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SYNTHESIS OF ACTIVE FORMS OF VITAMIN D VI[1]

SYNTHESIS OF (24R)- AND (24S)-24,25-DIHYDROXYVITAMIN D_3

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Recently, DeLuca et al.[2] have synthesized 24,25-dihydroxyvitamin D_3, a kidney metabolite of vitamin D_3 and found that the synthetic product, like the natural metabolite, elicits a pronounced and long-lasting intestinal calcium transport response, but has no effect on the bone calcium mobilization process. Although the stereochemistry at C-24 may be important for the expression of biological activity, both of natural and synthetic materials have not yet been elucidated on C-24 configuration. Now it is described in the present paper the synthesis of (24R)- and (24S)-24,25-dihydroxyvitamin D_3(1 and 2), from (24R)- and (24S)-24,25-dihydroxycholesterol derivatives, whose configurations at C-24 were unequivocally determined.

When 24ξ,25-dihydroxycholesterol(3), derived from its 3β-acetate 4[3], was treated with benzoylchloride/pyridine, it readily formed 3β,24ξ-dibenzoate 5 and, with difficulty[4] tribenzoates 6 and 7. Dibenzoate 5 was converted into 25-TMS ethers 8 and 9 on the action of trimethylsilylimidazole. Tribenzoates 6 and 7 and dibenzoate TMS-ethers 8 and 9 could be resolved into their 24-epimeric components[5] by silica gel column chromatography. Resolution of 24-epimer was also performed with 24,25-epoxides 10 and 11 obtained by oxidation of desmosterol benzoate with m-chloroperbenzoic acid.

Those three epimeric pairs (6;7, 8;9 and 10;11) were interrelated each other as shown in the scheme[6]. Identifications of respective compounds were

conveniently carried out by means of high pressure liquid chromatography[5], which efficiently resolves a various derivatives of C-24 epimer.

The stereochemistry at C-24 of epoxide 10 and 11 was determined by acid-catalyzed methanolysis followed by application of modified Horeau's method to the resulting methoxyalcohols 12 and 13[7]. This recently reported[8] modification is based on the gas chromatographic determination of diastereoisomeric amides of (+)- and (-)-α-phenylbutyric acid which permits the simple application of Horeau's method to small (10 μmole) of chiral secondary alcohols Taking advantage of this procedure C-24 stereochemistry of 12 and 13 was established as R and S respectively. In confirmation, C-24 configuration of tribenzoates 6 and 7 was also elucidated by the same method applied to the derived triols 14 and 15[9].

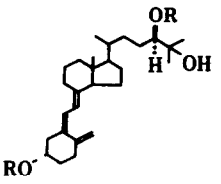
As the synthetic intermediates, we have selected dibenzoate TMS-ethers 8 and 9, which were prepared, as described above, from an epimeric mixture of triols 3 in a yield of 36% and 30%, respectively. Transformation of 8 and 9 into the corresponding vitamin D derivatives proceeded through the established sequences of vitamin D synthesis[10]. Thus, bromination of 8 with N-bromosuccinimide and then dehydrobromination with trimethylphosphite in refluxing xylene gave a mixture of 4,6- and 5,7-dienes, which was directly saponified with methanolic KOH. Pure 5,7-diene-triol 16, mp 203.5-205°, was obtained through triazoline adduct[11]. Irradiation of the diacetate 17[12] was performed in benzene-ethanol (2:1) solution with a high pressure mercury lamp (Ushio UM-102). The subsequent refluxing with benzene to effect the thermal isomerization of previtamin form to vitamin D derivative, afforded the vitamin D acetate 18[13], after purification by silica gel column chromatography. Saponification and final purification with high pressure liquid chromatography gave (24R)-24,25-dihydroxyvitamin D_3 (1). Uv and mass spectra of 1 were in a complete agreement with those of 24-racemate[2].

Essentially by the same manner, (24S)-TMS-ether 9 was converted, through 5,7-diene-triol 19, mp 214-215°, into (24S)-24,25-dihydroxyvitamin D_3 (2)[14].

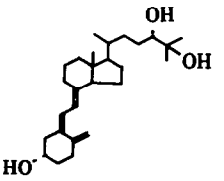
Biological activity of 1 and 2, and their identification with natural

metabolite are under investigation.

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



1, R=H
18, R=Ac



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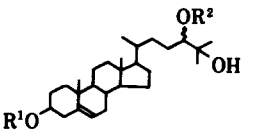
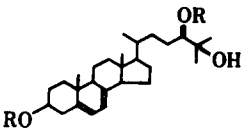
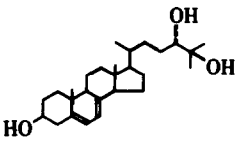


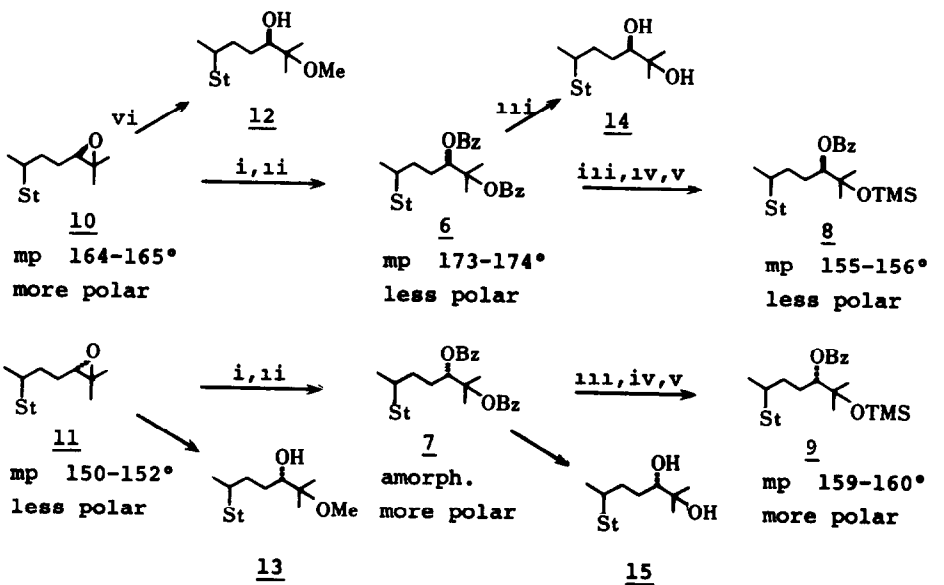
3, $R^1=R^2=H$
4, $R^1=Ac$; $R^2=H$
5, $R^1=R^2=Bz$

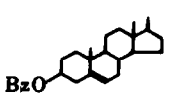


16, R=H
17, R=Ac



19



St = 

i, $HClO_4/H_2O$; ii, BzCl (2 mole), iii, KOH
iv, BzCl (3 mole) ; V, TMS-imidazole
vi, $HClO_4$/MeOH

REFERENCES AND FOOTNOTES

1. For Part V see M. Morisaki, J. Rubio-Lightbourn, N. Ikekawa and T. Takeshita, Chem. Pharm. Bull. (Tokyo), 21, 2568 (1973).
2. H-Yat Lam, H. K. Schnoes, H. F. DeLuca and T. C. Chen, Biochemistry, 12, 4851 (1973).
3. M. Seki, J. Rubio-Lightbourn, M. Morisaki and N. Ikekawa, Chem. Pharm. Bull., 21, 2783 (1973)
4. Under forcing benzoylation conditions, 3β,24ξ-Dihydroxycholesta-5,25-diene dibenzoate appeared as a by-product.
5. High pressure liquid chromatography (hlc) revealed the presence of 24-epimers in an almost 1:1 ratio, indicating non-stereoselectivity in OsO_4 oxidation of $\Delta^{24(25)}$-bond. The similar ratio was also observed with 24,25-epoxides 10 and 11. Hlc was done by a Dupont 840 equipped with uv detecter and Zorbax SIL column (25 cm x 2.1 mm), using n-hexane-CH_2Cl_2 (1:1) at 25 kg/cm^2.
6. It may be noted that acid-catalyzed ring opening of epoxides 10 and 11 occurred with retention of configuration at C-24, by attacking of nucleophile (e.g. OH^- or OMe^-) on C-25. This behavior is in accordance with the observations with the analogous epoxides: juvenile hormones, squalene-2,3-epoxide and lanosterol-24,25-epoxide.
7. By the analogous procedures, configuration of aglaiol, a (24S)-24,25-epoxy-triterpene was determined: R. B. Boar and K. Damps, J. C. S. Chem. Comm., 115 (1973).
8. C. J. W. Brooks and J. D. Gilbert, J. C. S. Chem. Comm., 194 (1973).
9. Gas chromatography of diastereoisomeric amides was carried out with an all-glass capillary column (0.25 mm x 30 m) coated with OV-17 at 260°. Estimated optical yields from 12, 13, 14 and 15 were 22, 7.2, 3.3 and 5.0 % respectively.
10. For example, L. F. Fieser and M. Fieser, "Steroids", Reinhold, New York, (1959), p. 90.
11. D. H. R. Barton, T.Shioiri and D. A. Widdowson, J. Chem. Soc., (C). 1968 (1971).
12. λmax (EtOH), 271, 282 and 293.5 nm; δ($CDCl_3$), 0.63 (3H, s, 18-Me), 0.98 (3H, s, 19-Me), 1.21 (6H, s, 26,27-Me), 2.07 and 2.13 (6H, two s, acetyl), 4.8 (2H, m, C-3,24-H), and 5.5 ppm (2H, AB type q, J=5 Hz, C-6,7-H).
13. λmax (EtOH), 266 nm; λmin, 228 nm; δ($CDCl_3$), 0.54 (3H, s, 18-Me), 1.18 (6H, s, 26,27-Me), 1.99 (3H, s, acetyl), 2.06 (3H, s, acetyl), 4.82 and 5.04 (2H, broad s, 19-CH_2), 6.00 and 6.22 ppm (2H, ABq, J=11 Hz, C-6,7-Hs).
14. An attempted separation of 1 from 2 by hlc using CH_2Cl_2-methanol (2 %) at 40 kg/cm^2, has been failed at present.