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SUBSTRATES TO STUDY THE MECHANISM OF VITAMIN D HYDROXYLATION: SYNTHESIS OF 25-R-[26-$^2H_3$]-CHOLECALCIFEROL

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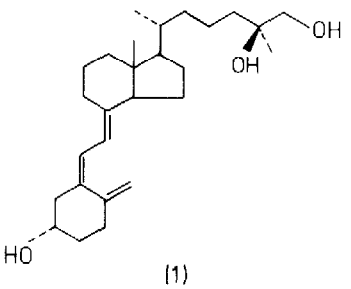
Summary: 25-R-[26-$^2H_3$]-Cholecalciferol has been synthesised from a C-22 aldehyde derived from ergosterol and R-3-[$^2H_3$]-methylbutyl bromide. This vitamin is designed to study the mechanism of enzymic hydroxylation at C-25 in the production of 25S, 26-dihydroxycholecalciferol.

It has recently been concluded that the vitamin D_3 metabolite, 25,26-dihydroxycholeciferol, possesses the 25S-configuration (1).[1] We now report the synthesis of 25-R-[26-$^2H_3$]-cholecalciferol (2) which is a substrate suitable to probe the stereochemistry of enzymic hydroxylation at C-25. If this process occurs with retention of configuration (insertion of oxygen into the C-H bond), then the 25S,26-dihydroxycholecalciferol will be produced without loss of deuterium. If C-25 hydroxylation occurs with inversion, then the metabolite will be formed with loss of a deuterium atom. These results will be differentiated by examination of the mass spectrum of the tris-trimethylsilyl ether of the metabolite.[5]

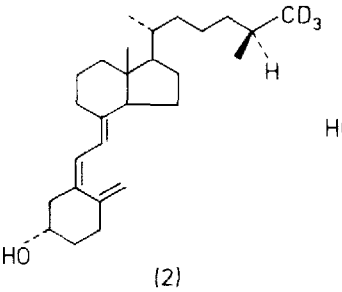
S(+)-Lactic acid (3) was converted to the R-[$^2H_3$]-isopropyl malonic acid (4)[6] via a route based on a procedure published by Mislow[7] and modified by Hill.[8] Briefly, 3 was converted to S-(-)-methyl O-benzyl lactate which was reduced by $LiAlD_4$ to the primary alcohol. The p-bromobenzenesulphonate of this alcohol was reduced with $LiAlD_4$ to give S-(+)-2-benzyloxy-1-[$^2H_3$]-propane. Hydrogenolysis (5% Pd on charcoal in toluene) of this product, tosylation of the resulting alcohol (with p-TsCl/pyridine) and reaction of the tosylate with the sodium salt of diethyl malonate in THF gave 4, which was hydrolysed (aqueous KOH) and then decarboxylated by heating at 140°. The resulting carboxylic acid was reduced with $LiAlH_4$ to give R-3-[$^2H_3$]-methylbutan-1-ol(5). The optical purity of this material was established by use of the chiral shift reagent tris(trifluoromethylhydroxymethylenecamphorate)-europium (III) [Eu(THC)$_3$]. With this shift reagent, isoamyl alcohol exhibits in the shifted spectrum (CCl_4 solution) two methyl doublets (J = 7Hz) near to 3ppm, one due to each enantiotopic methyl group. The correspondingly shifted spectrum of 5 exhibited only one methyl doublet.

On treatment with 48% HBr in concentrated H_2SO_4, 5 was converted to the corresponding bromide (6) which, as the corresponding Grignard reagent, was brought into reaction with the aldehyde 7 (readily available from ergosterol[9]). The resulting C-22 alcohol 8 (71% yield)

was converted to the mesylate (MsCl/pyridine, 76% yield, m.p. 129-133°) and this functionality then removed (86% yield) by reduction with $LiAlH_4$. The ring B protecting group was re-introduced prior to removal of the 3β-OH protecting group (p-TsOH in MeOH, reflux for 1.5h, 96% yield). Reaction of the product with $LiAlH_4$ gave the provitamin 25R-[26-$^2H_3$]-7-dehydrocholesterol (55% yield) which was converted to 2 in an overall yield of 24% by a previously published sequence of irradiation and thermal isomerisation.[10]



(1)



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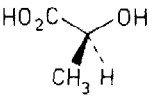

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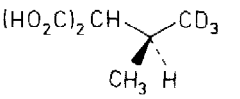

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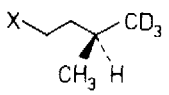
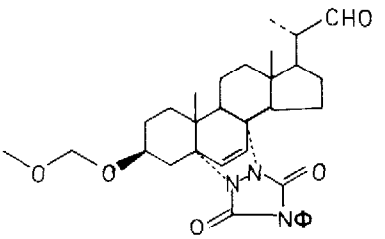


(5), X = OH

(6), X = Br



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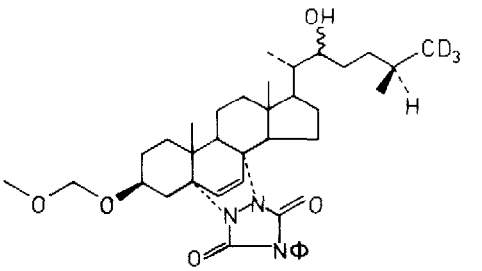

(8)

References

1 Because of an error in the determination by X-ray diffraction of the absolute configurations of C-25 epimers of 25,26-dihydroxycholecalciferol,[2] this metabolite was originally believed to possess the 25R configuration.[3] This error has now been corrected.[4]

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6 All isolated substances described in this paper were characterised by mass spectrometric and ^1H NMR data which were in full accord with the assigned structures.

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