[Chem. Pharm. Bull. 36(10)4144—4147(1988)]

Studies on Organic Fluorine Compounds. LII.[1] Synthesis and Biological Activity of 26,26,26,27,27-Pentafluoro-1α-hydroxy-27-methoxyvitamin D_3

YOSHIRO KOBAYASHI,*,[a] MASAHARU NAKAJIMA,[a] MASAKAZU NAKAZAWA,[a] TAKEO TAGUCHI,[a] NOBUO IKEKAWA,[b] HIROSHI SAI,[b] YOKO TANAKA,[c] and HECTOR F. DELUCA[c]

Tokyo College of Pharmacy,[a] Horinouchi, Hachioji, Tokyo 192-03, Japan, Department of Chemistry, Tokyo Institute of Technology,[b] Ookayama, Meguro-ku, Tokyo 152, Japan, and Department of Biochemistry, College of Agricultural and Life Science, University of Wisconsin,[c] Madison, Wisconsin 53706, U.S.A.

(Received February 17, 1988)

Replacement of one fluorine atom of the hexafluorocholesterol derivative (**4**) with a methoxyl group took place during the saponification of the acetyl groups of **4** with methanolic potassium hydroxide to give the pentafluoromethoxy compound (**5**), which was converted to the corresponding 1α-hydroxyvitamin D_3 form (**6**). The pentafluoromethoxy compound (**6**) was found to be about three times more potent than 1α-hydroxyvitamin D_3 in displacing radio-labeled 1,25-dihydroxyvitamin D_3 from the chick intestinal receptor, whereas the activity of **6** in response to bone calcium-mobilization in vitamin D-deficient rats was slightly lower than that of 1α-hydroxyvitamin D_3.

Keywords——vitamin D_3; trifluoromethyl; pentafluoro-1α-hydroxy-27-methoxyvitamin D_3; 2,2,4,4-tetrakis(trifluoromethyl)-1,3-dithietane; serum calcium

The crucial importance of the 1α-hydroxyl group of 1,25-dihydroxyvitamin D_3 (1,25(OH)$_2$ D_3), a hormonally active form of vitamin D_3, for eliciting its biological activity is well established.[2-4] Hydroxylation at C-24 and C-26 of 25-hydroxyvitamin D_3 (25-OH-D_3), and the production of 26, 23-lactone of 25-OH-D_3 are alternative metabolic pathways. On the basis of the metabolism and mode of function of vitamin D_3, and the characteristic properties of fluorinated compounds with respect to biological response, fluorinated vitamin D_3 analogs were designed and synthesized, and their biological responses were investigated to clarify the physiological significance of metabolic hydroxylation of vitamin D_3. The hexafluoro and 24,24-difluoro analogs of 1,25(OH)$_2$ D_3 showed higher activities than those of 1,25(OH)$_2$ D_3, presumably due to the metabolic stability of these fluoro analogs.[5-7] Moreover, these fluorinated analogs as well as 1,25(OH)$_2$ D_3 were found to show potent activity in inducing differentiation of human leukemia cells HL-60.[8]

Considering the therapeutic efficacy of 1α-hydroxyvitamin D_3[9] and the enhancement in the biological activities of the fluorinated analogs, attempts were made to prepare 26,26,26,27,27,27-hexafluoro-1α-hydroxyvitamin D_3. As a result we obtained the pentafluoromethoxy derivative (**6**), but not the hexafluoride.

For the synthesis of 1α-acetoxyhexafluorocholesterol 3β-acetate (**3**), the C_{24}-aldehyde (**1**) was reacted with triphenylphosphine and 2,2,4,4-tetrakis(trifluoromethyl)-1,3-dithietane[10] to give the hexafluoro-24-ene compound (**2**). It was found that reduction of the double bond was achieved by treating **2** with sodium borohydride in a mixture of THF and *tert*-butanol at room temperature to give **3** in 51% yield from **1**. Bromination of **3** with *N*-bromosuccinimide and subsequent dehydrobromination with collidine in boiling xylene gave the diacetate form

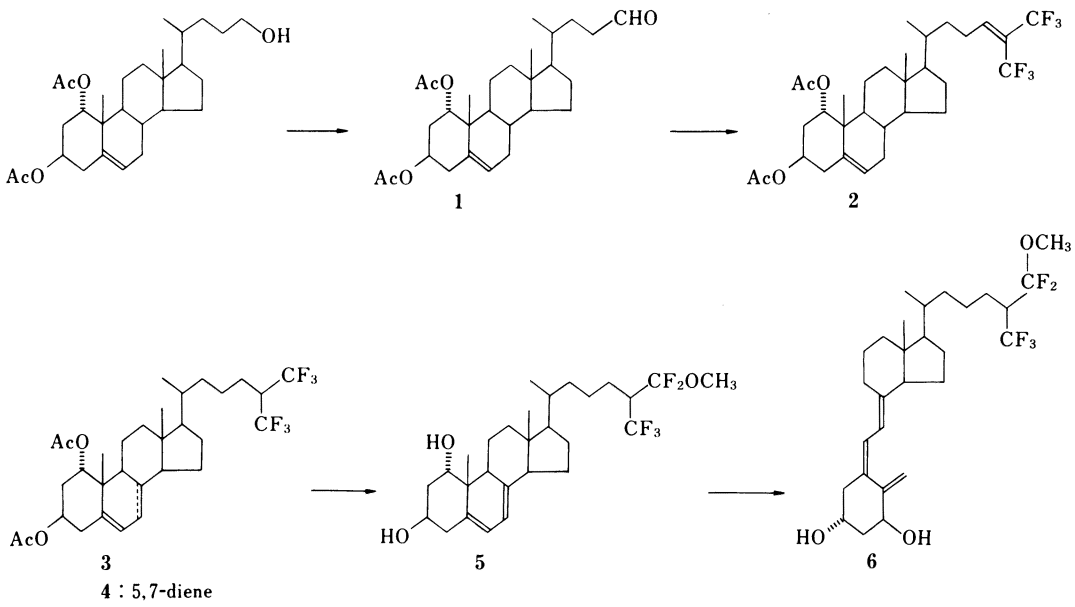

Chart 1

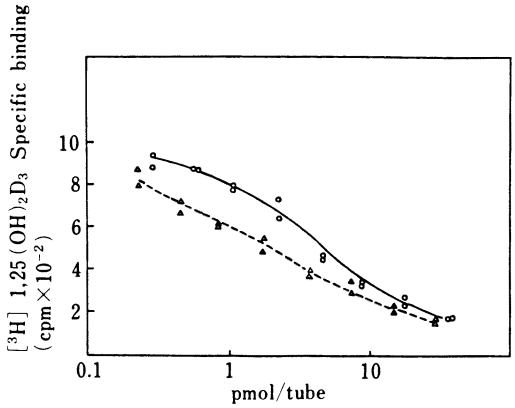

Fig. 1. Competitive Displacement of [26,27-^{3}H]1,25(OH)$_2$ D$_3$ from Chick Intestinal Cytosol Binding Protein for 1,25(OH)$_2$ D$_3$ by 1α-OH-D$_3$ (—○—) and by 1α-OH-26,26,26,27,27-F$_5$-27-OMe-D$_3$ (---△---)

TABLE I. Increase of Serum Calcium Concentration in Response to 1α-OH-D$_3$ or 1α-OH-26,26,26,27,27-F$_5$-27-OMe-D$_3$ (**6**)

Compound given	Serum calcium (mg/100 ml)	Number of rats
Vehicle	3.2±0.1[a)]	4
1α-OH-D$_3$	4.7±0.5[b)]	6
1α-OH-26,26,26,27,27-F$_5$-27-OMe-D$_3$ (**6**)	3.6±0.1[c)]	6

Standard deviation from the mean; significance of differences of *b*) and *c*) from *a*), and *b*) from *c*), $p<0.001$.

(**4**) of the 5,7-diene compound. Saponification of ester groups of **4** with 5% methanolic potassium hydroxide gave the pentafluoromethoxy derivative (**5**) in 20% yield from **3**. The 5,7-diene (**5**) may be a 1 : 1 epimeric mixture at C-25 from its ^{1}H-NMR spectrum, which showed a twin peak due to the methoxyl group. According to the standard method, the 5,7-diene (**5**) was transformed into the vitamin D$_3$ form (**6**) through photoirradiation followed by thermal isomerization.

In these procedures the strong electron-withdrawing nature of trifluoromethyl group facilitates the hydride reduction of the 1,1-bis(trifluoromethyl)ethene system in **2** and the elimination of hydrogen fluoride from **4** to form 1,1,3,3,3-pentafluoropropene structure,[11)]

which further reacts as a Michael acceptor with methanol to form **5**.

Biological Activity

The binding ability of **6** to the chick intestinal receptor protein was compared with that of 1α-hydroxyvitamin D_3.[12] The results obtained, which are shown in Fig. 1, demonstrate that the pentafluoro compound (**6**) is about three times more potent than 1α-hydroxyvitamin D_3 in displacing radiolabeled 1,25$(OH)_2$ D_3 from the receptor.

The biological activity of the pentafluoro compound (**6**) and 1α-hydroxyvitamin D_3 on bone calcium mobilization, measured in terms of the serum calcium concentration, was compared. The results are shown in Table I. Both the pentafluoro compound (**6**) and 1α-hydroxyvitamin D_3 at a dosage level of 650 pmol exhibited an increase of serum calcium concentration, but the activity of the pentafluoro compound (**6**) is slightly lower than that of 1α-hydroxyvitamin D_3. Since the increment of the biological activity of 26,26,26,27,27,27-hexafluoro-1,25$(OH)_2$ D_3 compared to that of 1,25$(OH)_2$ D_3 is considered as a result of metabolic stability by blocking the hydroxylation, but not due to the binding ability to the receptor protein,[5] it is of interest that the present fluoro derivative (**6**) showed a relatively strong binding ability. Further study is needed to examine the time course of the response and the precise effect of fluoride substitution.

Experimental

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were run on a Varian EM-390 or JEOL FX-100 spectrometer in $CDCl_3$ with Me_4Si as an internal standard. ^{19}F-NMR spectra were recorded on a Varian EM-360L spectrometer in $CDCl_3$ with benzotrifluoride as an internal standard. Mass spectra (MS) were obtained with a Hitachi RMU-7L double-focusing spectrometer. Ultraviolet(UV) spectra were obtained in ethanol solution with a Shimadzu UV-200 double-beam spectrophotometer. Column chromatography was effected with silica gel (Merck, 70—230 mesh). Preparative thin layer chromatography (TLC) was carried out on precoated plates of silica gel (Merck, Silica gel 60 F_{254}, 0.25 mm thickness). The usual work-up refers to dilution with water, extraction with an organic solvent, washing to neutrality, drying over magnesium sulfate, filtration, and removal of the solvent under reduced pressure. The following abbreviations are used: THF, tetrahydrofuran; ether, diethyl ether; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broadened singlet.

1α,3β-Diacetoxychol-5-en-24-al (1)——Dimethylsulfoxide (0.52 ml, 7.38 mmol) was added to a solution of oxalyl chloride (0.32 ml, 3.69 mmol) in dichloromethane (12 ml) at −78 °C under an argon atmosphere and the mixture was stirred at −78 °C (dry ice-acetone) for 10 min. Then 1α,3β-diacetoxychol-5-en-24-ol[13] (850 mg, 1.85 mmol) in dichloromethane (7 ml) was added and the mixture was stirred for 15 min. The reaction mixture was treated with triethylamine (2.05 ml) for 5 min at −78 °C, then warmed to room temperature. The usual work-up (ether) gave a crude product, which was chromatographed on silica gel. Elution with hexane–ethyl acetate (4 : 1, v/v) gave the aldehyde (**1**) as an amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 0.65 (3H, s, 18-H_3), 0.97 (3H, d, J=6 Hz, 21-H_3), 1.04 (3H, s, 19-H_3), 1.94 (3H, s, acetyl), 1.97 (3H, s, acetyl), 4.82 (1H, m, 3-H), 4.95 (1H, m, 1-H), 5.41 (1H, m, 6-H), 9.58 (1H, t, J=1.6 Hz, 24-H). MS m/z: 334 ($M^+ - 2 \times$ AcOH).

1α-Acetoxy-26,26,26,27,27,27-hexafluorocholesterol 3β-Acetate (3)——2,2,4,4-Tetrakis(trifluoromethyl)-1,3-dithietane (760 mg, 2.1 mmol) was added to a solution of the aldehyde (**1**) (134 mg, 0.29 mmol) and triphenylphosphine (1.024 g, 3.9 mmol) in ether (50 ml) at −78 °C, and the reaction mixture was stirred for 16 h while gradually being allowed to warm to room temperature. After removal of the solvent under reduced pressure, the residue was chromatographed on silica gel. Elution with hexane–ethyl acetate (5 : 1, v/v) gave a mixture of the hexafluoride (**2**) and triphenylphosphine sulfide (394 mg, molar ratio 1 : 1.77). **2**: ^{1}H-NMR ($CDCl_3$) δ: 0.68 (3H, s, 18-H_3), 0.95 (3H, d, J=6 Hz, 21-H_3), 1.08 (3H, s, 19-H_3), 2.02 (3H, s, acetyl), 2.05 (3H, s, acetyl), 4.95 (1H, m, 3-H), 5.02 (1H, m, 1-H), 5.58 (1H, m, 6-H), 6.80 (1H, m, 24-H). ^{19}F-NMR ($CDCl_3$) δ: 4.33 (3F, q, J=6.6 Hz), −2.67 (3F, q, J=6.6 Hz). This mixture (350 mg) was treated with sodium borohydride (100 mg) in THF (15 ml) and *tert*-butanol (7.5 ml) at room temperature for 22 h. After the usual work-up (ether–ethyl acetate (1 : 1, v/v) for extraction), the extracts were chromatographed on silica gel. Elution with hexane–ethyl acetate (10 : 1, v/v) gave crude hexafluorocholesterol (**3**), which was further purified on a column of silica gel. Elution with hexane–ethyl acetate (20 : 1, v/v) gave pure **3** (69 mg, 51%) as an amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 0.68 (3H, s, 18-H_3), 0.92 (3H, d, J=6 Hz, 21-H_3), 1.10 (3H, s, 19-H_3), 2.03 (3H, s, acetyl), 2.07 (3H, s, acetyl), 5.00 (1H, m, 3-H), 5.10 (1H, m, 1-H), 5.58 (1H, m, 6-H). ^{19}F-NMR ($CDCl_3$) δ: 3.3 (3F, d, J=10.3 Hz), 4.1 (3F, d, J=10.9 Hz). MS m/z: 454 ($M^+ - 2 \times$ AcOH − HF), 440, 335, 253. High-resolution MS Calcd for $C_{27}H_{35}F_5$, 454.2656. Found: 454.2539.

26,26,26,27,27-Pentafluoro-1α,3β-dihydroxy-27-methoxycholesta-5,7-diene (5)——A mixture of 26,26,26,27,27,27-

hexafluoro-1α,3β-diacetoxycholesta-5-ene (**3**, 26 mg, 0.04 mmol) and *N*-bromosuccinimide (11 mg, 0.062 mmol) in carbon tetrachloride (2 ml) was refluxed under an argon atmosphere for 25 min, then allowed to cool to 0 °C. The resulting precipitates were filtered off. The filtrate was concentrated below 40 °C to leave the residue. A solution of the residue in xylene (2 ml) was added dropwise to a refluxing solution of collidine (0.5 ml) and xylene (1.5 ml), and refluxing was continued for 20 min. The usual work-up (ethyl acetate for extraction) gave the crude diene (**4**). This in THF (5 ml) was treated with 5% methanolic potassium hydroxide (7.5 ml) for 60 min at room temperature. The usual work-up (ethyl acetate for extraction) gave a crude product, which was submitted to preparative TLC (benzene–ethyl acetate, 1 : 1 (v/v), developed three times). The band of $Rf=0.41$ was scraped off and eluted with ethyl acetate to give the pentafluoromethoxy-5,7-diene (**5**, 4.5 mg, 20%). ^{1}H-NMR ($CDCl_3$) δ: 0.68 (3H, s, 18-H_3), 0.93 (3H, d, $J=6$ Hz, 21-H_3), 1.08 (3H, s, 19-H_3), 3.786 and 3.789 (total 3H, each s, methoxy), 5.41 (1H, m), 5.55 (1H, m). UV λ_{max}^{EtOH}: 293, 282, 272 nm. MS *m/z*: 520 (M^+), 500 ($M^+ - HF$), 482, 466, 287, 269, 251, 233.

26,26,26,27,27-Pentafluoro-1α-hydroxy-27-methoxyvitamin D_3(6)——A solution of the 5,7-diene (**5**, 4.5 mg, 8.6 μmol) in benzene (90 ml) and ethanol (40 ml) was irradiated with a medium-pressure mercury lamp through a Vycor filter with ice cooling under an argon atmosphere for 5 min. Then, the reaction mixture was refluxed for 1 h under an argon atomosphere. Removal of the solvent under reduced pressure gave a crude product, which was submitted to preparative TLC (benzene–ethyl acetate, 1 : 1 (v/v), developed three times). The band of $Rf=0.50$ was scraped off and eluted with ethyl acetate to give the vitamin D_3 analog (**6**, 0.67 mg, 15%), UV λ_{max}^{EtOH}: 265 nm, λ_{min}^{EtOH}: 228 nm. MS *m/z*: 500 ($M^+ - HF$), 482, 466, 385, 287, 269, 251, 233, 213, 152, 134. The band of $Rf=0.43$ was scraped off and eluted with ethyl acetate to recover the 5,7-diene (1.3 mg, 29%).

Activity of 6 in Response to Bone Calcium-Mobilization——Weanling male rats obtained from Holtzman Co., Madison, Wisconsin, were fed a low calcium and vitamin D-deficient diet[14] for three weeks. They were then divided into three groups of four or six each and two groups were given 650 pmol of 1α-hydroxyvitamin D_3 or **6** dissolved in 0.05 ml of 95% ethanol intrajugularly. The rats in the control group were given the ethanol vehicle in the same manner. Sixteen hours later serum calcium concentration was measured as described by Tanaka *et al.*[15]

References

1) Part LI: K. Fujita, S. Kobayashi, I. Kudo, K. Inoue, S. Nojima, M. Ohno, Y. Kobayashi, M. Odagiri, and T. Taguchi, *Chem. Pharm. Bull.*, **35**, 647 (1987).
2) H. F. DeLuca and H. K. Schnoes, *Annu. Rev. Biochem.*, **45**, 611 (1976); *idem, ibid.*, **52**, 411 (1983).
3) A. W. Norman, J. Roth, and L. Orci, *Endocrine Rev.*, **3**, 331 (1982).
4) N. Ikekawa, *Med. Res. Rev.*, **7**, 333 (1987).
5) Y. Tanaka, H. F. DeLuca, Y. Kobayashi, and N. Ikekawa, *Arch. Biochem. Biophys.*, **229**, 348 (1984).
6) S. Okamoto, Y. Tanaka, H. F. DeLuca, Y. Kobayashi, and N. Ikekaewa, *Am. J. Physiol.*, **224**, E159 (1983).
7) Y. Kobayashi and T. Taguchi, *J. Synth. Org. Chem.*, **43**, 1073 (1985); R. Brommage and H. F. DeLuca, *Endocrine Rev.*, **6**, 491 (1985).
8) *a*) E. Abe, C. Miyaura, H. Sakagami, M. Takeda, K. Konno, T. Yamazaki, S. Yoshiki, and T. Suda, *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 4990 (1981); *b*) Z. Bar-Shavit, S. L. Teitelbaum, P. Reitsma, A. Hail, L. E. Pegg, J. Triai, and A. J. Kahn, *ibid.*, **80**, 5907 (1983); *c*) H. P. Koeffler, T. Amatruda, N. Ikekawa, Y. Kobayashi, and H. F. DeLuca, *Cancer Res.*, **44**, 5624 (1985).
9) M. F. Holick, E. J. Semmler, H. K. Schnoes, and H. F. DeLuca, *Science*, **180**, 190 (1973).
10) *a*) L. G. Anello and M. Van Der Puy, *J. Org. Chem.*, **47**, 377 (1982); *b*) D. J. Burton and Y. Inoue, *Tetrahedron Lett.*, **1979**, 3397.
11) I. L. Knunyants and Y. A. Cherburkov, *Izv. Akad. Nauk SSSR, Otdel. Khim. Nauk.*, **1960**, 2168 [*Chem. Abstr.*, **55**, 16413 (1961)].
12) *a*) J. A. Eisman, A. J. Hamstra, B. E. Kream, and H. F. DeLuca, *Arch. Biochem. Biophys.*, **176**, 235 (1976); *b*) R. M. Shepard, R. L. Horst, A. J. Hamstra, and H. F. DeLuce, *Biochem. J.*, **182**, 55 (1979).
13) Y. Kobayashi, T. Taguchi, S. Mitsuhashi, T. Eguchi, E. Ohshima, and N. Ikekawa, *Chem. Pharm. Bull.*, **30**, 4297 (1982).
14) T. Suda, H. F. DeLuca, and Y. Tanaka, *J. Nutr.*, **100**, 1049 (1970).
15) Y. Tanaka, H. Frank, H. F. DeLuca, N. Koizumi, and N. Ikekawa, *Biochemistry*, **14**, 3293 (1975).