IMPROVED SYNTHESIS OF 5β-CHOLESTAN-26-OIC ACIDS

A.K. Batta, G. Salen, G.S. Tint and S. Shefer

Department of Medicine, College of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ 07103; Veterans Administration Hospital East Orange, NJ 07019; Cabrini Medical Center, New York, NY 10003; The Public Health Research Institute of The City of New York, Inc., New York, NY 10016

Received 2-12-79

ABSTRACT

An improved method for the synthesis of 3α,7α-dihydroxy-5β-cholestan-26-oic acid and 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid is described. The method involves an Arndt-Eistert rearrangement of the corresponding diazoketone obtained by the action of diazoethane on 3α,7α-diformyloxy-5β-cholane-24-carboxylic or 3α,7α,12α-triformyloxy-5β-cholane-24-carboxylic acid chloride. The products are obtained in good yield and no isomeric 27-nor- 24-methyl acid contaminants are formed as encountered in the commonly employed Kolbe synthesis.

INTRODUCTION

3α,7α-Dihydroxy-5β-cholestan-26-oic acid (IIIa; Fig. 1) and 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (IIIb) have been postulated as intermediates in the biosynthetic pathway from cholesterol to chenodeoxycholic acid and cholic acid respectively (1,2). Both C_{27}-acids have been isolated from the bile of Alligator mississippiensis (3,4) and of man (5-7). Recently, an alternate mechanism for bile acid synthesis has been proposed involving intermediates hydroxylated at C-25 (8). In order to investigate the major biosynthetic pathway of bile acid formation, it is important to obtain the 26-carboxylated acids IIIa and IIIb in pure form.

Several methods are known for the synthesis of the 5β-cholestan-26-oic acids; the generally used procedure is the Kolbe synthesis (9)

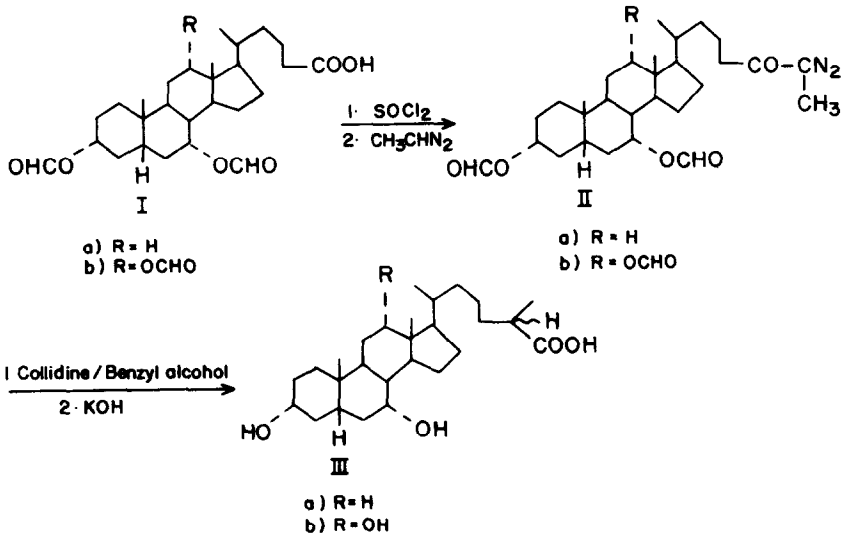

Fig. 1. Synthesis of 3α,7α-dihydroxy-5β-cholestan-26-oic acid and 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid.

which employs electrolytic coupling of a C_{24}-bile acid and the half ester of methylsuccinic acid. This method yields appreciable amounts (up to 40%) of isomeric 27-nor- 24-methyl-5β-cholestan-26-oic acids via cross coupling (9,10), which make it very difficult to obtain the desired 5β-cholestan-26-oic acids in pure form. In the present paper an improved method for the synthesis of 3α,7α-dihydroxy- and 3α,7α, 12α-trihydroxy-5β-cholestan-26-oic acids is described. This method employs an Arndt-Eistert rearrangement of the appropriate diazoketone (11a or 11b), obtained by the action of diazoethane on 3α,7α-diformyl-oxy-5β-cholane-24-carboxylic or 3α,7α,12α-triformyloxy-5β-cholane-24-

carboxylic acid chloride.

EXPERIMENTAL METHODS

Melting points were determined on a Thermolyne apparatus, model MP-12600, and are uncorrected.

Optical rotations were determined at 25°C in ethanol solution on a Carey model 60 spectropolarimeter.

Infrared spectra were recorded in chloroform solution on a Perkin-Elmer model 421 grating spectrophotometer.

GLC. The bile acids, as their methyl ester trimethylsilyl ether (TMSi) derivatives were analyzed on a 180 cm x 4 mm column packed with 3% QF-1 on 80/100 mesh Gas Chrom Q; column temp. 230°C (Hewlett-Packard model 7610 gas chromatograph).

Mass spectra were obtained with a Varian MAT-111 gas chromatograph-mass spectrometer (Varian Associates, Palo Alto, CA).

TLC was done on silica gel G plates (Brinkmann, 0.25 mm thickness). The spots were detected with phosphomolybdic acid (3.5% in isopropanol) and sulfuric acid (20%) with heating for 2 min at 110°C.

Preparation of diazoketone IIa (Fig. 1). 3α,7α-Diformyloxy-5β-cholane-24-carboxylic acid (Ia) [prepared from 0.9 gm of 3α,7α-dihydroxy-5β-cholane-24-carboxylic acid (II)] was dissolved in 3 ml of thionyl chloride and stirred under anhydrous conditions for 90 min at room temp. Excess thionyl chloride was removed by distillation with benzene under vacuum and the pale yellow semi-solid obtained was dissolved in 5 ml of benzene and poured over 50 ml of a cold ethereal solution of diazoethane [prepared from 5 gm of N-nitroso-N-ethylurea, synthesized as described for N-nitroso-N-methylurea (12)]. The reaction mixture was left overnight at 0°C and the solvent was evaporated under a current of dry N_2. The yellow oily product obtained showed a major spot on TLC, R_f 0.6 [solvent system, benzene-chloroform, 1:1 (v/v)] in addition to a minor spot, R_f 0.7. The faster moving compound had the same R_f value as ethyl 3α,7α-diformyloxy-5β-cholane-24-carboxylate but could not be removed by the conventional methods used for purification.

Preparation of 3α,7α-dihydroxy-5β-cholestan-26-oic acid (IIIa). The oily product (IIa) obtained above (1 gm) was dissolved in 3 ml of collidine and 3 ml of benzyl alcohol and refluxed for 25 min in an oil bath kept at 210-220°C. After cooling, 20 ml water was added and the reaction products were extracted three times with 50 ml ether each. The combined ether extracts were washed with 25 ml of 1 N HCl followed by a saturated solution of NaCl till neutral and then dried over anhydrous Na_2SO_4. After evaporation of the ether, the oily product was

refluxed with 25 ml of 10% alcoholic KOH for 3 hrs. The alcohol was removed with simultaneous addition of 25 ml of water and the neutral components were removed by extraction with ether. The aqueous solution was cooled and acidified to pH 1 with HCl. The pale yellow precipitate (0.8 gm) was filtered and washed with water till the washings were neutral. TLC in isooctane:ethyl acetate:acetic acid, 5:25:0.5 (v/v/v), showed the presence of two components, R_f 0.4 and 0.5. The slower moving compound had the same R_f value as 3α,7α-dihydroxy-5β-cholane-24-carboxylic acid, whereas the faster moving compound had the same R_f value as standard 3α,7α-dihydroxy-5β-cholestan-26-oic acid isolated from the bile of Alligator mississippiensis (4). The mixture was subjected to reversed-phase column chromatography [90 gm of Gas-Chrom Q; chloroform:heptane, 45:5 (v/v) as stationary phase and methanol:water, 165:135 (v/v) mobile phase]. The first 600 ml contained small amounts of polar impurities. The fraction 600 ml to 1050 ml yielded the compound corresponding to 3α,7α-dihydroxy-5β-cholane-24-carboxylic acid (0.19 gm) and the fraction 1550 ml to 2550 ml gave the compound with R_f value identical with that of 3α,7α-dihydroxy-5β-cholestan-26-oic acid (0.36 gm). It was crystallized from ethyl acetate, m.p. 155-158°C. Its methyl ester showed the same mass spectral fragmentation pattern (13) as the methyl ester of standard 3α,7α-dihydroxy-5β-cholestan-26-oic acid and the infrared spectrum of the free acid was superimposable over that of the standard.

Preparation of diazoketone IIb. This compound was prepared in the same manner as IIa, starting with 0.9 gm of 3α,7α,12α-trihydroxy-5β-cholane-24-carboxylic acid (14). The yellow oily product obtained failed to crystallize and showed one major spot, R_f 0.5, on TLC [solvent system, benzene:chloroform, 1:3 (v/v)] in addition to a minor spot, R_f 0.58, corresponding to ethyl 3α,7α,12α-triformyloxy-5β-cholane-24-carboxylate. The product could not be purified and was used as such for further reaction.

Preparation of 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (IIIb). 1 gm of (IIb) was reacted with 3 ml of collidine and 3 ml of benzyl alcohol in the same manner as described above for the preparation of IIIa. A pale yellow product 0.8 gm was obtained which showed two spots on TLC using heptane:ethyl acetate:acetic acid, 50:50:4 (v/v/v) as the solvent system (R_f 0.5 and 0.6). The mixture was subjected to column chromatography [80 gm of Celite 545; 70% aqueous acetic acid as the stationary phase and increasing proportions of benzene in petroleum ether (boiling range 67-69°C) as the mobile phase] as described by Carey and Haslewood (5). The column was eluted with 800 ml portions of petroleum ether containing 0, 20, 40, 60 and 80% benzene, respectively. The second half of the fraction eluted with 40% benzene and the first quarter of the fraction eluted with 60% benzene yielded 0.35 gm of a white amorphous powder, which was crystallized from ethyl acetate (m.p. 184-187°C). This material showed a single spot on TLC that agreed with that of standard 3α,7α,12α-trihydroxy-5β-cholestan-

26-oic acid, isolated from the bile of Alligator mississippiensis (4). The two acids had superimposable infrared spectra and their methyl esters showed identical mass spectral fragmentation patterns (13). The second half of the fraction eluted with 60% benzene and first quarter of the fraction eluted with 80% benzene yielded 0.2 gm of a white amorphous powder, identical with 3α,7α,12α-trihydroxy-5β-cholane-24-carboxylic acid.

DISCUSSION

This paper describes the synthesis of 3α,7α-dihydroxy-5β-cholestan-26-oic acid (IIIa, Fig. 1) and 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (IIIb) from 3α,7α-dihydroxy-5β-cholane-24-carboxylic acid and 3α,7α,12α-trihydroxy-5β-cholane-24-carboxylic acid, respectively. This method has the advantage over the electrolytic coupling procedure (9) that no isomeric 27-nor- 24-methyl-5β-cholestan-26-oic acids are produced. Consequently, pure 5β-cholestan-26-oic acids can be obtained without difficulty. The overall yield of the 3α,7α-dihydroxy- and 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acids obtained by this method was as high as 40%. Both acids contain an asymmetric center at C-25 and exist in the R and S configuration. These diastereoisomers can be separated by TLC (10,15) and then used for studies on the stereospecificity of the side-chain oxidation in bile acid biosynthesis.

ACKNOWLEDGMENTS

This work was supported by United States Public Health Service grants AM-18707, HL-17818 and AM-19696.

REFERENCES

1. Carey, Jr., J.B., J. Clin. Invest., 43, 1443 (1964).
2. Hanson, R.F., J. Clin. Invest., 50, 2051 (1971).
3. Haslewood, G.A.D., Biochem. J., 52, 583 (1952).
4. Dean, P.G.D., and Whitehouse, M.W., Biochem. J., 99, 9P (1966).
5. Carey, J.B. and Haslewood, G.A.D., J. Biol. Chem., 238, PC855 (1963).

6. Staple, E. and Rabinowitz, J.L., Biochim. Biophys. Acta, 59, 735 (1962).
7. Hanson, R.F. and Williams, G., Biochem. J., 121, 863 (1971).
8. Shefer, S., Cheng, F.W., Dayal, B., Hauser, S., Tint, G.S., Salen, G., and Mosbach, E.H., J. Clin. Invest. 57, 897 (1976).
9. Bridgwater, R.J., Biochem. J., 64, 593 (1956).
10. Kihira, K., Batta, A.K., Mosbach, E.H., Shefer, S., and Salen, G., J. Lipid Res., (in press, 1979).
11. Lettre, von H., Greiner, J., Rutz, K., Hofmann, L., Egle, A., and Bieger, W., Liebigs Ann. Chem., 758, 89 (1972).
12. Arndt, F., In: Organic Synthesis, collective volume II, Blatt, A.H., ed., John Wiley and Sons Inc., New York, N.Y., 1950, p. 461.
13. Dean, P.D.G. and Aplin, R.T., Steroids, 8, 565 (1966).
14. Pearlman, W.H., J. Amer. Chem. Soc., 69, 1475 (1947).
15. Batta, A.K., Salen, G., Blount, J.F., and Shefer, S., J. Lipid Res., (submitted, December 1978).