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LIPIDS OF COTTONSEED KERNELS

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The free, bound, and strongly bound lipids of the seed kernels of the cotton plant of variety 108-F have been studied. It has been shown that the free and bound lipids include 97.3% and 47.6% of neutral lipids, respectively. The polar lipids consist mainly of phospholipids and glycolipids. Two groups of glycolipids – sterol glycosides and glycosylglycerides – have been isolated and their compositions have been determined.

In order to obtain a food flour from cotton seeds, the kernels are dried to a moisture content of 2-3% [1]. In the drying process, the components of the kernel may change, and therefore for a comparison we first studied the compositions of the free (I), bound (II), and strongly bound (III) lipids of the initial kernels of the seeds of cotton plants of variety 108-F. The amount of (I) obtained by extraction with hexane in a Soxhlet apparatus was 39.7%; that of (II), extracted by Folch's method 6.3% [2], and that of (III) 0.3%. The amount of fraction (III) was judged from the amount of free fatty acids (FFAs) after alkaline hydrolysis [3]. By column chromatography (CC) with silicic acid, (I) and (II) were separated into polar lipids (PLs, yields 2.7 and 52.4%), and nonpolar or neutral lipids (NLs, yields 97.3 and 47.6%, respectively) [4]. The total yield of combined lipids from the column was 98-99%.

These figures indicate that the free lipids consist mainly of NLs, and the bound lipids contain almost equivalent amounts of NLs and PLs. A chloroform–methanol solution of fraction (II) was washed with 0.04% aqueous $CaCl_2$ to eliminate nonlipid components [5] and was then passed through a column; the NLs were eluted with chloroform, and the PLs, by successive elution first with acetone and then with methanol [6], were separated into glycolipids (GLs) and phospholipids (PhLs). The class composition of the NLs was determined by TLC on "Silufol" in solvent systems 1-4, and those of the GLs and PhLs in a thin layer of silica gel in system 5-7 (Table 1). Identification was carried out by specific reagents for individual groups of substances and from the R_f values, which corresponded to the rates of migration of known compounds.

In the neutral lipids of fractions (I) and (II) we detected carbohydrates, sterol esters, triacylglycerols (TAGs), FFAs, epoxyacylglycerols diacylglycerols (1,2- and 1,3-DAGs), oxyacylglycerols (OAGs), and traces of PhLs. The main component of fraction (I) consists of TAGs.

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TABLE 1. Compositions of the Glycosides and Phospholipids Isolated from the Bound Lipids

Spot No.	Rf in system 5	Rf in system 7	Identification	Visualizing reagent: iodine vapor	α-naphthol	Vaskovsky's reagent	ninhydrin	Dragendorff's reagent	perchloric acid
			Glycolipids						
1	0.91	0.90	A sterol glycoside ether	+	+	—	—	—	+
2	0,82	0,78	Monogalactosyl diglycerides (MGDGs)	+	+	—	—	—	—
3	0,72	0,53	A sterol glycoside	+	+	—	—	—	—
4	0,58	0,27	Digalactosyldiglycerides (DGDGs)	+	+	—	—	—	—
5	0,08	0,06	Unidentified GLs	+	+	—	—	—	—
6	0,03	0,02		+	+	—	—	—	—
			Phospholipids						
7	0,11	—	Lysophosphatidylcholines (LPGs)	+	—	+	—	+	—
8	0,30	—	Phosphatidylcholines + phosphatidylinositols (PGs + PIs)	+	—	+	—	+	—
9	0,52	—	Phosphatidylethanolamines (PEs)	—	—	+	+	—	—
10	0,85	—	Unidentified PLs	+	—	+	+	—	—

*The phosphatidylcholines and phosphatidylinositols were separated in system 7.

The glycolipids were separated by preparative TLC on silica gel in system 5, and the fractions obtained were subjected to acid hydrolysis [7]. A spot with R_f 0.72 in system 5, identified as a sterol glycoside, had the most intense coloration. Because of their small amount, the GLs with R_f 0.03 and 0.08 were analyzed together, and so were the MGDGs and DGDGs. The products of the hydrolysis of the sterol glycoside were galactose and β-sitosterol, and those of the MGDGs and DGDGs were galactose and FAs. A hydrolysate of the GLs with R_f 0.03 and 0.08 contained traces of mannose and also FAs, in addition to galactose and glucose. The ratio of galactose and glucose was 1:1.3. The presence of various sugar residues in the GLs has been reported previously [8]. Of the five PhLs isolated, four proved to be identical with compounds detected previously in cotton seeds [9].

For a comparative study of the fatty acid compositions of the individual classes of lipids we performed their GLC analysis. According to the results of the determinations, which are shown in Table 2, all the classes of lipids had the same set of fatty acids. There were, however, differences in their quantitative ratios. The strongly bound and polar fractions of the bound lipids, to which the GLs and PLs belonged, contained a 10-13% larger amount of saturated acids than the NLs, the main representative being the 16:0 acid. The amount of the 18:0 acid in the NLs was 2-3 times lower than in (III) and in the PLs isolated from (II). Similar characteristics have been reported by other authors in a study of rice and barley lipids [5]. It is interesting to note that (III) and the PLs from (II) had almost identical quantitative fatty acid compositions. It is probably just the PLs that tend to the formation in the plant organs of bonds with other components of the seeds that are stronger than those with the NLs.

Cerebrosides (CBs), which are 1-glycosylceramides, have been detected in the seeds of certain plants [10, 11], including the cotton plant [12]. We isolated the CBs by using a procedure suggested by El-Nockrashy and El-Shattory [12]. As markers we used the CBs of human brain purified as described by Bergel'son et al. [13]. In system 8 [14], a spot with R_f 0.42, corresponding in its retention time to one of the cerebrosides taken as markers, was detected.

EXPERIMENTAL

IR spectra were taken on a UR-10 instrument in a film, mass spectra on a MKh-1303 spectrophotometer at an energy of the ionizing electrons of 40 eV, and PMR spectra on JNM 4H-100/100 MHz instrument with HMDS as internal standard, using the δ scale. The GLC of the fatty acid methyl esters was carried on a Chrom-4 chromatograph with a flame-ionization detector, using a column filled with Chromatone N-AW DMS bearing 15% of Reoplex 400. For GLC, the monosaccharides were converted into the corresponding aldononitrile acetates [15] and these were recorded on A Tsvet 101 instrument. The liquid phase used was 5% of XE-60 on Chromatone N-AW. Analytical chromatography was performed on "Silufol" in the following solvent systems: 1) heptane—methyl ethyl ketone—acetic acid (43:7:1) [16]; 2) chloroform [11]; 3) hexane—ether

TABLE 2. Fatty-Acid Compositions of the Lipids of Cottonseed Kernels

Acid (GLC, %)	Class of lipids				
	NLs	MGDGs + DGDGs	PhLs	unidentified GLs	[illegible]
14:0	0,5	1,1	0.5	0,8	0,4
16:0	22,4	33.6	33,0	30,6	30.7
18:0	1,7	3,3	4,0	5,9	3.3
18:1	17,3	18 1	16,9	19,1	16,9
18:2	57,9	43.9	45 6	43,6	48,7
Sum of the fatty acids					
unsaturated	75,4	62,0	62,5	62,7	65,6
saturated	24,6	38,0	37,5	37 3	34,4

(6:4); and 4) hexane–ether (5:5) [17]; and on silica gel in the systems 4) and 5) chloroform–ethanol–water (65:25:4) [18]; 6) chloroform–acetone–methanol–acetic acid–water (65:20:10:10:3) [10]; 7) chloroform–methanol–25% ammonia (65:35:5) [9]; and 8) chloroform–methanol (85:15); and on paper in system 9) n-propanol–butanol–water (4:4:1). The GLs were desorbed from the plates with chloroform–methanol–water (50:45:5) and then with pure methanol [6].

Acid Hydrolysis of the GLs. An approximately 0.2 g sample of GLs was treated with 0.5 ml of 0.5 N H_2SO_4, and the mixture was boiled under reflux on the water bath for 10 h. The hydrolysis products were extracted twice with ether, and the extract obtained was washed with distilled water to neutrality. The aqueous fraction after hydrolysis was neutralized with dry $BaCO_3$, and the filtrate was evaporated to a volume of about 0.5 ml after which the sugars were identified with the aid of PC and GLC. The fatty acids were isolated from the ethereal extract after the hydrolysis of the glycosylglycerides, and they were analyzed in the form of their methyl esters by GLC. The ethereal extract after the hydrolysis of the sterol glycosides was subjected to TLC for its content of free sterols.

Diacylglycerols (1,3- and 1,2-DAGs) and Oxyacylglycerols (OAGs). (R_f 0.36 in system 1). By preparative TLC on silica gel in system 4, two zones corresponding to the 1,3- and 1,2-DAGs were isolated.

NMR spectrum of the 1,3-DAGs, ppm: 0.83 ($-CH_3$), 1.21 ($-CH_2-$), 1.53 ($-CH_2-CH_2COO-$), 1.93 ($-CH_2CH=$ and $-CH_2CH_2CH=$), 2.25 ($-CH_2COO-$), 2.68 ($=CHCH_2CH=$), superposed 4.16 [$-CH_2OCO-$, $-CH(OH)-$], 5.25 ($-CH=CH-$). Characteristic chemical shifts in the PMR spectrum of the 1,2-DAGs were, ppm: 3.6 ($-CH_2OH$), 4.16 ($-CH_2OCOR$), 4.9 (>CHOCOR) [17]. The alkaline hydrolysis of the OAGs yielded the sum of the normal and oxidized fatty acids, which were converted into their methyl esters. On TLC in system 4, a spot was detected having the same mobility as the methyl ester of ricinoleic acid.

The paper chromatography of the sugars was carried out in system 9. The spots were detected with methyl phthalate in ethanol.

The phospholipids were identified by comparison with authentic samples of PEs, PCs, and PIs, obtained from the Laboratory of the Chemistry of Phosphorus-Containing Plant Substances of the Institute of the Chemistry of Plant Substances of the Academy of Sciences of the Uzbek SSR.

The Sterol Glycoside (R_f 0.72 in system 5). Positive reaction with α-naphthol and 20% perchloric acid. IR spectrum (film): 3400 s, 2930 s, 2855 s, 1570 s, 1550 w, 1440 s, 1370 w, 1250 m, 1170 w, 1045 w, 1030 s. After acid hydrolysis the products were found to contain galactose (PC and GLC) and β-sitosterol (mol. wt. 414).

The MGDGs and DGDGs (R_f 0.82 and 0.58 in system 5). Positive reaction with α-naphthol. Identified by their mobilities in TLC by comparison with authentic samples isolated from wheat flour [19]. Acid hydrolysis yielded galactose and the combined FAs which were analyzed by GLC.

The GLs (R_f 0.03 and 0.08 in system 5). Positive reaction with α-naphthol. Acid hydrolysis yielded galactose, glucose, and traces of mannose and also FAs.

SUMMARY

The compositions of the free, bound, and strongly bound lipids of the seed kernels of the cotton plant of variety 108-F have been studied. It has been shown that the free and

bound lipids contain 97.3 and 47.6% of neutral (nonpolar) lipids, respectively. It has been established that the main components of the polar lipids are phospholipids and glycolipids. Two groups of glycolipids have been isolated — a sterol glycoside and glycosylglycerols — and their composition have been determined. The compositions of the fatty acids of the nonpolar lipids, the phospholipids, the glycolipids, and the strongly bound lipids have been determined. The polar lipids are distinguished from the nonpolar lipids by a higher content of saturated acids.

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