REDUCTION OF 12-OXO DERIVATIVES OF SOME BILE ACIDS

Růžena Míčková

Research Institute for Pharmacy and Biochemistry, 194 04 Prague 9

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Methyl ester of 3α-acetoxy-12-oxo-9 (11)-cholenic acid and some other analogous compounds were reduced to the corresponding 9(11)-unsaturated 12-hydroxy derivatives by the action of sodium borohydride in the presence of cerium(III) chloride.

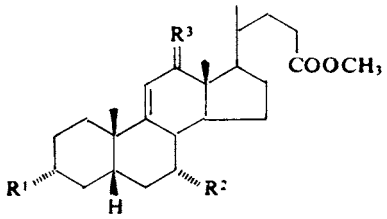
A part of our work, *viz.* preparation of steroid hormones from bile acids, was the reduction of 9(11)-unsaturated 12-oxo derivatives of some bile acids, with the view of obtaining the corresponding 9(11)-unsaturated 12-hydroxy derivatives. Hitherto, the only safe route has been hydrogenation of the starting 9(11)-unsaturated 12-oxo derivatives on platinum at elevated pressures, which, preserving the allyl double bond, yields a mixture of the two isomeric 12-hydroxy derivatives.

In attempts at the reduction by sodium borohydride we either obtained a mixture of the starting compound and the desired 9(11)-unsaturated 12-hydroxy derivatives, or, with an excess of the hydride, a mixture containing also the 9(11)-unsaturated product. Therefore, we tried other potential ways of the reduction. A little better selectivity (1,2 addition of hydrogen) was attained in the reduction with zinc borohydride, prepared *in situ* from anhydrous zinc chloride and sodium borohydride[1,2]. 3α-Acetoxydeoxycholic acid (*I*) thus gave a mixture, with 60% of the products having the desired allylalcoholic grouping (^{1}H NMR spectroscopy).

The best method proved to be that described by Luche[3]. This author has found that in the presence of chlorides of some lanthanides, especially cerium(III) and samarium chlorides, the reduction of α,β-unsaturated ketones affords almost exclusively the 1,2-addition products, *i.e.* the corresponding allyl alcohols. The reaction requires no other conditions than those used with sodium borohydride alone, and not even the presence of water (up to 5%) has any adverse effect on the selectivity. The mechanism of the action of lanthanides is obscure. In the use of less than one equivalent the selectivity disappears; it is possible that the carbonyl compound just forms a complex with the lanthanide, which is reduced by sodium borohydride in a consecutive reaction[3].

Even in the use of this method we obtained a mixture of the two isomeric allyl alcohols, but no contaminant with the reduced double bond was present (ascertained by ^{1}H NMR spectroscopy). The acetoxy derivatives *I* and *II* afforded a mixture of the corresponding two 9(11)-unsaturated 12-hydroxy derivatives *IIIa* and *IVb*,

which were converted into acetates *IIIb* and *IVc*. Only in the case of the allyl alcohol *IVb* did we partially succeed, by repeated crystallization from aqueous methanol, in separation and characterization of one of the two 12-hydroxy derivatives. Otherwise the mixture of the isomers would not be resolved by chromatography on alumina or silica gel. In the presence of hydrogen chloride as catalyst, the mixture of the two 12-hydroxy derivatives reacted with methanol with the formation of the previously described 12α-methoxy derivative, prepared in the same way[1].



I, $R^1 = CH_3COO$, $R^2 = H$, $R^3 = =O$

II, $R^1 = R^2 = CH_3COO$, $R^3 = =O$

IIIa, $R^1 = CH_3COO$, $R^2 = H$, $R^3 = \overset{OH}{H}$

IIIb, $R^1 = CH_3COO$, $R^2 = H$, $R^3 = \overset{OCOCH_3}{H}$

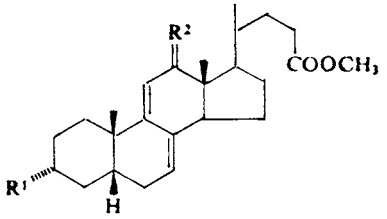
IVa, $R^1 = R^2 = CH_3COO$, $R^3 = \overset{OH}{H}$

IVb, $R^1 = R^2 = CH_3COO$, $R^3 = \overset{OH}{H}$

IVc, $R^1 = R^2 = CH_3COO$, $R^3 = \overset{OCOCH_3}{H}$

Va, $R^1 = C_6H_5COO$, $R^2 = H$, $R^3 = \overset{OH}{H}$

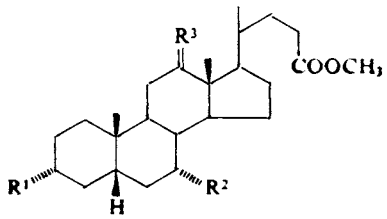
Vb, $R^1 = C_6H_5COO$, $R^2 = H$, $R^3 = \overset{OCH_3}{H}$



VI, $R^1 = CH_3COO$, $R^2 = =O$

VIIa, $R^1 = CH_3COO$, $R^2 = \overset{OH}{H}$

VIIb, $R^1 = CH_3COO$, $R^2 = \overset{OCOCH_3}{H}$



VIII, $R^1 = CH_3COO$, $R^2 = H$, $R^3 = =O$

IX, $R^1 = CH_3COO$, $R^2 = H$, $R^3 = \overset{OH}{H}$

X, $R^1 = R^2 = CH_3COO$, $R^3 = =O$

XI, $R^1 = R^2 = CH_3COO$, $R^3 = \overset{OH}{H}$

Analysis of the ^1H NMR spectra revealed that: *a*) the shifts of signals of the $C_{(18)}$, $C_{(19)}$ and $C_{(21)}$ hydrogen atoms of mixtures containing both 12-hydroxy derivatives fuse into one wide band from 0·60 to 1·2 ppm, *b*) the shifts of the $C_{(11)}$ and $C_{(12)}$ hydrogen atom signals are distinctly apart even with the mixtures and the magnitude of the signals suggests that the two 12-hydroxy derivatives are in the ratio 1 : 1.

By comparing the spectra of the 12α-methoxy dervative *Vb* and the 12-hydroxy derivative obtained by reduction of methyl ester of the 12-oxo acid *II* (separated by crystallization), on the basis of signal shifts of the $C_{(11)}$ hydrogen atoms, we ascribed the former the configuration 12α (*IVa*). The consequent pair values for the 12β-isomer are given in Table I.

In the same way we reduced the 7(8), 9(11)-unsaturated 12-oxo derivatives *VI* and the saturated 12-oxo derivatives *VIII* and *X*, which, according to the ^{1}H NMR spectra, afforded respectively almost pure 12α-hydroxy analogue *VIIa* (also characterised as 12-acetate *VIIb*), and 12α-hydroxy derivatives *IX* and *XI* with the natural configuration, although reduction of saturated 12-oxo derivatives by sodium borohydride was reported to produce both isomers[4].

EXPERIMENTAL

The melting points were determined on the Kofler block. Optical rotation was measured in chloroform with an accuracy of ±3°. The analytical samples were dried over phosphorus pentoxide at room temperature for 16 h. The UV spectra were measured in methanol employing a spectrophotometer Zeiss VSU-1. The ^{1}H NMR spectra were measured in deuteriochloroform with a spectrometer Tesla BS 487 C (80 Hz), with tetramethylsilane as internal standard. The chemical shifts are given in ppm. Thin-layer chromatography ran on Silufol UV 254 Kavalier Votice, the eluant being benzene containing dioxan (10%) and n-butyl acetate (2%). The spots were detected with concentrated sulphuric acid on heating the plates to 100°C. The samples were identified by mixed melting points, thin-layer chromatography and ^{1}H NMR spectra.

Methyl 3α-Acetoxy-12-hydroxy-9(11)-cholenate (*IIIa*)

To 1·3 g of 12-oxo derivative *I* (ref.[5]), dissolved in 50 ml of methanol, was added at room temperature 1·6 g of $CeCl_3.6\,H_2O$, then gradually 1·3 g of sodium borohydride. After 30 min the reaction was stopped by adding 50 ml of 5% hydrochloric acid. The mixture was agitated with chloroform; the extract was washed neutral with water, dried with calcium chloride and distilled *in vacuo*; yield 1·28 g of a single product (TLC); crystallization from methanol gave an analytical sample of *IIIa*, m.p. 120–123°C, $[\alpha]_D^{26} = +64°$ (*c* 1·1). For $C_{27}H_{42}O_5$ (446·6) calculated: 72·61% C, 9·48% H; found: 72·9% C, 9·76% H.

TABLE I

$C_{(11)}$ proton shifts of ^{1}H NMR spectra (ppm)

Compound	*IIIb*	*IVa*	*IVb*	*IVc*	*Vb*
12α	5·68	5·80	5·80	5·82	5·79
12β	5·30	—	5·35	5·81	—

Methyl 3α,12-Diacetoxy-9(11)-cholenate (*IIIb*)

12-Hydroxy derivative *IIIa* (1·0 g) in 40 ml of toluene was acetylated with 4 ml of acetic anhydride in 4 ml of pyridine for 6 h at 100°C. After working up the mixture there was obtained 1·05 g of the product (single by TLC): $[\alpha]_D^{23} = +96{\cdot}4°C$ (*c* 0·9); ^1H NMR spectrum: 0·6—1·2 (18-H, 19-H, 21-H), 2·09, 2·00 (3 H, s, s, 3-CH_3CO, 12-CH_3CO), 3·69 (3 H, s, —$COOCH_3$), 4·70 (1 H, bmt, 3β-H), 4·98 (1 H, d, 12β-H), 5·03 (1 H, bs, 12α-H), 5·30 (1 H, bs, 11-H, olef.), 5·68 (1 H, bd, 11-H olef.). For $C_{29}H_{44}O_6$ (488·6) calculated: 71·28% C, 9·08% H; found: 71·30% C, 9·22% H.

Methyl 3α,7α-Diacetoxy-12-hydroxy-9(11)-cholenate (*IVa, IVb*)

Adhering to the procedure described above, 2·0 g of 12-oxo acid *II* (ref.[6]) in 120 ml of methanol was reduced with 2 g of sodium borohydride in the presence of 3·2 g of $CeCl_3.6\,H_2O$; yield 1·8 g of a dry residue (single by TLC). Crystallization from aqueous methanol afforded the 12-hydroxy derivative *IVb*: m.p. 130—134°C, $[\alpha]_D^{22} = +52{\cdot}9°$ (*c* 1·2); ^1H NMR spectrum: 0·60 (3 H, 18-H), 0·75 (3 H, 19-H), 2·04 (3 H, s, 3α-CH_3CO), 3·70 (3 H, s, —$COOCH_3$), 3·95 (1 H, d, 12β-H), 4·15 (1 H, bs, 12α-H), 4·60 (1 H, bmt, 3β-H), 5·10 (1 H, bmt, 7β-H), 5·35 (1 H, bs, 11-H olef.), 5·80 (1 H, bd, 11-H olef.). For $C_{29}H_{44}O_7$ (504·6) calculated: 69·02% C, 8·79% H; found: 68·89% C, 8·94% H. Crystallization and two recrystallizations from aqueous methanol afforded almost pure 12-hydroxy derivative *IVa*: m.p. 169—172°C, $[\alpha]_D^{26} = +69{\cdot}40$ (*c* 1·7); ^1H NMR spectrum: 1·11 (3 H, s, 18-H), 0·59 (3 H, s, 19-H), 1·00 (3 H, bd, 21-H), 2·03 (6 H, s, 3α, 7α-CH_3CO), 3·70 (3 H, s, —$COOCH_3$), 3·94 (1 H, d, 12β-H, $J = 5{\cdot}50$ Hz), 4·60 (1 H, bmt, 3β-H ax), 5·10 (1 H, bmt, 7β-H eq), 5·80 (1 H, bd, 11-H olef., $J = 5{\cdot}51$ Hz). For $C_{29}H_{44}O_7$ (504·6) calculated: 69·02% C, 8·79% H; found: 68·67% C, 8·93% H.

Methyl 3α,7α,12-Triacetoxy-9(11)-cholenate (*IVc*)

0·5 g of *IVb* in 5 ml of toluene was acetylated as above by the addition of 2 ml of acetanhydride and 2 ml of pyridine; yield 0·45 g of a dry residue (a single product by TLC): $[\alpha]_D^{23} = 85{\cdot}8°$ (*c* 1·5). ^1H NMR spectrum: 2·10, 2·02, 2·00 (à 3 H, s, 3α, 7α, 12 —CH_3CO), 3·69 (3 H, s, —$COOCH_3$). 4·60 (1 H, bmt, 3β-H), 5·00 (1 H, d, 12β-H), 5·02 (1 H, bs, 12α-H), 5·15 (1 H, bmt, 7β-H), 5·31 (1 H, bs, 11-H olef.), 5·80 (1 H, bd, 11-H olef.). For $C_{31}H_{46}O_8$ (548·7) calculated: 67·85% C, 8·82% H; found: 68·04% C, 8·65% H.

Methyl 3α-Benzoyloxy-12α-methoxy-9(11)-cholenate (*Vb*)

1·3 g of *Va* (ref.[7]) was dissolved in 10 ml of methanol pre-saturated with hydrogen chloride and the mixture was left standing 24 h at room temperature and 8 h at −10°C. It was then diluted with water and agitated with dichloromethane. The extract was washed neutral with water, dried with calcium chloride and distilled *in vacuo*; yield 1·2 g of a non-crystalline residue (a single product by TLC). UV spectrum: $\lambda_{max} = 230$ nm ($\log \varepsilon = 4{\cdot}30$); ^1H NMR spectrum: 0·60 (3 H, s, 19-H), 0·95 (3 H, d, 21-H), 1·12 (3 H, s, 18-H), 3·41, 3·68 (à 3 H, s, 12-OCH_3, —$COOCH_3$), 5·00 (1 H, bmt, 3β-H), 5·79 (1 H, bd, 11-H olef.), 7·40 (3 H, m), 8·00 (2 H, m) (aromat. H).

Methyl 3α-Acetoxy-12α-hydroxy-7(8), 9(11)-choladienate (*VIIa*)

2 g of *VI* (ref.[6]) was reduced with 2 g of sodium borohydride in the presence of 3·2 g of $CeCl_3.6\,H_2O$ as described above; yield 1·8 g of hydroxy derivative *VIIa* (single by TLC). The analytical sample was obtained by crystallization from methanol; m.p. 125—128°C, $[\alpha]_D^{25} = +166{\cdot}5°$ (*c* 0·9). UV spectrum: $\lambda_{max} = 241$ nm ($\log \varepsilon = 4{\cdot}12$), 248 nm ($\log \varepsilon$) = 4·17), 256 nm ($\log \varepsilon =$

= 4·01). ^{1}H NMR spectrum: 0·502 (3 H, s), 1·03 (6 H, bs) (18-H) 19-H, 21-H), 1·99 (3 H, s, 3α-CH_3CO), 3·68 (3 H, s, $COOCH_3$), 4·00 (1 H, d, 12β-H, J = 5·5 Hz), 4·70 (1 H, bmt, 3β-H), 5·35 (1 H, bd, 7-H olef., J = 6·0 Hz), 5·75 (1 H, bd, 11-H olef., J = 5·5 Hz). For $C_{27}H_{40}O_5$ (444·6) calculated: 72·94% C, 9·07% H; found: 73·21% C, 9·18% H.

Methyl 3α,12α-Diacetoxy-7(8), 9(11)-choladienate (*VIIIb*)

Adhering to the procedure described above, 1·3 g of *VIIa* in 60 ml of toluene was acetylated with 2 ml of acetanhydride and 2 ml of pyridine; yield 1·29 g of *VIIb*; crystallization from methanol gave the analytical sample: m.p. 94–97°C, $[\alpha]_D^{25.5}$ = 79·4° (*c* 0·4). UV spectrum: λ_{max} = = 239 nm (log ε = 4·16), 247 (log ε = 4·21), 256 (log ε) = 4·06). ^{1}H NMR spectrum: 0·62 (3 H, s, 19-H), 0·92 (3 H, bd, 21-H), 1·02 (3 H, s, 18-H), 2·10, 2·00 (à 3 H, s, CH_3CO), 3·68 (3 H, s, $COOCH_3$), 4·68 (1 H, bmt, 3β-H), 5·10–5·50 (3 H, m, 7-H, 11-H, 12-H). For $C_{29}H_{42}O_6$ (486·63) calculated: 71·57% C, 8·70% H; found: 71·29% C, 8·73% H.

Methyl 3α-Acetoxy-12α-hydroxycholanate (*IX*)

Reduction of 2 g of *VIII* (ref.[7]) under the conditions specified above gave 1·7 g of the 12α-hydroxy derivative *IX*, m.p. 108–110°C, $[\alpha]_D^{23}$ = +67·6° (*c* 0·7). Reported[8] m.p. 128–130°C, $[\alpha]_D$ = = 66° ± 1°. ^{1}H NMR spectrum: 0·70$_t$ 0·95 (à 3 H, s, 18-H, 19-H), 1·00 (3 H, d, 21-H), 2·02 (3 H, s, CH_3CO), 3·65 (3 H, s, $COOCH_3$), 3·97 (1 H, bs, 12-H), 4·68 (1 H, bmt, 3-H); ^{1}H NMR spectrum of the natural 12α compound: 0·70 (3 H, s, 18-H), 0·91 (3 H, s, 18-H), 0·98 (3 H, d, 21-H), 2·00 (3 H, s, CH_3CO), 3·67 (3 H, s, $COOCH_3$, 3·98 (1 H, bs, 12-H), 4·70 (1 H, m, 3-H).

Methyl 3α,7α-Diacetoxy-12α-hydroxycholanate (*XI*)

Reduction of 2 g of *X* by the method described above gave 1·8 g of the 12α-hydroxy derivative *XI*, m.p. 175–179°C, $[\alpha]_D^{20}$ = +21·8° (*c* 0·8). Reported[9] m.p. 183–184°C, $[\alpha]_D$ = +23 ± 2°. ^{1}H NMR spectrum: 0·70, 0·95 (à 3 H, s, 18-H, 19-H), 0·99 (3 H, d, 21-H), 2·04, 2·00 (à 3 H, s, CH_3CO), 3·64 (3 H, s, $COOCH_3$), 3·98 (1 H, bmt, 12-H), 4·55 (1 H, bmt, 3-H), 4·85 (1 H, bmt, 7-H).

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