CROATICA CHEMICA ACTA CCACAA **64** (1) 59–63 (1991)

YU ISSN 0011-1643
CCA—1980 UDC 547
Original Scientific Paper

Synthesis of Methyl 3α,6β-Dihydroxy-5β-cholanate and the corresponding Ditosylate from Methyl 3α,6α-Dihydroxy-5β-cholanate

*Damir Medaković**, *Dušan Miljković and Jovan Hranisavljević*

Institute of Chemistry, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, YU-21000 Novi Sad, Yugoslavia

Received August 8, 1990

Methyl 3α,6α-dihydroxy-5β-cholanate (*1*) was regioselectively oxidized at position 6 with chromium(VI) oxide in acetic acid into methyl 3α-hydroxy-6-keto-5β-cholanate (*3*) and methyl 3,6-diketo-5β-cholanate (*4*) in 70 % and 30 % yields, respectively. Stereoselective reduction of monoketone *3* with sodium borohydride in benzene-ethanol-water gave epimeric methyl 3α,6β-dihydroxy-5β-cholanate (*5*) in 60 % yield. The same 3α,6β-diol (*5*) was obtained by reduction of the diketone (*4*). Tosylation of compound *5* with *p*-toluenesulfonyl chloride in dry pyridine afforded 88 % methyl 3α,6β-ditosyloxy-5β-cholanate (*7*).

INTRODUCTION

Methyl 3α,6α-dihydroxy-5β-cholanate (*1*) obtained from pig bile was regioselectively oxidized at position 6 with chromium(VI) oxide in acetic acid to give, after chromatographic separation, 70 % of methyl 3α-hydroxy-5β-cholanate (*3*) and 30 % of methyl 3,6-diketo-5β-cholanate (*4*). Stereoselective reduction of compound *3* afforded the epimeric 3α,6β-dihydroxy-5β-cholanate (*5*) in 60 % yield. Luckily, even the side product of the regioselective oxidation (*i.e.* the diketone *4*) may be reduced to the same 3α,6β-diol (*5*). By tosylation of compound *5*, methyl 3α,6β-ditosyloxy-5β-cholonate (*6*) was synthesized in 88 % yield. The synthesis of compound *6* is a part of our work on studying conformations of bile acids.

RESULTS AND DISCUSSION

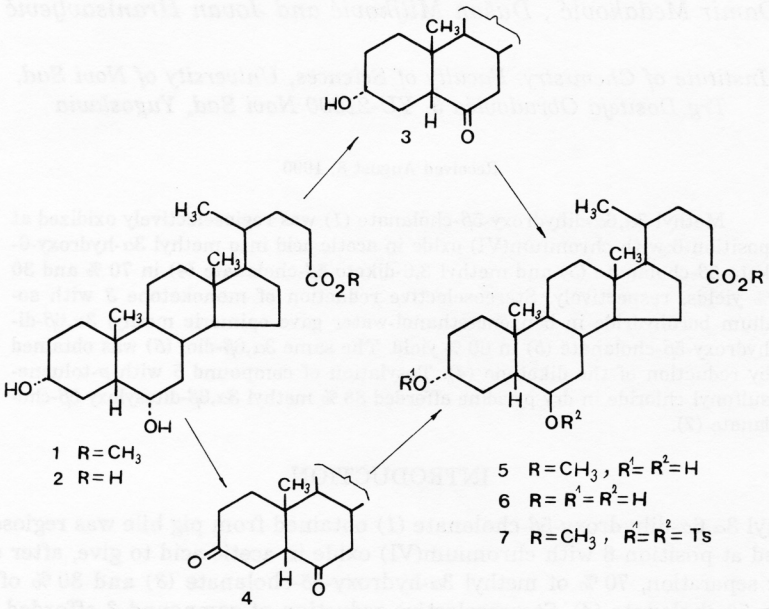
Bile acids are steroid compounds. They are constituents of bile and some of them can be isolated in commercially significant quantities.[1] In this respect, the most important are: ox bile with the main constituent 3α,7α,12α-trihydroxy-5β-cholanic acid, (cholic acid) and pig bile in which the main component is 3α,6α-dihydroxy-5β-cholanic acid (hyodesoxycholic acid).

The present work is a contribution to the chemistry of hyodesoxycholic acid, which is a useful starting compound for the synthesis of much more expensive steroids: progesterone, testosterone, cortisone *etc.*

Native bile acids are present in bile in the form of sodium salts of their conjugates with glycine and taurine. In the literature,[2] different procedures are described for isolation of bile acids from bile.

Methyl 3α,6α-dihydroxy-5β-cholanate (*1*) was prepared from stabilized bile (with formaldehyde) according to the published procedure,[3] with some modifications in a yield of 25 g per liter of pig bile, as a colourless crystalline aduct with benzene, from which it was recrystallized *m.p.* 86 °C (Lit.[4] 86 °C).

Saponification of analytical sample of compound *1* with sodium hydroxide in methanol afforded 3α,6α-dihydroxy-5β-cholanic acid (*2*) *m.p.* 202 °C (lit[3] *m.p.* 198 °C).



The first experiments, which were directed towards selective oxidation of 6α-hydroxy group in compound *1* with chromium(VI) oxide in acetic acid, failed. Namely, Wieland and Dane[5] described the procedure for the preparation of methyl 3α-hydroxy-6-keto-5β-cholanate (*3*) but, unfortunately, following their instructions did not give the expected result. A mixture of compound *1* and methyl 3,6-diketo-5β-cholanate (*4*) was obtained. Another procedure[6] for the preparation of compound *3* did not give satisfactory results either. This paper was criticized later,[7] because it was shown that oxidation of methyl 3α,6α-dihydroxy-5β-cholanate (*1*) with chromium(VI) oxide always affords a mixture of compounds *3* and *4*. For these reasons, we gave up further experiments directed towards preparation of pure compound *3*. Instead, we worked out a procedure for the preparation of *3* with the least admixture of *4*, whereby the products of oxidation were separated by column chromatography. Oxidation was performed with

chromium(VI) oxide in acetic acid at a temperature of 0–5 °C. After chromatographic separation, a pure compound *3*, *m.p.* 142–143 °C (Lit.[8] *m.p.* 141–142 °C) in 70 % yield, and 30 % of compound *4*, *m.p.* 139–140 °C (Lit[9] *m.p.* 139–140 °C) were isolated.

For the synthesis of diketone (*4*) we used the procedure described by Windaus[9], *i.e.* the oxidation of methyl 3α,6α-dihydroxy-5β-cholanate with chromium(VI) oxide in acetic acid at room tempt., whereupon diketone (*4*) was obtained in 90 % yield, *m.p.* 139–140 °C (Lit.[9] *m.p.* 139–140 °C).

Methyl 3,6-diketo-5β-cholanate (*4*) was reduced with sodium borohydride in benzene-ethanol-water at room temperature. The products were separated by column chromatography, whereby 3α,6β-dihydroxy-5β-cholanate (*5*) was obtained as the main product, in 60 % yield, *m.p.* 151–152 °C (Lit.[10] 114 °C), while the minor product was 3α,6α-dihydroxy-5β-cholanate (*1*) in 10 % yield.

Saponification of methyl ester *5* with sodium hydroxide in methanol afforded 3α,6β-dihydroxy-5β-cholanic acid (*6*), *m.p.* 212–213 °C (Lit.[10] 209–210 °C). Synthesis of compounds *5* and *6* was reported by Tukamoto[10], but in 15 % yield.

The large difference in melting points for compound *5* makes us suspect that Tukamoto did not get the same compound as we did, or that it was not pure enough, because he did not use chromatographic separation for the four obtained isomers by Meerwein-Pondorf-Verley reduction of methyl 3,6-diketo-5β-cholanate (*4*). In this way, by the described series of reactions, we succeeded, starting from methyl hydroxycholate (*1*), over diketone *4*, in getting epimeric compound *5*, *i.e.*, changing the configuration of hydroxyl group at position C-6.

Compounds *5* and *6* were also obtained by reduction of compound *3* under the same conditions as those described for reduction of *4*, followed by saponification of the obtained *5* to afford *6*. Also in this case, the ratio of the obtained 3α,6β-dihydroxy-5β-cholanate (*5*) and 3α,6α-dihydroxy-5β-cholanate (*1*) was the same as that obtained by reduction of compounds *4*, *i.e.*, 60 % and 10 %, respectively.

Tosylation of compound *5* with *p*-toluenesulfonyl chloride in dry pyridine at room temperature afforded 3α,6β-ditosyloxy-5β-cholanate (*7*), *m.p.* 103-104 ° in 88 % yield.

To our best knowledge, this compound has not been described in the literature. This compound contains bulky *p*-toluenesulfonyl group at position *6* which has axial orientation. Determination of its influence on the conformation of the steroid sceletion is a part of another work.

EXPERIMENTAL

Melting points are uncorrected. The ^{1}H-NMR spectra were recording on a Varian 60A spectrometer using $SiMe_4$ as internal standard, in $CDCl_3$ solutions. Chemicals shifts are given in ppm as δ values. Mass spectral data were obtained on a Varian CH-5 (70 eV) instrument. IR spectra were recorded on a Perkin-Elmer 457 spectrophotometer. Silica gel 0.063–0.200 mm was used for chromatographic separations. TLC were performed on silica gel G (Stahl) and detection was effected by charring with 50 % aq. H_2SO_4.

Synthesis of Methyl 3α-Hydroxy-6-keto-5β-cholanate (3)

Methyl hyodesoxycholate[3] (*1*) (3.00 g, 7.4 mmol) was dissolved in acetic acid (30 mL) and water (4.5 mL). To this solution, a solution of chromium(VI) oxide (0.70 g, 7 mmol) in 50 % acetic acid (9 mL) was added dropwise within 1.5 hrs at a temperature 0–5 °C, and the reaction mixture was kept at room temperature for another 6 hours. The residue (2.60 g) obtained after the usual work-up was chromatographed on silica gel (100 g). Benzene-acetone (90:10 and 80:20) elu-

ted methyl 3,6-diketo-5β-cholanate (*4*) (0,77 g, 29.6 %), *m.p.* 139–140 °C (from 96 % ethanol) (lit[9] *m.p.* 139–140 °C), followed by methyl 3α-hydroxy-5β-cholanate (*3*) (1.82 g, 70 %) *m.p.* 142–143 °C (from benzene-hexane) (lit[8] *m.p.* 141-142 °C); IR (KBr): 3500, 1725, 1690; ^{1}H-NMR: 0.65 (s, CH_3-18), 0.90 (s, CH_3-19), 3.70 (s, CH_3OCO). MS: m/z = 404 (M^+).

Anal. Calc. for $C_{25}H_{40}O_4$ (M.w. = 404.59): C 74.21, H 9.97 %; found C 74.25, H 9.72 %.

Synthesis of Methyl 3,6-Diketo-5β-cholanate (4)

Methyl hyodesoxycholate[3] (20.00 g, 50 mmol) was dissolved in acetic acid (200 mL) at room temperature and a solution of chromium(VI) oxide (20 g, 20 mmol) in water (20 mL) was added by stirring. The reaction mixture was kept at room temperature for 20 minutes. The product was extracted with benzene (3 × 200 mL). The benzene extracts were washed with 1.0 % sulfuric acid, dried (Na_2SO_4) and evaporated to dryness, affording an oily residue which crystallized after addition of 96 % ethanol. Recrystallization from 96 % ethanol gave methyl 3,6-diketo-5β-cholanate (*4*) (18 g, 90 %), *m.p.* 139–140 °C (Lit.[9] *m.p.* 139–140 °C); IR (KBr): 1740, 1720, 1690; ^{1}H-NMR: 0.65 (s, CH_3-18), 0.90 (s, CH_3-19), 3.65 (s, CH_3OCO). MS: m/z = 402 (M^+).

Anal. Calc. for $C_{25}H_{38}O_4$ (M.w. = 402.57): C 74.59, H 9.52 %; found: C 74.72, H 9.39 %.

Synthesis of Methyl 3α,6β-Dihydroxy-5β-cholanate (5)

Methyl 3,6-diketo-5β-cholanate (*4*) (3.15 g, 7.8 mmol) was dissolved in benzene (100 mL) and sodium borohydride (3.15 g, 85 mmol) dissolved in ethanol (45 mL) and water (12 mL) was added by stirring. The mixture was stirred for 1/2 h at room temperature, diluted with water, acidified with hydrochloric acid up to pH = 1 and extracted with ether. The ether extract was dried (Na_2SO_4) and evaporated *in vacuo*, affording the oily residue (3.10 g) which was chromatographed on silica gel (100 g). Benzene-acetone (90:10 and 80:20) eluted methyl 3α,6β-dihydroxy-5β-cholanate (*5*) (1.86 g, 60 %), *m.p.* 151–152 °C (benzene); IR (KBr): 3500, 1720; ^{1}H-NMR: 0.65 (s, CH_3-18), 1.15 (s, CH_3-19), 3.65 (s, CH_3OCO). MS: m/z = 406 (M^+).

Anal. Calc. for $C_{25}H_{42}O_4$ (M.w. = 406.60: C 73.89, H 10.34 %; found: C 74.13, H 10.12 %.

An analytical sample of methyl 3α,6β-dihydroxy-5β-cholanate (*5*) was saponified in the usual way (NaOH/MeOH) affording pure 3α,6β-dihydroxy-5β-cholanic acid (*6*), *m.p.* 212–213 °C (Lit.[10] *m.p.* 209-210 °C).

An analytical sample of methyl 3α-hydroxy-6-keto-5β-cholanate (*3*) was reduced under the same conditions as those described for the preparation of (*5*), affording the same ratio of compound (*5*) and (*1*).

Synthesis of Methyl 3α,6β-Ditosyloxy-5β-cholanate (7)

Methyl 3α,6β-dihydroxy-5β-cholanate (*5*) (590 mg, 1.4 mmol) was dissolved in dry pyridine (10 mL) and *p*-toluenesulfonyl chloride (1.1 g, 5.7 mmol) was added by stirring. The mixture was kept for two days at room temperature, followed by addition of another portion of *p*-toluenesulfonyl chloride (500 mg, 2.6 mmol) and by prolongation of the reaction time for one more day. After the usual work-up and evaporation of the solvent, the product was recrystallized from ether-hexane, affording methyl 3α,6β-ditosyloxy-5β-cholanate (*7*) (870 g, 88 %), *m.p.* 103–104 °C; IR (KBr): 3015, 1730, 1600, 1450, 1360, 1176; ^{1}H-NMR: 0.65 (s, CH_3-18), 1.023 (s, CH_3-19), 2.50 (s, 6H, 2 x CH_3-tosyl), 3.70 (s, CH_3OCO), 7.60 (m, 8H, 2 x tosyl).

Anal. Calc. for $C_{39}H_{54}O_8S_2$ (M.w. = 714.98): C 65.54, H 7.56, S 8.96 %; found: C 65.59, H 7.66, S 8.72 %.

REFERENCES

1. P. P. Nair and D. Kritchevsky, *The Bile Acids,* New York, Plenum Press, *1971.*
2. G. A. D. Haslewood, *Biochem. J.* **62** (1956) 637.
3. A. Windaus, *Z. Angew. Chem.* **36** (1923) 309.
4. R. E. Marker and J. Krueger, *J. Amer. Chem. Soc.* **62** (1940) 79.
5. H. Wieland and E. Dane, *Physiol. Chem.* **41** (1932) 212.

6. W. H. Hoehn, J. Linsk, and R. B. Moffett, *J. Amer. Chem. Soc.* **68** (1946) 1855.
7. F. Hodson, A.P. Gocan, and N. Serbon, *Rev. Roumanie Chim.* **10** (1965) 97.
8. R. Justoni and R. Pessina, *Il Farmaco, Ed. Sci.* **11** (1955) 73.
9. A. Windaus and A. Bohne, *Ann.* **433** (1923) 278.
10. M. Tukamoto, *J. Biochem.* (Japan) **32** (1940) 451.

SAŽETAK

Sinteza metil 3α,6β-dihidroksi-5β-holonata i odgovarajućeg ditosilata iz metil 3α,6α-dihidroksi-5β-holanata

Damir Medaković, Dušan Miljković i Jovan Hranisavljević

Metil 3α,6α-dihidroksi-5β-holanat (*1*) je regioselektivno oksidiran na poziciji 6 krom(VI)-oksidom u octenoj kiselini u metil-3α-hidroksi-6-keto-5β-holanat (*3*) sa iskorišćenjem od 70 % i metil 3,6-diketo-5β-holanat (*4*) sa iskorišćenjem od 30 %. Stereoselektivna redukcija monoketona (*3*) natrij borohidridom u otapalu benzen-etanol-voda priređen je epimerni metil 3α,6β-dihidroksi-5β-holanat (*5*) sa iskorišćenjem od 60 %. Identični 3α,6β-diol (*5*) priređen je redukcijom diketona (*4*). Tosiliranjem spoja *5* *p*-toluensulfonil kloridom u suhom piridinu priređen je metil-3α,6β-ditoziloks-5β-holanat (*7*) sa iskorišćenjem od 80 %.