Novel procedure for the preparation of 1α,3β-dihydroxy-2β-tritiated steroidal compounds

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A novel procedure for the preparation of 1α,3β-dihydroxy-2β-tritiated steroidal compounds by the reduction of the α-epoxide with sodium borotritiide in diglyme at 80 C is described. (Steroids **57:**444–446, 1992)

Keywords: steroids; 1α,3β-dihydroxy-2β-tritiated steroidal compound; sodium borotritiide; 22-oxacalcitriol

Introduction

During the course of our development of 20(S)-(3-hydroxy-3-methylbutyloxy)-9,10-secopregna-5,7,10(19)-triene-1α,3β-diol (22-oxacalcitriol) as an antihyperparathyroidism agent,[1] tritiated 22-oxacalcitriol was needed for pharmakokinetic and metabolic studies. It is well known that steroidal epoxides are readily cleaved by metal hydrides such as lithium aluminum hydride or lithium triethylborohydride.[2] The preparation of tritiated lithium aluminum hydride and lithium triethylborohydride is recorded in the literature,[3] but such tritiated reagents are not commercially available. Although tritiated sodium borohydride (NaB^3H_4) is a very stable and cheap tritiating reagent, reductive cleavage of epoxide with NaB^3H_4 has never, to our knowledge, been reported. We describe a procedure for tritiation by reductive cleavage of steroidal epoxide with NaB^3H_4.

Experimental

Melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded with a Hitachi 260-30 spectrometer, proton nuclear magnetic resonance (NMR) spectra with a JEOL FX-200, mass spectra (MS) with a Shimadzu GCMS-QP 1000, and ultraviolet (UV) spectra with a Shimadzu UV-240. The apparatus used for high-performance liquid chromatography (HPLC) was a Tosoh CCP with UV detector UV-8010 and RI detector RS-8000. High-performance liquid chromatography (HPLC) was carried out on a YMC A-312 at a flow rate of 1 ml/min with H_2O/MeOH (20 : 80). Radioactivity was measured with an Aloka LSC-900.

Preparative thin-layer chromatography (TLC) was performed on 20 × 20 cm plates coated with a 0.25-mm thickness of Merck Kieselgel 60 containing PF 254 indicator. NaB^3H_4 was purchased from Amersham Japan (code no. TRK 838). 1α,2α-Epoxy-20(S)-(3-hydroxy-3-methylbutyloxy)-pregna-5,7-diene-3β-ol (**1**) was prepared in our laboratories and will be reported elsewhere. The following values were found for **1**: colorless powder. IR (KBr): 3,350, 2,995, 2,905, 1,395, 1,380, 1,170, 1,110, 1,080, 1,065, 855 cm^{-1}. NMR ($CDCl_3$) δ: 0.61 (3H, s), 1.02 (3H, s), 1.22 (3H, d, J = 7.2 Hz), 1.24 (6H, s), 3.00 (1H, d, J = 3.6 Hz), 3.20 to 3.28 (1H, br), 3.32 (1H, d, J = 3.6 Hz), 3.44 to 3.56 (1H, m), 3.78 to 3.92 (2H, m), 5.32 to 5.40 (1H, m), 5.68 (1H, brd, J = 5.7 Hz). MS m/z: 416 (M^+), 68 (100%). UV (EtOH) λ_{max} nm: 289, 278, 267.

20(S)-(3-Hydroxy-3-methylbutyloxy)-pregna-5,7-diene-1α,3β-diol (2; R = H)

Reduction with $NaBH_4$. A mixture of the α-epoxide (**1**) (16.7 mg, 0.04 mmol) and $NaBH_4$ (1.7 mg, 0.04 mmol) in diglyme (0.9 ml) was stirred at 80 C for 15 hours under argon atmosphere. The mixture was then diluted with AcOEt (20 ml) and washed with H_2O (30 ml × 3). The aqueous layer was extracted with AcOEt (20 ml). The combined organic layer was dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by preparative thin-layer chromatography (TLC) with CH_2Cl_2/EtOH (10 : 1) to give the alcohol **2** (R = H) (10.6 mg, 63% yield) and the recovered epoxide **1** (7.3 mg, 44%, crude), which was purified by preparative TLC with *n*-hexane/AcOEt (2 : 1) to give pure epoxide **1** (5.9 mg, 35% yield). **2** (R = H): colorless prisms, mp 186.5 to

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Dedicated to Dr. Masatomo Hamana, Professor Emeritus of Kyushu University, on the occasion of his 75th birthday.

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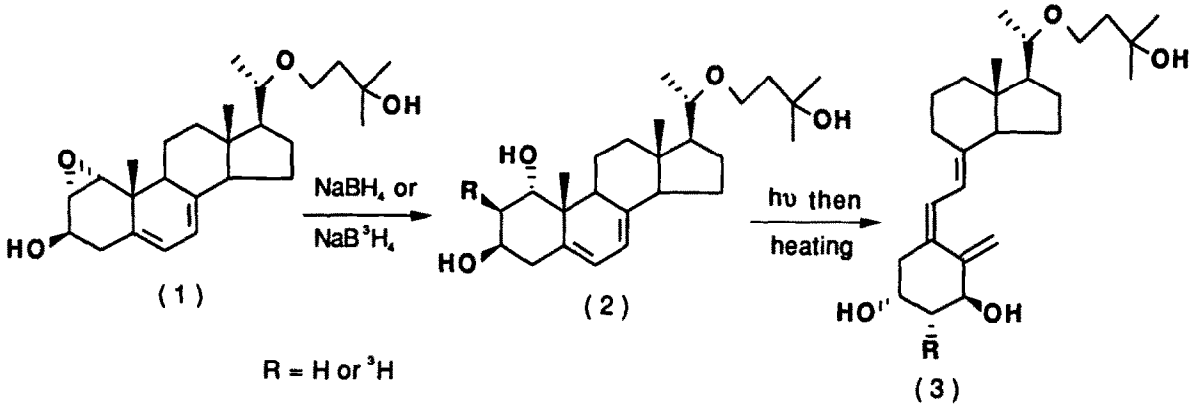


Scheme 1

187.5 C (acetone). IR (KBr): 3,500, 3,420, 3,000, 2,970, 2,910, 1,480, 1,390, 1,170, 1,100, 1,080, 1,065, 1,055 cm^{-1}. NMR ($CDCl_3$) δ: 0.62 (3H, s), 0.94 (3H, s), 1.22 (3H, d, J = 7.2 Hz), 1.24 (6H, s), 3.20 to 3.32 (1H, m), 3.42 to 3.56 (1H, m), 3.72 to 3.91 (2H, m), 4.06 (1H, br), 5.35 to 5.43 (1H, m), 5.72 (1H, brd, J = 5.7 Hz). MS m/z: 418 (M^+), 69 (100%). UV (EtOH) λ_{max} nm: 293, 281, 270. Analysis calculated for $C_{26}H_{42}O_4 \cdot 1/3H_2O$: C, 73.55; H, 10.13. Found: C, 73.75; H, 10.53. The retention time of **2** (R = H) in HPLC was 11.0 minutes.

20(S)-(3-Hydroxy-3-methylbutyloxy)-[2β-^{3}H]pregna-5,7-diene-1α,3β-diol(2; R = ^{3}H)

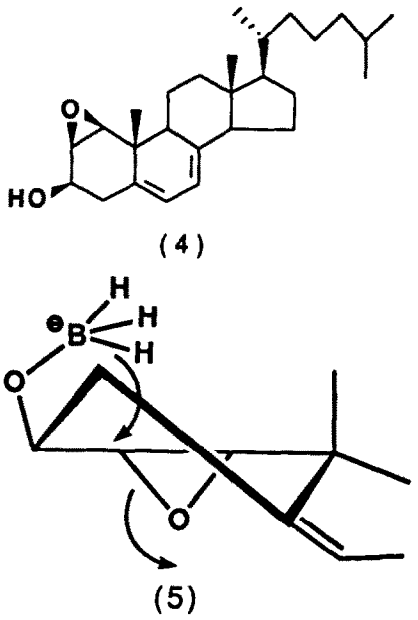
Reduction with NaB^3H_4. A mixture of the α-epoxide **1** (16.7 mg, 0.04 mmol) and NaB^3H_4 (2.0 Ci, 50 Ci/mmol) in diglyme (0.9 ml) was stirred at 80 C for 15 hours. The mixture was then treated as described above to give the alcohol **2** (R = ^{3}H) (103.5 mCi, 4.9 mg, 8.87 Ci/mmol, 29% chemical yield, 21% radiochemical yield). The retention time of **2** (R = ^{3}H) in HPLC was 11.0 minutes.

Results and discussion

Sodium borohydride ($NaBH_4$) is not commonly used in the reductive cleavage of epoxides due to sluggish reduction.[4] This is also the case of steroidal epoxides, only a few examples being available in the literature.[5-7]

While studying the synthesis of 20(S)-(3-hydroxy-3-methylbutyloxy)-[2β-^{3}H]-9,10-secopregna-5,7,10(19)-triene-1α,3β-diol (**3**; R = ^{3}H), we found that the α-epoxide **1** was reduced to the alcohol **2** (R = H) quantitatively with excess (10 eq.) $NaBH_4$ in hot (80 C) diglyme for 3 hours. Taking the tritiating experiment with NaB^3H_4 into consideration, the reduction of the α-epoxide **1** (0.04 mmol) was then carried out with a limited amount of $NaBH_4$ (0.04 mmol). The alcohol **2** (R = H) was obtained in 63% yield accompanied with the recovery of the epoxide **1** (35%). Having the results of $NaBH_4$ reduction, the same conditions were applied to prepare the tritiated alcohol **2** (R = ^{3}H). The reduction of **1** (0.04 mmol) with NaB^3H_4 (2.0 Ci, 50 Ci/mmol) in diglyme (0.9 ml) at 80 C for 15 hours afforded **2** (R = ^{3}H) (103.5 mCi, 8.87 Ci/mmol) in 29% chemical yield and 21% radiochemical yield (Scheme 1).

As 1β,2β-epoxy-20(S)-(3-methylbutyloxy)-pregna-5,7-diene-3β-ol (**4**)[8] was not cleaved under the same conditions used in excess $NaBH_4$ reduction, the stereospecificity seems to be attributable to the participation of 3β-hydroxy substituent as illustrated in **5**. Therefore, only the epoxides possessing trans hydroxy substituents could be reduced in this condition, and introduced tritiide or hydride must be approached from the same site as hydroxy substituent in a stereoselective manner (Scheme 2).



Scheme 2

This procedure is generally applicable in the preparation of 1α,3β-dihydroxy-2β-tritiated steroidal compounds, which are a very important moiety in vitamin D chemistry.

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