PHYTOCHEMICAL INVESTIGATION

ON THE PODS OF SENNA SEPTEMTRIONALIS

BEKURETSION WOLDEYESUS

BY

AUGUST

PHYTOCHEMICAL INVESTIGATION ON THE PODS OF *SENNA SEPTEMTRIONALIS*

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES, ADDIS ABABA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

BY

BEKURETSION WOLDEYESUS

AUGUST, 1996

ACKNOWLEDGMENTS

I would like to express my utmost appreciation to my research advisors, Dr. Berhanu Abegaz and Dr. Gizachew Alemayehu for their constant guidance and supervision. I would also like to thank Dr. Wendimagegn Mammo and Dr. Ermias Dagne who shared their experiences on this project. I am indebted to Senait Dagne and Beniam Kebede who were very resourceful and sincere to be of service. I also wish to express my thanks to all staff members of the Chemistry Department, Addis Ababa University and Food Research and Development Centre who in some way contributed to this work. SAREC, for the partial financial support it provided, is gratefully acknowledged.

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ABSTRACT

Phytochemical Investigation on the Pods of *S.septemtnonalis*.

The chloroform extract of the pods of *S.septemtriona/is* afforded anthraquinones: physcion, chrysophanol, emodin, floribundone-1, torosanin-9',10'-quinone, torosachrysone as well as 5,7'-pnyscion-fal!acinol. The isolation of the dimeric anthracene derivative 5,7'-physcion-fallacino! is reported here for the first time.

Quercetin, luteolin, a glycoside of quercetin and a number of unidentified pigments were isolated from the methanol extract.

The elucidations of the structures are based on spectroscopic techniques and by , comparison of the spectral data with those reported in the literature.

1. INTRODUCTION

1.1. GENERAL

Phytochemical studies of plants, especially of medicinal plants, are of great importance in developing drugs. They are useful in the study of chemotaxonomy and plant biodiversity as well as in documenting knowledge. Enhancing the knowledge of biological and pharmacological effects of plant constituents, and determination of the structures of the active principles, may help in sustaining the use of these products. The study of natural products offers challenging problems of stereospecific synthesis with a fascinating diversity of structures to synthetic organic chemists. These molecules of life have attracted the attention and efforts of the foremost organic chemists because of their economic importance in industry, their value in medicine, and their importance in regulating the interactions between plants and animals in nature[1]. The contribution of natural products to the development of medicine could be demonstrated by the amount of plant-derived drugs being used. In general 40% of modern drugs are said to be of natural origin[2]. Many antifeedants, plant growth regulating hormones, fungicides and agricultural chemicals are developed from natural products from time to time.

Natural products in fact do have a function in the organism from which they originate. Many of them are now considered to have vital roles as mediators of ecological interactions, thereby ensuring the continual survival of a particular organism. The ability to synthesize an array of secondary metabolites which may repel or attract other organisms is one facet of the survival strategy[3-5]. Flower

colour, pollination, deterrent properties in plants,[6] and chemical defence in termite[7] may explain some of the importance of secondary metabolites for the survival of an organism.

The genus *Senna,* which belongs to the Leguminosae family, has about 240 species mainly found in the tropical and subtropical zones of the world. In Ethiopia, there are eighteen species belonging to the genus[8]. These are: *S. petersiana, S. septemtrionalis, S. singueana, S. baccarinii, S. occidentalis, S. sophera, S. obtusifoiia, S. siamea, S. didymobotrya, S. ruspo/ii, S. longinacemosa, S. ellisae, S. truncata, S. italica, S. holosericea, S. multig/andulosa, S. alexanderiana,* and *S. bicapsularies.*

People living in the villages of Africa, Asia and other parts of the developing world are forced to resort to traditional practitioners In order to alleviate their sufferings and use traditional medicines for the continued maintenance of their health[6,9,10]. In this context, several species of *Senna,* having important medicinal properties, are also used as vital resort. They are used in the treatment of sexually transmitted diseases, skin diseases and are sources of the well known *Senna* purgative. Some of them are also useful as appetizer[11]. *Senna* species have been the subject of several pharmacological investigation. Some of them are known to be useful as a remedy for eye ailment[12]. Many of them are found to possess insecticidal properties and some exhibit antibiotic properties. S. *occidentalis* is used as an ascaricide, to relieve abdominal pains and against malaria. Its leaf and root parts are particularly useful for snake-bite, as pain-killer and for anthelmintic purposes[13,14]. The dried leaflets of 5. *acutifloia* and S. *angustifloia* are known as purgatives[15]. The aerial

Table 1

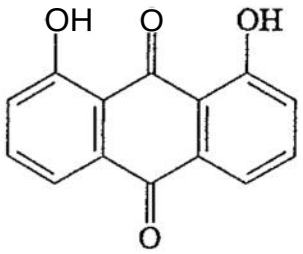
Anthraquinones of the Genus *Senna*[27].

Anthraquinone	R_1	R_2	R_3	R_4	R_6	R_8	R_7	R_{11}	$R_{\blacksquare}$	Isolated from
Sopheranin	OH	Me	OH	H	H	OH	$CH=CH_2$	OH	O	*S.sophera*
Chrysophanol	OH	H	Me	H	H	H	H	OH	O	*Senna* species
Physcion	OH	H	Me	H	H	OMe	H	OH	O	*Senna* species
Emodin	OH	H	Me	H	H	OH	H	OH	O	*Senna* species
1,2,7-trihydroxY-6,8-dimethoxy-3-methyl anthraquinone	OH	OH	Me	H	H	OMe	OH	OMe	O	*S.sophera*
1,2,6-trihydroxy-7,8-dimethoxy-3-methyl anthraquinone	OH	OH	Me	H	H	OH	OMe	OMe	O	*S.sophera*
Chrysophanhydroanthrone	OH	H	Me	H	H	H	H	OH	H,OH	*S.siamea*
Chrysophanol 9-anthrone	OH	H	Me	H	H	H	H	OH	H,H	*S.siamea*
Obtusifolin	OMe	OH	Me	H	H	H	H	OH	O	*S.obtusifolia*
Aurantio-obtusin	OMe	OH	Me	H	H	OH	OMe	OH	O	*S.obtusifolia*
Obtusin	OMe	OH	Me	H	H	OMe	OMe	OH	O	*S.obtusifolia*
Chryso-obtusin	OMe	OH	Me	H	H	OMe	OMe	OMe	O	*S.obtusifolia*
Questin	OH	H	Me	H	H	OH	H	OMe	O	*S.obtusifolia*, *S.occidentaiis*

All the dimeric anthraquinones so far reported have only C-C linkage in between the two units. Some of them are: aloe-emodin bianthrone, rhein bianthrone, 2,2'-bichrysophanol, 4,4'-bichrysophanol, chrysophanol bianthrone, 2,2'-chrysophanol-emodin bianthraquinone, 2,2'-biemodin, physcion bianthrone and 7,7'-biphyscion[25,26]. From preanthraquinones: torosachrysone, germichrysone, methyl germichrysone[28-31], germitorosone and methyl germitorosone[32] and from preanthraquinone dimers: singueanol-1 and singueanol-219 and dimers based on torosachrysone and physcion[33-36] are documented in the literature.

Flavonoids constitute one of the largest groups of naturally occurring phenols[37]. In plants flavonoids exist as aglycones, glycosides, sulphates and biflavonoid[38]. Flavonoids have important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors, precursors of toxic substances, as pigments and light screens[39].

Flavonoids occur as glycosides in which one or more sugar units are attached to the flavonoid nucleus. Glucose is the sugar most commonly involved; galactose, rhamnose, xylose and arabinose are uncommon but are known to occur. Flavonoid glycoside may be either O-glycosides or C-glycosides. O-glycosides are distinguished by the ease with which they are hydrolysed by acids. C-glycosides of flavonoids in which sugars are attached directly to the aromatic ring by a carbon bond are acid resistant. C-linked sugars so far reported are at positions 6 and 2 of the flavonoid nucleus[38,40]. Flavonoid glycosides are as abundant as their anthraquinone counter-parts in the *Senna* species[41].



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Some biological activities of anthraquinones also include antiviral[46,47], anthelmintic[48] and antifungal[49] properties. The anthraquinone ring system appears to be also metabolically active, and it has been suggested that anthraquinones may have a role in the process of fruit development in plants[5].

Flavonoid aglycones exhibit appreciable mutagenic activity. It has been reported that quercetin, kaempferol and rhamnetin were active as mutagen. Compounds belonging to different classes of flavonoids, including flavonois, flavone, flavanones, dihydroflavonols, dihydrochalcones and flavanol, as flavonoid pigments are responsible for the majority of flower colours present in nature and are implicated as insect feeding attractants. They also act as feeding deterrents[6].

The bioactivities of luteone as anti-fungi[50], 6-hydroxykaempferol as anticancer[51] and quercetin (27) as anti-hypertensive[52] are reported. The isolation of quercetin was reported from *S. obtusifolia, S. sophera* and S. *ita/ica*[35Ah53]. The effect of quercetin on the infectivity and replication of HSV-1 (Herpes simplex virus type-1), polio virus type-1, parainfluenza virus type-3 and respiratory syncytial virus (RSV) has been studied in cell culture. It was observed that quercetin produced a concentration-

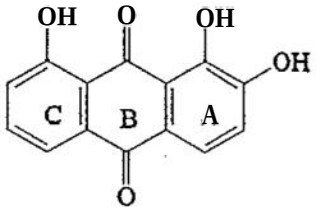
dependent reduction in the infectivity of each virus and retarded their intracellular replication[6].

1.3.2. Taxonomic Utility

Both taxonomy and chemistry are unifying fields. The former, based on evolutionary principles, provides a framework to account for morphological variation and its mechanism at the organism and population level, while the latter provides a systematic framework to describe, and in part comprehend, variations in the organization of elementary particles[54]. The description and classification of plants concern chemotaxonomy. If structurally similar compounds derived from different plant species are shown to share the same biosynthetic pathway, tentative assignment of both species to the same plant genus or family can be made. Chemotaxonomy is hence helpful in providing confirmations of the accuracy of the assignments[5].

At the present time at least it is clear that secondary compounds are primarily useful in taxonomic studies at the species and generic level. In favourable cases new information may accrue from chemical studies which will allow the revision of a group where morphological and other biological data may be at variance. The existence of chemical variation at the population level has to be borne in mind in all chemotaxonomic work. Where chemotypes occur, it is apparent that chemical studies can add a new dimension to our understanding of variation, structure and evolution within plant populations[54].

are mainly of polyketide origin. They are categorised depending on how one or both A and C rings bear particular substituents such as OH, OCH_3, CH_3, etc. It appears that, at least in higher plants, those anthraquinones with substituents on both A and C rings are derived via the acetate-malonate pathway. These compounds of polyketide origin, usually with a C-15 skeleton, are exemplified by emodin (5). Those compounds with only one ring substituted, such as alizarin (2) derive from shikimate and mevalonate route in some plant families. Shikimic acid (3) derived from phosphoenolpyruvic acid and erythro-4-phosphate is incorporated into alizarin together with the three central carbon of o-ketoglutaric acid, an intermediate of tricarboxylic cycle. However, the situation in lower organisms is not so clear-cut. Since pachybasin (4) which ought to be derived from shikimate, does in fact arise via the acetate-malonate pathway[5].



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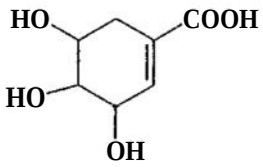

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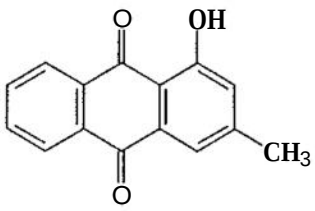

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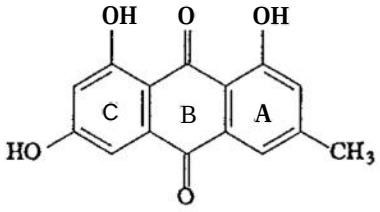

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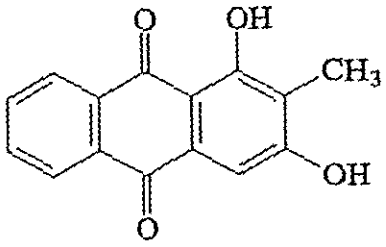
Anthraquinones of the Leguminosae have substituents on bo
exception of rubiadin (6) and domnacanthal (7). The most plausibl
for anthraquinones in the Leguminosae, therefore appears to be
origin.



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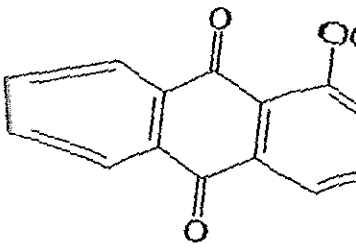

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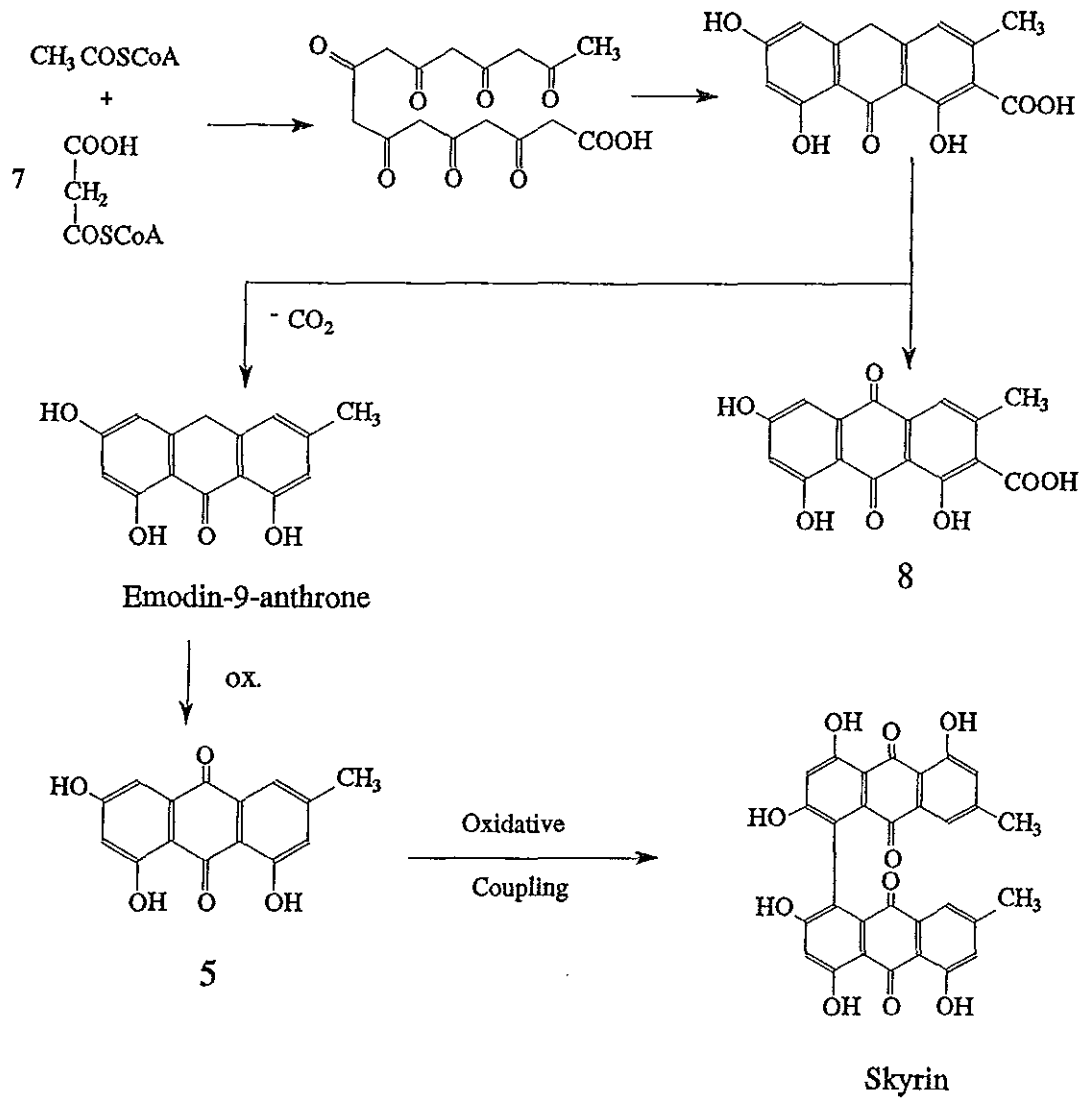
Anthrones are biosynthetic intermediates which lead to anthraquinor
and the oxidative coupling of anthraquinones leads to dimeric anthra
The biosynthetic pathway which leads to emodin (5) and endocrocin (
Scheme 1.

Scheme 1

Biosynthesis by the Polyketide Pathway[55].



The co-occurrence of torosachrysone, physcion and physcion anthrone with phlegmacines, anhydrophlegmacin-9-10-quinone and torosanin in *S. torosa*[30,31] suggests that, similar biogenetic route, (which involves the conversion of pre-anthraquinones to anthraquinones) may be followed in higher plants too. Experimental evidence indicated[31] that in the seedlings of *S. torosa*, germichrysone

is not derived from anthraquinone, but it is a product of *de novo* biosynthesis. Thus, it is possible to infer that, in plants the reduction of anthraquinones to pre-anthraquinones is least likely to occur.

The flavonoid variants are all related by a common biosynthetic pathway which incorporates precursors from both the shikimate and acetate-malonate pathways[38,40,58]. The flavonoid initially formed in the biosynthesis is thought to be chalcone (**9**) and all other forms are derived from this by a variety of routes. Further modification of flavonoids may occur at various stages resulting in additional hydroxylation, methylation of hydroxyl groups or of the flavonoid nucleus, dimerization, and glycosylation of hydroxyl groups or of the flavonoid nucleus.

1.3.4. Spectral Properties

Anthracene derivatives containing two hydroxyl groups in 1, 3 or 1, 8 positions give generally an orange-red or pink colour when treated with 0.5 % magnesium acetate[59,60], those in 1, 4 positions give a purple colour and those in 1, 2 positions produce a violet colour.

Generally, many dimeric anthraquinones display optical activity on account of the restricted rotation about the connecting C-C bond of their monomers[61,62].

The carbonyl stretching vibrational absorption region frequencies in infrared spectroscopy are important in indicating the presence of the fundamental anthraquinone unit and the hydroxylation pattern[63].

Table 2

Carbonyl Frequencies of Hydroxyanthraquinones in cm^{-1} [64]

Type of anthraquinone	Carbonyl frequencies
no *alpha* OH	1678 - 1653
1 OH (1)	1675-1647, 1637-1621
2 OH (1,4 & 1,5)	1645 - 1608
2 OH (1,8)	1678-1661, 1626-1616
3 OH (1,4,5)	1616 - 1592

The ultraviolet/visible spectra of anthraquinones show intense benzenoid absorptions at 240-260 nm, medium absorptions at 320-330 nm, a strong quinonoid electron transfer band at 270-290 nm accompanied by a weak quinonoid absorption band at 405 nm[65]. The 405 nm quinonoid absorption is susceptible to intensity increase and shifts to longer wave length by the introduction of nuclear hydroxyl groups[67-69].

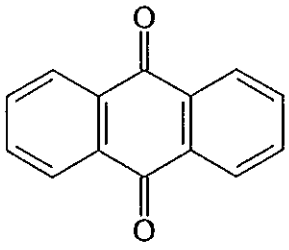
Nearly all flavonoids display two bands in their ultraviolet/visible spectra. Flavones absorb between 304 and 350 nm and flavonols show a band between 352 and 385 nm[41,70].

NMR spectroscopy furnishes information and serves as a powerful tool in the structural elucidation of anthraquinones. Chemical shifts, splitting patterns and

coupling constants greatly aid in structural assignment and determination of orientation of substituents. In proton NMR, the *alpha* and *beta* protons of 9,10-anthraquinone give multiplets centred at 8.07 and 7.67 ppm, respectively and are modified by substitution. The hydroxyl protons on 1, 4, 5 and 8 positions are easily distinguished from other types of hydroxyl protons by their appearance at unusually low field resonance between 11 and 14 ppm or above[71,72].

In ^{13}C NMR spectra of anthraquinones, carbon 9 and 10 are easily recognized by their resonance occurring between 180 and 185 ppm while carbons bearing hydroxyl groups appear at about 160 ppm[74].

Anthraquinones generally show similar tendency to eliminate neutral carbon monoxide in mass spectroscopy. In the mass spectra of anthraquinone (**13**), the most abundant ions are of mass 208 (M^+), 180 (M^+-CO) and 152 (M^+-2CO). Accurate mass measurements established that these two latter ions were produced by successive loss of two neutral CO fragments[75].



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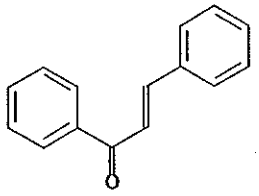
1.4. ***SENNA SEPTEMTRIONALIS***

S. septemtrionalis (syn. *S. floribunda, C. laevigata*) is a woody herb, shrub or small tree. Its height is in the range of 1-5 m and grows mainly between 1700 and 2400 m altitudes. It is wide spread through the tropics. In Ethiopia, it is found in Arssi, Harrarge, Illubabor, Kefa, Sidamo and Shewa regions[8].

The reports of earlier phytochemical investigations on the various parts of the plant including leaves, roots, flowers, and pods are summarized in Tables 3 & 4.



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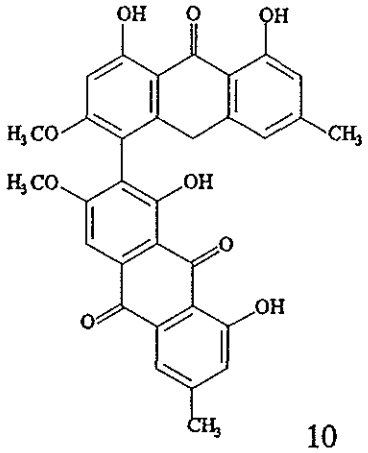

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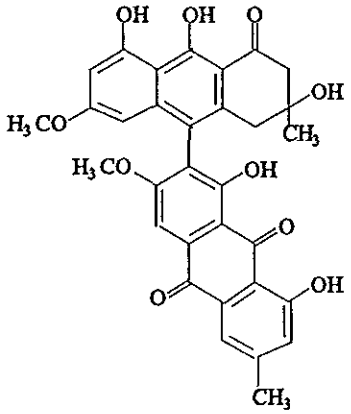

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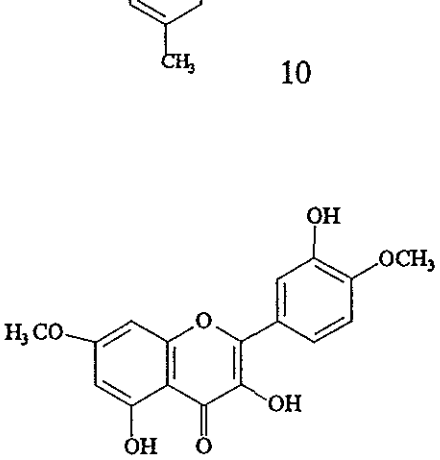

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Table 3

Anthraquinones and Flavonoids of *Senna septemtrionalis*

Compound	Structure	Part of plant from which the c'd is isolated	Ref.
Floribundone-1	16	leaves	77
Floribundone-2	10	leaves	77
Physcion	14	leaves, roots	77, 81
Chrysophanol	17	leaves, pods	77, 82
Anhydrophlegmacin-9',10'-quinone	11	leaves	78
Torosanin-9',10'-quinone	20	leaves	78
Emodin	5	roots, leaves	81
Quercetin	27	flower	76
Ombuin	12	roots, leaves	79, 81

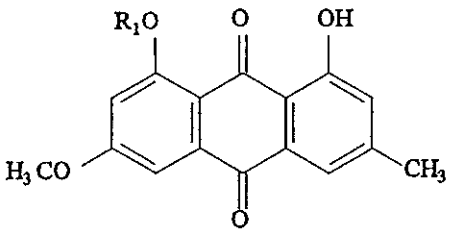


Fig. 1

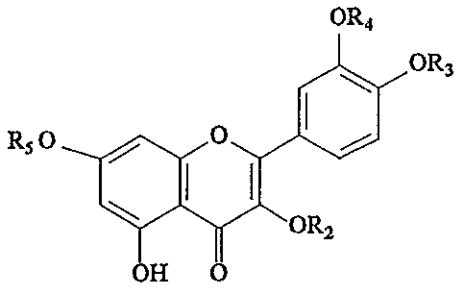


Fig. 2

Table 4

Glycosides of Physcion and Flavonoids of *Senna septemtrionalis*

Compound	Structure					Part of plant, (i.e., c'd isolated)	Ref.
	Fig	R_1 / R_2	R_3	R_4	R_5		
Physcion-8-*O*-galactoside	1	galactose	-	-	-	roots	81
Physcion-8-*O*-digalactoside	1	digalactose	-	-	-	pods	82
Rhamnetin 3-*O*-galactosyl galactopyranoside	2	digalactose, (1→4) bioside	H	H	Me	flower	76
Rhamnetin 3-*O*-galactosyl galactopyranoside	2	digalactose, (1→6) bioside	H	H	Me	flower	76
3-*O*-(2-rhamnosyglycosyl)-ombuin	2	rhamnose	Me	H	Me	leaves	79
Quercetin-3,7-di-rhamnopyranoside	2	rhamnose	H	H	rhamnose		40, 66
3',5 Dihydroxy-4',7-dimethoxy flavone-3-*O*-glucopyranoside	2	glucose	Me	H	Me		80
5-hydroxy-3',4',7-trimethoxy flavone-3-*O*-galactosyl galactopyranoside	2	digalactose	Me	Me	Me		80

1.5. OBJECTIVES OF THE PROJECT

This research work aims at extending further earlier studies. The foremost objectives of the research include solvent extractions, chromatographic isolation and structural elucidation of secondary metabolites particularly anthraquinones and flavonoids from the pods of *Senna septemtrionalis* which may have important pharmacological as well as chemotaxonomic values.

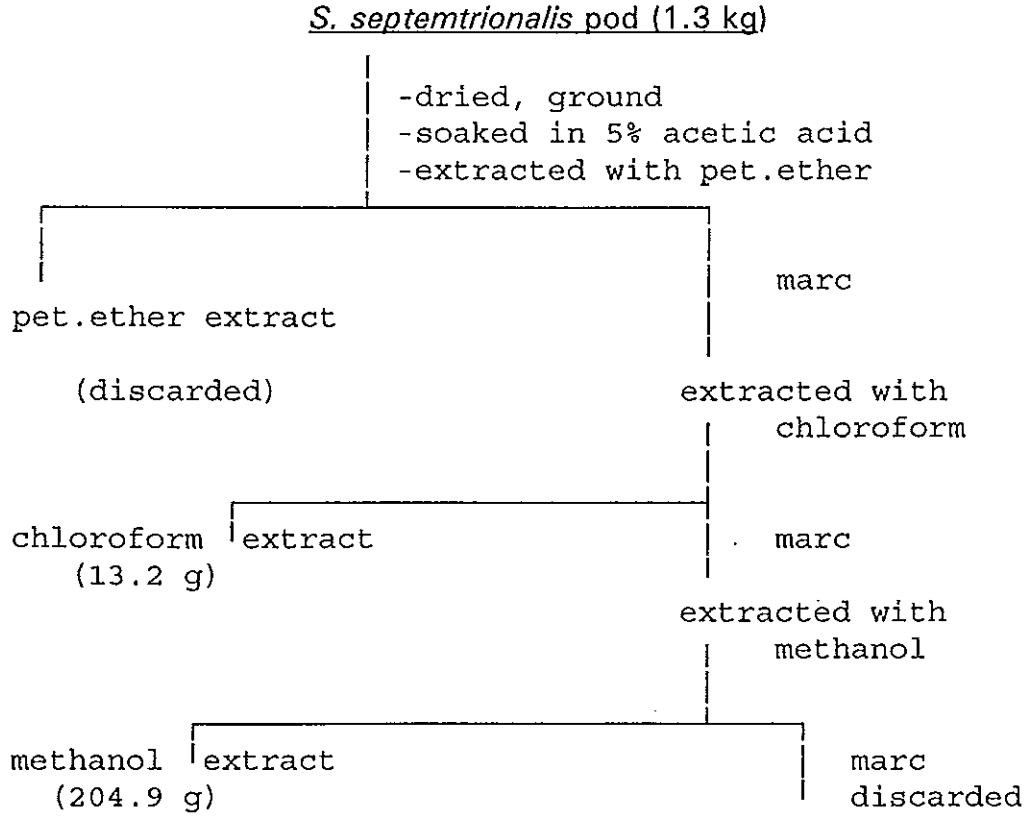
2. RESULTS AND DISCUSSION

In the course of this work, the pods of *Senna septemtrionalis* were investigated. This resulted in the isolation and identification of seven anthraquinones from the chloroform extract and three flavonoids from the methanol extract.

The dried and powdered pods of *S. septemtrionalis* were defatted with petroleum ether (40-60°C). The marc was extracted first with chloroform and then with methanol (scheme 2). The chloroform and methanol extracts when developed on TLC and sprayed with 5% methanolic KOH showed several spots that are positive for anthraquinones and flavonoids.

Scheme 2

Extraction of the Plant Material

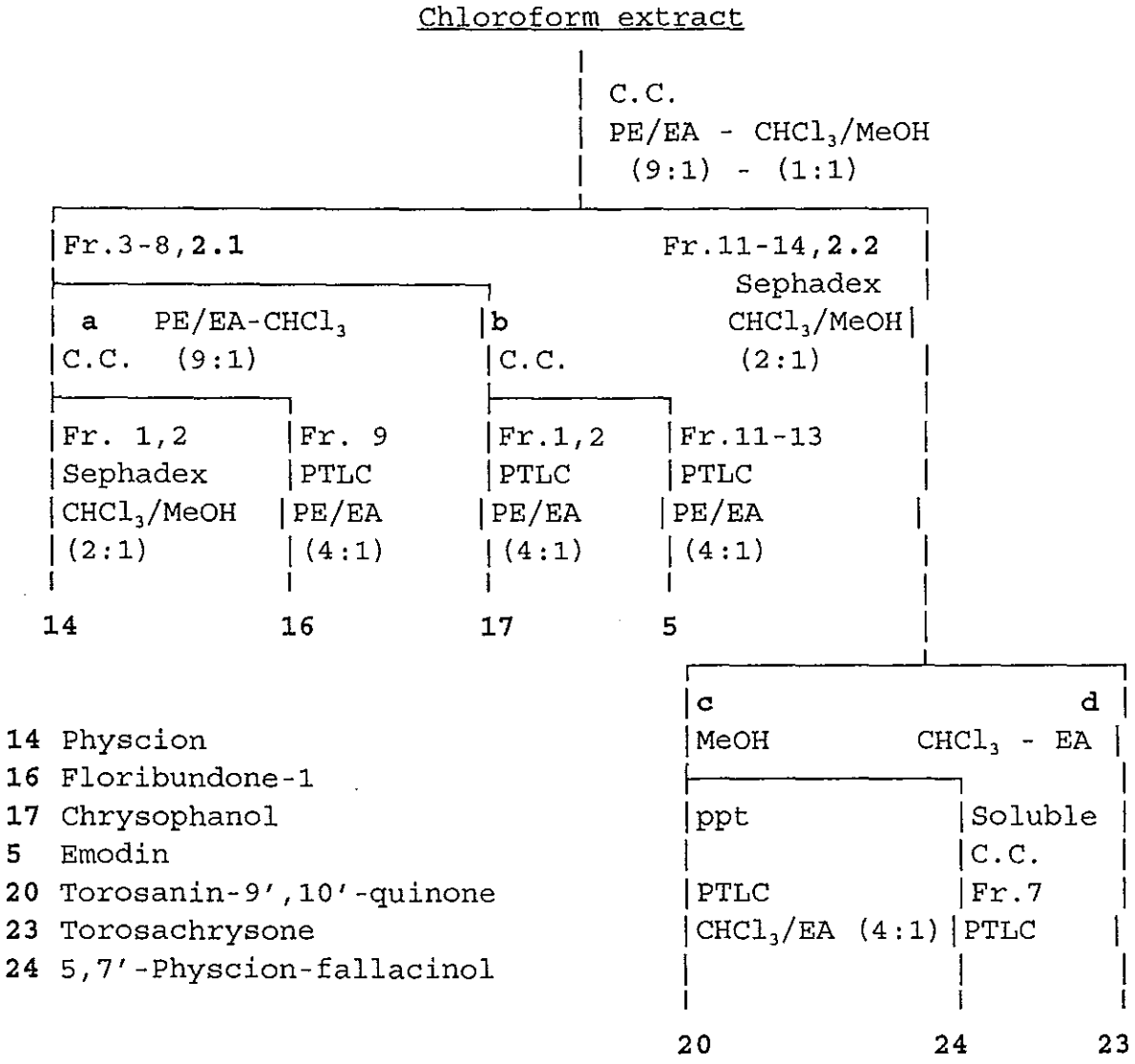


2.1. CHLOROFORM EXTRACT

The chloroform extract was chromatographed according to Scheme 3. The extract was applied on a column of silica gel and chromatographed using Petrol:EtOAc and $CHCl_3$:MeOH mixtures of increasing polarity. Fourteen fractions were collected. These fractions were combined into two parts, **2.1** and **2.2** based on similarity on TLC. **2.1** was chromatographed on a column of silica gel and on Sephadex. The progress of the separation was monitored by TLC. Further chromatography of fractions on PTLC afforded the common anthraquinones physcion (**14**), chrysophanol (**17**) and emodin (**5**) as well as the dimeric anthracene derivative floribundone-1 (**16**). The second part, **2.2** was first chromatographed on Sephadex and on a column of silica gel. Further separation on PTLC gave again emodin and floribundone-1 as well as torosanin-9,10-quinone (**20**), torosachrysone (**23**) and compound **24** (see Expt.).

Scheme 3

Chromatographic Separation of the Chloroform Extract



14 Physcion
16 Floribundone-1
17 Chrysophanol
5 Emodin
20 Torosanin-9',10'-quinone
23 Torosachrysone
24 5,7'-Physcion-fallacinol

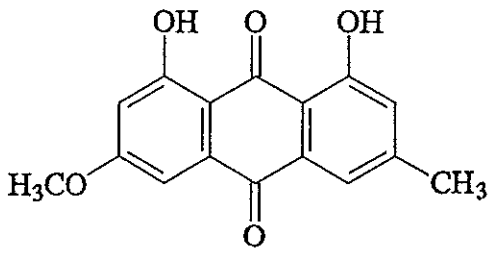
The six known anthraquinones were identified mainly on the basis of their ^{1}H NMR spectroscopic data and melting points in comparison with reported values[27,33,73,78] as well as their similarity on R_f values with authentic samples. The structural elucidation of hitherto unknown compound **24** was made possible by employing ^{1}H NMR (1D and 2D), MS as well as UV and IR spectroscopy.

2.1.1. Emodin (5)

This orange pigment was obtained from both the chloroform and methanol extracts (26 mg, 0.002%). The ^{1}H NMR spectrum of **5** revealed the presence of four aromatic protons (δ 7.13, 7.55, 7.25, 6.65) and an aromatic methyl group (δ 2.45). The above spectroscopic data led to the assignment of structure **5** to emodin. The identity of this anthraquinone was further confirmed by comparison with an authentic sample using TLC (solv. I, $CHCl_3$, solv. V). The melting point obtained agree very well with that reported for emodin in the literature (see Expt.).

2.1.2. Physcion (14)

This yellow pigment was isolated from the chloroform extract (11.9 mg, 0.0009%). The presence of two chelated hydroxyl groups (δ 12.30, 12.10), four aromatic protons (δ 7.05, 7.65, 7.35, 6.65), one methoxyl group (δ 3.90) and one aromatic methyl group (δ 2.40) were evident from the ^{1}H NMR spectrum.



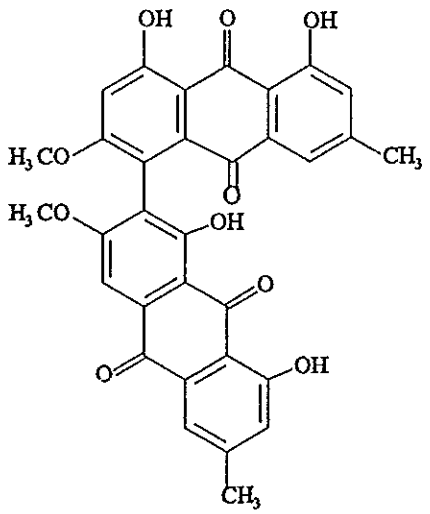
14

The above spectroscopic findings were found to be consistent with structure **14**. The isolation of this anthraquinone from the pods of *Senna septemtrionalis* was also reported previously[81]. Its melting point is in agreement with the reported value for

physcion (see Expt.). The identity of compound **14** was further established by comparison with an authentic sample using TLC (solv. I, $CHCl_3$).

2.1.3. Floribundone-1 (16)

This orange pigment was isolated from the chloroform extract (4.8 mg, 0.0004%). The ^1H NMR spectrum of **16** indicated the presence of four chelated hydroxyl groups (δ 12.15, 12.06, 12.25, 13.10), six aromatic protons (δ 7.04, 7.42, 6.80, 7.06, 7.67, 7.57), two methoxyl (δ 3.82, 3.85) and two aromatic methyl groups (δ 2.35, 2.45) (see Fig. 13).

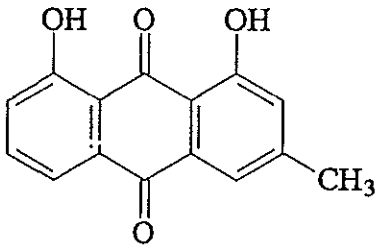


16

The spectrum was found to be similar with that of floribundone-1. The identity of this compound was further confirmed by comparison with an authentic sample using TLC (solv. I, $CHCl_3$, solv. V).

2.1.4. Chrysophanol (17)

This yellow pigment was obtained from the chloroform extract (5.1 mg, 0.0004%). The IR spectrum displayed absorption bands at 3420 cm^{-1}, 1675 cm^{-1} and 1630 cm^{-1} that indicate the presence of hydroxyl groups, non-chelated carbonyl and chelated carbonyl groups respectively.



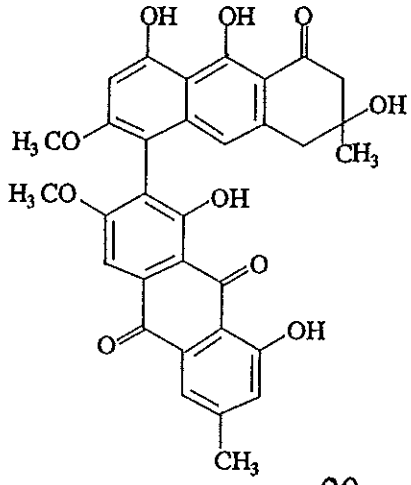
17

The presence of two chelated hydroxyl groups (δ 12.00, 12.10), five aromatic protons (δ 7.65, 7.80, 7.58, 7.10, 7.32) and one aromatic methyl (δ 2.43) was evident from the ^1H NMR spectrum. Its melting point is in agreement with the reported value for chrysophanol (see Expt.). The identity of compound **17** was further established by comparison with an authentic sample using TLC (solv. I, $CHCl_3$).

2.1.5. Torosanin-9',10'-quinone (20)

This anthraquinone is a dark red pigment, obtained from the chloroform extract (55.9 mg, 0.0043%). The ^1H NMR spectrum of **20** revealed the presence of four chelated and one non-chelated hydroxyl groups (δ 12.10, 12.34, 10.24, 16.40, 1.50), five aromatic protons (δ 7.10, 7.65, 7.55, 6.70, 6.42), two methoxyl (δ

3.89, 3.81), one aromatic methyl (δ 2.45), one non-aromatic methyl (δ 1.35) and two methylene groups (δ 2.80, 2.92).(see Fig.14).

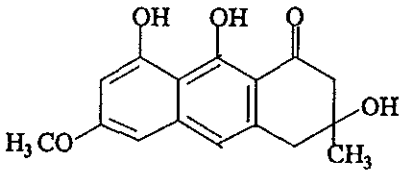


20

The above spectroscopic findings were found to be consistent with structure **20**. The identity of this anthraquinone was further confirmed by comparison with an authentic sample using TLC (solv. V).

2.1.6. Torosachrysone (23)

This yellow pigment was obtained from the chloroform extract (13.2 mg, 0.001%). The ^{1}H NMR spectrum of **23** indicated the presence of two chelated and one non-chelated hydroxyl groups (δ 9.70, 16.05, 2.05), three aromatic protons (δ 6.54, 6.47, 6.83), one methoxyl group (δ 3.85), two methylene groups (δ 2.80 and 3.00) and one non-aromatic methyl group (δ 1.42).



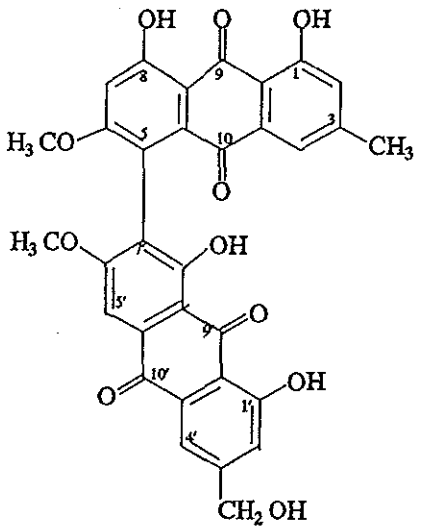
23

The above spectroscopic data led to the assignment of structure **23**. The spectrum was found to be similar with that of torosachrysone. The identity of this compound was further confirmed by comparison with an authentic sample using TLC (solv. V).

2.1.7. 5,7'-Physcion-fallacinol (24)

Compound **24** is a yellow pigment that turned from light yellow into pink colour on TLC when sprayed with 5% methanolic KOH. The colour change is characteristic for hydroxyanthraquinones[59] (8.5 mg, 0.0007%). It is homogenous on TLC (R_f 0.51, silica gel, $CHCl_3$-EtOAc, 4:1 and R_f 0.39, silica gel, $CHCl_3$-MeOH, 100:1). The IR spectrum, (see Fig. 3) showed absorption bands due to hydroxyl groups (3444 cm^{-1}), a non-chelated carbonyl group (1653 cm^{-1}) and a chelated carbonyl group (1623 cm^{-1}). The UV spectrum, (see Fig. 4) displayed absorption maxima at 244, 281 and 438 nm suggesting a quinonoid chromophore. The ^1H NMR, (300 MHz, $CDCl_3$) spectrum of **24**, (see Fig. 6) indicated four chelated hydroxyl groups (δ 13.06 *s*, 12.25 *s*, 12.20 *s* and 12.09 *s*). The aromatic region displayed six aromatic resonances: two protons have resonances at δ 7.57 *s* and 6.82 *s*, two *meta* coupled signals at δ 7.82 and 7.34 and another set of *meta* coupled signals at δ 7.42 and 7.04. From the 2D Correlated ^1H NMR spectrum, (see Fig. 7) the former

meta coupled resonances have cross peaks with each other as well as with a resonance at δ 4.85 and the latter ones also show cross peaks with each other and with a resonance at δ 2.36. The singlets at δ 3.84 and 3.89, integrating for three protons each, are attributable to aromatic methoxyl groups. The aromatic methyl group displayed a signal at δ 2.36. The singlet at δ 4.85 is attributable to a CH_2OH group. Compound **24** is a dimer and the proposed structure is shown below.



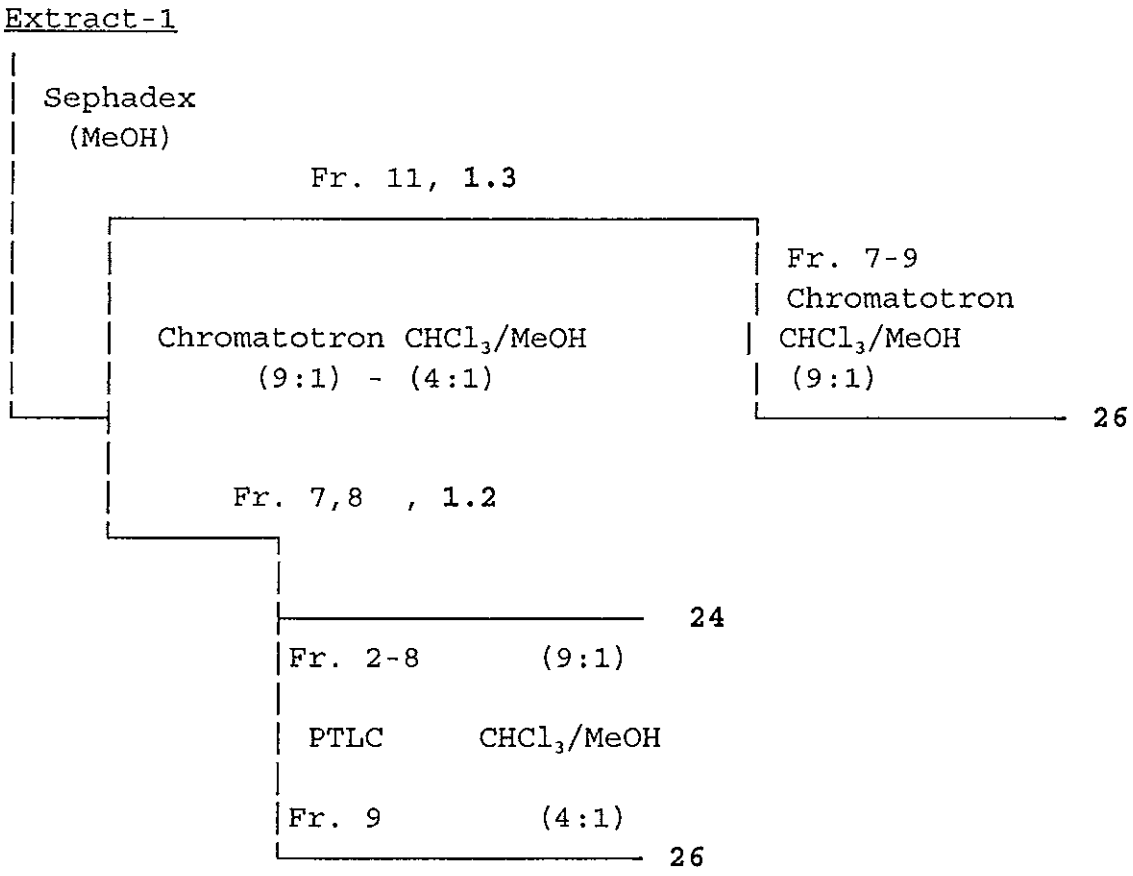
24

The alternative structure which would have the CH_2OH group at C-3 and the methyl group at C-3′ is ruled out by comparing the ^{1}H NMR spectrum of floribundone-1 (**16**) with that of compound **24**. The ^{1}H NMR spectrum of floribundone-1 (**16**) showed that Me-3 and Me-3′ resonate at δ 2.35 and 2.45 ppm respectively. Due to the twisted conformation of the dimer, Me-3 falls in the shielding zone of the lower anthraquinone moiety and hence showed a small upfield shift when compared to that of Me-3′. The ^{1}H NMR spectrum of compound **24** showed the aromatic methyl group signal at δ 2.36, indicating that it is attached to C-3 and not to C-3′. If it were at C-3′, the corresponding signal would have appeared at *ca.* δ 2.45 ppm.

Extract-1 was chromatographed according to Scheme 5. The extract was applied on Sephadex, chromatographed on chromatotron and multiply developed on PTLC, resulting the isolation of compound **24** and a glycoside of quercetin (**26**).

Scheme 5

Chromatographic Separation of Extract-1

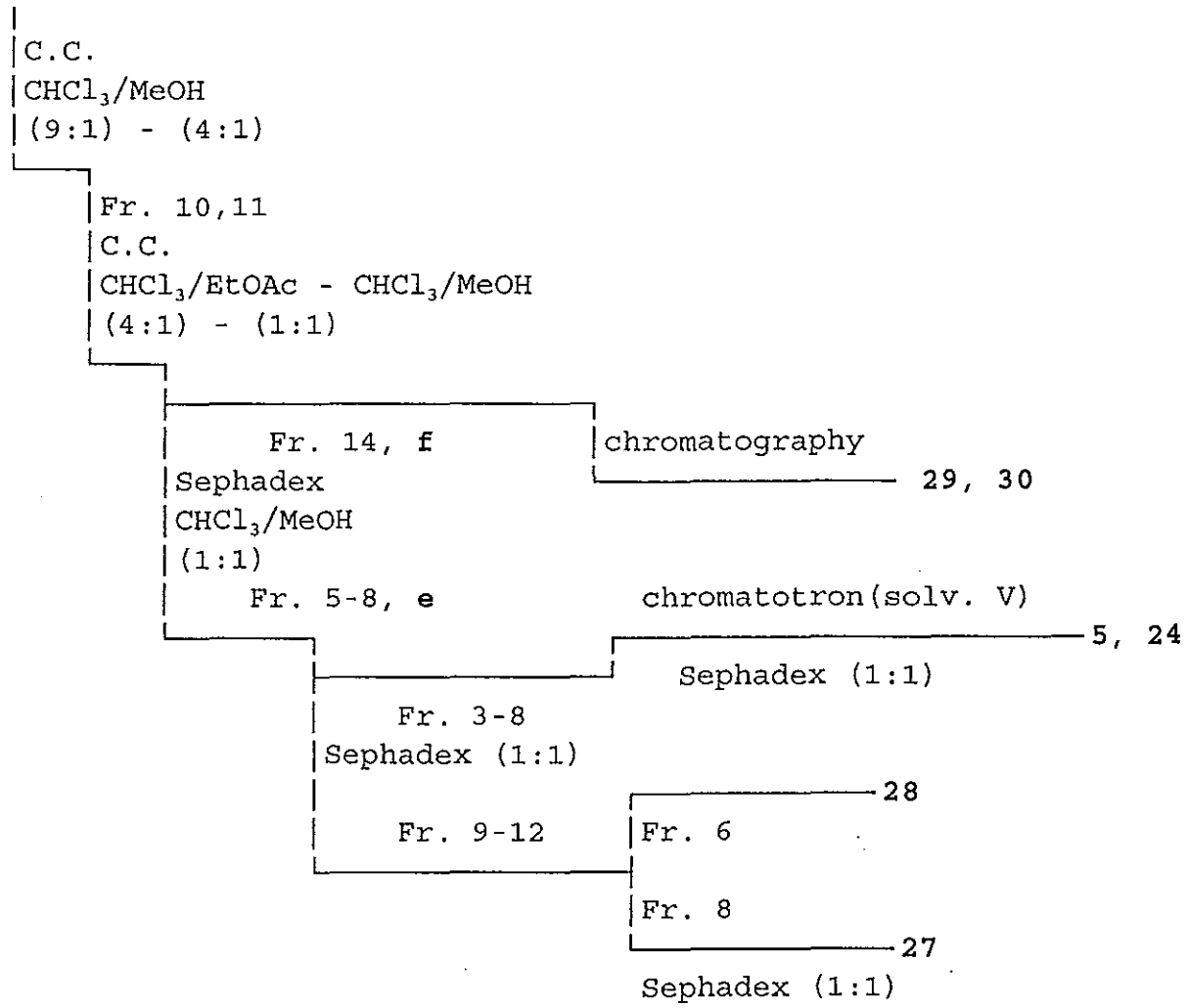


The benzene extract and extract-2 were combined and chromatographed according to Scheme 6. The combined extract was chromatographed several times on silica gel column to give **e** and **f**. Fraction **e** was subjected to Sephadex column

chromatography and purified on chromatotron to give again **5** and compound **24** of the chloroform extract as well as quercetin (**27**) and luteolin (**28**). Fraction **f** was chromatographed on Sephadex and then on chromatotron. Further purification on Sephadex and on PTLC afforded compound **29** and compound **30** (see Expt.).

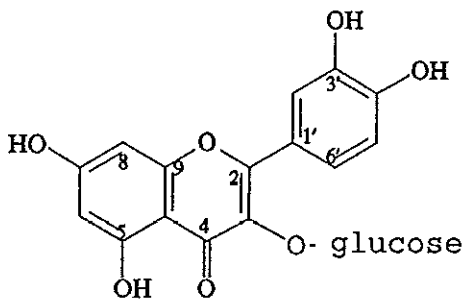
Scheme 6

Chromatographic Separation of Benzene Extract & Extract-2



2.2.1. Quercetin 3-*O*-glucoside (26)

This light yellow powder was isolated from the methanol extract (135 mg, 0.0104%). The ^{1}H NMR spectrum of compound **26** in DMSO-d_6 (see Fig. 8), revealed the presence of a chelated hydroxyl group at δ 12.65 and five aromatic protons (two *meta* coupled signals at δ 6.20 and 6.40, one *ortho* coupled signal at δ 6.82, one *ortho-meta* coupled signal at δ 7.60 and one *meta* coupled signal at δ 7.54). These resonances have strong resemblance with those of **27**. The spectrum also displayed additional non-distinctive and overlapped signals below 5.50, implying a glycoside moiety and quercetin appears to be the aglycone.



26

Table 5

^{13}C NMR Spectral Data of 26

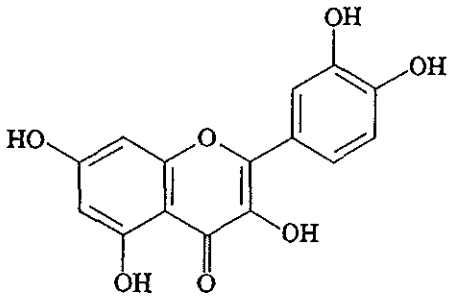
C	δ(ppm) **26** (22.5MHz, DMSO-d_6)
4	177.6
7	164.2
5	161.3
2,9	156.4

4′	148.5
3′	144.9
3	133.7
1′	121.7
6′	121.3
5′	116.4
2′	115.3
10	104.1
1″	101.5
6	98.9
8	93.6
3″	77.5
5″	76.8
2″	74.3
4″	70.3
6″	61.3

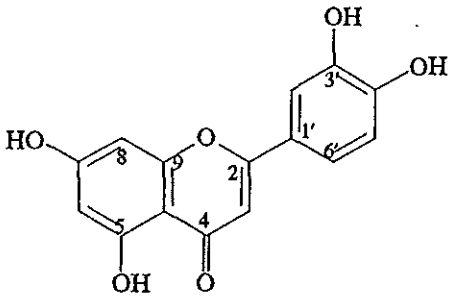
The ^{13}C NMR spectrum of **26** is in close agreement with that reported for quercetin 3-*O*-glucoside in the literature[40].

The carbon at the site of glycosylation is shifted to a higher field, in ^{13}C NMR, following glycosylation, whereas the *ortho* and *para* related carbons shift down field. Glycosylation of the 3 and 5 hydroxyls produces unusual effects. With the 3 hydroxyl, although the upfield shift of the C-3 signal is of the expected order (*ca.* 2 ppm), the down field shift of the *ortho* related C-2 signal is especially pronounced[83]. Accordingly, C-2 resonates at 156.4 in compound **26** while at 147.6 in quercetin. This is in agreement with the consideration of C-3 of **26** (i.e. *ortho* related to C-2) as the site of glycosylation.

The identity of compound **26** was further established using MS. The CIMS of compound **26** (quercetin 3-*O*-glucoside) revealed a molecular ion peak at m/z 487 (quercetin + glucose + Na). The signal at m/z 325 was due to loss of glucose, at m/z 303 was (quercetin + 1) and at m/z 185, the base peak, was (M^+ - quercetin + Na) (see Fig. 9).



27



28

2.2.2. Quercetin (27)

This flavonoid is a yellow powder, obtained from the methanol extract (85 mg, 0.0065%). The ^{1}H NMR spectrum of **27** revealed the presence of a chelated hydroxyl group at δ 12.47 and five aromatic protons (two *meta* coupled signals at δ 6.19 and 6.42, one *ortho* coupled signal at δ 6.87, one *ortho-meta* coupled signal at δ 7.55 and one *meta* coupled signal at δ 7.69), (see Fig. 10).

The ^{1}H NMR spectrum of **27** in DMSO-d_6 is very well consistent with that of quercetin. R_f comparison on TLC with authentic quercetin has further confirmed the identity of **27** (solv. VI).

2.2.3. Luteolin (28)

This yellow powder was isolated from the methanol extract (45 mg, 0.0035%). The presence of a chelated hydroxyl group at δ 12.96 and six aromatic protons, (two *meta* coupled signals at δ 6.19 and 6.45, one singlet at δ 6.68, one *ortho* coupled signal at δ 6.87, one *meta* coupled signal at δ 7.38 and one *ortho-meta* coupled signal at δ 7.43), were evident from the ^{1}H NMR spectrum of compound **28**, (see Fig. 11).

Table 6

^{13}C NMR Spectral Data of 28

C	δ(ppm) **28** (22.5MHz, DMSO-d_6)
4	181.6
7*	164.1
2*	163.9
5	161.5
9	157.3
4′	149.6
3′	145.7
1′	121.6
6′	118.9
5′	116.0
2′	113.4
10	103.7
3	102.8
6	98.8
8	93.8

* assignment interchangeable.

The ^{13}C NMR spectrum of **28**, (see Fig. 12) displayed 15 carbon resonances. The ^{13}C NMR data are consistent with that reported for luteolin in the literature[40].

2.2.4. Compound 29

Compound **29** and **30** are yellow pigments that turned into pink colour on TLC when they were sprayed with 5% methanolic KOH. (**29** has an R_f value 0.62 and **30** has 0.59 in $CHCl_3$/MeOH, 9:1). The colour change is characteristic for hydroxyanthraquinones.

^1H NMR spectrum of **29** in $CDCl_3$ indicated four chelated hydroxyl groups (δ 13.08, 12.35, 12.14 and 12.12). The aromatic region displayed the following resonances: δ 7.38, 7.04, 6.87, 6.79, 6.77 and 6.72. The singlets at δ 3.79 and 3.81, integrating for three protons each, could be attributed to aromatic methoxyl groups. The aromatic methyl groups displayed signals at δ 2.38 and 2.40.

2.2.5. Compound 30

^1H NMR spectrum of **30** in $CDCl_3$ showed highly similar resonances to that of **29** and indicated four chelated hydroxyl groups (δ 13.06, 12.32, 12.07 and 12.03). The aromatic protons resonated at δ 7.39, 7.02, 6.82, 6.81, 6.79 and 6.72. The signals at δ 3.80 and 3.84 could be attributed to methoxyl groups and those at δ 2.34 and 2.39 to aromatic methyl groups.

The available samples were hardly sufficient to generate enough spectroscopic data that would enable the full characterization of compounds **29** and **30**.

3. EXPERIMENTAL

3.1. GENERAL

Senna septemtrionalis pods were collected from Addis Ababa University (chem.dept.) garden on April 20, 1995 and identified by Dr. Sebsebe Demissew (voucher no. W-1). Specimen of the plant is deposited at the National Herbarium, A. A. U.

^{1}H NMR : a Joel Fx 90Q instrument at 90 MHz and an instrument at 300 MHz.

^{13}C NMR : Joel Fx 90Q (22.5 MHz)

UV : Milton Roy Spectronic 1001 Plus.

IR : Perkin-Elmer FTIR 1600 Series.

MS : CIMS & EIMS (Low Resolution).

MP : Bock-Monosc Op.

Analytical TLC : Silica gel 60 F_{254} (Merck) coated on aluminium sheets, 0.20 mm thickness.

Preparative TLC : Silica gel 60 $PF_{254+366}$ (Merck), 1 mm thickness and Pre-coated TLC plates SIL G-25, 0.25 mm silica gel with fluorescent indicator UV_{254}.

Column Chromatography : Silica gel 60, particle size 0.063-0.200 mm (70-230 mesh ASTM) and Sephadex (LH-20, $CHCl_3$-MeOH 2:1, 1:1 and 0:1).

Impregnation of Silica gel : with 5% oxalic acid.

Centrifugal TLC : Chromatotron model 8924, 2 mm thickness silica gel 60 PF_{254} containing gypsum.

Solvent system :

petroleum ether-EtOAc, 9:1 (solv. I); petroleum ether-EtOAc, 4:1 (solv. II); petroleum ether-EtOAc, 1:1 (solv. III); $CHCl_3$-EtOAc 9:1 (solv. IV); $CHCl_3$-EtOAc 4:1 (solv. V); $CHCl_3$-EtOAc 1:1 (solv. VI); $CHCl_3$-MeOH 9:1 (solv. VII); $CHCl_3$-MeOH 4:1 (solv. VIII); $CHCl_3$-MeOH 2:1 (solv. IX); $CHCl_3$-MeOH 1:1 (solv. X).

3.2. EXTRACTION AND ISOLATION

The dried and ground pods were soaked in 5% HOAc (3 l) for 24 hr and dried (1.3 kg). The dried and powdered plant material was defatted with petroleum ether (40-60°C), (9 l). The marc was extracted with cold chloroform (7 l) and the extract was freed of solvent to give 13.2 g of residue. Further extraction of the marc with cold MeOH (8 l) was done. The methanol extract was concentrated to yield 204.9 g of dark gummy residue (see Scheme 2).

3.2.1. Chloroform Extract

The chloroform extract was subjected to column chromatography in which 140 g of oxalic acid impregnated silica gel was packed with (solv. I), (0.2 l) into a glass column and the 13.2 g residue was adsorbed on silica gel and applied. Successive elution with increasing polarity gave in 16 fractions of *ca*. 75 ml each.

	eluent used				quantity (l)
	pet.ether :	EtOAc :	$CHCl_3$:	MeOH	
1.	9	1			0.25
2.	4	1			0.30
3.			1	0	0.25
4.			9	1	0.15
5.			4	1	0.20
6.			1	1	0.20

On the basis of TLC, fractions 3-8 were combined to give **2.1** and fractions 11-14 were combined to give **2.2.**

2.1 was divided into two equal portions a and b and each was subjected to column chromatography in which 60 g of silica gel was packed with (solv. I), (0.2 l) into a glass column and eluted with increasing polarity.

	eluent used			quantity (l)
	pet.ether :	EtOAc :	$CHCl_3$	
1.	9	1		0.35
2.	4	1		0.40
3.			1	0.60

Chromatography of portion a gave 10 fractions of 125 ml each.

Fractions 1 and 2 were combined and were subjected to separation on Sephadex using (solv. IX) resulting in the isolation of **14.**

Fraction 3 was subjected to separation on Sephadex (solv. IX) and then

chromatographed on PTLC, (solv. II) to give **15**, ^{1}H NMR spectrum of **15** did not have characteristic resonances.

Fraction 9 was subjected to separation on Sephadex (solv. IX) and then chromatographed on PTLC, (solv. II) to yield **16**.

Chromatography of portion **b** gave 15 fractions. The volume of each fraction was *ca.* 80 ml. Compounds **5**, **14**, **16** and **17** were isolated in the following manner:

Fractions 1 and 2 were combined and chromatographed on PTLC, (solv. II) resulting in the isolation of **17**.

Fractions 4-7 gave **14**.

Fractions 11-13 were combined and multiply developed on PTLC, (solv. II) resulting in the isolation of **5**.

Fraction 14 gave **16**.

2.2 was divided into three portions and each was fractionated on Sephadex using (solv. IX). TLC analyses were performed for the resulting 25 fractions and accordingly 15 of them were combined into two groups, **c** and **d**.

Column chromatography was done for each group making use of 60 g silica gel and neat chloroform for each packing.

	eluent used		quantity (l)	
	$CHCl_3$:	EtOAc	c	d
1.	1	0	0.6	0.2
2.	9	1	0.4	0.2
3.	4	1	0.3	0.3
4.	1	1	0.3	0.4
5.	0	1	0	0.2

Compounds **18**, **19**, **20**, **21**, **22**, **24** and **25** were isolated from **c**, as follows: **c** gave 18 fractions of 100 ml each. Fraction 5 was multiply developed on PTLC using (solv. III) so that clean separation of **18** and **19** were possible, however their ^1H NMR spectra were with no characteristic resonances.

Fraction 7 was chromatographed on PTLC using (solv. V) resulting the isolation of **24** and **22**. (^1H NMR of **22** appeared to be a mixture of floribundone-1 and floribundone-2).

Fraction 8 on PTLC using (solv. V) gave **21**, **22**, **24** & **25**. **21** gave a negative colour reaction for a hydroxyanthraquinone, and **25** on TLC, appeared to be the most polar of all but with too small an amount.

Fraction **c** had a methanol insoluble part which upon PTLC (solv. V) resulted in **24**, **20** and **22**.

Compounds **5**, **23** and **24** were isolated from **d**: 11 fractions of 100 ml each were obtained from **d**. Fractions 7 and 8 gave **5** and **24**.

Fraction 9 was chromatographed on PTLC using (solv. V) and resulted in the isolation of **23**.

3.2.2. Methanol Extract

Out of the 200 g methanol extract, 60 g was subjected to column chromatography. Successive elution resulted in 25 fractions. The volume of each fraction was *ca.* 200 ml.

	eluent used			quantity (l)
	$CHCl_3$: EtOAc : MeOH			
1.	1			1.75
2.	4	1		1
3.	1	1		1.25
4.	4	4	2	1
5		1	1	0.75

On the basis of TLC, fractions 5-7 were combined. These fractions were partitioned on Sephadex using (solv. IX) and then chromatographed on PTLC, using (solv. V) to give compound **24**.

The remaining 140 g methanol extract, as of scheme 4, was dissolved in water (1 l) and the suspension was centrifuged. The aqueous phase was extracted with ethyl acetate (1.5 l). The ethyl acetate freed dark residue was labelled extract-1 (2.8 g).

The water insoluble portion (ppt) was extracted with cold benzene (0.5 l) and the extract was concentrated to give 2.1 g. Further extraction of the ppt with cold ethyl acetate (0.5 l) resulted in a dark residue of extract-2 (9 g).

Extract-1 was subjected to separation on Sephadex (MeOH). 14 fractions were collected, each *ca.* 100 ml. By TLC monitoring, fractions 7 and 8 were combined

combined and chromatographed on column.

	eluent used			quantity (l)
	$CHCl_3$:	EtOAc :	MeOH	
1.	4	1		1
2.	1	1		1.25
3.	1	1	1	1.5
4.	1		1	2

Fourteen fractions, each *ca.* 200 ml, were collected. Fractions 5-8 were combined and labelled as **e** and fraction 14 as **f**.

Compounds **5**, **24**, **27** and **28** were isolated, from **e**, as follows:

Fraction **e** was subjected to separation on Sephadex, (solv. IX) and 12 fractions were obtained from which fractions 3-8 and fractions 9-12 were combined separately. Fractions 3-8 were applied on Sephadex, (solv. IX) and then purified on chromatotron, (solv. V) to give compound **5** and compound **24**. Fractions 9-12 were subjected to Sephadex using (solv. X) to obtain eight fractions. Fraction 6, passed through a purification step on Sephadex (solv. X), gave compound **28**. Fraction 7 gave **5** and fraction 8 resulted in compound **27**.

Fraction **f** furnished compounds **29** and **30** in the following manner: **f** was fractionated (in four portions) on Sephadex, (solv. X). TLC analyses were done for the 21 fractions, each *ca.* 150 ml and accordingly fractions 4-9 were combined and subjected to chromatotron.

	eluent used		quantity (l)
	$CHCl_3$:	MeOH	
1.	9	1	1.75
2.	4	1	1.50
3.	1	1	2

Seven fractions were obtained. The first three fractions were applied on Sephadex, (solv. X) to result in 14 fractions. Out of the 14 fractions, 9-11 were combined and fractionated again on Sephadex, (solv. X) to give four fractions. Fraction 3 furnished compound **29** and compound **30**, after it had been chromatographed and multiply developed on PTLC, $CHCl_3$/MeOH 19:1.

Compound **5** (Emodin):An orange pigment turning into deep red colour upon spraying with 5% methanolic KOH (R_f 0.22, solv. I, R_f 0.13, $CHCl_3$ and R_f 0.67, solv. V). MP 255-7, Lit. MP 259-60°C. ^{1}H NMR (90 MHz, $CDCl_3$ and MeOH-d_4) δ: 7.13 (1H, *br s*, 2-H), 7.55 (1H, *br s*, 4-H), 7.25 (1H, *d*, 5-H), 6.65 (1H, *d*, 7-H), 2.45 (3H, *s*, 3-Me).

Compound **14** (Physcion):A yellow pigment, showed a yellow to pink colour change on TLC when sprayed with 5% methanolic KOH (R_f 0.52, solv. I and R_f 0.62, $CHCl_3$). MP 206-8, Lit. MP 209-10°C. ^{1}H NMR (90 MHz, $CDCl_3$) δ: 12.10 (1H, *s*, 1-OH), 12.30 (1H, *s*, 8-OH), 7.65 (1H, *br s*, 4-H), 7.35 (1H, *d*, 5-H), 7.05 (1H, *br s*, 2-H), 6.65 (1H, *d*, 7-H), 3.90 (3H, *s*, 6-OMe), 2.40 (3H, *s*, 3-Me).

Compound **16** (Floribundone-1):An orange pigment, turned into red colour on TLC when sprayed with 5% methanolic KOH (R_f 0.22, solv. I, R_f 0.60, $CHCl_3$ and R_f 0.82, solv. V). Lit. MP >260°C. ^1H NMR (90 MHz, $CDCl_3$) δ: 12.06 (1H, *s*, 1-OH), 12.15 (1H, *s*, 8-OH), 12.25 (1H, *s*, 1'-OH), 13.10 (1H, *s*, 8'-OH), 7.04 (1H, *d*, 2-H), 7.42 (1H, *d*, 4-H), 6.80 (1H, *s*, 7-H), 7.06 (1H, *d*, 2'-H), 7.67 (1H, *d*, 4'-H), 7.57 (1H, *s*, 5'-H), 3.82 (3H, *s*, 6-OMe), 3.85 (3H, *s*, 6'-OMe), 2.35 (3H, *s*, 3-Me), 2.45 (3H, *s*, 3'-Me).

Compound **17** (Chrysophanol):A yellow pigment, showed a yellow to pink colour change on TLC when sprayed with 5% methanolic KOH (R_f 0.60, solv. I and R_f 0.67, $CHCl_3$). MP 195-7, Lit. MP 193-5°C. IR $\nu_{max}^{KBr}cm^{-1}$: 3420, 1675, 1630, 1610. ^1H NMR (90 MHz, $CDCl_3$) δ: 12.00 (1H, *s*, 1-OH), 12.10 (1H, *s*, 8-OH), 7.65 (1H, *br s*, 4-H), 7.80 (1H, 5-H), 7.58 (1H, 6-H), 7.10 (1H, *br s*, 2-H), 7.32 (1H, 7-H), 2.43 (3H, *s*, 3-Me).

Compound **20** (Torosanin-9',10'-quinone):It is a dark red pigment with a positive colour change on TLC when sprayed with 5% methanolic KOH (R_f 0.46, solv. V). ^1H NMR (90 MHz, $CDCl_3$) δ: 12.10 (1H, *s*, 1'-OH), 12.34 (1H, 8'-OH), 10.24 (1H, *s*, 8-OH), 16.40 (1H, *s*, 9-OH), 7.10 (1H, 2'-H), 7.65 (1H, 4'-H), 7.55 (1H, *s*, 5'-H), 6.70 (1H, *s*, 7-H), 6.42 (1H, 10-H), 3.89 (3H, *s*, 6'-OMe), 3.81 (3H, *s*, 6-OMe), 2.45 (3H, *s*, 3'-Me), 1.35 (3H, 3-Me), 2.80 (2H, 2-CH_2), 2.92 (2H, 4-CH_2), 1.50 (non-chelated OH).

Compound **23** (Torosachrysone):A yellow pigment, showed a light yellow to pink then gradually to violet colour changes on TLC when sprayed with 5% methanolic

KOH (R_f 0.42, solv. V). Lit. MP 191-4°C. ^{1}H NMR (90 MHz, $CDCl_3$ and a drop of MeOH-d_4) δ: 9.70 (1H, 8-OH), 16.05 (1H, 9-OH), 6.54 (1H, *s*, 5-H), 6.47 (1H, *s*, 7-H), 6.83 (1H, *s*, 10-H), 3.85 (3H, *s*, 6-OMe), 1.42 (3H, 3-Me), 2.80 (2H, 2-CH_2), 3.00 (2H, 4-CH_2), 2.05 (non-chelated OH).

Compound **24** (5,7′-Physcion-fallacinol):A yellow pigment, with a positive colour change for hydroxyanthraquinone (R_f 0.51, solv. V). MP dec>123°C. UV Λ_{max}^{MeOH}nm (log ε): 438(3.27), 281(2.68), 245(2.64), 203(2.85). IR ν_{max}^{KBr}cm^{-1}: 3444, 2919, 1653, 1623, 1381, 1270. EIMS 70 eV, (M^+ for $C_{32}H_{22}O_{11}$:582), m/z (rel. int.): 583[M^++1], 154, 136, 121, 107(100). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.09 (1H, *s*, 1-OH), 12.25 (1H, *s*, 8-OH), 12.20 (1H, *s*, 1′-OH), 13.06 (1H, *s*, 8′-OH), 7.04 (1H, *br s*, 2-H), 7.42 (1H, *br s*, 4-H), 6.82 (1H, *s*, 7-H), 7.34 (1H, *br s*, 2′-H), 7.82 (1H, *br s*, 4′-H), 7.57 (1H, *s*, 5′-H), 4.85 (2H, *s*, 3′-CH_2OH), 3.84 (3H, *s*, 6-OMe), 3.89 (3H, *s*, 6′-OMe), 2.36 (3H, *s*, 3-Me).

Compound **26** (Quercetin 3-*O*-glucoside or Isoquercitrin):A light yellow powder turning into deep yellow colour upon spraying with 5% methanolic KOH (R_f 0.35, solv. VIII, R_f 0.20, solv. VII). MP 225-7, Lit. MP 238-42°C. ^{13}C NMR: See Table 5. CIMS, (M^+ for $C_{21}H_{20}O_{12}$:464), m/z (rel. int.): 487[M^++Na], 325, 303, 185(100), 83, 57.

Compound **27** (Quercetin):It is a yellow powder with further colour deepening and then displaying a gradual colour change to light brown on TLC when sprayed with 5% methanolic KOH (R_f 0.27, solv. VI). Lit. MP 313-14°C. ^{1}H NMR (90 MHz, DMSO-d_6) δ: 12.47 (1H, *s*, 5-OH), 6.19 (1H, *d*, 6-H), 6.42 (1H, *d*, 8-H), 6.87 (1H,

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Appendix

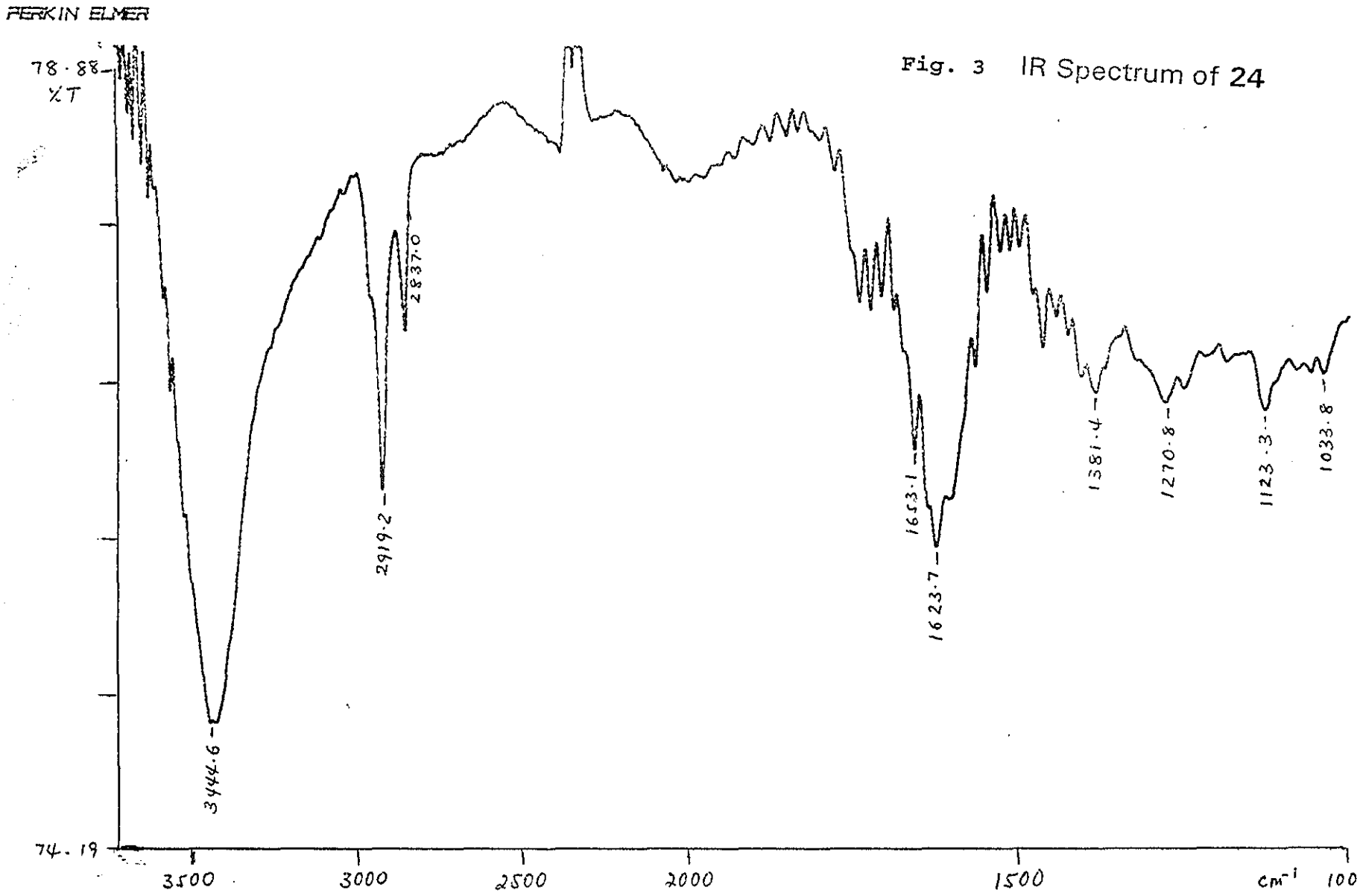
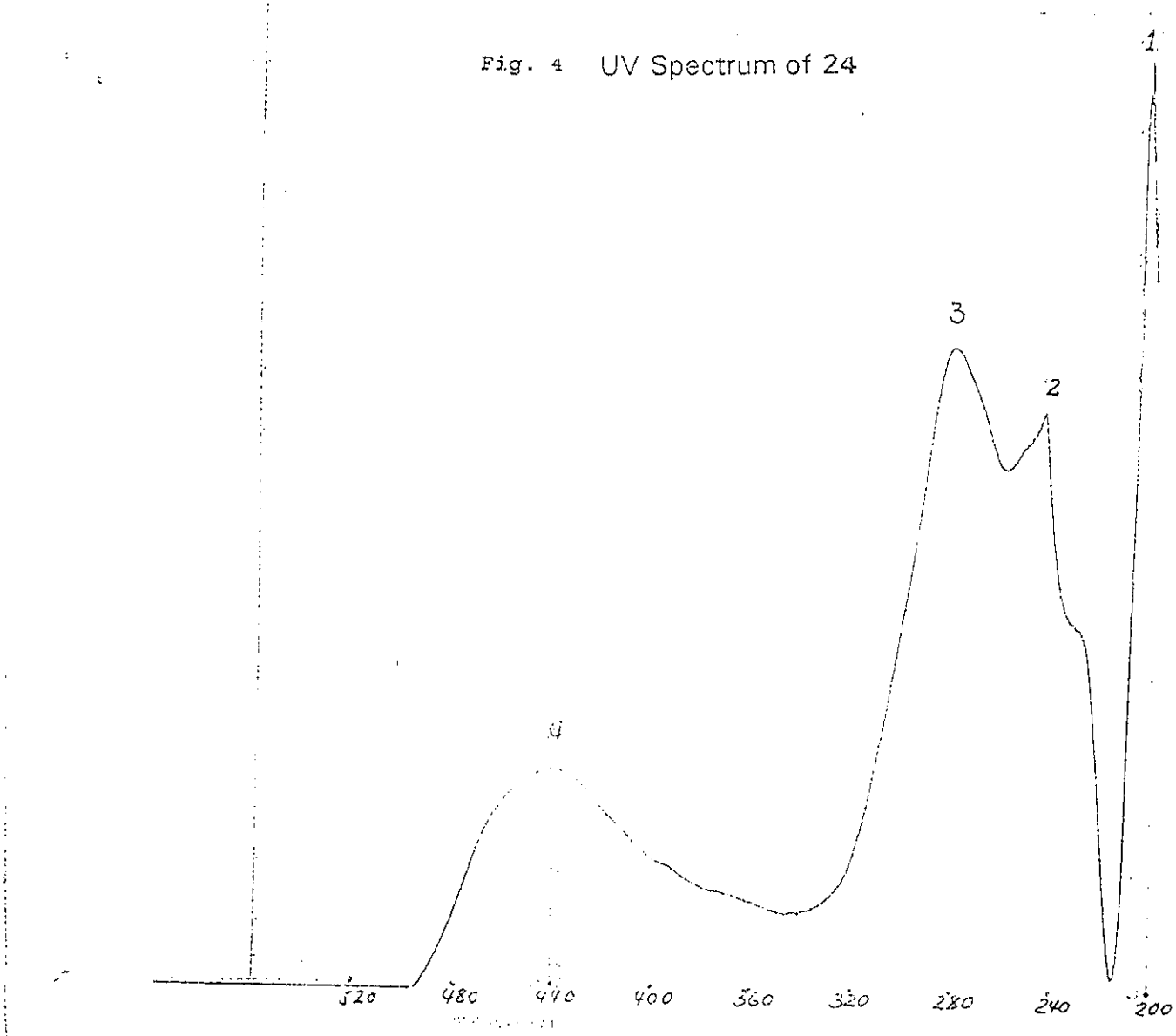


Fig. 3 IR Spectrum of 24

Fig. 4 UV Spectrum of 24



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NM/DIV
OVERLAY

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SCAN
RUN TIME
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2
IDA 1
SCAN
RUN TIME
244.6MM

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IDA 1
SCAN
RUN TIME
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4
IDA 1
SCAN
RUN TIME
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Fig. 6 ^{1}H NMR Spectrum of 24

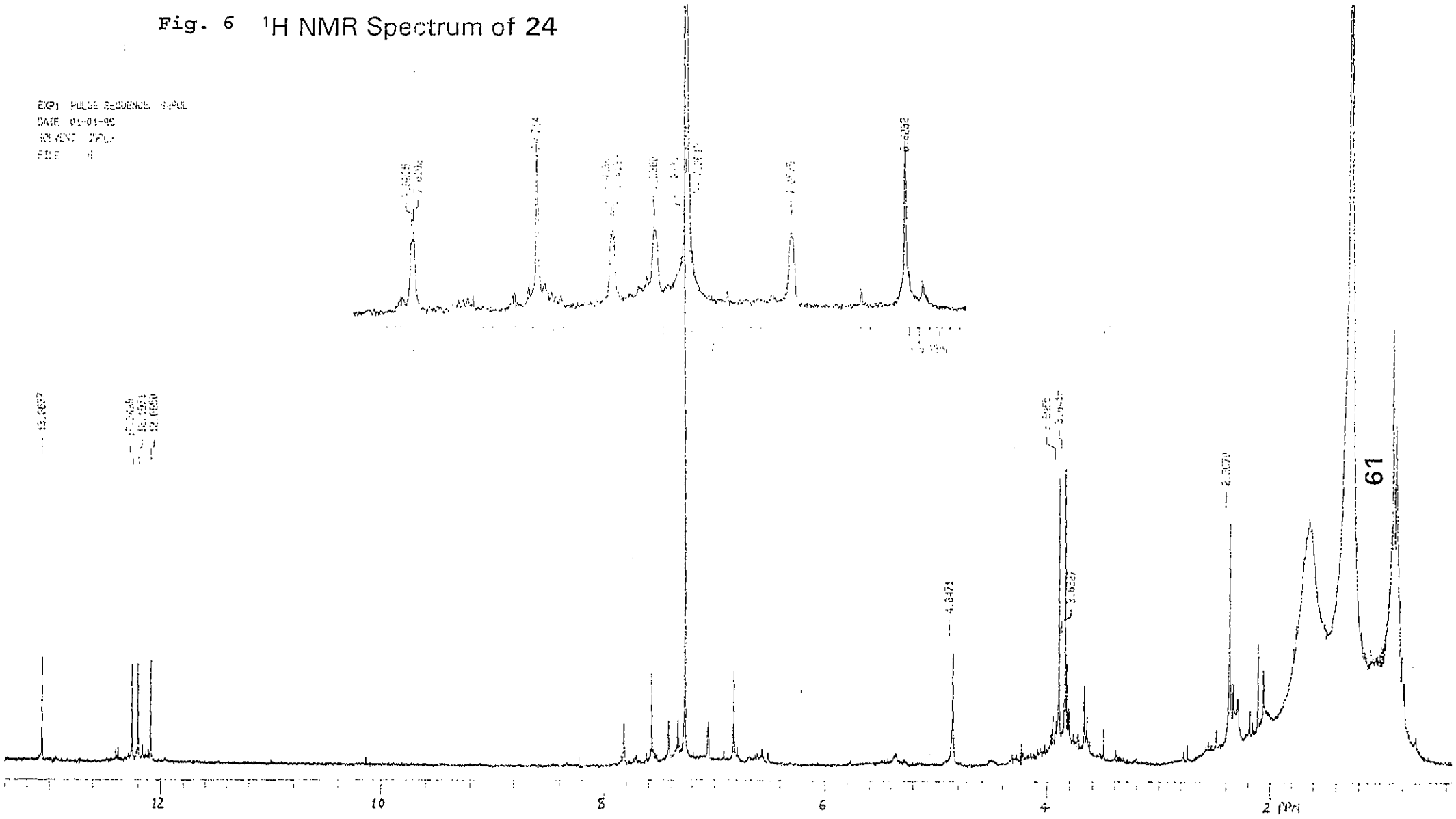


Fig. 7 2D Correlted Spectrum of 24

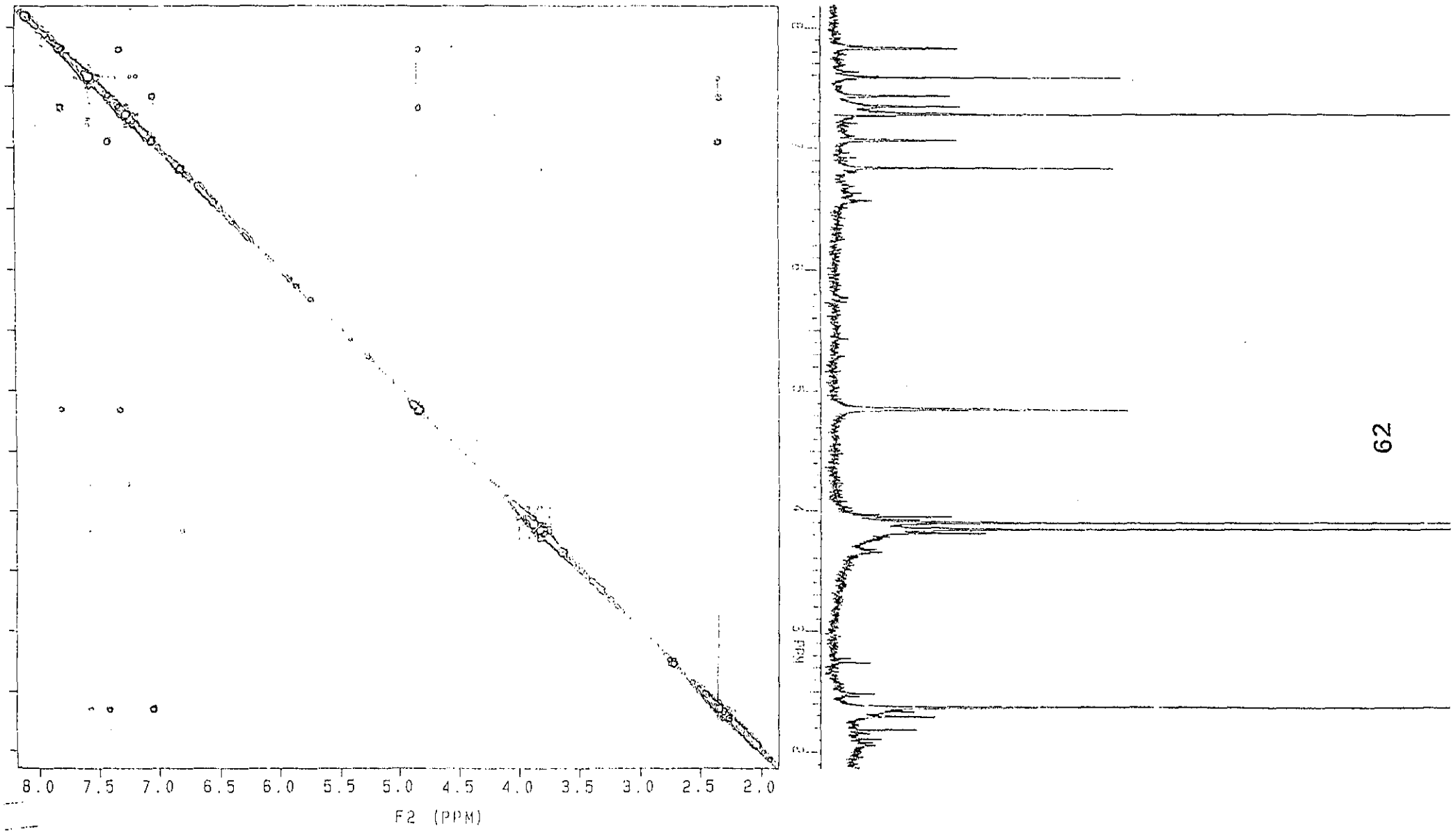
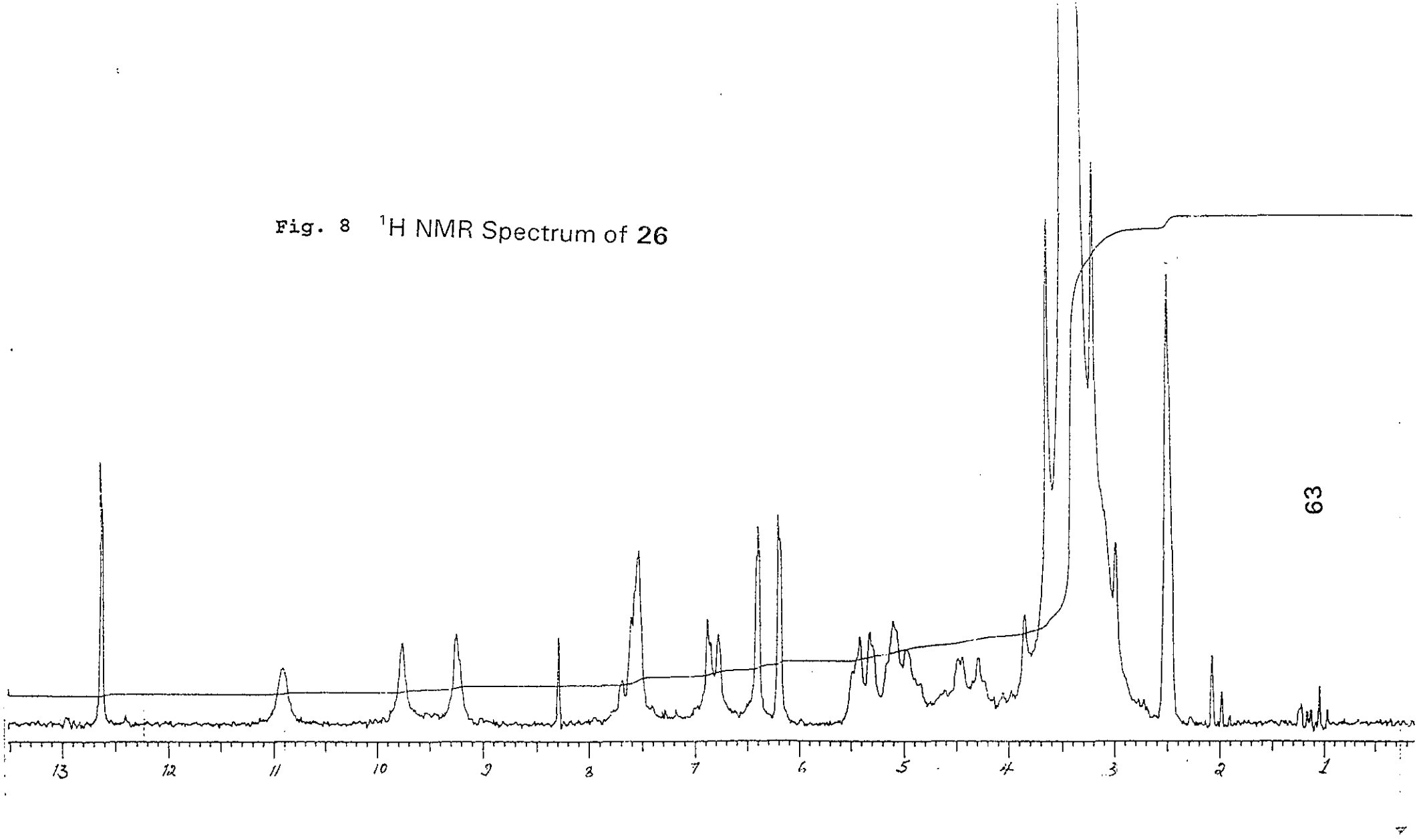


Fig. 8 ^{1}H NMR Spectrum of 26



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Comm: fia @ 10ul BW43 muk with 35V CID
Mode: ESI +Q1MS APICID LMR AVER [illegible] Study : esi pos fia
Oper: Muzi Client: BMA Inlet :
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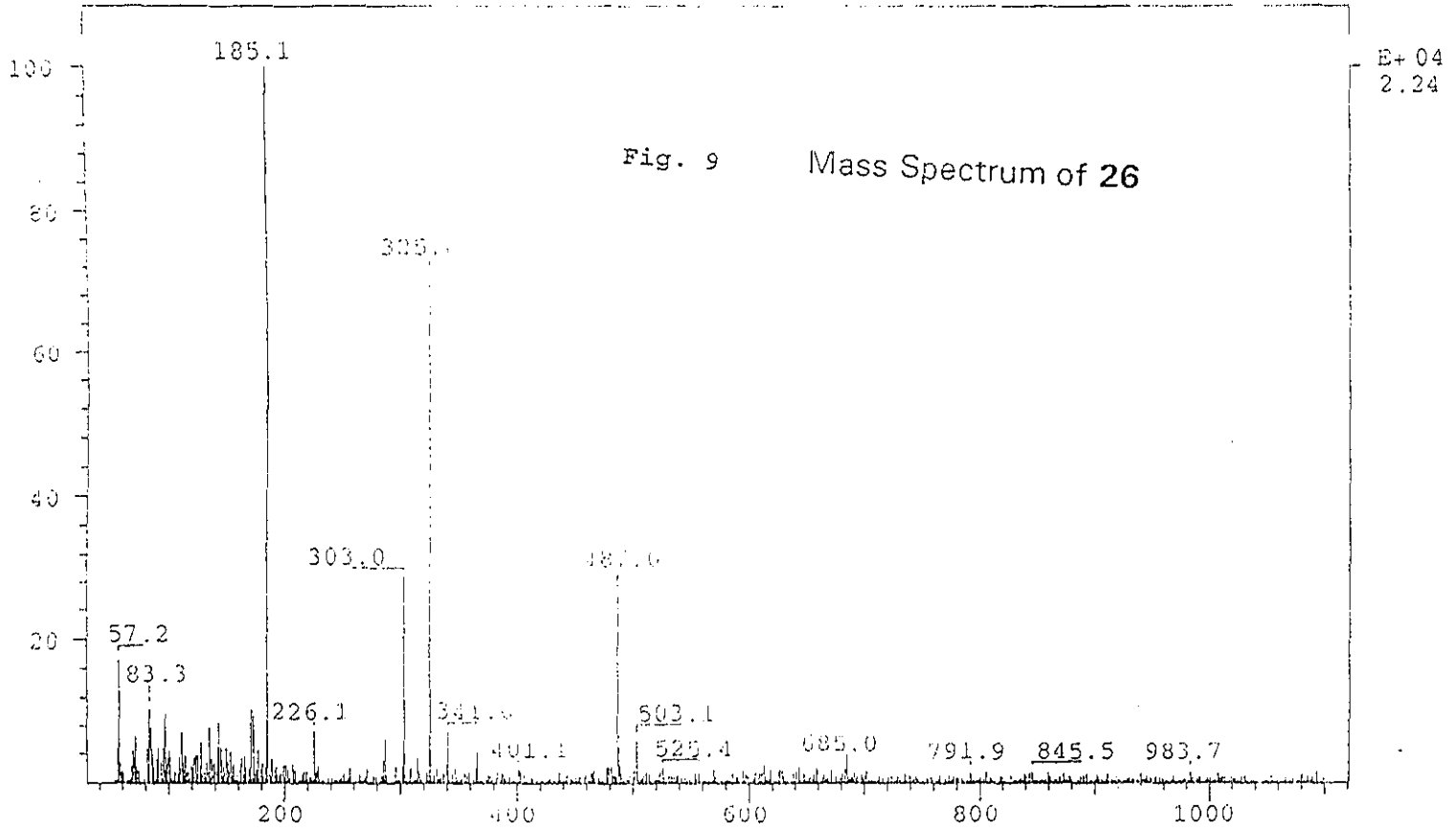


Fig. 9 Mass Spectrum of **26**

Fig. 10 ^{1}H NMR Spectrum of 27

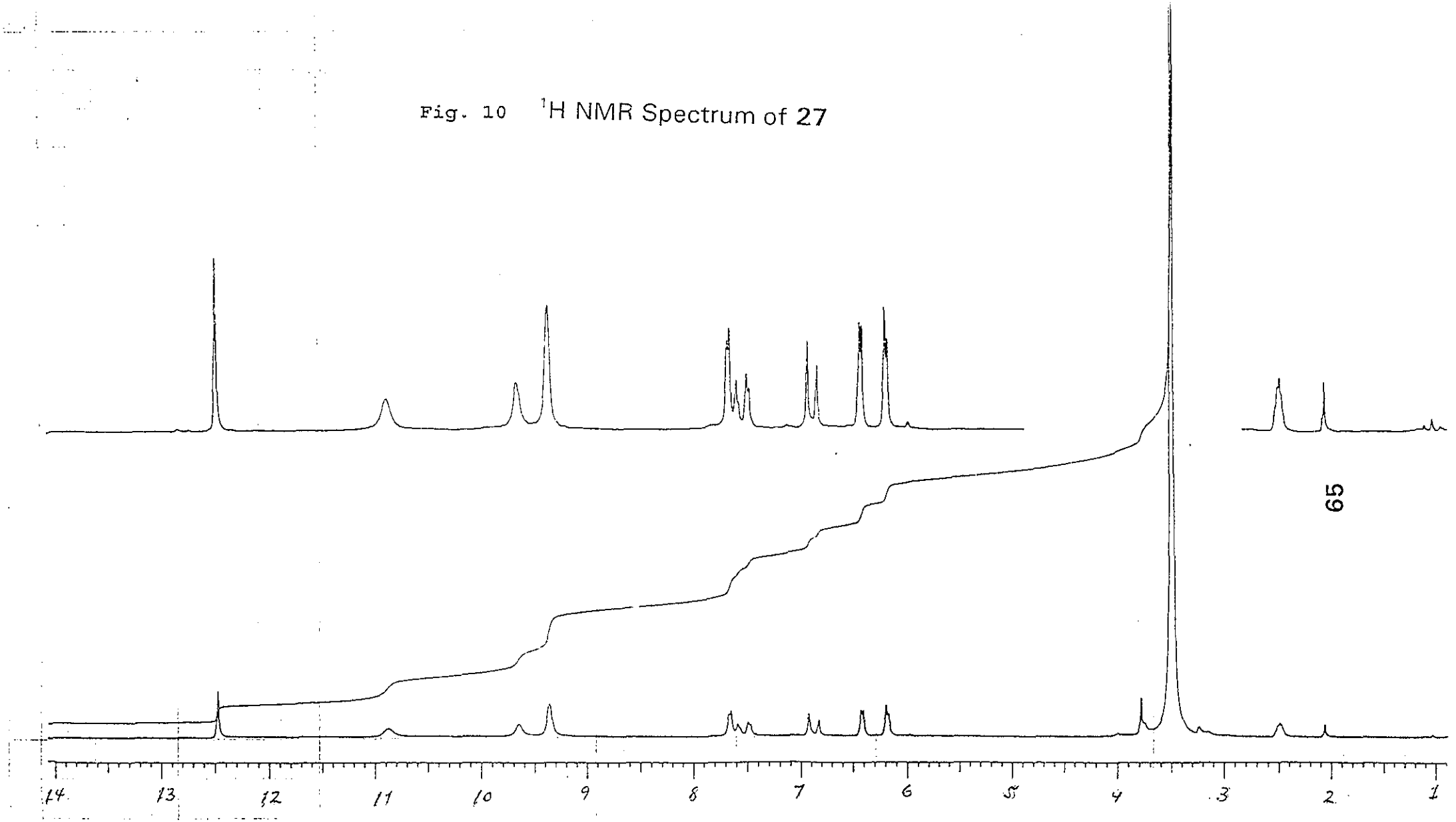
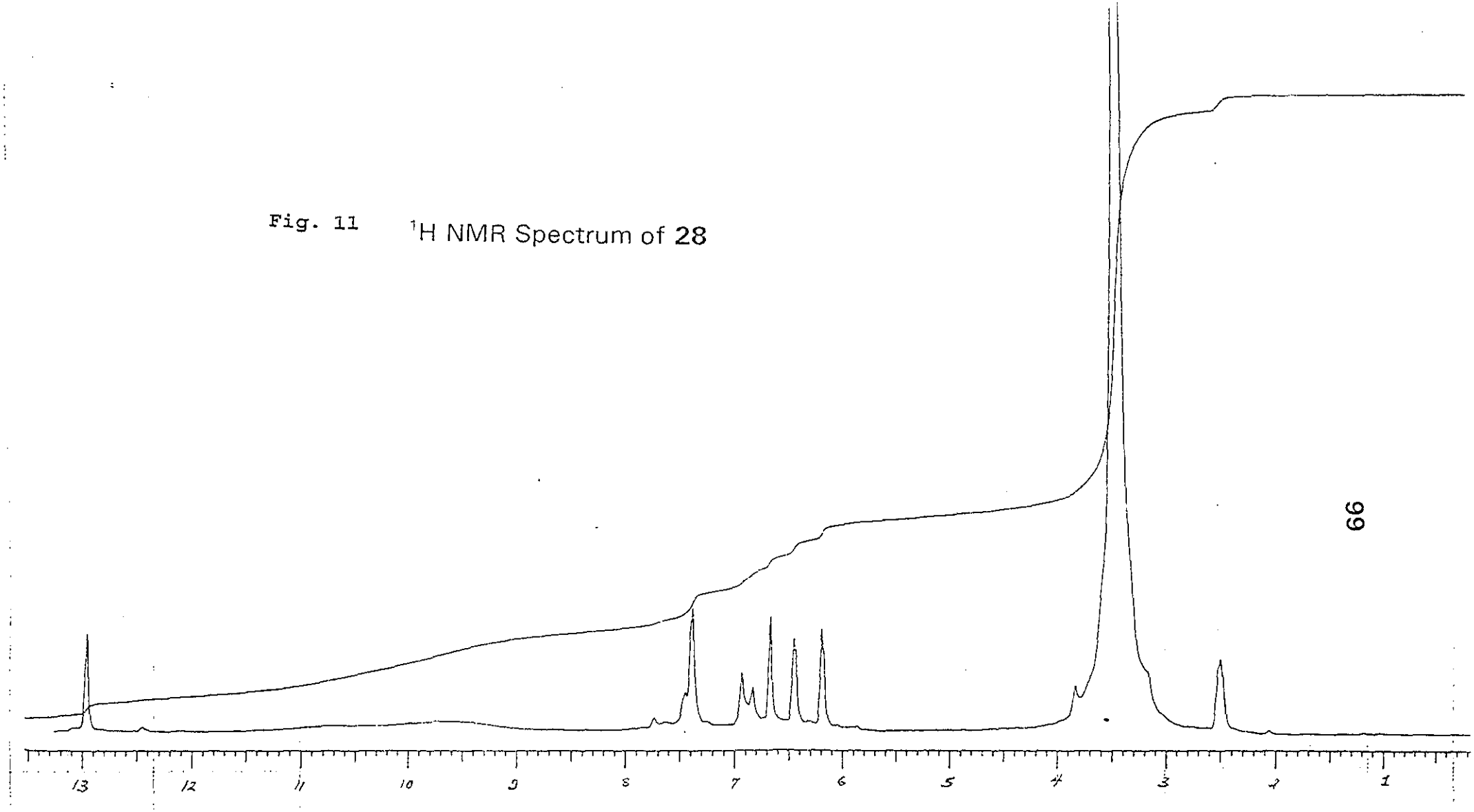


Fig. 11 ^{1}H NMR Spectrum of **28**



GAIN 11

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4	3638.07	161.450	1967
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Fig. 12 ^{13}C NMR Spectrum of 28

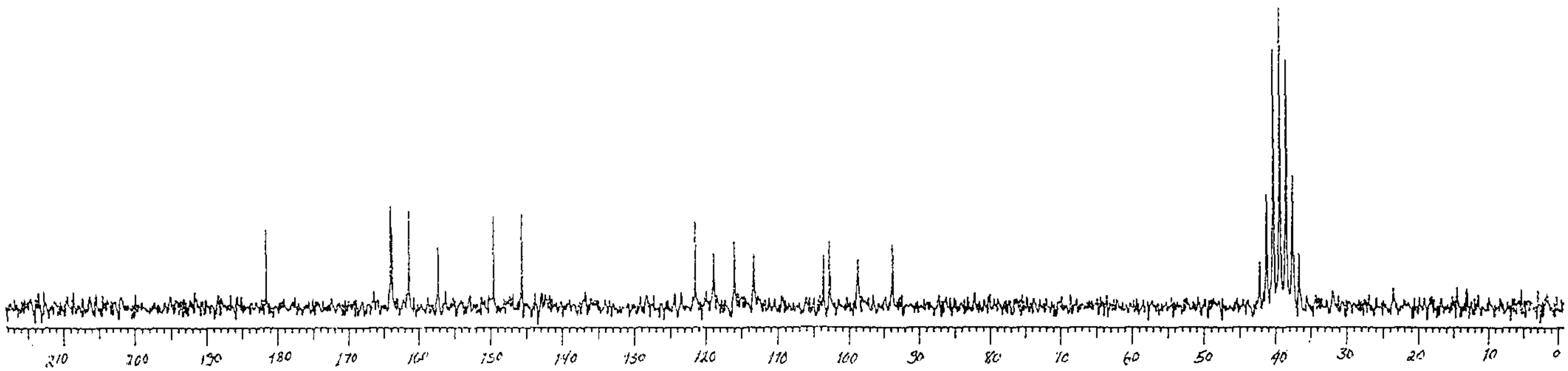

Fig. 13 ^{1}H NMR Spectrum of 16

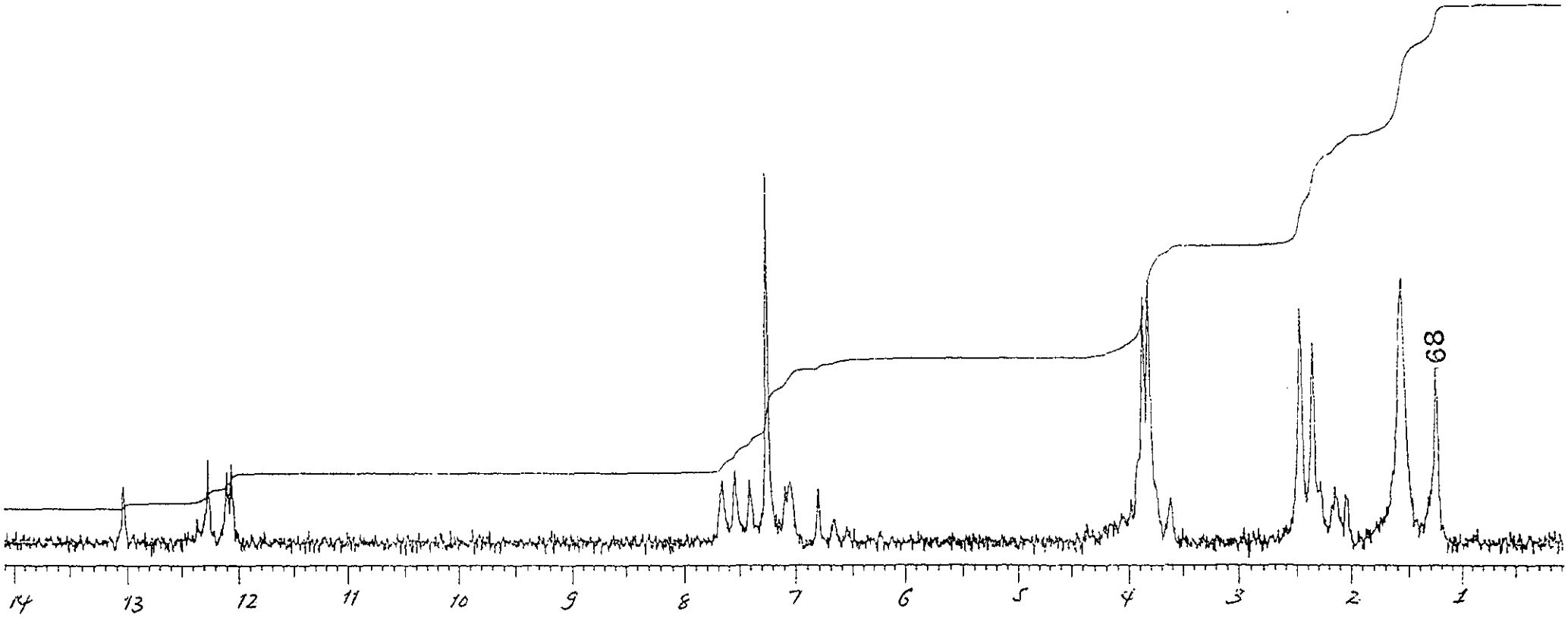

Fig. 14 ^{1}H NMR Spectrum of **20**