When the oil of *N. cataria* (17 g) was chromatographed on a column (22 × 0.5 cm) of $Al_2O_3$ (neutral, "Chemapol"), hexane (720 ml) eluted the TGs, diethyl ether (700 ml) eluted the DGs together with the free fatty acids and β-sitosterol, and chloroform (300 ml) eluted the MGs. The DGs were purified as described previously [5].


## SUMMARY

In a study of the position-species and fatty-acid compositions of the glyceride fractions of the oils of the seeds of *N. cataria* and *E. moluccelloides*, desorbed from silica gel and from neutral $Al_2O_3$, it was found that on $Al_2O_3$ isomerization of the TGs takes place with a change in their fatty acid composition consisting in the migration of acyl groups from positions 1 and 3 to position 2 both within the molecule and between the molecules of the TGs.

These properties exclude the use of this sorbent for the isolation and purification of the glyceride fractions with the aim of establishing their molecular structure.


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# VITAMINS OF THE OIL OF THE FRUIT OF *Hippophaë rhamnoides*


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The fat-soluble vitamins of *Hippophaë rhamnoides* L., which belongs to the family Elaeagnaceae, have been studied inadequately [1-5]. On the one hand, this is due to their extreme instability to oxidation during the isolation and storage of the oil and juice of the fruit and, on the other hand, to the fact that the oil of common sea buckthorn fruit has been of great interest to botanists, pharmacists, and technologists. The composition of the oils of the juice and the seeds have not been studied separately.

The causes of the high biological activity of the oil from the fruit of the sea buckthorn have not been elucidated [3]. The wide use of this oil [6, 7] in medicine and the shortage of it make it necessary to study its chemical composition in detail in order to search for or create an adequate substitute.

We have studied the oil of the juice and the oil for the seeds of the fruit of the plant collected at the end of August—beginning of September in the valley of the R. Paltau (Chatkal range).

The oil of the juice was isolated from the juice of the fruit by the method of Bligh and Dyer. The amount of oil was 7.4% of the weight of the juice, its refractive index $n_D^{22}$ 1.4646, and its acid No. 14.19 mg of KOH/g.

The seed oil consisted of the sum of the neutral lipids isolated from the seeds by hexane. The amount of oil was 11.8% of the weight of the absolutely dry seeds, $n_D^{22}$ 1.4740, acid No. 23.23 mg of KOH/g.


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Provitamins of the A Group (Carotenoids). The amount of carotenoids in the oil of the juice changed according to the conditions and the time of storage of the oil of the juice or of the fruit.

When the juice was stored (in the dark at 5°C) for 2 months, the amount of carotenoids in the oil isolated from it was 1.089 mg-%; for 3 months, 374; and for 4 months 298 mg-%.

The oil isolated directly from the seeds contained only 48 mg-% of carotenoids.

In order to determine how the carotenoids of the oil of the juice were preserved on storage in the fruit, we kept fruit in a place protected from the light at room temperature for 3 months in the air-dry state (5.6% moisture content). Then they were comminuted in an electric mill. The oil was extracted from the mass obtained with hexane. A mixture of the oil of the juice and the oil of the seeds was obtained in which the amount of carotenoids was 200 mg-%.

The amount of this mixture in the partially dried fruit was 23.7% on the absolutely dry weight. Consequently, in the partially dry juice (amount of oil in the seeds 11.8%) there was 11.9% of oil on the absolutely dry weight.

Now it is possible to calculate the total amount of carotenoids in the oil of the juice. Since 200 mg-% is the mean for the mixture of the two oils in equal amounts, and there is 48 mg-% of carotenoids in the seed oil, the oil of the juice contains $200 \cdot 2 - 48$, i.e., 350 mg-% of total carotenoids. Thus, the carotenoids are mainly concentrated in the oil of the juice.

In the natural material — fruit — the amount of carotenoids in the oil of the juice was considerably higher (1089 mg-%) than is known from literature sources (about 300 mg-%). When the oil was stored in the juice or the fruit, the amount of carotenoids fell more than threefold.

The total carotenoids in the oil of the juice were separated by column chromatography on magnesia with benzene containing 1% of methanol as described by B. G. Savinov [8]. Two carotenoid zones were obtained. Each zone was eluted. The volumes of the eluates in which carotenoids were found were determined, and they were identified from their absorption in the ultraviolet. In this way we found 49.81% (of the total carotenoids) of β-carotene and 50.19% of xanthophylls, which agrees with B. G. Savinov's results.

Without the addition of methanol to the eluent, six colored zones were distinctly separated on the same adsorbent. Because of the low amount of carotenoids, zone III was not identified. The composition of the carotenoids of the oil of the juice was as follows (% of total):

| Zone | Carotenoid ($\lambda_{max}$, nm; benzene) | Storage of the oil in the juice for 2 months | 3 months |
|---|---|---|---|
| I | β-Carotene (437, 462, 489) | 61.29 | 47.52 |
| II | 3,3'-Dihydroxy-β-carotene (zeaxanthin) (440, 463, 492) | 23.84 | 17.21 |
| III | Not identified | Tr. | Tr. |
| IV | 3,3'-Dihydroxy-retro-carotene (458, 485, 516) | 7.27 | 6.99 |
| V | 4,4'-Dioxo-β-carotene (canthaxanthin) (485) | 5.00 | 13.98 |
| VI | A dihydroxy-β-carotene (391, 411, 438) | 2.60 | 14.30 |

The main component of the carotenoids was β-carotene. After 3 months the ratio of the individual components in the oil of the juice had changed. A decrease in the amount of β-carotene and zeaxanthin was accompanied by an increase in the amount of canthaxanthin and of the dihydroxy-β-carotene of unknown structure. But, when the oil of the juice was stored the amount of β-carotene fell through a decrease both in the total number of carotenoids and of the relative amount of β-carotene in the combined carotenoids.

The component present in the second highest amount is zeaxanthin. Its presence in the fruit was not unexpected, since it has been found in them previously [8] in the bound state — in the form of zeaxanthin dipalmitate (physalin).

Vitamins of the E Group (Tocopherols). The total tocopherol content in the freshly isolated oil of the juice was 300 mg-%. After 6 months, it had fallen to 207 mg-%. The seed oil contained 227 mg-% of tocopherols. The combined E-group vitamins of the oil of the juice were separated by thin-layer chromatography and were identified as α-tocopherol (65% of the total tocopherols), δ-tocopherol (35%), and γ-tocopherol (traces). According to the results of gas–liquid chromatography [3], "sea buckthorn oil" (without the juice or the seeds being specified) was found previously to contain 12% of γ-tocopherol.

Vitamins of the F Group (Higher Unsaturated Fatty Acids). To determine the amount of unsaturated fatty acids present in the oil of the juice and the seed oil in the free and bound (ester) states, both oils were hydrolyzed by the cold saponification method. The fatty acids isolated were esterified with diazomethane, and the combined acyl esters of the fatty acids were separated by gas–liquid chromatography (GLC) under the conditions described previously [10]. From the GLC results we determined the fatty-acid compositions of the oil of the juice (wt.%: $C_{14:0}$, 0.39; $C_{16:0}$, 35.13; $C_{16:1}$, 52.96; $C_{18:0}$, 0.86; $C_{18:1}$, 9.71; $C_{18:2}$, 0.76; $C_{18:3}$, 0.19) and of the seed oil (wt.%: $C_{14:0}$, 0.15; $C_{16:0}$, 10.63; $C_{16:1}$, 5.88; $C_{18:0}$, 1.49; $C_{18:1}$, 26.64; $C_{18:2}$, 34.05; $C_{18:3}$, 21.16). As can be seen, only the seed oil contained essential fatty acids of the vitamin F group ($C_{18:2}$ and $C_{18:3}$ about 60% of the total fatty acids).

The oil of the juice is also distinguished from the seed oil by a high content of a component with a relative retention time (RRT) corresponding to the RRT of the $C_{16:1}$ acid, which is unusual for natural oils. These results were confirmed by paper chromatography (PC): the $R_f$ values of the $T_{16:1}$ acid and of a sample of myristic acid, which forms a "critical pair" with the $C_{16:1}$ acid, coincided. However, practice has shown that the RRT of the $C_{16:1}$ acid coincides with the RRTs of several unidentified components present in mixtures of the methyl esters of fatty acids isolated from other oils. Furthermore, the existence of "critical pairs" in PC also casts doubt on the presence of palmitoleic acid in the oil. In the sources available to us there is no proof of the structure of this acid in the oils studied by chemical methods of analysis.

On this basis, we separated the mixture of methyl esters of the fatty acids of the oil of the juice according to their degree of unsaturation by chromatography on a "silver column" [10]. In this way we isolated four fractions of esters.

By the GLC method we found traces of the $C_{20:0}$ and $C_{22:0}$ acids in the "unsaturateds" fraction.

In the "monoene" fraction we detected esters of $C_{18:1}$ and $C_{16:1}$ acids (according to RRTs, GLC). The IR spectra of the esters of the "monoenes" showed only the cis configuration of the ethylenic bonds.

The results of the oxidative degradation of the "monoenes" with the periodate–permanganate reagent showed that among the monocarboxylic and dicarboxylic fragments (GLC results after treatment of the degradation products with diazomethane) there were nonanecarboxylic, heptanecarboxylic, and nonanedicarboxylic acids

$$\begin{array}{l|l} CH_3(CH_2)_7CH= & CH(CH_2)_7COO^- \\ CH_3(CH_2)_5CH= & CH(CH_2)_7COO^- \\ CH_3(CH_2)_7COOH & HOOC(CH_2)_7COO^- \\ CH_3(CH_2)_5COOH & \end{array}$$

These results confirmed the $\Delta^9$ position of the ethylenic bond in both "monoenes."

Thus, the presence of cis-hexadec-9-enoic acid in the products of the hydrolysis of the oil of the juice has been demonstrated by chemical analysis in combination with physical methods.

The cis,cis-$C_{18:2}^{\Delta^{9,12}}$ (linoleic) and the cis,cis,cis-$C_{18:3}^{\Delta^{9,12,15}}$ (linolenic) acids were identified in trace amounts in the "diene" and "triene" fractions by GLC and IR spectroscopy. The RTTs of the isomer of these acids in relation to the position of the ethylenic bonds would differ from the RTTs of linoleic and linolenic acids.

## EXPERIMENTAL

Isolation of the Oil from the Juice. The lipids were extracted from the fruit juice with a mixture of chloroform and methanol (1:2). The chloroform part of the extract was dried over sodium sulfate. When the chloroform was distilled off in the vacuum of a water pump in a rotary evaporator at 40°C, a dark viscous oil was obtained. The oil was dissolved in hexane, and a voluminous precipitate formed, which was separated off by filtration. The hexane was distilled off and the oil was dried at 40°C in a vacuum of 680 mm Hg for 3 h.

Isolation of the Oil from the Seeds. The seeds were washed with water from residues of pericarp, dried to the air-dry state, and comminuted in an electric mill. The oil was extracted by cold (room temperature) steeping with hexane.

The acid numbers of the oils were determined by the standard procedure [11].

The total amount of carotenoids was determined by the procedure of FS-42-1011-75, which is based on the measurement of the optical density of a solution at a wavelength of 451 nm in comparison with the absorption of a standard solution of potassium dichromate, according to B. G. Savinov. The measurements were carried out on a Hitachi spectrophotometer.

The total amount to tocopherols was determined by the method of V. A. Devyatnin [12] from the capacity of the tocopherols for being oxidized with the formation of colored quinones We used a calibration curve reflecting the intensity of absorption of known amounts of the quinone derivative of a standard sample of α-tocopherol in the ultraviolet at 520 nm.

The tocopherols were identified and their amounts were determined by thin-layer chromatography on silica gel with chlorofrom as the mobile solvent in the presence of the α-, γ-, and δ-tocopherols of soybean oil as markers [13]. The tocopherols were extracted from the corresponding zones of the silica gel, and their amounts in the eluates were determined as described above.

The isolation of the total fatty acids and the determination of their composition, the separation of their esters on a "silver column," and the study of the structure of the acids were carried out by methods described previously [10].

## SUMMARY

A comparative study of the chemical composition of the oil of the juice and the seed oil of the fruit of *Hippophaë rhamnoides* has shown that vitamins of the A group are concentrated mainly in the oil of the juice (more than 1000 mg-%) and are represented mainly by β-carotene and zeaxanthin in a ratio close to 3:1.

The E-group vitamins are uniformly distributed in the oil of the juice and the seed oil (more than 200 mg-%). Of the vitamins of this group, the main representatives detected were α- and δ-tocopherols in a ratio of 2:1.

The vitamins of the F group are concentrated only in the seed oil (~60% of a mixture of linoleic and linolenic acids on the total fatty acids isolated from the oil, in a ratio of 1.5:1).

The position of the ethylenic bond in a $C_{16:1}$ acid isolated from products of the hydrolysis of the oil of the juice in a "monoenic" fraction has been shown by chemical analysis. Its cis configuration was determined by IR spectroscopy, which confirms the structure of this acid as cis-hexadec-9-enoic (palmitoleic) acid. An unusually high content of the $C_{16:1}$ acid is characteristic only for the oil of the juice.

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# THE STRUCTURE OF FETERIN — A NEW TERPENOID COUMARIN FROM *Ferula teterrima*

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The isolation from the roots of *Ferula teterrima* Kar. et Kir. (Umbelliferae) of four terpenoid coumarins — badrakemin acetate, samarcandin acetate, badrakemone, and badrakemin — has been reported by us previously [1]. On further study of the plant, we have isolated a new terpenoid coumarin which we have called feterin, with the composition $C_{26}H_{32}O_6$, mp 155-158°C, $[\alpha]_D^{20}$ −52° (c 1.02; $CHCl_3$), $M^+$ 440.

Feterin has a UV spectrum that is characteristic for umbelliferone derivatives [2]. In the IR spectrum (Fig. 1) there is a broad carbonyl band (1710-1720 $cm^{-1}$) showing the presence of an ester grouping, and the band of an OH group (3545 $cm^{-1}$). The hydroxy group in feterin is secondary, as is confirmed by its capacity for being acetylated under mild conditions, and also by the PMR spectrum (Fig. 2). From the empirical formula of feterin and the presence of one double bond in the terpenoid part (PMR spectrum) it follows that its sesquiterpene fragment has a bicyclic structure. The PMR spectrum of feterin is similar to that of badrakemin, which shows that their structures are similar, but from the multiplicity of the signal of the proton geminal to the hydroxy group it follows that, in contrast to badrakemin, it is equatorial. As also in the spectrum of badrakemin, there are the signals of three methyl groups attached to quarternary carbon atoms, and of >C=$CH_2$ and >CH—$CH_2$—OAr groups. Feterin also differs from badrakemin by the fact that it contains an acetoxy group, as is shown by the corresponding signals of a methyl group and of a proton geminal to an acetoxy group (one-proton sextet with its center at 5.18 ppm).

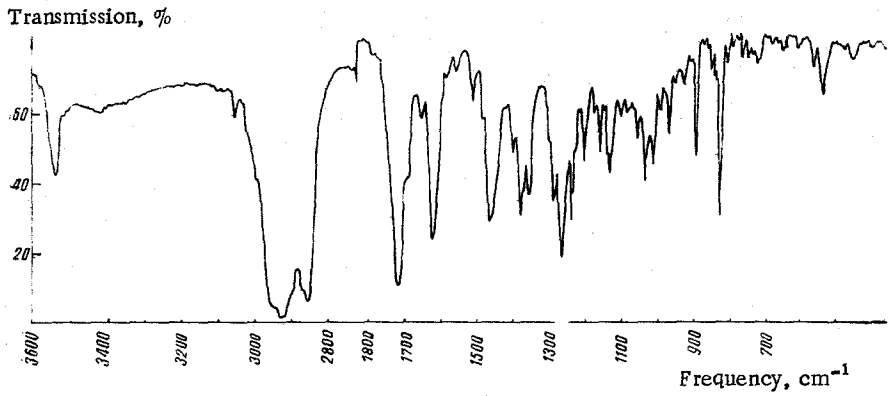


Fig. 1. IR spectrum of feterin (mull in paraffin oil).

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