On the Mechanism of Deoxycholic Acid Formation

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Recent work has shown that deoxycholic acid is formed from cholic acid by the action of intestinal microorganisms [1-3]. The aim of the present investigation was to study the reaction mechanisms of this transformation with the aid of cholic acid labelled with tritium in the 7β-position. The acid was prepared by reduction of 3α, 12α-dihydroxy-7-ketocholanic acid with sodium borohydride earlier exposed to tritium gas for 6 days. This compound was first administered together with cholic acid-24-^{14}C to rabbits. A bile fistula was made after two days. Practically all of the cholic acid had then been transformed into deoxycholic acid. The ratios of isotopes (^{3}H/^{14}C) in this acid and in the administered cholic acid were determined and found to be equal, thus demonstrating complete retention of the 7β-hydrogen during the elimination of the 7α-hydroxyl group.

The doubly labelled cholic acid was then given to rats and allowed to take part in the enterohepatic circulation for 1—7 days. Determinations of isotope ratios in the cholic acid samples isolated from the animals showed that a progressive loss of tritium had occurred.

It is known that in the rat deoxycholic acid is hydroxylated to cholic acid in the liver [4] and it was assumed that the loss of the 7β-^{3}H had taken place during the reaction sequence: cholic acid → deoxycholic acid → cholic acid. Norman and Sjövall [3] have isolated labelled deoxycholic and 3α, 12α-dihydroxy-7-ketocholanic acid from feces of rats that had received labelled cholic acid, and have further shown that the keto acid can be transformed into deoxycholic acid in the gut. If the 7-keto acid was an obligatory intermediate in the reaction cholic acid → deoxyholic acid this would explain the loss of the 7β-^{3}H. To clarify this point doubly labelled cholic acid was injected into the coecum of rats with a bile fistula and deoxycholic acid isolated from the feces. This acid retained 80 % of the tritium activity indicating that 3α,12α-dihyroxy-7-ketocholanic acid is only of minor importance as an intermediate in the formation of deoxycholic acid, the main path probably leading *via* the stereospecific elimination of water with the formation of an unsaturated acid as an intermediate.

The loss of tritium activity might also have occurred in the step deoxycholic acid → cholic acid which occurs in the liver. The doubly labelled deoxycholic acid obtained in the aforementioned experiments was therefore injected intraperitoneally into rats with a bile fistula. The ratio of T/^{14}C in the isolated cholic acid was then found to be less than 4 % of that in the injected deoxycholic acid.

The 7α-hydroxylation of cholesterol and the 11α- and 11β-hydroxylation in the pregnane-3,20-dione series have been found to proceed by direct replacement of the 7α-, 11α- and 11β-hydrogen, resp.[5-7] and it seems plausible that the 7α-hydroxylation of deoxycholic acid proceeds by a similar reaction, in which the 7β-hydrogen would not be labelized.

One explanation of these results is that the 7-hydrogen of the possible unsaturated intermediate formed by the elimination of water is brought into the 7α-position at the reduction of the double bond.

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Determination of the Position of Carbon-Carbon Double Bonds by Mass Spectrometry

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The position of the double bond in methyl oleate may be determined by comparing the mass spectra of methyl stearate and methyl 9,10-dideuterostearate. The latter compound was prepared by reduction of methyl oleate with deutero-hydrazine.

The extension of the method to poly-unsaturated fatty esters is under investigation.