*ORIGINAL RESEARCH*

# GC-MS analysis of penta- and tetra-cyclic triterpenes from resins of *Pistacia* species. Part I. *Pistacia lentiscus* var. Chia

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ABSTRACT: *Pistacia* species contain oleoresins with bioactive triterpenes. In this study triterpenes, including minor components, were identified and quantified in both neutral and acidic fraction of *Pistacia lentiscus* var. Chia resin, grown exclusively in Chios island (Greece), collected traditionally, as well as by the use of stimulating agents (liquid collection). It was proved that these two resin samples were composed of several different minor triterpenes. In the traditional collection of the resin, 36 triterpenes were identified, 23 of which are new minor compounds (five in the acidic and eighteen in the neutral fraction). In the liquid collection resin eight compounds were identified in the acidic and 11 in the neutral fraction, while seven compounds were not contained in resin traditionally collected. The main triterpenes in both resin samples collected traditionally and by use of stimulating agents were in the following order: isomasticadienonic acid (24 and 22.5% w/w of triterpenic fraction respectively), masticadienonic acid (9.3 and 14.7% w/w of triterpenic fraction) and 28-norolean-17-en-3-one (19 and 36% w/w of triterpenic fraction respectively). The aim of this study was to compare the qualitative and quantitative composition of triterpenes in the resin samples collected using the traditional and new liquid techniques, and examine whether the collection technique influences the contained triterpenes in *P. lentiscus* var. Chia resin samples. Finally, since there is confusion on interpreting mass spectra of triterpenes we present an analytical review on the base peaks, main fragments and fragmentation mechanism/pattern of several skeleton penta- and tetra-cyclic triterpenes reported in *P. lentiscus* resin. Also, a biosynthetic route for triterpene skeletons contained in *P. lentiscus* resin was approached. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: triterpenes; *Pistacia*; *Helicobacter pylori*; oleanane; tirucallane; dammarane; varnishes; oleanolic acid; mass spectra; mastic gum; terpenes; terpenoids; resin

## INTRODUCTION

In continuation of our research on natural resins containing triterpenes with biological properties and technological applications (adhesives, varnishes, sealant, water proofing, etc.), we herein report the chemical composition of triterpenes of the resins of *Pistacia* species. The biological properties of *Pistacia lentiscus* resin (mastic gum) are mainly attributed to their triterpenes.

*Pistacia lentiscus* var. Chia (Anacardiaceae family) is a tree exclusively grown on the Greek island Chios. Oleoresins are obtained traditionally by longitudinal incisions in the tree in the form of tears or droplets (Zohary, 1952). Recently, the Mastic Gum Growers Association (Chios, Greece, personal communication) has introduced a new collection technique (liquid collection), where the stimulating agent ethrel (ethephon) is used for resin excretion after incision of the tree, in order to increase resin (mastic gum) productivity from *P. lentiscus*. With that method mastic gum is produced in a fluid form with a characteristic odour. Therefore, in the present study triterpenes of both neutral and acidic fractions were also identified in resins collected using the stimulating agent ethrel.

Mastic gum has been reported to possess healing properties (known from the time of Dioscurides, 70ac) (1958), including anti-inflammatory (Duke, 1983), antimicrobial (Magiatis *et al.*, 1999) and anticancer properties (Duke, 1983; Hartwell, 1967). Our group proved that these resins have antioxidant (Assimopoulou and Papageorgiou, 2005) and radical scavenging activities (Assimopoulou and Papageorgiou, submitted). Recently, the interest of several researchers has been focused on the established activity of mastic gum to kill *Helicobacter pylori* (Huwez *et al.*, 1998; Bona *et al.*, 2001), and it also possesses gastric and duodenal anti-ulcer activity (Al Said *et al.*, 1986). Its medicinal and embalming properties were known in Egypt (Duke, 1983), while it is frequently cited in cancer folklore for tumours of breast, liver, spleen, stomach and uterus, and is reportedly used in cosmetics, for boils and as a haemostatic (Duke, 1983). Food supplements have appeared in the US market, as well as cosmetics, with active ingredient mastic gum.

In this paper, we return to the subject with a thorough study of the chemical composition of *P. lentiscus* var. Chia resin, since there is a great interest worldwide in its biological properties that are attributed mainly to

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the tetra- and penta-cyclic triterpenes it contains. Thus the qualitative identification and quantitative determination of triterpenes in *P. lentiscus* var. Chia resin is of great interest. Tetracyclic triterpenes (derivatives with tirucallane and dammarane skeleton) may, due to their structure, possess corticomimetic activity.

The triterpenoid resins dammar (Dipterocarpaceae family) and *Pistacia lentiscus* (mastic gum, Anacardiaceae family) are frequently used as picture varnishes. The identification of the materials in works of art is of fundamental importance for any conservation task. One of the major challenges of analytical chemists is the recognition of organic binding media and varnishes. Thus, biomarkers of *P. lentiscus* have to be evaluated.

The purpose of this study was to compare the triterpene composition in two resin samples, collected using the traditional and new techniques, and to examine whether the stimulated excretion of resin influences the qualitative and quantitative composition of resins. This is of great interest because these resins are used in pharmaceutical and cosmetic preparations, food supplements, and since its biological activity is highly associated with triterpene composition.

The chemical composition of triterpenes of both neutral and acidic fraction of *P. lentiscus* (traditionally collected) has been reported by several authors (Barton and Seoane, 1956; Seoane, 1956; Monaco *et al.*, 1973; Mills and White, 1989; Marner *et al.*, 1991; Stern *et al.*, 2003; Papageorgiou *et al.*, 1997; Colombini *et al.*, 2000; Dietemann *et al.*, 2001). The triterpenes of *P. lentiscus* resin liquid collection have never been reported. GC-MS analysis is a powerful technique for the analysis and characterization of complex triterpene mixtures with many derivatives of several ring skeletons and also a tool to distinguish different botanical sources containing biomarkers presenting very similar triterpenoid skeletons with different molecular weights.

*Pistacia lentiscus* resin was also identified by GC-MS, on the basis of the mass spectra of triterpenes contained, in archaeological art materials, adhesives and other archaeological residues and finds (Mills and White, 1989; Stern *et al.*, 2003; Colombini *et al.*, 2000; Regert and Rolando, 2002; Scalarone *et al.*, 2003; Hairfield and Hairfield, 1990; van der Doelen *et al.*, 1998). Finally aging of this resin was studied by GC-MS analysis (Stern *et al.*, 2003; Scalarone *et al.*, 2003; van der Doelen and Boon, 2000; Zumbuhl *et al.*, 1998).

Although there have been many papers presented on the characterization of triterpenes from *P. lentiscus* resin, only its main compounds have been identified by GC-MS and there is confusion on interpreting mass spectra of unidentified triterpenic compounds. In this paper we present an analytical review of published papers on the base peaks, main fragments and mass spectral fragmentation mechanism/pattern of several

skeletons of penta- and tetra-cyclic triterpenes reported in *P. lentiscus* resin. This review is crucial in order to facilitate the identification of triterpenes in *Pistacia* species and in every species that contains triterpenes, on the basis of mass spectral interpretation, according to molecular ion (M⁺), base peak and main fragments. This study will be helpful for archaeologists and archaeological chemists who need to analyse art objects.

The aim of this study was to present a complete study on the identification of penta- and tetra-cyclic triterpenes of *P. lentiscus* resin (both traditional and liquid collection), and both main and minor components will be characterized by mass spectra interpretation; minor components are reported herein for the first time in traditionally collected *P. lentiscus* var. Chia resin. It is also the first time that the chemical composition of mastic gum obtained by use of a stimulating agent (liquid collection) is reported and a comparison of the triterpenes is performed with that of traditionally collected resin. It is essential to analyse the triterpenes contained in liquid collection resin and compare them with the respective ones in the traditionally collected resin, since the biological activity of resin may be influenced by different triterpene composition.

## EXPERIMENTAL

**Materials and methods.** *Pistacia lentiscus* var. Chia resin, collected both traditionally and using ethrel (liquid collection) were kindly provided by the Chios Mastic Gum Growers Association (Chios, Greece). Both resins were fractionated to acidic and neutral fractions according to Barton and Seoane (1956). Methylation of the acidic fraction was performed in order to improve its chromatographic behaviour as follows: 800 mg of each acidic fraction was mixed with 9 mL iodomethane 10% in AcCN in the presence of 1.5 g potassium carbonate at 60°C for 3 h (Katritzky, 1995). All samples were diluted in chloroform. All solvent used were Merck products.

**Gas chromatography and mass spectrometry.** GC-MS analysis was carried out in a Hewlett Packard HP6890 gas chromatograph coupled with a MSD 5972 mass spectrometer and equipped with a HP 5 MS 30 m × 0.25 mm × 0.2 μm capillary column. Column constant flow was set at 1 mL/min. The system was operated under the following conditions: injection temperature 250°C; transfer line temperature 290°C; oven temperature program 80(1); rate 12°C/min; final temperature 290°C; final time 30 min. The mass spectrometer was monitored to scan *m/z* 35–650 with an ionizing voltage at 70 eV.

## RESULTS AND DISCUSSION

In the present study *P. lentiscus* var. Chia resin (mastic gum) collected with the traditional technique was subjected to subfractionation (Barton and Seoane, 1956)

and then these two fractions (acidic and neutral) were analysed by GC-MS in order to identify new constituents. The structure elucidation of the triterpenes was performed using mechanisms of skeleton fragmentation of triterpenes and their main fragments, reported below, and also retention times of each ingredient. *Pistacia lentiscus* var. Chia resin, which was collected using stimulating agents (liquid collection) was analysed in order to determine whether this new collection technique affects the qualitative and quantitative composition of triterpenes.

Confusion has been observed on the evaluation and interpretation of the mass spectra for the identification of triterpenic components of resin. In several already published papers molecular weight or molecular weight and the main fragment (base peak) was used as the only criterion for the characterization of a compound (Scalarone *et al.*, 2003; van der Doelen and

Boon, 2000). Such a characterization is arbitrary since there are several triterpenic structures with the same molecular weight but different skeletons.

In this study we will try to clarify the criteria for the evaluation and interpretation of mass spectra for the identification of triterpenes in several resins, natural or synthetic products or art objects, to review the main fragmentations and match them to each skeleton.

According to all previously published papers in triterpenes from *P. lentiscus* resin (Barton and Seoane, 1956; Seoane, 1956; Monaco *et al.*, 1973; Mills and White, 1989; Marner *et al.*, 1991; Stern *et al.*, 2003; Papageorgiou *et al.*, 1997; Colombini *et al.*, 2000; Dietemann *et al.*, 2001), but also with this study we observe that generally all *Pistacia* species contain triterpene derivatives with the following skeletons (Fig. 1): 12-oleanene (I; $R_3$=CH$_3$, $R_4$=H/H, $R_1$, $R_2$ varying), 18-oleanene (II; $R_3$=CH$_3$, $R_4$=H/H, $R_1$, $R_2$ varying),
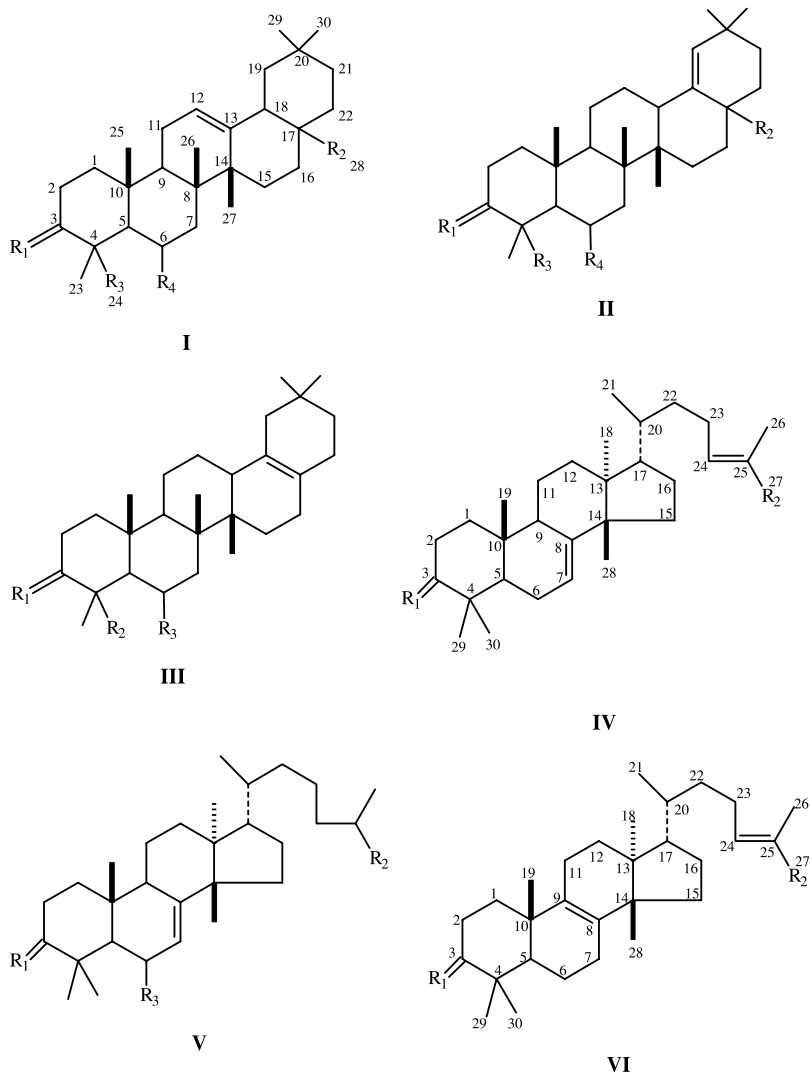


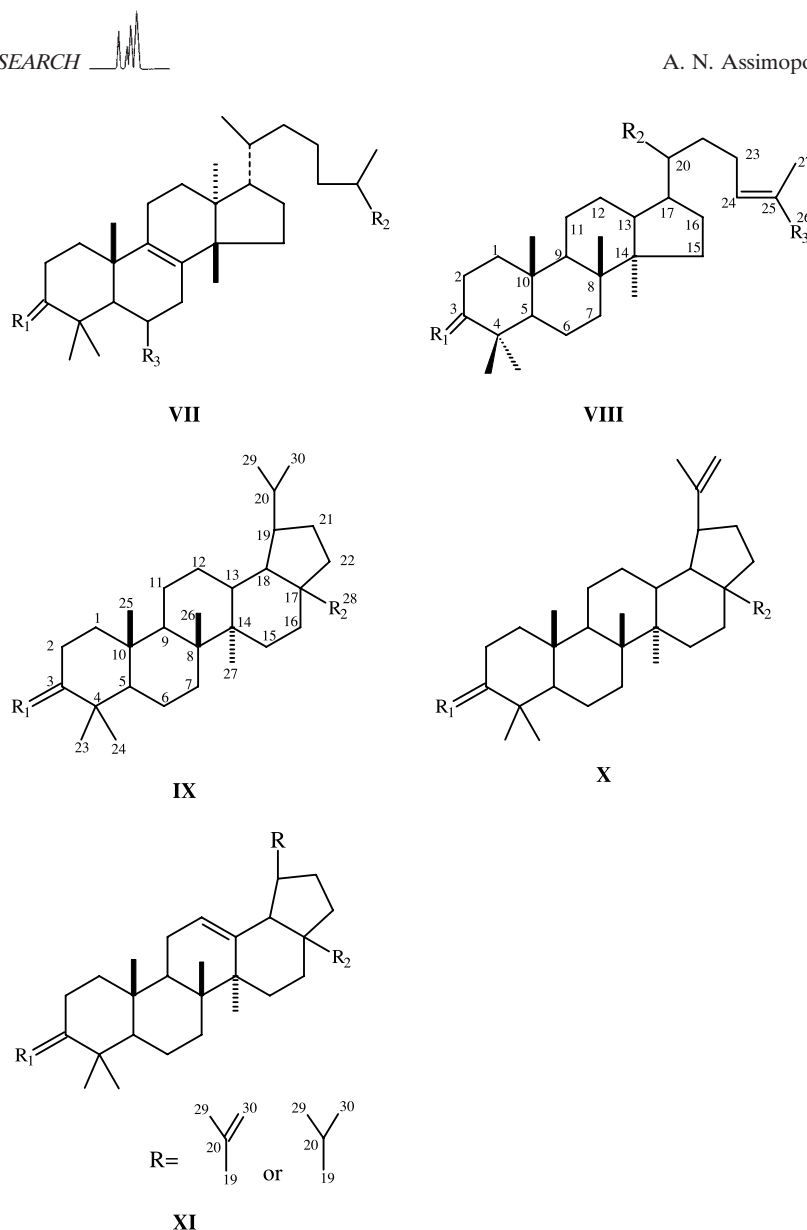**Figure 1.** Skeletons of penta- and tetra-cyclic triterpene derivatives identified in *Pistacia* species.

**Figure 1.** (*continued*)

28-nor-17-oleanene (III; R$_2$=CH$_3$, R$_3$=H/H, R$_1$ varying), 7-tirucallene (IV; R$_1$, R$_2$ varying), 24,25-dehydro-7-tirucallene (V; R$_3$=H/H, , R$_1$, R$_2$ varying), 8-tirucallene (VI; R$_1$, R$_2$ varying), 24,25-dehydro-8-tirucallene (VII; R$_3$=H/H, R$_1$, R$_2$ varying), dammarane (VIII; R$_1$, R$_2$, R$_3$ varying), lupane (IX; R$_1$, R$_2$ varying), lupene (X; R$_1$, R$_2$ varying) and 12-lupene (XI; R$_1$, R$_2$ varying), where R$_1$, R$_2$ and R$_3$ differ.

Each of the above-mentioned triterpene skeleton has its own MS fragmentation pattern, but also the nature of the R$_1$, R$_2$, R$_3$, R$_4$ substituents results in different *m/z* fragments. Thus an unidentified compound with M$^+$ 424 may present either 12-, 18-, 28-nor-17-oleanene type, lupane, lupene, 12-lupene, 7- or 8-tirucallene, or finally dammarane skeleton, and the main fragments can represent different R$_1$, R$_2$, R$_3$ or R$_4$ substituents in each skeleton.

Nortriterpenoids (mainly 28-nor; rings I or II where R$_2$=H; 12- or 18-noroleanene) present different fragmentation patterns compared with the respective triterpenes (12- or 18-oleanene). Thus main fragments, molecular ion (M$^+$), and also the characteristic *m/z* values have to be evaluated each time, in order to identify each triterpene by mass spectrometry. Below, the main fragment patterns, base peaks and mechanism of fragmentation will be analysed for each of the triterpene skeletons.

## 12-Unsaturated oleanenes and ursenes

According to Budzikiewicz *et al.* (1963), the most characteristic MS fragmentation of all compounds with a 12-unsaturated oleanene or ursene skeleton is attributed to a retro-Diels-Alder reaction in ring C, yielding
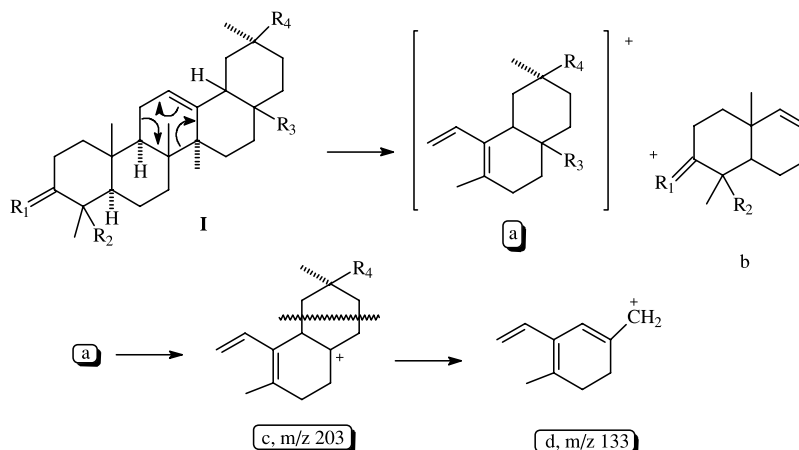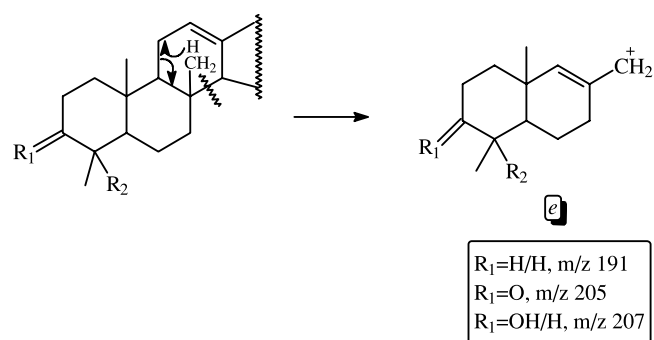
**Figure 2.** Characteristic mass fragment patterns from the upper right part of 12-unsaturated oleanene molecules, attributed to retro-Diels–Alder reaction.

species *a* (Fig. 2). This retro-Diels-Alder fragmentation is a characteristic diagnostic tool for the presence of a 12(13) double bond in triterpenes of the $\alpha$- and $\beta$-amyrin class. Ion *a* is subject to further fragmentation yielding species *c* ($m/z$ 203). Thus, in methyl oleanonate and methyl oleanolate ($R_3$=COOCH$_3$) species *a* corresponds to $m/z$ 262, while in compounds with $R_3$=$R_4$=CH$_3$ (e.g. $\beta$-amyrin, $\beta$-amyrone) it corresponds to $m/z$ 218. In each case species *c* ($m/z$ 203) is yielded, where 59 (COOCH$_3$) and 15 (CH$_3$) mass units are lost. In compounds with a methyl group as the C-17 substituent, species *a* is abundant ($m/z$ 218), whereas species *c* is not very pronounced; however in compounds with a carbomethoxy group at C-17, the intensity of species *c* equals or slightly exceeds that of species *a*. Hence, as reported (Budzikiewicz *et al.*, 1963), the relative intensities of fragments *a* and *c* offer an important indication about the nature of the substituent at C-17.

Species *c* is always accompanied by a less intense ion 14 mass units lower (203→189 $m/z$), while species *a* is accompanied by a fragment of low abundance containing 13 mass units less (e.g. $m/z$ 249 for methyl oleanonate). Species *c* suffers further decomposition by the loss of 70 mass units, yielding a fragment not abundant in most cases (fragment *d*). The above fragmentation pattern concerns the upper right of a 12-oleanene molecule. As shown in Fig. 2 (Budzikiewicz *et al.*, 1963), substitution in ring A and B (structure I) does not change the mass of fragment *a*.

There is one more fragment (*e*, Fig. 3) that is one of the most characteristic fragments of pentacyclic triterpenes and depends on the nature of the substituents bearing A and B rings (Budzikiewicz *et al.*, 1963). Species *e* is not abundant in the spectra of 12-unsaturated triterpenes. As shown, in triterpenes with $R_1$=H/H (e.g. $\beta$-amyrene), *e* is found at $m/z$ 191, while in derivatives with a 3-keto group (e.g. methyl



R$_1$=H/H, $m/z$ 191
R$_1$=O, $m/z$ 205
R$_1$=OH/H, $m/z$ 207

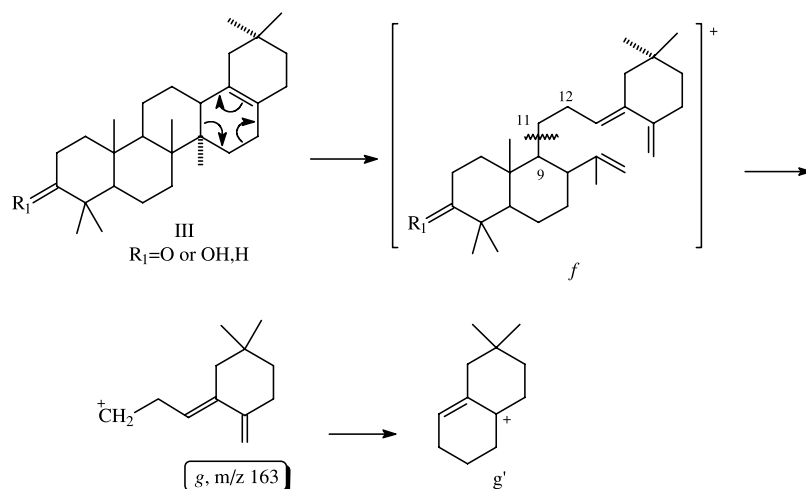**Figure 3.** Characteristic mass fragment from the left part of 12-unsaturated oleanene molecules.

oleanonate), *e* is shifted to $m/z$ 205. A 3-hydroxy and a 3-acetoxy group cause shifts to $m/z$ 207 and 249, respectively, while in both cases, an $m/z$ 189 fragment is observed, attributed to the further loss of water or acetic acid from species *e*.

Thus, 12-oleanenes present characteristic peaks at $m/z$: (i) 203, 189, 133 (upper right of the molecule, due to retro Diels Alder fragmentation; fragment *a*) and (ii) 207 for $R_1$=OH, 205 for $R_1$=O and 191 if $R_1$=H/H (not abundant fragments—left part of the molecule; fragment *e*).
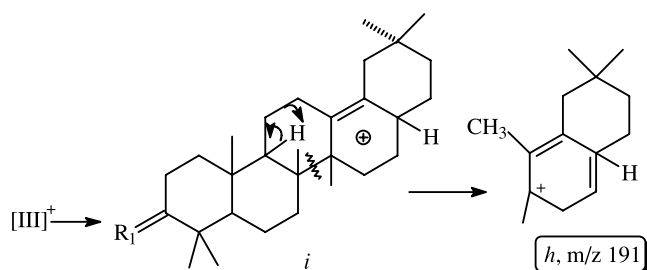
## 28-Nor-17(18)-oleanenes

In a paper by Budzikiewicz *et al.* (1963) 28-nor-17(18)-oleanen-3-one (III, Fig. 4) and 28-nor-17(18)-oleanen-3-ol are reported to exhibit their most abundant fragment ion at 163 $m/z$. The formation of the base peak at $m/z$ 163 is explained by a retro-Diels-Alder reaction involving the opening of ring D, yielding intermediate *f*. Two bonds are likely candidates for a further cleavage, 9–11 and 11–12, while fission of the 9–11 one is observed,

**Figure 4.** Characteristic mass fragmentation mechanism of 28-nor-17-oleanene molecules, attributed to retro-Diels–Alder reaction, leading to 163 $m/z$ (base peak).



**Figure 5.** Characteristic mass fragment pattern of 28-nor-17-oleanene molecules, leading to 191 $m/z$, the second strongest peak in the spectra.

possibly because the resulting ion, $g$ ($m/z$ 163, Fig. 4), rearranges to the more stable species $g'$.

The second strongest peak in the spectra of 28-nor-17(18)-oleanen-3-one appears at 191 $m/z$ ($h$, Fig. 5), which most likely comprises rings D and E, formed by rupture of the 11–12 and 8–14 bonds. Fragments corresponding in mass to the $m/z$ 191 ion of III ($h$, Fig. 5) are the most important cleavage products of 13(18)-unsaturated triterpenes.

As shown, whatever the substituents at skeletons A and B are, the base peak at 28-nor-olean-17-enes will be $m/z$ 163 and 191. Only substituents at rings D and E will shift the more intense fragments from $m/z$ 163 and 191.
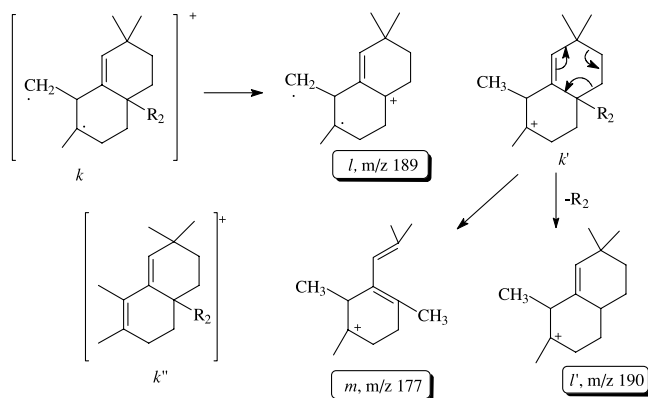
## 18-Oleanenes

Budzikiewicz *et al.* (1963) have elucidated the characteristic mass fragment patterns of this triterpene class that result from the most representative compounds: methyl moronate (Fig. 6; $R_1$=O, $R_2$=COOCH$_3$), methyl morolate ($R_1$=OH/H, $R_2$=COOCH$_3$), 18-oleanene ($R_1$=H/H, $R_2$=CH$_3$) and germanicol acetate ($R_1$=OAc/
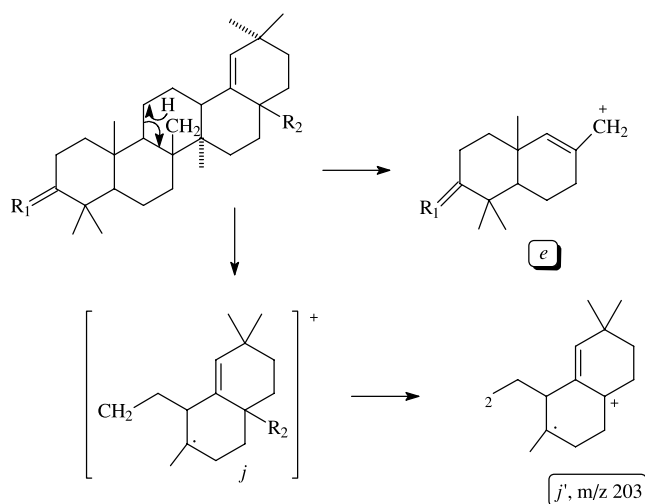


**Figure 6.** Characteristic mass fragment patterns of 18-unsaturated oleanene molecules.

H, $R_2$=CH$_3$). All the above compounds exhibit a pronounced loss of the C-17 substituent as expected by the allylic activation, due to the 18–19 double bond. Cleavage of ring C yields the characteristic fragment $e$ ($m/z$ 205 for derivatives with $R_1$=O, 207 for $R_1$=OH/H and 191 for $R_1$=H/H), as described above for 12-oleanenes, while an ion comprising the right-hand portion of the molecule ($j$, Fig. 6) is formed by the same type of cleavage without hydrogen rearrangement ($m/z$ 262 for methyl moronate and morolate, $m/z$ 218 for 18-oleanene). Fragment $j$ suffers further loss of the C-17 substituent giving $j'$ ($m/z$ 203 in all cases; Fig. 6). In 18-oleanenes $m/z$ 189 is the most abundant fragment, followed by 203, while the nature of the substituent $R_1$ is recognized by the $m/z$ 191, 207 or 205.

As reported (Budzikiewicz *et al.*, 1963), alternate fission of the 11–12 bond (with and without hydrogen

**Figure 7.** Alternative mass fragmentation mechanism of 18-unsaturated oleanene molecules.

rearrangement) yields species $k$ and $k'$ ($m/z$ 204/205 for 18-oleanenes with $R_2$=CH$_3$, $m/z$ 248/249 for $R_2$=COOCH$_3$), and $l$ and $l'$ ($m/z$ 189/190), respectively, by losing the C-17 substituent. Fragment $k$ rearranges to a more stable ion, $k''$ (Fig. 7). An $m/z$ 177 peak ($m$) is observed in the mass spectra of 18-oleanenes with a methyl group at C-17 ($R_2$).

## 12-Oleanenes containing a second double bond

Generally, Budzikiewicz *et al.* (1963) has reported that the fragmentation behaviour is determined by the presence of the 12–13 double bond, whereas additional centres of unsaturation exhibit only secondary effects.

## Saturated oleananes and ursanes

Introduction of a keto group at C-12 of oleananes leads to an abundant fragment at $m/z$ 234, the formation of which can be explained by a retro-Diels-Alder decomposition of the enol form which results in the ion $n$ (Fig. 8) (Budzikiewicz *et al.*, 1963; Karliner and Djerassi 1966).

## Lupane derivatives

The lupane series is characterized by a five-membered ring (E) to which an isopropyl or isopropenyl group is attached (Fig. 9). The loss of 43 mass units (C$_3$H$_7$) is

very pronounced in certain members, but becomes minimal in highly substituted derivatives or in the presence of an isopropenyl function (Budzikiewicz *et al.*, 1963; Heinzen *et al.*, 1996).

**Saturated lupanes.** Lupan-3-one (IX, Fig. 1, where $R_1$=O, $R_2$=CH$_3$), a main representative of this class, exhibits loss of methyl ($m/z$ 411) and isopropyl ($m/z$ 383) groups. The most abundant fragment for lupanes with both saturated and unsaturated side chain, occurs at 205 ($R_1$=O) or $m/z$ 207 ($R_1$=OH/H) and corresponds to species $e$ Fig. 9, as described for 12- and 18-oleanenes (Budzikiewicz *et al.*, 1963). In lupanes with saturated side chain the intense fragment at $m/z$ 191 is attributed to structure $o$ (Fig. 9), while in saturated lupanes with unsaturated side chains, such as lupen-3-one (X, Fig. 1, where $R_1$=O, $R_2$=CH$_3$) the $m/z$ 191 fragment is shifted to $m/z$ 189 (Fig. 9, species $o'$). Thus, when a peak at 191 $m/z$ is present, a saturated side chain in lupane series is proposed, while, when a $m/z$ 189 is observed, an unsaturated side chain is proposed. Hence, when in the mass spectrum of an unidentified triterpene the main fragments occur at $m/z$ 207 or 205 and $m/z$ 191 or 189, then a lupane skeleton is proposed with saturated or unsaturated side chain, respectively, with $R_1$=O ($m/z$ 205) or $R_1$=OH/H ($m/z$ 207). In the parent triterpene lupane (IX, Fig. 1, where $R_1$=H/H, $R_2$=CH$_3$), species $e$ and $o$ coincide so that $m/z$ 191 is by far the most abundant peak in the upper part of the spectrum (Fig. 9).

Lupanes can be distinguished from 12-oleanenes by mass spectral fragmentation, since the former yield from the upper part of the molecule an $m/z$ 191 or 189 (for saturated and unsaturated side chain respectively), while the latter ions at $m/z$ 203 and 189 and this consists a criterion for their identification. Also, species $e$ is abundant in lupanes, while in 12-oleanenes it is not. For the detection of lupanes with 18-oleanenes it can be stated that the former present their main peaks at 205 or 207 $m/z$ ($R_1$=O or OH/H respectively) followed by $m/z$ 191 (C$_3$H$_7$ side chain) or 189 (C$_3$H$_5$), while the latter are at $m/z$ 189, 203 followed by $m/z$ 205 or 207, respectively. The relative intensity of the peaks $m/z$ 189 and 205 or 207 for lupanes with unsaturated side chain is thus an identification criterion. The presence of an ion at 203 $m/z$ is also indicative of an 18-oleanene
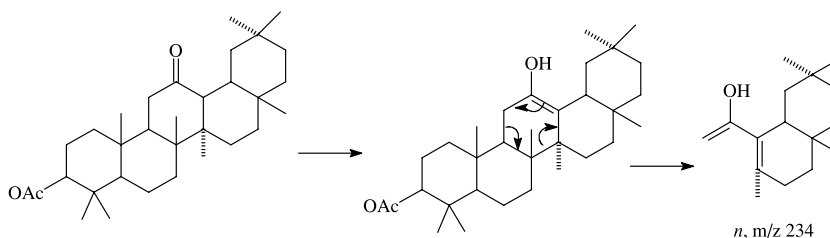


**Figure 8.** Fragmentation mechanism of 11-oxo-oleanane molecules, attributed to retro-Diels–Alder decomposition.
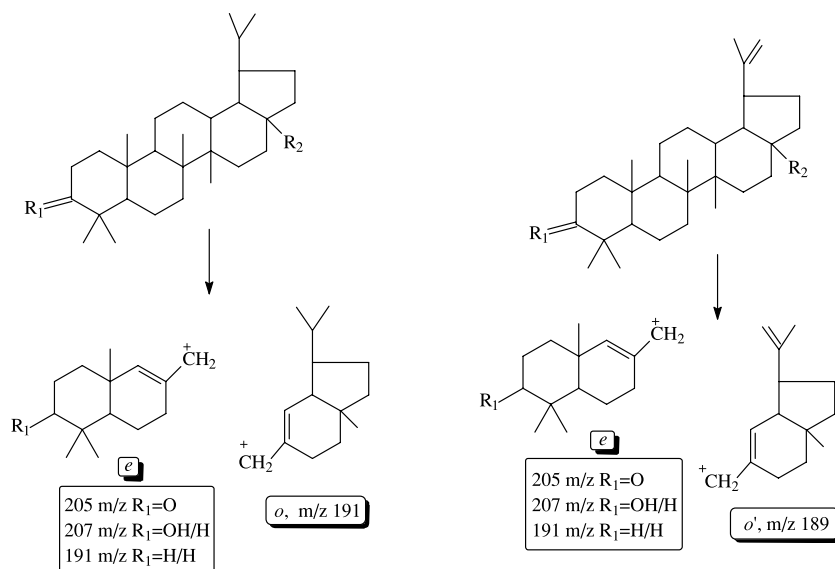
**Figure 9.** Characteristic mass fragment patterns of saturated lupane molecules, with saturated (left) and unsaturated (right) side chain.
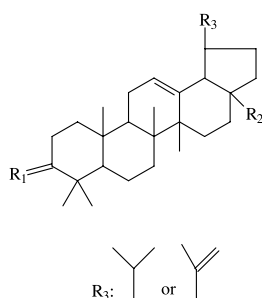


**Figure 10.** Structure of 12-unsaturated lupene molecules, with saturated and unsaturated side chain.

derivative, while it is not observed in lupanes. According to Nytoft (2002), the mass spectra of lupane and oleananes are very similar, except for the presence of an $m/z$ 369 fragment and larger $m/z$ 123 and 231 fragments in the lupane spectrum. The $m/z$ 369 fragment ion is generated by loss from the lupane structure of an isopropyl side chain, which is not present in oleanane.

**12-lupenes.** Although retro-Diels-Alder is a very characteristic type of fragmentation for 12-unsaturated oleananes and ursanes of the amyrin series, the members of the lupane series Fig. 10 exhibit this type of fragmentation only to a minor extent. The most characteristic peaks for 12-lupenes occur at $m/z$ 187, 189, 201 and 203 in both derivatives with $R_1=O$, $R_2=CH_3$, $R_3=C_3H_5$ and the corresponding 3-acetate ($R_1=OAc/H$, $R_2=CH_3$, $R_3=C_3H_5$), while in the derivative with $R_1=OH/H$, $R_2=CH_3$, $R_3=C_3H_7$) peaks at 189, 191 and 204 $m/z$ are noticed (Budzikiewicz *et al.*, 1963). According to Budzikiewicz *et al.* (1963), the formation of species *e* is not observed in either case.

## (8- and 7-)Tirucallene derivatives

**(isomastica-derivatives and mastica-derivatives).** The mechanism of mass fragmentation for 7- (mastica-) and 8- (isomastica-) tirucallene derivatives (IV, VI, Fig. 1) has been approached by Papageorgiou *et al.* (1997). These species are tirucallane derivatives and not lanostane, as wrongly characterized in previous papers (Papageorgiou *et al.*, 1997; Regert and Rolando, 2002). The main mass spectral patterns for 7- and 8-tirucallene derivatives are presented in Fig. 11(a) for $R_1=O$ and in Fig. 11(b) for $R_1=OH/H$ or OAc/H (Papageorgiou *et al.*, 1997).

Characteristic fragments for both 7- and 8-tirucallene compounds are $m/z$ 95, 55 257, 315 and 249. The ions at $m/z$ 121 and 241 suggest an isomastica-derivative (8-tirucallene), while $m/z$ 127 and 227 suggest mastica-ones ($\Delta 7$-). The ion at $m/z$ 257 corresponds to [M-side chain-part of ring D-CH$_3$]$^+$, while that at $m/z$ 313 arises from abstraction of the side chain [M-side chain]$^+$ [Fig. 11(a)] (Papageorgiou *et al.*, 1997).

The epimer types of the compounds differ from the normal ones only at the position of 3-hydroxyl group, which has to do with the ability of abstraction of the water molecule from the molecular ion. $m/z$ 189 is proposed for $\alpha$-, while $m/z$ 161 for $\beta$-epimer type (Papageorgiou *et al.*, 1997). When $m/z$ [M-18] is not significant, then an axial configuration ($\beta$-type) is proposed, whereas when this peak is abundant an $\alpha$-configuration is suggested. Iso-derivatives ($\Delta 8$-) elute before normal ones ($\Delta 7$-).

We herein propose the fragmentation pattern that yields ions with $m/z$ 95, and 55, which are characteristic for both 7- and 8-tirucallene compounds and in abundance in their mass spectra, while the ion at $m/z$ 121,
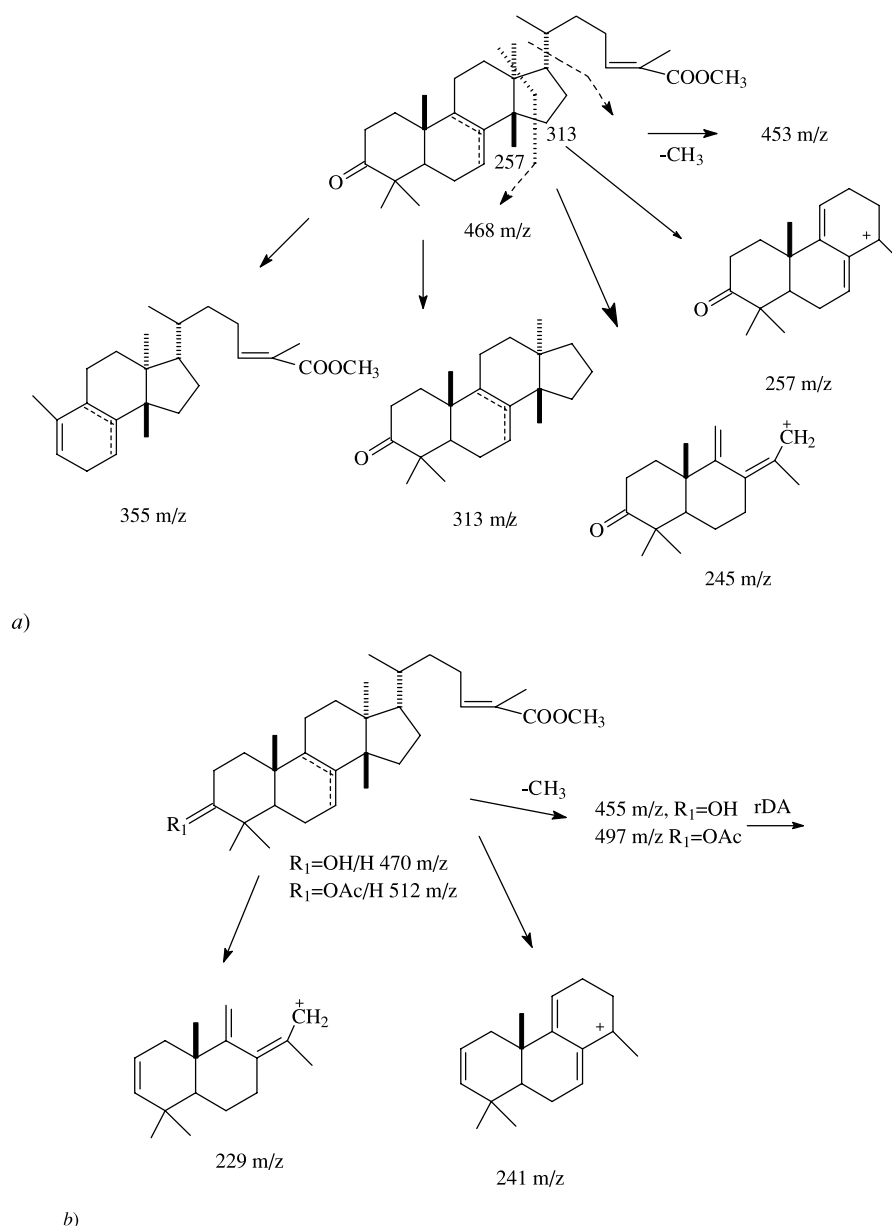
**Figure 11.** Characteristic mass spectral patterns of 7- and 8-unsaturated tirucallene molecules (mastica- and isomastica-) with (*a*) $R_1$=O and (*b*) $R_1$=OH/H or OAc/H.

which is characteristic for 8-tirucallene compounds, is further elucidated (Fig. 12).

### Dammarane

Cleavage of the side chain of the dammarane skeleton of hydroxydammarenone [VIII, Fig. 1, $R_1$=O, $R_3$=CH$_3$, $R_2$=(OH)CH$_3$] at C-17 results in an ion with $m/z$ 315 and not 355, as wrongly stated by van der Doelen *et al.* (1998), while ring C cleavage with concerted hydrogen transfer of the dammarane skeleton produces the fragment ion at $m/z$ 205 (Fig. 13). $m/z$ 109 is also a characteristic abundant fragment for dammarane

skeleton (van der Doelen *et al.*, 1998). In Fig. 13 we furthermore elucidate the formation of the mass fragment patterns for $m/z$ 69, 355 and 163, which are characteristic fragments for dammarane derivatives.

During resin aging several oxidized derivatives of dammarane skeleton were observed where the side chain was a furan derivative. The characteristic fragments of the ocotillone type molecule (oxidized dammarane skeleton) are presented in Fig. 14 (van der Doelen *et al.*, 1998). Cleavage of ring C with concerted hydrogen transfer occurs, resulting in a fragmention peak at $m/z$ 205. Fragmentation peaks at $m/z$ 399 and the base peak at $m/z$ 143 are characteristic in
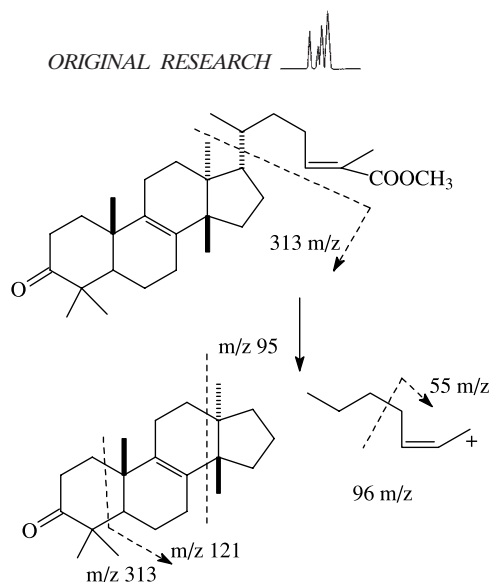
**Figure 12.** Proposed mass fragment patterns of 7- and 8-unsaturated tirucallene molecules that yield ions with 95, 55 and 121 *m/z*.

ocotillone-type molecules and can be explained by the side chain cleavage at two locations. The characteristic fragmentation at *m/z* 143 suggest the presence of a

hydroxyisopropyl-methyl-tetrahydroxyfuran side chain $[C_8H_{15}O_2]^+$ in the molecule (van der Doelen *et al.*, 1998).

As shown from the above analysis, the mass spectra of tetracyclic and pentacyclic triterpenes, based on an actual or modified oleanene or ursene, lupa(e)ne, tirucalla(e)ne and dammarane skeletons, offer valuable information and in many cases an unknown compound can be assigned to a certain structural type. In addition, the location and the nature of the substituents can be narrowed down considerably. In the present study, the identification of triterpenes contained in *P. lentiscus* var. Chia resin samples will be based on the above-described analysis.

The triterpenes that have been reported by several authors to date in *P. lentiscus* resin are presented in Table 1 (Seoane, 1956; Marner *et al.*, 1991; Colombini *et al.*, 2000; Dietemann *et al.*, 2001; Papageorgiou *et al.*, 1997; Scalarone *et al.*, 2003; van der Doelen and Boon, 2000). Triterpenes from the galls of *P. lentiscus* have also been reported (Monaco *et al.*, 1973).

In the present study the triterpenes of both acidic and neutral fraction of *Pistacia lentiscus* resin var.
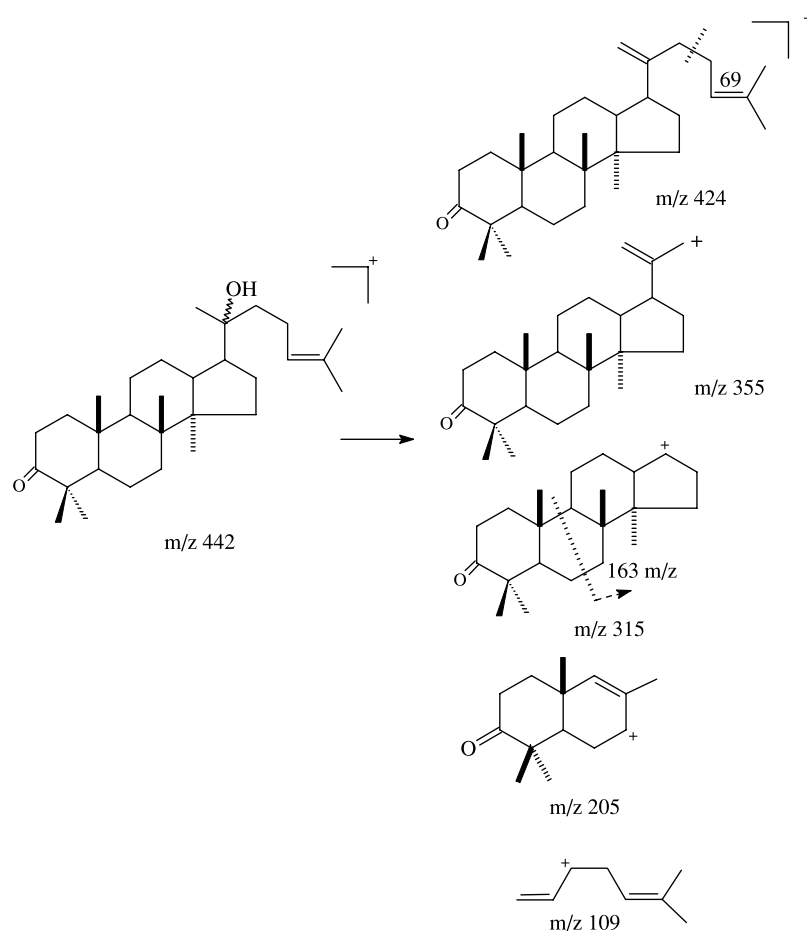


**Figure 13.** Characteristic mass fragment patterns of hydroxydammarenone and dammarane molecules in general.
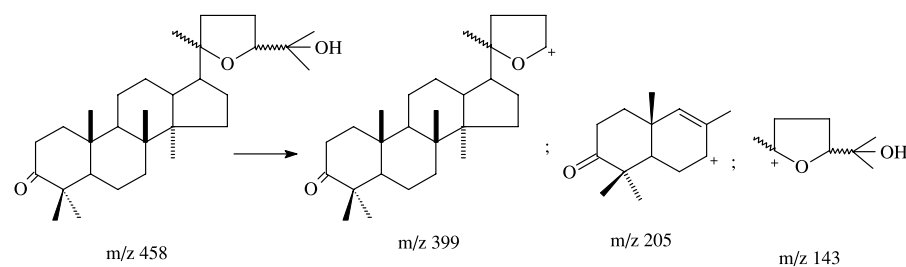
**Figure 14.** Characteristic mass fragment patterns of ocotillone type molecule (oxidized dammarane derivative with furan side chain).

**Table 1. Triterpenes identified in _Pistacia lentiscus_ resin to date**

| Acidic fraction | Neutral fraction |
|---|---|
| Oleanonic acid | β-Amyrin |
| Masticadienonic acid | Tirucallol |
| Isomasticadienonic acid | Dipterocarpol |
| Moronic acid | Lupeol |
| 3-_O_-Acetyl-3-epi-(iso)masticadienolic acid | β-Amyrone |
| 3,4-Seco-28-norolean-12-en-3-oic acid or | Oleanolic aldehyde |
| 3,4-seco-28-norolean-18-en-3-oic acid | |
| 18-α-H-Oleanonic acid | Germanicol |
| 3-Epi-(iso)masticadienolic acid | 3-Acetoxy-hydroxy-dammarenone |
| Masticadienolic acid | 3-Oxo-28-norolean-12-ene |
| Oleanolic acid | 3-Hydroxy-28-norolean-12-ene |
| | 3-Oxo-28-norlup-20(29)-ene |
| | (20S)-3β-Acetoxy-20-hydroxydammar-24-ene |
| | Dammaradienone |
| | Nor-β-amyrone |
| | Hydroxy-dammarenone |
| | 20,24-Epoxy-25-hydroxy-dammaren-3-one |
| | 28-Norolean-17-en-3-one |
| | 28-Norolean-18-en-3-one |
| | Norlupeone |
| | Nor-β-amyrin |

Chia were identified and quantified. Two samples were studied, one traditionally collected (semi-solid form) and the other collected with the addition of ethrel (ethephon, 2-chloroethylphosphinic acid), which is a stimulating agent used to prolong flow (liquid form). Semi-solid resin was obtained by incision of _P. lentiscus_ and it remained on the tree trunk until solidification (25–30 days). Liquid resin was collected using the stimulating compound ethrel (ethephon) after insicion of the tree trunk. Ethrel results in increased excretion of mastic resin, collected in a fluid state. This new technique has advantages compared with the traditional method, since the quantity of resin excreted is increased and it costs less (Mastic Gum Growers Association, personal communication). It is thus crucial to identify whether this new collection method influences the chemical composition, and specifically the bioactive triterpenes included, since the biological activity of _Pistacia_ resins is highly associated with the triterpenes contained. In a previous paper (Papanicolaou _et al._,

1995), the composition of the essential oil was proved to differ between the semi-solid and liquid resin and specifically myrcene proportion was reduced in the liquid collection sample.

The triterpenes identified in the present study in the two _P. lentiscus_ var. Chia resin samples collected traditionally and with the use of ethrel (liquid collection) (acidic and neutral fraction) are presented in Tables 2–5. In these Tables the molecular ion, main fragments and quantification of triterpenes are depicted. In the acidic fraction methyl esters are presented in Tables 2, 4.

Below, we will discuss how the triterpenes presented in Tables 2 and 3 were identified on the basis of the mass spectra of each peak and its retention time. Interpretation of mass spectra was performed according to the above-mentioned MS analysis. Total ion current chromatogram (TIC) of the acidic and neutral fraction of both resin samples are presented in the Appendix. The elution order is as presented in Tables 2–5, where
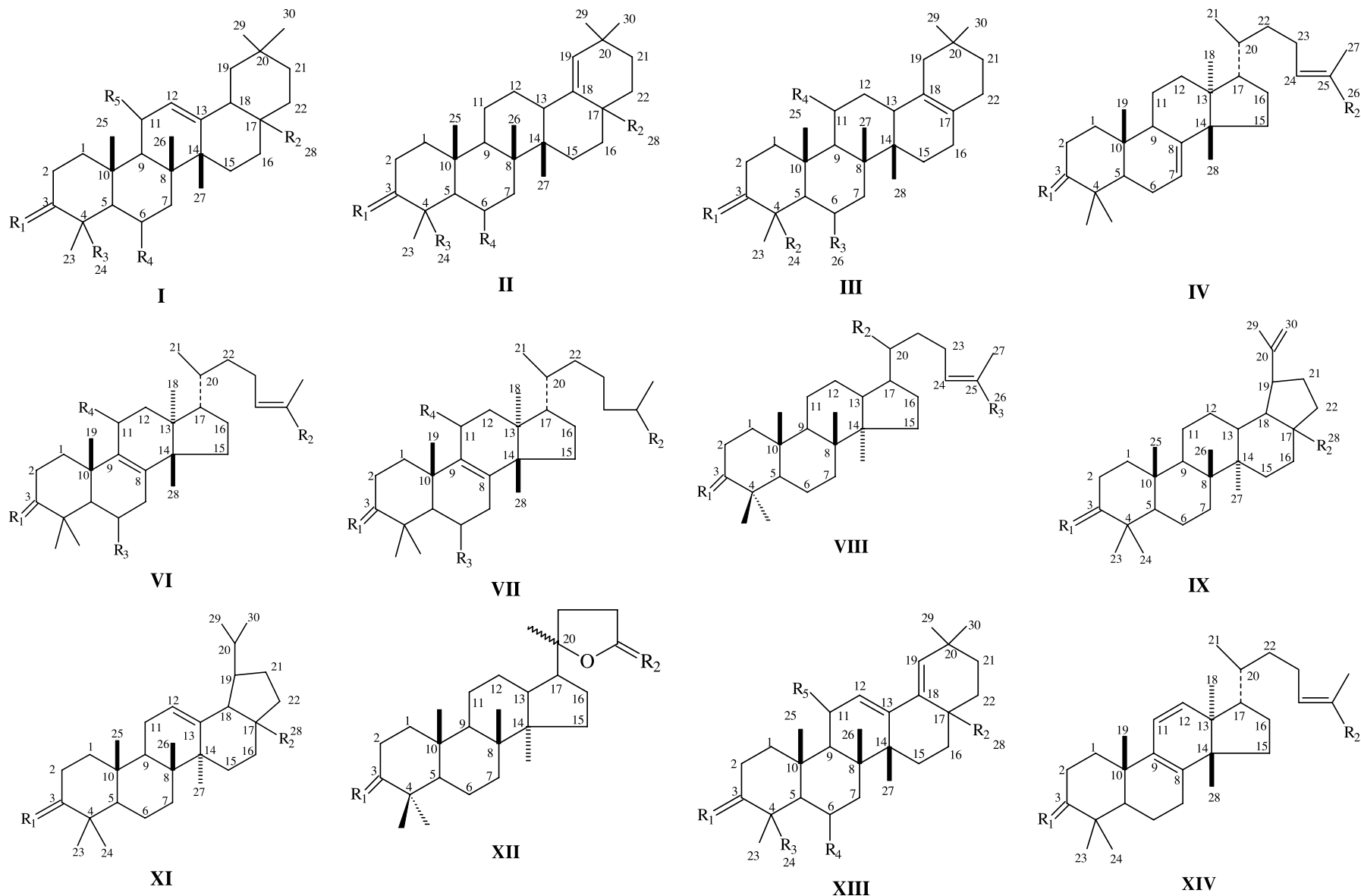
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**Table 2. Triterpenes identified by GC-MS analysis in the acidic fraction of *Pistacia lentiscus* var. Chia traditional resin collection**

**Table 2. (*continued*)**

| | Acidic fraction | Structure | Ri | M$^+$ | Main fragments (descending intensity) | Percentage w/w of triterpenic fraction | Percentage w/w of acidic fraction | Percentage w/w of resin |
|---|---|---|---|---|---|---|---|---|
| 1 | Methyl 11-Oxo-3$\beta$-hydroxy-28-norolean-17-en-6-oate | III | R$_1$=OH/H R$_2$=CH$_3$ R$_3$=COOCH$_3$ R$_4$=O | 484 | 163, 191, 207, 281, 410, 355, 424, 395 | 0.6 | 1.6 | 0.2 |
| 2 | Methyl moronate[a] | II | R$_1$=O R$_2$=COOCH$_3$ R$_3$=CH$_3$ R$_4$=H/H | 468 | 189, 205, 207, 163, 133, 105, 249, 281, 409, 355 | 0.5 | 1.3 | 0.2 |
| 3 | Methyl oleanonate[a] | I | R$_1$=O R$_2$=COOCH$_3$ R$_3$=CH$_3$ R$_4$=H/H=R$_5$ | 468 | 203, 189, 136, 262, 163, 424, 249, 409, 281 | 3.8 | 9.7 | 1.4 |
| 4 | Methyl 3$\beta$-acetoxy-6$\beta$-hydroxy-olean-18-en-28-olate | II | R$_1$=OAc/H R$_2$=COOCH$_3$ R$_3$=CH$_3$ R$_4$=OH | 530 | 207, 189, 281, 203,105, 133, 163, 253, 267, 355, 466, 451, 408, 327 | 0.2 | 0.4 | 0.06 |
| 5 | Methyl 3$\beta$-acetoxy-6$\beta$-hydroxy-dihydro-isomasticadienolate | VII | R$_1$=OAc/H R$_2$=COOCH$_3$ R$_3$=OH R$_4$=H/H | 530 | 207, 105, 121, 163, 257, 189, 281, 407, 466, 203, 267, 355, 309, 393, 435 | 0.1 | 0.4 | 0.05 |
| 6 | Methyl isomasticadienonate[a] | VI | R$_1$=O R$_2$=COOCH$_3$ R$_3$=H/H=R$_4$ | 468 | 453, 55, 95, 121, 119, 421, 257, 147, 159, 241, 187, 271, 435 | 24.4 | 63.3 | 8.9 |
| 7 | Methyl 3-epi-isomasticadienolate[a] | VI | R$_1$=OH/H ($\alpha$-) R$_2$=COOCH$_3$ R$_3$=H/H=R$_4$ | 470 | 95, 121, 119, 455, 437, 161, 147, 189, 205, 207, 229, 249, 339, 313, 281 | t | t | t |
| 8 | Methyl masticadienonate[a] | IV | R$_1$=O R$_2$=COOCH$_3$ | 468 | 453, 95, 55, 127, 145, 147, 257, 421, 189, 173, 227, 245, 271, 393, 355 | 9.3 | 24.1 | 3.4 |
| 9 | Methyl 3$\beta$-acetoxy-20,30-dehydro-12-lupen-28-oate | XI | R$_1$=OAc/H R$_2$=COOCH$_3$ | 512 | 437, 207, 191, 133, 281, 163, 253, 355, 411, 497 | 0.1 | 0.3 | 0.04 |
| 10 | Methyl olean-12,18-dien-3-olate | XIII | R$_1$=OH/H R$_2$=COOCH$_3$ R$_3$=CH$_3$ R$_4$=R5=H/H | 468 | 207, 189, 177, 147, 203, 119, 281, 233, 263, 341, 313 | 0.2 | 0.5 | 0.06 |

**Table 3. Triterpenes identified by GC-MS analysis in the neutral fraction of *Pistacia lentiscus* var. Chia traditional resin collection**

| | Neutral fraction | Structure | Ri | $M^+$ | Main fragments (descending intensity) | Percentage w/w of triterpenic fraction | Percentage w/w of acidic fraction | Percentage w/w of resin |
|---|---|---|---|---|---|---|---|---|
| 11 | Lupenone | IX | $R_1$=O<br>$R_2$=CH$_3$ | 424 | 69, 107, 205, 149, 133, 218, 177, 281, 231, 355, 327 | 0.2 | 0.7 | 0.1 |
| 12 | 3β-Hydroxy-6β-hydroxymethyl-28-norolean-17-ene | III | $R_1$=OH/H<br>$R_2$=CH$_3$<br>$R_3$=CH$_2$OH<br>$R_4$=H/H | 442 | 163, 93, 105, 207, 121, 133, 147, 409, 203, 191, 189, 281, 424, 257, 395, 355, 327, 313 | 0.6 | 1.7 | 0.2 |
| 13 | 3β-Hydroxy-28-norolean-17-en-6-al | III | $R_1$=OH/H<br>$R_2$=CH$_3$<br>$R_3$=CHO<br>$R_4$=H/H | 440 | 69, 163, 95, 119, 207, 189, 191, 281, 424, 355, 408, 313, 253 | 0.6 | 1.6 | 0.2 |
| 14 | Tirucallol[a] | VI | $R_1$=OH/H<br>$R_2$=CH$_3$<br>$R_3$=H/H=R$_4$ | 426 | 207, 135, 411, 121, 163, 281, 189, 253, 393, 327, 230 | 0.2 | 0.5 | 0.06 |
| 15 | 3-Methoxy-28-norolean-12-ene | I | $R_1$=OCH$_3$/H<br>$R_2$=H<br>$R_3$=CH$_3$<br>$R_4$=H/H=R$_5$ | 426 | 204, 189, 105, 121, 163, 133, 191, 411, 393, 355, 313, 281, 253, 221 | 0.7 | 1.9 | 0.2 |
| 16 | Dammaradienone[a] | VIII | $R_1$=O<br>$R_2$=(=)<br>$R_3$=CH$_3$ | 424 | 93, 109, 121, 205, 163, 189, 355, 313, 281, 245 | 0.9 | 2.6 | 0.3 |
| 17 | 28-Norolean-12-en-3-one[a] | I | $R_1$=O<br>$R_2$=H<br>$R_3$=CH$_3$<br>$R_4$=H/H=R$_5$ | 410 | 204, 189, 173, 133, 281, 393, 369, 313 | 1.1 | 3.1 | 0.4 |
| 18 | β-Amyrone[a] | I | $R_1$=O<br>$R_2$=CH$_3$=R$_3$<br>$R_4$=H/H=R$_5$ | 424 | 218, 203, 189, 119, 163, 207, 133, 281, 406, 409, 253, 327, 355 | 1.3 | 3.6 | 0.5 |
| 19 | Olean-18-en-3-one | II | $R_1$=O<br>$R_2$=CH$_3$=R$_3$<br>$R_4$=H/H | 424 | 93, 121, 205, 189, 147, 355, 177, 313 | 1.0 | 2.7 | 0.3 |
| 20 | 28-Norolean-17-en-3-one[a] | III | $R_1$=O<br>$R_2$=CH$_3$<br>$R_3$=H/H=R$_4$ | 410 | 163, 191, 55, 105, 119, 81, 147 | 19 | 53.4 | 6.9 |
| 21 | 28-Norolean-12,17-dien-3-one | III, Δ12 Double bond | $R_1$=O<br>$R_2$=CH$_3$<br>$R_3$=H/H=R$_4$ | 408 | 408, 163, 173, 191, 393, 203, 258, 281 | t | t | t |
| 22 | 6-Methyl-28-norolean-17-en-3-one | III | $R_1$=O<br>$R_3$=R$_2$=CH$_3$<br>$R_4$=H/H | 424 | 93, 55, 107, 163, 205, 191, 135, 313, 245, 355, 281 | 0.6 | 1.6 | 0.2 |
| 23 | 3-Methoxy-28-norolean-17-ene | III | $R_1$=OCH$_3$<br>$R_2$=CH$_3$<br>$R_3$=H/H=R$_4$ | 426 | 207, 163, 191, 141, 409, 281, 245, 221, 355, 327 | 0.7 | 1.9 | 0.2 |

**Table 3.** (*continued*)

| Neutral fraction | Structure | Ri | M⁺ | Main fragments (descending intensity) | Percentage w/w of triterpenic fraction | Percentage w/w of acidic fraction | Percentage w/w of resin |
|---|---|---|---|---|---|---|---|
| 24 3β-Acetoxy-28-norolean-17-ene | III | R₁=OAc/H R₂=CH₃ R₃=H/H=R₄ | 456 | 163, 191, 409, 105, 207, 281, 257, 355, 395, 425, 429 | t | t | t |
| 25 3-Oxo-28-norolean-17-en-6-al | III | R₁=O R₂=CH₃ R₃=CHO R₄=H/H | 438 | 163, 191, 105, 408, 393, 131, 258 | 0.5 | 1.3 | 0.2 |
| 26 3β-Hydroxy-6-methyl-28-norolean-17-ene | III | R₁=OH/H R₂=R₃=CH₃ R₄=H/H | 426 | 163, 207, 191, 189, 411, 281, 135, 355 | t | t | t |
| 27 Olean-18-en-3-ol | II | R₁=OH/H R₂=CH₃=R₃ R₄=H/H | 426 | 189, 207, 203, 119, 163, 147, 281, 408, 249, 327, 393, 355 | 0.2 | 0.5 | 0.07 |
| 28 3β-Hydroxy-dammarane-derivative | VIII | R₁=OH/H R₂=? R3=? | 424 | 141, 109, 163, 207, 191, 121, 281, 355, 373, 408, 327, 245, 149, 175 | t | t | t |
| 29 20,24-Epoxy-25-hydroxy-dammaren-3-one | XII | R₁=O R₂=CH₂(OH)(CH₃)₂ | 458 | 143, 163, 178, 121, 207, 191, 281, 399, 355, 253, 313 | 0.1 | 0.4 | 0.04 |
| 30 3β-Hydroxy-epoxy-dammarane-derivative | XII | R₁=OH/H R₂=? | 426 | 143, 178, 207, 163, 399, 281, 253 | 0.3 | 0.8 | 0.1 |
| 31 Hydroxydammarenone[a] | VIII | R₁=O R₂=OH/CH₃ R₃=CH₃ | 442 | 109, 69, 205, 203, 163, 135, 424, 355, 313, 395 | 0.8 | 2.2 | 0.3 |
| 32 28-Nor-17-oleanen-3-ol | III | R₁=OH/H R₂=CH₃ R₃=H/H=R₄ | 412 | 207, 191, 163, 105, 133, 409, 281, 253, 355, 327, 391, 229 | 0.1 | 0.4 | 0.05 |
| 33 Oleanonic aldehyde[a] | I | R₁=O R₂=CHO R₃=CH₃ R₄=R₅=H/H | 438 | 203, 189, 232, 175, 105, 119, 133, 409 | 5.4 | 15.2 | 2.0 |
| 34 Isomasticadienolic aldehyde | VI | R₁=OH/H R₂=CHO R₃=R₄=H/H | 440 | 423, 55, 95, 133, 119, 121, 173, 207, 257, 281, 405, 225, 327, 373, 395, 309 | 0.4 | 1.1 | 0.1 |
| 35 11-Oxo-β-amyrin acetate | I | R₁=OAc/H R₂=CH₃=R₃ R₄=H/H R₅=O | 482 | 385, 233, 207, 137, 135, 281, 253, 454, 327, 355, 422 | 0.3 | 0.8 | 0.1 |
| 36 Norlupenone[a] | IX | R₁=O R₂=H | 410 | 189, 234, 203, 119, 207, 249, 163, 281, 355, 327 | 0.7 | 2.0 | 0.2 |

[a] Compounds identified in previous papers in *P. lentiscus* traditional resin collection.

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**Table 4. Triterpenes identified by GC-MS analysis in the acidic fraction of *Pistacia lentiscus* var. Chia liquid resin collection**

| | Acidic fraction | Structure | R | $M^+$ | Main fragments (descending intensity) | Percentage w/w of triterpenic fraction | Percentage w/w of acidic fraction | Percentage w/w of resin |
|---|---|---|---|---|---|---|---|---|
| 1 | Methyl Oleanonate | I | $R_1$=O $R_2$=COOCH$_3$ $R_3$=CH$_3$ $R_4$=H/H=R$_5$ | 468 | 203, 189, 136, 262, 163, 424, 249, 409, 281 | 1.9 | 4.5 | 0.7 |
| 2 | Methyl 11-oxo-masticadienonate[a] | IV | $R_1$=O $R_2$=COOCH$_3$ $R_3$=O (C-11) | 482 | 259, 453, 468, 95, 119, 203, 159, 327, 281, 355, 426 | 1.1 | 2.7 | 0.4 |
| 3 | Methyl isomasticadienonate | VI | $R_1$=O $R_2$=COOCH$_3$ $R_3$=R$_4$=H/H | 468 | 453, 55, 95, 121, 119, 421, 257, 147, 159, 241, 187, 271, 435 | 22.5 | 53.8 | 8.2 |
| 4 | Methyl 3-epi-isomasticadienolate | VI | $R_1$=OH/H ($\alpha$-) $R_2$=COOCH$_3$ $R_3$=R$_4$=H/H | 470 | 95, 55, 121, 455, 437, 119, 147, 189, 161, 207, 241, 281, 315, 339, 301 | 0.9 | 2.2 | 0.3 |
| 5 | Methyl Masticadienonate | IV | $R_1$=O $R_2$=COOCH$_3$ | 468 | 453, 95, 55, 127, 145, 147, 257, 421, 189, 173, 227, 245, 271, 393, 355 | 14.7 | 35.0 | 5.4 |
| 6 | Methyl-3-acetoxy-3-epi-isomasticadienolate[a] | VI | $R_1$=OAc/H ($\alpha$-) $R_2$=COOCH$_3$ $R_3$=R$_4$=H/H | 512 | 437, 95, 55, 119, 121, 147, 187, 189, 207, 241, 281, 497, 315, 355 | 0.4 | 1 | 0.1 |
| 7 | Methyl isomastica-8,11(12)-dienolate[a] | XIV | $R_1$=OH/H $R_2$=COOCH$_3$ | 468 | 453, 55, 95, 207, 121, 147, 421, 173, 281, 253, 241, 257, 299, 355, 394 | t | t | t |
| 8 | Methyl-3-acetoxy-3-epi-masticadienolate[a] | IV | $R_1$=OAc/H ($\alpha$-) $R_2$=COOCH$_3$ | 512 | 437, 95, 55, 121, 135, 189, 207, 281, 497, 241, 327, 355 | 0.3 | 0.8 | 0.1 |

[a] Compounds not identified in *P. lentiscus* var. Chia traditional resin collection.

**Table 5. Triterpenes identified by GC-MS analysis in the neutral fraction of *Pistacia lentiscus* var. Chia liquid resin collection**

| | Neutral fraction | Structure | R | M$^+$ | Main fragments (descending intensity) | Percentage w/w of triterpenic fraction | Percentage of neutral fraction w/w | Percentage of resin w/w |
|---|---|---|---|---|---|---|---|---|
| 9 | Dammaradienone | VIII | $R_1$=O, $R_2$=(=) $R_3$=CH$_3$ | 424 | 93, 109, 121, 205, 163, 189, 355, 313, 281, 245 | 0.3 | 0.7 | 0.1 |
| 10 | 3-Methoxy-28-norolean-12-ene | I | $R_1$=OCH$_3$/H, $R_2$=H $R_3$=CH$_3$ $R_4$=H/H=$R_5$ | 426 | 204, 189, 107, 121, 135, 163, 191, 411, 393, 355, 313, 281, 253, 221 | 0.6 | 1.3 | 0.2 |
| 11 | 28-Norolean-12-en-3-ol[a] | I | $R_1$=OH/H $R_2$=H $R_3$=CH$_3$ $R_4$=H/H=$R_5$ | 412 | 204, 189, 175, 133, 119, 147, 281, 393, 355, 313 | 0.3 | 0.8 | 0.1 |
| 12 | β-Amyrin | I | $R_1$=OH/H $R_2$=CH$_3$=$R_3$ $R_4$=H/H=$R_5$ | 426 | 408, 218, 189, 203, 121, 147, 281, 245, 355, 313 | 0.3 | 0.7 | 0.1 |
| 13 | β-Amyrone | I | $R_1$=O $R_2$=CH$_3$=$R_3$ $R_4$=H/H=$R_5$ | 424 | 218, 203, 189, 119, 163, 207, 133, 281, 406, 409, 253, 327, 355 | 0.2 | 0.4 | 0.05 |
| 14 | 28-Nor-17-oleanen-3-one | III | $R_1$=O, $R_2$=CH$_3$ $R_3$=H/H=$R_4$ | 410 | 163, 191, 55, 105, 119, 81, 147 | 35.9 | 84.3 | 13.1 |
| 15 | Oleanonic aldehyde | I | $R_1$=O, $R_2$=CHO $R_3$=CH$_3$ $R_4$=H/H=$R_5$ | 438 | 203, 189, 232, 175, 105, 119, 133, 409 | 2.5 | 5.9 | 0.9 |
| 16 | 3β-Acetoxy-12-oleanene[a] | I | $R_1$=OAc/H $R_2$=CH$_3$=$R_3$ $R_4$=H/H=$R_5$ | 452 | 421, 203, 189, 133, 105 | 0.8 | 2.0 | 0.3 |
| 17 | 3β-Acetoxy-isomasticadienolic aldehyde[a] | VI | $R_1$=OAc/H $R_2$=CHO $R_3$=$R_4$=H/H | 482 | 453, 95, 55, 119, 121, 147, 203, 421, 207, 173, 189, 257, 281, 393, 355, 327 | 0.3 | 0.7 | 0.1 |
| 18 | 11-Oxo-β-amyrin acetate | I | $R_1$=OAc/H $R_2$=CH$_3$=$R_3$ $R_4$=H/H $R_5$=O | 482 | 137, 385, 233, 207, 259, 281, 253, 454, 435, 327, 355 | 0.3 | 0.8 | 0.1 |
| 19 | Norlupenone | IX | $R_1$=O $R_2$=H | 410 | 189, 234, 203, 119, 121, 163, 205, 249, 281, 355, 327 | 1.0 | 2.4 | 0.4 |

[a] Compounds not identified in *P. lentiscus* var. Chia traditional resin collection.

compounds presented first in the tables elute first in the chromatogram.

New compounds were identified both in the acidic and neutral fractions and the percentage of each compound was estimated in each fraction, in the total triterpenic fraction and in the resin sample. In the acidic fraction of traditionally collected *P. lentiscus* var. Chia resin 10 triterpenes (presented as methyl esters) were identified (Table 2). Methyl oleanonate (12-oleanene), methyl moronate (18-oleanene), methyl masticadienonate (7-tirucallene), methyl isomasticadienonate (8-tirucallene), which have all $M^+$ 468, were identified in the acidic fraction according to their main fragments (Table 2), the fragmentation mechanism proposed above and already published mass spectra. Methyl isomasticadienonate eluted in the chromatogram before masticadienonate. Methyl-3-epi-isomasticadienolate was also found in *P. lentiscus* var. Chia resin according to already published mass spectra (Table 2) (Papageorgiou *et al.*, 1997). All the above ingredients were found in previous analyses of *P. lentiscus* resin. Each of these compounds except methyl moronate was identified in a *P. lentiscus* var. Chia sample analysed in the present study obtained using ethrel (liquid form), but in different proportions in the triterpenic mixture (Table 4).

As established, isomasticadienonic acid seems to be the major constituent in both resin samples (semi-solid and liquid, 24.4 and 22.5% of total triterpenic fraction, respectively), followed by masticadienonic acid (9.3 and 14.7%, respectively) and oleanonic acid (3.8 and 1.9%, respectively). These three triterpenes comprise 37 and 39% of the total triterpenic fraction (neutral and acidic) of resin in semi-solid and fluid form, respectively, while the proportion of isomasticadienonic/masticadienonic acid is 2.6 and 1.5 in the two samples. Thus in each of the acidic fractions of *P. lentiscus* var. Chia resin samples tested, 8-tirucallene derivatives predominated.

Compound 1 (Table 2) with $M^+$ 484, main fragments at $m/z$ 163, 191, 207, was identified as methyl 11-oxo-3$\beta$-hydroxy-28-norolean-17-en-6-oate [$\Delta$17 $R_1$=OH, $R_2$=CH$_3$, $R_3$=COOCH$_3$, $R_4$=O], since the base peak 163 followed by 191 is indicative, according to our previous MS interpretation, of a 28-nor-17-oleanene skeleton. The mass fragment at $m/z$ 207 indicates the presence of a hydroxyl group at C-3; the loss of 60 $m/z$ shows a COOCH$_3$ group and thus a keto group is missing for the molecular weight to reach 484. 11-Oxo-12-oleanene derivatives have been reported as oxidation byproducts through the aging process of *P. lentiscus* resin and dammar resin (van der Doelen and Boon, 2000), as proposed in the biosynthetic route depicted in Fig. 15.

The only matter that has to be defined is the position of COOCH$_3$ group. As shown in Fig. 5, the base peak at 163 $m/z$ is produced from the D and E skeleton of 28-nor-$\Delta$17-oleanenes with a methyl group at C-20 (right upper part) and thus a COOCH$_3$ group cannot be placed at C-20, since the base peak should be shifted. Thus, COOCH$_3$ group will be placed on either the A or B skeleton. As far as we know, 28-norolean-17-ene derivatives with $R_2 \neq$ CH$_3$ and $R_3 \neq$ H/H (Fig. 1)
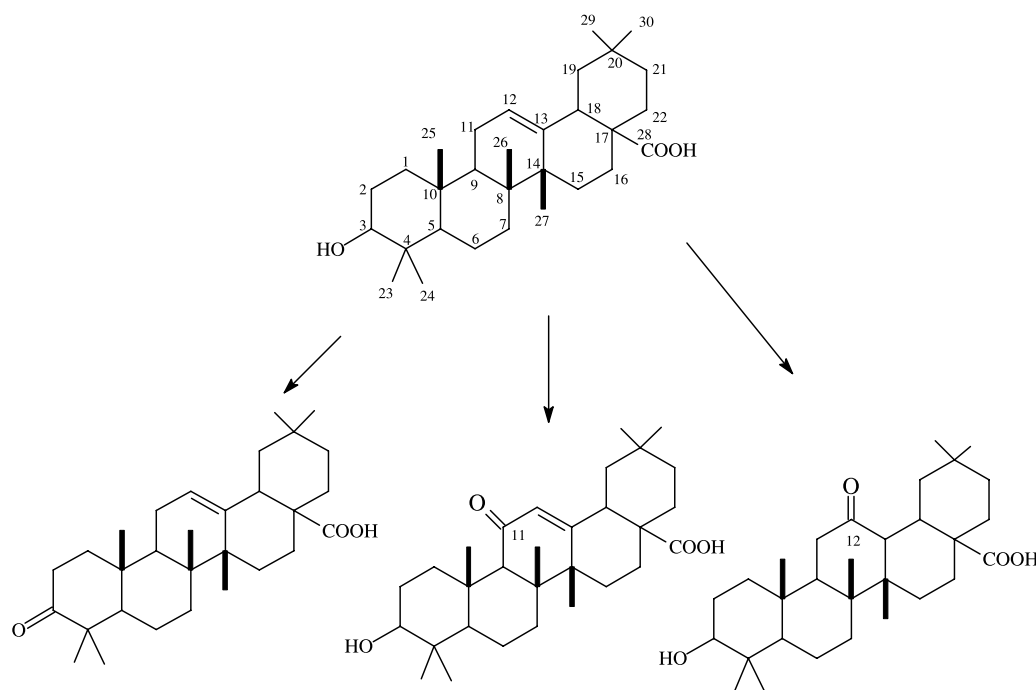


**Figure 15.** Proposed oxidation products of oleanolic acid.

have not been reported. Several 12-oleanene derivatives present a substituent other than H/H at C-6 (Akhtar and Malik, 1993; Ahmad and Atta-ur-Rahman, 1994) or C-2 (Ahmad and Atta-ur-Rahman, 1994). However, according to Budzikiewicz *et al.* (1963), the general fragmentation pattern of oleana(e)nes is not only affected when a keto or other group is placed at C-6, C-16 or C-21. Thus, $COOCH_3$ substituent is placed at C-6 and compound 1 is identified as 11-oxo-3$\beta$-hydroxy-28-norolean-17-en-6-oate. It is the first time that compound 1 is identified in *Pistacia* species. There is also a slight possibility that compounds identified as 28-norolean-17-ene derivatives (III, Fig. 1) may have as substituents $R_3$=H/H and $R_2$ varying (at C-4), depending on each $M^+$. This possibility is rejected since such mass fragments are not observed in the mass spectra of compounds identified as 28-norolean-17-ene derivatives. The difficulty in the identification of these compounds is that fragments presented to date concern the upper part of the molecule of 28-norolean-17-ene derivatives. 28-Norolean-17-ene derivatives with $R_3$=H/H and $R_2$ varying are rejected, since, as be described below, 28-norolean-17-enes are formed by decarboxylation of methyl oleanonate (12-oleanene) and 12-oleanene derivatives identified in *P. lentiscus* resin (I, Fig. 1) are only these with $R_3$=$CH_3$ and $R_4$ varying. Hence, it is likely that in the resultant 28-norolean-17-enes, $R_2$=$CH_3$ and $R_3$ vary. Each of the 28-norolean-17-ene derivatives that have been identified in the present study will have their substituent at C-6, according to the above discussion.

Compound 4 (Table 2) was identified as methyl 3$\beta$-acetoxy-6$\beta$-hydroxy-olean-18-en-28-olate ($M^+$ 530), since the main fragments descending (*m/z* 207, 189, 203) are indicative of an 18-oleanene structure, and a 3$\beta$-hydroxy or acetyloxy-group at C-3 (*m/z* 207). $COOCH_3$ group is reported at C-17 for $\Delta$-12 and $\Delta$-18 oleanene derivatives reported in *Pistacia* species. The hydroxyl group is placed at C-6, since, as reported, this does not affect the main fragments, and there have been reported several 6-hydroxy olean-12-ene derivatives. The mass spectra of 3$\beta$-acetoxy-6$\beta$-hydroxy-olean-12-en-28-olate (the respective 12-unsaturated derivative) have been presented in the online mass spectral database NIST 98 and also in the analysis of the Tolu balsam (Wahlberg and Enzell, 1971) with the same fragments, where the only difference is the proportion of *m/z* 203 and 189: in 12-unsaturated derivatives the fragment at *m/z* 203 is predominant where *m/z* 189 is predominant in 18-unsaturated derivatives. Thus compound 4 is characterized as 3$\beta$-acetoxy-6$\beta$-hydroxy-olean-18-en-28-olate since *m/z* 189 is more abundant than *m/z* 203 and is identified for the first time in *Pistacia* species.

Compound 5 was characterized as methyl 3$\beta$-acetoxy-6$\beta$-hydroxy-dihydro-isomasticadienolate ($M^+$ 530), since the main fragments are *m/z* 207, 121 and 257. From these fragments *m/z* 257 is characteristic of 7- and 8-tirucallene derivatives, while 121 is characteristic of $\Delta$8- (isomastica) derivative as reported. Another fragment (*m/z* 207) suggests a hydroxyl group or acetoxy derivative at C-3. Loss of *m/z* 59 (*m/z* 466, 407) suggests the presence of a $COOCH_3$ group ($R_2$, VII). The molecular ion peak at 530 *m/z* corresponds to an acetoxy-derivative of a triterpene methyl ester, which also has a hydroxyl group, since methyl-3$\beta$-acetoxy-dihydromasticadienolate has $M^+$ 514. Thus the hydroxyl group is placed most probably at C-6. It is the first time that compound 5 has been identified in *Pistacia* species.

Compound 9 from the acidic fraction was characterized as methyl 3$\beta$-acetoxy-20,30-dehydro-12-lupen-28-oate (512 $M^+$), since the main fragments, *m/z* 207 and 191, are indicative of a hydroxyl or acetoxy group at C-3 ($R_1$) and a lupane skeleton with saturated side chain (*m/z* 191), as previously discussed in the fragmentation mechanism of lupanes. 28-Norolean-17-ene skeleton is not possible since 163 *m/z* is not the base peak, while 12- and 18-oleanenes are excluded since their base peaks 203 and 189 are not present. A $COOCH_3$ group is placed at C-28. Since the $M^+$ is 512, compound 9 is a 12-lupene derivative with saturated side chain. This is also proved since in 12-lupenes retro-Diels Alder fragmentation does not appear (*m/z* 189, 203). Betulinic acetate could be a possible molecule (unsaturated side chain and saturated lupane skeleton), but must be rejected because the unsaturated side chain should give *m/z* 189 as the main fragment (Budzikiewicz *et al.*, 1963; Heinzen *et al.*, 1996). Thus methyl 3$\beta$-acetoxy-20,30-dehydro-12-lupen-28-oate is proposed. It is the first time that compound 9 has been identified in *P. lentiscus*.

Compound 10 is methyl olean-12,18-dien-3-olate [$M^+$ 468]. It was the last compound eluted at the chromatogram during analysis of the acidic fraction of traditionally collected resin. The main fragments *m/z* 207, 189 and 177 indicate the presence of a hydroxyl group at C-3 and an 18-oleanene structure. Loss of *m/z* 60 (341, 281) indicates the presence of a $COOCH_3$ group. A 3-hydroxy-18-oleanene derivative should have $M^+$ 470, while this compound has 468 $M^+$ and thus an olean-12,18-diene structure is proposed, since *m/z* 203 and 189 of 12-oleanene derivative are present. It is the first time that compound 10 and generally an oleandiene derivative has been identified in *Pistacia* species.

In the neutral fraction of the resin (traditionally collected) 26 triterpenes were identified, 18 of which have never been reported before (Table 3). During the analysis of the neutral fraction the following compounds were identified according to already published mass spectra and according to the fragmentation mechanisms presented before: tirucallol, dammaradienone, 28-norolean-12-en-3-one, $\beta$-amyrone, 28-norolean-17-en-3-one, hydroxy-dammarenone, oleanonic aldehyde

and nor-lupenone. These compounds have been already identified in *P. lentiscus* resin, analysed in several papers. The most abundant compound in the neutral fraction was found to be 28-norolean-17-en-3-one (53% of neutral fraction or 19% of total triterpenic fraction).

All the other compounds detected in the neutral fraction of *P. lentiscus* var. Chia (semi-solid form, Table 3) have not been reported before. Compound 12 was characterized as 3β-hydroxy-6β-hydroxymethyl-28-norolean-17-ene (a 28-norolean-17-ene derivative with $R_1$=OH/H, $R_3$=CH$_2$OH, $R_2$=CH$_3$), since the main fragments $m/z$ 163, 207 and 191 suggest that the ingredient possesses a 28-norolean-17-ene type skeleton with a 3β-hydroxyl group. The presence of a CH$_2$OH group was confirmed by the M$^+$ 442. According to the above analysis, this group was placed at C-6.

Compound 13 was suggested to be a 28-norolean-17-ene derivative with $R_1$=OH/H, $R_3$=CHO and $R_2$=CH$_3$ (named 3β-hydroxy-28-norolean-17-en-6-al). The analysis was the same as compound 12, whereas M$^+$ 440 indicates the presence of a CHO group at C-6 instead of CH$_2$OH. Compound 15 was characterized as 3-methoxy-28-norolean-12-ene (Δ12, $R_1$=OCH$_3$/H, $R_2$=H, M$^+$ 426), since the main fragments descending, $m/z$ 204 and 189, are indicative of a norolean-12-ene skeleton, as described above. Loss of 32 $m/z$ ($m/z$ 313 and 281) indicates a OCH$_3$ group. Compound 19 (M$^+$ 424) has been characterized as olean-18-en-3-one (an 18-oleanene derivative with substituents $R_1$=O, $R_2$=$R_3$=CH$_3$). Main fragments at $m/z$ 205, 189 and 177 are indicative of an 18-oleanene skeleton with a keto group at C-3 ($m/z$ 205). Thus, an 18-oleanen-3-one derivative with M$^+$ 424 is the one with $R_2$=CH$_3$. Compound 21 (M$^+$ 408) presented the main fragments of a 28-nor-17-oleanene derivative. Its molecular weight indicates the presence of two double bonds, a Δ17 and most probably a Δ12, and thus compound 21 was characterized as 28-norolean-12,17-dien-3-one.

Compound 22 was characterized as 6-methyl-28-norolean-17-en-3-one, a 28-nor-17-oleanene derivative with $R_1$=O, $R_2$=CH$_3$, $R_3$=CH$_3$ (M$^+$ 424). The main fragments, $m/z$ 163, 205 and 191, suggest the presence of a 28-norolean-17-ene skeleton with a keto group at C-3 (205 $m/z$). Thus, a methyl group was placed at C-6, according to the above analysis. Compound 23, with main fragments 207, 163 and 191 $m/z$ and M$^+$ 426, was proposed to be 3-methoxy-28-norolean-17-ene, since $m/z$ 221 indicates the presence of a OCH$_3$ group at C-3 (Budzikiewicz *et al.*, 1963). Compound 24 (Table 3) was proved to be a 28-nor-17-oleanene derivative with $R_1$=OAc/H, $R_2$=CH$_3$ and $R_3$=H/H (3β-acetoxy-28-norolean-17-ene, since the main fragments $m/z$ 163 and 191 indicate a 28-norolean-17-ene skeleton. $R_2$=CH$_3$ is the most common substituent for 28-nor-17-oleanene and thus $R_3$=H. The only derivative with M$^+$ 456 is the one with an acetoxy group at C-3.

Compound 25 (M$^+$ 438) was found to be according to the above analysis a 28-nor-17-oleanene derivative with $R_1$=O, $R_2$=CH$_3$ and $R_3$=CHO (3-oxo-28-norolean-17-en-6-al). The main $m/z$ 163 followed by 191 and 207 indicates a 28-norolean-17-ene skeleton. Loss of 30 $m/z$ (438, 408 $m/z$) shows a CHO group at C-6 as analysed above. Thus, compound 25 has a substituent $R_1$=O. Compound 26 was characterized as a 28-nor-17-oleanene compound with $R_1$=OH/H, $R_2$=$R_3$=CH$_3$, named 3β-hydroxy-6-methyl-28-norolean-17-ene, since $m/z$ 163, 207 and 191 indicate, according to our previous analysis, a 28-nor-17-oleanene skeleton with a 3β-hydroxyl group. Thus, from M$^+$ 426, $R_3$=CH$_3$.

Compound 27 in the neutral fraction of *P. lentiscus* var. Chia (semi-solid form) was established to be 18-oleanen-3-ol (Δ18, $R_1$=OH/H, $R_2$=CH$_3$). The main ions at $m/z$ 189, 207, 203 and 249 indicate according to the fragmentation mechanism reviewed above, an 18-oleanene skeleton. The $m/z$ 207 represents a 3-hydroxyl group and thus $R_2$=CH$_3$ (M$^+$ 426). Compound 28 (M$^+$ 424) is an unidentified dammarane derivative with a hydroxyl group at C-3 (β-epimer), since the characteristic fragments 109 and 207 $m/z$ are present. Its base peak was $m/z$ 141. It seems that 3β-hydroxy-dammarane compound 28 has M$^+$ 424 and base peak 141, while dammarane compound 30 M$^+$ 426 and base peak 143 and hence compound 28 will probably have a double bond more than compound 30 (probably at the side chain), since both of them have a 3-hydroxyl group ($m/z$ 207).

Compound 29 was characterized as 20,24-epoxy-25-hydroxy-dammaren-3-one according to the fragmentation mechanism presented above for dammarane compounds. This compound has been found in aged dammar resin (van der Doelen and Boon, 2000) and is an oxidized derivative of dammarane occurring during aging of the resin, its main fragment being $m/z$ 143. The characteristic fragmentation at $m/z$ 143 suggests the presence of a hydroxyisopropyl-methyl-tetrahydroxyfuran side chain [$C_8H_{15}O_2$]$^+$ in the molecule.

Compound 30 of the neutral fraction of traditionally collected resin was suggested as an unidentified epoxy-dammarene derivative with M$^+$ 426. Base peak 143 and $m/z$ 399 indicate the presence of a hydroxyisopropyl-methyl-tetrahydroxyfuran side chain, but the $R_1$, $R_2$ cannot be identified. Compound 32 was established to be 28-norolean-17-en-3-ol ($R_1$=OH/H, $R_3$=H/H, $R_2$=CH$_3$) (M$^+$ 412). Ions at $m/z$ 207, 163 and 191, which are the main fragments, are indicative of a 3β-hydroxyl group and a 28-nor-17-oleanene skeleton.

Isomasticadienolic aldehyde (M$^+$ 440, compound 34) was found in the neutral fraction of *P. lentiscus* var. Chia (traditional collection). Identification was performed according to the presence of 257, 121, 207 $m/z$, which are indicative of an 8-tirucallene skeleton with a 3β-hydroxyl group. Isomasticadienolic aldehyde

(R$_2$=CHO) has M$^+$ 440. 11-Oxo-$\beta$-amyrin acetate was proved to be included in the *P. lentiscus* resin var. Chia neutral fraction (compound 35, M$^+$ 482). Characterization was performed according to already published mass spectra not in *Pistacia* species (NIST 98 online mass spectra database, 1998). Loss of 60 *m/z* shows an acetoxy group, placed at C-3 (*m/z* 207). The base peak at 233 *m/z* is formed from the 218 base peak of amyrin plus oxygen *m/z*; 135 *m/z* is also a characteristic main fragment of 11-oxo-$\beta$-amyrin acetate. This product is also an oxidized derivative of $\beta$-amyrin acetate, probably formed during resin aging (such as compound 1 of the acidic fraction). 11-Oxo derivatives are most common in $\Delta$-12 oleanene derivatives. Such oxidative modifications are generally assumed to result from secondary reactions, which occur after the formation of the parent substances. The occurrence of this compound can be justified by oxidation mechanism of oleanonate derivatives as depicted in Fig. 15, where a CH$_3$ group is placed at C-17 instead of COOH.

In both Tables 2 and 3 where the results of our mass spectra analysis are presented for the neutral and acidic fraction of traditionally collected *P. lentiscus* var. Chia, the compounds that have already been identified in *Pistacia lentiscus* by other researchers are marked. As proved, in the acidic fraction five new triterpene compounds were elucidated, while in the neutral fraction 18 new minor triterpene compounds were also identified by GC-MS analysis. The major constituents of *P. lentiscus* resin var. Chia (semi-solid form) are, in the following order: isomasticadienonic acid, masticadienonic acid and 28-norolean-17-en-3-one. 8-Tirucallene derivatives predominated in the acidic fraction compared with $\Delta$7-, while in the neutral one only 8-tirucallene derivatives were present as minor

components. Several oleanene skeletons with different substituents were present in both the acidic and neutral fraction: 12-oleanene derivatives predominated in the acidic fraction, followed by $\Delta$18- and 28-nor-17-, whereas 28-nor-17-oleanene derivatives predominated in the neutral fraction followed by 12-oleanenes. 11-Oxo derivatives of $\Delta$-12 oleanene were present as minor components in both acidic and neutral fraction, probably due to oxidation of the parent 12-oleanene molecule. Thus it can be concluded that the resin sample analysed was not aged.

It is the first time that several minor new components have been identified in *P. lentiscus* var. Chia resin (semi-solid form). Methoxy-derivatives of oleanene skeletons have never been reported before in *P. lentiscus*. It is also significant that only oleanane and not ursane derivatives are biosynthesized in *P. lentiscus* resin.

28-Nor-17-oleanenes and mainly 28-nor-17-oleanen-3-one were the main components in the neutral fraction of traditionally collected *P. lentiscus* var. Chia. Trying to elucidate the biosynthetic route on which these oleanene derivatives are formed, it is proposed according to our findings and the observation of Pastorova (1997) that these are formed through decarboxylation at C-28 of 12-oleanen-28-oate (oleanonic acid methyl ester). This process leads preferentially to a $\Delta$-17 alkene with reduction of the $\Delta$12 double bond in oleanenes and double bond rearrangement, as depicted in Fig. 16. Such a decarboxylation procedure has been proposed by Ten Haven *et al.* (1992) as a diagenetic pathway to 28-nor-triterpenoids in sediments. 28-Norolean-17-en-3-ol did not appear in the chromatogram either because the respective $\Delta$12 methyl ester (methyl oleanolate) was not present or, less likely, because the 3-hydroxyl group was oxidized to 3-keto
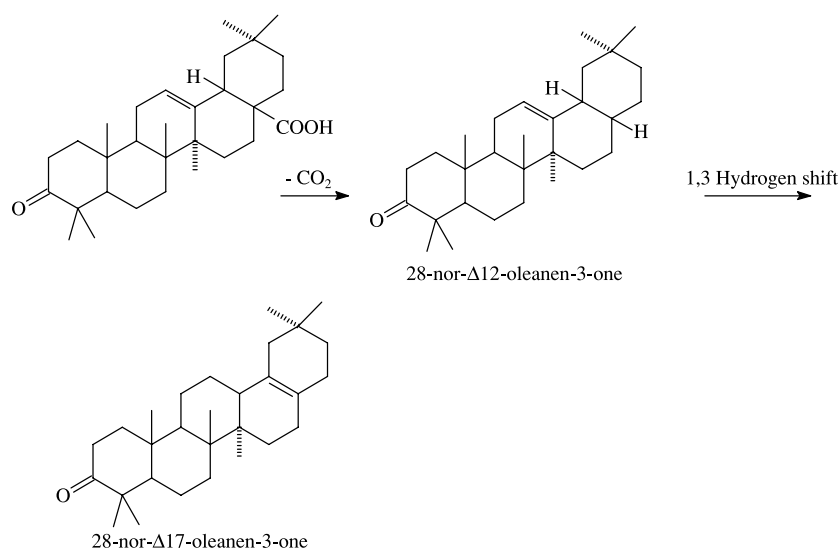


**Figure 16.** Biosynthetic formation of 28-nor-17-oleanen-3-one, the main component of neutral fraction of both resin samples, through decarboxylation of methyl oleanonate.

one. According to Ten Haven *et al.* (1992), C-28 alde-hydes can undergo loss of carbon monoxide during atmospheric oxidation and thus 28-nor-olean-17-ene derivatives can be formed alternatively from oleanonic aldehyde. The derivatives with 28-norolean-17-ene skeleton assigned in the present study to be contained in *P. lentiscus* var. Chia resin are probably biosynthetic transformation products of oleanane types (mainly 12-oleanene), which seem to be potential precursors for 28-nor-17-triterpenoids at the functionalized C-17. 28-Nor-17-oleanen-3-one has been reported to be stable during aging and temperature, while methyl oleanonate is not (Colombini *et al.*, 2000). It is most probable that 28-nor-17-oleanenes are formed during triterpene biosynthesis and not during aging of the resin.

In the second part of the paper we have analysed the resin of *P. lentiscus* var. Chia obtained using ethrel as a stimulating agent (liquid collection), which is a newly applied technique that increases resin productivity. The aim of this study was to compare the qualitative and quantitative composition of *P. lentiscus* var. Chia resin obtained by the two methods, traditional and liquid, since the biological activity that this resin possesses is attributed mainly to the triterpenes. As proved in the acidic fraction of liquid collection resin, eight triterpenes were identified (Table 4), four of which were not identified in traditionally collected *P. lentiscus resin* var. Chia, specifically methyl 11-oxo-masticadienonate, methyl isomastica-8,12-dienolate, methyl 3-acetoxy-3-epi-isomasticadienolate and methyl 3-acetoxy-3-epi-masticadienolate. The mass spectra of the last two compounds have been published before (Papageorgiou *et al.*, 1997), where the α-epimer type was identified due to the intense peak at *m/z* 437 ([M-CH$_3$-OAc]$^+$) and also as reported above due to the presence of an *m/z* 189 fragment.

Methyl-11-oxo-masticadienonate (M$^+$ 482) was identi-fied due to *m/z* 259, 95, 355 and 453, which indicated a Δ-7 tirucallene skeleton. The molecular ion at 482 sug-gests the presence of a keto group further to methyl masticadienonate, while the shift of the characteristic peak *m/z* 313 to 327 confirmed the presence of a keto group at the tetracyclic skeleton. The 11-keto derivative was proposed in the present study, since this derivative explains the *m/z* fragments presented in Table 4. As shown in Scheme 1, *m/z* 327 is formed from the
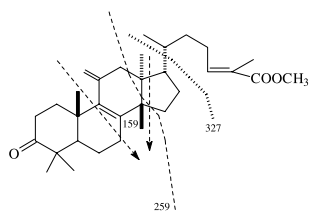
[M-side chain] ion. The respective fragment in methyl masticadienonate is *m/z* 315. Fragment at *m/z* 95 is the ion formed by the D skeleton together with the two CH$_3$ substituents, while *m/z* 159 (which is a fragment that is not present in all other mastica- or isomastica-derivatives) is attributed to ring C that contains the oxo-group, together with two methyl substituents. Finally, the characteristic fragment at *m/z* 259, which is also not present in other (iso)mastica-derivatives, is attributed to the fragment presented in Scheme 1 and contains an 11-oxo group at ring C. Keto derivatives of triterpenes and specifically of oleanonic acid have been found in mastic gum during resin aging (van der Doelen and Boon, 2000). This keto derivative of tirucallene skeleton may occur during aging of the resin and is reported for the first time.

Methylisomastica-8,11(12)-dienolate was character-ized due to the characteristic fragments: *m/z* 453, 121, 207, 241 and 257 that suggest the presence of a tirucallene derivative (257, 453, 55, 95, 121) as de-scribed above and specifically an 8-tirucallene deriv-ative (*m/z* 121 and 241). The molecular ion at 468 *m/z* and the presence of a hydroxyl group at C-3 (isomasticadienolate derivative, *m/z* 207) indicate the presence of an additional double bond. This is en-hanced since a hydroxyl group should give a 259 *m/z* fragment, while compound 7 has a fragment at *m/z* 257 and thus an additional double bond is present in the molecule. Since the *m/z* 257 fragment is formed from A, B and C rings and there is already a double bond in ring B, the additional double bond is placed at ring C and specifically at C-11. The interpretation of the mass spectra of the other triterpene compounds of the acidic fraction of *P. lentiscus* var. Chia resin liquid collection has already been reported in the analysis of triterpenes of traditionally collected *P. lentiscus* resin var. Chia.

In the neutral fraction of liquid collection *P. lentiscus* var. Chia resin, 11 compounds were identified (Table 5). From these, only 3-β-acetoxy-12-oleanene, 3-β-acetoxy-isomasticadienolic aldehyde and 28-norolean-12-en-3-ol were not contained in traditionally collected *P. lentiscus resin*. 3-β-Acetoxy-isomasticadienolic alde-hyde (M$^+$ 482) was identified since fragments at *m/z* 453, 95, 55, 257, 121 and 241 are indicative of an 8-tirucallene (isomasticadienolic) skeleton. M$^+$ 482 may corresponds either to an isomasticadienonate methyl ester with a keto group (R$_1$=O, R$_2$=COOCH$_3$, R$_3$=O at C-11 or C-12) or an isomasticadienolic aldehyde with a 3β-acetoxyl group (R$_1$=OAc, R$_2$=CHO). The fact that the main fragments were not shifted meant that the first derivative was rejected, since a keto group at C-11 or 12 causes shifts in the peaks. Thus, a 3β-acetoxy-isomasticadienolic aldehyde was proposed for com-pound 17. Loss of *m/z* 60 indicates a 3β-OAc group and thus R$_2$=CHO (C-17). 3-β-Acetoxy-12-oleanene (compound 16) has the main fragments *m/z* 203 and



**Scheme 1.**

189 (M⁺ 452), indicative of a 12-oleanene skeleton. The only Δ12-derivative with M⁺ 452 is the one with substituents $R_1$=OAc/H and $R_2$=CH₃.

Liquid collection *P. lentiscus* var. Chia resin contains fewer minor triterpene compounds in the neutral fraction than the traditionally collected one. As presented, liquid collection *P. lentiscus* resin contains in the acidic fraction mainly tirucallene derivatives with Δ8-predominating, while only Δ-12-oleanene derivatives are present in the acidic fraction. In the neutral fraction, 17-oleanene derivatives predominate with 28-norolean-17-en-3-one being the major compound, followed by 12-oleanene derivatives. 18-Oleanene derivatives are not present in the neutral fraction of resin liquid collection, not in traces, and this comprises a difference from the traditionally collected resin. As proved from the quantitative analysis of the resin samples, the one collected using ethrel contains about the same percentage of isomasticadienonic acid as the sample traditionally collected, increased percentage of masticadienonic acid, significantly increased percentage of 28-norolean-17-en-3-one (19 to 36%) and decreased percentage of methyl oleanonate. This means that the use of the stimulant agent ethephon probably stimulates the biosynthesis of 28-norolean-17-ene derivatives, and thus the decarboxylation of Δ12-oleanonates.

As reported in *Pistacia* species, penta- or tetra-cyclic triterpenes from the oleana(e)ne, dammarane, lupa(e)ne, tirucalla(e)ne skeletons are present. As reviewed (Mahato and Sen, 1997), biosynthesis of triterpenes proceeds via enzymatic controlled cyclization of (3S)-oxidosqualene and rearrangements, which affords the pentacyclic structure with an oxygenated functionality at C-3, and in most cases a double bond is also present. Cyclization to pentacyclic triterpenes proceeds via a chair–chair–chair conformation of the substrate. Thus, as shown in Fig. 17, the cyclization first produces the tetracyclic dammarenyl C-20 cation, followed by a rearrangement leading to the pentacyclic β-amyrin or α-amyrin systems, via the baccharenyl, lupenyl and oleanyl cation species. Tetracyclic and pentacyclic triterpenes generated from these cationic intermediates are widespread in nature. Squalene and oxidosqualene are converted to various skeletal types of triterpenes and sterols by different enzyme systems (Abe *et al.*, 1993). This scheme justifies the occurrence of oleana(e)ne, lupa(e)ne and dammarane derivatives in *Pistacia* species. As proved in our analysis, α-amyrin systems (ursane derivatives) do not occur in *Pistacia* species and thus biosynthesis is interrupted in oleanyl cations, which in turn give Δ12-, or Δ18-unsaturated oleanene derivatives, due to hydrogen transfer. The occurrence of Δ12- and Δ18-unsaturated oleanene derivatives in *Pistacia* species can be attributed to the stabilization of the oleanyl cation formed during biosynthesis, as proved in Fig. 17. Olean-12-ene is
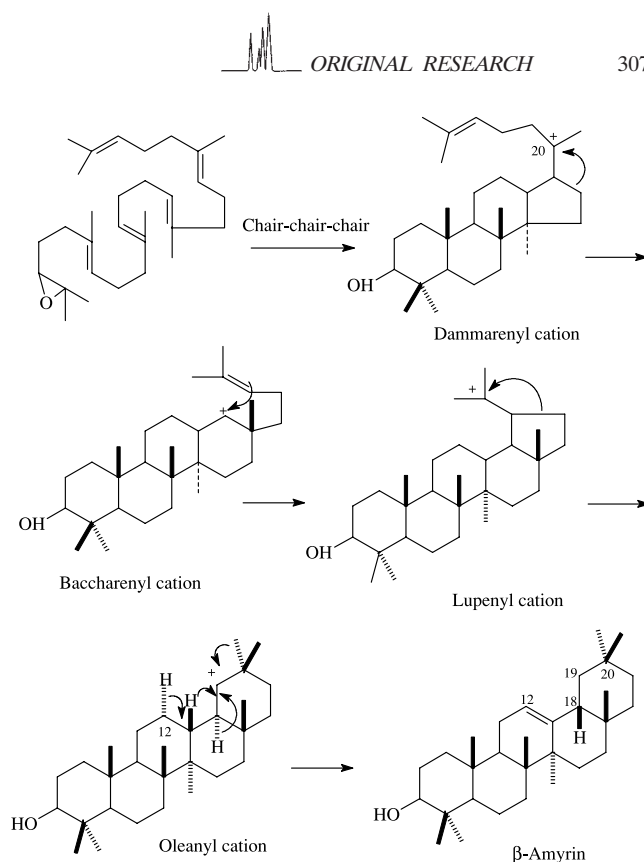


**Figure 17.** Biosynthetic pathway of triterpenes contained in *Pistacia* species from oxidosqualene.
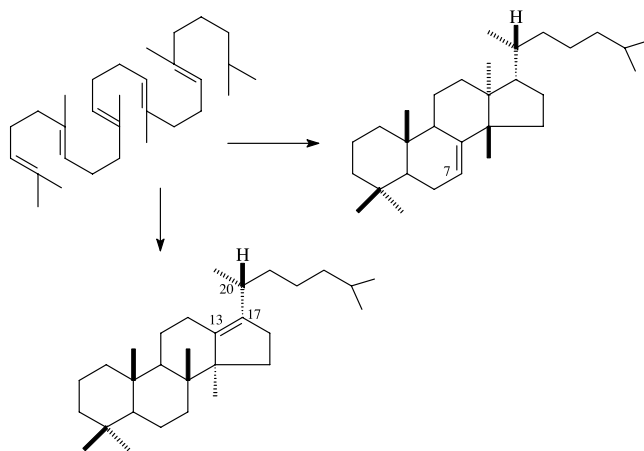


**Figure 18.** Biosynthetic pathway of euphen-7-ene, the epimer of tirucallene (parent molecule of mastica derivatives), from 2,3-dihydrosqualene.

isomerized to olean-18-ene and olean-13(18)-ene, but in *P. lentiscus* olean-13(18)-ene derivatives have never been reported and thus selective isomerization to Δ-18 derivatives probably occurs.

2,3-Dihydrosqualene is a molecule lacking one of the terminal double bonds of squalene, and is converted to the tetracyclic triterpene euphen-7-ene (Fig. 18) in good yield (Abe *et al.*, 1993). Squalene under the same pathway probably leads to euphen-7-ene with unsaturated

side chain (C-20), which is an epimer of tirucall-7-ene, the parent molecule for masticadienonate derivatives that occur in quite significant quantities in *Pistacia* resins. In the case of euph-7-ene formation, the enzyme seems to play a more active role in order to guide the course of reaction by strictly controlling the backbone rearrangement to yield the euphene skeleton with the C-20R stereochemistry and Δ-7 double bond rather than the most thermodynamically stable 13(17)-double bond. Thus in *Pistacia* species, a similar biosynthetic pathway is followed for tirucallane derivatives (starting with squalene for tirucallene compounds and with 2,3-dihydrosqualene for 20,24-dihydro-tirucallene derivatives) and this shows formation of 7-euphene or tirucallene derivatives compared with dammar-13(17)-ene.

The above biosynthetic routes prove the occurrence of oleana(e)ne (Δ12-, Δ18-), dammarane, lupa(e)ne and finally tirucalla(e)ne derivatives. Noroleanene-type molecules are reported to be formed by decarboxylation of compounds with a carboxylic acid at C-17. These compounds (28-norolean-17-enes) comprise a large proportion of the neutral fraction in *Pistacia lentiscus* resin. However, it is unknown whether this decarboxylation process takes place during biosynthesis by the action of enzymes after excretion or afterwards as a result of resin aging. As proposed by Scalarone *et al.* (2003), it is possible that decarboxylation is not taking place during aging, but during terpenoid biosynthesis in the plant *Pistacia lentiscus* var. Chia. 28-Norolean-17-en-3-one and each of the 28-norolean-17-ene derivatives identified in *P. lentiscus* resin samples can be formed from oleanonic acid methyl ester, using a mechanism of decarboxylation followed by a hydrogen shift, as shown in Fig. 16 (Pastorova, 1997). Thus, in the resin of *Pistacia lentiscus* 28-norolean-17-ene derivatives are formed from oleanonic (Δ12), oleanolic acid or oleanonic aldehyde (Ten Haven *et al.*, 1992) that are probably in abundance, and their percentage is reduced after the formation of noroleanenes. In liquid collection *P. lentiscus* resin the percentage of 28-nor-olean-17-enes was higher than in traditional collection. It can be assumed that the stimulating agent ethephon enhances the biosynthetic activity of enzymes and the decarboxylation procedure to noroleanenes is enhanced.

## CONCLUSION

In the present work a complete analysis of the identification of penta- and tetra-cyclic triterpenes of *P. lentiscus* var. Chia resin (both traditional and liquid collection) was approached, where both major and minor components were characterized by mass spectra interpretation; minor components are reported for the first time. It is also the first time that the chemical com-

position of *P. lentiscus* var. Chia obtained by use of a stimulating agent (liquid collection) has been reported and a comparison of the contained triterpenes has been performed with that of traditionally collected resin. Analysis of the triterpenes contained in liquid collection resin and comparison with the traditionally collected resin has been approached, since the biological activity of resin may be influenced by different triterpene composition.

Since the interpretation of mass spectral data of triterpenes is confusing and several authors have suggested triterpene derivatives only according to their molecular weight, with this study we present an analytical review of the base peaks, main fragments and fragmentation mechanism/pattern of several skeleton of penta- and tetra-cyclic triterpenes reported in *P. lentiscus* resin. This is helpful in order to facilitate the identification of triterpenes in *Pistacia* species and in every species that contains triterpenes, on the basis of mass spectral interpretation, according to M⁺, base peak and main fragments. This study will be also helpful for archaeologists and archaeological chemists who need to analyse art objects and identify their nature.

Ten triterpenes belonging to the acidic fraction of traditionally collected *P. lentiscus* resin var. Chia were identified, five of which are newly identified compounds. In the neutral fraction 26 triterpenes were characterized, 18 of which have not been reported before in that resin. Major constituents of the resin in semi-solid form were in the following order: isomasticadienonic acid, masticadienonic acid and 28-norolean-17-en-3-one. 8-Tirucallene derivatives predominated in the acidic fraction compared with Δ7-, while in the neutral one only 8-tirucallene derivatives were present as minor components. Several oleanene skeletons with different substituents were present in both the acidic and neutral fraction: Δ12-oleanene derivatives predominated in the acidic fraction, followed by Δ18- and 28-nor-17-, whereas 28-nor-17-oleanene derivatives predominated in the neutral fraction followed by 12-oleanenes. 11-Oxo derivatives of oleanenes were present in both acidic and neutral fraction, as minor components, probably due to oxidation of the parent Δ12 or Δ17-oleanene molecule. It is the first time that several minor new components of *P. lentiscus* var. Chia resin (semi-solid form) have been identified. Methoxy-derivatives of oleanene skeletons have never been reported in *P. lentiscus*. It is also significant that only oleanane and not ursane derivatives are biosynthesized in *P. lentiscus* resin.

Finally the triterpenes contained in *Pistacia lentiscus* resin var. Chia obtained by use of ethrel (stimulating agent) have been elucidated. In the acidic fraction eight triterpenes were identified, four of which were not identified in the traditionally collected resin. In the neutral fraction 11 compounds were identified, three of

which were not contained in traditionally collected resin. Liquid collection resin contained fewer minor triterpene compounds in the neutral fraction than the traditionally collected one. As presented, liquid collection resin contained mainly, in the acidic fraction, tirucallene derivatives with Δ8-predominating, while only Δ-12-oleanene derivatives were present in the acidic fraction. In the neutral fraction, 28-nor-17-oleanene derivatives predominated with 28-norolean-17-en-3-one being the major compound, followed by 12-oleanene derivatives. 18-Oleanene derivatives were not present in the neutral fraction of *P. lentiscus* var. Chia resin liquid collection, not in traces, and this is different from the traditionally collected resin. As proved from the quantitative analysis of the resin samples, liquid resin contains about the same percentage of isomasticadienonic acid with the sample traditionally collected, increased percentage of masticadienonic acid and significantly increased percentage of 28-norolean-17-en-3-one (19 to 36%). This means that the use of the stimulant agent ethrel probably stimulates the biosynthesis of norolean-17-ene derivatives, and thus the decarboxylation of 12-oleanonates is enhanced. A biosynthetic route of several triterpene skeleton presented in *P. lentiscus* resin is proposed.

## Acknowledgements

## REFERENCES

Abe I, Rohmer M and Prestwich GD. Enzymatic cyclization of squalene and oxidosqualene to sterols and triterpenes. *Chemical Reviews* 1993; **93**: 2189–2206.

Ahmad VU and Atta-ur-Rahman. *Handbook of Natural Products Data, Vol. 2. Pentacyclic Triterpenoids*. Elsevier: New York, 1994; 1–1556.

Akhtar N and Malik A. Oleanene type triterpenes from *Plumeria rubra*. *Phytochemistry* 1993; **32**(6): 1523–1525.

Al Said M, Ageel AM, Parmar NS and Tariq M. Evaluation of mastic obtained from *Pistacia lentiscus* crude drug for gastric and duodenal anti-ulcer activity. *Journal of Ethnopharmacology* 1986; **15**: 271–278.

Assimopoulou AN and Papageorgiou VP. Antioxidant activity of natural resins and bioactive triterpenes in oil substrates. Food Chemistry, in press, 2005.

Barton DHR and Seoane E. Triterpenoids. Part XXII. The constitution and stereochemistry of masticadienonic acid. *Journal of the Chemistry Society* 1956; 4150–4157.

Bona SG, Bono L, Daghetta L and Marone P. Bactericidal activity of *Pistacia lentiscus* gum mastic against *Helicobacter pylori*. *American Journal of Gastroenterology* 2001 (September suppl.); S49.

Budzikiewicz H, Wilson JM and Djerassi C. Mass spectrometry in structural and stereochemical problems. XXXII. Pentacyclic triterpenes. *Journal of the American Chemical Society* 1963; **85**: 3688–3699.

Colombini MP, Modugno F, Giannarelli S, Fuoco R and Matteini M. GC-MS characterization of paint varnishes. *Microchemical Journal* 2000; **67**: 385–396.

Dietemann P, Kalin M, Zumbuhl S, Knochenmuss R, Wulfert S and Zenobi R. A mass spectrometry and electron paramagnetic resonance study of photochemical and thermal aging of triterpenoid varnishes. *Analytical Chemisty* 2001; **73**: 2087–2096.

Dioscurides P. In *De Materia Medica*, Wellman M (ed.). Berloni Apud Weidmannos: Berlin, 1958.

Duke J. *Medicinal Plants of the Bible*. Trado-Medic Books: New York, 1983; 90.

Hairfield HH and Hairfield EM. Identification of a Late Bronze age resin. *Analytical Chemistry* 1990; **62**(1): 41–45.

Hartwell J. Plants used against cancer. *Lloydia* 1967; **30**(4): 395–397.

Heinzen H, de Vries JX, Moyna P, Remberg G, Martinez R and Tietze LF. Mass spectrometry of labelled triterpenoids-thermospray and electron impact ionization analysis. *Phytochemical Analysis* 1996; **7**: 237–244.

Huwez FU, Thirlwell D, Cockayne A and Ala'Aldeen DAA. Mastic gum kills *Helicobacter pylori*. *The New England Journal of Medicine* 1998; **339**(26): 1946.

Karliner J and Djerassi C. Terpenoids. LVII. Mass spectral and nuclear magnetic resonance studies of pentacyclic triterpene hydrocarbons. *Journal of the American Chemical Society* 1966; **31**: 1945–1956.

Katritzky AR. *Comprehensive Organic Functional Group Transformation*, Vol. 5. Cambridge University Press: Cambridge, 1995; 132.

Magiatis P, Melliou E, Skaltsounis AL, Chinou IB and Mitaku S. Chemical composition and antimicrobial activity of the essential oil of *Pistacia lentiscus* var. Chia. *Planta Medica* 1999; 65: 749–752.

Mahato SB and Sen S. Advances in triterpenoid research, 1990–1994. *Phytochemistry* 1997; **44**(7): 1185–1236.

Marner FJ, Freyer A and Lex J. Triterpenoids from gum mastic, the resin of *Pistacia lentiscus*. *Phytochemistry* 1991; **30**(11): 3709–3712.

Mills JS and White R. The identity of the resins from the Late Bronze Age shipwreck at Ulu Burun (Kas). *Archaeometry* 1989; **31**: 37–44.

Monaco P, Caputo R, Palumbo G and Mangoni L. Triterpenes from the galls of *Pistacia lentiscus*. *Phytochemistry* 1973; **12**: 2534–2537.

Nytoft HP, Bojeen-Koefoed JA, Christiansen FG and Fowler MG. Oleanane or lupane? Reappraisal of the presence of oleanane in Cretaceous-Tertiary oils and sediments. *Organic Geochemistry* 2002; **33**: 1225–1240.

Papageorgiou VP, Bakola-Christianopoulou MN, Apazidou KK and Psarros EE. Gas-chromatographic mass spectroscopic analysis of the acidic triterpenic fraction of mastic gum. *Journal of Chromatography A* 1997; **769**: 263–273.

Papanicolaou D, Melanitou M and Katsaboxakis K. Changes in chemical composition of the essential oil of Chios 'mastic resin' from *Pistacia lentiscus* var. Chia tree during solidification and storage. In *Food Flavours: Generation and Process Influence*, Charalambous G. (ed.). Elsevier Science: Oxford, 1995; 303–310.

Pastorova I. Chemically linking past and present: comparative studies of chars and resins. PhD thesis, Amsterdam University. 1997; 104–107.

Regert M and Rolando C. Identification of archaeological adhesives using direct inlet electron ionization mass spectrometry. *Analytical Chemistry* 2002; **74**: 965–975.

Scalarone D, van der Horst J, Boon JJ and Chiantore O. Direct-temperature mass spectrometric detection of volatile terpenoids and natural terpenoid polymers in fresh and artificially aged resins. *Journal of Mass Spectrometry* 2003; **38**: 607–617.

Seoane E. Further crystalline constituents of gum mastic. *Journal of the Chemical Society* 1956; 4158–4160.

Stern B, Heron C, Corr L, Serpico M and Bourriau J. Compositional variations in aged and heated *Pistacia* resin found in late bronze age Canaanite amphorae and bowls from Amarna, Egypt. *Archaeometry* 2003; **45**: 457–469.

Ten Haven HL, Peakman TM and Rullkotter J. Early diagenetic transformation of higher-plant triterpenoids in deep-sea sediments. *Geochimica et Cosmochimica Acta* 1992; **56**(5): 2001–2024.

van der Doelen GA and Boon JJ. Artificial ageing of varnish triterpenoids in solution. *Journal of Photochemistry and Photobiology A: Chemistry* 2000; **134**: 45–57.

van der Doelen GA, van den Berg KJ, Boon JJ, Shibayama N, de la Rie ER and Genuit WJL. Analysis of fresh triterpenoid resins and aged triterpenoid varnishes by high-performance liquid chromatography–atmospheric pressure chemical ionisation (tandem) mass spectrometry. *Journal of Chromatography A* 1998; **809**: 21–37.

Wahlberg I and Enzell CR. 3-Oxo-6β-hydroxyolean-12-en-28-oic acid, a new triterpenoid from commercial Tolu Balsam. *Acta Chemica Scandinavica* 1971; **25**: 70–76.

Zohary M. A monographical study of the genus *Pistacia*. *Palestine Journal of Botany, Jerusalem Series* 1952; **5**(4): 187–228.

Zumbuhl S, Knochenmuss R, Wulfert S, Dubois F, Dale MJ and Zenobi R. A graphite-assisted laser desorption/ionization study of light induced aging in triterpene dammar and mastic varnishes. *Analytical Chemistry* 1998; **70**: 707–715.
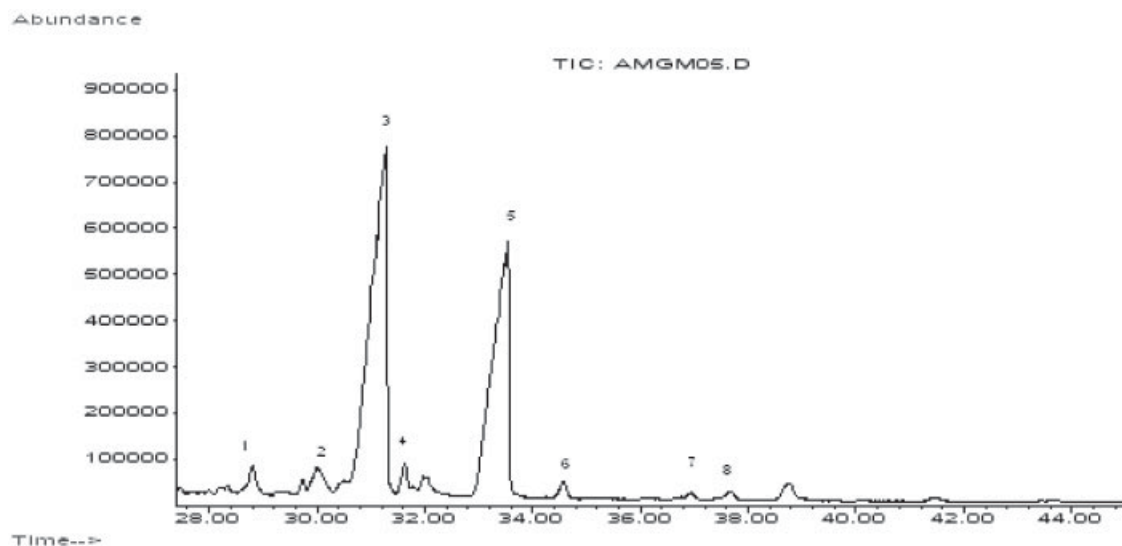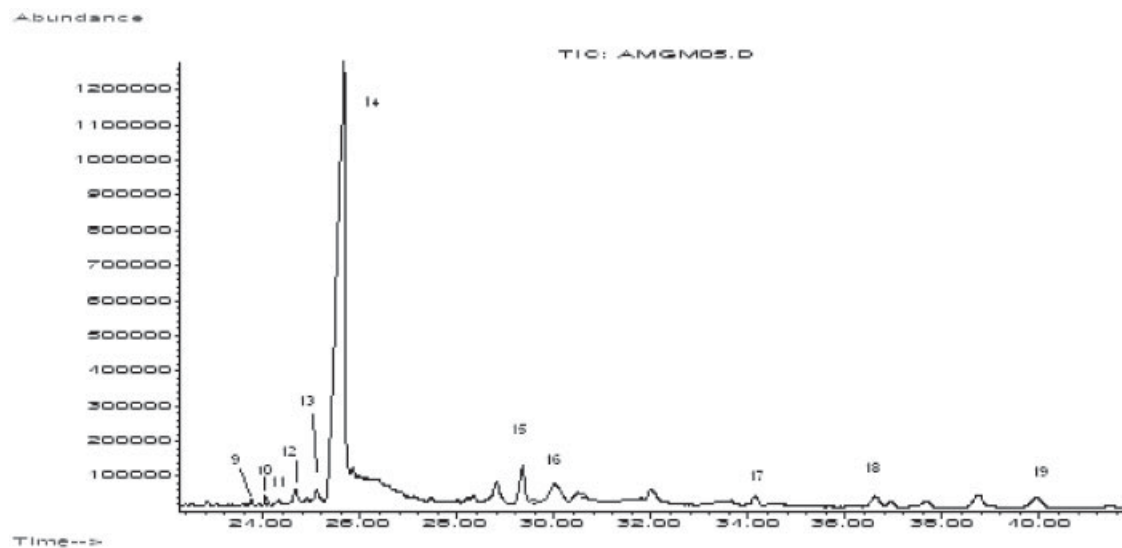
# APPENDIX
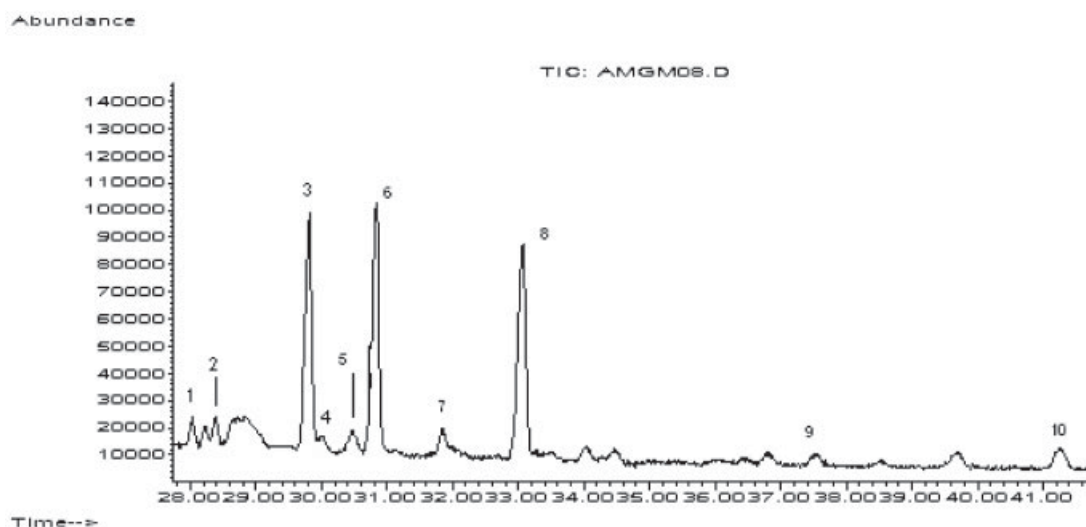


**Figure A1.** TIC of the region of the acidic fraction of *P. lentiscus* var. Chia traditionally collected resin.
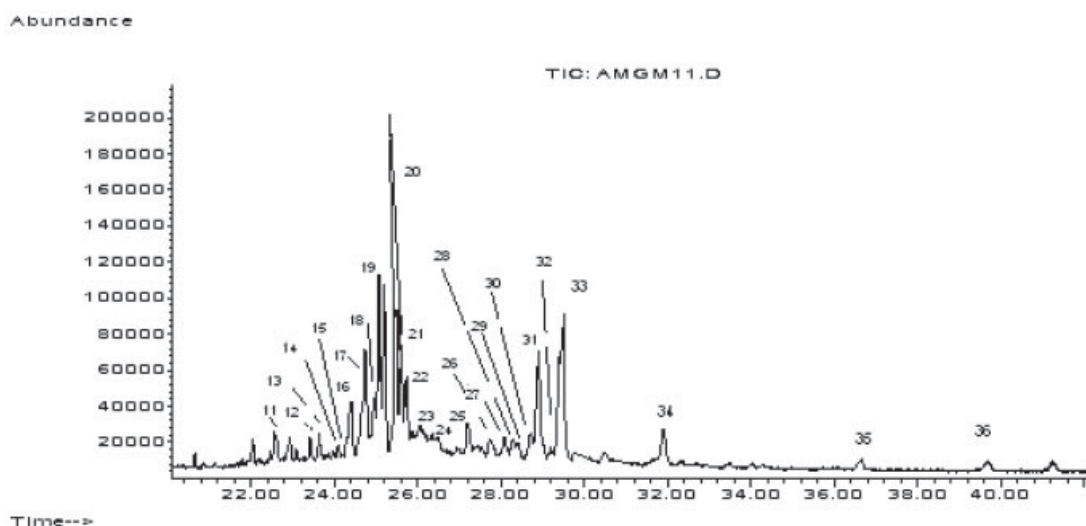


**Figure A2.** TIC of the region of the neutral fraction of *P. lentiscus* var. Chia traditionally collected resin.

**Figure A3.** TIC of the region of the acidic fraction of *P. lentiscus* var. Chia liquid collection resin.



**Figure A4.** TIC of the region of the neutral fraction of *P. lentiscus* var. Chia liquid collection resin.