was separated by extraction with chloroform; after washing, drying and evaporation of the chloroform solution there remained 3.64 g. (91%) of slightly discolored crystalline material, m.p. 135.5–138°. Recrystallization from ethanol provided colorless homodihydrocarbostyril, m.p. 142.5–143° (reported[7,13] m.p. 139–141°, 141°). The infrared absorption spectrum was in agreement with the expected structure. The aqueous solution remaining after chloroform extraction was made alkaline with 25% potassium hydroxide solution, and subjected to continuous ether extraction. A small amount (0.1 g.) of red oil was isolated which was not characterized, but which may have contained β-naphthylamine.

Rearrangement of Fluorenone Oxime.—A mixture of 2.00 g. of fluorenone oxime and 60 g. of polyphosphoric acid was heated with manual stirring to 175–180°, and maintained at this temperature for a few minutes. The resulting solution was cooled and treated with 300 ml. of water; the product separated in crystalline form and was removed by filtration. After washing and drying there was obtained 1.85 g. (93%) of phenanthridone, m.p. 286–289°.

Rearrangement of α-Benzil Monoxime.—A solution of 2.00 g. of α-benzil monoxime in 60 g. of polyphosphoric acid was heated with manual stirring to 110–115°, and was then maintained at 90–100° (steam) for 30 minutes. Treatment with 300 ml. of water gave a solution which was extracted well with ether. The ether solution was extracted with 5% sodium hydroxide solution to remove acidic products. The alkaline solution was acidified (hydrochloric acid) to provide 1.08 g. (quantitative yield, based on formation of one mole of benzoic acid) of colorless benzoic acid, m.p. 121–122°, identified by infrared spectrum and mixed melting point with an authentic sample.

A neutral fraction was isolated from the ether solution remaining after alkaline extraction. The ether solution was washed, dried and evaporated to yield 0.42 g. (40%) of colorless solid, m.p. 119–126°. Although the melting range was rather wide, no benzonitrile was detected in this product, and recrystallization from water gave pure benzamide, identified by infrared spectrum and mixed melting point.

Rearrangement of Phenylacetone Oxime.—A mixture of 15.0 g. of phenylacetone oxime and 300 g. of polyphosphoric acid was heated with manual stirring at 90–100° for five minutes. The mixture was treated with 2 l. of water, and the resulting solution was extracted with 1:1 ether–ethyl acetate. The organic solution was washed with saturated sodium chloride solution, dried, and evaporated with heating until the product came to constant weight. There was obtained 14.75 g. of non-basic yellow oil (which did not crystallize) indicating that the amount of basic material formed in the reaction was negligible. The non-basic product was distilled at 0.5 mm. to give the following fractions: (a) to 120°, 1.33 g., (b) 120–123°, 3.05 g., (c) 123–128°, 1.61 g., (d) 128–130°, 1.56 g. Fractions (b) and (c) solidified and were combined to yield 4.66 g. (29%) of colorless solid, m.p. 51–55°. This material was identified as N-benzylacetamide by a mixed melting point determination and by comparison of the infrared spectrum with that of an authentic sample prepared by acetylation of benzylamine. The yield may in fact have been higher than that indicated, since the last fraction (d) may also have contained a small amount of amide. The yield of crystalline material (29%) is smaller than usual for a ketoxime rearrangement.[1]

BETHESDA, MARYLAND

(13) J. V. Braun, *Ber.*, **40**, 1843 (1907); Briggs and DeAth, *J. Chem. Soc.*, 45 (1937).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF NEW YORK UNIVERSITY]

The Geometry of Sphingosine

BY KURT MISLOW

RECEIVED MAY 23, 1952

The infrared spectra of sphingosine, sphingosine sulfate, triacetylsphingosine and cerebron all exhibit a pronounced absorption maximum near 10.3 μ. These results provide strong evidence that sphingosine and its cerebroside precursors have the *trans* configuration.

Sphingosine, the common basic constituent of the sphingolipides,[1] has long been the object of structural investigations.[2] A variety of degradative studies,[3] and independent syntheses of dihydrosphingosine,[4] have lent strong support to the presently accepted formulation (I) of sphingosine.

$$CH_3(CH_2)_{12}—C^5H{=}C^4H—C^3HOH—C^2HNH_2—C^1H_2OH \quad (I)$$

However the presence of two asymmetric centers and of a double bond in the molecule offers a choice among eight possible stereoisomers, *cis–threo, cis–erythro, trans–threo, trans–erythro,* and the corresponding enantiomers. It has recently been shown[5] that the configuration around carbon atom 2 is D; the number of stereoisomers allowable for the naturally occurring compound has thereby been halved. There still remains the question of the configuration around the "allylic center" of the molecule, which is comprised of carbon atoms 3, 4 and 5.

With regard to the geometry of the double bond, the available literature reports are in conflict. Niemann[6] reported that naturally occurring "α"-sphingosine sulfate was isomerized under acid conditions to a thermodynamically more stable "β-sphingosine sulfate." On the basis of this observation, the *cis* configuration was assigned by him to the "α"-isomer. This result is in contradistinction to the findings of Ono,[7] who obtained *trans*-2-hexadecenal as a product of the lead tetraacetate oxidation of sphingosine; it must be remarked, however, that under these experimental conditions, any *cis*-2-hexadecenal, a possible product of cleavage, if initially formed would be expected to undergo rapid conversion to the *trans* isomer, *via* the enol form.

The present work was designed to yield more

(1) This term includes the cerebrosides, the sphingomyelins and the gangliosides (H. E. Carter, *et al.*, *J. Biol. Chem.*, **169**, 77 (1947)).

(2) H. Thierfelder and E. Klenk, "Die Chemie der Cerebroside und Phosphatide," J. Springer, Berlin, 1930.

(3) E. Klenk and W. Diebold, *Z. physiol. Chem.*, **198**, 25 (1931); H. E. Carter, F. J. Glick, W. P. Norris and G. E. Phillips, *J. Biol. Chem.*, **170**, 285 (1947); K. Ono, *J. Japan Biochem. Soc.*, **20**, 32 (1948) [*C. A.*, **43**, 6267 (1949)].

(4) G. I. Gregory and T. Malkin, *J. Chem. Soc.*, 2453 (1951); C. A. Grob, E. F. Jenny and H. Utzinger, *Helv. Chim. Acta*, **34**, 2249 (1951).

(5) H. E. Carter and C. G. Humiston, *J. Biol. Chem.*, **191**, 727 (1951).

(6) C. Niemann, THIS JOURNAL, **63**, 1763 (1941).

(7) K. Ono, *J. Japan. Biochem. Soc.*, **19**, 133 (1947) [*C. A.*, **44**, 10752 (1950)].

decisive information on the question of the geometry of the double bond.

There exists a growing body of evidence that the infrared spectra of 1,4-substituted 2-butenes, regardless of the nature of the substituents, are characterized by an intense absorption maximum (the "*trans*-peak") near 10.3 μ (970 cm.$^{-1}$) if the configuration is *trans*, and by the complete absence of this peak if the configuration is *cis*. Not only is this behavior exhibited by olefinic hydrocarbons,[8] but it is also shared by a variety of olefinic fatty acids[9] (vaccenic,[10,11] oleic,[11] elaidic,[11] linoleic,[12] *cis*- and *trans*-5-octenoic,[13] crotonic[14]), olefinic chlorides (*cis*- and *trans*-crotyl chloride,[15] *cis*- and *trans*-1,4-dichloro-2-butene[16]), and olefinic alcohols (*cis*- and *trans*-3-hexene-1-ol,[17] crotyl alcohol[14]), among others.[18] The presence or absence of the *trans*-peak may thus serve as a most reliable criterion on which to base a decision pertaining to the geometry of such substances.

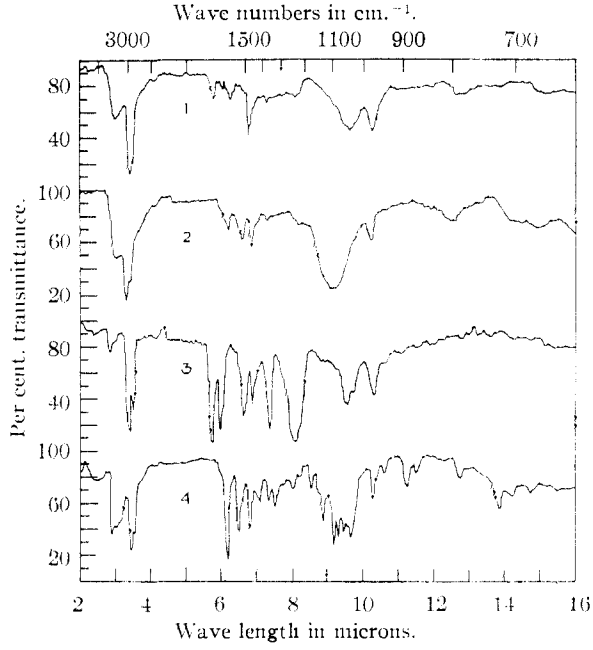


Fig. 1.—Infrared spectra of: 1, sphingosine, 5 wt. % solution in alcohol-free chloroform; 2, sphingosine sulfate, 2.9 wt. % solution in alcohol-free chloroform; 3, triacetylsphingosine, 5 wt. % solution in alcohol-free chloroform; 4, cerebron, film prepared by evaporation of a 2.5 wt. % gel in carbon tetrachloride.

The spectra of sphingosine, sphingosine sulfate and triacetylsphingosine, which are reproduced in Fig. 1 (curves 1, 2 and 3, resp.), have as their common striking characteristic a well defined *trans*-peak near 10.3 μ. It may thus be confidently concluded that sphingosine has the *trans* configuration. This result is in conflict with the inferences drawn by Niemann, and it serves to reopen the question of the identity of the "β-sphingosine sulfate" reported by that investigator.[6]

It may be argued that in the course of the hydrolysis of cerebrosides, the double bond suffers *cis–trans* isomerization, and that sphingosine, as isolated from the hydrolysis mixture, represents an altered species. However, the infrared spectrum of cerebron clearly exhibits a *trans*-peak (Fig. 1, curve 4): the double bond in the cerebrosides may therefore be considered *trans*,[19] and no *cis–trans* isomerization need be invoked.[20]

Experimental[21]

Cerebron (Phrenosin).—The starting material for this and all subsequent experiments was a beef brain cerebroside mixture.[22] When this material (10 g.) was refluxed with 30 ml. of a 10% solution of chloroform in methanol, and the resulting almost clear solution was allowed to come to room temperature, some cholesterol (m.p. 150–151°, $[\alpha]^{25}D$ −39.8° (*c* 7.86, chloroform)) immediately deposited in the form of long slender needles. It was noted that after two to five hours, cerebron started to deposit in the form of microscopic prismatic clusters which aggregated on the surface of the cholesterol crystals, and this difference in their rates of crystallization allowed a clean-cut separation of cholesterol and cerebron. The filtrate from the cholesterol precipitation was immediately diluted to 100 ml. with 10% chloroform–methanol and the cerebron (1.2 g.) which eventually deposited was recrystallized from 45 ml. of 10% chloroform–methanol. It was thus obtained in the form of a friable crystal powder (0.9 g.), whose rotation, $[\alpha]^{26}D$ −3.2° (*c* 14.90, pyridine), was in close agreement with previously reported values.[2]

Anal. Calcd. for $C_{48}H_{93}NO_9 \cdot H_2O$: C, 68.1; H, 11.32; N, 1.66. Found: C, 68.0; H, 11.15; N, 1.79.

Concentration and chilling in ice of the mother liquors produced a further precipitate (2.5 g.) of a somewhat waxy solid whose rotation, $[\alpha]^{27}D$ −9.3° (*c* 21.14, pyridine), large solubility in 10% chloroform–methanol as compared to that of cerebron, and analysis, were indicative of impure kerasin.[2]

Anal. Calcd. for $C_{48}H_{95}NO_8$: C, 71.0; H, 11.55; N, 1.73. Found: C, 72.1; H, 11.12; N, 2.2.

Sphingosine Sulfate.[23]—The hydrolysis of 30 g. of the cerebroside mixture (*vide supra*) in methanolic sulfuric acid[24] afforded 3.9 g. of crude sphingosine sulfate in the form of a friable, slightly yellow solid. Recrystallization of this material from 400 ml. of methanol gave a material (740 mg.)

(8) R. S. Rasmussen, R. R. Brattain and P. S. Zucco, *J. Chem. Phys.*, **15**, 136 (1947); J. A. Anderson, Jr., and W. D. Seyfried, *Anal. Chem.*, **20**, 998 (1948); N. D. Coggeshall, *ibid.*, **22**, 381 (1950).

(9) *Cf.* also R. G. Sinclair, A. F. McKay, G. S. Myers and R. N. Jones, THIS JOURNAL, **74**, 2578 (1952).

(10) K. Ahmad, F. M. Bumpus and F. M. Strong, *ibid.*, **70**, 3391 (1948).

(11) P. C. Rao and B. F. Daubert, *ibid.*, **70**, 1102 (1948).

(12) H. M. Walborsky, R. H. Davis and D. R. Howton, *ibid.*, **73**, 2590 (1951).

(13) D. R. Howton and R. H. Davis, *J. Org. Chem.*, **16**, 1405 (1951).

(14) A. R. Philpotts and W. Thain, to be published; *cf.* R. H. Hall, A. R. Philpotts, E. S. Stern and W. Thain, *J. Chem. Soc.*, 3341 (1951).

(15) L. F. Hatch and S. S. Nesbitt, THIS JOURNAL, **72**, 727 (1950).

(16) K. Mislow and H. M. Hellman, *ibid.*, **73**, 246 (1951).

(17) L. Crombie and S. H. Harper, *J. Chem. Soc.*, 873 (1950).

(18) It cannot be claimed that this listing exhausts all the available information.

(19) This result is of some interest also in view of the fact that the *trans* configuration is rare among naturally occurring lipide olefins (R. A. Raphael and P. Sondheimer, *J. Chem. Soc.*, 2100 (1950)).

(20) After the completion of this work, the author was informed in a private communication that Dr. Herbert E. Carter had completed similar studies on phrenosin (cerebron), a series of derivatives of sphingosine, dihydrosphingosine and the two 3-O-methylsphingosines. The *trans* configuration of these compounds was indicated by a peak at 10.3 μ in the unsaturated compounds and its essential absence in the saturated ones.

(21) Microanalyses by W. Manser (Zürich), and by Drs. G. Weiler and F. B. Strauss (Oxford). A Baird Model B instrument with 0.1-mm. cells was employed in the determination of the infrared spectra. In this connection, the assistance afforded by correspondence with Dr. Ralph Nusbaum and his staff, Spectroscopy Section, Atomic Energy Project, U.C.L.A., Los Angeles, Calif., is gratefully acknowledged.

(22) Purchased from Bios Laboratories, Inc., New York, N. Y.

(23) Mr. Sheldon Bleicher kindly assisted in this preparation.

(24) H. E. Carter, W. P. Norris, F. J. Glick, G. E. Phillips and R. Harris, *J. Biol. Chem.*, **170**, 269 (1947).

which had the properties of "α-sphingosine sulfate,"[6] *i.e.*, it gradually became yellow on exposure to air, and upon irradiation with ultraviolet light (Hanovia utility lamp) specimens exhibited a whitish fluorescence whose intensity considerably exceeded that of a sample of quinine sulfate (violet fluorescence) which was examined under comparable conditions.

Anal. Calcd. for $(C_{18}H_{37}O_2N)_2 \cdot H_2SO_4$: C, 62.0; H, 10.99; N, 4.02. Found: C, 61.4; H, 10.95; N, 3.83.

A sample of sphingosine, freshly prepared *via* the acid hydrolysis of beef cord, was kindly supplied by Dr. Irving Zabin, Physiol. Chem. Dept., the Medical School, U.C.L.A., Los Angeles, Calif. The sample, recrystallized from acetone, had m.p. 96°.

Triacetylsphingosine.—This compound was prepared from sphingosine sulfate according to the directions of Carter and co-workers[24] and purified by recrystallization from acetone. The material thus obtained had $[\alpha]^{27}D$ −10.9° (*c* 1.8, chloroform), m.p. 101–102° (repd.[24] $[\alpha]^{25}D$ −11.7° (chloroform), m.p. 101–102°).

Anal. Calcd. for $C_{24}H_{43}O_5N$: C, 67.7; H, 10.18; N, 3.29. Found: C, 67.5; H, 10.25; N, 3.02.

Acknowledgment.—A grant-in-aid from Research Corporation is gratefully acknowledged. The author also takes pleasure in thanking Dr. David R. Howton for stimulating discussions.

NEW YORK 53, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

On the Cytosine Deaminase of Yeast[1]

BY JACOB KREAM AND ERWIN CHARGAFF

RECEIVED MARCH 22, 1952

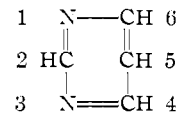
The description of the isolation of partially purified, stable cytosine deaminase preparations from bakers' yeast is followed by a study of the properties of the enzyme. It degrades cytosine with the production of equimolar quantities of uracil and ammonia in a unimolecular reaction at a *p*H optimum of 6.9. The effects of enzyme and substrate concentration and of temperature on the course of the enzymatic action have been investigated. The Michaelis constant has been found as 8.4×10^{-3} *M*, the temperature velocity constant as 19,500 cal./mole. Of many other pyrimidines studied, only 5-methylcytosine was converted to thymine by the enzyme preparations. Isocytosine acted as an inhibitor. Conclusions as to the specificity requirements of cytosine deaminase are discussed, and a procedure for the chromatographic separation of 5-methylcytosine from other pyrimidines and for its quantitative determination in minute amounts is described.

The present paper considers the cytosine deaminase of yeast in some detail. Brief accounts of some of the phases of the work have appeared.[2,3] Enzyme systems capable of deaminating cytosine to uracil have been encountered rather infrequently. Extracts of animal tissues appear in general to be unable to degrade cytosine itself, although the ribonucleoside cytidine is attacked.[4,5] That dietary cytosine can be converted to uracil in the body has been both denied[6] and affirmed.[7] Indications of the production of ammonia from cytosine, incubated with fowl blood, have been reported.[8]

The presence in bakers' yeast of enzymes able to deaminate cytosine and 5-methylcytosine to uracil and thymine, respectively, is well established.[2,3,9,10] A cytosine deaminase also has been found in *E. coli*[2,11,12] and obtained as a cell-free extract[12]; but this property does not seem to be inherent in all strains.[13] The development of convenient micro procedures for the study of purine and pyrimidine deaminases and the discovery that yeast was a good source for the isolation of cell-free preparations of cytosine deaminase[2,12] prompted a more detailed investigation of this enzyme, also with respect to its specificity characteristics.

Experimental

Material.—The following numbering system will be used in describing the pyrimidines.



Uracil either was used as a purified commercial preparation or synthesized here.[14] Cytosine was prepared from uracil,[15] as was 2-methoxy-6-aminopyrimidine. 5-Methylcytosine and 2-mercapto-6-aminopyrimidine were obtained through the courtesy of Dr. G. H. Hitchings of the Wellcome Research Laboratories, Tuckahoe, N. Y. For 2,6-diaminopyrimidine, 2-hydroxy-4,6-diaminopyrimidine and isoguanine (2-hydroxy-6-aminopurine) we are highly indebted to Dr. A. Bendich of the Sloan-Kettering Institute for Cancer Research, New York; for isocytosine (2-amino-6-hydroxypyrimidine) and 2,6-dihydroxy-5-aminopyrimidine to Dr. M. Hoffer of Hoffmann-La Roche, Inc., Nutley, N.J.; for 4-hydroxycytosine to Dr. M. Engelman of this college. The preparation of cytidylic acid has been described previously.[16] Cytidine was a purified commercial preparation.

Quantitative Determinations.—Cytosine and uracil were separated by filter paper chromatography and estimated by spectrophotometry, as described in a previous publication.[17] Essentially similar procedures served for the separation of thymine from 5-methylcytosine and the quantitative determination of the latter. For the techniques used, the recently published procedures for the estimation of xanthine and hypoxanthine should also be consulted.[12] The separa-

(1) Supported by a research grant from the National Institutes of Health, United States Public Health Service. This report is based on a dissertation submitted by Jacob Kream in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

(2) E. Chargaff and J. Kream, *J. Biol. Chem.*, **175**, 993 (1948).

(3) J. Kream and E. Chargaff, *Federation Proc.*, **9**, 192 (1950).

(4) G. Schmidt, *Z. physiol. Chem.*, **208**, 185 (1932).

(5) J. P. Greenstein, C. E. Carter, H. W. Chalkley and F. M. Leuthardt, *J. Natl. Cancer Inst.*, **7**, 9 (1946).

(6) L. B. Mendel and V. C. Myers, *Am. J. Physiol.*, **26**, 77 (1910).

(7) L. R. Cerecedo, *J. Biol. Chem.*, **75**, 661 (1927).

(8) E. J. Conway and R. Cooke, *Biochem. J.*, **33**, 457 (1939).

(9) A. Hahn and W. Lintzel, *Z. Biol.*, **79**, 179 (1923).

(10) A. Hahn and W. Haarmann, *ibid.*, **85**, 275 (1926).

(11) A. Hahn and L. Schäfer, *ibid.*, **83**, 511 (1925).

(12) J. Kream and E. Chargaff, THIS JOURNAL, **74**, 4274 (1952).

(13) T. P. Wang, H. Z. Sable and J. O. Lampen, *J. Biol. Chem.*, **184**, 17 (1950).

(14) D. Davidson and O. Baudisch, THIS JOURNAL, **48**, 2379 (1926).

(15) G. E. Hilbert and T. B. Johnson, *ibid.*, **52**, 1152 (1930).

(16) E. Vischer and E. Chargaff, *J. Biol. Chem.*, **176**, 715 (1948).

(17) E. Vischer and E. Chargaff, *ibid.*, **176**, 703 (1948).