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Presented by
Hadroug Aldjia

Topic

Separation, purification, identification and biological activities of iridoid glucosides from the Oleaceae plants family

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Contents

Introduction

Since time immemorial, medicinal plants have been the subjects of man's curiosity, because of they are sources of important therapeutic aids for alleviating human elements [1]. Almost every civilization has a history of medicinal plant use. Approximately 80 % of the people in the world's developing countries rely on traditional medicine for their primary health care needs [2].

In the past, most investigators have opted for synthetic compounds and have generally neglected what nature had to offer .However, many compounds used in today medicine have a complex structure and synthesizing these bioactive compounds chemically at a low price is not easy .Moreover , with increasing realization of the health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics, interest in the use of plants has revived throughout the world, and this can be attested from the fact that most of the pharmaceutical industry is highly dependent on wild populations for the supply of row material for extraction of medicinally important compounds [3].

The expansion of the market for herb demands strict standards for ingredients and manufacturing. The standardization of the herbal preparations requires a detailed study of their chemical composition and finding of the active components .Herb remedies that enjoy the greatest popularity are generally those that have been the most thoroughly investigated [4].

The genus Fraxinus (Oleaceae) comprises more than 40 species [5], mostly native in the north temperate zone, and are used for their wound healing and mild purgative as well as for the treatment of constipation and itching scalp in folk medicine [6,7].

In recent years, an increased interest in the phytochemistry of the genus Fraxinus has been motivated by the discovery of the secoiridoid compounds that constitute the major secondary metabolites and shown an interesting spectrum of biological activities [8].

Previous works on *Fraxinus xanthoxyloides Wall.* species [9,10] showed that it contains two hydroxycoumarins , cichoriin and fraxin . In the literature, there are no data on the isolation of secoiridoid compounds from this endemic species. So , the aim of the

present work is the isolation and structure elucidation of secoiridoid compounds from the Algerian species *Fraxinus xanthoxyloides Wall.*

The thesis has been divided into three chapters. A good deal of attention has been paid to provide accurate information on iridoid compounds and a detailed description of the investigated plant are described in chapter I. In chapter II, the results of a comprehensive phytochemical investigation carried on *Fraxinus xanthoxyloide*s species are reported . Finally, all the experimental work (isolation , purification and identification by means of spectroscopic techniques) is described in chapter III .

I . Literature survey.

I .1. Iridoids

Iridoids represent a large and still expending group of monoterpenoid comounds, which are almost exclusively of plant origin. However, the name iridoid is a generic term derived from the name iridodial (Fig.I.1), compound isolated from some species of Iridomyrmesc, a genus of ants, in which they occur as defensive secretions.The dialdehyde (1) may exist in equilibrium with the corresponding dihydropyran form (2) [11], as it is shown in Fig.I.1.

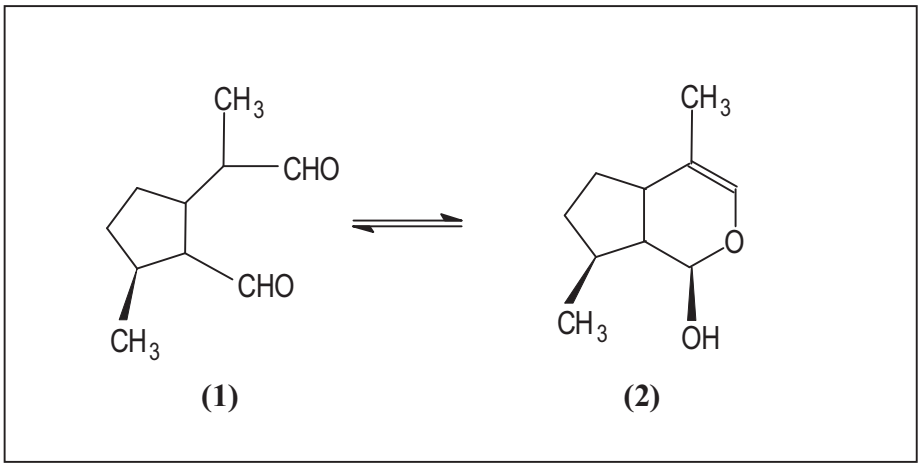


Fig .I.1. The tow isomeric forms of iridodial [11].

Iridoids were first isolated in the latter part of the nineteenth century, but the detailed structure of these compounds had not been determined until 1963. Compounds formed by the cleavage of the cyclopentane ring of iridoids, as shown by the dotted line in Fig .I.2, are called secoiridoids [12].

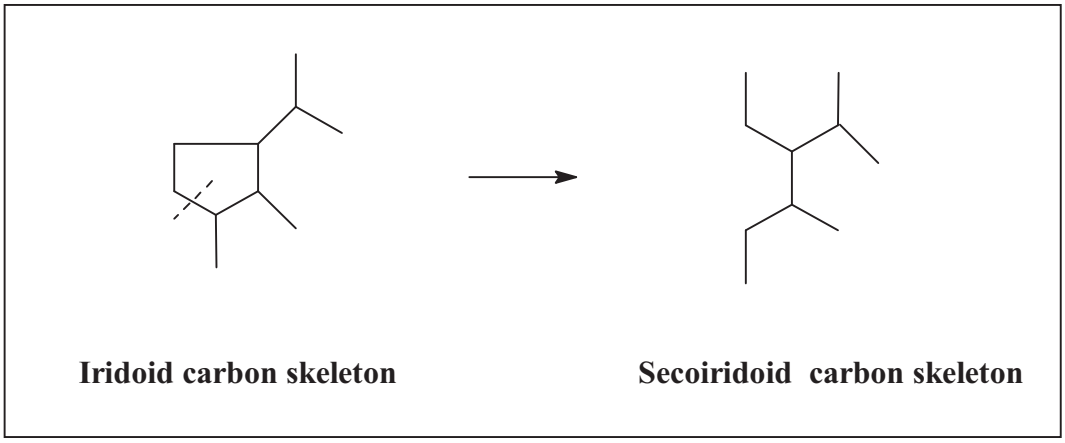


Fig.I.2. Carbon skeletons of iridoids and secoiridoids

The total number of iridoids of defined structure probably exceeds 500 comprising more than 300 iridoid glucosides, 100 secoiridoid glucosides and 100 non–glucosidic iridoids [13] .

All naturally occurring iridoid glucosides are characterized by a cyclopentano (C) pyran ring system as shown and numbered in Fig.I.3. Most of the known compounds till to date, have cis-linked rings and glucose as their sugar moiety [11].

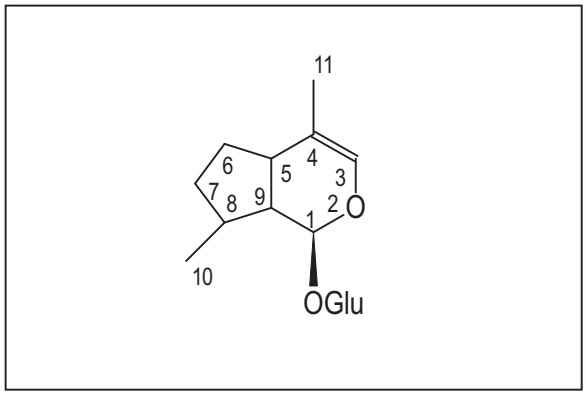


Fig.I .3 . Iridoid glucoside[11] .

The following general characteristics [14 ,15] can be underlined :
1) An enol-ether system involving C1, C3 and C4 ,where C3 is never substituted .
2) Lacking of C10 and /or C11 .
3) S- configuration at C1 , commonly substituted by an acetalic oxygen linked to a glucosidic moiety .
4) Cis - linkage involving C5 , sometimes substituted by an oxygen, and C9 substituted always with the hydrogen in β configuration .
5) Possible presence of an additional double bond between C6 and C7 or more rarely C7–C8 or C8 –C10 .

Also, it should be pointed out that in addition to sugar moieties already known to be constituent of iridoid glucosides, some further mono- and disaccharides have been encountered. Surprisingly, some of the rare monosaccharides were observed to occur only in valeriana –type iridoid glucosides, characterized by linkage of carbohydrate moieties to C11 and isovaleroyl group attached to C1 of the corresponding aglycone [16] .

Apiose has been confirmed to be the outer sugar moiety of the disaccharide unit of 6′-O-apiosylebuloside (3) [17], linked to C6 of the inner glucose unit . 3 – ketoglucose was found to occur as the sole sugar moiety of serruloside (4) and dihydroserruloside, both constituents of Penstemon species (Scrophulariaceae) [18,19] . Moreover, the two further iridoids serrulatoside (5) and penstemideaglucone -11-O-β–4′ – deoxymannopyranosido – 6′- O -β –D – glucopyranoside (6) were confirmed to contain disaccharide units consisting of 4 – deoxyaltrose glucose and 4 – deoxymannose glucose, respectively.In both cases the outer glucose moiety is linked to C6 of the inner 4 – deoxyhexose [18 , 19] . From Odontites species (Scrophulariaceae) aucubigenin – 1 – O - β - serotinoside (7) has been recognized to contain a disaccharide consisting of an α -D– xylopyranose moiety attached to C6 of the inner glucose unit [20].

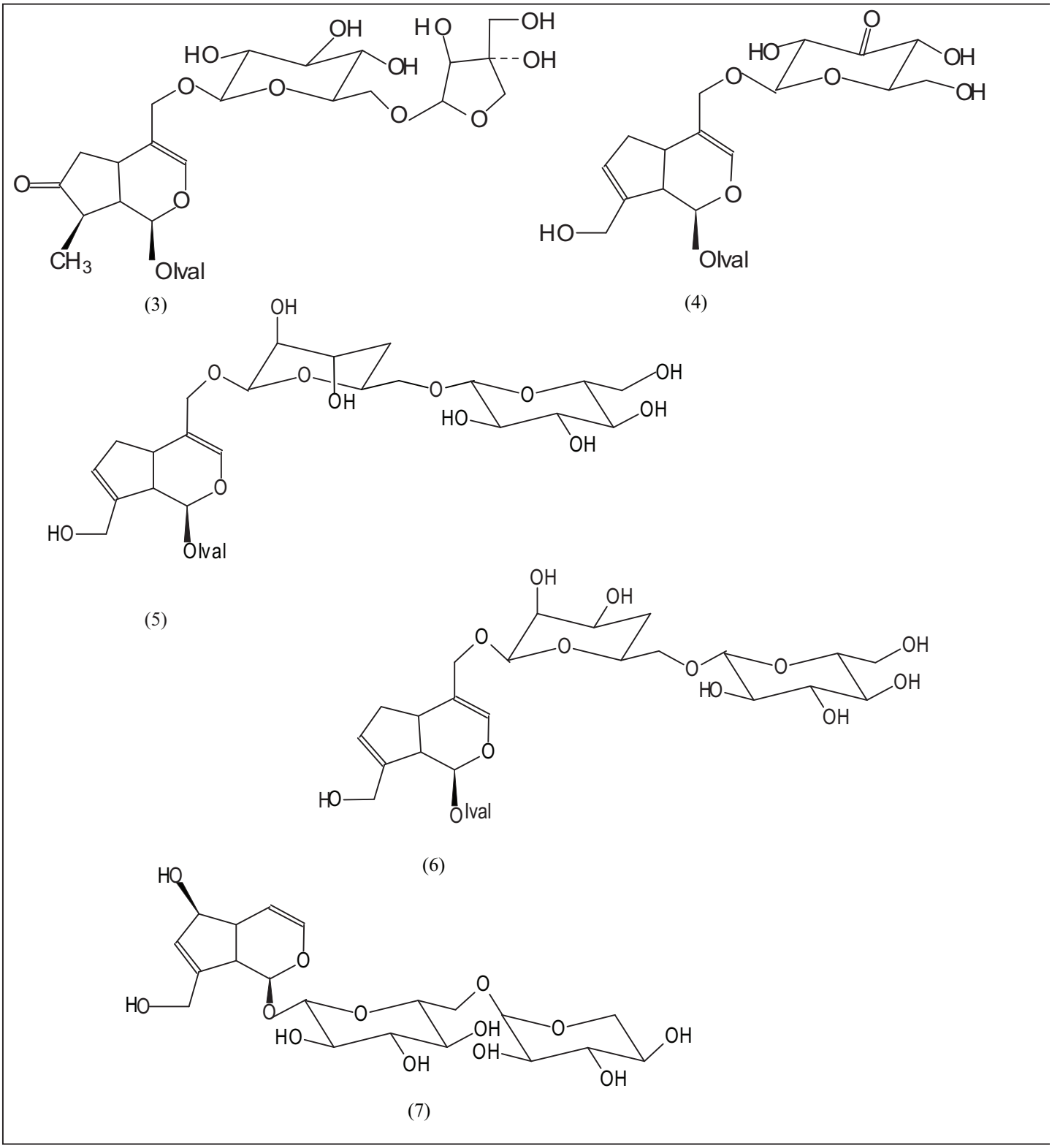


Fig.I.4. Examples of iridoids containing mono- and disaccharide units.

I.1.1- Nomenclature of iridoids.

A general system of nomenclature for the cyclopentanoid monoterpenes has not been developed and, consequently, trivial nomenclature can be employed for this class of terpenes [21].

The numbering system normally employed for the monocyclic cyclopentanoids is examplified by that shown for the dialdehyde form (1) of iridodial in Fig.I.5. Numbering of the heterocyclic systems is that shown for the hemiketal (2) of iridodial as displayed in Fig.I.5. A second numbering system, as outlined for loganin (8) in Fig.I.5 has been utilized for heterocyclic forms of the cyclopentanoid monoterpenes . However, we shall employ here the system described by structure (2) .An exception to the accepted numbering system is that used for the 14 –carbon iridoid glcosides (9). The numbering of the side chains is completed before numbering the carbomethoxy carbons of these compounds .The numbering system used for the secoiridoid glucoside compounds is examplified by that of structure (10)[22].

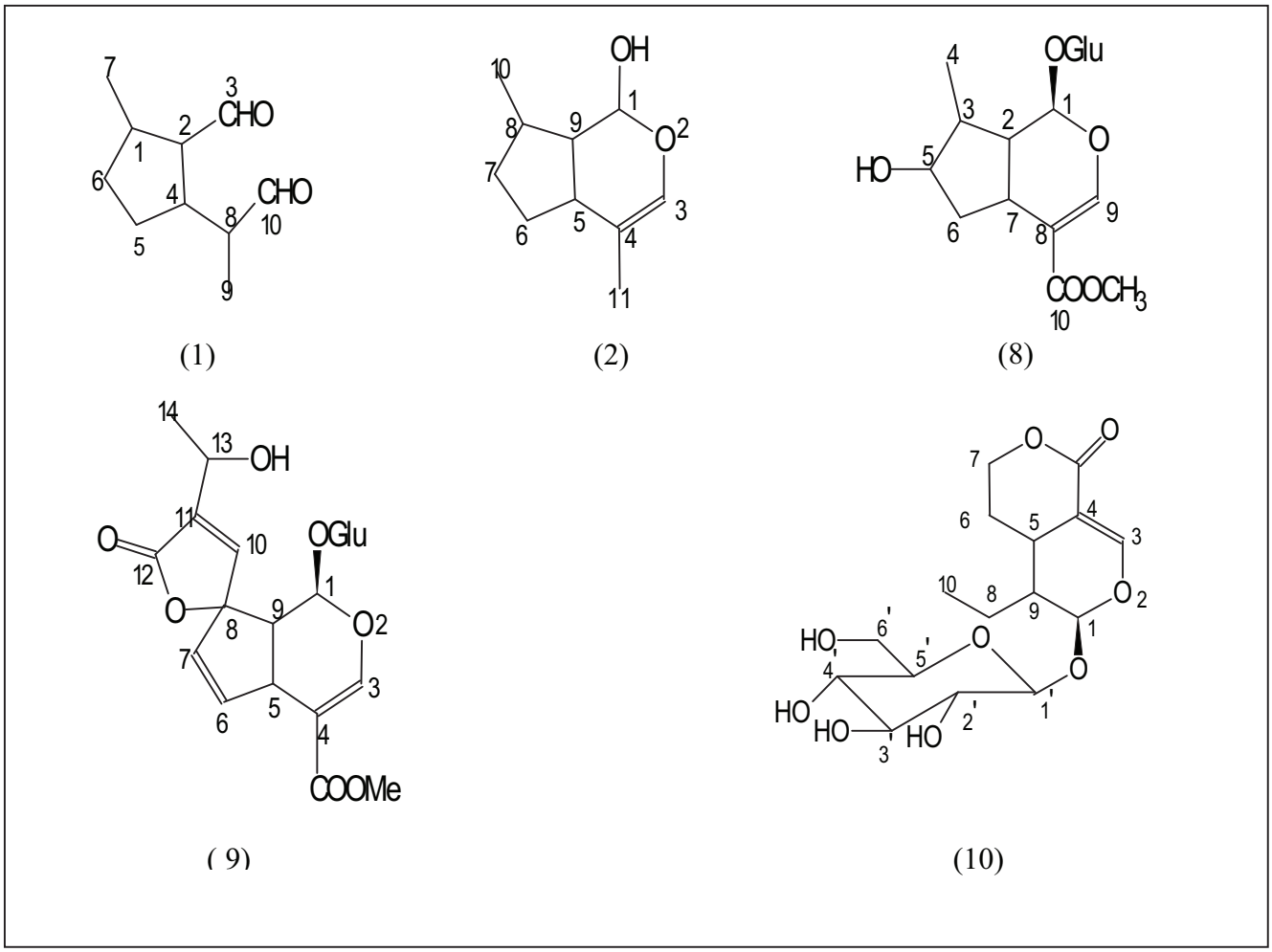


Fig .I . 5. Numbering of some iridoids

The numbering of the most common functionalities (Dihydrocaffeoyl (11) , Foliamenthoyl (12), Menthiafoloyl (13) , 2-Methyl-3-veratroyloxypropanoyl (14) , Vanilloyl (15) , Gentisoyl (16) ,Benzoyl (17) ,Coumaroyl (18) , Cinnamoyl (19), Caffeoyl (20), Isovaleroyl (21)) is given in Fig.I.6.

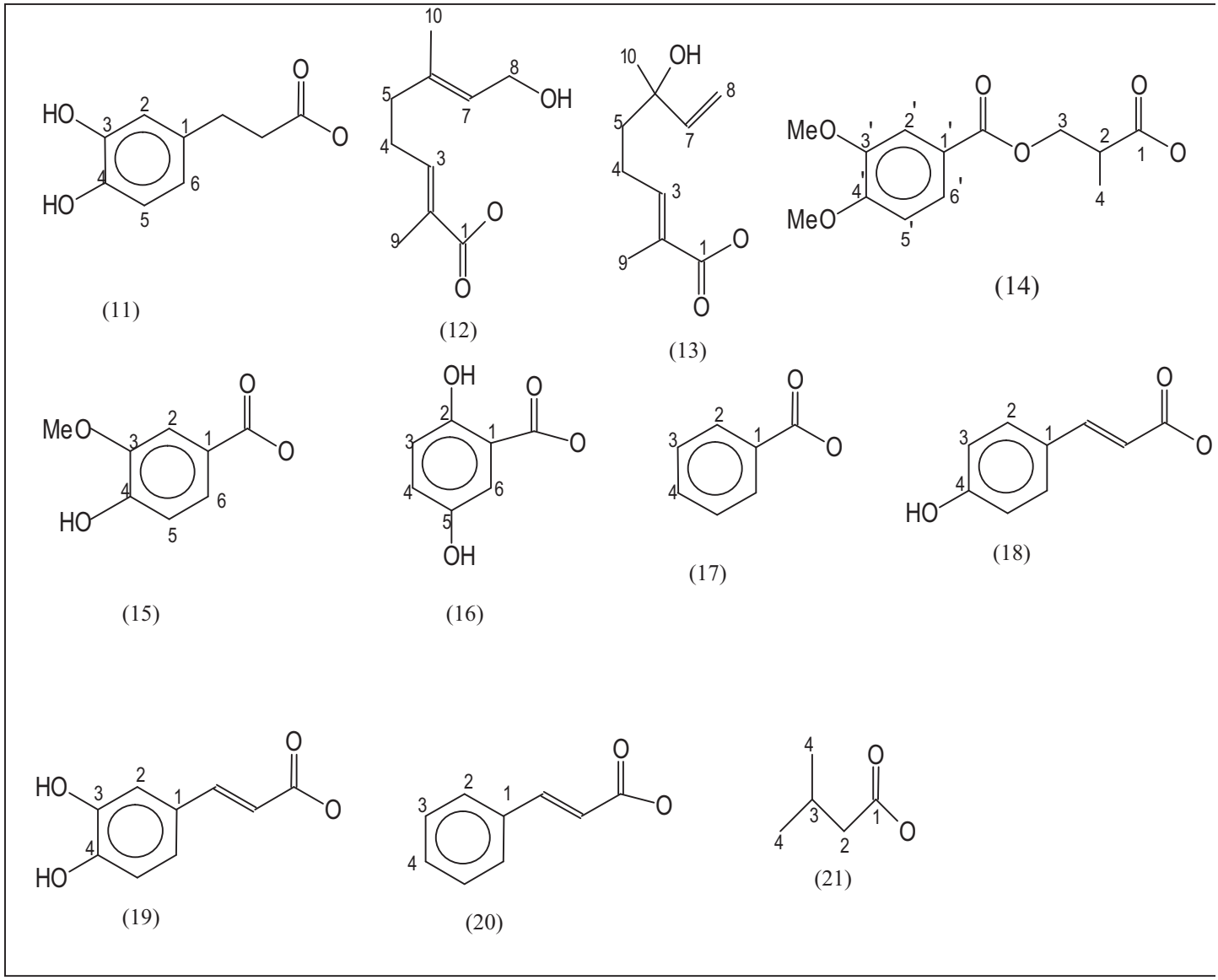


Fig.I.6.Numbering of some common substitutents in iridoids

I.1.2.Classification of iridoid compounds.

Naturally occurring iridoid compounds have been classified by several authors into different subgroups on the basis of their demonstrated or postulated biosynthesis as well as on the basis of chemical properties [23].

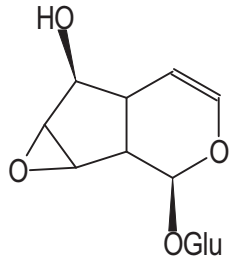
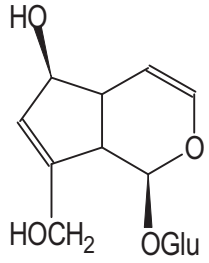
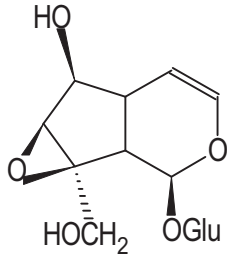
According to Sticher and Junod – Busch classification [24], natural iridoid compounds in the broadest sense are divided into five main groups :

1- Methyl cyclopentanoid (monoterpenes of the nepetalactone type) .
2- Iridoids
3- Monoterpene alkaloids
4- Secoiridoids
5- Nontryptophan portions of different indole and isoquinoline alkaloids

On the other hand, El-Naggar and Beal [25] have summarized only iridoid glucosides, secoiridoid glucosides and non glucosidic compounds , and omitting all nitrogen containing iridoids .

Iridoid glucosides are the most representative in naturally occurring substances. According to Mnasakanian [26] the carbon skeleton of these naturally substances in general consists of ten or nine and rarely eight carbons, but also compounds containing fourteen carbons are known . Iridoid glucoside division into subgroups, including examples of structures, is reported in table I.1.

Table I.1. Classification of iridoid glucosides according to their aglucone carbons number.

The structural formula	The representative of the subgroup	Subgroup type
	Unidoside	Type C-8
	Aucubin	Type C-9
	Catalpol	

TableI.1. Continued

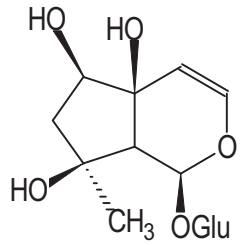
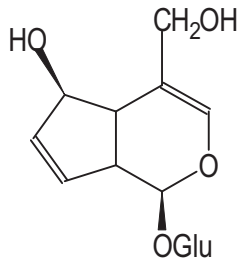
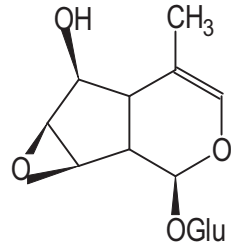
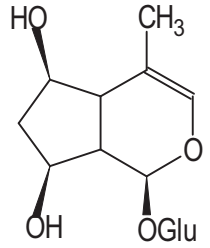
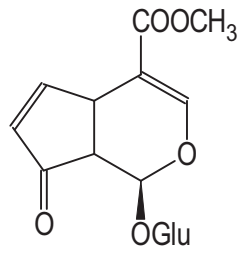
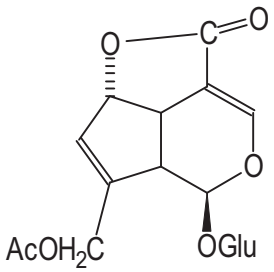
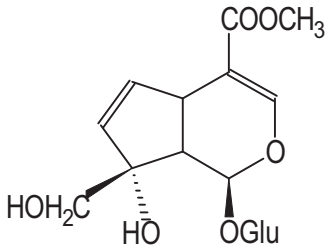
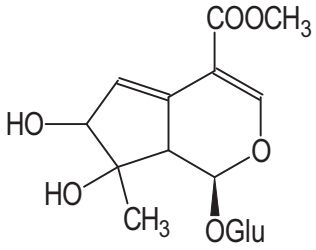
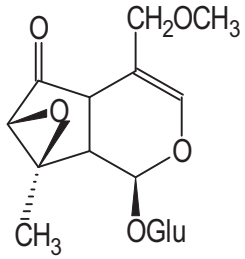
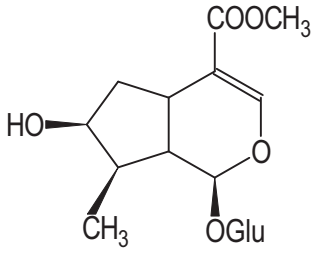
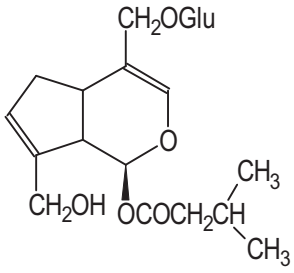
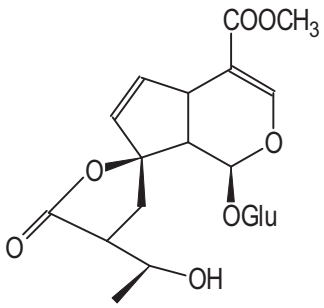
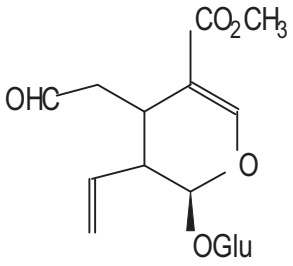
	Harpagide	Type C-9
	Decaloside	
	Deocioside	
	Deociol	
	Randioside	
	Asperioloside	Type C-10

Table I.1.Continued

	Monotropein	Type C-10
	Gentioside	
	Seringoxide	
	Loganin	
	Penstamide	
	Plumieride	Type C-14

Secoiridoid glucosides are derived from iridoid glucosides by oxidative cleavage of the cyclopentane ring and they can be further divided into the following four subgroups [25].

1. The simple secoirdoid glucosides: Secologanin (22), Sweroside (23).



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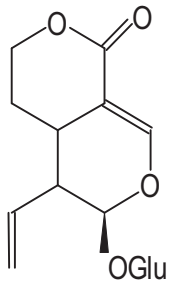
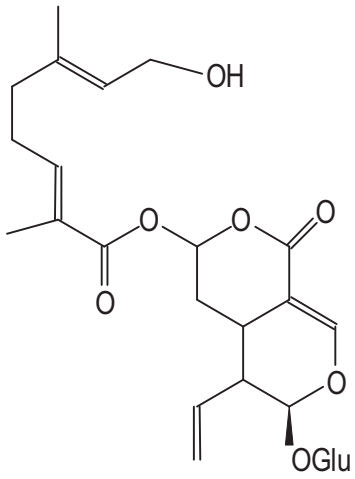


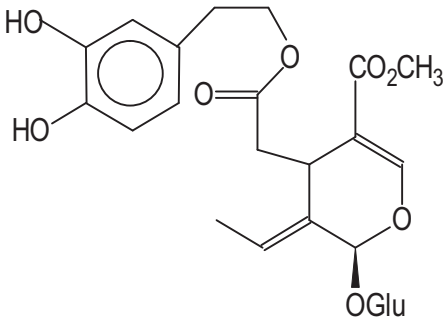
(23)

2. Secoiridoid glucosides conjugated with a terpene type moiety : Foliamenthin (24).



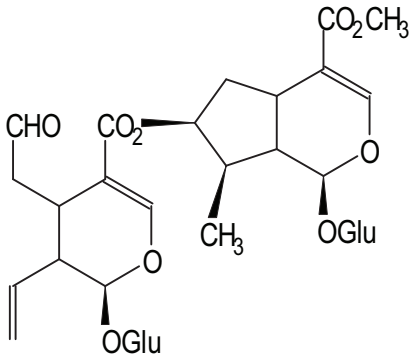
(24)

3. Secoiridoid glucosides carrying a phenolic moiety : Oleuropein (25).



(25)

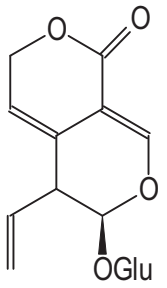
4. Bisiridoids : Sylvestroside III (26)



(26)

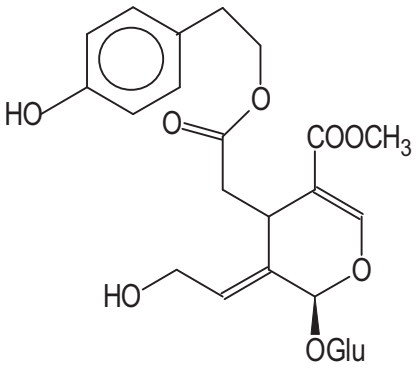
Inouye and Uesato [12] have surveyed the biosynthesis of iridoids and secoiridoids and presented another classification from the biogenetic point of view and hence dividing the compounds conventionally into non-glucosidic iridoids, iridoid glucosides and secoiridoid glucosides . The first tow groups are not further subdivided, whereas the third group is subdivided on the basis of biosynthetic pathways and structural similarities into the following four subgroups. For each subgroup, representative compounds are given .

1-The sweroside (23)-gentiopicroside (27) type bearing a vinyl group at C9



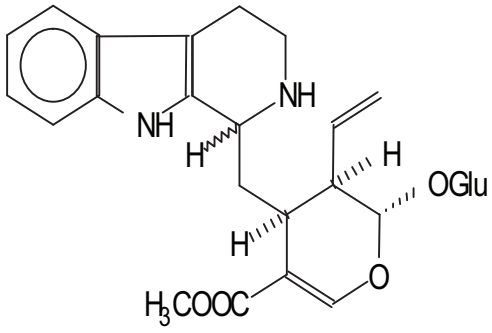
(27)

2-The oleoside – 10 –hydroxyoleoside type : oleuropein (25), 10-hydroxy-ligusroside (28).



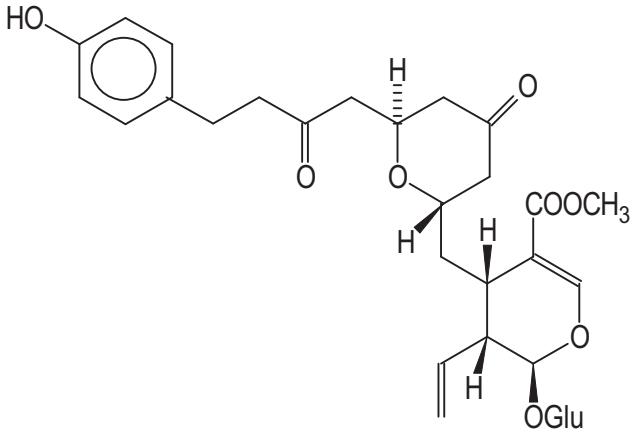
(28)

3-Alkaloidal glucosides containing a secoiridoid skeleton : strictosidine (29).



(29)

4- Hydrangenosides : hydrangenoside A (30) .

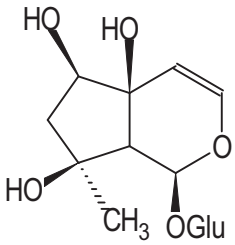


(30)

In contrast to previous classification, we arrive now to another type of classification, in which we can see that iridoids differing significantly in structure can be considered related.

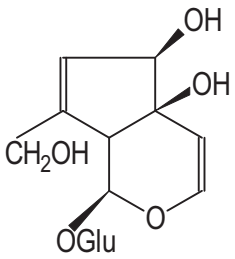
All known iridoids possess identical configuration at C1, C5 and C9 . Nevertheless, the $J_{1,9}$ values of the n.m.r. spectra of the iridoids vary between 0 and 10 Hz. The known iridoids can be divided into five groups according to their $J_{1,9}$ values [27,28] .

First group : with $J_{1,9}$ = 0-1Hz includes harpagide (31) and related compounds characterized with an α - methyl group at C8 and absence of an electron-acceptor substituent at C4. Iridoids containing such a substituent may belong to the same group, provided they have three hydroxy groups at C5 ,C8 and C6 or C7 in the cyclopentane ring or an acetyl group at C8 .



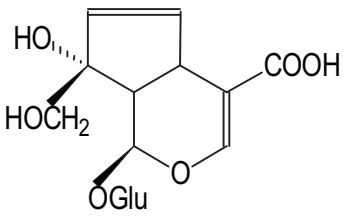
(31)

Second group : with $J_{1,9}$ = 1-2 Hz includes compounds of the above given type containing an electron-acceptor substituent at C4 and not more than two hydroxy groups in the cyclopentane ring . monomelittoside (32) as iridoids with a β-methyl and α –hydroxy group at C8 are related to the same group .

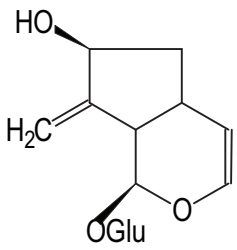


(32)

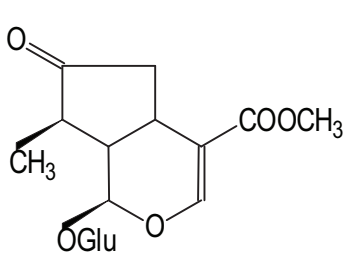
Third group : includes iridoids of monotropein type (33) , antirride (34) and ketologanin (35) have $J_{1,9}$ =3 Hz .



(33)

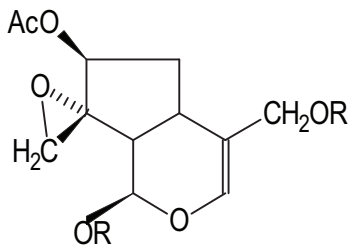


(34)

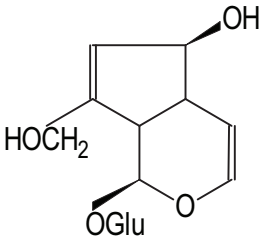


(35)

Fourth group : A great number of iridoids have their $J_{1,9}$ values in the range of 4 –7Hz. They can be divided into several subgroups .One of them includes iridoids analogous to those of the first group, differing only in the β-methyl group at C8 , with or without ketogroup at C6 . Iridoids related to didrovaltrate (36) in which a β –methyl group participates in an epoxy ring are analogous to those given above. Here should also be mentioned the Δ^7 -iridoids, such as aucubin (37) as well as iridoids with a β– C7-C8 epoxy ring and a methyl group at C8 ,e. g. antirrinoside (38)



(36)



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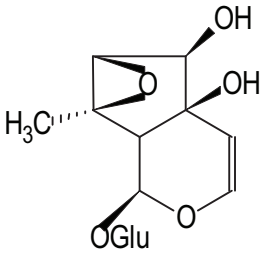
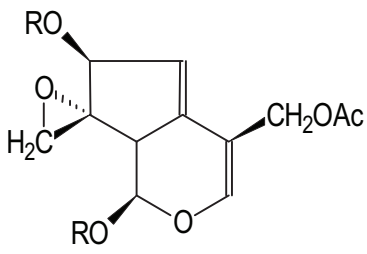


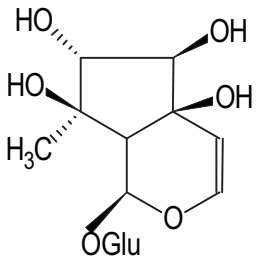
(38)

R=$COCH_2CH(CH_3)_2$

Fifth group : iridoids with a β-C7-C8 epoxy ring which have a proton or a hydroxymethyl group at C8 , valtrate (39) and procumbide (40) belong to the last group ($J_{1,9}$ = 8 –10 Hz) .All iridoids with a 6-α –OH group, except for those compounds in the first group belong to this group .



(39)



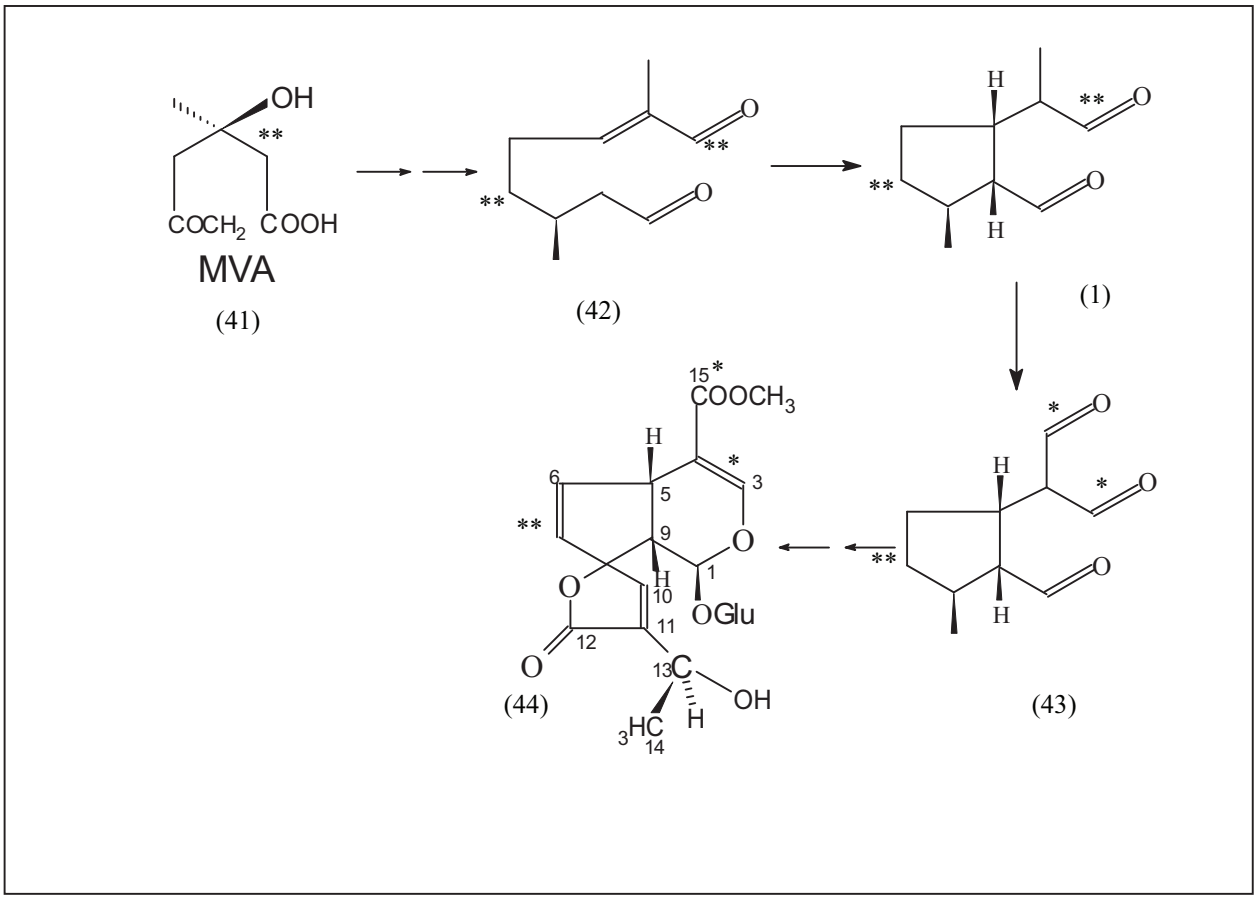
(40)

R = $COCH_2CH(CH_3)_2$

I.1.3. Biosynthesis of iridoid compounds.

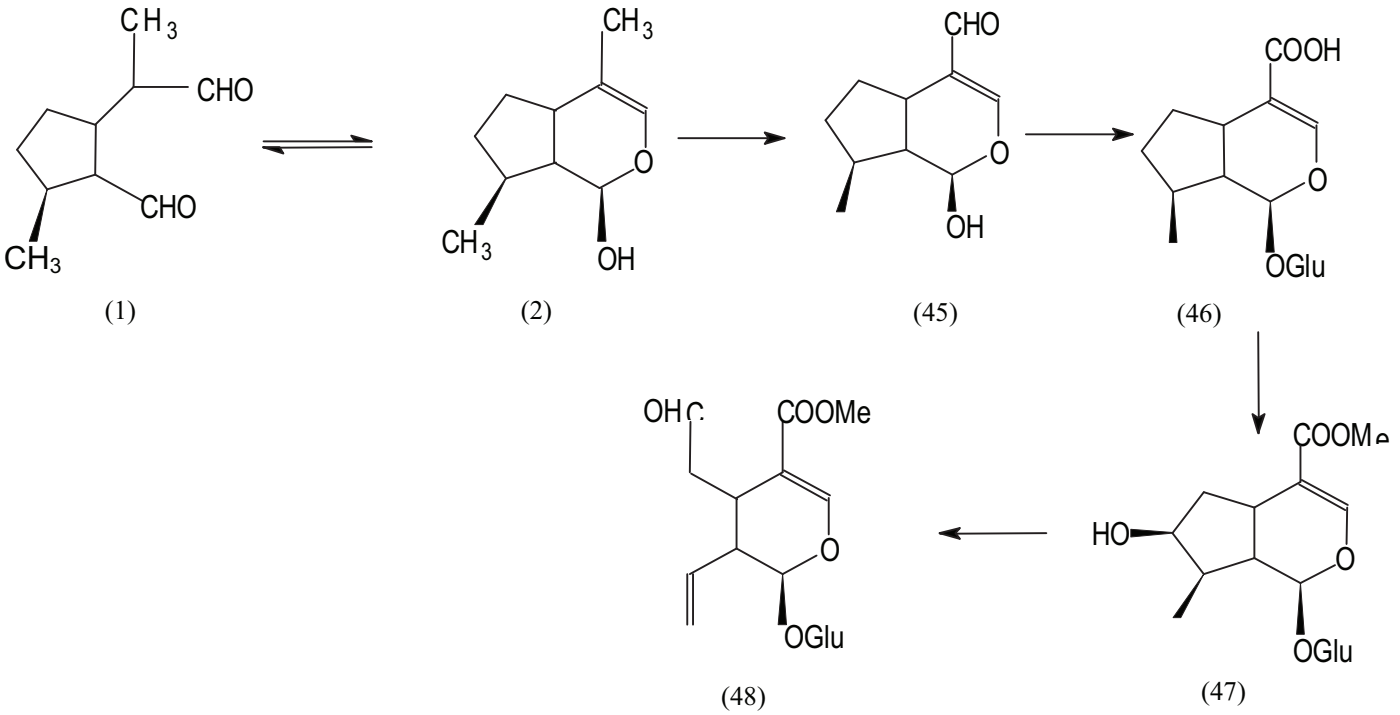
Through administration experiments using mevalonic acid (MVA) (41) labeled with ^{14}C, it was found that the ^{14}C label of [2- ^{14}C] MVA was incorporated into C7 , C3 and C15 of plumieride (44). Based on this finding , they inferred , as shown in scheme I.1 , that plumieride (44) is biosynthesized through a Michael – type cyclization of 10-oxocitronellal (42) to iridodial (1) , further oxidation to a substance like iridotrial (43) ,at which stage randomization takes place between C3 and C 15 [12,29,30].

Scheme I.1:



Recently , two main routes are known [31]. The first route (scheme I.2) is leading from iridodial via iridotrial (45) to deoxyloganic acid (46) which is the known precursor of many carbocyclic iridoids having the 8β- stereochemistry including loganin (47) and loganic acid, secologanin (42) and secologanic acid as well as the derived secoiridoids .Compounds from this route are found mainly in Cornales , Gentianales and Oleaceae .

Scheme I.2:



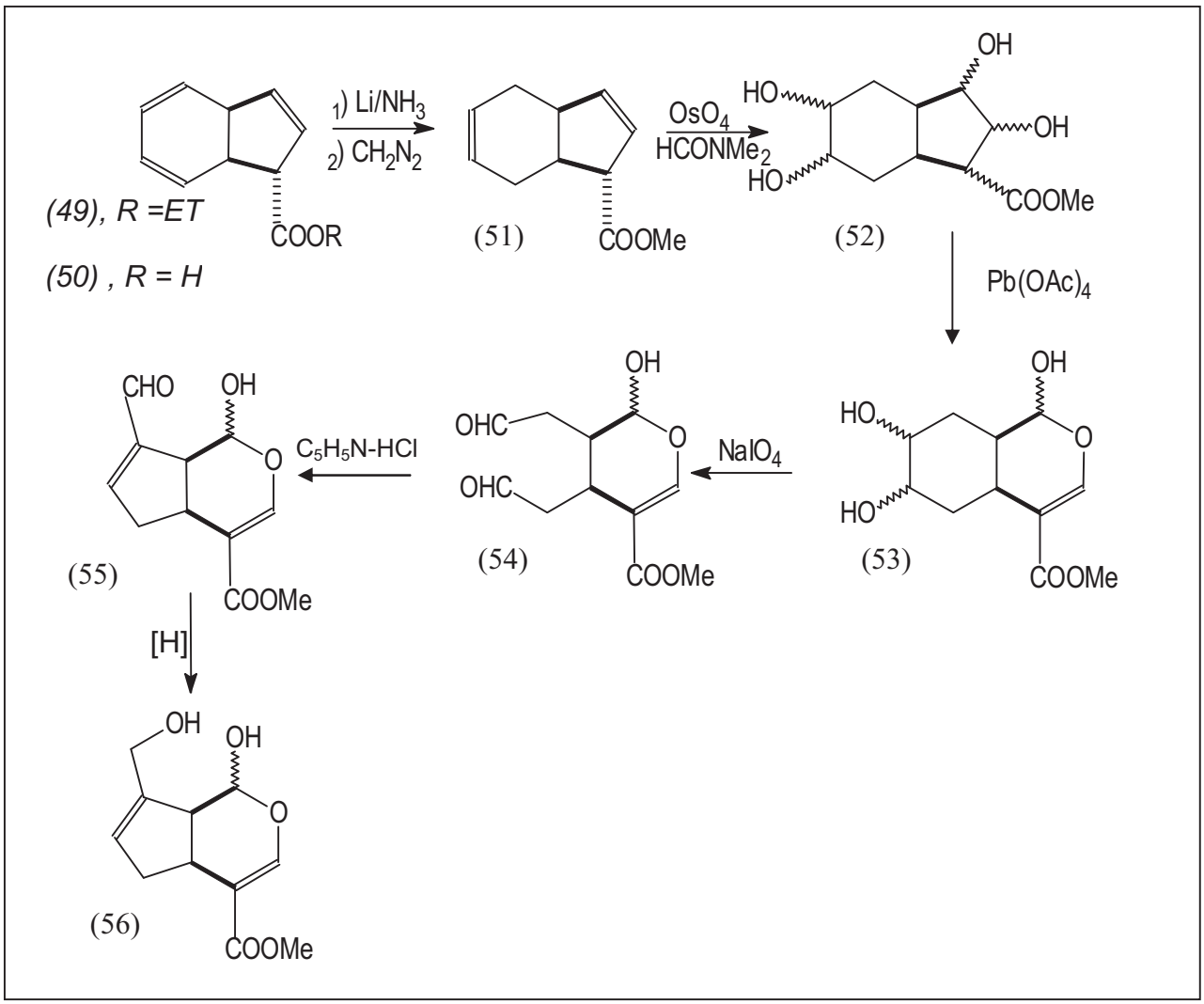
The second biosynthetic pathway (route II) involves 8- epi- iridodial ,8-epi- iridotrial and 8-epi-deoxyloganic acid . These are precursors for the decarboxylated carbocyclic iridoids, such as aucubin and catalpol . These compounds are never found in Oleaceae . A few unusual secoiridoids [32,33] are known to be formed by this route but they are different in structure from those derived from route I.

I.1.4. Synthesis of iridoids:

The total synthesis has been extremely important to confirm the proposed structures of natural products, to examine the feasibility of proposed biosynthetic pathways, and to provide compounds for study by the pharmaceutical industry[34].

The first contribution in the field of iridoids synthesis was by Buchi [35]. In this synthesis, in racemic form, of the iridoid genipin (scheme I.3) the ester (49) , prepared by thermal decomposition of the ethyldiazoacetate adduct of cyclooctatetraene, is hydrolyzed and the resulting acid (50) is reduced with lithium in ammonia and methylated with diazomethane to produce the ester (51) . Treatment of this ester with osmium tetroxide gives two isomeric tetraols (52) which are selectively cleaved with one mole of lead tetraacetate to produce the lactol (53). Three of the four possible cis-diols of (53) were isolated from the lead tetraaceate reaction and two of these were cleaved with sodium periodate to the dialdehyde (54) which undergoes cyclization to the aldehyde (55) (isolated in 68% yield) . Lithium tri-t-butoxy aluminium hydride reduction of (55) gives (+)- genipin (56).

Scheme I.3:



I.1.5. Conversion of iridoids .

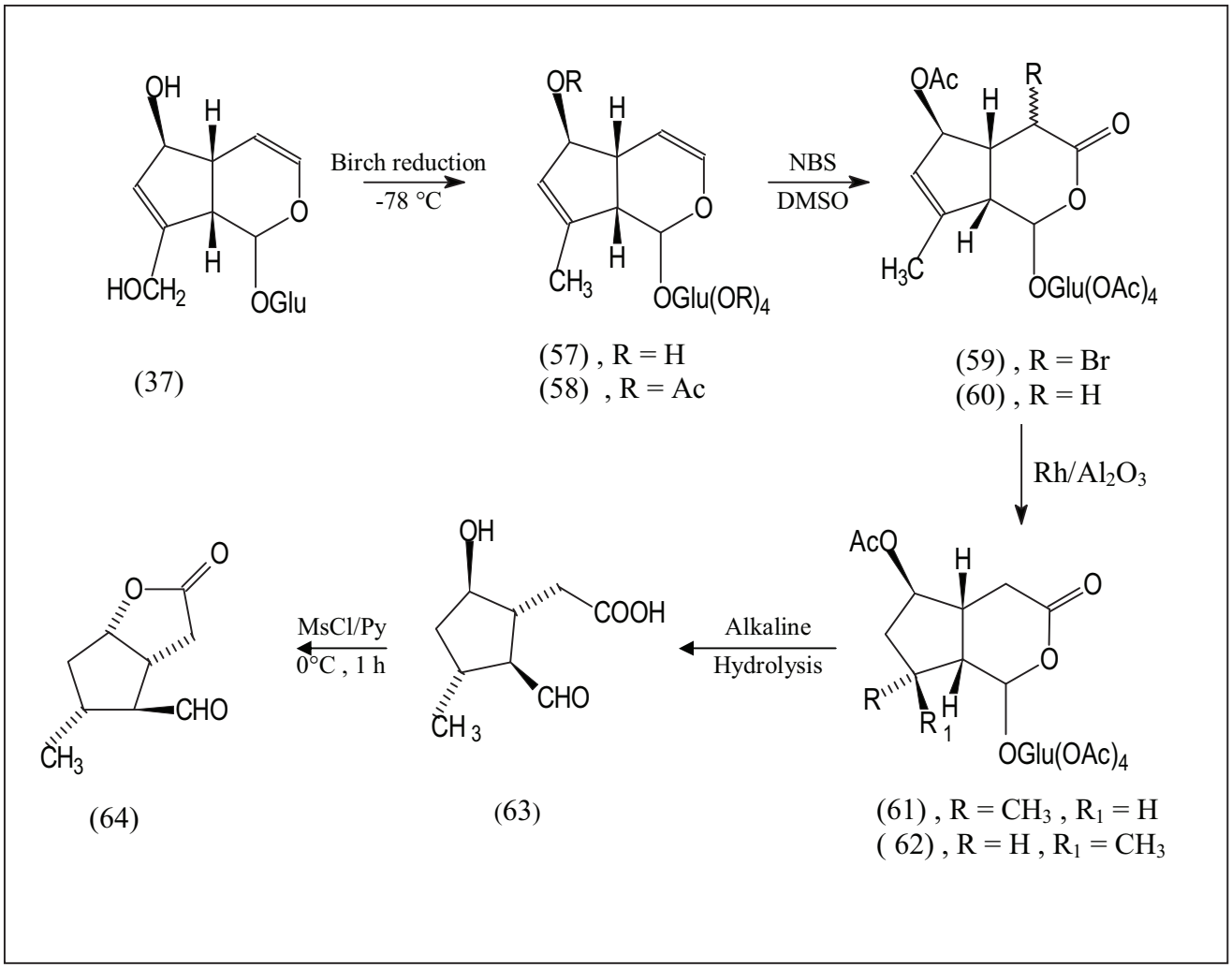
In the field of prostaglandins synthesis one of the main goals was to obtain pure products without expensive resolution methods. To this end, many fascinating approaches started with optically pure natural products . In particular ,the iridoid glucosides ,which undoubtely possess a potentially useful structure for the elaboration towards natural or modified prostaglandins were chosen as starting materials [35,36].

Bonadies and co-workers [37,38] have converted aucubin (37) to new Corey lactone analogue (64) as outlined in scheme I.4. Performing the Birch reduction of (37) at low temperature (-78°C) for 10 min ,they were able to obtain the monodeoxyaucubin (57) in good yield (80%) .

The oxidation of the enol-double bond of the acetyl derivative (58) was achieved by treatment with NBS in anhydrous DMSO, giving the lactone (59) .The reduction of (59) (Zn/CH_3COOH) afforded lactone (60) in excellent yield (95%) .

The hydrogenation of C7–C8 double bond in (60) (Rh/Al_2O_3 at one atmospheric pressure) afforded a mixture of (61) and (62) in a 2:1 ratio (95%) ,which was separated by chromatography .

Scheme I.4:



(37)

(57) , R = H
(58) , R = Ac

(59) , R = Br
(60) , R = H

(61) , R = CH_3 , R_1 = H
(62) , R = H , R_1 = CH_3

(63)

(64)

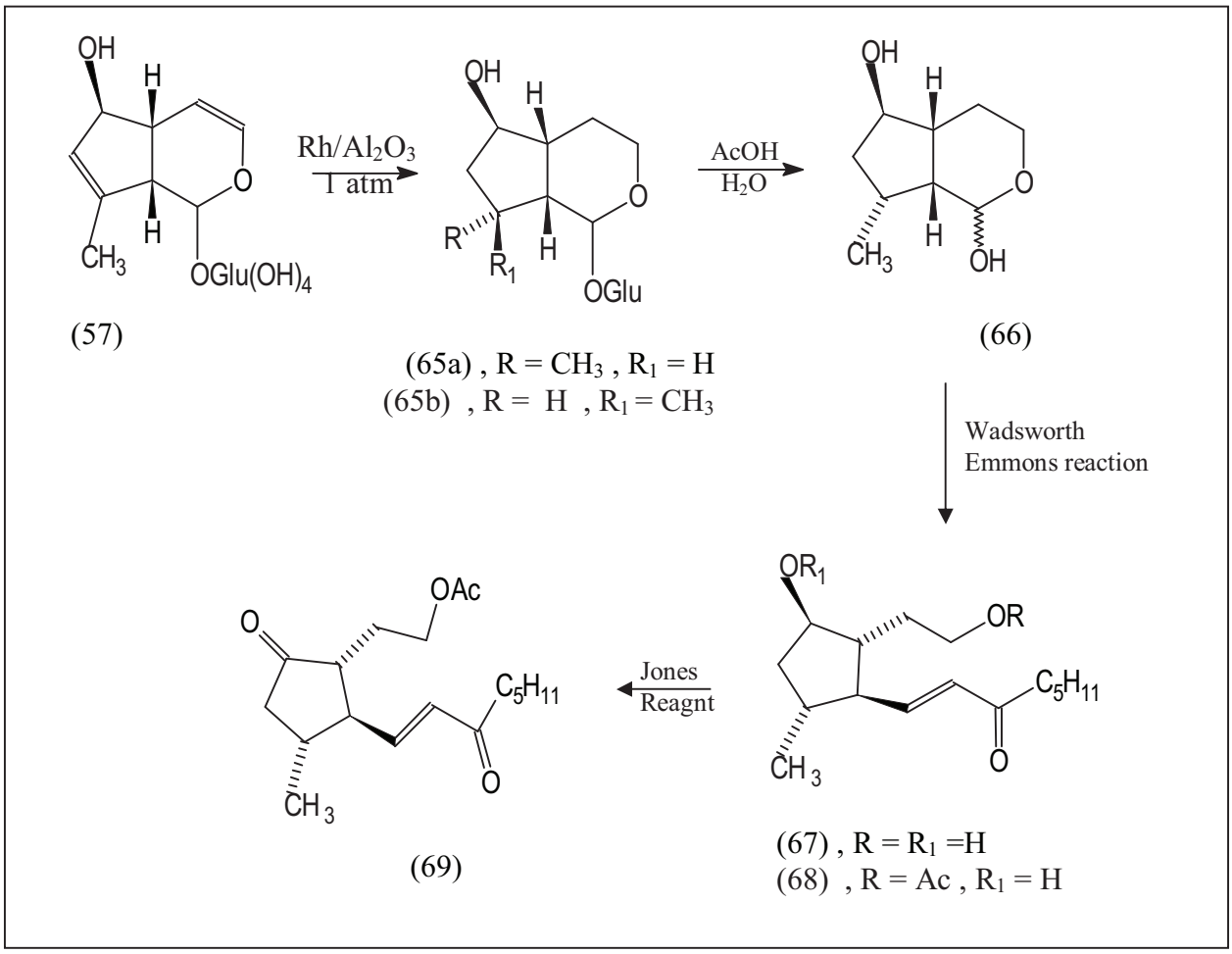
Subsequently, the lactone (61) was transformed into the hydroxy acid (63) .The alkaline hydrolysis of (61) caused loss of the glucose acetate groups and formation of the aldehyde in the more stable trans configuration of the cyclopentane ring.

Treatment of (63) with MsCl/Py at 0°C for 1h afforded the desired Corey lactone analogue (64), by intramolecular nucleophilic attack of the carboxylate anion to C9 with inversion of the configuration . (64) can be easily converted into 11-methyl – PG by established methods .

The approach outlined in scheme I.5, still used the monodeoxyaucubin (57) as starting material .The hydrogenation should lead to the tetrahydromonodeoxyaucubin (65). The hydrogenation was not a trivial matter, the best results were achieved with Rh/Al_2O_3, at one atmospheric pressure and room temperature, yielding approximately 60% and 40% of (65a) and (65b) . These products are separated by HPLC.

The subsequent hydrolysis of (65a) to the aglucone (66) was carried out both in acidic medium ($AcOH/H_2O$), in excellent yields (80-90%) . The Wadsworth-Emmons reaction of (66), with sodium dimethyl-2- oxoheptylphosphonate gave the desired enone (67) in 60% yield.

Scheme I.5:



Finally, the two hydroxyl functions were differentiated by selective acetylation (CH_3COOEt on Al_2O_3) of the primary one to the monoacetyl enone (68); subsequently, oxidation (Jones reagent) gave (69) which can be easily transformed into 11-deoxy-11α-methyl-PG series , by standard procedures.

I.1.6. Distribution of iridoids:

The iridoids show a restricted distribution to about 50 – 70 families [19], some of which are shown in Table I.2.

TableI.2. Iridoids distribution in plant families[39,40,41] .

Superorder	Order	Families
Rosiflorae	Hamamelidales	Hamamelidaceae
	Ruxales	Daphniphyllaceae
Loasiflorae	Loasales	Loasaceae
Corniflorae	Ericales	Ericaceae, Monotropaceae, Pyrolaceae
	Sarraceniales	Sarraceniaceae
	Eucommiales	Eucommiaceae
	Fouquieriales	Fouquieriaceae
	Cornales	Sambucaceae,Garryaceae, Alangiaceae,Aucubaceae,Cornaceae, Davidiaceae, Icacinaceae, Escalloniaceae, Stylidiaceae ,Hydrangeaceae, Adoxaceae, Symplocaceae, Montiniaceae
	Dipsacales	Calyceraceae,Caprifoliaceae, Viburnaceae,Valerianaceae,Dipsacaceae
Gentianiflorae	Goodeniales	Goodeniaceae
	Oleales	Oleaceae
	Gentianales	Gentianaceae, Loganiaceae, Rubiaceae, Menyanthaceae, Apocynaceae
Lamiiflorae	Lamiales	Lamiaceae ,Verbenaceae, Callitrichaceae.
	Hippuridales	Hippuridaceae
	Scrophulariales	Scrophulariaceae, Bignoniaceae,Myoporaceae, Buddlejaceae, Globulariaceae, Retziaceae, Plantaginaceae, Lentibulariaceae, Pedaliaceae, Martyniaceae, Acanthaceae

N.B :Families for which the name is underlined are reported to contain secoiridoids

I.1.7.Biological activities.

As the fact that Iridolacones (iridomyrmecin and isoiridomyrmecin) , the first structurally elucidated iridoids , are defensive substances ejected by iridomyrmex ants suggests that there are several ecologically interesting iridoids . Dolichodial, a secretion of Iridomyrmex and Dolichoderus species, and anisomorphal, that of Anisomorpha species, are repellents of these insects [40,42] . Iridolactones also occur in *Actinida polygama* and extracts are known as a powerful feline attractant [43].

On the other hand, iridoid glucosides often serve as feeding attractants and stimulants for larvae of some Lepidopterrae. The larvae of Ceratomia catalpae (Sphingidae) ,the most destructive insect of Catalpa trees [44] .

The two secoiridoid glucosides, oleuropein and its demethylated product demethyloleuropein, are known to stimulate oviposition of Dacus oleae Gmel., an insect which infests Mediterranean olive corps [45] .

In 1969, a compound was isolated by Fleming and co-workers from green olives that appeared to have antimicrobial properties noted earlier during the fermentation of brined olives. The compound, a bitter phenolic material was considered to be an enzymatic degradation product of oleuropein. Latter, they found that oleuropein was not inhibitory, but its hydrolysis product, the aglucone which possess the antimicrobial properties [46]. Also, according to Petkov and Manolov [46,47] ,oleuropein is the hypotensive principle of the leaves of the olive tree . In different pharmacological investigations including acute toxicity, effect on blood pressure, coronary action, antiarrhythmic action, effect on the intestinal smooth muscles, it was possible to demonstrate that oleuropein has a hypotensive action , as well as coronary dilating, antiarrhythmic and spasmolytic actions .

The seoiridoid glucosides , amarogentin , amaroswerin and amaropanin , isolated from gentianaceous plants, should be mentioned .They rank among the most bitter known compounds and crude drugs containing them are used as bitter tonics [46] .

Recently, the investigations carried out by kostova and co-workers [48] proved that the secoiridoid glucosides , ligstroside , insularoside , hydroxyornoside , oleuropein , framoside and 10 - hydroxy - ligstroside possess the antiinflammatory action .

I.1.8.Isolation and purification of iridoid compounds.

I.1.8.1. Detection.

A method of screening will be proposed in order to rapidly verify a possible presence of iridoids.Vanillin method: some leaves are extracted for one day with 95% aqueous ethanol at room temperature .A spot of the extract is overimposed on paper chromatography, run in n-BuOH/AcOH/H2O, 63:10:27 and sprayed with vanillin reagent, consisting of 4g of vanillin, 100ml of methanol and 4ml of concentrated HCl .Furthermore, the paper is heated at 100 - 110˚C for a few minutes . The colored spots, usually pink , pink-red ,brown ,blue or violet ,is the first indication on the presence of iridoids [49] .

I.1.8.2. Isolation of iridoids.

For the extraction of the usual iridoid compounds, a polar solvent such as methanol, ethanol, aqueous methanol or aqueous ethanol are ordinarily used at room temperature, and aqueous acetone is also applied in rare cases [50,51]. Whereas , Kostova and co-workers [52] have employed hot ethanol for the extraction of secoiridoid compounds from the bark of Fraxinus ornus species [50]. Then, in order to fractionate the concentrated extract it is suspended

in water and shaken with organic solvent successively in the order of increasing polarity e.g. diethyl ether,chloroform, ethyl acetate, n-butanol and so on. In this process coupled glucosides with lipophilic groups dissolve in diethyl ether, whereas most of glucosides are extracted by ethyl acetate or n- butanol and some glucosides of higher polarity remain in the aqueous layer [52,53]. For solvent – solvent partition, Iossifova and co-workers [54] have used PE, EtOAc and MeOH, while Tanahashi and co-workers[54] employed $CHCl_3$ and n-BuOH, successively. Nevertheless, subsequent chromatographic steps are necessary to obtain pure compounds. Various methods for the isolation of iridoid glucosides have been reviewed previously [24].

I.1.8.2.1. Column chromatography (CC).

Conventional chromatographic procedures have been used for the separation of iridoid mixtures. Certainly, column chromatography using silica gel as stationary phase is the most employed technique [56,57] . In this case a mixture of $CHCl_3$/MeOH is usually used with increasing MeOH content [58]. Chromatography on polyamide column is carried out by using H_2O /MeOH (or ethanol) and increasing the alcohol content in the eluant [59].

I.1.8.2.2. Vacuum liquid chromatography (VLC) .

Vacuum liquid chromatography seemed to be a suitable technique for the separation of iridoids [49]. Handjieva and co-workers [60] have used (VLC)on silica gel with $CHCl_3$ / MeOH mixtures for the separation of iridoids. Iossifova [53] has also used vacuum liquid chromatography (VLC) over silica gel, using dichloroethane (DCE) and DCE/MeOH with increasing polarity, for the separation of secoiridoids .

I.1.8.2.3. Thin layer chromatography (TLC) .

Analytical TLC (with silica gel absorbent) gives an important guide for the selection of the appropriate chromatographic methods and can also be used to determine the appropriate solvent system For PTLC .The following solvent systems can be used as eluants in varying proportions: $CHCl_3$/MeOH, EtOAc/Benzene /MeOH ,CH_2Cl_2/MeOH /H_2O ,n-BuOH /MeOH /H_2O, EtOAc /H_2O,etc. To detect iridoids on TLC, UV irradiation should first be tried .By this method spots of iridoids having a characteristic enol-carbonyl structure which can be located and detection by spraying with vanillin sulfuric acid reagent or by $FeCl_3$ in EtOH, followed by heating (at 120 °C for 5-10 min) is the most frequently used method [49, 24].

Preparative thin layer chromatography (PTLC) on silica gel absorbent is the most frequently used method for the purification of iridoid compounds [61].

I.1.8.2.4. High performance liquid chromatography (HPLC) .

Besides the classical methods mentioned before,semi-preparative and preparative liquid chromatography on reversed phase offers efficient separations in a short time, with very small amounts of solvents and a high resolution, though a preliminary purification is necessary in order to avoid column contamination. HPLC is the most effective for careful separation of iridoid compounds. Reversed phase columns such as µ Bondapak C_{18} are usually used in combination with eluants such as MeOH /H_2O in appropriate proportions [62,63].

I.1.8.2.5. Gas chromatography (GC) .

Gas chromatography is used nowadays only for identification in iridoid chemistry, since it requires tedious derivatisation of glucosides as trimethylsilyl . For this derivatives , columns such as OV-1,OV-17 ,OV-210 and OV-225 ,of several columns lengths ,are used at temperatures ranging from 215 to 270 °C, depending on the properties of the samples [62]..

For the purification of volatile non glucosidic iridoids such as the isomers of nepetalactone and iridomyrmycin, preparative GC is still useful, as is preparative HPLC [64,65]. Furthermore, in order to investigate a great number of plant species Frank and Rimpler [66]. have used GC/MS for the analysis of permethylated iridoids .

I.1.9.Structure elucidation of iridoid compounds.

The exceptional variability of iridoid glucosides in contrast to secoiridoid glucosides is caused mainly by the intact cyclopenta (c) pyran skeleton in cis-junction, and also by substitution with functional groups at the carbon atoms of the cyclopentane ring. Moreover, configurational changes and esterification of hydroxy group by various aliphatic and aromatic acids as well as the occasional occurrence of sugar moieties other than glucose contribute to the complexity of iridoid glucosides. However, in secoiridoid glucosides only glucose has been found as sugar moiety up to now. The variability of the 7,8-seco-skeleton only increases if ,e.g., lactone ring formation to compounds has taken place [16].

Up to 1978, structural information on naturally occurring iridoids was still obtained mainly by means of UV , IR and ^{1}H –NMR spectroscopy, as well as mass and ^{13}C-NMR spectroscopy to a lesser extent and by chemical methods, especially acetylation , methylation and chemical degradation as hydrogenation and enzymatic cleavage of the glucose [67,68].

I.1.9.1. Spectroscopic methods .

I.1.9.1.1. Ultra-violet (UV) and Infra-red (IR).

UV and IR spectroscopy have been little used for structural studies of iridoids. The most useful information to be gained from the UV and IR spectra of iridoids is concerned with unsaturation , hydroxyl and ester groups .

I.1.9.1.1.1– Ultra-violet spectra .

The only UV chromophore present in the basic skeleton of the iridoids, the double bond between C3-C4 is of diagnostic significance only if further conjugated with a C11 oxygenated function, in this case absorption maxima at about 230nm must be expected [69].

The absorption at 190-210 nm for compounds lacking C11 are somewhat suspicious, as they are probably due to solvent interference [49].

The data of UV spectrum of oleuropein (25) [70]: λ_{max}(MeOH) =233.5 , 284.0 nm. The first absorption is typical of a secoiridoids nucleus (an enol–ether system conjugated with a carbonyl group),while the second absorption due to a phenolic function.

I.1.9.1.1.2- Infra-red spectra.

Relatively few IR peaks are of diagnostic value in the spectra of iridoids. The double bond between C3-C4 is indicated by the presence of a band between 1620- 1660cm-1, which is very characteristic to these compounds [49].

The data of IR spectrum of oleuropein (25)[71],ν_{Max}(KBr) cm-1: 3550-3200 (OH) , 1700 (C=O) ,1620 (C=C), 1510 (aromatic ring).

I.1.9.1.2. Nuclear Magnetic Resonance (NMR).

Owing to the great importance of a correct use of NMR spectroscopy in the structure determination of iridoids most publications on these compounds include NMR data, mainly, ^{1}H and ^{13}C-NMR spectroscopy .

D_2O and CD_3OD are the most used solvents and it is recommended to employ the same solvent in a close comparison of similar structures to avoid possible erroneous interpretations due to a solvent shift of the peaks.

I.1.9.1.2.1- ^{1}H-NMR .

The acetalic proton H1 is one of the most typical signals in the iridoid spectrum. It is usually found between δ5.1-6.0ppm with a characteristic shape of a sharp doublet due to coupling with H9. Whereas, in the case of oleoside type secoiridoids it looks as a broad singlet [5,21] .

The value of the coupling constant J changes widely in a range of 1.5-8.5 Hz, as a consequence of the great flexibility of the dihydropyrane ring. H1 can be easily distinguished from the anomeric proton H1′ of the glucose on the basis of the chemical shift . It is usually found between δ4.79- 4.87 as a doublet and the value of the coupling constant (7 and 3.5 Hz for β and α glucosidic linkage , respectively [21].

The analysis of the H3 signal can give full information for dihydropyrane ring substitution .three cases may be distinguished [72] :

1) no substitution on C4 and C5: H3 appears as a doublet of doublet with $J_{3,4}$(6.0-7.0 Hz) and $J_{3,5}$ allylic coupling (0.5-1.5 Hz).
2) one substituent at C4 or C5: H3 is a sharp doublet with $J_{3,4}$ or $J_{3,5}$ coupling .
3) presence of substituents at both C4 and C5: H3 is a sharp singlet .

In the last two cases , very often there is unsaturated function present at C4 , e.g. aldehydic , carboxylic , carbomethoxy, which causes a low field shift of the H3 signal up to δ7.5, instead of the ordinary one between δ5.9 –6.4 [21,54].

The ^{1}H –NMR data of oleuropein (oleoside type secoiridoid glucosides), will be illustrated in Fig.I.7.

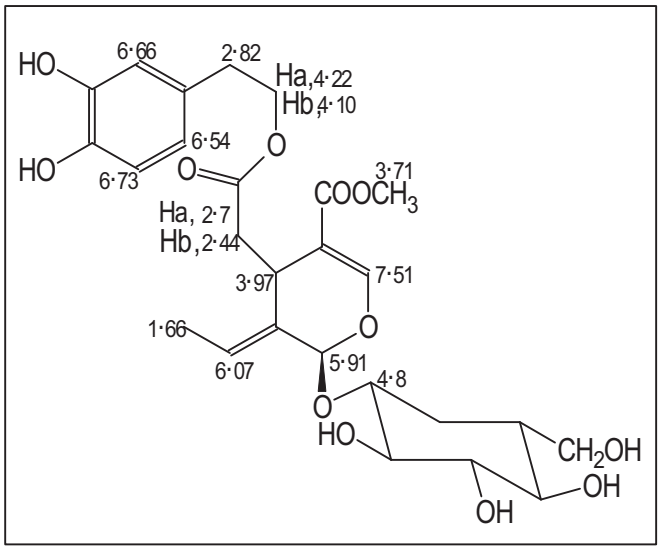


Fig.I.7.^{1}H-NMR (270 Hz,CD_3OD) of oleuropein [5].

I.1.9.1.2.2. ^{13}C -NMR .

Due to the complexity of the ^{1}H -NMR spectra, several signals were left unassigned. Thus,^{13}C –NMR spectroscopy is becoming much used as complemental technique to ^{1}H-NMR . This technique offers excellent possibilities for comparison between the isolated compounds .

C1: In this case,an acetalic C1 can not be easily distinguished from an anomeric C1′ , because they appear together near 99ppm . However, the value of the $^{1}J_{C,H}$ coupling constant can be used for the distinction: $J_{C(1),H(1)}$ is 177-172 Hz against 165-161 for $J_{C(1),H(1')}$ .The C1 chemical shift seems not to be significantly influences by C4 substitution. Whereas it is diagnostically dependent of the C6-OH configuration .When going from a 6-α-OH substituent to a 6-β-OH one , C1 is always shielded and H1 correspondingly deshielded [4,49]

The existence of a conformational mobility of the dihydropyrane ring, dependent of the substitution pattern in the fused cyclopentane ring . Furthermore, when the last one is rigidly held in a flat form due to the presence of a double bond, like in aucubin type compounds, or to an epoxide function, as in catalpol- type substances, the 6-α-OH generates a steric interaction with C4-C5 bond ,with a shielding effect on C4, C5 and C6 .While, in the saturated cyclopentane ring, the mobility of the two fused rings partially annuls the effects of the steric interaction and only a small effect on C6 is evident . As a consequence, possible changes on the dihydropyrane ring do not greatly affect the chemical shift data of the cyclopentane [50].

The typical shift values of C3 and C4 , in correlation with the different substitution at C4 , are reported in Table I.3 . Due to the linkage with oxygen C3 is always more deshielded than C4.

Table I.3. Chemical shifts values of C3 and C4 in correlation with the different substitution at C4 [46] .

Substituent at C(4)	C(3)	C(4)
___	143 – 139	109 –104
CH_3 ,CH_2OH	139 – 135	116 – 112
CO_2CH_3 , CO_2H	157 –151	114 –109
CHO	165 -163	125 – 124

Location of a hydroxy group at the 6 or 7 position can be assigned on the basis of the C5 chemical shift value : not substituted C5 will be deshielded up to 40 ppm by a 6-OH ; conversely , when the hydroxyl group is located in position 7 , C5 is found near 30 ppm [40].

Assignment of the C8 stereochemistry can be very important in saturated cyclopentane ring iridoids . Two main cases can be distinguishe [49]:

a) presence of C10 as only substituent : C10 in α vs. β –configuration appears deshielded ; thus ,methyl group in α configuration is found at 19.3 ppm against 15.8 in β configuration . This difference is the result of a steric interaction between the C10 and the C9 – C1 bond which affects also C9 but not C8.

b) C10 as a secondary alcoholic function geminal to a hydroxyl group : in this case also the α-CH_2OH vs. β epimers appears deshielded by 4 ppm . C9 is shielded , the steric interaction between the bulky hydroxyl group and C1 – C9 bond being now predominant .

Several compounds are simple esters (acetyl , benzoyl , coumaroyl , etc.) of very common iridoids. In these cases it is important to assign the esterification site. Esterification of primary and secondary hydroxyl functions causes a downfield shift of α carbon atoms (1.5 – 4 ppm) and an upfield shift of β carbon atoms (1 – 5 ppm) . For a tertiary function a larger downfield α effect is expected (10 ppm) [6] .

During the last years, several iridoids polyglucosidated have been isolated . The insertion of a glucosyl moiety causes a characteristic downfield shift of the carbon atom bearing this unit (α effects of 10 ppm) and downfield β effects on the adjacent carbons (0– 4 ppm) [40,59].The ^{13}C –NMR data of oleuropein will be illustrated below in Fig .1.8.

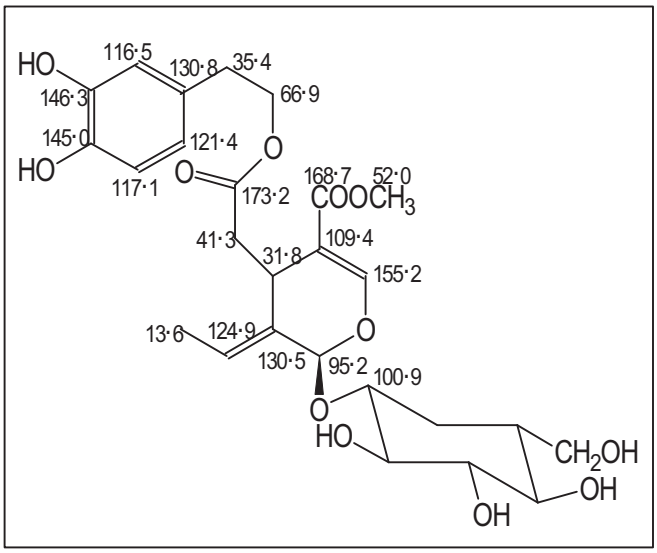


Fig.I.8. ^{13}C-NMR spectral data of oleuropein (67.8 MHz, CD_3OD)[47].

I.1.9.1.3.Mass Spectrometry (MS) .

Mass spectrometry has always been used in iridoids investigations. However, the structural properties and the sensitivity of these constituents frequently led to the thermal decomposition caused by the high temperatures needed for evaporation when using the EI technique . Better results are obtained with other ionization techniques [73,74,75]. Previous reports on the use of ammonia showed that it was preferred reactant gas for the chemical ionization CI mass spectrometry of natural compounds because of the soft ionization conditions . Whereas, reports on the use of amines as reactant gases showed more stable molecular ion adducts and a specific fragmentation[76] .

Mollova and co-workers [77] have used aliphatic amine as reactant gases in CI mass spectrometry of the secoiridoid glucoside gentiopicroside (27) and its tetraacetate. They found that the most suitable reagent gases are diethylamine (DEA) and trimethylamine (TEA) .Reprsentative spectra of gentiopicroside and its tetraacetate are given in Fig. I.9 . The mass spectral data are summarized in Table I .4.

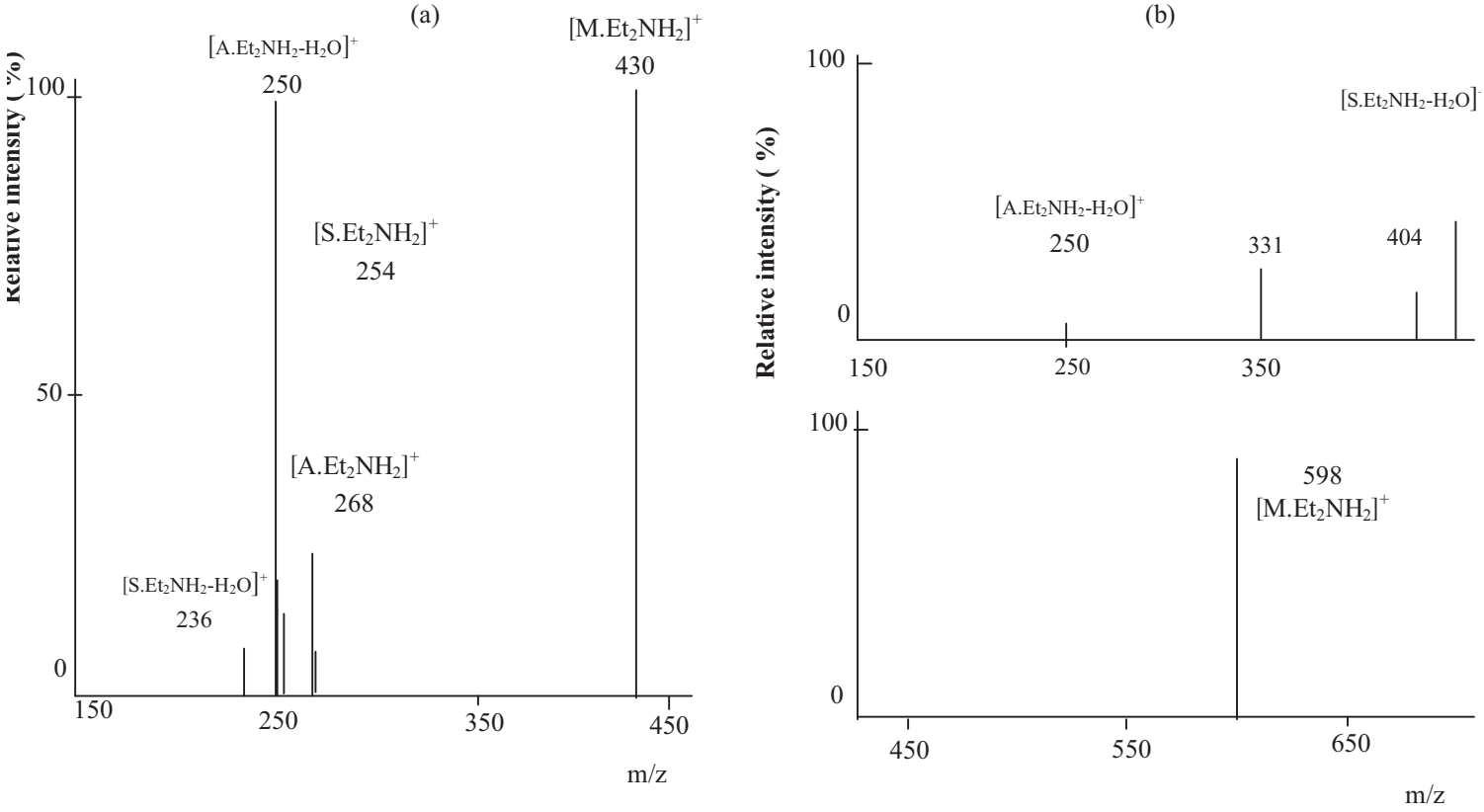


Fig. I. 9. CI mass spectrum (DEA) of gentiopicroside (a) and its tetraacetate (b)[77].

Table I .4. C I (DEA) mass spectral data for the studied secoiridoids [77] .

Compound	[$M.Et_2NH_2$]$^+$	[$S.Et_2NH_2$ $^+$	[$S.Et_2NH_2$- H_2O]$^+$	[$A.Et_2NH_2$]$^+$	[$A.Et_2NH_2$- H_2O]$^+$
a	430(94%)	254 (12%)	236(5%)	268(14%)	250 (100%)
b	598(100%)	422 (48%)	404 (21%)	-	250 (7%)

The main ions produced by (DEA) C I of gentiopictroside and its acetate can be divided into three groups: molecular adducts ions , aglucone adducts and their fragments and sugar adducts and their fragments .

The molecular adduct of gentiopicroside acetate is the most stable .

I.1.9.2. Chemical methods.

Iridoid glucosides are characterized by their instability in acid medium; subsequent steps involve hydrolysis toward the formation of an aglucone , conversion of the latter into coloured substances (mostly blue) , and finally degradation into polymeric blue –black products [19,21] .

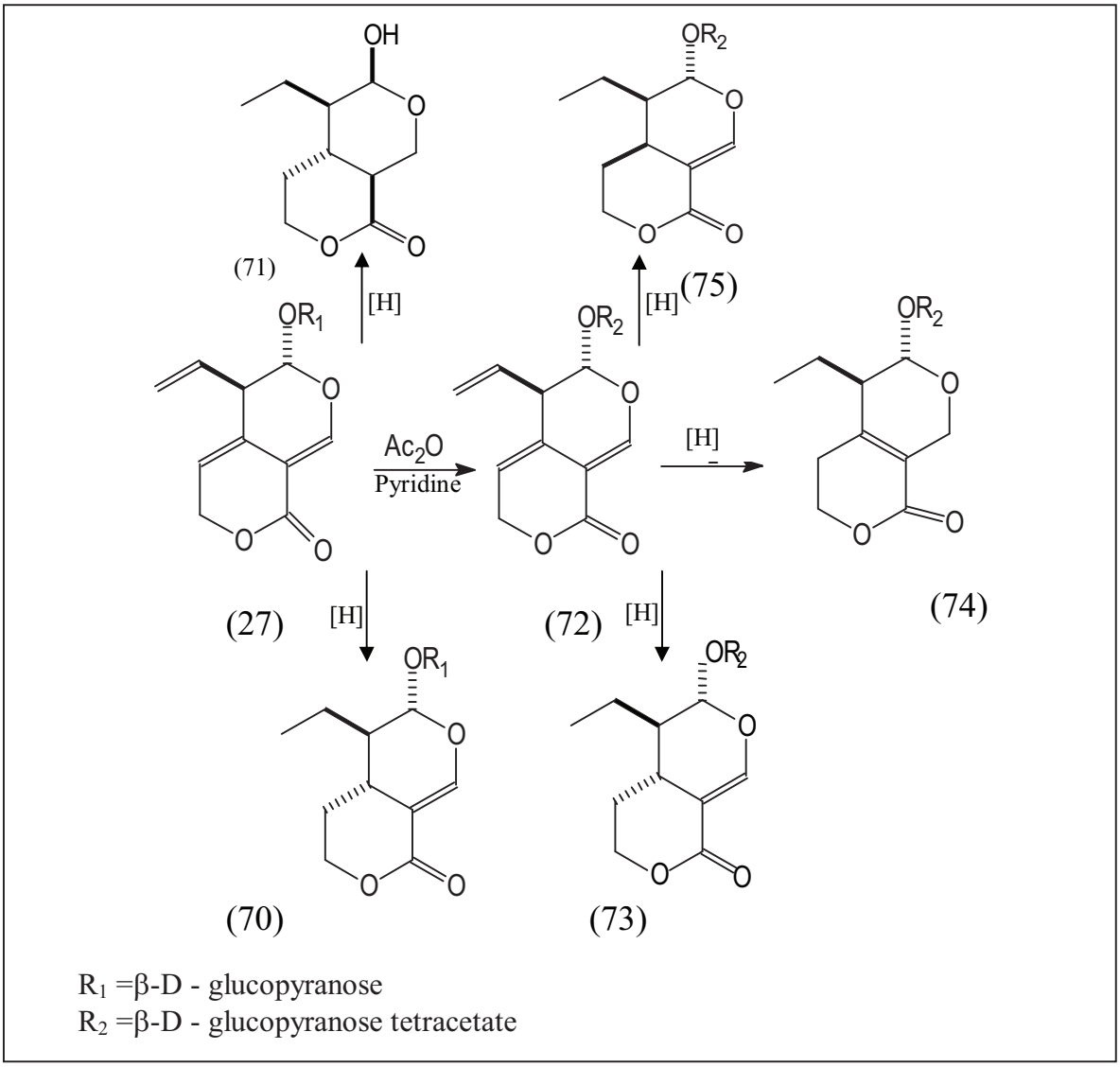
The aglucone decomposition products were used for the preparation of red and blue pigments,which are useful for color foods, Medicines, cosmetics, etc.[22].

The most employed derivative of iridoid glucosides is, without doubt, the acetyl derivative, which is useful in order to avoid separation problems due to the polar nature of these compounds.[17,28].

Acetylation of tertiary hydroxyl groups, like those in C5 and C8, must be naturally performed in harder conditions like in pyridine /Ac_2O at 37°C [20] .

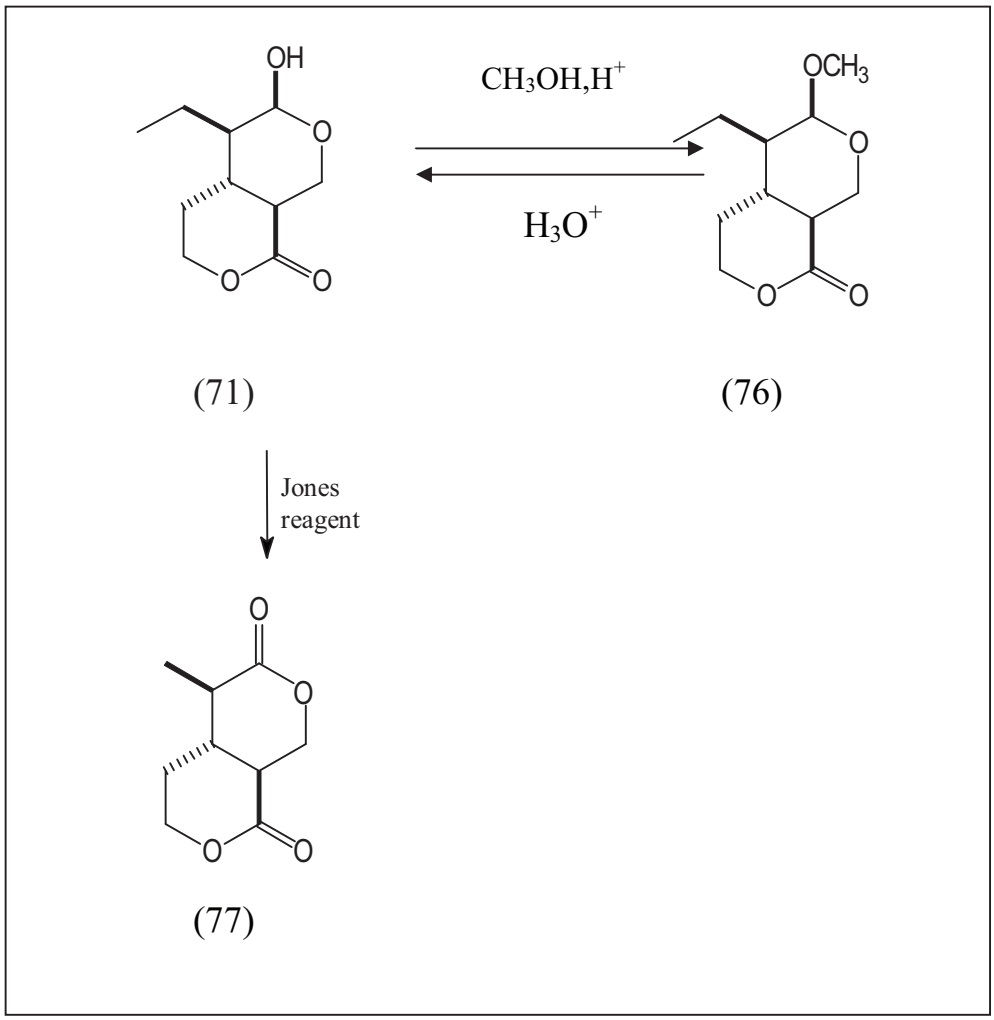
Hydrogenation of gentiopicroside (27)[22] at one atmosphere hydrogen pressure in ethyl acetate –water solvent and palladium on charcoal catalyst affords the tetrahydroderivative (70) . Extended hydrogenation at 30 - 40 atmospheres of hydrogen pressure in the same solvent system leads to concurrent reduction of the 3,4 -double bond and hydrolytic cleavage of the glucoside to produce the hexahydro -aglucone (71).Hydrogenation of gentiopicroside tetraacetate (72) in the absence of water at one atmosphere gives three tetrahydroderivatives (73) , (74) and (75) as shown in scheme I.6.

Scheme I.6:



Acid-catalyzed methanolysis of (71) leads to replacement of hydroxyl with methoxyl, while oxidation of (71) with Jones reagent produces the dilactone (77)[22] (as shown in scheme I.7) .

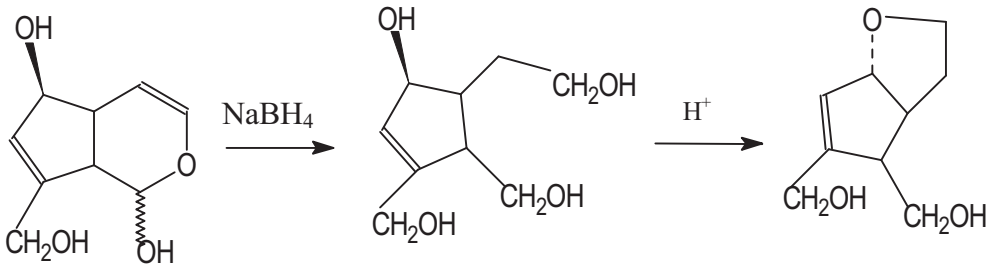
Scheme I.7:



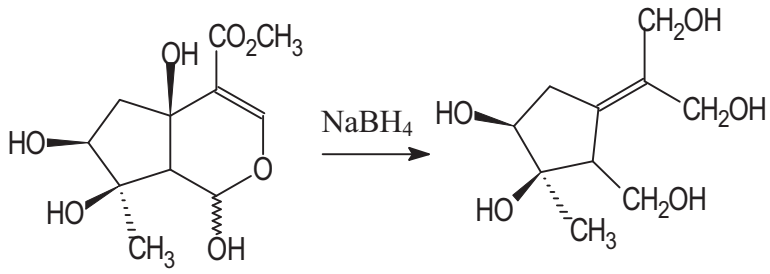
-Reaction of aglucones with $NaBH_4$[25].

Reduction of iridoid aglucones with $NaBH_4$ in aqueous solution gives rise to different products ,according to the substitution pattern of the dihydropyrane ring .

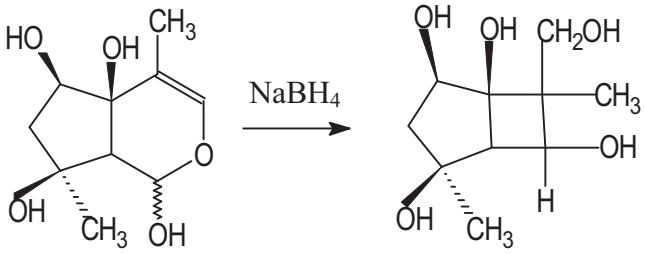
1) With no substituent at C(4) ,simple reduction of the dialdehydic from is obtained .



2) With 4-CO_2CH_3 and 5-OH ,4/5 dehydratation occurs.



3) With 4-CH_3 , a bicyclic derivative is obtained by intramolecular aldolic condensation .



I . 2 . The Oleaceae family .

The Oleaceae, the olive family, is a medium – sized family of about 600 species in 25 genera, the family is distributed on all continents except the Antarctic , from northern temperate to southern subtropical regions and from low to high elevations. According to the latest review of the entire family, it is divided into two subfamilies : Oleoideae and Jasminoideae [78,79] .

I . 2.1. The genus Fraxinus .

The genus Fraxinus, the Ashes, in Oleaceae, is a circumpolar genus of the northern hemisphere ,comprising ~ 50 species of mainly trees [79] .

I .2 .1.1 .Classification and morphology of Fraxinus species.

The most recent classification of the genus identified two sections : Ornus (subsections : Euornus And Ornaster) , and Fraxinaster (subsections : Dipetalae ,Pauciflorae , Sciadanthus , Melioides and Bumelioides) . Ornus is characterized by terminal inflorescence, insect pollinated Euornus have showy petalous flowers and wind pollinated Ornaster are apetalous . The species of section Fraxinaster are wind pollinated and characterized by apetalous flowers (except *Fraxinus dipetala* that has two petals) .The two main subsections Melioides and Bumelioides differ in that both calyx and corolla are wanting in Bumelioides [79] .
Varieties : *Fraxinus americana* , *Fraxinus holotricha* , *Fraxinus ornus , Fraxinus pennsylvanica lanceolata , Fraxinus velutina glabra , Fraxinus excelsior , Fraxinus angustifolia , Fraxinus oregona ,Fraxinus quadrangulata , Fraxinus nigra , Fraxinus longicuspis , Fraxinus mariesii , Fraxinus dimorpha, Fraxinus xanthoxyloides* .In Algeria , the genus Fraxinus is represented by two species *F. angustifolia* and *F. xanthoxyloides* [47,59,80] .
.
Fraxinus xanthoxyloides (Afghan Ash or Algerian Ash) :
This shrubby ash occurs from north Africa (Algeria , Moroco) to center and east Asia (Afghanistan [Hindukush mountain] ,Pakistan ,India , Kashmir , Himalayan mountain , and China) [80] .

I .2 .1.2. Description of Fraxinus species.

These hardy , deciduous and quick-growing trees are commonly known as Ash trees which are suitable for growing in yards and along roads, and survive in almost any soil in wind – swept areas and near the sea [79].
Ashes produce compound leaves with toothed leaflets that turn yellow and purple colors in autumn .Most of these trees produce unnoticeable , greenish-yellow , mal and female flowers on separate trees , which are borne in early spring [80].

I . 2. 1.3 . Medicinal value of Fraxinus species.

The bark of *Fraxinus japonic Blume* [81] has been used as a medicinal for rheumatism and gout in Japan.
In China, *Fraxinus malacophylla Hemst*. has long been used as a folk medicine .For example, the Bai tribe uses the whole plant in treating stomatitis ,haemostatic and urinary organ infection .Its root are used as an antipyretic , antimalaria , antirhinitis and antiinflammatory agent as well as a remedy for excretory organ infection . Its bark is used in treating stomatitis, toothache, pyrexia and urinary organ infection [82].

The bark of *Fraxinus ornus L* is used in the Bulgarian folk medicine for wound healing and as a remedy against various elements, including diarrhea and disentery [83]. Recently, the results obtained by Kostova and co–workers [4,84,85] support the claims of the folk medicine by presenting scientific proofs for the antimicrobial , antiinflammatory , skin –regenerating , antioxidant and antiviral properties of the bark extract and its components.

Generally, Fraxinus are used for their diuretic and mild purgative effects as well a for the treatments of constipation, dropsy, arthritis, rheumatic pain, cystitis and itching scalp in folk medicine[6] .

I.2.1.4. Secoiridoid compounds isolated from Fraxinus species.

From this genus 12 of the about 50 species have been investigated . A characteristic feature of Fraxinus plants is the high content of secoiridoid compounds of oleoside - type glucosides [35] . All the isolated secoiridoids hitherto from fraxinus species are listed In Table I.5.

Chapter II

Results and discussion

II. Results and discussion .

In this section, we will report and discuss the results of our phytochemical investigations, which threw light on the examination of two types of fractions:

- Non – volatile compounds .
- Volatile compounds .

II. 1. Identification of the isolated non – volatile compounds .

The ethyl acetate soluble portion of the total extract was separated by a combination of chromatographic procedures . Full details concerning the isolation and purification of the obtained compounds will be presented in the experimental section. Here, we will discuss the structure identification of the isolated components

Compound 1:

This compound was isolated as a colourless amorphous powder with $[\alpha]_D^{20}$ = -181.1° (MeOH). Its UV spectrum (Fig.II.1) reveals, besides the typical absorption at 227 nm of an iridoidic enol ether system conjugated with a carbonyl group, additional absorption at 280 nm due to a phenolic function . The IR spectrum (Fig.II.2) shows bands at 3550-3250 and 1700 cm^{-1} 1630 cm^{-1} which are corresponding to stretching vibration of hydroxyl and α, β – unsaturated ester groups . Furthermore, a band at 1504 cm^{-1} is due to aromatic ring.

The ^{1}H-NMR spectrum (Fig.II.3) of compound 1 displays typical signals of an oleoside nucleus : a singlet at δ 7.52ppm characteristic for the vinylic proton H3 of a secoiridoid glucoside , a doublet at δ 4.80 (1H,d, J = 7.6 Hz, H1′) for the anomeric proton H1′ . Olefinic proton at δ 6.06 (1H, br q , J = 7.1 Hz , H8) ,coupled to a methyl signal at δ 1.63 (3H , dd , J = 7.1 Hz and J = 1.4 Hz , H10) ,and an allylic acetal proton at δ 5.91 (1H, br s , H1) . The ^{1}H- NMR spectrum also suggested the presence of an aromatic AA'BB' spin system centered at δ 6.72 (2H ,d , J = 8.6 Hz , H5″ and H7″) and at δ 7.04 (2H ,d , J = 8.6 Hz , H4″ and H8″) , together with a ABX spin system of H_a6 , H_b6 and H5 at δ 2.69 (1H, dd , J = 14.1 Hz , and J = 4.5 Hz, H_a6) , δ 2.45 (1H, dd , J = 14.1 Hz and J = 9.2 Hz, H_b6) , and δ 3.95 (1H, dd , J = 9.2 Hz , J = 4.5 Hz, H5) ,respectively . Another ABX spin system appears at δ 4.21 (1H, dt , J = 10.7 Hz , and J = 7.0 Hz , H_a1″) , δ 4.12 (1H, dt , J = 10.7 Hz and J = 7.0 Hz , H_b1″), δ2.82 (2H , t , J = 7.0 Hz , H2″) , corresponding to the two methylene group of $–OCH_2CH_2Ph$ moiety. While, the singlet signal at δ 3.71 is assignable to the methoxy protons (3H, s , OMe) .

In addition , ^{13}C –NMR spectrum (Fig.II.4) shows two typical signals for carbonyl carbon at δ 173.3 and at δ 168.7 . These two signals are attributable to carbons at C7 and C11 , respectively . Carbon signals at δ 155.2 (C3) , δ 109.5 (C4) , δ 125.0 (C8) , δ 130.1 (C9) , as well as the methyl carbon at δ 13.6 (C10) and the anomeric carbon signal at δ 100.9 (C1′) together with allylic acetal carbon at δ 95.2 (C1) , indicate an oleoside type nucleus for compound 1 . The complete assignment of the ^{13}C- NMR spectrum of compound1 was performed on the basis of DEPT experiment (Fig.II.5). This procedure changes the intensities of ^{13}C signals based on the number of attached protons . Quaternary carbons are not observed and others are positive , negative or null depending on the angle used in the experiment . Here , we have used the 135° pulse which rapidly identifies fifteen positive signals for CH and CH_3 groups and four negative signals for CH_2 groups .

The direct comparison between the two spectra (^{13}C- NMR spectrum and DEPT ^{13}C- NMR spectrum) reveals the absence of six peaks in the DEPT spectrum, which are corresponding to quaternary carbons.

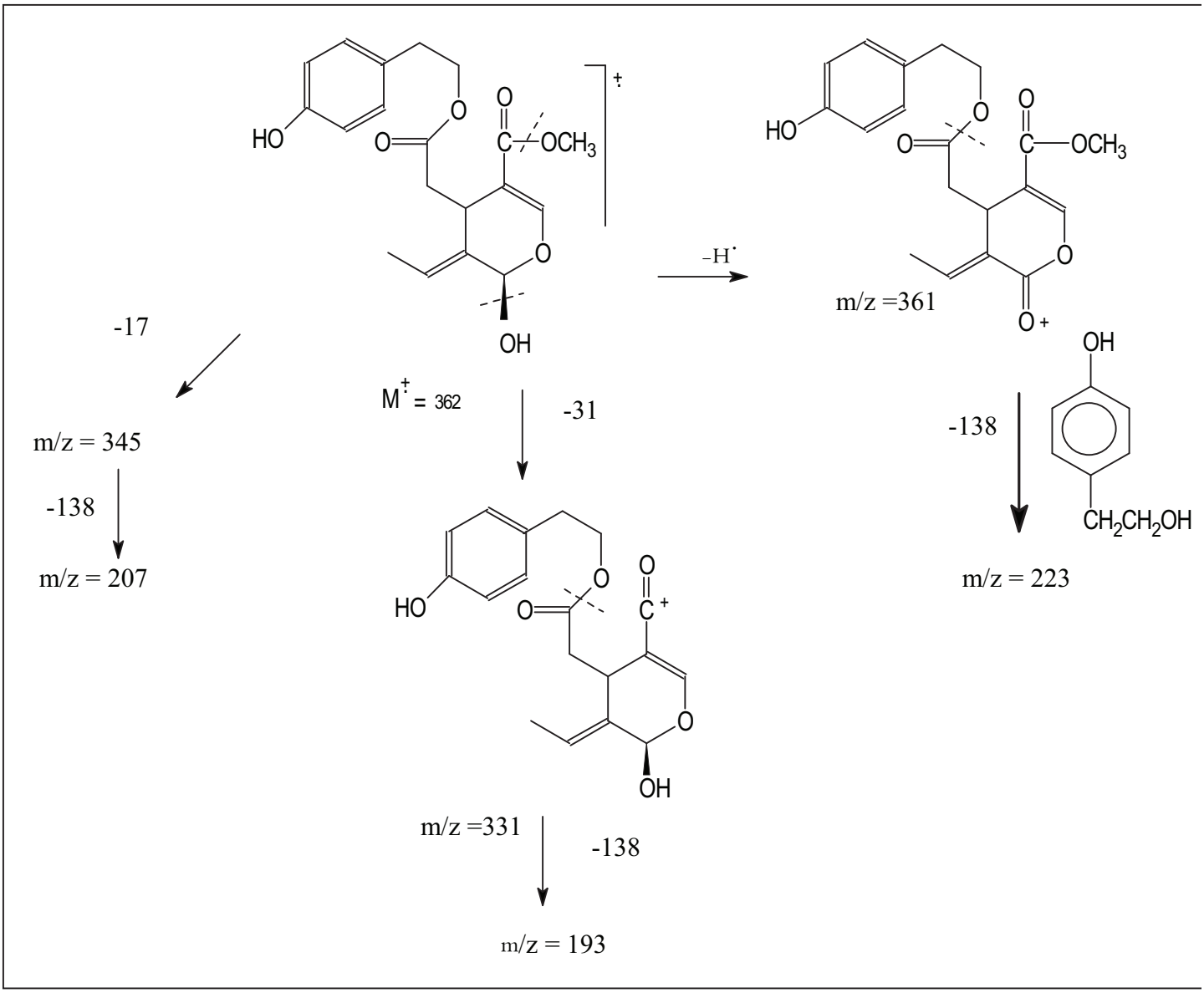
compound 1 is not volatile for direct electron impact (EI) mass spectrum , we silylated the sample and run GC/ MS analysis . Under the EI conditions we never get the molecular ion of the glucosides , because they split to aglucone and sugars . Usually, we get the mass spectrum and the molecular ion of the aglucone [73] .

In this case, under GC/ MS (EI) conditions , compound 1 is split into aglucone , glucose and 4-hydroxyphenylethanol and we can detect three of them. So, we have the MS spectrum of the aglucone (retention time 19.658 min , Fig.II.6), the MS of the glucose (retention time 10.069 min , Fig.II.7) and the MS of the 4- hydroxyphenylethanol (retention time 6.988 min , Fig.II.8).

Regarding the MS of the aglucone.

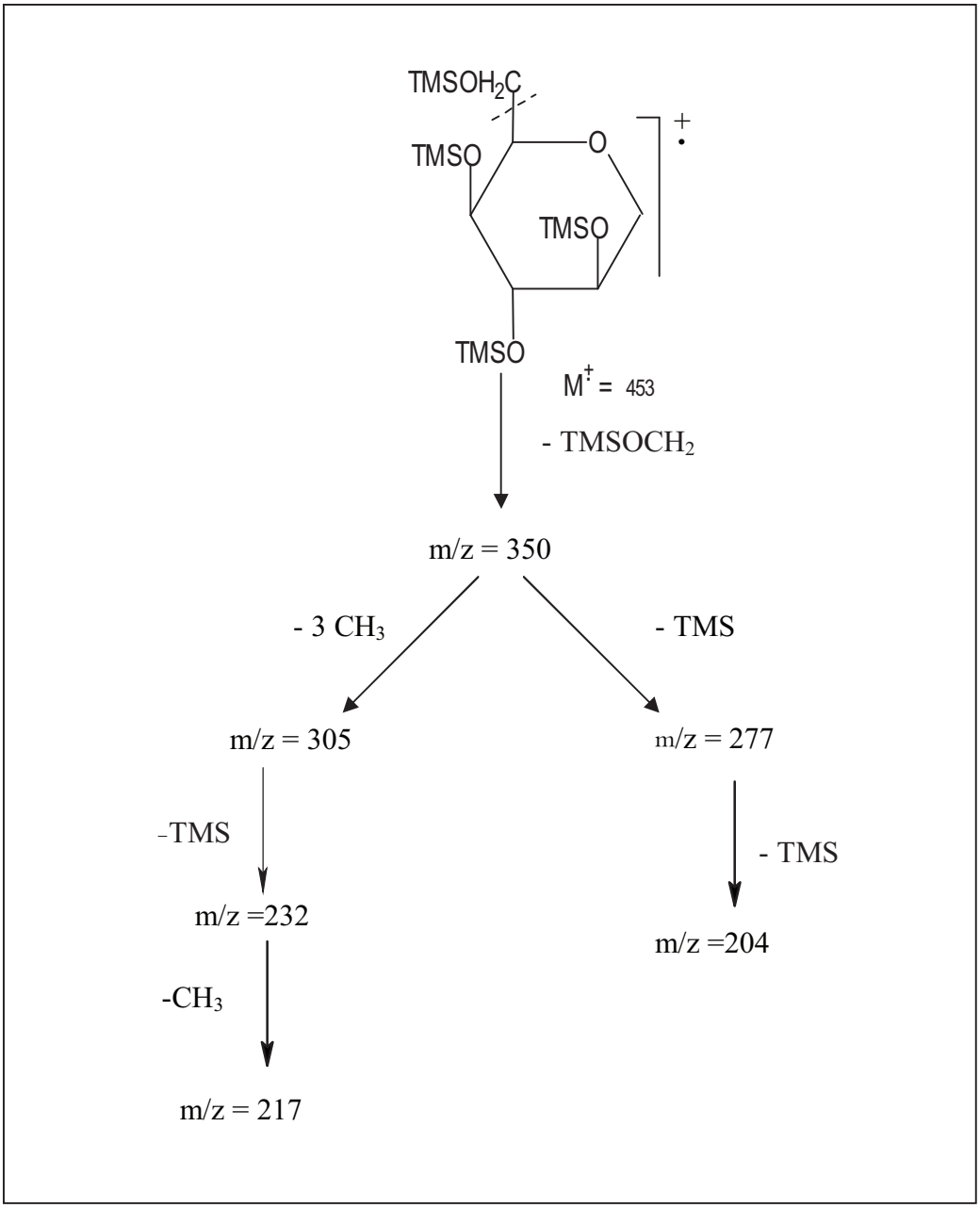
The aglucone ($M^{\cdot+}$ at m/z = 362) losses $H^{\cdot}$ to the formation of the stable ion at m/z =361 , or a methoxy radical ions with m/z = 331 , or a hydroxy radical to ions at m/z = 345. In addition to these peaks (362 , 361 , 331, 345) , peaks at m/z 223 , 207 and 193 are corresponding to the loss of 138 units (4- hydroxyphenylethanol) from m/z 361 , 345 and 331, respectively . The peak at m/z = 73 is characteristic of $^{+}Si(CH_3)_3$. A proposal of fragmentation for the aglucone of compound 1 will be given below in scheme II.1.

Scheme II.1:



The MS spectrum of the glucose (shown in Fig.II.7) exhibits a strong peak at m/z = 204 .The proposal pathway of fragmentation will be outlined below in scheme II.2.

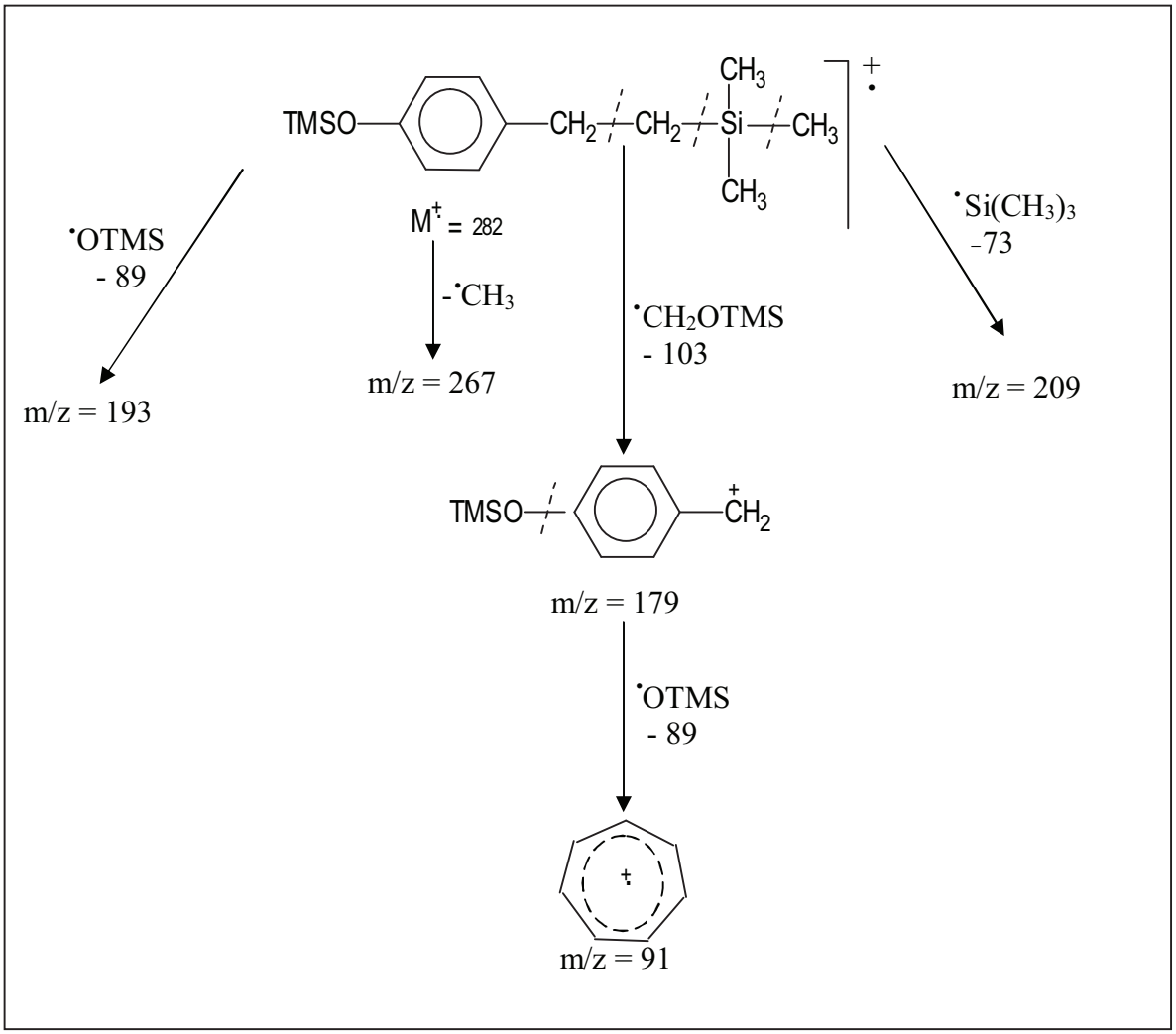
Scheme II.2:



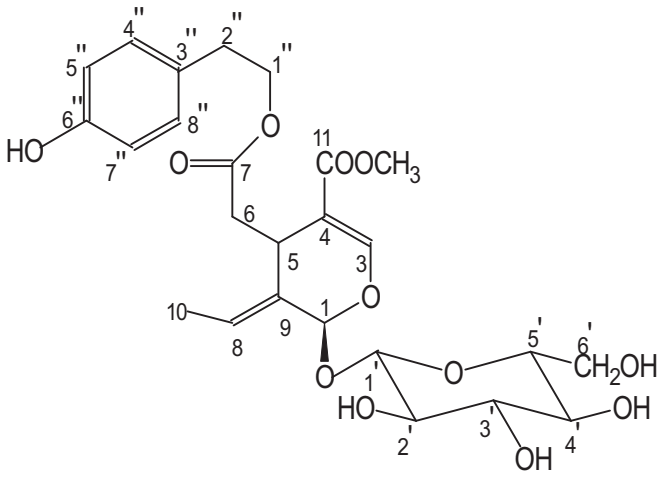
The MS spectrum of 4- Hydroxyphenylethanol (Fig.II.8) shows different ion peaks at m/z=267 ,175 ,193 and 91 could have resulted from the fragmentation of 4-hydroxyphenylethanol as it is illustrated below in scheme II.3.

Scheme II.3:

.



Additionally, the use of the spraying reagent $FeCl_3$ dissolved in EtOH have also confirmed to us that compound 1 contains a phenolic moiety. The spectral data of compound 1 was in full agreement with those reported for ligstroside [6] .Moreover, the co- TLC comparison with authentic samples gave proof that compound 1 is the secoiridoid glucoside ligstroside. The structure of compound1 is given below.



Ligstroside

Compound 2.

Compound was obtained as a powder with $[\alpha]_D^{28}$ = -143° (MeOH). The ^{1}H-NMR spectrum (Fig.II.9) of compound 2 exhibits a singlet at δ 7.57 which is characteristic of H3 of a secoiridoid ghrcoside , signals for methyl group at δ 1.75 (3H , dd , J = 7.1 and 1.4 Hz , H10) , a methoxy group at δ 3.74 (3H , s ,OMe) , an anomeric proton at δ 4.84 (1H ,d , J = 7.6 Hz , H1′) , an allylic acetal proton at δ 6.04 (1H , brs , H1) and an olefinic proton at δ 6.17 (1H , qd , J = 7.1 and 1.0 Hz , H8) ,indicating the presence of an oleoside - 11 –methyl ester moiety in the molecule. Furthermore, the ^{1}H – NMR spectrum shows an aromatic AA'BB' spin system at δ 7.01 (2H , J = 8.6 Hz , H5″ and H7″) , and at δ 7.25 (2H , J = 8.6 Hz , H4″ and H8″) , along with signals of a OCH_2CH_2Ph moiety , which appears as an A_2X_2 spin system at δ 3.75 (2H , t , J = 6.9 Hz , H1″) and δ 2.82 (2H , t , J = 6.9 Hz , H2″) , together with a ABX spin system of H_a6 , H_b6 and H5 at δ 2.96 (1H ,dd , J = 14.5 and 4.6 Hz , H_a6) , δ 2.73(1H ,dd , J = 14.5 and 9.1Hz , H_b6) and at δ 4.12 (1H ,dd , J = 9.1 and 4.6 Hz , H5),respectively .

The ^{13}C-NMR spectrum (Fig .II .10) shows two typical signals for carbonyl carbon at δ171.8 and δ 168.8 .These two signals are attributable to carbons at C7 and C11 , respectively. Carbon signals at δ 155.4 (C3), δ 109.4 (C4) , δ 125.2(C8), δ 130.7 (C9) as well as a methyl carbon at δ13.9 (C10) indicate an oleoside type nucleus for compound 2. These spectral features suggest that compound 2 is an ester of oleoside –11-methyl ester with tyrosol such as ligstroside (compound 1).

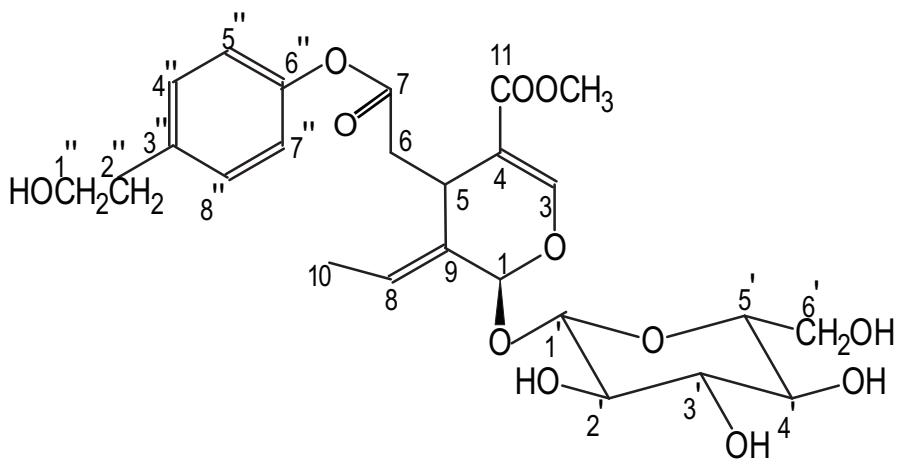
However, comparative studies of ^{1}H and ^{13}C-NMR spectra of compound 1 and compond 2 indicate that the nature of the ester linkage at C7 in compound 2 was obviously different from that in compound 1.

Furthermore, the downfield shifts of H6 and aromatic protons as well as the upfield shift of the C7 signal of compound 2, when compared with the corresponding signals of compound 1 [6,86] , strongly suggested that the C7 carbonyl group should be linked to a phenolic oxygen rather than an alcoholic one .This was also supported by an IR band at 1739 cm^{-1} due to a phenolic ester group (Fig.II.11) .

The GC/ MS analysis of compound 2 (silylated) shows that compound 2 is split into aglucone (retention time 13.21 min , Fig.II.12) ,glucose(retention time 8.07 min , Fig.II.13) and 4-hydroxyphenyl ethanol (retention time 5.09 min , Fig. II .14) .

The obtained mass spectra confirm definitely that compound 2 and compound 1 are isomeric compounds . So, the spectra of the two compounds are similar and we will observe the same peaks (362 , 361 , 331 , 345 , 207 , 223 , 193) in the spectrum of its aglucone .

Compound 2 was identified by direct comparison of its spectral data with those reported for formoside [86], as well as co-TLC comparison with authentic samples. So, compound 2 is formulated as shown below.

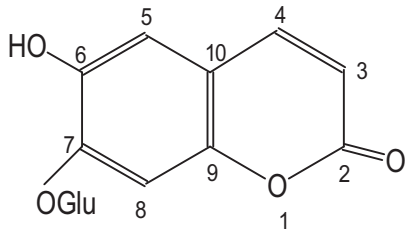


Formoside

Compound 3:

Compound 3 was obtained as a powder with $[\alpha]_D^{15}$ = -97.60° (MeOH) .The UV spectrum (Fig.II.15) shows absorptions maxima at 230 ,253 , 290 and 345 nm suggesting the presence of a conjugated aromatic system .

The ^{1}H – NMR spectrum of compound 3 (Fig.II.16) exhibits the presence of two doublets at δ 6.17 (H3) and at δ 7.72 (H4) together with two singlets at δ 6.89 (C8) and at δ 7.08 (C5) ,as well as the presence of doublet at δ 4.85 which is characteristic of the anomeric proton H1′ . These features suggest that compound 3 should be either cichoriin or esculin . The identity of compound 3 was confirmed by comparison of the obtained ^{1}H – NMR data with those reported in reference [90] and by comparison with cichoriin authentic sample (UV and co-TLC), they were in full agreement .Thus , glucoside 3 proved to be cichoriin .



Cichoriin

II.2. Identification of the volatile compounds.

The aim of the present study is to analyse and to compare the volatile constituents of the leaves and the stem bark of *Fraxinus xanthoxyloides Wall.* species . The constituents of the two samples (leaves and stem bark) were identified by analytical GC/ MS. The identification of components was accomplished by using computer. Some of the GC/MS peaks remained unidentified , because of the lack of authentic samples and library data of the corresponding compounds . The obtained results are summarized in table II.1. Aliphatic acids , alcohols and ketones , hydrocarbons , aromatic alcohols , diterpens and monterpenoides are the main groups of the compounds identified .

Table II.1. GC/MS analysis of the volatile compounds of leaves (L.F) and stem bark (S.b . F) of *F.xanthoxyloides* (in % of total ion current)

Volatile components	R_t(min)	S .b .F(%)	L .F (%)
Aliphatic acids and esters		**28.2**	**26.3**
2- Ethylhexanoic acid	11.46	-	tr[a]
3-Ethylheptanoic acid	13.56	tr	-
Octanoic acid	12.66	0.7	-
Nonanoic acid	14.93	2.6	0.6
Decanoic acid	16.99	2.0	0.2
Dodecanoic acid	20.83	0.5	-
Tetradecanoic acids	24.43	-	0.7
Tetradecanoic acid –1-methyl ethyl ester	25.44	0.2	-
Hexadecanoic acid	27.78	6.6	9.3
Hexadecanoic acid methyl ester	27.08	2.6	3.3
Hexadecanoic acid ethyl ester	28.17	0.8	2.2
Octadecanoic acid methyl ester	30.20	0.3	0.3
8-Octadecenoic acid methyl ester	29.88	0.6	-
11-Octadecenoic acid methyl ester	29.8	3.3	-
9,12 –Octadecadienoic acid	30.36	4.0	-
9,12-Octadecadienoic acid methyl ester	29.69	2.1	1.1
9,12,15-Octadecatrienoic acid methyl ester	29.82	-	7.1
9-Octadecenoic acid ethylester	30.78	1.2	1.5
9,11-Octadecadienoic acid ethyl ester	30.69	0.7	-
Aliphatic alcohols		-	**10.3**
1-Tridecanol	20.94	-	3.3
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	26.04	-	0.2
9,12,15-Octadecatrien-1-ol	30.52	-	6.8

Table II .1 continued

Aliphatic ketones		**0.9**	**1.0**
2,5-Cyclohexadien-1,4-dione	18.75	-	0.2
2,6-Di (t – butyl) – 4 – hydroxy – 4 – methyl –2,5-cyclohexadien -1-one	18.91	0.9	0.6
6,10,14-Trimethyl –2- pentadecanone	25.72	-	0.2
Hydrocarbons and derivatives		**1.3**	**0.6**
Pentacosane	35.30	0.3	-
Heptacosane	37.67	0.5	-
2,6,10,15,19,23- Hexamethyl –2,6,10,14,18,22-Tetracosahexane	39.25	0.5	0.6
Monoterpenoides		-	**0.3**
α -Terpineol	12.71	-	tr
L- Linalool	10.51	-	0.3
Linalool oxide	10.19	-	tr
Cis– Linalool oxide	9.79	-	tr
Sesquiterpenoides		-	**2.5**
Nerolidol	20.71	-	2.5
Diterpenes		**1.5**	**8.3**
Neophytadiene	25.62	-	0.7
Phytol	30.00	-	5.6
Isophytol	27.43	-	2.0
Ferruginol	33.09	1.5	-
Aromatic alcohols and ethers		**43.8**	**37.3**
Eugenol	16.5	-	0.6
2,5 – Bis (1,1 –dimethyl ethyl)-1,4-benzenediol	18.75	0.4	-
2,6-Bis (1,1-dimethyl ethyl)- 4- methyl phenol	19.72	42.8	36.7
Elemicin	20.60	0.6	-
Aromatic aldehydes and esters		**2.1**	**2.1**
Carbomethoxy –5- venyl pyridine	16.40	2.1	1.7
P- Carbomethoxy benzaldehyde	16.74	-	0.4
Others		**0.9**	**1.2**
Dihydroactinidiolide	20.08	-	1.2
Nonadecane	36.55	05	-
Dihydroabietan	29.12	0.4	-

[a] tr : trace

Table II.1 demonstrates some similarities and differences in the volatile composition of the leaves and stem bark of the studied plant .The presence of aliphatic acids is a characteristic feature of the two samples under investigation .The biggest amount of aliphatic acids is found in the stem bark (28.2%) . Nonanoic and Decanoic acid as well as Hexadecanoic acid and its methyl ester, methyl ester of Octadecanoic acid ,methyl ester of 9,12 – Octadecadienoic acid and ethyl ester of 9-Octadecenoic acid are common compounds in the volatiles of the two samples. The branched fatty acids , 2- Ethylhexanoic and 3-Ethylheptanoic acid , are detected in trace amount in the leaves and the bark ,respectively. Also, it interesting to not tat 9,12,15-Octadetrienoic acid methyl ester (7.1 %) was found only in the leaves .

Aliphatic alcohols are present in large amount (10.3 %) in the leaves, while aliphatic Ketones are found in low amount (1.0 %) in the leaves and (0.9 %) in the bark .

Hydrocarbons are observed in the volatiles of both leaves and bark (0.6 %) and (1.3 %) ,respectively . Mono- and sequiterpenoides are detected only in the leaves .

These results show a high content of aromatic alcohols and ethers, aliphatic acids and ester, aliphatic alcohols , diterpenes and in the leaves. Hexadecanoic acid its methyl ester , methyl ester of 9,12 ,15 –Octadecatrienoic acid ; 9,12,15 –Octadecatrien-1-ol , and phytol are the main volatile components of the leaves .

The high content of aromatic alcohols and ethers, aliphatic acids and esters and the presence of aliphatic Ketones and hydrocarbons is established for the stem bark . Hexadecanoic acid and its methyl ester , Nonanoic acid , the methyl ester of 11-Octadecenoic acid and 9,12 – Octadecadienoic acid are the main volailes of the stem bark .

The aromatic alcohol 2,6 – Bis (1,1- dimethyl ethyl) –4– methyl phenol is the main volatile cmponent of the two samples , it is found in big amount (36.7 %) and (42.8 %) in the volatiles of the leaves and the stem bark , respectively .

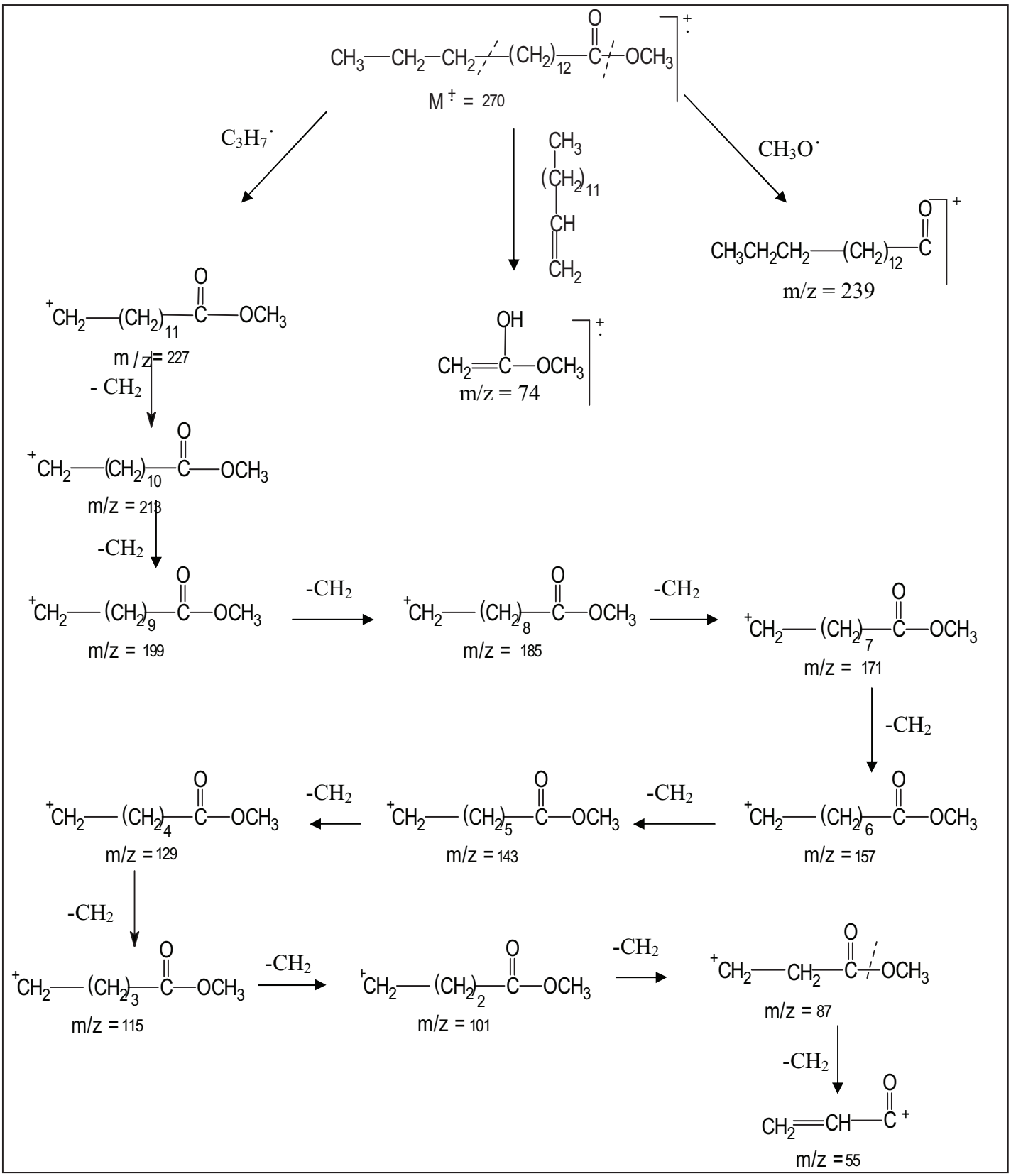
Some examples of MS spectra of the most abundant components of the volatiles are given together with their proposal pathways of fragmentation .

- Hexadecanoic acid methyl ester (R_T =27.08 min)

Chemical formula : $C_{17}H_{34}O_2$, M = 270 g/mol .

According to the mass spectrum of this compound (Fig.II.17) . The typical fragmentation pathways are illustrated below in scheme II.4 .

Scheme II.4 :

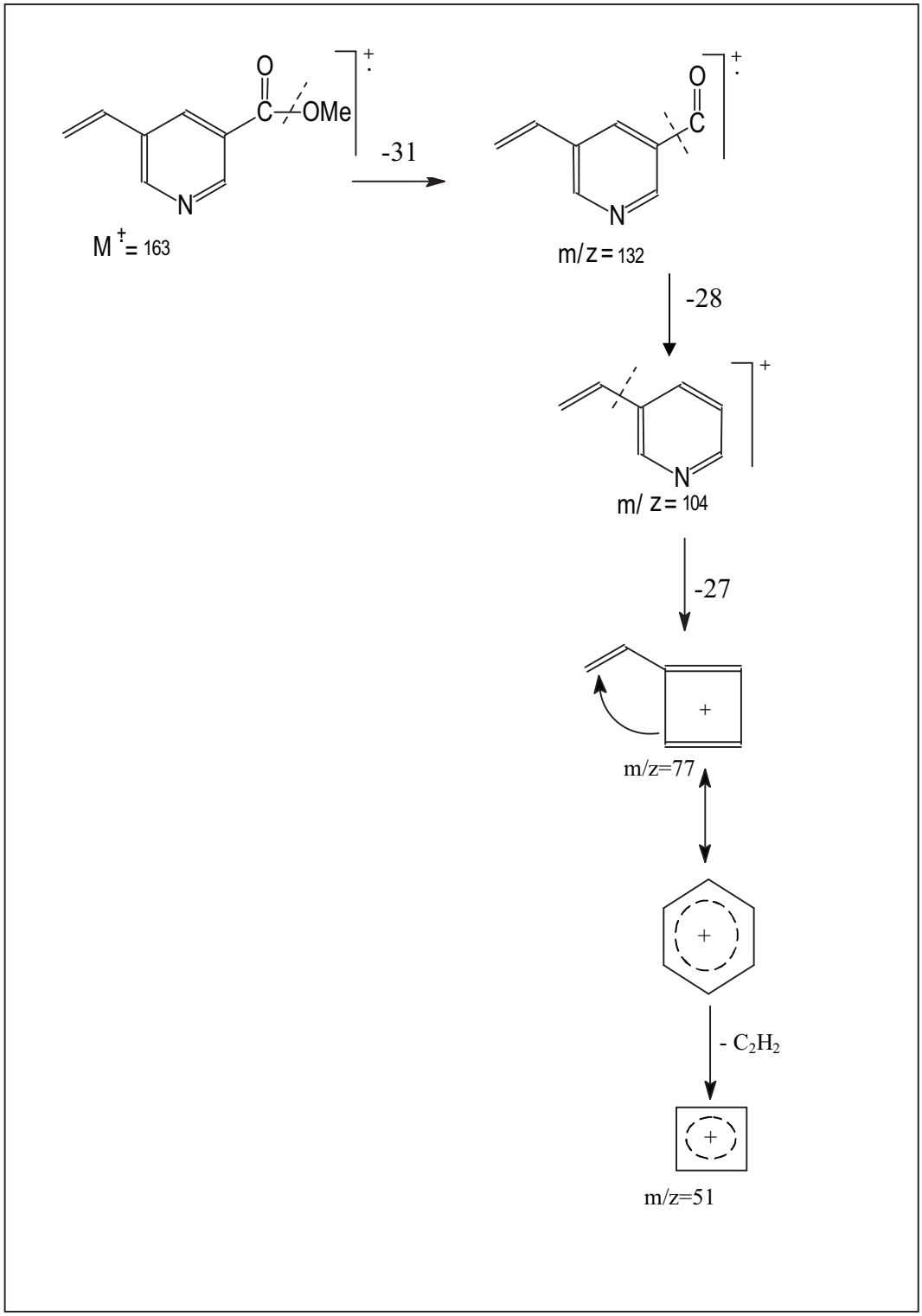


– Carbomethoxy - 5- vinylpyridine (R_T =16.40 min) .

Chemical formula : $C_9H_9O_2N$, M = 163 g/mol.

According to the mass spectrum of this compound ,shown in Fig.II.18, the proposed pathway of fragmentation is given below in scheme II.5.

Scheme II.5:

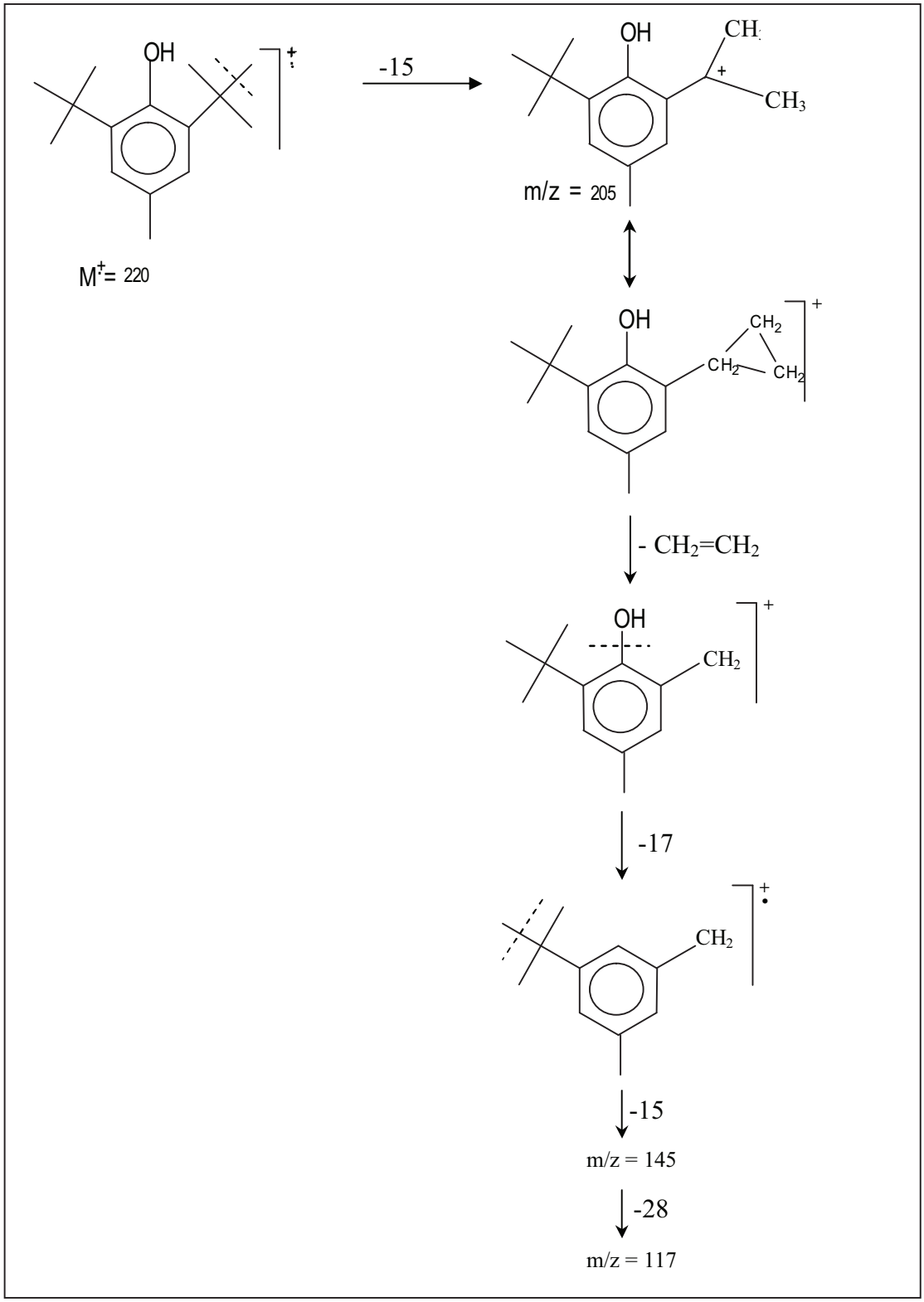


– 2,6 –Bis (1,1– dimethyl ethyl) 4 – methyl phenol (R_T = 19.72 min) :

Chemical formula : $C_{15}H_{24}O$, M = 220 g/mol.

According to the mass spectrum of this compound (Fig.II.19), the proposed pathway of fragmentation is illustrated bellow in scheme II.6.

Scheme II.6 :

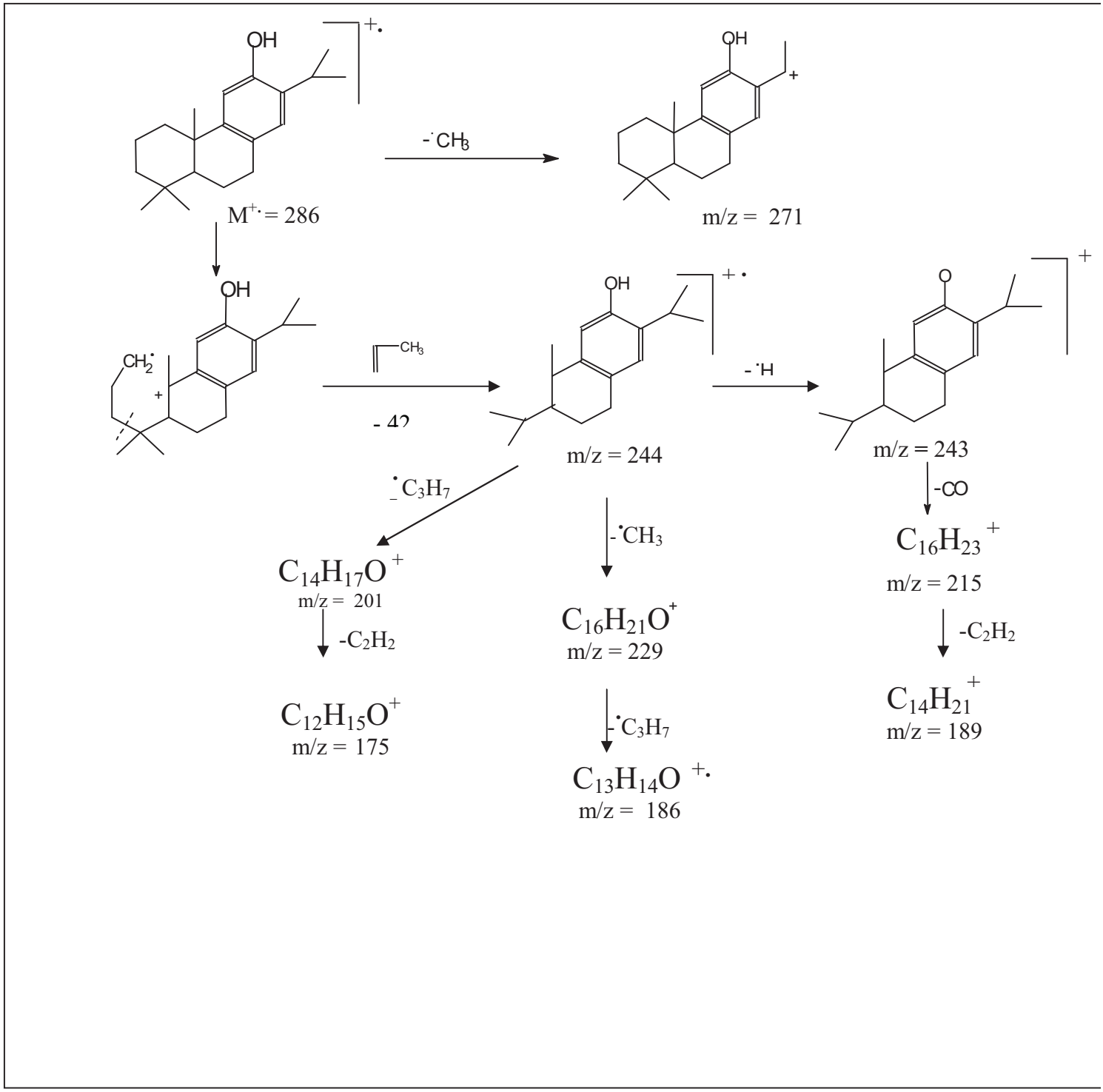


– Ferruginol (R_t = 33.09 min) :

Chemical formula : $C_{20}H_{30}O$, M = 286 g/mol.

Fig.II.20 represent the mass spectrum of ferruginol. The proposed pathways of fragmentation are given in scheme II.7 .

Scheme II.7:



Chapter III

Experimental section

III .Experimental section .

III.1. Materials and methods .

III.1.1. Plant material.

Plant material of *Fraxinus xanthoxyloides Wall*. species was collected from the region of Khenchela , Algeria. Leaves in June 2002 , stem bark in December 2003 . The plant was kindly authenticated by professor Mohamed Kaabeche , Setif University , Algeria .

III.1.2. Chromatography

1- Analytical TLC : In the following experiments , thin layer chromatography (TLC) was performed on percoated Merck Aluminium plates , silica gel 60 F_{254} , spots were detected under UV light first ,then exposure to I_2 vapor or by spraying with the ethanolic solution of $FeCl_3$ (2g of $FeCl_3$ in 60ml of EtOH) and heating .

The solvent systems used are :

P1.$CHCl_3$/ MeOH , 1:1
P2.$CHCl_3$/MeOH , 17:3
P3 . EtOAC

2– Preparative thin layer chromatography (PTLC) :

TLC percoated sheets , silica gel 60 F_{254} (Merck) were used .Bands were detected under UV light .

3 - Vacum liquid chromatography (VLC) :

Silica gel 60 (0.063-0.2 mm Merck) was used as adsorbent materials.The solvent systems used as eluants are :

1. $CHCl_3$
2. $CHCl_3$ / MeOH –with increasing polarity .

4– Column chromatography (CC) :

silica gel 60 (0.063 – 0.2 mm Merck) was used as adsorbent materials .The solvent systems used are :

1. $CHCl_3$
2. $CHCl_3$ /MeOH
3. EtOAc

III.1. 3 Spectroscopy :

UV and IR spectra were determined on Zeiss Specord UV/Vis and Bruker IFS 113V or Zeiss UR20 instruments , respectively . ^1H and ^{13}C-NMR spectra were obtained at 250 MHz (^1H – NMR) and at 63 MHz (^{13}C – NMR)(Bruker WM 250 instrument) .All spectra were obtained in CD_3OD using TMS as internal standard , with the chemical shifts quoted in δ (ppm) and the coupling constants J in (Hz) .

GC/MS spectra were obtained on Hewlett Packard Gas chromatograph 6890 equipped with a Hewlett Packard MS5973 detector. HP5 – MS capillary column (30m ×0.25 µm film thickness , Agilent technologies ,Wilmington , Delaware ,USA) was used . The temperature was programmed from 40 °C to 280 °C at rate of 6 °C /min . Helium was used as a carrier gas at 9 ml/min . The ion source was set at 250 °C and the ionization voltage was 70 eV . The identification of components was accomplished by computer searches in a HP Mass Spectral Library NIST 98 (Hewlett Packard , Palo Alto , California , USA) .

III.2. Isolation and purification of secoiridoids from the stem bark .

III.2.1. Extraction .

The aerial parts (stem bark) of *F. xanthoxyloides* (1kg) were air – dried and powdered by an electric mill .The plant material was subjected to exhaustive extraction with hot ethanol (3 × 6 l) and left at room temperature for tow days in each extraction (as it is described in [53]) .The combined extracts were concentrated to dryness by using a Rotavapor to afford the total ethanol extract1 (TE1) . TE1 (123.58 g) was further used for the isolation of pure components .

III.2.2. Isolation of secoiridoids .

20.7g of the residue TE1 was dissolved in 200ml of MeOH /H_2O ,1:1. Then , the solution was extracted successively with petroleum ether (3 ×100 ml) and ethyl acetate (3 ×100 ml) .Thereby , a little amount of Na_2SO_4 was added to each fraction in order to absorb water .

After solvent evaporation under reduced pressure to dryness, extracts weighted 5.23g (PE) and 2.936 g (EtOAc) .

The EtOAc extract (2.936 g) was further subjected to vacuum liquid chromatography over 30g silica gel , using chloroform ($CHCl_3$) and $CHCl_3$ / MeOH with increasing polarity as listed in Table III.1 .

Table III .1. The solvent systems used for VLC .

Fraction	Mobile phase	Volume used (ml)
1	$CHCl_3$	300
2	$CHCl_3$/ MeOH (10:1)	200
3	$CHCl_3$ / MeOH (10:1)	200
4	$CHCl_3$ / MeOH (5 :1)	200
5	$CHCl_3$ / MeOH (5 :1)	200
6	$CHCl_3$ / MeOH (5:1)	200
7	$CHCl_3$ / MeOH (3:1)	200
8	$CHCl_3$ / MeOH (3:1)	200

Fraction 4 was concentrated under reduced pressure to give residue R–4(579.02 mg). Thereby, R–4 was subjected to column chromatography over 80 g silica gel using $CHCl_3$ / MeOH with increasing polarity (10:1 , 8 :1 , 7 :1 ,6 :1 , 5:1 , 4:1 , 2 :1) .Fractions eluted with $CHCl_3$ / MeOH (6:1) were combined and concentrated under reduced pressure to give R – 5 (156.58 mg) .A part of the residue R- 5 (56 mg) was subjected to preparative TLC using $CHCl_3$ / MeOH (1: 1) as mobile phase to give residue R – 6 (44.58 mg) . The residue R – 6 was chromatographed on a silica gel column (9g) with ethyl acetate eluant solvent to give compound 1 (8.32 mg) and a residue R–7(14.32 mg) .

The residue R –7 was purified by preparative TLC in ethyl acetate system (3 developments), to obtain compound 2 (5.92 mg) .

III.2.3. Samples preparation for GC/MS :

3mg of the dry fractions (compound 1 and 2) were dissolved in 25 µl of dry pyridine . 40 µl N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) was added and the mixture heated at 80 °C for 20 min in a screw-cap vial.

Compound 1 and 2 were identified by comparison of their spectroscopic data with those described in the literature[6,86] and by spraying with the ethanolic solution of $FeCl_3$, as well as co-TLC comparison with ligstroside and formoside authentic samples .

The findings spectroscopic data of both compound 1 and 2 are given below.

Compound 1 (ligstroside): $[\alpha]_D^{20}$ = - 181.1° (MeOH) . UV λ_{max}^{MeOH} : 227 , 280nm ; IR (KBr) cm^{-1}: 3550- 3250 , 1700 , 1630 , 1504 . ^{1}H- NMR (CD_3OD)ppm : δ 7.52 (1H, s, H3), 4.80 (1H,d , J = 7.6 Hz , H 1′) , 6.06 (1H, br q , J = 7.1 Hz , H8) , 1.63 (3H , dd , J = 7.1 Hz and J = 1.4 Hz , H-10) , 5.91 (1H, br s , H1), 6.72 (2H ,d , J = 8.6 Hz , H5″ and H7″) , 7.04 (2H ,d , J = 8.6 Hz , H4″ and H8″) , 2.69 (1H , dd , J = 14.1 Hz , and J = 4.5 Hz , H_a6) , 2.45 (1H , dd , J = 14.1 Hz and J = 9.2 Hz , H_b6) , 3.95 (1H , dd , J = 9.2 Hz , J = 4.5 Hz , H5) , 4.21 (1H, dt , J = 10.7 Hz , and J = 7.0 Hz , H_a1″) , 4.12 (1H, dt , J = 10.7 Hz and J = 7.0 Hz , H_b1″) , 2.82 (2H , t , J = 7.0 Hz , H2″) , 3.71 (3H ,s , OMe) . ^{13}C – NMR (CD_3OD) ppm : δ 95.2 (C1) 155.2 (C3), 109.5 (C4) 31.9 (C5), 41.3 (C6), 173.3 (C7), 125.0 (C8), 130.1 (C9), 13.6 (C10),168.7 (C11),100.9 (C1′), 74.8 (C2′), 78.5 (C3′), 71.6 (C4′), 78.0 (C5′), 62.8 (C6′), 67.0 (C1″), 35.2 (C2″),130.5 (C3″), 131.1 (C4″), 116.4 (C5″), 157.2 (C6″), 116.4 (C7″), 131.1 (C8″).

Aglucone MS:

m/z (%): 362(30) $[M^{+\cdot}]$, 361(90) $[M^{+\cdot} –H]$, 331(11) $[M –CH_3O]^+$, 193(100) $[M – CH_3O – C_8H_{10}O_2]^+$, 223(1) $[M^{+\cdot} –H- C_8H_{10}O_2]$, 73(42) $[^+Si(CH_3)_3]$.

Glucose MS :

m/z (%) : 204(100) $[M.TMS – TMSOCH_2 -2TMS]^+$, 305(3) $[M.TMS- TMSOCH_2 – 3 CH_3]^+$, 217(22) $[M.TMS - TMSOCH_2 -3 CH_3 –TMS –CH_3]^+$, 73(25) $[^+Si(CH_3)_3]$.

4 –Hydroxyphenylethanol :

m/z (%) : 282(22) $[M.TMS]^+$, 267(18) $[M.TMS - CH_3]^+$, 193(15) $[M.TMS – OTMS]^+$, 179(100) $[M.TMS- TMSOCH_2]^+$, 91(1) $[M.TMS - TMSOCH_2 -OTMS]^+$, 73(26) $[^+Si(CH_3)_3]$.

Compound 2 (formoside) : $[\alpha]_D^{28}$ = - 143° (MeOH) . IR (KBr) cm^{-1}: 3410 , 1739 , 1703 , 1631 , 1508 . ^{1}H- NMR(CD_3OD)ppm : δ 7.57(1H, s, H3) , 1.75 (3H , dd , J = 7.1 and 1.4 Hz , H10) , 3.74 (3H , s ,OMe) , 4.84 (1H ,d , J = 7.6 Hz , H1′) , 6.04 (1H , brs , H1) , 6.17 (1H , qd , J = 7.1 and 1.0 Hz , H8) , 7.01 (2H , J = 8.6 Hz , H5″ and H7″) , 7.25 (2H , J = 8.6 Hz , H 4″ and H8″), 3.75 (2H , t , J = 6.9 Hz , H1″), 2.82 (2H , t , J = 6.9 Hz, H2″) , 2.96 (1H ,dd ,J = 14.5 and 4.6 Hz , H_a6), 2.73 (1H ,dd , J = 14.5 and 9.1Hz , H_b6), 4.12 (1H ,dd , J = 9.1 and 4.6 Hz, H5). ^{13}C-NMR (CD_3OD) : 95.4 (C1), 155.4 (C3), 109.4 (C4), 31.9 (C5), 41.2 (C6), 171.8 (C7), 125.2 (C8), 130.7 (C9), 13.9 (C10), 168.8 (C11),52.1(OMe), 101.1 (C1′), 71.5 (C2′),78.5 (C3′), 78.0 (C5′), 62.7 (C6′), 64.2 (C1″),39.6 (C2″), 138.3 (C3″), 131.0 (C4″),122.6 (C5″), 150.6 (C6″), 122.6 (C7″), 131.0 (C8″).

Aglucone MS:

m/z (%): 362(36) $[M^{+\cdot}]$, 361(100) $[M^{+\cdot} –H]$, 331(26) $[M –CH_3O]^+$, 193(1) $[M – CH_3O – C_8H_{10}O_2]^+$, 223(1) $[M^{+\cdot} –H- C_8H_{10}O_2]$, 73(28) $[^+Si(CH_3)_3]$.

Glucose MS :

m/z (%) : 204(100) $[M.TMS –TMSOCH_2 -2TMS]^+$,305(3) $[M.TMS- TMSOCH_2 –3CH_3]^+$, 217(22) $[M.TMS - TMSOCH_2 -3 CH_3 –TMS –CH_3]^+$, 73(34) $[^+Si(CH_3)_3]$.

4 –Hydroxyphenylethanol :
m/z (%) : 282(23) [M.TMS]$^+$, 267(19) [M.TMS – CH_3]$^+$, 193(16) [M.TMS –OTMS]$^+$, 179(100) [M.TMS- $TMSOCH_2$]$^+$, 91(1) [M.TMS - $TMSOCH_2$ -OTMS]$^+$, 73(31)[$^+Si(CH_3)_3$].

III.3. Isolation and purification of hydroxycoumarins from the leaves.

III.3.1. Extraction

Extraction of the air –dried and well ground plant material (300 g) was carried out with methanol for four times (4 × 500 ml) at room temperature for five days . The combined extracts were concentrated to dryness under reduced pressure to give 57.82g of the total methanol extract 2 (TE2) .

III.3.2. Isolation of hydroxycoumarins

30g of the residue (TE2) was dissolved in 300 ml of the solvent system MeOH /H_2O(1:2) .The resulting solution was extracted successively with Et_2O (3 × 300 ml) , EtOAc (3 × 200ml) and n – BuOH (3 × 200ml) . After solvent evaporation under reduced pressure , extracts weighted : 6. 57g (EtOAc) and 9.91g (n-BuOH).

The ethyl acetate extract (1g) was subjected to column chromatography over silica gel (100g) using $CHCl_3$ and $CHCl_3$ /MeOH with increasing amount of MeOH (39:1 , 37:3 , 36:4 , 34:6 , 32:8 , 30: 10) .Fractions of 100 ml were collected .Fractions eluted with $CHCl_3$ / MeOH (34 : 6) were monitored by TLC in P3 and thereby , similar fractions were combined to give fraction 1 which was concentrated under reduced pressure to a small volume and the deposited solid filtered to give the residue R –1 (12.9 mg), which was purified by preparative TLC in P3 (2 developments) to produce compound 3 (1.32mg) .

The identification of compound 3 was achieved by direct comparison with cichoriin authentic sample (UV , ^1H – NMR and CO – TLC) .
The findings data are given below.

Compound 3 (cichoriin) : $[\alpha]_D^{15}$ = -97.60° (MeOH) . UV λ_{max}^{MeOH} : 230 ,253 , 290 and 345 nm . ^1H- NMR(CD_3OD)ppm : δ 6.17 (1H, d, J = 9.5 Hz ,H3) , 7.7 (1H, d, J = 9.5 Hz , H4) , 6.89 (1H, s, H8) , 7.08 (1H, s, H5) , 4.85 (1H, d, J =7.17 Hz, H1′) .

III.4. GC/MS analysis of the volatiles of the leaves and the *bark of F . xanthoxyloides* .

TE1 (2g) and TE2 (1.83g) were dissolved in 150ml of MeOH / H_2O (2 :1) solvent system , respectively . Therefore, the resulting solutions were subjected to solvent - solvent partitioning using $CHCl_3$ (3 ×20 ml). Thereafter , the combined extract were concentrated to dryness under reduced pressure to give 0.486g (chloroform extract of TE1) and 1.28g (chloroform extract of TE2) .

A part of the chloroform extracts of the studied samples (300 mg from each sample) was subjected to a four hours distillation –extraction in Licknes –Nickerson apparatus [94] . The volatile compounds were extracted from the distillate with diethyl ether (3 × 50 ml) . Yields: leaves of *F .xanthoxyloiides* 15.37 mg (5.1 % of the lipophylic extract) .Stem bark of *F . xanthoxyloides* 9 mg (3 % of the lipophylic extract).

The two samples were investigated by analytical GC / MS . The identification of components was accomplished by computer searches in a HP Mass Spectral Library NIST 98 (Hewlett Packard , Palo Alto, California , USA) . Some of the GC/MS peaks remained

unidentified , because of the lack of authentic samples and library data of the corresponding compounds .

A listing of the components that have been observed in the volatiles of the two fractions along with their retention times is given in Table II.1.

– Hexadecanoic acid methyl ester (R_t = 27.08 min) .

m/z (%) : 270(12)$[M]^{+\cdot}$, 74(100) [M - CH_3 $(CH_2)11C_2H_3]^{+\cdot}$, 239(9) [M – CH_3O $]^+$, 227(20) [M – C_3H_7 $]^+$, 213(3) [M – C_3H_7-CH_2 $]^+$, 199(7) [M –C_3H_7 –$C_2H_4]^+$, 185(8) [M – C_3H_7- 3 CH_2 $]^+$, 171(7) [M – C_3H_7 – $4CH_2$ $]^+$, 157(2) [M – C_3H_7- $5CH_2$ $]^+$, 143(24) [M – C_3H_7-$6CH_2$ $]^+$, 129 (9) [M – C_3H_7-$7CH_2$ $]^+$, 115(3) [M – C_3H_7- $8CH_2$ $]^+$, 101(7) [M – C_3H_7- $9CH_2$ $]^+$, 87(79) [M – C_3H_7-$10CH_2$ $]^+$, 55(20) [M – C_3H_7-$11CH_2$ $]^+$.

-Carbomethoxy- 5 –vinylpyridine (R_t = 16.40) .

m/z (%) : 163(100) $[M]^{+\cdot}$, 132(100) [M –CH_3O] $^{+\cdot}$, 104(64) [M – CH_3O –CO]$^+$, 77(26) [M – CH_3O –CO–HCN]$^+$, 51(19) [M – CH_3O –CO-HCN –C_2H_2]$^+$, .

- 2,6- Bis(1,1-dimethylethyl) – 4- methylphenol (R_t = 19.72 min) .

m/z (%) : 220(28) $[M]^{+\cdot}$, 205(100) [M –CH_3]$^+$, 177(8) [M –CH_3–$C_2H_4]^+$, 145(10) [M –OH–CH_3]$^+$, 117(1) [M –OH-CH_3–$C_2H_4]^+$.

- Ferruginol (R_t = 33.09 min) .

m/z (%) : 286(100) $[M]^{+\cdot}$, 271(100) [M –CH_3]$^+$, 243(8) [M –C_3H_6-H]$^+$,229(16) [M –C_3H_6-CH_3]$^+$,215 (16) [M-C_3H_6-H-CO]$^+$,201(43) [M –C_3H_6-C_3H_7]$^+$,189(62) [M-C_3H_6-H-CO-C_2H_2]$^+$,175(57) [M-C_3H_6-C_3H_7-C_2H_2]$^+$.

Conclusion

This thesis was mainly concerned with the isolation and structure elucidation of secoiridoid glucosides active compounds from the Algerian plant *Fraxinus xanthoxyloides* Wall..This species which belong to the genus Fraxinus (Oleaceae family) is reported to contain oleoside type secoiridoid glucosides.

Our phytochemical investigations carried on F.xanthoxyloides species led to the isolation of two secoiridoid glucosides : ligstroside and formoside from the total ethanolic exctract of the stem bark .Together with the hydroxy coumarin glucoside cichoriin from the total methanolic extract of the leaves .

The identification of the isolated compounds was accomplished by direct comparison of the obtained spectral data (UV, IR ,^{1}H and ^{13}C-NMR) with those described in the literature as well as direct comparison with authentic samples .

Furthermore, a total of 31 compounds were identified from the leaves and 27 compounds from the stem bark by using analytical GC/MS for the analysis of the two volatile fractions.The major groups of constituents identified were aromatic alcohols and ethers (37.3%) ,aliphatic acids and esters (26.3%) ,aliphatic alcohols (10.3%), diterpenes (8.3%) and sesquiterpenoides (2.5%) from the leaves and aromatic alcohols and ethers (43.8%) , aliphatic acids and esters (28.2%) , diterpenes (1.5%) from the stem bark.

Spectre

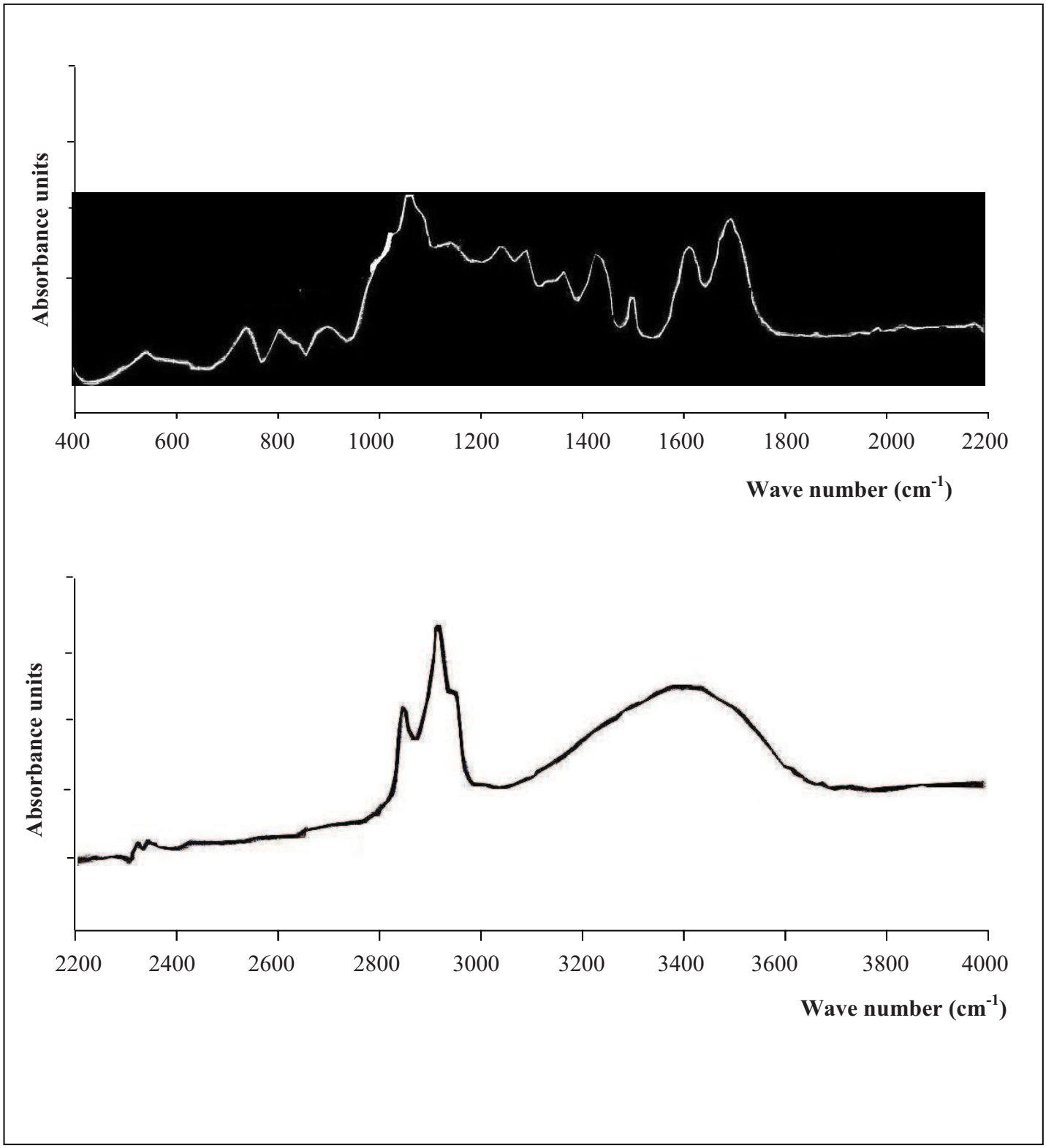


Fig.II. 2. IR (KBr) spectrum of compound 1(ligstroside).

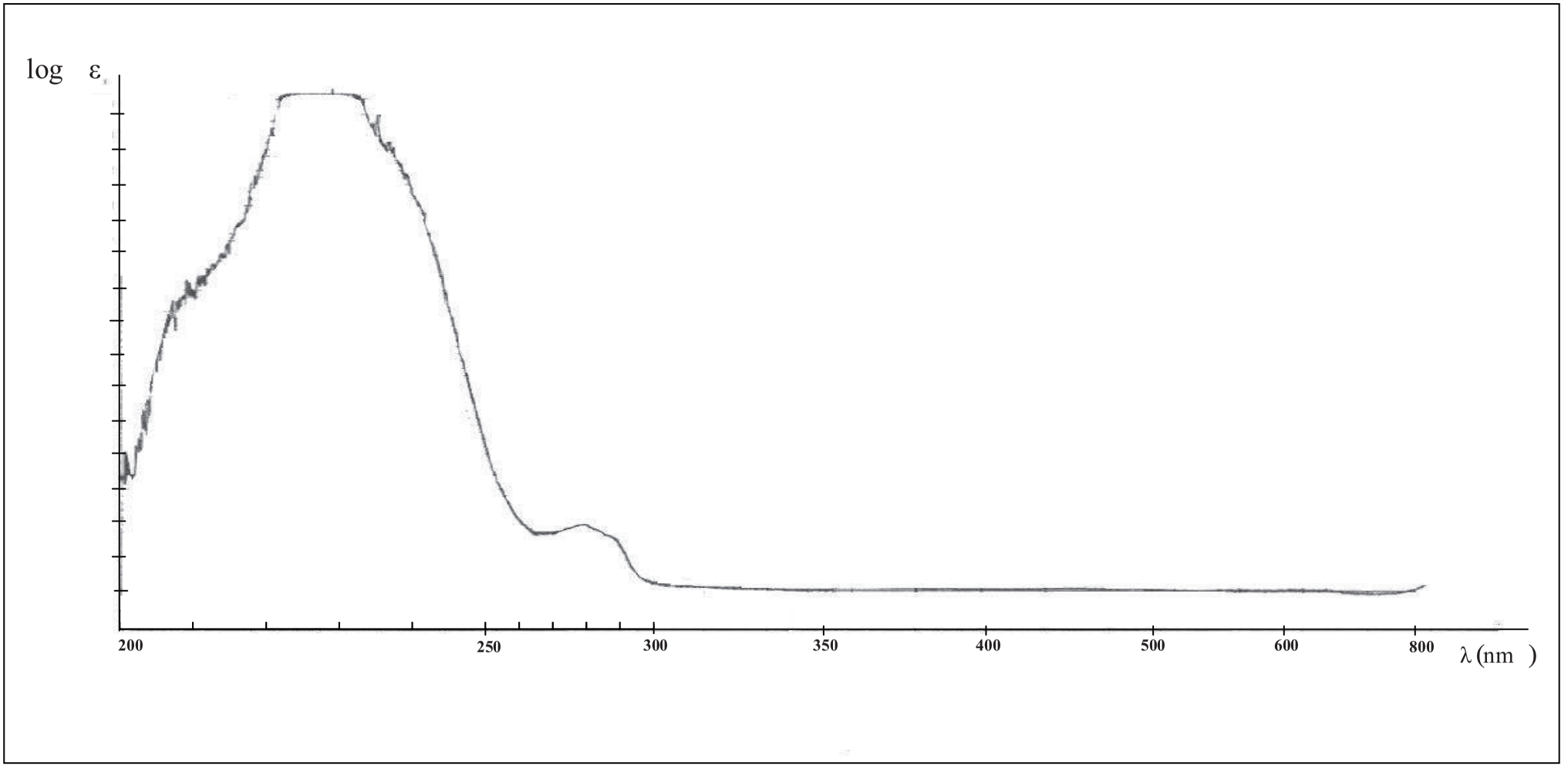


Fig .II .1 .UV spectrum of compound 1 (ligstroside) (MeOH)

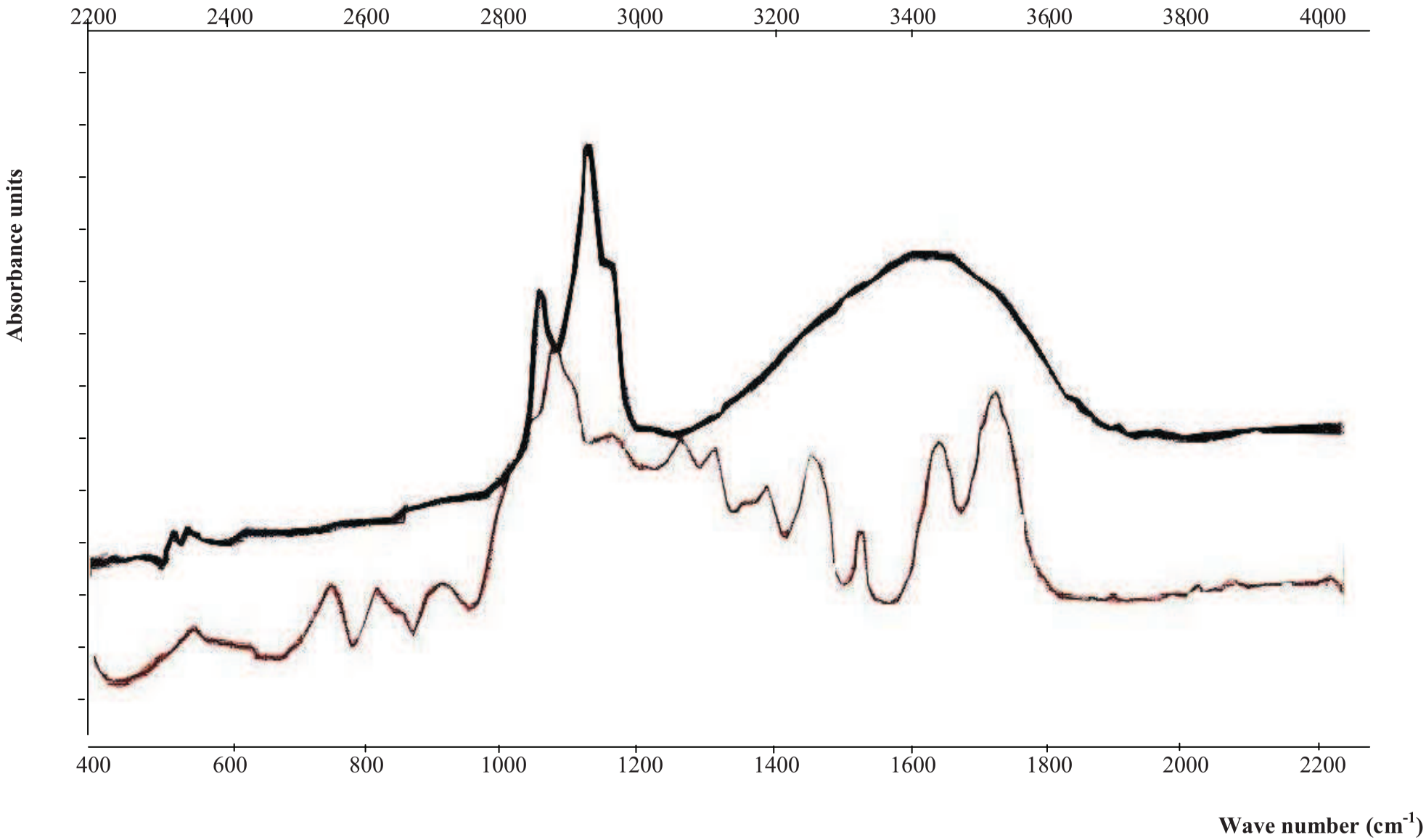


Fig.II. 2. IR (KBr) spectrum of ligstroside isolated compound

Table I. 5 : Secoidoich Isolated Hitherto From Framings species

Section	Species	Secoiricloid glycosides																																									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	
Ornus	**F.formosana Hayata**	+								+	+		+		+	+	+	+																										54,83
	F.malacophyllab Hemsl									+	+	+	+	+			+																											79,31
	F.ornus L.	+		+														+	+	+			+	+	+			+																52,84,85
	F.insularis Hemsl.			+																								+	+															31,86
	F.japonica Blume.			+																																								78
	F.chinensis Roxb.			+	+								+																						+									87
Fraxinaster	**F. excelsion L.**	+	+	+																						+	+						+	+										48,29,4
	F. americana L.	+	+	+			+		+												+	+				+						+	+										+	88,89
	F . whclei Lingelsh.	+	+			+							+															+	+	+	+					+								90,31
	F . Oxycarpa Wild.	+	+	+		+																															+	+	+	+	+			52,58
	F . angustifolia Vah	+		+				+																																+	+	+		46,58,6
	F. pallisiae Wilmott	+	+	+																						+																		31,52

1 Ligstroside
2 Hychoxgligstroside
3 Oleuropein
4 Neocleuropein
5 Hyclroxgoleuropein
6 Demethylligstroside
7 Ligsttral
8 Hychoxyoleuropein
9 Isoligustroside
10 Isoligustrosiche acid
11 Butylisoligusiosidate
12 Fraxiformoside
13 Fraximalacoside
14 Formoside
15 O-B-D- glucosylformoside
16 O-B-D- glucosylfraxiformoside
17 Framoside
18 Hydroxyframoside A
19 Hydroxyframoside B
20 Fraxamoside
21 Frameroside
22 Escuside
23 Ornoside
24 Hydroxyornoside
25 Oleoside chimethylester
26 B-D- glucosylpyranosyl oleoside
27 Insularoside
28 Insularoside-6-O-B-D-ghcoside
29 Uhdoside A
30 Uhdoside B
31 Gl3
32 Gl5
33 Excelsioside
34 frachinoside
35 Fraxuhdoside
36 Fraxicarboside A
37 Fraxicarboside B
38 Fraxicarboside C
39 Angustifolioside A
40 Angustifolioside B
41 Angustifolioside C
42 nirezhenide
43
44

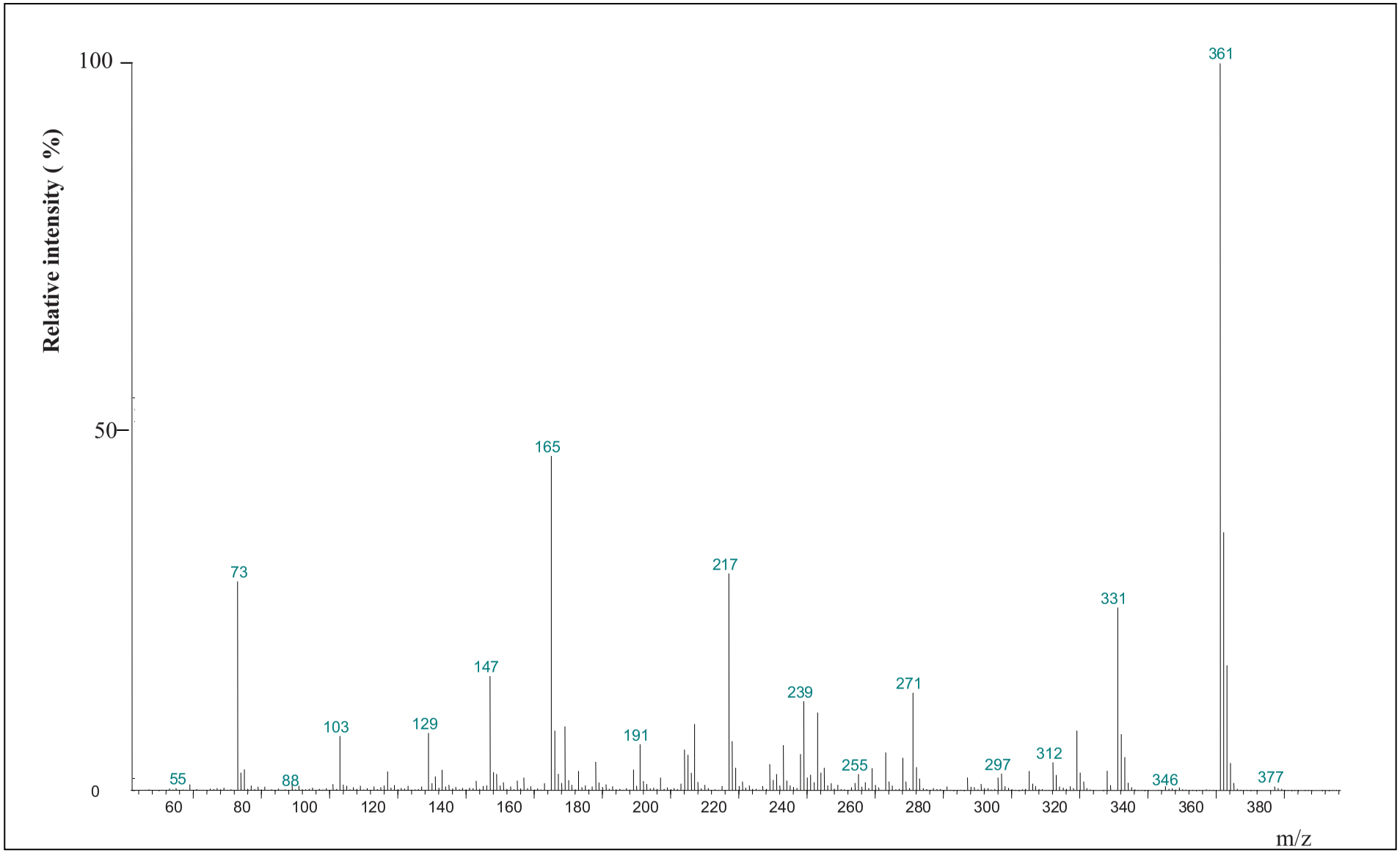


Fig. II. 12 . Mass spectrum of formoside aglucone moiety (EI , 70 eV)

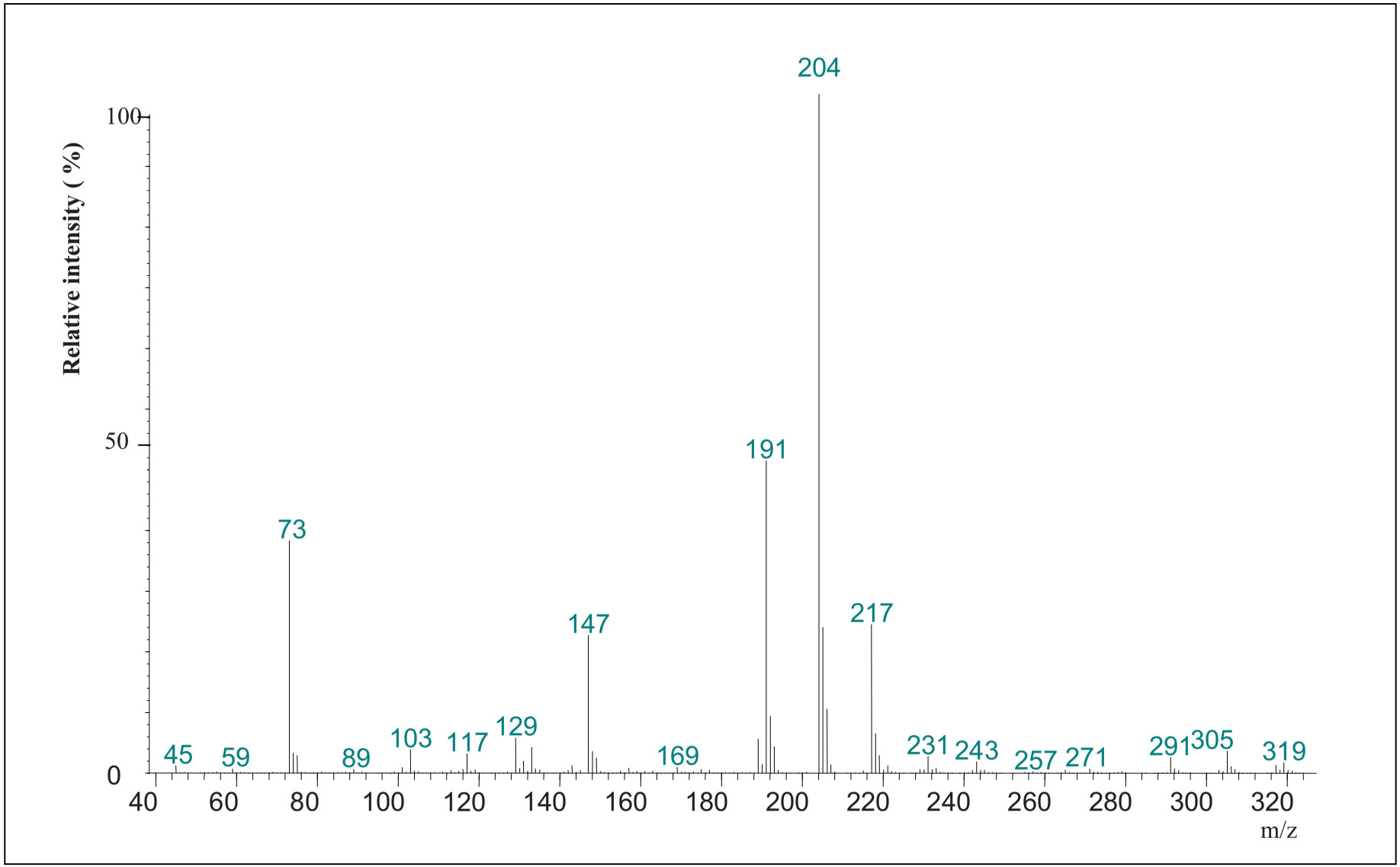


Fig. II. 13 . Mass spectrum of the glucose moiety (EI , 70 eV)

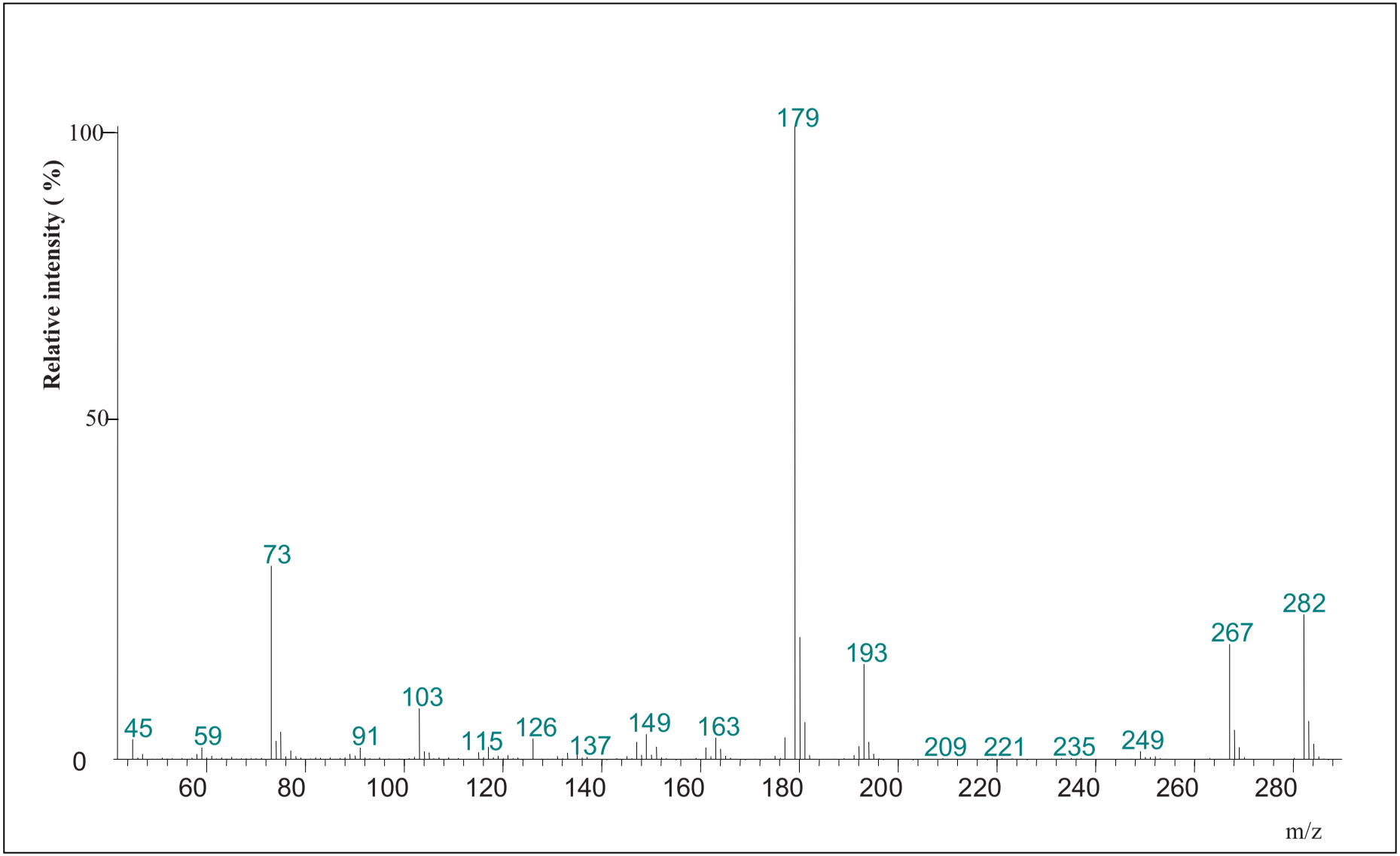


Fig. II. 14 . Mass spectrum of 4-Hydroxyphenyl ethanol moiety (EI , 70 eV)

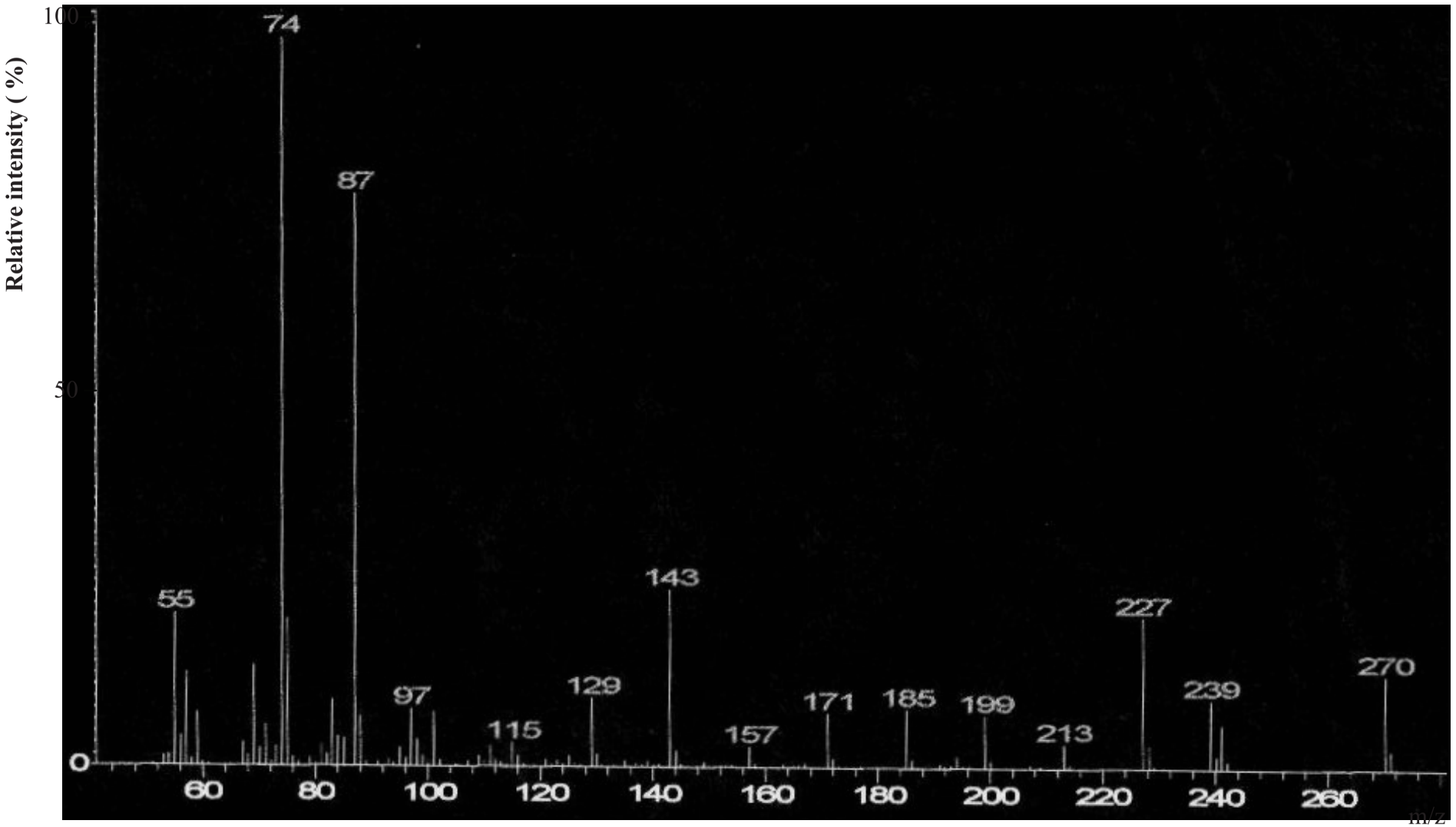


Fig. II. 17 . Mass spectrum of Hexadecanoic acid methyl ester volatile compound (EI , 70 eV)

Fig. II. 18. Mass spectrum of Carbomethoxy-5-vinyl pyridine volatile compound (EI , 70 eV)

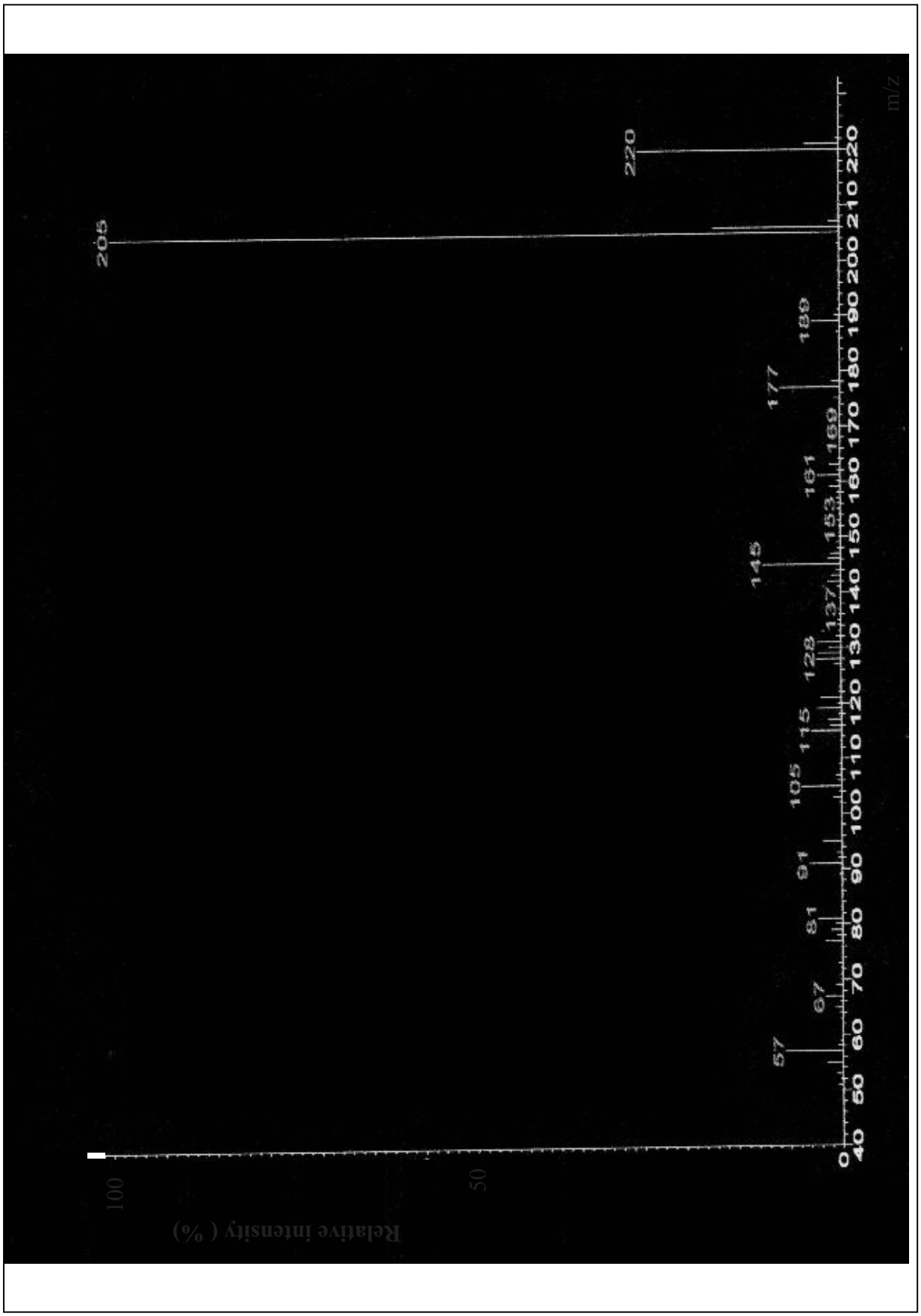


Fig. II. 19 . Mass spectrum of 2,6-Bis(1,1-dimithylethyl) –4-methylphenol volatile compound (EI , 70 eV)

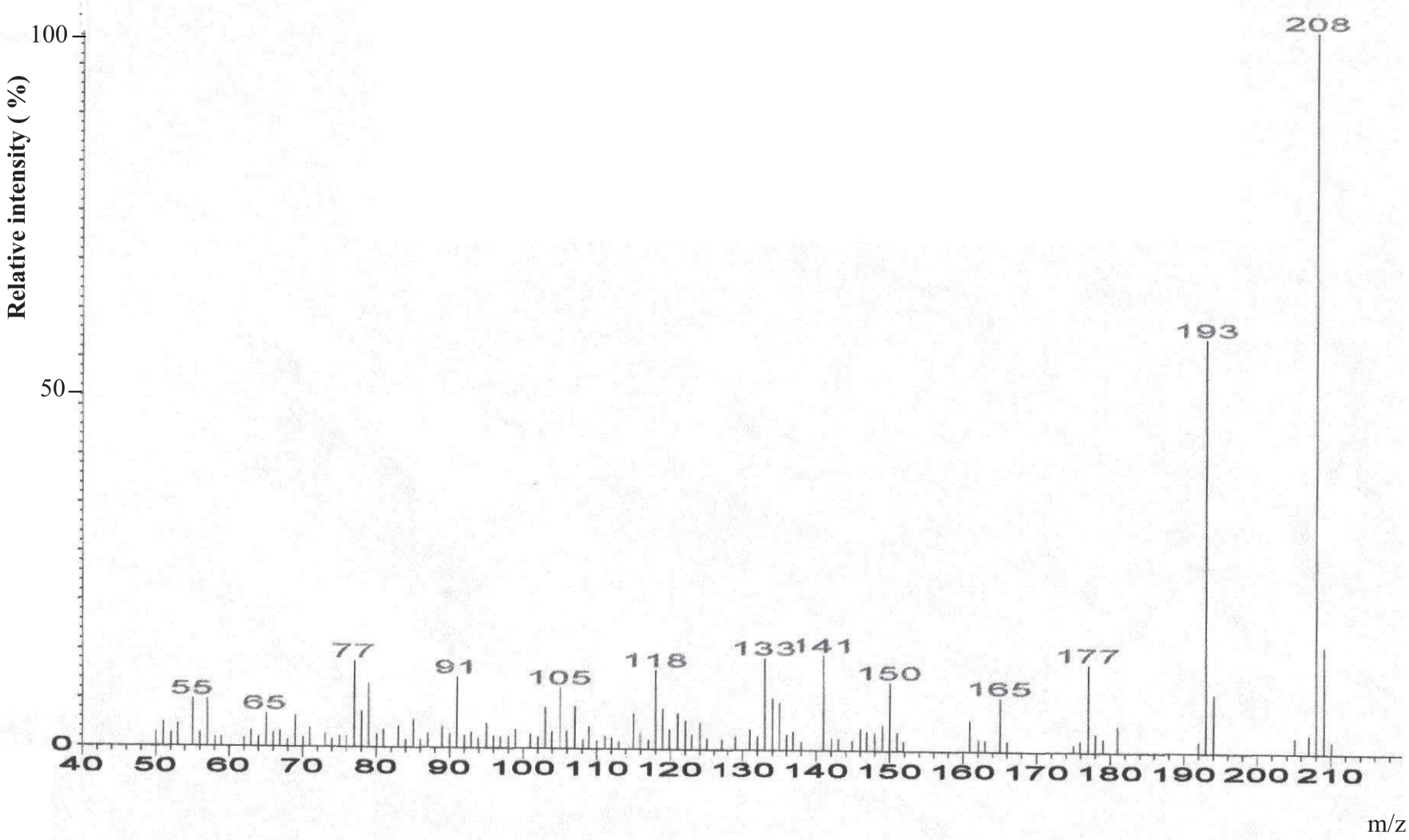


Fig. II. 20 . Mass spectrum of Elemicin volatile compound (EI , 70 eV)

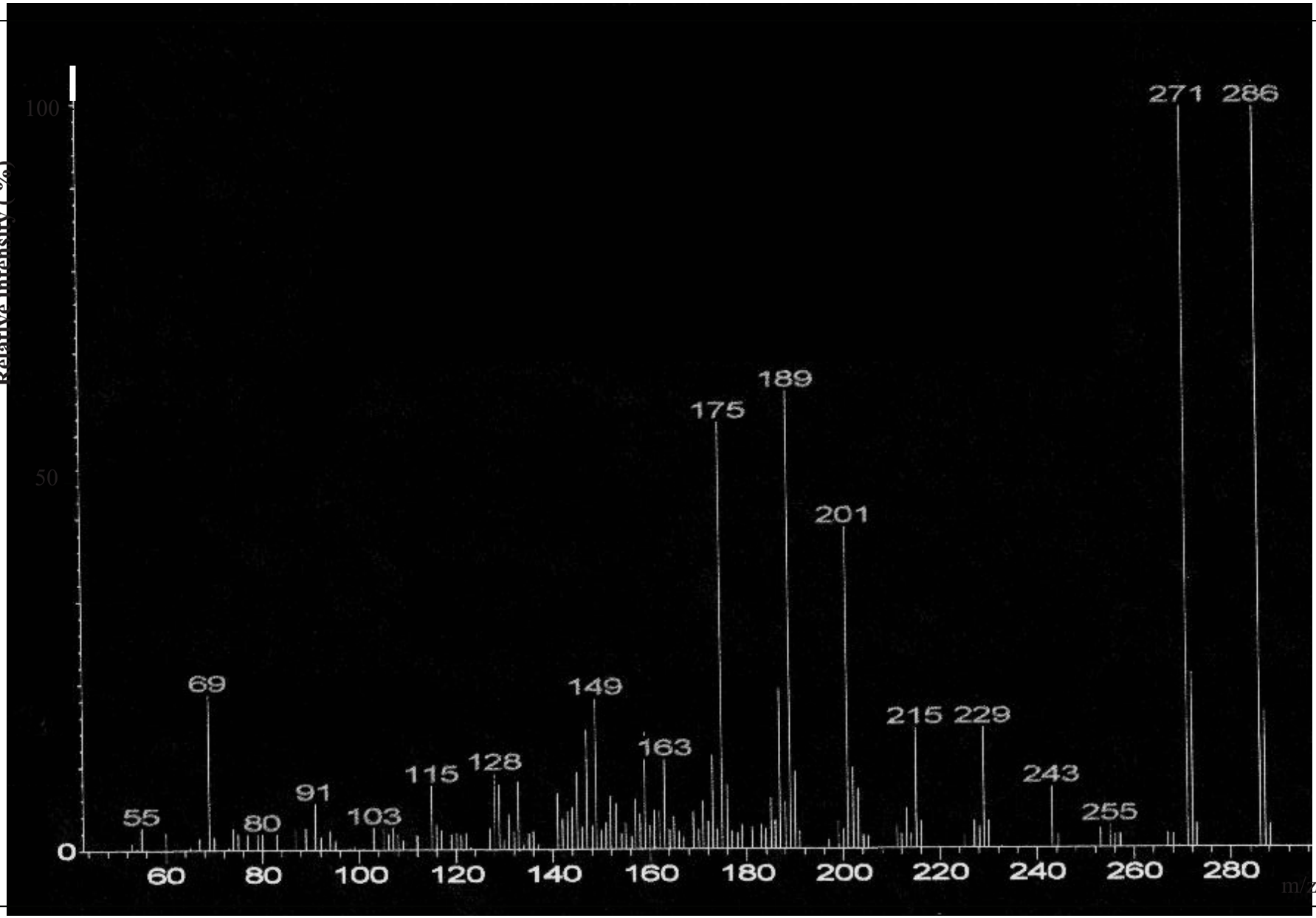


Fig. II. 20 . Mass spectrum of Ferruginol volatile compound (EI , 70 eV)

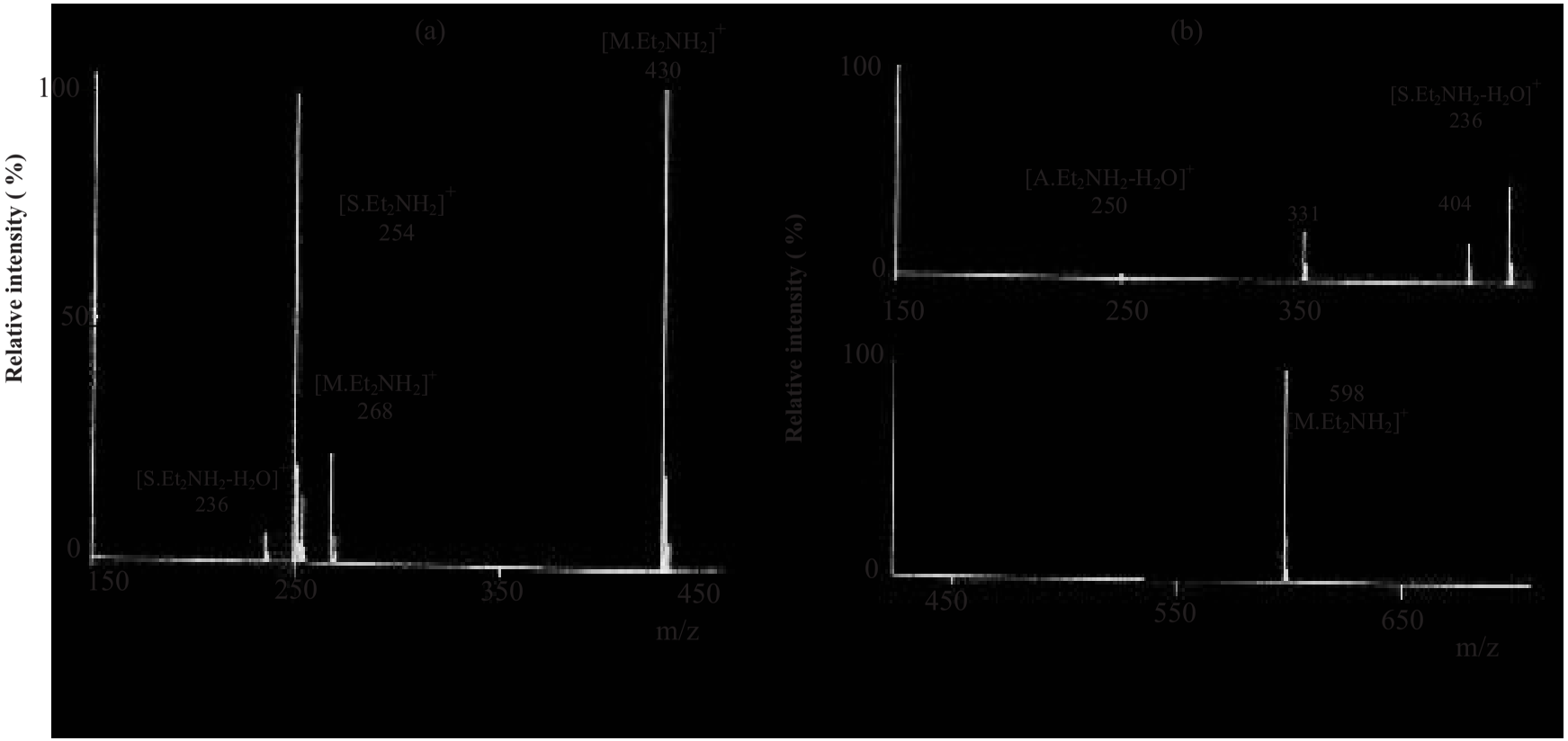


Fig. I. 13 . CI mass spectrum (DEA) of gentiopicroside (a) and its tetraacetate (b) .

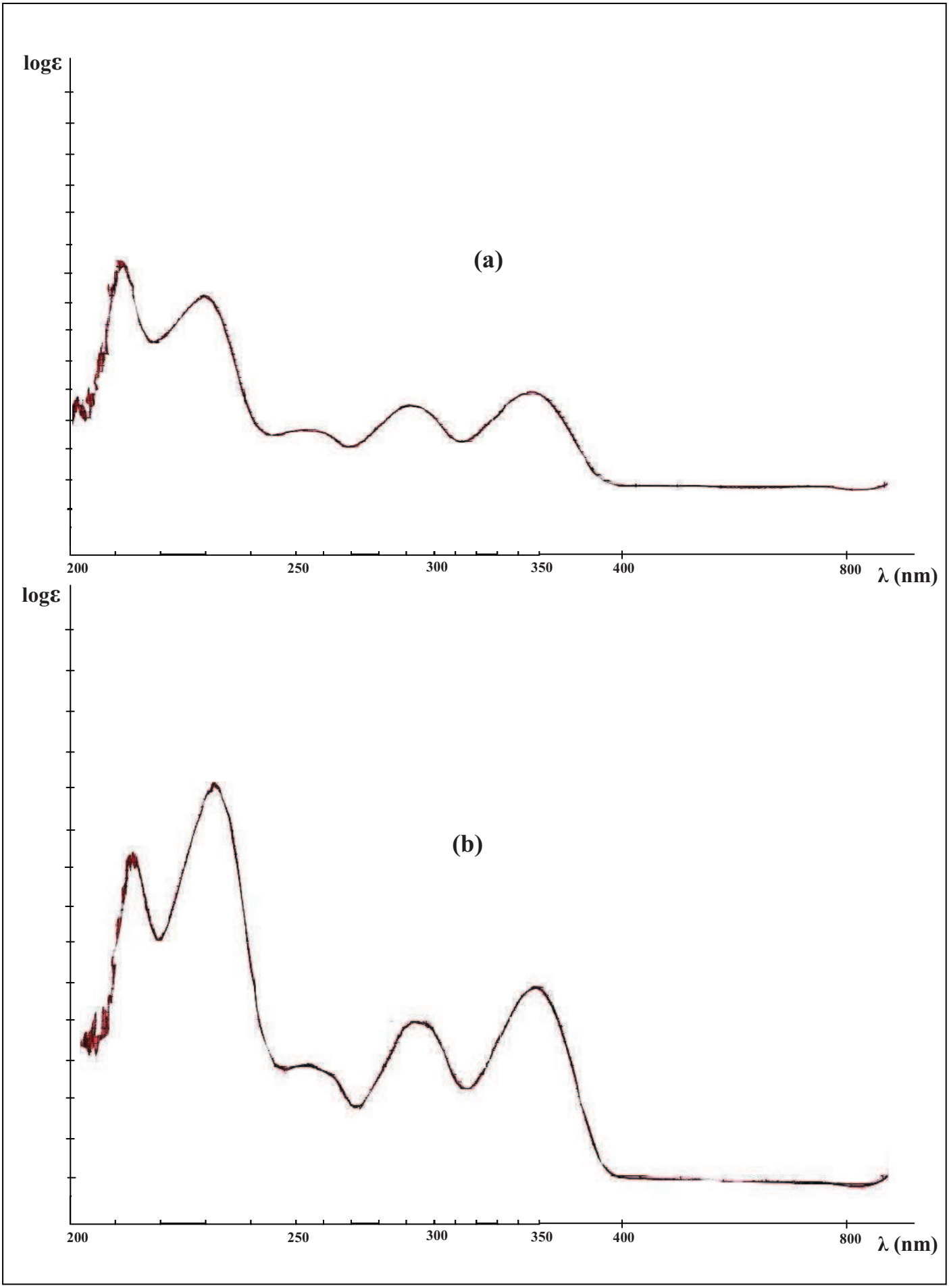


Fig .II.15. UV spectrum of cichoriin authentic sample (a) and compound -3 (MeOH) .

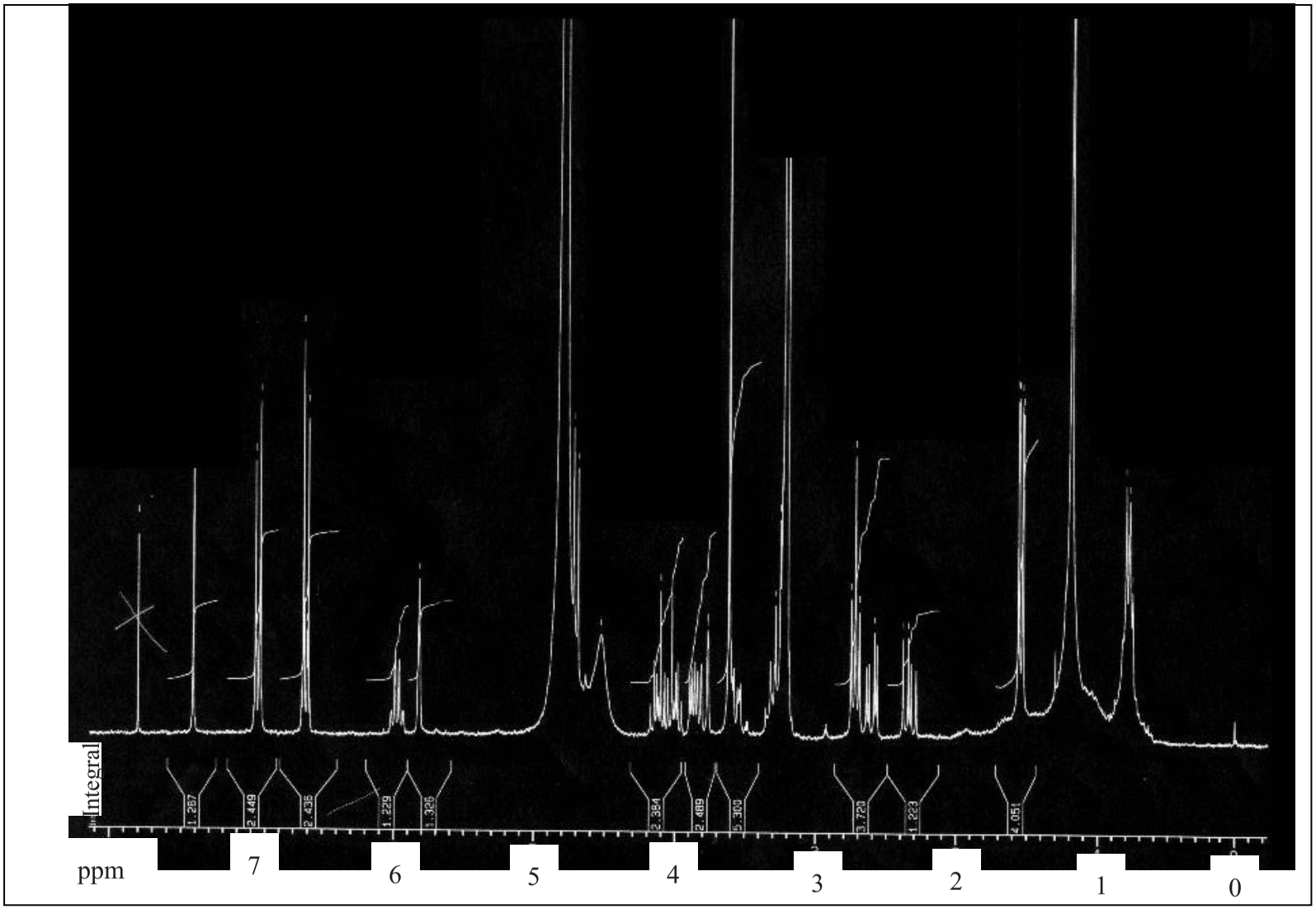


Fig. II. 3. ^{1}H-NMR spectrum of compound 1 (ligstrroside) (250 MHz , CD_3OD)

N.B : To obtain the correct chemical shifts (0.1ppm) is added to the spectrum values.

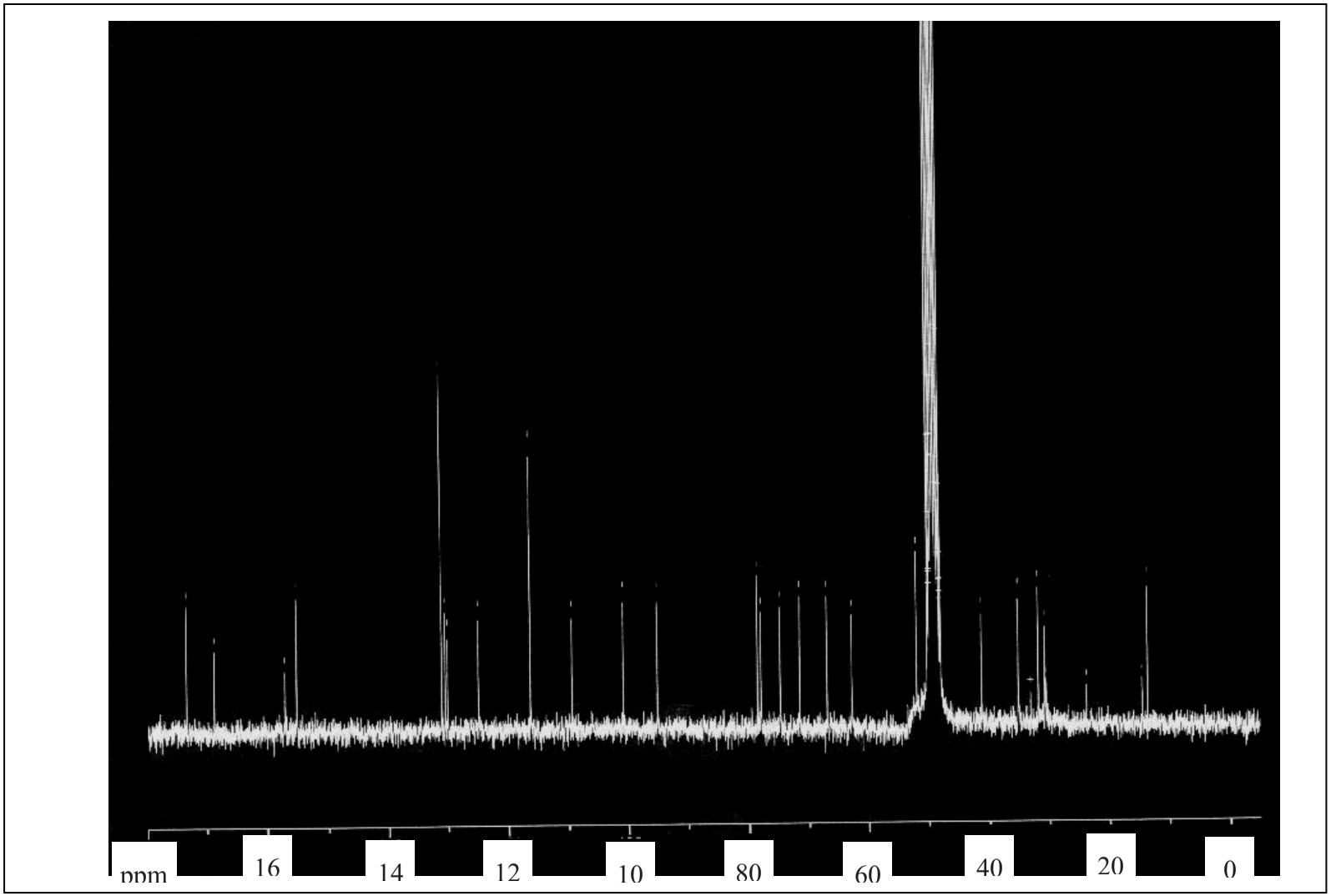


Fig. II.4 . ^{13}C-NMR spectrum of compound 1 (ligstrroside) (62.9 MHz , CD_3OD)

N.B : To obtain the correct chemical shifts (0.1ppm) is added to the spectrum values.

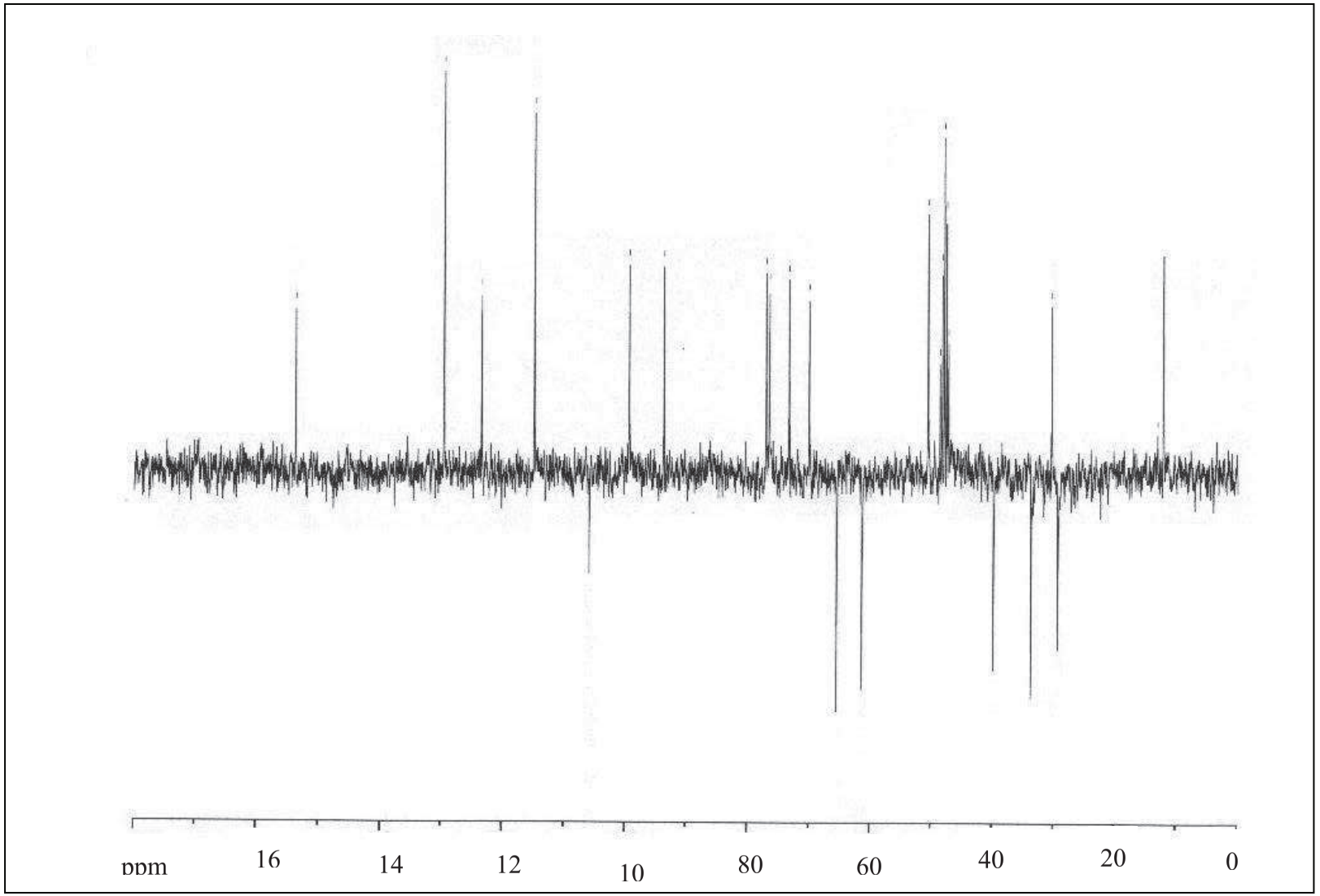


Fig. II. 5 . DEPT spectrum of compound 1 (ligstrroside) (62.9 MHz , CD_3OD)

N.B : To obtain the correct chemical shifts (0.1ppm) is added to the spectrum values.

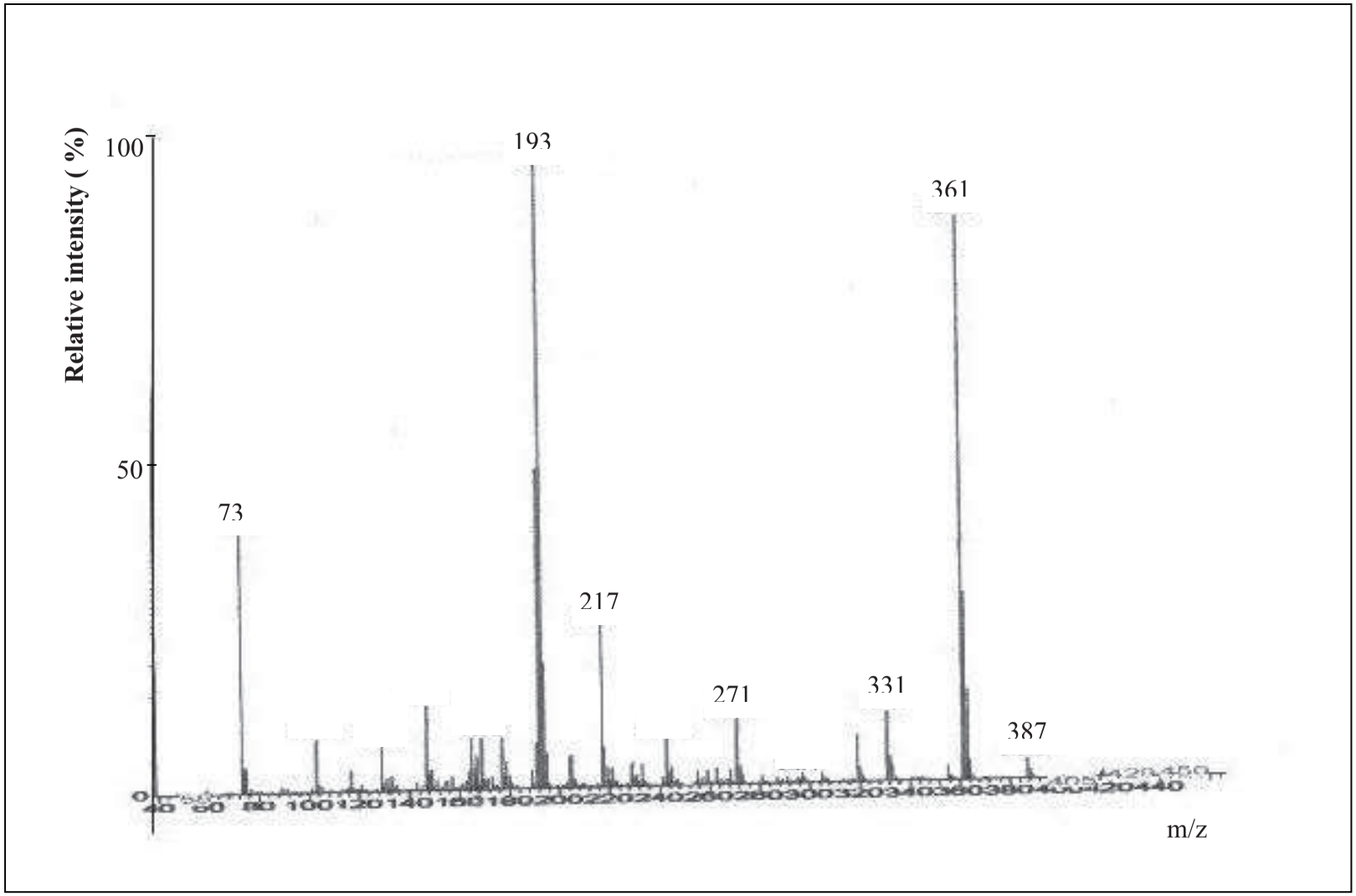


Fig. II. 6 . Mass spectrum of the aglucone moiety (EI , 70 eV)

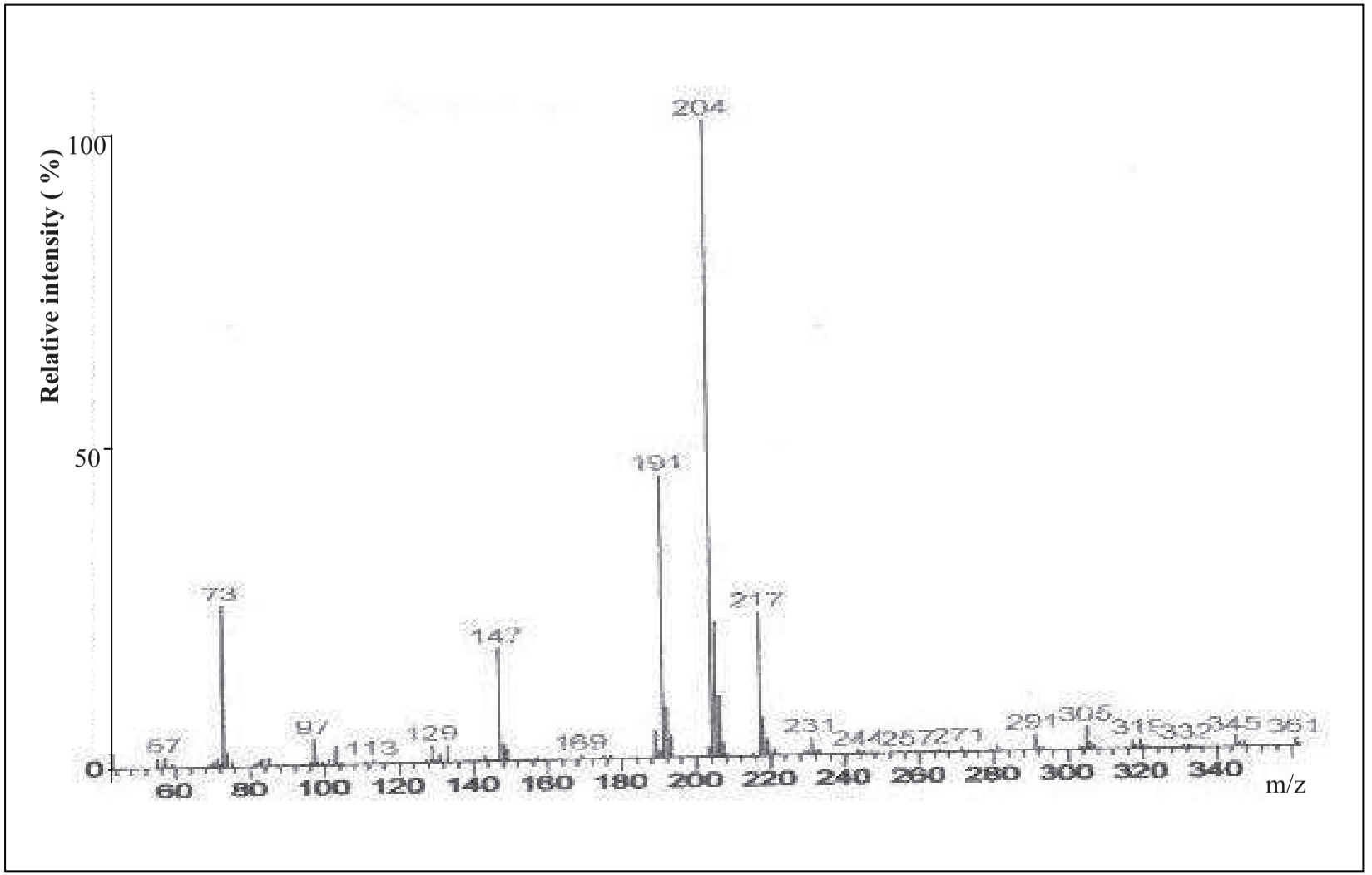


Fig. II. 7 . Mass spectrum of the glucose moiety (EI , 70 eV)

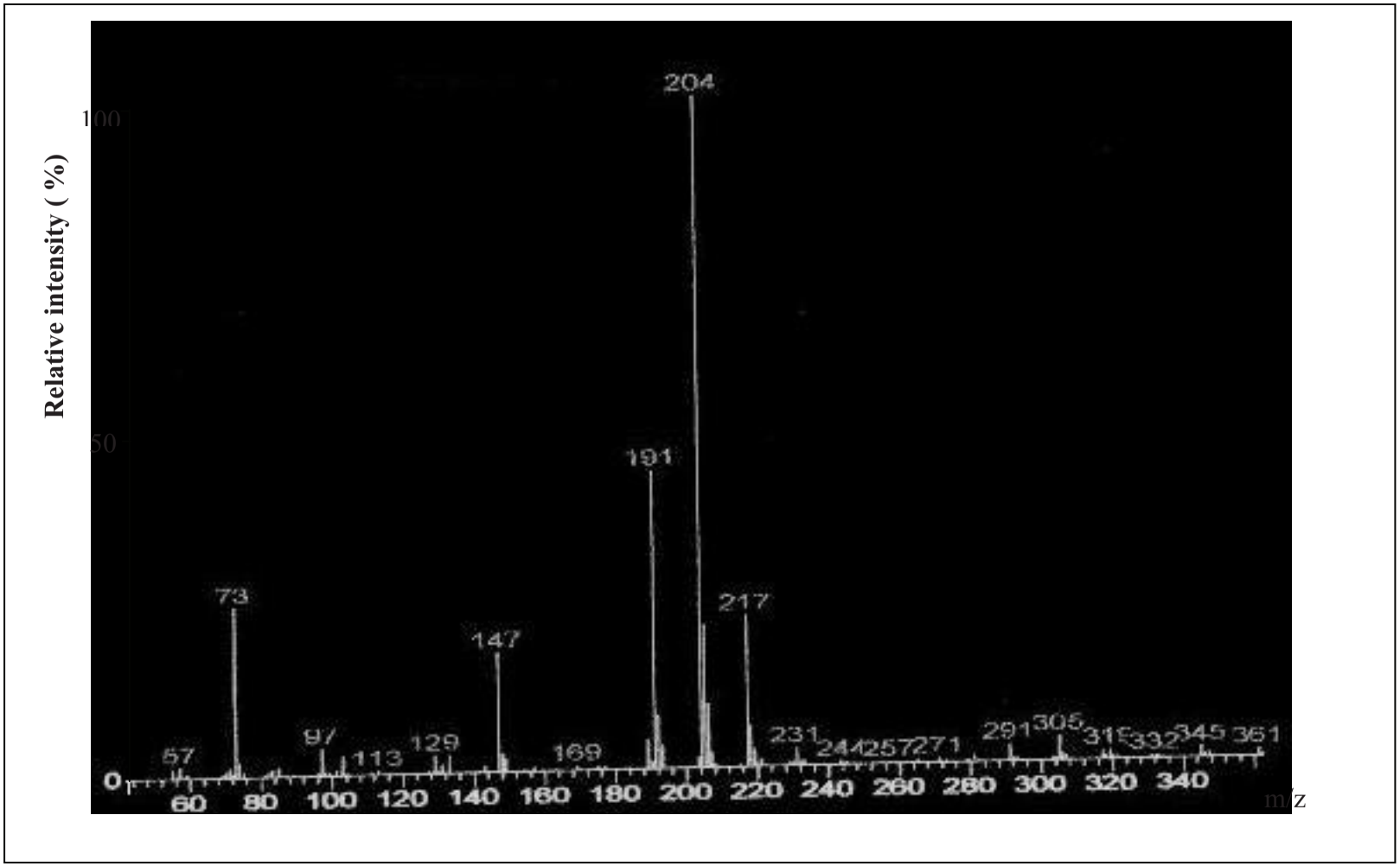


Fig. II. 8 . Mass spectrum of 4-Hydroxyphenyl ethanol moiety (EI , 70 eV)

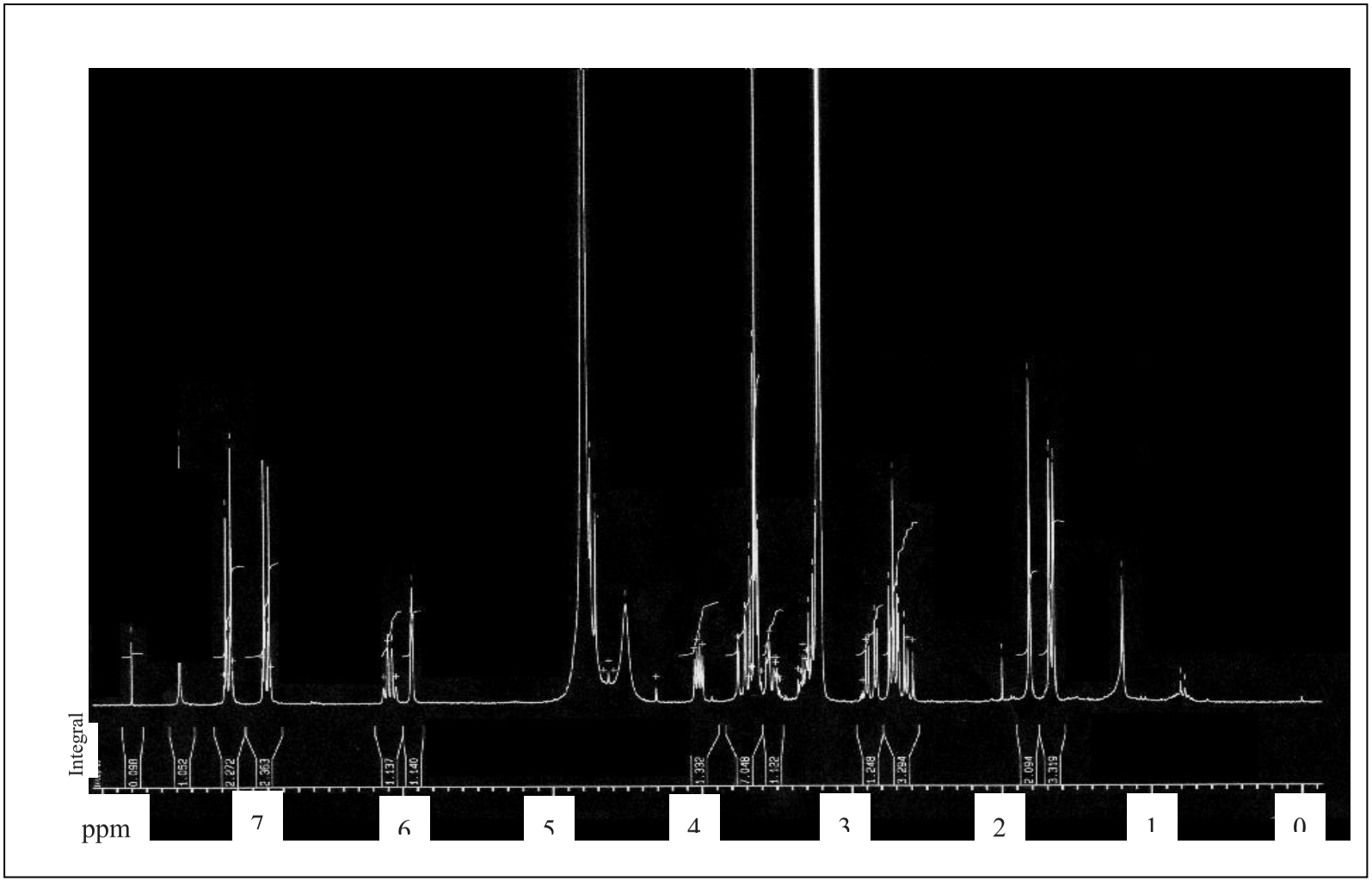


Fig. II. 9. ^{1}H-NMR spectrum of compound 2 (formoside) (250 MHz , CD_3OD)

N.B : To obtain the correct chemical shifts (0.1ppm) is added to the spectrum values.

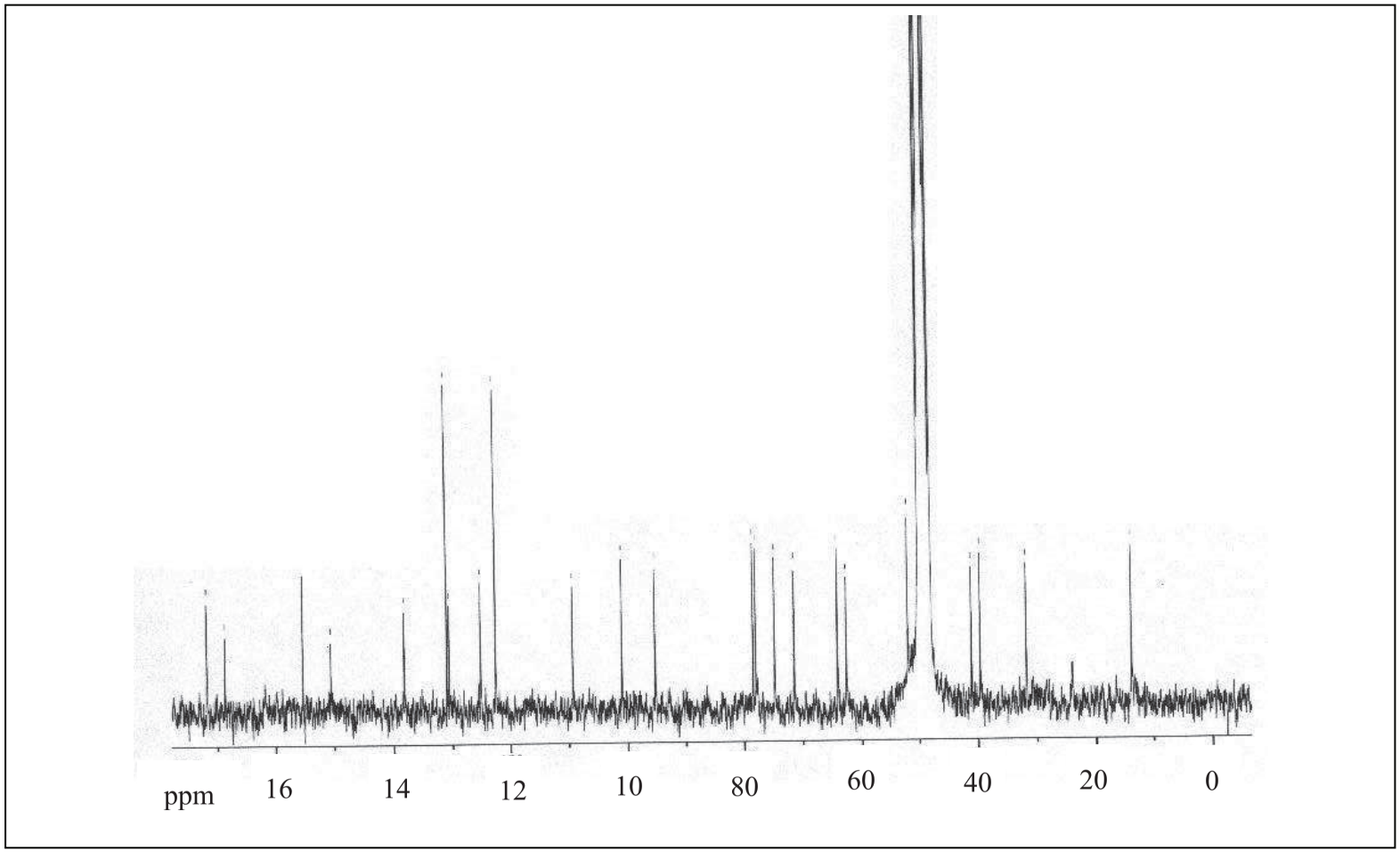


Fig. II.10 . ^{13}C-NMR spectrum of compound 2 (formoside) (62.9 MHz , CD_3OD)

N.B : To obtain the correct chemical shifts (0.1ppm) is added to the spectrum values.

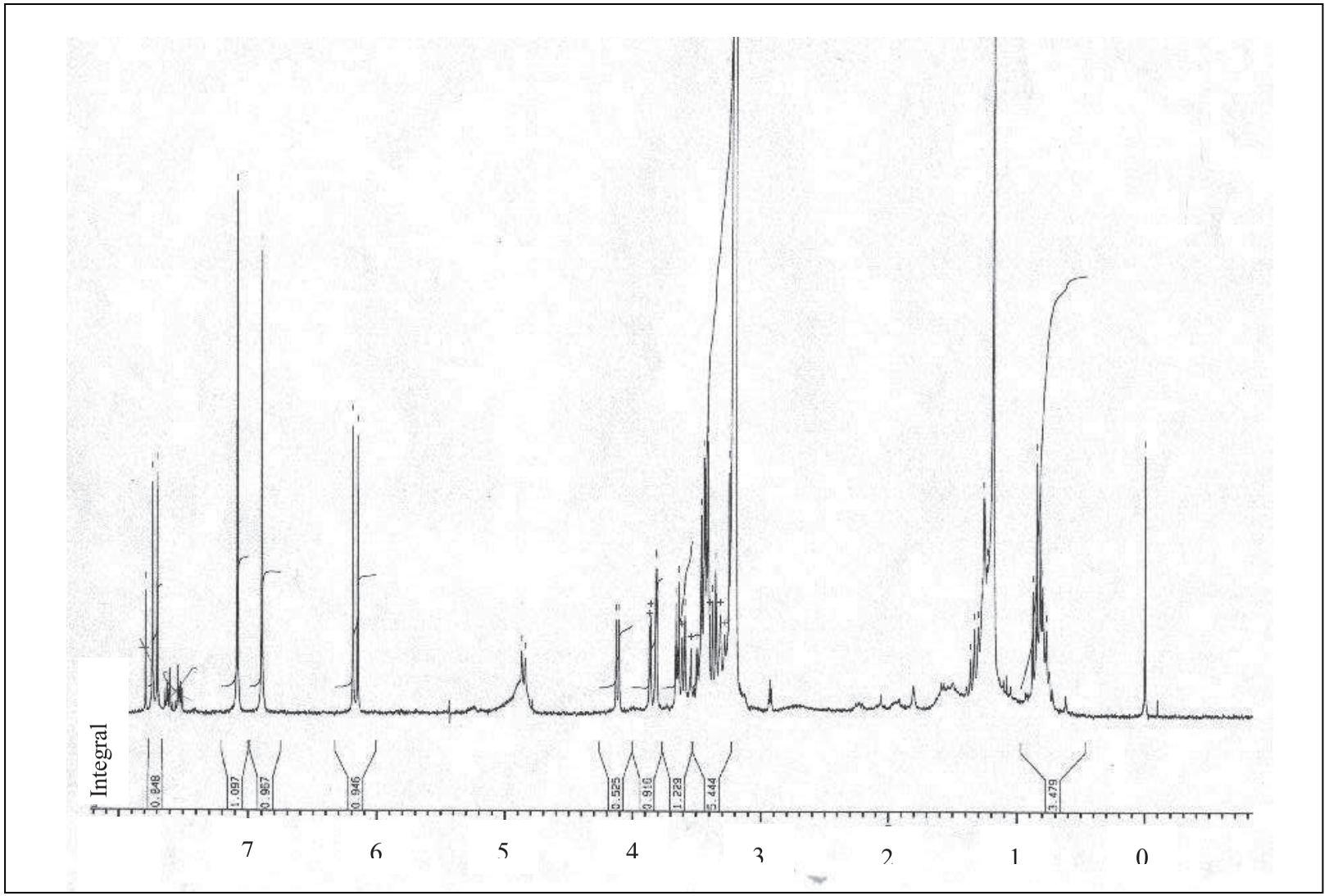


Fig. II. 16. ^{1}H-NMR spectrum of compound 3 (cichoriin) (250 MHz , CD_3OD)

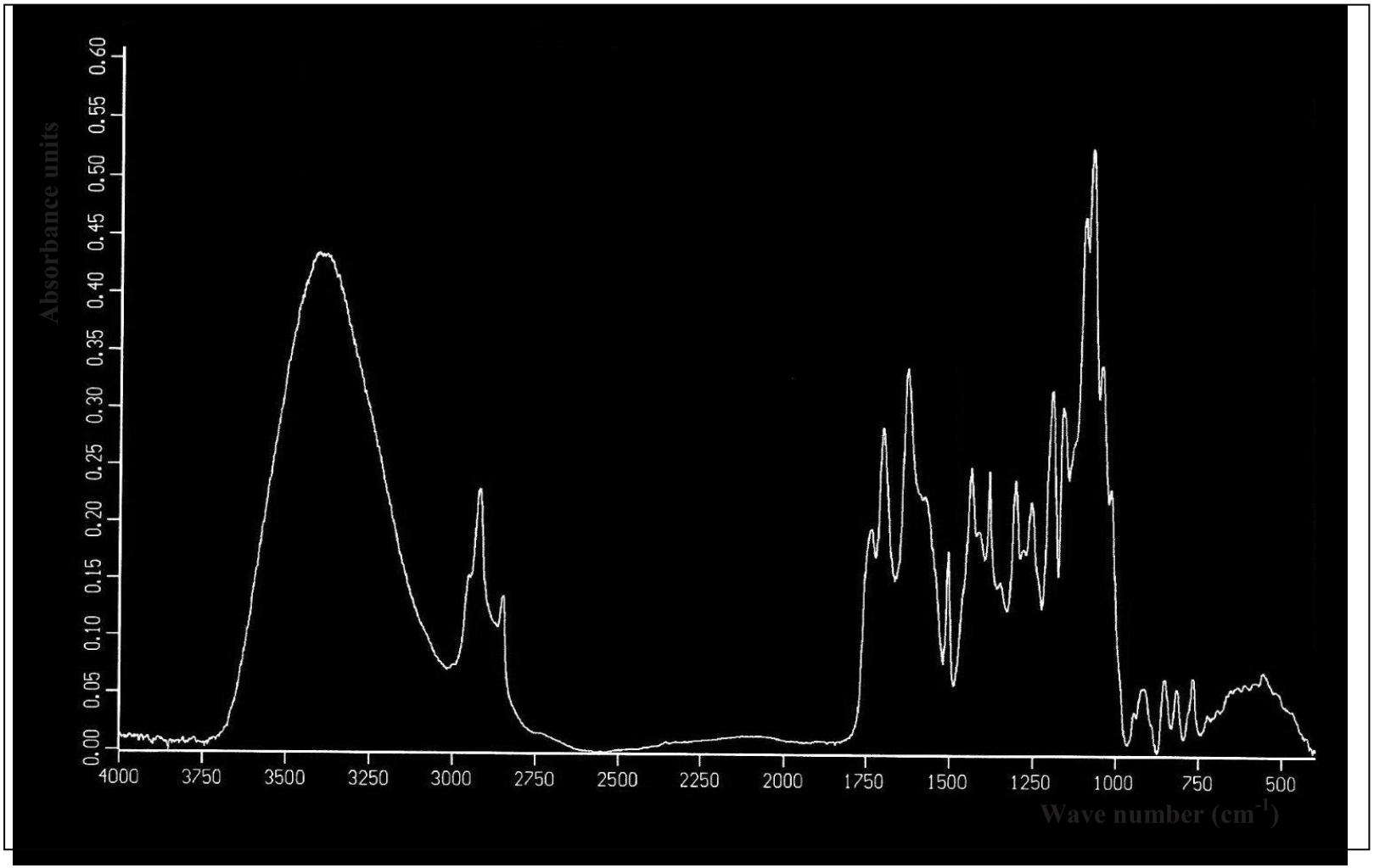


Fig.II. 11. IR (KBr) spectrum of compound 3 (formoside)

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Abbreviations

A	=	Aglucone
CC	=	Column Chromatography
CI	=	Chemical Ionization
DCE	=	Dichloroethane
DEA	=	Diethylamine
EI	=	Electron Ionization
GC	=	Gaz Chromatography
Glu	=	Glucose
HPLC	=	High Pressure Liquid Chromatography
IR	=	Infra-red
Ival	=	Isovaleroyl
L.F.	=	Leaves of Fraxinus
LVC	=	Liquid Vacuum Chromatography
M	=	Molecule
MS	=	Mass Spectroscopy
NMR	=	Nuclear Magnetic Resonance
Ph	=	Phenyl
PG	=	Prostaglandin
RP	=	Reversed Phase
TE1	=	Total Ethanol Extract 1
TE2	=	Total Methanol Extract 2
TEA	=	Triethylamine
TLC	=	Thin Layer Chromatography
PTLC	=	Preparative Thin Layer Chromatography
S	=	Sugar
S.B.F	=	Stem Bark of Fraxinus
TMS	=	Tetramethylsilyl
UV	=	Ultra-violet .

الملخص :

في هذا العمل درسنا المركبات السيكواريدويدية لنبات Fraxinus (عائلة الزيتونيات) المعروفة بفعاليتها البيولوجية المتعددة. لهذا الغرض تم اختبار كل من قشور الساق وكذلك أوراق النبات الجزائري *"Fraxinnus xanthoxyloides Wall."* أين تم فصل مركبين من السيكواريدويدات هما ligstroside و formoside من القشور في حين تم فصل الكومارين cichoriin من الأوراق. الصيغة المفصلة لهذه المركبات تمت معرفتها عن طريق الطرق الطيفية ***(UV , IR , ^{1}H and ^{13}C- NMR)***.

ومن جهة أخرى التحليل بواسطة **GC/MS** لمستخلصين أحدهما من الأوراق والآخر من قشور الساق سمح لنا بتشخيص 31 مركب من الأوراق و 27 مركب من القشور .

Abstract

In this work , we have been interested by secoiridoid compounds .These molecules are Known for their various biological activities .Our interest has been on the secoiridoid glucosides extracted from the genus Fraxinus on which many works were realized .

Our experimentation on the Algerian medicinal plan "*Fraxinnus xanthoxyloides Wall.*" permitted on one hand the isolation of two secoiridoid glucosides : ligstroside and formoside from the stem bark, together with the hydroxy coumarin glucoside cichoriin from the leaves .

The chemical structures of the isolated compounds were established by direct comparison of the obtained spectral data (UV , IR , ^{1}H and ^{13}C- NMR) with those previously reported .

On the other hand GC/MS analysis of the two fractions obtained from both leaves and stem bark of the title plant was carried out. As a result, a total of 31 compounds were identified from the leaves and 27 compounds from the stem bark .

Résumé.

L′interét de ce travail est l′étude des composés secoiridoidiques . Ces molécules sont connues par leurs différentes activités biologiques. Dans cette étude, on s'est intéressé spécialement aux secoiridoides extraites du genre Fraxinus sur lequel plusieurs travaux ont été réalisés .

Nos expériences sur la plante médicinal Algérienne *" Fraxinus xanthoxyloides Wall."* ,nous a permis d′une part d′isolé deux produits secoiridoidiques glucosidiques : ligstroside et formoside , des pelures de la tige avec le glucoside hydroxy coumarine ,cichoriin des feuilles .

Les structures chimiques de ces produits isolés sont déterminées en comparant les spectres obtenus (UV , IR , ^{1}H et ^{13}C-NMR) en basant sur les études précédentes .

D′ une autre part, on a exécuté l′analyse (GC/MS) de deux fractions obtenus l′ une des feuilles et l′autre des pelures,dont on a identifié 31 produits des feuilles , et 27 des pelures.