SYNTHESIS AND BIOLOGICAL ACTIVITY OF THE TRIMETHYLSILYL ETHER OF VITAMIN D_2

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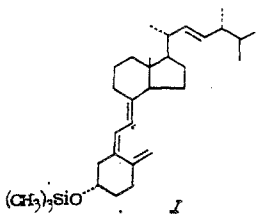
The application of ethers of the vitamins of the D group presents interest from the point of view of the increased stability of antirachitic preparations since it is known that their biological activity decreases sharply on prolonged storage and the application to feeds [7]. The introduction of the ether group into the molecule may increase the stability of the preparation. It was previously shown [5] that the esters of the vitamins of the D group and their metabolites with fatty acids (palmitic, acetic, butyric [2]) possess lower biological activity than the unesterified preparations. This is evidently associated with their hindered absorption in the small intestine of animals.

The biological action of the ethers of the vitamins of the D group has not been studied up to the present time; this is evidently due to the stability to hydrolysis of the majority of the compounds of this class. At the same time, there are data [6] to the effect that the trimethylsilyl derivatives of a related group of steroids – the sex hormones (19-nortestosterone) – do not possess toxicity, and their biological activity stays at the level of the non-etherified preparations.

The given communication was devoted to the study of the biological activity of the trimethylsilyl ether of vitamin D_2 (I), as well as the influence of the introduction of the trimethylsilyl group on the stability of the preparation ergocalciferol (II) on storage.



I

EXPERIMENTAL (CHEMICAL)

The UV spectrum was taken on a "Shimadzu UV-3000" spectrophotometer in hexane. The mass spectrum was obtained on an MX-1310 instrument with the direct input of the sample and the 70 eV energy of the ionizing electrons; the temperature of the ion source was 40°C. The purity of the substances was determined on a "Tsvet-105" chromatograph with a packed glass column 2.5 by 4500 mm with the phase OV-17 on "Chromatron N Super." The detector was the flame ionization type; the gas carrier was helium with the flow rate 30 ml/min. The temperature of the thermostat of the columns was 280°C. The purification of the reaction product was performed on a column 1.2 by 25 cm with silica gel L 100/160 (Czechoslovakia) with gradient elution (from hexane to the 50:1 mixture of hexane–ether).

Vitamin D_2 Trimethylsilyl Ether (I). To the solution of 200 mg of (II) in 20 ml of THF were added 1.5 ml of hexamethyldisilazane and 0.3 ml of trimethylsilyl chloride; the reaction mixture was boiled for 30 min with a reflux condenser. After the distillation of the solvent in vacuo and the purification on a column, 222 mg (94%) of (I), with the mp 44°C, were obtained. On its analysis by the method of GLC, it appears in the form of two peaks with the 10:3 ratio of the areas and the relative retention times (based on ergosterol) 0.57 and 0.67 correspondingly. The UV spectrum [λ_{max}, nm (ε)] is as follows: 265 (17,400). The mass spectrum [m/z (relative intensity, %)] is as follows: M^+ 468 (52), 453 (25), 378 (15), 343 (55), 253 (51), 211 (41), 208 (85), and 118 (100).

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TABLE 1. Vitamin D Activity of the Preparation Vitamin D_2 Trimethylsilyl Ether (I)

Group of chicks	Increase of living mass, g	Relative mass of the parathyroid gland, mg/100 g of living mass	Blood serum			Ash mass of the tibia, %	CaBP, μg per mg of total protein	Vitamin D activity of 1 kg as vitamin
			Ca, mg%	P, mg%	alkaline phosphatase activity (APA), *u			
1st basic ration	91.0±3.8	5.34±0.90	7.08±0.31	5.55±0.32	28.24±0.78	26.70±0.71	no	no
2nd basic ration + vitamin D_3	172.5±10.7	0.81±0.08	11.89±0.34	6.39±0.22	13.55±0.67	39.22±0.42	54.39±3.61	7500
3rd basic ration + (II)	171.5±8.3	0.91±0.05	11.46±0.16	6.98±0.31	11.20±0.70	38.97±0.66	53.87±4.01	7500
4th basic ration + (II)	114.4±9.6	4.16±0.70	8.97±0.47	5.57±0.35	24.84±0.51	29.10±0.67	11.67±3.70	750
5th basic ration + (I)	158.7±8.3	1.17±0.54	11.24±0.88	6.53±0.24	15.00±0.96	38.59±0.17	47.72±3.17	6000±300

*The APA is the amount of inorganic phosphorus (in milligrams cleaved from sodium glycerophosphate when 100 ml of blood serum is incubated at 37°C for 30 min).

In the study of the stability of the preparations, the samples of the non-etherified (II) and (I) were stored in a refrigerator at 4°C in vials with ground-glass stoppers for 6 months. After each month, the samples were removed, and the percentage content of the investigated substances in them was determined by the method of GLC.

EXPERIMENTAL (BIOLOGICAL)

Leghorn chicks were raised from the first days of life in cells without the admission of UV rays using a basic ration (BR) of the seed type, which contained all the required components except for vitamin D. At the age of 10 days, the total livestock was divided into five groups with 15 animals in each. The chicks of the first group continued to receive the BR, and served as the control. Standard amounts of vitamin D_3 (500 IU) and (II) (7500 and 750 μg) were added to the rations of three groups. These groups served as the standard. The chicks of the fifth group received the addition of the studied (I) in the amount of 200 μg per kg of feed to the basic ration. At the age of 30 days, the chicks were weighed and killed. Samples of the blood, tibias, parathyroid glands, and scrapings of the duodenal mucosa were taken from them.

The level of calcium in the blood serum was determined using the "Perkin-Elmer-403" atomic-absorption spectrometer; the inorganic phosphorus was determined by the molybdate method [9], and the alkaline phosphatase activity (APA) was determined by the reaction with sodium glycerophosphate as the substrate [4].

The tibia was dried, defatted in ether, and incinerated in a muffle furnace at 600°C. The duodenal mucosa was homogenized; the homogenate was centrifuged. The content of the specific vitamin D-dependent calcium-binding protein (CaBP) [1] in the supernatant fraction was determined using the method of radial immunodiffusion in agar, which was previously developed by one of us [3]. The vitamin D activity of the studied preparation (Table 1) was determined from the content of the CaBP in the duodenum, utilizing a standard curve.

The formation of (I) is confirmed by the mass of the molecular ion (cf. the mass spectrum). In other respects, the fragmentation of (I) on electron impact coincides with that of (II) [10], whereby the masses of the ions corresponding with the cleavage of trimethylsilanol [for (I)] and water [for (II)] are the same, and the others differ by the value m/z 72. The UV spectrum is identical to the spectrum of (II) [7]. It is known [8] that the thermal recyclization of the triene system of the previtamins and vitamins D proceeds at temperatures above 125°C with the formation of pyro- and isopyrovitamin D. The temperature of the ion of the mass spectrometer (40°C) is inadequate to carry out the isomerization. At the same time, such a process proceeds very rapidly in the chromatograph (280°C); this causes the appearance of the double peak in the chromatogram (see Experimental).

The results of the investigation of the biological action of (II) and (I), presented in Table 1, indicate that the antirachitic activity of the ether (I) corresponds to 80 ± 4% of the activity of the same mass of the non-etherified preparation (II). It should be stressed that, in spite of the difference in the molecular masses of (II) and (I) (396 and 468 correspondingly), the biological activity of equimolar amounts of these substances is virtually the same [for (I), it is 97 ± 4% of the value for (II)]. Therefore, the introduction of the trimethylsilyl group into the molecule of (II) does not exert a significant influence on its

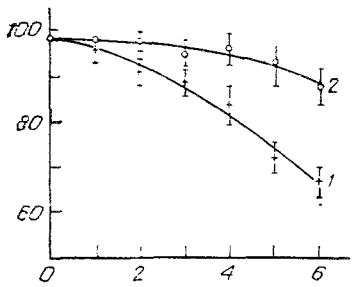

Fig. 1. Dependence of the content of (II) (curve 1) and (I) (curve 2) on the storage time of the preparation. The X-axis shows the time, months. The Y-axis shows the concentrations, %.

antirachitic activity; this is evidently explained by the ease of the hydrolysis of the indicated ether in the organism.

The results of the investigation of the stability of the preparations (II) and (I) on storage, presented in Fig. 1, indicated the significant increase of stability on the etherification of the hydroxyl group. Thus, after storage for 6 months, the content of (II) in the non-etherified preparation comprised 66 ± 4%, and the percentage of (I) in the etherified preparation comprised 88 ± 4. The increase in the stability of the vitamin on storage is evidently explained by the fact that the ether group inhibits the oxidation of the hydroxyl group. Moreover, the highly branched trimethylsilyl protecting group in the α-position may sterically hinder the degradation of the conjugated 5,7,10(19)-triene system. It is interesting to note that the kinetic curves for the decomposition of (II) and (I) (cf. Fig. 1) have the curvature facing upwards; this is characteristic of autocatalytic reactions. In other words, the accumulation of the degradation products increases the rate of decomposition of the vitamin and decreases the stability of the preparation.

The data obtained indicate that the application of silylated derivatives of the vitamins of the D group permits a decrease in the loss of this economically important preparation on storage.

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