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ABSOLUTE CONFIGURATIONS OF 24-HYDROXYCHOLESTEROL AND RELATED COMPOUNDS[1]

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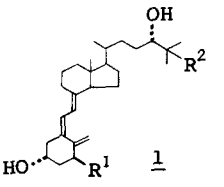
We have recently synthesized a series of C-24 epimers 1 and 2 of active forms of vitamin D, such as 24-hydroxy[2]-, 24,25-dihydroxy[3]-, 1α,24-dihydroxy[4]- and 1α,24,25-trihydroxy[5]-vitamin D_3. As a consequence of those studies, the stereochemistry of 24-hydroxy group in 24,25-dihydroxyvitamin D_3 which is one of the important metabolites of vitamin D_3 has been concluded as 24R, 2b[6]. Furthermore, some of the biological activity was observed with only one of the 24-hydroxy stereoisomers, suggesting functional importance of 24-hydroxylation of vitamin D[6,7]. However, C-24 configuration of the most fundamental analog, i.e. 24-hydroxyvitamin D_3 has been remained obscure due to the uncertainty[8] of the stereochemistry of the synthetic precursor, 24-hydroxycholesterol[9]. It is urgent need therefore, to determine the absolute configurations of 24-hydroxycholesterol and related compounds.

24S,25-Epoxycholesterol benzoate(3) whose configuration at C-24 has been determined previously[3], was refluxed with $LiAlH_4$-$AlCl_3$(3 : 1) in ether to give



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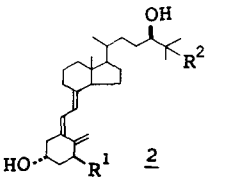

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a, $R^1 = R^2 = H$
b, $R^1 = H$; $R^2 = OH$
c, $R^1 = OH$; $R^2 = H$
d, $R^1 = R^2 = OH$

25-hydroxycholesterol (55 %) and 24S-hydroxycholesterol(5a)(35 %). The 24-ol 5a was isolated by chromatography and converted to the corresponding dibenzoate 5b During those procedures, epimerization at C-24 did not occur as evidenced from single peak of 5b on high pressure liquid chromatography(hplc) which resolves effectively C-24 isomers[10]. By a similar method, 24R,25-epoxide 4[3] was led to dibenzoate 6b. Each dibenzoates 5b and 6b were identified respectively, with the known[9] 3β,24ξ^1-dibenzoate(the more polar and higher mp isomer) and 3β,24ξ^2-dibenzoate(the less polar and lower mp isomer), in respect of mp, tlc and hplc. Thus, it is concluded that 24ξ^1-hydroxycholesterol(cerebrosterol) is 24S-hydroxycholesterol(5a) and 24ξ^2-isomer is 24R-hydroxycholesterol(6a).

A further confirmation was obtained by the modified Horeau's method[11] applied to THP ethers 5e and 6e. These were prepared from the corresponding dibenzoate 5b and 6b by the following sequence : (1) a partial hydrolysis with 1.1 eqiv. KOH in methanol-THF to form the monobenzoate 5c and 6c, (2) treatment with dihydropyran in CH_2Cl_2 containing tosyl acid to the THP ether 5d and 6d, (3) saponification with KOH in methanol-THF, affording 24ξ^1-isomer 5e mp 157-158.5° and 24ξ^2-isomer 6e, mp 154.5-155.5°. As shown in Fig. the configuration of 5e and 6e were determined as 24S-OH and 24R-OH, respectively, which are fully consistent with the above results obtained by chemical interrelations.

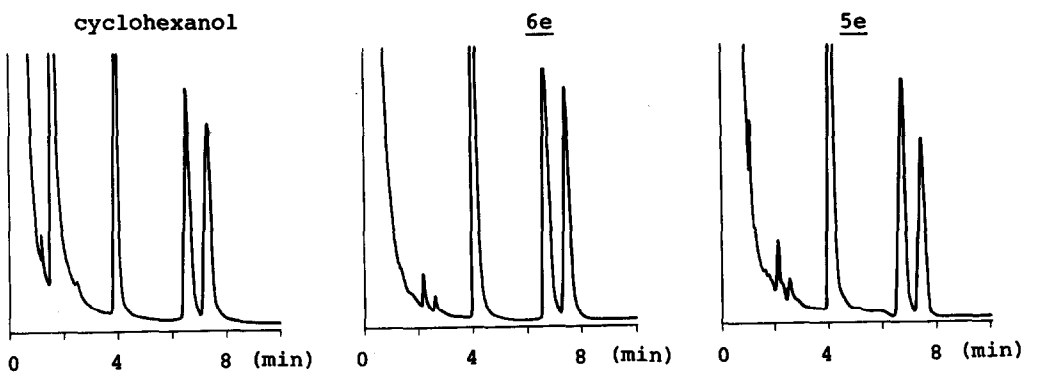
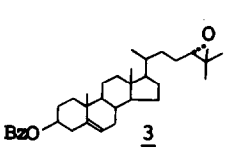


Fig. Gas liquid chromatograms of the (R)-α-phenylethylamides of excess (-)-(R)- and (+)-(S)-α-phenylbutyric acid after acylation of cyclohexanol, 6e and 5e. Apparatus: Shimadzu, 4BM-PF gas chromatograph. Column : all glass capillary column coated with OV-17, 20 m X 0.25 mm i.d., at 210°.



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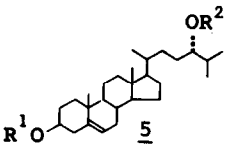

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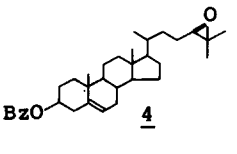

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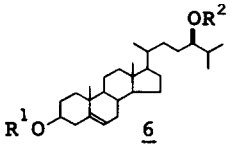


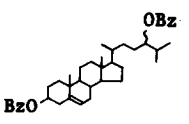
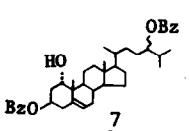
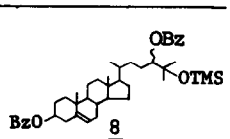
6

a, $R^1 = R^2 = H$
b, $R^1 = R^2 = Bz$
c, $R^1 = H$; $R^2 = Bz$
d, $R^1 = THP$; $R^2 = Bz$
e, $R^1 = THP$; $R^2 = OH$

From the above analogies, the stereochemistry of the analogous dibenzoates 7 which were the synthetic precursor of 1α,24ξ[1]- and 1α,24ξ[2]-dihydroxyvitamin D_3 (1c and 2c)[4] can be deduced : 24S and 24R configurations may be assigned to the more polar(24ξ[1]) and the less polar(24ξ[2]) compounds, respectively. Carbon-13 nmr analysis[12] of a series of C-24 epimers(Table) supported those supposition. It can be seen that signals of C-20, -21 and -24 of 24S-OH isomers always appeared at a lower field than those of 24R-OH isomers, presenting an useful diagnostic method for differentiation of 24-OH epimers.

It has now been established that 24ξ[1]-hydroxy- and 1α,24ξ[1]-dihydroxyvitamin D_3 (1a and 1c) have 24S-OH and 24ξ[2]-hydroxy- and 1α,24ξ[2]-dihydroxyvitamin D_3 (2a and 2c) have 24R-OH.

Table ^{13}C Chemical shift (ppm)*

						
	R(6b)	S(5b)	R	S	R	S
C-20	35.34	35.63	35.34	35.63	35.35	36.04
C-21	18.64	18.93	18.64	18.74	18.55	18.95
C-24	79.21	79.60	79.21	79.60	80.76	81.74

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* Recorded on PS/PFT-100(JEOL) in deuteriochloroform with tetramethylsilane as internal standard.

It is interesting to note that cerebrosterol isolated from brain is 24S-hydroxycholesterol, while the natural 24,25-dihydroxyvitamin D_3 (2b) has 24R-OH function and a series of 24R-OH-vitamin D analogs exert higher biological activity than 24S-OH congeners[6,7].

References and Footnotes

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3. M.Seki, N.Koizumi, M.Morisaki and N.Ikekawa, Tetrahedron Letters, 15 (1975).
4. M.Morisaki, N.Koizumi, N.Ikekawa, T.Takeshita and S.Ishimoto, J. Chem. Soc. Perkin I, in press.
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7. Y.Tanaka, H.Frank, H.F.DeLuca, N.Koizumi and N.Ikekawa, Biochemistry, submitted.
8. The configuration of 24-hydroxycholesterol[9] has been determined in 1954 (W.Klyne and W.M.Stokes, J. Chem. Soc., 1979) based on optical rotations data. However , it was suggested in 1970 (J.E.VanLier and L.L.Smith, J. Pharm. Sci., 59, 719) albeit with an undefinite evidence, that this assignment should be reversed. Therefore, we have retained the original nomenclature[9] for the previous papers[2,4].
9. A. Ercoli and P. de Ruggieri, J. Am. Chem. Soc., 75, 3284 (1953).
10. Retention times of 24S- and 24R-isomers were 10.1, 9.0 min., respectively, when analyzed with Shimadzu-DuPont 830 Liquid Chromatograph. Column, Zorbax SIL (25 cm X 2.1 mm); mobile phase, 10 % CH_2Cl_2 in hexane; pressure, 60 kg/cm^2; flow rate, 0.26 ml/min; detector, UV photometer.
11. C. J. W. Brooks and J. D. Gilbert, J. Chem. Soc. Chem. Comm., 194 (1973); J. D. Gilbert and C. J. W. Brooks, Anal. Letters, 639 (1973).
12. Although C-24 epimers of 8 were distinguished by proton nmr[5], this technique could not differentiate C-24 epimers of 5b-6b and 7.