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THE SYNTHESIS OF 1α,25-DIHYDROXYCHOLECALCIFEROL -- A METABOLICALLY ACTIVE FORM OF VITAMIN D_3

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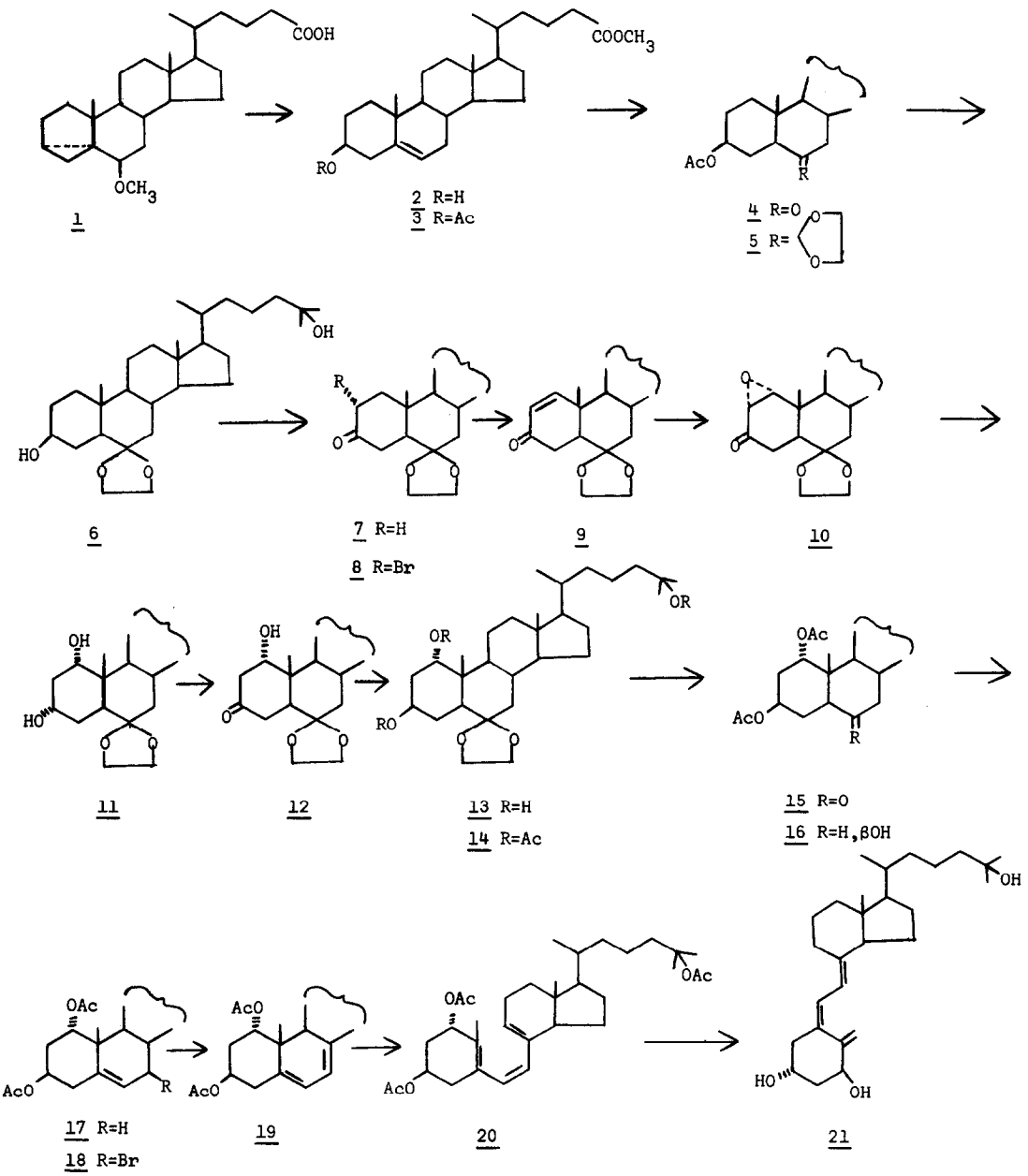
A year ago we reported (1, 2) the isolation and identification of 1,25-dihydroxycholecalciferol -- a metabolite of vitamin D_3, thought to be the active form of the vitamin in intestinal calcium transport and bone calcium mobilization. Independently, Kodicek and coworkers (3) obtained evidence for the same structure for a metabolite produced with kidney preparations in vitro. In view of the biochemical importance of this metabolite, the need for large quantities to investigate clinically its efficacy in the treatment of a number of vitamin D disorders, and the requirement for exact stereochemical definition of the 1-hydroxy function, we undertook a synthesis of 1α,25-dihydroxycholecalciferol (21).

Since maintenance of unambiguous stereochemistry at all centers of the desired vitamin metabolite was essential, a synthetic scheme involving the conversion of a suitable steroid precursor of known configuration to the vitamin appeared preferable (at least initially) to a total synthesis approach. The reaction series outlined in Scheme 1 was therefore adopted. The starting material, i-homocholanic acid methyl ether (1) (courtesy of Dr. John Babcock and Dr. Allan Campbell, the Upjohn Company), was readily converted to the cholenic acid derivative 2 (m.p. 114-115°, MW-402, 90% yield) by diazomethane esterification and acid hydrolysis (perchloric acid/acetone). From the corresponding acetate (3), the 6-ketone (4, m.p. 140-143°, MW-460, 55% yield) was prepared by Fieser's sequence (4) of nitration, reduction and acid hydrolysis. After protection of the ketone as the ketal (5), the 25-alcohol (6, m.p. 174-177°, MW-462) was obtained (81% from 4) by Grignard reaction. Oxidation of 6 (CrO_3/pyridine) afforded ketone 7 (81%, MW-460), which, via its 2-bromo derivative 8, was converted to the Δ^1-compound 9 (MW-458; λ_{max} 227; δ 5.80, d, J =10Hz and 7.05 d, J = 10Hz) by standard procedures (Br/CCl_4, and elimination with collidine or $CaCO_3$/DMF). Epoxidation of crude 9 (containing some saturated ketone 7, removed after step 11

Scheme 1: Synthesis of 1α,25-dihydroxycholecalciferol



1

2 R=H
3 R=Ac

4 R=O
5 R=

6

7 R=H
8 R=Br

9

10

11

12

13 R=H
14 R=Ac

15 R=O
16 R=H,βOH

17 R=H
18 R=Br

19

20

21

by chromatography) with H_2O_2 under basic conditions gave the expected (5) 1α,2α-epoxide (10, MW-474, δ 3.20, d, J = 4.2Hz, and 3.47, d, J = 4.2Hz) and reduction ($LiAlH_4$, ether) of the latter provided 1,3-diol (5). From this mixture, only 1α,3α-isomer (11, MW-478, Rf 0.44 (6)) was recovered by chromatography (7). To obtain the desired 1α,3β-compound (13), inversion of the hydroxyl stereochemistry at C-3 was necessary. This was accomplished (8) by selective oxidation (N-bromosuccinimide, t-butanol/pyridine) of 11 to the 3-ketone 12 (9) and reduction of the latter ($NaBH_4$/isopropanol) to the 1α,3β-isomer 13 (33% from 11, MW-478, Rf = 0.16 (6)). Chromatographic purification of 13, transformation (Ac_2O, pyridine, 90°) to the triacetate 14 (MW-604) and mild acid hydrolysis gave 6-ketone 15 (MW-560), which, upon reduction ($NaBH_4$/isopropanol), furnished the 6-alcohol (16, MW-562). Dehydration of 16 ($POCl_3$/pyridine) yielded the cholesterol intermediate 17 (MW-544; δ 5.1, m, C-1 and C-3-H; 5.6, m, C-6-H; 1.98, 2.05, 2.10, s, acetates). Conversion of 17 to the vitamin metabolite followed established procedures: the 7-bromo-derivative (18) was generated by reaction with N,N'-dimethyldibromohydantoin, and dehydrobromination of 18 with trimethylphosphite gave a mixture of dienes from which the desired 5,7-diene 19 (MW-542, λ_{max} 281, 272) was obtained by chromatography on $AgNO_3$-impregnated silicic acid. Irradiation of 19 and chromatography ($AgNO_3$-silicic acid) furnished the pre-vitamin D_3-derivative 20, from which the desired product -- 1α,25-dihydroxycholecalciferol (21), was obtained by hydrolysis (aqueous EtOH, KOH). Compound 21 and the natural product show identical ultraviolet absorption (λ_{max} 265 nm) and mass spectra (m/e 416 (M^+), 152, 134). Compound 21 and natural (radioactive) material co-chromatograph on Sephadex LH-20 columns. Synthetic and natural products also exhibit identical biological activities in tests of intestinal calcium transport, bone calcium mobilization and the line test for antirachitic activity (10). In addition, the 3α-hydroxy analog of 21 was synthesized (from 11) in a similar fashion and was found to be chromatographically distinct from the natural product as well as biologically inactive at dose levels which give marked biological activity in the case of the synthetic 1α,25-dihydroxyvitamin D_3-(21). Although insufficient pure natural metabolite is available for rigorous comparison with synthetic product the identical chromatographic and biological properties of both indicate strongly that the natural metabolite is 1α,25-dihydroxycholecalciferol (21) (11, 12).

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6. Tlc on silica gel G, ethylacetate-cyclohexane 3:1.

7. By analogy with published (ref. 5) and our own results on reduction of 1α,2α-epoxy-3-ketones of the cholesterol series, the 1α,3β-diol should have been obtained as the major product. It appears that this product may have been lost on chromatography because of its significantly greater polarity. Assignment of configuration to epoxide 10 and products 11 and 13 is based on published data (ref. 5), our own model experiments involving preparation and reactions of 1α,2α-epoxycholestan-3-one and 25-desoxy-10, and detailed analysis of the C-19 proton resonances of all derivatives, which in magnitude and direction of chemical shift are in accord with predictions based on published shift increments (e.g. A. I. Cohen and S. Rock, Jr. Steroids 3, 243 (1964)) for ring A substituents (e.g. C-19-H for 7, 67Hz; 10, 58Hz; 11, 52Hz; 12, 65Hz; 13, 55Hz; at 60 mc).

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9. Formation of the 3-ketone was proven by dehydration of 12 to the Δ^1-3-keto compound and comparison with purified intermediate 9.

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11. Intermediates were checked for homogeneity by tlc and/or glc. The elemental composition of most intermediates was determined by accurate mass measurement of the molecular ion at high resolution. For simple derivatives of the main intermediates (e.g. acetates) composition was inferred from the low resolution mass spectrum.

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