

Structure and Synthesis of 25-Hydroxycholecalciferol-26,23-lactone, a Metabolite of Vitamin D₃

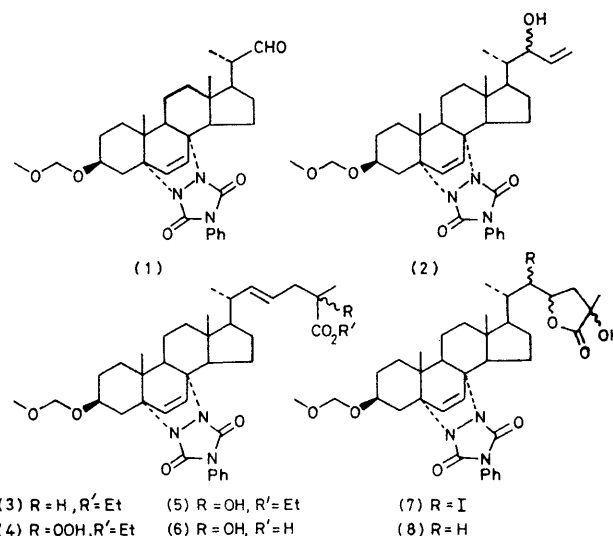
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Summary The four possible stereoisomers of a new metabolite of vitamin D₃, 25-hydroxycholecalciferol-26,23-lactone, have been synthesised; we establish that the natural product has the 23*R*, 25*S* stereochemistry, and that it has <1% of the activity of vitamin D₃ in bringing about intestinal calcium absorption.

In 1979, the isolation and identification of 25-hydroxycholecalciferol-26,23-lactone, a new metabolite of vitamin D₃ was reported.¹ A synthesis of all four possible diastereoisomeric lactones has recently been announced,² but the stereochemistries at C-23 and C-25 were not established. We now report independent syntheses of all four isomers, and show that the natural product has the 23*R*, 25*S* stereochemistry.

Ozonolysis of the 3β-methoxymethyl derivative of ergosterol, in which the ring B diene had been protected by prior reaction with 4-phenyl-1,2,4-triazoline-3,5-dione, gave the aldehyde (1).³ Reaction of (1) with 1.25 equiv. of vinyl magnesium bromide at -78 °C gave (2) (89% yield)† as a mixture of C-22 diastereoisomers, which was brought into reaction (Claisen rearrangement) with a 10-fold excess of triethyl orthopropionate and a catalytic quantity of propionic acid in benzene under reflux. The product (3) (88% yield) was converted into the enolate anion (lithium *N*-isopropylcyclohexylamide at -78 °C), which was in turn converted into the hydroperoxide (4) by reaction with O₂. Immediate reduction of (4) with triethyl phosphite at -78 °C gave the α-hydroxy-ester (5) in 93% yield from (3). The product (5) was shown to be a *ca.* 1:1 mixture of C-25 diastereoisomers by dispersion of its ¹H n.m.r. spectrum with the shift reagent Eu(fod)₃. Under these conditions, the 25-methyl resonance split into two signals of approximately equal intensity.

Alkaline hydrolysis of the mixture of diastereoisomers (5) gave the mixture of acids (6) (100% yield). This mixture was treated with I₂ and KI under basic conditions

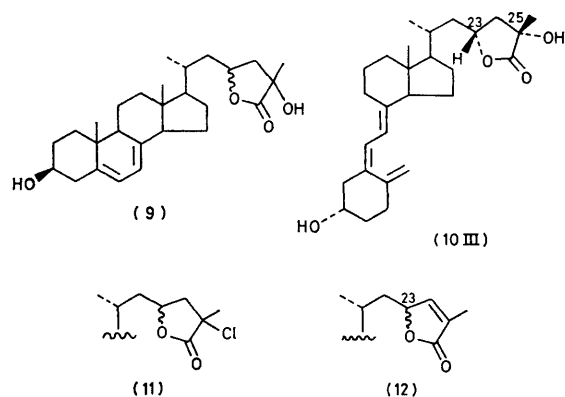


to give the iodolactones (7), which were separated into three components (iodolactones I, II, and III) by preparative h.p.l.c. Reduction of each of these iodolactones with an excess of tri-*n*-butyltin hydride at room temperature gave the de-iodinated lactones (8). Iodolactone I gave lactone I; iodolactone II gave two lactones, II and III, separable by preparative t.l.c.; and iodolactone III gave lactone IV. Thus, all four possible diastereoisomers of (8) were isolated. The yields of (8I), (8II), (8III), and (8IV) from (6) were 12, 5, 21, and 23% respectively (overall yield of lactones 61%).

The remaining synthetic steps were executed separately on the four separated diastereoisomers of (8). Removal of the 3β-methoxymethyl group (100% yield) with toluene-*p*-sulphonic acid in methanol, followed by deprotection of

† Extensive analytical data (¹H n.m.r., m.s., and where appropriate, u.v., i.r., and microanalysis) have been obtained for all the compounds described and are in complete agreement with the assigned structures.

the ring B diene (**54**—75% yields) by heating the product under reflux in collidine,⁴ gave (**9**). This 5,7-diene was irradiated with u.v. light in a mixed solvent (ether-THF-MeOH) (THF = tetrahydrofuran), after which fluorenone was added as a triplet sensitizer⁵ and irradiation continued. The resulting previtamins were isolated by preparative t.l.c., and subjected to thermal 1,7-sigmatropic shift of hydrogen to give the target molecules [(**10III**), and the other three possible stereoisomers at C-23 and C-25]. The yields of (**10I**), (**10II**), (**10III**), and (**10IV**) from the corresponding isomers of (**9**) were 23, 48, 55, and 51%.



Determination of the stereochemistry of all four metabolites (**10**) was possible from experiments carried out on the four corresponding lactones (**8**), all of which had been

obtained in crystalline form. Each lactone (**8**) was subjected to dehydration with SOCl_2 -pyridine at 60 °C. From each diastereoisomer two products [partial structures (**11**) and (**12**)] were obtained. Lactones (**8I**) and (**8III**) gave one butenolide (**12**), and lactones (**8II**) and (**8IV**) another butenolide (**12**). Thus, lactones (**8I**) and (**8III**) have one stereochemistry at C-23; and (**8II**) and (**8IV**) have the other at this centre. The stereochemistries of (**8I**) and (**8II**) [and hence of (**10I**) and (**10II**)] were determined as 23*R*, 25*R* and 23*S*, 25*S* by X-ray crystallography.⁶ Therefore (**8III**) [and (**10III**)] has the 23*R*, 25*S* stereochemistry and (**8IV**) [and (**10IV**)] the 23*S*, 25*R* stereochemistry.

Wichmann *et al.*^{1,2} report that the ^1H n.m.r. spectrum of the naturally occurring metabolite contains the C-23 proton resonance at δ 4.46. Only the spectra of (**10III**) and (**10IV**) are in accord with this observation [C-23 protons of (**10I**, **II**, **III**, and **IV**) are at δ 4.77, 4.72, 4.45, and 4.43, respectively]. Since it is established that 25,26-dihydroxy-cholecalciferol has the 25*S* absolute configuration,⁷ and is a biosynthetic precursor of the lactone metabolite,⁸ then the natural metabolite is 23*R*,25*S*-hydroxycholecalciferol-26,23-lactone (**10III**). A 35 mg sample of (**10III**) has been prepared by the described route [6% overall yield from (**1**)].

Biological testing of the natural metabolite, and its three diastereoisomers,[†] establishes that none of them has high physiological activity in bringing about intestinal calcium absorption (<1% of the activity of vitamin D_3).

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