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# A SYNTHESIS OF PHYTOSPHINGOSINE FROM D-GLUCOSAMINE

Roy Gigg, C.D. Warren and Jill Cunningham
National Institute for Medical Research, London, N.W.7.

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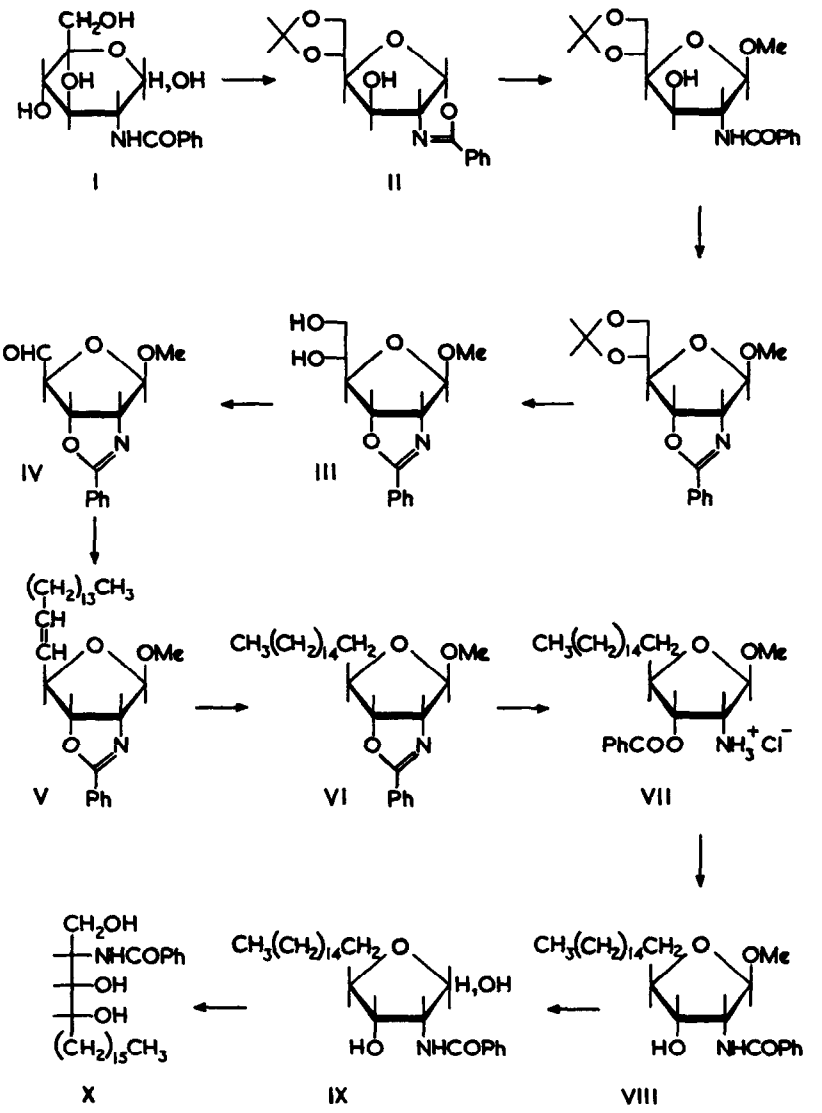
Phytosphingosine (cerebrin base) was first isolated[1] from the mushroom Amanita muscaria by Zellner in 1911 and has subsequently been isolated by other workers from various yeasts and moulds[2]. In 1952, Oda[3] established that phytosphingosine was one of the eight stereoisomers of 2-amino-1, 3, 4-trihydroxyoctadecane and in 1954, Carter and his coworkers[4] reported the isolation of phytosphingosine from plant seeds and arrived independently at the same structure. Further work by Carter and coworkers[5] has shown that phytosphingosine is present in plant seeds in the form of a complex glycolipid (phytoglycolipid) in which the phytosphingosine is joined through phosphate ester linkages to an oligosaccharide containing inositol, glucuronic acid, glucosamine, mannose, galactose, arabinose and (in some seeds) fucose. A tetra-acetate of phytosphingosine is produced extra-cellularly by the yeast Hansenula ciferrii[6] and cerebrosides containing phytosphingosine have been isolated from wheat flour[7] and plant leaves[8]. Recently Karlsson[9], has shown that phytosphingosine is a major component of the long-chain bases of human kidney cerebrosides and that it is also present in small quantities in human brain. He has suggested that phytosphingosine is an intermediate in the biosynthesis of sphingosine which is the major long-chain base of nervous tissue.

In 1963, Carter and Hendrickson[10] established by degradative studies that phytosphingosine had the D-ribo configuration (X) and

several investigators[11] have shown that natural phytosphingosine can have a chain length of 18 or 20 carbon atoms.

A previous synthesis[12] of 2-amino-1, 3, 4-trihydroxyoctadecane gave a mixture of stereoisomers and syntheses of the corresponding 1, 2, 3, 4-tetra-hydroxyalkanes[13] with some control over the stereochemistry have been reported. Recently[14] phytosphingosine has been prepared from synthetic sphingosine by reduction of the corresponding epoxide.

When considering a synthesis of phytosphingosine, carbohydrate intermediates seemed to be ideal since the stereochemistry of these compounds is already established. In previous synthetic work on the amino-sugar muramic acid[15], we had made use of the oxazoline (II) which is readily prepared[16] from N-benzoyl-D-glucosamine (I). Further investigations[17] showed that the oxazoline (II) could be converted into the oxazoline (III) which has the same configuration as phytosphingosine on carbon atoms 2, 3 and 4. Oxidation of the oxazoline (III) with sodium periodate, and condensation of the aldehyde (IV) with the Wittig reagent prepared from pentadecyl bromide[18], gave the crystalline product (V) (presumably the cis isomer) [needles, m.p. 43-44° from aqueous methanol; $[\alpha]_D^{22}$ + 11° (c, 1 in $CHCl_3$). Found: C 75.9; H. 9.7, N, 3.0; $C_{28}H_{43}NO_3$ requires C, 76.1; H, 9.8; N, 3.2%, $\nu_{max.}$ 1650 $cm^{-1}$ (C=N)]. On reduction with hydrogen in the presence of palladium on charcoal the corresponding saturated compound (VI) was formed [needles, m.p. 63-65° from aqueous methanol, $[\alpha]_D^{22}$ + 13° (c, 0.7 in $CHCl_3$). Found: C, 75.5, H, 10.3, N, 3.0 $C_{28}H_{45}NO_3$ requires C, 75.8; H, 10.2, N. 3.2%; $\nu_{max.}$ 1645 $cm^{-1}$ (C=N)]. Compound (VI) was readily hydrolysed with 0.05N acid in aqueous methanol to give the salt (VII) which was rapidly converted, in alkaline solution, to the amide (VIII) [needles, m.p. 112° from ethyl acetate-light petroleum, $[\alpha]_D^{22}$ + 6° (c, 1 in $CHCl_3$). Found. C, 72.7, H, 10.3, N, 3.1 $C_{28}H_{47}NO_4$ requires C, 72.8; H, 10.3; N, 3.0%)]. Compound (VIII) was hydrolysed by 0.1N acid in aqueous dioxan to give the furano-sugar (IX) [needles,


I
II
III
IV
V
VI
VII
VIII
IX
X

m.p. 107-109° from cyclohexane; $[\alpha]_D^{22}$ + 11° (c, 1 in $CHCl_3$). Found: C, 72.3, H, 10.0; N, 3.1 $C_{27}H_{45}NO_4$ requires C, 72.4, H, 10.1, N, 3.1%] and this on reduction with sodium borohydride gave the N-benzoyl derivative of $C_{20}$-phytosphingosine (X) [needles, m.p. 136-137° from ethyl acetate, $[\alpha]_D^{22}$ + 5° (c, 1 in pyridine). Found C, 72.3; H, 10.5, N, 3.2, $C_{27}H_{47}NO_4$ requires C, 72.1, H, 10.5, N, 3.1%, lit.[4] m.p. 135-136° for the $C_{18}$ compound, $[\alpha]_D$ + 5°, lit.[19] m.p. 137.8-138.8° for the $C_{18}$ compound].

By the action of acetic anhydride in pyridine, the amide (X) was converted to the triacetate [needles, m.p. 81-82° from aqueous ethanol; $[\alpha]_D^{22}$ + 11° (c, 0.53 in $CHCl_3$) Found C, 68.9, H, 9.4; N, 2.4; $C_{35}H_{53}NO_7$ requires C, 68.8; H, 9.3, N, 2.4%, lit.[10], m.p. 79.5-81° for the $C_{18}$ compound, lit.[19] m.p. 79-80° for the $C_{18}$ compound].

Compound (III) is also a suitable intermediate for the synthesis of dehydrophytosphingosine which has been isolated from plant seeds[10, 20] and the route to this compound is being investigated.

A further method for the synthesis of phytosphingosine via 1, 3, 4-tri-O-benzyl-2-deoxy-2-phthalimido-L-talitol and a method for preparing the various stereoisomers of phytosphingosine using the 1, 2, 3, 4-tetrahydroxyalkanes as intermediates have been investigated and will be published shortly. The 1, 2, 3, 4-tetrahydroxyalkanes are prepared from 1, 2, 3, 4-tetra-O-benzy hexitols which have been made available by using the allyl ether as a protecting group[21].

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