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.

LITERATURE CITED

1. G. A. Fokina and N. V. Belova, Khim. Prirodn. Soedin., 1, 31 (1971).
2. G. A. Fokina and N. V. Belova, Khim. Prirodn. Soedin., 429 (1971).
3. N. Ikekawa, D. Natori, H. Itokawa, S. Tobinaga, and M. Matsui, Chem. Pharm. Bull., 13, No. 3, 316 (1965).
4. R. Ikan and R. Gottlieb, Israel J. Chem., 8, 685 (1970).
5. E. C. Horning, W. I. A. Vanden Heuvel, and B. G. Creech, in: Methods of Biochemical Analysis, Vol. 11, Interscience, New York (1963), p. 86.
6. A. Kuksis, in: Methods of Biochemical Analysis, Vol. 14, Interscience, New York (1966), p. 359.

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

M. A. Baidarovtseva, B. A. Rudenko, M. I. Kuleshova, and V. F. Kucherov

UDC 542.91:543.544

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

N. D. Zelinskii Institute of Organic Chemistry, Academy of Sciences of the USSR.

Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 739-742, November-December, 1975. Original article submitted August 23, 1974.

This material is protected by copyright registered in the name of Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for $7.50.

TABLE 1. Retention Indices of Steroid Compounds in a Column Containing Polydimethylsiloxane SE-30 at 230°C

Compound	Peak	Kovat's retention indices	
		in steam	in nitrogen
Ergosterol	1	2867	2670
	2	3074	2757
Cholesterol		3024	3100
Diosgenin		3148	3150
Stigmasterol		3163	2990
β-Sitosterol	1	3140	3000
	2	3235	
Lanosterol	1	3163	
			3120
	2	3201	
Phytosterol	1	3113	3016
	2	3139	3120
	3	3198	—

improvement in the shape of the peak when steam has been used as the mobile phase in the chromatography of substances of low volatility and high polarity [2-4], we have performed the gas-chromatographic separation of free steroids under these conditions. In addition to steroid alcohols cholesterol, sitosterol, etc.) the group of compounds studied included some terpenoids (lanosterol) and steroid genins (diosgenin).

EXPERIMENTAL

The experiments on the gas-chromatographic separation of sterols in a current of steam were performed with a special all-glass chromatograph [5] fitted with a flame-ionization detector and two chromatographic columns differing in the polarity of the liquid phases used: a column 1.3 m long containing 1% of the weakly polar polydimethylsiloxane SKTB-K and a column 2 m long containing 5% of the polar polysiloxane XE-60 containing cyano groups. The solid support was Chromaton N with a grain size of 60-75 mesh or 75-90 mesh. In the experiments with the weakly polar liquid phase, the column temperature was 230°C, and with the XE-60 it was 260°C. The steam pressure at the inlet to the column was 0.5-1.3 atm. The samples were introduced in the form of solutions in chloroform or as dilute aqueous suspensions. In working with the column containing SKTB-K, the Kovats indices of all the compounds investigated were determined (Table 1). The table also gives the retention indices obtained under comparable conditions but using an inert gas – nitrogen – (the chromatograms are shown in Figs. 1 and 2.).

On comparing the results obtained, it may be concluded that the use of steam as mobile phase permits the retention times to be considerably shortened and the shape of the peaks arising to be improved. For three of the substances studied – β-sitosterol, lanosterol, and phytosterol – the nature of the chromatograms changed considerably on passing from nitrogen to steam. In the chromatogram with steam the number of peaks recorded proved to be greater: lanosterol and β-sitosterol were recorded in the form of two peaks each and phytosterol was eluted in the form of three peaks. Since the β-sitosterol and phytosterol isolated from natural sources are not homogeneous products [6], the fact that they gave chromatograms in the form of several peaks must apparently be considered as a result of the sharper separation achievable by using steam.

The results of a comparison of the retention indices of the compounds studied, measured in the column containing polydimethylsiloxane SE-30, showed that some of them change the value of their index by 100-150 units on passing from nitrogen to steam. This leads to a change in the order of issue of the peaks. For example, cholesterol, which is eluted in nitrogen after sitosterol, is eluted first in steam. On chromatograms of lanosterol and phytosterol obtained in a current of steam there is in each case a peak of a retention index close to 3200 (see Fig. 1), possibly corresponding to components of the same nature.

On passing to a more polar liquid phase – the cyano-group-containing polysiloxane XE-60 – the retention times of sterols falls considerably and their separation becomes somewhat less sharp (see Fig. 2).

Thus, gas–liquid chromatography in a current of steam considerably facilitates the analysis of steroid compounds as compared with chromatography in nitrogen and creates the prerequisite for their direct determination in aqueous biological media.

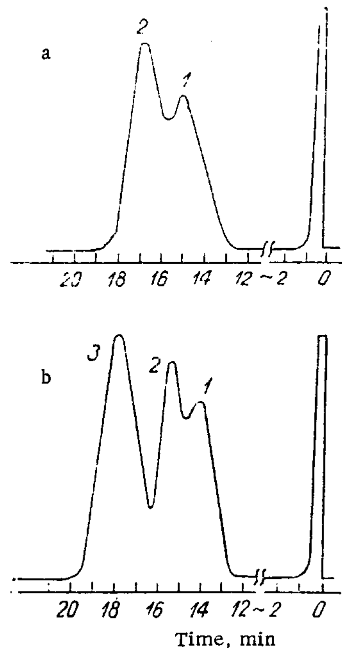

Fig. 1

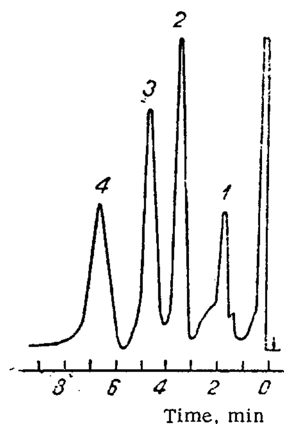

Fig. 2

Fig. 1. Chromatograms of natural sterols in a current of steam in a column containing 1% of polydimethylsiloxane SKTB on Chromaton at 230°C (length of the column 1 m, pressure of steam 0.5 atm): a) lanosterol (retention index of the 1st peak 3163 and of the second peak 3201); b) sitosterol (retention index of the first peak 3113, of the second peak 3139, and of the third peak 3198).

Fig. 2. Chromatogram of a mixture of sterols in a column of 5% of polysiloxane XE-60 containing nitrile groups (length of the column 2 m 230°C, pressure of steam 0.5 atm): peak 1) ergosterol; peak 2) cholesterol; peak 3) sigmasterol; peak 4) diosgenin.

SUMMARY

The gas-chromatographic separation of natural sterols using steam as the carrier gas has been performed.

LITERATURE CITED

1. L. D. Litvinov and G. A. Rudenko, Gas-chromatography in Medicine and Biology [in Russian], Moscow (1971).
2. C. Dumazert and C. Chiglione, Bull. Pharm. Marseilles, 11, No. 42, 119, 127 (1962).
3. G. A. Rudenko, M. A. Baidarovtseva, and V. F. Kucherov, Izv. Akad, Nauk SSSR, Ser. Khim., No. 11, 2634 (1972).
4. B. A. Rudenko, M. A. Baidarovtseva, and V. F. Kucherov, Izv. Akad. Nauk SSSR., Ser. Khim., No. 8, 1773 (1973).
5. B. A. Rudenko, M. A. Baidarovtseva, and V. F. Kucherov, Zavodsk. Lab., 40, No. 8, 931 (1974).
6. É. Heftmann, Steroid Biochemistry, Academic Press, New York (1970).