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Synthesis of (23*R*)-Calcidiol Lactone (25-Hydroxyvitamin D_3 26,23-Lactone)[1]

(23*R*)-Calcidiol lactone(25-hydroxyvitamin D_3 26,23-lactone) was synthesized from bisnorcholenic acid. The configuration at C-23 was determined by transformation to 22- and 23-hydroxycholesterols.

Keywords——calcidiol lactone; 25-hydroxyvitamin D_3 26,23-lactone; metabolite of vitamin D_3; 22-hydroxycholesterol; 23-hydroxycholesterol

Calcidiol lactone[2] is a new metabolite of Vitamen D_3. Although the preliminary tests of this metabolite showed interesting biological activity,[2,3] the configuration at C-23 and C-25 positions as well as the biological role of this metabolite are unknown yet. In order to confirm the reported structure and to elucidate the configuration at C-23 and C-25, it is necessary to synthesize four possible isomers. It would be also urged to prepare the natural calcidiol lactone for biological investigation. We describe herein a synthesis of (23*R*)-calcidiol lactone.

The 22-aldehyde 3-THP ether **1**[4] derived from 22,23-bisnorcholenic acid was coupled with vinylmagnesium bromide to give a mixture of the 22-alcohols (**2**) in a 6: 1 ratio. The less polar major alcohol, mp 155—156°, possesses the 22*R*-configuration according to the precedents for this mode of reaction.[5] The 22-alcohol **2** was reacted with ethyl orthopropionate and propionic acid as catalyst in refluxing xylene to give 22,23-*trans* 26-ethyl ester **3a** [mp 105—107.5°; NMR δ 1.11 ppm (3H, d, J=6.6 Hz, 27-H_3), 5.20—5.50 (3H, m, 6-H, 22-H, 23-H); IR 1720 cm^{-1}] in 96% yield. The 26-ester **3a** was hydrolysed to the 3-hydroxy-26-acid **3b**, mp 175—178°, by HCl-methanol and then KOH–methanol treatment. Iodolactonization[6] of the acid with iodine in acetonitrile at −0° gave regio- and stereoselectively a single product **4a** [mp 220—224°; 94% yield; NMR δ 1.28 (3H, d, J=6 Hz, 27-H_3), 2.50—3.10 (1H, m, 25-H), 4.10 (1H, dd, J=5.1, 5.7 Hz, 22-H), 4.60 (1H, m, 1/2w, 30 Hz, 23-H); IR 1768 cm^{-1}]. The iodolactone **4a** was then reduced by freshly distilled tributyltinhydride in dry THF at room temperature to the lactone **5a** [mp 223—224°; 85% yield; NMR δ 1.27 (3H, d, J=7.5 Hz, 27-H_3), 4.52 (1H, m, 1/2w 26 Hz, 23-H); IR 1760 cm^{-1}; CD (dioxane) $[\theta]^{20}$ (nm): −19 (249) (negative maximum).

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The C-23 configuration of the lactone **5a** was determined as *R* by transformation of the compound **4a** into 22- and 23-hydroxycholesterols as follows. The iodolactone 3-THP ether **4b** was converted to 22,23-epoxy-26-methyl ester **6** (mp 184—185°) with sodium carbonate in methanol. Reduction of the ester with $LiAlH_4$ in THF at room temperature gave 22,23-epoxy-26-ol **7a**. After iodination of the 26-ol by treatment with TsCl–pyridine and then NaI–acetone (reflux), the iodide **7b** was reduced with tributyltinhydride to 26-methyl compound **7c**. Reduction of the epoxide with $LiAlH_4$ and subsequent removal of the 3-THP group afforded a 1:1 mixture of 22- and 23-hydroxycholesterols. These hydroxycholesterols were identical with the authentic samples of (22*R*)-22-hydroxycholesterol (**8**)[7] and (23*S*)-23-hydroxycholesterol (**9**),[8] respectively, with respect to the retention times on GLC[9] and HPLC.[10]

Introduction of a hydroxy group at C-25 position was achieved by oxidation with MoOPH (MoO_5·Py·HMPA) of the enolate of lactone generated by lithium diisopropylamide[11] in THF at −78° to give 25-hydroxy-lactone **10a** [mp 184—193°, 65% yield; NMR δ 1.47 (3H, s, 27-H_3);

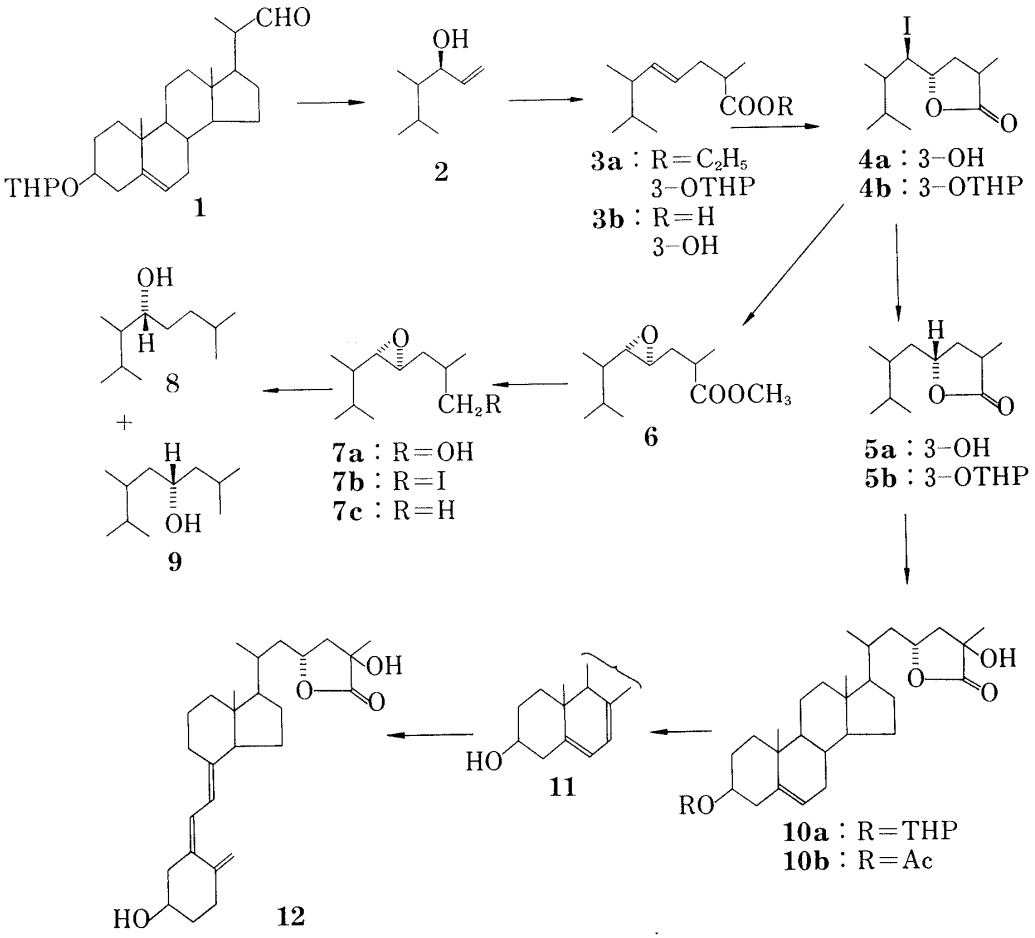

Chart 1

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9) The retention times on GLC using glass capillary column, 30 m × 0.25 mm i.d.; (22*R*)-22-OH (**8**) bis-trimethylsilyl ether (TMSi), 37.11 min; (22*S*)-22-OH bis-TMSi, 38.11 min; (23*R*)- and (23*S*)-23-OH (**9**) bis-TMSi, 41.22 min.
10) The retention times on HPLC using Zorbax SIL, 15 cm × 4.6 mm i.d., hexane–CH_2Cl_2 (6:1), 20 kg/cm²; (22*R*)-22-OH (**8**) bis-(+)-α-methoxy-α-trifluoromethyl-phenylacetate (MTPA), 7.4 min; (22*S*)-OH bis-MTPA, 9.0 min; (23*R*)-23-OH bis-MTPA, 9.4 min; (23*S*)-23-OH (**9**) bis-MTPA, 7.8 min.
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IR 1762 cm^{-1}; CD (dioxane) $[\theta]^{20}$ (nm) −49510 (230) negative maximum[12])] as a single product. After conversion of the 3-THP ether to 3-acetate, transformation of **10b** (mp 224—228°) into calcidiol lactone **12** was carried out by the standard procedure.[13] Bromination with N-bromosuccinimide in CCl_4, followed by debromination (collidine in refluxing xylene) gave a mixture of the 5,7-diene and 4,6-diene. Treatment with *p*-toluenesulfonic acid to convert the 4,6-diene into the much less polar 2,4,6-triene, purification by preparative TLC, alkaline hydrolysis of the acetyl group and then acidification gave the 5,7-diene **11** (30% yield, λ_{max} 262, 271, 281.5 and 293 nm). Irradiation of the diene **11** with a medium pressure mercury lamp in benzene–EtOH solution at 0° for 2.5 min, refluxing for 1 hr in the same solvent and purification by preparative TLC afforded the (23*R*)-calcidiol lactone **12** [23% yield, λ_{min} 226, λ_{max} 265 nm; MS *m/e*: 428 (M^+), 410, 395, 369, 271, 253, 211, 199, 197, 183, 171, 159, 158, 143, 136, 118]. The UV and MS spectra of the synthetic calcidiol lactone were superimposable with the data reported by DeLuca.[2] The signals in NMR spectrum [$CDCl_3$, 200 MHz; δ 0.58 (3H, s, 18-H_3), 1.05 (3H, d, J=5 Hz, 21-H_3), 3.99 (1H, m, 3α-H), 4.86 and 5.10 (2H, s pair of broad s, 19-H), 6.07 (1H, d, J=12 Hz, 7-H), 6.28 (1H, d, J=12 Hz, 6-H) are almost identical with the data reported for the natural metabolite, except one proton signal at δ 4.78(m) corresponding to C-23 proton, instead of the signal at δ 4.46.[2] This might suggest that the natural calcidiol lactone has 23*S*-configuration. However, it cannot be ruled out that this shift may be due to the difference of the C-25 stereochemistry.

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