Preparation and Biological Activity of 24-Epi-26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D₂

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A new fluorinated analog of vitamin D₂, 24-epi-26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D₂, was efficiently synthesized starting from (R)-4-isopropyl-3-propanyl-2-oxazolidinone with high stereochemical control. In all four physiological test systems, the fluorinated vitamin D₂ analog was found to be slightly less active than 1α,25-dihydroxyvitamin D₃.

Key words: vitamin D; fluorinated vitamin D analog; calcium regulation; 1α,25-dihydroxyvitamin D₃.

1α,25-Dihydroxyvitamin D₃ (1,25-(OH)₂D₃, 1), the hormonally active form of vitamin D₃, is essential to the regulation of calcium and phosphorus metabolism in animals. Among its functions are intestinal calcium transport, bone calcium mobilization, calcification of epiphyseal plate cartilage and elevation of plasma calcium and phosphorus concentration. These physiological properties and the application of 1 as a medicine to treat bone diseases such as osteoporosis have prompted the synthesis of vitamin D₃ analogs for enhancing and modifying biological activity. Kobayashi et al. reported the synthesis of a fluorinated analog, 26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D₃ (2), which displays enhanced and prolonged activity in various bioassays, compared to 1. Thus showing the ability of fluorine atoms to inhibit metabolic hydroxylation at the C-26 and C-27 positions, a step in the deactivation of 1. DeLuca et al. found that 24-epi-1α,25-dihydroxyvitamin D₃ (3) regulates intestinal calcium transport and calcification of epiphyseal plate cartilage but without promoting bone calcium mobilization or elevation of plasma calcium concentration. These findings prompted the authors to search for an efficient synthesis of the new fluorinated analog, 24-epi-26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D₂ (24-epi-26,27-F₆C₆H₂) (4). This paper describes in detail the stereorestricted synthesis of 4 along with preliminary results on its biological activities.

The synthesis was initiated by the enantioselective construction of the side chain. Diastereoselective reaction of the boron enolate derived from the chiral N-acyloxazolidinone 5 with hexafluoroacetone gave the carboximide 6 in 80% yield. The reduction of 6 with lithium borohydride (LiBH₄) in tetrahydrofuran (THF) at 0°C afforded the diol 7 in 64% yield. Treatment of 7 with (S)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl) in pyridine gave the corresponding (R)-α-methoxy-α-(trifluoromethyl)phenylacetyl ester 8, whose 1H- and 19F-NMR spectra showed 7 to be enantiomerically pure. The selective silylation of 7 with tert-butyldimethylsilyl chloride (TBDMSCl) and imidazole in dichloromethane (CH₂Cl₂) gave the tert-butyldimethylsilyl (TBDDS) ether 9 in 85% yield. The methoxymethylation of 9 with chloromethyl methyl ether (MOMCl) and diisopropylethylamine (iso-Pr₂NEt) in CH₂Cl₂ at room temperature provided the corresponding methoxymethyl (MOM) ether 10 in 81% yield. Desilylation of 10 with tetrabutylammonium fluoride (Bu₄NF) in THF afforded the alcohol 11 in quantitative yield. This alcohol 11 was treated with p-toluenesulfonyl fluoride (TsCl) in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP) in pyridine at room temperature for 16 h to give the tosylate 12 in 97% yield.

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Displacement of the tosyl group of 12 with sodium thiophenoxide (NaSPh) provided the sulfide 13 in 91% yield. The oxidation of 13 with m-chloroperbenzoic acid (MCPBA) in CH₂Cl₂ at 6°C gave the sulfone 14 in 98% yield.

To introduce a double bond at C-22 (vitamin D numbering) in a stereocontrolled manner, the condensation of the aldehyde 17 with the sulfone 14 was examined. The alcohol 15, prepared from vitamin D₃ according to the oxidation procedure of Toh and Okamura, was converted to the acetate 16 in 93% yield by acetylation with acetic anhydride (Ac₂O) in the presence of triethylamine (Et₃N) and DMAP in CH₂Cl₂. The reaction of 16 with ozone was carried out in the presence of pyridine in CH₂Cl₂ at 78°C. Reduction with zinc powder and acetic acid (AcOH) afforded the crude aldehyde 17, which was directly treated with the carbaniion derived from the sulfone 14 and butyllithium (BuLi), in the presence of magnesium bromide diethyl etherate (MgBr₂·Et₂O) in THF at −70 to 0°C to give the alcohol 18 as a mixture of diastereomers in 87% yield in two steps. Treatment of 18 with 5% sodium amalgam (5% Na-Hg) in the presence of sodium hydrogen phosphate (Na₂HPO₄) in THF-methanol (MeOH) at 0°C afforded the acetate 19 in 52% yield along with the alcohol 20 (22%). Deacetylation of 19 with methyllithium (MeLi) in THF and demethoxylation of the resulting alcohol 20 in 1,4-dioxane containing concentrated hydrochloric acid (HCl) at 60°C gave the corresponding diol 21 in 69% yield in two steps. The diol 21 was oxidized with pyridinium chlorochromate (PCC) in CH₂Cl₂ to give the ketone 22 in quantitative yield.

According to the general approach of Lythgoe et al., the Horner–Wittig reaction of ketone 22 with the phosphinoyl carbanion, prepared from the phosphine oxide 23 and BuLi, in THF at −78 to −40°C afforded the bis(TBDMS) ether 24 in 83% yield. After the desilylation of 24 with cation exchange resin (50W-X4) in

<table>
<thead>
<tr>
<th>Compound</th>
<th>VDR (IC₅₀) (M)</th>
<th>DBP (IC₅₀) (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,25-(OH)₂D₃ (1)</td>
<td>4.0 × 10⁻¹⁰</td>
<td>5.4 × 10⁻⁷</td>
</tr>
<tr>
<td>24-Epi-26,27,28-F₃-1,25-(OH)₂D₃ (4)</td>
<td>2.9 × 10⁻⁹</td>
<td>6.8 × 10⁻⁶</td>
</tr>
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</table>

Table 1: Binding Affinities of 1,25-(OH)₂D₃ (1) and 24-Epi-26,27,28-F₃-1,25-(OH)₂D₃ (4) to Chick Intestinal Cytosol Receptor (VDR) and Vitamin D-Deficient Rat Serum DBP

MeOH at room temperature, 24-epi-26,27,28-F₃-1,25-dihydroxyvitamin D₃ (4) was obtained as a colorless amorphous powder in 95% yield.

Table 1 shows the results of in vitro binding assays. The fluorinated vitamin D₃ analog 4 was 8-fold less effective than 1,25-(OH)₂D₃ (1) for binding to the chick embryonic intestinal 1,25-(OH)₂D₃ receptor (VDR). As regards binding affinity to vitamin D binding protein (DBP) from vitamin D-deficient rats, 4 was 13-fold less active than 1,25-(OH)₂D₃ (1). In in vivo experiments, each compound was given to 8-week-old Wistar male rats orally once a day for 3 weeks (0.04—0.625 µg/kg/d). The femurs were excised to measure specific gravity of bone, and blood samples were collected to measure the serum calcium level. As shown in Fig. 1, 4 did not increase the specific gravity of bone, thus indicating that 4 is less active than 1 in increasing bone content of calcium and phosphorus. Figure 2 shows that 4 was less effective than 1 for raising serum calcium in rats.

Experimental

Melting points were determined on a Yanako MP-500D hot stage microscope without correction. Optical rotations were measured in a 1.0-dm cell with a JASCO DIP-370 polarimeter. IR spectra were obtained on a Perkin Elmer 1600 FT-IR. ¹H-NMR and ¹³C-NMR spectra were recorded at 200 MHz and 188 MHz, respectively, on a Varian Gemini-200 instrument. ¹H-NMR data are given in parts per million (ppm) downfield
Fig. 1. Increasing Effects of 1,25-(OH)₂D₃ (1) and 24-Epi-26,27-F₄-1,25-(OH)₂D₃ (4) on Specific Gravity of Bone in Rats

Each compound was given orally once a day for 3 weeks. Doses are given in mg/kg. d. The rats were killed and the femurs were excised. The weight and volume of the femurs dissected free of soft tissues were measured. Specific gravity was calculated as weight (mg)/volume (ml). * p < 0.05 vs. control.

Fig. 2. Effects of 1,25-(OH)₂D₃ (1) and 24-Epi-26,27-F₄-1,25-(OH)₂D₃ (4) on Serum Calcium in Rats

Each compound was given orally once a day for 3 weeks (0.04-0.0625 mg/kg/d). Blood samples were collected from the main artery to measure the serum Ca level. * p < 0.01 vs. control.

from tetracyselinal (TMS) as the internal standard. ¹⁸F-NMR data are given in ppm upfield from CDCl₃ as the internal standard. The abbreviations used are as follows: s = singlet, d = doublet, t = triplet, q = quartet, sep = septet, m = multiplet, br = broad. Coupling constants (J values) are given in parentheses (Hz). Low- and high-resolution MS analyses were performed using a Kratos Concept-1H double-focusing magnetic sector spectrometer. Elemental analysis was conducted at Toray Research Center, Inc., Tokyo. Kieselgel 60 (Merck, 230-400 mesh) was used for column chromatography. Thin-layer chromatography (TLC) was carried out with pre-coated Kieselgel 60F₂₅₄ plates (Merck). All reactions were carried out under an argon atmosphere with magnetic stirring in oven-dried glassware.

(2,5,4R)-4-Isopropyl-3-[(4',4',4'-trifluoro-3'-hydroxy-2'-methyl-3'- trifluoromethylbutyl)oxy]-2-oxazolidinone (6) To a solution of (R)-4-isopropyl-3-propenyl-2-oxazolidinone (5) (776 mg, 4.19 mmol) in dry CH₃Cl (5 ml) was added dibutylboron triflate (Bu₂BOT) (1.32 g, 4.81 mmol) at -78 °C over 2 min. After 10 min at this temperature, Et₂N (760 ml, 5.45 mmol) was added over 10 min and the reaction mixture was warmed to 0 °C. After 1 h at 0 °C, the solution was cooled to -78 °C and gaseous hexfluorocouzen (1.5 ml at -78 °C, 11.9 mmol) was added with a cannula. After 0.5 h at -78 °C, the reaction mixture was brought to and held at 0 °C for 2 h, then the reaction was quenched with 0.7 mol phosphate buffer (0.1 M, pH 7.0) and MeOH (10 ml), followed by the addition of 30% H₂O₂ MeOH (5 ml 25 ml). After 1 h at 0 °C, the mixture was concentrated in vacuo. The residue was diluted with 10% aqueous NaHCO₃ and extracted with CH₂Cl₂. The combined extracts were washed with brine, dried over MgSO₄ and filtered. After evaporation of the solvent, the chromatography of the residue with hexane:EtOAc (3:1, v/v) gave 6 (1.18 g, 80%). 6 colorless needles (hexane:EtOAc), mp 96.1-97.9 °C. [α]D₂⁰ = -47.9° (c = 0.98, CHCl₃). IR (KBr):

13H-NMR (CDCl₃): δ = 0.88 (3H, d, J = 6.9 Hz), 0.94 (3H, d, J = 7.0 Hz), 1.43 (3H, d, J = 7.1, 2.7 Hz), 2.37 (1H, dcp, J = 7.1, 2.8 Hz), 4.28-4.49 (3H, m), 4.75 (1H, q, J = 6.7 Hz), 6.53 (1H, s). ¹⁸F-NMR (CDCl₃): δ = 73.32 (3F, d, J = 11.5, 1.5 Hz), 76.17 (3F, q, J = 11.5 Hz). MS m/z: 351 (M⁺), 282, 223, 175, 86, 69. Anal. Calcd for C₁₇H₁₀F₂₆O₆N: C 41.0, H 4.3, N 4.1. Found: C 41.1, H 4.5; N 4.4.

(4R,4A,3R)-4-Trifluoro-2-methyl-3-trifluoromethylaniline-1,3-diol (7) A solution of 6 (1.12 g, 3.19 mmol) in dry THF (10 ml) was treated with LiH (350 mg, 16.0 mmol) in five portions at 0 °C. The mixture was stirred at 0 °C for 3.5 h, and the reaction was quenched with 0.7 mol phosphate buffer. The whole was washed with brine, dried over MgSO₄ and filtered. After evaporation of the solvent, the chromatography of the residue with hexane:EtOAc (1:1, v/v) gave 7 (846 mg, 84%). 7: colorless oil. [α]D₂⁰ = -7.5 (c = 0.76, CHCl₃). IR (KBr): 3300-3000 cm⁻¹. ¹H-NMR (CDCl₃): δ = 1.12 (3H, d, J = 7.3 Hz), 2.27 (1H, s), 2.48-2.70 (1H, m), 3.87 (1H, dd, J = 11.0, 4.1 Hz), 4.10 (1H, t, J = 11.0 Hz), 6.21 (1H, s). ¹⁸F-NMR (CDCl₃): δ = 72.34 (3F, q, J = 10.0 Hz), 76.59 (3F, q, J = 10.0 Hz). MS m/z: 208 (M⁺ - 18), 139. High-resolution MS (HRMS) Calcd for C₁₂H₁₀F₂O₄ (M - H₂O): 208.032. Found: 208.031.

(R)-MTPA Ester of 7 (8) A solution of 7 (20 mg, 88 mmol) in dry pyridine (0.5 ml) was treated with (S)-MTPAC (23 mg, 91 mmol) at 0 °C. The reaction mixture was stirred to and left at room temperature for 16h, diluted with H₂O and extracted with EtOAc. The combined extracts were washed with 2 x aqueous HCl, saturated aqueous NaHCO₃ and brine, dried over MgSO₄ and filtered. After evaporation of the solvent by evaporation, the chromatography of the residue with hexane:EtOAc (30:1, v/v) gave 8 (564 mg, 85%). colorless oil. [α]D₂⁰ = -12.9 (c = 1.07, CHCl₃). IR (KBr): 3318-3000 cm⁻¹. ¹H-NMR (CDCl₃): δ = 0.12 (6H, s), 0.91 (9H, s), 1.08 (3H, d, J = 7.1 Hz), 2.43-2.62 (3H, m), 3.75 (1H, dd, J = 10.4, 4.4 Hz), 3.97 (1H, dd, J = 10.7, 10.4 Hz), 6.74 (1H, s). ¹⁸F-NMR (CDCl₃): δ = 72.24 (3F, q, J = 10.0 Hz), 76.62 (3F, q, J = 10.0 Hz). MS m/z: 389 (M⁺), 328 (M⁺ - H₂O) (HRMS) Calcd for C₁₅H₁₈F₂O₂ (M - H₂O): 389.043. Found: 389.043.

(R)-[1-(tert-Butyl dimethylsilyloxy)]-1,1-trifluoro-3-methyl-2-trifluoromethylaniline (9) Imidazole (441 mg, 6.48 mmol) and TBDMSCl (370 mg, 2.45 mmol) was added to a solution of 9 (444 mg, 1.96 mmol) in dry CH₂Cl₂ (5 ml) at room temperature. The reaction mixture was stirred for 1h, diluted with CH₂Cl₂, washed with saturated aqueous NH₄Cl and brine, dried over MgSO₄ and filtered. After evaporation of the solvent by evaporation, chromatography of the residue with hexane:EtOAc (30:1, v/v) gave 9 (546 mg, 85%). colorless oil. [α]D₂⁰ = -12.9 (c = 1.07, CHCl₃). IR (KBr): 3318 cm⁻¹. ¹H-NMR (CDCl₃): δ = 0.12 (6H, s), 0.91 (9H, s), 1.08 (3H, d, J = 7.1 Hz), 2.43-2.62 (3H, m), 3.75 (1H, dd, J = 10.4, 4.4 Hz), 3.97 (1H, dd, J = 10.7, 10.4 Hz), 6.74 (1H, s). ¹⁸F-NMR (CDCl₃): δ = 72.24 (3F, q, J = 10.0 Hz), 76.62 (3F, q, J = 10.0 Hz). MS m/z: 389 (M⁺), 328 (M⁺ - H₂O) (HRMS) Calcd for C₁₅H₁₈F₂O₂ (M - H₂O): 389.043. Found: 389.043.

(1R,1,3,6-trifluoro-2-[(methoxymethyl)oxy]-3-methyl-4-(p-toluenesulfonyl)phenylbutanate (14) 14

(1R,1,3,6-trifluoro-2-[(methoxymethyl)oxy]-3-methyl-4-(p-toluenesulfonyl)phenylbutanate (14), after re-distillation from TiCl4 (680 mg, 3.57 mmol) and DMAP (20 mg, 0.16 mmol) were added to a solution of 11 (476 mg, 1.76 mmol) in dry pyridine (1.0 mL). The reaction mixture was stirred at room temperature for 10 h and diluted with Et2O. The organic layer was washed with 0.5 N aqueous HCl, saturated aqueous NaHCO3 and brine, dried over MgSO4 and filtered. After evaporation of the solvent, chromatography of the residue with hexane-EtOAc (10:1, v/v) gave 12 (723 mg, 6986 μmol) as colorless oil (2.16 g, 20% yield). 1H-NMR (CDCl3) δ: 1.32 (3H, d, J = 6.6 Hz, 2.46 (3H, s), 2.55–2.78 (1H, m), 3.39 (3H, s), 3.83–3.93 (1H, m), 4.38 (1H, dd, J = 10.0, 2.8 Hz), 4.86 (1H, d, J = 6.4 Hz), 4.91 (1H, d, J = 6.4 Hz), 7.20–7.35 (2H, m), 7.75–7.83 (2H, m). 13F-NMR (CDCl3) δ: 69.24 (3F, q, J = 9.8 Hz), 69.51 (3F, q, J = 9.8 Hz). MS m/z: 424 (M+).


(5S,1R,1,3,6-trifluoro-2-[(methoxymethyl)oxy]-3-methyl-4-phenylbutanate (13) A solution of PhSO3 (300 μL, 2.92 mmol) in DMF (2 mL) was added dropwise to a suspension of Na2O (60% Na2O, 110 mg, 2.75 mmol) in dry THF (2 mL) at 0°C. The mixture was stirred at room temperature for 10 min, then a solution of 12 (723 mg, 1.70 mmol) in dry DMF–THF (2–4 mL) was added to the Na2SO3 solution at 0°C. The mixture was stirred at room temperature for 14 h, the reaction was quenched with saturated aqueous NaHCl and brine, and the whole was washed with water and evaporated. The crude products were then washed with brine, dried over MgSO4 and filtered. After evaporation of the solvent, chromatography of the residue with hexane-EtOAc (60:1, v/v) gave 13 (163 mg, 69%). 13F-NMR (CDCl3) δ: 1.28 (3H, d, J = 6.6 Hz, 2.46 (3H, s), 2.38–2.55 (1H, m), 2.67 (1H, J = 12.2 Hz), 3.42 (2H, s), 3.48 (1H, d, J = 6.4 Hz, 4.79 (2H, s), 7.27–7.39 (1H, m). 13F-NMR (CDCl3) δ: 68.27 (3F, q, J = 9.8 Hz), 69.50 (3F, q, J = 9.8 Hz). MS m/z: 362 (M+). 317. HRMS Caled for C19H18F2O3S (M+): 362.077. Found: 362.078.

(4R)-Benzensulfonyl-1,1-trifluoro-2-[(methoxymethyl)oxy]-3-methyl-4-trifluorobutylbutanate (14) A solution of 13 (543 mg, 1.50 mmol) and NaH2PO4 (3.06 g, 37.4 mmol) in dry CH2Cl2 (10 mL) was treated with MCPBA (70%, 920 mg, 3.70 mmol) at 0°C. After 40 min at 6°C, the reaction mixture was stirred with Et2O, washed with saturated aqueous NaHCO3, 5% aqueous NaOH and brine, dried over MgSO4 and filtered. After evaporation of the solvent, chromatography of the residue with hexane-EtOAc (10:1, v/v) gave 14 (579 mg, 98%). 14F-NMR (CDCl3) δ: 1.32 (3H, d, J = 6.6 Hz, 2.46 (3H, s), 2.38–2.55 (1H, m), 3.42 (3H, s), 3.62 (1H, d, J = 6.4 Hz), 4.28 (1H, d, J = 6.4 Hz, 4.95 (1H, d, J = 6.2 Hz), 7.25–7.75 (1H, m), 7.91–8.75 (2H, m). 13F-NMR (CDCl3) δ: 68.92 (3F, m, MS m/z: 394 (M+)) 363. HRMS Caled for C19H18F2O3S (M+): 394.068. Found: 394.067.

[1R-(1α,3α,4α,7α)-4-Acetoxyoctahydro-1,1-dimethyl-1H-indene-1-carboxylic acid] 4-Acetoxyoctahydro-1,1-dimethyl-1H-indene-1-carboxylic acid] (21) A solution of 19 (150 mg, 0.32 mmol) in dry THF (1 mL) was treated with MeI (1.40 mL in ether, 340 μL, 0.48 mmol) at −78°C. After 5 min in the same temperature, the reaction mixture was allowed to warm to room temperature, then the reaction was quenched with saturated aqueous NaHCO3 and the whole was extracted with Et2O. The combined extracts were washed with brine, dried over MgSO4 and filtered. After evaporation of the solvent, chromatography of the residue with hexane-EtOAc (30:1, v/v) gave 19 (161 mg, 52%). 19F-NMR (CDCl3) δ: 72.18 (3F, q, J = 9.4 Hz), 74.83 (3F, q, J = 9.4 Hz). MS m/z: 488 (M+). 457, 443. HRMS Caled for C21H20F2O3S (M+): 488.236. Found: 488.237. 218. 220. 13F-NMR (CDCl3) δ: 49.9 (3F, q, J = 9.5 Hz, 7.20–7.35 (2H, m), 7.75–7.83 (2H, m). 13F-NMR (CDCl3) δ: 68.92 (3F, m, MS m/z: 446 (M+). 428, 415. HRMS Caled for C21H20F2O3S (M+): 446.226. Found: 446.225.

[1R-(1α,3α,4α,7α)-4-Octahydro-1,1-dimethyl-1H-indene-1-carboxylic acid] 4-Octahydro-1,1-dimethyl-1H-indene-1-carboxylic acid] (21) A solution of 19 (150 mg, 0.32 mmol) in dry THF (1 mL) was treated with MeI (1.40 mL in ether, 340 μL, 0.48 mmol) at −78°C. After 5 min in the same temperature, the reaction mixture was allowed to warm to room temperature, then the reaction was quenched with saturated aqueous NaHCO3 and the whole was extracted with Et2O. The combined extracts were washed with brine, dried over MgSO4 and filtered. After evaporation of the solvent, chromatography of the residue with hexane-EtOAc (30:1, v/v) gave 19 (161 mg, 52%). 19F-NMR (CDCl3) δ: 72.18 (3F, q, J = 9.4 Hz), 74.83 (3F, q, J = 9.4 Hz). MS m/z: 488 (M+). 457, 443. HRMS Caled for C21H20F2O3S (M+): 488.236. Found: 488.237. 218. 220. 13F-NMR (CDCl3) δ: 49.9 (3F, q, J = 9.5 Hz, 7.20–7.35 (2H, m), 7.75–7.83 (2H, m). 13F-NMR (CDCl3) δ: 68.92 (3F, m, MS m/z: 446 (M+). 428, 415. HRMS Caled for C21H20F2O3S (M+): 446.226. Found: 446.225.
Binding Affinity with Vitamin D-Deficient Rat Serum DBP

DBP, prepared from a 2000-fold-diluted serum from a vitamin D-deficient rat using 0.05 μM phosphate buffer (pH 7.4), was incubated at 25°C for 1 h with [3H]-25(OH)D3 and various concentrations of 1,25(OH)2D3 (1) and 24-epi-26,27-F-1,25(OH)2D3 (4). At the end of incubation, dioxan-chloroform was added to the assay mixture and this preparation was mixed for 15 s. The mixture was left for 30 min and then centrifuged at 4°C. The supernatant was transferred into a scintillation vial to measure the radioactivity.

In Vivo Testing

Compounds 1 and 4 were each given to 8-week-old Wistar male rats orally once a day for 3 weeks (0.04-0.625 μg/kg/d). Blood samples were collected from the main artery and the serum Ca level was measured by the method of Connerety and Briggs.13 The rats were killed, and the right and left femurs were excised. The weight and volume of the femurs dissected free of soft tissues were measured. Specific gravity was calculated as weight (mg)/volume (ml).

Acknowledgments

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References and Notes