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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 ß-sitosterol.

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

INTRODUCTION

The 7ß-hydroxysterols stigmast-5-ene-3ß,7ß-diol, cholest-5-ene-3ß,7ß-diol, the 7α-hydroxysterols stigmast-5-ene-3ß,7α-diol, cholest-5-ene-3ß,7α-diol and the 7-oxosterols 3ß-hydroxystigmast-5-en-7-one, 3ß-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-3ß,7ß-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.

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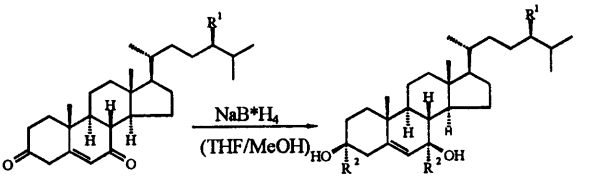
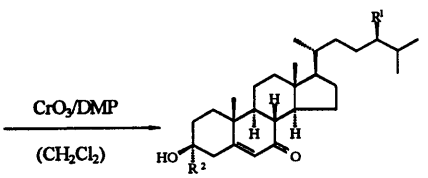
SYNTHESIS

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 7ß-hydroxysterols **3** - **5** in a yield of 87 - 90% besides approximately 5% of the epimeric 7α-hydroxysterols, separated by TLC or column chromatography (CC) on silica gel. The specific radioactivity of [3α,7α-$^3H_2$]-stigmast-5-ene-3ß,7ß-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 ^{1}H-NMR signals for H-3α (δ 3.52) / H-7α (δ 3.82, **3**, δ 3.83, **4**). The allylic oxidation of the ^{2}H-labelled 7-hydroxysterols **3**, **4** with CrO_3/DMP (3,5-dimethylpyrazole) [5] gave the 7-oxosterols **6**, **7**. [3α,4β,7β-$^2H_3$]-Cholest-5-ene-3β,7α-diol was previously prepared from cholest-4-en-3-one by equilibration in tert.-BuOH and tert.-BuOK followed by addition of [1-^{2}H]-acetic acid and reduction with NaB^2H_4. The formed [3α,4β-$^2H_2$]-cholest-5-en-3β-ol was oxidized with chromic acid to [3α,4β-$^2H_2$]-3β-hydroxycholest-5-en-7-one reduced with $LiAl^2H_4$ to [3α,4β,7β-$^2H_3$]-cholest-5-ene-3β,7α-diol [6].



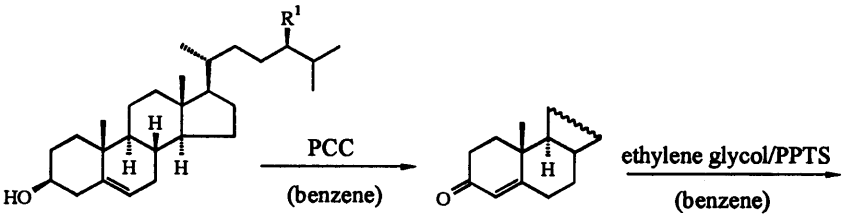
1 R^1 = H
2 R^1 = Et

3 R^1 = H R^2 = ^{2}H (90%)
4 R^1 = Et R^2 = ^{2}H (87%)
5 R^1 = Et R^2 = ^{3}H (90%)



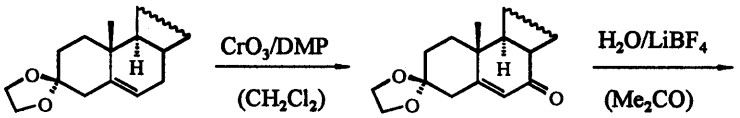
6 R^1 = H R^2 = ^{2}H (63%)
7 R^1 = Et R^2 = ^{2}H (64%)

The preparation of cholest-5-ene-3,7-dione (**1**) by Jones oxidation of cholest-5-ene-3ß,7ß-diol (without information on the yield) is described by Shoppee and Newman [7]. In our hands the Jones oxidation of 3ß-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**), 3α,4α-epoxycholest-5-en-7-one, 3α,4β-dihydroxycholest-5-en-7-one and 3α-formyloxy-4β-hydroxy-cholest-5-en-7-one [8]. So the 3,7-dioxosterols **1, 2** were synthesized in the following way. Cholesterol and ß-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 CrO_3/DMP to **12, 13**. The ketals were hydrolyzed with $LiBF_4/H_2O$/MeCN



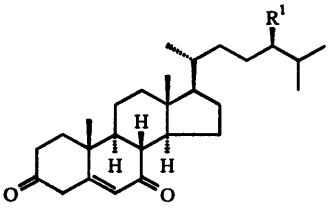
$R^1 = H$ **8** (85%)

$R^1 = Et$ **9** (80%)



10 (97%) **12** (64%)

11 (95%) **13** (67%)



1 $R^1 = H$ (82%)

2 $R^1 = Et$ (79%)

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

[3α,7α-$^{3}H_2$]-Stigmast-5-ene-3ß,7ß-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 $NaB^{3}H_4$ 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 ^{3}H-labelled sterol **5** showed identical chromatographic behaviour with the authentic non-labelled compound

[3α,7α-$^{2}H_2$]-Cholest-5-ene-3ß,7ß-diol (3), [3α,7α-$^{2}H_2$]-stigmast-5-en-3ß,7ß-diol (4)

80 mg (0.20 mmol) of cholest-5-ene-3,7-dione (**1**) and 42 mg (1.0 mmol) of $NaB^{2}H_4$ were added to 10 ml of a 0.4 M solution of $CeCl_3 \cdot 7\ H_2O$ 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 (M^{+}-H_2O, 100). ^{1}H-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^+-H_2O, 100), 285 (15), 190 (20). ^{1}H-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-^{2}H]-3ß-Hydroxycholest-5-en-7-one (6), [3α-^{2}H]-3ß-hydroxystigmast-5-en-7-one (7)

50 mg (0.50 mmol) of CrO_3 (dried with P_4O_{10}) was suspended in 3 ml of anhydrous CH_2Cl_2 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-$^2H_2$]-cholest-5-ene-3ß,7ß-diol (**3**) was added and the reaction mixture stirred for 1h maintaining a temperature between -10 and -20°C. 30 ml of CH_2Cl_2 and 5 ml of 5% hydrochloric acid were added, the organic phase was separated, dried with Na_2SO_4 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). ^{1}H-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). ^{1}H-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 Et_2O was added and the mixture filtered. The organic phase was treated with saturated NaCl-solution, dried with Na_2SO_4 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). ^{1}H-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 ß-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). ^{1}H-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-ethylenedioxystigmast-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). ^{1}H-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. ^{1}H-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-ethylenedioxystigmast-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}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}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}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}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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