J Chem Crystallogr (2014) 44:487–492
DOI 10.1007/s10870-014-0543-1

ORIGINAL PAPER

Synthesis, NMR Characterization and Crystal Structure of Methyl 3α,7α-Dihydroxy-12-oxo-5β-cholanate

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Received: 16 April 2014 / Accepted: 22 August 2014 / Published online: 2 September 2014

Abstract The crystal structure and NMR characterization of methyl 3α,7α-dihydroxy-12-oxo-5β-cholanate are described. The title compound which was obtained from methyl cholanate in a 3-step synthetic sequence that does not alter the starting chirality, crystallizes in the monoclinic system with P 2_1 space group. While despite the substitution pattern rings A, B and C adopt chair conformations, the 5 membered D ring, that bears the side chain attached to C-17, shows a twisted conformation on C-13–C-14. In the crystal array, classical hydrogen bond interactions O–H···H and intermolecular contacts C–H···O of hydrogen bond type are observed.

Keywords Cholic acid · Methyl 3α,7α-dihydroxy-12-oxo-5β-cholanate · NMR · Crystal structure

Introduction

Bile acids constitute a subfamily of steroid that has attracted intensive attention for nearly one century [1, 2]. In addition to their intrinsic biological activity [3], most of naturally occurring bile acids have been employed as starting materials in the synthesis of different compounds that includes sex and adrenocortical hormones [4, 5], brassinosteroids and their analogues [6, 7], quasinoids [8], juvenoid conjugates [9] and supramolecular hosts [10] among many others.

In connection with our program on the synthesis of potentially bioactive steroids, we have become interested in the synthesis and unambiguous structural characterization of bile acid derivatives bearing different functionality in the steroid framework. Herein we report on the NMR characterization and crystalline structure of methyl 3α,7α-dihydroxy-12-oxo-5β-cholanate.

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Experimental

General Conditions

Reactions were monitored by TLC on ALUGRAM® SIL G/UV254 plates from MACHEREY-NAGEL. Chromatographic plates were sprayed with a 1 % solution of vanillin in 50 % $HClO_4$ and heated until color developed. Chromatographic purifications were carried out in columns packed with silica gel 60 (size 0.04–0.063 mm). employing mixtures of hexane/ethyl acetate. Melting points were measured on a Melt-Temp II apparatus. Mass spectra were registered in a Thermo-Electron spectrometer model DFS (Double Focus Sector). NMR spectra were recorded in $CDCl_3$ solution in a Varian INOVA 400 MHz spectrometer using the solvent signals as references. NMR signal assignments were made with the aid of a combination of 2D homonuclear (^1H–^1H) and heteronuclear (^1H–^{13}C) correlation techniques, which included ^1H–^1H COSY, ^1H–^1H Nuclear Overhauser Effect Spectroscopy (NOESY), Heteronuclear Single Quantum Correlation (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC). All 2D NMR spectra were recorded using the standard pulse sequences and parameters recommended by the manufacturer and were processed employing the MestreNova NMR processing program (see http://mestrelab.com/).

Methyl 3α,7α-diacetoxy-12α-hydroxy-5β-cholanate (**2**). Pyridin (7.5 ml) and acetic anhydride (7.5 ml) were added

Table 1 ^{13}C NMR signals of compound **2–4** (δ ppm)

	2	3	4
C-1	34.5	34.5	35.3
C-2	26.7	26.5	30.4
C-3	74.1	73.5	71.6
C-4	34.8	34.8	39.6
C-5	42.1	40.4	41.1
C-6	31.3	31.3	34.9
C-7	70.8	70.4	67.9
C-8	38.1	37.8	39.3
C-9	28.2	37.8	36.9
C-10	34.4	35.5	35.8
C-11	28.6	37.5	37.7
C-12	72.7	213.9	214.8
C-13	46.6	57.0	56.9
C-14	40.9	53.0	53.3
C-15	23.0	23.7	23.8
C-16	27.3	27.3	27.5
C-17	47.2	46.3	46.3
C-18	12.5	11.5	11.5
C-19	22.5	22.1	22.2
C-20	35.0	35.4	35.6
C-21	17.4	18.5	18.5
C-22	30.8	30.4	30.5
C-23	31.0	31.1	31.2
C-24	174.6	174.5	174.7
CH_3 acetyl	21.5, 21.7	21.4, 21.4	–
C=O acetyl	170.5, 170.6	170.1, 170.6	–
OCH_3	51.5	51.5	51.4

to a suspension of methyl cholate (**1**) (6.4 g, 15 mmol) in benzene (30 ml) and the mixture was stirred for 24 h before pouring into water (300 ml). Ethyl acetate (100 ml) was added and the organic layer was washed with water (4 × 30 ml) and with 50 ml portions of 10 % aq. $CuSO_4$ (until no change in the color of $CuSO_4$ was observed), washed again with water (3 × 50 ml), dried (anh. Na_2SO_4) and evaporated to afford 5.17 g (68 %) of the desired diacetate (**2**) after purification in a chromatographic column packed with silica gel employing hexane/ethyl acetate 10/1 to 4/1 as eluent. Mp 183–185 °C (*from ethyl acetate*) Lit. 185–187 °C [11]. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.89 (dd, $J = 5.9$, 3.1 Hz, 1H), 4.58 (tt, $J = 11.4$, 4.4 Hz, 1H), 4.00 (s, 1H), 3.65 (s, 3H, CH_3O), 2.37 (ddd, $J = 15.0$, 10.0, 5.0 Hz, 1H), 2.28–2.16 (m, 2H), 2.06 (s, 3H), 2.02 (s, 3H), 0.97 (d, $J = 6.3$ Hz, 3H), 0.92 (s, 3H), 0.68 (s, 3H). For ^{13}C NMR (100.5 MHz) see Table 1.

Methyl 3α,7α-diacetoxy-12-oxo-5β-cholanate (**3**) An excess of Jones reagent was added dropwise to a solution of compound **2** (506 mg, 1 mmol) in acetone (60 ml) at 0 °C and the mixture was stirred until starting material was consumed and the color of the oxidant remained. Isopropanol (3 ml) was added, the mixture was stirred for 15 min, poured into cold aqueous NaCl solution and extracted with ethyl acetate (3 × 30 ml). The organic layer was washed with saturated NaCl solution (2 × 30 ml), dried (anh. Na_2SO_4) and evaporated to afford 978 mg (97%) of the desired ketone. Mp 174–175 °C (*from ethyl acetate*) Lit. 177–179 °C [11]. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.96 (d, $J = 2.7$ Hz, 1H), 4.60–4.49 (m, 1H), 3.64 (s, 3H, CH_3O), 2.49 (t, $J = 12.6$ Hz, 1H), 2.01 (s, 3H), 1.99 (s, 3H), 1.01 (s, 3H), 1.00 (s, 3H), 0.82 (t, $J = 8.0$ Hz, 3H). For ^{13}C NMR (100.5 MHz) see Table 1.

Methyl 3α,7α-dihydroxy-12-oxo-5β-cholanate (**4**). The acetylated ketone (**3**) (1.008 g, 2 mmol) was stirred in a 10 % $NaOCH_3$ solution (8 ml) for 24 h at room temperature. After neutralization with concentrated HCl, water (20 ml) was added and the mixture was extracted with ethyl acetate (2 × 25 ml). The organic layer was washed with saturated NaCl solution, dried (anh. Na_2SO_4) and evaporated to afford the desired dihydroxylated ketone. Chromatographic purification in a column packed with silica gel employing hexane/ethyl acetate 10/1 to 4/1 as eluent afforded 722 mg (86 %). Mp 157–158C °C (*from ethyl acetate*) Lit. 154.5–161.5 °C [12]. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.91 (d, $J = 1.9$ Hz, 1H), 3.64 (s, 3H, CH_3O), 3.41 (qd, $J = 11.2$, 5.7 Hz, 1H), 2.44 (dd, $J = 20.3$, 8.0 Hz, 1H), 1.00 (s, 3H), 0.97 (s, 3H), 0.83 (d, $J = 6.6$ Hz, 3H). For ^{13}C NMR (100.5 MHz) see Table 1.

X-ray Crystallography

A suitable single crystal of compound **4** grown by slow evaporation of a hexane/ethyl acetate solution was mounted on a glass fiber and crystallographic data were collected with an Oxford Diffraction Gemini "A" diffractometer with a CCD area detector ($\lambda_{MoK\alpha} = 0.71073$ Å, monochromator: graphite) at 130 K. Unit cell constants were determined with a set of three runs of 15 frames (1° in ω). The collected data set consisted of 3 runs of 325 frames of intensity (1° in ω), and a crystal-to-detector distance of 55.00 mm. The double pass method of scanning was used to exclude any noise. The collected frames were integrated by using an orientation matrix determined from the narrow frame scans.

CrysAlisPro and CrysAlis RED software packages [13] were used for data collection and data integration. Analysis of the integrated data did not reveal any decay. Final cell constants were determined by a global refinement of 2,193 reflections ($\theta < 26.0°$). Collected data were corrected for absorption effects by using an Analytical numeric absorption

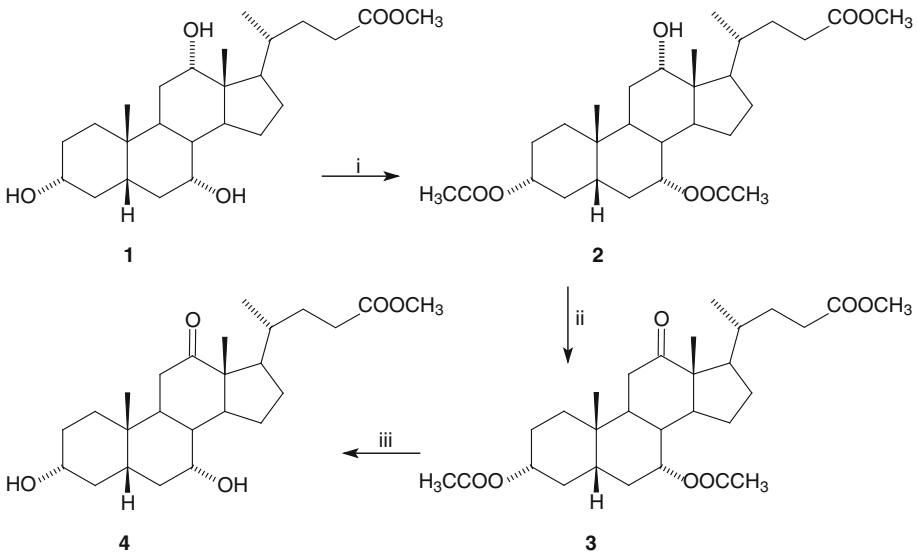
Table 2 Crystal data and structure refinement for **4**

Empirical formula	$C_{25}H_{40}O_5$
Formula weight	420.57
Temperature	130(2) K
Wavelength (MoK α)	0.71073 Å
Crystal system	Monoclinic
Space group	P 2_1
Unit cell dimensions	a = 9.2238(6) Å
	b = 7.4909(6) Å
	c = 17.2270(13) Å
	β = 103.865(7)°
Volume	1,155.6(2) $Å^3$
Z	2
Density (calculated)	1.209 Mg/m^3
Absorption coefficient	0.082 mm^{-1}
F(000)	460
Crystal size	0.23 × 0.09 × 0.07 mm^3
Theta range for data collection	3.546–25.347°
Index ranges	$-11 \le h \le 11$, $-9 \le k \le 8$, $-20 \le l \le 17$
Reflections collected	8,414
Independent reflections	3,861 [R(int) = 0.0707]
Completeness to theta = 25.242°	99.7 %
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3,861/1/281
Goodness-of-fit on F^2	1.043
Final R indices [$I > 2\sigma(I)$]	R1 = 0.0661, wR2 = 0.1466
R indices (all data)	R1 = 0.0911, wR2 = 0.1674
Largest diff. peak and hole	0.201 and −0.294 e·$Å^{-3}$

correction [14] using a multifaceted crystal model based on expressions upon the Laue symmetry using equivalent reflections. Structure solution and refinement were carried out with the programs SHELXS97 and SHELXL97 respectively [15]. ORTEP-3 for Windows [16] was employed for molecular graphics and the software used to prepare material for publication was WinGX [17].

Full-matrix least-squares refinement was carried out by minimizing $(Fo^2 - Fc^2)^2$. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms in hydroxyl groups were localized on difference Fourier maps and refined as riding on their parent atoms, with O–H distance = 0.86 Å and U_{iso} (H) = 1.5 U_{eq}(O). Hydrogen atoms attached to carbon atoms were placed in geometrically idealized positions and refined as riding on their parent atoms, with C–H = 0.98–1.00 Å with U_{iso} (H) = 1.2U_{eq}(C) for methylene and methyne groups respectively, and U_{iso} (H) = 1.5 U_{eq}(C) for methyl group. Crystal data and experimental details of the structure determination are listed in Table 2.

The absolute configuration of the title compound (**4**) can be assumed without risk, as that known for the starting material considering that the synthetic transformations carried out do not affect the chirality in the naturally occurring steroid framework. Additionally, the assumed absolute configuration was corroborated by analysis of 1,831 Bijvoet pairs. Although the refined Flack parameter x = −2.3 (10) [18] is not definitive, the Hooft parameter y = 0.5 (2) and Hooft P2(true) = 0.506 [19] are strong indicators that the reported configuration is correct.



Scheme 1 Synthetic sequence. (*i*) $Ac_2O/pyr/C_6H_6$, R.T; (*ii*) CrO_3/H_2SO_4/acetone, R.T; (*iii*) $NaOCH_3$/MeOH, R.T

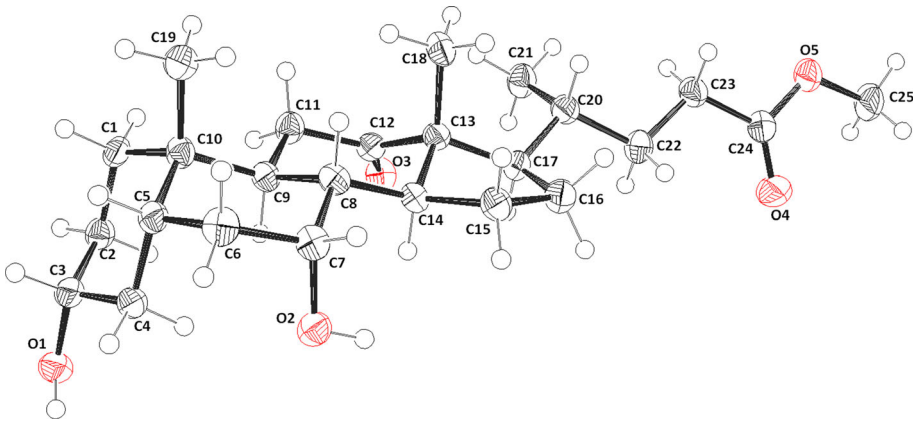


Fig. 1 Crystal structure of compound **4** with the thermal ellipsoids drawn at 50 % of probability

Crystallographic data have been deposited at the Cambridge Crystallographic Data Center as supplementary material number CCDC 996968. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK. E-mail:deposit@ccdc.cam.ac.uk.

Results and Discussion

Partial acetylation of methyl cholate (**1**) afforded the diacetate **2** in moderate yield after column chromatography purification. The Jones oxidation of the hydroxyl function at C-12 in compound **2** produced nearly quantitative yield of the diacetylated ketone **3** that was hydrolyzed to the title compound **4** (Scheme 1).

Crystal Structure Discussion

The asymmetric unit of compound **4** corresponds to one molecule of methyl 3α,7α-dihydroxy-12-oxo-cholanate. The steroid ABCD fused framework bears axial methyl groups attached to C-10 and C-13, a carbonyl function at C-12 and *cis* A/B, *trans* B/C and *trans* C/D ring junctions. The equatorial hydroxy group attached to C-3 does not disturb the *A* ring, which presents a perfect chair conformation [Cremer and Pople parameters: [20] Q = 0.567(5) Å, θ = 177.0(5), φ = 268(11)°, if the calculation is done following the sequence C1–C10–C5–C4–C3–C2 (clockwise)]; all asymmetric parameters less than 5.3 (5)° [21]. Rotational symmetry is dominant; a pseudo-C2 axis bisects the C-3–C-4 bond with asymmetric parameters ΔC_2(C-3–C-4) = 5.3 (5)° and ΔC_s (C-3) = 2.6 (4)°, with a weighted average absolute torsion angle of 55.35 (20)° and weighted average ring bond distance of 1.5297(29) Å. In the *B* ring, the axial hydroxyl group bonded to C-7 slightly disturbs the chair conformation [Cremer and Pople parameters: Q = 0.557(5) Å, θ = 6.9 (5), φ = 266(4)°, if the calculation is done following the sequence C5–C10–C9–C8–C7–C6 (clockwise)]; all asymmetric parameters less than 7.0 (6)°, with asymmetric parameters ΔC_2(C-6–C-7) = 2.0 (5)° and ΔC_s(C-7) = 3.8 (4)°, with a weighted average absolute torsion angle of 54.10(22)° and weighted average ring bond distance of 1.541(3) Å. The

Table 3 Selected bond lengths and angles for compound **4**

Bond	Distance (Å)
C1–C2	1.527(7)
C1–C10	1.540(7)
C2–C3	1.497(7)
C3–O1	1.441(6)
C7–O2	1.429(6)
C10–C19	1.545(7)
C12–O3	1.229(6)
C17–C20	1.531(7)
C23–C24	1.486(7)
C24–O4	1.200(6)
C24–O5	1.347(6)
C25–O5	1.435(6)
Angles	**(°)**
C2–C1–C10	114.0(4)
O1–C3–C2	108.2(4)
O1–C3–C4	111.0(4)
O2–C7–C6	106.6(4)
O2–C7–C8	113.2(4)
O3–C12–C13	122.5(4)
O3–C12–C11	121.4(5)
C20–C17–C13	117.3(4)
C20–C17–C16	113.4(4)
O4–C24–O5	122.6(4)
O4–C24–C23	126.4(5)
O5–C24–C23	111.0(4)
C24–O5–C25	115.7(4)

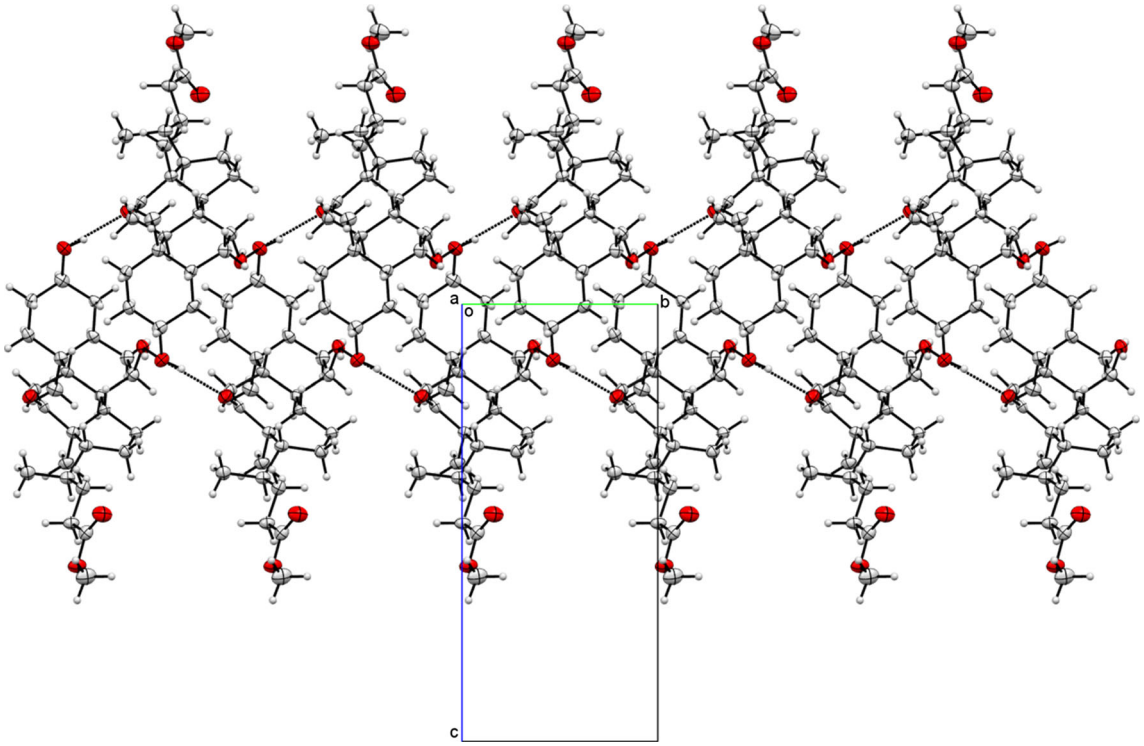


Fig. 2 Crystal array of compound **4**; view along the *a* axis; with perspective to plane formed by *b–c* axes emphasizing the *C(8)*, *C(10)*, $C_2^2(10)$ and $R_2^2(18)$ motifs

presence of the carbonyl function at C-12 does not disturb the conformation of ring C that assumes an almost perfect chair shape [Cremer and Pople parameters Q = 0.579(5) Å, θ = 12.5 (5) and ϕ = 253 (2)° if the calculation is done following the sequence C8–C14–C13–C12–C11–C10 (counter clockwise)], with asymmetric parameters: ΔC_2(C-8–C-9) = 11.5(5), ΔC_2(C-11–C-12) = 16.9(5), ΔC_s(C-8) = 13.4(4), ΔC_s(C-9) = 2.5(4)° with a weighted average absolute torsion angle of 55.52 (2)° and weighted average ring bond distance of 1.532 (3) Å.

The five-membered D-ring that bears the side chain attached to position C-17 shows a twisted conformation on C-13–C-14 [Cremer and Pople parameters: q2 = 0.447(6) Å and ϕ_2 = 197.9(7)°] with asymmetric parameters [22]: Δ = 0.2, τ_m = 45.2(3), ΔC_s(C-13) = 17.2(5), ΔC_s(C-14) = 16.9(5) and ΔC_2(C-13–C-14) = 0.2(3)°, with a weighted average absolute torsion angle of 30.91(21)° and weighted average ring bond distance of 1.545(3) Å. Figure 1 shows the crystal structure of compound **4**. Selected bond lengths and angles are presented in Table 3.

In the crystal structure there are classical hydrogen bond interactions O–H···H and intermolecular contacts C–H···O of hydrogen bond type. The hydrogen bond O-2–H-2O···O-1 (1.97(7) Å, 158(6)°), O-1–H-1O···O-3 (1.99(7) Å, 172(7)°) with symmetry operation-x+1, y+1/2, −z forms the *C(8)*, *C(10)*, $C_2^2(10)$ and $R_2^2(18)$ motifs [23] and together with the intermolecular C-2–H-2B···O2 2.62 Å, C-4–H-4A···O2 2.37 Å interactions lead to infinite laminar array with base vector [0 1 0] along the *b-c* plane. Figure 1 shows the crystal array of compound **4** (Fig. 2).

Acknowledgments The authors acknowledge the financial support provided by Dirección General de Asuntos del Personal Académico (Project DGAPA-IN211714) and the Faculty of Chemistry (PAPIIT-5000-9063). Thanks are due to Rosa I. del Villar Morales (USAI-UNAM) for recording NMR spectra and to Dr. Carlos Cobas from Mestrelab® for assistance with the MestreNova NMR processing program.

References

1. Fieser L, Feiser M (1959) Steroids. Reinhold, New York and references therein
2. Setchell KDR, Kritchevsky D, Nair PP (1971) The bile acids, vol 1. Plenum, New York, pp 1–25 249–283
3. Montse MJ, Marin JGC, Antelo A, Vázquez-Tato J (2009) World J Gastroenterol 21(15):804–816
4. Lemos TLG, McChesney JD (1990) J Nat Prod 53:152–156
5. Hirschmann R (1992) Steroids 57(12):579–592
6. Zhou W-S, Huang L-F (1992) Tetrahedron 48:1837–1852
7. Perez-Gil R, Iglesias-Arteaga MA, Perez-Martinez C, Coll-Manchado F, Coll-Garcia D, Rosado A (1998) Eur J Org Chem 2405–2407
8. Dias JR, Nassim B (1980) Steroids 35:405–417
9. Jurček O, Wimmer Z, Svobodová H, Bennettová B, Kolehmainen K, Drašar P (2009) Steroids 74:779–785

10. Tamminen J, Kolehmainen E (2001) Molecules 6:21–46
11. Fieser LF, Rajagopalan S (1950) J Am Chem Soc 72:5530–5536
12. Chang F (1979) J Org Chem 44(25):4567–4572
13. Oxford Diffraction (2009) CrysAlis CCD and CrysAlis Red. Oxford Diffraction, Abingdon
14. Clark RC, Reid JS (1995) Acta Cryst A51:887–897
15. Sheldrick GM (2008) Acta Cryst A64:112–122
16. Farrugia LJ (1997) J Appl Crystallogr 30:565
17. Farrugia LJ (1999) J Appl Crystallogr 32:837–838
18. Flack HD (1983) Acta Cryst A 39:876–881
19. Hooft RWW, Straver LH, Spek AL (2008) J Appl Crystallogr 41:96–103
20. Cremer D, Pople JA (1975) J Am Chem Soc 97:1354–1358
21. Duax WL, Weeks CM (1976) Rohrer DC. In: Eliel EL, Allinger N (eds) Topics in stereochemistry, vol 2. Wiley, New York, pp 271–283
22. Altona C, Geise HJ, Romers C (1968) Tetrahedron 24:13–32
23. Etter MC (1990) MacDonald JC. Acta Cryst B 46:256–262