Monatshefte für Chemie 135, 1137–1141 (2004)
DOI 10.1007/s00706-004-0197-3

Monatshefte für Chemie
Chemical Monthly
Printed in Austria

Synthesis of 6-Hydroxy Derivatives of Steroidal Hormones by SeO_2 Mediated Oxidation

Rainer Strommer[1], **Claudia Hödl**[1], **Wolfgang Strauss**[2], **Reinhard Sailer**[2], **Ernst Haslinger**[1], **Hans Wolfgang Schramm**[1], and **Christoph Seger**[1,*,†]

[1] Institute of Pharmaceutical Chemistry and Pharmaceutical Technology, University of Graz, A-8010 Graz, Austria
[2] Institute for Laser Technology in Medicine and Metrology, University of Ulm, D-89081 Ulm, Germany

Received March 2, 2004; accepted March 8, 2004
Published online June 30, 2004

Summary. Selenium dioxide oxidation of molecules with cyclopentanoperhydrophenanthrene skeleton and allylic moieties, such as the well known human steroidal hormones progesterone and testosterone enables the syntheses of potential active 6β-hydroxysteriods.

Keywords. Selenium dioxide; NMR; Progesterone; Testosterone.

Introduction

A wide variety of steroidal hormone derivatives have been synthesized over the past decades, many of them by introducing oxygen bearing functionalities in a highly stereoselective and regioselective manner. Because of their biological importance [1, 2], there has been an immense amount of work on the selective oxidation of steroid hormones. Nevertheless, only little attention has been paid to the introduction of functionalities into position 6 of the steroidal backbone. *Terasawa et al.* [3] oxidised a variety of halogenated corticosteroids with SeO_2 under different reaction conditions and prepared regioselectively the corresponding 6-hydroxydiastereomers. In this case, the stereospecifity of the reaction was driven by the steric influence of halogen substituents in position 9 directing the new hydroxy group in opposite direction to the halogen atom. We have shown [4], that

* Corresponding author. E-mail: christoph.seger@uibk.ac.at
† Current address: Institute of Pharmacy, Department of Pharmacognosy, University of Innsbruck, A-6020 Innsbruck, Austria

in the case of mifepristone [5] a member of the steroidal 4-ene-3-one derivative family, the well known and thoroughly documented selenium dioxide [6–8] promoted allylic oxidation of double bonds [9–11] leads to 6α-hydroxy and 6β-hydroxy mifepristone. Based on these findings we decided to investigate the general scope of this reaction for similar compounds and present here the reaction schemes for two prominent biogenic members – progesterone and testosterone.

Results and Discussions

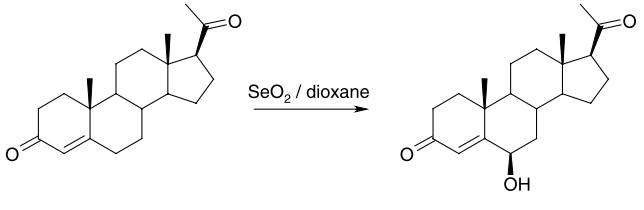
The allylic hydroxylation reaction was carried out by refluxing a suspension of the steroid under investigation with SeO_2 yielding a mixture of desired derivatives and by-products in moderate yields. In accordance with the proposed reaction mechanism [12, 13], 6β-hydroxy compounds are formed predominantly in the cases of progesterone and testosterone.

Progesterone

The reaction of progesterone (**1**) with SeO_2 proved that a selective introduction of a hydroxy group on position 6 of the steroid skeleton is possible. The main product, 6β-hydroxyprogesterone (**2**) (Scheme 1), was isolated from the reaction mixture and purified by preparative HPLC and its structure was determined by NMR spectroscopy. The resonance of the olefinic proton H-4 shifted to lower field compared to the resonance of the proton in the starting material progesterone (from 5.72 to 5.80 ppm), and the CH_2 signal of C-6 was replaced by a downfield shifted CH signal (72.9 ppm). The configuration of the new stereogenic centre in position 6 was determined from the coupling constant between H-6 and the H-7 protons. The signal of H-6 (4.38 ppm) showed no significant line splitting due to scalar coupling, thus excluding an axial coupling partner. Therefore this proton is located in the pseudoequatorial α position at C-6.

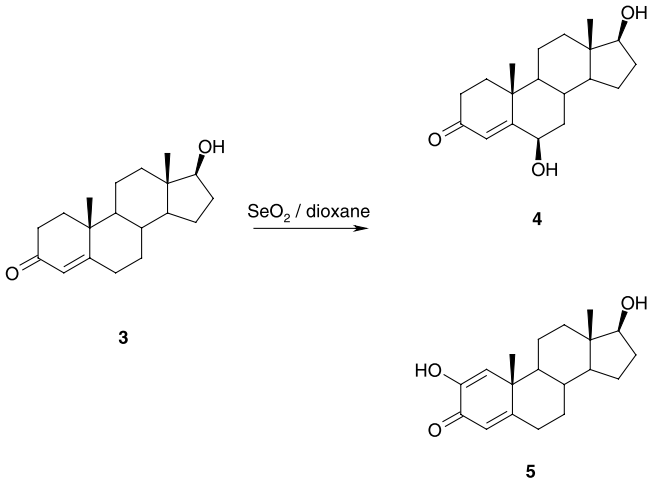
Testosterone

Oxidation of testosterone (**3**) with SeO_2 yielded two major products in the ratio of 2:1 (Scheme 2). The main product was 6-hydroxytestosterone (**4**) and the NMR spectrum showed features similar to **2**, thus allowing to assing the stereochemistry of the introduced hydroxy function as β, too. The structure of the other isolated compound **5** was determined to be 2,17β-dihydroxy-1,4-androstadiene-3-on. An olefinic singulett at



1 2

Scheme 1



Scheme 2

7.05 ppm together with two carbon resonances at 126.8 (s, C-2) and 151.8 ppm (d, C-1) allowed to determine the position of the introduced double bond unequivocally to positions 1 and 2. An independent proof was possible by observing an N*O*E correlation signal between H-1 (7.15 ppm) and both H-11 (1.60 ppm) and CH_3-19 (1.18 ppm).

In conclusion, these results demonstrate that SeO_2 oxidation enables selective hydroxylation in position 6 of certain steroidal 4-en-3-ones.

Experimental

Mp: *Büchi/Tottoli* melting point apparatus; analytical TLC: precoated Al-backed 0.2 mm silicagel 60 F_{254} plates (E. Merck), mobile phase: cyclohexane:*Ac*O*Et* = 1:1; UV/Vis: Shimadzu UV-160 A (*Me*OH); IR: Perkin Elmer 1720-X (KBr); MS: Varian MAT-711 (70 eV); NMR: Varian Unity Inova 400 NMR spectrometer equipped with either tuneable broad band inverse probe tuned to ^{13}C for the inverse detected gradient selected 2D experiments or dual $^{1}H/^{13}C$ probe for the 1D experiments. The measurements were carried out in $CDCl_3$. The sample temperatures were kept at 300 K. Standard commercial pulse sequence programs were used. HMBC experiments were optimized for a long range coupling constant of 8 Hz. Shift values were referred to *TMS*.

Selenium Dioxide Oxidation of Allylic Steroidal 4-En-3-ones

Allylic steroidal 4-en-3-ones (0.25 mmol) were dissolved in 50 cm^3 of dry dioxane in a 100 cm^3 three-neck flask with a reflux condenser. After addition of SeO_2 (33 mg) the mixture was stirred at 80°C under Ar until the slightly yellowish reaction mixture became red. The reaction was stopped after 20 h by addition of 30 cm^3 of 5% aqueous KOH. Thereafter, the solution was extracted four times with *Ac*O*Et*, and the combined organic layers were washed with H_2O to neutral *pH*, dried over Na_2SO_4, and evaporated. A yellowish oil was obtained, which was further purified by column chromatography on silica (mobile phase: cyclohexane:*Ac*O*Et* = 1:1). Separation of the single fractions was performed by preparative HPLC (Labomatic-HD-200/high pressure metering pump) on a Bischoff prep 3250, Prontoprep-120-10 C 18 HS 10 µm HPLC column (l = 500 mm, d = 32 mm); mobile phase: H_2O:acetonitril = 65:35, flow: 25 cm^3/min, detection: preparative UV-detector, 277 nm (Labocord 700 UV-VIS-spectrophotometer), pressure: 75 bar, injection volume: 5 cm^3, amount of substance: up to 50 mg. Fractions containing desired products were lyophilized.

6β-Hydroxy-4-pregnen-3,20-dion (**2**, $C_{21}H_{30}O_3$)

Yield 23%; mp 172°C; ^{1}H NMR ($CDCl_3$): $\delta = 0.68$ (s, 18-CH_3), 0.95 (m, 9-H), 1.15 (m, 14-H), 1.24 (m, 7α-H), 1.30 (m, 15β-H), 1.37 (s, 19-CH_3), 1.41 (m, 12α-H), 1.50 (m, 11α-H), 1.60 (m, 11β-H), 1.66 (m, 16β-H), 1.70 (m, 15α-H), 1.70 (m, 1α-H), 2.00 (m, 8-H), 2.01 (m, 7β-H), 2.08 (m, 1β-H), 2.10 (m, 12β-H), 2.15 (s, 21-CH_3), 2.20 (m, 16β-H), 2.39 (m, 2α-H), 2.50 (m, 2β-H), 2.55 (m, 17α-H), 4.38 (m, 6α-H), 5.80 (s, 4-H) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 13.2$ (C-18), 19.4 (C-19), 20.8 (C-11), 22.7 (C-16), 24.2 (C-15), 29.6 (C-8), 31.3 (C-21), 34.0 (C-2), 37.0 (C-1), 37.8 (C-10), 38.3 (C-7), 38.5 (C-162), 43.8 (C-13), 53.4 (C-18), 55.9 (C-14), 63.4 (C-17), 72.9 (C-6), 126.3 (C-4), 167.7 (C-5), 199.9 (C-3), 209.0 (C-20) ppm; IR (KBr): $\bar{\nu} = 3421$ (m, υ_{OH}), 2926 (w, υ_{CC}), 2866 (w, υ_{CH}), 1699, 1681 (s, υ_{CO}) cm^{-1}; UV/Vis (*Me*OH): $\lambda_{max} = 230$ nm; $R_f = 0.25$; MS (70 eV): m/z (%) = 330.5 (M^+, 100), 315 (28), 227 (20), 152 (55), 91 (35).

6β,17β-Dihydroxy-4-androsten-3-on (**4**, $C_{19}H_{28}O_3$)

Yield 24%; mp 205°C; ^{1}H NMR ($CDCl_3$): $\delta = 0.80$ (s, 18-CH_3), 0.92 (m, 9-H), 0.98 (m, 14-H), 1.10 (m, 12α-H), 1.22 (m, 7α-H), 1.37 (m, 15β-H), 1.39 (s, 19-CH_3), 1.39 (m, 16-H), 1.45 (m, 11β-H), 1.55 (m, 11α-H), 1.62 (m, 15α-H), 1.72 (m, 1β-H), 1.89 (m, 12β-H), 1.99 (m, 8-H), 2.00 (m, 7β-H), 2.03 (m, 1α-H), 2.04 (m, 16-H), 2.39 (m, 2α-H), 2.56 (m, 2β-H), 3.65 (t, $J = 6.7$ Hz, 17α-H), 4.36 (m, 6α-H), 5.81 (s, 4-H) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 11.1$ (C-18), 19.6 (C-19), 20.6 (C-11), 23.3 (C-15), 29.8 (C-8), 30.5 (C-16), 34.2 (C-2), 36.4 (C-12), 37.1 (C-1), 38.1 (C-7), 38.2 (C-10), 42.9 (C-13), 50.5 (C-14), 53.8 (C-9), 73.1 (C-6), 81.7 (C-17), 126.3 (C-4), 168.0 (C-5), 200.1 (C-3) ppm; IR (KBr): $\bar{\nu} = 3411$ (m, OH-valence), 2938 (m, υ_{CC}), 1663 (s, υ_{CO}) cm^{-1}; UV/Vis (*Me*OH): $\lambda_{max} = 236$ nm; $R_f = 0{,}19$; MS (70 eV): m/z (%) = 304.2 (M^+, 100), 289 (32), 152 (10), 91 (24), 57 (68).

2,17β-Dihydroxy-1,4-androstadien-3-on (**5**, $C_{19}H_{26}O_3$)

Yield 11%; mp 174°C; ^{1}H NMR ($CDCl_3$): $\delta = 0.78$ (s, 18-CH_3), 0.88 (m, 9-H), 0.90 (m, 14-H), 0.95 (m, 12-H), 0.98 (m, 7-H), 1.18 (s, 19-CH_3), 1.35 (m, 15-H), 1.44 (m, 16-H), 1.52 (m, 11-H), 1.56 (m, 12-H), 1.60 (m, 11-H), 1.62 (m, 8-H), 1.80 (m, 15-H), 1.96 (m, 7-H), 2.05 (m, 16-H), 2.42 (m, 6-H), 3.54 (m, 17-H), 6.09 (s, 4-H), 7.15 (s, 1-H) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 10.8$ (C-18), 18.6 (C-19), 22.4 (C-11), 23.3 (C-15), 30.3 (C-16), 32.2 (C-6), 33.0 (C-7), 35.3 (C-8), 36.2 (C-12), 43.0 (C-13), 46.2 (C-10), 50.2 (C-14), 53.0 (C-9), 81.4 (C-17), 121.8 (C-4), 126.8 (C-2), 151.8 (C-1), 170.6 (C-5), 183.6 (C-3) ppm; IR (KBr): $\bar{\nu} = 3447$ (b, υ_{OH}), 2935 (m, υ_{CC}), 1644 (m, υ_{CO}) cm^{-1}; UV/Vis (*Me*OH): $\lambda_{max} = 215$, 257 nm; $R_f = 0{,}17$; MS (70 eV): m/z (%) = 302.2 (M^+, 100), 286 (16), 122 (95), 91 (35), 57 (24).

Acknowledgements

This work was supported by the Interdisciplinary Center of Medical Research (IZKF, project C-3) of the University of Ulm (grant number 01 KS 9605/2, Federal Ministry of Education and Research). The authors gratefully thank Dr. *O. Kunert* for measuring the NMR spectra.

References

[1] Herrmann W, Wyss R, Riondel A, Philibert D, Teutsch G, Sakiz E, Baulieu E (1982) Cr Acad Ser (Paris) **294**: 933
[2] Bertagna X, Bertagna C, Luton JP, Husson MJ Girard F (1984) J Clin Endocr Metab **59**: 25
[3] Terasawa T, Okada T (1991) Synth Commun **21**: 307
[4] Strommer R, Strauß W, Sailer R, Emmert H, Steiner R, Reisinger E, Haslinger E, Schramm HW (2001) Monatsh Chem **132**: 387

[5] Teutsch G, Ojasoo T, Raynaud JP (1988) J Steroid Biochem **31**: 549
[6] Rabjohn N (1976) In: Dauben WG (ed) Organic Reactions, vol 24. Wiley, New York, p 261
[7] Dunn AD (1999) Org Prep Proc Int **31**: 120
[8] Hong-Seok K, Jung-Ho K (2001) Bull Korean Chem Soc **22**: 1390
[9] Meystre C, Frey H, Voser W, Wettstein A (1956) Helv Chim Acta **39**: 734
[10] Rosenheim O, Starling WW (1937) J Chem Soc 377
[11] Shibuya K (1994) Synth Commun **24**: 2923
[12] March J (1992) Reactions, Mechanisms and Structure. Wiley, New York, 698
[13] Fieser LF, Fieser M (1967) Reagents for Organic Synthesis, vol 1. Wiley, New York, p 992