

Synthesis of Palmitaldehyde-1,2-³H*

BENJAMIN WEISS

From the Departments of Biochemistry, New York State Psychiatric Institute and
College of Physicians and Surgeons, Columbia University, 722 W. 168th St.,
New York, N. Y. 10032

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Palmitoin was oxidized to the diketone with CrO₃ in glacial acetic acid. Both the diketone and palmitoin were hydrogenated over Pt in glacial acetic acid in the presence of ³H₂O. The glycol was degraded with periodic acid to palmitaldehyde-1,2-³H, and the isotope distribution on carbon atoms 1 and 2 was determined by derivatization to the thiosemicarbazone and by oxidation and conversion to palmitamide. The aldehyde obtained by reduction of the diketone was of higher specific activity than that derived from acyloin, but the C-1/C-2 ratios of tritium activity were similar. Reduction of the diketone with [³H]LiAlH₄ yielded aldehyde with high specific activity and with isotope predominantly on carbon atom 1.

An enzyme system in rat brain catalyzes *in vitro* the formation of sphingosine from palmitaldehyde and L-serine in the presence of pyridoxal phosphate and Mn²⁺ (Brady *et al.*, 1958). The α - and β -hydrogen atoms of serine are retained during incorporation as shown by the close agreement between the β/α ratio of tritium in the DL-serine-2,3-³H administered to suckling rats and the C-1/C-2 ratio of tritium in the isolated sphingosine (Weiss, 1963). In order to determine the origin of the hydrogen atom on carbon atom 3 of sphingosine it is necessary to employ palmitaldehyde labeled with isotope in the 1-position. The procedure chosen for the synthesis of this compound was via the corresponding acyloin (Gaullitz and Malins, 1960), followed by oxidation to the diketone, reduction to the glycol, and, finally, periodate oxidation of the glycol to the aldehyde. The merit of this method is in the ease of synthesis, the homogeneity of the product, and the stability of the glycol intermediate which can be rapidly degraded to the aldehyde when needed, thus eliminating the hazard of storage of the aldehyde which polymerizes on standing.

Palmitoin was obtained from methyl palmitate according to a slightly modified procedure of Hansley (1935). In the purification of the product from methanol, an oil separated from the hot solution. This by-product material melted at 58–74°, was soluble in ethyl acetate, gave an infrared spectrum similar to that of the acyloin, and yielded palmitoin upon prolonged refluxing with methanol.

Palmitoin was not susceptible to the usual oxidants for enediols but was smoothly oxidized to the diketone with excellent yields by CrO₃ in glacial acetic acid. Complete reduction of either diketone or acyloin to the glycol by LiAlH₄ could not be effected under a variety of conditions such as the use of a large excess of hydride or by a change in solvent from diethyl ether to *p*-dioxane or dimethyl ether of diethylene glycol to give higher operating temperatures. In either case the product was a mixture of glycol and acyloin with a melting point of, usually, 97–111°. The infrared absorption at 1725 cm⁻¹ disclosed in the mixture an acyloin content of less than 10%; fractionation of the mixture with methanol yielded pure glycol but with large losses in the desired product. It has been reported that the reduction of polyunsaturated long-chain acyloins with LiAlH₄ can be accomplished

quantitatively but the evidence presented is insufficient since the product of the reaction was examined by thin-layer chromatography and by the consumption of periodate (Gaullitz and Malins, 1960). The criterion of periodate uptake as an indication of the purity of glycol in the presence of contaminating acyloin is inadequate since it was found in this study that under the conditions employed for the oxidation of the glycol the acyloin is cleaved by periodic acid to palmitaldehyde and palmitic acid. This finding is in agreement with that observed for the periodate oxidation of benzoin to benzaldehyde and benzoic acid (Jackson, 1946). The failure to reduce acyloin (or diketone, which stops at the acyloin stage) completely to the glycol with LiAlH₄ is attributed to the presence of enediol (Fig. 1,C) in which the olefinic linkage is not attacked by the hydride.

The reduction of these compounds over Pt was also examined. Of the many solvents tried, only glacial acetic acid was effective; owing to the insolubility of these compounds, the operating temperature had to be maintained near 75°. Reduction of the acyloin proceeded more easily than that of the diketone and, in each instance, the product was exclusively glycol as indicated by melting point and the lack of an infrared-absorption band at 1725 cm⁻¹. It is assumed that in both the chemical and catalytic reductions the glycol contains all possible isomers.

Cleavage of the glycol was effected easily with periodic acid in methanol after 15 minutes at 60–65°. The course of the reaction was followed conveniently by the absence of a precipitate after cooling the reaction mixture to room temperature; the glycol is insoluble in cool methanol. The aldehyde gave the correct melting point for the dinitrophenylhydrazone and thiosemicarbazone derivatives, and showed a single peak on the gas chromatograph. When palmitaldehyde was synthesized via reduction of the corresponding acyl chloride with lithium aluminum tri-*tert*-butoxy hydride, many side-products were obtained.¹

Two procedures were employed for the preparation of palmitaldehyde-1,2-³H. In the first, labeling was effected by a combination of exchange and reduction (Bloch and Rittenberg, 1944) by hydrogenating diketone or acyloin over Pt in glacial acetic acid in the presence of 10 mc (0.1 ml) of ³H₂O. Under these conditions the reduction did not proceed to completion; the products isolated from the diketone and acyloin melted at 97–108° and 117–120°, respectively. In the second method, isotope was introduced directly

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¹ B. Weiss and R. L. Stiller, unpublished results.

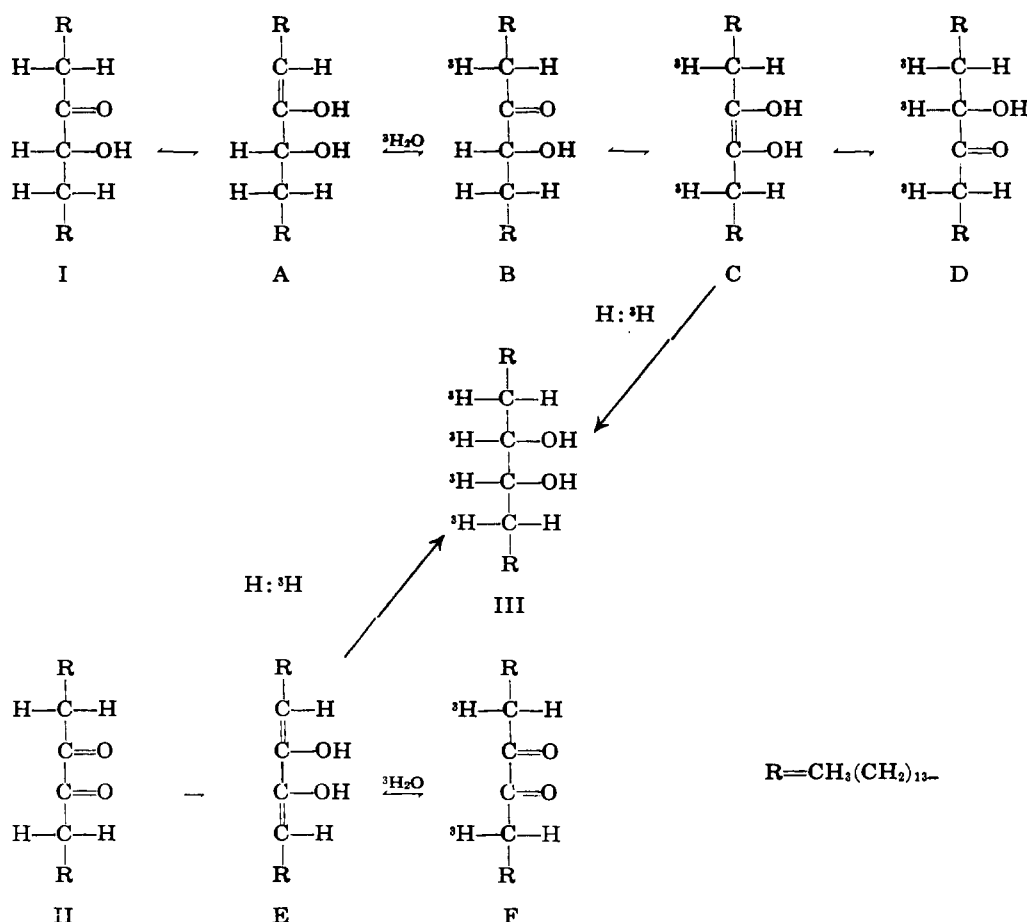


FIG. 1.—The reduction of 16-hydroxy-17-ketodotriacontane (I) and 16,17-diketodotriacontane (II) over Pt in glacial acetic acid in the presence of ³H₂O yields labeled glycol (III). The isotope distribution in the palmitaldehyde obtained after periodate oxidation of the glycol is accounted for by (1) exchange of hydrogen by tautomerism and (2) by direct addition of hydrogen. The major species A, B, C, D, E, and F, that presumably participate in the labeling process are shown. See text for details.

by reduction of the diketone with one-half the stoichiometric amount of [³H]LiAlH₄, followed by an excess of unlabeled hydride. The yellow color of the diketone was promptly discharged upon reaction with LiAlH₄, but with [³H]LiAlH₄, this failed to occur. The isotopic hydride was considerably more insoluble in diethyl ether and *p*-dioxane than the unlabeled compound. The products obtained in each procedure, after dilution with carrier, were degraded with periodate; the *n*-heptane extract containing the aldehyde was washed with dilute alkali and water to remove the palmitic acid formed from the small amounts of acyloin present in the glycol.

The extent of isotope incorporation into the 1- and 2-positions of palmitaldehyde was determined by derivatization of a portion of the aldehyde to the thiosemicarbazone; the remainder was oxidized in good yield with CrO₃ in glacial acetic acid to the acid which was converted to the amide via the acyl chloride. The radioactivity of each derivative, dissolved in *p*-dioxane, was determined in the Packard Tri-Carb liquid scintillation spectrometer (Weiss, 1963). The tritium activity on carbon atom 1 was obtained by subtracting the specific activity of palmitamide (tritium activity on carbon atom 2) from the specific activity of the thiosemicarbazone (tritium activity on carbon atoms 1 and 2). It was assumed that little or no labeling occurred beyond the second carbon atom. In the exchange procedure, diketone is superior to acyloin as an intermediate in the synthesis of palmital-

TABLE I
DISTRIBUTION OF ISOTOPE IN PALMITALDEHYDE-1,2-³H

Starting Compound	Palmityl-thiosemicarbazone (³ H on carbon atoms 1 and 2) (μc/mm)	Palmitamide (³ H on carbon atom 2) (μc/mm)	Tritium on Carbon Atom 1 ^a (μc/mm)
16-Hydroxy-17-ketodotriacontane ^b	14.5	5.7	8.8
16,17-Diketodotriacontane ^b	46.5	18.9	27.6
16,17-Diketodotriacontane ^c	1081.8	54.2	1027.6

^a Values were obtained by difference. ^b Compounds were labeled by exchange with ³H₂O and H:³H. ^c Compound was labeled with [³H]LiAlH₄.

dehyde since about three times as much isotope is found in the final product (Table I). The C-1/C-2 ratios of tritium activity in the palmitaldehyde obtained with diketone and acyloin as intermediates were 1.46 and 1.54, respectively. It is assumed from similar reactions with periodic acid (Weiss, 1963) that the hydrogen atoms on carbon atoms 16 and 17 are not rendered labile during oxidation. The pattern of labeling is due to hydrogenation and to tautomerism involving the methylene carbon atoms and the carbon

atoms bearing oxygen substituents (Fig. 1, A, B, C, D, E, and F); the contribution made by each pathway to the labeling process was not determined. When $[^3\text{H}]\text{LiAlH}_4$ was employed in the synthesis of the aldehyde, the tritium activity was predominantly on carbon atom 1 (Table I).

In the course of this study several homologous unlabeled acyloins were prepared; their melting points agreed with those from the same compounds prepared earlier by Hansley (1935). The osazones of these acyloins were also prepared (Hansley, 1935), but no analytical values were given. Since their preparation was difficult, the 2,4-dinitrophenylosazones of these compounds were made along with the corresponding diketones.

EXPERIMENTAL

16-Hydroxy-17-ketodotriacontane (I).—Into a 4-necked 500-ml flask, equipped with an Alihn condenser and CaSO_4 drying tube, mechanical stirrer with mercury seal, N_2 inlet tube and thermometer, and dropping funnel, were placed 200 ml of freshly distilled *p*-xylene and 7.14 g of Na. The reaction mixture was heated as high purity N_2 was bubbled in through a $\text{CaSO}_4\cdot\text{P}_2\text{O}_5$ drying tube; when the temperature reached 110° , the mixture was stirred and, after 15 minutes, 40 g of freshly distilled methyl palmitate in 40 ml of warm *p*-xylene was added over a 90-minute interval. The temperature of the reaction mixture was maintained between 106 and 114° . After 30 minutes of additional stirring the reaction mixture was allowed to cool to about 80° and the excess Na was destroyed by addition of 50 ml of methanol. After 75 ml of water had been added the mixture was treated with 500 ml of ether; the ether layer was washed successively with several 100-ml portions of water, 100 ml of dilute HCl, and with water until neutral. The ether was removed, and the residue was dried by repeated addition of methanol with distillation. The dry product was crystallized several times from 300 ml of methanol at room temperature; yield, 16.8 g; mp, $73\text{--}75^\circ$.

Anal. Calcd. for $\text{C}_{32}\text{H}_{64}\text{O}_2$ (480.5): C, 79.92; H, 13.43; Active H, 0.21. Found: C, 80.97; H, 13.43; Active H, 0.17.

Infrared-absorption bands are (KBr disk) 1725 cm^{-1} (s), 1480 cm^{-1} (s), 1407 cm^{-1} (w), 1380 cm^{-1} (w), 1113 cm^{-1} (m), 1087 cm^{-1} (m), 945 cm^{-1} (w) and 918 cm^{-1} (w). The 2,4-dinitrophenylosazone melted at $124\text{--}126^\circ$.

Anal. Calcd. for $\text{C}_{44}\text{H}_{70}\text{O}_8\text{N}_8$ (838.6): N, 13.36. Found: N, 13.42.

16,17-Diketodotriacontane (II).—A solution of 45 ml of glacial acetic acid containing 961 mg of compound I and 150 mg of CrO_3 was heated in a water bath at 70° for 15 minutes with stirring. The reaction mixture was diluted with 3 volumes of water and the product was removed by treatment with three 100-ml portions of ether. After washing with several 50-ml portions of water, the ether was removed and the residue was transferred to a Buchner funnel with several portions of methanol. The precipitate, free of water and glacial acetic acid, was crystallized from 200 ml of *n*-heptane. The solution was allowed to cool to room temperature and was filtered; yield of yellow crystalline product was 742 mg; mp, $81\text{--}82^\circ$. The filtrate, after chilling, deposited a mixture of compounds I and II; yield, 120 mg.; mp, $75\text{--}76^\circ$.

Anal. Calcd. for $\text{C}_{32}\text{H}_{62}\text{O}_2$ (478.5): C, 80.25; H, 13.06; Active H, 0. Found: C, 80.11; H, 12.99; Active H, 0.

Infrared-absorption bands are (KBr disk) 1725

cm^{-1} (s), 1480 cm^{-1} (s), 1407 cm^{-1} (m), 1380 cm^{-1} (m), and $935\text{--}918\text{ cm}^{-1}$ (m) doublet.

16,17-Dihydroxydotriacontane via Catalytic Reduction (III).—Either compound I or II (200 mg) was hydrogenated with magnetic stirring over 100 mg of PtO_2 in 10 ml of glacial acetic acid for 5 hours at 75° . After addition of 10 ml of *n*-heptane, the reaction mixture was heated and filtered, and the catalyst was washed successively with small hot portions of *n*-heptane and ethanol. The combined filtrates were concentrated to near dryness and the product was recrystallized several times from 35 ml of ethanol; yield, 134 mg; mp, $124\text{--}125^\circ$.

Anal. Calcd. for $\text{C}_{32}\text{H}_{66}\text{O}_2$ (482.5): C, 79.58; H, 13.79; Active H, 0.42. Found: C, 80.18; H, 13.86; Active H, 0.38.

Infrared-absorption bands are (KBr disk) 1480 cm^{-1} (s), 1075 cm^{-1} (s), 1042 cm^{-1} (w), 1025 cm^{-1} (w), 945 cm^{-1} (m), 918 cm^{-1} (m), and 885 cm^{-1} (m).

16,17-Dihydroxydotriacontane via LiAlH_4 (IV).—Dry ether (50 ml) containing 480 mg of either compound I or II and 60 mg of LiAlH_4 were refluxed for 4 hours. After the successive addition of 150 ml of ether, 25 ml of methanol, and 100 ml of 1 *N* NaOH, the separated lower phase was re-treated with the same volume of ether. The combined ether extracts were washed with water and concentrated to dryness, and the residue was crystallized from 125 ml of ethanol; yield, 432 mg; mp from reduction of compound I, $106\text{--}115^\circ$; mp from reduction of compound II, $103\text{--}112^\circ$. In either case, no infrared absorption was found at 1725 cm^{-1} .

Infrared examination of graded amounts of compound I or II in the presence of compound III disclosed that the lowest limit of detection of the former, from the absorption at 1725 cm^{-1} , is 10%.

Periodate Oxidation of 16,17-Dihydroxydotriacontane (V).—Periodic acid (900 mg) in 1 ml of water was added to 200 mg of compound III in 20 ml of methanol; the reaction mixture was held at $60\text{--}65^\circ$ for 10 minutes with constant stirring. Upon cooling to room temperature, the solution was treated with several 15-ml portions of *n*-heptane; the combined *n*-heptane extracts were washed with water, filtered, and concentrated to a colorless sirup. Gas-liquid chromatography of a sample from the sirup revealed only one peak with diethylene glycol adipate polyester (15% on Chromosorb W, 60/80 mesh) as the stationary phase. The column temperature was 197° and the argon flow rate was 65 ml/min. One-half the aldehyde was derivatized with thiosemicarbazide (Weiss, 1963); yield of palmitylthiosemicarbazone, 105 mg; mp, $106\text{--}107^\circ$.

The remainder of the aldehyde was dried over P_2O_5 and oxidized with 75 mg of CrO_3 in 10 ml of glacial acetic acid at $40\text{--}45^\circ$ for 15 minutes with constant stirring. The reaction mixture was diluted with an equal volume of water and treated with several 15-ml portions of *n*-heptane. The combined *n*-heptane extracts were washed with water, filtered, and concentrated, and the product was dried over P_2O_5 . The palmitic acid was refluxed 1 hour with 1 ml of freshly distilled SOCl_2 in 4 ml of dry benzene; after removal of the excess SOCl_2 , 10 ml of benzene saturated with NH_3 was added. The solution was concentrated to about 3 ml and decolorized with Norit A after the addition of 15 ml of hot ethanol. The filtrate was concentrated to dryness and 20 ml of *n*-heptane and 5 ml of water were added; the solution was heated to boiling and the hot *n*-heptane layer was withdrawn and concentrated to approximately 5 ml. The chilled solution was centrifuged and the precipitate was

recrystallized from the same solvent; yield of palmitamide, 24 mg; mp, 103–105°.

Periodate Oxidation of 16-Hydroxy-17-ketodotriacontane.—Compound I (200 mg) was oxidized in the same manner as described for compound V; yield of palmitylthiosemicarbazone, 96 mg; mp, 106–107°; yield of palmitic acid, 27 mg; mp, 62°.

Palmitaldehyde-1,2-³H via ³H₂O.—Either compound I or II (200 mg) was treated as described in the synthesis of compound III except that 10 mc (0.1 ml) of ³H₂O was added to the reaction mixture. The isolated glycol was degraded as described for compound V and the distribution of isotope in positions 1 and 2 of the aldehyde (Table I) was determined via the thiosemicarbazone and amide derivatives. The radioactive glycol was stored until needed at which time it was cleaved to the aldehyde with periodate.

Palmitaldehyde-1,2-³H via [³H]LiAlH₄.—Compound II (252 mg) and 5.0 mg of [³H]LiAlH₄ (25 mc) in 50 ml of dry ether were refluxed for 12 hours; unlabeled LiAlH₄ was added until the yellow color of the diketone was discharged. The reaction mixture was refluxed for 6 hours and the remainder of the procedure was carried out as described for compound IV; yield, 206 mg; mp, 97–107°.

The glycol was degraded as described for compound V with the exception that the *n*-heptane extract containing the palmitaldehyde was washed with several 5-ml portions of 0.1 N NaOH before the final wash with water. The distribution of activity (Table I) was determined as described for compound V.

14-Hydroxy-15-ketooctacosane (VI).—This compound was prepared from 7.6 g of Na and 40 g of methyl myristate in the same manner as described for the preparation of compound I; yield, 14.7 g; mp, 70–71°.

Anal. Calcd. for C₂₈H₅₆O₂ (424.4): C, 79.16; H, 13.30. Found: C, 79.06; H, 13.20.

The 2,4-dinitrophenylosazone melted at 122–123°.

Anal. Calcd. for C₄₆H₉₂O₈N₈ (782.5): N, 14.31. Found: N, 14.13.

14,15-Diketooctacosane.—This compound was pre-

pared from 1 g of compound VI according to the procedure described for the preparation of compound II; yield, 860 mg; mp, 81–82°.

Anal. Calcd. for C₂₈H₅₄O₂ (422.4): C, 79.54; H, 12.89; Active H, 0. Found: C, 80.02; H, 13.06; Active H, 0.

12-Hydroxy-13-ketotetracosane (VII).—This compound was prepared from 8.6 g of Na and 40 g of methyl laurate under the conditions described for the preparation of compound I; yield, 19.2 g; mp, 61–62°.

Anal. Calcd. for C₂₄H₄₈O₂ (368.4): C, 78.18; H, 13.13. Found: C, 78.38; H, 12.99.

The 2,4-dinitrophenylosazone melted at 163–164°.

Anal. Calcd. for C₃₆H₇₄O₈N₈ (726.4): N, 15.42. Found: N, 15.12.

12,13-Diketotetracosane.—This compound was prepared from 1 g of compound VII according to the procedure described for the preparation of compound II; yield, 680 mg; mp, 72–73°.

Anal. Calcd. for C₂₄H₄₆O₂ (366.4): C, 78.61; H, 12.66; Active H, 0. Found: C, 79.01; H, 12.67; Active H, 0.

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Immunochemical Studies on Blood Groups. XXXII. Immunochemical Properties of and Possible Partial Structures for the Blood Group A, B, and H Antigenic Determinants*

GERALD SCHIFFMAN,† ELVIN A. KABAT, AND WILLIAM THOMPSON

From the Departments of Microbiology and Neurology, College of Physicians and Surgeons, Columbia University and the Neurological Institute, Presbyterian Hospital, New York

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The immunochemical properties of oligosaccharide fragments A₂, B₂, and H₄, obtained from human ovarian cyst blood group A, B, and H substances by cleavage with NaOH in the presence of NaBH₄, have been studied. These fragments account with one exception for all the immunochemical reactivities of the intact blood group substances, including P1 specificity previously thought to reside in the interior of the molecule. Possible partial structures for the A, B, and H determinants are inferred.

In an earlier study (Schiffman *et al.*, 1964), a method for cleaving blood group mucopolysaccharides using

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NaOH in the presence of sodium borohydride was described. Paper chromatography of the fragments obtained yielded partially purified compounds having the properties expected for the antigenic determinants of blood group A, B, and H substances. These prod-

† Present address: Department of Research Medicine, University of Pennsylvania, Philadelphia 4, Pennsylvania.