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LIPIDS FROM SEEDS OF *Amaranthus* sp.

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The composition of free, bound, and strongly bound lipids of Amaranthus *sp. is studied. The fatty-acid composition of separate lipid classes is determined. Unsaponified sterols that were fractionated by TLC into five components were isolated.*

The chemical composition of lipids from the vegetative and generative organs of *Amaranthus* (Amaranthaceae) has recently attracted much attention [1-3]. The oil of amaranth seeds is used in food and medicine owing to the large amount of squalene, sterols, triterpenes, and other biologically active substances found in it.

We investigated the lipids in seeds of *Amaranthus* sp. collected from the experimental plot of the Institute of the Chemistry of Plant Substances. In contrast with the *Amaranthus caudathus* seeds that we studied previously, which are black, the seeds studied were grayish-yellow; the leaves, green. The plants were greater than 1.5 m high.

The lipids of the *Amaranthus* sp. seeds consisted of 6.04% free (FL), 0.89% bound (BL), and 0.99% strongly bound (SBL) lipids.

The BL were separated by preparative TLC (PTLC) into neutral (NL, 44.8%) and polar (PoL, 55.2%) lipids, the latter contained phospho- (PL) and glucolipids (GL) at 53.1 and 46.9% of the PoL mass, respectively. The protein content of seeds defatted by hexane was 19.2%. The FL of amaranth were separated by TLC on silica gel in systems 1-4. The following classes of lipids were identified: paraffinic, olefinic and isoprenic hydrocarbons, fatty acid esters and high-molecular-weight alcohols, triacylglycerides, tocopherols, free fatty acids, isoprenic alcohols, triterpenes, sterols, and diacylglycerides. The acid number of the FL was 7.7 mg KOH.

Seven classes were observed by studying PL by two-dimensional TLC in systems 5 and 6. Five of these were identified: lysophosphatidylcholine (LPC), phosphatidylinosites (PI), phosphatidylcholines (PC), phosphatidylethanolamines (PE), and N-acyllysophosphatidylethanolamines (LPE). It was found visually that the PC, PI, and PE fractions were predominant.

The GL were separated on silica gel using system 7. The following compounds were identified from qualitative reactions on the GL and R_f values of known substances: digalactosyldiglycerides (DGDG), cerebrosides (CB), sterylglycosides (SG), monogalactosyldiglycerides (MGDG), sterylglycoside esters (SGE).

The sterylglycoside fraction that was separated by PTLC was identical in chromatographic behavior to β-cytosterol β-*D*-glucopyranoside, which was observed previously in other plants [4].

Fatty acids were isolated from the separate lipid classes. Their composition was determined (Table 1). Table 1 shows that nine fatty acids with predominantly the 16:0, 18:1, and 18:2 isomers were present in all classes except for the esters. The FL and PL are highly unsaturated. The NL from the BL, the glycolipids, and the esters contain >30% acid 16:0 and are more saturated compared with the other classes.

Qualitative reactions on the GL and PL in the SBL were negative. However, five substances that were formed during strong alkaline hydrolysis of GL and PL were visualized with H_2SO_4 after TLC using system 5.

The total PL from the FL were separated by PTLC on silica gel. The PC, PE, and minor fractions of PL, which were found higher than PE in system 5, were isolated. Fatty acids were isolated from the PL. Their composition was determined (Table 2).

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TABLE 1. Fatty Acid Composition of Individual Lipid Classes from *Amaranthus* sp. Seeds (%, GLC)

Acids	Bound lipids			SBL	PL	Esters
	NL	GL	PL			
12:0	0.1	1.6	0.5	0.5	0.4	-
14:0	0.2	1.7	0.6	0.4	0.4	-
15:0	0.2	2.4	0.7	0.7	0.3	-
16:0	34.2	35.6	19.7	25.4	22.7	32.2
17:0	0.7	2.6	1.4	0.8	1.4	-
18:0	3.0	1.6	Tr.	3.6	2.1	-
16:1	1.3	Tr.	0.4	2.3	0.7	-
18:1	23.1	33.2	26.5	40.8	25.6	25.5
18:2	37.2	21.3	50.2	25.5	46.4	42.3
$\Sigma_{sat.}$	48.4	45.5	22.9	31.4	27.3	31.2
$\Sigma_{unsat.}$	51.6	54.5	77.1	68.6	72.7	67.8

TABLE 2. Fatty Acid Composition of PL (%, GLC)

Acids	Total PL	PC	PE	Minor PL
16:0	26.6	30.9	42.1	37.1
18:1	27.7	24.7	30.9	26.3
18:2	45.7	44.4	27.0	36.6
$\Sigma_{sat.}$	26.6	30.9	42.1	37.1
$\Sigma_{unsat.}$	73.4	69.1	57.9	62.9

The PC fraction is distinguished by a high degree of unsaturation compared with the PE and minor PL. The situation is analogous for the PL of cotton seeds [5].

The FL of amaranth were saponified by 10% KOH in alcohol. The unsaponified fraction (UF, 8.4%) was isolated by ether. The total UF was separated by TLC on silica gel in systems 1-4. Hydrocarbons, tocopherols, isoprenic alcohols, triterpenes, and sterols were identified. Squalene (40.7%) and sterols (16.2%) predominated.

The sterols were studied in more detail. A strong band at 3449 cm^{-1} was found in the IR spectrum. This is characteristic of hydroxyl groups. An absorption band at 1050 cm^{-1} corresponds to C–O vibrations of hydroxyl groups. A band at 1595 cm^{-1} corresponds to primary alcohols groups [6]. The UV spectrum of a $CHCl_3$ solution of the UF had absorptions at 434, 456, and 488 nm, which are consistent with the monoepoxide of β-carotene [7]. The sterol fraction was acetylated with acetic anhydride in the presence of pyridine [8]. The homogeneity of the resulting acetates was checked by TLC.

It should be noted that the sterols of the studied amaranth and their acetates after TLC in system 2 gave R_f values of 0.19 and 0.84, i.e., less than the R_f value of the references, which were β-cytosterol and its acetate (R_f = 0.21 and 0.86, respectively). The spot of the sterols was mottled lilac-gray after visualization with 50% H_2SO_4, in contrast with β-cytosterol, which is purplish.

Sterol acetates were separated by PTLC on silica gel with 20% $AgNO_3$ by four-fold development using system 8, which has been used by many researchers to separate triterpene and 4-monomethylsterol acetates [9, 10]. We observed five substances with R_f values of 0.06, 0.17, 0.25, 0.58, and 0.59, which were eluted by a $CHCl_3$:ether (1:1, v/v) mixture. The amount of each substance was 34.6, 14.1, 10.9, 30.6, and 9.6%, respectively.

The homogeneity of the isolated acetates was checked by TLC using system 2. One spot was seen. The reference was β-cytosterol acetate. The isolated sterol acetates were separated using system 9. The calculated R_s values of the spots relative to the reference, cholesterol acetate, were compared with the literature data [11]. The acetates were identified as stigmasterol (R_s = 0.87) and sitosterol (R_s = 1.0). However, the R_s values of the other amaranth sterol acetates were different from those

given in the literature [11].

The compositions of sterols from several amaranth species have been reported [12]. It was noted that α-spinosterol predominates and reaches 52% in *A. tricolor*. A substantial quantity (28%) of this sterol is found in the unsaponified fraction of pumpkin lipids [12].

We have previously [1] identified by mass spectrometry four sterols in lipids from the seeds of *Amaranthus caudatus*: β-sitosterol, stigmasterol, campesterol, and cholesterol. Further investigation revealed that the sterols also contain α-spinosterol with M^+ 412 and a $\Delta^{7,22}$ double bond, stigmasterol with $\Delta^{5,22}$, and avenosterol with $\Delta^{5,22}$.

EXPERIMENTAL

UV, IR, and mass spectra were recorded on the instruments described previously [1].

Analytical and preparative chromatography were performed on silica gel with 10% $CaSO_4$; Ag^+-TLC, with 20% $AgNO_3$.

The solvent systems were: 1)-3) $C_2H_5OC_2H_5$—C_6H_{14} (3:7, 6:4, 5:5, respectively); 4) $CH_3(CH_2)_5CH_3$—C_6H_6 (9:1); 5) $CHCl_3$—CH_3OH—NH_4OH (28%) (65:35:5); 6) $CHCl_3$—CH_3COCH_3—CH_3OH—CH_3COOH—H_2O (5:2:1:1:0.5); 7) $CHCl_3$—CH_3COCH_3 —CH_3OH — CH_3COOH — H_2O (65:20:10:10:3); 8) CH_2Cl_2—CCl4 (1:5); 9) $CHCl_3$—C_6H_{14}—CH_3COOH (25:75:0.5).

The FL, BL, and SBL were separated according to the literature method [13].

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