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A Convenient Synthesis of 1α-Hydroxyvitamin D_2

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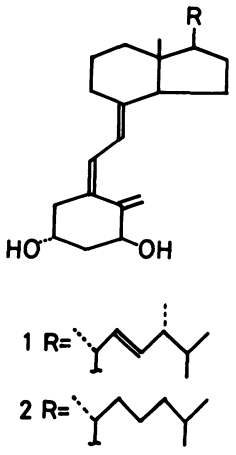
The enol acetylation of ergosta-1,4,6,22-tetraen-3-one (**4**) by treatment with isopropenyl acetate under acidic condition gave 3-acetoxy-1,3,5,7,22-ergostapentaene (**5**). The reduction of **5** with calcium borohydride afforded ergosta-1,5,7,22-tetraen-3β-ol (**6**). The *t*-butyldimethylsilyl ether derivatives (**8**) of the Diels–Alder cyclo adduct (**7**) derived from **6** and 4-phenyl-3*H*-1,2,4-triazole-3,5-dione was oxidized with *m*-CPBA to the diepoxide adduct (**9**). The 1α,2α-epoxide of the diepoxy alcohol (**10**) obtained after the removal of the silyl group of **9** was selectively reduced with $LiAlH_4$ to yield the 22,23-epoxy-1α,3β-diol (**11**) and **11** was subsequently converted to the corresponding diacetate (**12**). The deoxygenation of the 22,23-epoxide of **12** with a combination of sodium iodide and trifluoroacetic anhydride gave the 1α-acetoxyergosteryl acetate (**13**). Irradiation of **13** with a high-pressure mercury lamp followed by thermal isomerization, saponification, purification on silica gel and crystallization gave 1α-hydroxyvitamin D_2 (**1**) as colorless crystals.

It is reported that 1α-hydroxyvitamin D_2 (**1**)[1] is about ten times less toxic than 1α-hydroxyvitamin D_3 (**2**),[2] and yet appears to be almost equally potent in vitamin D activity.[3] This suggests that **1** might represent a therapeutically superior compound. DeLuca et al. reported the synthesis of **1** by the irradiation of 1α-acetoxyergosteryl acetate (**13**) followed by the thermal isomerization and saponification. However, concerning the synthesis of **13**, their preparative process utilizing ergost-5-en-3β-ol as a key intermediate generally gave a poor yield of **13** due to the side reactions involving the formation of a considerable amount of the 4,6-diene isomer.[4]



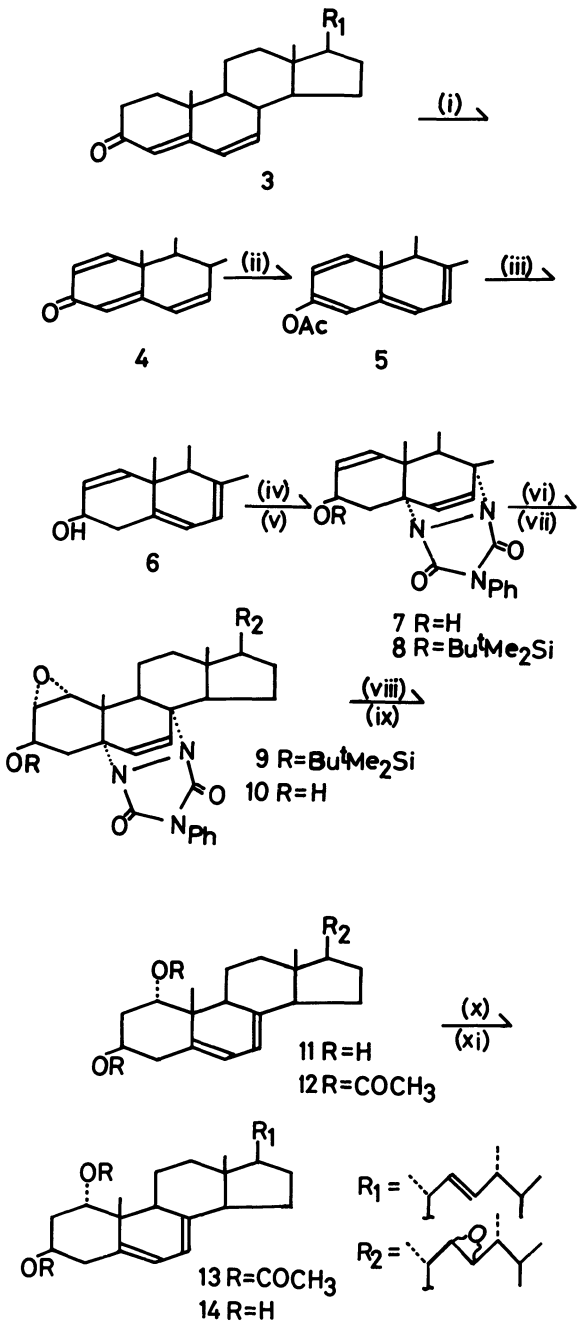
Thus, it is desirable to obtain **13** in higher yiled for the preparation of **1**. We have reported the conversion of cholesta-1,4,6-trien-3-one to cholesta-5,7-diene-1α,3β-diol via 3-acetoxy-1,3,5,7-cholestatetraene as a key intermediate.[5] This procedure seemed to be applicable to the synthesis of **13**. In the present paper, an alternative convenient method for the preparation of 1α-acetoxyergosteryl acetate (**13**) and its conversion to 1α-hydroxyvitamin D_2 (**1**) are described.

Ergosta-4,6,22-trien-3-one (**3**)[6] was dehydrogenated to ergosta-1,4,6,22-tetraen-3-one (**4**) by the modified method of Pelć et al.[7] Treatment of the 4,6,22-trienone (**3**) with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in the presence of a catalytic amount of 5-sulfosalicylic acid gave the tetraenone (**4**) in a fairly good yield. The tetraenone (**4**) was converted to 3-acetoxy-1,3,5,7,22-ergostapentaene (**5**) by the enol acetylation in the presence of a combination of isopropenyl acetate and *p*-toluenesulfonic acid. Longer time is required to complete this enol acetylation compared with that of cholesta-1,4,6-trien-3-one.[5] The pentaenyl acetate (**5**) was subsequently reduced with calcium borohydride[8] to yield ergosta-1,5,7,22-tetraen-3β-ol (**6**) in good yield.

After the 5,7-diene of **6** was protected with 4-phenyl-3*H*-1,2,4-triazole-3,5-dione[9] as a Diels–Alder adduct (**7**), the hydroxyl group was protected by conversion to *t*-butyldimethylsilyl derivative (**8**) for performing the selective α-epoxidation of the double bond (C-1).[10] In the next stage, the reactivity of the oxidizing reagent to the two different double bond (C-1 and C-22) was examined. When an equimolar amount of *m*-chloroperbenzoic acid (*m*-CPBA) was allowed to react with **8**, the ^1H NMR spectrum of the product showed that the signals due to the double bond at C-22 disappeared completely, indicating the double bond at C-22 was epoxidized selectively. Therefore, we planned to regenerate the double bond by removal of the epoxide after the hydroxyl group was introduced at the α position of C-1.

Theatment of **8** with excess *m*-CPBA afforded the diepoxide derivative (**9**) as diastereomeric mixtures with respect to the 22,23-epoxide, showing two spots on thin-layer chromatography.[11] On the other hand, the orientation of the 1,2-epoxide was confirmed as α-configuration by comparison of the ^1H NMR spectra of the compounds **9** and **10** (described later) with those of corresponding established 1α,2α-epoxide derivatives obtained from cholesterol.[8,10] Removal of the silyl group of **9** by treatment with tetrabutylammonium fluoride tetrahydrofuran solution gave the diepoxy

alcohol (**10**). The resulting **10** was reduced with excess $LiAlH_4$ to yield 22,23-epoxy diol (**11**). Crump et al. reported the reduction of the 22,23-epoxide of ergostery acetate protected with 4-phenyl-3*H*-1,2,4-triazole-3,5-dione ($LiAlH_4$ in tetrahydrofuran-ether, reflux for 3 days).[12] However, the 22,23-epoxide of **11** was not reduced under the conditions employed here (in tetrahydrofuran, reflux for 1 h).[13] The epoxy diol (**11**) was treated with pyridine–acetyl chloride to afford the corresponding diacetate (**12**). After trying several deoxygenating reagents,[14] we found that a combination of sodium iodide and trifluoroacetic anhydride[15] successfully deoxygenated the 22,23-epoxide of **12** to yield the desired 1α-acetoxyergosteryl acetate (**13**). The deoxygenation should be carried out at relatively low temperature (0—5 °C) to avoid side reactions involving the formation of the cis isomer. The *E* stereochemistry of the regenerated double bond (C-22) in **13** was supported by the ^1H NMR spectrum, where the coupling pattern of the olefinic protons at the side chain was in quite agreement with that of 1α-acetoxyergosteryl acetate obtained by the method described in the literature.[1] The diacetate (**13**) was hydrolyzed upon treatment with ethanolic potassium hydroxide to give the corresponding diol (**14**).



Scheme 1. (i) DDQ-5-sulfosalicylic acid. (ii) Isopropenyl acetate-*p*-TsOH. (iii) $Ca(BH_4)_2$, 0 °C in MeOH–EtOH. (iv) 4-Phenyl-3*H*-1,2,4-triazole-3,5-dione. (v) Bu^tMe_2SiCl–imidazole, 35 °C in DMF. (vi) *m*-CPBA. (vii) Bu_4NF, 5 °C in THF. (viii) $LiAlH_4$–THF. (ix) CH_3COCl–pyridine. (x) NaI–$(CF_3CO)_2O$, 5 °C in THF–acetonitrile. (xi) KOH–EtOH.

The irradiation of the diacetate (**13**) with high-pressure mercury lamp using aq. 1.2% KNO_3 solution as a filter followed by the thermal isomerization of precalciferol to calciferol and the subsequent saponification afforded the crude 1α-hydroxyvitamin D_2 (**1**). Purification by column chromatography on silica gel and crystallization gave **1** as colorless crystals.

Since 1α-acetoxyergosteryl acetate (**13**) is obtainable readily from ergosterol as described above, the present study provides an efficient method for the preparation of 1α-hydroxyvitamin D_2 (**1**).

Experimental

Melting points are uncorrected. UV spectra were taken on a Hitachi 320 spectrometer. ^1H NMR spectra were recorded in $CDCl_3$ on a JEOL JNF-FX200 spectrometer with TMS as an internal standard. Mass spectra were measured on a Hitachi M-80 mass spectrometer. Optical rotations were determined with a JASCO DIP-4 polarimeter. Solvents were removed under reduced pressure.

Ergosta-1,4,6,22-tetraen-3-one (4). A mixture of ergosta-4,6,22-trien-3-one (**3**) (65 g, 165.0 mmol), DDQ (65 g, 286.3 mmol) and 5-sulfosalicylic acid (12 g, 47.2 mmol) in dioxane (500 ml) was heated under reflux for 3 h. The solution was filtered to remove the hydroquinone formed and the filtrate was concentrated to dryness. The mixture was extracted with ethyl acetate. The extract was washed with aq. 10% KOH solution and brine, dried over sodium sulfate and evaporated. The residue was chromatographed on silica gel. Elution with ethyl acetate–hexane (1/9, v/v) gave crude tetraenone (**4**) (45 g), which was crystallized from acetone to afford pale yellow needles. 35 g (54%): mp 112—114 °C (lit,[6] 104—106 °C).

3-Acetoxy-1,3,5,7,22-ergostapentaene (5). A mixture of **4** (10 g, 25.5 mmol), *p*-toluenesulfonic acid (10 g, 52.6 mmol), and isopropenyl acetate (100 ml, 900.0 mmol) in butyl acetate (100 ml) was allowed to reflux for 16 h. The mixture solution was washed with water, aq. $NaHCO_3$ solution and brine, dried over sodium sulfate and evaporated. The residue was crystallized from acetone to give the pentaene (**5**) as yellow needles. 7.6 g (69%): mp 154—156 °C; λ_{max} 252 nm

(ε=9200, EtOH); *m/z* 434 (M^+); ^1H NMR δ 5.64—5.98 (m, 5H, H-1, H-2, H-4, H-6, H-7), 5.21 (m, 2H, H-22, H-23), 2.20 (s, 3H, $COCH_3$). Found: C, 82.53; H, 9.87%. Calcd for $C_{30}H_{42}O_2$: C, 82.88; H, 9.76%; $[\alpha]_D^{25}$ −508° (*c* 0.2, $CHCl_3$).

Ergosta-1,5,7,22-tetraen-3β-ol (6). The ether solution (100 ml) of the pentaene (**5**) (10 g, 23.0 mmol) was added dropwise at −10 °C to a stirred solution of calcium borohydride in ethanol–methanol (calcium chloride (30 g, 333.3 mmol) in methanol (300 ml) and sodium borohydride (15 g, 405.4 mmol) in ethanol (150 ml)). The mixture was stirred at 0 °C for 3 h and at room temperature overnight. After addition of 50% acetic acid to dissolve the resulted precipitates, the product was extracted with ethyl acetate. The extract was washed with water, aq. $NaHCO_3$ solution, brine and dried over sodium sulfate. The ethyl acetate was evaporated to dryness and the residue was chromatographed on silica gel. The product, which was eluted with hexane–ethyl acetate (4/1, v/v) was crystallized from acetone to give the tetraene (**6**). 7.7 g (85%): mp 156—157 °C; λ_{max} 282 nm (ε=11000, EtOH); *m/z* 394 (M^+); ^1H NMR δ=5.47—5.78 (m, 4H, H-1, H-2, H-6, H-7), 5.21 (m, 2H, H-22, H-23), 4.31 (1H, m, H-3). Found: C, 85.06; H, 10.71%. Calcd for $C_{28}H_{42}O$: C, 85.19; H, 10.75%.

The Diels-Alder Adduct of Ergosta-1,5,7,22-tetraen-3β-ol (7). The tetraenol (**6**) (9.6 g, 24.4 mmol) was dissolved in ethyl acetate (100 ml). To the solution, 4-phenyl-3*H*-1,2,4-triazole-3,5-dione (4.8 g, 27.4 mmol) was added by portion until a faint pink color persisted. The solvent was removed and the residue was purified by silica-gel column chromatography (eluted with chloroform) to give the adduct (**7**). 12.5 g (90%): mp 170—171 °C (crystallized from methanol); *m/z* 394 (M^+−175); ^1H NMR δ=7.40 (5H, m, C_6H_5), 6.44, 6.26 (2H, AB q, *J*=8 Hz, H-6, H-7), 6.04 (2H, m, H-1, H-2), 5.20 (2H, m, H-22, H-23), 5.06 (1H, m, H-3). Found: C, 75.74; H, 8.34; N, 7.29%. Calcd for $C_{36}H_{47}N_3O_3$: C, 75.87; H, 8.33; N, 7.37%.

The 3β-*O*-(*t*-Butyldimethylsilyl) Derivative (8) of the Diels-Alder Adduct (7). To the solution of the Diels–Alder adduct (**7**) (8.4 g, 14.8 mmol) in *N*,*N*-dimethylformamide (20 ml), *t*-butyldimethylsilyl chloride (3.6 g, 23.8 mmol) and imidazole (3.6 g, 52.9 mmol) was added. The mixture solution was warmed at 35° for 1 h and extracted with ether. The ether solution was washed with brine, dried over sodium sulfate and evaporated. The residue was crystallized from methanol–ether to give the silyl derivative (**8**). 9.1 g (90%): mp 186—188 °C; ^1H NMR δ=7.40 (5H, m, C_6H_5), 6.44, 6.26 (2H, AB q, *J*=8 Hz, H-6, H-7), 5.69 (2H, m, H-1, H-2), 5.18 (2H, m, H-22, H-23), 4.99 (1H, m, H-3). Found: C, 73.71; H, 9.07; N, 6.15%. Calcd for $C_{42}H_{61}N_3O_3Si$: C, 73.73; H, 9.01; N, 6.14%.

1α,2α:22,23-Diepoxide Derivative (9). The silyl derivative of the Diels–Alder adduct (**8**) (7.5 g, 10.7 mmol) was dissolved in chloroform (100 ml) and *m*-chloroperbenzoic acid (*m*-CPBA) (7.5 g, 43.5 mmol) was added. After the mixture was stirred at room temperature for 24 h, the solution was washed with sodium carbonate and brine, dried over sodium sulfate and evaporated to give the diepoxide (**9**). 7.1 g (90%): mp 194—195 °C (crystallized from methanol); *m/z* 541 (M^+−175); ^1H NMR δ=7.45 (5H, m, C_6H_5), 6.42, 6.20 (2H, AB q, *J*=8 Hz, H-6, H-7), 4.94 (1H, m, H-3), 3.17—3.34 (2H, m, H-1, H-2). Found: C, 70.34; H, 8.61; N, 5.85%. Calcd for $C_{42}H_{61}N_3O_5Si$: C, 70.43; H, 8.60; N, 5.87%.

22,23-Epoxy-ergosta-5,7-diene-1α,3β-diol (11). A tetrahydrofuran solution (50 ml) of the crude **9** (5.6 g, 7.8 mmol) and 1 M (1 M=1 mol dm^{-3}) tetrabutylammonium fluoride tetrahydrofuran solution (12 ml) was allowed to react at 5 °C for 16 h. The mixture was extracted with ethyl acetate, washed with brine and dried over sodium sulfate. Evaporation to dryness left a residue, which was chromatographed on silica gel with chloroform as eluent to afford the diepoxy alcohol **10** as oil. 3.7 g (79%): *m/z* 427 (M^+−175); ^1H NMR δ=7.45 (5H, m, C_6H_5), 6.43, 6.21 (2H, AB q, *J*=8 Hz, H-6, H-7), 5.04 (1H, m, H-3), 3.27 (2H, m, H-1, H-2).

To the tetrahydrofuran solution (400 ml) of $LiAlH_4$ (8.1 g, 213.7 mmol), **10** (2.7 g, 4.5 mmol) in tetrahydrofuran (150 ml) was added dropwise. The mixture was heated under reflux for 1 h. After decomposition of the excess $LiAlH_4$ by addition of water, dil. hydrochloric acid was added to give a clear solution. The solution was extracted with ethyl acetate, washed with aq. $NaHCO_3$ solution and brine, and dried over sodium sulfate. Chromatography on silica gel of the crude oil obtained after evaporation of ethyl acetate (eluted with chloroform–ethyl acetate) (4/1, v/v) yielded the epoxy diol (**11**). 1.2 g (63%): mp 155—157 °C (crystallized from acetone); *m/z* 428 (M^+); λ_{max} 282 nm (ε=10900, EtOH); ^1H NMR δ=5.70, 5.38 (2H, m, H-6, H-7), 4.05 (1H, m, H-3), 3.73 (1H, bs, H-1). Found: C, 78.11; H, 10.31%. Calcd for $C_{28}H_{44}O_3$: C, 78.44; H, 10.37%.

1α-Acetoxyergosteryl Acetate (13). The epoxy diol (**11**) (2.0 g, 4.7 mmol) was treated with pyridine (5 ml)–acetyl chloride (1 ml, 14.0 mmol) at room temperature for 2 h. The mixture was extracted with hexane, washed with dil. hydrochloric acid and brine, and dried over sodium sulfate. Chromatography on silica gel (eluted with hexane) gave the epoxy diacetate (**12**) as oil. 1.9 g (81%): *m/z* 512 (M^+); ^1H NMR δ=5.67, 5.39 (2H, m, H-6, H-7), 4.99 (2H, m, H-1, H-3), 2.09, 2.03 (6H, s, $COCH_3$).

Trifluoroacetic anhydride (1.5 ml, 10.6 mmol) was added to the solution of sodium iodide (6.0 g, 40.0 mmol) in 30 ml of acetonitrile–tetrahydrofuran (1/1, v/v). The mixture was stirred at room temperature for 30 min. After the solution was cooled to 0 °C, the epoxy diacetate (**12**) (900 mg, 1.8 mmol) in 10 ml of acetonitrile–tetrahydrofuran (1/1, v/v) was added dropwise. The mixture solution was kept at 5 °C for 48 h. The reaction mixture was extracted with hexane, washed with 5% sodium hydrogensulfite and brine, and dried over sodium sulfate. The residue after evaporation of the solvent was purified by column chromatography on silica gel (eluted with hexane–ethyl acetate, 95/5, v/v) to afford the crude **13**, which was crystallized from ethanol to yield the diacetate as colorless crystals. 350 mg (40%): mp 129—130 °C; *m/z* 496 (M^+); λ_{max} 282 nm (ε=10800, EtOH); ^1H NMR δ=5.68, 5.40 (2H, m, H-6, H-7), 5.19 (2H, m, H-22, H-23), 5.01 (2H, m, H-1, H-3), 2.08, 2.03 (6H, s, $COCH_3$). Found: C, 77.14; H, 9.78%. Calcd for $C_{32}H_{48}O_4$: C, 77.36; H, 9.76%.

The diacetate (**13**) (300 mg, 0.6 mmol) was treated with ethanolic potassium hydroxide (KOH 150 mg, EtOH 10 ml) under reflux for 10 min. The mixture was extracted with ether and washed with brine. Evaporation of the solvent followed by the crystallization from ethanol yielded the diol (**14**) as colorless crystals. 210 mg (84%): mp 197—198 °C; λ_{max} 282 nm (ε=11000, EtOH); *m/z* 412 (M^+); ^1H NMR δ=5.74,

5.37 (2H, m, H-6, H-7), 5.19 (2H, m, H-22, H-23), 4.07 (1H, m, H-3), 3.78 (1H, bs, H-1). Found: C, 80.92; H, 10.72%. Calcd for $C_{28}H_{44}O_2$: C, 81.48; H, 10.77%.

1α-Hydroxyvitamin D_2 (1). The ether solution of the diacetate (**13**) (200 mg, 0.4 mmol) was irradiated at room temperature for 2 min (high pressure mercury lamp, Ushio UM-452; aq. 1.2% KNO_3 solution as filter). After the ether was removed, the resulted residue was dissolved in ethanol (10 ml) and the solution heated under reflux for 1 h. To the solution, 1 M ethanolic potassium hydroxide solution (2 ml) was added and the mixture refluxed for a further 10 min. The solution was extracted with ether, washed with brine, and dried over sodium sulfate. Column chromatography on silica gel after evaporation of ether (eluted with chloroform–ethyl acetate, 9/1, v/v) afforded the crude hydroxyvitamin D_2 (**1**). Crystallization from hexane–ethyl acetate gave **1** as colorless crystals. 28 mg (17%): mp 136—138 °C (lit,[16] 138—140 °C); λ_{max} 265 nm (ε=18500, EtOH); $[\alpha]_D^{25}$ +47.5° (*c* 0.5, EtOH).

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