Communications to the Editor

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SYNTHESIS OF THE 1β-HYDROXYLATED BILE ACIDS AND IDENTIFICATION OF 1β,3α,7α-TRIHYDROXY- AND 1β,3α,7α,12α-TETRAHYDROXY-5β-CHOLAN-24-OIC ACIDS IN HUMAN MECONIUM

Masahiko Tohma,*[,a] Reijiro Mahara,[a] Hiromi Takeshita,[a] Takao Kurosawa,[a] Shigeo Ikegawa,[a] and Hiroshi Nittono[b]

Faculty of Pharmaceutical Sciences, Higashi-Nippon-Gakuen University,[a] Ishikari-Tobetsu, Hokkaido 061-02, Japan and Department of Pediatrics, School of Medicine, Juntendo University,[b] Bunkyo-ku Hongo, Tokyo 113, Japan

The 1β-hydroxylated lithocholic (**1**), deoxycholic (**2**), chenodeoxycholic (**3**) and cholic acids (**4**) were synthesized from the corresponding bile acids as starting materials. These unusual bile acids in human meconium were determined by the selected ion monitoring in gas chromatography-mass spectrometry, and novel 1β-hydroxychenodeoxycholic (**3**) and 1β-hydroxycholic acids (**4**) were identified in significant amounts of 10.6% and 5.8%, respectively, of the total bile acids.

KEYWORDS —— bile acid; gas chromatography-mass spectrometry; selected ion monitoring; human meconium; 1β,3α-dihydroxy-5β-cholan-24-oic acid; 1β,3α,7α-trihydroxy-5β-cholan-24-oic acid; 1β,3α,12α-trihydroxy-5β-cholan-24-oic acid; 1β,3α,7α,12α-tetrahydroxy-5β-cholan-24-oic acid

Almé *et al.*[1] found the unusual bile acids hydroxylated at the C-1 position in urine from patients with hepatobiliary diseases by means of gas chromatography-mass spectrometric (GC-MS) analysis. The structures of these bile acids were tentatively assigned from their mass spectra to 1,3,12-trihydroxy- and 1,3,7,12-tetrahydroxycholanoic acids. They were also detected in urine of late pregnant women,[2] newborn infants,[3] and in human meconium.[4] Further quantitative investigation of the bile acids has been delayed because of a lack of the standard samples, although 1β,3α,12α-trihydroxy-5β-cholanoic acid was synthesized by microbial hydroxylation[5] and methyl 1α,3α-dihydroxy-5β-cholanoate was obtained by chemical synthesis.[6] We wish to describe here the synthesis of the 1β-hydroxylated lithocholic (**1**), deoxycholic (**2**), chenodeoxycholic (**3**) and cholic acids (**4**), and the identification of significant amounts of these unusual bile acids (**2-4**) in human meconium.

Four 1β-hydroxylated bile acids (**1-4**) were synthesized from the corresponding lithocholic (**5**), deoxycholic (**6**), chenodeoxycholic (**7**) and cholic acids (**8**) as starting materials according to the reaction scheme as shown in Chart 1. The methyl esters of the bile acids (**5-8**) were selectively oxidized to the 3-oxo compounds with Jones reagent or silver carbonate on Celite in boiling toluene. The Δ^1-unsaturated ketones (**I**) were prepared from the corresponding 2,4-dibromoketones by reductive debromination at C-4 with chromous acetate in ethanol and dehydrobromination with calcium carbonate in dimethylformamide. The enones (**I**) were oxidized with alkaline hydrogen peroxide to give the 1β,2β-epoxyketones (**II**). Reductive cleavage of the epoxides (**II**) to the 1β-hydroxyketones (**III**) was carried out by the treatment with chromous acetate in ethanol. Subsequent reduction with sodium borohydride gave the 1β,3α-dihydroxy compounds, NMR δ: 3.90 (t, J=3 Hz, 1α-H), 3.84-4.17 (m, 3β-H). Finally, these bile acid esters were hydrolyzed with methanolic potassium hydroxide to the respective desired

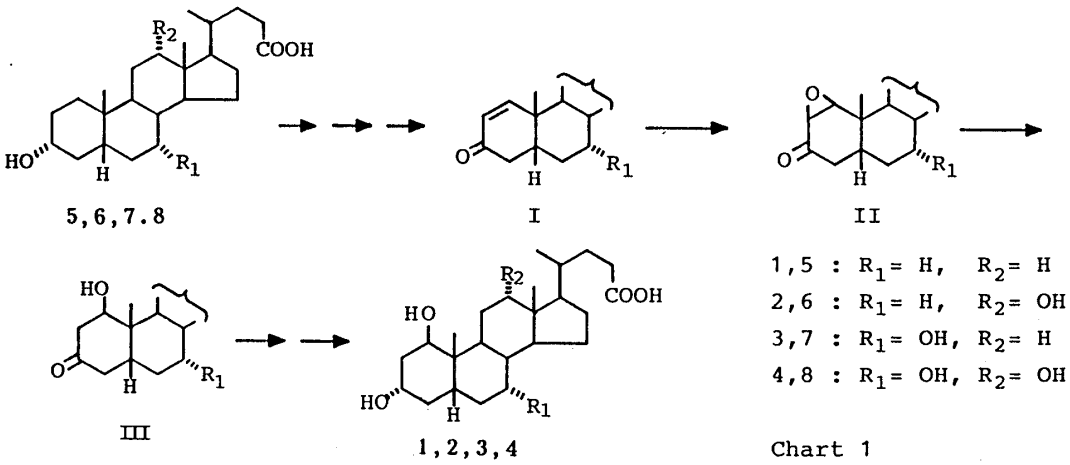

Chart 1

1β,3α-dihydroxy- (1), mp 274-278°C, 1β,3α,12α-trihydroxy- (2), mp 262-264°C, 1β,3α,7α-trihydroxy- (3), mp 266-270°C, and 1β, 3α,7α,12α-tetrahydroxy-5β-cholan-24-oic acids (4), mp 288-290°C.

The glycine, taurine and sulfuric acid conjugates of the bile acids (1-4) were synthesized in order to develop a quantitative analysis of these bile acids in biological fluids. The glyco- and tauro-conjugates were prepared by mixing of each bile acid and ethyl glycinate or taurine with diethylphosphoryl cyanide in the presence of triethylamine. The bile acid sulfates were synthesized from the corresponding free bile acids (1-4) or their glycoconjugates with chlorosulfonic acid in pyridine.

The mass spectra of the methyl ester-trimethylsilyl ether derivatives of the synthesized 1β-hydroxy bile acids (1-4) are shown in Fig. 1. They exhibit similar simple fragmentation patterns having a characteristic base peak at m/z 217 based on a bis-trimethylsilyl ether of the 1β,3α-diol,[7] together with the corresponding M-15 and M-90 ion peaks of very low intensities. The spectra of the 2 and 4 derivatives were identical with those of the 1-hydroxylated bile acids proposed by Almé *et al.*[1]

A highly sensitive and specific quantitative assay for these bile acids (1-4) in biological fluids has been developed by selected ion monitoring of the characteristic fragments at m/z 217 and m/z 210 in GC-MS analysis, using [2,2,3,4,4,23,23-$^{2}H_{7}$]deoxycholic acid as an internal standard. Prior to GC-MS analysis, the bile acid fraction was extracted from biological samples on a Bond Elut cartridge together

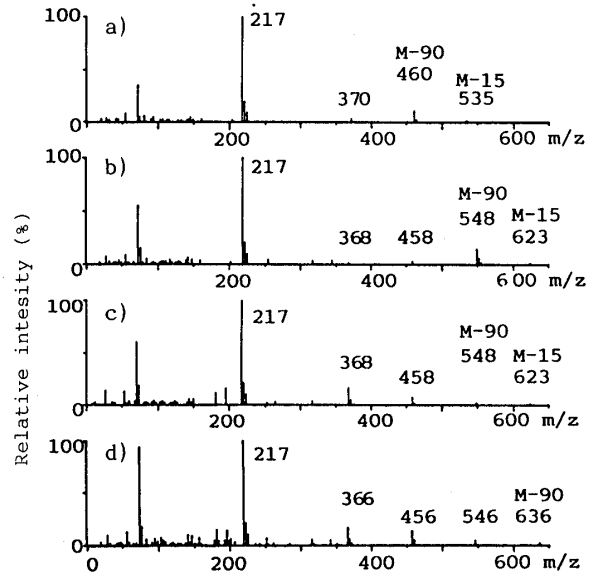

Fig. 1. Mass Spectra of the Methyl Ester-Trimethylsilyl Ether Derivatives of the 1β-Hydroxylated Bile Acids

a) 1β-Hydroxylithocholic acid (1).
b) 1β-Hydroxydeoxycholic acid (2).
c) 1β-Hydroxychenodeoxycholic acid (3).
d) 1β-Hydroxycholic acid (4).

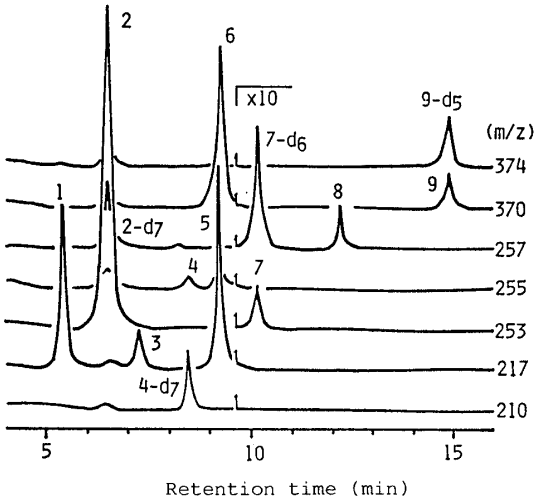

Fig. 2. Gas Chromatography-Mass Spectrometric Analysis of the Methyl Ester-Trimethylsilyl Ether Derivatives of the Bile Acids from Human Meconium, Column : 1.5% Poly I-110 on Gas Chrom Q, 250°C.

1) 1β-Hydroxycholic acid (**4**); 2) cholic acid; 3) 1β-hydroxydeoxycholic acid (**2**); 4) deoxycholic acid; 5) 1β-hydroxychenodeoxycholic acid (**3**); 6) chenodeoxycholic acid; 7) 3β,12α-dihydroxy-5-cholen-24-oic acid; 8) lithocholic acid; 9) 3β-hydroxy-5-cholen-24-oic acid.

with the added internal standard. After solvolysis with hydrochloric acid in ethanol-acetone (1:9) at pH 1 and alkaline hydrolysis in 4N sodium hydroxide-methanol (1:1), the free bile acids were methylated with diazomethane-ether and converted into the trimethylsilyl ethers with N-trimethylsilylimidazole. This method gave good linearity on calibration curves over the range of 0.1-100 ng for each bile acid, and reasonable recoveries of 92-101% of the free and conjugated bile acids.

The bile acids (**1-4**) in human meconium were quantitatively determined by the developed GC-MS analysis as shown in Fig. 2. The new unusual bile acids, 1β,3α,7α-triol (**3**) and 1β,3α,7α,12α-tetrol (**4**) were identified in significant amounts of 0.328 and 0.179 μg/mg, respectively, accompanied by the 1β,3α,12α-triol (**2**, 0.062 μg/mg), and these 1β-hydroxylated bile acids constituted 18.4% of the total bile acids in meconium. We also have been able to identify other unusual bile acids: 3β-hydroxy-[8] (0.052 μg/mg) and 3β,12α-dihydroxy-5-cholen-24-oic acid[9] (0.033 μg/mg). The 1β,3α,12α-triol (**2**) has sometimes been found in healthy urine and serum in trace amounts (0.02 μg/ml), but the 1β,3α-diol (**1**) has not yet been detected in any human biological fluids.

These results suggest that the metabolism of bile acids in fetal liver differs from that of healthy adults, and the activity of the 1β-hydroxylase is probably elevated in the juvenile liver cell. A more detailed investigation is now in progress and the results will be reported elsewhere.

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