Synthesis of phytanic acid

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SUMMARY Preparation of methyl phytanate from phytol by catalytic hydrogenation and chromic oxide oxidation is described. The method was applied to the preparation of methyl phytanate-^{14}C.

KEY WORDS phytanic acid · methyl phytanate · improved synthesis · uniformly labeled · phytol · dihydrophytol

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PHYTANIC ACID (3,7,11,15-tetramethyl hexadecanoic acid) is a branched-chain fatty acid of biological significance. Reports pertaining to the identification and occurrence of this acid in edible fats, including its involvement in the human disorder known as Refsum's syndrome, have been published (1–4). Its precursor, phytol (3,7,11,15-tetramethyl 2-hexadecenol) may play an important role in positioning the chlorophyll molecule in the chloroplast membrane(5). The methyl groups may enhance the hydrophobic interactions of phytol with the hydrocarbon side chains of chloroplast protein and thus stabilize lipid–protein association. Phytanic acid should be an excellent model for studying this interaction. It should also be a highly effective agent for inhibiting the orientation of hydrocarbon chains, reducing bilayer lipid membrane stability, and impeding fat crystallization. Toward the exploration of these matters, we undertook the synthesis of phytanic acid. The procedures, patterned after those of Karrer, Epprecht, and König (6), Klenk and Kahlke (7), and Lough (8), were relatively simple and efficient.

Phytol (Mann Research Laboratories, New York, N.Y. and Calbiochem, Los Angeles, Calif.) was converted to dihydrophytol in essentially quantitative yield by hydrogenation of 5 g of compound in 50 ml of absolute ethanol in the Parr apparatus at 33 psi hydrogen, room temperature, and 0.1 g of platinic oxide catalyst (Matheson, Coleman, & Bell, Norwood, Ohio). The total yield (5 g) was dissolved in 33 ml of glacial acetic acid. This solution was treated dropwise, with shaking, with a reagent composed of 50 ml of 80% acetic acid to which 2.33 g of $KHSO_4$ and then 2.33 g of CrO_3 had been added. A mild exothermic reaction occurred and was allowed to continue for 2 hr. This produced a greenish-gray solution containing a precipitate. Water (100 ml) was added and the mixture was then extracted 3 times with 100-ml quantities of petroleum ether (bp 35–65°C). The extract was washed several times with 50-ml quantities of water and then evaporated to give a residue (5.1 g) which was saponified under reflux for 15 min in 20 ml of 1.4 N KOH in ethanol. Dilution with 25 ml of water and extraction with hexane yielded 1.2 g of unsaponifiable matter. Gas chromatographic analysis revealed this to be mainly dihydrophytol.

The soap was decomposed by adding 7 ml of concentrated HCl in 14 ml of water and the crude phytanic acid was recovered by hexane extraction; yield, 3.8 g of light brown oil. Methyl phytanate of 90% purity was prepared by refluxing the crude acid in 1% v/v H_2SO_4 in methanol. A hexane solution of the crude acid was kept at 2°C to precipitate most of the impurities. The purified acid, recovered by evaporating the filtered hexane solution, is a viscous liquid at 0°C. Methyl

phytanate (99% pure by gas chromatography) prepared from this acid had $[\alpha]_D^{24}$ −0.20°, in reasonable agreement with $[\alpha]_{500}^{20}$ −0.35° for the same synthetic product by Lough (8), but at variance with the optical activity of naturally occurring forms (2, 8, 9). Infrared spectrum and gas chromatographic retention data for the methyl ester were in precise agreement with published values (see references 8, 9).

By an analogous procedure, methyl phytanate-^{14}C was prepared from phytol-^{14}C derived from chlorophyll of ^{14}C-labeled *Chlorella pyrenoidosa*.

Uniformly labeled phytol-^{14}C was prepared from *Chlorella pyrenoidosa* cultured by the method of Maruo, Takahashi, and Hattori (10) in inorganic medium in a cotton-plugged 125 ml Erlenmeyer flask containing a Teflon-coated magnetic stirrer bar within a small vacuum desiccator, into which were introduced successive 5-mc portions of $^{14}CO_2$ (35% ^{14}C) in air. The culture was illuminated between two banks of fluorescent lamps at 500 foot-candles. After three days' stirring at 20°C, with periodic monitoring of ^{14}C content by aspiration of an aliquot into a tube containing a filter paper wetted with dilute alkali and addition of two subsequent portions of $^{14}CO_2$, the algae had increased in mass by 20-fold. When the $^{14}CO_2$ content was found to be reduced to <1% of the dose, the algae were removed and centrifuged in a 15 ml tube. Addition of hot 80% ethanol to such CO_2-deficient cells failed to extract chlorophyll with the normal ease. It was found practical to extract the cell mass with a small amount of 90% formic acid. The chlorophyll was recovered by dilution of the formic acid solution in the presence of chloroform or ethanol–toluene. The dry solution of lipids was saponified with methanolic sodium methoxide in the usual manner. The phytol and unsaponifiable components were recovered by extraction with ethyl ether.

The phytol-^{14}C was separated on a preparative thin-layer chromatographic plate, from which a radioautogram was prepared on Kodak Blue-sensitive single coated X-ray film with a thin Mylar protective film over the radioactive plate. The phytol was extracted with ether from the area thus indicated. The product cochromatographed precisely with authentic phytol on gas–liquid and thin-layer chromatography. The uniformly labeled phytol was hydrogenated and oxidized by the above method. The radioactive intermediates and methyl phytanate-U-^{14}C cochromatographed precisely with the unlabeled substances.

Manuscript received 13 December 1965; accepted 10 February 1966.

We are indebted to Dr. P. Haverkamp Begemann for a sample of authentic methyl phytanate and to Dr. T. S. Oakwood for measurements of optical activity.

This work was supported by National Science Foundation grant GM-590 and Public Health Service Grant HE 03632-07 (MET).

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