Chemical Synthesis of 1-^{14}C-Octadecyldihydroxyacetone Phosphate and 1-^{14}C-Octadecyldihydroxyacetone

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Abstract ☐ The synthesis of 1-^{14}C-octadecyldihydroxyacetone phosphate of high specific activity was accomplished *via* the cyclohexylammonium salt of 1-*O*-(1-^{14}C-octadecyl)-2,2-dimethoxypropane-3-phosphate.

Keyphrases ☐ 1-^{14}C-Octadecyldihydroxyacetone and phosphate—synthesis ☐ 1-*O*-(1-^{14}C-Octadecyl)-2,2-dimethoxypropane-3-phosphate, cyclohexylammonium salt—used in synthesis of high specific activity 1-^{14}C-octadecyldihydroxyacetone and phosphate

Piantadosi *et al.* (1, 2) previously reported the total chemical synthesis of unlabeled *O*-alkyl dihydroxyacetone phosphate, *O*-alkyl dihydroxyacetone, and the derivatives. However, to study the metabolism of these compounds, it became necessary to synthesize them with radioactive labels. In biological systems, the *O*-alkyl dihydroxyacetone phosphate is a key intermediate in the biosynthesis of complex ether-linked lipids (3–11). This report describes the preparation of 1-^{14}C-octadecyldihydroxyacetone and its phosphate derivative having high radiopurities and high specific activities.

The sequence of reactions used to synthesize the labeled compounds was based on the previous procedure for the synthesis of unlabeled *O*-alkyl dihydroxyacetone phosphate and derivatives (2). In the present synthetic procedure (Scheme I), 3-*O*-benzylglycerol (I) was esterified with *p*-nitrobenzoyl chloride in the presence of pyridine, resulting in a crude mixture of mono- and diesters (II); this mixture contained over 70% of the monoester derivative as determined by TLC. The Compound II mixture was oxidized to III with dimethyl sulfoxide and dicyclohexylcarbodiimide in the presence of trifluoroacetic acid. Compound III was ketalized to IV, which was hydrolyzed to V with sodium hydroxide. Compound V was then reacted with 1-^{14}C-octadecyl bromide in the presence of powdered potassium hydroxide in benzene, resulting in VI. After purification by silica gel column chromatography, Compound VI was debenzylated with palladium black to VII, which was phosphorylated to VIII. Compound VIII was hydrogenated with platinum oxide to remove the phenyl groups, and finally the cyclohexylammonium salt (X) was isolated. Compounds X and VII were treated with dilute hydrochloric acid to release XI and XII, respectively.

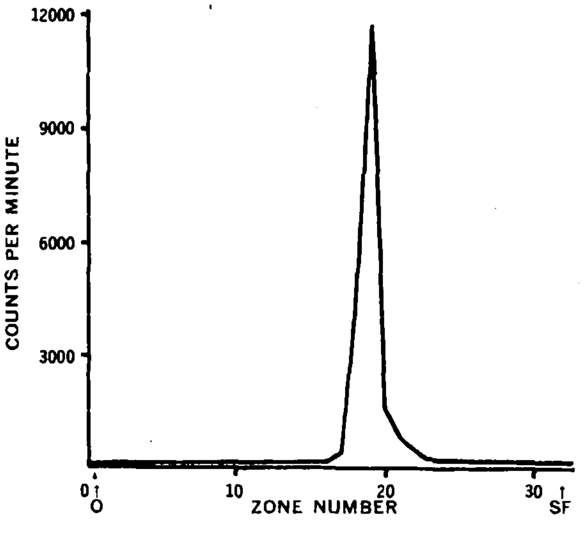

Figure 1—*TLC ^{14}C-zonal profile scans of 1-^{14}C-octadecyldihydroxyacetone phosphate. The solvent system used was chloroform–methanol–acetic acid–water (50:25:8:4 v/v). O = origin, and SF = solvent front.*

EXPERIMENTAL[1]

3-*O*-Benzylglycerol (I)—3-*O*-Benzylglycerol was prepared according to the procedures of Sowden and Fischer (13) and Howe and Malkin (14).

1-*O*-(*p*-Nitrobenzoyl)-3-*O*-benzyl-2-propanone (III)—Compound I was esterified with *p*-nitrobenzoyl chloride in the usual manner to give Compound II. TLC, using a solvent system of chloroform–diethyl ether (60:40 v/v), revealed a 70% yield of the mono-*p*-nitrobenzoyl derivative. Compound II was subjected to the Pfitzner–Moffatt oxidation for a period of 5 hr. (15, 16). The filtrate was extracted three times with 200-ml. portions of an ice-cold saturated sodium bicarbonate solution, two 200-ml. portions of water, and finally dried over anhydrous sodium sulfate. The ether was removed under reduced pressure, and the residue was taken up in 300 ml. of warm ether and left overnight at 5°. The resulting 25 g. of product was recrystallized from 200 ml. of ether at 5° and yielded 21 g. (32% based on the reacted 3-*O*-benzylglycerol ether), m.p. 92–93°. TLC in chloroform–ether (60:40 v/v) revealed one spot having R_f 0.68.

Anal.—Calc. for $C_{17}H_{15}NO_6$: C, 62.00; H, 4.59; N, 4.25. Found: C, 62.01; H, 4.68; N, 4.28.

1-*O*-(*p*-Nitrobenzoyl)-2,2-dimethoxy-3-*O*-benzylpropane (IV)—Compound III was ketalized in the usual manner in the presence of absolute methanol, trimethylorthoformate, and a catalytic amount of concentrated sulfuric acid. TLC in *n*-hexane–ether (80:20 v/v) revealed a single component with R_f 0.51, indicating that complete ketalization of Compound III had occurred to give Compound IV.

1-Hydroxy-2,2-dimethoxy-3-*O*-benzylpropane (V)—Seven grams of Compound IV was dissolved in 100 ml. of methanol containing 10 ml. of 4 *N* sodium hydroxide solution, and the mixture was stirred at room temperature for 24 hr. The methanol was evaporated and the remaining aqueous mixture was extracted three times with 150-ml. portions of ether. The combined ethereal extracts were washed once with 100 ml. of water and dried over anhydrous sodium sulfate. After removing the ether under reduced pressure, 3.2 g. (80% yield) of the syrupy product was obtained. TLC in *n*-hexane–ether (50:50 v/v) revealed one spot with R_f 0.25.

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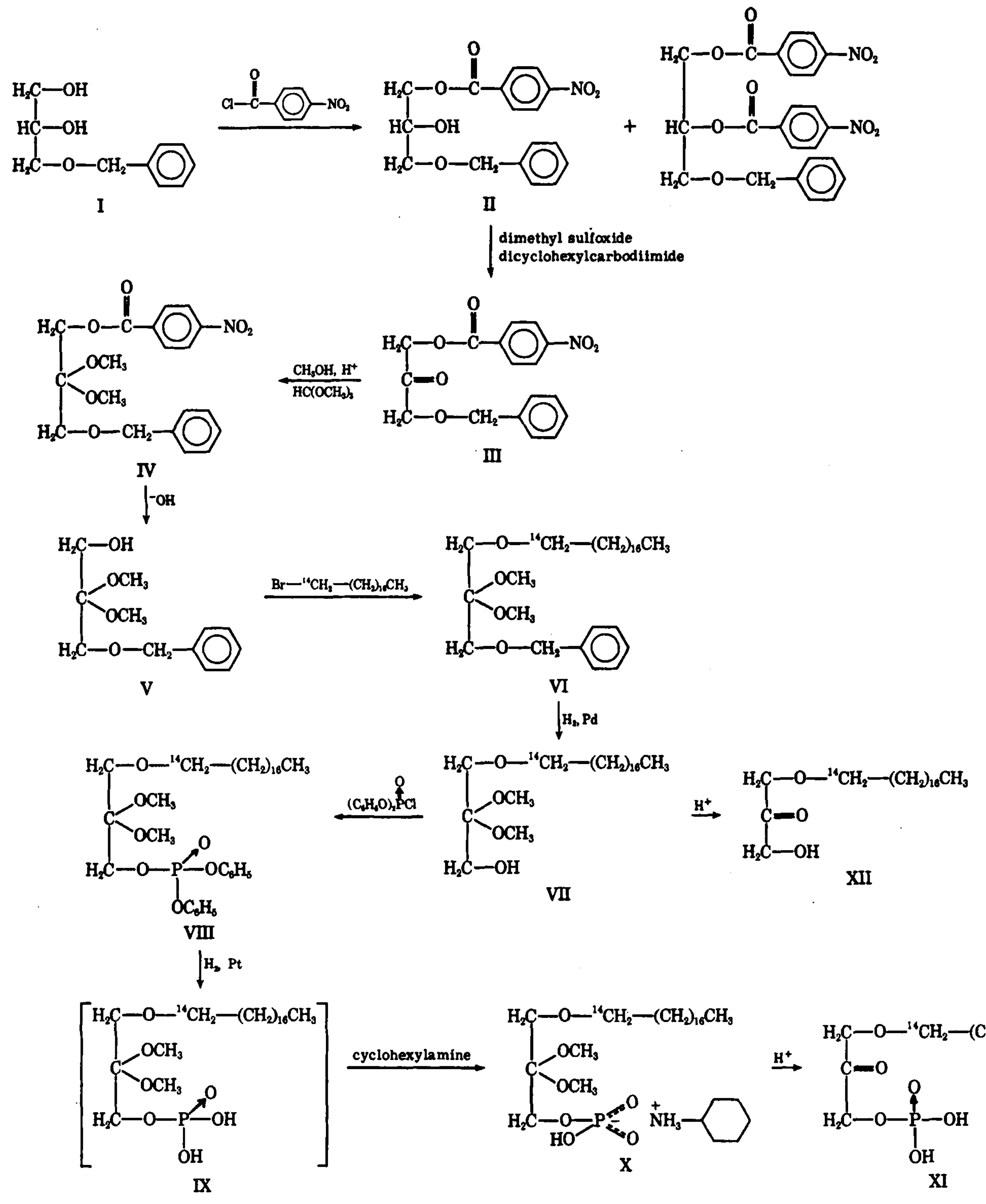
[1] The 1-^{14}C-octadecyl bromide (2.5 mc./mmole), which was purchased from International Chemical and Nuclear Corp., Irvine, Calif., had a radiopurity >99%. Radioactive intermediates were purified on columns (1.9 × 12 cm.) containing 15–20 g. silica gel (70–325 mesh, Brinkmann). Melting points were taken with a Mel-Temp melting-point apparatus and are corrected. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga. The specific radioactivities were determined with a Packard Tri-Carb scintillation spectrometer (model 3320) which had a ^{14}C counting efficiency of 79%. The radiopurity of the labeled compounds was determined by a zonal profile scanning method (12).



Scheme I

Anal.—Calc. for $C_{12}H_{18}O_4$: C, 63.69; H, 8.01. Found: C, 63.60; H, 7.98.

1-*O*-(1-^{14}C-Octadecyl)-2,2-dimethoxy-3-*O*-benzylpropane (VI)—In a 30-ml. reaction filter flask, equipped with a magnetic stirrer, Dean-Stark receiver, reflux condenser, and anhydrous calcium sulfate[2] drying tube, were placed 200 mg. of powdered potassium hydroxide and 340 mg. (1.5 mmoles) of Compound V dissolved in 10 ml. of dry benzene. Then 333 mg. of 1-^{14}C-octadecyl bromide (1.0 mmole) in 15 ml. benzene was added, and the mixture was refluxed in a sand bath at 83–87° for 24 hr. The cooled mixture was filtered under nitrogen to remove the potassium bromide, and the filtrate was collected in a 100-ml. round-bottom flask. After the precipitate was thoroughly washed with chloroform, the combined filtrate was evaporated under reduced pressure. The oily product (750 mg.) was dissolved in 5 ml. of warm *n*-hexane and was applied on a column (1.9 × 12 cm.) containing 15 g. of silica gel. The column was eluted with the following sequence of solvents: Solvent I, 100 ml. *n*-hexane; Solvent II, 100 ml. *n*-hexane-ether (7:3 v/v); and Solvent III, 100 ml. *n*-hexane-ether (1:1 v/v); 20-ml. fractions were collected.

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[2] Drierite.

Fractions 1–5 contained the alkyl halide and hydrocarbon breakdown products. The desired product, Compound VI, appeared in fractions 7 and 8 (425 mg., 89% yield).

Anal.—Calc. for $C_{30}H_{54}O_4$: C, 75.26; H, 11.37. Found: C, 75.23; H, 11.53.

1-*O*-(1-^{14}C-Octadecyl)-2,2-dimethoxy-3-hydroxypropane (VII)—Compound VI (425 mg.) in 50 ml. of *n*-hexane was debenzylated with 250 mg. of palladium black in a Parr hydrogenator at 20 p.s.i. at room temperature for 5 hr. The catalyst was filtered and washed thoroughly with chloroform. The combined filtrate was evaporated under reduced pressure. Then the white solid product (350 mg.) was dissolved in about 5 ml. of warm *n*-hexane and applied on a column (1.9 × 12 cm.) containing 20 g. of silica gel. The column was eluted with the following solvents: fraction 1, 150 ml. *n*-hexane; fraction 2, 100 ml. *n*-hexane–ether (9:1 v/v); fraction 3, 100 ml. *n*-hexane–ether (8:2 v/v); and fraction 4, 100 ml. *n*-hexane–ether (7:3 v/v). Fractions 1–3 contained some of the hydrocarbon breakdown products, and fraction 4 contained Compound VII (230 mg., 66% yield).

Anal.—Calc. for $C_{23}H_{48}O_4$: C, 71.08; H, 12.44. Found: C, 71.21, H, 12.30.

Cyclohexylammonium Salt of 1-*O*-(1-^{14}C-Octadecyl)-2,2-dimethoxypropane-3-phosphate (X)—A solution of 200 mg. of Compound VII (0.51 mmole) in 10 ml. of dry pyridine was placed in a 30-ml. reaction filter flask and cooled to 0° in an ice bath. Over 5 min., 250 mg. of diphenyl chlorophosphate was added with stirring. The flask was stoppered and allowed to stand at 5° for 24 hr. The precipitated pyridine hydrochloride was removed by filtration under nitrogen pressure and the filtrate was collected. After the precipitate was thoroughly washed with benzene, the combined filtrate was evaporated to dryness under reduced pressure. The oily product (320 mg.) was dissolved in 5 ml. of benzene and was applied to a column (1.9 × 12 cm.) containing 15 g. of silica gel. The column was eluted with 150-ml. portions of benzene, benzene–chloroform (3:1 v/v), and benzene–chloroform (1:1 v/v); 50-ml. fractions were collected. Fractions 5–8 contained 255 mg. (80%) of the phosphorylated intermediate, VIII. Compound VIII was dissolved in 50 ml. of absolute ethanol containing 150 mg. of platinum oxide and hydrogenated at 15 p.s.i. at room temperature for 3 hr. to remove the phenyl groups. The catalyst was removed by filtration and washed with absolute ethanol. Subsequently, 100 mg. of cyclohexylamine was added to the filtrate, and the mixture was left overnight at room temperature. After the solvent was removed under reduced pressure, a white solid product (325 mg.) appeared which was dissolved in 15 ml. of chloroform and stored at −15°.

1-*O*-(1-^{14}C-Octadecyl)dihydroxyacetone Phosphate (XI)—1-*O*-(1-^{14}C-Octadecyl)dihydroxyacetone phosphate was prepared from Compound X by the method described previously (2). About 10 mg. of Compound X was dissolved in 5 ml. of 0.1*N* HCl and shaken at 37° for 2 hr. The mixture was immediately extracted by a modification of the Bligh and Dyer (17) procedure, the solvent was removed under reduced pressure, and the product was dissolved in chloroform. TLC in chloroform–methanol–acetic acid–water (50:25:8:4 v/v) showed the product to be over 99% pure; the specific radioactivity was 2.46 mc./mmole. Figure 1 shows the ^{14}C-zonal profile scan of 1-*O*-(1-^{14}C-octadecyl)dihydroxyacetone phosphate after treatment of the cyclohexylammonium salt with dilute hydrochloric acid.

1-*O*-(1-^{14}C-Octadecyl)dihydroxyacetone (XII)—1-*O*-(1-^{14}C-Octadecyl)dihydroxyacetone was prepared from Compound VII by the same method used to prepare Compound XI. The radiopurity was 98–99%, as determined by TLC using a solvent system of hexane–ether–methanol–acetic acid (70:30:5:1 v/v).

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