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Synthesis of 19-Acetylthio-3-β-acetoxy-9,10-secochola-1(10),5*Z*,7*E*-triene, and 1-β-Acetylthio-3-β-acetoxy-9,10-secochola-5*Z*,7*E*,10(19)-triene. An Approach to 1β- and 19-Thiolation of the Vitamin D Skeleton

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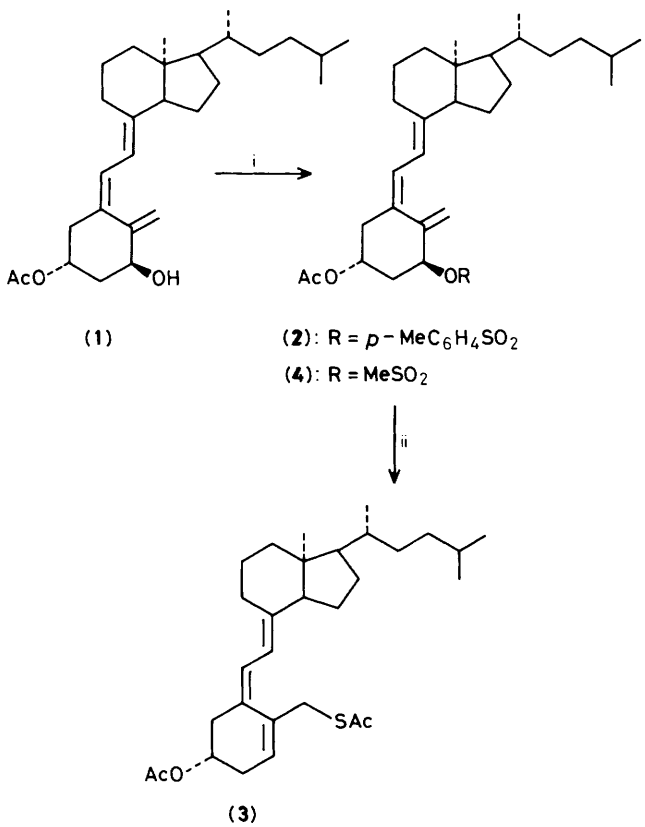
Reaction of 1-α-methylsulphonyloxy or 1-α-(*p*-tolylsulphonyloxy) derivatives of vitamin D_3-acetate with KSAc in dimethyl sulphoxide resulted exclusively in 19-acetylthiolation; the same reaction with 1-α-*p*-tolylsulphonyloxy-(6*R*)-methoxycyclovitamin D_3 and its corresponding 25-hydroxy derivative offered a selective route to 1-β-thiol analogues of vitamin D_3 and 25-hydroxyvitamin D_3, both of which were capable of competing with 1α,25-dihydroxyvitamin D_3 for chick intestinal cytosolic receptor.

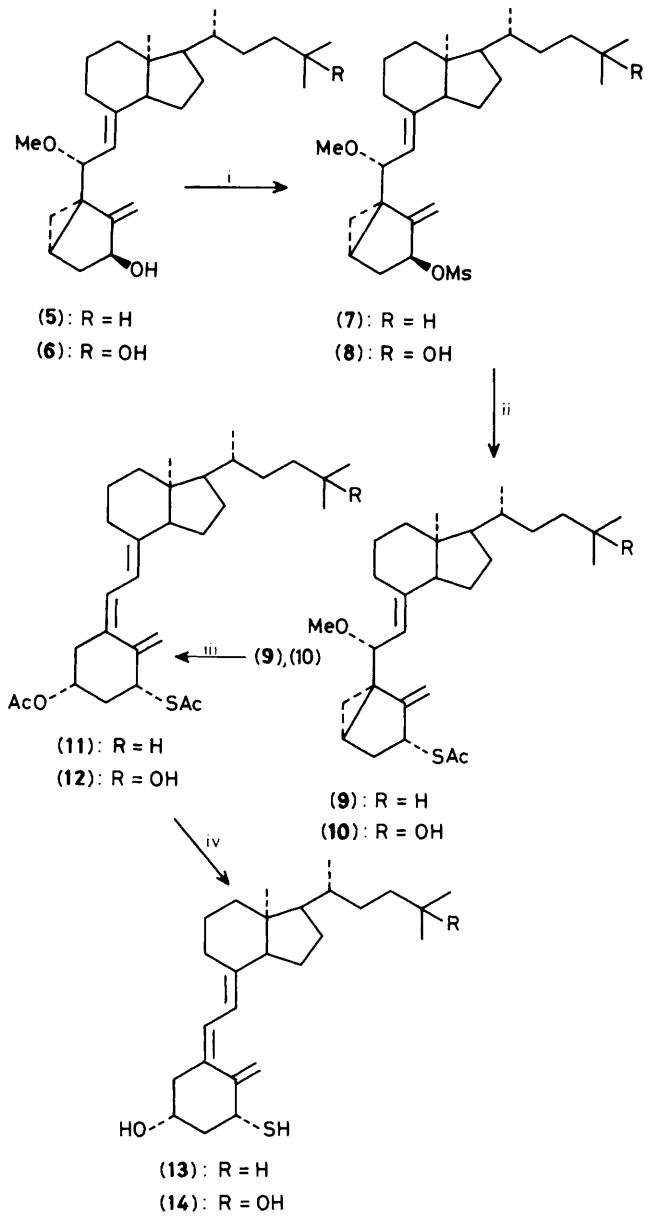
1α,25-Dihydroxyvitamin D_3 [1,25$(OH)_2$-D_3] has been established as the hormonally active metabolite of vitamin D_3 and is the most active form of vitamin D_3 known,[1] having a potent effect on intestinal calcium transport and bone remodelling.[2] The 1α-hydroxy group of 1,25$(OH)_2$-D_3 has been implicated as a very important group for biological activity, whilst the 25-hydroxy group is of secondary importance in this respect.[2] The configuration of the 1α-hydroxy group also governs the biological activity of 1,25$(OH)_2$-D_3 since the epimer, 1β,25-dihydroxyvitamin D_3, has been shown to be devoid of vitamin D-like activity.[3]

Therefore, functionalization of the 1-position in either the 1α or 1β configurations is of interest to investigate further the structure–activity relationships of this important position. We were primarily interested in the effect of 1-thiolation on vitamin D-like activity since sulphur and oxygen possess chemical similarities. It has previously been shown that replacement of the 3β-OH of vitamin D_3 with a 3β-thiol made the vitamin biologically inert *in vivo*.[4] 1α-Fluorovitamin D_3 has also been synthesized and shown to possess biological activity *in vivo*, possibly as a result of 25-hydroxylation of this compound in the liver.[5]

Initially, we chose 1α-hydroxyvitamin D_3-3-acetate (**1**)[6] as a starting material in order to see if 1α-(*p*-tolylsulphonyloxy)-vitamin D_3-3-acetate (**2**) would serve as an intermediate for 1-thiolation. Treatment of (**1**) with *p*-toluenesulphonyl chloride (14 equiv.) in pyridine at 0 °C overnight gave (**2**) in a quantitative yield. Reaction of (**2**) with *S*-potassium thioacetate (52 equiv.) in dimethyl sulphoxide (DMSO) for 10 min at 20 °C afforded 19-acetylthio-3-β-acetoxy-9,10-secochola-1(10),5*Z*,7*E*-triene (**3**) (76%) without trace of 1-substituted products. The same result was obtained with the methanesulphonyl ester (**4**). The reaction was interesting because it was exclusively S_N2 (with rearrangement). Furthermore, this



Scheme 1. *Reagents and conditions*: i, *p*-$MeC_6H_4SO_2Cl$ or $MeSO_2Cl$, pyridine, 0 °C, overnight; ii, KSAc, DMSO, room temp., 10 min.



Scheme 2. *Reagents and conditions*: i, $MeSO_2Cl$, pyridine, 0 °C, overnight; ii, KSAc, DMSO, room temperature, 10 min; iii, AcOH, 60 °C, 15 min; iv, 5% KOH, MeOH, room temp.

reaction offers a route to selective thiolation of the 19-position of the vitamin D backbone, and the subsequent development of interesting 19-substituted thiol analogues. Thioester (**3**) exhibited strong i.r. absorbances for acetoxy (1725 cm^{-1}) and thioacetoxy (1680 cm^{-1}). The 250 MHz ^1H n.m.r. ($CDCl_3$) of (**3**) was as follows: δ 0.57 (s, 3H, 18-Me), 0.86, 0.88 (d, 6H, *J* 5.8 Hz, 26, 27-Me), 0.92 (d, 3H, *J* 6.0 Hz, 21-Me), 1.56 (s, 2H, 19-CH_2), 2.03 (s, 3H, OAc), 2.32 (s, 3H, SAc), 5.04 (m, 1H, OAc), 5.79 (t, 1H, *J* 3.5 Hz, H-1), 5.95, and 6.17 (ABq, *J* 11.5 Hz, H6, 7). The u.v. spectrum (MeOH) showed an absorbance maximum at 288 nm and a minimum at 250 nm characteristic of a fully conjugated triene. It is likely that the driving force for formation of the 19-acetylthiol is an increase in conjugation in going from (**2**) to (**3**).

We next considered 1α-hydroxy-(6*R*)-methoxycyclovitamin D_3 (**5**)[6] and 1α,25-dihydroxy-(6*R*)-methoxycyclovitamin D_3 (**6**)[6] as starting materials since increased conjugation of the exocyclic 10(19) bond could not act as a driving force for 19-substitution in this case. Compounds (**5**) and (**6**) reacted quantitatively with methanesulphonyl chloride (1.2 equiv.) in pyridine at 0 °C to give the corresponding methanesulphonates (**7**) and (**8**), respectively. Reaction of (**7**) and (**8**) with a 20-fold excess of KSAc in dry DMSO gave, after 10 min at 20 °C, 1-β-acetylthio-(6*R*)-methoxycyclovitamin D_3 (**9**)† (76%) and 1-β-acetylthio-25-hydroxy-(6*R*)-methoxycyclovitamin D_3 (**10**)† (40%), with the expected spectral properties. An interesting feature of this reaction was that the 19-acetylthiol isomers of (**9**) and (**10**) were not formed and there was no evidence of any 1α-thioacetoxy substituted products. Treatment of (**9**) and (**10**) with glacial acetic acid for 15 min at 60 °C[6] afforded (**11**) (40%) and (**12**) (22%), respectively, after chromatographic separation from their corresponding 5,6-*trans* isomers. The 250 MHz ^1H n.m.r. spectrum ($CDCl_3$) of (**11**) was as follows: δ 0.54 (s, 3H, 18-Me), 0.87 (d, 6H, *J* 6.7 Hz, 26,27-Me), 0.91 (d, 3H, *J* 6.1 Hz, 21-Me), 2.07 (s, 3H, OAc), 2.32 (s, 3H, SAc), 4.42 (m, 1H, H-1), 4.92 (br. s, 1H, H-19*Z*), 5.04 (m, 1H, H-3), 5.36 (br. s, 1H, H-19*E*), 5.91 and 6.33 (ABq, 2H, *J* 11 Hz, H-6,7). Compound (**12**) exhibited an identical ^1H n.m.r. spectrum to (**11**), except that the 26- and 27-methyls exhibited a singlet at δ 1.23. Compounds (**11**) and (**12**) showed u.v. absorbance maxima at 265 nm (MeOH) characteristic of the vitamin D triene system; all other spectral properties were consistent with their assigned structures. Careful hydrolysis (5% KOH/MeOH) of (**11**) and (**12**) afforded the target compounds (**13**) and (**14**), each with the expected spectral characteristics.

Preliminary bioassays with compounds (**11**)—(**14**) indicated that they were inactive at stimulating intestinal calcium transport and bone calcium mobilisation in vitamin D and calcium-deficient rats.[7] Binding studies of (**11**)—(**14**) using chick intestinal cytosolic receptor indicated that they were capable of competing with [^3H]-1,25$(OH)_2$-D_3.[7] Compound (**12**) was about five times more potent than 25-hydroxyvitamin D_3 at displacing [^3H]-1,25$(OH)_2$-D_3 from the above receptor system. The lack of activity of (**11**)—(**14**) *in vivo* may either be a result of metabolic inactivation, or an indication that the compounds are vitamin D antagonists.

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References

1 H. F. DeLuca and H. K. Schnoes, *Ann. Rev. Biochem.*, 1976, **45**, 631; J. Roth and L. Orci, *Endocrine Rev.*, 1982, **3**, 331.
2 A. W. Norman in 'Vitamin D, Basic and Clinical Nutrition,' Marcel Dekker, inc., 1980, vol. 2, p. 224.
3 S. A. Holick, M. F. Holick, and J. A. MacLaughlin, *Biochem. Biophys. Res. Comm.*, 1980, **97**, 1031.
4 S. Bernstein and K. L. Sax, *J. Org. Chem.*, 1951, **16**, 685.
5 J. L. Napoli, M. A. Fivizzani, H. K. Schnoes, and H. F. De Luca, *Biochemistry*, 1979, **18**, 1641.
6 H. E. Paaren, D. E. Hamer, H. K. Schnoes, and H. F. De Luca, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 2080.
7 M. F. Holick, M. Garabedian, and H. F. De Luca, *Biochemistry*, 1972, **11**, 2715.

† The configuration of the 1-thioacetoxy group in compounds (**9**) and (**10**) was assigned as '1β' from the observation that the major product formed, albeit in low yield, from the reaction of 1α-hydroxy-(6*R*)-methoxycyclovitamin D_3[6] with thioacetic acid in the presence of diethyl azodicarboxylate and triphenylphosphine was identical to (**9**). The reaction procedure was as described in G. Grynkiewicz and H. Burnznska, *Tetrahedron*, 1976, **32**, 2109, substituting thioacetic acid for benzoic acid. This procedure is described to lead to substitution with inversion of configuration for allylic alcohols, without allylic rearrangement.