TRITERPENOIDS FROM Rhododendron caucasicum AND Rh. Ledebourii

GAS-LIQUID CHROMATOGRAPHY OF RHODODENDRON EXTRACTS

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We have previously reported the wide distribution of triterpenes in plants of the genus Rhododendron, family Ericaceae [1].

The present paper gives the results of a study of the triterpene composition of two domestic species of rhododendron – Rh. caucasicum Pall., collected in the region of Pyatigorsk, and Rh. ledebourii Pojark, introduced into the Leningrad Botanical Garden.

Crystalline substances were obtained from the neutral fraction of the extract of Rh. caucasicum Pall. by column chromatography on alumina. Two of them were identified as campanullin and friedelin from their IR spectra and melting points. A third substance, with mp 274°C, proved to be identical with epifriedelinol, a sample of which we obtained by the reduction of friedelin over a platinum catalyst in acetic acid [2].

From the neutral fraction of a chloroform extract of Rh. ledebourii Pojark we isolated substances which were identified as campanullin, β-sitosterol, and betulin.

Gas-Liquid Chromatography of the Triterpenes [3, 4]. We studied the chromatographic mobility of the triterpenoids isolated from rhododendrons [5], and also some other samples available to us.

In order to calculate the relative retention volumes, cholestane was used as the standard. The results of the analysis are given below.

Relative retention volumes of triterpenoids*

Cholestane	1 (5.1 min)	Lupeol	3.38	$\Delta^{5(10)}$-Alnusenol acetate	4.12
Taraxerol	2.97	Lupeol acetate	4.65	Campanullin	2.48
Taraxerol acetate	3.88	Betulin	6.91	β-Amyrin	3.08
Taraxerone	2.86	Betulin acetate	10.65	α-Amyrin	3.32
Simiarol	4.13	Epifriedelinol	4.27	Zeroin	5.05
Simiarol acetate	5.62	Friedelin	4.06	Myricadiol	6.36
Simiarone	4.09	$\Delta^{5(10)}$-Alnusenol	3.18	Uvaol	6.65

*3% of E-30; carrier gas H_2; 50 ml/sec.

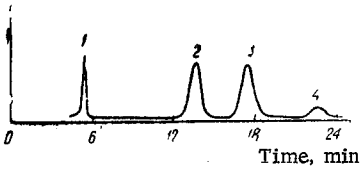

Fig. 1. GL chromatogram of $\Delta^{5(10)}$-alnusenol and its derivatives: 1) cholestane; 2) campanullin; 3) $\Delta^{5(10)}$-alnusenol; 4) $\Delta^{5(10)}$-alnusenol acetate.

As an example, Fig. 1 gives a GL chromatogram of a mixture of $\Delta^{5(10)}$-alnusenol, campanullin, and $\Delta^{5(10)}$-alnusenol acetate.

A rise in the relative retention volumes is found in the sequence ketone–alcohol–acetate. These values are very close for the alcohols and the ketones corresponding to them (on chromatography, mixtures of them were not separated, forming a single common peak).

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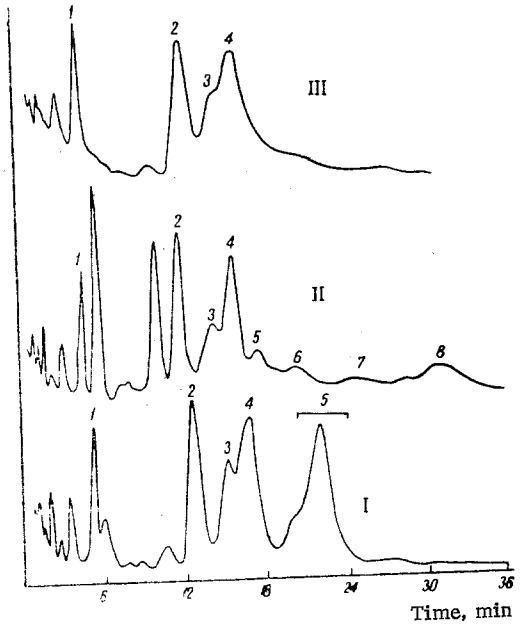

Fig. 2. GL chromatograms of rhododendron extracts: I. Rh. caucasicum Pall. 1) cholestane; 2) campanullin; 3,4) unidentified substances; 5) epifriedelinol and friedelin. II. Rh. aureum Georgi. 1) cholestane; 2) campanullin; 3) unidentified substance; 4) taraxerol and taraxerone; 5,6,7,8) unidentified substances. III. Rh. ledebourii Pojark. 1) cholestane; 2) campanullin; 3,4) unidentified substances.

Then, under the same conditions, chloroform extracts of the rhododendrons investigated and an extract of Rh. aureum Georgi, the triterpene composition of which we have studied previously [5], were chromatographed.

The peaks on the chromatograms (Fig. 2, curves I, II, and III) were identified by comparing the relative retention volumes given in the present work and those obtained by the method of adding authentic samples.

On curve I (see Fig. 2), peak 2 (2.55) is ascribed to campanullin, and peak 5 (4.23) corresponds to a mixture of epifriedelinol and friedelin. On curve II (see Fig. 2), peak 2 (2.46) corresponds to campanullin, and peak 4 (3.03) is ascribed to a mixture of taraxerol and taraxerone which, like epifriedelinol and friedelin, have very similar relative retention volumes and are practically incapable of separation. On curve III (see Fig. 2), peak 2 (2.55) was identified as campanullin.

The use of the GLC method for the analysis of such complex mixtures as plant extracts makes it possible to obtain some preliminary information on their triterpene composition.

EXPERIMENTAL

The rhododendron extracts were chromatographed on alumina (Brockmann activity III). The eluents used were petroleum ether, benzene, chloroform, and mixtures of them.

The IR spectra were taken in paraffin oil on a UR-10 instrument. The analyses of all the compounds corresponded to the calculated figures.

Isolation of Triterpenoids from Rh. caucasicum. Comminuted branches and leaves (128 g) were extracted with chloroform until the Liebermann–Burchard reaction was negative. The chloroform was distilled off, and the acid compounds were separated from the resin obtained. The weight of the neutral fraction of the resin was 3.5 g. Chromatography of the neutral fraction of the resin (with benzene as the eluent) gave a substance $C_{30}H_{50}O$ with mp 201°C (from acetone), which was identical with campanullin. IR spectrum, cm^{-1}: 915, 960, 948, 1000, 1010, 1028.

From the same column, a mixture of benzene and chloroform (1 : 1) eluted friedelin, $C_{30}H_{48}O$, with mp 241°C (from ethanol). IR spectrum: 1715 cm^{-1} (C=O).

Subsequent elution of the column with a mixture of benzene and chloroform yielded a substance $C_{30}H_{52}O$ with mp 274°C identified as epifriedelinol (from $CHCl_3$–C_2H_5OH).

Reduction of Friedelin. 0.05 g of the ketone was hydrogenated in acetic acid (5 ml) over PtO_2 (0.1 g). The reaction mixture was diluted with water and extracted with chloroform. The reaction product melted at 270-271°C (from a mixture of $CHCl_3$ and C_2H_5OH). IR spectrum: 3480 cm^{-1} (–OH).

Isolation of the Triterpenes from Rh. ledebourii. The process described above was applied to 100 g of dried and comminuted branches and leaves. The weight of the neutral fraction on the resin was 3 g. When this resin was chromatographed [petroleum ether–benzene (1 : 1)], a substance with mp 200°C (from acetone) was obtained, which was identical with campanullin.

Further elution of the column with chloroform yielded a substance with mp 128°C. The IR spectrum of the substance was identical with that of β-sitosterol.

The subsequent chloroformic eluates yielded betulin, $C_{30}H_{50}O$, with mp 247°C (from ethanol). IR spectrum, cm^{-1}: 885, 1643 (>C=CH_2), 3390 (−OH).

GLC was performed in a Pye series 104 instrument with a flame ionization detector (rate of flow of gas 50 ml/sec) in glass columns (100 × 0.4 cm) with 3% of SE-30 on Gas-Chrom Q (100 × 100 mesh). The column temperature was 240°C. Samples of pure markers, 3-10 μliter of 0.5% solutions in acetone were introduced into the column by means of a Hamilton syringe.

Preparation of Samples of Rhododendron Extracts. One gram of the comminuted branches and leaves was extracted with 10 ml of chloroform. The chloroform extract was passed through a thin layer of alumina and then evaporated.

SUMMARY

1. From the stems and leaves of Rh. caucasicum Pall. campanullin, friedelin, and epifriedelinol have been isolated and identified, and from the stems and leaves of Rh. ledebourii Pojark campanullin, β-sitosterol, and betulin.

2. The chromatographic mobility of 20 triterpenoids on the stationary phase SE-30 has been studied.

3. It has been shown that the GLC method permits some preliminary conclusions to be obtained concerning the triterpene composition of rhododendron extracts.

LITERATURE CITED

1. N. V. Belova, Rast. Res., 1968, No. 4.
2. P. R. Jefferies, J. Chem. Soc., 473 (1954).
3. P. Capella, E. Fedell, and M. Cirimele, Chem. Ind. (London), 1963, 1590.
4. N. Ikekawa, S. Natori, H. Itokawa, S. Tobinaga, and M. Matsui, Chem. Pharm. Bull., 13(3), 316-325 (1965).
5. N. V. Belova and G. A. Fokina, Khim. Prirodn. Soedin., 137 (1970).