Synthesis of DL-1,2-Dihydroxyhexadecane, DL-*erythro*-1,2,3,-Trihydroxyhexadecane, and DL-1,2-Dihydroxy[1,3,4-^{3}H]hexadecane. Metabolism of [1,2-^{3}H]Palmitaldehyde and DL-1,2-Dihydroxy[1,3,4-^{3}H]hexadecane

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ABSTRACT: DL-1,2-Dihydroxyhexadecane was synthesized by reacting *trans*-2-hexadecenoic acid successively with diazomethane, perbenzoic acid, and $LiAlH_4$. The epoxide was opened at the least-substituted carbon atom to give the more highly substituted alcohol. DL-*erythro*-1,2,3-Trihydroxyhexadecane was prepared by refluxing DL-*trans*-2,3-epoxyhexadecanoic acid in glacial acetic acid, followed by treatment with alkali and $LiAlH_4$. Periodate oxidation of the diol and triol yielded pentadecanal and tetradecanal, respectively; the water-soluble cleavage product in each case was formaldehyde. DL-1,2-Dihydroxy[1,3,4-^{3}H]hexadecane was synthesized, and the isotope distribution was determined by periodate oxidation. The tritium content was greatest on carbon atom 1, whereas no activity was present on carbon atom 2. The similar activity on carbon atoms 3 and 4 may be due to the formation of a new carbonium ion center as the result of a hydride shift from carbon atom 4 to carbon atom 3, which is the carbonium ion center originally formed from scission of the epoxide ring by $LiAlH_4$. After intracranial injection of [1,2-^{3}H]palmitaldehyde and DL-1,2-dihydroxy-[1,3,4-^{3}H]hexadecane, the sphingosine and cholesterol isolated from the brain contained little radioactivity.

In order to test the hypothesis that sphingosine is formed by the condensation of palmitaldehyde and L-serine (Brady *et al.*, 1958; Sprinson and Coulon, 1954) Weiss (1964a) synthesized [1,2-^{3}H]palmitaldehyde. It was hoped that by determining the ratio of tritium on carbon atoms 3 and 4 of the isolated sphingosine the mechanism of the biosynthesis of the base would be better understood. After intracranial injection of [1,2-^{3}H]palmitaldehyde, the sphingosine obtained by hydrolysis of the brain sphingolipids (Carter *et al.*, 1947, 1961) contained little radioactivity (Table I); cholesterol, also, was only slightly radioactive (Table I). The low level of isotope incorporation into sphingosine and cholesterol was attributed to trimerization of the aldehyde (Stephen, 1925; Weiss, 1964a); transition from monomer (yellowish oil, soluble in ethanol) to trimer (white solid, insoluble in ethanol) is extremely rapid and may be seen easily during sublimation of the radioactive aldehyde. The normal instability of the aldehyde carbonyl group is considerably accentuated by the presence of tritium on carbon atoms 1 and 2. The administration of the thiosemicarbazone and diethyl acetal (Gray, 1960) derivatives of [1,2-^{3}H]palmitaldehyde did not improve the isotope utilization (Table I).

The possibility was considered that 1,2-dihydroxyhexadecane (Figure 1A) might serve as a precursor of palmitaldehyde if the secondary hydroxyl group were removed enzymatically by dehydration to form the vinyl alcohol (Figure 1B). That this was not the case was shown by the slight incorporation of isotope into sphingosine after injection of DL-1,2-dihydroxy-[1,3,4-^{3}H]hexadecane (Table I).

The base isolated from brain tissue was degraded with lead tetraacetate instead of with periodic acid, which caused the loss of some base (dihydrosphingosine) by formation of an insoluble salt. The degradation results were inconclusive as to the amount of tritium on carbon atoms 1 through 4 because of the low specific activity of the base.

DL-1,2-Dihydroxyhexadecane was synthesized by treating *trans*-2-hexadecenoic acid successively with diazomethane, perbenzoic acid, and $LiAlH_4$. The epoxide was opened at the least-substituted carbon atom to give the more highly substituted alcohol (Eliel and Delmonte, 1958; Weiss, 1965). The same result was achieved by the hydride reduction of DL-*trans*-2,3-epoxyhexadecanoic acid. Vapor-phase chromatography and conversion to the 2,4-dinitrophenylhydrazones of the long-chain aldehydes formed by periodate oxidation of the diol disclosed a single component, pentadecanal.

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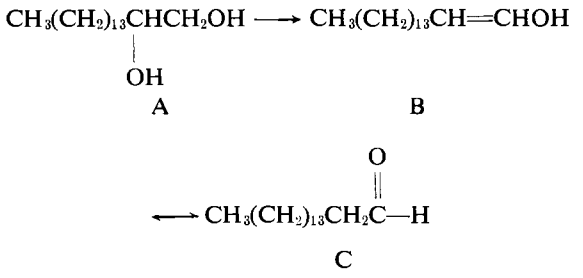

FIGURE 1: (A) DL-1,2-Dihydroxyhexadecane; (B) tautomeric form of aldehyde; (C) palmitaldehyde. See text for details.

TABLE I: Utilization of [1,2-^{3}H]Palmitaldehyde and DL-1,2-Dihydroxy[1,3,4-^{3}H]hexadecane by Rat Brain.

Experiment	Compound Counted: Triacetylsphingosine (cpm/mg)	Compound Counted: Cholesterol (cpm/mg)
[1,2-^{3}H]Palmitaldehyde[a]	66	27
Palmitaldehyde thiosemicarbazone	76	41
Palmitaldehyde diethyl acetal	22	10
DL-1,2-Dihydroxy-[1,3,4-^{3}H]hexadecane	50	11

[a] Values are the average of four experiments. See text for details.

The water-soluble cleavage product was formaldehyde, which was identified as the dimedon derivative.

DL-*erytho*-1,2,3-Trihydroxyhexadecane was prepared by refluxing DL-*trans*-2,3-epoxyhexadecanoic acid in glacial acetic acid; this was followed successively by treatment with alkali and $LiAlH_4$. The hydroxyl groups on carbon atoms 2 and 3 are *cis* owing to inversion which occurs during opening of the epoxide ring by the glacial acetic acid (Swern, 1948). Periodate oxidation of the triol yielded tetradecanal and formaldehyde; the formic acid was not isolated.

The addition of diazomethane to *trans*-2-hexadecenoic acid until the yellow color of the reagent persisted, i.e., a ratio of diazomethane to acid of 2:1 or more, yielded a crystalline product corresponding to a substituted pyrazoline. Mild alkaline treatment of this compound gave, presumably, DL-3-carboxy-4-tridecyl-2-pyrazoline.

DL-1,2-Dihydroxy[1,3,4-^{3}H]hexadecane was prepared in the same manner as described for the preparation of unlabeled diol. The distribution of isotope in the tritium-labeled diol, DL-1,2-dihydroxy[1,3,4-^{3}H]hexadecane, was determined by periodate oxidation. The formaldehyde (tritium on carbon atom 1) was counted as the dimedon derivative. The pentadecanal, a portion of which was counted as the thiosemicarbazone (tritium on carbon atoms 2, 3, and 4), was oxidized to the acid with CrO_3 in glacial acetic acid. The acid (tritium on carbon atoms 3 and 4) was converted to tetradecylamine (tritium on carbon atoms 3 and 4) via the azide and then to tetradecanoic acid (tritium on carbon atom 4). It is assumed that little or no isotope is present beyond carbon atom 4. As anticipated, the greatest activity resided on carbon atom 1, whereas no activity was present on carbon atom 2 (Table II). The similar activity on carbon atoms 3 and 4 (Table II) may be due to the formation of a new carbonium ion center as the result of a hydride shift from carbon atom 4 to carbon atom 3, which is the carbonium ion center originally formed from scission of the epoxide ring by $LiAlH_4$. The presence of less activity in tetradecylamine than in pentadecanoic acid may have resulted from the loss of isotope from carbon atom 3 during conversion of the pentadecanoic acid to amine.

TABLE II: Distribution of Isotope in DL-1,2-Dihydroxy-[1,3,4-^{3}H]hexadecane.[a]

Compound	Tritium on Carbon Atom[b] (No.)	Activity (μc/mmole)
Formaldimedon	1	923 (61%)
n-Pentadecanal thiosemicarbazone	2, 3, 4	592 (39%)
n-Pentadecanoic acid	3, 4	598
n-Tetradecylamine	3, 4	512
n-Tetradecanoic acid	4	284 (19%)

[a] Total activity is 1515 μc/mmole. [b] No tritium is present on carbon atom 2 (activity of pentadecanal thiosemicarbazone minus activity of pentadecanoic acid). The similar activity of 308 (20%) μc/mmole (activity of thiosemicarbazone minus activity of tetradecanoic acid) and 284 μc/mmole on carbon atoms 3 and 4, respectively, may be due to a hydride shift. See text for details.

Experimental

trans-2-Methylhexadecenoate (Compound I). Diazomethane (formed from the alkaline treatment of 24.7 g of Diazald[1] [Tenny *et al.*, 1963]) in 275 ml of ether-

[1] Diazald, *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide, was obtained from Aldrich Chemical Co., Inc.

methanol (10:1) was added slowly to 25.4 g of *trans*-2-hexadecenoic acid (Shapiro *et al.*, 1958). The solution was concentrated, and after reconcentration with several portions of ether the sirup was dried over P_2O_5.

Methyl DL-*trans-2,3-Epoxyhexadecanoate* (*Compound II*). Perbenzoic acid, 13.3 g (Swern, 1953) in 200 ml of benzene was added to 10.7 g of compound I in 25 ml of chloroform. After the material had remained overnight at room temperature, 50 ml of ethyl acetate was added, and the reaction mixture was washed successively with several 50-ml portions of 1.5% $NaHCO_3$ and water. The solvent was removed under reduced pressure, and the dried residue was dissolved in petroleum ether (bp 60–70°). The precipitate that formed in the chilled solution was discarded, and the filtrate was concentrated to a sirup; yield, 7.3 g.

DL-*1,2-Dihydroxyhexadecane* (*Compound III*). To a chilled solution of 4.0 g of compound II in 50 ml of dry ether was added 1.5 g of $LiAlH_4$. The reaction mixture was refluxed 5 hours. After the successive addition of 50 ml of methanol and 100 ml of 2.5 N NaOH, the product was removed with two 200-ml portions of ether–ethyl acetate (1:1). The combined organic layers were washed with water, filtered, and concentrated. The residue was crystallized from petroleum ether; yield, 1.9 g; mp 57–59°.

Anal. Calcd for $C_{16}H_{34}O_2$ (258.3): C, 74.34; H, 13.27. Found: C, 74.06; H, 13.17.

DL-*trans-2,3-Epoxyhexadecanoic Acid* (*Compound IV*). A solution of 2.0 g of compound II in 10 ml of methanol and 2 ml of saturated KOH, after standing overnight at room temperature, was chilled and acidified with 6 N HCl. The product was removed with ether, and the solution was washed with water. After removal of the solvent, the dried solid was crystallized from petroleum ether; yield, 1.4 g; mp 82–83°.

Anal. Calcd for $C_{16}H_{30}O_3$ (270.2): C, 71.05; H, 11.19. Found: C, 70.72; H, 11.11.

Reduction of 4.0 g of compound IV with 1.5 g of $LiAlH_4$ yielded 2.4 g of DL-1,2-dihydroxyhexadecane; mp 57–59°.

DL-*erythro-2,3-Dihydroxyhexadecanoic Acid* (*Compound V*). Compound IV, 3.90 g, was refluxed in 50 ml of glacial acetic acid for 4 hours. The solvent was removed under reduced pressure, and the residue was treated with 50 ml of 1.0 N KOH in 95% methanol. After about 10 hours at room temperature, the chilled solution was diluted with an equal volume of water and acidified with 6 N HCl. The product was extracted into ether, and the washed ether solution was concentrated. The dried residue was crystallized from petroleum ether–ethanol (14:1); yield, 3.1 g; mp 101–103°.

Anal. Calcd for $C_{16}H_{32}O_4$ (288.3): C, 66.61; H, 11.19. Found: C, 66.82; H, 11.08.

DL-*erythro-1,2,3-Trihydroxyhexadecane* (*Compound VI*). To 2.0 g of compound V in 60 ml of cold dry *p*-dioxane was added 0.9 g of $LiAlH_4$. When the initial reaction subsided, the mixture was refluxed 4 hours, and the product was isolated as described in the preparation of compound III. The residue was crystallized from ethanol; yield, 1.2 g; mp 98–102°.

Anal. Calcd for $C_{16}H_{34}O_3$ (274.3): C, 70.00; H, 12.49. Found: C, 70.02; H, 12.37.

DL-*3-Carboxymethyl-4-tridecyl-2-pyrazoline* (*Compound VII*). The diazomethane from 18.0 g of Diazald was added to 7.5 g of *trans*-2-hexadecenoic acid; the yellow color of the reagent persisted. The dried product, isolated in the same manner as described for the preparation of compound I, crystallized from petroleum ether in glistening yellow plates; yield, 4.0 g; mp 64–66°.

Anal. Calcd for $C_{18}H_{34}O_2N_2$ (310.3): C, 69.62; H, 11.05; N, 9.02. Found: C, 69.50; H, 11.13; N, 9.17.

The infrared absorption bands in chloroform are: 3500 cm^{-1}(w), 3020 cm^{-1}(s), 1760 cm^{-1}(s), 1585 cm^{-1} (m), 1460 cm^{-1}(s), 1375 cm^{-1}(m), 1335 cm^{-1}(w), 1240 cm^{-1}(s), and 1115 cm^{-1}(s).

DL-*3-Carboxy-4-tridecyl-2-pyrazoline* (*Compound VIII*). Compound VII, 1.0 g, in 10 ml of methanol and 2 ml of saturated KOH, after standing overnight at room temperature, was chilled and acidified with 6 N HCl. The product was extracted into ether, and the ether solution was washed with water and concentrated. The dried residue was crystallized from petroleum ether–ethanol (100:1); yield, 660 mg; mp 97–99°.

Anal. Calcd for $C_{17}H_{32}O_2N_2$ (296.3): C, 68.86; H, 10.89; N, 9.45. Found: C, 68.54; H, 10.73; N, 9.39.

The infrared absorption bands are (KBr disk): 3450 cm^{-1}(w), 3000 cm^{-1}(m), 1710 cm^{-1}(s), 1530 cm^{-1}(s), 1485 cm^{-1}(s), 1350 cm^{-1}(m), 1250 cm^{-1}(m), 1200 cm^{-1}(w), 1112 cm^{-1}(s), and 930 cm^{-1}(m).

Periodate Oxidation of Diol and Triol. Compounds III and VI, 100 mg of each, were oxidized with 230 mg of periodic acid in 15 ml of 90% methanol at 55° for 1 hour. Vapor-phase chromatography of the long-chain aldehydes, separated as previously described (Weiss, 1964b), showed a single peak corresponding to pentadecanal, retention time 8.0 minutes, and tetradecanal, retention time 4.75 minutes, for the diol and triol, respectively. The 2,4-dinitrophenylhydrazones of pentadecanal and tetradecanal melted at 103–104° and 101–102°, respectively. The water-soluble cleavage product from the oxidation of the diol and triol was formaldehyde, which was identified as the dimedon derivative, mp 189–190°; the formic acid from the triol was not isolated. Periodate oxidation of DL-*erythro*-2,3-dihydroxyhexadecanoic acid gave tetradecanal and, presumably, glyoxylic acid, which was not determined.

DL-*1,2-Dihydroxy*[*1,3,4-*^{3}H]*hexadecane* (*Compound IX*). To 1.1 g of compound II in 60 ml of cold dry ether was added 12 mg (25 mc) of [^{3}H]$LiAlH_4$.[2] After refluxing for 3 hours, 140 mg of $LiAlH_4$ was added to the chilled reaction mixture, which was refluxed for an additional 3 hours. The product was isolated in the same manner as described for the preparation of compound III; yield, 570 mg; mp 57–59°.

Isotope Distribution in DL-*1,2-Dihydroxy*[*1,3,4-*^{3}H]-

[2] The [^{3}H]$LiAlH_4$ was obtained from New England Nuclear Corp.

hexadecane. Compound IX, 3.2 mg, and compound III, 398.3 mg, were oxidized together with excess periodate in the same manner as described for the oxidation of compounds III and VI. Formaldehyde was counted as the dimedon derivative, mp 189–190°. The long-chain aldehyde, after conversion of a portion to the thiosemicarbazone, mp 95–96°, was oxidized to the acid with CrO_3 in glacial acetic acid (Weiss, 1964a). The acid was degraded via the azide to tetradecylamine, which was oxidized to tetradecanoic acid with alkaline permanganate (Aronoff, 1957).

Administration of Substrates. [1,2-^{3}H]Palmitaldehyde was prepared by the periodic acid oxidation of 16,17-dihydroxy[15,16,17,18-^{3}H]dotriacontane (Weiss, 1964a). The [1,2-^{3}H]palmitaldehyde, 3.9 mg (18.1 μc), with tritium on carbon atoms 1 and 2 of 1028 and 54 μc/mmole, respectively, was dissolved in 0.4 ml of Tween 20–water (1:3, v/v) which was held in a water bath at about 60°. DL-1,2-Dihydroxy[1,3,4-^{3}H]hexadecane, 6.9 mg (40.5 μc, Table II), was kept, also, under the same conditions in 0.4 ml of Tween 20–ethanol (1:10, v/v). Forty rats, 13–15 days old and lightly anesthetized with ether, were injected intracranially (Lindberg and Ernster, 1950) with 0.01 ml of substrate per animal. The rats were killed 7–10 days after the injections and the sphingosine (counted as the triacetyl derivative) and cholesterol were isolated from the pooled brains as previously described (Carter *et al.*, 1947, 1961; Weiss, 1963).

Degradation of Sphingosine with Lead Tetraacetate. Sphingosine, 150 mg, was degraded with 487 mg (10% excess) of lead tetraacetate (recrystallized from glacial acetic acid; Fieser, 1957) in 9 ml of dry benzene and 2 ml of glacial acetic acid at 55–60° for 1 hour. The reaction mixture was diluted with 3 ml of water and the hexadecenal was removed with *n*-heptane. After reduction over platinum in ethanol, the hexadecanal was converted to the thiosemicarbazone (Weiss, 1963) (tritium on carbon atoms 3–18) and palmitamide (Weiss, 1964a) (tritium on carbon atoms 4–18) derivatives. Formaldimedon (tritium on carbon atom 1) was obtained from the distillate after lyophilization of the combined aqueous acetic acid lower phase and water washes from the *n*-heptane extracts. The tritium on carbon atom 2 was determined by difference.

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