NEUTRAL LIPIDS AND POLYSACCHARIDES OF KENAF PROCESSING WASTES

I. Tolubaev, Kh. S. Mukhamedova, D. A. Rakhimov, and A. I. Glushenkova

UDC 547.953.917;665.37

The class composition and fatty-acid composition of the neutral lipids of kenaf processing wastes have been studied. The presence of acetates of triterpene alcohols and of free triterpenols has been established by physicochemical methods of analysis in the neutral lipids of kenaf. Hemicelluloses and cellulose predominate in the polysaccharide complex of kenaf chaff. Among the hemicelluloses, the xylans predominate.

Continuing an investigation of the lipids of kenaf processing wastes (chaff, flowers, leaves, and ripe seeds) [1], we have studied the composition of the neutral lipids (NLs) and of the carbohydrate complex. The total NLs were obtained as in [2] (1.0% of the weight of the dry raw material) and were separated by column chromatography into individual classes by solvent systems 1. The fractions containing two and more classes of lipids were separated by preparative TLC. The assignment of the substances of the chromatographically pure fractions to definite classes was made on the basis of the mobilities of model samples (systems 1-4), and from qualitative reactions and spectral characteristics. The following classes were identified (in % of the weight of the NLs): hydrocarbons – 0.3, carotenoids + sterol esters – 30.6, triacylglycerols (TAGs) – 39.4, free fatty acids (FFAs) – 20.1, acetates of triterpene alcohols – 1.0, triterpenols – 0.8, sterols – 5.1, diacylglycerols (DAGs) – 0.6, monoacylglycerols (MAGs) – 0.8, chlorophyll – 0.9, polar lipids (PolLs) – 0.4.

As can be seen from the figures given, the main class of lipids was the TAGs.

The fatty acids isolated from the acylglycerols by alkaline hydrolysis [3] were analyzed by the GLC method (Table 1). The set of fatty acids of individual classes of NLs consisted of 10-12 components. Among the saturated acids the 16:0 species predominated in all cases, and among the unsaturated acids the 18:1 or 18:2 species. In terms of total amount, the unsaturated acids in the individual classes were predominant except for the FFAs and the acylsterols. In order to establish which acids esterified the central position of the TAG molecule, we made use of pancreatic hydrolysis (see Table 1). The results showed that the sn-2 positions of the TAGs of the kenaf production wastes were esterified predominantly (61.9%) by the 18:1 acid. In the mass spectra both of the sterols and of the products of the severe saponification of the acylsterols intense peaks of the molecular ions with m/z 414, 412, and 400 were detected, which were assigned to β-sitosterol, stigmasterol, and campesterol, respectively [4]. On the basis of TLC results and their mass spectra, the spots with R_f 0.75 and 0.45 (system 2) were assigned to acetates of triterpene alcohols and triterpenols [4, 5]. We have found nothing in the literature relating to the study of such compounds in the lipids of plants of the family Malvaceae.

The amounts of chlorophyll-like compounds and carotenoids were determined by a spectrophotometric method [7, 8]. It was found that the amount of chlorophyll *a* was 27 mg/100 g and of chlorophyll b 3.6 mg/100 g; the carotenoids amounted to 1.5 mg/100 g of NLs. According to TLC results (system 4), the PL fraction consisted of a mixture of glyco- and phospholipids (PLs) in which the glycolipids (GLs) predominated visually. Oxidized carotenoids were identified from their chromatographic mobilities in TLC (system 2), R_f 0.1.

The alcohol-soluble sugars of the kenaf wastes were extracted with 82% ethanol, and then the polysaccharides were isolated by fractional extraction successively with water, a mixture of oxalic acid and ammonium oxalate, and alkali [9]. The yield of alcohol-soluble sugars was 1.1% on the weight of the raw material; glucose, arabinose, xylose, and galactose were detected with the aid of PC. The qualitative carbohydrate composition of the polysaccharide fraction was determined after complete acid hydrolysis and was analyzed by the PC and GLC methods (Table 2). The water-soluble polysaccharides consisted of a white amorphous powder which possessed no reducing capacity and gave no reaction with iodine, i.e., it did not contain a glucan of the starch type.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 25-27, January-February, 1990. Original article submitted March 6, 1989.

0009-3130/90/2601-0019$12.50

TABLE 1. FA Compositions of the Sum and the Individual Classes of Neutral Lipids from Kenaf Production Wastes

Neutral lipids	Fatty acid													
	10 : 0	12 : 0	14 : 0	16 : 0	16 : 1	17 : 0	17 : 1	18 : 0	18 : 1	18 : 2	18 : 3	20 : 0	ΣS	ΣU
Sum of NLs	Tr.	1,0	0,8	35,1	2,0	Tr.	Tr.	2,8	24,0	34,3	Tr.	Tr.	39,7	60,3
TAGs	0,4	0,6	0,4	33,3	2,6	Tr.	Tr.	2,2	25,3	35,2	Tr.	—	36,9	63,1
sn-2-MAGS		0,6	0,4	9,3	1,9	Tr.	Tr.	3,4	61,9	21,2	1,3	—	13,7	86,3
FFAs	—	1,0	1,3	48,3	3,0	3,0	2,3	9,0	27,5	3,2	Tr.	1,4	64,0	36,0
Acylsterols	1,4	2,1	3,8	32,4	6,5	5,1	10,2	10,0	12,6	7,1	8,8	—	54,8	45,2
DAGs	Tr.	1,5	1,8	23,8	3,1	Tr.	Tr.	4,3	30,1	35,8	0,6	—	30,4	69,6
MAGs	0,3	2,0	1,3	21,2	3,5	Tr.	Tr.	5,0	31,3	35,1	0,3	—	29,8	70,2

TABLE 2. Amounts and Compositions of the Polysaccharides of Kenaf Wastes

Extractant	Yield of polysaccharide on the weight of the raw material, %	Amounts of sugars, %					
		Gal	Glc	Man	Xyl	Ara	Rham
Water, 20°	2.6	29,92	5,44	5,44	11 96	18,49	28,0
Water, 90°	1,3	13,48	22,77	3,85	24,19	16,37	19,2
0,5% $C_2H_2O_4$ + $+C_2H_2(NH_4)_2$, 70°	4 3	16,42	30,59	16,27	0,97	13.74	22,0
15% NaOH, 20°							
HMCs A	5,3	—	—	—	100	—	—
HMCs B	4,03	—	3,42	3,3	92,0	—	1,2

The acid-soluble polysaccharides were present in considerably larger amounts than the water-soluble polysaccharides. In the products of acid hydrolysis, in addition to the monosaccharides mentioned above, galacturonic acid was detected, being identified by PC and electrophoresis [9]. Among the alkali-soluble hemicelluloses (HMCs A and B) there was a larger amount of fraction A, for which a polymer constructed of xylose units is characteristic. Thus, it has been established that in the carbohydrate complex of kenaf wastes there are water-soluble polysaccharides, an acid polysaccharide, and hemicelluloses. Among the hemicelluloses xylans predominate. In our opinion, kenaf wastes can be used as a source of D-mannose, and the hemicelluloses as a source of xylose.

EXPERIMENTAL

Mass spectra were taken on a MKh 1303 instrument at an energy of the ionizing electrons of 40 eV, and UV spectra on a Hitachi spectrophotometer in cyclohexane. The kenaf wastes were obtained from the experimental station for tanning crops of the Central Asian branch of BASKhNIL [Lenin All-Union Academy of Agricultural Sciences]. The total NLs were extracted by steeping the comminuted raw material with hexane. The lipids were separated on a column of silica gel with subsequent subfractionation of the coarse fractions into individual classes by TLC. Silica gel 100-160 μm was used for CC and silica gel 5/40 and also Chemapol Silufol (Czechoslovakia) for TLC. The spots were identified with iodine vapor and with 50% H_2SO_4/CH_3OH followed by heating at 100-110°C. GL spots were revealed with α-naphthol, and PLs with the Vas'kovskii reagent. The descending chromatography of the carbohydrates was performed on type FN-11 paper (GDR) in system 5. Sugars were revealed with a solution of acid aniline phthalate at 105-110°C. Monosaccharides were analyzed in the form of the corresponding aldonitrile acetates [10].

Solvent systems: 1) hexane—ether (95:5), (90:10), (85:15), (80:20), (70:30), (60:40), and (50:50), diethyl ether, acetone, and chloroform; 2) hexane—ether—acetic acid (70:30:1); 3) heptane—methyl ethyl ketone—acetic acid (43:7:1); 4) chloroform—methanol—25% ammonia (65:35:5); and 5) butanol—pyridine—water (6:4:3).

In TLC, the triterpene alcohol acetates and triterpenols were revealed with sulfuric acid and heating. Mass spectrum: M^+ 468, m/z 218, 203, 189; M^+ m/z 218, 403.

The hydrolysis of the polysaccharides was carried out in 2 N sulfuric acid in the boiling water bath for 8-24 h. The hydrolysate was neutralized with $BaCO_3$ and, after appropriate working up, was analyzed by PC and GLC. GLC was performed on a Chrom-4 instrument with a flame-ionization detector. For the fatty acid methyl esters we used a stainless steel column filled with 17% of PEGS on Celite 545. The dimensions of the column were 4 mm × 2.5 m and the temperature 198-200°C. For the analysis of sugars we used a 3 × 200 cm glass column. It was packed with 5% of XE-60 on Chromaton N-AW (0.200-

0.160 mm). The rate of flow of helium was 60 ml/min, and the thermostat temperature 210°C. The alkaline hydrolysis of the combined HLs, TAGs, 2-MAGs, DAGs, and MAGs was carried out as in [2].

LITERATURE CITED

1. I. Tolibaev, Kh. S. Mukhamedova, A. I. Glushenkova, A. S. Tursunkhodzhaev, and T. B. Azizov, *Khim. Prir. Soedin.*, 686 (1986).
2. I. Tolibaev, Kh. S. Mukhamedova, and A. I. Glushenkova, *Khim. Prir. Soedin.*, 558 (1986).
3. E. Stahl, *Thin Layer Chromatography*, Springer/Academic Press, New York (1965) [Russian translation from the German: Moscow (1965), p. 147].
4. T. Itoh, T. Tamura, and T. Matsumoto, *J. Am. Oil Chem. Soc.* **50**, 122 (1973).
5. S. N. Hooper, R. F. Chandler, E. Lewis, and W. D. Jamieson, *Lipids* **17**, No. 1, 60 (1982).
6. R. F. Chandler, S. N. Hooper, W. D. Jamieson, and E. Lewis, *Lipids* **17**, No. 2, 102 (1982).
7. T. G. Maslova, I. A. Popova, and O. F. Popova, *Fiziol. Rast.* **33**, No. 3, 615 (1986).
8. *Handbook of Methods of Investigation, Technical and Chemical Control, and the Accounting of Production in the Oils and Fats Industry* [in Russian], Vol. 1, Book 1, Leningrad (1967), p. 509.
9. A. O. Arifkhodzhaev, D. A. Rakhimov, and Z. F. Ismailov, *Khim. Prir. Soedin.*, 246 (1980).
10. D. A. Rakhimov, N. P. Yuldasheva, S. A. Khamidkhodzhaev, and E. S. Kondratenko, *Khim. Prir. Soedin.*, 21 (1985).
11. D. G. Lange and L. K. N. Jones, *Can. J. Chem.* **45**, 1995 (1967).

PREPARATIVE FRACTIONATION OF CEREBRAL GANGLIOSIDES WITH THE AID OF ION-EXCHANGE CHROMATOGRAPHY ON SPHERON CH 300

K. Yu. Gordeev and S. G. Batrakov

UDC 543.86:547.952

A new preparative method is proposed for the separation of natural mixtures of gangliosides into fractions of mono-, di-, and trisialogangliosides which is based on ion-exchange chromatography on a weak anion-exchange resin — Spheron CH 300. The latter ensures a considerably faster separation than the ion-exchange resins usually employed.

The demand for effective methods for the preparative separation of complex mixtures of nerve-tissue gangliosides is due to the modern development of investigations of the physiological functions and biochemical properties of this class of glycolipids [1, 2]. According to the existing methodology, the isolation of individual gangliosides is carried out in several stages [2]. In the first stage, fractions consisting of components with the same number of sialic acid residues are usually used. For this purpose, ion-exchange chromatography on anion-exchange resins containing amino or diethylamino groups is frequently employed [2-4]. With the use of the ion-exchange materials proposed in the cited papers in high-performance liquid chromatography, a rapid and satisfactory separation of small amounts (up to 15 mg) of combined gangliosides is ensured. However, in large-scale (1 g and more) fractionation the process lasts several days and a risk of the modification of the native compounds appears.

The authors of the present paper have used for this purpose Spheron CH 300 — an anion-exchange resin containing hydrazine groups produced in Czechoslovakia (Lachema) and have found that preparative chromatography on this sorbent requires a considerably shorter time and permits the isolation of mono-, di-, and trisialogangliosides in satisfactory yield. Thanks to the relatively low basicity of Spheron CH 300, the probability of the appearance of artefacts in the chromatographic

Scientific-Research Laboratory of Biologically Active Substances of Hydrobionts, USSR Ministry of Health, Moscow.

Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 28-30, January-February, 1990. Original article submitted February 10, 1989.

0009-3130/90/2601-0021$12.50