macromolecular glycolipids[2-4] which contain neuraminic acid, sphingosine,[5] fatty acid (stearic),[6] hexoses, and a hexosamine. Earlier studies have demonstrated the hexoses to be exclusively glucose and galactose and the hexosamine to be exclusively galactosamine.[4,8] However, little is known of the manner in which these constituents are linked. A cerebroside frequently has been postulated to be the basic unit in the structure of the gangliosides. The present communication reports the successful hydrolytic degradation of a purified preparation of brain ganglioside to a glucocerebroside.

Bovine brain ganglioside was prepared as an ash-free fraction (I) homogeneous by electrophoretic and ultracentrifuge studies (minimal molecular weight 250,000), completely water-soluble, free of phosphatides (P 0.07%) and of dialyzable contaminants.[4,7,8] (I) contains nitrogen 2.9%, hexose 24.0% (expressed as galactose), and galactosamine 10.0%.

Optimal conditions for the isolation of the constituent glucocerebroside have been defined as follows: a 2% aqueous solution of I is autohydrolyzed at 100° for a critical time interval of 25 minutes, then exhaustively dialyzed. The dialyzate contains 66% of the neuraminic acid and 5.2% of the galactosamine of I. The non-dialyzable residue (II) accounts for 80% by weight of I. II is hydrolyzed with 0.09 *N* HCl (sealed tube, 100°, 16 hours) then exhaustively dialyzed. The resulting non-dialyzable residue (III), still water-soluble, is further hydrolyzed (0.09 *N* HCl, sealed tube, 100°, 5 hours). The hydrolyzate, which now contains a brown insoluble material (IV), is exhaustively dialyzed. IV is separated by centrifugation, dried *in vacuo*, and taken up in clear solution with warm methanol:chloroform (9:1). 12% of IV remains as an insoluble black residue which is discarded. When this solution (40 mg./cc.) is allowed to cool to room temperature, birefringent spherocrystals (MCS) are formed. To date, yields of MCS represent between 26 and 30% by weight of I. MCS crystals contain no neuraminic acid and no galactosamine. The analysis, compared with that expected for a glucostearocerebroside crystallized with one mole of water,[9] is

	N, %	C, %	H, %	Hexose (as glucose), %
Found	1.86	67.13	10.95	21.8
Calcd. for $C_{42}H_{81}O_8N(H_2O)$	1.88	67.65	11.14	22.0

MCS is insoluble in water, slightly soluble in pure chloroform, very soluble in mixtures of chloroform:methanol. MCS shows slight liquefication at 165°, then melts sharply between 172–174°. The iodine number is 23.2. In chloroform:methanol (2:1) (1 mg./cc.), it is levorotatory ($[\alpha]^{20}D$ −2.08°). The infrared spectrum is consistent with that of a cerebroside.[10] A sample of crystalline MCS gave these spacings by X-ray diffraction[11]: 2.41, 4.10, 9.1, 10.4, 15.6, 21.1, 30.6, 49.2, 55.5, and 63.5 Å.

Hydrolysis of MCS in *N* HCl (sealed tube, 100°, 16 hours) liberated the hexose quantitatively. This was shown to be exclusively glucose by paper chromatography. The remaining water-insoluble residue contained 2.3% nitrogen, as expected for a ceramide. It is yet to be established whether the base is sphingosine or dihydrosphingosine.[12]

The melting point, specific rotation, iodine number and the elementary analysis, distinguish MCS from the previously described cerebrosides phrenosine, cerasine and nervone.[13] A glucostearocerebroside has not been described previously. Its presence as the basic constituent of brain ganglioside would be consistent with the demonstration by Klenk that the constituent fatty acid of brain ganglioside is stearic acid.[6] The isolation of this glucocerebroside is of interest with regard to the over-all structure and biosynthesis of the gangliosides.

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SYNTHESIS OF ±-CRYPTOPLEURINE[1]

Sir:

The vesicant alkaloid cryptopleurine[2] has interesting physiological properties[3] and has been stated to possess a skeletal structure of a novel type. Although the alkaloid could not be degraded to compounds of known structure,[3] chemical,[3,4] spectroscopic,[4] and, in particular, crystallographic (X-ray)[5] evidence was adequate to allow the base to be designated as 2′,3′,6′-trimethoxyphenanthro-[9′,10′,2,3]quinolizidine (I).

By the use of the general methods described earlier[6] we have accomplished the first synthesis of ±-cryptopleurine. Condensation of 6-nitroveratraldehyde with homoanisic acid yielded 6-nitro-3,4,4′-trimethoxy-α-phenylcinnamic acid (m.p. 185–186°; *Anal.* Calcd. for $C_{18}H_{17}NO_7 \cdot H_2O$: C,

(1) This investigation was supported by a research grant (H-2170) from the National Heart Institute of the National Institutes of Health, Public Health Service.
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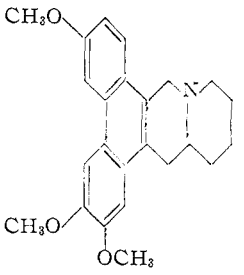
57.29; H, 5.08; N, 3.71. Found: C, 57.11; H, 4.77; N, 3.90). Reduction yielded the corresponding amino derivative (m.p. 206–207°) which when diazotized underwent the Pschorr cyclization to yield 2,3,6-trimethoxyphenanthrene-9-carboxylic acid (m.p. 222°; Calcd. for $C_{18}H_{16}O_5,H_2O$: C, 65.44; H, 5.49. Found: C, 65.54; 5.36). The phenanthroic acid was converted to the ethyl ester (m.p. 136.5–137°), which was reduced to the corresponding carbinol (m.p. 184–187°; *Anal.* Calcd. for $C_{18}H_{18}O_4$: C, 72.56; H, 6.17. Found: C, 72.46; H, 6.08) by the action of lithium aluminum hydride.



I, Base II, Methiodide

The bromide obtained from 2,3,6-trimethoxyphenanthryl-9-carbinol was not purified, but allowed to react immediately with pyridine-2-aldehyde. The salt thus obtained was cyclized by heating it in polyphosphoric acid at 80° for five hours. The cyclization product, presumably a 2,3,6-trimethoxydibenzo[h,j]acridizinium salt, could not be recrystallized satisfactorily and therefore was converted to the chloride by means of ion exchange and hydrogenated directly using platinum oxide catalyst. The product, purified by chromatography, melted at 199–200° (lit.[4] m.p. 197–198°; *Anal.* Calcd. for $C_{24}H_{27}NO_3$: C, 76.36; H, 7.21; N, 3.71. Found: C, 75.85; H, 7.17; N, 4.00) did not depress the melting point of an authentic sample,[7] and had ultraviolet and infrared spectra like those reported for cryptopleurine. The methiodide (II, m.p. 272–274°, lit.[4,8] 270–272° did not depress the melting point of an authentic sample of ±-cryptopleurine methiodide, and the infrared spectra (potassium bromide plate method) of the two substances were identical.

(7) We are indebted to Dr. E. Gellert for making this comparison for us and for furnishing us with a sample of ±-cryptopleurine methiodide.

(8) E. Gellert, *Australian J. Chem.*, **9**, 489 (1956). Since Dr. Gellert's method for obtaining the ± base from natural (optically active) cryptopleurine is somewhat involved, he has taken pains to demonstrate rigorously that the optically inactive methiodide is actually ±-cryptopleurine methiodide.

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PHASE EQUILIBRIUM IN THE HYDROTHERMAL SHRINKAGE OF COLLAGEN

Sir:

The shrinkage of collagen has been shown to denote a first order transition between crystalline and amorphous phases.[1,2] This being the case, the stress τ_{eq} required to maintain a state of equilibrium between the phases should be related to the temperature T according to an analog of the Clausius–Clapeyron equation[3,4]

$$d(\tau_{eq}/T)/d(1/T) = (\Delta\overline{\overline{H}}/V_c)/(\Delta\overline{\overline{L}}/L_c) \qquad (1)$$

where $\Delta\overline{\overline{H}}$ and $\Delta\overline{\overline{L}}$ are the latent enthalpy and length changes accompanying melting and subsequent swelling in the excess of surrounding solvent, V_c and L_c are the volume and length of the (dry) crystalline fiber, and τ_{eq} is the stress referred to the cross section of the fiber in the same state. Tobolsky, Haselkorn and Catsiff[5] have attempted recently to apply this relationship to the spontaneous shrinkage temperature T_s for bovine tendon at various stresses τ_s, the sample being immersed in water. Their results, like those similarly obtained a number of years ago by Wöhlisch,[6] yield a value of about 1.7 cal. $g.^{-1}$ for the latent enthalpy.[7]

We wish to point out that the temperature for the onset of shrinkage is substantially higher than that required for equilibrium between crystalline and amorphous phases. The values of τ_s and T_s which pertain to the shrinkage phenomenon as ordinarily determined should not therefore be employed in the foregoing equation, which is applicable only at thermodynamic equilibrium. We have found, however, that the equilibrium stress τ_{eq} at a given temperature T_m may be determined readily by the procedure outlined below.

Fresh rat-tail tendons were cross-linked (*i.e.*, tanned) with formaldehyde in order to suppress flow in the amorphous phase. A single tanned fiber about 10 cm. in length was suspended vertically between a fixed lower clamp and an upper clamp attached to a strain gage. The fiber and clamps were immersed in thermostated 3 *M* KCNS solution and the fiber was allowed to undergo partial contraction, such that one or more totally amorphous (shrunken) regions formed adjacent to (native) crystalline regions. The length was then adjusted to yield a force which remained constant for at least 30 minutes. Close approach to equilibrium is indicated by the fact that a small change in force, induced by slight alteration of the length, was succeeded by a change in the force toward the equilibrium value. The observed force is independent of the degree of shrinkage of the fiber, provided that a crystalline section remains. By measuring the thus established τ_{eq} as a function of temperature, we have obtained the results summarized below for two samples differing in degree of cross-linking.

The first two rows of the table emphasize the disparity between T_s and T_m^i, the equilibrium melting temperature established by extrapolation to zero force. The latent enthalpies $\Delta\overline{\overline{H}}/V_c$ per unit volume of collagen given in the fifth row have been calculated from eq. (1) using the results in the

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(7) Professor Tobolsky has informed us that the results in their paper (ref. 5) are referred to 1 g. of *swollen* collagen, and hence that a factor of approximately 4 must be applied to convert their results to a "per unit of dry collagen" basis. Such correction has been introduced in the value 7 cal. $g.^{-1}$ quoted above.