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A Revisitation of the Clemmensen Reduction of Diosgenin. Characterization of Byproducts and their Use in the Preparation of (25R)-26-Hydroxysterols.

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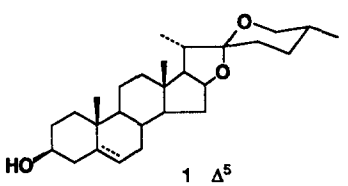
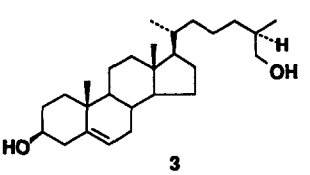
Abstract: *Four major byproducts of the Clemmensen reduction of diosgenin have been isolated and identified: (25R)-17β-methyl-18-nor-17α-cholesta-5,13-diene-3β,26-diol, dihydrodiosgenin, (22E,25R)-cholesta-5,22-diene-3β,16β,26-triol, and its 22Z isomer. These byproducts were used to prepare deuterium- and tritium-labeled (25R)-26-hydroxycholesterol (3) and Δ^{22} analogs of 3.*

Mitochondrial 26-hydroxylation of sterols is an obligatory reaction in major pathways of bile acid formation[3] and is an initial reaction in the metabolism of 3β-hydroxy-5α-cholest-8(14)-en-15-one, a potent hypocholesterolemic agent.[4] The products of 26-hydroxylation, i.e. (25R)-26-hydroxycholesterol (**3**)[5] and (25R)-26-oxygenated-15-ketosterols,[6] are highly active in the regulation of sterol synthesis in cultured mammalian cells. The initial step in the chemical synthesis of (25R)-C-26 functionalized sterols is generally the Clemmensen reduction of a sapogenin,[6a,7] a reaction that often proceeds in modest or variable yield. In the case of diosgenin (**1**), this reaction affords an almost quantitative recovery of steroid material, of which the desired 3β,16β,26-triol **4** and recovered diosgenin constitute at most ~75%. Clemmensen reduction byproducts of sapogenins have been investigated only for the case of tigogenin (**2**), which was reported to give a 63:37 ratio of (25R)-5α-cholestane-3β,16β,26-triol and the 22S isomer of dihydrotigogenin.[8] The latter was said to decompose readily to Δ^{22}- and Δ^{23}-3β,16β,26-triols, from which it could not be separated. Additionally, the main triol product was shown to be transformed to (25R)-17β-methyl-18-nor-5α,17α-cholest-13-ene-3β,26-diol under acidic conditions. In the Clemmensen reduction of diosgenin, we have observed the corresponding 18-norsterol but have found different structures and reactivities for the other byproducts. We describe here the isolation and identification of four major byproducts in the Clemmensen reduction of diosgenin. Deuterium- and tritium-labeled 3 and analogs of 3 with Δ^{22} unsaturation were prepared from these byproducts.



1 Δ^5
2 Δ^0



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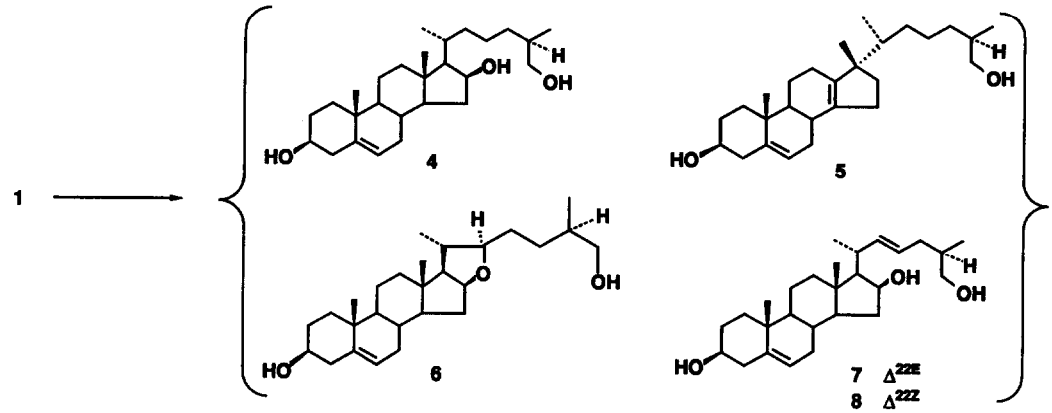
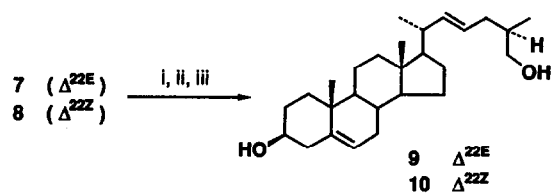
Clemmensen reduction of diosgenin as described previously[6a] gave a crude product consisting of triol **4**, diosgenin, and several byproducts that were not readily separated by recrystallization or chromatography on silica gel. A much better separation was obtained after the crude product mixture was silylated with $tBuSiMe_2Cl$ to mask the 3β- and 26-hydroxyl groups.[9] Four byproducts were isolated as their bis-TBDMS ethers and hydrolyzed to (25*R*)-17β-methyl-18-nor-17α-cholesta-5,13-diene-3β,26-diol (**5**), (22E,25R)-cholesta-5,22-diene-3β,16β,26-triol (**7**), its 22Z isomer **8**, and dihydrodiosgenin (**6**).



Structures of **5**, **6**, **7**, and **8** were established by ^{1}H and ^{13}C NMR.[10] Spectra of a 1:1 mixture of **6** derived from the Clemmensen reduction and dihydrodiosgenin prepared[11] by treatment of diosgenin with $LiAlH_4$-$AlCl_3$ showed no doubling of peaks under conditions capable of resolving signals differing by only 0.03 ppm (^{13}C) or 0.005 ppm (^{1}H). This result together with other supporting physical constants established the identity of **6** as dihydrodiosgenin (22R isomer). For the structure elucidation of **7** and **8**, the bis-TBDMS ether of each $\Delta^{5,22}$-3β,16β,26-triol was converted to the 16β-mesylate and reduced with $LiAlH_4$ to give, after hydrolysis of the silyl ether groups, the $\Delta^{5,22}$-3β,26-diols **9** and **10**, respectively (Scheme 1).[12] The ^{13}C NMR spectra of **9** and **10** were in agreement with those reported[13] for the 22E and 22Z isomers of cholesta-5,22-dien-3β-ol. The structure of **5** was based on the similarity of its ^{1}H and ^{13}C NMR and mass spectra with those reported for other Δ^{13}-17β-methyl-18-norsterols.[8,14]

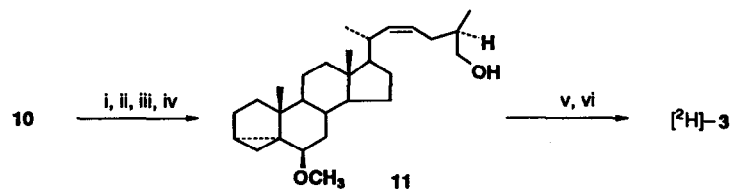


Scheme 1. *Reagents and conditions*: i, MsCl, pyridine, room temperature, overnight; ii, $LiAlH_4$, ether, 1-h reflux, followed by purification on $AgNO_3$-silica gel; iii, pTsOH, acetone-chloroform (~10:1), room temperature, overnight.

Isolation and characterization of each of the reaction byproducts by ^{1}H and ^{13}C NMR and HPLC permitted a precise evaluation of the composition of the crude Clemmensen reduction products. For this experiment, concentrated HCl (100 ml) was added over 1 h to a refluxing solution of diosgenin (5 g) in absolute ethanol (500 ml) containing amalgamated zinc (417 g). Refluxing was continued for an additional 4 h, and samples for analysis were taken at 0.5, 1, 1.5, 2.5, and 5 h after addition of HCl was begun; samples were poured into water, and the precipitate was collected by filtration. HPLC and ^{13}C NMR analysis of a sample taken at 1.5 h indicated a composition of 59% triol **4**, 18% diosgenin, 3% 18-norsterol **5**, 8% dihydrodiosgenin (**6**), 5% Δ^{22E}-triol **7**, and 7% Δ^{22Z}-triol **8**.[15] The relative amounts of these components did not change significantly with time except that **5** increased to 9% at 5 h at the expense of **4** (52% at 5 h). Virtually all the ^{13}C NMR signals of >2% intensity in the spectrum of the 2.5-h sample were accounted for by these products. NMR signals attributable to yamogenin or the 22S isomer of dihydrodiosgenin were not detected in any samples.

The foregoing results cast serious doubt on previously proposed structures and mechanisms for formation of Clemmensen reduction byproducts.[8] The constant ratio observed for **6**:**7**:**8** from 0.5 to 5 h suggests that $\Delta^{5,22}$ dienes **7** and **8** do not arise from 22R-dihydrodiosgenin (**6**). More importantly, treatment of **6** with refluxing ethanol-conc. HCl (5:1) for 5 h gave (22R,25R)-furosta-3,5-dien-26-ol (25%), recovered **6** (60%), and no detectable **7** or **8**. Olefins are known byproducts of Clemmensen reductions.[16] The formation of dihydrodiosgenin (**6**) under Clemmensen reduction conditions is not surprising since sapogenins are converted to dihydrosapogenins by catalytic hydrogenation in acidic media.[11] The remaining byproduct, the 18-norsterol **5** appears to arise from dehydration of triol **4** to the Δ^{16} sterol, followed by rearrangement to **5** as proposed previously.[8] The decomposition of **4** to **5** is significant in the case of prolonged refluxing of the reaction mixture or refluxing in a higher-boiling solvent such as n-butanol.

Diol **10** was exploited as the starting material for the preparation of deuterium- and tritium-labeled **3** (Scheme 2). The 26-TBDPS ether of **10** was converted via the 3β-tosylate to the i-steroid. Removal of the 26-silyl group gave (22*Z*,25*R*)-3α,5-cyclo-5α-cholest-22-en-26-ol (**11**), which was reduced with deuterium over PtO_2 and hydrolyzed to [^{2}H]-**3**.[17] NMR and MS analysis indicated that the bulk of the deuterium label was located at C-22, C-23, and C-24. Analogous catalytic reduction of **11** in the presence of tritium gas gave, after appropriate purification, [^{3}H]-**3** of high specific activity (16.9 Ci/mmol). This represents an efficient synthesis of **3** labeled in positions that are chemically and metabolically stable for the purposes of many biological experiments.



Scheme 2. *Reagents and conditions*: i , $tBuSiPh_2Cl$, imidazole, CH_2Cl_2-DMF (1:1), 22 °C, 12 h ; ii, pTsCl, pyridine, 22 °C, 12 h; iii, KOAc, refluxing MeOH, 12 h; iv, Bu_4NF, THF, 22 °C, 20 h; v, PtO_2, EtOAc-CH_3COOD (1:1), 1 atm $^2H_2$, 22 °C, 0.5 h; vi, pTsOH, refluxing dioxane-water (4:1), 1 h. All yields were ≥70%.

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9. The crude product (5 g, obtained from 5 g of diosgenin) was silylated by heating at 70 °C for 1 h with imidazole (10 g), dry dimethylformamide (9 ml), benzene (45 ml), and (after azeotropic removal of water) t-butyldimethylsilyl chloride (4.32 g). The components were separated by medium-pressure liquid chromatography (37 x 440 mm Lobar silica column eluted with 1% ethyl acetate in hexane).
10. These compounds were fully characterized (^{1}H NMR (500 MHz, $CDCl_3$), ^{13}C NMR (75 MHz, $CDCl_3$), MS (70 eV), mp, $[\alpha]_D$, IR, TLC, and HPLC) and showed satisfactory elemental analysis or high-resolution MS. Selected analytical data: **5**: mp 146–148 °C; ^{13}C NMR δ 22.84 (C-12), 136.16 and 140.23 (C-13 and C-14), 24.91 (17-Me), 14.61 (C-21); ^{1}H NMR δ 1.00 (s, 17-Me), 1.34 (m, H-20), 0.71 (d, 6.8 Hz, H-21); MS *m/z* 400 (1, M^+), 271 (100, M–SC), 253 (27, M–SC–H_2O); **6**: mp 173.5–174.5 °C; ^{13}C NMR δ 18.84 (C-21), 90.27 (C-22); ^{1}H NMR δ 1.76 (dqd, 8.4, 6.8, 5.1 Hz, H-20), 1.00 (d, 6.8 Hz, H-21), 3.33 (ddd, 8.5, 8.0, 3.9 Hz, H-22); MS *m/z* 416 (3, M^+), 271 (100), 253 (20); **7**: mp 166–167 °C; ^{13}C NMR δ 34.94 (C-20), 139.12 (C-22), 127.06 (C-23), 36.58 (C-24); ^{1}H NMR δ 1.07 (d, 6.7 Hz, H-21), 5.50 (ddt, 15.4, 8.9, 1.1 Hz, H-22), 5.58 (dt, 15.4, 6.9 Hz, H-23); MS *m/z* 416 (2, M^+), 401 (10), 383 (13), 272 (51); **8**: mp 184.5–185 °C; ^{13}C NMR δ 29.34 (C-20), 137.62 (C-22), 127.04 (C-23), 31.23 (C-24); ^{1}H NMR δ 1.03 (d, 6.6 Hz, H-21), 5.44 (ddt, 11.1, 9.5, 1.6 Hz, H-22), 5.36 (ddd, 10.9, 7.2, 6.6 Hz, H-23); MS *m/z* 416 (2, M^+), 401 (11), 383 (17), 272 (55).
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12. Selected analytical data: **9**: mp 172–174 °C; ^{13}C NMR δ 40.14 (C-20), 36.53 (C-24); **10**: mp 181–183 °C; ^{13}C NMR δ 34.26 (C-20), 31.38 (C-24).
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15. HPLC detector responses at 210 nm were measured for the major components and used in the calculation of the percent composition. Minor byproducts included Δ^{22E} and Δ^{22Z} analogs of **5** and $\Delta^{3,5}$ sterols from dehydration of **4**, **5**, **6**, **7**, and **8**. Most of these very minor byproducts were obtained in sufficient purity to determine their ^{13}C NMR chemical shifts and HPLC retention times. Under other reaction conditions (e.g. EtOH-$CHCl_3$ or butanol solvent), much larger amounts of **5** or **6** were observed.
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17. The synthetic intermediates were fully characterized as above and showed satisfactory elemental analysis or high-resolution MS. Selected analytical data: **11**: oil; ^{13}C NMR δ 13.02 (C-4), 137.96 (C-22), 124.31 (C-23), 31.36 (C-24); ^{1}H NMR δ 0.43 (dd, 8.0, 5.1 Hz, H-4α), 0.65 (dd, 5.0, 3.8 Hz, H-4β), 0.96 (d, 6.6 Hz, H-21); MS *m/z* 414 (18, M^+), 399 (39), 382 (39), 359 (62), 255 (28); [^{2}H]-**3**: mp 175–177 °C; MS: molecular ion showed d_0 (1%), d_1 (8%), d_2 (36%), d_3 (28%), d_4 (18%), d_5 (5%), d_6 (4%), and small amounts of d_7, d_8, and d_9 species, whereas m/z 273 (M–SC) showed complete absence of deuterium; ^{13}C NMR: no signal for C-22 or C-23, strongly diminished signal for C-24; ^{2}H NMR δ 1.05 (broad, H-22, H-24), 1.25 (H-23), 1.33 (strong, broad signal, H-22, H-23, H-24), and minor signals (~5% each of total intensity) at δ 0.90 (H-21, H-27) and 1.60 (H-25).

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