Synthesis of 22,23-Epoxyvitamin D_2 (22,23-Epoxyergocalciferol)

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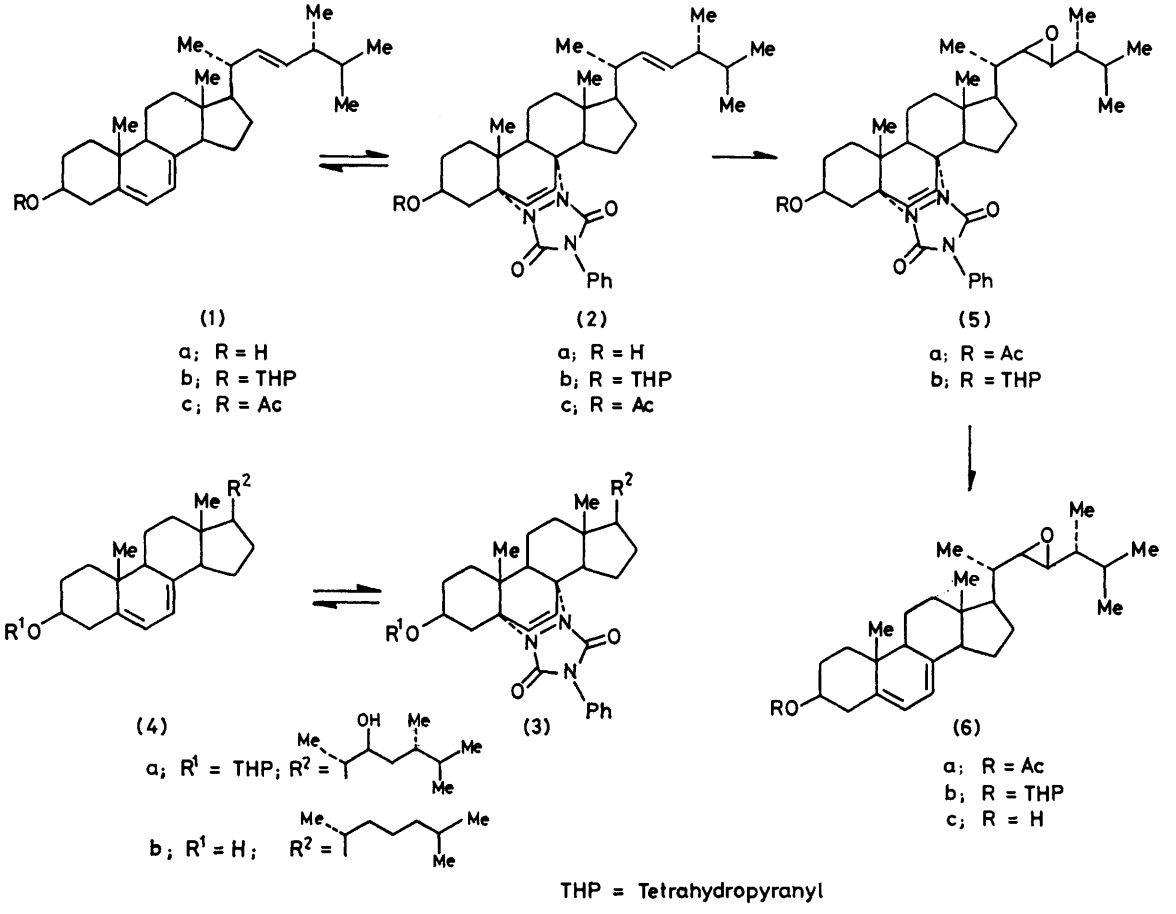
Heating Diels–Alder adducts of steroidal-5,7-dienes and 4-phenyl-1,2,4-triazoline-3,5-dione with mineral base in an appropriate solvent at 120 °C gives the corresponding steroidal-5,7-dienes in high yields. This method is applied to a synthesis of 22,23-epoxyergosterols. The irradiation of this compound followed by thermal isomerization gives 22,23-epoxyvitamin D_2.

VITAMIN D has recently received much attention because its hydroxylated derivatives have characteristic physiological activities.[1] Many 'artificial' hydroxyvitamin D analogues have been prepared in an effort to increase and modify the activity of the vitamin.[2-7] An epoxy-group appears to be one of the important precursors of a hydroxy-group, and thus the introduction of an epoxy-group in the side-chain of vitamin D might be useful in obtaining active compounds. As part of the study of synthetic vitamin D derivatives with potential physiological activities, the synthesis of epoxyvitamin D_2 will be reported here in detail.

The use of 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) for protection of steroidal ring-B dienes has been reported,[4,8-10] in which several ways of reversing the cycloaddition were attempted. Barton *et al.*[9] reported that the diene system could be regenerated in high yield by treatment of these adducts with lithium aluminium hydride, but naturally this method can not be applied to a compound having an $LiAlH_4$-sensitive group which is required in the final product. We established a new method to generate the steroidal diene from the Diels–Alder adduct without affecting an $LiAlH_4$-sensitive group,[11] and this method is applied to the synthesis of the title compound.



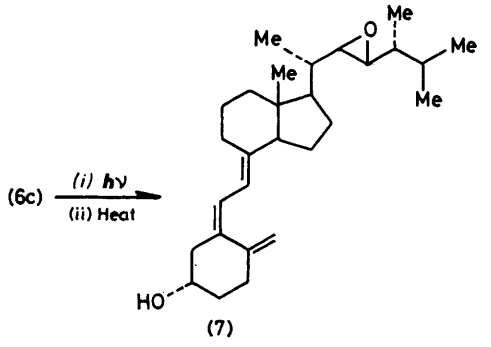
RESULTS AND DISCUSSION

Ergosterol (1a) was treated with PTAD to give the adduct (2a),[8,9] which was then treated with dihydropyran in the presence of $POCl_3$ to afford the ether (2b); treatment of (2b) with acid then gave (2a). Compound (2b) was also obtained from ergosteryl tetrahydropyranyl

ether (1b) [12] by 1,4-cycloaddition with PTAD in 81% yield. The adduct (2b) (1 mmol) and anhydrous K_2CO_3 (1 mmol) in anhydrous dimethyl sulphoxide were heated at 120 °C for 7 h with stirring to give (1b) in a quantitative yield. An analogous result was obtained with the ergosterol adduct (2a). The retro-1,4-cycloaddition of the ergosteryl acetate adduct (2c) [9] in the presence of anhydrous K_2CO_3 in Me_2SO afforded ergosteryl acetate (1c) in high yield. When pyridine was used in place of anhydrous K_2CO_3, the retro-addition of adducts (2) proceeded so slowly that about 90% of (2) was recovered. In the conversion of (2) into (1), Me_2SO and $HCONMe_2$ were better solvents than ethylene glycol or methyl cellosolve. In order to obtain the optimum conditions, these retro-additions were carried out under various reaction conditions (at 50, 70, 100, 120, and 140 °C; and for 2, 5, 7, and 10 h). Yields were optimum at 120 °C and for 7 h reaction. The cleavage of (2c) using a hydrous solvent system was accompanied by removal of the acetyl group of (2c) to furnish ergosterol (1a) in 88% yield. Adduct (3a), derived from 22,23-dihydro-22-hydroxyergosteryl tetrahydropyranyl ether (4a) [11] and PTAD, was treated in an analogous fashion to give a 95% yield of (4a). The 1,4-cycloadduct (3b) [of 7-dehydrocholesterol (4b) and PTAD] was converted into (4b) in 52% yield under similar conditions.

Treatment of the epoxy-adduct (5a) [4] [derived from (2c)] under similar conditions resulted in retro-cycloaddition without affecting the epoxide ring to afford 22,23-epoxyergosteryl acetate (6a) in 83% overall yield from (2c). Adduct (5b) was prepared by epoxidation of (2b) with *m*-chloroperbenzoic acid in CH_2Cl_2. The analogous conversion of (5b) gave 22,23-epoxyergosteryl tetrahydropyranyl ether (6b) in high yield. Hydrolysis of (6b) with dilute HCl in ethanol furnished 22,23-epoxyergosterol (6c), which was also obtained in 95% yield by heating (6a) for 10 min with 2N-KOH in refluxing aqueous methanol.

This retro-addition method is useful because of its simplicity and the high yields obtained, as well as the wide applicability to compounds having groups which are sensitive to $LiAlH_4$.



(7)

Irradiation of the 5,7-diene (6c) with a high-pressure mercury lamp for 1 min gave a mixture of photoisomers, which was then refluxed to effect thermal isomerization of previtamin D into the vitamin. The desired vitamin D (7) was isolated by preparative t.l.c. in 45% yield. Eyley and Williams have reported that fluorenone can be used in a convenient and practical method of increasing the yield of vitamin D_2 from the provitamin.[13] Under similar conditions, irradiation of the diene (6c) in the presence of fluorenone increased the yield of (7) (58%).

The biological activity of (7) is currently under investigation.

EXPERIMENTAL

M.p.s were determined on a hot-stage apparatus. Absorption spectra were recorded with a Hitachi model EPI-S_2 i.r. spectrophotometer and a Hitachi model 124 spectrophotometer. N.m.r. spectra (100 MHz) were recorded in $CDCl_3$ (internal $SiMe_4$ as standard) with a JEOL model PS PFT-100. Mass spectra were run with Shimadzu LKB-9000 spectrometer, operating at an electron-beam energy of 70 eV and with direct inlet. T.l.c. and p.l.c. were carried out with Kieselgel F 254. The developing solvent was of CH_2Cl_2–ethanol (96 : 4).

1,4-*Cycloadditions of PTAD*.—The addition reaction of PTAD to steroidal 5,7-dienes was conducted according to a modification of the method of Barton *et al.*[9]

(*a*) *With ergosteryl tetrahydropyranyl ether* (1b). To a freshly prepared solution of PTAD (1.05 g, 6 mmol) in dry acetone (60 ml), a solution of (1b) (2.41 g, 5 mmol) was added dropwise at −70 °C. After the mixture had been stirred at −70 °C for 3 h, alumina (Grade V) (4 g) was added. The resulting mixture was stirred for a further 1 h and then set aside at room temperature. The mixture was filtered and the filtrate was concentrated to afford an oil, which solidified on trituration with ether to give the adduct (2.66 g; 81%). Recrystallization from ethanol afforded (2b) (2.32 g; 71%) as colourless needles, m.p. 154—155 °C; $\nu_{max.}$ (KBr) 1 760 and 1 704 cm^{-1}; $\lambda_{max.}$ (MeOH) 256 nm (log ε 3.60) (Found: C, 75.05; H, 8.90; N, 6.65. Calc. for $C_{41}H_{57}N_3O_4$: C, 75.08; H, 8.75; N, 6.41%).

(*b*) *With* (22S)-22,23-*dihydro*-22-*hydroxyergosteryl tetrahydropyranyl ether* (4a). A mixture of (4a) (5 g, 10 mmol) and PTAD (2.1 g, 12 mmol) was treated as in (*a*) to give the adduct (3a) (4.96 g; 74%), which was recrystallized from acetone to give colourless needles, m.p. 142—144 °C; $\nu_{max.}$ (KBr) 3 500, 1 760, and 1 710 cm^{-1}; $\lambda_{max.}$ (MeOH) 255 nm (log ε 3.86) (Found: C, 72.65; H, 9.0; N, 6.05. Calc. for $C_{41}H_{59}N_3O_5$: C, 73.07; H, 8.82; N, 6.24%).

(*c*) *With 7-dehydrocholesterol* (4b). A mixture of (4b) (3.85 g, 10 mmol) and PTAD (2.1 g, 12 mmol) was treated in a similar manner as in (*a*) to afford (3b) (4.03 g; 72%) as colourless needles, m.p. 156—157 °C; $\nu_{max.}$ (KBr) 3 450, 1 762, and 1 710 cm^{-1}; $\lambda_{max.}$ (MeOH) 255 nm (log ε 3.68) (Found: C, 75.25; H, 9.1; N, 7.90. Calc. for $C_{35}H_{49}N_3O_3$: C, 75.10; H, 8.82; N, 7.51%).

The 1,4-*Cycloadduct* (2a) *of Ergosterol* (1a).—(*a*) *From ergosterol* (1a). A mixture of (1a) (1.98 g, 5 mmol) and PTAD (1.05 g, 6 mmol) was treated, following the procedure used in the preparation of (2b), to afford adduct (2a) (2.5 g; 87%), m.p. 191—193 °C (lit.,[8] 190—191.5 °C), which was identified by t.l.c. and i.r.

(*b*) *From the* 1,4-*cycloadduct* (2b). To a solution of (2b) (0.2 g) in ethanol (6 ml), concentrated HCl (3 drops) was added. The resulting solution was warmed for 3 min on a water bath. After addition of water, the crystals that

separated were washed with water and recrystallized from aqueous acetone to give (2a) (0.18 g; 90%).

(*c*) *From the* 1,4-*cycloadduct* (2c). To a solution of KOH (0.7 g) in 95% ethanol (30 ml), (2c) (3.2 g) was added. The mixture was refluxed for 4 h to afford (2a) (2.7 g; 97%).

The 1,4-*Cycloadduct* (2b) *from* (2a).—To a solution of (2a) (1.0 g) and 2,3-dihydropyran (0.6 ml) in CH_2Cl_2 (30 ml), $POCl_3$ (1 drop) was added. The mixture was stirred and cooled to keep its temperature below 20 °C. The resulting solution was poured into ether (100 ml), and the ether solution was washed twice with 5% Na_2CO_3, once with saturated NaCl, dried with anhydrous Na_2SO_4, and concentrated to give an oil. The oil solidified on trituration with ethanol to afford an ether (1.0 g; 87%) identical to (2b) on the basis of t.l.c. and u.v. and i.r. spectra.

Retro-1,4-*cycloaddition*.—In these retro-1,4-cycloaddition reactions, all solvents were dried with molecular sieves unless otherwise stated.

(a) *Of the PTAD adduct* (2a). (*i*) A mixture of (2a) (570 mg, 1 mmol) and anhydrous K_2CO_3 (140 mg, 1 mmol) in Me_2SO or $HCONMe_2$ (50 ml) was stirred and heated at 120 °C for 7 h. After addition of alumina (1 g), the reaction mixture was stirred for 10 min at room temperature and filtered. The filtrate was poured into water and the crystals which separated were washed with water to give the product, identical with (1a) by t.l.c. and absorption spectrum.

(*ii*) A solution of (2a) (570 mg) in Me_2SO (50 ml) was heated at 120 °C for 7 h. The same treatment gave (1a) (120 mg; 30%), and (2a) (180 mg; 18%) was recovered.

(*b*) *Of the PTAD adduct* (2b). A mixture of (2b) (660 mg, 1 mmol) and anhydrous K_2CO_3 (140 mg, 1 mmol) in Me_2SO (50 ml) was treated as in (*a*)—(*i*) above to give (1b) (465 mg; 97%).

(*c*) *Of the PTAD adduct* (2c). (*i*) *In anhydrous solvents*. A mixture of (2c) (61 mg) and anhydrous K_2CO_3 (14 mg) in Me_2SO (5 ml) was treated, following the procedure used in (*a*)—(*i*), to give (1c) (43 mg; 98%). A solution of (2c) (61 mg) and pyridine (0.5 ml) in Me_2SO (2.5 ml) was treated in an analogous fashion to give a 10% yield of (1c). A mixture of (2c) and anhydrous K_2CO_3 in ethylene glycol or methyl cellosolve was treated as in the case described above to give (1c) (57 and 52%, respectively).

(*ii*) *In hydrous solvent*. A mixture of (2c) and K_2CO_3 in commercial ethylene glycol was treated as in the case described above to give ergosterol (1a) (88%).

(*d*) *Of the PTAD adduct* (3a). A mixture of (3a) (0.1 mmol) and anhydrous K_2CO_3 in $HCONMe_2$ or Me_2SO was treated in an analogous fashion to give a 94% yield of (4a).

(*e*) *Of the PTAD Adduct* (3b). The treatment of (3b) under similar conditions gave (4b) in 52% yield.

22,23-*Epoxyergosteryl Acetate* (6a).—The epoxide (5a) of (2c) was prepared by a method similar to that described by Crump *et al.*[4] The crude epoxide (5a) [derived from (2c) (2.0 g, 3.3 mmol)] was dissolved in anhydrous Me_2SO (50 ml), and anhydrous K_2CO_3 (115 mg, 3 mmol) was added. The resulting mixture was heated at 120 °C for 7 h with stirring and treated, following the procedure used in retro-1,4-cycloaddition of (2a), to give the product, which was recrystallized from methanol–acetone (2 : 1) to give (6a) [1.24 g; 83% based on (2c)] as colourless needles, m.p. 133—134 °C; $\nu_{max.}$ (KBr) 1 734, 1 652, 1 600, 1 250, 905, and 835 cm^{-1}; $\lambda_{max.}$ (MeOH) 271 (log ε 4.10), 281 (4.12), and 293 (3.89) nm (Found: C, 79.20; H, 10.60. Calc. for $C_{30}H_{46}O_3$: 79.25; H, 10.20%).

22,23-*Epoxyergosteryl Tetrahydropyranyl Ether* (6b).—To a solution of (2b) (1.98 g, 3 mmol), in CH_2Cl_2 (30 ml) was added *m*-chloroperbenzoic acid (780 mg, 4.5 mmol). The resulting solution was set aside at 22 °C for 20 h, passed through an alumina column, and eluted with CH_2Cl_2 to give crude (5b) (2.43 g). A mixture of crude (5b) and anhydrous K_2CO_3 (415 mg, 3 mmol) in anhydrous Me_2SO (50 ml) was treated, following the procedure used in (6a), to afford (6b) [1.36 g; 91% based on (2b)] as silky needles [from methanol–acetone (1 : 1)], m.p. 138—139 °C; $\nu_{max.}$ (KBr disk) 1 652, 1 602, 1 260, 906, and 830 cm^{-1}; $\lambda_{max.}$ (MeOH) 271 (log ε 4.10), 282 (4.11), and 293 (3.89) nm (Found: C, 80.10; H, 10.80. Calc. for $C_{33}H_{52}O_3$: C, 79.79; H, 10.55%).

22,23-*Epoxyergosterol* (6c).—(*a*) *From the acetate* (6a). To a solution of (6a) (228 mg, 0.5 mmol) in ethanol (20 ml), was added 2N KOH (1 ml). The mixture was refluxed for 15 min, diluted with water, and the crystals which separated were recrystallized from 95% ethanol to give (6c) (195 mg; 95%) as colourless needles, m.p. 138—139 °C; $\nu_{max.}$ (KBr) 3 420, 1 654, 1 600, 1 240, 906, and 830 cm^{-1}; $\lambda_{max.}$ (MeOH) 271 (log ε 4.05), 281 (4.06), and 293 (3.85) nm; δ 0.64 (3 H, s, 13-Me), 3.60 (2 H, m, 22-H and 23-H), 4.65 (1 H, m, 3α-H), and 5.5 (2 H, AB quartet, *J* 6 Hz, 6-H and 7-H); *m/e* 412 (M^+), 394 ($[M - H_2O]^+$), 379 ($[M - H_2O - Me]^+$), 299 {$[M$ — (side-chain except Et)]$^+$}, 271 ($[M$ — side-chain]$^+$), 253 (271 — H_2O), 141 (side-chain), 113 (141 — Et), 95 (113 — H_2O), and 42 (113 — C_5H_{11}) (Found: C, 79.60; H, 10.75. Calc. for $C_{28}H_{44}O_2 \cdot 0.5H_2O$: C, 79.76; H, 10.76%).

(*b*) *From the tetrahydropyranyl ether* (6b). A solution of (6b) (497 mg, 1 mmol) in 90% ethanol (30 ml) and concentrated HCl (0.1 ml) was warmed on a water-bath for 5 min and then cooled. The solution was diluted with water and neutralized with $NaHCO_3$, and the crystals which separated were recrystallized to afford needles, identical with (6c) (388 mg; 94%) on the basis of t.l.c. and i.r. and u.v. spectra.

22,23-*Epoxyvitamin* D_2 (7).—(*a*) *Without fluorenone*. A solution of (6c) (41 mg, 0.1 mmol) in a mixture of benzene (100 ml) and ethanol (30 ml) was transferred into a quartz apparatus which was placed in an ice-bath. The solution was agitated by bubbling dry nitrogen through a sintered glass disc in the base of the irradiation vessel. The solution was irradiated for 1 min with a 450 W high-pressure mercury lamp (Ushio UM-453) and the reaction mixture was then refluxed for 1.5 h. After evaporation of the solvent, the residue was applied as a streak to a p.l.c. plate, the plate was developed for *ca.* 3 h, and the band corresponding to the vitamin was scraped off and extracted with ether. The ether solution was evaporated to give 22,23-epoxyvitamin D_2 (7) as a colourless foam in 45% yield [based on the u.v. spectrum (ε 18 300), taken as the standard for calculation [14]]; $\lambda_{max.}$ (MeOH) 263 nm, $\lambda_{min.}$ (MeOH) 227 nm; δ 0.67 (3 H, s, 13-Me), 0.96 (6 H, d, *J* 7 Hz, 25-Me_2), 3.70 (2 H, m, 22-H and 23-H), 3.83 (1 H, m, 3α-H), 4.80 (1 H, d, *J ca.* 2 Hz) and 5.00 (1 H, d, *J ca.* 2 Hz) (19-H_2),5.38 (1 H, m, 7-H or 6-H), and 6.12 (1 H, dd, *J* 13 and 11 Hz, 6-H or 7-H); *m/e* 412 (M^+), 394 ($[M - H_2O]^+$), 379 (394 — Me), 299 {$[M$ — (side-chain — Et)]$^+$}, 271 {$[M$ — side-chain)]$^+$}, 253 (271 — H_2O), 141 side-chain, 136 (ring A), 113 (141 — Et), 118 (136 — H_2O), 95 (113 — H_2O), and 42 (113 — C_5H_{11}).

(*b*) *With fluorenone*. A solution of (6c) (41 mg, 0.1 mmol) in a mixture of benzene (100 ml) and ethanol (30 ml) was irradiated for 1 min with the mercury lamp. After addition of fluorenone (18 mg, 0.1 mmol), the reaction mixture was irradiated for a further 1 min. The resulting

photoreaction mixture was refluxed for 1.5 h and then purified as in (*a*) to afford (7) in 58% yield.

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