electrons per molecule. The solution was evaporated nearly to dryness *in vacuo* in a slow current of air at a bath temperature not exceeding 40°C. To the residue were added 200 ml of butanol; the butanol solution was concentrated *in vacuo* to 30 ml. A small precipitate of potassium chloride (from the agar bridge) was filtered off and dry ether added to the filtrate. A precipitate, 940 mg, was filtered off, dissolved in alcohol and reprecipitated with ether. (Found: C 61.60; H 5.52; N 4.54; Cl^- 11.12. Calc. for $C_{15}H_{14}NO_3Cl,1/2\ C_2H_5OH$: C 61.07; H 5.44; N 4.45; Cl^- 11.26). On heating in a closed capillary the compound turned yellow at about 100°, decomposed around 170° and was completely melted at 178°. The I.R.-spectrum contained a.o. bands at (cm^{-1}): 3300—2500, 1705 (s), 1640 (ms), and 1594 (ms).

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Received December 21, 1963.

Studies on Sphingosines

3. C_{20}-Dihydrosphingosine, a hitherto Unknown Sphingosine *

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Three sphingosines have so far been reported, namely C_{18}-sphingosine,[3] C_{18}-dihydrosphingosine [4,5] and C_{20}-sphingosine.[6] A fourth sphingosine, C_{20}-dihydrosphingosine, is the subject of this communication.

The new compound has been isolated as a dinitrophenyl (DNP) derivative from a mixture of DNP-sphingosines, prepared as described earlier.[1] The DNP-sphingosines are freed from impurities and by-products from the acid hydrolysis [2] by chromatography on silicic acid using increasing proportions of diethyl ether in light petroleum (b.p. 60°—70°). The mixture of DNP-sphingosines is then fractionated on paper on a preparative scale. This procedure is a modification of an earlier described analytical method based on reversed phase chromatography.[1] The separated compounds are extracted from the paper with ethanol, rechromatographed and finally freed from solvent (tetralin) by-products on a silicic acid column. In this way five hitherto unknown sphingosines have been prepared in a pure form. Of these C_{20}-dihydrosphingosine is separated from the known sphingosines as shown in Fig. 1.

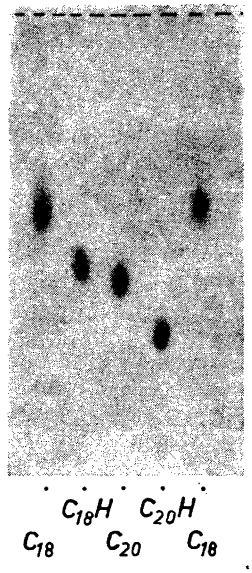

Fig. 1. Chromatogram showing from left to right pure dinitrophenyl derivatives of C_{18}-sphingosine, C_{18}-dihydrosphingosine, C_{20}-sphingosine, C_{20}-dihydrosphingosine and C_{18}-sphingosine. Solvent: Upper phase of methanol-tetralin-water 90—10—10 (v/v). Time for development: 6 h. For further details, see Ref.[1] The chromatogram was photographed with »Kodak, Photographic Plates, B 10». (C_{20}-sphingosine, which has not been isolated before, will be subject of a later communication by the author.)

The pure DNP-C_{20}-dihydrosphingosine (Found: C 61.38; H 8.77; N 8.42. Calc. for DNP-C_{20}-dihydrosphingosine,

* Communications 1 and 2 in this series are Refs. 1 and 2, respectively.

$C_{26}H_{45}O_6N_3$: C 63.24; H 8.78; N 8.52.) is identical in thin layer and analytical paper chromatography [1] with DNP-C_{20}-dihydrosphingosine, obtained from C_{20}-sphingosine through catalytic hydrogenation. Its infrared spectrum is identical with that of DNP-C_{18}-dihydrosphingosine. After oxidation with potassium permanganate octadecanoic acid is the longest acid identified. Under the same conditions DNP-C_{18}-sphingosine yields tetradecanoic acid while DNP-C_{18}-dihydrosphingosine and DNP-C_{20}-sphingosine yield hexadecanoic acid.

The new sphingosine has been isolated from human and bovine brain and from hair. In human brain it is found only in gangliosides. Cerebrosides, sulfatides and sphingomyelins contain C_{18}-sphingosine and a few per cent of C_{18}-dihydrosphingosine but no C_{20}-sphingosines. The total ganglioside fraction is composed of about one third of C_{18}-sphingosines and two thirds of C_{20}-sphingosines. Of these the proportion of saturated to unsaturated sphingosines is about 1 to 10.

Recently, a small amount of stearic acid was reported as an oxidation product from a mixture of ganglioside long chain bases.[7] This is probably derived from the sphingosine here described.

Details of this work will be published later. Different natural and synthetic substances used as references during this work were kindly supplied by Herbert E. Carter, University of Illinois, Paul W. O'Connell, The Upjohn Company, Michigan, and David Shapiro, Rehovoth, Israel.

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Received February 10, 1964.

Some New 1,2,3,4-Thiatriazoles

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In connexion with some investigations on the thiosemicarbazides (Studies of Thioacids IX, *To be published*) we have prepared several new 1,2,3,4-thiatriazoles. The thiatriazoles listed in Table 1 were prepared from thiosemicarbazides and nitrous acid by the usual procedure.[1] The main reason for preparing these compounds was to complete the series, the methyl, ethyl, butyl, benzyl and phenyl derivatives being known.[1] The ethoxycarbonylmethyl derivative (N-(1,2,3,4-thiatriazol-5-yl)glycine ethylester) was prepared as an example of a 1,2,3,4-thiatriazole with a functional group. This compound was, however, rather unstable and decomposed on attempts to transform it into the free acid or the amide.

5-Acylamino-1,2,3,4-thiatriazoles could not be prepared by acylation of 5-amino-1,2,3,4-thiatriazole, since fission of the thiatriazole ring took place. Acylisothiocyanates were found to react with hydrazoic acid to form 5-acylamino-1,2,3,4-thiatriazoles, but difficulties were encountered when purifying these compounds. These reactions are now being studied in more detail.

Furthermore, some 5-*o*-alkoxyphenyl-1,2,3,4-thiatriazoles (Table 2) have been prepared from the corresponding dithioates and sodium azide. These reactions were studied because it was known from other experiences that an *o*-alkoxy group with secondary or large alkyl groups could exert a steric hindrance.[2] However, all the compounds investigated reacted in the normal way.

None of the compounds described here exhibited the azide band near 2200 cm^{-1} in their infrared spectra and are therefore true thiatriazoles and not thioazides.

All thiatriazoles listed in Table 2 show a medium strong infrared band near 1575 cm^{-1}. Infrared spectra were further recorded for 8 of the 13 thiatriazoles described in our earlier paper [3] (with the substituent in 5-position = phenyl, *o*-tolyl, *m*-chlorophenyl, *o*-methoxyphenyl, *p*-hy-