NON-STEREOSPECIFIC FORMATION OF 3α,7α,24-TRIHYDROXY-5β-CHOLESTAN-26-OIC ACID DURING CHENODEOXYCHOLIC ACID BIOSYNTHESIS

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Incubation of 3α,7α-dihydroxy-5β-cholestan-26-oic acid and its Δ^{24}-analog with rat liver homogenate produced a mixture of C-24,25 diastereoisomers of 3α,7α,24-trihydroxy-5β-cholestan-26-oic acid, a key intermediate of chenodeoxycholic acid biosynthesis.

KEYWORDS chenodeoxycholic acid; β-oxidation; bile acid; 3α,7α-dihydroxy-5β-cholestan-26-oic acid; 3α,7α,24-trihydroxy-5β-cholestan-26-oic acid

Chenodeoxycholic acid (**5**) constitutes the primary bile acid, together with cholic acid (12α-hydroxy analog of **5**). The final stage of its biosynthesis in liver involves β-oxidation of 3α,7α-dihydroxy-5β-cholestan-26-oic acid (DHCA, **1**), of which coenzyme A (CoA) thioester would be transformed into **5** via Δ^{24}-DHCA (**2**), 24-hydroxy-DHCA (**3**) and 24-oxo-DHCA(**4**) or their CoA derivatives (Chart 1).[1] As for biosynthesis of cholic acid, we have recently demonstrated using rat liver homogenate that all four diastereoisomers (at C-24,25) of 3α,7α12α,24-tetrahydroxy-5β-cholestan-26-oic acid (TeHCA) are produced from (25R)- and (25S)-3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid,[2] and all of them in turn are transformed into cholic acid.[3] These non-stereoselective behaviors of TeHCA in cholic acid biosynthesis have prompted us to carry out the similar experiments on chenodeoxycholic acid biosynthesis.

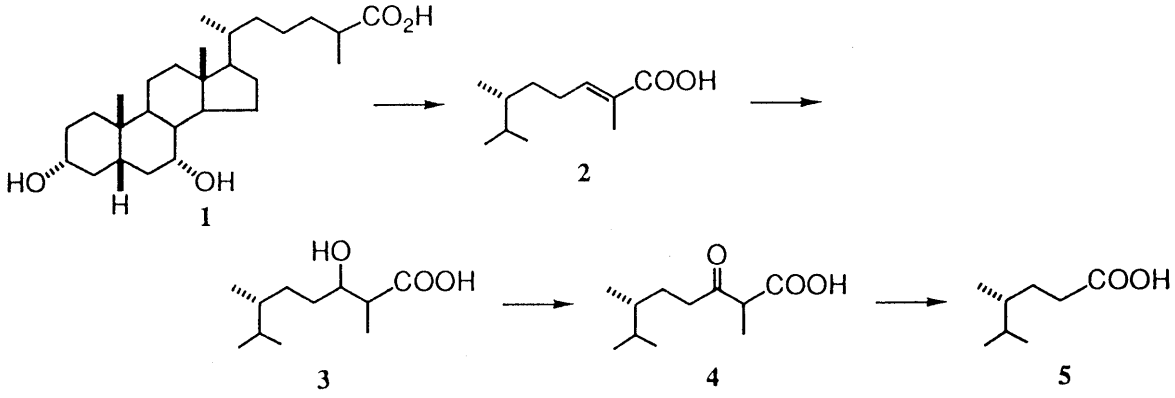

Chart 1. Probable Biosynthetic Sequence of Chenodeoxycholic Acid
The acid residue could be CoA form in a biological system.

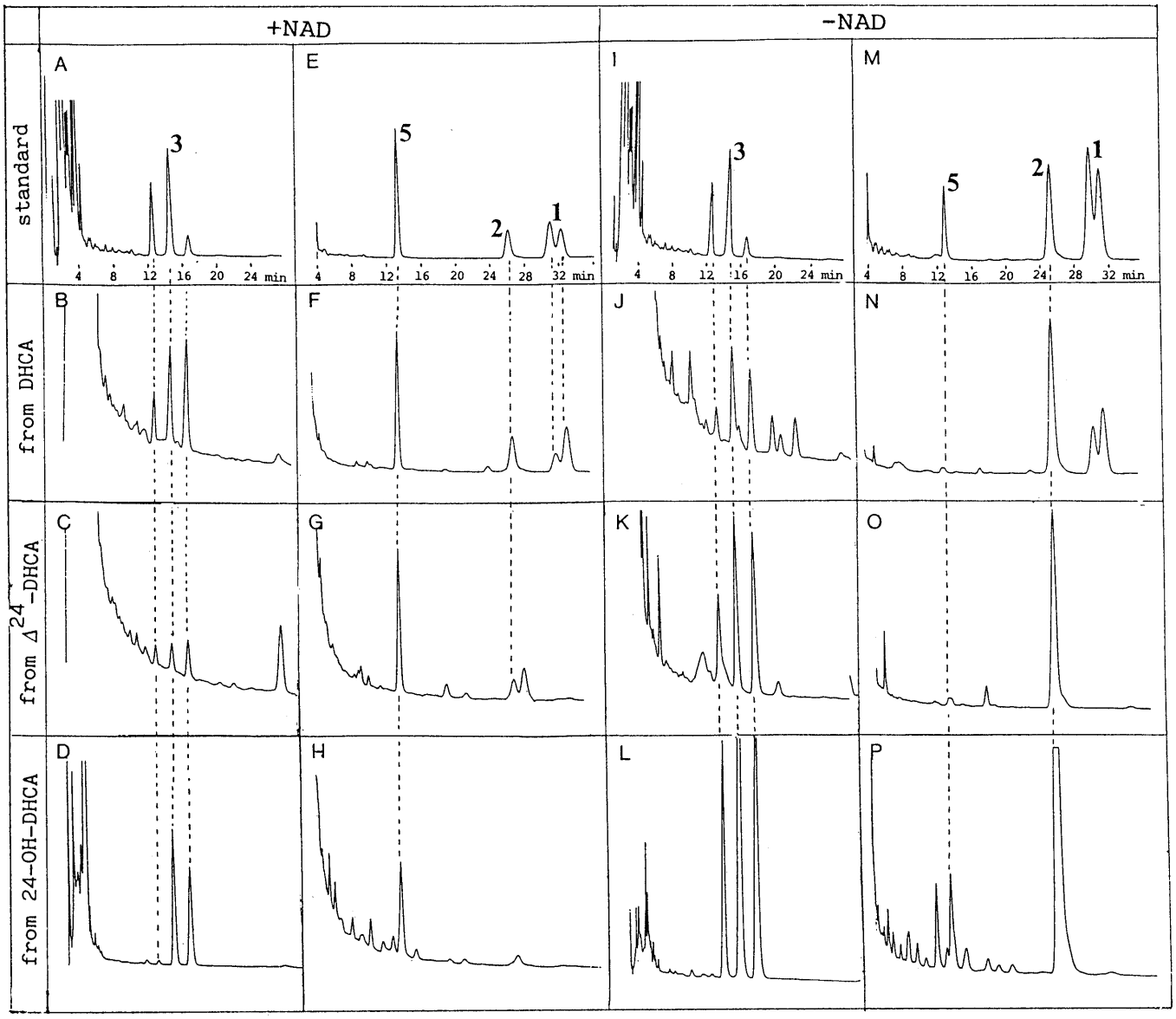

Fig. 1. HPLC Profiles of the Incubation Products. Incubation of DHCA (**1**), Δ^{24}-DHCA (**2**) and 24-hydroxy-DHCA (**3**) with rat liver homogenate was carried out in the presence (B, C, D, E, F, G and H) or the absence (I, J, K, L, M, N, O and P) of NAD, and the incubation products were processed as described in the text.

Authentic samples of (25RS)-DHCA (**1**), (24E)-Δ^{24}-DHCA (**2**) and the four diastereoisomeric mixture of 24-hydroxy-DHCA (**3**) were chemically prepared from the commercially available chenodeoxycholic acid.[4] HPLC analysis of the corresponding p-bromophenacyl ester derivatives revealed, as expected, twin and single peak for **1** and **2**, respectively (Fig. 1E and M). However, only three peaks appeared for **3** (Fig. 1A and I), and the preparatively separated middle peak was found to be a mixture of the two stereoisomers.[4]

Incubation of **1**,**2** and **3** (each 0.1 mg) with rat liver homogenate (10,000g precipitate, 5 ml Tris-HCl buffer pH 8.5 containing ATP, CoA and $MgCl_2$) was carried

out in the presence or absence of NAD as described previously.[2] Incubation mixtures were successively treated with ethanol, 10% NaOH and 3N HCl, and extracted with ethyl acetate. The concentrated extract was purified with a Sephadex LH-20 (CH_2Cl_2-MeOH 10:1) and derivatized to the p-bromophenacyl ester. This was then preparatively resolved by HPLC (Shim-pack CLC-SIL, CH_2Cl_2-MeOH 40:1) into the more polar fraction containing **3** and the less polar one containing **1**, **2** and **5**. Each fraction was finally analyzed by HPLC (Shim-pack CLC-ODS, 85% aqueous MeOH, 2.0 ml/min, UV detection at 254 nm), whose profiles are shown in Fig. 1.

In the presence of NAD, **1**, **2** and **3** were efficiently converted to **5** as shown in Fig. 1 F, G and H. The more polar fraction of the incubation products from **1** and **2** showed three peaks corresponding to the four stereoisomers of **3** (Fig. 1 B and C). This strongly suggests the transformations of **1** and **2** into the four (or three) stereoisomers of **3**. These transformations were also observed in the absence of NAD (Fig. 1 J and K), where the further conversion to **5** via **4** was not observed (Fig. 1 N and O). The facts are in accord with expectation, since NAD should be an essential cofactor of oxidation of **3** to **4** (Fig. 1L). It can be concluded that DHCA (**1**) and Δ^{24}- DHCA (**2**) were converted to the stereoisomeric mixtures of 24-hydroxy-DHCA (**3**) by rat liver homogenate, and these observations are in close parallel with those obtained with TeHCA during cholic acid biosynthesis.[2]

It should be mentioned that the preferential consumption of one (or two) of the stereoisomers of **3** (Fig. 1D) suggests a stereoselective conversion of **3** into **5**. Further, in the absence of NAD, **3** appears to be efficiently dehydrated to give **2** (Fig. 1P). Confirmation of these points and identification of the genuine intermediate of chenodeoxycholic acid biosynthesis should await further studies.

REFERENCES AND NOTES

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4) The synthesis and spectral data of these compounds will be described elsewhere. In Fig. 1A, the first, second and third peaks (approximately 3 : 5 : 1) correspond to (24S,25R)-, (24S,25S)-/(24R,25S), and (24R,25R)-isomers, respectively. This diastereoisomeric mixture of **3** was used as such for incubation substrate (Fig. 1D, H, L and P). In Fig. 1B, the more mobile and the less mobile peaks of **1** correspond to (25S)- and (25R)-isomers. The stereochemistries at C-24 and/or C-25 of **1** and **3** were assigned by comparing their NMR data with those reported.[2,5]

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(Received May 16, 1994; accepted June 8, 1994)