Finally, we have examined the chloroform extractive of the lichen thalli after the ether extraction and have isolated two neutral substances. The one obtained in a 0.1% yield was found identical with zeorin (V),[8] while the more polar compound (0.15%) was identified with ergosterol peroxide (VI)[9] which was prepared by photosensitized oxygenation of ergosterol.[10]

Acknowledgement The authors wish to express their sincere thankness to Emeritus Professor Y. Asahina and Dr. M. Nuno for identification of the lichen material.

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Received December 11, 1974

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[Chem. Pharm. Bull. 23(3) 695—697 (1975)]

UDC 547.92.04 : 615.356.011.5

Synthesis of Active Forms of Vitamin D. VIII.[1] Synthesis of [24*R*]- and [24*S*]-1α,24,25-Trihydroxyvitamin D_3[2]

24ξ,25-Dihydroxycholesterol was converted, through 1,4,6-trien-3-one and its 1α,2α-epoxide, into 1α,24ξ,25-trihydroxycholesterol. After resolution of C-24 epimers and determining their configurations, both isomers were led to [24*R*]- and [24*S*]-1α,24,25-trihydroxyvitamin D_3 by bromination, dehydrobromination and ultraviolet-irradiation.

Vitamin D_3 is first hydroxylated in the liver on C-25 before it travels to the kidney to be hydroxylated either on C-1 or C-24.[3] Under normal or hypercalcemic conditions the major circulating metabolite of 25-hydroxyvitamin D_3 is 24,25-dihydroxyvitamin D_3.[4] A polar metabolite of the latter has recently isolated by Holick, *et al.*[5] and identified as 1,24,25-trihydroxyvitamin D_3, although the stereochemistry at C-1 and C-24 has been remained to be determined. They have also reported that this vitamin D analog appears to have preferential action on the intestine.

In our continuing efforts of synthesis of vitamin D analogs having useful specific and/or enhanced activities and also in the hope of determining the absolute configurations of the natural 1,24,25-trihydroxyvitamin D_3,[5] we have now synthesized [24*R*]- and [24*S*]-1α,24,25-trihydroxyvitamin D_3 (**11** and **12**).

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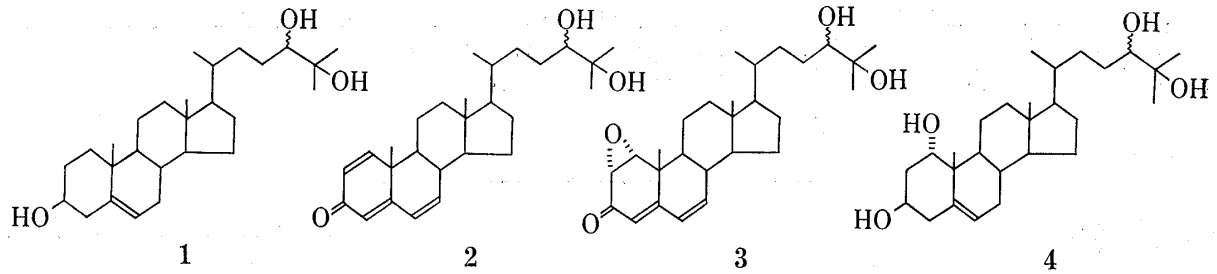
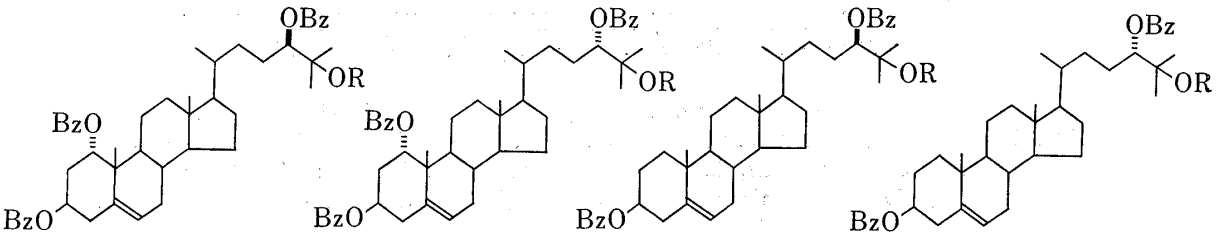
24ξ,25-Dihydroxycholesterol **1**, mp 184—187° (AcOEt) derived from its 3-acetate[6] was subjected to the procedures of Barton, *et al.*,[7] to introduce 1α-hydroxy function. Oxidation of **1** with DDQ/dioxane (reflux, 21 hr) gave trienone **2**, mp 193—197° (acetone) in 65% yield. By reaction of **2** with 30% H_2O_2/NaOH–MeOH, 1α,2α-epoxide **3** was obtained in 76% yield; **3**, mp 164—166° (ether), M^+ 428.2900 (Calcd. 428.2926). Epoxide **3** was reduced with a large excess of Li in liquid ammonia-THF, followed by treatment with NH_4Cl, affording 1α,24ξ,25-trihydroxycholesterol (**4**) in 55% yield; **4**, mp 198—201° (acetone), NMR (C_5D_5N), δ 0.81 (3H, s, 18-Me), 1.07 (3H, s, 19-Me), 1.38 (6H, s, 26,27-Me), 3.47 (1H, m, C-24-H), 4.01 (1H, m, C-1-H), 4.50 (1H, m, C-3-H) and 5.50 ppm (1H, m, C-6-H).

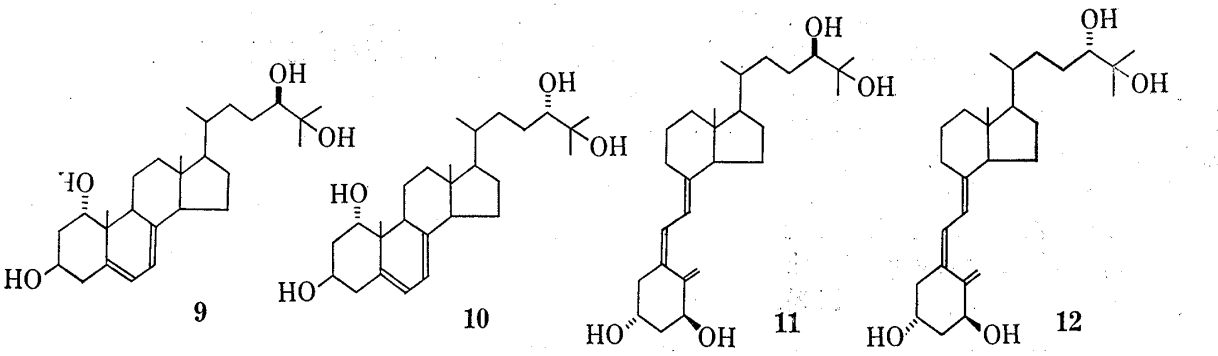
At this stage, resolution of C-24 epimers was performed with dibenzoate trimethylsilyl ether **5** and dibenzoate acetate **6**, by means of silica gel column or thin-layer chromatography. Nuclear magnetic resonance (NMR) spectra of the less polar isomers **5a** and **6a** showed 18-methyl signals at the considerably lower field than those of the more polar ones **5b** and **6b** (see Table I). Comparable behaviors have been observed with 1α-dehydroxy analogs **7** and **8**, whose configurations at C-24 were firmly established.[8] It can be concluded from the data



1 2 3 4



5a : R=TMS **5b** : R=TMS **7a** : R=TMS **7b** : R=TMS
6a : R=Ac **6b** : R=Ac **8a** : R=Bz **8b** : R=Bz



9 10 11 12

TMS=tetamethylsilyl
BZ=benzoyl

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Table I. Chemical Shifts of C-18 Methyl (ppm)

Less polar compd.	18-CH_3	More polar compd.	18-CH_3
5a	0.68	**5b**	0.62
6a	0.66	**6b**	0.60
7a(24*R*)	0.65	**7b**(24*S*)	0.60
8a(24*R*)	0.68	**8b**(24*S*)	0.63

of Table I, that the more polar compounds should have [24*S*] and the less polar ones, [24*R*] configuration.

Bromination of **5a** with dibromodimethylhydantoin in hexane (reflux, 15 min) and dehydrobromination of the crude product with *s*-collidine in *p*-xylene (140°, 15 min) gave a mixture of dienes. This was saponified and purified with column chromatography on silica gel and then with $AgNO_3$-impregnated thin-layer chromatography (TLC) to afford [24*R*]-5,7-diene **9** mp 191—193° λ_{max} (EtOH), 272, 282 and 294 nm, *m/e* M^+ 432, NMR (deuterioacetone), δ 0.67 (3H, s, 18-Me), 0.91 (3H, s, 19-Me), 1.12 (6H, s, 26,27-Me), 3.32 (1H, m, C-24-H), 3.80 (1H, m, C-1-H), 4.08 (1H, m, C-3-H) and 5.52 ppm (2H, ABq, J=6 Hz, C-6,7-Hs).

Irradiation of **9** was carried out with a 200 W medium pressure mercury lamp (Hanovia 654A 36) in ether–EtOH (1: 1) solution (5°, 60 sec). The subsequent refluxing with benzene–EtOH to effect thermal isomerization and the purification with $AgNO_3$-impregnated TLC afforded [24*R*]-1α,24,25-trihydroxyvitamin D_3 (**11**), λ_{min} 228 nm, λ_{max} 265 nm, NMR (deuterioacetone), δ 0.58 (3H, s, 18-Me), 1.12 (6H, s, 26,27-Me), 3.25 (1H, m, C-24-H), 4.15 (1H, m, C-1-H), 4.39 (1H, m, C-3-H), 5.30 and 4.93 (2H, two s, 19-CH_2), and 6.18 ppm (2H, BAq, J= 11 Hz, C-6,7-Hs). Mass spectrum indicated prominent peaks at *m/e* 432 (M^+), 414, 396, 287, 269, 251 and 134 which are in good agreement with those of the metabolic product.[5] Essentially by the same methods, [24*S*]-TMS ether **5b** was converted through 5,7-diene **10** mp 148—154° into [24*S*]-1α,24,25-trihydroxyvitamin D_3 (**12**). The spectral (UV, mass and NMR) properties of **12** were indistinguishable from those of [24*R*]-isomer **11**.

Biological activity of **11** and **12**, and their identification with the metabolic product[5] are under investigation.

This work was supported by a grant from the Ministry of Education, for which we greatly appreciate.

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Received December 17, 1974

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