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Stero-bile Acids and Bile Alcohols*

XCV. Synthesis of 3α, 7α, 12α-Trihydroxy-5β-cholestane-24-carboxylic Acid and the Chemical Structure of Trihydroxybufosterocholenic Acid Isolated from Toad Bile

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1. 3α, 7α, 12α-Trihydroxy-5β-cholestane-24-carboxylic acid was synthesized.

2. It was verified synthetically that trihydroxybufosterocholenic acid isolated from toad bile is 3α, 7α, 12α-trihydroxy-5β-cholest-22-ene-24-carboxylic acid.

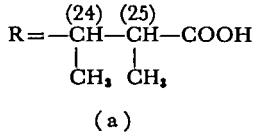
From the bile of the toad, *Bufo vulgaris formosus*, Shimizu and Oda (*1*) isolated an unsaturated bile acid to which they assigned the formula $C_{28}H_{46}O_5$ and the name "trihydroxybufosterocholenic acid".

Shimizu and Kazuno (*2*) clarified later the nuclear structure (the cholic acid nucleus) and the location of the double bond (C_{22}-C_{23}) of this acid, from the fact that the substance on ozonization gives bisnorcholic acid, $C_{23}H_{36}O_5$. The structure of the terminal part of the side chain had not been established conclusively but was proposed tentatively to be (a) or (b).

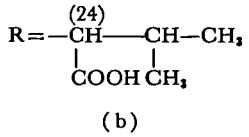
3α, 7α, 12α-Trihydroxy-24-methyl-5β-cholestanoic acid having the structure (a) form was prepared by Hoshita (*3*), but the properties of this acid were different from those of the saturated acid(trihydroxybufosterocholanic acid) obtained from trihydroxybufosterocholenic acid by catalytic hydrogenation.

Thus, it seemed likely that the side chain structure of trihydroxybufosterocholenic acid may have the structure (b) form, although the possibility that its saturated acid may be a stereoisomer at C-24 and/or C-25 of the synthetic trihydroxy-24-methyl-5β-cholestanoic acid still remained.

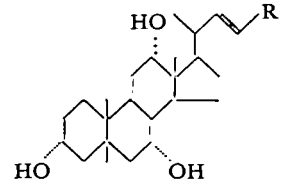
In the present paper, the synthesis of 3α, 7α, 12α-trihydroxy-5β-cholestane-24-carboxylic acid which has the postulated structure (b) is reported.



(a)



(b)



* XCIV. Hoshita, T., Amimoto, K., Nakagawa, T., and Kazuno, T., *J. Biochem.*, 61, 750 (1967)

EXPERIMENTAL

1. *3α, 7α, 12α-Trihydroxy-5β-cholest-24-ene-24-carboxylic Acid* (IV)—A solution of 10.5 g. of 3α, 7α, 12α-trihydroxy-5β-cholestan-24-one (I) (*4*) in 150 ml. of acetic anhydride which contained anhydrous sodium acetate was heated on a water bath for 20 hours. The reaction mixture was diluted with water and extracted with ether. The ether extract was washed with 4% aqueous solution of sodium bicarbonate and then with water, dried and concentrated.

To the acetylated material (11 g.) in a mixture of 450 ml. of ethanol, 100 ml. of water and 200 ml. of acetic acid, 90 g. of potassium cyanide were added with stirring. The mixed solution was stirred for 6 hours and after 3 days was poured into a large amount of ice water. The precipitate was collected, washed with water and then dissolved in ether. The ether solution was washed with water and concentrated. The residue (II, cyanohydrin) dissolved in 200 ml. of pyridine was cooled in an ice bath, and 75 ml. of freshly distilled phosphorus oxychloride was added with shaking. The reaction mixture was left at room temperature for 12 hours, and then poured into ice water. The precipitate was extracted with ether, and the ether extract was washed with dilute hydrochloric acid, sodium bicarbonate solution and water, successively, and then dried with sodium sulfate and evaporated to dryness.

The residue (III, the unsaturated cyano compound) was refluxed with 10% potassium hydroxide in triethylene glycol for 8 hours. The saponification mixture was diluted with water and repeatedly extracted with ether to remove neutral material. The alkaline layer was acidified with dilute hydrochloric acid and extracted with ethyl acetate. The extract was washed with water until free from hydrochloric acid, and evaporated to dryness. The residue showed, on thin layer chromatograms, a spot corresponding to trihydroxy-C_{25}-acid, and another spot with somewhat slower running rate. This residue (950 mg.) was put on a Hostalene column (90 g.) as described by Bergström and Sjövall (*5*) with the following solvent system: chloroform-heptane-methanol-water (45 : 5 : 165 : 135, v/v). The eluate with the moving phase (methanol-water) was automatically collected in fractions (20 ml. each) in test tubes, and part of each fraction was subjected to thin layer chromatography. Fractions eluted from 400 ml. to 560 ml. gave a single spot with the mobility corresponding to a trihydroxy-C_{25}-acid.

The material present in these fractions was recrystallized from acetone and then from methanol-water to give crystals melting at 204—205°C. The infrared and ultraviolet spectral data and chromatographic behaviors (paper, thin layer, gas and column chromatography) of this acid showed that it may be 3α, 7α, 12α-trihydroxy-5β-cholest-23-ene-24-carboxylic acid (IV). Analysis: Calcd. for $C_{25}H_{40}O_5$, C 72.69, H 10.02. Found C 72.27, H 9.87.

2. *3α, 7α, 12α-Trihydroxy-5β-cholestane-24-carboxylic Acid* (V)—The unsaturated acid (IV) dissolved in ethanol was hydrogenated with platinum oxide catalyst at room temperature. One molecule equivalent of hydrogen was absorbed within 10 minutes. After removal of the platinum catalyst from the reaction mixture, the solvent was distilled off. Recrystallization of the residue from methanol-ethyl-acetate mixture and then from acetone gave crystals, melting at 200°C, of 3α, 7α, 12α-trihydroxy-5β-cholestane-24-carboxylic acid (V). Analysis: Calcd. for $C_{25}H_{48}O_5$, C 72.37, H 10.41. Found C 72.22, H 10.24.

The melting point of the crystals was not depressed when admixed with trihydroxybufosterocholanic acid,

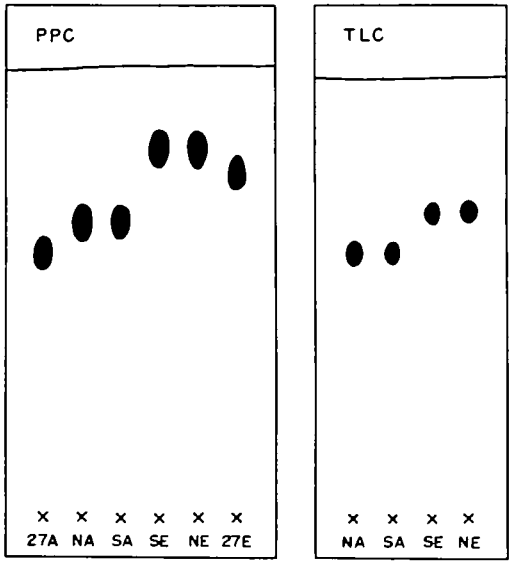


Fig. 1. Paper (PPC) and thin layer (TLC) chromatograms of synthetic and natural acids.
Solvent system of PPC: Isopropyl ether-*n*-heptane-acetic acid-water (60 : 40 : 70 : 30, v/v)
Solvent system of TCL: Benzene-isopropanol-acetic acid (30 : 10 : 1, v/v)
27A: 3α, 7α, 12α-Trihydroxy-5β-cholestanoic acid
27E: Methyl 3α, 7α, 12α-trihydroxy-5β-cholestanoate
NA: Trihydroxybufostercocholanic acid
NE: Methyl trihydroxybufosterocholanate
SA: 3α, 7α, 12α-Trihydroxy-5β-cholestane-24-carboxylic acid
SE: Methyl ester of SA

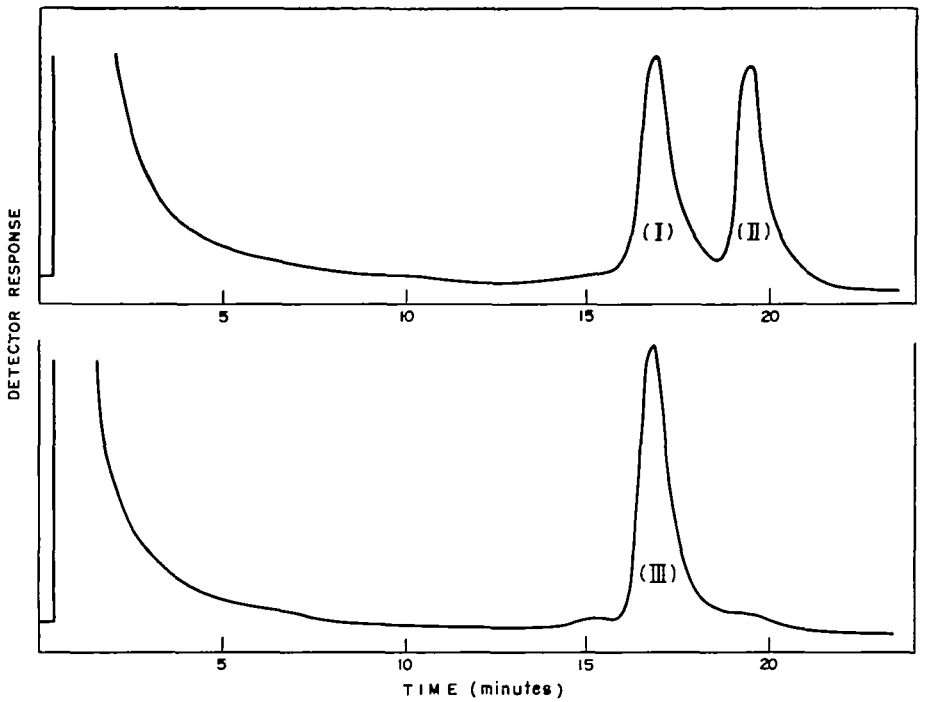
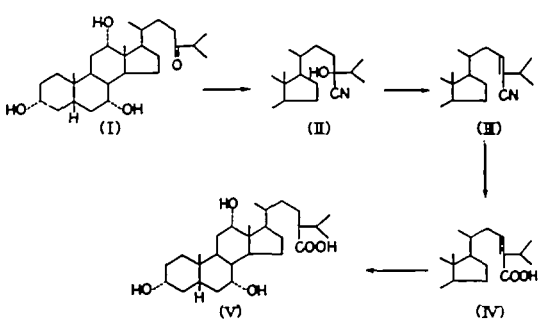


FIG. 2. Gas chromatograms of trimethylsilyl ether derivatives of methyl esters of 3α, 7α, 12α-trihydroxy-5β-cholestane-24-carboxylic acid (I), 3α, 7α, 12α-trihydroxy-24-methyl-5β-cholestanoic acid (II), and trihydroxybufosterocholanic acid (III).

1% SE-30, 100 cm. column. Column temperature, 245°C.



m.p. 200°C. Paper and thin layer chromatographic behaviors of the synthetic acid and its methyl ester were identical respectively with those of trihydroxybufosterocholanic acid and its methyl ester as shown in Fig. 1. On gas chromatography, the retention time of the trimethylsilyl ether derivative of the methyl ester of the synthetic acid was the same as that of the trimethylsilyl ether of methyl trihydroxybufosterocholanate, but differed from that of the corresponding derivative of methyl trihydroxy-24-methyl-5β-cholestanoate previously synthesized by Hoshita (*3*) (Fig. 2). Infrared and nuclear magnetic resonance spectra of the synthetic acid were found to be identical with those of trihydroxybufosterocholanic acid.

DISCUSSION

As shown in "EXPERIMENTAL," comparison of the properties of the synthetic acid, 3α, 7α, 12α-trihydroxy-5β-cholestane-24-carboxylic acid and trihydroxybufosterocholanic acid derived from the natural acid isolated from the toad bile makes it clear that both specimens are identical. The possibility that trihydroxybufosterocholanic acid may be one of a stereo-isomer of trihydroxy-24-methyl-5β-cholestanoic acid previously synthesized has been excluded by gas chromatographic data.

Thus, it is assumed that chemical structure of trihydroxybufosterocholenic acid is 3α, 7α, 12α-trihydroxy-5β-cholest-22-ene-24-carboxylic acid (form b). This structure may not be expected as a metabolite of cholesterol, for the extra carbon atom at C-24 would not

directly be derived from this C-27 sterol. Indeed, in our previous investigation (*6*), labelled trihydroxybufosterocholenic acid was not isolated from the toad that had been treated by injection with cholesterol-4-^{14}C.

It was demonstrated that guinea pig (*7*) and rat livers (*8*) are capable of modifying β-sitosterol and ergosterol respectively to give substances analogous to bile acids. These results suggest that, if sterols other than cholesterol did enter the liver they would be converted, like cholesterol, into bile acid analogues.

As a small amount of campesterol has been found in the toad liver (*9*), it is possible that this C-28 sterol is a precursor of the C-28 bile acid present in the toad bile.

The chemical evidence available at present suggests another possible mechanism for the formation of trihydroxybufosterocholenic acid. The methylation mechanism for C-28 sterol formation has not been established, but the possibility that a steroid with a hydroxymethyl group at C-24-position is an intermediate in the methylation process has been proposed (*10*). The fact that the carboxyl group of trihydroxybufosterocholenic acid is not at the end carbon atom of the side chain as in C-27-bile acids but as an extra carbon atom at C-24 suggests that this C-28 acid might be directly formed from such an intermediate without prior formation of a C-28 sterol.

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