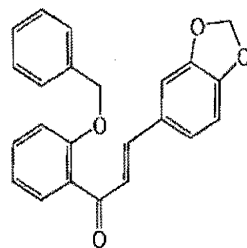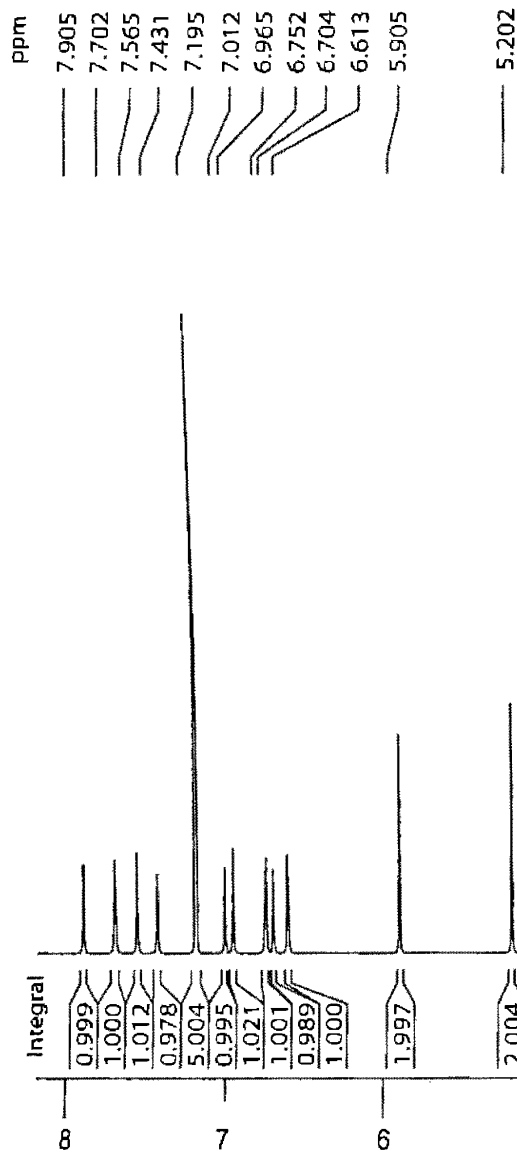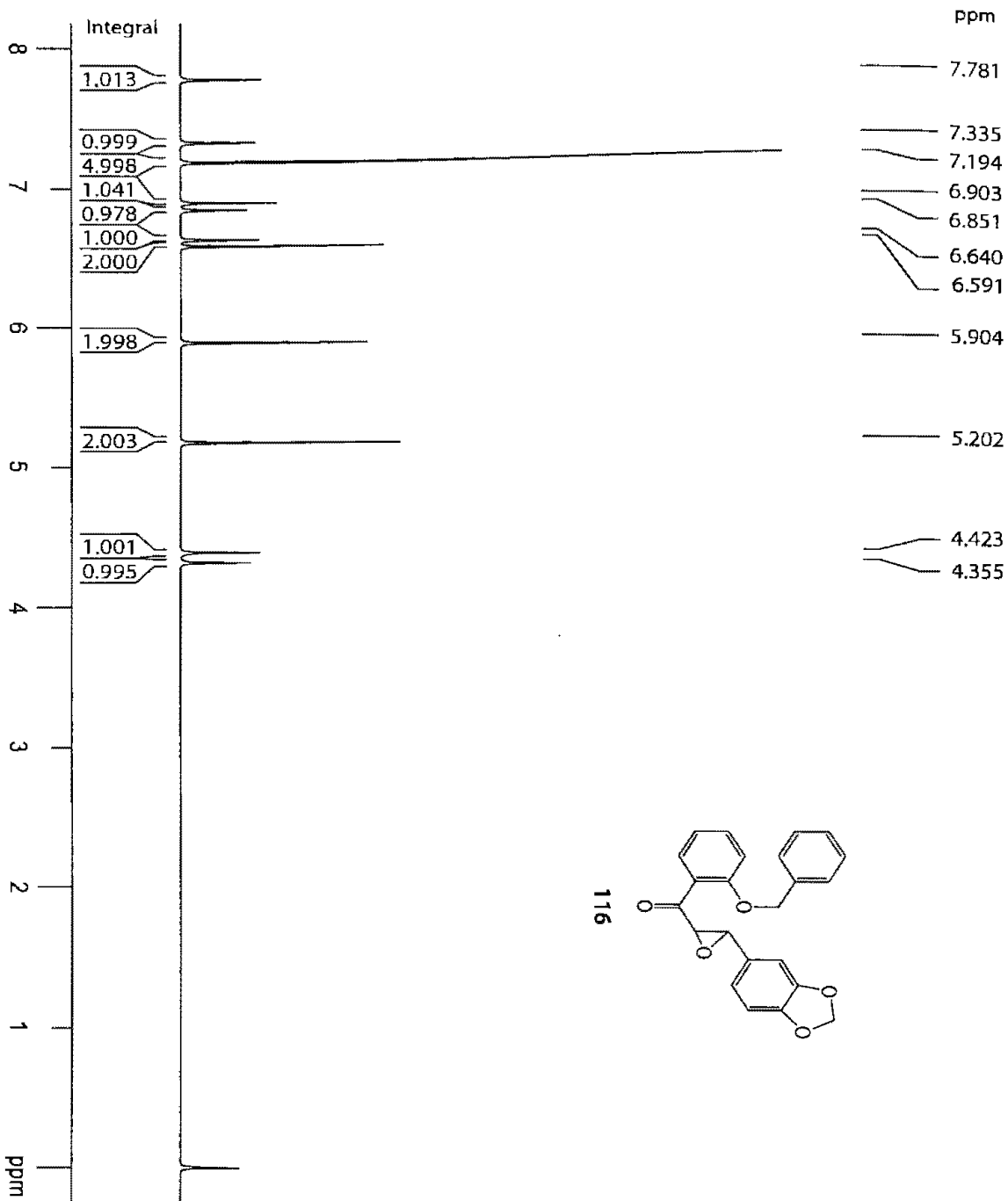Current Data Parameters
NAME   MRAI-110
EXPNO   1
PROCNO   1

F2 - Acquisition Parameters
Date_   20080902
Time   17.43
INSTRUM   spect
PROBHD   5 mm Dual 13C/
PULPROS   zg30
TD   65536
SOLVENT   CDC13
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
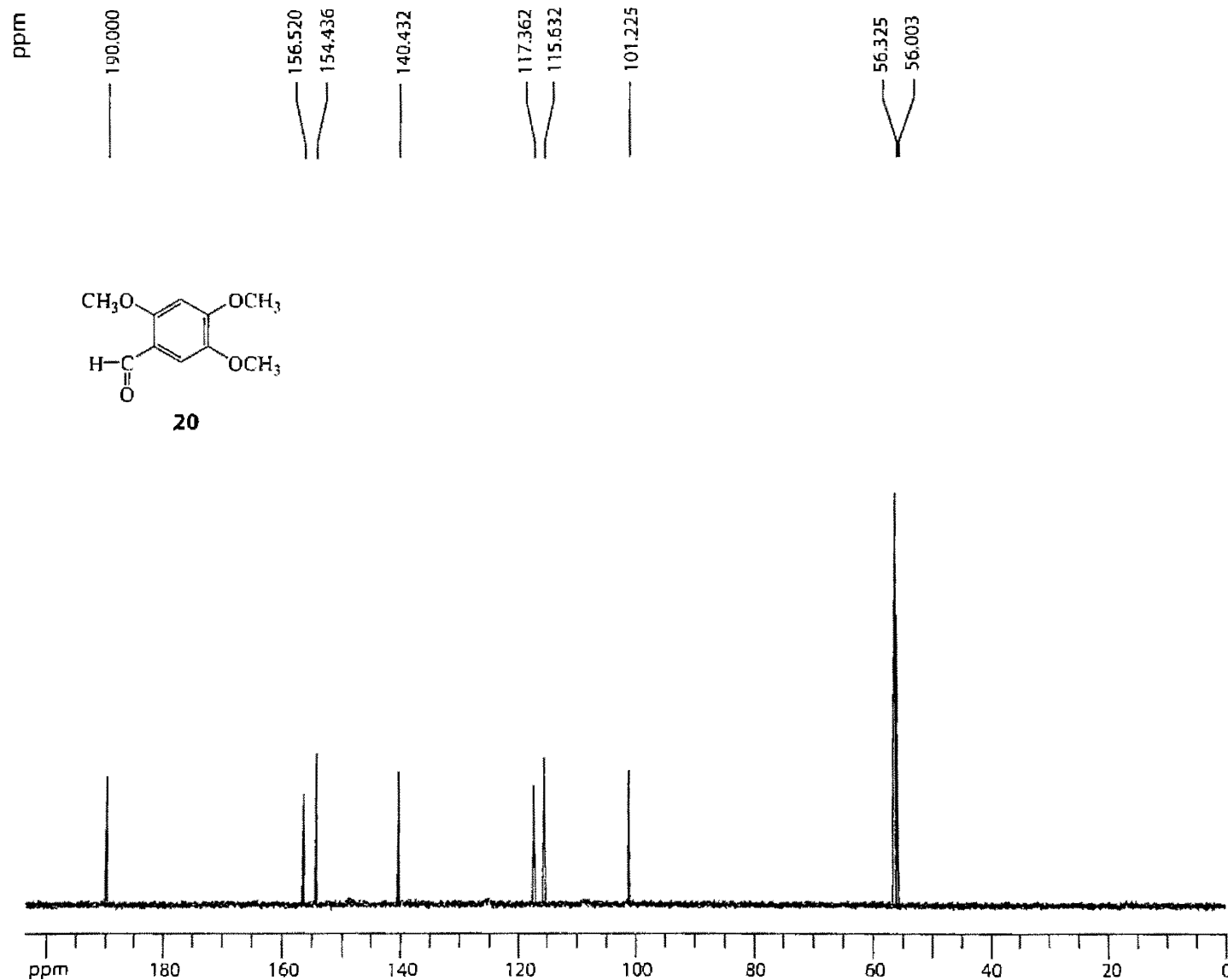SF   250.1300060 MHz
WDW   EM
SSB   0
LB   0.30 Hz
GB   0
PC   1.00

Integral

0.990

1.989
0.998
2.003
1.000
1.004
1.021

1.001
0.997

12   10   8   6   4   2   ppm

ppm
9.877
7.703
6.962
3.731

CH₃O— (benzaldehyde structure, 4-methoxybenzaldehyde)

27

Integral

0.998

1.999
2.000

3.001

10   9   8   7   6   5   4   3   2   1   ppm

Current Data Parameters
NAME   MRA-11-
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   6.330277 sec
RG   574.7
DW   96.600 usec
DE   6.00 usec
TE   292.2 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   250.1300056 MHz
WDW   EM
SSB   0
LB   0.30 Hz
GB   0
PC   1.00

3.732

5.201

6.723
6.964
7.013
7.192
7.434
7.562
7.701
7.905

2.998

2.002

1.990
1.004
0.997
6.987
1.001
1.003
1.033
1.000

ppm

Integral

7.781

7.334
7.193
7.084
6.901
6.852
6.706

5.201

4.423
4.355

3.732

112

Integral

1.021

0.999
5.002
2.001
1.020
0.990
2.000

1.998

1.010
1.001

3.001

8   7   6   5   4   3   2   1   ppm

7.906
7.645
7.561
7.375
7.015
6.926
6.750
6.704
6.612
5.903

113

Integral

1.000
0.996
0.988
0.978
1.002
1.011
0.998
1.004
0.989
1.000
1.999

13 12 11 10 9 8 7 6 5 4 3 2 1 ppm

7.724
7.271
6.905
6.812
6.641
6.590
5.902

4.426
4.354

114

Integral

1.000

0.999
1.004
0.997
1.014
0.988
1.997
2.000

1.001
0.995

12   10   8   6   4   2   ppm

ppm 7.905 7.702 7.565 7.431 7.195 7.012 6.965 6.752 6.704 6.613 5.905

5.202

115

Integral
0.999 1.000 1.012 0.978 5.004 0.995 1.021 1.001 0.989 1.000

1.997

2.004

8   7   6   5   4   3   2   1   ppm

ppm

190.000

156.520
154.436

140.432

117.362
115.632

101.225

56.325
56.003

CH₃O / OCH₃ / OCH₃ structure labeled **20** (aldehyde, H–C=O)

Current Date Parameters
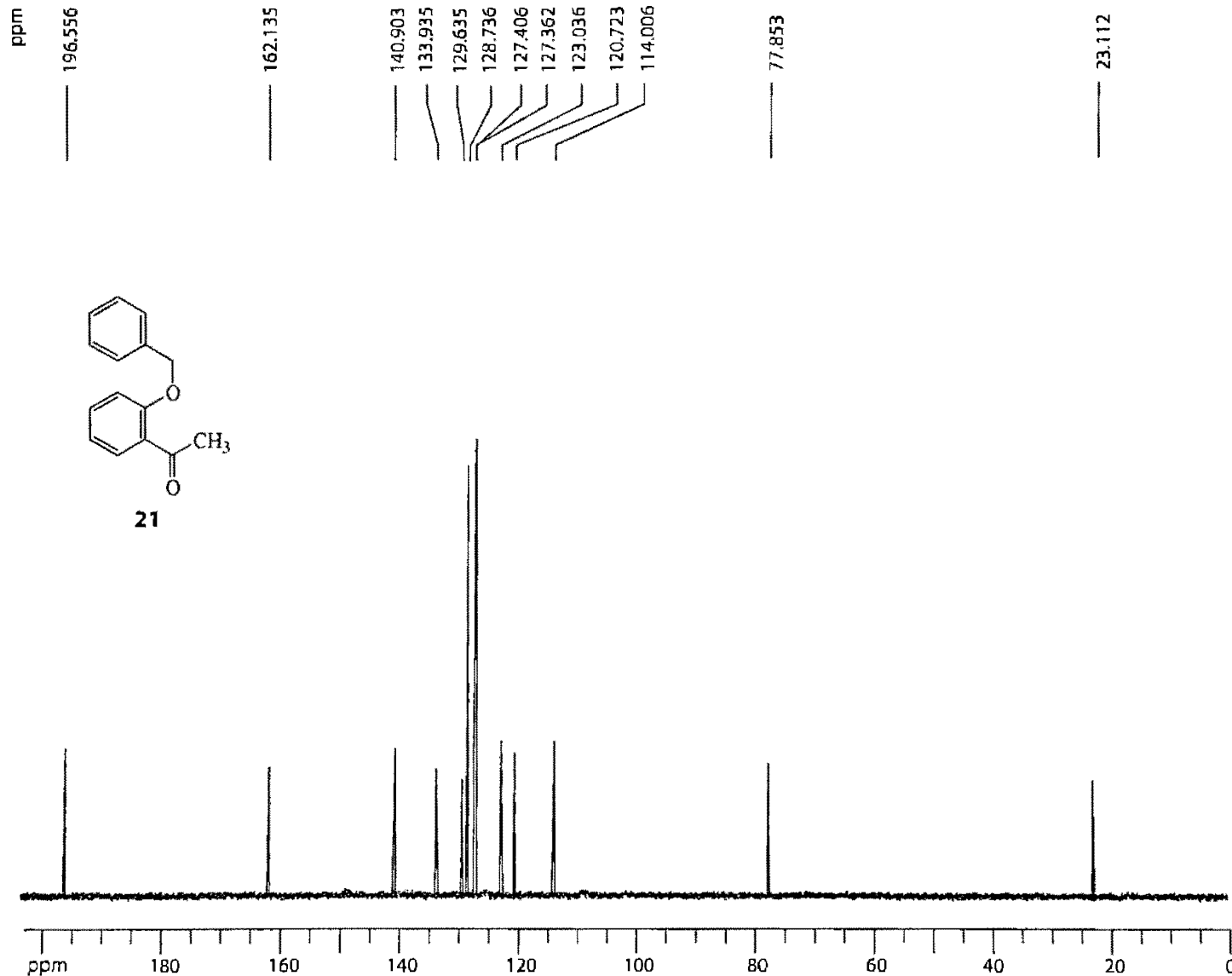NAME   WRAI-20
EXPNO   2
PROCNO   1

F2 - Acquisition Parameters
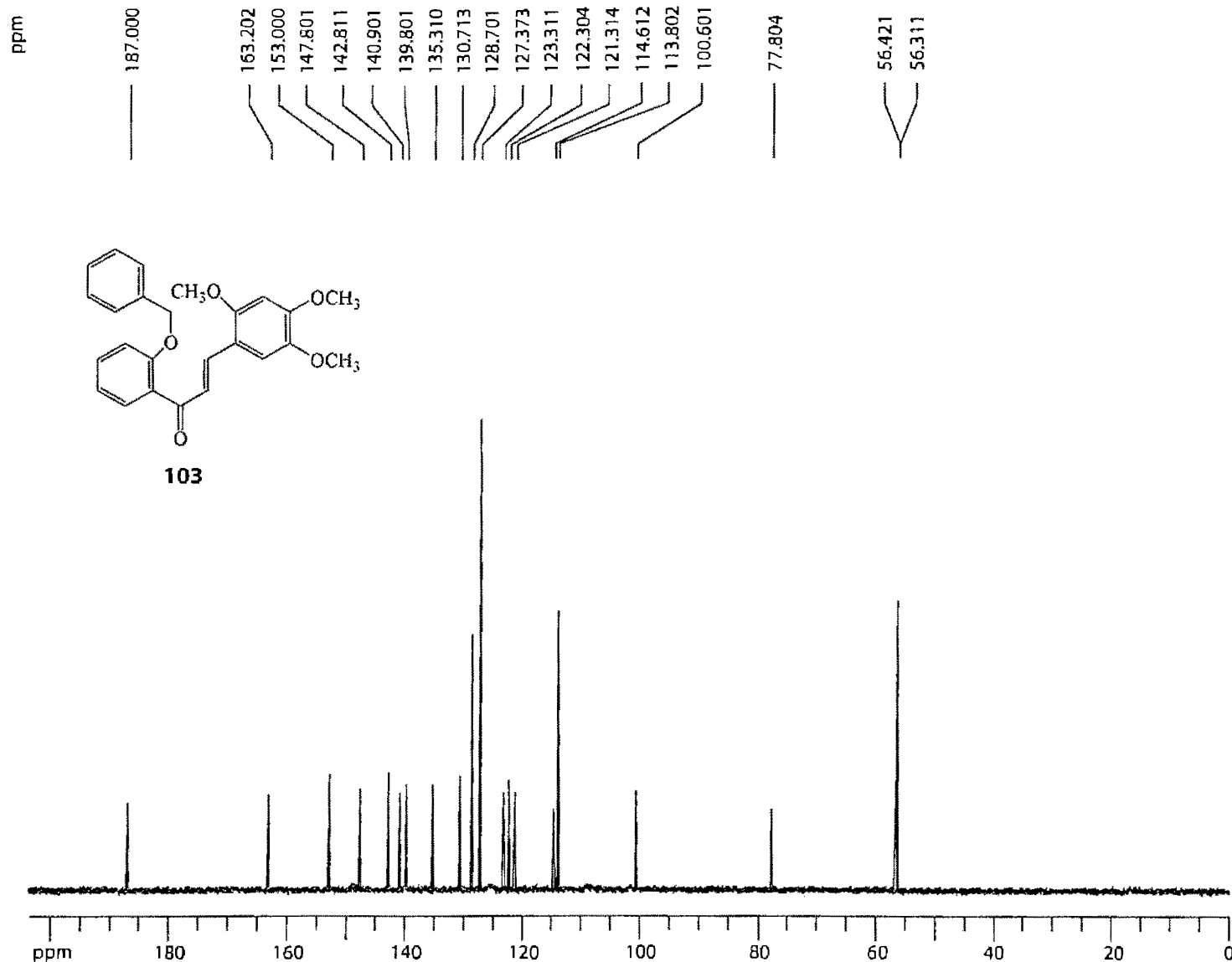Date_   20080903
Time   12.44
INSTRUM   spect
PROBHD   5 mm Dual 13
PULPROG   zgpg30
TD   65536
SOLVENT   DMSO
NS   300
DS   0
SWH   19041.270 Hz
FIDRES   0.302754 Hz
AQ   1.6515572 sec
RG   15384
DW   25.200 usec
DE   6.00 usec
TE   303.0 K
D12   0.00002000 sec
PL13   22.00 dB
D1   2.00000000 sec
CPDPRG2   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   62.8952423 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   12625.13 Hz
F2P   5.826 ppm
F2   366.11 Hz
PPMCM   9.74389 ppm/cm
HZCM   612.85060 Hz/cm

ppm 180 160 140 120 100 80 60 40 20 0

187.301

158.503
153.001
147.804
142.803
139.812
135.711
131.112
123.901
123.301
121.612
116.201
113.811

100.613

56.400
56.301

CH₃O and OCH₃ structure labeled **101**

ppm   180   160   140   120   100   80   60   40   20   0

ppm

197.601

157.403
152.204
148.101
139.810
134.004
130.003
124.602
121.001
116.612
115.611
113.002
100.602

70.001

56.304
48.501

CH₃O — drawn as CH$_3$O, OCH$_3$, OCH$_3$, OH, O

102

Current Data Parameters
NAME   MRAI-102
EXPNO   2
PROCNO   1

F2 - Acquisition Parameters
Date_   20030005
Time   12.44
INSTRUM   spect
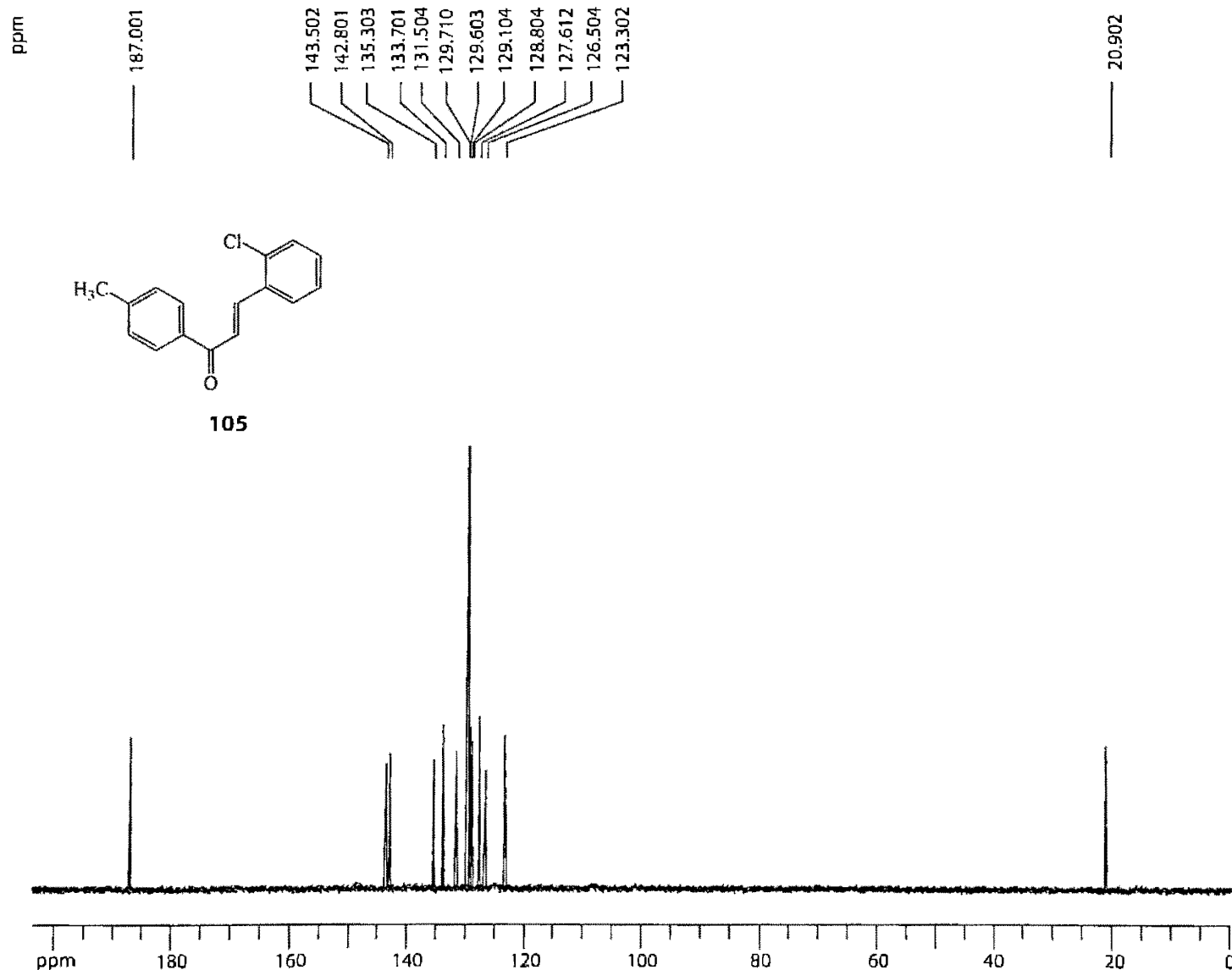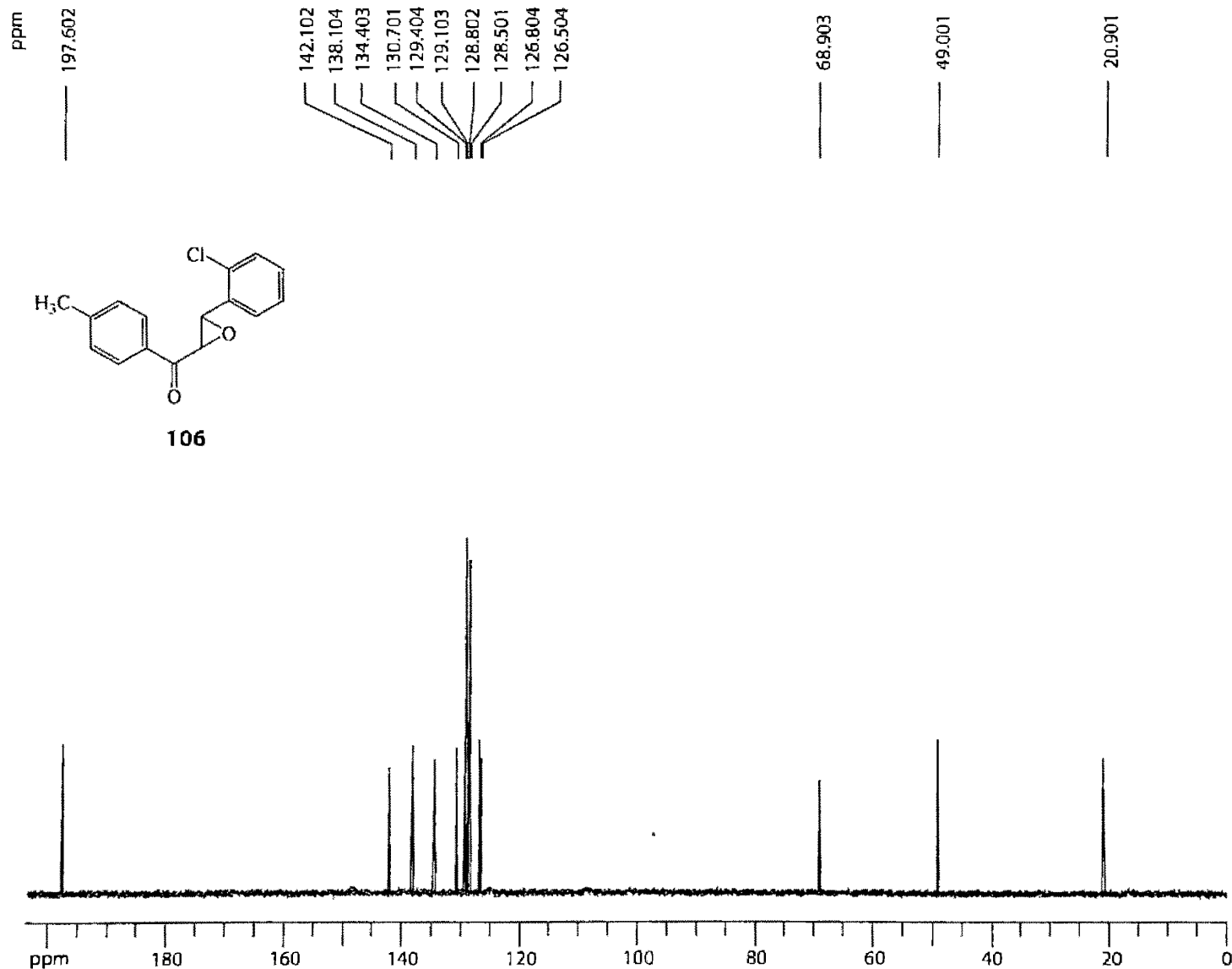PROBHD   5 mm Dual 13
PULPROG   zgpg30
TD   65536
SOLVENT   DMSO
NS   300
DS   0
SWH   19841.270 Hz
FIDRES   0.302754 Hz
AQ   1.6515577 sec
RG   16384
DW   25.200 usec
DE   6.00 usec
TE   300.0 K
D12   0.00002000 sec
PL13   22.00 dB
D1   2.00000000 sec
CPDPRG2   waltz16
PCPD2   50.00 usec
SFO2   250.1312345 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   62.8952425 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   12625.13 Hz
F2P   5.821 ppm
F2   366.11 Hz
PPMCM   5.74395 ppm/cm
HZCM   612.85080 Hz/cm

ppm   180   160   140   120   100   80   60   40   20   0

ppm

196.556

162.135

140.903
133.935
129.635
128.736
127.406
127.362
123.036
120.723
114.006

77.853

23.112

21

Current Data Parameters
NAME   HRAI-21
EXPNO   7
PROCNO   1

F2 - Acquisition Parameters
Date_   20030303
Time   12.44
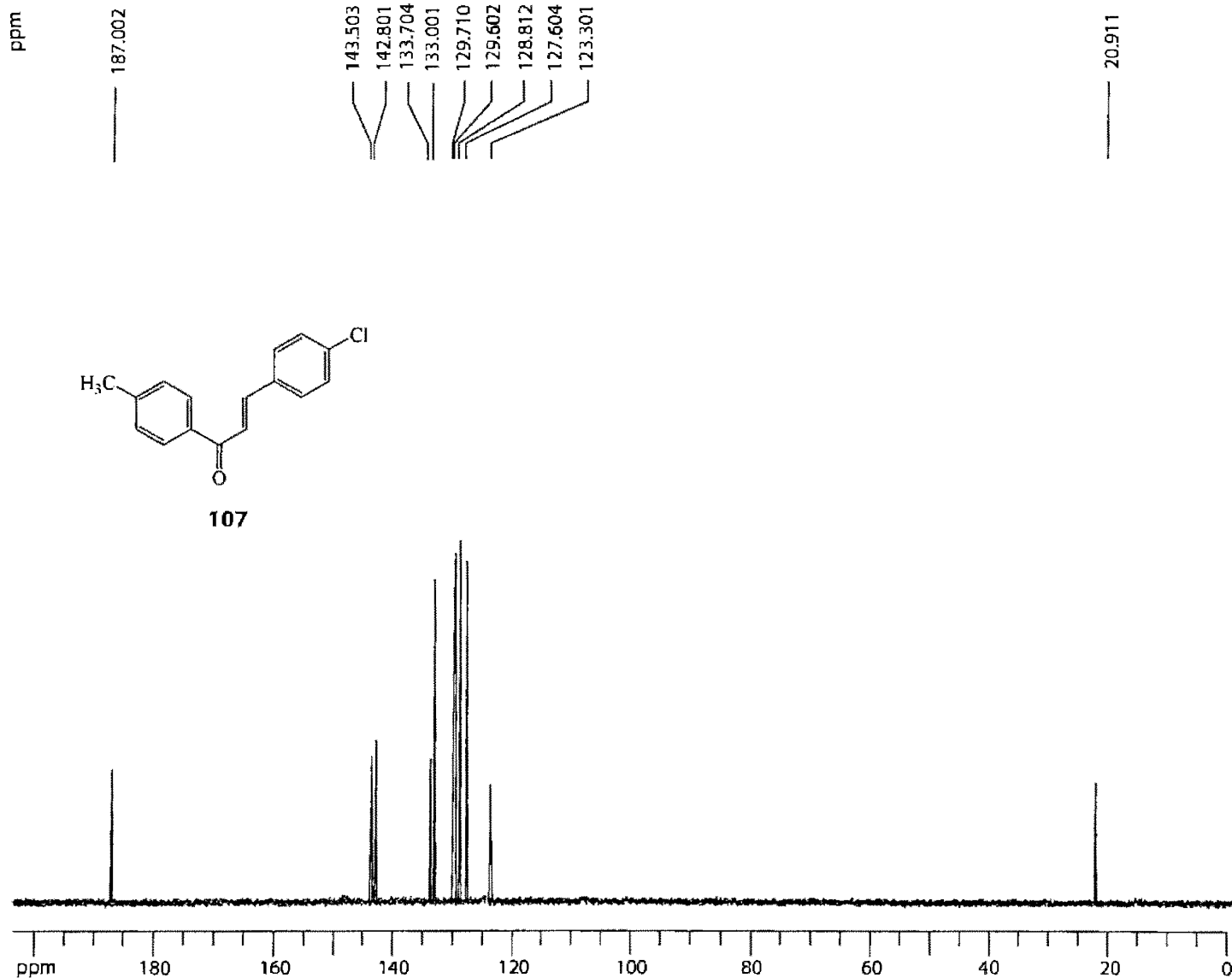INSTRUM   spect
PROBHD   5 mm Dual 13
PULPROG   zgpg30
TD   65536
SOLVENT   DMSO
NS   300
DS   t
SWH   19841.270 Hz
FIDRES   0.302754 Hz
AQ   1.6515572 sec
RG   16384
DW   25.200 usec
DE   6.00 usec
TE   300.0 K
D12   0.00002000 sec
PL13   22.00 dB
D1   2.00000000 sec
CPDPRG2   waltz16
PCPD2   90.00 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   62.8953429 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   5.824 ppm
F2   366.11 Hz
PPMCM   9.74389 ppm/cm
HZCM   612.85063 Hz/cm

ppm   180   160   140   120   100   80   60   40   20   0

ppm

187.000

163.202
153.000
147.801
142.811
140.901
139.801
135.310
130.713
128.701
127.373
123.311
122.304
121.314
114.612
113.802
100.501

77.804

56.421
56.311

CH$_3$O

OCH$_3$

O

OCH$_3$

O

103

ppm 180 160 140 120 100 80 60 40 20 0

ppm

197.613

162.103
152.202
148.001
140.903
139.804
133.902
129.600
128.704
127.390
123.001
120.705
116.602
114.503
113.001
100.611

77.802
70.000

56.312
48.501

CH₃O — OCH₃

OCH₃

O

104
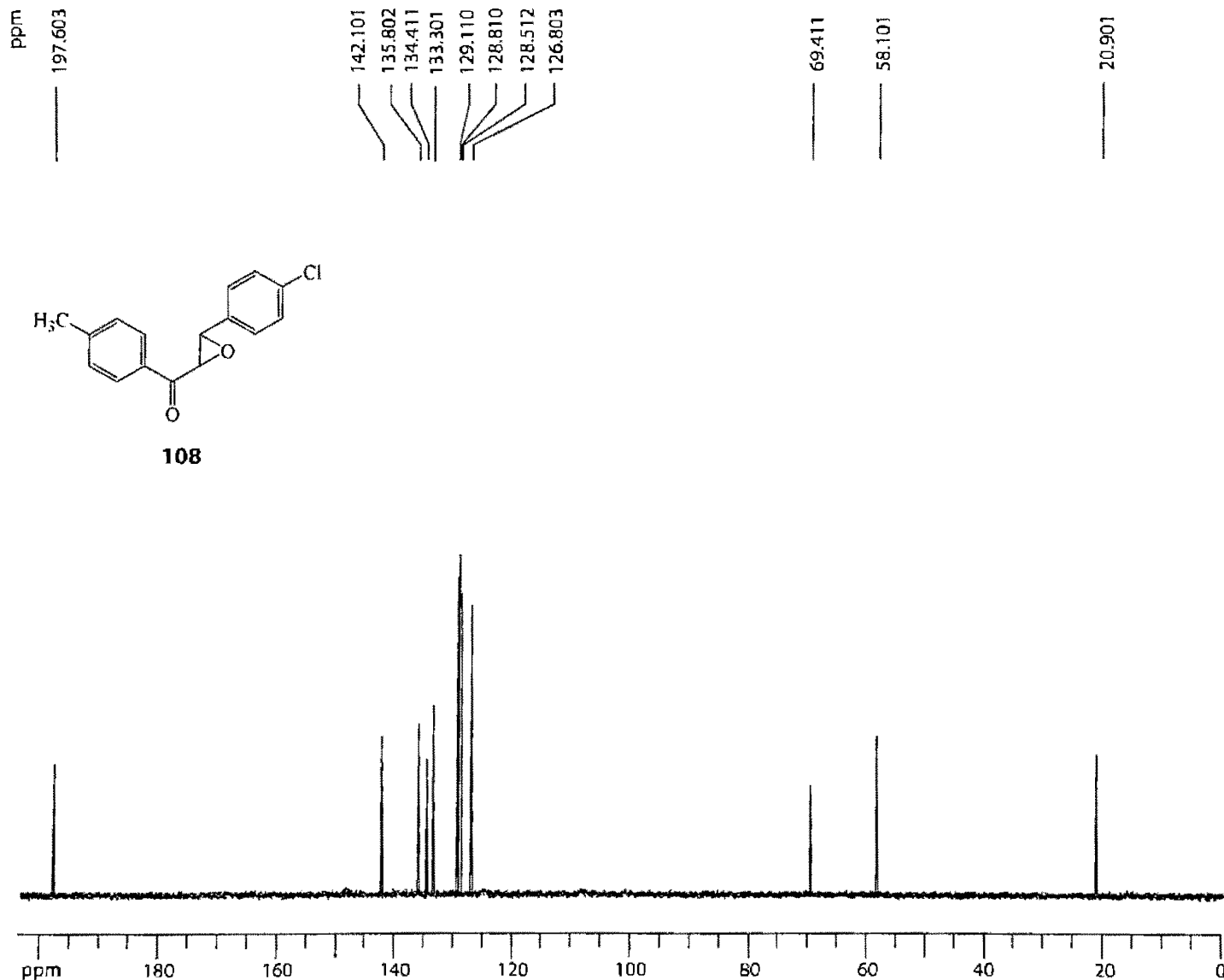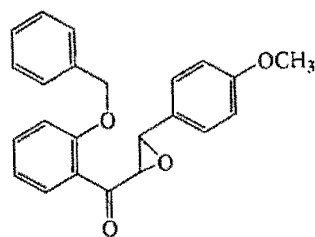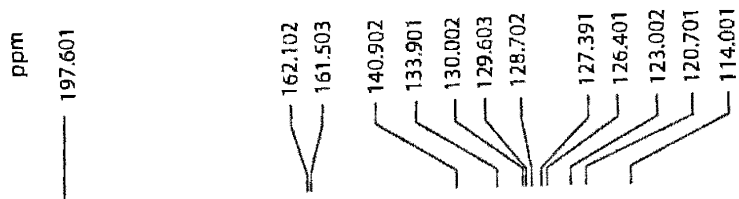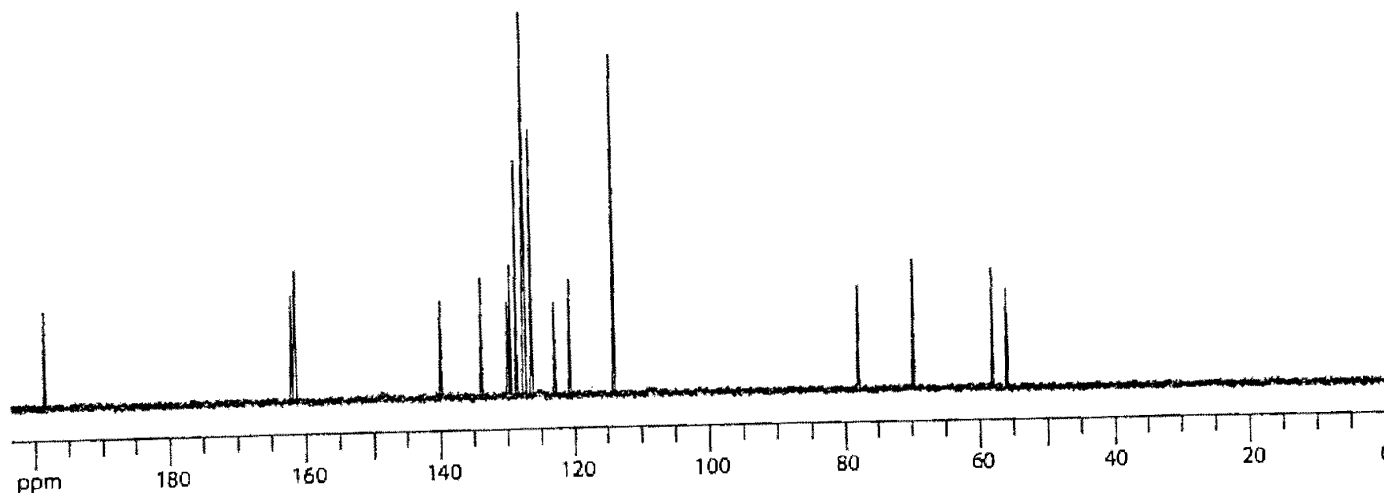
Current Data Parameters
NAME   MBAT-104
EXPNO   2
PROCNO   1

F2 - Acquisition Parameters
Date_   20060614
Time   15.10
INSTRUM   spect
PROBHD   5 mm Dual 13
PULPROG   zgpg30
TD   65536
SOLVENT   CDCl3
NS   86
DS   0
SWH   19841.270 Hz
FIDRES   0.302754 Hz
AQ   1.6515672 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
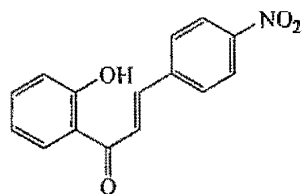NUC1   13C
PL1   0.00 dB
D11   0.03000000 sec

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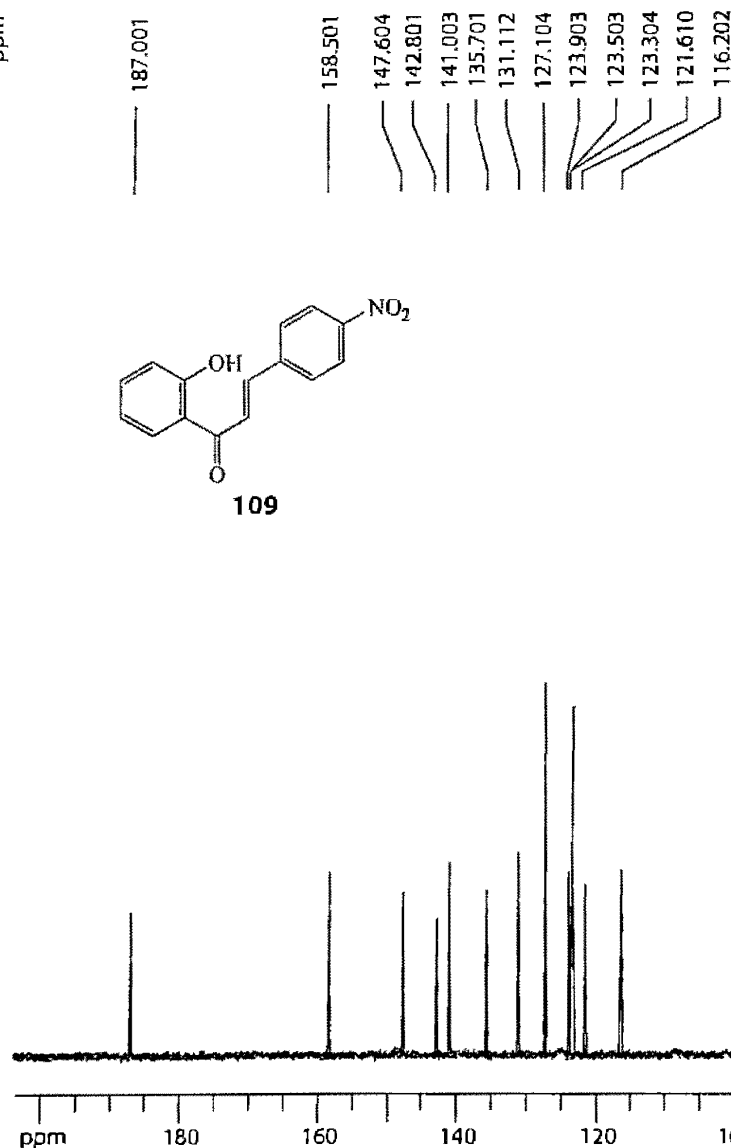WDW   EM
SSB   0
LB   1.00 Hz
GB   0
PC   1.40

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

ppm   180   160   140   120   100   80   60   40   20   0

ppm

187.001

143.502
142.801
135.303
133.701
131.504
129.710
129.603
129.104
128.804
127.612
126.504
123.302

20.902

105

Current Data Parameters
NAME   MRAI-105
EXPNO   2
PROCNO   1

F2 - Acquisition Parameters
Date_   20060520
Time   12.44
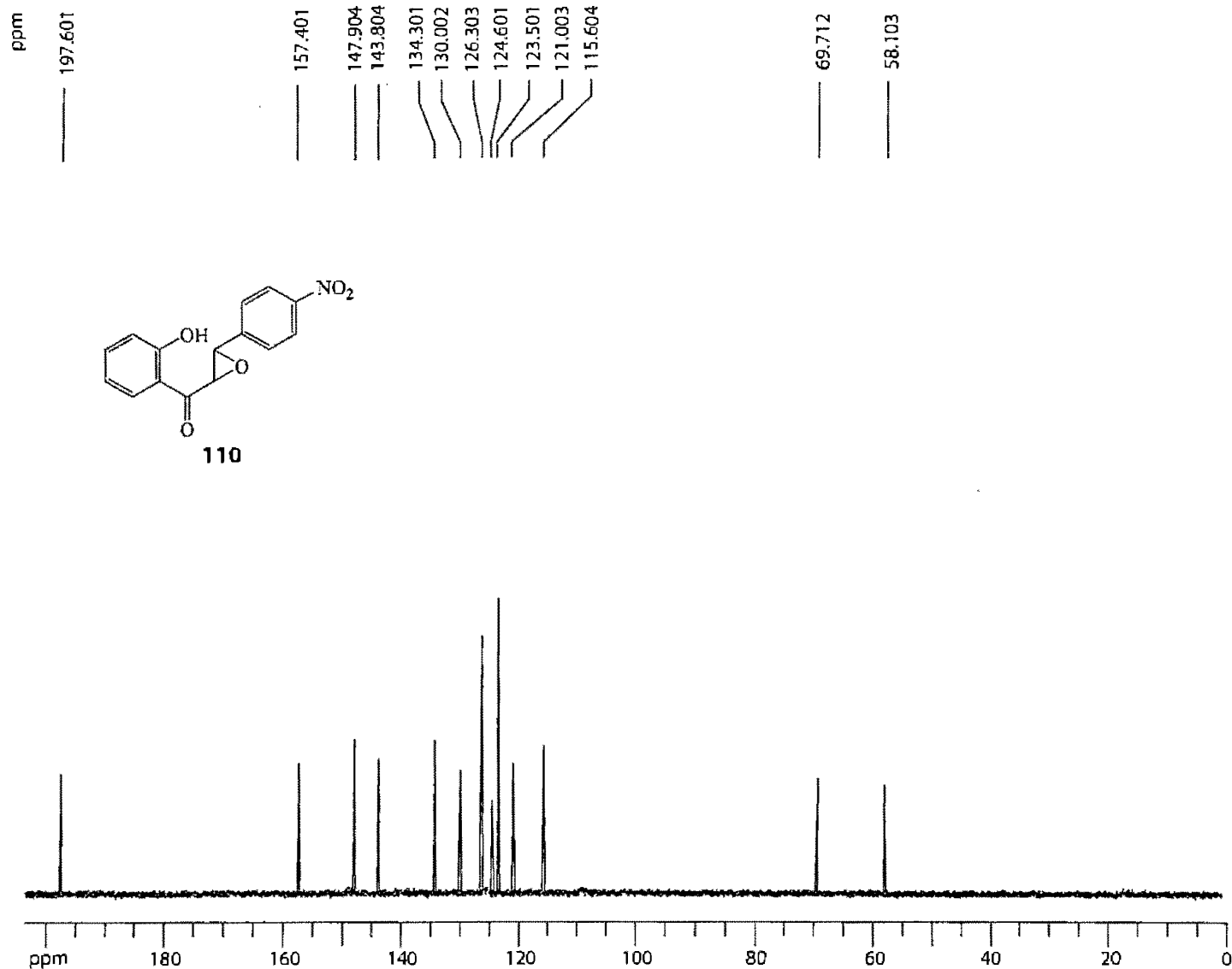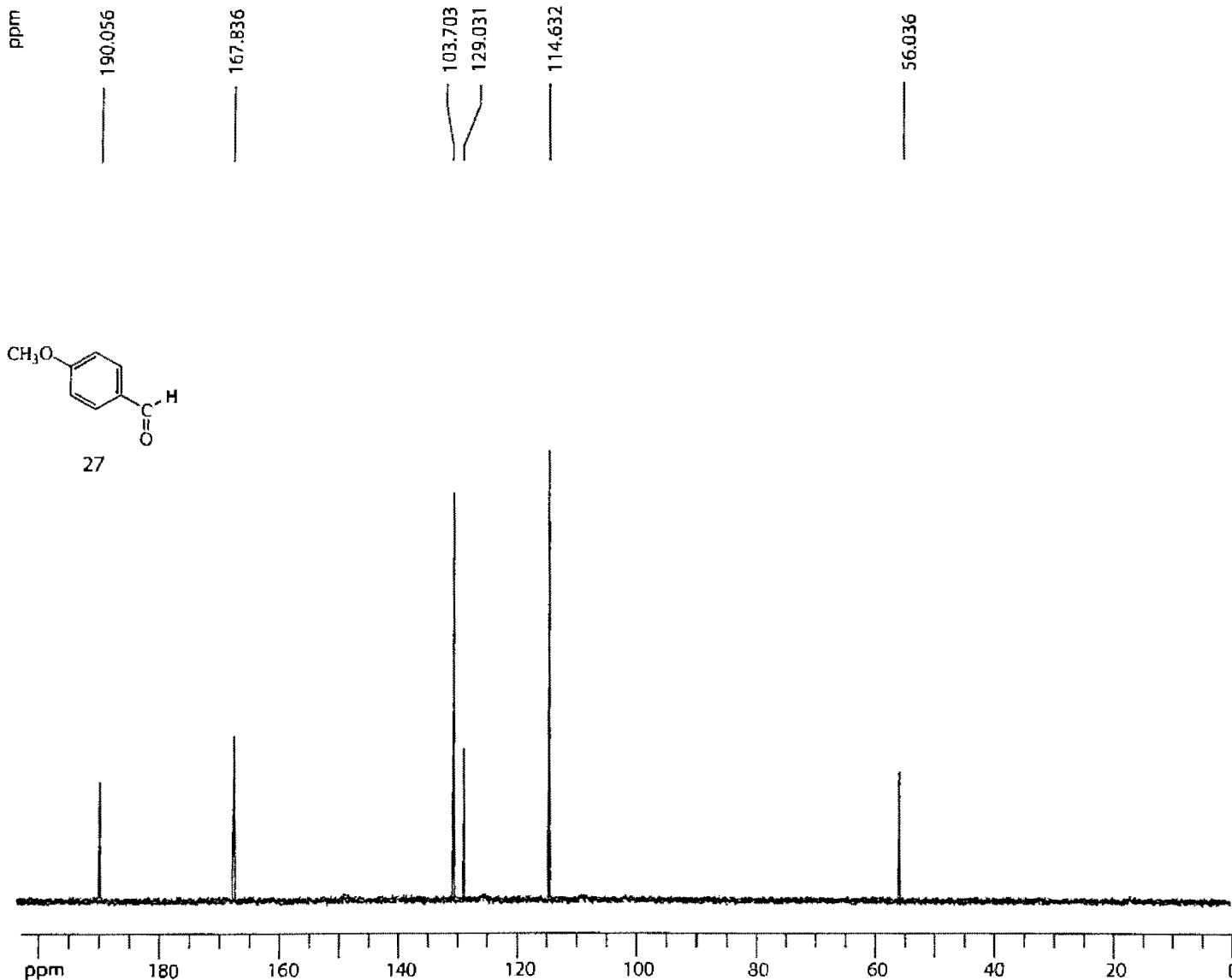INSTRUM   spect
PROBHD   5 mm Dual 13
PULPROG   zgpg30
TD   65536
SOLVENT   DMSO
NS   300
DS   0
SWH   19841.270 Hz
FIDRES   0.302754 Hz
AQ   1.6515572 sec
RG   16384
DW   25.200 usec
DE   6.00 usec
TE   300.0 K
D12   0.00002000 sec
PL13   22.00 dB
D1   2.00000000 sec
CPDPRG2   waltz16
PCPD2   90.00 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   200.702 ppm
F1   12625.13 Hz
F2P   5.824 ppm
F2   366.11 Hz
PPMCM   5.74395 ppm/cm
HZCM   612.85080 Hz/cm

ppm   180   160   140   120   100   80   60   40   20   0

ppm

197.602

142.102
138.104
134.403
130.701
129.404
129.103
128.802
128.501
126.804
126.504

68.903

49.001

20.901

106

ppm   180   160   140   120   100   80   60   40   20   0

ppm

187.002

143.503
142.801
133.704
133.001
129.710
129.602
128.812
127.604
123.301

20.911

107

ppm   180   160   140   120   100   80   60   40   20   0

197.603

142.101
135.802
134.411
133.301
129.110
128.810
128.512
126.803

69.411

58.101

20.901

108

ppm   180   160   140   120   100   80   60   40   20   0

ppm

187.001

158.501
147.604
142.801
141.003
135.701
131.112
127.104
123.903
123.503
123.304
121.610
116.202

OH

NO$_2$

O

109

ppm   180   160   140   120   100   80   60   40   20   0

ppm

197.601

157.401
147.904
143.804
134.301
130.002
126.303
124.601
123.501
121.003
115.604

69.712
58.103

110

Current Data Parameters
NAME   MRA1-110
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
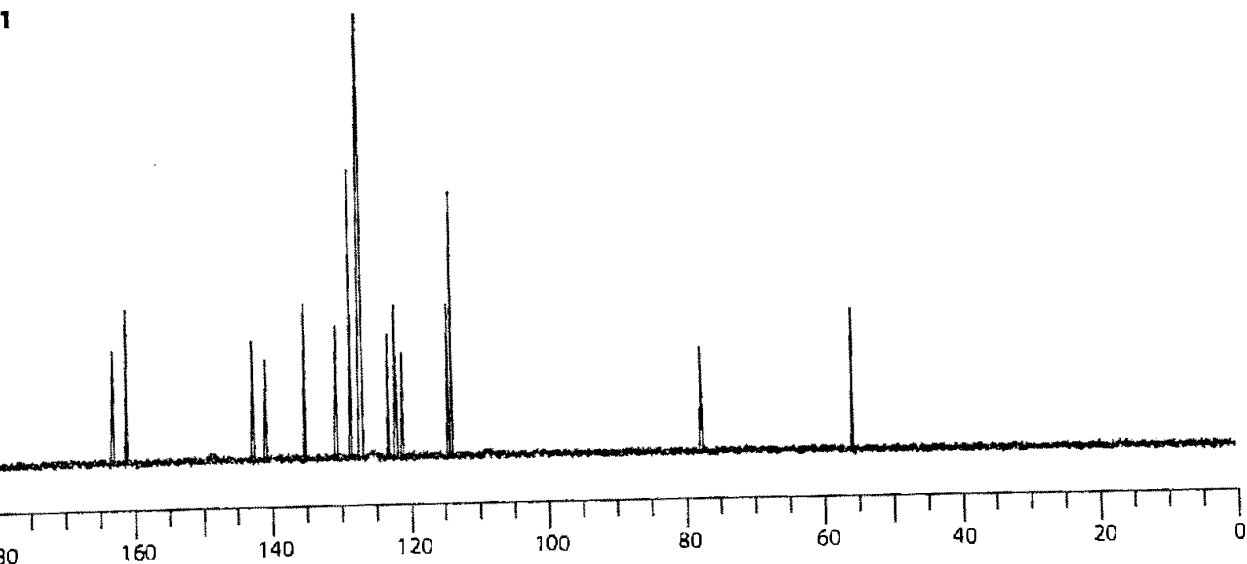D11   0.03000000 sec

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

10 NMR plot parameters
CX   20.00 cm
F1P   210.252 ppm
F1   13226.40 Hz
F2P   -0.491 ppm
F2   -30.85 Hz
PPMCM   10.53915 ppm/cm
HZCM   662.86252 Hz/cm

ppm   180   160   140   120   100   80   60   40   20   0

ppm

190.056

167.836

103.703
129.031

114.632

56.036

CH₃O—⟨benzene⟩—CHO

27

Current Data Parameters
NAME   MRAI-27
EXPNO   2
PROCNO   1

F2 - Acquisition Parameters
Date_   20090003
Time   12.44
INSTRUM   spect
PROBHD   5 mm Dual 13
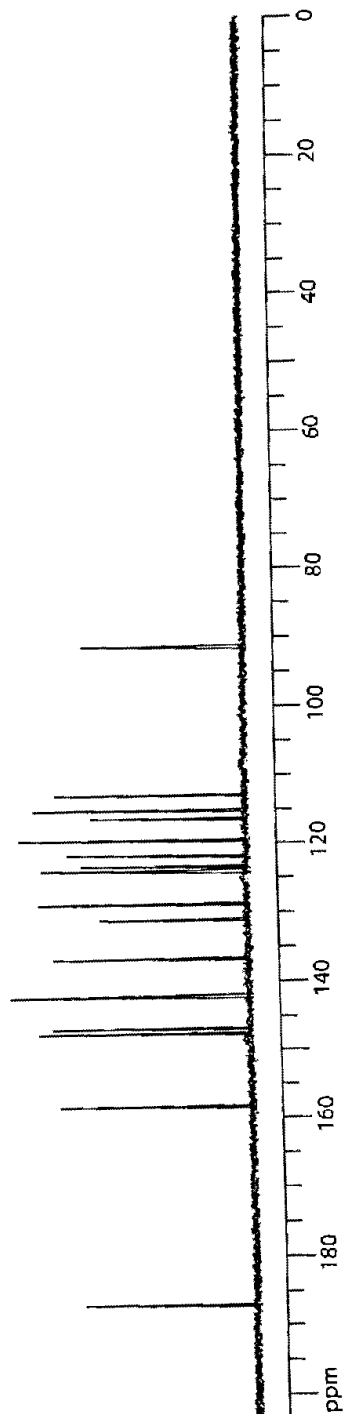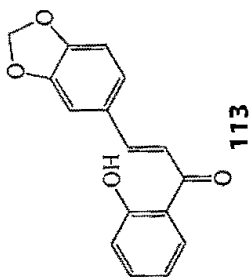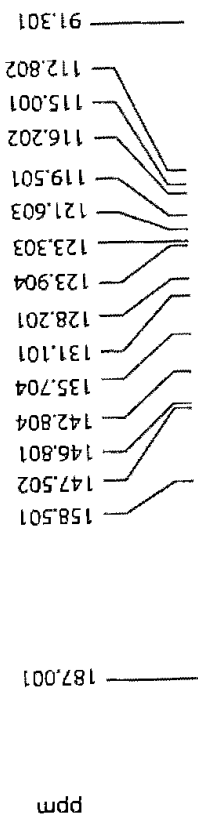PULPROG   zgpg30
TD   65536
SOLVENT   DMSO
NS   300
DS   0
SWH   19841.270 Hz
FIDRES   0.302754 Hz
AQ   1.6515572 sec
RG   16384
DW   25.200 usec
DE   6.00 usec
TE   300.0 K
D12   0.00002000 sec
PL13   22.00 dB
D1   2.00000000 sec
CPDPRG2   waltz16
PCPD2   90.00 usec
SFO2   250.1312346 MHz
NUC2   1H
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   62.8952425 MHz
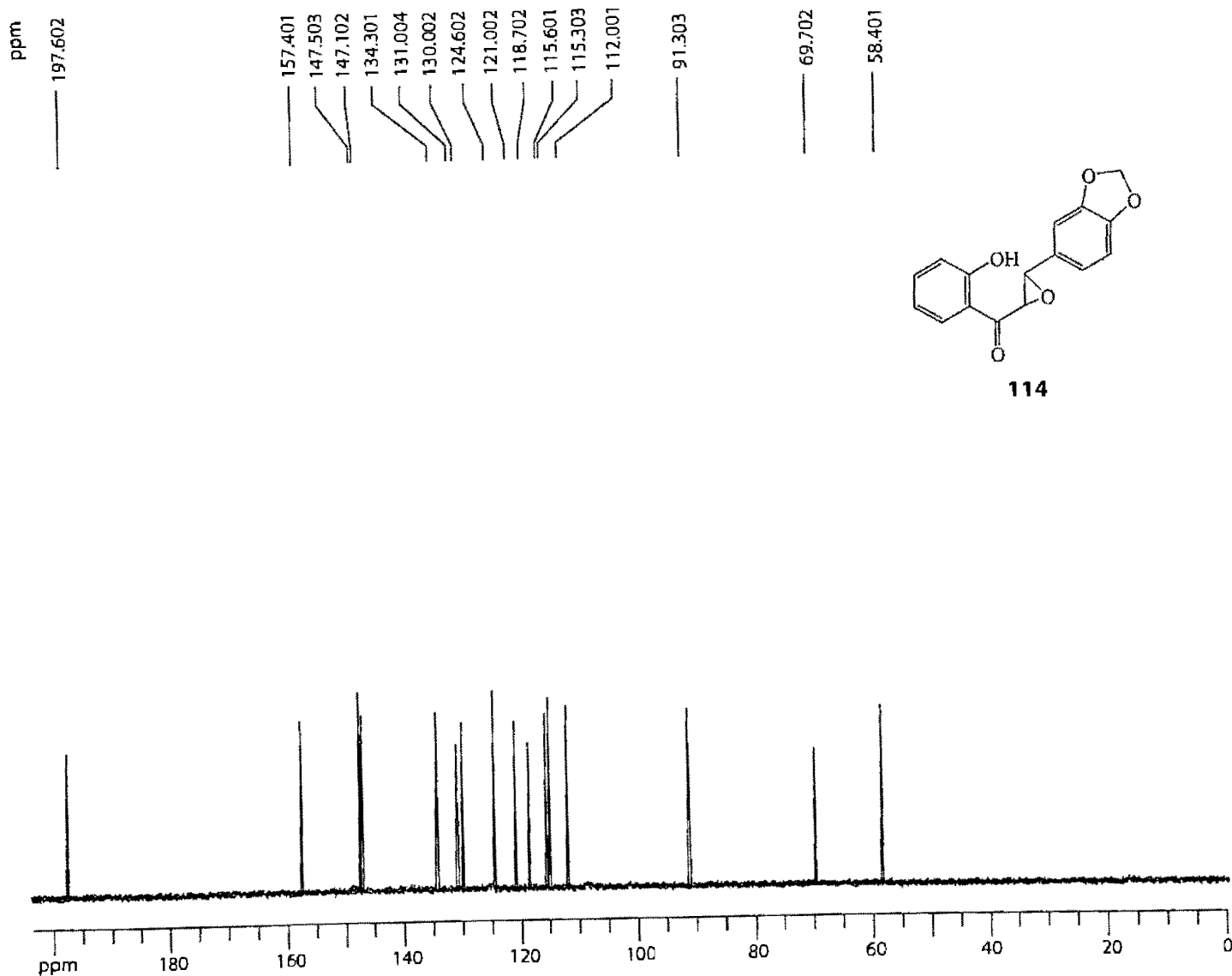WDW   EM
SSB   0
LB   1.00 Hz
GB   0
PC   1.40

1D NMR plot parameters
CX   20.00 cm
F1P   200.701 ppm
F1   12625.13 Hz
F2P   5.821 ppm
F2   366.11 Hz
PPMCM   5.74395 ppm/cm
HZCM   613.85080 Hz/cm

ppm   180   160   140   120   100   80   60   40   20   0

ppm

197.601

162.102
161.503
140.902
133.901
130.002
129.603
128.702
127.391
126.401
123.002
120.701
114.001

77.803

69.704

58.103
56.002

OCH₃

112

ppm   180   160   140   120   100   80   60   40   20   0

113

187.001
158.501
147.502
146.801
142.804
135.704
131.101
128.201
123.904
123.303
121.603
119.501
116.202
115.001
112.802
91.301

ppm

ppm

197.602

157.401
147.503
147.102
134.301
131.004
130.002
124.602
121.002
118.702
115.601
115.303
112.001

91.303

69.702

58.401

114

Current Data Parameters
NAME   MRA1-114
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
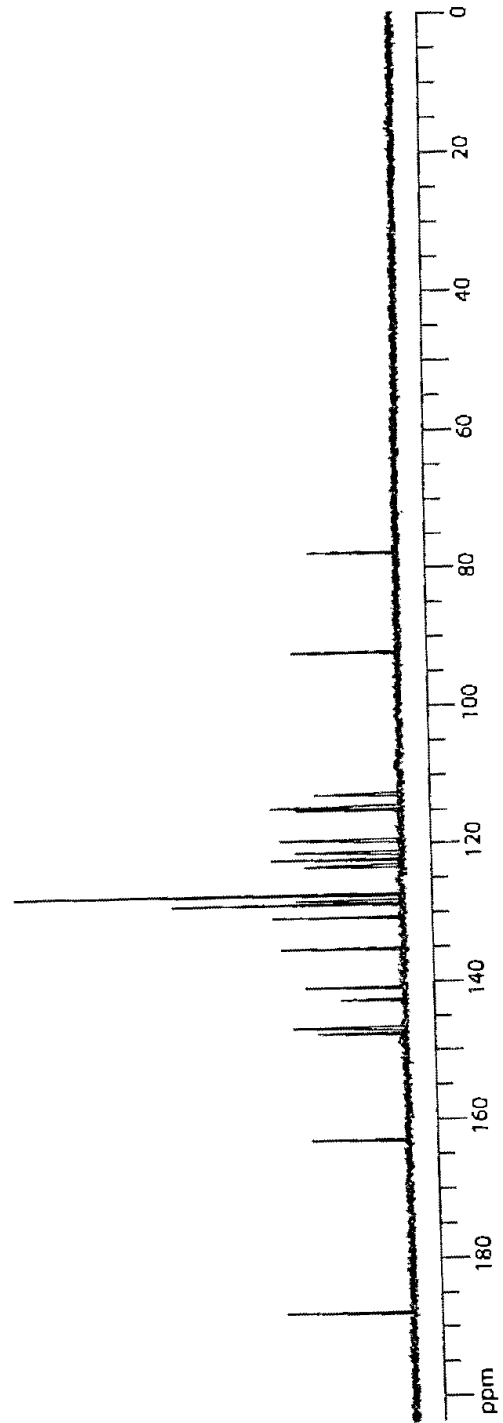DE   6.00 usec
TE   300.0 K
D12   0.00002000 sec
PL13   22.00 dB
D1   2.00000000 sec
CPDPRG2   waltz16
PCPD2   90.00 usec
SFO2   250.1315445 MHz
NUC2   1H
PL2   0.00 dB
PL12   22.00 dB
P1   10.20 usec
SFO1   62.9031573 MHz
NUC1   13C
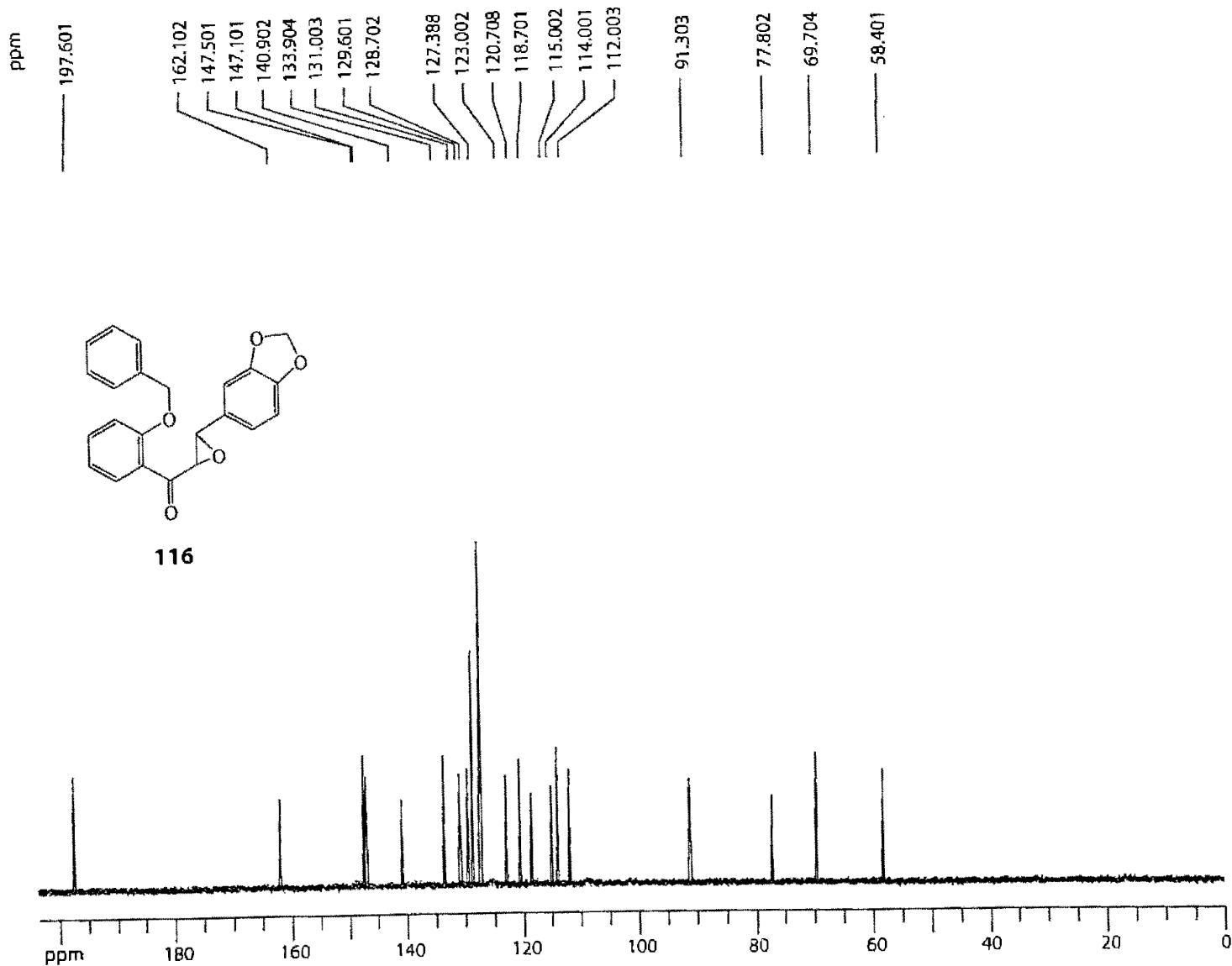PL1   0.00 dB
D11   0.03000000 sec

F2 - Processing parameters
SI   32768
SF   62.8962481 MHz
WDW   EM
SSB   0
LB   1.00 Hz
GB   0
PC   1.40

1D NMR plot parameters
CX   25.00 cm
F1P   210.252 ppm
F1   13226.40 Hz
F2P   -6.491 ppm
F2   -30.85 Hz
PPMCM   10.53916 ppm/cm
HZCM   662.86255 Hz/cm

ppm

77.801

91.303
112.803
114.610
115.001
119.521
121.301
122.303
123.304

127.380

128.201
128.702
130.701
135.303
140.903
142.801
146.802
147.504
163.203

187.001

115

197.601

162.102
147.501
147.101
140.902
133.904
131.003
129.601
128.702

127.388
123.002
120.708
118.701
115.002
114.001
112.003

91.303

77.802

69.704

58.401

116

ppm   180   160   140   120   100   80   60   40   20   0