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Chemical Modifications of Androsta-1,4-diene-3,17-dione. II.[1] Synthesis of 17-nor-Vitamin D Analogues having a Hydroxy Group at 1α- or 2β-Position and Biological Activity of 1α-Hydroxy-17-nor-17,17-ethylenedioxyvitamin D

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Using 1α- and 2β-hydroxy derivatives of 3β-hydroxy-17,17-ethylenedioxyandrosta-5,7-diene obtained in our previous work as starting materials, 1α- and 2β-hydroxylated analogues of 17-nor-17,17-ethylenedioxyvitamin D have been prepared *via* (i) photochemical conrotatory opening of the B-ring and (ii) the subsequent thermal 1,7-antarafacial hydrogen shifts.

1α-Hydroxy-17,17-ethylenedioxyvitamin D showed no significant antirachitic activity in rats. This fact seems to suggest that, in addition to the 1α-hydroxy function, the presence of the side chain in the vitamin D system plays also an important role in the biological action of the D analogues.

Recent studies indicate that the essential requisite of vitamin D to produce its characteristic physiological activity is the presence of 1α-hydroxy function. Thus, 1α,25-dihydroxy-,[3–5] and 1α-hydroxycholecalciferol[6–8] are known to exhibit vitamin D activity (stimulation of intestinal calcium transport and bone mineral mobilization) in anephric rats. Also, 3-deoxy-1α-hydroxycholecalciferol,[9] recently synthesized by DeLuca's group, showed similar biological activity to intact rats, though its effectiveness to anephric rats has not been reported as yet.[9] Furthermore, it is now generally observed that the analogues which have the 3-hydroxy function in a *pseudo* 1-position[10] such as 5,6-*trans*-D_3[11] and dihydroxytachysterol$_3$[12] (DHT_3) also exhibit similar biological activity. In our continuing studies of the structure/activity relationships of the vitamin D system, we have interested to prepare 17-nor-analogues of vitamin D having 1α-hydroxy function so as to examine if side chain is

1) Part I: H. Sakamoto, A. Sugimoto, and C. Kaneko, *Chem. Pharm. Bull.* (Tokyo), **22**, 2903 (1974).
2) Location: *a) 9–500–1, Nagareyama-shi, Chiba-ken, 270–01, Japan; b) Surugadai 2–3–10, Kanda, Chiyoda-ku, Tokyo, 101, Japan; c) 1–5–47, Yushima, Bunkyo-ku, Tokyo, 113, Japan.*
3) M.F. Holick, H.K. Schnoes, H.F. DeLuca, T. Suda, and R.J. Cousins, Biochemistry, **10**, 2799 (1971).
4) E.J. Semmler, M.F. Holick, H.K. Schnoes, and H.F. DeLuca, *Tetrahedron Letters*, **1972**, 4147.
5) D.E. Lowson, D.R. Fraser, E. Kodicek, H.R. Morris, and D.H. Williams, *Nature*, **230**, 228 (1971).
6) M.F. Holick, E.J. Semmler, H.K. Schnoes, and H.F. DeLuca, Science, **180**, 190 (1973).
7) D.H.R. Barton, R.H. Hesse, M.M. Pechet, and E. Rizzardo, *J. Amer. Chem. Soc.*, **95**, 2748 (1973).
8) C. Kaneko, S. Yamada, A. Sugimoto, Y. Eguchi, M. Ishikawa, T. Suda, M. Suzuki, S. Kakuta, and S. Sasaki, *Steroids*, **23**, 75 (1974).
9) H.Y. Lam, B.L. Onisko, H.K. Schnoes, and H.F. DeLuca, *Biochem. Biophys. Res. Comm.*, **59**, 845 (1974).
10) Trans isomerization of vitamin D which rotates the A ring 180° places the 3β-hydroxy function geometrically in a pseudo 1α-hydroxy position making the *trans* isomer an analogue of 1α,25-dihydroxycholecalciferol [1α,25-$(OH)_2$-D_3] in its ring A moiety.
11) M.F. Holick, M. Garabedian, and H.F. DeLuca, *Biochemistry*, **11**, 2715 (1972).
12) S. Sagar, R.L. Estrada, and M. Kaye, *Arch. Intern. Med.*, **130**, 768 (1972).

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needed for biological activity once a 1α-hydroxy function is provided. The research along this line have led us prepare two new analogues of 17-nor-vitamin D. The results presented in this paper established that 1α-hydroxy-17-nor-17,17-ethylenedioxyvitamin D does not exhibit any significant vitamin D activity as measured by the intestinal calcium transport and bone mineral mobilization assays and thus demonstrate an absolute importance of the side chain in the vitamin D system for its biological activity.

In part I of this series,[1] three of the present authors reported the synthesis of 1α- and 2β-hydroxylated analogues (**1a** and **1b**) of 3β-hydroxy-17,17-ethylenedioxyandrosta-5,7-diene in each three isolation steps starting from 17,17-ethylenedioxyandrosta-1,4-dien-3-one.[13] In the present synthesis, we have utilized these compounds (**1a** and **1b**) for the starting materials. The approaches outlined in Chart 1 were developed (the arrow symbolism used in the Chart is that developed recently by one (C.K.) of the present authors in order to depict stereospecificity of pericyclic reaction in terms of electronic theory).[14]

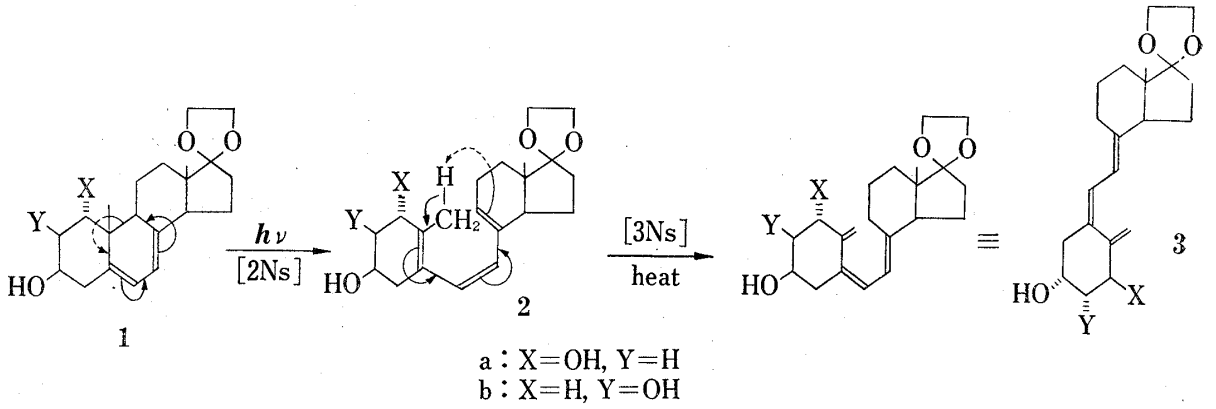

Chart 1

The compounds (**1a** and **1b**) upon irradiation in ether furnished the corresponding previtamin D derivatives (**2a** and **2b**). The latter compounds were then equilibrated to the corresponding D analogues (**3a** and **3b**) at room temperature under argon. Two 17-nor-vitamin D analogues (**3a** and **3b**) exhibited the expected ultraviolet spectrum (UV) and mass spectra and their structures were established further by their nuclear magnetic resonance (NMR) spectra (Figure 1). Obviously, the existence of the extra-hydroxy group in the A ring in these compounds does not affect the photochemical ring opening step and subsequent thermal hydrogen migration step, since the yield (*ca.* 20%) of the 17-nor-vitamins D was comparable with that reported for vitamin D itself.[15]

Biological Activity of the Analogue 3a

For intestinal calcium transport and bone mineral mobilization activity, weanling male rats of the Wistar strain were fed for 3 weeks a vitamin D-deficient, low calcium (0.003%) diet.[16] At the end of the third week, groups of rats received intrajugularly the appropriate dose of the analogue **3a** or 1α-OH-D_3 in 0.05 ml of 95% ethanol. Control rats received the same amounts of the vehicle. Eighteen hours later the animals were decapitated, and the

13) By essentially the same procedure, cholesta-1,4-dien-3-one was also successfully converted to 1α- and 2β-hydroxylated analogues of cholesta-5,7-dien-3β-ol. See, C. Kaneko, A. Sugimoto, Y. Eguchi, S. Yamada, M. Ishikawa, S. Sasaki, and T. Suda, *Tetrahedron*, **30**, 2701 (1974).

14) An odd or even Ns (number of solid arrow used in a given pericycle) determines whether the reaction is allowed or forbidden. See, C. Kaneko, *Tetrahedron*, 28, 4915 (1972); *idem*, *Rept. Res. Inst. Med. Engi.*, Tokyo Medico-Dental Univ., **7**, 7 (1973).

15) F. Hunziker and N.L. Mulner, *Helv. Chim. Acta*, **41**, 70 (1958).

16) T. Suda, H.F. DeLuca, and Y. Tanaka, *J. Nutrition*, **100**, 1049 (1970).

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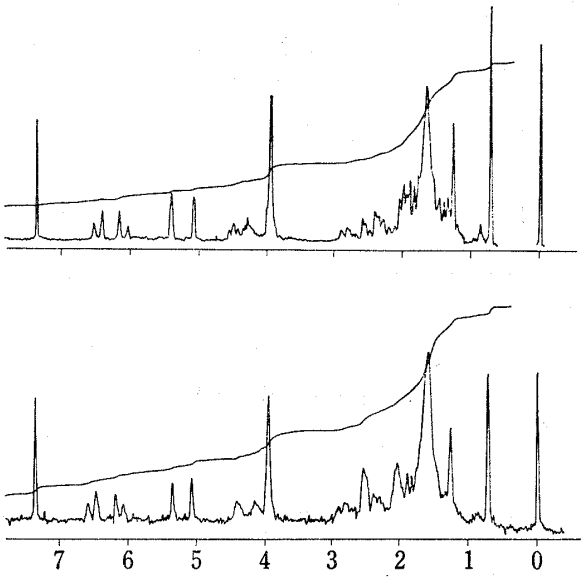

Fig. 1. H^1-NMR Spectra of 1α- and 2β-Hydroxylated Analogues (3a and 3b) of 17-Nor-17,17-ethylenedioxyvitamin D in Deuterochloroform (100 MHz, δ ppm: TMS).

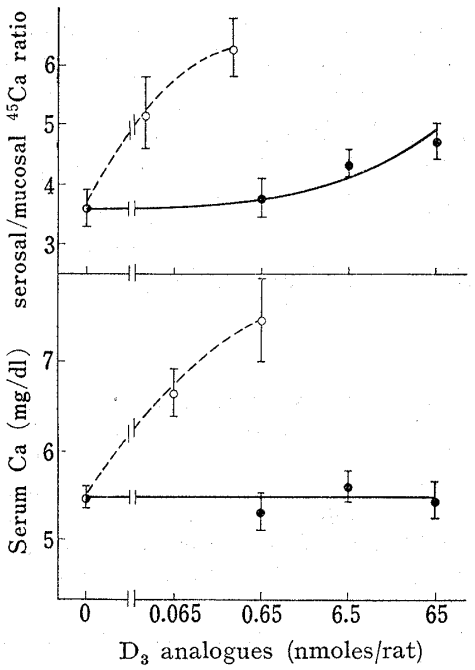

Fig. 2. Comparative Effectiveness of Analogue **3a** (Solid Line) and 1α-OH-D_3 (Dotted Line) in Intestinal Calcium Transport and Bone Mineral Mobilization Activity

The vertical bars represent the standard error of the means. Five rats were used for each determination.

···ϕ··· :1α-OH-D_3, —●— :analogue 3a

duodena and the blood were collected. The everted sacs were prepared according to the procedure of Martin and DeLuca[17] for measuring intestinal calcium transport activity. For the assay of bone mineral mobilization activity, serum calcium concentration from rats were determined with a Perkin Elmer Model 403 atomic absorption spectrometer. The rise in serum calcium concentration in rats on this low calcium diet reflects increased mobilization of bone mineral.

Figure 2 shows the comparative effectiveness of the analogue **3a** and 1α-OH-D_3 in intestinal calcium transport as well as in bone mineral mobilization activity. As already suggested by the preliminary report of Holick and DeLuca,[18] the analogue **3a** did not elicit any significant biological response, suggesting an importance of the side chain for its biological activity. Even high dose (65 nmoles) of the analogue **3a** was totally ineffective in bone mineral mobilization activity, while as little as 0.065 nmoles of 1α-OH-D_3 was able to stimulate bone mineral mobilization. On the other hand, more than 6.5 nmoles of the analogue **3a** seemed to elicit slight response in intestinal system, though 0.065 nmoles of 1α-OH-D_3 showed much higher response in this system. Differential responses of the intestinal and bone systems to various vitamin D metabolites have been observed before.[19,20] Lam, *et al.*[9] suggested that the bone mineral mobilization system appears more sensitive to structural departures from the 1α,25-

17) D.L. Martin and H.F. DeLuca, *Amer. J. Physiol.*, **216**, 1351 (1969).
18) M.F. Holick and H.F. DeLuca, *Federation Proceedings*, **32**, 467 (1973).
19) H.Y. Lam, H.K. Schnoes, H.F. DeLuca, and T.C. Chen, *Biochemistry*, **12**, 4851 (1973).
20) M.F. Holick, A. Kleiner-Bossaller, H.K. Schnoes, P.M. Kasten, I.T. Boyle, and H.F. DeLuca, *J. Biol. Chem.*, **248**, 6691 (1973).

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dihydroxy substitution pattern than the intestinal calcium transport mechanism. Our results appear to fit with their explanation.

Recently, several research groups[9,21,22] have reported that 1α-OH-D_3 might have to undergo hydroxylation at C-25 position before it functions on target tissues. Since the analogue **3a** does not possess any side chain of vitamin D_3, it can not be converted to 1α,25-dihydroxy derivative. Although it is not known at present that what part of the side chain of vitamin D_3 is most important for biological activity, our present results suggest that at least a part of the side chain (probably the hydroxy function at C-25 position) is also important for biological activity of the vitamin D system.

Experimental

UV spectra were measured with a Hitachi Model-323 spectrometer. NMR spectra were recorded on a JNM-PS-100 (100 Mcps) spectrometer with tetramethylsilane as internal standard and the chemical shifts are given in δ (ppm) values; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were determined on a Hitachi double-focus RMU-7 spectrometer.

Photochemical Conversion of 1α-Hydroxy-17-nor-17,17-ethylenedioxyprevitamin D (1a) to 1α-Hydroxy-17-nor-17,17-ethylenedioxyprevitamin D (2a)——Twenty five milligrams of 1α,3β-dihydroxy-17,17-ethylenedioxyandrosta-5,7-diene (**1a**) was dissolved in 600 ml of distilled ether. After bubbling of argon for 5 min, the whole was irradiated by 200 W high-pressure Hg immersion lamp (Hanovia 654A-36) through a Vycor filter under argon atmosphere. After 13 min, both the irradiation and argon bubbling were terminated. After evaporation of the solvent *in vacuo* below 20°, the whole residue was chromatographed on Sephadex LH-20 (17 g) with hexane–$CHCl_3$ (35:65 v/v). 1α-Hydroxy-17-nor-17,17-ethylenedioxyprevitamin D (**2a**) was eluted first which was followed by two fractions containing *ca.* 9 mg of the starting material (**1a**) and *ca.* 4 mg of 1α-hydroxy-17-nor-17,17-ethylenedioxytachysterol (its definite purification and identification were unsuccessful due to its instability and insufficiency in amount, but it showed a UV maximum at the expected position: 281 nm in ether[23]). The combined previtamin fractions amounted to 5 mg (oil) and showed its absorption maximum at 261 nm in ether.

Thermal Conversion of 1α-Hydroxy-17-nor-17,17-ethylenedioxyprevitamin D (2a) to 1α-Hydroxy-17-nor-17,17-ethylenedioxyvitamin D (3a)——The previtamin fraction (*ca.* 5 mg) obtained above was dissolved in 100 ml of distilled ether and the whole was stored in dark place (20—25°) under argon atmosphere. During the storage, the thermal 1,7-antarafacial hydrogen shift of **2a** to **3a** was followed by the periodical measurements of its UV spectra. Thus, the absorption maximum shifted gradually from 261 nm to 264 nm and the intensity increased up to 1.8 times than that of the original solution. After 10—14 days (by that time, the UV spectrum of the solution showed its maximum at 264 nm with a constant intensity), the solvent was removed *in vacuo*. The residue was finally purified on a Sephadex LH-20 column (8 g) with hexane–$CHCl_3$ (35:65 v/v). The UV spectrum of each fraction was measured and pure 1α-hydroxy-17-nor-17,17-ethylenedioxyvitamin D (**3a**) (*ca.* 3 mg) was obtained. The yield of **3a** was calculated as 3.4 mg [λ_{max}: 264 nm (ε 18300 taken as standard for calculation)[23]]. The NMR spectrum ($CDCl_3$) of **3a** showed the characteristic resonances of the olefinic protons with the vitamin D chromophore and the chemical shifts of these and C_1-protons almost identical with those observed in 1α-hydroxyvitamin D_3,[8] δ 6.02 (1H, d, J_{AB} 12 Hz) and 6.38 (1H, d, 12 Hz) (6- and 7-H), 5.33 (1H, broad s) and 5.01 (1H, broad s) (19-H_2), 4.44 (1H, m) and 4.24 (1H, m) (1- and 3-H), 3.90 (4H, s) (–O–CH_2–CH_2–O–), 0.712 (3H, s) (CH_3-protons). The mass spectrum of **3a** showed a molecular ion at *m/e* 346 and fragment ion peaks at *m/e* 328, 310 284, and 99.

Preparation of 2β-Hydroxy-17-nor-17,17-ethylenedioxyprevitamin D (2b) and its Conversion to 2β-Hydroxy-17-nor-17,17-ethylenedioxyvitamin D (3b)——Forty seven milligrams of 2β,3β-dihydroxy-17,17-ethylenedioxyandrosta-5,7-diene (**1b**) in 650 ml of distilled ether was irradiated for 25 min by the same light source used in the above experiment under a current of argon. The residue obtained by evaporation of the solvent *in vacuo* was chromatographed on 20 g of Sephadex LH-20 with the same solvent system as above. 2β-Hydroxy-17-nor-17,17-ethylenedioxyprevitamin D (**2b**) was eluted first which was followed by *ca.* 6 mg of the tachysterol fraction (λ_{max}: 281 nm in ether). The combined previtamin fraction (*ca.* 5.5 mg: λ_{max} 258 nm in ether) was dissolved in 100 ml of ether and was stored for 2 weeks as described above. The final purification on a Sephadex LH-20 column (8 g) with hexane–$CHCl_3$ (35:65 v/v) afforded 3.7 mg of the vitamin D (**3b**) [λ_{max} (ether) 266 nm (ε 18300 taken as standard for calculation)[23]]. The NMR spectrum of **3b** showed the resonances of the olefinic as well as C_1-protons with chemical shifts almost identical with those observed in 2β-

21) E. Kodicek, *Lancet i*, **1974**, 325.
22) D.J. Cork, M.R. Haussler, M.J. Pitt, E. Rizzardo, R.H. Hesse, and M.M. Pechet, *Endocrinology*, **94**, 1337 (1974).
23) L.F. Fieser and M. Fieser, "Steroids," Reinhold Pub. Co., New York, 1959, p. 148.

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hydroxycholecalciferol (2β-OH-D_3),[24] δ 6.46 (1H, d, $J=11$ Hz) and 6.07 (1H, d, 11 Hz) (6- and 7-H), 5.30 (1H, broad s) and 5.02 (1H, broad s) (19-H_2), 4.36 (1H, m) and 4.11 (1H, m) (2- and 3-H), 3.90 (4H, s) (–O–CH_2–CH_2–O–), 0.72 (3H, s) (CH_3-protons). The mass spectrum of **3b** showed a molecular ion at *m/e* 346 and the fragment ion peaks as observed in the spectrum of **3a**.

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24) C. Kaneko, S. Yamada, A. Sugimoto, and M. Ishikawa, *Chem, Pharm. Bull.* (Tokyo), **23**, 1616 (1975). See also, C. Kaneko, *J. Syn. Org. Chem. Japan*, **33**, 75 (1975).

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