GAS–LIQUID CHROMATOGRAPHY OF TRITERPENOIDS.

SEPARATION OF THE METHYL ESTERS OF OLEANOLIC AND URSOLIC ACIDS

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We have reported on the use of the GLC method for studying the triterpene composition of the acid fractions of plant extracts and for the identification of oleanolic and ursolic acids in such mixtures [1]. The aim of the present investigation was to determine the optimum conditions for the quantitative chromatographic analysis of methyl oleanolate (I) and methyl ursolate (II).

Chromatography was carried out on a Pye series 104 chromatograph with a flame-ionization detector. Glass columns (180 × 0.4 cm) with 3% of OV-17 and SE-30 on Chromaton N-AW-DMCS (0.16-0.20 mm) were used.

The individual compounds (I) and (II) and a mixture of them in a 1:1 ratio were analyzed in the form of 1.5% solutions in chloroform with the injection of a dose of 0.4 μl.

For the comparative evaluation of the columns we calculated from the parameters of the peaks of the individual substance the selectivity coefficient K_s and the height equivalent to a theoretical plate, H. As the characteristics of the separation process for compounds (I) and (II) we used the effective separation criterion K_{eff}, and for their model mixture the degree of separation K [2]. We also took into account the time of analysis T.

The parameters K_{eff}, K, and T were determined as functions of the temperature of the column T_{col} and the rate of flow of carrier gas (argon) V_{Ar} (Table 1).

The two chromatographic columns proved to be approximately identical in selectivity and efficiency. The lack of separation of compounds (I) and (II) on SE-30 recorded previously [1] is probably due to the quality of the sorbent with a low concentration of stationary phase.

A comparison of the areas of the peaks on the chromatogram of a model mixture of (I) and (II) at the maximum achievable degree of separation shows that the compounds investigated have the same affinity for the stationary liquid.

Under the experimental conditions selected, a satisfactory degree of separation (K = 1.38) on OV-17 was obtained with a time of analysis of 26 min; K = 1.42 was observed on SE-30 after 42 min. At higher degrees of separation of methyl oleanate and ursolate the time of analysis increased considerably.

TABLE 1. Characteristics of the GLC Separation of the Methyl Esters of Oleanolic and Ursolic Acids

Phase	T_{col}, °C	V_{Ar}, ml/min	K_s	H for I	H for II	K_{eff}	K	Time, min
OV-17	290	30	0,14	0,98	0,94	1,48	1,38	26
	280	40	0,14	1,01	0,93	1,38	1,35	28
	270	30	0,15	1,00	0,93	1,41	1,37	30
	260	50	0,16	1,00	0,98	1,46	1,35	50
	240	50	0,16	1,19	0,88	1,50	1,44	117
SE-30	290	20	0,13	0,93	0,87	1,41	1,33	38
	280	30	0,14	0,87	0,76	1,47	1,42	42
		40	0,14	1,03	0,92	1,35	1,33	34
		50	0,14	0,98	1,09	1,24	1,19	28
	260	30	0,15	0,84	0,78	1,54	1,50	86
		50	0,15	1,03	0,96	1,33	1,30	62
		75	0,15	1,37	1,33	1,25	1,22	44
	240	50	0,15	0,98	1,09	1,47	1,45	162

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STRUCTURE OF EDPETISIDININE

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We have previously reported the isolation from *Petilium eduardi* (Rgl.) Vved. of a base with mp 263-265°C (methanol) $[\alpha]_D$ −15.3° (c 0.39; methanol–chloroform (9:1)) [1], composition $C_{27}H_{43}NO_3$ (I). The alkaloid is new and we have called it edpetisidinine. IR spectrum of (I), ν_{max}, cm^{-1}: 3300 (OH), 2770 (trans-quinolizidine) [2], 1670 (>C=C<).

The acetylation of (I) with acetic anhydride in pyridine gave a triacetyl derivative (I), M^+ 555. IR spectrum of (II), ν_{max}, cm^{-1}: 1745, 1255 (ester C=O). The oxidation of (I) with chromium trioxide gave edpetisidininedione (III), with mp 216-218°C (benzene–petroleum ether), M^+ 425. It was impossible to obtain a triketone derivative of edpetisidinine. An acid solution of (I) instantaneously decolorized a solution of potassium permanganate. However, it was impossible to reduce edpetisidinine by the Adams method under various conditions.

The mass spectrum of (I) had the peaks of ions with m/e 98, 111, 112, 123, 124, 125, 149, 164, 178, 373, 400, 414 $(M - 15)^+$, and 429 M^+ (100%), which are characteristic for the decomposition of the C-nor,D-homosteroid alkaloids of the cevine group [3-5].

The NMR spectrum of (I) showed the signals from one tertiary methyl group at 0.90 ppm (19-CH_3) and from two secondary methyl groups at 0.87 and 0.79 ppm (21-CH_3, 27-CH_3) (in CD_3OD + $CDCl_3$). The difficulty in the reduction of the double bond and the absence of the signal of an olefinic proton in the NMR spectrum of edpetisidinine show that the double bond in the molecule of (I) is tetrasubstituted. The NMR spectrum of (II) contained singlets at 0.90 ppm (19-CH_3) and 1.95, 1.98, and 2.0 ppm (9 H, $OCOCH_3$), doublets from secondary methyl groups at 0.86 and 0.79 ppm, and multiplets from protons geminal to acetoxy groups at 5.07 ppm (2 H, $\underline{H}C-OCOCH_3$) and 4.78 ppm (1 H, $\underline{H}C-OCOCH_3$) ($CDCl_3$).

Judging from the facts given above, (I) has the heterocyclic skeleton of cevanine and contains three secondary hydroxy groups and one doublet bond.

A comparison of the chemical shifts (CSs) of the protons of the 19-CH_3 group in the NMR spectra of (I) and (II) with those of edpetisinine, triacetyledpetisinine [1], and diacetylkorseveriline [6] showed that two of the OH groups in (I) were present at C_3 and C_6, while positions of the double bonds between C_8 and C_9 and between C_8 and C_{14} and of the third OH group at C_1 and C_{11} were excluded. Edpetisidinine is not oxidized by periodic acid. This means that the third OH group cannot be located at C_2, C_4, or C_7. In the mass spectrum of (I), the peaks of ions with m/e 111, 112, 164, and 178, which are formed by a known pathway [3, 5, 8], exclude the possibility that the OH group and the double bond are located on the carbon atoms of rings E/F. The impossibility of obtaining a triketone by the oxidation of (I) and the presence in the mass spectrum of (I) of the peaks of an ion with m/e 178 [6] show that the third OH group is located at C_{15}.

The close values of the CSs of the protons of the 19-CH_3 group in the NMR spectra of edpetisidinine and edpetisinine [1] show that the double bond is probably between C_{12} and C_{13}, since the presence of a double bond in this position in the C-nor,D-homosteroid alkaloids does not affect the CSs of the protons of the 19-CH_3 group [7]. According to the CSs of the protons of the 19-CH_3 group in the NMR spectrum of (I), rings A/B and B/C are trans-

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