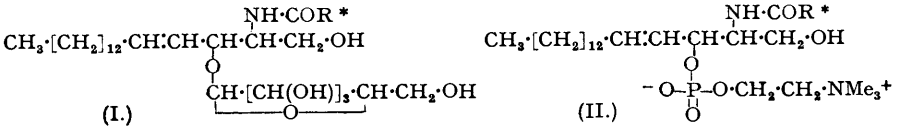
541. *The Sphingolipid Field. Part I. Synthesis of Racemic 2-Amino-octadecane-1 : 3-diols.*

By G. I. Gregory and T. Malkin.

The synthesis of a mixture of racemates of 2-amino-octadecane-1 : 3-diol is recorded, of which one form is known as dihydrosphingosine, obtained by hydrolysis of phospholipid sphingomyelin (Carter, Glick, Norris, and Phillips, *J. Biol. Chem.*, 1947, **170**, 285). The triacetyl, tribenzoyl, and *N*-acetyl derivatives of the synthetic amino-diol are described.

Whilst investigating the products formed by the hydrolysis of the phospholipid sphingomyelin, Thudichum (*J. pr. Chem.*, 1882, **25**, 19) isolated a base to which he gave the name sphingosine and the empirical formula $C_{17}H_{35}O_2N$. Later workers (Levene and Jacobs, *J. Biol. Chem.*, 1912, **11**, 547; Levene and West, *ibid.*, 1914, **16**, 549; Lapworth, *J.*, 1913, 1029) supported the C_{17} formula and also showed that sphingosine was a straight-chain compound containing two hydroxyl groups and a primary amino-group attached severally to the first three carbon atoms, and a double bond between the fourth and the fifth carbon atom; but Klenk and Diebold (*Z. physiol. Chem.*, 1929, **185**, 169; 1931, **198**, 25) adduced evidence, now accepted, in favour of a C_{18} chain, and proposed the formula 3-amino-octadec-4-ene-1 : 2-diol.

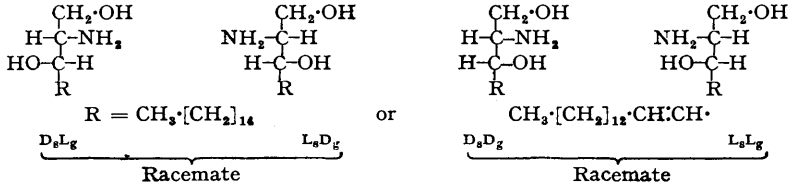
Carter, Glick, Norris, and Phillips (*J. Biol. Chem.*, 1947, **170**, 285) sought to confirm Klenk and Diebold's conclusions by investigating the degradation of dihydrosphingosine by periodic acid. Sphingosine, obtained by hydrolysis of a sphingomyelin–cerebroside mixture (Carter, Haines, Ledyard, and Norris, *J. Biol. Chem.*, 1947, **169**, 77; *ibid.*, **170**, 269), was *N*-benzoylated and reduced to give *N*-benzoyldihydrosphingosine, but this could not be induced to react with periodic acid, thus indicating the absence of the 1 : 2-glycol system postulated by Klenk and Diebold. On the other hand, evidence for a 2-amino-octadecane-1 : 3-diol structure was given by the formation of a benzylidene derivative of *N*-benzoyldihydrosphingosine and was supported by the reaction of periodic acid with dihydrosphingosine to yield hexadecanal, formaldehyde, formic acid, and ammonia. This work has also established the structure of sphingosine as 2-amino-octadec-4-ene-1 : 3-diol.



(I.) (II.)

* The amino-group is acylated by a variety of acids, most frequently by lignoceric or α-hydroxylignoceric acid.

Dihydrosphingosine has been isolated directly from the hydrolysate of the cerebroside (I) fraction obtained from *Cysticerus fasciolaris* (Lesuk and Anderson, *ibid.*, 1941, **139**, 457) and, more recently, from the hydrolysate of a pure sphingomyelin (II) (Thannhauser and Boncoddo, *ibid.*, 1948, **172**, 141), where it occurs with the unsaturated base sphingosine.

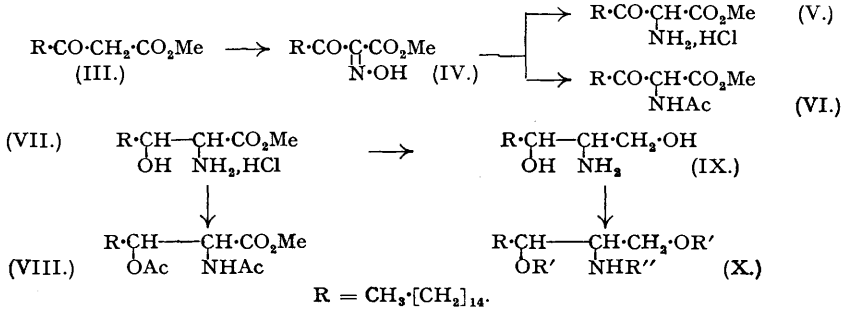


Racemate Racemate

The synthesis of dihydrosphingosine has not yet been described, although Carter (*ibid.*, 1947, **170**, 295) suggested that a possible synthetic route might lie through the intermediate 2-amino-3-hydroxyoctadecanoate esters,† and discussed the problems arising from the existence of two racemates of 2-amino-octadecane-1 : 3-diol. The configuration of the naturally occurring 2-amino-octadecane-1 : 3-diol, which we may continue to call dihydrosphingosine, has not yet been determined, and the present synthesis was undertaken in the hope of obtaining information on this.

† Geneva numbering (CO_2H = 1.)

Several routes to methyl 2-amino-3-keto-octadecanoate or its more stable hydrochloride having been investigated with no success, it was found possible to oximinate methyl 3-keto-octadecanoate (III) (Ställberg-Stenhagen, *Arkiv Kemi, Min., Geol.*, 1945, **20**, *A*, No. 19) in 55% yield. An attempt to reduce the oxime with lithium aluminium hydride gave a mixture which appeared to be long-chain amines; however, attempts to separate a pure substance were unsuccessful (cf. the attempted reduction of methyl α-oximinofuroylacetate by Hayes and Gever, *J. Org. Chem.*, 1951, **16**, 269). Stepwise reduction was next employed; first, the oxime (IV) was reduced with palladium–charcoal in the presence of 2 mols. of hydrogen chloride, to prevent the formation of secondary amines (cf. Hartung and Hamlin, *J. Biol. Chem.*, 1942, **145**, 349). The product was shown to be methyl 2-amino-3-keto-octadecanoate hydrochloride (V) which gave an acetyl derivative (VI), identical with the product obtained



by reductive acetylation of the oxime. Reduction of the hydrochloride (V) over platinum oxide gave methyl 2-amino-3-hydroxyoctadecanoate hydrochloride (VII) in good yield. The *NO*-diacetyl derivative (VIII) was prepared and was identical with the product obtained by a similar reduction followed by acetylation, of methyl 2-acetamido-3-keto-octadecanoate (VI). The final stage was carried out by reducing the free base, methyl 2-amino-3-hydroxy-octadecanoate, with lithium aluminium hydride to give 2-amino-octadecane-1 : 3-diol (IX), isolated as the hydrochloride. The *N*-acetyl (X; R′ = H, R″ = Ac), triacetyl (X; R′ = R″ = Ac), and tribenzoyl derivatives (X; R′ = R″ = Bz) were prepared and had melting points within a few degrees of the corresponding derivatives prepared from dihydrosphingosine obtained from naturally occurring sphingolipids (Carter *et al.*, *loc. cit.*, p. 269), despite the fact that the synthetic product is probably a mixture of the two possible racemates. It has, however, been suggested (Fodor, Bruckner, Kiss, and Ōhegyi, *J. Org. Chem.*, 1949, **14**, 337) that the orientation of the keto- and oxime groups predetermines the position of the hydroxy- and amino-groups in the reduction product, leading to the formation of one racemate only.

The salts (V) and (VII), and the hydrochloride of (IX) were submitted to paper-partition chromatography and found to have R_F values 0·12, 0·88, and 0·73 respectively. Under similar conditions, a sample of natural dihydrosphingosine hydrochloride had R_F 0·72.

Experimental.

Methyl 3-Keto-octadecanoate (III).—This was prepared according to Ställberg-Stenhagen's method (*loc. cit.*) in 55% yield, and had m. p. 48·5—49° (from methanol).

Oximination of Methyl 3-Keto-octadecanoate.—Of the several methods tried for introduction of an oximino-group into methyl 3-keto-octadecanoate, the following was the most successful. A solution of hydrogen chloride in dry ether (150 ml.) was added dropwise, with stirring, to an ice-cooled solution of methyl 3-keto-octadecanoate (12·5 g., 0·04 mole) and butyl nitrite (8·25 g., 0·08 mole) in dry ether (60 ml.) during 45 minutes. The mixture was allowed to warm to room temperature during 1 hour, then poured into ice-cold water (300 ml.), and the ethereal solution separated, washed free from acid, and dried ($MgSO_4$). Removal of the solvent gave a solid, m. p. 57—59°. This was not purified, but was reduced directly.

When the oximination was carried out by passing a rapid stream of gaseous hydrogen chloride into an ethereal solution of methyl 3-keto-octadecanoate and butyl nitrite, a solid, m. p. 88—90°, separated and was removed by filtration. Recrystallisation from alcohol, ether, and finally light petroleum (b. p. 60—80°) gave *3-keto-2-oximino-octadecanoic acid*, m. p. 91—91·5° (Found : C, 66·2; H, 9·7; N, 4·3. $C_{18}H_{33}O_4N$ requires C, 66·1; H, 10·1; N, 4·3%).

Methyl 2-Amino-3-keto-octadecanoate Hydrochloride (V).—The crude oximination product (m. p. 57—59°) in ethyl alcohol (50 ml.) was shaken with hydrogen at 100 lb./sq. in., palladium–charcoal (2 g.; 10%; Ott and Schröter, *Ber.*, 1927, **60**, 633), palladium chloride (0·4 g.), and concentrated hydrochloric acid (6 ml.; 35%) for 6 hours. The precipitated crystalline solid was redissolved by

addition of a further 150 ml. of alcohol and warming; the catalyst was removed by filtration and the filtrate evaporated to dryness. The solid was refluxed with dry ether for 30 minutes, filtered off, and recrystallised twice from ethyl alcohol, to give *methyl 2-amino-3-keto-octadecanoate hydrochloride*, m. p. 115° (Found: C, 62·2; H, 10·2; N, 4·1; Cl, 9·8. $C_{19}H_{38}O_3NCl$ requires C, 62·7; H, 10·5; N, 3·9; Cl, 9·7%). The yield, from methyl 3-keto-octadecanoate, was 7·4 g. (55%). This compound gives a red-violet colour with ninhydrin and a cherry-red colour with alloxan.

The hydrochloride (1 g.), sodium acetate (0·1 g.; anhydrous), and acetic anhydride (5 ml.), heated at 100° for 1 hour, cooled, and poured into ice-cold water (100 ml.), gave an oil which quickly solidified. Recrystallised from alcohol and then acetone the *N-acetyl* derivative gave rosettes, m. p. 85° (Found: C, 68·1; H, 10·4; N, 3·4. $C_{21}H_{39}O_4N$ requires C, 68·2; H, 10·6; N, 3·8%). When a melted specimen was viewed under a polarising microscope, large feathery spherulites were seen. The 2 : 4-*dinitrophenylhydrazone* of this derivative separated from alcohol and was recrystallised from ethyl acetate; it had m. p. 114° (Found: C, 58·75; H, 7·6; N, 12·5. $C_{27}H_{43}O_7N_5$ requires C, 58·95; H, 7·9; N, 12·75%). It is interesting that this analysis corresponds to the open-chain derivative, whereas ethyl α-acetamidoacetoacetate forms a pyrazolone derivative with 2 : 4-dinitrophenylhydrazine (Albertson *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 1150).

Methyl 2-acetamido-3-keto-octadecanoate was also prepared directly from the crude oximination product by reductive acetylation as follows. To the crude oximination product (2·0 g.) (m. p. 57—59°) in acetic acid (20 ml.)–acetic anhydride (12 ml.), zinc dust (3 g.) was added with stirring during 20 minutes, the temperature rising to about 40°. The mixture was stirred at 50° for 3 hours and then at room temperature for 2 hours, after which water (30 ml.) was added and the zinc removed by filtration. The zinc cake was washed with chloroform (3 × 20 ml.) and this extract added to the filtrate, the chloroform layer separated, washed with water followed by saturated sodium hydrogen carbonate, and dried ($MgCl_2$), and the solvent removed. The solid was recrystallised from acetone eight times, whereafter it had m. p. 83—84°; the mixed m. p. with the previously prepared specimen was 84°.

Methyl 2-Amino-3-hydroxyoctadecanoate Hydrochloride (VII).—Methyl 2-amino-3-keto-octadecanoate hydrochloride (5·5 g.) in ethyl alcohol (200 ml.) was shaken with hydrogen at 100 lb./sq. in. at room temperature for 10 hours with platinum oxide (Adams's catalyst; 0·25 g.). The catalyst was removed by filtration, a further quantity of fresh platinum oxide added, and the reduction continued for a further 10 hours. The solution, after removal of catalyst, was concentrated to 50 ml. and cooled to 0°, whereupon some unchanged material crystallised and was recovered. On evaporation of the solution to dryness, 4·7 g. of material were obtained and refluxed with dry ether for 30 minutes; removal of the solid (4·5 g.) by filtration gave *methyl 2-amino-3-hydroxyoctadecanoate hydrochloride*, m. p. 122—125° (82%) (Found: C, 62·3; H, 10·9; N, 4·0; Cl, 10·0. $C_{19}H_{40}O_3NCl$ requires C, 62·3; H, 11·0; N, 3·8; Cl, 9·7%). This material gave a red-violet colour with alcoholic ninhydrin.

This hydrochloride (0·5 g.), with sodium acetate and acetic anhydride, gave *methyl 2-acetamido-3-acetoxyoctadecanoate*, m. p. 103·5—104·5° after recrystallisation from ethyl alcohol and acetone (Found: C, 66·4; H, 10·5; N, 3·7. $C_{23}H_{45}O_5N$ requires C, 66·8; H, 10·5; N, 3·4%). When viewed under the polarising microscope, a melted layer gave very large spherulites easily distinguishable from methyl 2-acetamido-3-keto-octadecanoate.

Reduction of Methyl 2-Acetamido-3-keto-octadecanoate.—A solution of the ester (0·5 g.) in ethyl alcohol (30 ml.) was shaken with platinum oxide (0·05 g.) and hydrogen at 100 lb./sq. in. for 10 hours; the catalyst was removed, a further quantity added (0·05 g.), and the hydrogenation continued for a further 10 hours. Removal of the catalyst and concentration of the solution gave a crystalline solid, m. p. 80—83°. This was acetylated in the usual manner with sodium acetate and acetic anhydride to give a product, m. p. 92—95°. Two recrystallisations from alcohol and one from acetone gave methyl 2-acetamido-3-acetoxyoctadecanoate, m. p. 102·5—103·5°; the mixed m. p. with a sample prepared as above was 103°.

2-*Amino-octadecane*-1 : 3-*diol* (IX).—Methyl 2-amino-3-hydroxyoctadecanoate hydrochloride (3·7 g., 0·01 mole) was shaken with ether (30 ml.) and a 5% aqueous solution of sodium acetate (100 ml.) containing one equivalent of sodium hydroxide (10 ml. of N.) until all the solid had disappeared. The ethereal solution was then separated and washed repeatedly with dilute aqueous alcohol until free from alkali. After being dried, the ether was removed, leaving the free base, methyl 2-amino-3-hydroxyoctadecanoate (3·0 g., 90%). The base was added in sodium-dried ether (20 ml.) dropwise with stirring to a solution of lithium aluminium hydride (1·15 g., 0·03 mole) in anhydrous ether (30 ml.) during 15 minutes. The reaction mixture was refluxed with stirring for 4 hours, after which water (10 ml.) was carefully added to decompose excess of lithium aluminium hydride. The ethereal layer was separated and shaken with 10% sodium hydroxide solution (20 ml.). A solid appeared at the interface and was removed by filtration; this appears to be sodium 2-amino-3-hydroxyoctadecanoate, obtained by hydrolysis of the original methyl ester. The ethereal solution was washed with water and dried ($MgSO_4$). Dry hydrogen chloride was passed in until precipitation was complete. Recrystallisation of the product gave 0·9 g. (30%) of 2-*amino-octadecane*-1 : 3-*diol hydrochloride* (Found: C, 63·7; H, 11·5; N, 4·5; Cl, 10·8. $C_{18}H_{40}O_2NCl$ requires C, 64·0; H, 11·9; N, 4·2; Cl, 10·5%). This product is probably a mixture of the two racemates.

A further quantity of amino-diol was recovered as the *N*-acetyl derivative (see below) from the residues of the reduction experiment, the total yield (from methyl 2-amino-3-hydroxyoctadecanoate) being 45%.

2-*Acetamido-octadecane*-1 : 3-*diol* (X; R′ = H, R″ = Ac).—To 2-amino-octadecane-1 : 3-diol hydrochloride (0·4 g.) in ether (30 ml.)–10% aqueous sodium hydroxide (20 ml.), acetic anhydride (0·3 g.) was added in three portions with cooling and shaking, a precipitate being formed in the ethereal layer. The precipitate was dissolved by addition of methanol, and the ether–methanol solution washed free

from alkali, dried, and concentrated to 5 ml., whereupon a precipitate formed; this was removed by filtration and recrystallised from methyl alcohol, to give *2-acetamido-octadecane*-1 : 3-*diol*, m. p. 123—125° (Found : C, 70·0; H, 11·8; N, 4·1. $C_{20}H_{41}O_3N$ requires C, 69·9; H, 12·0; N, 4·1%). A melted specimen showed characteristic large spherulites when viewed under the polarising microscope. *N*-Acetyldihydrosphingosine, prepared by Carter *et al.* (*loc. cit.*, p. 269) from dihydrosphingosine sulphate obtained by hydrolysis of sphingolipids, had m. p. 126°.

Both 2-amino-octadecane-1 : 3-diol hydrochloride and 2-acetamido-octadecane-1 : 3-diol, when acetylated in the usual manner with acetic anhydride and sodium acetate, gave *2-acetamido*-1 : 3-*diacetoxy-octadecane*, m. p. 98—100° (Found : C, 67·7; H, 10·7; N, 3·1. $C_{24}H_{45}O_5N$ requires C, 67·4; H, 10·6; N, 3·3%). Carter gives 102—103° as the m. p. of triacetyldihydrosphingosine.

The amino-diol (from 0·2 g. of the hydrochloride), benzoylated in pyridine (5 ml.) with benzoyl chloride (0·35 g.) at room temperature for 3 hours, gave an oil which quickly solidified. Three recrystallisations from ethyl alcohol gave the *tribenzoyl* derivative, m. p. 138—140° (Found : C, 76·3; H, 8·1; N, 2·4. $C_{39}H_{51}O_5N$ requires C, 76·4; H, 8·4; N, 2·3%). Carter records the m. p. of tribenzoyldihydrosphingosine as 144°.

Paper-partition Chromatography of the Hydrochlorides.—Alcoholic solutions (5%) of methyl 2-amino-3-keto- (V) and methyl 2-amino-3-hydroxy-octadecanoate hydrochloride (VII), and 2-amino-octadecane-1 : 3-diol hydrochloride were spotted, according to the technique of Consden, Martin, and Gordon (*Biochem. J.*, 1944, **38**, 224), on sheets of Whatman No. 1 filter paper. The solvent used was butanol–ethanol–water (40 : 19 : 11). Before the solvent had reached the bottom, the paper was removed, dried, and sprayed with a 0·2% alcoholic solution of ninhydrin. On warming, the methyl 2-amino-3-keto-octadecanoate hydrochloride was observed as purple spots ($R_F = 0·12$), and the 3-hydroxy-ester gave purple spots if left at room temperature for some time ($R_F = 0·88$). 2-Amino-octadecane-1 : 3-diol hydrochloride is best detected by spraying the chromatogram with ammoniacal silver nitrate, which gives brown spots (R_F 0·73). A specimen of dihydrosphingosine hydrochloride, obtained from the sphingolipids of beef brain, was subjected to paper-partition chromatography under similar conditions and had R_F 0·72.

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