Characterization of the C₁₅ Branched-Chain Fatty Acids of Corynbacterium acnes by Gas Chromatography

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In a limited number of microorganisms, branched-chain fatty acids have been reported to be major constituents of lipids (M. Kates, p. 27, in R. Pauletto and D. Kritchevsky [ed.], Advances in Lipid Research, vol. 2, Academic Press, Inc., New York, 1964). The point of branching of the single methyl group in the fatty acid chain occurs at the penultimate carbon atom in the iso-acids and at the antipenultimate carbon atom in the anteiso-acids. Several members of the homologous series of both iso- and anteiso-acids have been reported in various microorganisms [S. Akashi and K. A. Saito, J. Biochem. (Tokyo) 47: 222, 1960; R. W. Walker and I. S. Fagerson, Can. J. Microbiol. 11:229, 1965; T. Kaneda, J. Bacteriol. 93:894, 1967]. In a recent study, we found that the principal fatty acid of Corynebacterium acnes, Propionibacterium freudenreichii, and P. shermanii was a saturated C₁₅ branched-chain acid (C. W. Moss et al., J. Bacteriol. 94:1300, 1967). However, the position of the methyl group was not determined. The presence of large amounts of C₁₅ branched-chain acid (range: 24 to 49% of total fatty acids) in each of 27 isolates of C. acnes and in single strains of each of the above Propionibacterium species suggested a close relationship among strains and possibly among species. Since this finding may be of some value in the identification and taxonomy of these bacterial species, we wanted to determine the position (iso or anteiso) of the methyl group in the C₁₅ branched-chain acid. This would enable us to assess the possibility of an even closer relationship among these organisms than was shown previously by us and by others (H. C. Douglas and S. E. Gunter, J. Bacteriol. 52:15, 1946).

Twenty-two strains of C. acnes, two strains of P. acnes, and one strain each of P. freudenreichii and P. shermanii were utilized in these experiments. C. acnes cultures were isolated from a variety of clinical sources at diverse geographical locations; all Propionibacterium cultures were obtained from the American Type Culture Collection. A description of the cultural characteristics

![Fig. 1. Gas-liquid chromatography (GLC) of reference branched-chain fatty acids and bacterial fatty acids. Top GLC chromatogram represents the reference iso-C₁₅ (i-C₁₅) and anteiso-C₁₅ (a-C₁₅) fatty acids; middle GLC chromatogram represents bacterial fatty acids from Corynebacterium acnes; bottom GLC chromatogram represents bacterial fatty acids from Propionibacterium freudenreichii and P. shermanii.](http://jb.asm.org/)
and major fatty acid composition of each culture was reported previously (C. W. Moss et al., J. Bacteriol. 94:1300, 1967). Media, conditions of growth, saponification procedures, and extraction and methylation of acidic components were described earlier (C. W. Moss et al., J. Bacteriol. 94:1300, 1967; C. W. Moss and V. J. Lewis, Appl. Microbiol. 15:390, 1967). Fatty acid methyl esters were analyzed by use of a Barber-Colman model 5000 gas chromatograph fitted with a 2.4 m × 5 mm (inner diameter) U-tube glass column of 15% ethylene glycol succinate coated on 80/100 mesh Chromosorb W (Applied Science Laboratories, Inc., State College, Pa.). Operating parameters of the instrument were: injection temperature, 230 C; detector temperature, 240 C; column temperature, 160 C; carrier gas, nitrogen. Fatty acid methyl ester standards, including 12-methyltetradecanoic acid (anteiso-C_{15}), were obtained from Applied Science Laboratories; 13-methyltetradecanoic acid (iso-C_{15}) was provided through the courtesy of Toshi Kaneda.

By use of the above conditions of gas-liquid chromatography (GLC), the reference standard iso- and anteiso-C_{15} fatty acid esters were sufficiently separated to permit GLC characterization of the bacterial C_{15} branched-chain acid. Although the two reference acids were not completely resolved, a 1 min difference in retention time was noted (top GLC tracing in Fig. 1). The middle GLC tracing in Fig. 1 is a representative fatty acid profile of the C. acnes cultures. It is apparent that the iso-C_{15} acid was the single most abundant acid in each of the 22 strains tested. Also present in each strain, but in a much smaller amount, was the anteiso-C_{15} acid. The ratio of iso-C_{15} to anteiso-C_{15} was approximately 7:1. Fatty acid profiles, showing a similar ratio of iso-C_{15} to anteiso-C_{15} acid, were also observed in the two ATCC cultures of P. acnes (ATCC 6919 and 6921). An example of the fatty acid profile obtained from P. freudenreichii (ATCC 6207) and P. shermanii (ATCC 6207) is illustrated in the bottom GLC tracing. In contrast to the results with the C. acnes and P. acnes cultures, the single most abundant acid in these two strains was anteiso-C_{15} acid. The ratio of iso- to anteiso-C_{15} acid in these two strains was approximately 1:8.

Occurrence of iso-C_{15} as the most abundant acid in each of the 22 isolates of C. acnes supports our previous study, which indicated homogeneity among strains of this species. Moreover, these isolates appear to be essentially identical to the two ATCC strains of P. acnes in fatty acid composition as well as in other characteristics (C. W. Moss et al., J. Bacteriol. 94:1300, 1967). The observation that P. freudenreichii and P. shermanii contained the anteiso-C_{15} acid as the single most abundant acid may provide an additional criterion for the differentiation of these two species from C. acnes, provided that other strains of each species are found to have similar fatty acid profiles. However, all strains used in this study were processed under uniform conditions after cultivation in a single growth medium. Others have shown that the nature of the growth medium (i.e., availability of precursors of the terminal portions of the branched-chain acids) markedly influences the relative abundance of iso- and anteiso-acids in other bacteria (T. Kaneda, Can. J. Microbiol., 12:501, 1966; T. G. Tornabene, E. O. Bennett, and J. Oró, J. Bacteriol. 94:344, 1967). Studies are now in progress to determine the effects of this variable on the branched-chained fatty acids of cultures used in this investigation.

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