LIPIDS OF *Bryonia alba*

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The lipid composition of the roots of *Bryonia alba* (Cucurbitaceae) have been studied: It consists of fractions of 3-acyloxy-24-alkyl(alkenyl)-cholest-7-enes (I), triacylglycerols (II), 1,2-diacyl-3-monoglycopyranosyl-sn-glycerols (III), 1,2-diacyl-3-diglycopyranosyl-sn-glycerols (IV), 1,3-bis(3-sn-phosphatidyl)glycerols (V), 3-sn-phosphatidylethanolamines (VI), 3-sn-phosphatidylcholines (VII), and methyl esters of fatty acids (VIII). The amount of unsaturated fatty acids in the lipid fractions (I-VIII) is 60-94%, the main component being linolenic acid.

The aim of the present work was a study of the lipids of white bryony (*Bryonia alba* L., Cucurbitaceae). We were impelled to this by information in the literature on the bacteriostatic [1] and bactericidal [2] properties of oil of bryony roots, and also the question of the precursor of the trihydroxyoctadecadienoic acids isolated previously, which are the main components of the fraction exhibiting prostaglandin-like activity [3]. Their precursor may be either free linolenic acid or a lipid containing a hydroxy acid analogous to the phosphatidylcholines of soybeans [4] and to the triglycerides of *Securidaca longipedunculata* [5], containing hydroxylated and epoxidated fatty acids in the bound state.

The results of a structural investigation of individual lipid fractions isolated from a chloroform–methanol extract of bryony roots showed the presence of nine components, the alkaline deacylation of which led to the formation of fatty acids. In this investigation we detected no esters of hydroxy acids. As a result of an analysis of the spectral characteristic of the compounds isolated, of their derivatives, and of the products of their chemical degradation, eight* components of the extract were identified as fractions of 3-acyloxy-24-alkyl-(alkenyl)cholest-7-enes (I), triacylglycerols (II), 1,2-diacyl-3-monoglycopyranosyl-sn-glycerols (III), 1,2-diacyl-3-diglycopyranosyl-sn-glycerols (IV), 1,3-bis(3-sn-phosphatidyl)glycerols (V), 3-sn-phosphatidylethanolamines (VI), 3-sn-phosphatidylcholines (VII), and methyl esters of fatty acids (VIII). It is not excluded that the latter are formed in the extraction process. Below we give information on the composition and amounts (%) of the fatty acids in lipid fractions I-IX:

Acid	Fraction								
	I	II	III	IV	V	VI	VII	VIII	IX
Palmitic	8.1	25.1	4.6	20.3	26.5	36.1	26.5	1.2	67.1
Palmitoleic	1.2	2.7	1.4	3.7	2.7	3.4	2.3	—	2.4
Stearic	—	1.4	1.2	5.0	2.3	2.8	3.9	—	1.9
Oleic	—	9.0	9.0	18.8	9.6	9.7	10.2	0.9	1.7
Linoleic	15.0	12.3	6.8	12.2	12.2	10.0	12.8	0.9	4.7
Linolenic	74.7	48.6	76.4	39.9	45.2	36.1	43.1	98.0	20.9
Others	1.0	0.9	0.6	0.1	1.5	1.9	1.2	—	2.0

the composition and amount (%) of the carbohydrates in the glycolipid fractions III and IV:

Hexapyranose	Fractions	
	III	IV
D-Glucopyranose	6.0	27.2
D-Galactopyranose	94.0	72.8

*The structure of the most polar phospholipid fraction with an acidic nature (IX) present in minor amounts will be reported in a separate communication.

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and the composition and amounts of the phytosterols in the fraction consisting of their esters (I):

	%
24-Methylcholest-7-en-3β-ol	3
24-Ethylcholest-7-en-3β-ol	40
24-Methylidenecholest-7-en-3β-ol	3
24-Ethylidenecholest-7-en-3β-ol	47
24-Ethylidene-4-methylcholest-7-en-3β-ol	5
24-Ethyl-4-methylcholest-7-en-3β-ol	2

The phytosterols from the fraction consisting of their esters (I) were practically identical in composition with the fraction of free 24-alkyl(alkenyl)cholest-2-en-3β-ols and their β-D-glycopyranosides that we have described previously [6].

The lipid composition of the roots of *B. alba* is, therefore, typical for nonphotosynthesizing plant tissues [7-9]. This also relates, in particular, to the fatty acid composition where, however, a comparatively high content of linolenic acid, reaching 74-77% in the fractions of phytosterol esters (I) and of monoglycopyranosyldiglycerols (III), must be noted.

As is well known, linolenic acid suppresses the growth of *Staphyloccoccus aureus* [10], which is possibly an explanation, if only a partial one, of the bacteriostatic action of bryony in relation to this species of microorganisms [1]. It is also obvious that the trihydroxydecadienoic acids [3] are formed from free linolenic acid and not as the result of the hydrolysis of lipids.

EXPERIMENTAL

The methods of extracting the roots of *B. alba* and of preparing the silica gel for column chromatography and thin-layer chromatography (TLC) have been described in one of our proceding communications [1]. For TLC we used the following solvent mixtures: 1) hexane–ether–acetic acid (98:2:0.2), 2) (80:2:1), and 3) (85:15:1); 4) chloroform–methanol–water (60:10:1), 5) (60:2:1), and 6) (60:20:2); 7) chloroform–benzene–ethylacetate (3:2:1); and 8) chloroform–methanol–concentrated ammonia (70:20:2).

The substances were detectd on the chromtogram with: a) 50% H_2SO_4 followed by carbonization at 200-250°C; b) a 0.5% solution of vanillin in 15% H_3PO_4 followed by heating the plates at 100°C for 5 min; c) a 0.2% solution of morin in ethanol (spots observed in UV light); d) a 0.3% solution of ninhydrin in ethanol followed by heating the chromatograms at 100-110°C; e) the reagent for phospholipids [11]; f) the α-naphthol reagent [12]; g) the Dragendorf reagent [13]; h) the periodate–Schiff reagent [14]; and i) iodine vapor.

Nonlipid impurities were eliminated on Sephadex LH-20 [15]. Ion-exchange chromatography was performed on a column (2.5 × 30 cm) of DEAE-cellulose in the AcO^- form [16]. Severe acid hydrolysis and the acid and alkaline methanolysis of the lipid were carried out by the methods generally adopted [17]. Water-soluble products of alkaline methanolysis and of acid hydrolysis of the polar lipids was identified on paper (PC) in the following solvent systems: 9) phenol–water–acetic acid–ethanol (50:22:3:3); 10) isopropanol–water–concentrated ammonia (7:2:1); and 11) butanol–benzene–pyridine–water (51:3:3), upper phase. On PC, the substances were revealed by the molybdate reagent [13], the ninhydrin reagent, the Dragendorf reagent, and analine phthalate, and by the periodate–benzidine method. The amount of lipid phosphorus was determined by Gerlack's method [18] with the aid of an SF-16 spectrophotometer, and sugars by the GLC method (Chrom-41, Czechoslovakia) in the form of the trimethylsilyl (TMS) ethers of their methyl glycosides on a column (1200 × 3 mm) containing 3% Se-30 on Gas-Chrom Q (125-150 μ) at 170°C, using mannitol as internal standard and helium as the carrier gas. The phytosterol fraction was analyzed in the form of the TMS derivatives on the same column at 250°C [6]. The fatty acid methyl esters were analyzed by GLC on a column containing 8% of PEGA on Chromosorb W (100-125 μ) at 170°C using methyl heptadecanoate as internal standard. The mass-spectrometric analysis (MKh-1320; 70 eV) of the glycolipids was carried out in the form of their acetates and deuteroacetates [19, 20], and of the glycerophospholipids (V) in the form of the TMS derivative of the product of its alkaline deacylation [21]. The IR (UR-20) and PMR (Varian-60T) spectra of fractions I-VIII agreed with those given in the literature [17, 22].

Isolation of the Lipid Fractions of Bryony. A chloroform–methanolic extract freed from nonlipid impurities was evaporated and the residue (22 g) was deposited on column (A) containing 100 g of silica gel (100-150 μ) which had been activated at 180°C for 7 h.

The substances were eluted successively with benzene, benzene–chloroform (4:1), chloroform, chloroform–methanol (24:1), (19:1), (14:1), (9:1), (3:1), (2:1), and (1:4), and methanol (1000 ml each). Fractions with a volume of 20 ml were collected and were analyzed by TLC in systems 1-8 with revealing agents a-h.

The 3-Acyloxy-24-alkyl(alkenyl)cholest-7-ene Fractions (I). The first 500 ml of benzene eluted from column (A) 205 mg of acylphytosterols (I), revealed in system 1 by reagents a-c in the form of a homogeneous spot with R_f 0.64.

Fatty Acid Methyl Ester Fraction (VIII). The next 500 ml of benzene (fraction 25-50) eluted from column (A) 76.2 mg of a mixture of acylphytosterols (I) with a more polar fraction (IX). The mixture was rechromatographed on a column containing 7 g of silica gel with elution by benzene and the collection of 2-ml fractions. As a result 34 mg of the fatty acid methyl ester fraction (IX) was obtained with R_f 0.52 (1: a-c).*

Triacylglycerol Fraction (II). The benzene–chloroform (4:1) system eluted from column (A) 445 mg of substances containing fraction II, which was separated by preparative TLC in system 2. This gave 334 mg of triacylglycerols with R_f 0.67 (3: a-c).

1,2-Diacyl-3-monoglycosyl-sn-glycerol Fraction (III). The first 600 ml of chloroform–methanol (24:1) eluted from column (A) 4394 mg of a mixture of substances containing fraction III. The mixture was subjected to rechromatography on a column of silica gel (23 g, 80-100 μ) impregnated with 0.5 g of H_3BO_4 with elution by chloroform and chloroform–methanol (400:1, 200:1, and 130:1), 20-ml fractions being collected. The (200:2) system eluted 207 mg of a mixture of substances which was separated further by preparative TLC in system 4. The final purification of the 80 mg of a mixture of substances obtained in this way and containing as the main component fraction III was carried out with the aid of partition column chromatography on Sephadex LH-20. For this purpose, 10 ml of the sorbent was impregnated with a few milliliters of the lower phase of system 12 [methanol–water–chloroform–t-butanol (120:180:15:15)] and was suspended in a mixture of the same solvent taken in a ratio of 174:126:15:15 (system 13). The suspension was filled into a column and washed with 50 ml of system 13, and then the mixture to be separated in 1 ml of the above-mentioned system 13 was deposited on it and the column was eluted with the same mixture, 10-ml fractions being collected and monitored with the aid of TLC in system 4. As a result, 16 mg of pure fraction III was obtained with R_f 0.6 (4; a-c, f, h). Fatty acids: hexoses - 2:1.

Mass spectrum of the tetraacetate of fraction III [R_f 0.88 (6; a-c)] m/e: 331, 169, 573, 575, 595, 597, 599, 601, 603. Mass spectrum of the [D_{12}]tetraacetate of III, m/e: 343, 172, 573, 575, 595, 597, 601, 603.

1,2-Diacyl-3-diglycosyl-sn-glycerol Fraction (IV). The first 600 ml of the chloroform–methanol (1:1) system eluted 231.8 mg of a mixture of substances containing fraction IV, which was purified further by column chromatography on DEAE-cellulose [15], by preparative TLC in system 8, and by partition chromatography on a column of Sephadex LH-20 using as eluents in this process successively the upper phases of system 13 and of system 14 – methanol–water–chloroform–t-butanol (150:150:15:15) – and system 12. As a result, 24.6 mg of fraction 4 was obtained with R_f 0.55 (5: a-c, h, f). Fatty acid: hexoses = 1:1.

Mass spectrum of the heptaacetate of IV [R_f 0.7 (6; a-c)], m/e: 619, 331, 169, 573, 575, 595, 547, 594, 601, 603. Mass spectrum of the [D_{27}]hepta-acetate of III, m/e: 640, 343, 172, 573, 575, 595, 597, 599, 601, 603.

1,3-Bis-(3-sn-phosphatidyl)glycerols Fraction (V). The chloroform–methanol (3:1) system eluted from column (A) 411 mg of a mixture of substances containing fraction V which was further purified on DEAE-cellulose, elution by the ammoniacal system giving 96.8 mg of fraction V with R_f 0.70 (4: a-c, e). Ratio of fatty acids to phosphorus: 4:1. The mass spectrum of the TMS ether of the glycerophosphate corresponded to that of pentakis (trimethylsilyl)-sn-glycerophosphorylglycerol, m/e: 591, 516, 503, 461, 445, 351, 299, 73.

3-sn-Phosphatidylethanolamine Fraction (VI). The last 400 ml of the chloroform–methanol (1:1) system eluted from column (A) 137 mg of a mixture of substances containing fraction VI, which was additionally purified by chromatography on DEAE-cellulose [16] [chloroform–methanol (7:3) system] and preparative TLC in system 8. As a result, 10.8 mg of fraction V was obtained with R_f 0.55 (5; a-e), not differing in its chromatographic mobility in systems 4-6 and

*Here and below the words "solvent systems" and "revealing agent" are omitted.

8 from rat liver phosphatidylethanolamine [23]. Glycerol and ethanolamine were identified in the water-soluble products of the deacylation of VI. The product of alkaline-deacylation of VI was identical with glycerylphosphorylethanolamine obtained from rat liver. The fatty acids:phosphorus ratio was 2:1.

3-sn-Phosphatidylcholine Fraction (VII). Methanol eluted from column (A) fraction VII (39 mg), appearing in the form of a homogeneous spot on TLC in systems 4-6 and 8 (revealing agents a-c, e, g) and not differing in its R_f values from rat liver phosphatidylcholine [23]. Glycerol and choline were identified in the water-soluble products of the severe acid hydrolysis of VII. The product of the alkaline deacylation of VII was identical with the glycerylphosphorylcholine obtained from rat liver phosphatidylcholine. The fatty acids:phosphorus ratio was 2:1.

Fraction IX. A mixture of chloroform and methanol (1:4) eluted from column (A) 128 mg of a mixture containing fraction IX, which was additionally purified by chromatography on DEAE-cellulose (ammoniacal system) and by preparative TLC in system 6. This gave 9.6 mg of fraction IX with R_f 0.24 (6; a-c, e).

The mass spectra were obtained by R. G. Grigoryan.

SUMMARY

Extracts of the roots of *Bryonia alba* L. (Cucurbitaceae) (white bryony) contain as the main lipid fractions 3-acyloxy-24-alkyl(alkenyl)cholest-7-enes (I), triacylglycerols (II), 1,2-diacyl-3-monoglycopyrano-sn-glycerols (III), 1,2-diacyl-3-diglycopyranosyl-sn-glycerols (IV), 1,3-bis(3-sn-phosphatidyl)glycerols (V), 3-sn-phosphatidylcholines (VII), and fatty acid methyl esters (VIII). The proportion of unsaturated fatty acids in the lipid fractions I-VIII is 60-94%, the main component being linolenic acid.

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