tion, just as aspirin and indomethacin, anti-inflammatory drugs which are well known as inhibitors of prostaglandin biosynthesis,[11] have no effect on ADP. Thus, these compounds like aspirin and indomethacin may be expected to have some effect on prostaglandin biosynthesis and anti-inflammatory activity.

It is also of pharmacological interest that the two dopamine metabolites (compound I and 3,4-dihydroxyphenylethanol) exhibited anti-aggregatory activity and were about two times as effective as aspirin in AA (Table I).

Studies are now in progress on the effects of the above compounds on the prostaglandin biosynthesis and anti-inflammatory activity and on the structural elucidation of compounds IV and V.

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Stereoselective Synthesis and Structure Proof of a Metabolite of Vitamin D_3, (23*S*, 25*R*)-25-Hydroxyvitamin D_3 26,23-Lactone (Calcidiol Lactone)

Stereochemical configurations of biologically prepared 25-hydroxyvitamin D_3 26,23-lactone (calcidiol lactone) at C-23 and C-25 are determined to be *S* and *R*, respectively, by comparison of its high performance LC retention time with those of (23*S*,25*R*)- and (23*R*,-25*S*)-25-hydroxyvitamin D_3 26,23-lactone which have been synthesized stereoselectively starting from C-22 steroid aldehyde and (*R*)- or (*S*)-citramalic acid.

Keywords——vitamin D metabolite; calcidiol lactone; stereoselective synthesis; determination of stereochemistry; iodolactonization

Recently 25-hydroxyvitamin D_3 26,23-lactone (calcidiol lactone) has been isolated and identified[1] as one of the major metabolites of vitamin D_3. Although syntheses of all of the four possible diastereomers of the metabolite have been reported[2] and two of the four isomers have been shown to have the spectroscopic properties in accord with the natural calcidiol lactone, one of which being demonstrated to co-migrate with the natural product,[2a] the stereochemistries of the metabolite at C-23 and C-25 have still not been clarified. In the previous paper,[3] we reported the stereoselective synthesis of 23*R*,25*S*-calcidiol lactone using readily available (*S*)-citramalic acid as the chiral template to construct the side chain, because 25*S*,26-dihydroxyvitamin D_3 was expected to be a biosynthetic precursor of calcidiol lactone[4] and showed that the compound was the only isomer with 25*S* configuration whose spectroscopic properties were in agreement with those of the natural calcidiol lactone. While preparing this manuscript, another synthetic work of calcidiol lactone was appeared.[5] In the paper the stereochemistries of all of the four diastereomeric calcidiol lactones synthesized *via* the non-stereospecific routes were determined unequivocally based on the X-ray analysis and

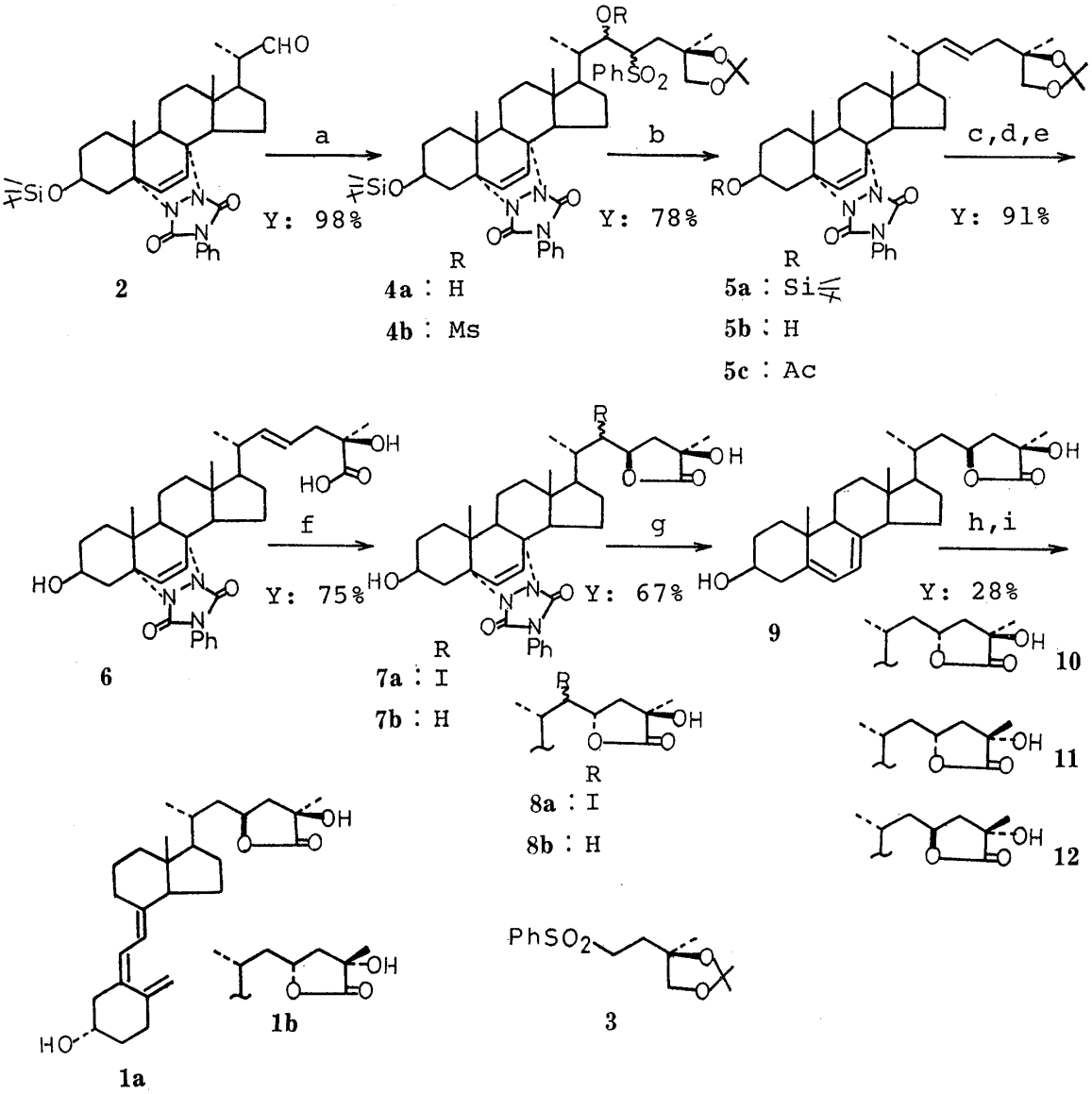

Chart 1

a) Lithium diipopropyl amide (LDA), **3**. b) Na–Mg, MeOH, Na_2HPO_4. c) Pyridinium *p*-toluenesulfonate(PPTS), EtOH. d) Dimethyl sulfoxide(DMSO), pyridine-SO_3, Et_3N. e) I_2, KOH, MeOH, H_2O. f) I_2, CH_2Cl_2, pyridine. g) K_2CO_3, DMSO, 120—130°C. h) *hν*. i) EtOH, room temp. Y=yield.

the 23*S*,25*R*- and 23*R*,25*S*-isomers were shown to be the ones whose spectral data were in accord with those of the natural metabolite. From biosynthetic point of view, they concluded that 23*R*,25*S*-calcidiol lactone was the natural product. Since we have also come to the conclusion that 23*R*,25*S*- and 23*S*,25*R*-calcidiol lactone are two of the four diastereomers, spectral properties of which are in compatible with those of the natural product, we have proceeded the synthesis of the two isomers for the direct comparison with the metabolite to determine the stereochemistries. We now accomplished the synthesis and wish to report, for the first time, that the configurations of the natural calcidiol lactone at C-23 and C-25 were determined to be *S* and *R*, respectively, by the direct comparison with the natural metabolite.

Synthesis of 23*S*,25*R*-calcidiol lactone (**1a**) was performed *via* essentially the same synthetic pathway as described for the 23*R*,25*S*-isomer (**1b**)[3] starting from the C-22 steroid aldehyde (**2**) and (*R*)-1,2-isopropylidene-2-methyl-4-phenylsulphonylbutane-1,2-diol (**3**) (mp 61—62°C, $[\alpha]_D^{24}$ +8.1, $CHCl_3$, c=1.5) which was obtained from (*R*)-(−)-citramalic acid in 65% overall yield. Each step proceeded similarly to that of the corresponding 25*S*-isomer as shown in Chart 1 (**2**→**6**). Iodolactonization of the γ,δ-unsaturated carboxylic acid (**6**) was studied in more detail in order to obtain the desired 23*S*,25*R*-lactone. Under the conditions (I_2, MeCN, 0°C) where the same carboxylic acid with 25*S* configuration yielded exclusively 23*S*-iodolactone (corresponds to 23*R*-lactone) (90% stereoselectivity) iodolactonization of the 25*R*-carboxylic acid (**6**) exhibited poor stereoselectivity and the iodolactone **7a** and **8a** were obtained in 3: 4 ratio. Variation of the solvent (CH_2Cl_2, Et_2O, and AcOEt) did not affect the product ratio appreciably, however, addition of pyridine found to cause pronounced effect in the stereoselectivity and the desired isomer (**7a**) was obtained as the major product. Thus, by treatment with iodine (6 eq.) in CH_2Cl_2 in the presence of pyridine (15 eq.) at 0°C, **6** afforded **7a** and **8a** in 4: 1 ratio in 75% total yield. The epimers were separated readily after converted to the lactone (**7b** and **8b**) by silica gel column chromatography. Stereochemistry at C-23 of the epimers were determined by comparison of the ^1H NMR spectra of the provitamin D (**9** and **10**) [δ in $CDCl_3$ **9**: 0.63 (3H, s, H-18), 0.95 (3H, s, H-19), 1.50 (3H, s, H-27), 4.44 (1H, m, H-23); **10**: 0.65 (3H, s, H-18), 0.95 (3H, s, H-19), 1.50 (3H, s, H-27), 4.78 (1H, m, H-23)] with those of the corresponding 23*R*,25*S*- (**11**) and 23*S*, 25*S*-isomers (**12**).[3] The major isomer whose spectral property was similar to that of the 23*R*,25*S*-isomer (**11**) was assigned to the 23*S*,25*R*-lactone (**9**)[6] because of the stereochemical resemblance of the two isomers. The provitamin D (**9**) was converted to the vitamin (**1a**) by the usual method. Spectral data of the 23*S*,25*R*-calcidiol lactone [MS *m/e* 428 (M^+), 410, 395, 369, 136, 118; IR ($CHCl_3$) 1772 cm^{-1}, ^1H NMR ($CDCl_3$) δ 0.56 (3H, s, H-18), 1.49 (3H, s, H-27), 3.97 (1H, m, H-3), 4.44 (1H, m, H-23), 4.83 (1H, bs, H-19), 5.05 (1H, bs, H-19), 6.13 (2H, ABq, J=11 Hz, H-6 and 7); UV (95% EtOH) 265 nm] thus synthesized were in good agreement with those reported for the natural metabolite.[1]

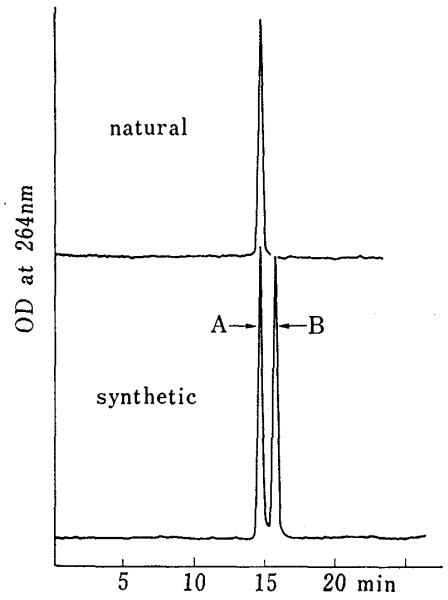

Fig. 1. HPLC Profile of Natural (upper) and Synthetic (bottom) 23*S*, 25*R*- (**A**) and 23*R*,25*S*-Calcidiol Lactone (**B**)

Column, 4.6 mm × 25 cm Zorbax-SIL; eluent, 9% isopropanol in hexane; flow rate, 1 ml/min; detector, 264 nm.

To determine the configuration of the natural calcidiol lactone, the high performance liquid chromatography (HPLC) elution times of the natural metabolite[7] and the synthetic 23*S*,25*R*- and 23*R*,25*S*-calcidiol lactone (**1a** and **1b**) were compared. As shown in Fig. 1, the

natural product was eluted with the same retention time as that of the 23*S*,25*R*-isomer (**1a**) demonstrating the configurations of the natural calcidiol lactone at C-23 and C-25 to be *S* and *R*, respectively.

This results clearly ruled out 25*S*,26-dihydroxyvitamin D_3 as a biosynthetic precursor of the natural calcidiol lactone. Recently isolation and identification of 23*S*,25-dihydroxyvitamin D_3 have been announced as an *in vitro* metabolite of vitamin D_3.[8] The new metabolite may probably be the true biosynthetic precursor of calcidiol lactone.

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