OXIDATION OF FUROST-20(22)-ENES WITH 3-CHLOROPEROXYBENZOIC ACID AND OSMIUM TETROXIDE

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Dedicated to the memory of Professor Václav Černý.

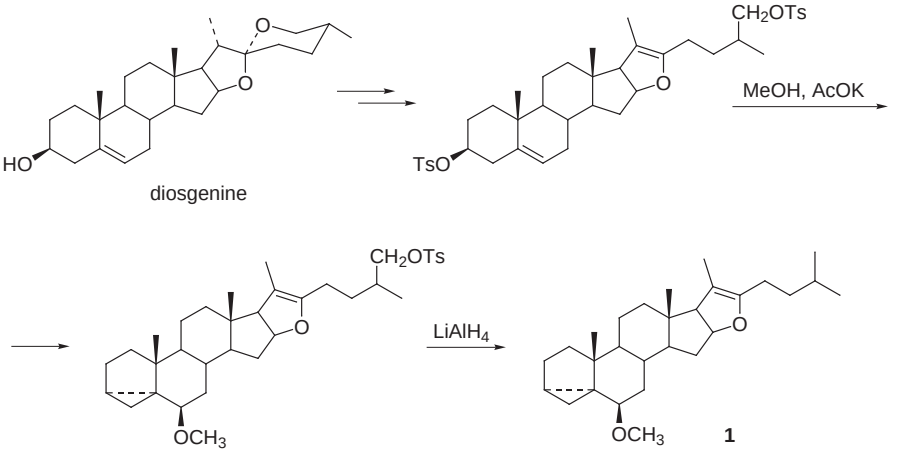
6β-Methoxy-3α,5-cyclo-5α-furost-20(22)-ene and its 17α-hydroxy derivative were oxidized with 3-chloroperoxybenzoic acid and OsO_4. The former compound afforded hydroxylated products, whereas the latter underwent degradation to C_{21} or C_{19} steroids.
Keywords: Pseudosapogenins; Furostanes; Oxidations; Steroids; Steroidal saponins.

Steroidal saponins comprise a diverse class of plant glycosides which possess a broad range of interesting biological activities[1,2]. The most common are saponins with the steroid aglycone (sapogenin), such as spirostane or furostane derivatives[3]. A number of polyhydroxy cholestane saponins with cytostatic activity was recently reported[4–7].

When treated with carboxylic acid anhydrides, steroid sapogenins undergo cleavage of ring F (refs[8,9]). The products are furost-20-enes called pseudosapogenins. These compounds are intermediates in the transformation of spirostanes to medicinally useful pregnane derivatives. Oxidation of pseudosapogenins with various reagents, such as CrO_3/AcOH, $KMnO_4/NaIO_4$ or H_2O_2/AcOH, has been intensively studied[10,11]. These reactions led to cleavage of ring E. Interestingly, a similar process also takes place in nature and the corresponding saponins were recently found in some Chinese medicinal plants[12]. It is postulated that 20,22-epoxide is an intermediate in this enzymatic oxidation. In the chemical oxidation of pseudosapogenins various compounds, such as diols, epoxides or allylic alcohols, were proposed as intermediates[3]. However, the final reaction products were 16β-hydroxypregnan-20-one esters or pregn-16-en-20-one derivatives. In the oxidation of pseudosapogenins containing a free 26-hydroxy group with 3-chloroperoxybenzoic acid (MCPBA), the products were 20-hydroxyspirostanes[13].

RESULTS AND DISSCUSSION

The aim of this study was to explore the possibility of application of furost-20(22)-enes oxidation under mild conditions to the synthesis of polyhydroxy cholestane derivatives. The starting materials for this study were 6β-methoxy-3α,5-cyclo-5α-furost-20(22)-ene (**1**) and its 17α-hydroxy derivative, *i.e.* 6β-methoxy-3α,5-cyclo-5α-furost-20(22)-en-17α-ol (**2**). The latter compound is available by epoxide ring cleavage in the 16α,17α-epoxy-20-oxo precursor as recently described[14]. Compound **1** was prepared from diosgenine (Scheme 1). Buffered methanolysis of pseudodiosgenine ditosylate followed by lithium aluminum hydride reduction afforded compound **1**.

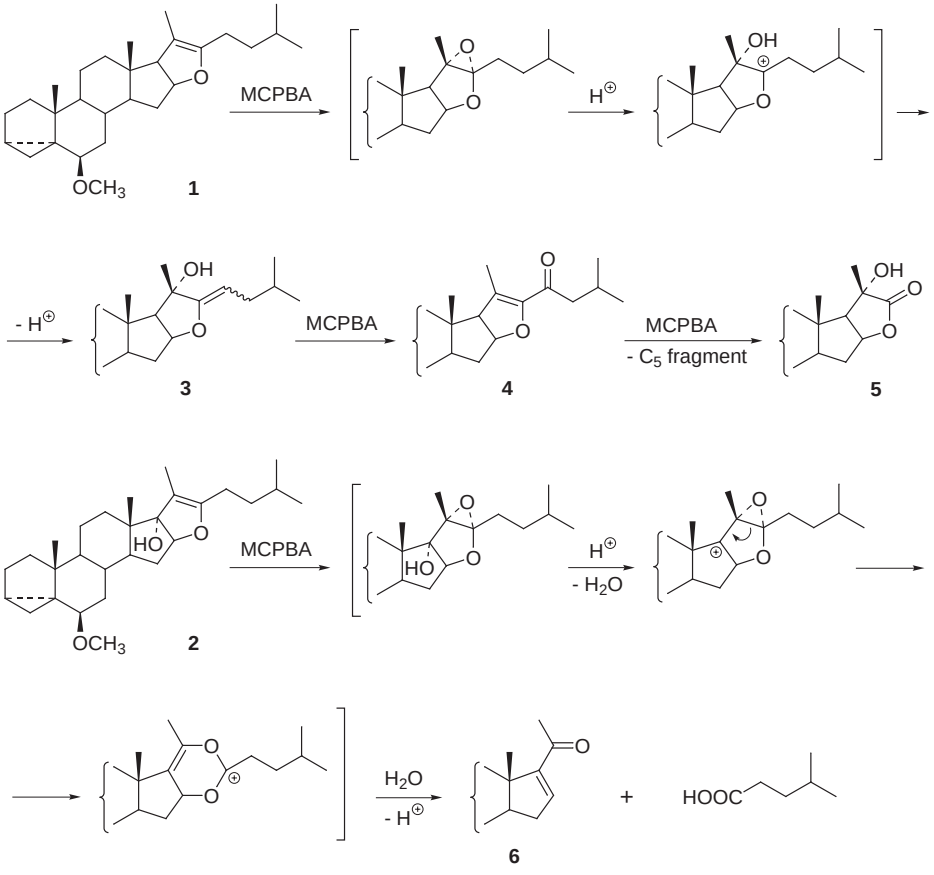


SCHEME 1

Both furost-20(22)-enes were subjected to oxidation with MCPBA in dichloromethane or osmium tetroxide in pyridine. The results of this study are shown in Schemes 2 and 3.

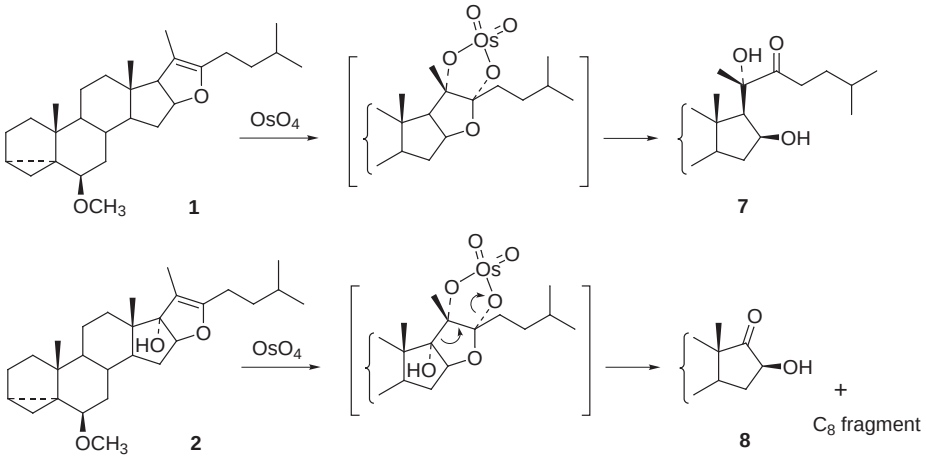
The reaction of compound **1** with MCPBA yielded the allylic alcohol **3**, 23-ketone **4**, and several minor products. TLC monitoring of the reaction mixture proved fast formation of the allylic alcohol **3** (a single stereoisomer was obtained but configuration at the C22–C23 double bond could not be elucidated from its spectra). The presumed initial reaction product, 20,22-epoxide, readily undergoes rearrangement to the allylic alcohol **3** *via* the stabilized carbocation. Further oxidation of **3** with MCPBA gives the α,β-unsaturated ketone **4**. Slow disappearance of both products **3** and **4** on prolonging the reaction time was observed in favor to the formation of the α-hydroxylactone **5**.

The reaction of compound **2** with the same reagent resulted in degradation to 6β-methoxy-3α,5-cyclo-5α-pregn-16-en-20-one[15,16] (**6**). A tentative mechanism of the reaction is shown in Scheme 2. The driving force for cleavage of the C20–C22 single bond is presumably the formation of a highly stabilized oxocarbenium ion. The low-molecular-weight reaction product was probably 4-methylpentanoic acid.



SCHEME 2

Oxidation of 6β-methoxy-3α,5-cyclo-5α-furost-20(22)-ene (**1**) with osmium tetroxide (Scheme 3) afforded the corresponding osmate which was found to be extraordinarily stable. The osmium derivative can be reduced with sodium hydrogen sulfite/pyridine but this takes 2 days to complete the reaction. The final product was compound **7** as expected. The compound exists in its open-chain form probably due to an intramolecular hydrogen bond.



SCHEME 3

Osmylation of compound **2** led to an unusual fragmentation into the C_{19} steroidal α-hydroxy ketone **8** and the C_8 product. The mechanism of this reaction is not clear but most likely cleavage of the C17–C20 single bond is assisted by formation of 17-oxocarbenium ion. It seems that the mechanism of the rearrangement is similar to that of a fragmentation of compound **2** promoted by MCPBA. Compound **8** proved resistant to autoxidation in contrast to the 16α-hydroxy-17-ketones[17].

Of the four reactions studied only osmylation of 6β-methoxy-3α,5-cyclo-5α-furost-20(22)-ene (**1**) proceeded as expected and may be useful for the synthesis of some natural products.

EXPERIMENTAL

Melting points were determined on a Kofler apparatus of the Boetius type. NMR spectra were recorded with a Bruker AC 200F spectrometer using $CDCl_3$ solutions with TMS as the internal standard (only selected signals in the ^1H NMR spectra are reported). Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. Infrared spectra (wavenumbers in cm^{-1}) were recorded on a Nicolet series II Magna-IR 550 FT-IR spectrometer as chloroform solutions. Mass spectra were obtained at 70 eV with an AMD-604 spectrometer. The reaction products were isolated by column chromatography performed on 70–230 mesh silica gel (Baker). Compound **2** was prepared from the commercially available 3β-hydroxypregn-5-en-20-one *via* 3β-hydroxycholesta-5,16-dien-22-one[18] according to the procedure previously described[14].

Synthesis of 6β-Methoxy-3α,5-cyclo-5α-furost-20(22)-ene (**1**)

The title compound was prepared from diosgenine as shown in Scheme 1. Ring E of diosgenine was opened with aluminum chloride in acetic anhydride according to the de-

scribed procedure[19]. The resulting 3β,26-diacetate (500 mg, 1 mmol) was hydrolyzed with potassium hydroxide (280 mg, 5 mmol) in methanol (100 ml) and the diol was treated with TsCl (1 020 mg, 15.3 mmol) in pyridine (30 ml). Buffered methanolysis[20] (0.31 g of potassium acetate in 70 ml of MeOH) of the crude pseudodiosgenine ditosylate followed by lithium aluminum hydride (380 mg, 10 mmol) reduction in refluxing THF (100 ml) and silica gel column chromatography afforded compound **1** (282 mg, 68%; eluted with 5% ethyl acetate–hexane) as an oil. ^{1}H NMR: 4.73 m, 1 H (H-16α); 3.33 s, 3 H (OCH_3); 2.78 m, 1 H (H-6α); 2.48 d, 1 H, J = 5.7 (H-17α); 1.59 s, 3 H (3 × H-21); 1.04 s, 3 H (3 × H-19); 0.89 d, 6 H, J = 6.5 (3 × H-26 and 3 × H-27); 0.74 s, 3 H (3 × H-18); 0.65 m, 1 H (H-4β); 0.44 dd, 1 H, J_1 = 7.9, J_2 = 5.1 (H-4α). For $C_{28}H_{44}O_2$ (412.7) calculated: 81.50% C, 10.75% H; found: 81.31% C, 10.66% H.

Oxidation with 3-Chloroperoxybenzoic Acid

To a stirred solution of a steroid (0.22 mmol) in dichloromethane (5 ml) 3-chloroperoxybenzoic acid (43 mg of a commercial 50–60% MCPBA; 0.14 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, quenched by addition of aqueous solution of sodium sulfide and extracted with chloroform. The extract was dried over anhydrous magnesium sulfate and the solvent was evaporated *in vacuo*. The reaction products were separated by silica gel column chromatography.

6β-Methoxy-3α,5-cyclo-5α-furost-22-en-20-ol (**3**). Amorphous solid eluted with hexane–ethyl acetate (10%), yield 30%. IR: 3 589, 3 437, 1 690, 1 096. ^{1}H NMR: 4.91 m, 1 H (H-16α); 4.33 dd, 1 H, J_1 = 8.1, J_2 = 6.6 (23-H); 3.32 s, 3 H (OCH_3); 2.78 m, 1 H (H-6α); 1.51 s, 3 H (3 × H-21); 1.02 s, 3 H (3 × 19-H); 0.88 and 0.86 2 × d, 6 H, J(25,26) = J(25,27) = 6.5 (3 × H-26 and 3 × H-27); 0.85 s, 3 H (3 × 18-H); 0.65 t, 1 H, J = 4.9 (H-4β); 0.44 dd, 1 H, J_1 = 8.0, J_2 = 5.1 (H-4α). ^{13}C NMR: 161.9 (C), 93.2 (CH), 83.9 (CH), 82.1 (CH), 77.6 (C), 66.3 (CH), 56.6 (CH_3), 56.6 (CH), 47.8 (CH), 43.4 (C), 40.6 (C), 39.5 (CH_2), 35.2 (C), 35.0 (CH_2), 34.0 (CH_2), 33.3 (CH_2), 33.1 (CH_2), 29.6 (CH), 28.8 (CH), 24.9 (CH_2), 22.5 (CH_3), 22.3 (CH_3), 21.9 (CH_2), 21.5 (CH), 21.2 (CH_3), 19.3 (CH_3), 13.5 (CH_3), 13.1 (CH_2). EI MS, *m/z* (rel.%): 428 (27) [M^+], 385 (70) [$M^+ - C_3H_7$], 335 (12), 253 (37), 121 (100). HR-MS (*m/z*) for $C_{28}H_{44}O_3$, found: 428.3294; calculated: 428.3291.

6β-Methoxy-3α,5-cyclo-5α-furost-20(22)-en-23-one (**4**). Eluted with hexane–ethyl acetate (4%) as an oil, yield 17%. IR: 1 683, 1 615, 1 091. ^{1}H NMR: 4.83 m, 1 H (H-16α); 3.33 s, 3 H (OCH_3); 2.79 m, 1 H (H-6α); 2.62 dd, 1 H, J_1 = 10.3, J_2 = 1.1 (H-17α); 2.43 dd, 2 H, J_1 = 7.0, J_2 = 5.4 (24-H); 2.02 d, 3 H, J = 1.1 (3 × H-21); 1.04 s, 3 H (3 × H-19); 0.93 and 0.92 2 × d, 6 H, J(25,26) = J(25,27) = 6.6 (3 × H-26 and 3 × H-27); 0.77 s, 3 H (3 × H-18); 0.67 t, 1 H, J = 3.9 (H-4α); 0.45 dd, J_1 = 8.0, J_2 = 5.2, 1 H (H-4β). ^{13}C NMR: 196.6 (C), 148.5 (C), 123.1 (C), 84.4 (CH), 82.1 (CH), 65.6 (CH), 56.6 (CH_3), 54.8 (CH), 49.3 (CH_2), 47.9 (CH), 44.7 (C), 43.4 (C), 39.9 (CH_2), 35.4 (CH_2), 35.2 (C), 34.3 (CH_2), 33.3 (CH_2), 29.9 (CH), 24.9 (CH_2), 24.3 (CH), 22.7 (CH_3), 22.6 (CH_2), 22.6 (CH_3), 21.4 (CH), 19.3 (CH_3), 14.3 (CH_3 × 2), 13.2 (CH_2). EI MS, *m/z* (rel.%): 426 (32) [M^+], 394 (18) [M^+ – MeOH], 180 (100). HR-MS (*m/z*) for $C_{28}H_{42}O_3$, found: 426.3138; calculated: 426.3134.

20α-Hydroxy-6β-methoxy-3α,5-cyclo-5α-pregnane-20,16β-carbolactone (**5**). Amorphous solid eluted with hexane–ethyl acetate (25%), yield 22% (achieved when the reaction time was extended to 2 days). IR: 3 575, 3 347, 1 766, 1 097. ^{1}H NMR: 5.09 m, 1 H (H-16α); 3.34 s, 3 H (OCH_3); 2.79 m, 1 H (H-6α); 2.27 m, 1 H; 2.11 d, 1 H, J = 8.6 (H-17α); 1.59 s, 3 H (3 × H-21); 1.03 s, 3 H (3 × H-19); 0.87 s, 3 H (3 × H-18); 0.67 t, 1 H, J = 3.9 (H-4α); 0.47 dd, J_1 =

8.0, J_2 = 5.2, 1 H (H-4β). EI MS, *m/z* (rel.%): 374 (18) [M^+], 359 (21) [$M^+ - CH_3$], 342 (47) [M^+ – MeOH], 319 (100). HR-MS (*m/z*) for $C_{23}H_{34}O_4$, found: 374.2464; calculated: 374.2457.

6β-Methoxy-3α,5-cyclo-5α-pregn-16-en-20-one (**6**). Eluted with hexane–ethyl acetate (11%) as an oil, yield 32%. IR: 1 707, 1 662, 1 587, 1 090. ^{1}H NMR: 6.69 m, 1 H (H-16); 3.35 s, 3 H (OCH_3); 2.80 m, 1 H (H-6β); 2.25 s, 3 H (3 × H-21); 1.05 s, 3 H (3 × H-19); 0.94 s, 3 H (3 × H-18); 0.67 t, 1 H, *J* = 4.9 (H-4α); 0.45 dd, 1 H, J_1 = 8.0, J_2 = 5.1 (4β-H). ^{13}C NMR: 196.7 (C), 155.5 (C), 144.4 (CH), 82.1 (CH), 56.6 (CH), 56.5 (CH_3), 48.4 (CH), 46.4 (C), 43.5 (C), 35.3 (CH_2), 35.1 (CH_2), 35.0 (C), 33.0 (CH_2), 32.2 (CH_2), 28.8 (CH), 27.1 (CH_3), 24.8 (CH_2), 22.3 (CH_2), 21.2 (CH), 19.1 (CH_3), 16.1 (CH_3), 13.1 (CH_2).

Oxidation of **1** with Osmium Tetroxide

To a stirred solution of compound **1** (58 mg, 0.14 mmol) in pyridine (3 ml), a solution of osmium tetroxide (38 mg, 0,15 mmol) in pyridine (2 ml) was added. The reaction mixture was stirred at room temperature for 18 h, additional 6 ml of pyridine, 8 ml of water, and aqueous solution of sodium hydrogen sulfite (2 ml of 40% $NaHSO_3$) were added in order to reduce osmium complex. The reaction mixture was stirred for 2 days, poured into water and extracted with ether. The extract was washed with water, solvent was removed *in vacuo* from the extract, and crude product was purified by silica gel chromatography.

16β,20α-Dihydroxy-6β-methoxy-3α,5-cyclo-5α-cholestan-22-one (**7**). Eluted with hexane–ethyl acetate (25%), yield 49%, m.p. 179–182 °C (dichloromethane–hexane). IR: 3 689, 3 446, 1 702, 1 093. ^{1}H NMR: 4.71 m, 1 H (H-16α); 4.43 s, 1 H (OH); 3.87 brs, 1 H (OH); 3.32 s, 3 H (OCH_3); 2.77 m, 1 H (H-6α); 2.65 t, *J* = 7.5, 2 H (2 × H-23); 2.30 m, 1 H; 1.56 s, 3 H (3 × H-21); 1.05 s, 3 H (3 × H-19); 1.02 s, 3 H (3 × H-18); 0.93 d, *J* = 6.1, 6 H (3 × H-26 and 3 × H-27); 0.65 t, *J* = 4.4, 1 H (H-4β); 0.44 dd, J_1 = 8.0, J_2 = 5.1, 1 H (H-4α). ^{13}C NMR: 214.5 (C), 82.1 (CH), 81.5 (C), 73.5 (CH), 59.6 (CH), 56.5 (CH_3), 54.4 (CH), 48.0 (CH), 43.4 (C), 42.6 (C), 39.2 (CH_2), 37.1 (CH_2), 35.3 (C), 35.2 (CH_2), 34.8 (CH_2), 33.3 (CH_2), 32.4 (CH_2), 29.4 (CH), 27.7 (CH), 26.7 (CH_3), 24.9 (CH_2), 22.4 (CH_3 × 2), 22.1 (CH_2), 21.5 (CH), 19.3 (CH_3), 15.2 (CH_3), 13.0 (CH_2). EI MS, *m/z* (rel.%): 428 (12) [$M^+ - H_2O$], 385 (30) [$M^+ - C_3H_7$], 347 (100). HR-MS (*m/z*) (ESI) for $C_{28}H_{46}O_4Na$, found: 469.3288; calculated: 469.3294.

Oxidation of **2** with Osmium Tetroxide

To a stirred solution of compound **2** (120 mg, 0.28 mmol) in pyridine (3 ml), a solution of osmium tetroxide (71 mg, 0.28 mmol) in pyridine (5 ml) was added. The reaction mixture was stirred at room temperature for 3 days, 20 ml of pyridine–water (1 : 1 v/v) was added, then osmium complex was reduced with 3 ml of 40% aqueous solution of sodium hydrogen sulfite for 1 h. The reaction mixture was extracted with ether, solvent was removed *in vacuo*, and product was purified by silica gel chromatography.

16β-Hydroxy-6β-methoxy-3α,5-cyclo-5α-androstan-17-one (**8**). Eluted with hexane–ethyl acetate (28%) as an oil, yield 20%. IR: 3 558, 3 463, 1 744, 1 090, 1 008. ^{1}H NMR: 4.38 d, 1 H, *J* = 7.6 (H-16α); 3.37 s, 3 H (OCH_3); 2.84 m, 1 H (H-6α); 1.05 s, 3 H (3 × H-19); 1.02 s, 3 H (3 × H-18); 0.68 t, 1 H, *J* = 3.9 (H-4β); 0.47 dd, 1 H, J_1 = 8.0, J_2 = 5.1 (H-4α). ^{13}C NMR: 219.5 (C), 81.8 (CH), 71.4 (CH), 56.7 (CH_3), 48.3 (CH), 47.8 (C), 43.5 (C), 34.9 (CH_2), 33.9 (CH_2), 33.3 (C), 31.6 (CH_2), 30.5 (CH_2), 30.2 (CH), 24.8 (CH_2), 21.6 (CH_2), 21.2 (CH), 19.2 (CH_3), 14.3 (CH_3), 13.2 (CH_2). For $C_{20}H_{30}O_3$ (318.5) calculated: 75.43% C, 9.50% H; found: 75.21% C, 9.41% H.

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