HYDROBORATION OF 5α-ERGOST-8-EN-3β-OL

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ABSTRACT

Hydration via hydroboration of 5α-ergost-8-en-3ß-ol affords 5α-ergostane-3ß,15α-diol, 5α,14ß-ergostane-3ß,15ß-diol, and 5α-ergostane-3ß,7ß-diol, and not 5α,9ß-ergostane-3ß,7ß-diol, as previously reported by others.

INTRODUCTION

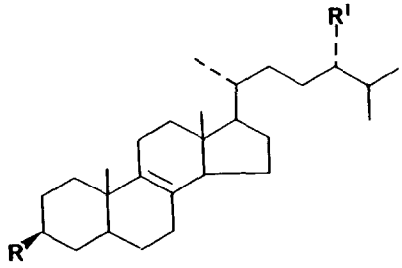
In connection with a program leading to the synthesis of steroids with an unnatural stereochemistry (1-3) which have been shown to possess inhibitory activity on sterol biosynthesis (4,5), we have previously investigated the hydroboration at 50-60 C of 5α-cholest-8-ene 1a (6) and have shown that this reaction parallels the results obtained starting with the 5α-cholest-8(14)-ene 2a. In light of these results, previous reports, which elucidated a different reaction sequence in the

ergosterol series (7-9), appear improbable. Thus, we have reexamined the hydroboration followed by oxidation of the 5α-ergost-8-en- 3ß-ol 1b and of 5α-ergost-8(14)-en-3ß-ol 2b in various conditions and report here the results, which are in complete agreement with those we have obtained for the cholestane series. In all cases the obtained products were 5α-ergostane-3ß,15α-diol 3a and 5α,14ß-ergostane-3ß,15ß-diol 4a. Minor amounts of 5α-ergostane-3ß,7ß-diol 5 were also obtained. The ratio of the 14α,15α and 14ß,15ß isomers 3a and 4a could be changed by heating the hydroboration mixture at 120 C for a proper time. The amount of the 7ß-hydroxy compound 5, on the contrary, was independent from the temperature. Our results represent a correction of those previously reported (7).

EXPERIMENTAL

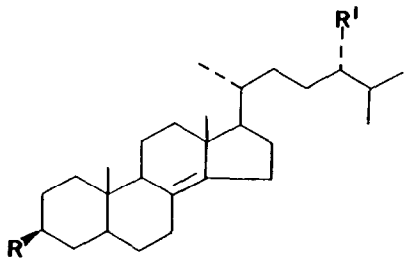
All mps are uncorrected. IR spectra were recorded for solutions in chloroform. ^{1}H-NMR spectra were recorded on a Bruker AM-500 instrument for solutions in ^{2}H chloroform and are reported as values relative to Me_4Si at 0.0 ppm. Mass spectra were determined on a Varian 112 S mass spectrometer by direct inlet. Routine optical rotations were recorded with a Perkin-Elmer 141 spectropolarimeter for 1% solutions in chloroform, and $[\alpha]_D^{20}$ values are given in degrees. GLC-mass spectrometry analyses were performed using an LKB 2091 mass spectrometer equipped with a PYE UNICAM gas-chromatograph and a 2-m silanized glass column of 1% SE30 on Gaschrom Q support, operating at 260 C. TLC was performed on precoated silica gel G plates (E. Merck, HF_{254}), visualized by spraying with 70% sulfuric acid, followed by heating. Column chromatographies were performed by Still's method (flash chromatography) (10).

5α-Ergost-8-en-3ß-ol 1b and 5α-ergost-8(14)-en-3ß-ol 2b were prepared according to reference 11. The purity of each



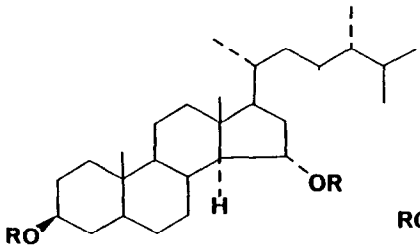
1 a: R = R' = H

b: R = OH : R' = CH_3



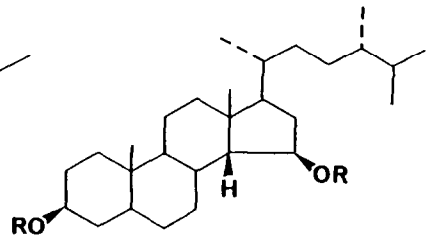
2 a: R = R' = H

b: R = OH : R' = CH_3



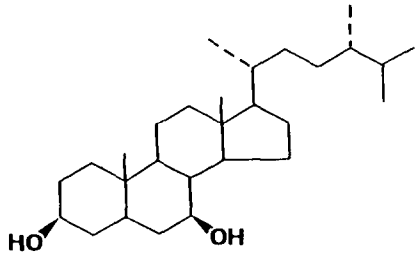
3 a: R = H

b: R = $COCH_3$



4 a: R = H

b: R = $COCH_3$



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product was proved by GLC-mass spectrometry and by ^{1}H-NMR at 500 MHz.

Hydroboration Procedures. The hydroboration was performed, generating the diborane in two different ways, and in the same conditions reported for the cholestane series (6).
Method a: Diborane was generated by adding a solution of sodium borohydride (6 g, 15.9 mmol) in diglyme (300 mL) to a solution of boron trifluoride etherate (50 g, 35.2 mmol) in diglyme (200 mL), in the type of apparatus described by Brown and Subba Rao (12). The gas was passed into a solution of the steroidal olefin (1 g in 40 mL of dry diglyme) at 50-60 C during ca. 3 h by means of a slow stream of nitrogen. Water was added to destroy excess diborane and the mixture was oxidized directly.
Method b: Diborane was generated by adding to a solution of sodium borohydride (6 g) in diglyme (300 mL) a solution of iodine (20 g) in diglyme (200 mL) (13). This procedure was used in order to avoid contamination by boron trifluoride etherate.

Oxidation of Organoboranes. All oxidations were carried out by the following procedure. Aqueous sodium hydroxide (20 mL of a 10% solution) was added to a solution of the organoboranes (derived from 1 g of olefin) in tetrahydrofuran (40 mL). The solution was cooled in ice-water, and aqueous hydrogen peroxide (15 mL of a 30% solution) was added dropwise with stirring; cooling was continued. The mixture was stirred for 1 h at 0 C and diluted with water and diethyl ether; the organic layer was washed with sodium bisulfite solution and water. The extract was then dried over sodium sulfate and evaporated.

Hydration of 5α-ergost-8-ene-3ß-ol 1b. Hydroboration (methods a and b) of this olefin (1 g) affords, after oxidation, a mixture of alcohols which were chromatographed to give two different products. The first eluted (779 mg) material formed microcrystals with mp 158-160 C (from hexane-diethyl ether); $[\alpha]_D^{20}$ +30. GLC-mass spectrometry analysis and ^{1}H-NMR spectroscopy of this material showed that it was a 1:3 mixture of two separable components, as shown forward, after acetylation and chromatography. A product showing similar mp and $[\alpha]_D^{20}$ was isolated previously in the same reaction, and the structure of a 5α,9ß-ergostane-3ß,7ß-diol was assigned (7). The last eluted compound was 5α-ergostane-3ß,7ß-diol 5 (110 mg): mp 190-191 C (from hexane-diethyl ether); $[\alpha]_D^{20}$ +32; (lit. (14) 178-182 C; $[\alpha]_D^{20}$ +26.5); δ 0.672 (3 H, s, 18-CH_3; calcd (16) 0.700), 0.769 (3 H, d, J 6.8 Hz), 0.776 (3 H, d, J 6.8 Hz), 0.825 (3 H, s,

19-CH_3; calcd (16) 0.833), 0.848 (3 H, d, J 6.8 Hz), 0.910 (3 H, d, J 6.8 Hz), 3.352 (1 H, ddd, J 9.8, 9.8, and 4.9, 7α-H), and 3.582 (1 H, dddd, J 9.8, 9.8, 4.9, and 4.9 Hz, 3α-H); M (mass spectrum) 418 (M^+), 400 (100%), 385, 302, 273, and 250 (Found: C, 80.4; H, 12.1. $C_{28}H_{50}O_2$ requires C, 80.3; H, 12.0%).

In order to separate the less polar mixture of compounds, part of the material (100 mg) was dissolved in pyridine (2 mL) containing 4-(dimethylamino)pyridine (50 mg) and treated with acetic anhydride (1 mL) at room temperature for 12 h. Work-up afforded a mixture which was chromatographed to afford two diacetates. The first was 5α-ergostane-3ß,15α-diol diacetate 3b (25 mg): mp 125-126 C (from methanol); $[\alpha]_D^{20}$ +39; (lit. (8) 123-126 C; $[\alpha]_D^{20}$ +35); δ 0.700 (3 H, s, 18-CH_3; calcd (16) 0.734), 0.750 (3 H, d, J 6.8 Hz), 0.758 (3 H, d, J 6.8 Hz), 0.808 (3 H, s, 19-CH_3; calcd (16) 0.825), 0.834 (3 H, d, J 7.0 Hz), 0.880 (3 H, d, J 6.3 Hz), 1.989 (3 H, s, $COCH_3$), 2.008 (3 H, s, $COCH_3$), 4.673 (1 H, dddd, J 9.8, 9.8, 4.9, and 4.9 Hz, 3α-H), and 4.788 (1 H, ddd, J 9.8, 9.8, and 3.5 Hz, 15ß-H); M (mass spectrum) 442 (M^+-60), 427, 400, 382, 367, 343, and 315 (100%) (Found: C, 76.4; H, 10.6. $C_{32}H_{54}O_4$ requires C, 76.45; H, 10.8%).

Saponification of 3b (100 mg) with 0.2 N aqueous methanolic KOH (5 mL) regenerated the 5α-ergostane-3ß,15α-diol 3a (85 mg): mp 190-92 C (dec) $[\alpha]_D^{20}$ +35; δ 0.678 (3 H, s, 18-CH_3; calcd (16) 0.700), 0.765 (3 H, d, J 6.8 Hz), 0.776 (3 H, d, J 6.8 Hz), 0.814 (3 H, s, 19-CH_3; calcd (16) 0.816), 0.845 (3 H, d, J 6.8 Hz), 0.892 (3 H, d, J 6.5 Hz), 3.588 (1 H, dddd, J 9.8, 9.8, 4.9, and 4.9 Hz, 3α-H), and 3.938 (3 H, ddd, J 9.0, 9.0, and 3.5 Hz, 15ß-H); M (mass spectrum) 418 (M^+), 400 (100%), 385, 302, 273, and 250 (Found: C, 80.2; H, 12.0. $C_{28}H_{50}O_2$ requires C 80.3; H, 12.0%).

The more polar component of the mixture was 5α,14ß-ergostane-3ß,15ß-diol diacetate 4b (80 mg): glass; $[\alpha]_D^{20}$ -21; (lit. (8) glass; $[\alpha]_D^{20}$ -18); δ 0.786 (3 H, s, 19-CH_3), 0.787 (3 H, d, J 7.0 Hz), 0.794 (3 H, d, J 6.3 Hz), 0.818 (3 H, d, J 7.0 Hz), 0.861 (3 H, d, J 6.3 Hz), 1.015 (3 H, s, 18-CH_3), 1.304 (1 H, ddd, $J_{16\alpha,16\beta}$ 14.7, $J_{15\beta,16\beta}$ 5.2, and $J_{16\beta,17\alpha}$ 4.0 Hz, 16ß-H), 1.868 (1 H, dd, $J_{14\beta,15\alpha}$ 9.0 and $J_{8\beta,14\beta}$ 4.2 Hz, 14ß-H), 1.990 (3 H, s, $COCH_3$), 2.003 (3 H, s, $COCH_3$), 2.422 (1 H, ddd, $J_{16\alpha,16\beta}$ 14.7, $J_{16\alpha,17\alpha}$ 9.0, and $J_{15\alpha,16\alpha}$ 9.0 Hz, 16α-H), 4.652 (1 H, dddd, J 9.8, 9.8, 4.9, and 4.9 Hz, 3α-H), and 5.120 (1 H,

ddd, $J_{15\alpha,16\alpha}$ 9.0, $J_{14\beta,15\alpha}$ 9.0, and $J_{15\alpha,16\beta}$ 5.2 Hz, 15α-H); M (mass spectrum) 442 (M^+-60), 427, 400, 382, 367, 343, and 315 (100%) (Found: C, 76.5; H, 10.7. $C_{32}H_{54}O_4$ requires C, 76.45; H, 10.8%).

Saponification of 4b, (100 mg) with 0.2 N aqueous methanolic KOH (5 mL) regenerated the 5α,14ß-ergostane-3ß,15ß-diol 4a (87 mg): mp 152-154 C; $[\alpha]_D^{20}$ +19; δ 0.784 (6 H, d, J 6.8 Hz), 0.786 (3 H, s, 19-CH_3; calcd (16,17) 0.783), 0.846 (3 H, d, J 6.5 Hz), 0.852 (3 H, d, J 7.0 Hz), 0.991 (3 H, s, 18-CH_3; calcd (16,17) 0.963), 1.368 (1 H, ddd, $J_{16\alpha,16\beta}$ 13.3, $J_{15\alpha,16\beta}$ 5.6, and $J_{16\beta,17\alpha}$ < 1 Hz, 16ß-H), 1.500 (1 H, dd, $J_{14\beta,15\alpha}$ 9.0 and $J_{8\beta,14\beta}$ 4.2 Hz, 14ß-H), 2.279 (1 H, ddd, $J_{16\alpha,16\beta}$ 13.3, $J_{15\alpha,16\alpha}$ 9.0, and $J_{16\alpha,17\alpha}$ 9.0 Hz, 16α-H), 3.590 (1H, dddd, J 9.8, 9.8, 4.9, and 4.9 Hz, 3α-H), and 4.270 (1 H, ddd, $J_{15\alpha,16\alpha}$ 9.0, $J_{14\beta,15\alpha}$ 9.0, and $J_{15\alpha,16\beta}$ 5.6 Hz, 15α-H); M (mass spectrum) 418 (M^+), 400 (100%), 385, 302, 273, and 250 (Found: C, 80.4; H, 12.0. $C_{28}H_{50}O_2$ requires C, 80.3; H, 12.0%).

Thermal equilibration of steroidal boranes from 5α-ergost-8-en-3ß-ol 1b. The hydroboration mixture obtained (method a or b), starting with ergostenol 1b (1 g), was heated at 120 C for 1 h and then oxidized as reported above. After chromatography, the 5α-ergostane-3ß,7ß-diol 5 (107 mg) was obtained with physicochemical properties identical to that described above, accompanied by an unseparable crystalline mixture of the isomeric diols 3a and 4a (769 mg) in a ratio of 3.5:1 (GLC-mass spectrometry, and ^{1}H-NMR at 500 MHz). Acetylation, chromatographic separation of the mixture, and regeneration of the hydroxy groups afforded pure 3a (459 mg) and 3b (129 mg) which were identical in all respects (^{1}H-NMR, mp, mixed mp) with those reported above.

Hydration of 5α-ergost-8(14)-en-3ß-ol 2b. Hydroboration (method a or b) of this olefin (500 mg) affords, after oxidation, a mixture of alcohols which were chromatographed to give, first a mixture (GLC-mass spectrometry and ^{1}H-NMR at 500 MHz) of the diols 3a and 4a in a ratio of 1:3 (390 mg), and then the 5α-ergostane-3ß,7ß-diol 5 (54 mg), identical in all respects (mass spectrum, ^{1}H-NMR, mp, and mixed mp) with that described above.

RESULTS AND DISCUSSION

Hydroboration of 5α-ergost-8-en-3ß-ol 1b was performed generating the diborane by interaction of sodium borohydride with boron trifluoride etherate or iodine. The second procedure was used in order to rigorously exclude the presence of the boron halide, which could isomerize the ^{8} double bond to the

$^{8(14)}$ or ^{14} position (15). However, in both cases, when the hydroboration was performed at 50-60 C and the boron derivatives were oxidized with hydrogen peroxide in sodium hydroxide, a mixture of compounds to which now we have assigned the structure of 5α-ergostane-3ß,15α-diol 3a and 5α,14ß-ergostane-3ß,15ß-diol 4a was obtained, accompanied by minor amounts of 5α-ergostane-3ß,7ß-diol 5. The last compound could be separated by column chromatography, while the 15-hydroxy isomers 3a and 4a were unseparable by column chromatography, and crystallized together, affording a product which showed melting point and optical rotation similar to that reported for a supposed 5α,9ß-ergostane-3ß,7ß-diol obtained in the same reaction (7). However, GLC-mass spectrometry analysis of the crystalline mixture showed two peaks (in about 1:3 ratio) responsible for two mass spectra, which differed only in the relative intensities of the most significant peaks.

The structure of the 5α-ergostane-3ß,7ß-diol 5 was

attributed by comparison of its physicochemical properties (mass spectrum, elemental analysis, mp, and mixed mp) with that of the sample prepared according to reference (14). Its ^{1}H-NMR spectrum showed the typical 3α-proton signal and a signal showing the multiplicity of a ddd at 3.352 ppm (J 9.8, 9.8, and 4.9 Hz), attributable to the 7α-proton geminal with the equatorial hydroxy group. In addition, the position of the 18- and 19-methyl proton signals were in good agreement with the values calculated by Zürcher's rules (16).

In order to separate the mixture of 3ß,15α and 3ß,15ß diols, 3a and 4a, they were acetylated to afford the corresponding diacetates, 3b and 4b, which could be separated by column chromatography. Each diacetate showed correct physicochemical properties and was identical with that obtainable by similar hydration of 5α-ergost-8(14)-en-3ß-diol 2b followed by acetylation (8). Saponification of the acetates 3b and 4b afforded the corresponding diols 3a and 4a with physicochemical properties in agreement with the assigned structures. In particular, in the ^{1}H-NMR spectrum of 3a, the positions of the 18- and 19-methyl proton signals were in good agreement with the values calculated by Zürcher's rules (16), while the ^{1}H-NMR spectrum at 500 MHz of 4a supported the cis geometry of C/D ring and the ß orientation of the 15-hydroxy

group. In fact, the analysis of the 14ß-proton signal showed a 9.0-Hz coupling constant with 15α-proton (the dihedral angle between these and protons measured on Dreiding models is about 140 °) and a 4.2-Hz coupling constant with 8ß-proton, indicative of an equatorial-axial relationship for 14ß and 8ß protons. In addition, the positions of 18- and 19-methyl proton signals of 4a were in agreement with the values calculated (16,17). The ratio of 15α to 15ß boranes increases when the hydroboration was effected at higher temperatures, and at 120 C, the 15α-borane was the major component. On the contrary, the relative amount of the 3ß,7ß-diol 5 was unaffected by temperature.

The behavior of 5α-ergost-8(14)-en-3ß-ol 2b toward the hydroboration at 50-60 C parallels that of the 5α-ergost-8-en-3ß-ol 1b, affording the same diols 3a, 4a, and 5 in an identical reciprocal ratio.

In conclusion, the hydration via hydroboration of the 5α-ergost-8-en-3ß-ol 1b affords a mixture of the diols 3a, 4a, and 5 identical to that obtainable starting with 5α-ergost-8(14)-en-3ß-ol 2b instead of the reported 5α,9ß-ergostane-3ß,7ß-diol (7). The course of the reaction is well superimposable to that observed in cholesterol series and rationalized (6) by an initial isomerization of the double bond from the 8(9) to the 8(14) (as shown by GLC-mass spectrometry), and to a lesser

extent, to the 7(8) positions. Successive hydration of the formed 7(8) and 8(14) ergostenes then affords the final products.

Our results represent a revision of previously reported literature (7).

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