MASS-SPECTROMETRIC INVESTIGATION OF STEROL AND TRITERPENOID ESTERS FROM LEAVES OF THE COTTON PLANT

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The qualitative composition of two fractions of extractive substances isolated from the leaves of a cotton plant of the variety 108-F and containing sterol and triterpenoid esters with saturated and unsaturated aliphatic acids having chain lengths of from C_{12} to C_{30} has been studied by a group of mass-spectrometric methods. A total of 45 compounds was detected.

Continuing an investigation of the biologically active components of cotton-plant leaves, we have separated on a silica gel column the total extractive substances of the leaves of variety 108F gathered in the ripening period in September, 1985, and have isolated fractions consisting mainly of mixtures of sterol esters (fraction A) and triterpenoid esters (fraction B). In these mixtures, free sitosterol, stigmasterol, campesterol, 24-ethylidenecholesterol, cholesterol, and the triterpenoid amyrin were detected mass-spectrometrically, being identified from the peaks of their molecular and fragmentary ions and also by comparison of the linking scanning (B/E = const.) spectra of the molecular ions.

After the saponification of the total material with 6% alcoholic caustic soda, the same free sterols and amyrin were detected in the mixture by mass spectrometry. The main acidifying acids proved to be palmitic and linolenic, which were identified in the form of their ethyl esters.

The aim of the present work was to determine the composition of the mixture of esters and to describe features of their breakdown under electron impact.

Sections of the mass spectra in the m/z interval of 200-700 (A) and 200-900 (B) are given in Fig. 1. In the spectrum of fraction A the peaks of ions with m/z 396 and 397 corresponding to $(M - ROH)^+$ and $(M - RO)^+$ of sitosterol (where R is the acyl radical of the esterifying acid) are characterized by the highest intensities. The peak next in height is that of an ion with m/z 296 ($C_{22}H_{32}$), which is characteristic for acylated sterols with a $\Delta^{24/28}$-bond. It is formed as the result of the processes of the successive elimination of ROH and a rearrangement of the McLafferty type taking place with the cleavage of the C-22–C-23 bond [1]. An ion with m/z 255 represents the result of the splitting out of ROH and the substituent at C-17 without migration of hydrogen atoms, which is more characteristic of sterols with saturated side chains [2]. Characteristic of the spectra of free sterols of the same type are intense peaks of the $(M - 85)^+$ and $(M - 111)^+$ ions arising by the splitting out of the elements of rings A and B [3]. However, in view of competition on the part of the process of splitting out the voluminous substituent from C-3, these fragmentation pathways are not realized in sterol esters.

In the molecular region of the spectrum of fraction A peaks with m/z 676 ($C_{47}H_{80}O_2$), 674 ($C_{47}H_{78}O_2$), 664 ($C_{46}H_{80}O_2$), 650 ($C_{45}H_{78}O_2$), 636 ($C_{44}H_{76}O_2$), 622 ($C_{43}H_{74}O_2$), 608 ($C_{42}H_{72}O_2$), and 594 ($C_{41}H_{70}O_2$) stand out. Since the numbers of carbon atoms in the molecule of the main sterols are 27-29 and the length of the carbon chain of the esterifying acids may also differ, these M^+ ions may correspond to a whole set of isomeric compounds. An additional complication is introduced by a change in the degree of saturation of the steroid and acylating parts of the molecules. To determine the composition of the mixture of esters we therefore made an analysis of the metastable defocusing (MD) spectra of the ions $(M - ROH)^+$ which enabled the nature of R to be determined by comparing the compositions of the daughter and parental ions.

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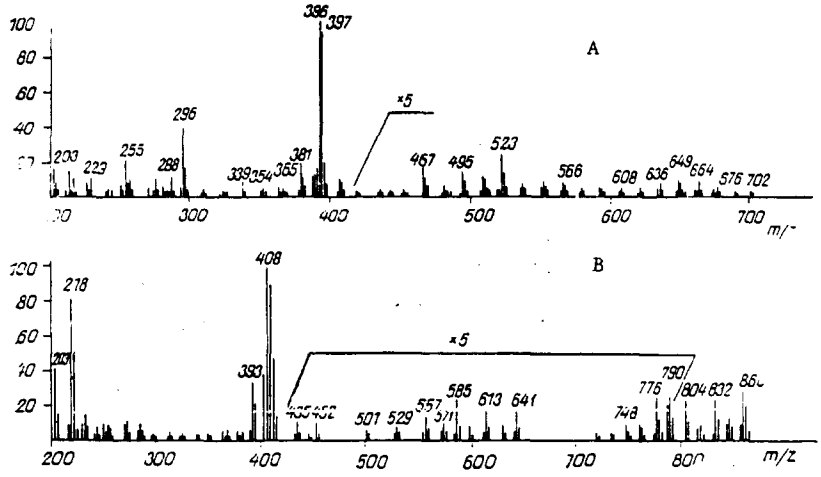


Fig. 1

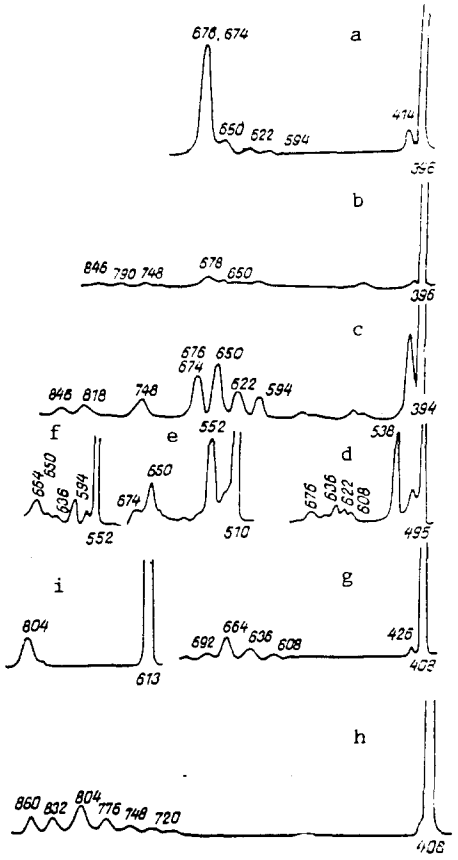


Fig. 2

To establish the set of substituents at C-17 of the steroid nucleus, we determined the elementary compositions of the ions in the 495-566 a.m.u. interval and studied their MD spectra. In analyzing the results, we started from features of the fragmentation of the side chain characteristic of each type of sterols. These features are: for sterols with a saturated side chain the most characteristic ions are a; for Δ^{22}-sterols the ions b – H and the triplet of ions a, a – H, and a – 2H the last of which is the strongest; and for the $\Delta^{24}/^{28}$-sterols the ions f – H and a – 2H.

To check the reliability of the results obtained we recorded the linked scanning spectra of some of the molecular ions, which permitted the opposite problem to be solved: to establish which daughter ions were formed directly from given parental ions.

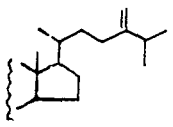
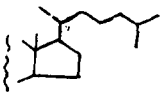
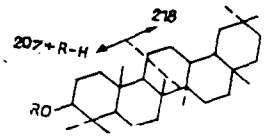
TABLE 1. Types of Esterifying Acids, Mass Numbers of the Molecular and the Daughter Ions, and Structures of the Steroid Moieties of the Ester Molecules with an Indication of Fragmentation Pathways

Acid	m/z of the M^+ ion	m/z of daughter ions and their origin	Structure
12:1	594	396 $(M-ROH)^+$	
14:1	622	.	
15:1	636	495 (a)	
16:1	650	396 $(M-ROH)^+$, 509 (a)	
17:1	664	523 (a)	
18:1	678	. 537 (a)	
18:2	676	. 535 (a)	
18:3	674	. 533 (a)	Sitosterol esters
23:1	748	.	
26:1	790	.	
28:2	816	.	
30:1	846	.	
12:0	594	551 (e)	
16:0	650	538 (b—H)	
17:0	664	552 (b—H)	
			Stigmasterol esters
14:0	622	394 $(M-ROH)^+$	
18:0	678	. 537 (a—2H)	
18:1	676	. 510 (c), 495 (d—H)	
18:2	674	.	
23:0	748	.	
28:0	818	.	
30:0	846	.	
15:0	636	495 (a—2H)	
15:0	650	552 (f—H)	24-Ethylidenecholesterol esters
17:0	664	566 (f—H)	
15:1	622	495 (a)	
18:1	664	382 $(M-ROH)^+$	Campesterol esters
12:0	580	537 (e)	
16:0	636	538 (b—H)	Brassicasterol esters

Table 1 gives the types of esterifying acids, the mass numbers of the molecular and daughter ions obtained by the MD and B/E = const. methods of scanning the spectra, and the structures of the steroid moieties of the molecules of the sterol ester with letter indications of their characteristic fragmentation pathways.

The sitosterol esters formed the largest group of compounds detected. The MD spectrum of the ion $(M - ROH)^+$ with m/z 396 at a temperature of the inlet system of 150°C gave a series of precursor ions showing the presence of monoenic acyls with chain lengths of C_{12}, C_{14}, and C_{16}–C_{18} (Fig. 2, a). With a rise in the temperature of the experiments, weaker

TABLE 1 (continued)...

Acids	m/z of the M + ion	m/z of daugher ions and their region	Structure
15:0	622	538 (f—H)	
16:0	636	552 (f—H)	
17:0	650	566 (f—H)	23-Methylenecholesterol esters
15:1	608	495 (a)	
17:1	636	523 (a)	
18:1	650	368 $(M-ROH)^+$	Cholesterol esters
12:0	608	408 $(M-ROH)^+$ 207 + R—H	
14:0	636	.	
16:0	664	. 445	
18:0	692	.	
20:0	720	. 501	
22:0	748	. 529	
24:0	776	. 557	
26:0	804	. 585	
28:0	832	. 613	
30:0	860	. 641	

Amyrin esters (the Δ^{12}-bond has been omitted from the formula of amyrin).

metastable ions appeared that characterized the presence of acyls with longer chains (Fig. 2, b). Some of the molecular ions of the esters appeared in the MD spectra of ions of type a with m/z 495, 509, 523, 533, 535, and 537 (see Table 1).

The MD spectra of the $(M - ROH)^+$ ions with m/z 294 of sterol esters of the C_{29} series monounsaturated in the side chain at C-17 each contains eight metastable peaks corresponding to transitions from M^+ (Fig. 2, c). The majority of these M^+ ions can be referred to esters of either of the two most common sterols of this type — stigmasterol and 24-ethylenecholesterol (seven transitions). However, some transitions to daughter ions formed with the cleavage of bonds of the C-17 substituent enable the nature of a number of components of the mixture to be determined. Thus, the loss of a 112-a.m.u. fragment with the composition C_8H_{16} in the transitions 650 → 538 and 664 → 552 (Fig. 2, f) showed that these daughter ions are b — H ions and that the corresponding compounds are esters of stigmasterol with the 16:0 and 17:0 acids (see Table 1). On recording the MD spectra of the ions with m/z 552 and 566, transitions were also obtained from parental ions with m/z 650 and 664. The loss of 98 a.m.u in the form of a C_7H_{14} fragment is characteristic for 24-ethylidenecholesterol [1]. In this process the f — H ions are formed, the esterifying acids being the same as in the case of stigmasterol.

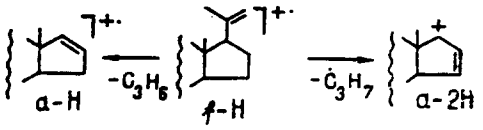
The Δ^{22}-sterols — stigmasterol and brassicasterol — form the $(M - 43)^+$ ions by the splitting out of $C_3H_7^{\cdot}$ in the cleavage of the C-24—C-25 bond (the ions e). Part of the MD spectra showed the presence of subsidiary transitions — for example, the 594 → 551 and 580 → 537 transitions — which indicates the presence of stigmasterol and brassicasterol esterified with the 12:0 acid.

Ions of type a — 2H are equally characteristic of sterols with different positions of the π-bond in the substituent at C-17. Part of the MD spectra — for example, ions with m/z 495 and 510 — showed the occurrence of transitions from daughter ions with m/z 676, which indicates the reality of the cleavage of the bonds of ring D by pathways c and d.

Some of the transitions recorded with the aid of the MD spectra permit a dual interpretation. This is connected primarily with the fact that the spectra of sterols with saturated

side chains are characterized by the ions a. and those with unsaturated side chains by the ions a − 2H. Therefore the existence of the transitions 622 → 495, 636 → 495, and 678 → 537 can be considered as a consequence of the fragmentation of isomeric esters with a π-bond either in the acylating residue or in the C-17 side chain.

One of the features of the MD spectra of the ions a − H with m/z 510 and 524 was the presence of the transitions 552 → 510 and 566 → 525 with the loss of C_3H_6. The heights of the corresponding metastable peaks were comparable with the heights of those of the daughter and parental ions (Fig. 2, e), which indicates the rearrangement nature of the process. It was due to the passage of the f − H ions into the a − H ions. The MD spectra of the ions a − 2H with m/z 495 (Fig. 2, d) and 523 possessed a similar property: they included intense metastable peaks of precursor ions with m/z 538 and 566, respectively, which was connected with the loss of an isopropyl radical by the ions f − H:



These transitions confirmed the presence of sterols with a $\Delta^{24/28}$ bond. Thus, the 622 → 538, 636 → 552, and 650 → 566 transitions indicated the presence of esters of 24-methylenecholesterol with the 15:0, 16:0, and 17:0 acids.

The greatest difficulty was presented by the identification of esters of sterols of the C_{27} and C_{28} series with saturated side chains. The peaks of the $(M - ROH)^+$ ions of these sterols with m/z 368 and 382 in the review spectra of fraction A were weak and were masked by the peaks of ions with a different origin, including the isotropic peaks of ions the mass numbers of which were smaller by 1 a.m.u. This situation is illustrated for example, by the MD spectrum of the ion with m/z 368 which shows metastable peaks equidistant from one another corresponding to precursors with m/z 816, 748, 680, 612, 544, and 476 differing from one another by 68 units, i.e., there may have been a polyisoprenoid of the type of plastoquinone here as an impurity. The only MD peak of the spectrum not obeying this rule related to the 650 → 368 transition corresponding to the fragmentation of an ester of cholesterol and oleic acid. Esters of cholesterol with the 15:1 and 17:1 acids were shown by the 608 (M^+) → 495 (a) and 636 (M^+) → 532 (a) transitions satisfying only the given variants.

Analysis of the B/E = const. spectrum of the m/z 664 ion (Fig. 3, a) showed the presence of the daughter ions $(M - RO)^+$ from sitosterol (m/z 397) and stigmasterol or 24-ethylidenecholesterol (m/z 395) and of the $(M - ROH)^+$ ion from campesterol (m/z 382). The last transition was evidence of the presence of an ester of the sterol with oleic acid. In addition, the spectrum showed transitions to the following daughter ions: m/z 649 $(M - 15^+)$, 621 (e), 552 (f − H), and 523 (a).

Thus, analysis of the mass-spectrometric results permitted the detection of more than 30 esters of seven sterols and acids with chain lengths of from 12 to 30. The predominant coupling of sterols saturated in the side chain with unsaturated acids and conversely is characteristic.

In an analysis of the mass spectrum of fraction B (Fig. 1), in addition to the peaks of the ions $(M - ROH)^+$ and $(M - RO)^+$ with m/z 408 and 409, a high intensity was observed of the peaks of the ions $(408 - CH_3)^+$ and m/z 218 − the product of the retrodiene breakdown of ring C of a pentacyclic triterpenoid of the oleanene type [4]. At a temperature was the inlet system of the order of 150°C the molecular ions of esters of amyrin with relatively short-chain fatty acids appeared with m/z 608, 636, 664, and 692. When the temperature was raised to 200°C the peaks of M^+ ions appeared with m/z 720, 748, 776, 804, 832, and 860. Each of these ions contained from 42 to 60 carbon atoms and two oxygen atoms, i.e., they corresponded to amyrin esters with all the even-carbon saturated acids from 12:0 to 30:0.

To confirm this, the MD spectra of the $(M - ROH)^+$ ion were taken under various conditions (Fig. 2, g, h), and the transitions from all the molecular ions mentioned were recorded. The MD spectrum of the $(408 - CH_3)^+$ ion gave a similar pattern, i.e., in this process there was a synchronous elimination of ROH + CH_3.

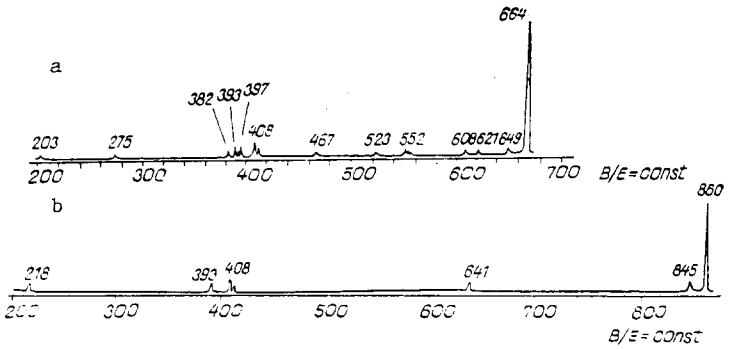


Fig. 3

In the spectrum of B (see Fig. 1), between the molecular region and the $(M - ROH)^+$ ions there was a homologous series of peaks with odd mass numbers: 501, 529, 557, 585, 613, and 641. The last of them has the composition $C_{44}H_{81}O_2$, and each preceding one is smaller by C_2H_4. Using the m/z 613 ion as an example (Fig. 2, i) the MD spectra of these ions showed as precursors the M^+ ions of esters of amyrin with the acids from 20:0 to 30:0. These ions, according to their method of formation, were analogous to the m/z 207 ions of amyrin [4] additionally including a substituent at C-3 (207 + R − H). With a lowering of the temperature of the experiment, in the spectrum of fraction B the peak of an ion with m/z 445 appeared, representing an analogous fragment with a palmitic acid residue.

The linked scanning spectra of the ions with m/z 664 showed, in addition to daughter ions characteristic for sterol ester impurities, ions with m/z 408, 393, and 203 (Fig. 3, a), corresponding to triterpenoid esters. This once again confirmed the multicomponent composition of the compounds with M^+ 664.

The analogous spectrum of the M^+ ion with m/z 860 of amyrin triacontanoate (Fig. 3, b) showed all the main daughter ions characteristic of this compound: m/z 218, 393, 408, and 641.

Thus, a mixture of amyrin esters with ten saturated fatty acids has been detected.

EXPERIMENTAL

MKh 1310 mass spectrometer, SVP 5 system for direct sample introduction, ionizing voltage 60 V, collector current 50 μA, temperature of the evaporator bulb and the ionization chamber 150-200°C. For the measurements of the elementary composition and the conditions of obtaining the MD spectra, see [5]. Linked scanning was carried out on a device made in SKB AP NTO AN SSSR [Special Design Bureau of Analytical Instrument Construction of the Scientific and Technical Division of the USSR Academy of Sciences] (B. M. Voronin and G. V. Fridlyanskii). The selected daughter ion was recorded at the parameters of the mass analyzer B_0 and E_0 and in the subsequent scanning of the magnetic and electricity fields the ratio $B/E = B_0/E_0$ was maintained. The mass numbers of the daughter ions M_d were calculated from the formula

$$M_d = \sqrt{M_0 \cdot M'_d},$$

where M'_d is the mass number of the metastable ion determined from the scale; and

M_0 is the mass number of the parental ion.

Isolation of Fractions A and B. The finely comminuted freeze-dried cotton-plant leaves (120 g) were steeped at room temperature with 600 ml of acetone four times, with shaking for 2 h each time. The combined extract was evaporated in a rotary evaporator. This gave 10 g of total material, which was chromatographed on a column (3.6 × 120 cm) of KSK silica gel, 60-100 mesh, with a ratio of adsorbent to substance of 40:1. Elution was performed with the hexane–chloroform (1:1) system. Fraction A (140 mg), fraction B (60 mg), and an intermediate fraction (130 mg) containing sterol, and triterpenoid esters were isolated.

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STEROID COMPOUNDS OF MARINE SPONGES.

XI. STEROIDS OF THE AUSTRALIAN SPONGE *Trachyopsis sp.*

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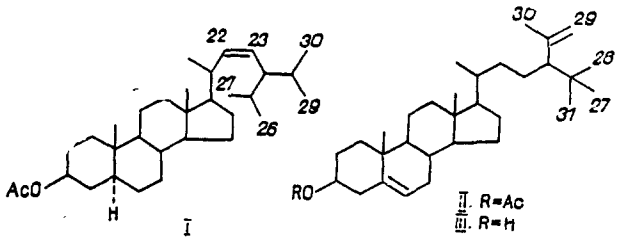
The compositions of two steroid fractions from the Australian sponge *Trachyopsis sp.* have been investigated. The fractions of steroid sulfates consisted of the trisulfates of halistanol (76%) and of 24-isopropyl-5α-cholest-22-ene-2β,3α,6α-triol (20%). In the sterol fraction, axinyssasterol (64%) and 24-isopropyl-5β-cholest-22Z-en-3 -ol (30%) were identified.

Continuing a study of physiologically active substances from sponges of the family *Halichondridae* [1], we have established the steroid composition of fractions of sulfated polyols and free sterols from the Australian sponge *Trachyopsis sp.* Both the fractions mentioned were isolated by methods that we have described previously [2].

These steroid sulfates were hydrolyzed, giving the previously known halistanol (76%) and 24-isopropyl-5α-cholest-22-ene-2β,3α,6α-triol (20%) which were identified in the form of the triacetates (GLC-MS) [2]. Analysis of the free sterols, performed with the aid of GLC-MS, showed the presence in this fraction of two main components (64 and 30% of the weight of the fraction). The two substances differed from the usual steroids of the majority of invertebrates and had 31 and 30 carbon atoms, respectively. The sterols were isolated, after acetylation, by chromatography on silica gel impregnated with silver nitrate.

Although these compounds were known previously as minor components of the sponges *Halichondria sp.* and *Pseudoaxinyssa sp.* [3, 4], their spectral properties have remained little studied.



I

The acetate of sterol (I) proved to be identical with the acetate of 24-isopropyl-5α-cholest-22Z-en-3β-ol from *Halichondria sp.* [3]. The assignment of the signals of the carbon atoms in its ^{13}C NMR spectrum was made with the aid of J-modulation, using INDOR and by comparing the spectrum with the spectra of model cholestanols [5, 6] (Table 1).

The acetate of sterol (II) was identical in structure with the acetate of axinyssasterol from *Pseudoaxinyssa sp*. In actual fact, the deacetylation of (II) gave this sterol (III), which was shown by a comparison of its ^{13}C NMR spectrum (in C_6D_6) with that described in the literature [5].

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