

SYNTHESIS OF DEUTERIUM AND TRITIUM LABELLED 7-HYDROXY- AND 7-OXOSTEROLS

H. Schabdach, J. Schröder, K. Seifert

University of Bayreuth, Organic Chemistry I/2, NW II, D-95440 Bayreuth, Germany

SUMMARY

Deuterium and tritium 3,7-bis-labelled 7-hydroxysterols **3** - **5** were prepared by reduction of the 3,7-dioxosterols **1**, **2** with NaB^2H_4 and NaB^3H_4 . Regioselective oxidation of the deuterium 3,7-bis-labelled 7-hydroxysterols **3**, **4** with CrO_3/DMP led to the 3-labelled 7-oxosterols **6**, **7**. The 3,7-dioxosterols **1**, **2** were synthesized starting from cholesterol and β -sitosterol.

Key words: 7-Hydroxysterols, 7-oxosterols, specific labelling, deuterium, tritium, plant disease resistance inducing compounds

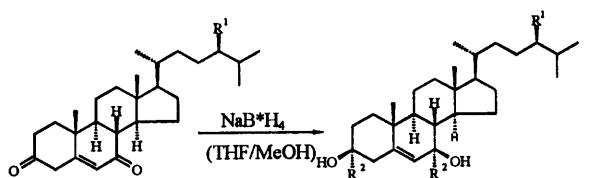
INTRODUCTION

The 7 β -hydroxysterols stigmast-5-ene-3 β ,7 β -diol, cholest-5-ene-3 β ,7 β -diol, the 7 α -hydroxysterols stigmast-5-ene-3 β ,7 α -diol, cholest-5-ene-3 β ,7 α -diol and the 7-oxosterols 3 β -hydroxystigmast-5-en-7-one, 3 β -hydroxycholest-5-en-7-one induce resistance toward the fungal pathogens *Puccinia striiformis* West. and *Puccinia hordei* Otth in barley and wheat [1]. Since we were interested in the investigation of the uptake, translocation and metabolism of **3** - **7** in barley and wheat, the compounds were regioselectively labelled in positions 3 and 7 with deuterium and tritium.

Cholest-5-ene-3 β ,7 β -diol shows significant cytotoxic activity toward tumor cells [2, 3] as well as inhibition of the HMG-CoA-reductase activity [4]. So specific double labelling (deuterium, tritium) of this compound is also of interest in the field of pharmacological and biochemical investigations.

SYNTHESIS

The 3,7-dioxosterols **1**, **2** were reduced with NaB^2H_4 and NaB^3H_4 in THF/MeOH to the deuterium and tritium labelled 7 β -hydroxysterols **3** - **5** in a yield of 87 - 90% besides approximately 5% of the epimeric 7 α -hydroxysterols, separated by TLC or column chromatography (CC) on silica gel. The specific radioactivity of [3 α ,7 α - $^3\text{H}_2$]-stigmast-5-ene-3 β ,7 β -diol (**5**) was 193 MBq/mmol. The introduction of deuterium in position 3/7 followed from the lack of the ^{13}C -NMR signals for C-3 (δ 71.4) / C-7 (δ 73.3) in a proton broad band-decoupled ^{13}C -NMR spectrum and the ^1H -NMR signals for H-3 α (δ 3.52) / H-7 α (δ 3.82, **3**, δ 3.83, **4**). The allylic oxidation of the ^2H -labelled 7-hydroxysterols **3**, **4** with CrO_3/DMP (3,5-dimethylpyrazole) [5] gave the 7-oxosterols **6**, **7**. [3 α ,4 β ,7 β - $^2\text{H}_3$]-Cholest-5-ene-3 β ,7 α -diol was previously prepared from cholest-4-en-3-one by equilibration in tert.-BuOH and tert.-BuOK followed by addition of [1 - ^2H]-acetic acid and reduction with NaB^2H_4 . The formed [3 α ,4 β - $^2\text{H}_2$]-cholest-5-en-3 β -ol was oxidized with chromic acid to [3 α ,4 β - $^2\text{H}_2$]-3 β -hydroxycholest-5-en-7-one reduced with LiAl^2H_4 to [3 α ,4 β ,7 β - $^2\text{H}_3$]-cholest-5-ene-3 β ,7 α -diol [6].



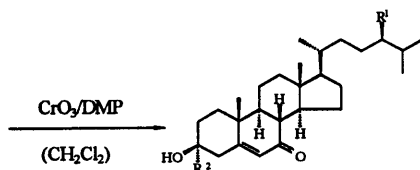
1 $\text{R}^1 = \text{H}$

2 $\text{R}^1 = \text{Et}$

3 $\text{R}^1 = \text{H}$ $\text{R}^2 = ^2\text{H}$ (90%)

4 $\text{R}^1 = \text{Et}$ $\text{R}^2 = ^2\text{H}$ (87%)

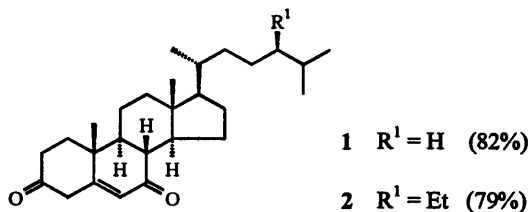
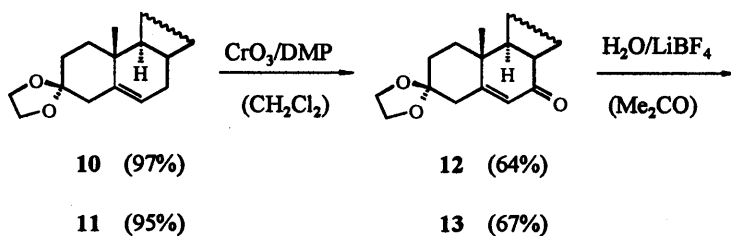
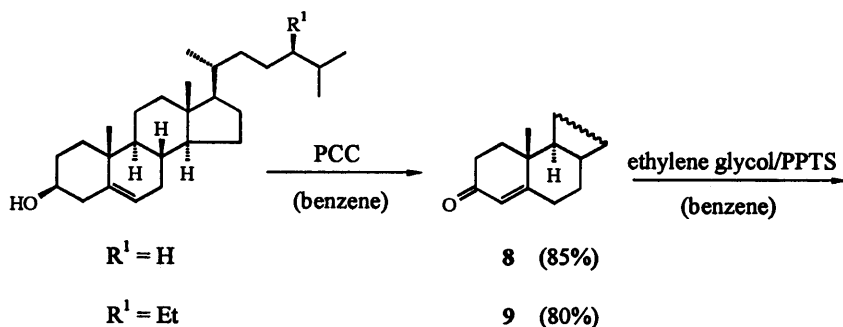
5 $\text{R}^1 = \text{Et}$ $\text{R}^2 = ^3\text{H}$ (90%)



6 $\text{R}^1 = \text{H}$ $\text{R}^2 = ^2\text{H}$ (63%)

7 $\text{R}^1 = \text{Et}$ $\text{R}^2 = ^2\text{H}$ (64%)

The preparation of cholest-5-ene-3,7-dione (**1**) by Jones oxidation of cholest-5-ene-3 β ,7 β -diol (without information on the yield) is described by Shoppee and Newman [7]. In our hands the Jones oxidation of 3 β -hydroxycholest-5-en-7-one gave **1** in a yield of only 20%. The oxidation of cholest-3,5-dien-7-one with performic acid gave a mixture of cholest-5-ene-3,7-dione (**1**), 3 α ,4 α -epoxycholest-5-en-7-one, 3 α ,4 β -dihydroxycholest-5-en-7-one and 3 α -formyloxy-4 β -hydroxycholest-5-en-7-one [8]. So the 3,7-dioxosterols **1**, **2** were synthesized in the following way. Cholesterol and β -sitosterol were oxidized with PCC (pyridinium chlorochromate) to cholest-4-en-3-one (**8**) and stigmast-4-en-3-one (**9**). Ketalization of the 3-oxo-group of **8**, **9** with ethylene glycol in the presence of PPTS [pyridinium p-toluenesulphonate] led in nearly quantitative yields to **10**, **11** which were oxidized with CrO₃/DMP to **12**, **13**. The ketals were hydrolyzed with LiBF₄/H₂O/MeCN



[9] to the 3,7-dioxosterols 1, 2. The assignments of the ^1H - and ^{13}C -NMR data for the sterols were based on ^1H -, ^1H ^1H COSY-, ^{13}C -, ^{13}C APT-, and ^1H ^{13}C COSY-experiments.

According to UV measurements in CHCl_3 [7] and EtOH [10] cholest-5-ene-3,7-dione (1) was described as 3-hydroxycholest-3,5-dien-7-one. The ^{13}C -NMR spectrum of 1 in CDCl_3 shows 2 CO signals at δ 206.3 (C-3) and 198.3 (C-7). So the 3,7-dioxosterol 1 can not exist in the enol form.

EXPERIMENTAL

[3 α ,7 α - $^3\text{H}_2$]-Stigmast-5-ene-3 β ,7 β -diol (5)

32.0 mg (0.075 mmol) of stigmast-5-ene-3,7-dione (2), dissolved in 1.2 ml of THF/MeOH (2:1) was added to 1.4 mg (0.037 mmol, 51 MBq) of NaB^3H_4 and stirred for 1 hr at room temperature. The reaction was stopped by addition of 500 μl of 3% hydrochloric acid. After 15 min 1.2 ml of CH_2Cl_2 was added and the mixture stirred for 45 min. The organic phase was separated and evaporated under reduced pressure to dryness. Preparative TLC (cyclohexane/EtOAc (1:1)) of the residue yielded 29 mg (90%) of 5 with a specific radioactivity of 193 MBq/mmol. The ^3H -labelled sterol 5 showed identical chromatographic behaviour with the authentic non-labelled compound

[3 α ,7 α - $^2\text{H}_2$]-Cholest-5-ene-3 β ,7 β -diol (3), [3 α ,7 α - $^2\text{H}_2$]-stigmast-5-en-3 β ,7 β -diol (4)

80 mg (0.20 mmol) of cholest-5-ene-3,7-dione (1) and 42 mg (1.0 mmol) of NaB^2H_4 were added to 10 ml of a 0.4 M solution of $\text{CeCl}_3 \cdot 7 \text{H}_2\text{O}$ in THF/MeOH (2:1). The mixture was stirred for 1 hr at room temperature. The reaction was stopped by addition of 10 ml of H_2O and 30 ml of Et_2O . The organic phase was separated, dried with Na_2SO_4 and evaporated. Column chromatography of the residue on 30 g of silica gel with cyclohexane/EtOAc (1:1) yielded 73 mg (90%) of 3. R_f 0.28, cyclohexane/EtOAc (1:1). m. p. 174 - 175°C. MS 70 eV m/z (rel. int.): 386 ($\text{M}^+ - \text{H}_2\text{O}$, 100). ^1H -NMR: δ 5.27 (d, $J = 1.4$, H-6), 0.67 (s, 3H-18), 1.05 (s, 3H-19), 0.99 (d, $J = 6.9$, 3H-21), 0.85 (d, $J = 6.5$, 3H-26, 3H-27).

The same procedure was used for the preparation of 4. The reduction of 70 mg (0.16 mmol) of 2 gave 62 mg (87%) of 4. R_f 0.28, cyclohexane/EtOAc (1:1). m. p. 169-171°C. MS 70 eV m/z (rel.

int.): 414 (M^+ -H₂O, 100), 285 (15), 190 (20). ¹H-NMR: δ 5.26 (d, J = 1.4, H-6), 0.67 (s, 3H-18), 1.03 (s, 3H-19), 0.90 (d, J = 6.5, 3H-21), 0.79 (d, J = 6.6, 3H-26*), 0.81 (d, J = 6.6, 3H-27*), 0.82 (m, 3H-29).

[3α-²H]-3β-Hydroxycholest-5-en-7-one (6), [3α-²H]-3β-hydroxystigmast-5-en-7-one (7)

50 mg (0.50 mmol) of CrO₃ (dried with P₄O₁₀) was suspended in 3 ml of anhydrous CH₂Cl₂ at -20°C under nitrogen and 48 mg (0.50 mmol) of DMP was added. The resulting mixture was stirred for 15 min at -20°C. 35 mg (0.087 mmol) of [3α,7α-²H₂]-cholest-5-ene-3β,7β-diol (3) was added and the reaction mixture stirred for 1h maintaining a temperature between -10 and -20°C. 30 ml of CH₂Cl₂ and 5 ml of 5% hydrochloric acid were added, the organic phase was separated, dried with Na₂SO₄ and evaporated. Column chromatography of the residue on 10 g of silica gel with cyclohexane/EtOAc (1:1) gave 22 mg (63%) of 6. R_f 0.36, cyclohexane/EtOAc (1:1). m. p. 169-170°C. MS 70 eV m/z (rel. int.): 401 (M^+ , 100), 368 (18), 288 (10), 193 (12). ¹H-NMR: δ 5.65 (d, J = 1.5, H-6), 2.24 (dd, J = 11.6, H-8), 0.64 (s, 3H-18), 1.16 (s, 3H-19), 0.89 (d, J = 6.5, 3H-21), 0.83 (d, J = 6.6, 3H-26, 3H-27).

Starting from 30 mg (0.069 mmol) of 4 19 mg (64%) of 7 was obtained. R_f 0.37, cyclohexane/EtOAc (1:1). m. p. 149-150°C. MS 70 eV m/z (rel. int.): 429 (M^+ , 100), 396 (10), 288(10), 193(20). ¹H-NMR: δ 5.65 (d, J = 1.5, H-6), 2.21 (dd, J = 11.0, H-8), 0.66 (s, 3H-18), 1.17 (s, 3H-19), 0.90 (d, J = 6.5, 3H-21), 0.78 (d, J = 6.6, 3H-26*), 0.80 (d, J = 6.6, 3H-27*), 0.83 (m, 3H-29).

Cholest-4-en-3-one (8), stigmast-4-en-3-one (9)

1.55 g (4.0 mmol) of cholesterol and 21.6 g (100 mmol) of PCC were dissolved in 70 ml of benzene and refluxed for 4h. 100 ml of Et₂O was added and the mixture filtered. The organic phase was treated with saturated NaCl-solution, dried with Na₂SO₄ and evaporated. The residue was chromatographed on 70 g of silica gel with cyclohexane/EtOAc (2:1) and yielded 1.31 g (85%) of 8. R_f 0.49 cyclohexane/EtOAc (3:1). m. p. 89-91°C ([11] m. p. 80-81.5°C) MS 70 eV m/z (rel. int.):

384 (M^+ , 20), 271 (10), 242 (30), 124 (100). 1H -NMR: δ 5.69, (d, $J = 1.1$, H-4), 0.68 (s, 3H-18), 1.15 (s, 3H-19), 0.88 (d, $J = 6.5$, 3H-21), 0.84 (d, $J = 6.6$, 3H-26, 3H-27).

The same procedure starting from 1.66 g (4.0 mmol) of β -sitosterol gave 1.32 g (80%) of 9. R_f 0.49 cyclohexane/EtOAc (3:1). m. p. 94-95°C ([12] m. p. 95-96.5°C). MS 70 eV m/z (rel. int.): 412 (M^+ , 10), 370 (30), 289 (35), 229 (50) 124 (100). 1H -NMR: δ 5.68 (d, $J = 1.5$, H-4), 0.68 (s, 3H-18), 1.14 (s, 3H-19), 0.88 (d, $J = 6.5$, 3H-21), 0.80 (d, $J = 6.5$, 3H-26*), 0.79 (d, $J = 6.5$, 3H-27*), 0.81 (m, 3H-29).

3,3-Ethylenedioxycholest-5-ene (10), 3,3-ethylenedioxytigmast-5-ene (11)

1.20 g (3.1 mmol) of cholest-4-en-3-one (8), 1 ml (18 mmol) of ethylene glycol and 150 mg (0.6 mmol) of PPTS were dissolved in 50 ml of benzene and refluxed for 12 h by means of a Dean - Stark trap. Benzene was evaporated, the residue dissolved in CH_2Cl_2 and washed with water. The organic phase was dried with Na_2SO_4 , evaporated and yielded 1.30 g (97%) of 10. R_f 0.55 cyclohexane/EtOAc (7:1). m. p. 131-132°C ([13] m. p. 134-135 °C). 1H -NMR: δ 2.08 (dd, $J = 14.2$, $J = 2.7$, H-4), 2.54 (m, H-4), 5.32 (m, H-6), 0.65 (s, 3H-18), 0.99 (s, 3H-19), 0.88 (d, $J = 6.5$, 3H-21), 0.83 (d, $J = 6.6$, 3H-26, 3H-27), 3.91 (m, 2H-1', 2H-2').

Starting from 1.25 g (3.0 mmol) of 9 1.32 g (95%) of 11 was obtained. R_f 0.55 cyclohexane/EtOAc (7:1). m. p. 135-137°C. 1H -NMR: δ 2.07 (dd, $J = 14.2$, $J = 2.8$, H-4), 2.53 (m, H-4), 5.32 (m, H-6), 0.65 (s, 3H-18), 0.99 (s, 3H-19), 0.90 (d, $J = 6.5$, 3H-21), 0.80 (d, $J = 6.5$, 3H-26*), 0.82 (d, $J = 6.5$, 3H-27*), 0.81 (m, 3H-29), 3.90 (m, 2H-1', 2H-2').

3,3-Ethylenedioxycholest-5-en-7-one (12), 3,3-ethylenedioxytigmast-5-en-7-one (13)

2.80 g (28 mmol) of CrO_3 (dried with P_4O_{10}) was suspended in 40 ml of anhydrous CH_2Cl_2 at -20°C under nitrogen and 2.69 g (28.0 mmol) DMP was added. The mixture was stirred for 20 min maintaining a temperature between -10 and -20°C. 1.20 g (2.8 mmol) of 3,3-ethylenedioxycholest-5-ene (10) was added and the mixture stirred for 5 h at -20°C. 100 ml of CH_2Cl_2 and 30 ml of 5% hydrochloric acid were added. The organic phase was separated, dried with Na_2SO_4 and evaporated.

The chromatography of the residue on 130 g of silica gel with cyclohexane/EtOAc (3:1) gave 806 mg (64%) of **12**. R_f 0.32 cyclohexane/EtOAc (7:1). m. p. 147-148°C ([14] m. p. 146-147.5°C) MS 70 eV m/z (rel. int.): 442 (M^+ , 10), 99 (100). $^1\text{H-NMR}$: δ 2.28 (dd, $J = 14.8$, $J = 2.8$, H-4), 2.63 (dd, $J = 14.8$, $J = 1.8$, H-4), 5.62 (d, $J = 1.8$, H-6), 2.18 (dd, $J = 10.9$, H-8), 0.64 (s, 3H-18), 1.16 (s, 3H-19), 0.88 (d, $J = 6.4$, 3H-21), 0.82 (d, $J = 6.5$, 3H-26, 3H-27), 3.92 (m, 2H-1', 2H-2').

Starting from 1.20 g (2.6 mmol) of **11** 832 mg (67%) of **13** were obtained. R_f 0.32 cyclohexane/EtOAc (7:1). m. p. 137-139°C. MS 70 eV m/z (rel. int.): 470 (M^+ , 10), 99 (100). $^1\text{H-NMR}$ δ 2.26 (dd, $J = 14.7$, $J = 2.9$, H-4), 2.62 (dd, $J = 14.7$, $J = 1.8$, H-4), 5.61 (d, $J = 1.8$, H-6), 2.18 (dd, $J = 10.6$, H-8), 0.64 (s, 3H-18), 1.16 (s, 3H-19), 0.88 (d, $J = 6.5$, 3H-21), 0.78 (d, $J = 6.7$, 3H-26*), 0.80 (d, $J = 6.7$, 3H-27*), 0.81 (m, 3H-29), 3.91 (m, 2H-1', 2H-2').

Cholest-5-ene-3,7-dione (1), stigmast-5-ene-3,7-dione (2)

100 mg (0.23 mmol) of **12**, 94 mg (1.00 mmol) of LiBF_4 , 0.5 ml of H_2O and 10 ml of MeCN were stirred for 24 h at 50°C. 10 ml of H_2O was added and the aqueous phase was extracted 2 x with 60 ml of CH_2Cl_2 . The CH_2Cl_2 phase was dried with Na_2SO_4 , evaporated and the residue chromatographed on 20 g of silica gel with cyclohexane/EtOAc (1:1). 74 mg (82%) of **1** was obtained. R_f 0.24 cyclohexane/EtOAc (3:1). m. p. 185-187°C ([7] m. p. 185°C). MS 70 eV m/z (rel. int.): 398 (M^+ , 100), 285 (20), 190 (35). $^1\text{H-NMR}$: δ 3.02 (d, $J = 15.1$, H-4), 3.44 (dd, $J = 15.1$, $J = 2.3$, H-4), 5.68 (d, $J = 2.3$, H-6), 2.55 (dd, $J = 11.6$, H-8), 0.68 (s, 3H-18), 1.39 (s, 3H-19), 0.89 (d, $J = 6.5$, 3H-21), 0.83 (d, $J = 6.6$, 3H-26, 3H-27).

100 mg (0.21 mmol) of **13** was hydrolyzed in the same way to 72 mg (79%) of **2**. R_f 0.24 cyclohexane/EtOAc (3:1). m. p. 165-166°C. MS 70 eV m/z (rel. int.): 426 (M^+ , 100), 285 (15), 190 (20). $^1\text{H-NMR}$: δ 3.00 (d, $J = 15.0$, H-4), 3.44 (dd, $J = 15.0$, $J = 2.4$, H-4), 5.67 (d, $J = 2.4$, H-6), 2.54 (dd, $J = 11.6$, H-8), 0.67 (s, 3H-18), 1.38 (s, 3H-19), 0.89 (d, $J = 6.4$, 3H-21), 0.77 (d, $J = 6.5$, 3H-26*), 0.79 (d, $J = 6.5$, 3H-27*), 0.80 (m, 3H-29).

* Assignments may be reversed.

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