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Non-stereoselective Conversion of the Four Diastereoisomers at the C-24 and C-25 Positions of 3α,7α,12α,24-Tetrahydroxy-5β-Cholestan-26-oic Acid into Cholic Acid*

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In the biosynthesis of cholic acid in animals, cholesterol is first converted into 3α, 7α,12α-trihydroxy-5β-cholestane and then the latter compound is oxidized to 3α,7α, 12α-trihydroxy-5β-cholestan-26-oic acid (THCA). The acid is presumed to be transformed into cholic acid by a sequence of reactions analogous to that involved in the β-oxidation of fatty acids. 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid (TeHCA) has been postulated as an intermediate of this biologically important C–C bond cleavage reaction. To answer the question of which stereoisomer of TeHCA is the true intermediate in cholic acid biosynthesis, it is essential to know the stereochemical specificity of the four isomers in the C–C bond cleavage reaction.

Previously, we have prepared the four stereoisomers of TeHCA. In this report the four stereoisomers of TeHCA were incubated with rat liver mitochondrial fraction supplemented with adenosine triphosphate, coenzyme A, nicotinamide adenine dinucleotide, and $MgCl_2$. The incubation mixture was saponified, and after addition of THCA as an internal standard, were extracted with ethyl acetate under acidic condition. The concentrated extracts were treated with ethereal diazomethane and the cholic acid methyl ester fraction was separated by TLC. This was then derivatized with trymethylsilylimidazole. The resultant tris-trimethylsilyl ether of cholic acid methyl ester was analyzed by gas liquid chromatography-mass spectrometry. It has been found that (24S, 25R)-, (24S, 25S)-, (24R, 25S)- and (24R, 25R)-TeHCA were converted into cholic acid in 9.8%, 8.8%, 4.6%, 6.3% yield respectively.

Hoshita et al. demonstrated that the (24R, 25R)-isomer is the sole TeHCA produced on incubation of THCA with 700×g supernatant fraction, while our present data indicate all the four isomers of TeHCA are equally well transformed into cholic acid on incubation with mitochondrial fraction.

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