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ORIGIN OF CYCLOHEXANECARBOXYLIC ACID IN *BACILLUS ACIDOCALDARIUS*

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Abstract—In *Bacillus acidocaldarius*, shikimic acid is converted into the cyclohexanecarboxylic acid precursor of fatty acids by way of cyclohexene-1-carboxylic acid, but not by way of cyclohexene-3- or -4-carboxylic acid or benzoic acid.

Bacillus acidocaldarius, an acidophilic thermophilic bacterium[1,2] contains lipids largely derived from ω-cyclohexyl fatty acids (11-cyclohexylundecanoic, C_{17}, and 13-cyclohexyltridecanoic, C_{19}).[3,4] These arise by C_2-elongation from the CoA derivative of cyclohexanecarboxylic acid, which in turn is formed by the complete reduction of shikimate.[5] We have shown[6] that in this organism the initiating transacylase of the enzyme system for fatty acid synthesis will accept a variety of acyl initiators—normal, branched-chain, or cyclic—provided that their overall molecular length is similar to that of n-C_5 to n-C_7 acyl species; the specificity of this initiating step was established by observing the effect of both "natural" and "non-natural" acids, added to the cultures on the fatty acid composition of the cells.

Alternatively the same method can be used to determine whether an added acid will serve as precursor for the "natural" acyl–CoA species from which a particular type of fatty acid is formed; if so, the proportion of that type of fatty acid will be increased, and if not, it will be either unchanged or diminished through competition with the synthesis of an un-natural fatty acid. We have employed this method to explore somewhat further the origin of the cyclohexanecarboxylic acid in *B. acidocaldarius*.

Experimental techniques have already been described in full.[6] Shikimic acid, benzoic acid, cyclohexanecarboxylic acid, cyclohexene-4-carboxylic acid and cyclohexene-1-carboxylic acid were purchased, and the latter was converted into cyclohexene-3-carboxylic acid (satisfactory IR and NMR data) by the method of Moppett and Sutherland.[7]

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Under standard growth conditions (60°, pH 3·5) the *B. acidocaldarius* cells yielded 19 mg/g (lyophilized weight) of the C_{17} and C_{19} ω-cyclohexyl acids (with 14 mg/g of other fatty acids). Additions of 0·1–0·5 mM cyclohexane–carboxylic acid raised the yield of ω-cyclohexyl acids to 43–44 mg/g (other acids negligible). The effects of shikimic acid (with 0·1 mM, 24 mg/g; with 0·5 mM, 39 mg/g) and of cyclohexene-1-carboxylic acid (with 0·1 or 0·5 mM, 40 mg/g) were similar and almost as large. Benzoic acid, in contrast, had no effect at 0·1 mM and was toxic at 0·5 mM.

With additions of either cyclohexene-3-carboxylic or -4-carboxylic acids the yields of all the usual fatty acids were depressed and in each case a new component was present in the derived methyl esters. This was separated from the usual esters (which are entirely saturated) by chromatography on 5% argentized silica gel plates in 5% Et_2O–hexane. Cultures to which cyclohexene-3-carboxylic acid had been added thus afforded (with 0·1 mM, 13 mg/g; with 0·5 mM, 23 mg/g) a mixture of methyl 11-(cyclohex-2′-enyl)-undecanoate and methyl 13-(cyclohex-2′-enyl)-tridecanoate [M^+/e 274, 308; equivalent chain lengths for GLC on diethyleneglycol succinate 21·39, 23·39 (methyl stearate, 18·00)], convertible into the corresponding ω-cyclohexyl acids by hydrogenation (Pt in MeOH).

Similarly, cultures to which cyclohexene-4-carboxylic acid had been added afforded (with 0·1 mM, 16 mg/g; with 0·5 mM, 22 mg/g) a mixture of methyl 11-(cyclohex-3′-enyl)-undecanoate and methyl 13-(cyclohex-3′-enyl)-tridecanoate (M^+/e 274, 308; equivalent chain lengths 21·41 and 23·41). In each case the cyclohexenyl ester of M^+/e 308 was purified by preparative GLC and gave satisfactory NMR data.

These data establish that the final step in the formation of cyclohexanecarboxylic acid from shikimate in *B. acidocaldarius* is the reduction of cyclohexene-1-carboxylic acid, and that neither benzoic acid nor the other cyclohexenecarboxylic acids are intermediates in the overall process. The route by which shikimate is deoxygenated to the intermediate cyclohexene-1-carboxylate remains unexplored.