Synthesis of Phytosphingosine[*]

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ABSTRACT: Phytosphingosine was synthesized from sphingosine by the following sequence of reactions: (1) benzoylation to the tribenzoyl derivative; (2) epoxidation with perbenzoic acid; (3) reduction with $LiAlH_4$; (4) resolution of the diastereoisomeric *N*-benzyl bases; and (5) hydrogenolysis of the *N*-benzyl group. The *N*-benzoyl derivatives of the bases obtained from the resolution of the diastereoisomers melted at 135–136° and 101–104°. The same derivative of the natural base melted at 135–136°. The conversion of sphingosine into phytosphingosine is further proof that carbon atoms 2 and 3 of each base have the same configuration.

The proof of the structure of phytosphingosine as D-*ribo*-1,3,4-trihydroxy-2-aminooctadecane was completed recently by Carter and Hendrickson (1963). Nothing is known about the biosynthesis of this compound. By analogy with the formation of sphingosine in mammalian tissues, it could arise from the condensation of pentadecanal with L-2-hydroxy-3-amino-4-hydroxybutyric acid or of D-2-hydroxyhexadecanal with L-serine. Phytosphingosine may be formed, also, by the hydration of sphingosine. The potential existence of such a biosynthetic pathway is suggested by the chemical synthesis of phytosphingosine from sphingosine.

Sphingosine was carried through the following sequence of reactions (Figure 1A–G): (1) benzoylation to the tribenzoyl derivative; (2) epoxidation with perbenzoic acid in chloroform-benzene; (3) reduction with $LiAlH_4$; (4) resolution of the diastereoisomeric *N*-benzyl bases with petroleum ether–ethanol; and (5) hydrogenolysis of the *N*-benzyl group over palladium in ethanol. Vapor-phase chromatography of the aldehydes obtained after periodate oxidation of the *N*-benzyl bases (Figure 1D,E) disclosed that the epoxide ring was opened by $LiAlH_4$ exclusively on carbon atom 5. This is in agreement with the hypothesis of Eliel and Delmonte (1956) that $LiAlH_4$ attacks unsymmetrically substituted epoxides predominantly at the least substituted carbon atom to give the more highly substituted alcohol. Scission of the epoxide ring at carbon atom 4 of the C_{20} homolog of tribenzoylsphingosine by hydrogenation over platinum in 95% ethanol has been reported (Proštenik and Majhofer-Oresčanin, 1960); in the present study, under essentially the same conditions and with many variations, the epoxide of tribenzoylsphingosine (Figure 1C) was unaffected. Attempts to reverse the direction of ring opening by conducting the reduction in the presence of $AlCl_3$ (Eliel and Delmonte, 1958) were unsuccessful. Some debenzylation occurred during reduction of the epoxide of tribenzoylsphingosine with $LiAlH_4$ since the product gave a slightly positive ninhydrin reaction.

The *N*-benzoyl derivatives of bases F and G (Figure 1), obtained from the resolution of the diastereoisomeric mixture, melted at 135–136° and 101–104°, respectively. The same derivative of the natural base prepared by Carter *et al.* (1954) melted at 135–136°. The conversion of sphingosine into phytosphingosine is further proof that carbon atoms 2 and 3 of each base have the same configuration (Carter and Humiston, 1951; Carter and Hendrickson, 1963).

Experimental

4,5-trans-Epoxytribenzoylsphingosine (*C*). Sphingosine was isolated from beef spinal cord and converted to the tribenzoyl derivative according to the procedure of Carter *et al.* (1947). To 6.1 g of tribenzoylsphingosine in 20 ml of chloroform was added 2.76 g of perbenzoic acid (Swern, 1953) in 100 ml of benzene. After 24 hours at room temperature in the dark, the reaction mixture was washed with 5% $NaHCO_3$ and with water until neutral. The solution was filtered, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was crystallized from ethanol–petroleum ether, bp 60–70° (1:10) at room temperature; mp 135–137°; yield 4.34 g.

Anal. Calcd for $C_{39}H_{49}NO_6$ (627.8): C, 74.55; H, 7.87. Found: C, 75.07; H, 8.00.

The infrared-absorption bands in chloroform are: 3450 cm^{-1}(w), 2970 cm^{-1}(s), 1760 cm^{-1}(s), 1675 cm^{-1}(m), 1610 cm^{-1}(w), 1590 cm^{-1}(w), 1550 cm^{-1}(m), 1490 cm^{-1}(m), 1460 cm^{-1}(m), 1280 cm^{-1}(s), 1185 cm^{-1}(w), 1120cm^{-1}(s), 1080cm^{-1}(m), and 1040 cm^{-1}(m).

Diastereoisomeric Mixture of 1,3,4-Trihydroxy-2-benzylaminooctadecanes (*D,E*). To compound C,

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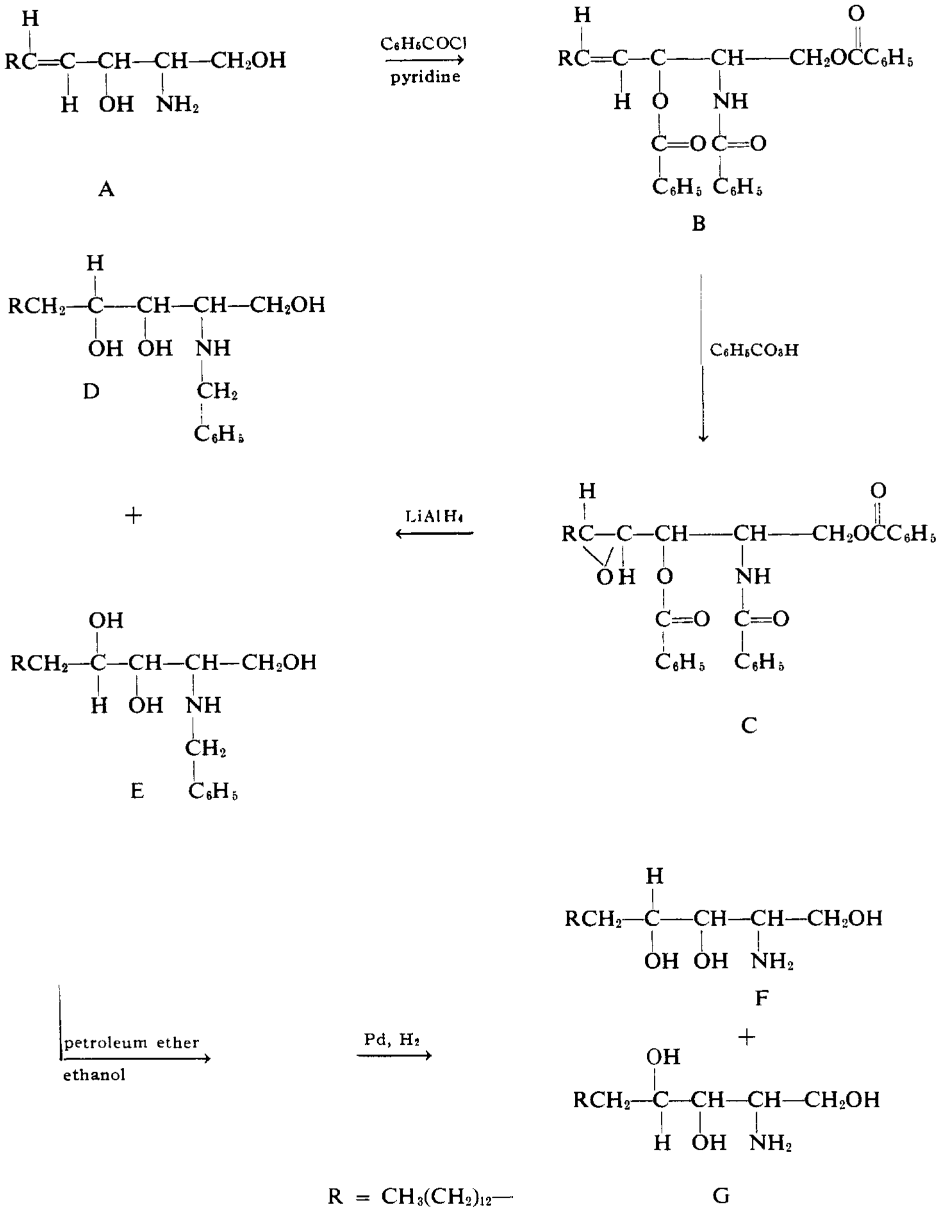

Figure 1: Sphingosine was carried through the above sequence of reactions to yield phytosphingosine. See text for details.

4.34 g, in 50 ml of cold dry ether, was added 1.1 g of $LiAlH_4$. After the initial reaction subsided, the mixture was refluxed 4 hours and then treated successively with 50 ml of methanol, 100 ml of 2.5 N NaOH, and three times with 150-ml portions of ether–ethyl acetate (1:1). The combined organic layers were washed with water until neutral, filtered, and concentrated. The residue, dried over P_2O_5, melted at 47–84°.

erythro Diastereoisomer (*D*). The diastereoisomeric mixture (D,E) was dissolved in 80 ml of hot petroleum ether. The precipitate, obtained by centrifugation of the chilled solution, was recrystallized three times from 70 ml of petroleum ether–ethanol (70:1) at room temperature; mp 91–92°; yield 1.23 g; ninhydrin reaction, negative.

Anal. Calcd for $C_{25}H_{45}O_3N$ (407.4): C, 73.64; H, 11.13. Found: C, 73.26; H, 11.21.

The infrared-absorption bands in chloroform are: 3400 cm^{-1}(m), 2970 cm^{-1}(s), 1470 cm^{-1}(m), 1230 cm^{-1}(m), and doublet 1070–1035 cm^{-1}(s).

threo Diastereoisomer (*E*). The initial supernatant from the chilled solution, after removal of the *erythro* compound, was concentrated. The dried, waxy residue melted at 48–58° and gave a slightly positive ninhydrin reaction; yield 1.36 g. Except for a slight variation in the doublet band, the infrared-absorption spectrum is essentially the same as that for compound D.

Analysis of Aldehydes. Periodate oxidation of both compounds D and E and vapor-phase chromatography of their long-chain aldehydes (Weiss, 1964) disclosed in each instance one major peak, pentadecanal, at 7 minutes; a peak at 10.5 minutes, hexadecanal, was due to small amounts of dihydrosphingosine in the sphingosine preparation.

The 2,4-dinitrophenylhydrazones of the aldehydes from compounds D and E melted at 104–106°; this is in agreement with the melting point of the same derivative for pentadecanal (Carter *et al.*, 1954).

erythro- and threo-1,3,4-Trihydroxy-2-aminooctadecanes (F and G). Compound D or E, 1.0 g, was reduced over 100 mg of palladium oxide in 20 ml of ethanol for 4 hours at room temperature. The filtered reaction mixture was treated with several portions of ether after the addition of 20 ml of water and 1 ml of 5 N NaOH. The combined and washed ether layers were concentrated, and the residue was dried over P_2O_5. The base from compound D melted at 97–107° and gave a positive ninhydrin reaction; the base from compound E was a wax and showed the same reaction toward ninhydrin.

N-Benzoylphytosphingosine. Base F or G, 500 mg, was dissolved in 40 ml of acetone, previously dried over P_2O_5 and distilled. After 18 hours at 5°, the solution was centrifuged. The slight precipitate from base G was discarded. The precipitate from base F was washed twice with small portions of acetone at room temperature and centrifuged each time; the dihydrosphingosine residue was discarded. The combined supernatants from base F and the supernatant from base G were concentrated to dryness and the products were benzoylated in aqueous alkali and ether according to the procedure of Carter *et al.* (1954). The *N*-benzoyl derivatives of bases F and G, recrystallized from ether-methanol, melted at 135–136° and 101–104°, respectively; yield 284 mg and 254 mg, respectively.

Anal. Calcd for $C_{25}H_{43}NO_4$ (421.4): C, 71.20; H, 10.29. Found for *N*-benzoyl base F and G, respectively: C, 71.29; H, 10.33; and C, 71.27; H, 10.35.

The infrared-absorption bands (KBr disk) for either product are: 3500 cm^{-1}(s), 3000 cm^{-1}(s), 1640 cm^{-1}(s), 1590 cm^{-1}(w), 1550 cm^{-1}(s), 1500 cm^{-1}(w), 1470 cm^{-1}(m), 1350 cm^{-1}(w), 1305 cm^{-1}(w), 1115 cm^{-1}(w), 1080 cm^{-1}(s), and 1045 cm^{-1}(m).

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