which moves toward the anode (Table I). Thus, a fraction resembling electrophoretically the supernatant form is still present up to this step. However, if the enzyme from step 3 (procedure B) is chromatographed on Amberlite XE-64, 98 % of the MDH appears as a single peak. The total recovery of MDH is again over 90 %. When eluted from the resin, the enzyme is shown to be electrophoretically homogeneous. Thus, both treatment with butanol and chromatography on Amberlite XE-64 are necessary for the complete conversion of the enzyme to a single form.

The conversion of the "supernatant" form (procedure C) follows the same pattern. Electrophoresis of the butanol-treated "supernatant" shows that 30–40 % has been converted to the "mitochondrial" form. However, the butanol-treated "supernatant" binds to Amberlite XE-64, and the eluted enzyme moves toward the cathode (Table I).

The well known ability of butanol to break up lipid complexes suggests a possible explanation of the results. The "supernatant" form may be a lipoprotein complex of MDH. Treatment with butanol dissociates part of the complex. The remaining complex may be sensitized enough so that the resin can now resolve it completely.

The authors wish to acknowledge the technical assistance of Barbara Cunningham. This investigation was supported by a research grant (C-1856) from the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

Division of Biochemistry, Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Ill. (U.S.A.)

A. J. SOPHIANOPOULOS
CARL S. VESTLING

[1] G. S. CHRISTIE AND J. D. JUDAH, *Proc. Roy. Soc. (London)*, *B* 141 (1953) 420.
[2] C. J. R. THORNE, *Biochim. Biophys. Acta*, 42 (1960) 175.
[3] C. S. VESTLING, T. HUANG, F. C. GRIMM, A. C. BOYER, A. K. KHETTRY AND R. H. HOOK, *Federation Proc.*, 18 (1959) 343.
[4] A. J. SOPHIANOPOULOS AND C. S. VESTLING, *Abstr. 137th Meeting Am. Chem. Soc., Cleveland, Ohio, April 1960*, p. 43C.
[5] T. WIELAND, G. PFLEIDERER, I. HAIPT AND W. WORNER, *Biochem. Z.*, 332 (1959) 1.
[6] W. C. SCHNEIDER, *J. Biol. Chem.*, 176 (1948) 259.

Received October 4th, 1960

Biochim. Biophys. Acta, 45 (1960) 400–402

The isolation and structure of cerebrosides from wheat flour

Our interest in the chemistry and biochemistry of plant lipids led us to a study of the benzene-extractable lipids of wheat flour[1], from which we have now been able to isolate and characterize a mixture of cerebrosides.

Unbleached wheat flour was extracted with benzene. Addition of acetone caused precipitation of an insoluble fraction. The benzene-acetone-soluble material was distributed between *n*-heptane and methanol and the methanol-soluble lipids were purified using a silicic acid column. The fraction eluted with chloroform–methanol (94:6) was rich in cerebrosides, as established by color tests, elementary analyses and infrared spectrum.

Acid hydrolysis of this material and extraction of the basic components by the method of CARTER *et al.*[2] gave a mixture of long-chain bases, which were purified by

silicic acid chromatography. The fraction eluted with chloroform–methanol (1:4) was ninhydrin-positive and in the infrared had a pattern very similar to that of brain sphingosine. The maximum at 970 cm^{-1} was observed, indicating the presence of a *trans*-double bond. Periodate oxidation of this material, followed by vapor-phase chromatography of the long-chain aldehydes by the method of SWEELEY AND MOSCATELLI[3], gave the results reported in Table I. On the basis of the retention time of aldehydes from the unknown mixture compared with these of known standards,

TABLE I

THE RETENTION TIME OF ALDEHYDES ISOLATED AFTER PERIODATE OXIDATION OF THE CORRESPONDING BASE*

	Base(s)	Peak Retention time (min) of aldehyde				
		a	*b*	*c*	*d*	*e*
1	From wheat-flour cerebrosides	6.6	7.2	8.2	9.3	—
2	From wheat-flour cerebrosides** (hydrogenated aldehydes)	6.6	—	8.2	—	—
3	Dihydrosphingosine	—	—	8.2	—	—
4	Sphingosine	—	—	—	—	13.2
5	Phytosphingosine	6.8	—	—	—	—
6	Dehydrophytosphingosine	—	7.4	—	—	—
7	Mixture of 3, 4, 5, and 6	6.8	7.4	8.5	—	13.2

* Column as described in ref. 3. Temperature, 199°; 12 lb/in^2.

** Unsaturated aldehydes in mixture of aldehydes, were hydrogenated to fully saturated aldehydes, as described in ref. 3.

we were able to identify the presence of following bases: (1) phytosphingosine (peak a); (2) dehydrophytosphingosine* (peak b); (3) dihydrosphingosine (peak c); (4) a new, long-chain base, with isolated double bond (peak d). To this base we have assigned the formula:

$$CH_3\cdot(CH_2)_x\cdot CH{=}CH\cdot(CH_2)_{12-x}\cdot \underset{\displaystyle OH}{\underset{|}{CH}}\cdot \underset{\displaystyle NH_2}{\underset{|}{CH}}\cdot CH_2OH$$

This structure was confirmed by the following studies. The crude cerebroside fraction, isolated as described above, was hydrolyzed with 1 *N* NaOH at 37°, fatty acids were removed using Dowex-2 (OH-phase) and Amberlite-MB-3 columns, and the cerebroside fraction was purified by crystallization, yielding a white powder with elementary analysis: C, 66.72; H, 11.09; N, 2.07 %. The infrared spectrum of this sample showed the presence of hydroxyl-, amide- and *trans*-double-bond absorptions. The material was oxidized with sodium periodate in aqueous methanol, followed by $NaBH_4$ reduction and then by mild acidic hydrolysis (0.1 *N* HCl at room temperature). Chloroform extraction of the reaction mixture gave ceramides, free of phytosphingosine and dehydrophytosphingosine-containing material. After purification on silicic acid, a substance with C, 73.92; H, 12.02; N, 2.02 %, and an infrared spectrum typical for

* Isolated from soybean in this laboratory and tentatively assigned the structure

$$CH_3\cdot(CH_2)_8\cdot CH{=}CH\cdot(CH_2)_3\cdot \underset{\displaystyle OH}{\underset{|}{CH}}\cdot \underset{\displaystyle OH}{\underset{|}{CH}}\cdot \underset{\displaystyle NH_2}{\underset{|}{CH}}\cdot CH_2OH$$

ceramide, was obtained. A double bond at 970 cm^{-1} was detected. Periodate consumption of this material was zero. On the assumption that this ceramide was a mixture of dihydrosphingosine and the new ceramide containing the long-chain base, catalytic hydrogenation (in presence of Adams catalyst) was performed. A white powder with m.p. 109–114° was obtained with C, 73.77; H, 12.42; N, 2.25 % (calcd. for hydroxystearoyl-dihydrosphingosine: C, 74.04; H, 12.60; N, 2.40 %).

Alkaline hydrolysis of the reduced ceramide in aqueous dioxane followed by acidification and extraction with chloroform gave a mixture of fatty acids, from which using silicic acid chromatography, α-hydroxystearic acid was isolated as the major component. The melting point (93–94.5°), elementary analysis (C, 72.50; H, 12.15; neutralization equivalent, 296; calcd.: C, 71.94; H, 12.08; neutralization equivalent, 300) and infrared spectrum confirmed this structure, although the presence of other fatty acids was also observed.

From aqueous solution, after adjusting the pH to 12, the long-chain base was extracted with ether. The white powder obtained had the elementary composition: C, 71.81; H, 12,67; N, 4.48 %. The periodate oxidation of this base yielded formic acid and formaldehyde (isolated in form of dimedone derivative in 49 % yield of theory). In addition, the free base was converted to the tribenzoyl-derivative, which after recrystallization had m.p. 146–148.5° (undepressed with authentic tribenzoyl-dihydrosphingosine), infrared spectrum identical with authentic tribenzoyldihydrosphingosine and with elementary analysis: C, 76.29; H, 8.62; N, 2.31 % (calcd.: C, 76.31; H, 8.38, N, 2.28 %).

The sugar component of this cerebroside was identified after acidic hydrolysis as glucose by paper chromatographic technique as well as by the method of ROSENBERG AND CHARGAFF[4].

The discovery in plant sources of dihydrosphingosine and of an isomer of sphingosine represents a novel development in the knowledge of long-chain bases.

Full details of these investigations will be published elsewhere. The authors wish to express their appreciation to the Procter and Gamble Co. for a research grant and for a supply of wheat-flour lipids.

H. E. CARTER
R. A. HENDRY
S. NOJIMA
N. Z. STANACEV

The W. A. Noyes Chemical Laboratory, University of Illinois, Urbana, Ill. (U.S.A.)

[1] H. E. CARTER, R. H. MCCLUER AND D. E. SLIFER, *J. Am. Chem. Soc.*, 78 (1956) 3735.
[2] H. E. CARTER, W. P. NORRIS, F. J. GLICK, G. E. PHILLIPS AND R. HARRIS, *J. Biol. Chem.*, 170 (1947) 269.
[3] C. C. SWEELEY AND E. A. MOSCATELLI, *J. Lipid Research*, 1 (1959) 40.
[4] A. ROSENBERG AND E. CHARGAFF, *J. Biol. Chem.*, 232 (1958) 1031.

Received October 10th, 1960