[Chem. Pharm. Bull. 34(4)1568—1572(1986)]

Studies on Organic Fluorine Compounds. L.[1] Synthesis and Biological Activity of 2α-Fluorovitamin D_3

YOSHIRO KOBAYASHI,*,[a] MASAKAZU NAKAZAWA,[a] ITSUMARO KUMADAKI,[a] TAKEO TAGUCHI,[a] ETSUO OHSHIMA,[b] NOBUO IKEKAWA,*,[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 September 18, 1985)

Reaction of 3,6β-diacetoxycholest-2-ene (**2**) with cesium fluoroxysulfate gave 6β-acetoxy-2α-fluorocholestan-3-one (**3**), which was successfully converted to 2α-fluorovitamin D_3 (**10**). A single dose of 500 ng of **10** produced an intestinal calcium transport response and a bone calcium mobilization response in vitamin D-deficient rats equivalent to those of vitamin D_3.

Keywords——vitamin D_3; 2α-fluorovitamin D_3; cesium fluoroxysulfate; fluorination; 3,6β-diacetoxycholest-2-ene; 6β-acetoxy-2α-fluorocholestan-3-one

The crucial importance of the 1α-hydroxyl function of 1α,25-hydroxyvitamin D_3, a hormonal active form of vitamin D_3, for eliciting its biological activity is well established.[2] A number of fluorinated steroidal hormones have so far been reported as antiinflammatory agents with augmented and/or differential activities.[3,4] The effects of the fluorine in these molecules are cosidered to be the result of retardation of the functional conversion of the hydroxyl group to the ketone, and an enhancement of the binding ability to the enzyme receptor site due to increase of the acidity of the hydroxyl group by the fluorine atom or to conformational change through hydrogen bond formation. On the basis of similar considerations, 2-fluorovitamin D_3 analogs are of interest. 2β-Fluoro-1α-hydroxyvitamin D_3 was synthesized through *trans*-diaxial ring opening reaction of the α-epoxide with fluoride ion[5] and its biological activity was found to be higher than that of 1α-hydroxyvitamin D_3.[6]

In this report the synthesis and the biological activity of 2α-fluorovitamin D_3 (**10**) are described.

It was reported that introduction of fluorine into the 2α-position of 3-ketosteroids was effected by electrophilic fluorination of 3-acetoxy-, 3-methoxy- or 3-trimethylsilyloxy-2-ene-steroids with fluoroxytrifluoromethane (CF_3OF)[7] or xenon difluoride (XeF_2).[8] Although these reactions gave satisfactory results in terms of yield and selectivity, reactions with CF_3OF require stringent safety precautions because of the high reactivity and toxicity of this gaseous reagent, while the relatively high price of XeF_2 and difficulty in the preparation of XeF_2 are the disadvantages of this reagent.[9] Cesium fluoroxysulfate ($CsSO_4F$), which is easily prepared by treating cesium sulfate with fluorine is a mild electrophilic fluorinating reagent with olefinic compounds and is a solid material which can be isolated and stored.[10,11] We found that the reaction of 3,6β-diacetoxycholest-2-ene (**2**) with $CsSO_4F$ afforded the desired 2α-fluoro-3-keto derivative (**3**), which was successfully converted to **10**.

Treatment of 6β-acetoxycholestan-3-one (**1**)[12] with isopropenyl acetate in the presence of *p*-toluenesulfonic acid gave the enol acetate (**2**) in 95% yield. The enol acetate (**2**) was reacted

with $CsSO_4F$ in dichloromethane at room temperature to give the desired 2α-fluoro-3-keto derivative (**3**) in 23% yield, along with recovery of **2**. The α-configuration of the fluorine atom at C-2 in the fluoroketone (**3**) was confirmed by its nuclear magnetic resonance (NMR) spectrum, in which the 2β-proton (axial) signal appears at 5.02 ppm as ddd, $J_{gem}=48.7$, $J_{aa}=12.4$ and $J_{ae}=7.0$ Hz.[13]

Reduction of the fluoroketone (**3**) with sodium borohydride gave a mixture of the 2α-fluoro-3β-ol (**4**) and the 3α-epimer (**5**) in 53% and 20% yields, respectively. A similar result was reported in $NaBH_4$ reduction of 3-keto steroids.[7,14] Saponification of **4** with 5% methanolic potassium hydroxide followed by acetylation of the 3β-hydroxyl group with acetic anhydride in pyridine at −15 °C afforded the monoacetylated compound (**7**), which was converted to 3β-acetoxy-2α-fluorocholest-5-ene (**8**) by treatment with $POCl_3$ in pyridine.

Transformation of **8** into **10** was achieved by the standard procedure from the cholesterol derivative.[15] Thus, allylic bromination of **8** with *N*-bromosuccinimide and subsequent dehydrobromination with n-Bu_4NF gave a mixture of the 4,6-diene and the 5,7-diene (**9**), from which the desired 5,7-diene was isolated by treatment with *p*-toluenesulfonic acid in acetone followed by purification by thin layer chromatography (TLC). The 5,7-diene (**9**) was irradiated with a medium-pressure Hg lamp in benzene–ethanol (2 : 1) for 2.5 min and then refluxed for 1 h to give the vitamin D_3 acetate in 18% yield. Saponification and purification by high-performance liquid chromatography (HPLC) gave **10**.

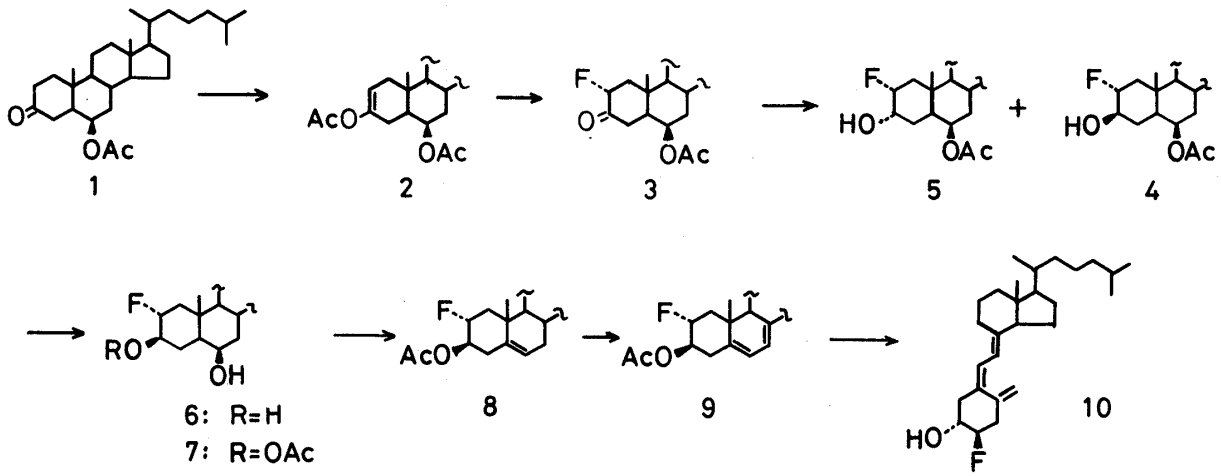

Chart 1

TABLE I. Increase of Intestinal Calcium Transport and Serum Calcium Concentration in Response to 2α-Fluorovitamin D_3 (**10**) and Vitamin D_3

Compound given	Amount of compound	Intestinal Ca transport (Ca serosal/Ca mucosal)	Serum Ca (mg/100 ml)
EtOH	—	$2.4\pm0.2^{a)}$	$4.1\pm0.1^{c)}$
Vitamin D_3	250 ng/rat	$5.8\pm0.4^{b)}$	$5.6\pm0.5^{d)}$
10	500 ng/rat	$5.9\pm1.1^{b)}$	$5.6\pm0.5^{d)}$

Standard deviation from the mean significance of difference *b*) from *a*) $p<0.001$, *d*) from *c*) $p<0.001$.

Biological Activities

The biological activities of **10** and vitamin D_3 on intestinal calcium transport and bone calcium mobilization measured in terms of the serum calcium concentration were compared. The results are shown in Table I. Compound **10** at a dosage level of 500 ng exhibits

stimulation of intestinal calcium transport or bone calcium mobilization essentially equivalent to that of vitamin D_3 itself.

The importance of the 1α-hydroxyl group of vitamin D_3 for eliciting the biological response is well established.[2] Fluoride substitution at the 2 position was found to increase the activity in the case of 2β-fluoro-1α-hydroxyvitamin D_3.[5,6] We are now carrying out the synthesis of 2α-fluoro-1α-hydroxyvitamin D_3 in order to compare the effect of fluoride substitution at the 2 position (α *vs.* β) on the vitamin D activity.

Experimental

Melting points were determined with a hot-stage microscope and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken with a Hitachi R-24-A or JEOL PS-100 spectrometer in $CDCl_3$ with Me_4Si as an internal standard. Mass spectra (MS) were determined with a Shimadzu LKB-9000S or a Hitachi M-80 mass spectrometer at 70 eV. Ultraviolet (UV) spectra were obtained with a Shimadzu UV-200 double-beam spectrophotometer in EtOH solution. Preparative thin layer chromatography was carried out on precoated plates of silica gel (E. Merck, 0.25 mm thickness). 10% fluorine in nitrogen was purchased from Matheson Co.

$CsSO_4F$[10]——About 20 mmol of F_2 (as a 10% mixture in nitrogen) was passed during 1 h into a solution of Cs_2SO_4 (4 g) in water (8 ml) in Teflon tube which was cooled in an ice-salt bath. The reaction mixture was centrifuged and the supernatant was removed by decantation. The remaining solid material was washed with cold water (2 ml) and then dried *in vacuo* to leave a pale yellow precipitate (1.9 g).

3,6β-Diacetoxycholest-2-ene (2)——A solution of 6β-acetoxycholestan-3-one (**1**) (1215 mg, 2.74 mmol) in isopropenyl acetate (15 ml) was refluxed in the presence of *p*-toluenesulfonic acid (20 mg) under argon for 3 h. Most of the solvent was removed and the concentrated mixture was extracted with ethyl acetate. The extract was washed with saturated $NaHCO_3$, and brine, and dried over $MgSO_4$. The residue obtained upon evaporation of the solvent was purified by column chromatography on silica gel (40 g). Elution with benzene gave the enol-acetate (**2**) (1265 mg, 95%), glass. ^{1}H-NMR δ: 0.71 (3H, s, 18-H_3), 0.88 (6H, d, J=6 Hz, 26- and 27-H_3), 0.91 (3H, d, J=6 Hz, 21-H_3), 1.02 (3H, s, 19-H_3), 2.08 and 2.11 (6H, s×2, 3- and 6-acetyl), 5.03 (1H, m, 6α-H), 5.23 (1H, dd, J=5 and 2 Hz, 2-H).

6β-Acetoxy-2α-fluorocholestan-3-one (3)——A solution of the enol-acetate (**2**, 1.4 g) in methylene chloride (8 ml) was added to a suspension of cesium fluoroxysulfate (713 mg) in dry methylene chloride (8 ml) at room temperature, and the mixture was stirred at room temperature for 24 h. Water was added and the resulting precipitate was filtered off. The filtrate was extracted with methylene chloride, and the extract was washed with brine and dried over $MgSO_4$. The residue obtained upon evaporation of the solvent was purified on a silica gel (130 g) column. Elution with hexane–ether (1:1) gave a mixture containing **2** as a major component (397 mg). Further elution with the same solvent system gave the fluoroketone (**3**) (308 mg, 23%), mp 153—156 °C (from MeOH). ^{1}H-NMR δ: 0.73 (3H, s, 18-H_3), 0.87 (6H, d, J=6 Hz, 26- and 27-H_3), 0.93 (3H, d, J=6 Hz, 21-H_3), 1.27 (3H, s, 19-H_3), 2.07 (3H, s, acetyl), 4.97 (1H, m, 6α-H), 5.02 (1H, ddd, J=48.7, 12.4, 7.0 Hz, 2β-H). High-resolution MS Calcd for $C_{27}H_{43}FO$ ($M^+ - AcOH$): 402.3296. Found: 402.3277.

6β-Acetoxy-2α-fluoro-3β-hydroxycholestane (4)——Sodium borohydride (90 mg) in methanol (6 ml) was added to a solution of the fluoroketone (**3**, 270 mg) in THF (24 ml) at room temperature, and the mixture was stirred at room temperature for 1 h. Then water and ethyl acetate were added, and the excess reagent was quenched with 2 N HCl. The organic phase was washed with saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The crude mixture obtained upon evaporation of the solvent was separated by column chromatography on silica gel (10 g). Elution with benzene–ethyl acetate (10:1) gave 6β-acetoxy-2α-fluoro-3α-hydroxycholestane (**5**) (54 mg, 20%), mp 148—150 °C ($CHCl_3$–MeOH). ^{1}H-NMR δ: 0.79 (3H, s, 18-H_3), 2.20 (3H, s, acetyl), 4.22 (1H, m, $W_{1/2}$=13 Hz, 3β-H), 4.65 (1H, m, 2β-H), 4.98 (1H, m, 6α-H). MS *m/z*: 404 ($M^+ - AcOH$), 389, 384, 371, 369.

Further elution with the same solvent system gave the 3β-alcohol (**4**) (144 mg, 53%), mp 142—143 °C ($CHCl_3$–MeOH). ^{1}H-NMR δ: 0.70 (3H, s, 18-H_3), 0.86 (6H, d, J=6 Hz, 26- and 27-H_3), 0.91 (3H, d, J=6 Hz, 21-H_3), 1.03 (3H, s, 19-H_3), 2.02 (3H, s, acetyl), 3.70 (1H, m, $W_{1/2}$=30 Hz, 3α-H), 4.49 (1H, dm, J=54 Hz, 2β-H), 5.02 (1H, m, 6α-H). MS *m/z*: 404 ($M^+ - AcOH$), 389, 384, 371, 369.

3β-Acetoxy-2α-fluoro-6β-hydroxycholestane (7)——The 3β-alcohol (**4**) (144 mg, 0.309 mmol) was treated with 5% KOH–methanol (15 ml) in THF (30 ml) at 60 °C for 4 h, then water and ethyl acetate were added to the reaction mixture. The organic phase was washed with 2 N HCl, saturated $NaHCO_3$, and brine and dried over $MgSO_4$. The residue obtained upon evaporation of the solvent gave the diol (**6**). This was dissolved in a mixture of pyridine (10 ml) and acetic anhydride (2 ml), and the solution was stirred at −15 °C for 6 h, then extracted with ethyl acetate. The extract was washed with 2 N HCl, saturated $NaHCO_3$, and brine, and dried over $MgSO_4$. The residue obtained upon evaporation of the solvent was purified on a silica gel column (10 g). Elution with benzene–ethyl acetate (10:1) gave the acetate (**7**) (132 mg, 92%), glass. ^{1}H-NMR δ: 3.90 (1H, m, 6α-H), 4.96 (1H, m, 3α-H).

3β-Acetoxy-2α-fluorocholest-5-ene (8)——Phosphorus oxychloride (0.06 ml) was added dropwise to a solution of the acetate (**7**) (92 mg, 0.198 mmol) in pyridine (2 ml) at 0 °C. The mixture was stirred at room temperature for 2 h, then ice and ethyl acetate were added. The organic phase was washed with 2 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The residue obtained upon evaporation of the solvent was purified by column chromatography on silica gel (5 g). Elution with benzene–ethyl acetate (20 : 1) gave the olefin (**8**) (90 mg, 98%), mp 116—119 °C (MeOH). ^{1}H-NMR δ: 0.68 (3H, s, 18-H_3), 0.87 (6H, d, J=6 Hz, 26- and 27-H_3), 0.93 (3H, d, J=6 Hz, 21-H_3), 1.06 (3H, s, 19-H_3), 2.08 (3H, s, acetyl), 4.10—5.00 (1H, br, 2β-H), 4.86 (1H, m, 3α-H), 5.42 (1H, m, 6-H). High-resolution MS Calcd for $C_{27}H_{43}F$ ($M^+ - AcOH$): 386.3347. Found: 386.3370.

3β-Acetoxy-2α-fluorocholesta-5,7-diene (9)——*N*-Bromosuccinimide (19.2 mg) was added to a refluxing solution of the olefin (**8**) (34.5 mg, 0.077 mmol) in carbon tetrachloride (2 ml), and the mixture was refluxed under argon. After 50 min, the reaction mixture was cooled and the precipitate was filtered off. The filtrate was concentrated under reduced pressure to give the crude bromide, which was treated with THF (5 ml) containing a small amount of tetra-*n*-butylammonium bromide at room temperature under argon in the dark. After 50 min, tetra-*n*-butylammonium fluoride (1 M THF solution, 0.5 ml) was added, and the whole was stirred at room temperature for 30 min under argon in the dark. Water and ethyl acetate were added and the organic phase was washed with 2 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. Evaporation of the solvent gave the crude diene, which was treated for 11 h under argon in the dark with acetone (15 ml) containing a catalytic amount of *p*-toluenesulfonic acid. The reaction mixture was extracted with ethyl acetate. The extract was washed with saturated $NaHCO_3$, and brine, and dried over $MgSO_4$. The residue obtained upon evaporation of the solvent was applied on silica gel TLC-plate. The plate was developed with hexane–ethyl acetate (20 : 1) three times and elution of the scraped-off band (Rf=0.6) with ethyl acetate followed by evaporation of the solvent, gave the 5,7-diene (**9**) (8.4 mg, 25%), λ_{max} 264, 272, 282, and 293 nm.

2α-Fluorovitamin D_3 (10)——A solution of the 5,7-diene (5 mg) in benzene (80 ml) and ethanol (40 ml) was irradiated with a medium pressure mercury lamp through a Vicor filter for 2.5 min at 0 °C under argon. The reaction mixture was then refluxed for 1 h under argon. Evaporation of the solvent gave a crude mixture, which was applied to TLC-plates and developed with hexane–benzene (3 : 1) three times. The band at Rf=0.31 gave **10** (0.914 mg, 18.3%). This was treated with a mixture of 5% KOH–methanol (2 ml) and THF (2 ml) overnight at room temperature under argon in the dark. The reaction mixture was extracted with ethyl acetate. The extract was washed with 2 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by HPLC (Shimadzu LC-3A; column, Zorbax-SIL, 4.6 mm i.d. × 15 cm; elution, hexane–methylene chloride (2 : 1); flow rate, 2.2 ml/min; t_R, 3.2 min) to give **10**, λ_{max} 265, λ_{min} 226 nm. ^{1}H-NMR δ: 0.54 (3H, s, 18-H_3), 0.87 (6H, d, J=6 Hz, 26-, 27-H_3), 0.92 (3H, d, J=6 Hz, 21-H_3), 3.88 (1H, m, 3α-H), 4.46 (1H, dm, J=48 Hz, 2β-H), 4.96 and 5.16 (2H, br s × 2, 19-H), 5.91 and 6.30 (2H, AB q, J=11 Hz, 6-, 7-H). High-resolution MS Calcd for $C_{27}H_{43}FO$: 402.3295. Found: 402.3272.

Biological Activity of 10——Weanling male rats were obtained from Holtzman Co., Madison, Wis. and fed water and a low-Ca, vitamin D-deficient diet[17] *ad libitum* for 3 weeks. They were then divided into three groups of five or six rats each and two groups were given 250 ng of vitamin D_3 or 500 ng of **10** dissolved in 0.1 ml of 95% ethanol intrajugularly. The rats in the control group were given the ethanol vehicle in the same manner. Twenty-four hours later, intestinal Ca transport and serum Ca concentration were measured as described by Martin and DeLuca[18] and Tanaka *et al.*[19]

References

1) Part IL: T. Nagai, I. Kumadaki, T. Miki, Y. Kobayashi, and G. Tomizawa, *Chem. Pharm. Bull.*, **34**, 1546 (1986).
2) H. F. DeLuca and H. K. Schnoes, *Ann. Rev. Biochem.*, **45**, 611 (1976); *idem, ibid.*, **52**, 411 (1983).
3) R. Filler, "Organofluorine Chemicals and Their Industrial Application," ed. by R. E. Banks, Ellis Horwood Ltd., Chichester, 1979, p. 123.
4) A. Wettstein, "Carbon–Fluorine Compounds," A Ciba Found. Symp., Elsevier, Amsterdam, 1972, p. 281.
5) J. Oshida, M. Morisaki, and N. Ikekawa, *Tetrahedron Lett.*, **21**, 1755 (1980).
6) Y. Tanaka, H. F. DeLuca, N. Ikekawa, and J. Oshida, unpublished data.
7) D. H. R. Barton, L. S. Godinho, R. H. Hesse, and M. M. Pechet, *J. Chem. Soc., Chem. Commun.*, **1968**, 804; D. H. R. Barton, *Pure Appl. Chem.*, **21**, 285 (1970).
8) T. Tsushima, K. Kawada, and T. Tsuji, *Tetrahedron Lett.*, **23**, 1165 (1982).
9) M. Zupan and B. Sket, *J. Org. Chem.*, **43**, 698 (1978); R. Filler, *Israel J. Chem.*, **17**, 71 (1978).
10) E. H. Appelman, L. J. Baside, and R. C. Thompson, *J. Am. Chem. Soc.*, **101**, 3384 (1979).
11) S. Stauber and M. Zupan, *J. Chem. Soc., Chem. Commun.*, **1981**, 148; *idem, ibid.*, **1981**, 795; *idem, J. Fluorine Chem.*, **17**, 579 (1981).
12) B. Pelc and E. Kodicek, *J. Chem. Soc.* (*C*), **1970**, 1624.
13) The 2β-proton signal of the reported 2α-fluoro-3-keto steroids appears as ddd, J_{gem}=47—50, J_{aa}=8—10 and J_{ae}=4—8 Hz.[7,8,14]

14) M. Neeman and Y. Osawa, *J. Am. Chem. Soc.*, **85**, 232 (1963).
15) M. Morisaki, K. Bannai, and N. Ikekawa, *Chem. Pharm. Bull.*, **21**, 1853 (1973).
16) N. Koizumi, M. Ishiguro, M. Yasuda, and N. Ikekawa, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 1401.
17) T. Suda, H. F. DeLuca, and Y. Tanaka, *J. Nutr.*, **100**, 1049 (1970).
18) D. L. Martin and H. F. DeLuca, *Am. J. Physiol.*, **216**, 1351 (1969).
19) Y. Tanaka, H. Frank, H. F. DeLuca, N. Koizumi, and N. Ikekawa, *Biochemistry*, **14**, 3293 (1975).