Tetrahedron Vol. 40, No. 5, pp. 851 to 854, 1984
Printed in Great Britain.

0040-4020/84 $3.00 + .00
Pergamon Press Ltd.

BILE ACIDS LXIX.
SELECTIVE K-SELECTRIDE REDUCTION OF 3,7-DIKETO STEROIDS[1]

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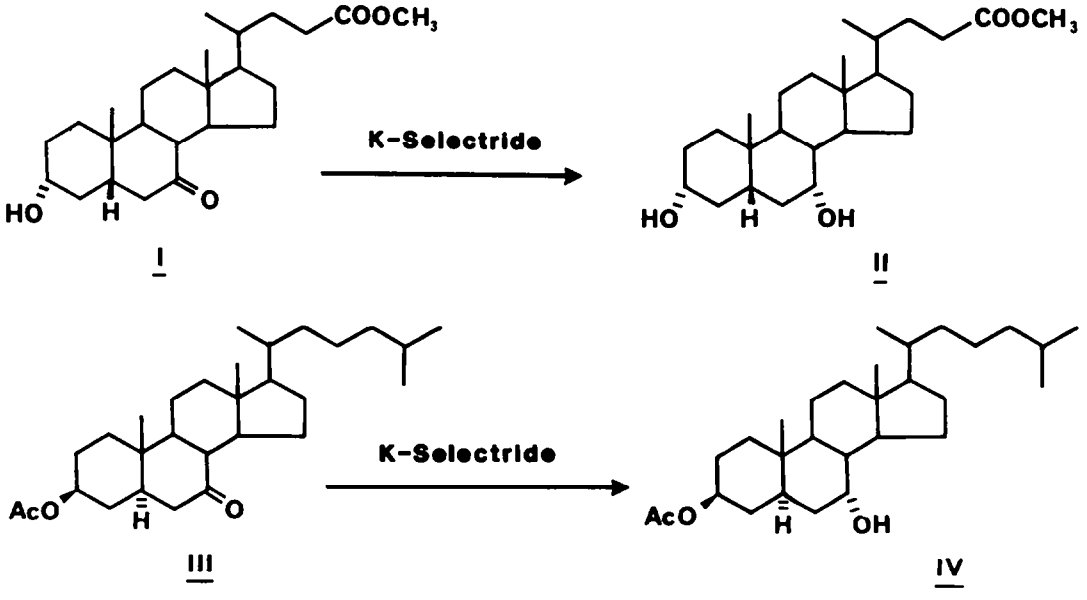
(*Received in USA* 12 *September* 1983)

Abstract - The K-Selectride reduction at low temperature (-45°C) of 7-oxo-5α-cholestan-3β-yl acetate and methyl 7-oxo-3α-hydroxy-5β-cholanoate resulted in almost quantitative yield of the 7α-alcohol in the 5α-compound but only moderate yield of the 5β-analog. The simultaneous reduction of two carbonyl groups in the 3 and 7 positions afforded good to excellent yields of the diaxial diol in planar steroids (methyl 3,7-dioxo-5α-cholanoate, 3,7-dioxo-5α-cholestane and methyl 3,7-dioxo-5α-cholestan-27-oate) and only 14% of 3α,7α-$(OH)_2$ from methyl 3,7-dioxo-5β-cholanoate.

Following reports that certain borohydrides reduce carbonyl compounds highly stereoselectively[2], the reduction of 3-keto steroids was successfully attempted using potassium[3] or lithium tri-sec-butylborohydride[4] to provide the axial alcohols as major products. To acquire diaxial diols from existing diols or monohydroxymonoketones requires isomerization of the equatorial alcohol[5] or reduction of the ketone to the axial alcohol. Since diaxial 3α,7α-diols of sterols or bile acids in the 5α-series are important to our biochemical studies, and current methods of preparation[6,7] require improvement, we have inves-



tigated the stereoselective reduction of 7-oxo- and 3,7-dioxo-5α-sterols and bile acid derivatives. Such a procedure would be useful since 3,7-dioxo-5α-sterols and bile acid derivatives are quantitatively an important by-product of the Raney nickel allomerization of 3α,7α-dihydroxy-5β-bile acids or sterols[8]. This paper demonstrates the utility of K-Selectride (potassium tri-sec-butylborohydride) in reduction of diketones to diaxial alcohols and describes the reduction of some 7-keto and 3,7-diketosteroids by means of this reagent.

RESULTS AND DISCUSSION

In preliminary experiments the effectiveness of K-Selectride as a reducing agent was tested for the 7-keto steroidal group with methyl 7-ketolithocholate (I) as a suitable reactant.

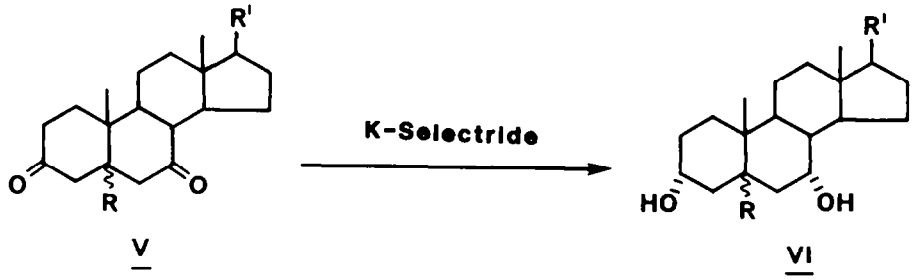
After a series of experiments at different temperatures (from -78°C to 0°C) and for different periods of time (from 2 to 6 hrs.) the optimal conditions were selected (-45°C and 5 hrs). The distribution of products was 52% of the 3α,7α-diol (II) and 20% of impurities, while the remaining 28% was unchanged starting material.

The reaction was carried out similarly on a 7-ketosteroid with 5α-configuration, 7-oxocholestanyl acetate (III); a 96% yield of 5α-cholestane-3β,7α-diol 3-acetate (IV) was obtained. This result can be compared with the reductions of 3-keto-5α- and 5β-cholestanes with the same reagent[4], at 0 or 5°C, where yields of 90% or 93% of the 3-axial alcohol were obtained respectively. At -78° reduction of a 3-keto-5α-cholestane derivative[3] afforded 84% of the axial 3α-alcohol.

The scope of the reaction was then extended to the simultaneous reduction of positions 3 and 7. For this purpose, a few C_{24} or C_{27} 3,7-dioxosteroids (V) with cis- or trans-A/B rings and with or without terminal methyl ester were synthesized. These diketones were derived from 3,7-diols or monoketone-monoalcohols which were oxidized with pyridinium chlorochromate.

Methyl 3,7-dioxo-5α-cholanoate (Va) and its 5β-isomer (Vb) were reduced in equal quantities and under the same conditions to facilitate comparison. The former compound yielded 62% of the desired 3α,7α-$(OH)_2$ (VIa), and 15% of unchanged starting material. The 5β-isomer Vb afforded a complex mixture of products, including 14% of VIb, 25% of its isomer methyl 3β,7α-dihydroxy-5α-cholanoate, 30% of the partially reduced 3α-hydroxy-7-keto product and 15% of unchanged 3,7-dioxo Vb. 3,7-Dioxo-5α-cholestanes were reduced in good yields to the corresponding diaxial dihydroxy products. Methyl 3,7-dioxo-5α-cholestan-27-oate (Vc) gave 82% of 3α,7α-diol VIc, and its analog 3,7-dioxo-5α-cholestane (Vd) afforded an almost quantitative yield of the diaxial diol VId.

These results suggest that the coplanarity of ring system in 5α-steroids may be responsible for improved yields in the production of 3α,7α-diols, and that the presence of an ester in the molecule has little effect on the yield. Despite the rigorous anhydrous conditions, it was observed that the reaction proceeds better with reagent from an unopened bottle rather than from a bottle previously



V VI

a R=α-H R'=$CH(CH_3)CH_2CH_2COOCH_3$
b R=β-H R'=$CH(CH_3)CH_2CH_2COOCH_3$
c R=α-H R'=$CH(CH_3)CH_2CH_2CH_2CH(CH_3)COOCH_3$
d R=α-H R'=$CH(CH_3)CH_2CH_2CH_2CH(CH_3)_2$

used. In general, it is more effective to use K-Selectride in two or more portions rather than adding all the reagent at once.

EXPERIMENTAL SECTION

^{1}H NMR spectra of $CDCl_3$ solutions were recorded on a Jeol FT-100MHz spectrometer, with TMS as internal standard. Mass spectra were measured using an LKB Model 9000 mass spectrometer at 70 eV. Gas chromatograms were obtained from an HP 402 high efficiency gas chromatograph, and RRT values[9] (relative retention time) are related to methyl deoxycholate unless specified. RRT's for certain C_{27} derivatives were calculated from reported RRT's for requisite C_{24} and C_{27} compounds[10]. The ratios of solvents for TLC are given as v/v. Analytical HPLC was carried out with a Waters Assoc. Model ALC-201 apparatus as described[11]; semiprep HPLC utilized the same apparatus with a Waters semi-prep column. All mp's are uncorrected. All solvents were analytical grade or were redistilled before use. K-Selectride (Aldrich) is a 1.0 M solution of potassium tri-sec-butylborohydride in tetrahydrofuran.

Reduction of methyl 7-oxo-3α-hydroxy-5β-cholanoate (I): The title compound (50 mg, 0.12 mmol) was vacuum dried and placed in a flame-dried 35 ml round bottom flask. Tetrahydrofuran (5 ml) was freshly distilled over $LiAlH_4$ into the flask, under N_2; the flask was closed with a rubber septum, and the temperature was brought to -45°C with a slurry of chlorobenzene and liquid nitrogen. K-Selectride (600 µl of 1 M solution, 0.6 mmol) was added with a syringe through the septum, and the mixture was magnetically stirred under N_2, at -45°C for 5 hours. After the reaction was quenched with 0.5 ml of 30% H_2O_2 and 0.2 ml of 5 M KOH, water was added, and the organic solvent was evaporated. The aqueous suspension was extracted three times (3x35 ml) with ether, the ether washed to neutrality, dried over $MgSO_4$ and evaporated. TLC on silica gel (toluene: acetone: methanol, 16/6/0.2) showed a mixture of products containing the desired 7α-hydroxy derivative (II, R_f 0.23), and some starting material (I, R_f 0.38). By gas-chromatography on a 3% OV-210 column at 240°C, the distribution was shown to be 52% of 7α-hydroxy product (II, RRT 1.18), 28% starting material (I, RRT 1.88) and 20% impurities. These compounds were separated on a preparative TLC plate using the same solvent mixture as before, and compared with authentical samples. The 7α-hydroxy compound (II) was found to be identical in its physical properties to methyl chenodeoxycholate. The main impurity (R_f 0.07) was not methyl ursodeoxycholate (the 3α,7β-diol) (R_f 0.26); similarly in a more polar TLC system the impurity did not migrate with 7-ketolithocholic acid, chenodeoxycholic acid, or 5β-cholane-3α,7α,24-triol.

Reduction of 7-oxo-5α-cholestan-3β-yl acetate (III): In a similar procedure, the title compound (25 mg, 56 µmol) was dissolved in 4 ml of freshly distilled THF, cooled to -45°C and reacted under N_2 with K-Selectride (400 µl of 1 M solution in THF, 0.4 mmol). After 5 hours the reaction was stopped and extracted as before. From TLC on silica (toluene:ethyl acetate, 9/1) the reaction appeared to have gone to completion (R_f 0.33); by GC (3% OV-1, 260°C) 5α-cholestane-3β,7α-diol 3-acetate (IV) represented 96% of the total. Physical properties of this product coincided with those of an authentic sample[12].

For the preparation of 3,7-diketo compounds the corresponding 3,7-diols (or monoketo monohydroxy compounds) were oxidized by pyridinium chlorochromate. Where applicable, the terminal carboxylic acid group was methylated with methanol and HCl, prior to oxidation. For example: to a solution of methyl 3β,7α-dihydroxy-5α-cholanoate (167 mg, 0.41 mmol) in methylene chloride (80 ml), pyridinium chlorochromate (300 mg, 1.39 mmol, Aldrich) was added at room temperature and stirred for 90 min. Three drops of methanol quenched the reaction; after the solvent was evaporated, the solid was redissolved in a minimum amount of 40% ethyl acetate in hexane and passed through a short silica column with the same eluant to remove salts and color. The eluate was extracted three times with saturated aqueous NaCl solution, dried over $MgSO_4$ and filtered to provide a clear colorless solution. On standing, crystals of Va separated (123 mg, 75%) with m.p. 158°C (lit.[8] 160°-1°C). These crystals had the same physical properties (TLC, GC) as an authentical sample[8].

Reduction of methyl 3,7-dioxo-5α-cholanoate (Va): This compound (51 mg, 0.13 mmol) was reduced with 1.2 ml solution of 1 M K-Selectride (1.2 mmol) at -45° and under N_2 as described, to yield 45 mg of a mixture rich in methyl 3α,7α-dihydroxy-5α-cholanoate (VIa), 62% by GC on 1% OV-17, 260°, RRT 1.27; 15% of the mixture was starting material (Va, RRT 1.50). Separation of VIa by prep-TLC, in the conditions described below, gave a compound with the following properties: NMR: δ (ppm) 0.66 (3H, s, C18), 0.79 (3H, s, C19), 3.66 (3H, s, CH_3 ester), 3.83 (1H, sharp m, β-H at C7) and 4.03 (1H, sharp m, β-H at C3). TLC; R_f 0.24 on silica gel (toluene: acetone: methanol, 16/6/0.1), coincident with authentic methyl allocholanoate.

Reduction of methyl 3,7-dioxo-5β-cholanoate (Vb): The title compound was synthesized by oxidation of chenodeoxycholic acid and subsequent esterification with methanol and hydrochloric acid. The resulting mixture was separated by semi-prep HPLC with a silica column and hexane as eluant to yield methyl 3,7-dioxo-5β-cholanoate (Vb), pure by analytical HPLC, GC and TLC. GC: 3% OV-1, 260°, RRT 1.04[9]. TLC: R_f 0.16 on silica gel (hexane:ethyl acetate:acetic acid, 15/5/0.3). MS: comparable to reported spectrum[13] (m/e) 402 (31%, M^+), 384 (10%, M-H_2O), 370 (16%, M-CH_3OH), 287 (100%, M-side chain), 269 [41%, M-(side chain+ H_2O)], and 192 (30%, ion u[13]).

Reduction was carried out in the same quantity and conditions as the 5α-isomer. By TLC on silica gel carried out exactly as after reduction of compound I, the following were identified by R_f: VIb, R_f 0.23 (the 3α,7α-diol), I (0.38), the 3β,7α-diol (0.30) and Vb (0.78). By GC on OV-210 the quantities of each were VIb (14%), I (30%), the 3β,7α-diol (25%), Vb (15%).

Reduction of methyl 3,7-dioxo-5α-cholestan-27-oate (Vc): The starting material for this reaction was prepared similarly from 3β,7ξ, 27-trihydroxy-5α-cholestane and esterified

with 2,2-dimethoxypropane, methanol and concentrated hydrochloric acid[14] to provide compound Vc. m.p.: 124-125°C (from hexane). NMR: δ (ppm) 0.67 (3H, s, C18), 0.90 (3H, d, J=6Hz, C21), 1.14 (3H, d, J=7Hz, C26), 1.25 (3H, s, C19), 1.80-2.60 (8H, m, C2+C4+C6+C25) and 3.67 (3H, s, CH_3 ester). IR: ν (cm^{-1}) 2940 and 2860 (aliph. C-H str.), 1739 (ester, C=O str.), 1723 and 1708 (two ketones, C=O str.), 1164 (ester, C-O str.). TLC: R_f 0.42 on silica (acetone:hexane, 2/8). MS: (m/e) 444 (47%, M^+), 426 (9%, M-H_2O), 412 (18%, M-CH_3OH), 384 (17%, M-$HCOOCH_3$), 287 (30%, M-side chain), 269 [42%, M-(side chain+H_2O)], 260 [17%, M-(side chain+C16+C17)], 247, 246 and 245 [29%, 54% and 40%, M-(side chain+C15+C16+C17 and H-migration)] and 192 (100%, ion u[13]). GC: 3% OV-1, 260°, RRT 2.78.

Methyl 3,7-dioxo-5α-cholestan-27-oate (Vc, 4.5 mg, 10 μmoles) was reduced with K-Selectride (0.1 ml, 0.1 mmol) for 5½ hr. in the same manner as before. Since GC analysis showed that the reaction was incomplete, another 0.1 ml of K-Selectride was added, and the reaction was continued for an additional 5 hours. After the usual work-up, methyl 3α,7α-dihydroxy-5α-cholestan-27-oate (VIc) was identified[15] by GC (82%, 3% OV-1, RRT 2.19) and MS. MS: 448 (7%, M^+), 430 (100%, M-H_2O), 415 [19%, M-(CH_3+H_2O)], 412 (48%, M-$2H_2O$), 397 [23%, M-($2H_2O$+CH_3)], 291 (9%, M-side chain), 273 [34%, M-(H_2O+side chain)], 255 (23%, M-($2H_2O$+side chain)], 249 (43%), 246 (43%), 231 (22%), 228 (33%) and 213 (45%). NMR: δ (ppm) 0.65 (3H, s, C18), 0.78 (3H, s, C19), 0.88 (3H, d, J=6Hz, C21), 1.13 (3H, d, J=7Hz, C26), 3.67 (3H, s, CH_3 ester), 3.82 (1H, sharp m, β-H at C7) and 4.06 (1H, sharp m, β-H at C3). TLC: R_f 0.23 on silica (toluene:acetone: methanol, 16/6/0.1).

Reduction of 3,7-dioxo-5α-cholestane (Vd): The title compound (9 mg, 22.5 μmoles) was reduced in exactly the same conditions as compound Vc with two batches of 0.23 mmoles each of K-Selectride. Work-up of the mixture yielded 95% of 3α,7α-dihydroxy-5α-cholestane (VId). NMR: δ (ppm) 0.63 (3H, s, C18), 0.78 (3H, s, C19), 0.86 (6H, d, J=6Hz, C26,27), 0.90 (3H, d, J=6Hz, C21), 3.83 (1H, sharp m, β-H at C7) and 4.05 (1H, sharp m, β-H at C3); these data are in complete accord with those of a previous study[5]. TLC: R_f 0.31 on silica (toluene: acetone:methanol, 16/6/0.1); MS: 386 (22%, M-H_2O), 368 (30%, M-$2H_2O$), 353 [8%, M-($2H_2O$+CH_3)], 273 [5%, M-(H_2O+side chain)], 255 (31%, M-($2H_2O$+side chain)], 249 (11%), 246 (59%), 231 (25%), 228 (6%) and 213 (19%). (A similar fragmentation pattern has been reported[16]); GC: (3% OV-1, 260°), RRT 1.07.

ACKNOWLEDGEMENT

This investigation was supported by U.S. Public Health Service Grant HL-07878. The assistance of Dr. Ata Schirazi of Washington University NMR Facility in acquiring spectra is gratefully appreciated.

REFERENCES

1 Presented in part at the 18th American Chemical Society Midwest Regional Meeting, November 1983, Lawrence, Kansas.
2 C.A.Brown, J. Am. Chem. Soc., 95:4100 (1973); S.Krishnamurthy, Aldrichimica Acta, 7:55 (1974) and references cited therein.
3 S.V. Hiremath and W.H. Elliott, Steroids, 38(4):465 (1981).
4 R. Contreras and L. Mendoza, Steroids, 34:121 (1979).
5 R. Shaw and W.H. Elliott, J. Biol. Chem., 254(15):7177 (1979).
6 W.H. Elliott. Allo bile acids. In: The Bile Acids. Vol. 1: Chemistry, edited by P.P. Nair and D. Kritchevsky. New York, Plenum, 1971, p. 47-93.
7 W.H. Elliott. The Allo Bile Acids. In: The Hepatobiliary System, edited by W. Taylor, New York, Plenum, 1976, p. 469-481.
8 M.N. Mitra and W.H. Elliott, J. Org. Chem., 33:2814 (1968) and ref. 2 above.
9 W.H. Elliott, L.B. Walsh, M.M. Mui, M.A. Thorne and C.A. Siegfried, J. Chromatogr., 44:452 (1969).
10 S. Ali, E. Stephenson and W.H. Elliott, J. Lipid Res., 23:947 (1982).
11 R. Shaw and W.H. Elliott, J. Chromatogr., 177:289 (1979).
12 B.W. Noll. Ph.D. Dissertation, Saint Louis University, 1970.
13 W.H. Elliott. Bile Acids. In: Biochemical Applications of Mass Spectrometry, edited by G.R. Waller, New York, Wiley, 1972, p. 291.
14 R. Shaw and W.H. Elliott, J. Lipid Res., 19(6):783 (1978).
15 S.Y. Kamat and W.H. Elliott, Steroids, 20(3):279 (1972).
16 M.M. Mui and W.H. Elliott, Biochemistry, 14:2712 (1975).