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SYNTHESIS OF [25R]- AND [25S]-25,26-DIHYDROXYVITAMIN D_3[1]

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In addition to the 1α-hydroxy group essential for eliciting biological activity, vitamin D_3 metabolites bear the hydroxyl function in side chain at C-24, C-25 and/or C-26.[2] Among those, the absolute configuration at C-24 in the 24-hydroxylated metabolites, 24,25-dihydroxyvitamin D_3[3] and 1,24,25-trihydroxyvitamin D_3[4] have previously been determined as 24R. However, the stereochemistry of C-25 in 25,26-dihydroxyvitamin D_3 has remained to be elucidated. Here, we describe synthesis of [25R]- and [25S]-25,26-dihydroxyvitamin D_3 (12 and 17).[5] Comparison of these synthetic materials with the natural one in respect of chromatographic behavior and biological activity would be expected to establish the absolute configuration of natural 25,26-dihydroxyvitamin D_3.

25ξ,26-Dihydroxycholesterol, a progenitor of 25ξ,26-dihydroxyvitamin D_3 was previously prepared as a 1 : 1 mixture of C-25 epimers.[6] Attempted resolution of those epimers in the form of various derivatives was fruitless. Now, starting from cholenic acid (1) [24R,25S]- and [24S,25R]-24,25-epoxycholest-5-ene-3β,26-diol dibenzoate (6 and 7) were prepared and separated from each other, their stereochemistry at C-24 and C-25 were determined by interrelation to the known[7] [24S]- and [24R]-3,24-dibenzoyloxycholest-5-en-25-ol trimethylsilyl ether (9 and 14), and finally elaborated to [25R]- and [25S]-25,26-dihydroxyvitamin D_3 (12 and 17).

Tetrahydropyranyl ether of cholenic acid (1) was reduced ($LiAlH_4$/THF) and then oxidized (pyridinium chlorochromate/CH_2Cl_2) to give the aldehyde 2. React-

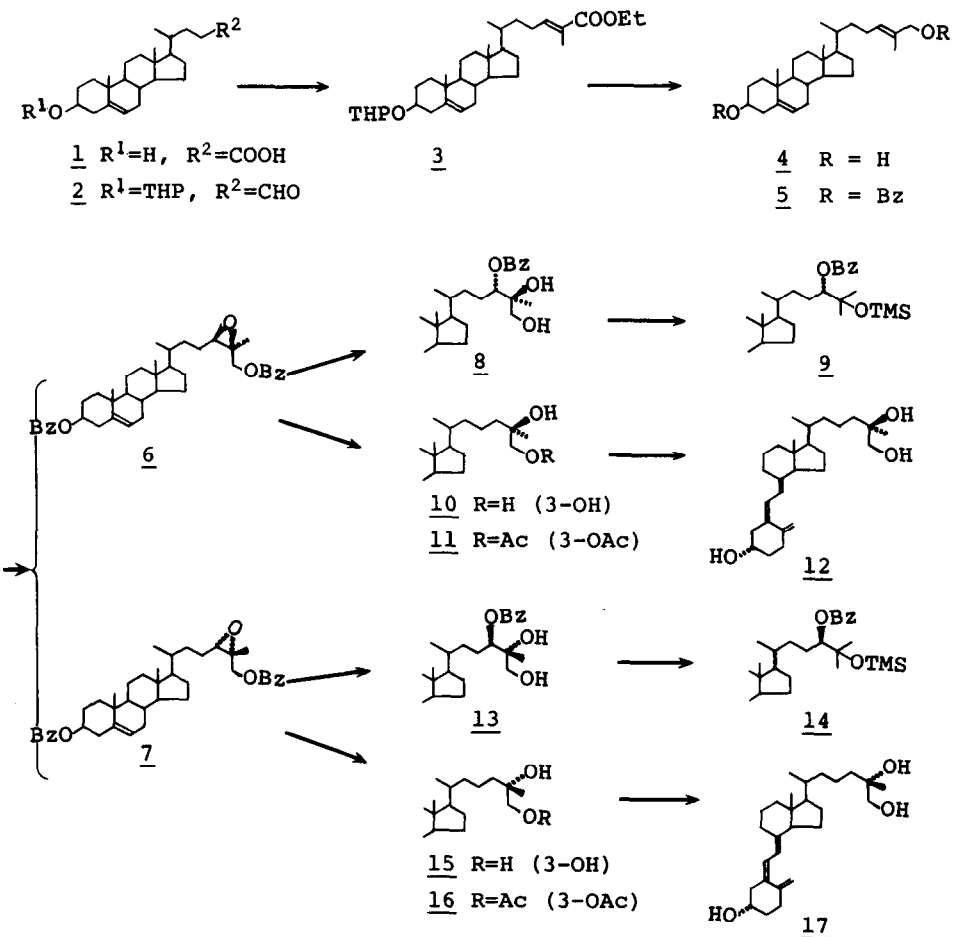
ion of 2 with α-ethoxycarbonyl ethylidene phosphorane in dimethylsulfoxide afforded the (E)-ester 3 (δ, 6.7 ppm, 24-H, characteristic to β-cis-H of α,β-unsaturated ester) in 62% yield from 1. Reduction ($LiAlH_4$/THF) of 3 followed by deprotection (HCl/methanol-THF) gave the diol 4, mp 178°. The corresponding dibenzoate 5 was oxidized with OsO_4/N-methylmorpholin oxide[8] to yield the 24,25-glycol, which on treatment with tosylchloride/pyridine and then with K_2CO_3, afforded a diasteromeric mixture of the 24,25-epoxide (6 and 7) in 86% yield from 4. In view of the E-orientation of the starting olefin 5 and the established mechanism of the subsequent reactions, the configuration of these epoxides should be 24R,25S or 24S,25R. Resolution of the epoxides was accomplished by preparative thin layer chromatography on silica gel developed with benzene several times, to give the less polar epoxide 6 and the more polar epoxide 7 (1 : 1). Their ^1H NMR spectra (100 MHz) were practically indistinguishable, exhibiting the signals at 1.45(25-Me), 2.9(24-H) and 4.4 ppm (AB J=11 Hz, 26-methylene). Acid treatment ($HClO_4$/THF-H_2O) of the more polar epoxide 7 gave the 25,26-glycol 13 (35%) : δ 3.5(AB, J=10 Hz, 26-methylene) and 5.3 ppm(24-H), and 3,26-dibenzoyloxycholest-5-ene-24,25-diol(45%) : δ 3.6(1H, 24-H), and 4.38(2H, AB, J= 11 Hz, 26-methylene). Benzoyl group participation on C-24 during this ring opening reaction was revealed from the parallel experiment carried out in the presence of [^{18}O]-water, when ^{18}O was found to be incorporated into benzoyl carbonyl group.[9] It was deduced that the configuration at C-24 was inverted in the conversion of the epoxide 7 into the glycol 13.

The 26-hydroxyl function of 13 was removed by tosylation and then reduction ($LiAlH_4$/THF). The resulting 24,25-dihydroxycholesterol was derivatized into the [24R]-dibenzoate trimethylsilyl ether 14, identical with an authentic sample.[7] Thus, the configurations of 24S and therefore, 25R were assigned to the more polar epoxide 7. Analogous transformation of the less polar epoxide 6 via the glycol 8 into the [24S]-dibenzoate trimethylsilyl ether 9[7] established the stereochemistry of 6 to be 24R,25S.

Reduction($LiAlH_4$/THF) of 6 and 7 produced the [25R]-25,26-diol 10 and the [25S]-25,26-diol 15, respectively.[10] Subsequent transformations of 10 and 15 to the corresponding vitamin D system were performed in the similar manner used[5b)]

for synthesis of 25ξ,26-dihydroxyvitamin D_3. The obtained [25R]- and [25S]-25,26-dihydroxyvitamin D_3 (12 and 17) show the same thin-layer mobility and identical mass and ultraviolet spectra as those of an authentic sample[6] of 25ξ,26-dihydroxyvitamin D_3.

Biological activity of 12 and 17 is now being examined at Professor Hector F.DeLuca's laboratory.



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5. For the synthesis of 25ξ,26-dihydroxyvitamin D_3 as a mixture of C-25 epimers see : a) J.Redel, P.A.Bell, N.Bazely, Y.Calando, F.Delbarre and E.Kodicek, Steroids, 24, 463 (1974) ; b) H-Y.Lam, H.K.Schnoes and H.F.DeLuca, Steroids, 25, 247 (1975) ; c) S.C.Eyley and D.H.Williams, J. Chem. Soc., Perkin I, 731 (1976). Recently synthesis of 25ξ^1,26- and 25ξ^2,26-dihydroxyvitamin D_3 was reported : d) J.Redel, L.Miravet, N.Bazely, Y.Calando, M.Carrè and F.Delbarre, C.R.Acad. Sci. Paris, 285, Sèrie D, 443 (1977). Subsequently they have determined by x-ray diffraction analysis the stereochemistry of a synthetic precursor of 25ξ^1,26-dihydroxyvitamin D_3 and concluded that 25ξ^1, and 25ξ^2 epimer has 25S- and 25R-configuration, respectively : e) M.Cesario, J.Guilhem, C.Pascard and J.Redel, Tetrahedron Lett., 1097 (1978).

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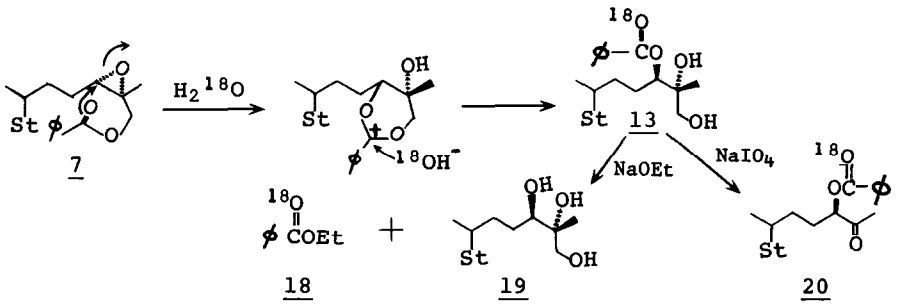
9. The location of the incorporated ^{18}O was determined by mass spectrometric analysis of the dibenzoate 13 and its degradation products (18, 19 and 20). That 13 contains one [^{18}O]-benzoate moiety per molecule was indicated by ions at m/e 522 (M + 2 - BzOH) and m/e 398 (M - 2BzOH) with no significant ion at 400. Similarly 20 showed a prominent ion at m/e 490(M + 2 - BzOH). Absence of ^{18}O in 19 was clear from no peak appeared at m/e 436 (M + 2). Ethyl benzoate 18 showed m/e 152 (M + 2) and 150 (M) in nearly 1 : 1 intensity. It may be considered that the former came from the ^{18}O-containing 24-benzoate and the latter from 3-benzoate.



10. The physical data of the corresponding diacetates 11 and 16 were in agreement with those described in ref. 5d): 11, mp 118-120°, δ(CCl_4, 270 MHz) 3.864 ppm (2H, AB, δ_A-δ_B=0.047 ppm, J=11.40Hz, 26-methylene) ; 16, mp 147-149°, δ 3.870 ppm(2H, AB, δ_A-δ_B=0.063 ppm, J=11.40Hz). We are indebted to Professor T.Miyazawa (The University of Tokyo) for taking NMR spectra (Bruker 270).