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SYNTHESIS AND ISOLATION OF MONOESTERS OF SUCROSE AND ARACHIDONIC ACID

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Monoesters of sucrose and arachidonic acid have been synthesized by the transesterification of ethyl arachidonate with unprotected sucrose, and the structural isomers – 6-O-arachidonoylsucrose, 6'-O-arachidonoylsucrose, and 3-O-arachidonoylsucrose – have been separated chromatographically. The positions of the ester bonds in these compounds were determined by the ^{13}C NMR method.

The esters of sucrose and higher fatty acids are finding use in pharmacy, the food industry, cosmetics, and other sectors of the national economy as nonionic surface-active agents [1-4]. The use of these compounds in medicine may also prove to be promising due to their antimicrobial and antitumoral activity [5-8]. The preparation of esters of sucrose and saturated or slightly unsaturated fatty acids or mixtures of higher fatty polyenoic acids has been described [1, 4, 9]. However, it is known that with an increase in the degree of unsaturation of the hydrocarbon moiety the solubility of esters of sucrose in water rises [1], which is important for their use in medicine. Furthermore, interest in the preparation of esters of sucrose and polyunsaturated fatty acids is also due to the fact that these compounds may prove to be more active because of the high biological activity of the free polyunsaturated fatty acids [10].

We have synthesized monoesters of sucrose and arachidonic acid with the subsequent isolation of the individual monoesters.

Arachidonic acid obtained from the lipids forming waste products from the production of insulin [10] was esterified with absolute ethanol in the presence of a catalytic amount of thionyl chloride [11]. To obtain monoesters of sucrose and arachidonic acid, which possess a higher solubility in water than di- and polyesters of sucrose, we used the transesterification of ethyl arachidonate with an eightfold excess of unprotected sucrose in N,N-dimethylformamide in the presence of an alkaline catalyst. Here the different reactivities of the hydroxy groups in the sucrose were taken into account. To separate the resulting monoesters from the unchanged sucrose after the elimination of the dimethylformamide we used extraction with ethyl acetate. The use of ethyl acetate was due to the good solubility of the monoesters in it and also to its rapid separation from the aqueous phase. To free the monoesters from unchanged ethyl arachidonate, the sodium arachidonate formed, and dimethylformamide residues we used adsorption chromatography on silica gel.

The purified reaction product had a band of vibrations at 1730 cm^{-1} in the IR spectrum, which corresponds to an ester bond, and was an individual substance according to TLC on Silu-

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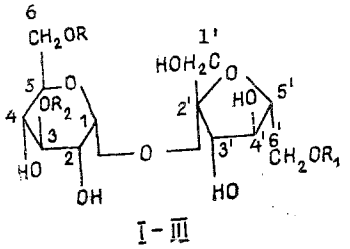
TABLE 1. Chemical Shifts for the Carbon Atoms of the Carbohydrate Moieties of Compounds (I)-(IV) (DMSO-d_6, δ, ppm) and Δδ

Carbon atom	Compound						
	I	Δδ	II	Δδ	III	Δδ	IV
1	91,55	—0,25	91,73	—0,07	91,48	—0,32	91,80
2	71,56	—0,14	71 65	—0,05	69,66	—2,04	71,70
3	72,71	—0,29	72,89[a]	—0,11	75,68	+2,68	73,00[a]
4	70,01[a]	—0,02	70,12	+0.09	67,69	—2,34	70,03
5	70,15[a]	—2,72	72,75[a]	—0,12	72,49	—0,38	72,87[a]
6	63,54	+2,85	60,81	+0,12	60,17	—0,52	60,69
1′	62,56[b]	+0.36	61,80	—0,40	62 20	0.00	62,20[b]
2′	103,95	—0,15	104.26	+0,16	104,14	+0,04	104,10
3′	77,13	—0,18	76,64	—0,67	76,95	—0,36	77,31
4′	74,61	+0,13	74,92	+0,44	74,30	—0.18	74.48
5′	82,72	+0,12	79,17	—3,43	82,67	+0.07	82,60
6′	62,26[b]	—0,01	65.62	+3.35	62,20	—0,07	62,27[b]

Note. a, b — Values marked similarly may change places within a given compound.

fol. However, on Merck plates it separated into three compounds with different mobilities which it was possible to separate chromatographically on fine-grained silica gel by elution under pressure in a gradient of chloroform with rectified alcohol containing increasing concentrations of the latter. These compounds were 6-O-arachidonoylsucrose (I), 6'-O-arachidonoylsucrose (II), and 3-O-arachidonoylsucrose (III), the main reaction products being compounds (I) and (II), while compound (III) was formed in very small amount. Compounds (I) and (II) were obtained in the form of crystals. They consisted of highly hygroscopic substances readily soluble in ethyl acetate, chloroform, and water at room temperature.



I-III

I. R=—CO—CH₂—(CH₂)₂—
—(CH=CH—CH₂)₄—(CH₂)₃—CH₃,
R₁=R₂=H

II. R₁=—CO—CH₂—(CH₂)₂—
—(CH=CH—CH₂)₄—(CH₂)₃—CH₃,
R=R₂=H

III. R₂=—CO—CH₂—(CH₂)₂—
—(CH=CH—CH₂)₄—(CH₂)₃—CH₃,
R=R₁=H

The structures of compounds (I)-(III) were confirmed by the ^{13}C NMR method. For comparison we obtained the ^{13}C NMR spectrum of unsubstituted sucrose (IV), the assignment of the signals in which was made on the basis of a comparison of the spectra which we obtained for this compound under the conditions of monoresonance and of selective $\{^1H\}$—^{13}C heteronuclear double resonance. The assignments obtained correspond to those given in the literature [12, 13].

Table 1 gives the ^{13}C chemical shifts for the compounds studied and their differences for the corresponding carbons of monoesters (I)-(III) relative to (IV) (Δδ). The values given for Δδ permit the conclusion that in compounds (I)-(III) the ester groups are present in position 6, 6', and 3, respectively. In actual fact, it follows from a paper in which the monoacetate esters of galactose and of α-L-rhamnopyranosyl-D-galactose are considered [14] that in comparison with free sucrose the carbon atoms in the α-position to the ester group are descreened by 2.5-4.0 ppm while those in the β-position are screened by 2.0-2.7 ppm. The chemical shifts of the hydrocarbon moiety of compounds (I)-(III) are given in the Experimental section and agree with the shifts of arachidonic acid [10].

EXPERIMENTAL

TLC was performed on Silufol UV 254 using the systems: 1) hexane—ether (20:1), and 2) chloroform—methanol—water (16:4.5:1). For a check by TLC of the separation of the monoesters

of sucrose and arachidonic acid we used Merck plates (Kieselgel 60 F_{254}, Art. 5715) and system 2. On Silufol, the substances were detected by treating the plates with 10% ethanolic molybdophosphoric acid and on the Merck plates by treatment with sulfuric acid followed by heating the chromatograms to 100-120°C. The ^{13}C NMR spectra were obtained on a Bruker WM-250 spectrometer with a working frequency of 62.9 MHz. The signal of the solvent (DMSO-d_6) was used as an internal standard, its chemical shift on the δ scale being taken as 39.6 ppm. The concentration of the solutions studied was 0.05 M. IR spectra were recorded on a Perkin–Elmer 682 instrument in KBr tablets. Specific rotations were determined on an A1-EPO-01 polarimeter.

Preparation of Ethyl Arachidonate. To 2 g (6.5 mmole) of arachidonic acid obtained from the lipid wastes from the production of insulin were added 5.6 ml of absolute ethanol and 0.1 ml (1.4 mmole) of thionyl chloride. The reaction mixture was stirred at room temperature for 4 h, the formation of the ethyl arachidonate being monitored by TLC on Silufol (R_f 0.62, system 1). The reaction mixture was poured into water cooled to 10°C and was extracted with hexane (2 × 50 ml). The solvent was evaporated from the organic layer and the residue was passed through a column containing 10 g of silica gel. After elution with hexane, 1.59 g of ethyl arachidonate was obtained in the form of a colorless liquid (yield 73%).

Preparation of 6-O-Arachidonoylsucrose, 6'-O-Arachidonoylsucrose, and 3-O-Arachidonoylsucrose. At 80°C, 1 g (3.3 mmole) of ethyl arachidonate and 0.03 g (0.66 mmole) of sodium hydroxide were added to a solution of 9 g (26.4 mmole) of sucrose in 30 ml of N,N-dimethylformamide. The reaction mixture was stirred at 100-110°C for 12 h. The course of the formation of the sucrose monoesters and of arachidonic acid was monitored by TLC on Silufol [R_f 0.56 (system 2)]. The dimethylformamide was evaporated in vacuum to dryness, the residue was treated with 100 ml of ethyl acetate, the mixture was stirred at room temperature, and the solvent was decanted off. The residue was dissolved in 25 ml of water and the solution was extracted with ethyl acetate (3 × 100 ml).

The solvent was evaporated off from the combined ethyl acetate extracts, and the residue was deposited on a column 76 cm high containing 80 g of silica gel, mark L with a grain size of 0.04-0.06 mm. The substances were eluted from the column under a pressure of 0.5 atm with a mixture of chloroform and rectified alcohol in the following ratios and amounts: 100:0 – 300 ml; 95:5 – 100 ml; 90:10 – 200 ml; 85:15 – 200 ml; 80:20 – 200 ml; 70:30 – 100 ml. The following were obtained successively: 0.35 g of a mixture of ethyl arachidonate and arachidonic acid, 0.06 g of a mixture of di- and polyesters of arachidonic acid and sucrose, 0.03 g of 3-O-arachidonoylsucrose (III), 0.55 g of 6-O-arachidonoylsucrose (I), 0.25 g of a mixture of compounds (I) and (II), and 0.24 g of 6'-O-arachidonoylsucrose (II). The total yield of monoesters of sucrose and arachidonic acid was 55%. Compounds (I) and (II) were lyophilized with benzene and were crystallized from hexane.

Compound (I): R_f (system 2), $[\alpha]_D^{20}$ +26.7° (c 2.5; chloroform); ^{13}C NMR (hydrocarbon moiety of the molecule); 172.74 (C-1"), 32.86 (C-2"), 24.43 (C-3"), 26.05 (C-4"), 25.20; 25.23 (C-7", C-10", C-13"), 26.62 (C-16"), 28.70 (C-17"), 30.88 (C-18"), 21.95 (C-19"), 13.90 (C-20"), 127.50; 127.67; 127.82; 127.95; 128.11; 128.28; 129.04; 129.93 (C=C).

Compound (II): R_f 0.50 (system 2), $[\alpha]_D^{20}$ +33.9° (c 3.0, chloroform); ^{13}C NMR (hydrocarbon moiety of the molecule); 172.61 (C-1"), 32.86 (C-2"), 24.39 (C-3"), 26.02 (C-4"), 25.19; 25.23 (C-7", C-10", C-13"), 26.61 (C-16"), 28.68 (C-17"), 30.87 (C-18"), 21.94 (C-19"), 13.89 (C-20"), 127.49; 127.66; 127.83; 127.93; 128.10; 128.30; 129.00; 129.93 (C=C).

Compound (III): R_f 0.64 (system 2), $[\alpha]_D^{20}$ +42.2° (c 2.0; chloroform), ^{13}C NMR (hydrocarbon moiety of the molecule): 172.34 (C-1"), 33.44 (C-2"), 24.63 (C-3"), 26.08 (C-4"), 25.20; 25.24 (C-7", C-10", C-13"), 26.63 (C-16"), 28.71 (C-17"), 30.88 (C-18"), 21.95 (C-19"), 13.90 (C-20"), 127.50, 127.67; 127.83; 128.00; 128.11; 128.20; 129.20; 129.94 (C=C).

CONCLUSIONS

Monoesters of sucrose and arachidonic acid have been synthesized. A method is proposed for the separation which enables the individual structural isomers – 6-O-arachidonoylsucrose, 6'-O-arachidonoylsucrose, and 3-O-arachidonoylsucrose – to be obtained. The positions of the ester groups in the compounds obtained have been established by the ^{13}C NMR method.

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COMPOSITION OF COTTONSEED SOAPSTOCK FATS

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The compositions of cottonseed soapstocks from first-grade and low-grade cotton seeds have been studied by the methods of CC on polyamide and on silica gel, TLC, GLC, ESR, and UV, IR, and mass spectrometry. It has been found that saturated, and also oxygenated, fatty acids, nonpolar acylglycerols, glycolipids, sterols, arylalkanes, gossypol pigments, and ions of metals of variable valence are concentrated in the acid fat of the soapstock from low-grade seeds. The soapstock contains a very small amount of tocopherols and phospholipids, mainly phosphatidylinositols. The combined gossypol pigments of the soapstocks include stabilized gossypol radical ions.

Cottonseed oil for food purposes is freed from undesirable impurities by treatment with aqueous solutions of sodium hydroxide. The waste formed in this process – soapstock – consists of a complex mixture of nonfatty substances (mucilages, traces of metals, alkali) and fatty, lipophilic, substances (soap, neutral fat, phospholipids, pigments, and other unsaponifiable substances).

Soapstock fats are a traditional raw material for soap boiling, although the use of cottonseed soapstocks for these purposes is limited because of their dark-brown coloration. Other methods of utilizing soapstocks are possible [1], but the most rational use is being hindered by the inadequacy of information about their chemical composition.

This paper gives the results of the analysis of samples of cottonseed soapstocks obtained in the processing of high-grade seeds (soapstock-1) and seeds of mixed grades (soapstock-2) in the course of the batch refining of the oil in the Tashkent Oil and Fats Combine.

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