LIPIDS OF Hibiscus cannabinus SEEDS

I. Tolibaev, Kh. S. Mukhmedova,
and A. I. Glushenkova

UDC 547.953:655.37

The neutral and polar lipids of kenaf seeds of the variety Uzbetskii-1574 from the 1983 crop have been studied. The fatty acid compositions of six acyl-containing classes of neutral lipids have been established for the first time. The acids in the sn-2 positions of the triacylglycerols and of the main classes of phospholipids have been determined. The main triacylglycerol species are those in which the sn-2 position is esterified by linoleic acid.

The seeds of Hibiscus (kenaf), family Malvaceae, belong to the medium-oil type with 18-21% of soil [1-3]. H. esculentus L. growing in various countries of the world has been investigated in fairly great detail. The main indices and the composition of the fatty acids (FAs) of the seed oil of this species have been determined, and a high level of amino acid, proteins, and tocopherols and the possibility of their utilization for the production of protein isolates, edible oil, etc., have been noted [3].

There is no information on the neutral lipids of H. cannabinus L. We have investigated the seeds of H. cannabinus, Uzbetskii-1574 variety, 1983 crop. The neutral and polar lipids extracted from the comminuted seeds by hexane and by chloroform–methanol (2:1) were studied. The hexane extract consisted of a yellow oil, its yield being 20%. On TLC (Silufol, solvent systems 1-4) with the aid of model samples of plant lipids, literature information on the chromatographic mobilities of lipid classes, and also characteristic color reaction of the separated compounds, the following classes of lipids were detected in the hexane extract: hydrocarbons (HCs), sterol esters (SEs), triacylglycerols (TAGs), free fatty acids (FFAs), epoxyacylglycerols (EAGs), diacylglycerols (DAGs), free sterols (FSs), monoacylglycerols (MAGs) and polar lipids (PLs).

The quantitative composition of the lipid was judged from the results of column chromatography (CC) of the extracts followed by the preparative separation of narrow fractions in TLC (Table 1).

The main lipid class of kenaf seed oil, as of other plant oils, is represented by the TAGs, and the other classes of lipids are present in only small amounts.

The HC fraction was analyzed with the aid of GLC. The C_{27}, C_{29}, and C_{31} hydrocarbons were predominate (10.6, 18.2, and 26.6%, respectively).

The products of the severe hydrolysis of the SEs and also the FSs were studied by mass spectrometry. The kenaf seed lipids contained four sterol components, mainly in the free form and a smaller amount in the bound form. Three of these were β-sitosterol, stigmasterol, and campesterol – characteristic compounds for the seed lipids of higher plants [4]. β-Sitosterol predominated. A fourth, unidentified, component was present in an insignificant amount.

DAGs and MAGs are not usually present or are present in only small amounts in the oils of ripe seeds [5, 6]. In the kenaf seeds, these components were detected in amounts of 0.2 and 0.1%, respectively.

When the GL [glycolipid] fraction was separated by TLC, spots of MGDGs and DGDGs were detected [7]. The severe hydrolysis of the GLs yielded GAs and a water-soluble fraction which gave a positive reaction with aniline phthalate for sugars.

Institute of the Chemistry of Plant Substances of the UzSSR Academy of Sciences, Tashkent.
Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 558-564, September-October, 1986. Original article submitted April 21, 1986.

0009-3130/86/2205-0523$12.50

TABLE 1. Class Composition of the Lipids of Kenaf Seed Oil

Neutral lipids		Phospholipids	
Class	Amt., % on weight of extract	Class	Amt., % on weight of extract
Carbohydrates	0,2	PCs	39,2
Sterol esters	0,4	PIs	30,4
TAGs	97,5	PEs	16,0
FFAs	0 6	N-Acyl-PEs	9,6
EAGs	Tr.		
DAGs	0,2	N-Acyllyso-PEs	3,2
Sterols	0,6	Lyso-PCs	0 8
MAGs	0,1	Lyso-PIs	0,8
Glycolipids	0,2		
Phospholipids	0,2		

TABLE 2. Fatty Acid Compositions of the Acyl-Containing Neutral Lipids [NLs] of Kenaf Seeds

Fatty acid	Sum of the NLs	Sterol esters	FFAs	TAGs		DAGS	MAGs	GLs
				total	sn-2-MAGs			
10:0	Сл.	1,2	—	0,5	—	—	0,9	—
12:0	0,2	1,0	—	0,5	0,5	1,2	0,6	0,8
14:0	0,5	1,4	1,0	0,9	0,5	1,0	1,1	1,0
16:0	27,5	25,4	31,4	26,9	5,7	42,3	40,4	49,5
16:1	1,2	1,5	1,3	0,9	1,4	1,4	1,1	1,5
17:0	0,4	0,8	1,0	Tr.	Tr.	0,5	Tr.	—
17:1	0,5	0,6	1,0	0,5	1,4	—	Tr.	—
18:0	4,2	8,0	6,5	3,8	1,1	5,7	7,0	8,8
18:1	30,0	37,5	29,7	28,4	34,2	29,2	31,2	32,4
18:2	34,1	12,4	20,4	35,8	54,2	18,7	16,2	6 0
18:3	1,4	10,2	1,7	1,8	1,0	Tr.	1,5	—
$\Sigma_{П}$	32,8	37,8	45,9	32,6	7,8	50,7	50,0	60,1
$\Sigma_{Н}$	67,2	62,2	54,1	67,4	92,2	49,3	50,0	39,9

To evaluate the acyl-containing classes of lipids, their FA compositions were determined (Table 2).

In all the acyl-containing classes except the TAGs, oleic acid predominated among the unsaturated acids. The amount of linoleic acid was smallest in the TAGs. Linolenic acid was present in all the samples with the exception of the GLs, in amounts ranging from traces (in the DAGs) to 10.2% (in the sterol esters). The total amount of saturated acids in the FFAs, DAGs, MAGs, and GLs was almost 1.5-2 times greater than in the TAGs.

Thus, by comparing our results on the composition of the FAs of the neutral lipids of kenaf seeds with literature information for another type of kenaf [3] and also cotton seeds [8], it may be concluded that they are similar to one another with respect to the sum of the saturated and unsaturated FAs. In contrast to the lipids of the seeds of H. esculentus from various geographical zones (USA, Sudan, Canada, India, etc.), the lipids of the Central Asian species H.cannabinus contain the 18:3 acid.

In order to establish the composition of the FAs, in the central position of the TAGs we made use of the method of lipase hydrolysis. The TAGs were hydrolyzed with the aid of pancreatic lipase, and the composition of the FAs from the sn-2-MAGs are given in Table 2.

The results of the analysis showed that the central position of the TAGs was esterified mainly with linoleic (54.2%) and oleic (34.2%) acids. From these results, using Coleman's method as modified by A. L. Markman [9] we calculated the species composition of the TAGs (Table 3).

Of the 67 TAG species given, 23 were represented in an amount of 0.1% and 16 in amounts of 0.2-0.5%. The main species were PLL (11.8%), PLO (11.6%), and PLP (8.9%), these figures being close to those for cottonseed oil [8].

By summing the results relating to saturation (S) and unsaturation (U) of the fatty acid radicals, the following position-species composition of the TAGs of kenaf seed oil

TABLE 3. Position-Species Composition of the TAGs of Kenaf Seed Oils (%)

TAGs	Amount	TAGs	Amount	TAGs	Amount
PPP	0,1	LPoL	0,2	POLe	0,6
PPS	0,3	PPoL	0,6	SOS	0,1
PPO	1,5	PPoLe	0,1	SOL	0,9
PPL	1,5	OPoP	0,6	SOO	0,8
		LPS	2,2	SoLe	0,1
PPLe	0,1	LPO	11,6	OOO	2,1
SPO	0,2	PLL	11,8	LOO	2,2
SPL	0,2	PLLe	0,9	LOL	4,5
LPO	0,9	OLLe	0,5	LoLe	0,4
LPL	0,5	LeLs	0,1	LeOO	0,3
LPLe	0,1	OLO	3,3	OOPo	0,1
LePO	0,1	PoLO	0,1	PLP	8,9
PPoP	0,5	PoLL	0,1	PLPo	0,3
PoPoO	0,1	SLO	1,3	SLS	0,1
SPoO	0,1	OPoO	0,2	LLS	1,4
SPoP	0,1	PSP	0,1	OLL	7,1
SPoP	0,1	PSO	0,2	LLL	3,7
LPoP	0,6	PSL	0,2	LLLe	0,5
LPoO	0,4	OSO	0,1	PLeP	0,1
LSL	0,1	OSL	0,1	PLEO	0,2
		POS	1,4	PLeL	0,2
POP	5,7	POO	7,6	OLeL	0,1
POPo	0,2	POL	7,3	LLeL	0,1

Symbols for acids: P – palmitic; Po – palmitoleic; S – stearic; O – oleic; L – linoleic; Le – linolenic. Species present in amounts of less than 0.1% are not given in the table.

was obtained (%): SSS–1.6; SSU–3.9; USU–1.7; SUU–47.9; SUS–19.1; UUU–25.8.

The phospholipids were extracted from defatted kenaf seed meal by Folch's method [10]. The quantitative class composition of the total PLs, established with the aid of two-dimensional TLC in systems 3 and 6, are given in Table 1.

The combined PLs were separated on a column of silica gel. Narrow fractions of the PLs were analyzed by TLC using systems 3 and 5, depending on the polarities of the individual components. For detailed analysis, homogeneous classes of PLs were isolated from narrow fractions by preparative TLC on silica gel. The structures of two minor PLs of kenaf seeds of the Uzbetskii-1574 variety (lyso-PCs and lyso-PIs) were confirmed by their IR spectra [11], by determinations of the N and P contents, and by the identification of the products of acid hydrolysis.

The fatty acid compositions of the total PLs freed from impurities and of individual classes of PLs were established after mild acid hydrolysis, isolation, methylation, and GLC analysis. The position distribution of the fatty acid radicals in the glyceride moieties of the molecules of the quantitatively main classes of PLs were determined by enzymatic hydrolysis using snake venom as a source of phospholipase A_2.

An analysis of the GLC results (Table 2) showed that the combined PLs contained no 17:0 and 17:1 acids. It must be mentioned that linolenic acid is mainly localized in the N-acetylated PLs. With respect to increasing saturation the individual classes of PLs form the following sequence: PEs < PCs < lyso-PCs < N-acyl-PEs < N-acyllyso-PEs < PIs < lyso-PIs.

On the basis of the PMR spectrum (absence of a multiplet at 5.3 ppm) [12] and also of the FA composition, the structures of sn-1-acylglycerophosphorylcholines and sn-1-acylglycerophosphorylinositols may be proposed for the lyso-PCs and lyso-PIs respectively.

In a determination of the possible position-species composition of the PFs, PIs, and PEs we based ourselves on the experimental results of the position distribution of the fatty acid radicals and their molecules and a mathematical method. The number of species calculated for

TABLE 4. Fatty Acid Composition of the Total Phospholipids of Kenaf Seeds and Individual Classes of Them

Phospholipids		Fatty acid									
		12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	ΣП	ΣН
Sum of the PLs		2,4	2,8	28,8	3,0	6,9	23,3	31, 3	1,5	40,9	50,1
PLs	total	3,2	4 0	21,1	1,4	8,0	29 0	32,8	0,5	36,3	63,7
	sn-1	7,6	7,1	36,8	Tr.	11,1	24,9	12,5	Tr.	62,6	37,4
	sn-2	Tr.	1,1	4,5	1,1	1,0	36,3	54 7	1,3	6,6	93,4
PEs	total	1,0	1,5	24,1	2,1	6 6	26,0	38,7	Tr.	33,2	66,8
	sn-1	1,1	1,4	47,5	—	6,7	15 9	27,4	—	56,7	43,3
	sn-2	1,4	2,0	3,0	1,8	2,7	35,4	52,6	1,1	9,1	90,9
PIs	total	1,5	1,4	41,2	3,2	5,4	24,2	23,1	Tr.	49,5	50,5
	sn-1	4,1	2,0	66,5	Tr.	7,2	8,4	11,8	—	79,8	20,2
	sn-2	Tr	0,6	14,8	1,5	3,9	45,5	30,9	2 8	19,3	80,7
N-Acyl-PEs		2,6	3 6	29,4	4,8	5,5	18,9	21,3	13,9	41,1	58,9
N-Acyllyso-PEs		6,9	6 3	27,2	2,2	8,0	22,4	12,4	14,6	48,4	51,6
Lyso-PCs		3,3	2,0	31,7	1,1	3,3	33,6	25,0	—	40,3	59,7
Lyso-PIs		1,6	3,7	46,7	1,8	13,2	22,6	9,4	—	65,2	34,8

the PEs was 50, for the PIs 43, and for the PEs 37, and they were combined into the following types (%):

		PCs	PIs	PEs
Disaturated	(SS)	4.1	15.3	5.2
Saturated-unsaturated	(SU)	2.5	4.0	3.6
Unsaturated-saturated	(US)	58.5	64.4	51.8
Diunsaturated	(UU)	34.9	16.3	39.4

As we see, with respect to their amounts of all four types the PE and PC molecules are similar to one another while the PIs differ by higher amounts of the SS and US types and a considerably smaller amount of the UU type.

EXPERIMENTAL

The kenaf seeds were obtained from the Uzbek Experimental Station for Bast Crops of the VASKhNIL [V. I. Lenin All-Union Academy of Agricultural Sciences) branch. The neutral lipids were extracted from the comminuted seeds by repeated steeping with hexane at room temperature. The extracts were combined and the solvent was driven off in a rotary evaporator.

The lipids were separated into classes and the individual fractions were monitored by the methods of PC and TLC. Silufol and Chemapol 5/40 μ silica gel (Czechoslovakia) were used for TLC. The spots of the NLs were detected with iodine vapor, and by spraying with 5% H_2SO_4 followed by heating to 120°C for 3-5 min, the PLs by the Dragendorff and Vas'kovskii reagents, and the GLC with α-naphtol [7]. For the qualitative detection of epoxy compounds we used a 0.05 M ethanolic solution of picric acid [13]. Column chromatography was carried out on Chemapol 100/160 μ silica gel at a ratio of the total NLs to adsorbent of 1:20 and of the total PLs to adsorbent of 1:40.

The following solvent systems were used: 1) hexane–ether–acetic acid (70:30:1); 2) hexane–ether–acetic acid (90:10:1); 3) chloroform–ethanol–ammonia (65:35:5); 4) hexane–ether (95:5); 5) chloroform–methanol–water (65:35:5); 6) chloroform–ethanol–acetone–acetic acid–water (10:5:4:2:1); 7) hexane–ether (9:1); 8) hexane–ether (4:1); 9) heptane–methyl ethyl ketone–acetone acid (43:7:1); 10) hexane–ether (1:1); 11) hexane–ether (3:2); 12) propanol–butanol–water (4:4:1); and 13) butanol–pyridine–water (6:4:3).

IR spectra were taken on a UR-10 instrument of the samples in the form of films, and mass spectra on a MKh 1303 spectrometer at an energy of the ionizing electrons of 40 eV. GLC was performed on a Chrom-4 instrument with a flame ionization detector. For FAMEs we used a stainless steel column filled with Chromaton N-AW-DMCS and with 17% of PEGS on Celite 545. The dimensions of the column were 4 mm × 2.5 m and its temperature 198°C for the sterols and HCs we used a 4 mm × 1.2 m column filled with 5% of SE-30 on Chromaton N-AW-DMCS at a temperature of 260°C.

NMR spectra were recorded on a Geol C-60-HL/60 MHz instrument in deuterochloroform solution. The alkaline hydrolysis of the total NLs and of the PLs, TAGs, and DAGs was carried out as in [14].

Hydrocarbons were eluted from the column with hexane, R_f 0.95 (systems 7 and 8).

Sterol esters were eluted from the column with hexane, R_f 0.9-0.95 (system 9).

Triacylglycerols were eluted by solvent system 7, R_f 0.8 (system 8). Their IR spectrum was similar to that given in the literature.

Free fatty acids were eluted by solvent system 7; R_f 0.5 in system 9. After methylation with diazomethane, R_f 0.9 in system 9.

Diacylglycerols were eluted by solvent system 10. R_f 0.4 in system 9.

Monoacylglycerols were eluted with ether; R_f 0.1 in system 8.

Glycolipids were eluted with acetone and ethanol (with phospholipids). When freed from impurities, the GLs had R_f 0.8 (MGDs) and 0.6 (DGDs) in system 5.

Saponification of the Sterol Esters. A mixture of 50 mg of the sample and 5 ml of 20% methanolic KOH solution was boiled for 24 h. The reaction was monitored with the aid of TLC in system 1.

The solvent was evaporated off and the residue was dissolved in water. The unsaponifiable substances were eliminated from the alkaline solution by three extractions with petroleum ether (40-60°C), the solution was acidified with 10% hydrochloric acid, the fatty acidswere extracted with diethyl ether, and the extract was washed with water and was dried over anhydrous sodium sulfate. The ethereal solution was evaporated to dryness, and the FAs were methylated with diazomethane and were analyzed by GLC. Sterols with R_f 0.35 (in system 1) were isolated from the unsaponificable fraction, and when the chromatogram was sprayed with 50% sulfuric acid and was then heated they appeared in the form of lilac spots. Mass spectra (125°C; 40 eV; 0.5 mA), m/z: M^+ 414, M^+ 412, M^+ 400 – strong peaks of the molecular ions which were assigned to β-sitosterol, stigmasterol, and campesterol, respectively. Characteristic peaks of the ions of these compounds were as follows: m/z 399, 396, 394, 315, 303, 301, 300, 273, 255, 231, 213. Among the bound sterols the following were detected with the aid of GLC (%): β-sitosterol, 88.8; campesterol, 7.3; stigmasterol, 3.0; and an unidentified compound, 0.9.

Free sterols were eluted with system 10. The identification and analysis of these compounds were carried out as described above. The following were detected (%): β-sitosterol, 89.9; stigmasterol, 5.2; campesterol, 3.9; and unidentified, 1.0.

Enzymatic Hydrolysis of the TAGs with Pancreatic Lipase [9]. To 1 g of the TAGs were added 0.5 ml of a 1% solution of polyvinyl alcohol, 1 ml of a solution of calcium chloride, and 7 ml of ammoniacal buffer (pH 8.0). The mixture was kept in a thermostat at 40°C for 10 min. Then, with constant stirring, 0.25 g of lipase previously defatted with acetone was added to the flask.

The reaction was performed at 40°C for 30 min with constant stirring, 1-2 drops of 4 M NH_4OH being added to the mixture every 5 min. The course of the reaction was monitored with the aid of TLC, attention being directed to the intensity of spot of the sn-1,2(2,3)-DAGs in order not to cause the splitting of the DAGs into FAAs and glycerol. When hydrolysis had reached the desired depth, the reaction was stopped by the addition of 1-2 ml of 4 N HCl to the reaction mixture. The hydrolysis products were extracted with ether, and the ethereal solution was washed with ether and dried with sodium sulfate. The products were separated preparatively in solvent system 11.

Acid Hydrolysis of the Glycolipids [7]. The hydrolysis of 50 mg of the sample was carried out in 2 ml of 5% sulfuric acid by boiling for 20 h. The course of hydrolysis was monitored by TLC in system 12. The hydrolysis products were extracted with ether. The ethereal solution was washed with water and was dried with sodium sulfate.

The fatty acids were methylated with diazomethane and were analyzed by GLC. The aqueous fraction was neutralized with dry barium carbonate. The precipitate was filtered off and the filtrate was concentrated to a volume of ∿1 ml. The residue was analyzed with the aid of PC in system 13. The spots were revealed with aniline phthalate. Galactose was identified.

The enzymatic hydrolysis of the main classes of the PLs was performed with the aid of kufi venom phospholipase A_2 in Tris buffer (pH 8.15). The hydrolysis products were separated by preparative TLC in system 3. The FAs that had been split out from the sn-2 position were

desorbed from the silica gel, methylated, and analyzed by GLC; lyso compounds were subjected to alkaline saponification and the FAs in the sn-1 position were analyzed as described above.

SUMMARY

1. The compositions of the neutral and polar lipids of Hibiscus cannabinus L. seeds have been studied.

2. Eight classes of neutral lipids, of which six are acyl-containing, have been isolated and characterized for the first time.

3. With respect to the fatty acid composition of the total neutral lipids and the distribution of the fatty acids in the triacylglycerols, kenaf seed oil is similar to cottonseed oil.

4. In the triacylglycerols the main species are those in which the sn-2 position is esterified with linoleic acid.

5. Minor phospholipids of kenaf seeds have been identified as lyso-PCs and lyso-PIs.

LITERATURE CITED

1. P. A. Karakoltsidis and S. M. Constantinidis, J. Ag. Food Chem., 23, 1204 (1975).
2. F. M. Martin, L. Telek, R. Ruberta, and A. G. Santiago, J. Food Sci., 44, 1517 (1979).
3. C. L. Kalra and J. S. Pruthi, Indian Food Packer, 38, 37 (1984).
4. T. Itoh, T. Tamura, and T. Matsumoto, J. Am. Oil Chemists' Soc., 50, 122 (1973).
5. R. V. Madrigal, R. D. Plattner, and C. R. Smith, Lipids, 10, 208 (1975).
6. G. V. Panekina, S. D. Gusakova, M. Ya. Tabak, and A. U. Umarov, Khim. Prikl. Soedin., 44 (1978).
7. M. Kates, Techniques of Lipidology, North-Holland, Amsterdam/American Elsevier, New York (1972).
8. S. G. Yunusova, I. P. Nazarova, S. D. Gusakova, and A. I. Glushenkova, Khim. Prikl. Soedin., 319 (1980).
9. A. L. Markman, T. V. Chernenko, and A. U. Umarov, Prikl. Biokhim. Mikrobiol., No. 6, 616 (1969).
10. J. Folch, M. Lees, and S. H. Sloane-Stanley, J. Biol. Chem., 226, 497 (1957).
11. L. J. Bellamy, Infrared Spectra of Complex Molecules (2nd edn.), Methuen, London/Wiley, New York (1958).
12. D. Chapman and A. Morrison, J. Biol. Chem., 24, 5044 (1966).
13. J. A. Fioriti and R. J. Sims, J. CHromatogr., 32, 761 (1968).
14. E. Stahl, Thin-Layer Chromatography (2nd English edn.). Allen and Unwin, London/Springer, New York (1969) [Russian translation] (from the German), Moscow (1965), p. 147.