Structure and Synthesis of 25-Hydroxycholecalciferol-26,23-lactone, a Metabolite of Vitamin D_3

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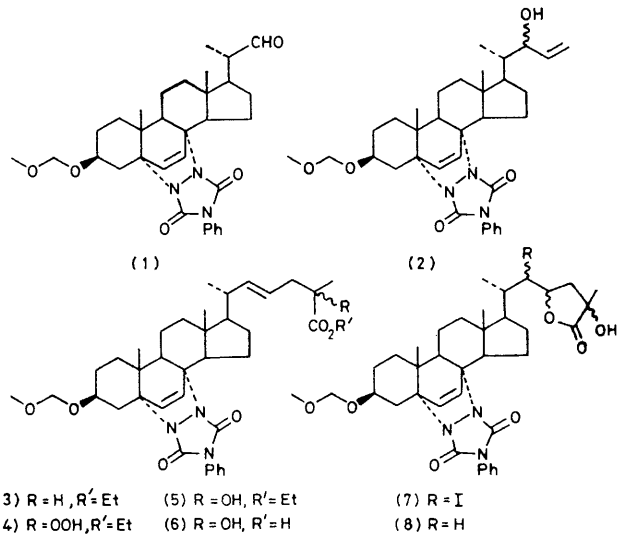
Summary The four possible stereoisomers of a new metabolite of vitamin D_3, 25-hydroxycholecalciferol-26,23-lactone, have been synthesised; we establish that the natural product has the 23*R*, 25*S* stereochemistry, and that it has $<1\%$ of the activity of vitamin D_3 in bringing about intestinal calcium absorption.

IN 1979, the isolation and identification of 25-hydroxycholecalciferol-26,23-lactone, a new metabolite of vitamin D_3 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 O_2. 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 ^{1}H 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 I_2 and KI under basic conditions

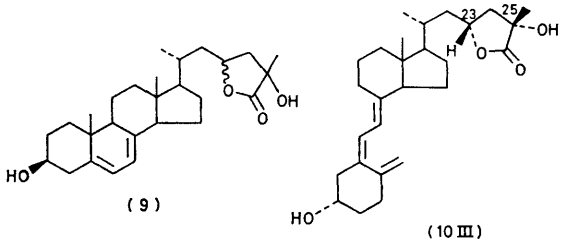


to give the iodolactones (**7**), which were separated into three components (iodolactones I, II, and III) by preparative h.p.l.c. Reduction of each of these iodolactones with an excess of tri-n-butyltin hydride at room temperature gave the de-iodinated lactones (**8**). Iodolactone I gave lactone I; 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 (**8**I), (**8**II), (**8**III), and (**8**IV) 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

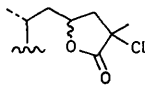
† Extensive analytical data (^{1}H n.m.r., m.s., and where appropriate, u.v., i.r., and microanalysis) have been obtained for all the compounds described and are in complete agreement with the assigned structures.

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 sensitizer[5] 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 [(**10**III), and the other three possible stereoisomers at C-23 and C-25]. The yields of (**10**I), (**10**II), (**10**III), and (**10**IV) from the corresponding isomers of (**9**) were 23, 48, 55, and 51%.



(9)

(10 III)



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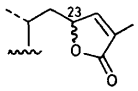

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Determination of the stereochemistry of all four metabolites (**10**) was possible from experiments carried out on the four corresponding lactones (**8**), all of which had been obtained in crystalline form. Each lactone (**8**) was subjected to dehydration with $SOCl_2$–pyridine at 60 °C. From each diastereoisomer two products [partial structures (**11**) and (**12**)] were obtained. Lactones (**8**I) and (**8**III) gave one butenolide (**12**), and lactones (**8**II) and (**8**IV) another butenolide (**12**). Thus, lactones (**8**I) and (**8**III) have one stereochemistry at C-23; and (**8**II) and (**8**IV) have the other at this centre. The stereochemistries of (**8**I) and (**8**II) [and hence of (**10**I) and (**10**II)] were determined as 23*R*, 25*R* and 23*S*, 25*S* by *X*-ray crystallography.[6] Therefore (**8**III) [and (**10**III)] has the 23*R*, 25*S* stereochemistry and (**8**IV) [and (**10**IV)] 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 (**10**III) and (**10**IV) are in accord with this observation [C-23 protons of (**10**I, II, III, and IV) are at δ 4·77, 4·72, 4·45, and 4·43, respectively]. Since it is established that 25,26-dihydroxycholecalciferol 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* (**10**III). A 35 mg sample of (**10**III) 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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