by sodium tetrahydroborate, performed by the method of Chanley et al. [5], led to a compound identical with the holothurinogenin (2).

SUMMARY

A new triterpene oligoside, holothurin B_1, has been isolated from the holothurin *H. floridana*. It has been shown that the native aglycone of holothurin B_1 has the structure of holosta-9(11)-ene)3β,12α,17α-triol (2). Two new holostane derivatives have been isolated and characterized — holosta-8,11-diene-3β,17α-diol, and 3β,17α-dihydroxyholost-9(11)-en-12-one. A scheme of the transformation of the holothurinogenin (2) under the conditions of acid hydrolysis has been put forward.

LITERATURE CITED

1. V. A. Stonik, A. D. Chumak, V. V. Isakov, N. I. Belogortseva, V. Ya. Chirva, and G. B. Elyakov, Khim. Prir. Soedin., 522 (1979).
2. I. Kitagawa, T. Nishino, T. Matsuno, H. Akutsu, and Y. Kyogoku, Tetrahedron Lett., No. 11, 985 (1978).
3. G. Habermehl and G. Volkwein, Ann. Chem., 731, 53 (1970).
4. L. Dorfman, Chem. Rev., 53, 47 (1953).
5. J. D. Chanley, T. Mezzetti, and H. Sobotka, Tetrahedron, 22, 1857 (1966).
6. I. Kitagawa, T. Nishino, and Y. Kuogoku, Tetrahedron Lett. No. 16, 191 (1979).
7. G. F. H. Green, J. E. Page, and S. E. Staniforth, J. Chem. Soc., (B), 807 (1966).
8. J. D. Chanley and C. Rossi, Tetrahedron, 25, 1911 (1969).
9. J. D. Chanley and C. Rossi, Tetrahedron, 25, 1897 (1969).
10. W. Auf der Heyde and W. Lutke, Chem. Ber., 111, 2384 (1978).
11. S. A. Knight, Org. Magn. Reson., 6, 603 (1974).
12. I. Rothberg, B. M. Tursch, and C. Djerassi, J. Org. Chem., 98, 209 (1973).

CHEMICAL INVESTIGATION OF *Hippophaë rhamnoides*.

I. THE MAIN COMPONENTS OF THE UNSAPONIFIABLE PART OF AN EXTRACT OF THE FRUIT PULP

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It has been established that the sterol fraction of the unsaponifiable part of a pentane extract of the fruit pulp of common sea buckthorn contains not only sterol but also triterpene alcohols and higher fatty alcohols. β-Sitosterol, 24-methylenecycloartanol, citrostadienol, and uvaol have been isolated from it. β- and α-Amyrins, 24-ethylcholest-7-en-3β-ol and erythrodiol have also been identified in it by chromato-mass spectrometry.

The high value of common sea buckthorn as a natural polyvitamin concentrate and the source of an oil possessing a broad pharmacological action spectrum [1] has served as the basis for a far-ranging chemical investigation of this plant with the aim of finding the biologically active components in it.

In a chemical and pharmacological investigation of common sea buckthorn it was observed that its biological activity is determined by the unsaponifiable fraction, the most active part of which is the "sterols." The main component of the "sterol" fraction proved to be β-sitosterol [2]. A preliminary attempt to investigate the "sterol" fraction of pharmacopoeial sea-buckthorn oil recently made with the aid of GLC and TLC led to the identification of one more component — stigmasterol [3].

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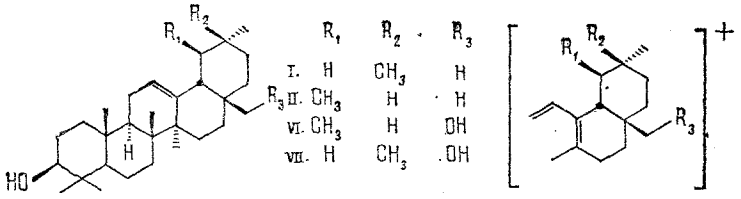
We have investigated the "sterol" fraction with the aid of column chromatography on silica gel, GLC, and chromato-mass spectrometry. The unsaponifiable residue obtained from a pentane extract of the fruit pulp is according to TLC, a mixture of previously studied carotenoids and tocopherols [3, 4] and also of "sterols," which, on a chromatogram after its treatment with sulfuric acid, gives several overlapping bright orange spots. The combined "sterols" were separated by column chromatography into seven fractions (1-7).

According to GLC, fractions 1 and 2 had the same qualitative composition and consisted of two groups of compounds differing appreciably in their relative retention times (RRTs). The first consisted of a mixture of fatty alcohols (RRT in the interval from 0.05 to 0.66; here and below the RTTs are given relative to β-sitosterol), among which the C_{18}, C_{20}, C_{24}, and C_{26} primary alcohols of normal structure predominated. They were identified by GLC with the addition of authentic samples. The mass spectra of these compounds correspond to the nature of the fragmentation in the spectra of analogous higher fatty alcohols [5].

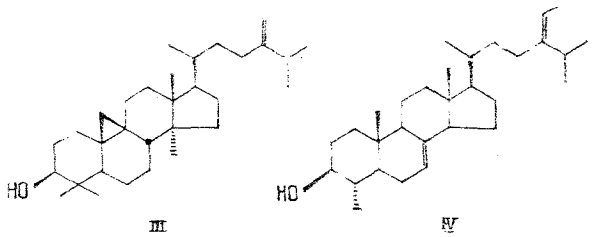
The second group of compounds, the amount of which was greater in fraction 2, appeared on GLC in the form of three peaks with RRTs of 1.07, 1.16, and 1.32. The first two peaks coincided with the peaks of a mixture of β- and α-amyrins which we used as a standard, that with the smaller RRT (1.07) corresponding to β-amyrin (I) and that with the larger (1.16) to α-amyrin(II). According to the literature [6], this is the order in which they issue in GLC from a column with the stationary phase SE-30 which we also used in our work. The fact that the peaks with RRTs of 1.07 and 1.16 corresponded to β- and α-amyrins was also confirmed by the results of chromato-mass spectrometry. The mass spectra of these compounds were similar to one another, containing the peaks of the molecular ion (m/z 426) corresponding the the same formula $C_{30}H_{50}O$. For olean-12-ene and urs-12-ene derivatives, to which β- and α-amyrins respectively belong, fragmentation with the formation of ion A [7] (reverse Diels—Alder reaction) having a mass of 203 for both amyrins is very characteristic. In actual fact, this ion appeared in the mass spectrum of the substances with RRTs of 1.07 and 1.16, and, just as in the mass spectra of other compounds of this type, it was one of the main ions. When fractions 1 and 2 were subjected to TLC, they each gave two overlapping spots, that with the larger R_f value coinciding with that of a mixture of β- and α-amyrins used as marker. The spot with the smaller R_f value corresponded to a substance with a RRT of 1.32.

Thus, on the basis of the results of GLC, TLC, and mass spectra it can be stated that the peaks with RRTs of 1.07 and 1.16 belonged to β- and α-amyrins respectively.



A substance corresponding to a peak with an RRT of 1.32 was isolated after the additional chromatography of fraction 2 on silica gel. Its melting point and PMR spectra practically coincided with those for 24-methylenecycloartanol (III) [8]. This identification was confirmed by the mass spectrum, which shows the peaks of ions with m/z 440 (M^+, $C_{31}H_{52}O$) (4%), 425 (9%) $(M - CH_3)^+$, 422 (11%), $(M - H_2O)^+$, 407 (18%), $(M - H_2O - CH_3)^+$, 315 (4%) (M − side chain)$^+$, and 300 (12%) (M − side chain − CH_3)$^+$. In the nature of its fragmentation, this spectrum is similar to that of the acetate of compound (III) which has been given by Itoh et al. [9].

According to GLC, fraction 3, like 1 and 2, contained two groups of substances. The first group included the same aliphatic alcohols as the preceding fractions, but their relative amount was now considerably smaller (see the Experimental part). The second group was represented on a chromatogram by four main peaks with RRTs of 0.95, 1.07, ∿1.16, and 1.32. The first peak corresponded to an unidentified triterpene alcohol (m/z 326, $C_{30}H_{50}O$), and the second and fourth to β-amyrin and to 24-methylenecycloartanol (confirmed by chromato-mass spectrometry). As was established by the method of fragmentography [10], the unsymmetrical peak with an RRT of ∿1.16 corresponded to two substances difficult to separate by GLC. One of them was α-amyrin and the other an unidentified homotriterpene isomeric with 24-methylenecycloartanol (m/z 440, $C_{31}H_{52}O$).

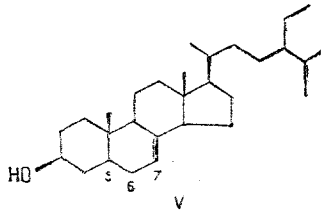


III IV

Fraction 4 proved to be a complex mixture. By rechromatography it was possible to isolate its two main components, one of which was identified from its melting point, mass spectrum, and a GLC comparison with an authentic sample as β-sitosterol (RRT 1.00). The second component (RRT 1.32, m/z 426) was identified as citrostadienol (IV) (4α-methylstigmast-7, 24(28)Z-dien-3β-ol) on the basis of its PMR spectrum, coinciding with that given in the literature [11]. The mass-spectrometric fragmentation of this component is in agreement with the known laws for the decomposition of steroid 7,24(28)-dienes [12].

In addition to β-sitosterol and citrostadienol, fraction 4 contained at least another four, unidentified, compound.

Fraction 5 consisted mainly of β-sitosterol with an admixture of other sterols, for one of which (RRT 1.14) it was possible to obtain a fairly good mass spectrum. The high intensity of the peak of the molecular ion (m/z 414, 45%) and the low intensities of the $(M - H_2O)^+$ (9%) and $(M - H_2O - CH_3)^+$ (6%) ions permitted the assumption that the compound under investigation was a Δ^7-sterol [12]. The intensities of the lines in its mass spectrum were close to the intensities of the corresponding lines in that of the acetate of 24-ethylcholest-7-en-3β-ol described in the literature [12]. On this basis, we assumed that the sterol with an RRT of 1.14 was 24-ethylcholest-7-en-3β-ol (V). The greater retention time in GLC for this compound than for its Δ^5 isomer — β-sitosterol — is in harmony with known facts concerning the relative retention times of isomeric Δ^5 and Δ^7 sterols [12].



V

It was impossible to determine the percentage amount of compound (V) in fraction 5, because of the low efficiency of the packed column that was used for the quantitative evaluation of the fractions in comparison with the capillary column used in the chromato-mass spectrometer.

Fraction 6 contained four compounds, with RRTs of 1.00, 1.14, 1.98, and 2.17. The first two were β-sitosterol and the sterol (V), and, as was found, the two others were the main components in the last, seventh, fraction. The substance with an RRT of 2.17 was isolated after the rechromatography of fraction 6 and was identified by a comparison with an authentic sample by TLC GLC, and melting point, as uvaol (VI).

The component with RRT 1.98 could not be isolated in the pure form. On the basis of the coincidence of its mass spectrum with that described in the literature [13], it was identified as erythrodiol (VII).

The quantitative analysis and mass-spectrometric identification of the components of the fractions showed that the "sterol" part of the unsaponifiable residue consisted of fatty alcohols (35%) and a mixture of sterols and triterpene alcohols (65%). The latter contained β-sitosterol (40%), 24-methylenecycloartanol (29%), β-amyrin (7%), α-amyrin (6%), erythrodiol (5%), uvaol (5%), citrostadienol (1%), 24-ethylcholest-7-en-3β-ol (1%), and an unidentified compound (6%).

We were unable to detect appreciable amounts of stigmasterol. Its detection by Koshelev et al. [3] can be explained by the fact that they studied pharmacopoeial sea buckthorn oil in the form of a solution of the components of the fruit pulp of the sea buckthorn in sunflowerseed oil. As is well known [14], the latter contains a large amount of stigmasterol.

TABLE 1. Composition of the "Sterol" Fractions Isolated from the Unsaponifiable Fraction of a Pentane Extract of Common Sea Buckthorn Fruit Pulp

Number of the fraction and its weight, %	Amounts of the components, % (according to GLC)														
	fatty alcohols						unsaturated polycyclic alcohols								
	C_{18}	C_{20}	C_{22}	C_{24}	C_{26}	other	β-sitosterol	I	II	III	IV	V	VI	VII	other
1 1,09	4	2	4	27	37	2		3	3	15					
2 2,90	3	2	2	25	27	1		5	6	27					
3 3,20		2	2	20	17			11	7	36					5
4 1,18							84				6				9
5 1,57							97								3
6 0,57							72					14	3	9	3
7 0 80							4					2	46	41	7

EXPERIMENTAL

Melting points were determined on a Kofler block. The PMR spectra were recorded on a XL-200 instrument (200 MHz) for solutions in deuterochloroform with tetramethylsilane as internal standard. High-resolution mass spectra were recorded (for individual compounds) on a MS-902 instrument at energies of the ionizing electrons of 12 and 70 eV. The quantitative analysis of the fractions was carried out by GLC using the method of internal normalization from the areas of the peaks on a Chrom-4 instrument (Czechoslovakia) with a glass column, 250 × 0.3 cm, containing 5% of SE-30 on Chromaton N-AW DMCS, column temperature 260°C, temperature of the evaporator 300°C, carrier gas nitrogen. To identify the compounds through their retention times, we also used a column with the stationary phase Lukopren G-1000 (5%) deposited on Chromaton N-AW, glass column, 250 × 0.3 cm, column temperature 250°C, evaporator temperature 300°C, carrier gas, nitrogen.

The chromato-mass spectrometric analysis and the identification of the components in the mixtures were performed on a LKB-2091 instrument with a 2130 data-processing system. Glass capillary column 35 m long with an internal diameter of 0.25 mm, stationary phase SE-30, temperature of the column 270-300°C, of the evaporator 300-330°C, and of the separator 300°C; carrier gas helium. Energy of the ionizing electrons 70 eV. During the time of issuance of the separated components of the fractions being analyzed, a sequence of 10 spectra was fed into the computer at each moment. The heaviest (molecular) ion in the mass spectrum corresponding to each chromatographic peak was determined by the fragmentographic method [10].

Before the recording of the GLCs, the fractions under investigation were freed from contaminating colored substances by passage through a column of silica gel impregnated with silver nitrate (5%), the eluent being petroleum ether–diethyl ether (1:1).

For column chromatography we used air-dry type KSK silica gel with a grain size of 0.140-0.315 mm. The eluent was petroleum ether with increasing concentrations (from 0 to 20%) of diethyl ether. Silufol plates were used for TLC.

The material investigated was industrial common sea buckthorn fruit pulp from the Biisk vitamin factory.

Preparation of the Unsaponifiable Residue. The sea buckthorn fruit pulp was extracted with pentane in a Soxhlet apparatus. The yield of extract, which had the form of a mobile red-brown oil, was 28%. A solution of 390 g of this extract in 2200 g of 10% ethanolic potassium hydroxide solution was boiled under reflux for 1.5 h. From the mixture so obtained, 0.7 liter of ethanol was distilled off in a rotary evaporator, and the residue was diluted with three liters of distilled water and was extracted with diethyl ether (6 × 0.5 liter). The combined ethereal extracts were washed with saturated aqueous sodium chloride solution and were dried with anhydrous sodium sulfate. After the solvent had been distilled off, 16.36 g of unsaponifiable residue was obtained (yield 4.2% on the initial pulp extract).

Separation of the "Sterol" Fraction. The chromatography of 16.36 g of unsaponifiable residue on a column (350 × 65 mm) of silica gel yielded successively 2.04 g of a white waxy substance (mixture of unidentified hydrocarbons), 1.70 g of a solid orange substance (mixture of carotenoids), and 1.19 g of a yellow substance (mixture of tocopherols). Further chromatography using mixtures of petroleum ether and diethyl ether with the concentration of the

latter rising from 10 to 20% led to the successive elution of seven fractions corresponding to the total "sterol" fraction. The yield of the fractions and their compositions are given in Table 1.

24-Methylenecycloartanol (III). This was isolated by the chromatography of fraction 2 on a column (1800 × 18 mm) of silica gel. It had the form of white crystals with mp 121-122.5°C (from acetonitrile); according to the literature [8]: mp 121-122.5°C.

Citrostadienol (IV). This was isolated, together with β-sitosterol, after the chromatography of fraction 4 on a column (1800 × 18 mm) of silica gel. White crystals (from acetonitrile) with mp 165-167°C; according to the literature [11]: 166-167°C, 165°C, 162-164°C (depending on the source of isolation).

24-Ethylcholest-7-en-3β-ol (V). Mass spectrum (m/z) 414 (M^+, 45%), 399 (15%), 396 (9%), 381 (6%), 329 (3%), 275 (3%), 273 (16%), 255 (52%), 246 (9%), 231 (22%), 229 (20%), 213 (25%).

Uvaol (VI). After recrystallization from ethanol, it had mp 231-232°C; according to the literature [15]: mp 232°C.

SUMMARY

1. The composition of the "sterol" fraction of the unsaponifiable part of an extract of common sea buckthorn fruit pulp has been investigated.

2. It has been established that the "sterol" fraction contains as the main components fatty alcohols, β-sitosterol, and 24-methylenecycloartanol, and also β- and α-amyrins, erythrodiol, uvaol, citrostadienol, and 24-ethylcholest-7-en-3-β-ol.

LITERATURE CITED

1. M. D. Mashkovskii, Drugs [in Russian], Part I (1977), p. 515.
2. V. N. Chukaeva, A. I. Gurvich, M. T. Ushakova, A. Z. Efimov, and T. Ya. Apokatonodi, in: Proceedings of a Conference on Vitamins from Natural Raw Material, September 4-5, 1963 [in Russian], Kuibyshev (1964), p. 172.
3. Yu. A. Koshelev, L. D. Ageeva, and Z. G. Grebtsova, in: Vitamin Plant Resources and Their Utilization [in Russian], Moscow (1977), p. 304.
4. B. G. Savinov and L. D. Protsenko, Ukr. Khim. Zh., 20, 399 (1954); T. G. Zhmyrko, E. I. Gigienova, L. D. Protsenko, and A. U. Umarov, Khim. Prir. Soedin., 313 (1978).
5. H. Budzikiewicz, C. Djerassi, and D. Williams, Interpretation of Mass Spectra of Organic Compounds, Holden-Day, San Francisco (1964).
6. N. Ikekawa, S. Natori, H. Itokawa, S. Toninaga, and M. N. Matsui. Chem. Pharm. Bull., 13, 316 (1965).
7. H. Budzikiewica, J. H. Wilson, and C. Djerassi, J. Am. Chem. Soc., 85, 3688 (1963).
8. T. J. Halsall and J. A. Troke, J. Chem. Soc., Perkin Trans. I, 1758 (1963).
9. T. Itoh, T. Tamura, and T. Matsumoto, Phytochemistry, 16, 1723 (1977).
10. R. A. Khmel'nitskii, I. M. Lukashenko, and E. S. Brodskii, The Pyrolytic Mass Spectroscopy of High-Molecular-Weight Compounds [in Russian], Moscow (1980), p. 66.
11. J. S. Pyrek and A. Schmigt-Szolowska, Rocz. Chem., 51, 951 (1967).
12. B. A. Knigts, J. Gas Chrom., 5, 273 (1967).
13. S. R. Heller and G. W. A. Milne, EPA-NIH Mass-Spectral Data Base, National Bureau of Standards, Washington (1979).
14. T. Milkova, N. Msrekov, S. Popov, N. Wulfson, and I. Bogdanova, Die Nahrung, 12, 1 (1977).
15. T. K. Devon and A. I. Scott, Handbook of Naturally Occurring Compounds, Vol. 2, Academic Press, New York (1962), p. 326.