424. *The Sphingolipid Field. Part II.* An Improved Synthesis of Racemic 2-Amino-octadecane-1 : 3-diols, and an X-Ray Examination of Its Derivatives.*

By M. J. Egerton, G. I. Gregory, and T. Malkin.

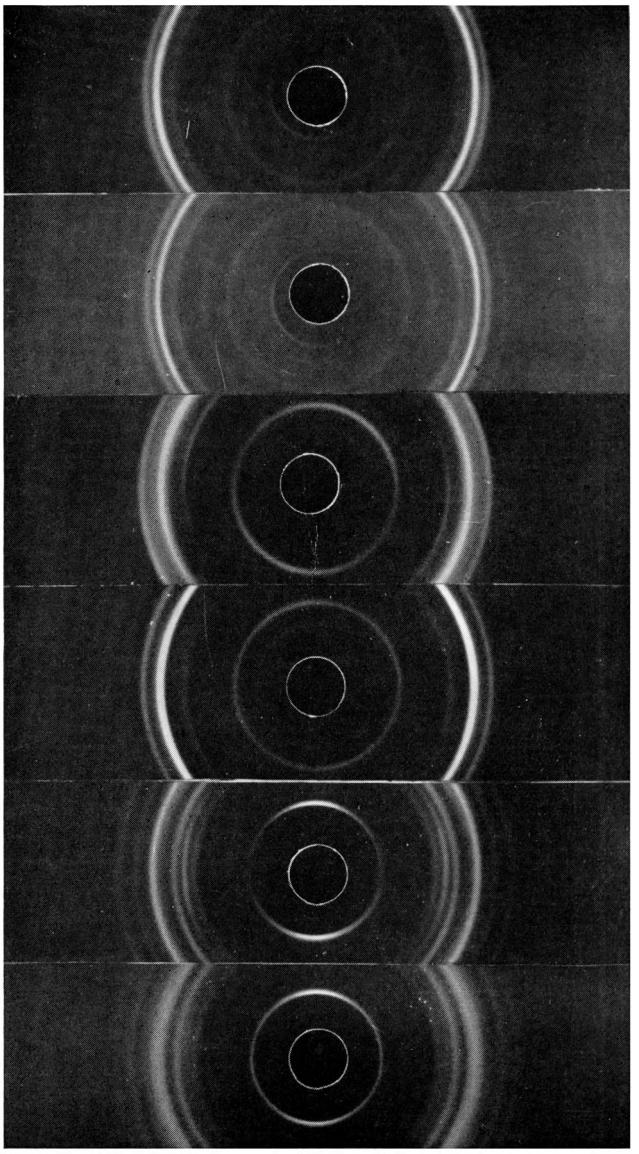
An improved synthesis of racemic 2-amino-octadecane-1 : 3-diols is described, in which hexadecanal is condensed with 2-nitroethanol, and the racemic 2-nitro-octadecane-1 : 3-diols so formed are catalytically reduced to the amino-diols. *X*-Ray data are given for the *N*-acetyl, triacetyl, and tribenzoyl derivatives of the synthetic amino-diols and dihydrosphingosine.

Since the publication of Part I,* which described the synthesis of racemates of 2-amino-octadecane-1 : 3-diols from methyl 3-keto-octadecanoate,† Grob, Jenny, and Utzinger (*Helv. Chim. Acta*, 1951, **34**, 2249) have described a new synthesis in which they condense hexadecanal with 2-nitroethanol, and reduce the product to the amino-diol. Unaware of this work, we had ourselves carried out this synthesis and, as our method differs considerably at each stage, with an advantage in overall yield (44%, compared with 10·5%), we thought it desirable to publish our results before our study of the racemates was completed.

The main loss by the above workers is in the Rosenmund conversion of palmitoyl chloride into hexadecanal (35% yield compared with our 85%), where they appear to have overlooked, *inter alia*, the necessity for the use of a quinoline–sulphur poison (see also Experimental).

In the condensation of hexadecanal with 2-nitroethanol, we adopted the method used by Controulis, Rebstock, and Crooks for the synthesis of chloramphenicols (*J. Amer. Chem. Soc.*, 1949, **71**, 2462), namely, the use of sodium methoxide in methanol, and isolation of the sodium salt of *aci*-2-nitro-octadecane-1 : 3-diol (I). The latter was obtained in 78% yield in a few hours, compared with 60% after four days by Grob *et al.*, who used sodium hydroxide in methanol.

* Part I, *J.*, 1951, 2453. † Geneva numbering (CO_2H = 1).



Racemic 2-acetamido-octadecane-1 : 3-diols.

N-*Acetyl-dihydrosphingosine.*

Racemic triacetyl derivative of 2-amino-octadecane-1 : 3-diols.

Triacetyl-dihydrosphingosine.

Racemic tribenzoyl derivative of 2-amino-octadecane-1 : 3-diols.

Tribenzoyl-dihydrosphingosine.

[*To face p.* 2273.

Our final reduction was carried out with Adams's platinum oxide, which gives an advantage of some 20% in yield over the Raney nickel method used by Grob *et al.*

$$C_{15}H_{31}\cdot CHO + H_2C(NO_2)\cdot CH_2\cdot OH \xrightarrow{NaOMe} C_{15}H_{31}\cdot CH(OH)\cdot C(NO\cdot ONa)\cdot CH_2\cdot OH \text{ (I)} \xrightarrow[PtO_2]{H_2} C_{15}H_{31}\cdot CH(OH)\cdot CH(NH_2)\cdot CH_2\cdot OH$$

In a reduction with palladium black we found that only two-thirds of the required amount of hydrogen was taken up; the product did not appear to contain any of the required amino-diol, and was presumably the hydroxylamine derivative. It was not purified, but was further reduced in the presence of Adams's catalyst, whereupon the remaining third of hydrogen was taken up, to yield the amino-diol.‡

N-Acetyl, triacetyl, and tribenzoyl derivatives of the synthetic diol were prepared as described in Part I, as were similar derivatives of dihydrosphingosine, which was isolated from beef brain, essentially by the method of Carter, Norris, Glick, Phillips, and Harris (*J. Biol. Chem.*, 1947, **170**, 269; see also Carter, Haines, Ledyard, and Norris, *ibid.*, 1947, **169**, 77). The synthetic material is, of course, a mixture of two racemates, and although the product was crystallised as far as possible to constant melting point, m. p.s show slight divergencies from those reported in Part I. Of the three derivatives the *N*-acetyl shows the greatest constancy in this respect. This compound also exhibits the greatest similarity to the natural optically active *N*-acetyl derivative (*N*-acetyldihydrosphingosine) in both melting point and *X*-ray spacings, the *X*-ray photographs being practically indistinguishable. Triacetyl and tribenzoyl derivatives of the natural and the synthetic product exhibit small but real differences in both melting points and *X*-ray structure (see Table and Plate).

Melting points and X-ray spacings of derivatives of dihydrosphingosine and of racemic 2-amino-octadecane-1 : 3-diols.

Derivative	$[\alpha]_D^{20}$	M. p.	Long spacing, Å	Short spacings,* Å
N-Acetyl (natural)	+9·5°	125—126°	28·6	3·23w, 3·50w, 3·84s, 4·10vs, 4·56m
,, (synthetic) ...	—	124—125	28·5	3·29w, 3·51w, 3·86s, 4·10vs, 4·56m
Triacetyl (natural)	+16·2°	102—103	32·3	3·40w, 3·76s, 4·20vs, 4·96m
,, (synthetic) ...	—	94—96	31·7	3·41w, 3·76s, 4·26vs, 4·96m
Tribenzoyl (natural) ...	−28·8	144—145	30·8	3·29w, 3·37w, 4·06vs, 4·66s, 5·01w, 5·39m
,, (synthetic)...	—	147—148	30·3	3·32w, 3·44w, 4·04vs, 4·63s, 4·99m, 5·39

vs = very strong, s = strong, m = moderate, w = weak.

* For historical reasons, these spacings have hitherto been referred to as side spacings. The above terminology is, however, more consistent.

Experimental

X-Ray Investigation.—Long spacings were determined as described earlier (*J.*, 1934, 666), by using a Müller spectrograph. Exposures of ½ hour each side of the film were required, the tube being run at 12—15 milliamp. Short spacings were determined on a Philips *X*-ray diffraction apparatus (Cu). Exposures of ½ hour were required, the tube being run at 20 milliamp. and 30 kv.

Hexadecanal.—In a three-necked flask equipped with a stirrer, condenser, and hydrogen-inlet tube, were placed xylene (90 c.c.; sodium-dried), hexadecanoyl chloride (20 g.; freshly prepared), palladium on barium carbonate (8 g.; cf. Vogel, " Practical Organic Chemistry," Longmans, Green and Co., p. 990), and quinoline–sulphur poison (0·8 c.c.; *op. cit.*, p. 669). The reaction mixture was heated in an oil-bath at 150° with constant stirring, and a rapid stream of hydrogen was passed through the mixture and into a flask of distilled water to absorb the hydrogen chloride produced. The aqueous acid was titrated with N-sodium hydroxide from time to time, and the end of the reaction was indicated by the persistence of the pink colour of phenolphthalein (100 minutes). The catalyst was filtered off and washed with a little dry xylene, and the solvent was removed under reduced pressure. Distillation of the residue gave hexadecanal (15 g., 86%), b. p. 142—145°/1 mm., m. p. 36°.

‡ Using hexadec-2-enal instead of hexadecanal, and reducing the product with lithium aluminium hydride, we have now obtained racemic 2-aminoctadec-4-ene-1 : 3-diols, one isomer of which is sphingosine.—G. I. G., T. M.

85—90% of the theoretical amount of alkali is normally required. Barium carbonate is preferable to barium sulphate as catalyst support. Sodium-dried xylene is satisfactory, and we do not recommend the use of aluminium chloride for drying. A longer time of reaction usually results in a lower yield, and the time required increases with the molecular weight of the aldehyde; thus for comparable experiments, the times are dodecanal 60 and tetradecanal 90 minutes. Purification through the bisulphite compound, the method adopted by Grob *et al.*, results in loss of the aldehyde trimer, which is invariably formed to some extent. On distillation, as described above, the trimer is reconverted into monomer. There is, indeed, evidence that the use of bisulphite favours formation of trimer (Le Sueur, *J.*, 1904, 827).

Sodium Salt of aci-2-*Nitro-octadecane*-1 : 3-*diol*.—To hexadecanal (5·25 g.) and 2-nitroethanol (2·0 g.) in dry methanol (100 c.c.) was added with stirring methanolic sodium methoxide (0·505 g. of sodium in 50 c.c. of methanol) during 20 minutes, a white precipitate being formed. Stirring was continued for a further 2 hours, after which the white precipitate was filtered off and washed with a little cold methanol and with ether. The yield was 6 g. (78%). The salt is not stable and gradually becomes orange-brown; after some weeks it fails to give good yields of the amino-diol when reduced as described below.

2-*Amino-octadecane*-1 : 3-*diol*.—To the above sodium salt (4·0 g.) in glacial acetic acid (60 c.c.) was added platinic oxide (0·2 g.), and reduction was effected at room temperature, the theoretical amount of hydrogen being taken up in 4 hours. After removal of the catalyst, the solution was concentrated to 10 c.c. under reduced pressure, and ether (100 c.c.) was added. The resulting ethereal suspension of amino-diol acetate was shaken with 2N-sodium hydroxide (100 c.c.) and methanol (20 c.c.), and the ethereal layer of free base was separated and dried (Na_2SO_4). Removal of the solvent yielded 2·4 g. (71%) of racemic 2-amino-octadecane-1 : 3-diols, m. p. 82—85° which did not change appreciably on crystallisation from hexane (Found: C, 71·5; H, 13·0; N, 4·5. Calc. for $C_{18}H_{39}O_2N$: C, 71·7; H, 13·0; N, 4·65%).

Reduction of the above sodium salt (1 g.) with palladium black resulted in the uptake of only 2 mols. of hydrogen. The product did not appear to contain any amino-diol, and was presumably the intermediate hydroxylamine derivative. The product was not purified, but was further reduced in ethanol with Adams's platinum oxide, whereupon a further mol. of hydrogen was taken up. After removal of the catalyst the solution was concentrated to 5 c.c. and brought to pH 4 with N-methanolic sulphuric acid; 2-*amino-octadecane*-1 : 3-*diol sulphate* was precipitated, having m. p. 228—232° after two crystallisations from acetic acid (Found: C, 61·2; H, 11·25; N, 4·0; S, 5·0. $C_{36}H_{80}O_8N_2S$ requires C, 61·6; H, 11·5; N, 4·0; S, 4·6%).

2-*Acetamido-octadecane*-1 : 3-*diol*.—This was prepared from the amino-diol as described in Part I. Several crystallisations from acetone yielded rosettes, m. p. 124—125°.

The triacetyl derivative was prepared from the free base or from the *N*-acetyl derivative as described in Part I, or by acetylation with acetic anhydride and pyridine. It was not easily purified, but after crystallisation from hexane and from ethanol (twice) it melted at 94—96°.

The tribenzoyl derivative was prepared as described in Part I and was crystallised first from methanol and then from acetone, yielding long, fine needles, m. p. 147—148°.

Although the above compounds are almost certainly mixtures of racemates, further crystallisation did not appreciably affect the m. p.s.

Dihydrosphingosine.—Sphingolipid mixtures were obtained from beef brain (approx. 5-lb. batches) by using the procedure described by Carter, Haines, *et al.* (*loc. cit.*). The crude material was crystallised once from acetic acid, washed thoroughly with acetone, and hydrolysed, isolated, and purified through the sulphates, as described by Carter, Norris, *et al.* (*loc. cit.*). *N*-Acetyl, triacetyl, and tribenzoyl derivatives of dihydrosphingosine were prepared as described for the corresponding synthetic compounds.

Our thanks are offered to the Department of Scientific and Industrial Research (G. I. G.) and to the Gloucester Education Authority (M. J. E.) for maintenance grants, to Professor C. W. Ottaway, of our Veterinary Science Department, who kindly arranged for the provision of beef brain, and to Messrs. Price (Bromborough) Ltd. for gifts of fatty acids.

THE UNIVERSITY, BRISTOL, 8. [*Received, February 2nd,* 1952.]