SUMMARY

1. The structure of the main groups of phospholipids isolated from the seed kernels of the thin-fibered cotton plant of variety S-6029 has been studied.

2. The fatty-acid compositions and the position distributions of the fatty acids have been determined, and from the results the possible molecular compositions of the phosphatidylcholines, phosphatidylethanolamines, and phosphatidylinositols have been calculated.

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THE SEED OIL OF Rindera oblongifolia

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Among the acids present in the glycerides of the seed oils of the family Boraginaceae, attention is being attracted by the simultaneous presence of two isomeric trienoic acids, $18:3^{6,9,12}$ and $18:3^{9,12,15}$, and one tetraenoic acid, $18:4^{6,9,12,15}$ [1-5]. Furthermore, common components of Boraginaceae oils are the 20:0, 20:1, 20:2, 22:0, 22:1, and 24:1 acids [3-5], and in one of them the $18:1^{11}$ isomer of oleic acid has been found [3].

Some workers have not found the C_{20} acid series in the seed oils of a number of species [6], and therefore we decided to study those oils in relation to the fatty-acid composition of which doubt has arisen [7]. The present paper deals with one of them – the seed oil of Rindera oblongifolia. The Rindera seeds (round, ovate nuts with wings) were collected on the southern slopes of the Ugamskii range in 1974. The weight of 1000 seeds was 2.7 g, their size 0.1 × 0.4 × 0.55 mm, and their oil content calculated on the absolutely dry substance 12.14%.

The seeds yielded a light yellow oil with density d_4^{20} 0.9124 g/cm^3, viscosity 11.65° E, n_D^{20} 1.4770, saponification No. 179.54 mg KOH/g, Hehner No. 95.88%, Reichert–Meissl No. 1.37 mg KOH/g, Polenske No. 9.81%, acid No. 4.96 mg KOH/g, iodine No. 151.49%, thiocyanogen No. 91.23%, unsaponifiables content 1.88%, tocopherols 0.3 mg-%, phosphatides 0.39%.

The mixture of fatty acids isolated from the oil by saponification at room temperature had a neutralization No. of 203.28 mg KOH/g, a mean molecular weight of 275.97, an iodine No. of 159.59%, and a thiocyanogen No. of 95.05%, and they contained 8.43% of saturated acids with a neutralization No. of 198.88 mg KOH/g and a mean molecular weight of 282.08.

The mixture of fatty-acid methyl esters obtained by means of diazomethane was analyzed by thin-layer

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chromatography (TLC), paper chromatography (PC), and gas–liquid chromatography (GLC). The TLC method showed the presence in the mixture of five types of acids of which one was saturated and four were unsaturated (monoenoic, dienoic, trienoic, and tetraenoic).

Of the seven zones on a paper chromatogram of the mixture of acids revealed by the reagents for the carboxy group, the slowest showed no unsaturation, which enabled it to be ascribed unambiguously to the 22:0 acid (behenic). The six following zones showed the possibility of the presence of 22:1, 20:1, 18:1, 18:2, 18:3, and 18:4 acids, since they showed unsaturation and migrated in the same way as markers. However, the unambiguous assignment of these zones to the acids mentioned is impossible. Similar zones can also be produced by such "critical pairs" of acids as 24:2, 22:2, 20:2, 20:3, 20:4, etc., which cannot be detected by GLC when their concentration is low because of their long retention times.

The results of a comparison of the PC and GLC of the methyl esters of the mixture of acids enabled four types of unsaturated acids to be ascribed unambiguously to the tetraenoic, trienoic, dienoic, and monoenoic acids of the C_{18} series, and two types of unsaturated acids to the 20:1 and 22:1 monoenoic acids.

The presence of the 22:0 acids was confirmed by the GLC of a mixture of the methyl esters of the saturated acids isolated by Bertram's method supplementing the results obtained above: The 16:0, 18:0, and 20:0 acids were detected, of which the first two produced individual peaks while the peak of the third was masked under the 18:3 peaks of the GLC of the total mixture of acids.

The methyl esters of the combined fatty acids were separated by the CC/$AgNO_3$ method [5] according to their degree of unsaturation into five fractions. In the saturated-acid fraction all the fatty acids detected were determined quantitatively (GLC), and so were traces of three iso acids – 14:0, 15:0, and 17:0. In the monoenoic fraction by GLC, PC, and TLC/$AgNO_3$ we found the 18:1, 20:1, and 22:1 acids in a ratio of 65.09; 9.83; 25.09 mol. %, respectively.

The oxidative degradation of the monomers by periodate-permanganate mixture followed by saponification yielded three dicarboxylic acids – azelaic, undecanedicarboxylic, and brussylic acid (63.16, 12.33, and 24.5 mol. %, respectively; GLC of the methyl esters). The change in the ratio in favor of undecanedicarboxylic acid as compared with that expected from the GLC results shows the presence in the mixture of about 2% of a position isomer of oleic acid, the $18:1^{11}$ isomer.

Thus, in the seed oils studied, of the monoenoic acids the $18:1^{11}$, $18:1^{9}$, $20:1^{11}$, and $22:1^{13}$ compounds are present. The graphical dependence of the logarithm of the retention time on the number of carbon atoms is expressed by a straight line for the last three acids, which shows the similarity of the terminal carbon chain in the structure of these acids, $CH_3(CH_2)_7CH=$, and confirms the results obtained.

To separate the methyl esters of the acids of the monoenoic fraction, column chromatography was repeated and narrower fractions were collected.

Using PC as a control, three fractions of esters were obtained: the first contained the ester of the 22:1 (maximum), 24:1, and 26:1 acids, the second contained those of the 22:1 and 20:1 (maximum) acids, and the third contained those of the 20:1 and 18:1 (maximum) acids.

The results of the oxidative degradation and the GLC of the dicarboxylic acid products of each fraction confirmed the positions of the ethylenic bonds that had been found in the monoenoic acids and, additionally, showed the positions of the double bonds in the tetracosenoic acid ($24:1^{15}$) and the hexacosenoic acid ($26:1^{17}$).

The oxidative degradation of the dienoic fraction showed the presence of only one acid – $18:3^{9,12}$. Among the fragments from the degradation of the trienoic fraction were found azelaic and adipic acids, which confirmed the GLC results on the presence of two isomeric trienoic acids – $18:3^{6,9,12}$ and $18:3^{9,12,15}$. In the products of the degradation of the tetraenoic fraction we found only adipic acid, from the decomposition of the $18:4^{6,9,12,15}$ fatty acid.

The results obtained enabled the composition of the fatty acids of the oil of Rindera oblongifoira to be calculated on the basis of the results of four gas–liquid chromatograms of the methyl esters (the mixture of fatty acids; the total saturated acids; the total monoenoic acids; and mixtures of the dicarboxylic products of the oxidative degradation of the total monoenoic acids) (mol. %): iso-14:0 – traces; iso-15:0 – traces; 16:0 – 6.41; iso-17:0 – traces; 18:0 – 1.47; $18:1^{9}$ – 28.39; $18:1^{11}$ – 2; $18:2^{9,12}$ – 23.97; $18:3^{6,9,12}$ – 2.58; $18:3^{9,12,15}$ – 12.99; $18:4^{6,9,12,15}$ – 7.44; 20:0 – 0.62; $20:1^{11}$ – 3.89; 22:0 – 0.32; $22:1^{13}$ – 9.92; $24:1^{15}$ – traces; and $26:1^{17}$ – traces.

The IR and UV spectra of the mixtures of methyl esters showed no conjugated and trans-ethylenic bonds or hydroxy and epoxy groups. The absence of hydroxy compounds among the fatty acids was also confirmed by the PC of the mixtures of the acids.

EXPERIMENTAL

Oil was extracted from the freshly ground seeds by extraction with light petroleum ether by steeping at room temperature. The miscella obtained was rapidly filtered, and the ether was distilled off under vacuum in a rotary evaporator. The oil (2 g) was saponified with 20 ml of a methanolic solution of 1.74 g of caustic potash with vigorous shaking for 30 min. The methanol was distilled off immediately under vacuum at a water-bath temperature of 40°C, and the residue was highly diluted with water. The unsaponifiables were extracted three times with petroleum ether. The soaps were decomposed under a layer of diethyl ether with 10% sulfuric acid added until the aqueous soap solution had a weak acid reaction. The combined ethereal extracts of the mixture of fatty acids was washed with distilled water, dried with sodium sulfate, and filtered, and the ether was driven off completely under vacuum in a rotary evaporator. All the operations lasted no longer than 3 h, after which the mixture of fatty acids or their methyl esters was rapidly analyzed by the GLC, PC, and TLC/$AgNO_3$ methods.

The methyl esters of the fatty and dicarboxylic acids were obtained by reaction with diazomethane.

The oxidative destruction of the individual fractions of the unsaturated fatty acids was carried out by Rudloff's method [8-11] with some modifications in the ratio of the oxidizing mixture and the weight of the sample.

The separation of mixtures of homologs of methyl esters of fatty mono- and dicarboxylic acids was effected on a UKh-2 chromatograph with a thermal conductivity detector at 203°C. The column (3 m × 4 mm) was filled with Chromaton N-AW-HMDS, impregnated with 15% of Reoplex-400. The pressure of helium at the outlet was 2.4 atm.

The ratio of the methyl esters was calculated in molar percentages [11], and they were identified from their relative retention times [12, 13]. For this purpose we used the linear dependence of the logarithms of this magnitude on the number of carbon atoms in the chains of esters of fatty acids having the same chain length but different numbers of ethylenic bonds or the same number of ethylenic bonds and different chain lengths [12, 13, 16-18].

Paper chromatography [14] in combination with technical analysis [17] was used to separate both the mixtures of free fatty acids and the mixtures of the methyl esters of these acids.

The thin-layer chromatography of the methyl esters of the fatty acids was performed on glass plates (6 × 9 cm) with a layer (270 μm) of KSK silica gel (150 mesh) fixed with 5% of gypsum and containing 20% of $AgNO_3$ in the petroleum ether–benzene (6 : 4 by volume) system.

SUMMARY

The oil of the seeds of Rindera oblongifolia, family Boraginaceae, growing in Central Asia, has been studied for the first time. Among the acids of the triglycerides of the oil five types of monoenoic acid differing by the length of the carbon chain but having the same length of the terminal carbon chain – $18:1^{9}$, $20:1^{11}$, $22:1^{13}$, $24:1^{15}$, and $26:1^{17}$ – have been detected for the first time. This is the first time that the last of these acids has been found in a seed oil of this family.

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THE USE OF THE ACETOLYSIS REACTION FOR THE INVESTIGATION OF NATURAL PHOSPHOLIPIDS

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The dephosphorylation of glycerophosphatides by heating with a mixture of acetic acids and acetic anhydride leads to acetates of the corresponding diglycerides [1-7]. The acetolysis reaction has been used repeatedly for determining glycerol in phospholipids [4, 5] and for studying their molecular composition on the basis of a structural analysis of the diglycerides formed [3-5, 8, 9]. Nutter and Privett [10] have questioned the suitability of this method for determining molecular types of phospholipids, since they found that acetolysis is accompanied by a redistribution of the acyl residues. At the same time, other authors [3, 8] have not observed acyl migration and have used acetolysis to determine the molecular types of the lecithins isolated from egg yolk.

The exhaustive identification of the products of the acetolysis of phospholipids has not hitherto been performed. Such an attempt is described in the present paper. The results obtained have shown that the acetolysis of glycerophosphatides isolated from various mammalian tissues followed by the methanolysis of the acetolysis products is accompanied by the formation of acylals of fatty aldehydes and of some dihydric alcohols, which complicates the interpretation of gas–liquid chromatograms (GLC) and distorts the results of the determination of glycerol.

Since literature information relating to the optimum temperature and time of acetolysis is self-contradictory (see [3-5, 7]), we first studied the quantitative side of this reaction. The total phospholipids isolated from various mammalian tissues was subjected to acetolysis at 150°C [3] for 1-10 h. The acetolysis products were evaporated, the residue was distributed in the chloroform–methanol–water (8 : 4 : 3) system [3], and the completeness of the reaction was judged from the amount of phosphorus passing into the aqueous methanolic layer. The results of the dephosphorylation of natural phospholipids with a mixture of acetic acid and acetic anhydride [1 ml of a mixture of acid and anhydride (2 : 3) to 25 mg of phospholipids] showed that the process is practically complete after 5 h.

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