# THE SYNTHESIS OF METHYL 3α,7α-DIACETOXY-11-OXO-5β-CHOLAN-24-OATE

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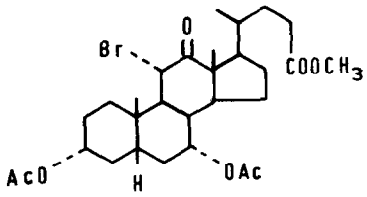
## ABSTRACT

Reaction of methyl-3α-7α-diacetoxy-11α-bromo-12-oxo-5β-cholan-24-oate with sodium borohydride in pyridine solution containing sodium acetate gave the corresponding 11β, 12β-epoxide in 65% yield. The epoxy-ring was opened with hydrobromic or hydroiodic acid to give the corresponding 12α-halo-11β-alcohols, which were converted to the halo-ketones and finally to methyl 3α,7α-diacetoxy-11-oxo-5β-cholan-24-oate.

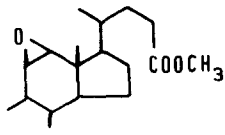
## RESULTS AND DISCUSSION

The procedure for the preparation of 7-deoxy-$C_{11}$-oxygenated-steroids from 3-acetoxy-12-oxosteroids as introduced by Conforth et al. (1) included the following steps: 11α-bromo-12-ketone → 11α-bromo-12β-alcohol → 11β,12β-epoxide → 12α-bromo-11β-alcohol → 11-oxo-12α-bromide → 11-oxosteroid.

In the present study this procedure was chosen for the preparation of the 3α,7α-diacetoxy-11-oxo-steroid VIII, using the available (2) methyl 3α,7α-diacetoxy-11α-bromo-12-oxo-5β-cholan-24-oate (I) as a starting material. However, borohydride reduction of I in ethanolic (1) or methanolic (3) solutions under the conditions used for 7-deoxy-steroids (1) yielded complex mixtures including unbrominated compounds




I

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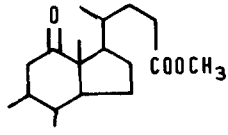


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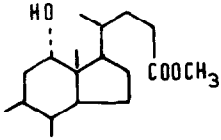
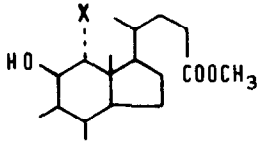
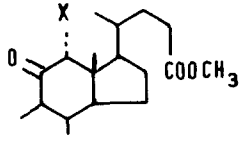



IV




V, x = Br
VI, x = I




VII, x = Br
VIII, x = H

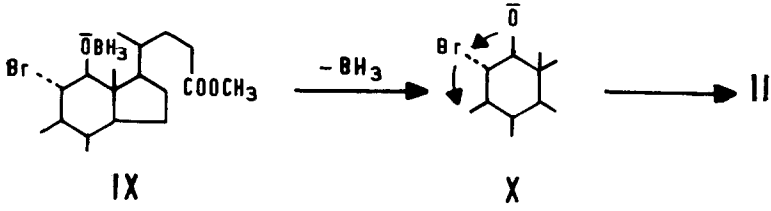
instead of the desired bromohydrin methyl 3$\alpha$, 7$\alpha$-diacetoxy-11$\alpha$-bromo-12$\beta$-hydroxy-5$\beta$-cholan-24-oate. Similar mixtures were also obtained when the reductions were carried out in iso-propanol or tert-butanol solutions with or without the addition of bicarbonate buffer.

When pyridine was used as a solvent for borohydride reduction for 2 hr. the bromoketone I was not reduced. However when the reaction time was prolonged and sodium acetate added, the epoxide II was directly formed (>60% yield). Lower yields were also obtained when the sodium acetate was omitted or when the temperature exceeded 25°C. Under these conditions the epoxide II obtained was accompanied with at least four by-products of which the 12-ketone III and the 12$\alpha$-hydroxy-compound IV were identified. Compound III may originate from the reduction of the 11$\alpha$-bromo moiety of I prior to reduction of the oxo-group

(3), giving a 12-keto-steroid which has been shown to resist further reduction in pyridine solutions (4). The 12$\alpha$-by-product may be produced by the borohydride reduction of the 11$\alpha$-bromine of the cis-bromohydrin methyl 3$\alpha$,7$\alpha$-diacetoxy-11$\alpha$-bromo-12$\alpha$-hydroxy-5$\beta$-cholan-24-oate. This bromohydrin was not isolated in the present study but cis-bromohydrins of the 7-deoxy-steroids have been previously shown to be formed as by-products in borohydride reductions (1, 3), being converted to 12-keto-steroids under alkaline conditions during the epoxy-ring closure of the corresponding trans-bromohydrins (1, 3).

The failure to obtain the 12$\beta$-hydroxy-analog of I under conditions suitable for the production of the trans-bromohydrins of 7-deoxy-steroids indicates that the bromo moiety of the 7-OAc-steroid is more reactive towards borohydride reduction.

In pyridine solutions the lability of the bromo-moiety is less pronounced compared to solutions of alcohols, which accelerate the removal of the $Br^-$ leaving-group in SN-reactions (5), and do not favor the retention of bromine-atom during the 12-ketone reduction of 7-OAc-steroids. Although the final product of the reaction in pyridine, the epoxide II is also an unbrominated compound, the presumed direct product of the borohydride reduction IX (6) can be formed only if the 11-bromo-substituent is retained in the initial step. The epoxy-ring closure is probably affected by the ability of the pyridine molecule to




IX X

form 'Lewis-salts' with $BH_3$-group (7), withdrawing it from the negatively charged complex IX; the resulting product is the alkoxide X, which is the intermediate in the intramolecular epoxy-ring closure (8). The desired effect of the acetate ion in the reaction may be attributed to the 'buffer effect' previously proposed (1), namely: inhibition of the C—Br bond cleavage of the bromoketone.

In the mass spectrum of the epoxide II the ion fragments 415 and 355 were represented, corresponding to 89 and 89 + 60 (acetic acid) loss, respectively. This fragmentation was not observed in the 11$\alpha$, 12$\alpha$-epoxy (2), 11-oxo (VIII) or 12-oxo (III) analogs having the same molecular weight 504 (table 1), neither was it observed in compounds IV and VII nor in all the 7-OAc steroids studied previously (2). Low relative intensities of m/e 415 and 355 did appear in the spectra of halohydrins V and VI, however, the spectra of the two compounds showed no $M^+$ values and the highest mass number observed was 504. This may have been attributed to the rapid loss of a hydrohalide molecule from the halohydrins under the conditions of the mass spectrometry, and the formation of the epoxide II. The structure of the ion

TABLE 1

Comparison of the Mass Spectra of Four Analogs of Methyl 3α,7α-Diacetoxy-5β-cholan-24-oate having the Molecular Weight 504

| Ion Fragment, m/e | Relative Intensities (% of base peak) | | | |
|---|---|---|---|---|
| | β-Epoxide II | α-Epoxide (2)* | Compound III | Compound VIII |
| $M^+$ (504) | 0.3 | 78 | 27 | 7 |
| M - 60 | 8.5 | 36 | 100 | 25 |
| 429 | — | — | — | 15 |
| M - (60 + 18) | 4.5 | — | — | — |
| M - 89 (415) | 22 | — | — | — |
| M - 120 | 25 | 100 | 33 | 100 |
| M - (60 + 89)(355) | 44.5 | — | — | — |
| 351 | — | 53 | — | 37 |
| 291 | — | — | — | 79 |
| 269 | 100 | 27 | 29 | — |

* Methyl 3α,7α-diacetoxy-11α,12α-epoxy-5β-cholan-24-oate.

fragments 415 and 355 is subject to further investigation.

The epoxide II was converted to VIII in the usual way (1, 3).

## EXPERIMENTAL

Ultraviolet spectra were determined with a Unicam ultraviolet spectrometer (Model Sp 800A) in ethanol. Infrared spectra were measured in potassium bromide disks using a Perkin-Elmer spectrophotometer (Model 337). Nmr spectra were recorded on a Jeol C-60-H

high resolution nmr spectrometer in $CDCl_3$ solutions with tetramethylsilane as an internal standard. Mass spectra were recorded on a CH5 Varian MAT mass spectrometer. Cd spectra were obtained in ethanol solutions using a Cary 60 recording spectropolarimeter. Optical rotations were determined with a Perkin-Elmer 141 polarimeter in chloroform solutions. TLC was performed on silica gel G in the system chloroform : ethyl acetate 95 : 5, the spots were located with concentrated sulfuric acid at 100°C for 15 minutes. Silicic acid for column chromatography was pretreated as previously described (9).

Methyl 3α,7α-diacetoxy-11β,12β-epoxy-5β-cholan-24-oate (II). A mixture of 2 g of I (2), sodium acetate (2 g) and sodium borohydride (0.6 g) in dry pyridine (25 ml) was stirred under protection of $CaCl_2$ for four days. Appropriate precautions were taken that the temperature would not exceed 25°C. The reaction mixture was transferred into a separatory funnel with ether (200 ml) and a mixture of concentrated sulfuric acid (12 ml) in ice (approx. 150 g) was added in portions during a period of 60 minutes, adjusting the pH of the water phase to 3.0. The ether phase was washed twice with water (the pH of the second wash reached 5.0) and the ether was dried and evaporated. The residue (1.98 g) was treated with diazomethane in ether. The solvent was removed and the residue was acetylated with pyridine-acetic anhydride (overnight, room temperature). Reextraction with ether and washing (diluted $H_2SO_4$, $H_2O$, concentrated aqueous bicarbonate, $H_2O$) gave 1.95 g of material which proved by TLC to be a mixture of a major product and at least 4 by-products. The mixture was chromatographed on 24 g of silicic acid with 400 ml fractions of increasing concentrations of chloroform in hexane (Table 2).

The residue of fractions II - IV (1.09 g, 65% yield) was crystallized from ether : hexane for 3 days at 4°C giving 550 mg of II, m. p. 166°C. The mother liquor yielded another 283 mg of crystals after one week at 4°C. $[\alpha]_D$ +18.8; ir 2925, 1730, 1253, 885 (epoxy) 860 $cm^{-1}$; nmr δ 0.75 (s, 3, 18-$CH_3$), 1.09 (s, 3, 19-$CH_3$), 2.03 (s, 6, 3 and 7-$OCOCH_3$), 3.08 (m, 2, 11-CH and 12-CH), 3.63 (s, 3, 24-$OCH_3$), 4.62 (m, 1, 3-CH), 4.90 (m, 1, 7-CH) ppm; mass spectrum: Table 1.

Anal. Calcd. for $C_{29}H_{44}O_7$ : C, 69.05; H, 8.73;
Found: C, 69.14; H, 8.65.

The residue from fractions V and VI (350 mg) was crystallized from methanol to give 71 mg of crystals. Recrystallization yielded 31 mg of material being uniform in TLC ($R_f$ 0.35; color orange-brown). The compound was identical to III in all respects, m.p. 179-180°; reported m.p. 177-179° (10). Nmr 1.04 (s, 6, 18 and 19-$CH_3$), 2.03 (s, 6, 3 and 7-$OCOCH_3$), 3.65 (s, 3, 24-$OCH_3$), 4.59 (m, 1, 3-CH), 5.03 (m, 1, 7-CH) ppm; the mass spectrum is given in Table 1.

TABLE 2

Chromatography of Sodium Borohydride-Pyridine Reduction Products of Methyl 3α,7α-diacetoxy-11α-bromo-12-oxo-5β-cholan-24-oate (I)

| Fraction | Chloroform in hexane (%) | Residue (mg) | $R_f$ in TLC | Spot colors |
|---|---|---|---|---|
| I | 0 | — | — | — |
| II | 20 | 240 | 0.4 | Yellow |
| III | 20 | 500 | 0.4 | Yellow |
| IV | 22.5 | 350 | 0.4 | Yellow |
| V | 22.5 | 130 | 0.3 and 0.35 | Yellow and orange-brown, respectively |
| VI | 25 | 220 | 0.4 and 0.35 | Yellow and orange-brown, respectively |
| VII | 100 | 480 | 0.1, .09 and .08 | Yellow-orange, yellow, and yellow, respectively |

The mother liquor consisted of II and III and another unidentified compound as judged by nmr.

Crystallization of fraction VII from methanol at 4°C for one month yielded 73 mg of crystals being uniform in TLC ($R_f$ 0.1, color yellow-orange). The compound was identical with IV in all respects; m.p. 186-187°C; reported m.p. 185-186°C (10); nmr δ 0.69 (s, 3, 18-$CH_3$), 0.96 (s, 3, 19-$CH_3$), 2.02 (s, 3, 3-$OCOCH_3$), 2.05 (s, 3, 7-$OCOCH_3$), 3.23 (s, 1, 12-OH), 3.63 (s, 3, 24-$OCH_3$); 4.0 (m, 1, 12-CH), 4.53 (m, 1, 3-CH), 4.88 (m, 1, 7-CH) ppm; mass spectrum m/e: 428 (M − $CH_3COOH - H_2O$), 368, 313, 253 (base peak).

The epoxide II was prepared in pyridine solutions (5 ml) using batches of 0.5 g bromoketone I and 0.15 g borohydride under various conditions, purifying the product with Girard reagent P(3) and chromatography on alumina (10 g) with 4% chloroform in hexane (150 ml).

| Temperature (°C) | Reaction time (days) | Sodium acetate (grams) | Mean yield (%) calculated from three reactions |
|---|---|---|---|
| <25 | 4 | 0.5 | 65 |
| 4 | 7 | 0.5 | 60 |
| 42 - 45 | 4 | 0.5 | 42 |
| <25 | 4 | — | 53 |

Methyl 3α,7α-diacetoxy-12α-bromo-11β-hydroxy-5β-cholan-24-oate (V). 48% HBr (4 ml) was added to a solution of β-epoxide II (2 g) in acetic acid (50 ml). After 25 minutes at room temperature the reaction mixture was poured into water (200 ml) containing crushed ice and $NaHSO_3$ (0.5 g). The solid (2.1 g) was filtered and crystallized from ether : hexane to give 1.12 g of V, m.p. 179-180°C; $[\alpha]_D$ +34.8°; ir 1735, 1250, 728, 715 (C - Br) $cm^{-1}$; nmr δ 1.06 (s, 3, 18-$CH_3$), 1.15 (s, 3, 19-$CH_3$), 2.85 (s, 3, 3-$OCOCH_3$), 2.88 (s, 3, 7-$OCOCH_3$), 3.65 (s, 3, 24-$OCH_3$), 4.47 (2, m. 12-CHBr and 7-CH), 5.0 (1, m. 3-CH) ppm; mass spectrum m/e (%): 586, 584 ($M^+$, 0), 504(4.7), 486(6.3), 444(18.8), 426(20.3), 415 (9.4), 385(12.5), 384(14.1), 371(100), 367(50), 311(82), 269(72), 251(88).

Anal. Calcd. for $C_{29}H_{45}O_7Br$: C, 59.49; H, 7.69; Br, 13.7
Found: C, 59.34; H, 7.63; Br, 14.1

Methyl 3α,7α-diacetoxy-12α-iodo-11β-hydroxy-5β-cholan-24-oate (VI). A solution of 95% hydroiodic acid (0.5 ml) was added to β-epoxide II (300 mg) in acetic acid (25 ml) as described for V. The product (340 mg) was crystallized from ether : hexane to give 200 mg of VI, m.p. 140-142°C; $[\alpha]_D$ +8.3°; ir 1728, 1250, 1018 and 1070 $cm^{-1}$; nmr δ 0.72 (s, 3, 18-$CH_3$), 1.09(s, 3, 19-$CH_3$), 2.06(s, 3, 3-$OCOCH_3$), 2.09(s, 3, 7-$OCOCH_3$), 2.8(m, 1, 11-OH), 3.67(s, 3, 24-$OCH_3$), 4.06(m, 1, 11-CH), 4.50(m, 1, 12-CH), 4.68(m, 1, 3-CH), 4.86(m, 1, 7-CH) ppm; mass spectrum m/e (%): 632($M^+$, 0), 504(5.3), 444(51), 415(5.3), 385(40), 384(38), 368(85), 367(100), 355(16), 353(90).

Anal. Calcd. for $C_{29}H_{45}O_7I$: C, 55.06; H, 7.12; I, 20.1
Found: C, 55.24; H, 7.15; I, 19.6.

Methyl 3α,7α-diacetoxy-12α-bromo-11-oxo-5β-cholan-24-oate (VII). $CrO_3$ (0.5 g) in water (1 ml) and acetic acid (5 ml) was added to a solution of bromohydrin V (0.5 g) in acetic acid (5 ml). After 2.5 hr. at room temperature the product was extracted with ether and washed with $H_2O$ (3 times), sat. aq. $NaHCO_3$ and $H_2O$. The solvent was removed

and the residue (490 mg) was crystallized from methanol at room temperature for 4 days with eventual scraping with a glass rod, giving 272 mg of cubes; after 2 days another 136 mg of VII was obtained from the mother liquor. M.p. 132°C; $[\alpha]_D$ -7.9°. uv 310 nm ($\epsilon$ 210); ir 1735, 1245, 735 and 730 (C—Br) $cm^{-1}$; nmr $\delta$ 0.80 (s, 3, 18-$CH_3$), 1.13 (s, 3, 19-$CH_3$), 2.04 (s, 3, 3-$OCOCH_3$), 2.13 (s, 3, 7-$OCOCH_3$), 3.65 (s, 3, 24-$OCH_3$), 4.24 (s, 1, 12-CH), 4.56 (m, 1, 3-CH), 4.90 (m, 1, 7-CH) ppm; cd $[\theta]_{255}$ 0°, $[\theta]^{max}_{314}$ - 18.990°, $[\theta]_{360}$ 0°; mass spectrum m/e (%): 584, 582 ($M^+$, 1, 1), 524, 522 (10, 11), 509, 507 (24, 25), 478(15), 464, 462(28, 29), 443(50), 383(100), 367(33), 351(40).

Anal. Calcd. for $C_{29}H_{43}O_7Br$: C, 59.69; H, 7.38; Br, 13.7
Found: C, 59.94; H, 7.53; Br, 14.0

Methyl 3$\alpha$,7$\alpha$-diacetoxy-11-oxo-5$\beta$-cholan-24-oate (VIII). Bromoketone VII (540 mg), sodium acetate (2 g) and Zn dust (2 g) were refluxed in acetic acid (120 ml) for 2 hr. The solution was filtered and water (500 ml) was added. The solid (430 mg) was filtered and crystallized from isopropanol to give 340 mg VIII, m.p. 204°C; $[\alpha]_D$ +30.8; uv 290 nm (flat peak) ($\epsilon$ 68); ir 1732, 1703, 1255 $cm^{-1}$; nmr $\delta$ 0.62 (s, 3, 18-$CH_3$), 1.15 (s, 3, 19-$CH_3$), 2.03 (s, 3, 3-$OCOCH_3$), 2.12 (s, 3, 7-$OCOCH_3$), 3.65 (s, 3, 24-$OCH_3$), 4.53 (m, 1, 3-CH), 5.0 (m, 1, 7-CH) ppm; cd $[\theta]_{250}$ +1150°; $[\theta]^{max}_{292}$ +2160°, $[\theta]_{313}$ 0°, $[\theta]^{max}_{329}$ -2160°; $[\theta]_{357}$ 0°; mass spectrum: Table 1.

Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.05; H, 8.73
Found: C, 68.79; H, 8.80.

10 mg of VIII was dissolved in ethanol (2 ml) and 4 M KOH (1 ml). The mixture was kept on a water bath for 6 hr. and additional 2 days at room temperature. The product was extracted with chloroform and the solvent was removed. The residue was successively treated with etheral solution of diazomethane and N,O-trimethylsilyl-trifluoroacetamide containing 1% trimethylchlorosilane (0.2 ml) for 12 hr. at room temperature. An aliquot was analyzed in the mass spectrometer (direct inlet), giving the following spectrum: m/e (%) 564 (M-diTMSi) (14%), 474(50), 459(70), 402(47), 387(50), 384(61), 348(60), 229(100).

The 11-ketone VIII was prepared directly from the epoxide II via the iodohydrin route as follows: a solution of 95% HI (5 ml) was added to $\beta$-epoxide II (2 g) in acetic acid (50 ml). The mixture was poured into water-ice containing $NaHSO_3$ as described for V and the solid (2.2 g) was dissolved in acetic acid (50 ml). $CrO_3$ (1.5 g) in water (10 ml) and acetic acid (50 ml) was added and the mixture was again poured into water - ice - $NaHSO_3$, after 30 minutes at room temperature. The solid (2.1 g) was refluxed for 15 minutes in acetic acid containing sodium acetate and Zn dust as outlined above. The product (1.6 g) was identical to VIII in all respects.

An Unsuccessful Attempt to Reduce the Bromoketone I for a Short Period.

100 mg of the bromoketone I was dissolved in pyridine (2 ml) containing sodium borohydride (30 mg) and sodium acetate (100 mg). The mixture was stirred for 120 minutes and was treated as described above. The product (100 mg) was crystallized from isopropanol to give 72 mg of crystals, being identical to I in all respects (2).

Attempts to Reduce the Bromoketone I with Sodium Borohydride in Alcohols

100 mg of bromoketone I was subjected to reduction with sodium borohydride in ethanol-bicarbonate as described by Conforth et al. (1). The resulting oil (85 mg) was analyzed on TLC showing at least 6 spots ($R_f$ values ranged from .05 - 0.4). The main peaks in nmr and mass spectra consisted with those obtained from the borohydride reduction of the 12-ketone III in ethanol, namely a mixture of IV and its 12β-epimer; δ 0.69 and 0.72 ppm corresponding to the 18-$CH_3$ of the former and the latter respectively; 0.96 ppm, 19-$CH_3$ of the two C-12-alcohols; 0.53, 0.62, 1.02, 1.06 ppm, low intensities, not identified. Mass spectrum m/e: 428 ($M-CH_3COOH-H_2O$), 368, 313, 253 (base peak).

Reduction of I with solid borohydride in absolute ethanol (60 min., room temp.) did not change significantly the above pattern.

Similar results were also obtained when 100 mg batches of I were reduced in methanol, isopropanol or tert.-butanol with or without aq. bicarbonate buffer.

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