GAS – LIQUID CHROMATOGRAPHY OF TRITERPENOIDS

II. DERIVATIVES OF PENTACYCLIC ALCOHOLS AND ACIDS. ANALYSIS OF ACIDS FROM PLANT EXTRACTS

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Continuing a study of the chromatographic mobility of triterpenoids under the conditions of gas-chromatographic analysis, we have used the phase OV-17 together with the phase SE-30 for the first time. In the present paper we give the results obtained for various triterpene pentacyclic alcohols and acid derivatives belonging to the ursane, lupane, and oleanane groups and the modified oleanene group (Tables 1 and 2).

TABLE 1. Relative Retention Volumes of Pentacyclic Alcohols and Their Derivatives

Substance	C=C	OH	C=O	OSi (CH_2)	Relative retention volume
					1.5% OV-17, Chromosorb W, 80-100 mesh
Cholestane					1.00 (2.6 min)
Compounds of the oleanane group					
β-Amyrin	12	3β			4.73
β-Amyrin acetate	12	3βOAc			6.25
epi-β-Amyrin acetate	12	3αOAc			5.85
Compounds of the modified oleanane group					
a)D-Friedo (taraxerane) derivatives					
Taraxerol	14	3β			4.82
Taraxerol acetate	14	3βOAc			5.85
TMS derivative of taraxerol	14			3	3.45
Epitaraxerol	14	3α			4.55
Epitaraxerol acetate	14	3αOAc			5.61
TMS derivative of epitaraxerol	14			3	2.55
Taraxerone	14		3		4.50
b)D: A-Friedo (friedelane) derivatives					
Friedelinol		3α			7.78
TMS derivative of friedelinol				3	6.04
Epifriedelinol		3β			7.17
TMS derivative of epifriedelinol				3	5.32
Friedelin			3		8.32
c) E: B-Friedo (Nhopane) derivatives					
Simmiarol	5	3β			8.05
TMS derivative of simmiarol	5			3	5.04
Episimmiarol	5	3α			7.91
TMS derivative of episimmiarol	5			3	5.58
Simmiarone	5		3		7.69
Compounds of the ursane group					
α-Amyrin	12	3β			5.55

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TABLE 2. Relative Retention Volumes of Derivatives of Triterpene Acids

Substance	C=C	OH	C=O	OCH_3	$OSi(CH_3)_3$	Relative retention volume: 1,5% SE-30, Gas-chrom G, 60–80 mesh	Relative retention volume: 1,5 % OV-17, Chromosorb W, 80–100 mesh
Compounds of the oleanane group							
Methyl oleanolate	12	3β		28		1,00 (17,8 min)	1,00(27,2 min)
Methyl katonate	12	3α		29		1,38	0,36
Acetate of methyl katonate	12	3αOAc		29		1,43	1,50
TMS derivative of methyl katonate	12			29	3	1,08	0,18
Methyl epikatonate	12	3β		29		1,44	0.55
Acetate of methyl epikatonate	12	3βOAc		29		1,83	—
TMS derivative of methyl epikatonate	12			29	3	1,59	0,38
Methyl macedonate	11,13(18)	3β,21α		29		1,54	2,29
Methyl dioxomacedonate	11,13(18)		3,21	29		1,36	1,89
Methyl meristotropate	11,13(18)	3β	22	29		—	2,34
Methyl dioxomeristotropate	11,13(18)		3,22	29		—	2,30
Methyl machaerate	12	3β	21	28		1,68	2,29
Methyl morolate	18	3β		28		1,12	0,93
Methyl echinocystate	12	3β,16α		28		1,56	2,03
Diacetate of methyl echinoocystate	12	3βOAc		28		2,39	—
Methyl α-glycyrrhetate	12	16αOAc					
Acetate of methyl α-glycyrrhetate	12	3β	11	30		1,90	—
Ethyl α-glycyrrhetate	12	3βOAc	11	30		2,64	2,87
Acetate of ethyl α-glycyrrhetate	12	3β	11	30 OEt		2,95	—
Dimethyl gypsogenate	12	3βOAc	11	30 OEt		3,00	—
Acetate of dimethyl gypsogenate	12	3β		23,28		1,68	2,04
		3βOAc		23,28		1,93	—
Compounds of the ursane group							
Acetate of methyl ursulate	12						
Acetate of methyl bryonolate	12	3βOAc		28		1,41	1,29
		3βOAc		29		1,68	—
Compounds of the lupane group							
Methyl betulinate	20(29)	3β		28		1,02	0,97
Acetate of methyl betulinate	20(29)	3βOAc		28		1,35	1,15
TMS derivative of methyl betulinate	20(29)			28	3	—	0,69
Methyl epibetulinate	20(29)	3α		28		—	0,91
TMS derivative of methyl epibetulinate	20(29)			28	3	—	0,50

Note. A dash means that no analysis was performed.

TABLE 3. Relative Retention Volumes of Derivatives of Ursolic and Oleanolic Acids

Substance	1,5% SE-30, Gas-chrom G, 60–80 mesh	1,5 % SE-30, Chromosorb W, 80–100 mesh	1,5 % OV-17, Chromosorb W, 80–100 mesh
Methyl oleanolate	1,00 (17,8 min)	1,00 (10.8 min)	1,00 (27,2 min)
TMS derivative of methyl oleanolate	1,14	1,08	—
Methyl ursolate	1,15	1,09	1,15
TMS derivative of methyl ursolate	1,24	1,25	—

To calculate the relative retention volumes of the alcohols and some of their derivatives, as the standard we took cholestane (see Table 1), and in the calculations of the corresponding values for esters of triterpene acids we used methyl oleanate as standard (Tables 2 and 3).

The dependence of the retention time of triterpene compounds on the number and nature of the substituents reported previously [2, 3, 4] was observed in chromatography on both SE-30 and on OV-17.

We traced this relationship clearly for derivatives of α-glycyrrhetic acid (the methyl ester, the ethyl ester, and the acetate of the ethyl ester) in chromatography using SE-30 and for derivatives of gypsogenic acid (dimethyl ester, and the acetate of the dimethyl ester) and of echinocystic acid (methyl ester, diacetate of the methyl ester) in gas–liquid analysis using a column containing OV-17 as the stationary phase (see Table 2).

While the replacement in triterpene compounds of a hydroxy group by an acetyl group led to an increase in the retention time in chromatography both on SE-30 and on OV-17, the relative retention volumes of the TMS ethers of the triterpenoids proved to be smaller on OV-17 than those of the corresponding initial compounds; for example, for friedelinol (7.78) and for the TMS ether of friedelinol (6.04) (see Table 1).

The sequence of retention times (acetates > free triterpenoids > TMS ethers) in GLC on OV-17 is similar to the analogous relationship established for steroids in chromatography on XE-60 [6].

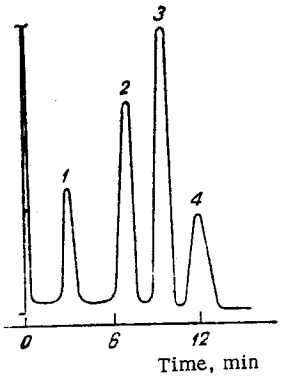

Fig. 1

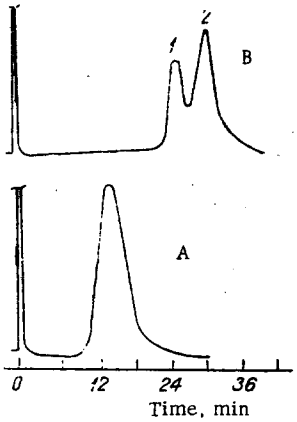

Fig. 2

Fig. 1. GL chromatogram of taraxerol derivatives (1.5% OV-17, T 240°C, V_{Ar} 75 ml/min): 1) cholestane; 2) TMS derivative of epitaraxerol; 3) TMS derivative of taraxerol; 4) taraxerone.

Fig. 2. GL chromatograms of methyl esters of triterpene acids (T 240°C; V_{Ar} 75 ml/min): a) 1.5% SE-30; mixture of methyl oleanate and methyl ursolate; b) 1.5% OV-17; 1) methyl oleanolate; 2) methyl ursolate.

There is information in the literature [3, 4] on the GLC of some triterpene ketones and alcohols on SE-60, from which it follows that retention times of ketones are greater than those of the corresponding alcohols.

In the GLC of triterpene compounds on OV-17, the relative retention volumes of ketones proved to be either smaller than those of the corresponding alcohols (taxerone, simmiarone) or very slightly greater (friedelin) (see Table 1).

On the whole, however, the differences between the relative retention volumes for triterpenoids with hydroxy groups and for their oxo derivatives are small both on OV-17 and on SE-30 [1, 2]. This fact can probably be explained by the "nonselectivity" of SE-30 and OV-17 with respect to these compounds, by analogy with steroids [5, 6].

We have also studied the chromatographic behavior of a number of epimeric alcohols and derivatives of epimeric acids (see Tables 1 and 2). It was found that the retention times of triterpenoids with equatorial substituents in chromatography both with SE-30 and OV-17 are greater than for substances with axial substituents, which is in harmony with literature information obtained for the stationary phase XE-60 [4].

It was impossible to separate a mixture of epimers on either of the two stationary phases used, and separation could be achieved only in the case of the silylated product. As an example, Fig. 1 gives a chromatogram of a mixture of TMS derivatives of taraxerol, epitaraxerol, and taraxerone on OV-17.

We have reported [2] the chromatography of a mixture of α- and β-amyrins on SE-30. When these compounds were chromatographed on OV-17 it was possible to achieve the separation of a mixture of these compounds, but a mixture of α- and β-amyrins and β-sitosterol was scarcely separated.

The chromatographic behavior of methyl ursolate and methyl oleanolate has been studied by the GLC method on the stationary phases SE-30 and OV-17 (see Table 3). It was impossible to separate a mixture of these substances on SE-30 (Fig. 2A), and only the silyl derivatives of the methyl esters were separated. At the same time, on OV-17 good separation of the methyl esters themselves of these acids was observed, the retention times of the substances being relatively small and the peaks sharp (Fig. 2B). Then, under the same conditions, we investigated the acid fractions of chloroform extracts of several species of rhododendrons: gold mat rhododendron, Ledebour's rhododendron, and the Caucasian rhododendron, in which oleanolic and ursolic acids have been identified by GLC.

Samples of triterpenoids were kindly given to us by L. G. Matyukhina, I. A. Saltykova, and A. D. Zorin, and samples of methyl epibetulinate and of the acetate of 3-epi-β-amyrin by Dr. Herz (State University, Florida, USA) and Dr. Ito (Sendai University, Japan).

EXPERIMENTAL

Gas–liquid chromatography was performed on a Pye series 104 instrument with a flame ionization detector at a rate of flow of Ar of 75 ml/min and a column temperature of 240°C. Glass columns (100×0.4 cm) containing 1.5% of SE-30 on Gas-chrom G (60-80 mesh), 1.5% of SE-30 on Chromosorb W (80-100 mesh), and 1.5% of OV-17 on Chromosorb W (80-100 mesh) were used. Samples of pure markers in the form of 1 μl of a 0.5% solution in chloroform were injected into the column with a Hamilton syringe. The trimethylsilyl derivatives were obtained by the method of Ikekawa et al. [3] and were introduced directly into the chromatograph.

To prepare samples of the methyl esters of the acids from the rhododendron extracts, the extracts obtained after the treatment of the plant raw material with chloroform were separated into acidic and neutral fractions. The acidic fraction (0.5 g) was treated with a solution of diazomethane in diethyl ether. After the elimination of the solvent, the residue was dissolved in 3 ml of chloroform, and 1-2 μl of the mixture was introduced directly into the chromatograph.

SUMMARY

1. The chromatographic mobilities of 52 triterpenoids on the stationary phases SE-30 and OV-17 have been studied.

2. It has been established that the retention times of triterpenoids depend on the number, nature, and steric orientation of the functional groups.

3. It has been shown that the GLC method can be used for the investigation of the triterpene composition of the acidic fractions of plant extracts. The use of OV-17 in the analysis of methylated products gives the best results.

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GAS-CHROMATOGRAPHIC SEPARATION OF FREE STEROLS USING STEAM AS THE MOBILE PHASE

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At the present time, the gas-chromatographic analysis of steroid compounds is one of the analytical methods used both in the study of the metabolism and chemical transformations of steroids and in the production of steroid drugs. As stated in the majority of publications, steroid compounds are analyzed after their previous conversion into the more volatile trimethylsilyl, acetyl, or trifluoroacetyl derivatives [1]. This is due to the high polarity and exceptionally low vapor pressure of the majority of compounds of the group under discussion. Furthermore, the gas chromatography of the free steroids under the usual conditions is complicated by the formation of diffuse "tails" which markedly impair resolution and reduce the sensitivity of the chromatographic system. In view of the fact that in a number of cases a considerable reduction in retention time and

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