The rate of elution was such as to give a 15-ml fraction every hour, and the fractions were analyzed by the phenol–sulfuric acid method and by their absorption at 280 nm. The results are given in Fig. 3.

Chromatography on Cation-Exchange Resin. The total carbohydrate-protein fraction (160 mg) was deposited on a column (40 × 1.5 cm). Elution was performed with phosphate buffer, pH 6, and then with phosphate buffer, pH 6, in 1 M NaCl solution. The fractions were analyzed as described above. The results are given in Fig. 4.

The determination of the hemagglutinating activity of the initial carbohydrate-protein compounds of maize stigmata and of the fractions isolated by chromatography on various adsorbents were determined in a dilution series by the method of Dubois et al. [3].

SUMMARY

It has been shown that the phytohemagglutinins of maize stigmata consist of carbohydrate-protein compounds and that the carbohydrate and protein components have a fairly strong bond. The nature of this bond remains unknown.

The phytohemagglutins isolated possess a well-defined agglutinating and mitotic activity.

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A NEW PREPARATIVE METHOD FOR ISOLATING CEREBROSIDES AND SPHINOGOSINE BASES FROM CATTLE BRAIN

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The majority of preparative methods for isolating the cerebrosides of the brain described hitherto are based on the poor solubility of these compounds in ether and cold ethanol and their good solubility in hot ethanol [1, 2]. For the final purification of the cerebrosides, crystallization from glacial acetic acid has been recommended [3]. The isolation of cerebroside fractions has also been performed by the chromatography of the total lipids on silica gel or on alumina [4-6]. Another method for the preparative isolation of the cerebrosides of the brain [7, 8] is based on the elimination of the bulk of the phospholipids by precipitation with acetone and filtration through a column packed with Florisil. The acidic and basic phospholipids remaining in the extract were removed by ion-exchange chromatography on a column containing a mixture of cation- and anion-exchange resins. The final stage of purification of the cerebrosides was crystallization from a mixture of chloroform and methanol [7, 8]. Finally, the chromatography of the total lipids of the brain on DEAE-cellulose and on magnesium silicate has been recommended for the isolation of the cerebrosides [9, 10]. The best of the preparative methods described in the literature is that of Wells and Dittmer [11], which successfully combines the preliminary purification of the total sphingolipids by extraction with cold ether, the cleavage of the phospholipids by mild alkaline hydrolysis [12], and column chromatography on silica gel, leading to the isolation of the three main types of brain sphinogolipids: cerebrosides, sulfatides, and sphingomyelin.

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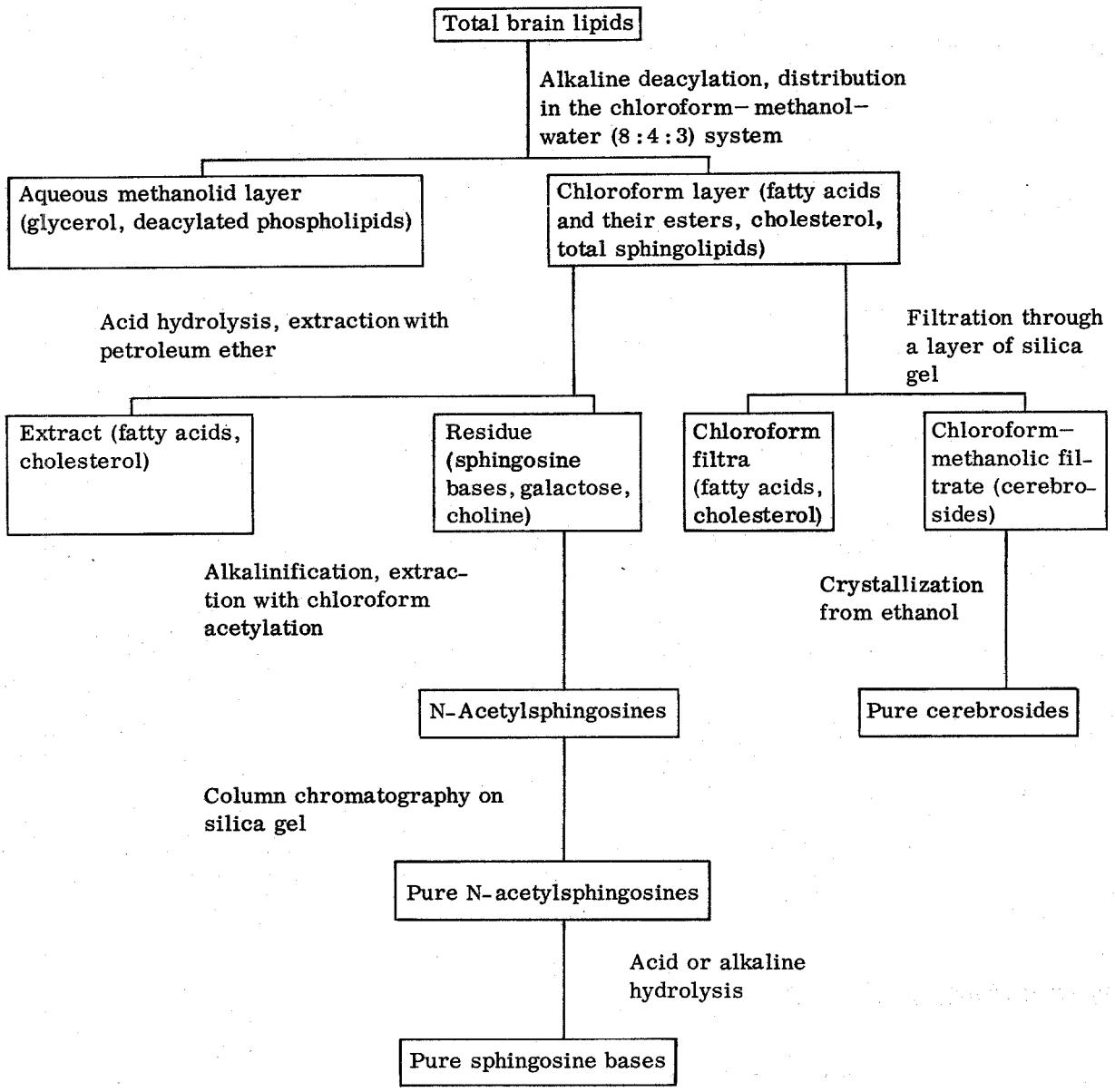
Scheme 1



The isolation of the unsaponified sphingosine bases from the products of acid hydrolysis of brain cerebrosides has been described by various authors [13–15]. In view of the fact that the fractionation of the sulfates of the sphingosine bases by their precipitation with methanol did not lead to individual substances, Carter et al. [16] isolated C_{18}-sphingosine and C_{18}-dihydrosphingosine from brain cerebrosides in the form of the corresponding triacetates. The use of column chromatography to isolate sphingosine bases has been described by Karlsson [17]. Unfortunately, this method cannot be recommended for preparative work, since more than 50% of the sphingosines deposited on the silica gel cannot be eluted from the column.

It can be seen from the brief review given that the preparative methods for isolating cerebrosides and sphingosine bases are in need of further improvement. The main defect of the methods of isolating cerebrosides described in the literature is that they include complex procedures for freeing them from phospholipids or stepwise extraction with solvents, which does not lead to the complete separation of the lipids and is connected with the risk of contaminating the fractions isolated with products of the oxidation of unsaturated fatty acids.

In order to create a simple method for isolating cerebrosides from cattle brain, we have proposed to isolate these compounds without the use of column chromatography and prolonged stepwise extraction with solvents

in the way shown in Scheme 1. The total brain lipids were subjected to mild alkaline decylation and the reaction products were distributed between chloroform and aqueous methanol. The aqueous methanolic layer, containing glycerol and deacylated phospholipids, was discarded and the chloroform layer was rapidly filtered through a layer of silica gel. The filtrate contained the fatty acids (and their methyl esters) and cholesterol. Subsequent washing of the adsorbent with chloroform–methanol (3 : 2) gave a cerebroside fraction containing small amounts of cholesterol as impurity. Under these conditions the whole of the sphingomyelin remained on the silica gel.

The isolation of the C_{18}-sphingosines was also made directly from the total deacylated brain lipids (see Scheme 1). The total sphingolipids present together with the fatty acids and cholesterol in the chloroform solution of the deacylation products were subjected to acid hydrolysis [18]. The hydrolyzate was extracted with petroleum ether, and the residue was made alkaline and was reextracted with chloroform (see Scheme 1). The unpurified sphingosine bases obtained by the evaporation of the chloroform extract were converted into N-acetylsphingosines by the method of Klenk and Diebold [14]. These compounds were purified by column chromatography on silica gel. The pure C_{18}-sphingosines were regenerated by the alkaline or acid hydrolysis of the N-acetate. A high degree of purity of the preparations was ensured by the column chromatography of the N-acetyl derivatives of the sphingosines on silica gel which, unlike the column chromatography of the sphingosines themselves, is not associated with losses of the substances being isolated.

EXPERIMENTAL

Extraction of the Total Lipids. Cattle brain (3.0 kg) was freed from membranes and blood vessels and was homogenized with 3 liters of chloroform–methanol (2 : 1). The homogenate was treated with another 3 liters of the same mixture of solvents, and the contents of the flask were carefully stirred and were left overnight at 0 to +5°C. The comminuted tissue was separated from the chloroform–methanol solutions by centrifuging (4000 rpm, 30 min) and was reextracted with another 3 liters of chloroform–methanol (2 : 1). The combined extract was diluted with an amount of water such that the ratio of the aqueous methanolic and chloroform layers was approximately 2 : 3. The chloroform layer was separated off and evaporated in vacuum. The yield of total lipids was 480 g.

Alkaline Deacylation of the Total Brain Lipids. With stirring, 2 liters of a 0.1 N solution of potassium hydroxide in 98% methanol were added to a solution of 480 g of the total lipids in 250 ml of chloroform. The reaction mixture was kept at 40°C for 2 h and was then neutralized with a 1 N solution of hydrochloric acid in methanol to pH 7.5 and was diluted with 3.25 liters of chloroform and 1.5 liters of water and left at room temperature until the layers had separated completely. The chloroform layer was separated off and the aqueous methanolic layer was extracted with chloroform (3 × 1 liter). The combined chloroform extract was evaporated in vacuum. The residue, consisting of fatty acids, cholesterol, cerebrosides, and sphingomyelin ("unsaponified sphingolipid" fraction), weighed about 260 g.

Isolation of the Cerebrosides. The unpurified sphingolipids isolated by the method described above (130 g) were dissolved in 1 liter of chloroform and the solution was filtered in vacuum through 1 kg of KSK silica gel (75-100 mesh) placed in a Büchner funnel with a glass filter having a diameter of 25 cm. The layer of silica gel was washed with 5 liters of chloroform. The chloroform filtrate was discarded and the adsorbent was rapidly washed with 5 liters of chloroform–methanol (3:2). The chloroform–methanol filtrate was analyzed by thin-layer chromatography (TLC) in the chloroform–methanol–water (65 : 25 : 4) system and was evaporated in vacuum. The residue (40 g), consisting of the cerebrosides with a small amount of cholesterol and more polar impurities, was crystallized three times from ethanol. The yield of chromatographically pure cerebrosides was 16 g; the ratio of the sphingosine bases (determined by the method of Lauter and Trams [19]), fatty acids, and galactose was 1:1:1. The fatty-acid composition of the cerebrosides is given in Table 1.

Acid Hydrolysis of the Unsaponified Brain Sphingolipids. The unsaponified sphingolipids obtained by the alkaline deacylation of the total lipids of brain as described above (130 g) were extracted with 2 liters of a solution consisting of methanol, concentrated hydrochloric acid, and water (82 : 8.6 : 9.4) [19]. The reaction mixture was boiled for 18 h, cooled, and extracted with petroleum ether (4 × 500 ml). The aqueous methanolic solution was made alkaline with 49% potassium hydroxide to pH 13 and was extracted with chloroform (1 × 2 liters and 2 × 500 ml). The chloroform extract was evaporated in vacuum to a volume of 500-600 ml and was washed with water to neutrality. The washed extract was evaporated. The yield of unpurified sphingosine bases was 20 g.

Purification of the Sphingosine Bases via the N-Acetylsphingosines. A solution of 20 g of the unpurified sphingosine bases in 500 ml of ether was treated with 150 ml of water and 15 ml of acetic anhydride. The reac-

TABLE 1. Fatty-Acid Composition of the Cerebrosides of Cattle Brain

Symbol	Fatty acid	α-Hydroxy acid	Lit. information	
			fatty acid	α-hydroxy acid
16:0	—	—	0,3	0,2
16:1	—	—	0,9	—
18:0	2,8	9,8	13,0	19,6
18:1	—	—	0,4	—
19:0	1,2	—	—	—
20:0	—	—	0,7	0,3
20:1	—	—	0,1	0,8
22:0	3,4	4,7	3,9	4,5
22:1	—	—	0,3	—
23:0	5,3	7,7	5,8	8,2
23:1	—	—	0,6	0,3
24:0	25,8	36,3	22,2	32,9
24:1	33,7	14,5	24,3	7,8
25:0	16,0	10,0	13,8	12,9
25:1				
26:0	11,8	17,0	12,1	10,0
26:1				
27:0				
27:1	—	—	1,0	1,9
28:0				
28:1	—	—	0,6	0,7

Note. The methyl esters of the fatty acids and the methyl esters of the α-acetoxy acids were analyzed by gas–liquid chromatography. The column had dimensions of 1500 × 4 mm with 3% of silicone SE-30 on Chromosorb W (80-100 mesh). The temperature was programmed from 160 to 250°C at the rate of 4 deg/min. The instrument was a Pye-Unicam series 104, model 64, chromatograph.

tion mixture was shaken for 5 min, the ether layer was separated off, and the aqueous layer was extracted with ether (2 × 200 ml). The combined ethereal extract was evaporated to dryness. The residue (9 g) was chromatographed on a column with dimensions of 500 × 40 mm containing 250 g of KSK silica gel (80-100 mesh). The substances were eluted successively with 1 liter of chloroform and 1 liter of 2% methanol in chloroform, 40-ml fractions being collected. The fractions containing, according to TLC [17], pure N-acetylsphingosines [Rf 0.7 in the chloroform–methanol–water (65 : 25 : 4) system; revealing agent a 25% solution of sulfuric acid in methanol] were combined and evaporated. The yield of chromatographically pure N-acetylsphingosine was 5 g.

Alkaline Hydrolysis of Brain N-Acetylsphingosines. The N-acetylsphingosines (55 mg) were boiled with 5 ml of a 1 N solution of caustic soda in 90% methanol for 3 h [21]. The reaction products were distributed in the chloroform–methanol–water (8 : 4 : 3) system. The chloroform layer contained the sphingosine bases. Yield: 48 mg. A single spot with R_f 0.5 was observed.

SUMMARY

A new preparative method for the isolation of the cerebrosides and sphingosine bases from cattle brain is proposed.

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A STUDY OF THE PHOSPHOLIPIDS OF KENAF

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In the present paper we give the results of an investigation of the structure of the main components of the total phospholipids of kenaf of variety Kuban'-333, grown in the Uzbek experimental station for bast crops [1]. The previously ground seed kernels were defatted with acetone. The total phospholipids were extracted by Folch's method [2] and were freed from carbohydrates by filtration through Molselekt G-25 [3]. The combined phospholipids were separated into ethanol-soluble and ethanol-insoluble fractions and each separately was passed through a column of silica gel. The neutral lipids were eluted with chloroform, and the phospholipids with mixtures of chloroform and methanol of increasing polarity. The final purification of the main fractions (phosphatidylcholines, phosphatidylethanolamines, and phosphatidylinositols) and the two minor fractions (X_1 and X_2) of phospholipids was performed by preparative chromatography in a thin layer of silica gel in solvents systems 1 and 2. This gave homogeneous fractions with the following constants:

Phosphatidylcholines (PCs): molar ratio N/P = 0.9; $[\alpha]_D^{20} + 6.3^0$ (c 2.0; $CHCl_3$);

Phosphatidylinositols (PIs): no N; P 3.3%;

Phosphatidylethanolamines (PEs): molar ratio N/P = 1.1.

The IR spectra of the fractions obtained coincided with those of glycerophospholipids [4, 5]. To confirm the structure of the main components, we carried out acid hydrolysis. The following products were found in the hydrolyzate: from the PCs, choline; from the PIs, inositol; from the PEs, ethanolamine; and, in the hydrolyzates of all the fractions, glycerol. The amines and polyols were identified from their Rf values with markers in a thin layer of silica gel in systems 3 and 4. The revealing agents were a solution of ninhydrin, Dragendorff's reagent, a 1% solution of potassium metaperiodate, and benzidine solution. To determine their fatty-acid compositions, the phospholipids were subjected to alkaline hydrolysis. The fatty acids were analyzed in the form of methyl esters by GLC. The results of the analysis of the fatty acid compositions of the total phospholipids and of the individual fractions are given in Table 1. The fatty acids of the total phospholipid and its components are similar qualitatively, but the ratios of the individual acids differ. In the individual fractions of the phospholipids of kenaf seeds, from 21.4 to 37% of saturated fatty acids containing mainly palmitic acid and from 63 to 78.6% of unsaturated acids with a predominance of linoleic acid are found. The degree of unsaturation of the phospholipid molecules rises in the following sequence: PIs → X_1-PL → PEs → PCs → X_2-PL.

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