SYNTHESIS OF 1α-HYDROXYERGOCALCIFEROL

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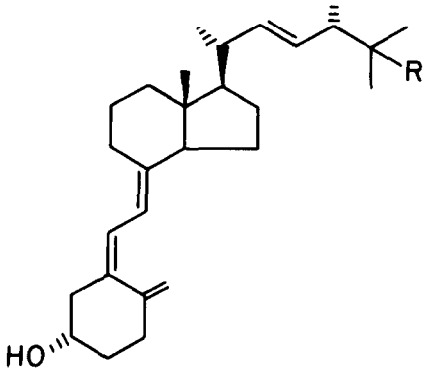
ABSTRACT

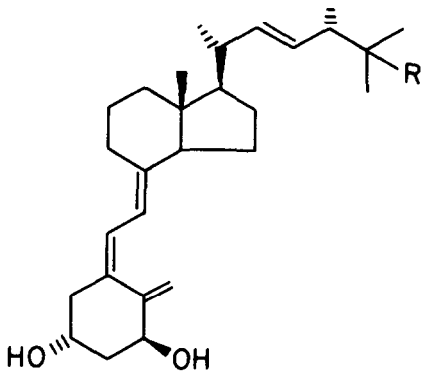
A synthesis of 1α-hydroxyergocalciferol (1α-hydroxyvitamin D_2), a potent analog of vitamin D_2, is described. The preparative route involves conversion of ergosterol in two steps (60%) to the known ergosta-4,6,22-trien-3-one and dehydrogenation of the triene with SeO_2 to ergosta-1,4,6,22-tetraen-3-one (30%). Epoxidation of the tetraenone to the corresponding 1α,2α-epoxide followed by Li/NH_3 reduction gave ergosta-5,22-diene-1α,3β-diol in 26% yield from the tetraenone. After conversion to the corresponding diacetate and allylic bromination/dehydrobromination 1α-acetoxyergosteryl acetate was obtained. Irradiation of this intermediate gave the previtamin which was converted to the new vitamin analog by thermal equilibration and hydrolysis of the acetates. Characteristic uv, nmr and mass spectral patterns confirmed the structure of the product.

INTRODUCTION

The demonstration that expression of biological activity of vitamin D_3 requires prior metabolic conversion (in two steps) to the 1α,25-dihydroxy derivative has prompted synthetic efforts in several laboratories aiming, in part, at a definition of structure/activity relationships of the vitamin D system. Prime preparative targets have been analogs with modified hydroxyl substitution patterns, and their in vivo and in vitro assay has indeed furnished considerable insight into the relative functional importance of specific structural parameters [2]. Since metabolic activation of vitamin D_2 (ergocalciferol, 1) proceeds by an analogous hydroxylation sequence [3,4], i.e. 1→2→3, a comparative study of activity patterns between members of the D_2 and D_3 series appeared desirable. Of particular interest in this regard were derivatives of 1 that might represent useful probes for elucidating the molecular basis for the low activity of vitamin D_2 in birds (ca. 1/10 that of D_3). An obvious initial objective was 1α-hydroxyvitaminD_2 (4), a compound that

would allow direct activity comparisons with the corresponding very potent D_3-analog, 1α-hydroxyvitamin D_3 [2], and thereby help define the site and mechanism of avian discrimination against D_2. We have previously documented [5] the high in vivo potency of analog 4 and offer here experimental details of its preparation.





1 R = H

2 R = OH

3 R = OH

4 R = H

EXPERIMENTAL METHODS

Melting points, determined with a Thomas/Hoover apparatus, are uncorrected. Both nominal and high resolution mass spectra (at 70 eV) were measured on an A.E.I. MS-902 instrument, using direct probe introduction at 120-150°; nmr spectra (in $CDCl_3$ and with TMS as internal reference) were recorded on Varian T-60 or Brüker 90 MHz spectrometers, and Beckman DB or Cary-15 spectrophotometers were used for ultraviolet spectroscopy. Elemental analyses were provided by Microtech Laboratories, Skokie, Ill.

1,4,6,22-Ergostatetraen-3-one (7): To a solution of 5 g of 6 [mp 105-106°, λ_{max} 280 nm, prepared from 5 as described [6]] in 80 ml t-butyl alcohol and 1 ml acetic acid, 1.5 g SeO_2 was added and the mixture was reluxed under N_2 for 16 hr. The solvent was then evaporated in vacuo, the residue redissolved in 150 ml ethanol and 7 ml of 28% aqueous $(NH_4)_2S$ was added. This solution was refluxed for 1.5 hr and then kept at room temperature overnight. After evaporation of the solvent under reduced pressure and addition of $CHCl_3$, the resulting slurry was filtered through a short Al_2O_3-column to remove the Se powder. Concentration of the filtrate and separation of the products on a silicic acid (300 g)

column eluted with 20% ethyl acetate in Skellysolve B gave 1.5 g (30% yield) of the ergostatetraen-3-one 7. Crystallization from Skellysolve B gave pale yellow crystals of mp 110-112°; $[\alpha]_D^{20}$ -66° (c 1.3, $CHCl_3$); uv (EtOH) λ_{max} 301, 257, 225 nm (ε = 11,700; 9000; 10,500); nmr, δ 5.22 (2H, m, C-22,23), 5.90-6.45 (4H, multiplets, C-2,4,6,7), 7.12 (1H, d, J = 10 Hz, C-1); mass spectrum: m/e (relative intensity) 392 (M^+, 54), 377 (5), 349 (6), 268 (100), 265 (13), 173 (42), 171 (48), 159 (28), 147 (31). Anal. calcd. for $C_{28}H_{40}O$: C, 85.66; H, 10.27; found: C, 85.97; H, 10.47.

1α,2α-Epoxy-4,6,22-ergostatrien-3-one (8): A solution of 1.5 g of 7 in 200 ml of MeOH and 50 ml dioxane was treated with 1 ml of 10% NaOH and 6 ml of 30% H_2O_2 and kept at room temperature overnight. Partial evaporation of solvent and addition of water precipitated the product which was filtered, washed with water, dried in vacuo, redissolved in $CHCl_3$, and applied to a column of 100 g silicic acid prepared in $CHCl_3$. Elution with $CHCl_3$ gave 1.2 g (77%) of epoxide (8); crystallization from methanol/acetone yielded material of mp 143-145°; $[\alpha]_D^{20}$ +128.5° (c 1.4, $CHCl_3$); nmr ($CDCl_3$) δ 3.23 (1H, dd, J = 4 and 2 Hz, C-2), 3.60 (1H, d, J = 4 Hz, C-1) 5.22 (2H, m, C-22,23), 5.67 (1H, broad singlet, C-4), 6.10 (2H, s, C-6,7); mass spectrum: m/e (relative intensity) 408 (M^+, 17), 392 (5), 365 (5), 338 (4), 284 (100, M-sidechain + H), 171 (38), 125 (52). Anal. calcd. for $C_{28}H_{40}O_2$: C, 82.30; H, 9.87; found: C, 82.00; H, 10.02.

5-Ergostene-1α,3β-diol (9) and Diacetate (10): A solution of 600 mg of epoxide (8) in 70 ml freshly distilled THF was added (in one batch) to 70 ml liquid ammonia containing 2 g of a 30% lithium dispersion in mineral oil. After 10 min reflux, 15 g NH_4Cl was added in small portions over a 20 min period. Evaporation of NH_3, addition of water, extraction with ether, drying (Na_2SO_4), and evaporation of the ether gave a residue which was chromatographed on silicic acid (120 g) poured as a slurry in 20% ether in Skellysolve B. Elution (250 ml batches of 20%, 50%, 70% ether in Skellysolve B followed by pure ether, then 20% and 50% ethyl acetate in ether) gave 200 mg (30% yield) of 9. Crystallization from Skellysolve B/ethyl acetate gave material of mp 180-182°; nmr, δ 3.85 (2H, broad multiplet, C-1,3), 5.22 (2H, m, C-22,23), 5.60 (1H, m, C-6); mass spectrum: m/e (relative intensity) 414 (68, M^+), 396 (100), 378 (11), 363 (8), 289 (26), 271 (40), 253 (38); high resolution mass analysis: calcd. for $C_{28}H_{46}O_2$, 414.3497; found 414.3497.

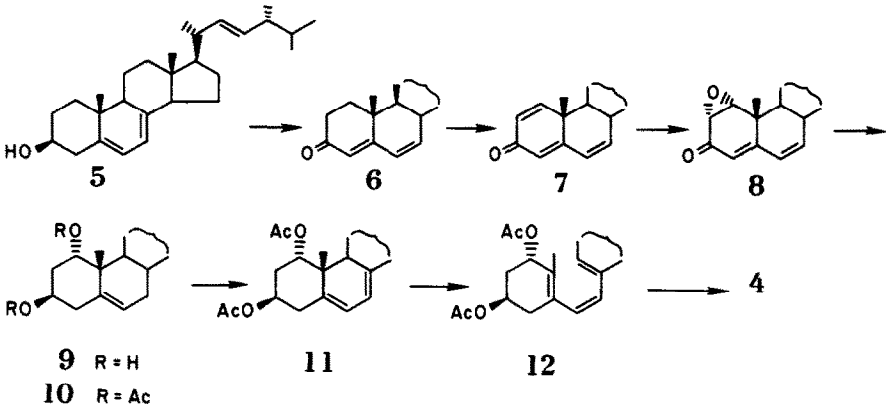
Reaction of 200 mg of 9 in pyridine (10 ml) and acetic anhydride (10 ml) at 80° for 24 hr gave after the usual workup and silicic acid column chromatography, 130 mg (54% yield) of the pure diacetate 10, homogenous on the tlc (R_f = 0.75; ethyl acetate/cyclohexane, 1:1). Crystallization from acetone gave white needles, mp 134.5-135°; nmr, δ, 2.03, 2.06 (6H, singlets, C-1, 3-OAc), 5.1 (4H, m, C-1,3 and C-22,23), 5.56 (1H, m, C-6); mass spectrum: m/e (relative intensity) 378 (77, M-60-60), 363 (5), 235 (4), 253 (14), 157 (27), 125 (29), 118 (100); high resolution mass analysis: calcd. for $C_{28}H_{42}$, 378.3287; found, 378.3279. Anal. calcd. for $C_{32}H_{50}O_4$: C, 77.06; H, 10.10; found: C, 76.99; H, 10.22.

1α-Acetoxyergosteryl acetate (11): To 100 mg of 10 dissolved in 6 ml Skellysolve B, at 70°, 43.0 mg of N,N'-dibromo-5,5-dimethylhydantoin (1.5 eq. of Br) was added. The solution was refluxed with stirring for 15 min then cooled in an ice bath and filtered. The filtrate, taken up in 2 ml xylene, was added dropwise to a solution of 0.2 ml trimethylphosphite and 1 ml xylene preheated to 135°, and was kept at 135-140° for 2 hr. After evaporation of the solvent under reduced pressure the residue was chromatographed on $AgNO_3$-impregnated silicic acid. Elution with 10% ether in Skellysolve B gave ca. 10 mg (10% yield) of compound 11: uv (EtOH) λ_{max} 295, 283, 272 nm; mass spectrum: m/e (relative intensity) 496 (2, M^+), 463 (11), 376 (100), 251 (26).

1α-Hydroxyergocalciferol (4): A solution of 4 mg of 11 in 200 ml ether was irradiated (high pressure Hg vapor lamp, Hanovia Model 654A, vycor filter) at 0° under N_2 for 2 min. The products were separated into two fractions on $AgNO_3$-impregnated silicic acid eluted with 50 ml of 5% and 200 ml of 10% ether in Skellysolve B. The nonpolar fraction contained the desired pre-D derivative 12, exhibiting uv absorption at λ_{max} 260 nm, and λ_{min} 235 mn. After heating in 95% EtOH (ca. 3 ml) at 80° for 2 h, the absorption shifted to λ_{max} 265 nm, λ_{min} 228 and the absorbance was enhanced indicating conversion of the previtamin to the vitamin. Two drops of 0.9 N KOH in MeOH were then added and the mixture was kept at 60° for 10 min. Evaporation of ethanol under a stream of N_2, addition of H_2O and extraction with $CHCl_3$, drying (Na_2SO_4) and evaporation of $CHCl_3$ solvent gave a residue which was applied to a 20 g LH-20 column in $CHCl_3$/Skellysolve B (1:1) and eluted with the same solvent. Collection of 3.2 ml fractions gave 0.52 mg (16%) of 4 in fractions 25-33. Purity was established by hplc analyses which have been documented previously [7]; uv (EtOH) λ_{max} 265 nm, λ_{min} 228 nm; mass spectrum: m/e (relative intensity), 412 (M^+, 24), 394 (19), 376 (10), 287 (12), 269 (15), 251 (14), 152 (35), 135 (71), 134 (100); nmr (90 MHz), δ6.40 and 6.00 (2H, AB quartet, J = 12 Hz, C-6,7), 5.32, 5.00 (2H, narrow multiplets, C-19), 5.20 (2H, m, C-22,23), 4.40 (1H, m, C-1), 4.20 (1H, m, C-3), 0.55 (3H, s, C-18). Original spectra are included in our earlier report [5]. High resolution mass analysis: m/e (composition, m/e calcd.) 412.3305 ($C_{28}H_{44}O_2$, 412.3337), 287.2041 ($C_{19}H_{27}O_2$, 287.2031) 152.0835 ($C_9H_{12}O_2$, 152.0837), 134.0740 ($C_9H_{10}O$, 134.0732).

RESULTS AND DISCUSSION

The new vitamin D analog was prepared from ergosterol (5) by a nine-step route, key stages of which are summarized by structures 5→12. Given the ready availablity of the starting material and the feasibility of regio-and stereoselective epoxidation [8,9] followed by direct reduction [10] of dienone-epoxide 8 to diol 9, the scheme appeared to offer a relatively convenient approach to the required 1α-hydroxylated sterol intermediate.



Oppenauer oxidation of 5 (to 4,7,22-ergostatrien-3-one) followed by acid catalyzed double bond conjugation according to the procedures of Shepherd et al. [6] gave 4,6,22-ergostatrien-3-one (6) in 60% yield. Selenium dioxide oxidation [11,12] of 6 then led to the required tetraenone 7 in 30% yield. Attempts to effect this conversion by DDQ oxidation proved even less rewarding. Upon treatment of 7 with alkaline hydrogen peroxide the α-epoxide 8 was obtained (77%) in accord with analogous preparations of 1α,2α-oxides from trienones of the cholestane[9,10,13, 14], pregnane [15] and androstane [9] series. Reduction of 8 with lithium in liquid NH_3/THF [10] furnished, after chromatography and crystallization, a 30% yield of diol 9, which, via its diacetate (10), was converted to the required 5,7-diene 11 by the bromination/dehydrobromination procedure of Hunziker and Müllner [16]. Disappointing yields (ca. 10%) of pure diene were obtained in this instance, in part due to side reactions involving the unsaturated sidechain of 10. Irradiation of 11 in ether solution

and chromatography of the products yielded the previtamin diacetate derivative 12, from which, after isomerization to the vitamin skelton, saponification and rechromatography, analog 4 (16% from 11) was obtained in pure form (by hplc [7]) exhibiting the expected spectral characteristics.

The synthetic route outlined here could, of course, be adapted to the preparation of other 1α-hydroxyvitamin analogs. Diol 9, for example, could serve as a general intermediate for the elaboration of a variety of side chain analogs, since protection of the Δ^5-unsaturation by i-ether formation [yielding 6β-methoxy-3α,5-cyclo-5α-ergost-22-en-1α-ol; unpublished results] would allow ozonolytic degradation and side chain resynthesis.

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References and Notes

1. Present address: Manitoba Institute of Cell Biology, 700 Banatyne Ave., Winnipeg, Canada R3E 0V9

2. DeLuca, H. F. and Schnoes, H. K., ANN. REV. BIOCHEM. 45, 631 (1976).

3. Suda, T., DeLuca, H. F., Schnoes, H. K., and Blunt, J. W., BIOCHEMISTRY 8, 3115 (1969).

4. Jones, G., Schnoes, H. K., and DeLuca, H. F., BIOCHEMISTRY 14, 1250 (1975).

5. Lam, H.-Y. P., Schnoes, H. K., and DeLuca, H. F., SCIENCE 186, 1038 (1974).

6. Shepherd, D. A., Donia, R. A., Campbell, J. A., Johnson, B. A., Holysz, R. P., Slomp, G., Jr., Stafford, J. E., Pederson, R. L., and Ott, A. C., J. AM. CHEM. SOC. 77, 112 (1955).

7. Jones, G., and DeLuca, H. F., J. LIPID RES. 16, 448 (1975).

8. Pelc, B., Hodkova, J., and Holubek, J., COLL. CZECH. CHEM. COMMUN. 31, 1363 (1966).

9. Pelc, B., and Kodicek, E., J. CHEM. SOC. (C) 1568 (1971).

10. Barton, D. H. R., Hesse, R. H., Pechet, M. M., and Rizzardo, E., J. AM. CHEM. SOC. 95, 2748 (1973).

11. Beard, C. C. in "Organic Reactions in Steroid Chemistry", (J. Fried and J. A. Edwards, eds.). pp. 318-319.Van Nostrand Reinhold Corp., New York, Vol. 1, 1972.

12. Fieser, L. F., and Fieser, M., "Reagents for Organic Synthesis," John Wiley & Sons, New York, 1967, vol. 1, p. 998.

13. Barton, D. H. R., Hesse, R. H., Pechet, M. M., and Rizzardo, E., J. CHEM. SOC. CHEM. COMMUN. 203 (1974).

14. Morisaki, M., Rubio-Lightbourn, J., Ikekawa, N., and Takeshita, T., CHEM. BULL. 21, 2568 (1973).

15. Lam, H.-Y., Schnoes, H. K., DeLuca, H. F., Reeve, L., and Stern, P. H., STEROIDS 26, 422 (1975).

16. Hunziker, F., and Müllner, F. X., HELV. CHIM. ACTA 41, 70 (1958).