Isomerization of Sphingosine Sulfate[1]

BY CARL NIEMANN

Sphingosine sulfate obtained by the hydrolysis of crude cerebroside-sphingomylin fractions, derived from bovine brain or spinal cord, is moderately soluble in hot absolute ethanol.[2] However, if this salt is heated in the presence of absolute ethanol containing a trace of sulfuric acid, one can isolate from the reaction mixture two substances of markedly different solubility but of apparently identical composition. As both compounds give analytical values which are in excellent agreement with those demanded by the empirical formula $(C_{18}H_{37}O_2N)_2H_2SO_4$, it appears that the two substances are isomeric sphingosine sulfates. I shall designate the salt which possesses the greater solubility in hot ethanol as α-sphingosine sulfate (the original sphingosine sulfate) and the other as β-sphingosine sulfate. By proper choice of solvent each of these sulfates can be recrystallized without conversion to the other or change in composition. Aside from their different solubilities the α- and β-sphingosine sulfates can be distinguished by their rates of hydrogenation, the α-sphingosine sulfate being hydrogenated much more rapidly than the β-sphingosine sulfate. Both salts are white crystalline solids when freshly prepared but, upon exposure to light and the atmosphere, the α-sphingosine sulfate gradually becomes colored. A striking difference between the two isomeric sulfates is revealed when they are exposed to ultraviolet light. Upon irradiation, freshly prepared solid α-sphingosine sulfate exhibits an intense blue-white fluorescence whereas β-sphingosine sulfate shows a weak violet fluorescence.

On the basis of the above facts it seems likely that the two sulfates are geometrical isomers and that the α-sphingosine sulfate possesses the *cis* configuration and the β-sphingosine sulfate the *trans* configuration.

Experimental[3]

The crude cerebroside-sphingomylin fractions (C-S fractions) were prepared from fresh and desiccated bovine brain and spinal cord by successive extraction of the tissue with acetone, ether and hot ethanol and isolation of the desired fraction from the latter solvent.[2a]

Hydrolysis of C-S Fractions.—One hundred grams of C-S fraction was refluxed for thirty-six hours with 1 liter of 2 *N* methanolic hydrogen chloride. The hydrolyzate was chilled, centrifuged, the supernatant liquid decanted from the solid esters, and the latter washed with cold methanol. The supernatant liquid and washings were distilled until 250 ml. of residue remained. To this concentrate was added 200 ml. of 5 *N* sodium hydroxide and 1.5 liters of water. The emulsion was extracted with ether and the ether phase washed with half-saturated salt solution and dried over sodium sulfate. The dried ethereal extract was freed of solvent, the residue taken up in 30 ml. of methanol and 10% methanolic sulfuric acid added until the solution was acid to litmus. After standing for some days over concd. sulfuric acid the crystalline precipitate was collected with the aid of acetone. A second crop was obtained from the mother liquor. The precipitates were combined to give fraction C-S-H_1; average yield 17.2 g.

α-Sphingosine Sulfate.[4]—Fraction C-S-H_1 (51 g.) was recrystallized from 2.5 liters of absolute ethanol to give 20.6 g. of α-sphingosine sulfate (α_1). Recrystallization of 20.5 g. of the latter product from 1.25 liters of absolute ethanol gave 12.8 g. of α-sphingosine sulfate (α_2). Twice recrystallized α-sphingosine sulfate (12.7 g.) was refluxed with 600 ml. of absolute ethanol containing 1 drop of concd. sulfuric acid. Some of the material failed to go into solution, and after refluxing for thirty minutes the hot solution was filtered and the filtrate allowed to stand at 25° for three days. The crystalline precipitate that had formed in this interval was collected and designated as α-sphingosine sulfate (α_3). This latter product possessed all of the characteristics of the original twice-recrystallized α-sphingosine sulfate. Recrystallization of α-sphingosine sulfate (α_3) from absolute ethanol gave α-sphingosine sulfate (α_4).

Anal. Calcd. for $(C_{18}H_{37}O_2N)_2H_2SO_4$ (697.0): C, 62.0; H, 11.0; N, 4.0. Found: (α_1); C, 61.8; H, 10.7; N, 4.2; (α_2); C, 61.8; H, 10.9; N, 4.2; (α_3); C, 62.2; H, 11.0; N, 4.0; (α_4); C, 62.1; H, 11.1; N. 4.2.

β-Sphingosine Sulfate.[4]—The residue remaining after the filtration of the hot acidulated ethanol solution (above) was refluxed with 250 ml. of absolute ethanol, filtered, and the residue dried *in vacuo* over sulfuric acid. The β-sphingosine sulfate (β_1) so obtained (3.5 g.), in contrast to the twice recrystallized α-sphingosine sulfate, was only sparingly soluble in hot absolute ethanol. A portion was recrystallized from glacial acetic acid to give β-sphingosine sulfate (β_2). The filtrate resulting from the extraction of β-sphingosine sulfate with boiling ethanol (above) deposited 0.7 g. of β-sphingosine sulfate (β_3). Recrystallization of this product from glacial acetic acid gave β-sphingosine sulfate (β_4).

Anal. Calcd. for $(C_{18}H_{37}O_2N)_2H_2SO_4$ (697.0): C, 62.0; H, 11.0; N, 4.0. Found: (β_1); C, 62.2; H, 11.0; N, 4.2; (β_2); C, 61.9; H, 11.3; N, 4.2; (β_3); C, 62.2; H, 11.0; N, 4.2; (β_4); C, 61.9; H, 11.1; N, 4.2.

Hydrogenation Experiments.[4]—Samples of the two sulfates (12.40 mg.) were dissolved in 10 ml. of glacial acetic acid, 9.60 mg. of platinic oxide was added to each solution

(1) Contribution No. 804 from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology.

(2) (a) H. Thierfelder and E. Klenk, "Die Chemie der Cerebroside und Phosphatide," J. Springer, Berlin, 1930; (b) M. Guggenheim, "Die biogenen Amine," S. Karger, Basel, 1940.

(3) Microanalyses by H. Lanz, Jr., and G. A. Swinehart.

(4) Parallel experiments were conducted using C-S-H_1 fractions obtained from both brain and spinal cord. As the results obtained in these experiments were identical, only those experiments using C-S-H_1 fractions prepared from brain will be described.

and the hydrogen uptake measured in an appropriate apparatus.[5] The data, reduced to a common basis, are given in Table I.

TABLE I

HYDROGENATION OF SPHINGOSINE SULFATES

	Hydrogen absorbed in t minutes[a]									
Substance	t = 15	30	45	75	105	135	165	255	315	375
α-Sulfate (α_2)	56	81	89	94	95	95				
β-Sulfate (β_2)	8	22	28	41	53	62	67	80	87	90

[a] Expressed as per cent. of the theoretical quantity required for the conversion of sphingosine sulfate into dihydrosphingosine sulfate.

Examination with Ultraviolet Light.[4]—Specimens of all of the α- and β-sphingosine sulfates described above were examined with a Hanovia "Luxor Scientific" lamp. All of the β-sphingosine sulfate preparations exhibited a weak violet fluorescence which was less intense than that shown by quinine sulfate under comparable conditions. In the case of α-sphingosine sulfate the color of the fluorescence, but not the intensity, varied with the age of the sample. With freshly prepared α-sulfate the color was a blue-white but with the older, cream-colored samples the color lost its bluish character and appeared as white or ivory. The intensity of the fluorescence was very much greater than that exhibited by quinine sulfate.

(5) A. N. Prater and A. J. Haagen-Smit, *Ind. Eng. Chem., Anal. Ed.*, **12**, 705 (1940).

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The Preparation of Trichloromethanesulfonyl Chloride

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A recent article by Pelikh[1] describes the use of trichloromethanesulfonyl chloride, Cl_3CSO_2Cl, for the control of the sugar-beet weevil and gives a method for its preparation. In response to a request for some of the material by one of the entomologists of this Bureau, attempts were made to obtain the Russian journal. These having been unsuccessful, some of the compound was prepared by the method described by Rathke.[2] In this procedure trichloromethanesulfenyl chloride (perchloromethyl mercaptan), Cl_3CSCl, is oxidized at room temperature with nitric acid (sp. gr. 1.2). The reactants are immiscible so that the reaction is very slow; after two to three weeks a low yield of the acid chloride was obtained.

It has now been found that the oxidation proceeds smoothly and rapidly in acetic acid. The trichloromethanesulfonyl chloride is obtained in a yield of about 50%.

According to Richter-Anschütz[3] the compound possesses a penetrating camphoraceous odor, and Pelikh[1] states that it has a beet-like smell. The pure product obtained by us had a penetrating odor and was a lachrymator. The use of calcium trichloromethanesulfonate[4] as specified by Pelikh may circumvent the lachrymatory effect.

The trichloromethanesulfenyl chloride can be prepared readily from carbon disulfide and chlorine, with iodine as a catalyst, by the procedure of Helfrich and Reid[5] or that described in "Organic Syntheses."[6] It has now been found that this reaction also can be carried out conveniently by shaking the carbon disulfide plus iodine in an apparatus such as the Burgess-Parr shaker used in catalytic hydrogenation, while passing chlorine into the bottle under slight pressure at such a rate that the temperature of the solution does not exceed 30°. The reaction is completed when the absorption of chlorine practically ceases. The volume of solution should approximately be doubled. Over-chlorination decreases the yield owing to the formation of carbon tetrachloride. Allowing the solution to stand for a day or two increases the yield.[5] The reaction product is then subjected to fractional distillation under reduced pressure. The fraction boiling at 65–68°, p = 50 mm., was collected and used for the subsequent oxidation experiments.

The oxidation procedure given below was chosen as the best after a number of experiments had been tried in which the concentrations of trichloromethanesulfenyl chloride, acetic acid, and nitric acid were varied.

Ten grams of trichloromethanesulfenyl chloride dissolved in 30 cc. of glacial acetic acid is refluxed gently, and 15 cc. of concentrated nitric acid is added drop by drop over a period of ten to fifteen minutes. The reaction is exothermic and brown fumes are evolved. The amount of heat supplied may have to be decreased. After all the nitric acid has been added, the solution is refluxed for twenty minutes longer, cooled and diluted with several volumes of water. The separated trichloromethanesulfonyl chloride is filtered, washed well with water, and dried. Such a preparation is fairly pure, but if necessary it may be recrystallized by dissolving in warm ethanol and diluting with water. It may also be purified by sublimation or steam distillation. The melting point is 140–140.5°

(1) N. D. Pelikh, *Sakhar* (U. S. S. R.), No. 6, 40 (1937). *Khim. Referat. Zhur.*, **1**, No. 8–9, 45 (1938). Through *C. A.*, **33**, 7031 (1939).

(2) B. Rathke, *Ber.*, **3**, 858 (1870); *Ann.*, **167**, 195 (1873).

(3) Richter-Anschütz, "Chemie der Kohlenstoffverbindungen," Ed. 12, Vol. I, p. 541, 1928.

(4) N. D. Pelikh and S. I. Lyukin, Russian patent 52,159, Nov. 1937. Through *C. A.*, **34**, 3008 (1940).

(5) O. B. Helfrich and E. E. Reid, THIS JOURNAL, **43**, 591 (1921).

(6) Henry Gilman, "Organic Syntheses," Coll. Vol. I, p. 493.