SYNTHESIS OF VITAMIN D_3 FROM ERGOSTEROL

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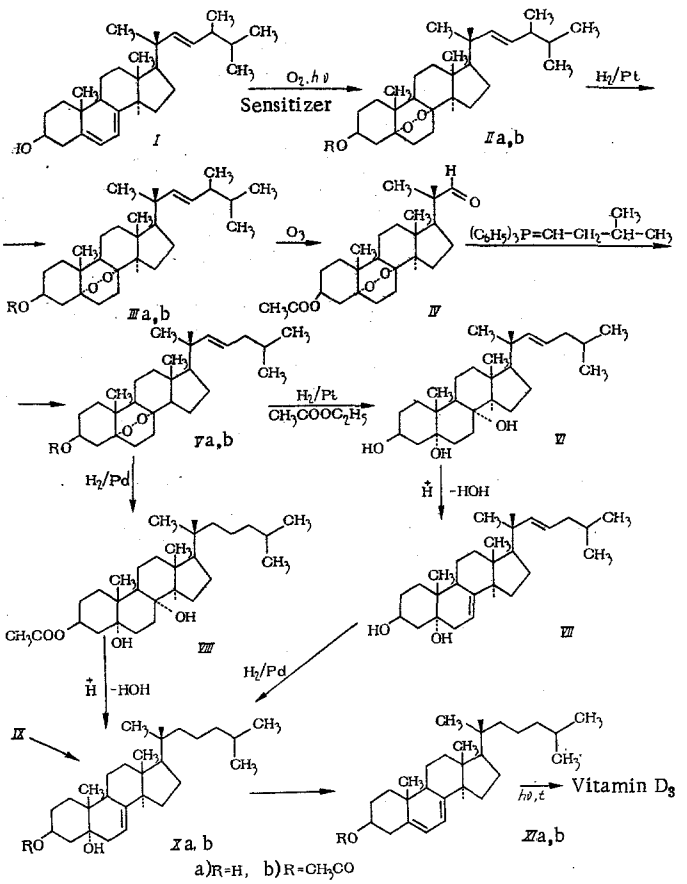
In the synthesis of vitamin D_3, the main raw material is cholesterol, the sources of which are limited. We have investigated the possibility of obtaining vitamin D_3 from ergosterol. Ergosterol may also prove to be a convenient starting material for the synthesis of metabolites of vitamin D modified in the side chain and possessing a high biological activity [1].

The general scheme for the synthesis of provitamins D modified in the side chain from ergosterol may include the following main steps: 1) protection of the double-bond system of ergosterol in ring B, 2) oxidative cleavage at the double bond in the side chain to form the aldehyde, 3) addition of a side chain of the desired structure, and 4) regeneration of the system of conjugated double bonds in ring B.



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It is known that ergosterol can form adducts with dienophiles, for example, with maleic anhydride [2] and with 4-phenyl-1,2,4-triazoline-3,5,-dione [3, 4], from which it can be regenerated. However, the first adduct is obtained in low yield, and 4-phenyl-1,2,4-triazolidine-3,5-dione is difficult of access.

To prese the system of conjugated double bonds in ergosterol (I) we used the photosensitized addition of oxygen with the formation of ergosterol peroxide (IIa) [5]. This reaction can be considered as the dienophilic addition of oxygen to ergosterol [6].

The selective hydrogenation of (IIa) in the presence of platinum and acetylation led to the acetate of 5,8-epidioxyergost-22-en-3β-ol (IIIb), the double bond in the side chain of which can be cleaved by ozonolysis with a yield of the aldehyde (IV) of about 70% [7].

To build on the required side chain, we used the Wittig condensation of (IV) with isopentylenetriphenylphosphorane [8], which gave the acetate of 5,8-epidioxycholest-22-en-3-β-ol (Vb) with a yield of about 50%. The structure of (Vb) was confirmed by the similarity of its PMR spectrum to that of (IIIb), and also by the similarity of the polarographic behaviors of these compounds on reduction at a dropping mercury electrode [9].

In order to regenerate the system of double bonds in ring B, compound (Vb) was hydrogenated in the presence of platinum oxide or palladium to the acetate of cholestane-3β,5α,8α-triol (VIII), which was not isolated from the reaction mixture [the presence of (VIII) in the hydrogenation products was shown by thin-layer chromatography]. The triol (VIII) readily dehydrates under the influence of small amounts of mineral acid [7] with the formation mainly of the acetate of cholest-7-ene-3β,5α-diol (Xb).

The partial hydrogenation of (Va) in ethyl acetate in the presence of platinum oxide and the subsequent dehydration of the (VI) leads to cholestane-7,22-diene-3β,5α-diol (VIII) which, by the hydrogenation of the double bond in the side chain, gives cholest-7-ene-3β,5α-diol (Xa). The latter compound has also been synthesized from 7-dehydrocholesterol peroxide [10]. The identity of the samples of (Xa) obtained by the different methods shows the absence of epimerization at the asymmetrical C-20 atom during the synthesis.

Dehydration at the expense of the hydroxy group of C-5 in (Xb) takes place not only with the formation of the acetate of cholesta-5,7-dien-3β-ol (XIb) containing a system of conjugated double bonds, but also with the elimination of the hydrogen atom at C-4. When (Xb) was dehydrogenated with p-toluenesulfonyl chloride in dimethylformamide in the presence of 2,6-lutidine and sulfur dioxide [11], the amount of (XIb) in the dehydration products determined from the UV spectrum was 46%. Chromatography on silicic acid treated with silver nitrate permitted the isolation, in addition to the (XIb), of a substance which was characterized as cholesta-4,7-dien-3β-ol acetate.

Compound (Xa) was subjected to photoisomerization by the action of radiation with λ =280-320 nm. From the irradiation products, after thermal treatment by means of thin-layer chromatography, vitamin D_3, identical with a standard sample of vitamin D_3 from the results of UV spectroscopy and biological activity [12], was isolated.

EXPERIMENTAL

The UV spectra were measured on a SF-4A spectrophotometer; the IR spectra were taken on a UR-10 spectrophotometer; and the PMR spectra were taken on a Hitachi R-200A instrument using carbon tetrachloride as the solvent, in the δ scale relative to hexamethyldisiloxane (δ = 0.05 ppm). The angles of rotation and the optical rotatory dispersion spectra were measured on a JASCO ORD/UV-5 spectropolarimeter. Thin-layer chromatography was performed on plates coated with silica gel in the cyclohexane–ether (1 : 4) system [13]. The melting points were determined in accordance with GFX [State Pharmacopoeia of the USSR, 10th ed.]. The half-wave reduction potentials, $E_{1/2}$ (against a mercury anode) were determined in aqueous dimethylformamide [9].

Ergosterol Peroxide (5,8-Epidioxyergosta-6,22-dien-3β-ol) (IIa). Compound (I) was oxidized with atmospheric oxygen in the light [5] in isopropanol in the presence of methylene blue as a sensitizer. Yield 76%, mp 175-178°C (according to the literature, mp 178°C [5], 178-185°C [7]). IR spectrum, cm^{-1}*: 865 (m), 975 (s, trans-CH=CH), 1055 (s), 1660 (m, C=C in ring B). Acetate of ergosterol peroxide (IIb), mp 200-202°C (according to the literature, mp 202°C [5], 199-200°C [7]), $E_{1/2}$ = 1.41 V.

*Here and below, the following abbreviations are used to denote the intensities of the absorption bands: w – weak; m – medium; s – strong; v.s. – very strong.

5,8-Epidioxyergost-22-en-3β-ol (IIIa). This was obtained by the partial hydrogenation of (IIa) in the presence of platinum oxide. Yield 93%, mp 174-175°C (from methanol) (according to the literature, mp 175-178°C). Acetate (IIIb): mp 210-215°C, $[\alpha]_D = -112°$ (according to the literature mp 209-215°C $[\alpha']_D = -121$ [7]). IR spectrum, cm^{-1}: 875 (m), 975(s), 1040, 1050 (s), 1253 (v.s); 1735 (v.s); $E_{1/2} = -1.36$ V.

Acetate of 3β-Hydroxy-5,8-epidioxybisnorcholan-22-al (IV). A current of oxygen containing about 2.5% of ozone was passed through a solution of 0.2 g of (IIIb) in 7 ml of chloroform and 0.07 ml of pyridine cooled to −(65-70)°C until ozone was detected in the gas leaving the apparatus (test with a solution of potassium iodide). The solution was washed with 2% sulfuric acid, sodium bicarbonate solution, and water, and was dried and evaporated to dryness. The residue was chromatographed on a column of silicic acid. Elution with benzene gave 0.11 g of (IV) (65%), mp 195-200°C (according to the literature, mp 189-199°C [7]). IR spectrum, cm^{-1}: 0.875 (w), 975 (w), 1040, 1050 (s), 1255 (v.s), 1740 (v.s), 2690, 2740 (w, CHO). $E_{1/2} = -1.24$ V.

Acetate of 5,8-Epidioxycholest-22-en-3β-ol (Vb). To a vigorously-stirred suspension of 4.13 g of isopentyltriphenylphosphonium bromide (mp 150-152°C [8]) in 20 ml of dry ether was added 5 ml of an ethereal solution of phenyllithium (T = 0.00125 g/ml). Dry argon was passed over the reaction mixture throughout the reaction. After 10 min, to the orange-red solution of phosphorane that had been formed was added a solution of 2.02 g of (IV) in 25 ml of dry benzene. The reaction mixture became almost colorless, and after 10 min it was treated with 10 ml of water and 20 ml of ether. The resulting solution was washed with 2% sulfuric acid and with sodium bicarbonate solution and was dried and evaporated to dryness. Thin-layer chromatography showed the presence of hydrolysis products. The residue was acetylated with acetic anhydride in pyridine. The product was purified by chromatography on silicic acid and was eluted with a mixture of hexane and benzene (2 : 1). This gave 1.18 g of (Vb), yield 47.6%, mp 161-163°C (from methanol). PMR spectrum, ppm*: 0.72 (3 H s, 13-CH_3), 0.86-0.96 (12 H, poorly-resolved signal, d, 25-$CH_3 \times 2$, s, 10-CH_3, and d, 20-CH_3), 4.78 (1 H, m, 3α-H), 5.11 (2 H, m, 22-H, 23-H). Found, %: C 75.73; H 9.64. $C_{29}H_{46}O_4$. Calculated, %: C 75.94; H 10.01. $E_{1/2} = -1.36$ V.

Acetate of Cholest-7-ene-3β,5α-diol (Xb). A solution of 780 mg of (Vb) (0.0017 mole) in 20 ml of ethyl acetate was hydrogenated in the presence of 100 mg of palladium black. After 150 min, 0.0034 mole of hydrogen had been absorbed, and hydrogenation ceased. The catalyst was filtered off and the solution was evaporated to dryness. The residue consisted mainly of (VIII) with R_S relative to (Vb) 0.26. The dry residue of (VIII) was dissolved in the minimum amount of methanol and a drop of hydrochloric acid was added. After a few minutes, crystals of (Xb) deposited. The mass was cooled to 10°C and filtered. This gave 730 mg of a substance (mainly (Xb), R_S relative to (VIII) 2.28), which was purified by chromatography on silicic acid, being eluted with benzene. This gave a fraction of 160 mg, mp 175-180°C (according to PMR, its content of (Xb) was about 50%) and a fraction of 440 mg, mp 180-182°C (content of (Xb) about 80%). The yield of (Xb) was 55%; after recrystallization from methanol, mp 193-195°C. PMR spectrum, ppm: 0.54 (3 H s, 13-CH_3), 0.86 (6 H d, J 5.4 Hz, 2×25-CH_3), 0.90 (3 H s, 10-CH_3), 0.91 (3 H d, J 6.2 Hz, 20-CH_3), 1.17 (4 H m, 2×22-H, 2×23-H), 1.92 (3 H s, CH_3CO), 4.75 (1 H m, 3α-H), 4.95 (1 H m, 7-H). Found, %: C 78.47; H 10.86. $C_{29}H_{48}O_3$. Calculated, %: C 78.32; H 10.85.

Cholesta-7,22-diene-3β,5α-diol (VII). A solution of 0.85 g of (Vb) in 10 ml of benzene was treated with 10 ml of a 5% solution of caustic potash in methanol, and after 2 h the solution was washed with water, the solvent was evaporated off, and the residue was dissolved in 10 ml of ethyl acetate. The resulting solution of (Va) was hydrogenated in the presence of 70 mg of platinum oxide, absorbing 45 ml of hydrogen; a precipitate of (VI) deposited and hydrogenation ceased. The residue was separated off, and the resulting triol (VI) (700 mg, mp 172-180°C) was dissolved in 1 ml of methanol and a drop of hydrochloric acid was added. After 2 h, the solvent was evaporated off and the residue was chromatographed on a column of silicic acid, the (VII) being eluted with benzene−ether (1 : 1). This gave 340 mg of (VII), mp 185-190°C. PMR spectrum, ppm: 0.55 (3 H s, 13-CH_3), 0.85 (6 H d, J 5.4 Hz, 2×25-CH_3), 0.90 (3 H s, 10-CH_3), 0.99 (3 H d, J 6.9 Hz, 20-CH_3), 3.98 (1 H m, 3α-H), 4.94 (1H m, 7-H), 5.15 (2 H m, 22-H and 23-H). Found, %: C 80.40; H 11.01. $C_{27}H_{44}O_2$. Calculated, %: C 80.94; H 11.07.

Cholest-7-ene-3β,5α-diol (Xa). a. This was obtained from the acetate of the peroxide of 7-dehydrocholesterol, mp 154-155°C [10] by a method similar to that for obtaining (VIIa); mp 196-200°C (from methanol), $[\alpha]_D = +27.5°$, $[\alpha]_{299} = +95°$, $[\alpha]_{281} = 0°$. PMR spectra, ppm: 0.54 (3 H s, 13-CH_3), 0.85 (6 H d, J 5.3 Hz,

*Here and below, the following abbreviations are used to denote the signals: s − singlet; d − doublet; q − quartet; m − multiplet.

2×25-CH_3), 0.89 (3 H s, 10-CH_3), 0.91 (3 H d, J 6.0 Hz, 20-CH_3), 1.17 (4 H m, 2×22-H, 2×23-H), 3.97 (1 H m, 3α-H), 5.00 (1 H m, 7-H).

b. A solution of 60 mg of (VII) in 3 ml of ethanol was hydrogenated in the presence of 3 mg of palladium black, 3.5 ml of hydrogen being absorbed. The catalyst was filtered off and the solvent was evaporated off to give 52 mg of (Xa), mp 198-203°C (from methanol), $[\alpha]_D = +31°$, $[\alpha]_{299} = +103°$, $[\alpha]_{280} = 0°$. PMR spectrum, ppm: 0.54 (3 H s), 0.85 (6 H d, J 5.3 Hz), 0.89 (3 H s), 0.91 (3 H d, J 6.0 Hz), 1.17 (4 H m), 3.97 (1 H m), 5.00 (1 H m).

Acetate of Cholesta-5,7-dien-3β-ol (XIb). To a solution of 130 mg of (Xb) in 5 ml of 2,6-lutidine and 20 ml of dimethylformamide were added 0.5 ml of a 5% solution of sulfur dioxide and 800 mg of p-toluenesulfonyl chloride. After 40 min, 100 ml of ether was added to the solution and it was washed with water, 2% sulfuric acid, sodium bicarbonate solution, and again with water, the solvent was evaporated to dryness, and the residue was chromatographed on a column containing 9 g of silicic acid with 15% by weight of silver nitrate. The column was eluted with a mixture of hexane and benzene with the concentration of benzene increasing from 0 to 40% by volume. The hexane–benzene mixtures containing 0 to 25% of benzene eluted 25 mg of a substance which was identified as the acetate of cholesta-4,7-dien-3β-ol showing no absorption in the UV region of the spectrum, mp 106-108° (from methanol). PMR spectrum, ppm: 0.55 (3 H s, 13-CH_3), 0.85 (6 H d, J 5.4 Hz, 2×25-CH_3), 0.91 (3 H d, J 5.6 Hz, 20-CH_3), 1.04 (3 H s, 10-CH_3), 1.20 (4 H m, 2×22-H, 2×23-H), 1.93 (3 H s, CH_3CO), 5.00 (2 H m, 3α-H and 7-H), 5.31 ppm (1 H d, J 3.7 Hz, 4-H). Hexane–benzene mixtures with 25-40% of benzene eluted 64 mg of a substance from which, after crystallization from methanol, 46 mg of (XIb) was obtained with mp 126-127°C (according to the literature, mp 130°C [14]). UV spectrum: λ_{max} 293.5, 282, and 272 nm, $\varepsilon_{272} = 12{,}700$; PMR spectrum, ppm: 0.61 (3 H s; 13-CH_3), 0.86 (6 H d, J 5.4 Hz, 2×25-CH_3), 0.91 (3 H s, 10-CH_3), 0.91 (3 H d, J 5.7 Hz, 20-CH_3), 1.20 (4 H m, 2×22-H, 2×23-H), 1.04 (3 H s, CH_3CO), 4.63 (1 H m, 3-H, 5.19, 5.37 (2 H AB KB, J 6.4 Hz, 6-H, 7-H).

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