products were formed. This observation should provide a simple assay system without resorting to TLC resolution of a mixture of radioactive products and subsequent counting of the gel co-chromatography with 3-keto-dihydrosphingosine. The inability of stearoyl CoA to support the incorporation of serine into sphingosine base would suggest that the synthesis of eicososphingosine (the C_{20} homologue) which is found in gangliosides (13) may arise by a different pathway.

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Unsaturated Fatty Acids of Mycobacteria

ABSTRACT

The double bond locations have been determined for the mono-unsaturated fatty acids, C_{14} to C_{26} of M. smegmatis and M. bovis BCG. The 14:1 and 16:1 fatty acids from M. smegmatis are principally \Delta^{10}, while the 17:1, 18:1 and 19:1 fatty acids from both organisms are \Delta^{9}. In the case of M. smegmatis, the 20:1, 22:1 and 24:1 fatty acids are principally \Delta^{11}, \Delta^{13} and \Delta^{15}, respectively, while the 22:1, 24:1 and 26:1 fatty acids of BCG are principally \Delta^{13}, \Delta^{15} and \Delta^{17}, respectively.
The double bond locations of mycobacterial mono-unsaturated fatty acids have been determined for only a few fatty acids. Scheuerbrandt and Bloch (1) showed that the C16 fatty acid consisted principally of the Δ10 isomer and the C18 fatty acid the Δ9 isomer. Burke (2), using the avirulent Mycobacterium tuberculosis strain H37Rv, found that C16 monoene consisted of a mixture of 10-, 6- and 9-hexadecenoic acids in a proportion of 65, 15 and 10. The C17 and C18 fatty acids were mostly the Δ9 isomer. The C20 acid appeared to be trans-2-eicosenoate.

This report describes the double bond location of the series of mono-unsaturated fatty acids from C14 to C26 in M. smegmatis ATCC 19420 and M. bovis BCG (Glaxo strain).

The organisms were grown and harvested as described previously (3). Cells were hydrolyzed in 95% methanol containing 20% potassium hydroxide (4). Fatty acid methyl esters were prepared by refluxing the crude fatty acid mixture in methanol-benzene-concentrated sulfuric acid 20:10:1 for 2 hr. Mercuric acetate adducts were prepared (5) and the esters were separated by column chromatography on Florisil [Fisher Scientific Co., Medford, Mass.; 100-200 mesh; deactivated with 7% water, w/w] (6). The solvent sequence petroleum ether-diethyl ether 95:5, petroleum ether-diethyl ether 85:15 and methanol-concentrated hydrochloric acid 90:10 was used. The last fraction, which contained the unsaturated fatty acids, was further purified by thin layer chromatography on Florisil [Fisher Scientific Co., Medford, Mass.; 100-200 mesh; deactivated with 7% water, w/w] (6). The solvent sequence petroleum ether-diethyl ether 95:5, petroleum ether-diethyl ether 85:15 and methanol-concentrated hydrochloric acid 90:10 was used. The last fraction, which contained the unsaturated fatty acids, was further purified by thin layer chromatography on Florisil [Fisher Scientific Co., Medford, Mass.; 100-200 mesh; deactivated with 7% water, w/w] (6). The solvent sequence petroleum ether-diethyl ether 95:5, petroleum ether-diethyl ether 85:15 and methanol-concentrated hydrochloric acid 90:10 was used. The last fraction, which contained the unsaturated fatty acids, was further purified by thin layer chromatography on Florisil [Fisher Scientific Co., Medford, Mass.; 100-200 mesh; deactivated with 7% water, w/w] (6). The solvent sequence petroleum ether-diethyl ether 95:5, petroleum ether-diethyl ether 85:15 and methanol-concentrated hydrochloric acid 90:10 was used.

The periodate-permanganate cleavage products were analyzed by GLC on a Perkin Elmer 900 gas chromatograph using three sets of columns: 5 ft x 1/8 in., 2.5% OV-225 on 80-100 mesh AW Chromosorb G; 6 ft x 1/8 in., 15% 20 mesh AW Celite; and 6 ft x 1/8 in., 2.5% OV-1 on 80-100 mesh AW, DMCS Chromosorb G (high performance). Double bond location was assigned principally on the basis of the identification of the dicarboxylic acid oxidation product.

Table I lists the relative yields of dicarboxylic acids obtained from periodate-permanganate oxidation. The C14 and C15 fatty acids consist of a wide range of isomers and may reflect diverse biosynthetic origins. The C16 fatty acids consist principally of the Δ9, and Δ10 isomers, while the C17, 18 and 19 acids are mostly the Δ9 isomers, an indication of a common desaturase system.

The C20,22,24 fatty acids of M. smegmatis and C22,24,26 acids of BCG are a related series reflecting a common origin from the Δ9 C18 fatty acid with C-2 elongation. The C20 fatty acid of BCG is a mixture of 9, 10 and 11 isomers and may have originated by a combination...