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Gas chromatographic–mass spectroscopic analysis of the acidic triterpenic fraction of mastic gum

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Abstract

The acidic fraction of mastic gum, the resin of *Pistacia lentiscus* L. *var. Chia*, was analyzed using GC–MS. Ten triterpenoid acids – three already known – were tentatively identified as their methyl esters i.e., moronic, oleanonic, isomasticadienonic, masticadienonic, 18αH-oleanonic, oleanolic, 3-epiisomasticadienolic, masticadienolic, 3α-acetoxy-isomasticadienolic and 3α-acetoxymasticadienolic acids.

Keywords: *Pistacia lentiscus* L. *var. Chia*; Mastic gum; Triterpenoid acids; Terpenoids

1. Introduction

The genus *Pistacia* of the *Anacardiaceae* family has some eleven recognised species [1], many of which yield resin in some degree. However, only two of them, namely *Pistacia atlantica* Desf. and *Pistacia lentiscus* L. *var. Chia*, are major sources of resin that can be collected, traded and used and which has been an important article of commerce for centuries. Both species are cultivated on the Greek island of Chios [2] and yield the resins known as Chian turpentine and mastic gum, respectively.

Pistacia lentiscus L. is a small evergreen shrub native to the Mediterranean countries [3]. In contrast, its variety, *Pistacia lentiscus* L. *var. Chia*, is a small tree, growing particularly and almost exclusively in the south region of the Chios island [4].

Mastic gum is obtained by shallow incisions of the bark or the trunk and main branches of *Pistacia lentiscus* L. *var. Chia* with special tools [5].

Although the chemical composition of the resins extracted from insect galls on the plant of almost all species of *Pistacia* L. has been analyzed [6–14], only a few constituents of mastic gum have been isolated and identified. Early attempts to analyze mastic gum [15–17] were either unreliable or incomplete [18]. In 1956, Barton and Seoane [18,19] isolated and identified three crystalline compounds (masticadienonic, isomasticadienonic and oleanonic acids) from the acidic fraction and tirucallol from the neutral fraction of mastic gum. However, they did not mention the source of the resin analyzed in order to verify its authenticity. Recently, four novel triterpenoids were identified in the neutral fraction of mastic gum by Marner et al. [20]. Analyses carried out by Mills and White [4] and Hairfield and Hairfield [21] of the resin found in a Bronze Age shipwreck in Southern Turkey are referred to as

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chian turpentine and although the origin of the resin (chian turpentine or mastic gum) was to be defined, the study of mastic gum was limited to a TLC analysis [21].

The study of a series of natural resins, including mastic gum, forms a part of our laboratory programme which deals with the isolation of their active agents. Hence, extensive analyses of the volatile ingredients of mastic gum have been performed [22,23].

In a continuation of our efforts we herein report on the analysis of the acidic fraction of mastic gum using GC–MS. The identification of the GC peaks was performed by the use of the published mass spectra and mass spectral studies and retention characteristics.

2. Experimental

2.1. Materials

All solvents used were Merck products. The mastic gum analyzed was provided by the Chios Gum Mastic Growers Association.

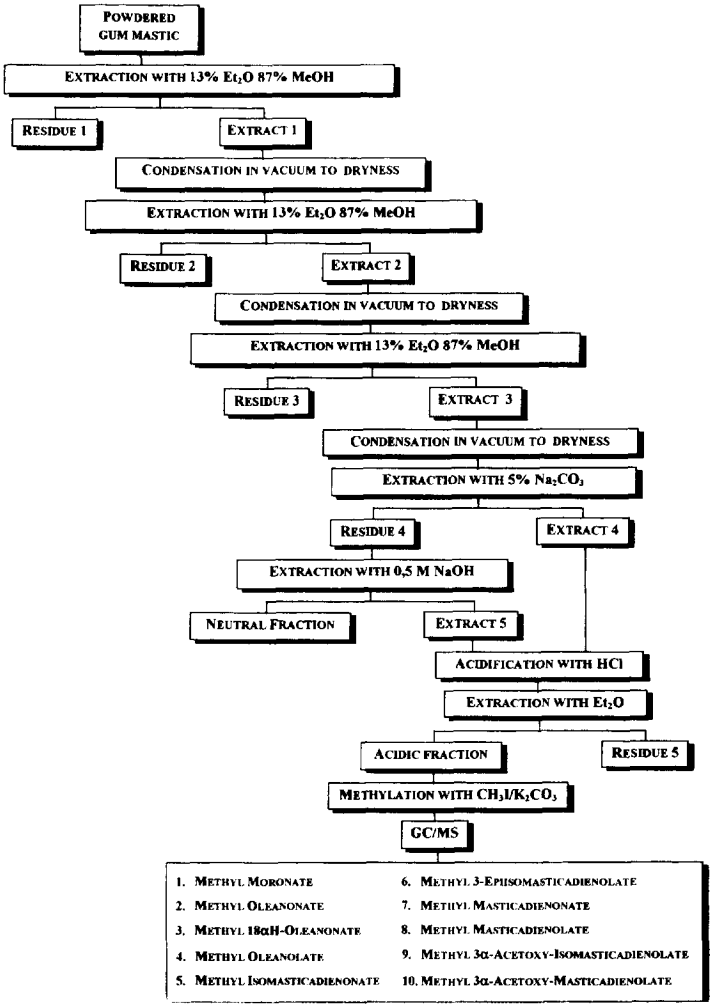


Fig. 1. Scheme for the triterpenoids separation from the acidic fraction of mastic gum.

2.2. Gas chromatography and mass spectrometry

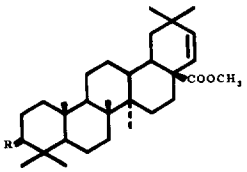
GC–MS analysis was carried out in a Hewlett-Packard Model HP 5890 gas chromatograph coupled with a HP 5989 mass spectrometer and equipped with a 30 m HP-5 capillary column of 0.25 mm I.D. and 0.11 μm film thickness. The system was operated under the following conditions: injector temperature, 280°C; GC column temperature, 180°C for 0.5 min, 180–240°C at 5°C min^{-1}, 240–300°C at 2°C min^{-1} and a final temperature of 300°C for 15 min. The mass spectrometer was set to scan 50–700 u per nominal s with an ionizing voltage at 70 eV.

2.3. Preparation of the acidic fraction

The acidic fraction was obtained as follows [18]: 45 g of powdered mastic gum were dissolved in 50 ml diethyl ether and the resulting solution was diluted with 330 ml methanol. The insoluble ingredients of the resin were discarded and the solution was condensed under vacuum to a viscous liquid. This liquid was then dissolved in 50 ml diethyl ether and the solution was diluted with 330 ml methanol and filtered. The complete solution of the resin in the diethyl ether–methanol system indicated the end of the repetitions. The product of the last evaporation was dissolved in 380 ml diethyl ether and extracted with 94 ml of 5% sodium carbonate solution. The ethereal phase was collected and extracted with 66 ml of 0.5 *M* sodium hydroxide solution. The aqueous extracts were combined, acidified with hydrochloric acid and extracted with diethyl ether. The ethereal phase which contained the fraction of the resin richest in triterpenes, was evaporated to dryness to afford the acidic fraction of mastic gum (Fig. 1).

Table 1
Gas chromatographic and mass spectrometric data for the triterpenes of the acidic fraction of mastic gum

Structure types



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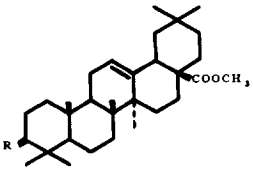

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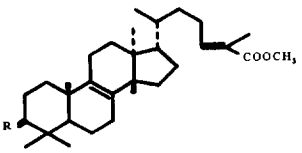

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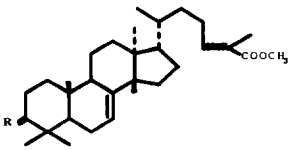

IV

No.	Triterpenes		Structure type	*R*	R_t (min)	$M^{+\cdot}$ (rel. int. %)
	Common name	Systematic name				
1	Methyl moronate	3-oxo-olean-18-en-28-oic methyl ester	I	O	38.007	468(11)
2	Methyl oleanonate	3-oxo-olean-12-en-28-oic methyl ester	II	O	38.591	468(10)
3	Methyl 18α*H*-oleanate	3-oxo-18α*H*-olean-12-en-28-oic methyl ester	II	O	38.740	468(18)
4	Methyl oleanolate	3β-hydroxy-olean-12-en-28-oic methyl ester	II	β-OH,H	39.081	470(2)
5	Methyl isomasticadienonate	3-oxo-13α,14β,17β*H*,20α*H*-lanosta-8,24-dien-26-oic methyl ester	III	O	40.877	468(7)
6	Methyl 3-epi-isomasticadienolate	3α-hydroxy-13α,14β,17β*H*,20α*H*-lanosta-8,24-dien-26-oic methyl ester	III	α-OH,H	41.279	470(25)
7	Methyl masticadienonate	3-oxo-13α,14β,17β*H*,20α*H*-lanosta-7,24-dien-26-oic methyl ester	IV	O	42.867	468(10)
8	Methyl masticadienolate	3β-hydroxy-13α,14β,17β*H*,20α*H*-lanosta-7,24-dien-26-oic methyl ester	IV	β-OH,H	43.474	470(16)
9	Methyl 3-acetoxy-3-epiisomasticadienolate	3α-acetoxy-13α,14β,17β*H*,20α*H*-lanosta-8,24-dien-26-oic methyl ester	III	α-CH_3COO	43.833	512(7)
10	Methyl 3-acetoxy-3-epimasticadienolate	3α-acetoxy-13α,14β,17β*H*,20α*H*-lanosta-7,24-dien-26-oic methyl ester	IV	α-CH_3COO	46.199	512(5)

2.4. Derivatization procedure

A 800-mg amount of the acidic fraction were methylated with 9 ml iodomethane 10% in acetonitrile, in the presence of 1.5 g potassium carbonate, in 60°C for 3 h, to improve its chromatographic behaviour and analyzed by GC–MS [24,25].

3. Results and discussion

MS, alone or in combination with chemical methods of analysis, i.e., computerized capillary GC–MS, can be a valuable tool in medicinal and biological research for the separation and identification of complex organic mixtures. GC–MS has already been used successfully to identify triterpenoids in resins [26,27].

The GC–MS analysis of the acidic fraction of mastic gum led to the separation of ten ingredients. Their structures were tentatively identified on the basis of MS properties. Table 1 presents their systematic nomenclature and GC relative retention times. The total ion current (TIC) chromatogram of the acidic fraction of mastic gum is illustrated in Fig. 2.

The mass spectra of ingredients 1, 2, 4, 5 and 7 (Fig. 3; panels 1, 2, 4, 5, and 7) are identical to the published spectra of methyl moronate, oleanonate, isomasticadienonate, masticadienonate [4,21] and oleanolate [28], respectively.

The mass spectra of ingredients 2 and 3 (Fig. 3; panels 2 and 3) are identical, differing only slightly in the relative intensity of some fragments. Therefore, ingredient 3 is probably an isomer of methyl oleanonate. Since migration of the double bond to other positions at the oleanane skeleton drastically alters the general fragmentation [29,30], and the observed peaks at m/z 262, 203, 408 and 205 are indicative of a double bond at C-12 (Δ^{12}), a carbomethoxy substituent at C-17 and an oxo one at C-3 (Fig. 4) [29], methyl 18αH-oleanonate is the most proper structure for 3. This assumption is reinforced by the somewhat more intense ions at m/z 468, 409 and 408 and the abundant ion at m/z 189, the formation of which may be favoured by the 18αH-stereochemistry [31,32]. Furthermore, 18αH-oleanonic along with oleanonic, moronic and olean-13(18)-en-28-oic acid are the most common natural products of the oleanane series [33].

The mass spectra of ingredients 5 and 7 (Fig. 3; panels 5 and 7) are virtually identical with slight quantitative differences. The origin of the most important peaks in the light of published MS studies on steroids is discussed below (Fig. 5). The assignment of various structural fragments to the principal

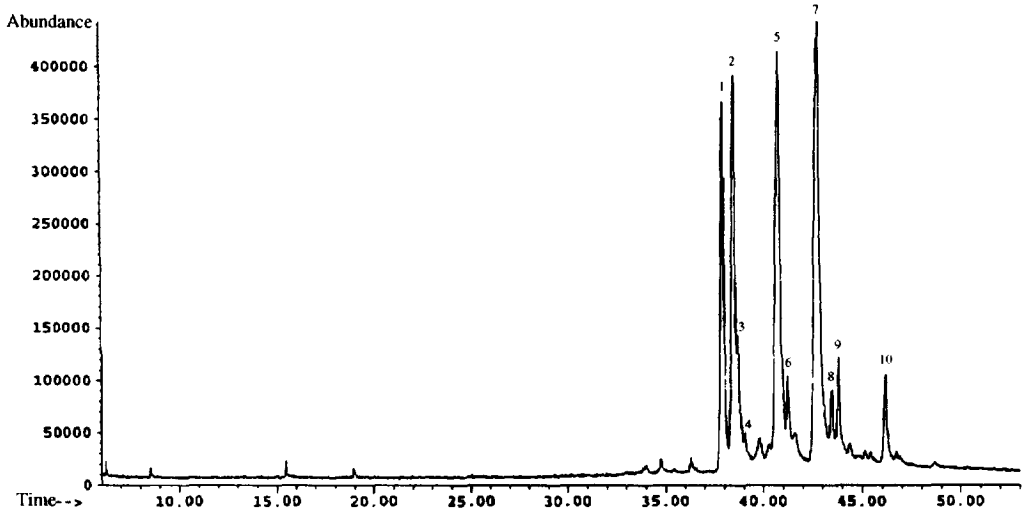

Fig. 2. Total ion current chromatogram (TIC) of the acidic fraction of mastic gum. Conditions as in Section 2. For peak identifications see Table 1.

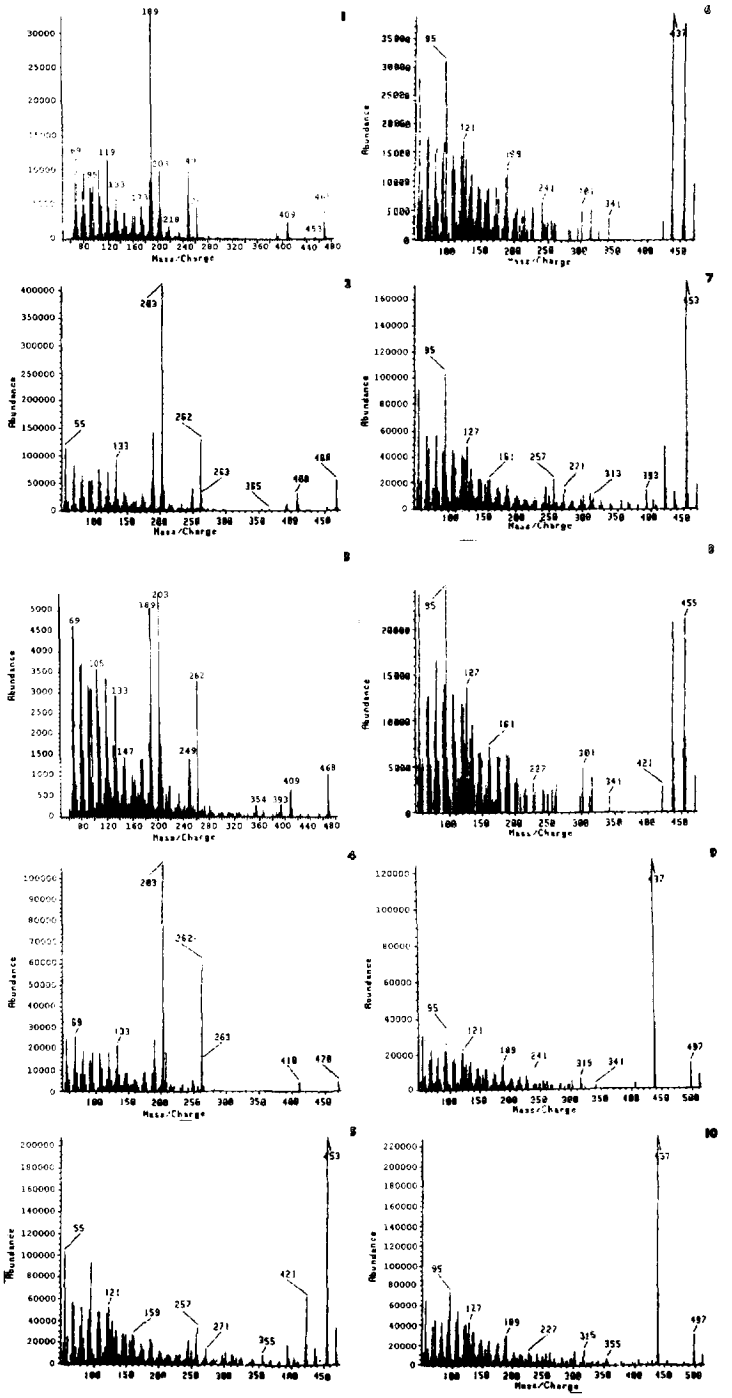

Fig. 3. Electron impact mass spectra of methyl moronate (**1**), methyl oleanonate (**2**), methyl 18α-oleanonate (**3**), methyl oleanolate (**4**), methyl isomasticadienonate (**5**), methyl 3-epiisomasticadienolate (**6**), methyl masticadienonate (**7**), methyl masticadienolate (**8**), methyl 3α-acetoxy-isomasticadienolate (**9**), methyl 3α-acetoxy-masticadienolate (**10**).

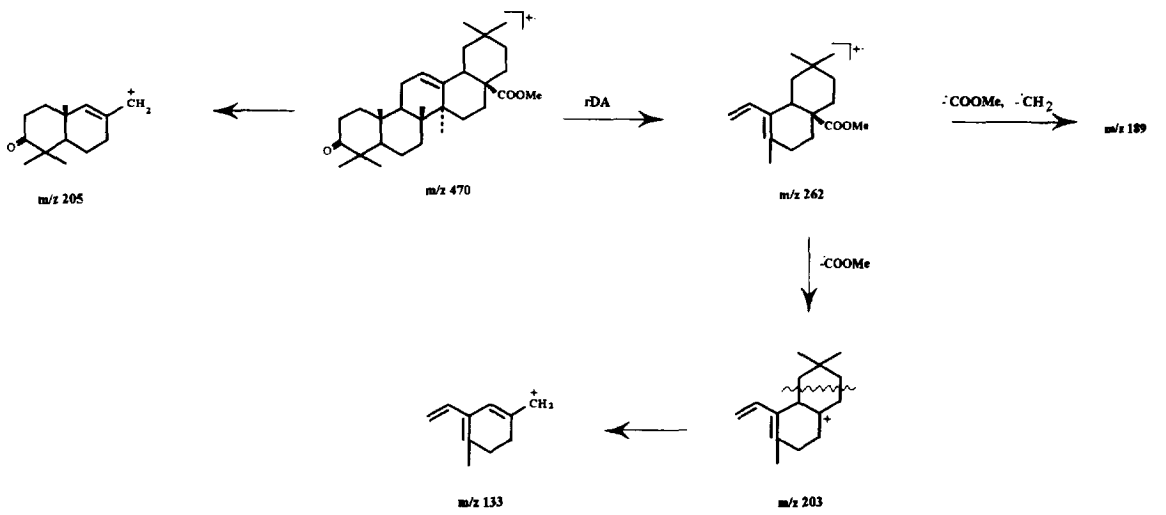

Fig. 4. Principal mass spectral fragments of methyl oleanonate.

peaks does not imply any mechanistic preferences but is largely based on a comparison with the recorded spectra of analogue steroids.

Equatorial (α) configuration of C-5 hydrogen is supported by the presence of the strong peak at m/z 453, attributed to methyl elimination from the molecular ion [$M^{+\cdot}$] and the absence of any abundant rDA fragments (m/z 330, 315) [34]. Increased methyl group loss in 5α-steroidal olefins reflects the absence of the low energy concerted retro Diels–Adler (rDA) fragmentation as a competitive pathway.

Indicative of the increased substitution around ring D, expulsion of the side chain together with a C_3H_6 moiety (m/z 271) without hydrogen transfer is a characteristic feature of the mass spectra of ingredients 5 and 7. The dominant ring D cleavage process seems to be a favourable one since it relieves the strain inherent in the hydridan system and also the steric crowding between the C-18 methyl group and the C-17 side chain. Furthermore, it is of considerable importance as a diagnostic tool in determining the nature of the C-17 substituent [35,36]. As can be visualized from the mass spectra of 4 and 6, Δ^7 facilitates ring D cleavage in contrast to Δ^8 [35,36].

The minor fragment at m/z 313 is characteristic of the structure of the side chain of 5 and 7, possessing a double bond at C-24 [37,38]. Moreover, its relative intensity provides some evidence for the location of the double bond at the steroid nucleus. Δ^7 isomers facilitate loss of the side chain and the corresponding peak (m/z 313) is of greater intensity compared to that of Δ^8 steroids as is apparent in Fig. 3 (panels 5 and 6). It is noteworthy that no hydrogen transfer accompanied the fragmentation of their side chain.

Although rupture of ring D predominates in the mass spectra of 5 and 7, the loss of the six carbon atoms and one oxygen from ring A is noticeable (m/z 355) [39].

As well as exhibiting the previously discussed ions, the mass spectra of 5 and 7 also display prominent peaks at m/z 257, 249 and 245, that essentially constitute the main diagnostic fragment ions of the double bond location at the steroid nucleus. The formation of ions at m/z 257 requires the migration of the double bond to $\Delta^{9(11)}$, therefore it is of diminished intensity in the mass spectrum of 7 (Fig. 3.7) [29,40]. Additionally, Δ^8 slightly favours the formation of m/z 245 ions [29,40].

In the lower mass region two characteristic peaks are observed at m/z 121 and 127. Nevertheless, the nature or the origin of both fragment ions has not been established; they seem to be indicative of the Δ^8 and Δ^7, respectively.

The structures of ingredients 5 and 7 with two extra hydrogen atoms are possible for ingredients 6 and 8, respectively, as judged by the close resemblance of their mass spectra and the molecular ions of 6 and 8 at m/z 470 (Fig. 3; panels 6 and 8).

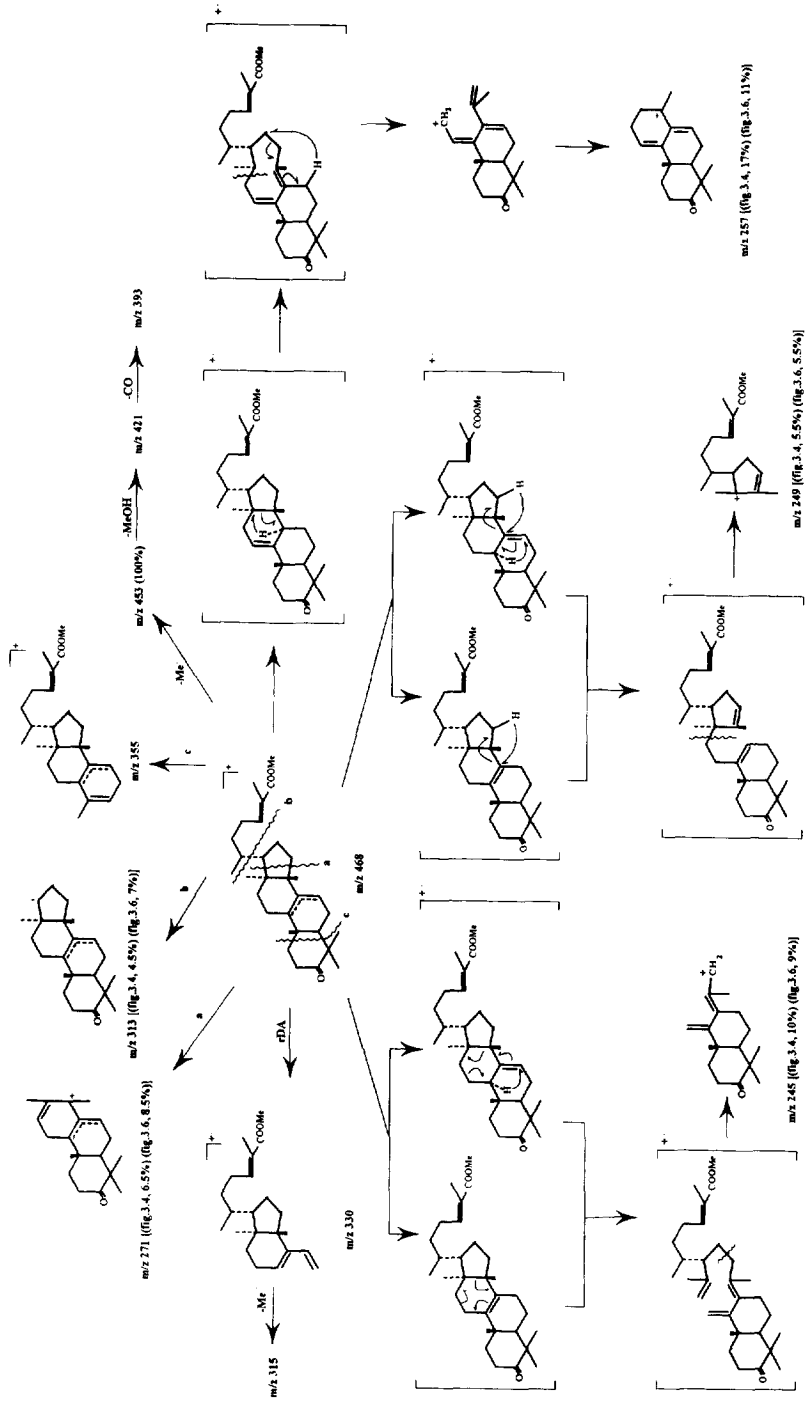

Fig. 5. Proposed mass spectral fragmentation pattern for the ingredients **5** and **7**. (**5**: 8-en, **7**: 7-en).

The characteristic feature of the mass spectra of 6 and 8 is the presence of two strong peaks at m/z 455 and 437, indicative of the location of the two extra hydrogen atoms to C-3 and of the equatorial configuration of C-5 hydrogen. The difference in the degree of dehydration (Fig. 3; panels 6 and 8) supports the conclusion that the hydroxyl linkage at the C-3 atom resembles in character the axial and equatorial bonds in the structures of 8 and 6, respectively. The explanation must lie in the competitive character of water abstraction in 5α-steroidal 3-oxygenated olefins in the 3β-configuration, to methyl radical expulsion, since the C-19 methyl is both, the possible source for the abstraction of a hydrogen atom and the major source along with C-18, of methyl group loss. In contrast, this dehydration is promoted by the close proximity of the 3α-OH group and the 5α-H atom in both chair and boat conformations of ring A, in the $[M-Me]^+$ ion [41–44].

The predominant loss of the water molecule rules the subsequent fragmentation process in the mass spectra of 6 and 8. Therefore, we expected to observe the characteristic fragmentation patterns we experienced in the mass spectra of 5 and 7, after the initial loss of the water molecule (Fig. 6).

Indeed the characteristic m/z 271 ion, emanated from the ring D rupture of ingredients 5 and 7, is shifted to m/z 255, whereas the diagnostic of the double bond location fragment ion at m/z 257 and 245 in the mass spectra of 5 and 7 is shifted to m/z 241 and 229, respectively. Additionally, the m/z 313 ion derived from side chain loss in the mass spectra of 4 and 6 is shifted to m/z 297 in the mass spectra of 6 and 8.

As expected, only those peaks were shifted which still retain C-3 and its substituents. Thus, the m/z 249 ion, as well as the m/z 315 ion remain unchanged on going from mass spectra of 5, 7 to those of 6, 8. The peak at m/z 315 owes its origin to the presence of the double bond at the steroid nucleus. It is of interest that rDA is an important fragmentation process in the mass spectra of 6 and 8, in contrast to those of 5 and 7.

The peaks at m/z 341 and 301 have no analog in the mass spectra of 5 and 7. An attractive rationale for their formation involves expulsion of 29 mass units as $^{\cdot}CH_2CH_3$ or $^{\cdot}CHO$ and the rupture of ring A and ring B, respectively, due to rDA fragmentation. Rupture of ring A is induced by the $\Delta^{2(3)}$ double bond derived from the water molecule elimination and is not stereochemically dependent on the presence of Δ^7 or Δ^8 double bonds [34].

The assignment of Δ^7 and Δ^8 to the ingredients 8 and 6, respectively, was confirmed by the greater intensity of ions at m/z 229 and 241 and the diminished one of ions at m/z 255 and 297 in the mass spectrum of 6, while the opposite phenomenon is observed in the mass spectrum of 8 (Fig. 6). Furthermore, as an additional proof, an intense peak at m/z 121 and one at m/z 127 is present in the mass spectra of 6 and 8, respectively.

The qualitatively identical picture of the mass spectra of ingredients 6 and 8 is observed in the mass spectra of 9 and 10, respectively, in the mass region beyond m/z 437. Besides, a typical $[M-15]^+$ ion at m/z 497 and a strong peak at m/z 437 are present in the mass spectra of 9 and 10 (Fig. 3; panels 9 and 10). Therefore, the structures of 6 and 8 are possible for ingredients 9 and 10, respectively, with the addition of an acetoxy group at C-3 instead of the existing hydroxyl group (Fig. 6).

The correctness of this assignment is demonstrated by the fact that all principal peaks observed in the mass spectra of 6 and 8 are retained by the mass spectra of 9 and 10, i.e., m/z 437, 341, 315, 301, 255, 249, 241 and 229.

The greater intensity of ions at m/z 249 and 241 in the mass spectrum of 9 and of ions at m/z 255 and 297 in the mass spectrum of 10, point to Δ^8 and Δ^7 locations of the double bond in the structure of 9 and 10, respectively. This assignment is reinforced by the presence of peaks at m/z 121 and 127 in the mass spectra of 9 and 10, respectively, and by the fact that the iso-form has a smaller t_R than the normal one, as in the case of ingredients 5 and 7 [4].

In general, the acetoxy derivatives show stronger substituent elimination than the hydroxyl compounds due to the larger size of the acetoxy group, compared with that of the hydroxyl one. However, the 3α-acetoxy group facilitates elimination of the acetic acid molecule from the $[M-Me]^+$ ion in the 5α-steroids. Therefore, the increased intensity of the m/z 436 peak is indicative of an equatorial configuration for the 3-acetoxy group [28].

In conclusion, the composition of the resin yielded by *Pistacia lentiscus* L. *var. Chia*, is quite different

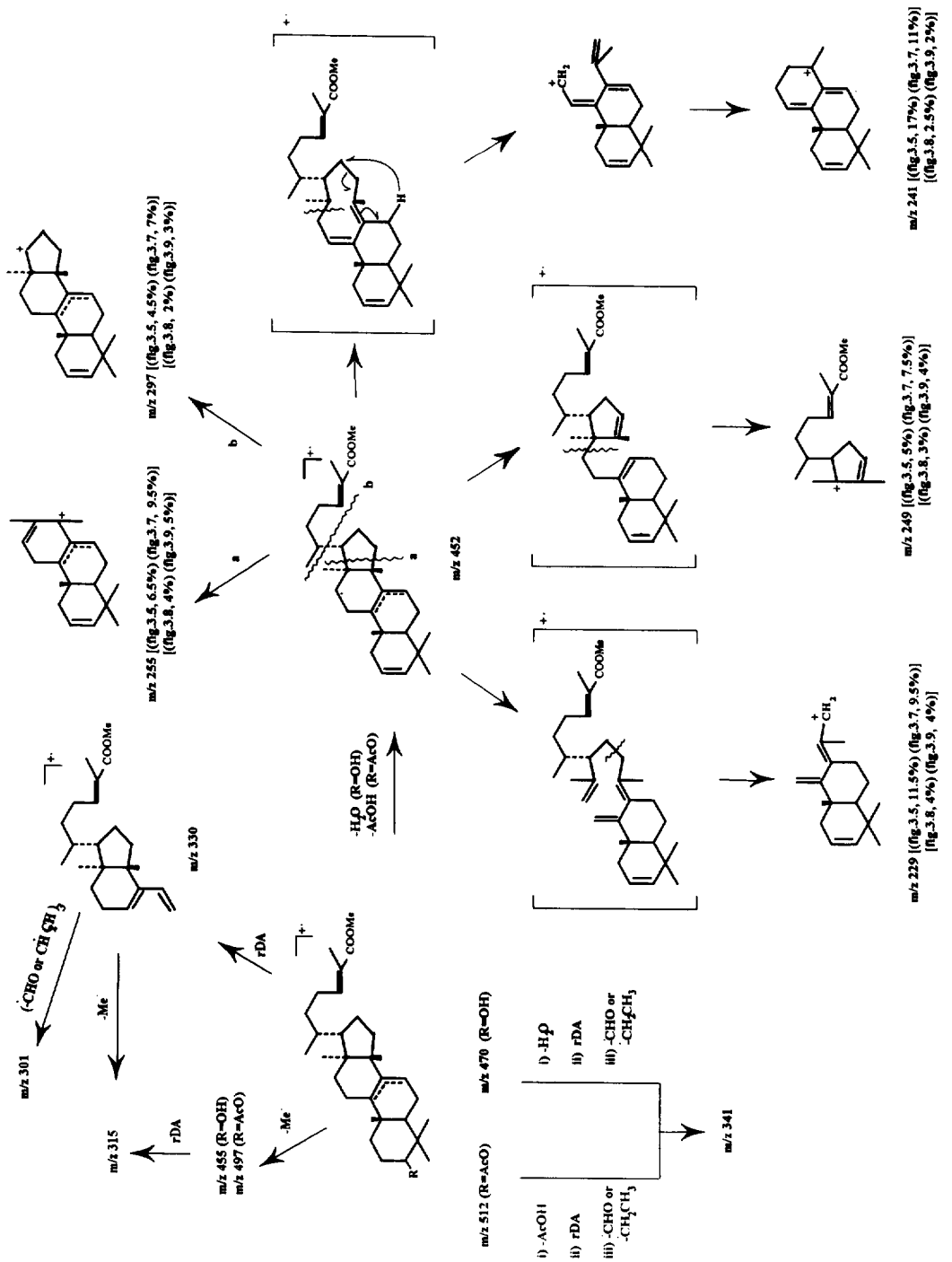

Fig. 6. Proposed mass spectral fragmentation pattern for the ingredients **6**, **8**, **9** and **10**. (**6**, **9**: 8-en, **8**, **10**: 7-en).

Table 2
Triterpenes identified in the acidic fraction of the galls extracted from various species of *Pistacia* L.

Triterpenes	M.W.	*Pistacia lentiscus* L. [7]	*Pistacia terebinthus* L. [9]	*Pistacia palestina* L. [14]	*Pistacia vera* L. [12]
Me isomasticadienonate	468			x	x
Me masticadienonate	468	x	x	x	x
Me oleanonate	468	x	x	x	x
Me dihydromasticadienonate	470	x			x
Me isomasticadienolate	470		x		
Me masticadienolate	470	x	x	x	x
Me 3-epiisomasticadienolate	470		x	x	
Me 3-epimasticadienolate	470	x	x	x	x
Me oleanolate	470	x	x	x	
Me 3-epioleanolate	470	x	x		
Me dihydroisomasticadienolate	472		x		
Me dihydromasticadienolate	472	x	x		
Me dihydro-3-epimasticadienolate	472	x			x
Me 3α-acetoxyisomasticadienolate	512			x	x
Me 3α-acetoxymasticadienolate	512			x	x

from that extracted from the galls of *Pistacia lentiscus* L., since methyl isomasticadienonate and 3-epi-isomasticadienolate as well as methyl 3α-acetoxy-isomasticadienolate and 3α-acetoxymasticadienolate were not found in the latter resin (Table 2). The two former constituents of mastic gum were found in the resin extracted from the galls of *Pistacia Vera* L. and *Pistacia Palestina* L., whereas the two latter constitute the resin extracted from the galls of *Pistacia Terebinthus* L. and *Pistacia Palestina* L.

Acknowledgments

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