

## Microbial Metabolism of the Isoprenoid Alkane Pristane

( $\alpha$ -methylglutarate/4,8,12-trimethyltridecanoate/fatty acid metabolism)

E. J. McKENNA\* AND R. E. KALLIO\*

Department of Microbiology, University of Iowa, Iowa City, Iowa

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**ABSTRACT** The "inert" hydrocarbon pristane (2,6,10,14-tetramethylpentadecane) can be utilized as the sole source of carbon and energy for growth of a coryneform soil isolate. Identification of the metabolites 4,8,12-trimethyltridecanoic acid and  $\alpha$ -methylglutaric acid indicates that two pathways of fatty acid metabolism operate in this bacterial strain.

The widespread use of pristane as a biological marker appears to be predicated on its structural similarity to phytol and its apparent stability, which may be only a reflection of the inability of microorganisms to carry out its anaerobic destruction.

Isoprenoid hydrocarbons are ubiquitous natural products. Pristane (2,6,10,14-tetramethylpentadecane), presumably derived from the phytol moiety of chlorophyll present in photosynthetic organisms, has been detected in bacteria, algae (1), and higher plants (2); traces of pristane also have been reported in human sebaceous lipids (3), in various tissues of the human and cow, in rat liver, and in wool wax (2). Marine sources of pristane include zooplankton, lobster, fish, sharks, sperm whale (4), and recent sediments (5). Fossil fuels such as coal and petroleum contain this isoprenoid alkane, as do ancient sediments (6). The stable structure persists even in Precambrian rocks (6) and perhaps is indigenous to extraterrestrial meteorites (1).

Pristane's relative inertness accounts for its usefulness as a biological marker. The coexistence of microfossils with pristane and phytane (2,6,10,14-tetramethylhexadecane) in Precambrian rocks is significant to the paleobotanist investigating the antiquity of life on earth (7). In detection of these markers, geologists have a new tool for determining the environment of the earth in past geological epochs (8). Meinschein *et al.* (9) have employed long-chain alkanes ( $C_{15}$ - $C_{30}$ ) as biological markers in exobiological research. Neither pristane nor any other alkane in the indicated molecular-weight range was found at a concentration exceeding one part per billion by weight in a 50-g sample of lunar fines. The pristane content of the digestive tract of the basking shark is considered to be indicative of food sources and feeding grounds of the shark; thus the marine biologist finds hydrocarbon analysis potentially useful in studies of the movement of marine animals (10). The gradation in proportions of phytanic (3,7,11,15-tetramethylhexadecanoic), pristanic (2,6,10,14-tetramethylpentadecanoic), and 4,8,12-trimethyltridecanoic

(TMTD) acids from Antarctic whale oil through North Atlantic whale oil to seal oil has suggested to Ackman and Hooper (11) a correlation with change in food intake for marine mammals from a primarily zooplankton diet through a partial fish diet to an exclusive fish diet. More recently, Blumer *et al.* (12) have isolated three phytol-derived olefinic hydrocarbons (the 2,10- and 5,10-diene and the 2,6,10-triene analogs of pristane) from marine zooplankton and fishes. Since these olefins are not present in ancient sediments and petroleum, they are considered to be valuable markers for the distinction between marine oil pollution and hydrocarbons derived from organisms.

Despite pristane's "inertness", we had found in a growth survey of bacteria known to utilize *n*-alkanes that under proper conditions pristane is readily utilized by a variety of organisms (13). In addition to the mycobacteria, nocardiae, and one moraxella strain tested in the growth study, several soil isolates also utilized pristane as a sole carbon and energy source for growth. One strain, tentatively identified as a species of *Corynebacterium* and designated RTMP-S, was chosen for further study because of its consistently profuse growth on pristane.

The soil isolate was grown in a mineral-salts medium at the expense of 0.2% pristane in shake culture for 80 hr, the time of maximum metabolite accumulation. Culture fluids (30 liters) were concentrated under reduced pressure and the crude concentrate (1 g), when fractionated by column chromatography on silicic acid, yielded a minor fraction, eluted with hexane-diethyl ether-acetic acid (90:10:1, Solvent 1), and a major fraction, which was eluted when the proportion of ether was increased (hexane-diethyl ether-acetic acid, 60:40:1, Solvent 2). The major fraction crystallized readily from benzene. Recrystallization from hexane-ether and final purification by sublimation yielded 155 mg of colorless crystals, mp (uncor) 74.5°C; elemental analysis: C, 49.38; H, 6.75 ( $C_6H_{10}O_4$  requires C, 49.32; H, 6.85%); neutralization equivalent, 72.9. Gas-liquid chromatography on Apiezon L (180°C) and on LAC-2-R 446 (115°C) gave equivalent chain lengths (ECL) of 5.3 and 5.5, respectively, while thin-layer chromatography on silica gel G (Solvent 2) revealed an  $R_f$  (relative to palmitic acid) of 0.14. Both cochromatography and mixed melting points of this fraction and authentic  $\alpha$ -methylglutaric acid were identical to that of the reference compound alone; infrared and nuclear magnetic resonance spectra of the two compounds further confirmed the identity (13).

The minor fraction (19 mg) exhibited an  $R_f$  value of 0.96 relative to palmitic acid upon thin-layer chromatography on silicic acid in Solvent 1, and this monocarboxylic acid fraction

Abbreviations: ECL, equivalent chain length; TMTD, 4,8,12-trimethyltridecanoate.

\* Present address: Department of Microbiology, University of Illinois, Urbana, Ill. 61801.



carboxylic acid cycle to provide energy and carbon for cell synthesis.

The possibility that the isolated acids were synthesized *de novo* from short-chain (normal and/or branched) fatty acid metabolites must be ruled out before the proposed metabolic pathway for pristane can be considered proved.

Growth studies in our laboratory have shown that highly branched alkanes are much less readily assimilated than linear ones (13). However, if multiple substituent groups are small and are properly spaced along the alkane chain, they need not be a deterrent to microbial utilization, as evidenced by the profuse growth of many bacterial strains on pristane. To undergo biological oxidation, the aliphatic hydrocarbon must possess a structure that will permit metabolism of the oxygenated intermediate, for example,  $\beta$ -oxidation of pristanic acid. Thus, at least one hydrogen atom must be present on both the  $\alpha$ - and  $\beta$ -carbon atoms of the fatty acid for  $\beta$ -oxidation to occur. Other modes of fatty acid oxidation,  $\alpha$ - and  $\omega$ -oxidation, presumably in similar fashion require the presence of hydrogen atoms for fatty acid dehydrogenation to occur.

Isolation and identification of TMTD and  $\alpha$ -methylglutarate indicate that two pathways of fatty acid oxidation operate in the organism studied:  $\beta$ -oxidation and  $\omega$ -oxidation followed by  $\beta$ -oxidation. The accumulation of pristane in the biosphere appears to be a consequence of the lack of proper conditions for oxidation rather than a refractory chemical structure.

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