SYNTHESIS OF VITAMIN D_3 ESTERS

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UDC 615.356:577.161.2

The study of vitamin D esters commenced in 1927-1928 [18, 21], and up to the present, they remain of interest to many researchers [8, 13, 17]. There are many reasons for this interest. First of all, some of the investigated vitamin D_2 and D_3 esters have antirachitic activity [3, 7]. It has also been shown that part of the vitamin D_3 in the organism is esterified to give aliphatic acid esters [11, 13, 15, 22], which possibly play a definite role in biochemical processes. Vitamin D esters such as the sulfate and phosphate, dicarboxylic acid monoesters, etc. are water-soluble forms of vitamin D_3 [16, 17], and may be the basis for the creation of new D-vitamin preparations. The toxic effect of some vitamin D_3 esters (the acetate, butyrate, and palmitate) is lower than for nonesterified vitamins [2]. In addition, vitamin D_2 and D_3 esters have higher stability during prolonged storage than the corresponding vitamins [12].

However, vitamin D esters have not yet been studied systematically. Even the compounds described in the literature were characterized incompletely, and their biological activity has been studied using only a limited number of biochemical tests. It is therefore difficult to draw a conclusion regarding the dependence of the biological activity of vitamin D esters on their structures.

In the present research we set out to synthesize a number of vitamin D_3 esters of low- and high-molecular-weight aliphatic acids, as well as some aromatic acids, for their comparative study using modern biochemical investigative methods.

The production of vitamin D_3 esters is hindered by a number of side processes – acidic isomerization of the triene system of vitamin D_3 when acid chlorides are used as the acylating agents [14], hydrolysis of the resulting esters during their isolation from the reaction mixtures, and the complexities involved in their purification and crystallization. In some cases this leads to significant decrease in the yields of the final products and the need to very carefully select the conditions for carrying out the process and identifying the compounds obtained.

The individuality of the synthesized vitamin D_3 esters was established by means of thin-layer chromatography on Silufol UV-254 plates, the melting points, and polarimetric measurements for the compounds described. The structures of the substances obtained were confirmed by UV, IR, and PMR spectroscopic data.

The presence of a lone absorption band at 265 nm in the UV spectra of the esters (except for the 3,5-dinitrobenzoate [12]) constituted evidence that the triene structure of vitamin D_3 was retained.

The most characteristic feature of the IR spectra of the vitamin D_3 esters (except for the tosylate) is the presence of a rather narrow but very intense absorption band of C=O stretching vibrations at 1730-1740 cm^{-1}, as well as two weak $\nu_{>C=CH_2}$ bands of vitamin D_3 at 1625 and 1650 cm^{-1}. In the 3030 and 3090 cm^{-1} regions one observes one to two weak but characteristic absorption bands of ν_{C-H} stretching vibrations (=CH_3–19, =Ch–7, and =CH–6), while ν_{C-H} absorption bands of aromatic rings are present in the spectra of the p-nitrobenzoate, the 3,5-dinitrobenzoate, and the tosylate in the same region. Very intense ν_{C-H} absorption bands of CH, CH_2, and CH_3 groups are observed at 2840-2890 cm^{-1}. The spectra of the esters with aromatic acids contain a band of in-plane C–H deformation vibrations of the benzene rings at 820 cm^{-1} [1].

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0091-150X/91/2509-0661$12.50 © 1992 Plenum Publishing Corporation

Lines characteristic for vitamin D_3 are present in the PMR spectra of all of the esters obtained (δ, ppm): 0.54 (s, CH_3–18), 0.88 (d, J = 7 Hz, CH_3–26, CH_3–27), 0.91 (d, J = 7 Hz, CH_3–21),4.84 and 5.04 (both d, J = 2 Hz, =CH–19(E) and =CH–19(Z)], 6.21 (both d, J = 11 Hz, CH–6, CH–7) [10]. The locations and forms of these signals were virtually unchanged for all of the synthesized compounds.

EXPERIMENTAL

The UV absorption spectra of solutions of the compounds in ethanol were recorded with an SF-46 spectrophotometer over the 200-400 nm range. The IR spectra were recorded with an IKS-29 spectrometer over the 400-4000 cm^{-1} range. The PMR spectra of solutions in deuterochloroform were recorded with a Bruker WP-100 spectrometer with tetramethylsilane (TMS) as the internal standard. The polarimetric measurements of solutions (0.2%) were carried out at 20°C with a Polamat-A polarimeter. The melting points of the substances were determined with a Kofler–Böetius stage. The progress of the reaction was monitored by means of thin-layer chromatography on Silufol UV-254 plates in a benzene–acetone (98:2) system or a hexane–benzene (60:40) system.

Esters of Vitamin D_3 and Lower Carboxylic Acids

Vitamin D_3 Acetate. This ester was obtained by the reaction of the vitamin with acetic anhydride by the method in [9] or with acetyl chloride. In the latter case 1.0 g (2.6 mmole) of vitamin D_3 resin was dissolved in 10 ml of benzene, 10 ml of pyridine was added, and 1 ml (11.7 mmole) of the acid chloride was added dropwise with continuous stirring and cooling. Esterification was complete after several hours maintenance of the reaction mixture at room temperature. A threefold excess of 5% $NaHCO_3$ solution was then added to the reaction mixture, and it was allowed to stand for 3 h at room temperature. The benzene layer was then separated from the aqueous layer, which was extracted three times with 25-ml portions of ether. The benzene extract was combined with the ether extract, washed successively with water, 2% HCl solution, 5% aqueous $NaHCO_3$ solution, and water until the wash water had pH 7, and then dried (Na_2SO_4). After removal of the solvents in vacuo, the residue (a resin) was chromatographed with a column packed with Al_2O_3 using hexane as the eluent, followed by a hexane–benzene mixture (2:1). A 1.1-g sample of the crude product yielded 0.8 g of a transparent resin, which could not be crystallized and had $[\alpha]_D$ + 33° (chloroform), +36.8° (acetone), and +56.5° (ethanol). UV spectrum: λ_{max} 265 nm, ε 18,700. PMR spectrum, δ, ppm: 2.04 [s, –C(O)**CH_3**], 4.94 (m, J = 3.9 Hz, H–3) [[$\alpha]_D$ + 37.4° (acetone), +30.6° (chloroform) [8]].

The remaining esters were similarly obtained by the reaction of vitamin D_3 with the chlorides of the corresponding acids.

Vitamin D_3 Propionate. The reaction gave 0.89 g of a colorless resin that could not be crystallized from acetone. After prolonged standing at −15°C, the resinous mass began to crystallize. The colorless crystals had mp 55-56°C and $[\alpha]_D$ +35.6° (acetone) and +30° (chloroform). UV spectrum: λ_{max} 265 nm, ε 18,300. PMR spectrum, ppm: 1.14[t, J = 7 Hz, –C(O)CH_2-**CH_3**], 2.31 [q, J = 7 Hz, –C(O)**CH_2**CH_3], 4.94 (m, J = 3.9 Hz, H–3) [mp 36-37°C (acetone, −10°C), $[\alpha]_D$ + 30.4° (chloroform) [3]].

Vitamin D_3 Butyrate. A 0.7-g sample of colorless resin, after three crystallizations from acetone at −15°C, gave 0.3 g of crystals with mp 64.5-65.0°C and $[\alpha]_D$ + 39° (chloroform) and +4° (benzene). UV spectrum: λ_{max} 265 nm, ε 19,350. PMR spectrum, δ, ppm: 1.60 [t, J = 7 Hz, –C(O)$(CH_2)_2$=**CH_3**], 2.28 [t, J = 7 Hz, –C(O)**CH_2**CH_2CH_3], 4.95 (m, J = 3.9 Hz, H–3) [mp 62.5-64.5°C (acetone–methanol) [5] and $[\alpha]_D$ +39° (chloroform) [3]]. UV spectrum: λ_{max} 265 nm, ε 19,300 [4].

Vitamin D_3 Isobutyrate. The colorless resin did not crystallize from organic solvents. After prolonged maintenance at −15°C, the resinous mass crystallized to give colorless crystals with mp 39-41°C and $[\alpha]_D$ + 24° (chloroform) and 0° (benzene). UV spectrum: λ_{max} 265 nm, ε 19,100. PMR spectrum, δ, ppm: 1.10 [d, J = 7 Hz, C(O)–CH(**CH_3**)$_2$], 4.94(m, J = 3.9 Hz, H–3).

Vitamin D_3 Isovalerate. A 1.2-g sample of a colorless resin crystallized after prolonged maintenance at −5°C and had mp 66-67°C and $[\alpha]_D$ + 31.8° (chloroform) and +36.8° (acetone). UV spectrum: λ_{max} 265 nm and ε 17,300. PMR spectrum, δ, ppm: 1.12 [d, J = 7 Hz, (**CH_3**)$_2$CH], 1.65 [0, [sic] J = 7 Hz, –C(O)**CH_2**$CH(CH_3)_2$], 4.94 (m, J = 3.9 Hz, H–3) [mp 66-68°C (acetone) [3]].

Vitamin D_3 Caproate. A 1.2-g sample of the colorless resin, with $[\alpha]_D$ + 35.4° (chloroform), yielded after recrystallization from acetone at −15°C for 2 days, gave 0.8 g of crystals with mp 41.5-42.0°C and $[\alpha]_D$ + 36° (chloroform), +40.5° (acetone), and 0° (benzene). UV spectrum: λ_{max} 265 nm, ε 18,400. PMR spectrum, δ, ppm: 2.30 (t, J = 7 Hz, $-C(O)-\mathbf{CH_2}-$), 4.94 (m, J = 3.9 Hz, H-3).

Esters of Vitamin D_3 and Higher Carboxylic Acids

These compounds were obtained as described above. Purification of the resinous products obtained was accomplished by high-performance liquid chromatography (HPLC) with a Ø 22 column (ℓ = 300 mm) packed with silica gel (10-15 μm) using UV detection (λ = 290 nm) and 0.05% isopropyl alcohol in hexane as the eluent.

Vitamin D_3 Laurate. This compound was obtained as a colorless resin with $[\alpha]_D$ + 26.3° (chloroform) and +20.5° (acetone). UV spectrum: λ_{max} 265 nm, ε 19,700. PMR spectrum, δ, ppm: 1.23 [t, J = 7 Hz, $-(CH_2)_{10}-\mathbf{CH_3}$], 2.24 [t, J = 7 Hz, $-C(O)-\mathbf{CH_2}-$].

Vitamin D_3 Myristate. This compound was obtained as a resin with $[\alpha]_D$ + 21.6° (chloroform), +16.7° (acetone), and −2° (benzene). UV spectrum: λ_{max} 265 nm, ε 18,300. PMR spectrum, δ, ppm: 1.23 [t, J = 7 Hz, $-(CH_2)_{12}-\mathbf{CH_3}$], 2.24 [t, J = 7 Hz, $-C(O)-\mathbf{CH_2}$].

Vitamin D_3 Palmitate. This compound was obtained as a resin and had $[\alpha]_D$ + 19.5° (chloroform) and +17.9° (acetone). UV spectrum: λ_{max} 265 nm, ε 19,300. PMR spectrum, δ, ppm: 1.23 [t, J = 7 Hz, $(CH_2)_{14}-\mathbf{CH_3}$], 2.24 [t, J = 7 Hz, $-C(O)-\mathbf{CH_2}$].

Vitamin D_3 Stearate. After two crystallizations from acetone, the product had mp 127-129°C and $[\alpha]_D$ +18.9° (chloroform) and 14.3° (acetone). UV spectrum: λ_{max} 265 nm, ε 18,900. PMR spectrum, δ, ppm: 1.23 [t, J = 7 Hz, $(CH_2)_{16}\mathbf{CH_3}$], 2.24 [t, J = 7 Hz, $-C(O)-\mathbf{CH_2}-$].

Esters of Vitamin D_3 and Aromatic Acids

Vitamin D_3 Benzoate. This ester was obtained by the reaction of the vitamin and benzoyl chloride in pyridine. After purification on Al_2O_3 (elution with benzene), the oily product was crystallized twice from methanol–dioxane. The colorless crystals had mp 109-111°C and $[\alpha]_D$ +106° (chloroform). UV spectrum: λ_{max} 229, 265 nm, ε 22,000, 19,400. PMR spectrum, δ, ppm: 5.21 (m, J = 3.9 Hz, H-3), 7.48 and 8.02 (both m, benzene ring protons) [mp 110-111°C (dioxane–methanol), $[\alpha]_D$ +105.2° (chloroform). UV spectrum: λ_{max} 222, 227.5, 265 nm, ε 24,400, 24,000, 19,700 [6]].

Vitamin D_3 Phenylacetate. This ester was obtained as described above. The reaction proceeded with resinification. Workup gave a light-yellow resin, which could not be crystallized and had $[\alpha]_D$ +35° (acetone) and 0° (benzene). UV spectrum: λ_{max} 265 nm, ε 18,100.

Vitamin D_3 Tosylate. This ester was obtained by reaction with p-TsCl in pyridine at room temperature for 2 h; the reaction mixture was then allowed to stand overnight at 0°C. The reaction mixture was poured into water, and the aqueous mixture was extracted with ether, followed by workup as described above. The resulting resin was crystallized twice from ether at −15°C to give a product with mp 107-109°C and $[\alpha]_D$ = 27.8° (benzene), 0° (chloroform), and −32° (acetone). UV spectrum: λ_{max} 265 nm, ε 19,000. PMR spectrum, δ, ppm: 2.45 (s, $\mathbf{CH_3}$Ts), 4.67 (m, J = 3.9 Hz, H-3), 7.31 and 7.80 (both d, benzene ring protons) [mp 107-109°C (ether [19]].

Vitamin D_3 p-Nitrobenzoate. This ester was obtained by reaction of the vitamin with p-nitrobenzoyl chloride in pyridine–benzene. Despite an excess amount of the chloride, the esterification took several days at room temperature with significant resinification of the reaction mixture. Workup as described above gave a yellow resin. Three crystallizations from methanol–ether at −15°C gave crystals with mp 126-128°C and $[\alpha]_D$ +114° (chloroform) and +60° (benzene). UV spectrum: λ_{max} 265 nm, ε 28,700. PMR spectrum, δ, ppm: 5.25 (m, J = 3.9 Hz, H-3), 8.23 (d, H-m,o) [mp 125-126°C (methanol–ether), $[\alpha]_D$ +114.6° (chloroform), UV spectrum: λ_{max} 261 nm, ε 30,800 [12]].

Vitamin D_3 3,5-Dinitrobenzoate. This ester was obtained in the same way as the p-nitrobenzoate. It was crystallized from methanol–benzoate to give a product with mp 134°C and from ether at −15°C to give a product with mp 140-141°C and $[\alpha]_D$ + 98° (chloroform), +97° (acetone), and +62° (benzene). The UV spectrum at 230-300 nm was a descending curve with ε 23,500 at 265 nm. Similar results were presented in [12], (ε 22,800). PMR spectrum, δ, ppm: 5.30 (m, J = 3.9 Hz, H-3), 9.13 and 9.21 (both d, j = 1-2 Hz, H–o,p) [mp 142°C (ether) [20] and 132°C (methanol–benzene) [12], $[\alpha]_D$ +97° (chloroform), +62° (benzene) [20], +96.3° (acetone) [12]].

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