CHEMICAL INVESTIGATION OF Hippophaë rhamnoides.

II. MAIN COMPONENTS OF THE NEUTRAL REACTION OF THE SAPONIFICATION PRODUCTS OF AN EXTRACT OF THE LEAVES OF THE SEA BUCKTHORN

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The qualitative and quantitative compositions of the neutral fraction of the products of the saponification of a diethyl ether extract of a sea buckthorn leaves have been investigated. Fourteen triterpene compounds and eight aliphatic alcohols have been identified from the results of GLC, CMS, and PMR and also from their melting points.

Continuing the chemical investigation of sea buckthorn [1], we have studied the compositions of the sterols and triterpenes from the leaves of the plant, concerning which there is no information in the literature.

By chromatographing the neutral fraction of the saponification products of an extract of the leaves (NFSP) on a column of silica gel we isolated fractions (23.4%)* containing mixtures of hydrocarbons, of carotenoids, of tocopherols, of polyprenols, and of phytol, which were identified by TLC in comparison with authentic samples.

By subsequent elution we obtained 11 more polar fractions containing aliphatic alcohols and polycyclic alcohols (sterols and triterpenoids).

On gas-liquid chromatography, the aliphatic alcohols gave peaks not overlapping with the peaks of the other components of the fractions obtained. This enabled both their total amount in the neutral fraction of the saponification products of an extract of the leaves (19.0%) and also their qualitative and quantitative compositions to be determined. They were represented by n-alkanols with the following numbers of carbon atoms: 18 (0.4%), 20 (1.4%). 22 (4.0%), 23 (0.1%), 24 (8.6%), 25 (0.2%), 26 (3.5%) and 28 (0.8%).

Of polycyclic alcohols we found the following groups of triterpene compounds: dimethylsterols (11.6%), methylsterols (0.8%), sterols (6.8%), triterpene aldehydes (1.1%), and diols (9.1%). The compositions of the fractions were calculated from the GLC results. The polycyclic alcohols were identified with the aid of chromato-mass spectrometry, by the isolation of individual compounds in the form of their acetates, and by comparing their melting points and PMR and mass spectra with literature information or with the corresponding characteristics for authentic samples.

In the least polar group of substances under investigation, including monohydric triterpene alcohols and dimetylsterols we detected and identified from their relative retention times (RRTs) five components: α-amyrin (1.15), β-amyrin (1.05), 24-methylenecycloartanol (1.27), cycloartenol (1.14), and lupeol (1.15). The last two alcohols, not previously detected in the pulp of the fruit of the sea buckthorn [1], were isolated and characterized in the form of acetates, since their RRTs coincided with the RRT of α-amyrin.

The methylsterol fractions eluted after the triterpene alcohols proved, according to GLC, to be fairly complex – they contained the remainder of the dimethylsterols, and also compounds with RRTs of 0.94, 1.29, 1.00, 1.60, and 1.78. The mass spectrum of the compound with

*The yield is give as a percentage on the initial neutral fraction.

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an RRT of 0.94 was represented by the molecular ion with m/z 426 (29%) and fragmentary ions with m/z 411 (99%), 393 (16%), 309 (6%), and 245 (26%). A comparison of the RRT and mass spectrum with the corresponding characteristics of obtusifoliol [2, 3] showed their complete coincidence.

The compound with the RRT of 1.29 was identified as citrostadienol on the basis of its mass spectrum: 426 (13%) (M^+), 411 (12%), 393 (5%), 328 (59%), 310 (9%), 285 (100%). Furthermore, the acetate of this compound was isolated in the pure form and its characteristics coincided with those given in the literature.

The compound with the RRT of 1.00 was identified as β-sitosterol by comparing its melting point and its PMR and mass spectra with those given in the literature.

Triterpene aldehydes (RRTs 1.60 and 1.78) were identified on the basis of their mass-spectral characteristics. The mass spectrum of the first compound had the lines of ions with m/z 440 (3%) (M^+) and 422 (2%), and also 232 (45%), 207 (20%), 203 (100%), 189 (26%), and 133 (17%). The mass spectrum of the second contained lines with m/z 440 (3%), 422 (2%), 232 (16%), 203 (100%), 207 (23%), 189 (18%), 133 (35%).

The presence of each of the spectra of the strongest line with m/z 203 is characteristic for compounds with olean-12-enic and urs-12-enic structures with substituents at carbon atom 17 which were readily split off from the fragmentary ions formed in a process of breakdown described by a reversed Diels–Alder reaction [4]. In this case, such ions are those with m/z 232, ejecting particles with 29 mass units. Consequently, the compounds with RRTs of 1.60 and 1.78 can be assigned to the triterpene alcohols with olean-12-enic and urs-12-enic skeletons containing an aldehyde group at carbon atom 17 in each cases. To confirm the structures of the compounds iolated, a mixture of these components was reduced with sodium tetrahydroborate and a mixture of two compounds was obtained the RRTs and mass spectra of which coincided completely with those for erythrodiol and uvaol [1, 2]. These facts show that the compounds were oleanolic aldehyde and ursolic aldehyde.

In addition to the compounds mentioned, from the sterol fragment we isolated, in the form of their acetates, two other substances not previously detected in the pulp sea buckthorn fruit. On the basis of their melting points, PMR spectra, and mass spectra they were identified as 28-norolean-12,18(17)-dien-3β-ol and 28-norurs-12,18(17)-dien-3β-ol.

The most polar fractions contained erythrodiol and uvaol. As a result of the qualitative and quantitative analysis of the fractions of higher aliphatic and polycyclic alcohols of the NFSP of a diethyl ether extract of sea buckthorn leaves that had been performed, the composition of the aliphatic alcohols was established and 14 triterpenoid compounds were identified and their amounts were determined: cycloartenol (2.6%), 24-methylenecycloartenol (1.3%), α-amyrin (3.4%), β-amyrin (1.7%), lupeol (2.6%), obtusifoliol (0.3%), oleanolical dehyde (0.5%), ursolic aldehyde (0.6%), β-sitosterol (6.8%), nordiene I (0.2%), nordiene II (0.3%), erythrodiol (5.8%), uvaol (3.3%), and citrostadienol (0.5%).

In a study of the main triterpene compounds of the NFSP of an extract of the pulp of sea buckthorn fruit we identified eight polycyclic alcohols: 24-methylenecycloartanol (13%), α-amyrin (2.7%), β-amyrin (3.2%), citrostadienol (0.4%), β-sitosterol (18%), 24-ethylcholest-7-en-3β-ol (0.4%), erythrodiol (2.3%), and uvaol (2.3%) [1].

EXPERIMENTAL

Melting points were determined on a Kofler stage. PMR spectra were recorded on a Bruker WP 200 SY instrument for solutions in deuterochloroform, with tetramethylsilane as internal standard. The quantitative analysis of the fractions was performed by GLC using the method of internal normalization from the areas of the peaks on a Chrom-4 instrument (Czechoslovakia) with a 0.3 × 250 cm glass column containing 5% of SE-30 on Chromaton N-AW, DMCS (temperature of the column 260°C and of the evaporator 300°C; carrier gas nitrogen), and also on the phase Lukopren under the same conditions.

The chromato-mass spectrometric analysis and identification of the components in the mixtures were performed on a LKB-2091 instrument with a 2130 data-processing system. The column was a glass capillary 25 m long and 0.25 mm in internal diameter, the stationary phase SE-30, the temperature of the column 270-300°C, that of the evaporator 310°C, and that of the separator 306°C, with helium as the carrier gas. The energy of the ionizing electrons was 70 eV. During the time of issuance of the separated components of the fractions being analyzed, of the order of ten spectra for each component were fed into the computer.

TABLE 1. Quantitative Amounts of Aliphatic and Polycyclic Alcohols in the Neutral Fraction of the Saponification Products of an Extract of Sea Buckthorn Leaves

Fractions		Aliphatic alcohols	Phytol	Cycloartenol	24-Methylenecycloartanol	α-Amyrin	β-Amyrin	Lupeol	Obtusifoliol	Citrostadienol	Oleanolicaldehyde	Ursolicaldehyde	β-Sitosterene	Nordiene I	Nordiene II	Erythrodiol	Uvaol	Other compounds
number	weight																	
1	1,40	38,3	34,7	18,4	4,4	—	—	—	—	—	—	—	—	—	—	—	—	4 7
2	7,22	54,6	6 8	6,1	5 0	11,7	6 3	10,3 †		—	—	—	—	—	—	—	—	Tr.
3	1 57	72,1	2,0	—	—	9,0	4,6	6 4	3,5	2,3	—	—	—	—	—	—		Tr.
4	0,58	52,6	0 7	—	—	12,5	5 6	—	6,3	8,9	4,0	5,2	—	—	—	—	—	4,2
5	0,48	36,2	1,3	—	—	5,0	0,5	—	—	10,2	22,0	21,5	3,2	—	—	—	—	Tr.
6	0,28	9,9	—	—	—	—	—	—	—	5,2	23,5	14,9	46,4	—	—	—	—	Tr.
7	1,69	—	—	—	—	—	—		—	—	—	—	95,0	2,0	3,0		—	Tr.
8	0,51	—	—	—	—	—	—		—			—	86,0	7,0	7,0			Tr.
9	1,72	—	—	—	—	—	—	—	—	—	—	—	—	—	—	83,9	16,1	Tr.
10	1,15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	28,1	55,2	16,7
11	0.60	—	—	—	—	—	—	—	—	—	—	—	—	—	—	16,9	24,8	58,3
	%	19,0	3,5	2,6	1,3	3,4	1,7	2 6	0,3	0 5 1,29	0 6	0,5	6 8	0,2	0,3	5,8	3,3	
	OBY*			1,14	1,27	1,15	1 05	1,15	0,94		1.6	1,78	1,00	1,39	1,49	1,94	2,09	

*The RRT of β-sitosterol was taken as 1.

† The percentages of α-amyrin and lupeol were calculated after additional chromatography.

For column chromatography we used air-dry silica gel of type SKS with a grain size of 0.140-0.315 mm. The eluent system consisted of petroleum ether with increasing concentrations (from 0 to 50%) of diethyl ether. Air-dry silica gel of type L 0.04-0.10 mm (Czechoslovakia) impregnated with silver nitrate (10%) was used to separate the acetates. The eluting system consisted of hexane with increasing concentrations (from 1.5 to 5.0%) of diethyl ether. Silufol plates were used for TLC. The air-dry sea buckthorn leaves were obtained from the Biisk vitamin factory.

Isolation of the Neutral Fraction of the Saponification Products. The comminuted sea buckthorn leaves were extracted with diethyl ether in a Soxhlet apparatus for 12 h. The yield of extract was 4.85% on the weight of the initial raw material. Of this extract, 73 g was saponified by the usual procedure [5]. This gave 32.3 g of NFSP, which amounted to 44% on the weight of the extract.

Isolation of Fractions of Aliphatic and Polycyclic Alcohols. By chromatographing 32.2 g of NFSP on a volumn (36 × 1060 mm) of silica gel (with, as the eluant, petroleum ether with increasing amounts, from 1 to 15% of diethyl ether) we obtained, successively, 1.15 g of a white waxy product (mixture of hydrocarbons), 0.37 g of an orange solid substance (mixture of carotenoids),0.83 g of an oily product (mixture of tocopherols), and 4.00 g of a viscous product (mixture of polypyrenols). Continuing elution with petroleum ether with the addition of diethyl ether in amounts of from 15 to 50%, we isolated another 13 fractions. The yields of fractions 1-11 and their compositions are given in Table 1. Fractions 12 and 13 with a total weight of 3.20 g, containing more polar compounds, were not investigated.

Cycloartenol Acetate. Fraction 1 was acetylated by the usual procedure [5], and the products were then chromatographed on a column of silica gel impregnated with silver nitrate. A fraction that was pure according to GLC with a weight of 0.22 g was identified as cycloartenol acetate. Melting point 117-119°C (from ethanol). According to the literature [6]: mp 122-124°C.

Mass spectrum: 468 (M^+) (5%), 453 (4%), 408 (22%), 393 (18%), 339 (8%), 297 (6%), 286 (10%), 271 (7%). PMR spectrum, δ, 0.33 (1 H, d, C-19); 0.56 (1 H, d, C-19); 0.84 (3 H, s, C-3); 0.89 (6 H, s, C-30, 31); 0.95 (3 H, s, C-18); 1.60 (3 H, s, C-26); 1.68 (3 H, s, C-27); 2.05 (3 H, s, 3β-OAc); 4.55 (1 H, m, C-3α); 5.09 (1 H, m, C-24). The spectra coincided with those given in the literature [2, 6].

Chromatography of the Acetates of the Alcohols of Fraction 2. Fraction 2 was dissolved in hot hexane and the precipitate that deposited (according to GLC, a mixture of aliphatic alcohols) was separated off. The mother liquor was evaporated, giving 4.50 g of a yellow solid residue (according to GLC, a mixture of triterpene alcohols with a small amount of aliphatic alcohols). When 0.82 g of this residue was acetylated, 0.85 g of a mixture of acetates was obtained which was chromatographed on a column of silica gel impregnated with silver nitrate. On elution with hexane containing from 1.5 to 5% of diethyl ehter, five fractions (a-e) were obtained.

Fraction a (weight 0.54 g) consisted of a mixture of acetates of aliphatic alcohols, of phytol, of β-amyrin, and of cycloartenol (results of chromato-mass spectrometry).

Fraction b (weight 0.22 g) consisted of a mixture of acetates of aliphatic alcohols, of phytol, and of α-amyrin (results of GLC and chromato-mass spectrometry).

Fraction c (weight 0.09 g), according to GLC and CMS, contained a mixture of acetates of aliphatic alcohols, of phytol, α-amyrin, and of lupeol.

Fraction d (weight 0.04 g) consisted of lupeol acetate, mp 212-214°C (from ethanol). According to the literature [6]: 216-218°C. Mass spectrum: 468 (21%), 408 (32%), 393 (16%), 365 (24%), 299 (8%), 297 (7%), 249 (11%), 218 (21%), 203 (40%), 189 (98%). PMR spectrum, δ, 0.78 (3 H, s, C-28), 0.84 (9 H, s, C-23, 24, 25); 0.94 (3 H, s, C-27); 105 (3 H, s, C-26); 1.68 (3 H, s, C-30); 2.04 (3 H, s, C-3β-OAc); 4.57 (1 H, m, C-29); 4.68 (1 H, m, C-29); 4.48 (1 H, m, C-3α-H). The spectra coincided with those given in the literature [2, 6].

Fraction e (weight 0.09 g) consisted of a mixture of the acetates of 24-methylenecycloartanol and of lupeol, and trace amounts of unidentified components.

β-Sitoterol. This was isolated by the chromatography of fractions 5 and 6, mp 136-137°C (from hexane), mp according to the literature [7] 137°C. The results of PMR and mass spectrometry confirmed the correctness of the identification [2, 8].

Chromatography of the Acetates of the Alcohols of Fractions 7 and 8. Fractions 7 and 8 were acetylated and the mixture of acetates was chromatographed on a column of silica gel impregnated with silver nitrate. On elution with hexane containing from 1.5 to 5% of diethyl ether, β-sitosterol acetate was obtained with mp 126-128°C (from hexane), and further elution gave two more fractions.

The first of these fractions (weighing 0.04 g) consisted of the acetate of "nordiene I" with mp 176-181°C from ethanol, mp according to the literature [9], 179-181°C. PMR spectrum, δ, 0.86, 0.87, (6 H, d, C-23, 24); 0.89 (3 H, s, C-25); 0.94 (9 H, s, C-26, 29, 30); 1.12 (3 H, s, C-27); 2.04 (3 H, s, C-3β-OAc); 4.49 (1 H, m, C-3α-H); 5.29 (1 H, m, C-12). Mass spectrum: 452 (77%), 392 (18%), 202 (64%), 190 (44%), 189 (53%), 133 (16%), 132 (18%).

The second fraction (weighing 0.04 g) consisted of the acetate of "nordiene II," with mp 189-192°C (from ethanol). PMR spectrum, δ, 0.80, 0.84 (3 H, d, C-30); 0.86, 0.87 (9 H, s, C-23, 24, 25); 0.93, 0.97 (3 H, d, C-29); 0.98 (3 H, s, C-26); 1.07 (3 H, s, C-27); 2.04 (3 H, s, C-3β-OAc); 4.49 (1 H, m, C-3α-H); 5.30 (1 H, m, C-12). Mass spectrum: 452 (69%), 392 (12%), 202 (67%), 189 (56%), 133 (20%), 132 (29%) [10].

Triterpenediols. The mass spectra of the diols were obtained in the CMS analysis of fractions 9-11 (see Table 1). Erythrodiol – mass spectrum: 442 (M^+) (2%), 424 (3%), 234 (15%), 207 (11%), 203 (100%), 133 (14%). Uvaol – mass spectrum: 442 (M^+) (2%), 424 (4%), 234 (12%), 207 (15%), 203 (100%), 133 (29%) [2].

Reduction of the Aldehydes. A mixture of aldehyde isolated by the additional chromatography of fractions 5 and 6 on a volumn of type L silica gel (Czechoslovakia), in an amount of 0.30 g, was dissolved in 2 ml of ethanol, and the solution was treated with 0.10 g of sodium tetrahydroborate and stirred at 10°C for 1 h. After the end of the reaction, the mixture was evaporated and the residue was deposited on a column of silica gel. Elution with hexane with the addition of from 25 to 50% diethyl ether gave 0.26 g of a mixture of erythrodiol and uvaol (GLC and chromato-mass spectrometry).

Citrostadienol Acetate. The residue of fractions 5 and 6 after the separation of the aldehyde was acetylated, and the acetates were deposited on a column of silica gel impregnated with silver nitrate. On elution with hexane containing from 3 to 5% of diethyl ether, 0.05 g of citrostadienol acetate was obtained with mp 140-147°C (from hexane). According to the literature [11]: mp 147-149°C. The mass spectrum and PMR spectrum coincided with those given in the literature.

SUMMARY

The composition of the neutral fraction of the products of the saponification of an extract of Hippophaë rhamnoides L. leaves has been investigated. Fourteen triterpene compounds have been identified from GLC, PMR, and chromato-mass spectrometric results.

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