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GAS–LIQUID CHROMATOGRAPHY OF TRITERPENE ALCOHOLS OF THE DAMMARANE SERIES FROM THE LEAVES OF THE GENUS *Betula*

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UDC 543.544.45+547.597+581.192

The relative retention times have been determined and the stability has been shown of tetracyclic alcohols of the dammarane series from leaves of Far Eastern species of the genus *Betula*, and of their TMS ethers, on the stationary liquid phases OV-1 and OV-225 under GLC conditions. The conditions of obtaining the TMS ethers of the compounds under investigation, and the properties of these ethers, have been studied with the aid of GLC analysis, IR spectroscopy, and mass spectrometry.

From the unsaponifiable part of ethereal extracts of the leaves of Far Eastern species of genus *Betula*, in addition to known triterpenoids of the dammarane series, we have isolated a number of compounds of this class, have established their structure, and have shown that they are present in considerable amount [1], and this has enabled some Far Eastern species of birch to be regarded as promising sources of initial material for the synthesis of physiologically active analogs of the panaxosides.

In this connection, the necessity arose for developing a method for the qualitative and quantitative estimation of the triterpenoid composition of the raw material.

The well-known advantages of GLC showed the desirability of using it for solving the problem posed.

There is an extremely limited amount of information in the literature on the gas-chromatographic properties of triterpene alcohols of the dammarane series [2-5], and therefore the natural approach to the development of method was the study of the behavior of these compounds under the conditions of gas-chromatographic analysis.

Here we give the results for triterpene alcohols the side chains of which are closed to form tetrahydrofuran rings (Fig. 1a) differing from one another by the number (from 2 to 4), the positions (at C atoms 3, 11, 12, 17, and 25), and configurations of their hydroxy groups (Table 1, compounds (I-VIII)).

The gas-chromatographic behavior was studied on two high-temperature stationary phases (SLPs) of different polarities: OV-1 and OV-225. Each of the alcohols was recorded on the chromatogram as a single peak and they were stable under the conditions of chromatographic separation, as was shown by the complete coincidence of the mass spectra of compounds recorded by direct introduction and after passage through the chromatographic column.

It can be seen from Table 1 that the gas-chromatographic properties of the alcohols are determined mainly by the numbers of hydroxy groups, while the positions and configurations of

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 592-596, September-October, 1983. Original article submitted September 16, 1982.

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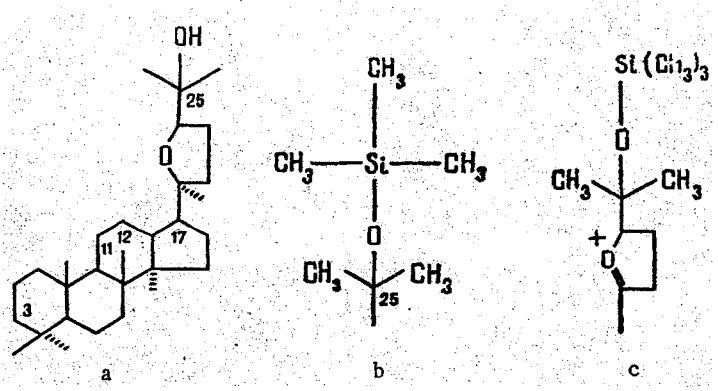

Fig. 1. Structures of the epoxydammarane alcohols (a) and of the main fragments of their TMS ethers (b and c) formed in the decomposition of the molecular ions.

TABLE 1. Relative Retention Times of Triterpenoids of the Dammarane Series and of Their TMS Ethers

20(S), 24(R)-Epoxydammarane	OV-1		OV-225	
	alcohol	TMS ether	alcohol	TMS ether
I. 3α, 25-diol	2,41	2,37	2,83	2,03
II. 3β, 25-diol	2,51	3,16	3,06	2,86
III. 3α, 12β, 25-triol	3,66	2,66	6,76	1 96
IV. 3β, 12β, 25-triol	3,85	3,85	6,75	2,91
V. 3α, 11α, 25-triol	3,78	2,57	6,83	1,89
VI. 3β, 11α, 25-triol	4,00	3,46	7,18	2,61
VII. 3α, 12β, 17α, 25-tetraol	4,98	3,12*	11,75	2,67*
VIII. 3α, 17α, 25-triol	3,52	3,24*	5,63	3,71*
Cholesterol (standard)	1 (4,0 min)	1 (4,8 min)	1 (2,1 min)	1 (3,9 min)
Cholestanol	0,55	0,46	0,40	0,56

*TMS ethers with free hydroxy groups at C_{17}.

these groups have no appreciable influence on the relative retention times (RRTs). For example, compounds (III-VI) have similar RRTs. This circumstance and the deficient symmetry of the peaks can considerably complicate the identification and subsequent quantitative analysis of the alcohols.

Consequently, as derivatives we selected the trimethylsilyl (TMS) ethers, and we investigated their preparation and properties. It was established that the full TMS ethers of the alcohols (I-VI) can be obtained at room temperature in 5-1 min when using as silylating agent trimethylsilylimidazole (TMSI) and as catalyst chlorotrimethylsilane (CTMS) in pyridine or dimethylformamide or without a solvent. In the absence of a catalyst, the reaction takes place slowly and is complete only after 20 h. Here it is possible to observe differences in the rates of esterification of the hydroxyls according to their positions in the molecule. Thus, for alcohols (I) and (II), having hydroxy groups at C_3 and C_{25}, in the first few hours of the reaction the mono-TMS ether at C_3 is formed predominantly, with only a small amount of the di-TMS ether. In these circumstances, two peaks are recorded on the chromatogram (Fig. 2a). As the reaction proceeds further the peak with the smaller RRT corresponding to the mono-TMS ether diminishes, and the peak of the di-TMS with the greater RRT increases (Fig. 2b). After 20 h, only one peak, corresponding to the di-TMS ether is recorded on the chromatogram (Fig. 2c).

The hydroxyls at C_{11} and C_{12} of the alcohols (III)-(VI) are silylated just as rapidly as the hydroxyl at C_3. At first the di-TMS ether at C_3 and C_{11} or C_{12} is formed, and only after 20 h is the esterification of the hydroxyl at C_{25} completed. Thus, for alcohols (I-VI), the group limiting the silylation is the hydroxyl at C_{25}. The appearance in alcohols (VII)

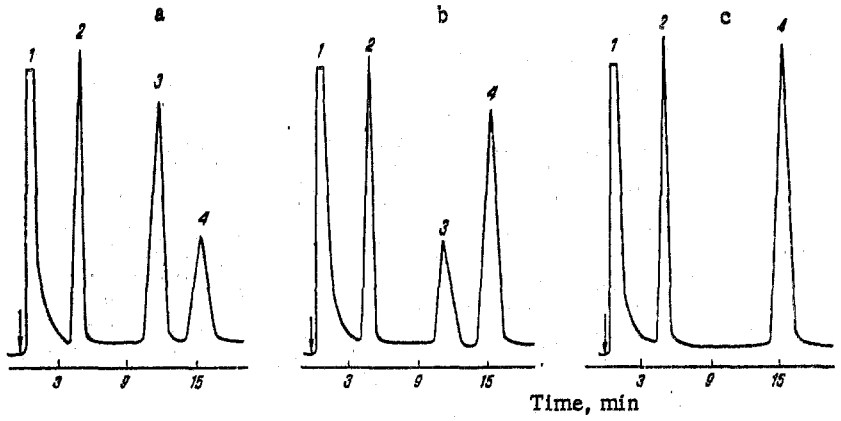

Fig. 2. Occurrence of the silylation reaction of 20(S),24(R)-epoxydammarane-3β,25-diol at room temperature and in the absence of a catalyst. Chromatograms of the reaction mixture on OV-1 (245°C, helium, 65 ml/min): a) after 1 h; b) after 5 h; c) after 20 h from the beginning of the reaction; 1) pyridine; 2) TMS ether of cholesterol; 3) mono-TMS ether of the diol; 4) di-TMS ether of the diol.

TABLE 2. Relative Intensities of the Peaks in the Mass Spectra of the TMS Ethers of Triterpenoids of the Dammarane Series, %

Compound	M	M−CH_3	M−90	M−CH_3−90	M−131	M−131−90	M−131−2×90	M−215	M−215−90	M−215−2×90	M−215−90−H_2O
I	0,05	0,62	0,18	5,94	0,99	16,83	—	—	0,27	0,20	—
II	—	0,16	—	0,50	0,28	2,72	—	—	—	1,61	—
III	0,07	0,20	0,09	0,47	0,89	6,43	44,29	0,41	0,14	—	—
IV	—	0,67	0,13	0,87	1,74	12,39	32,60	0,48	0,22	—	—
V	1,38	1,69	1,38	1,69	2,77	3,69	16,90	0,61	—	—	—
VI	0,22	0,38	0,28	0,49	0,70	0,68	2,05	0,16	—	—	—
VII*	—	0,24	—	2,58	1,17	2,23	—	52,94	28,89	100,00	—
VIII*	—	0,21	0,26	0,35	0,18	0,45	—	—	3,71	—	23,81

*TMS ethers with free OH groups at C_{17}.

and (VIII) of an additional OH group at C_{17} does not change the silylation process described but the final reaction products are the tri-TMS ether of (VII) and the di-TMS ether of (VIII) with free hydroxyls at C_{17}.

It is possible to accelerate the reaction in the absence of a catalyst by raising the temperature. The full TMS ethers of alcohols (I)-(VI) can be obtained in 1.5 h at 100°C. Attempts to obtain the full TMS ethers of the alcohols (VII) having hydroxyls at C_{17} were unsuccessful even under very severe reaction conditions (140°C, 25 h, in the presence of TMS). TMS ethers with free hydroxy groups at C_{17} were obtained which gave peaks entirely suitable for their quantitative estimation of chromatograms.

The TMS ethers of alcohols (I-VIII) are fairly stable. They remain in the reaction mixture for more than a month, and under the conditions of GLC analysis they undergo no changes, as is shown by the identity of their mass spectra taken at the outlet from the gas-chromatographic column and on direct introduction into the mass spectrometer.

When extracted from the reaction mixture with n-hexane in the presence of water, they can be kept in the refrigerator without changes for two months, forming amorphous or crystalline substances.

The gas-chromatographic properties of the TMS ethers are suitably distinguished from those of the initial alcohol. They are recorded on chromatograms in the form of four symmetrical peaks (Figs. 2a, b, and c). It can be seen from Table 1 that the esterification of the alcohols permitted a considerable improvement in the separation of epimers (compounds (I) and (II); (III) and (IV); and (V) and (VI)) and of isomers (compounds (III) and (VII);

(IV) and (VI); and (V) and (VIII)). The only critical pair remaining for the GLC separation is formed by compounds (III) and (V), the RRTs of which are the same on both SLPs. We found no definite correlations in the change of RRT on passing from the alcohols to their TMS ethers for this series of compounds.

The preparation and properties of the TMS ethers of the alcohols investigated were monitored throughout the investigation by GLC analysis, IR spectroscopy, and mass spectrometry. The relative intensities of the peaks in the mass spectra of the TMS ethers are given in Table 2. A characteristic ion of the mass spectra of the TMS ethers is that with m/z 131 (Fig. 1b). In the mass spectra of compounds (I-VI) and (VIII) this peak is the main one. Furthermore, typical losses from the molecular ions in each case are caused by the splitting out of the tetrahydrofuran ring with m/z 215 (Fig. 1c), of the methyl group, and of the silanol $(CH_3)_3SiOH$ (m/z 90).

EXPERIMENTAL

Gas–liquid chromatography was carried out on a Tsvet 104 instrument with a phase-ionization detector under the conditions: carrier gas helium (65 ml/min); glass columns (200 × 0.3 cm) filled with Chromaton N-super (0.250-0.315) with 1.5% of OV-1 (245°C) and with 3% of OV-225 (288 and 245°C on chromatographing the alcohols and the ether, respectively), deactivated by the introduction of several portions of hexamethyldisilazane (10 μl each) under the working conditions. The samples to be analyzed (4-8 μl) were introduced directly into the column.

IR spectra were recorded on a Specord 751 R spectrophotometer in n-hexane solution.

Mass spectra were recorded on a LKB 9000 spectrometer at an ionizing voltage of 70 V using a chromatographic column with a nonpolar liquid phase (column temperature 280°C).

Preparation of the TMS Ethers. In a test-tube with a ground-in stopper, 0.1-0.2 mg of one of the initial alcohols was dissolved in 80 μl of dry pyridine. Then 80 μl of TMSIM and 40 μl of CTMS were added. The reaction mixture was left at room temperature for 30 min. In the absence of CTMS, the reaction mixture was kept at 100°C for 90 min.

Isolation of the TMS Ethers. The reaction mixture was diluted with distilled water (∿1 ml). The TMS ethers were extracted with n-hexane (2 × 0.3 ml), with subsequent washing of the extract by water (3 × 0.4 ml), and the solvent was evaporated in a current of argon.

Preparation of Trimethylsilylimidazole. TMSIM was obtained from imidazole and hexamethyldisilazane as described by Bielby et al. [6].

SUMMARY

The relative retention times of tetracyclic alcohols of the dammarane series from the leaves of Far Eastern species of the genus *Betula* and of their TMS ethers on the stationary liquid phases OV-1 and OV-225 have been determined, and their stability under GLC conditions has been shown.

The conditions for the silylation of the compounds investigated have been studied. The products of this reaction have been characterized by the use of IR spectroscopy and mass spectrometry.

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