A STUDY OF THE PRODUCTS OF THE AUTOOXIDATION OF VITAMIN D

I. NONPOLAR PRODUCTS OF THE OXIDATIVE DEGRADATION OF VITAMIN D_2

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In the study of the metabolism and the mechanism of the action of vitamin D, it was established that in the organism it undergoes transformation leading to the formation of active and inactive metabolites [1].

Subsequent investigations have shown that the active metabolites of vitamin D are the products of its oxidation – 25-hydroxycholecalciferol (vitamin D_3) [2], 25-hydroxyergocalciferol (vitamin D_2) [3], and 21, 25-dihydroxycholecalciferol [4]. It has been observed that vitamin D oxidizes at the C_1 carbon atom [5].

In addition, in a consideration of the mechanism of the action of vitamin D_2 on the structure and functional activity of lipoprotein membranes it was found that the observed effects are apparently due to the action of highly reactive peroxide compounds obtained from vitamin D_2 as a result of its autooxidation [6, 7].

In view of this, it appeared of interest to make a detailed study of the oxidative transformations of vitamin D – in particular, its autooxidative transformations in an aqueous medium – and also to determine the nature and structure of the products formed under these conditions.

The thin-layer chromatography on alumina of the products of the oxidation of an aqueous ethanolic suspension of vitamin D_2 incubated with stirring at 50° C for 4 h showed the presence of several spots (Fig. 1a).

One of these spots (1), which is probably the main polar oxidation product of vitamin D_2 (R_S 0.7 relative to vitamin D_2) has a peroxide nature, gives a specific coloration with starch-iodine and does not absorb in UV light, and on a polarogram it shows the diffuse reduction wave with $E_{1/2} = -1.5$ V characteristic of cyclic peroxides [8].

Spot 2 is due to vitamin D_2 which has remained unchanged under the experimental conditions. Spot 3 (R_S 1.2) is a mixture of substances which, as a polarographic analysis has shown ($E_{1/2} = -2.66$ V), contains precalciferol [8]; however, it was impossible to separate it from the accompanying compounds.

The nonpolar oxidation products, represented on the chromatogram by spot 4 (R_S 2.1) gave a coloration with 2,4-dinitrophenylhydrazine, which is characteristic for a carbonyl group.

A more detailed investigation of this fraction by thin-layer chromatography on plates with "Silufol" silica gel showed that it consisted of three substances of carbonyl nature (see Fig. 1b). Compounds (A and B) were not colored on the chromatogram with a solution of antimony trichloride, while compound C was colored. On the plate, compounds A and C gave a characteristic blue coloration with Savitskii's reagent (N-methylbenzothiazolone hydrazone hydrochloride), which shows the presence of an aldehyde group in it [9].

These compounds were obtained individually by chromatography on alumina.

The compounds isolated had λ_{max} (in ethanol, nm) 255 (A), 235 (B), and 245 (C) (Fig. 2).

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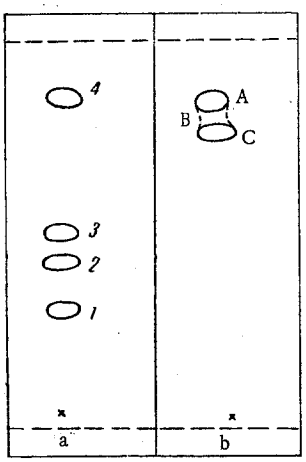


Fig. 1

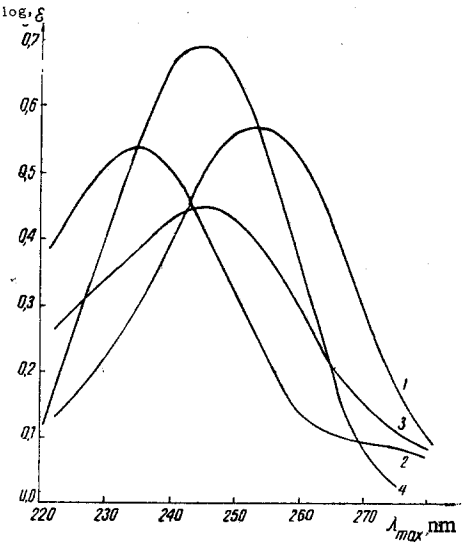


Fig. 2

Fig. 1. Thin-layer chromatogram of the products of the oxidation of vitamin D_2 in the isopropanol–benzene (5 : 95) system on alumina (a) and of the carbonyl fraction in the petroleum ether – diethyl ether (1 : 1) system on plates (b).

Fig. 2. Absorption spectra in ethanol of the γ compound A (1), compound B (2), compound C (3), and the C_{21} aldehyde (4).

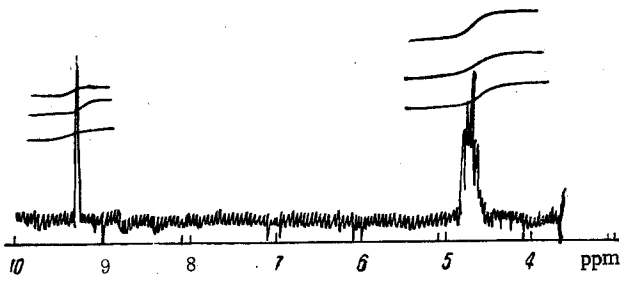


Fig. 3. NMR spectrum of compound A.

The IR spectra exhibited characteristic absorption bands: for compound A in the 975 cm^{-1} region (trans-CH = CH of a side chain), 1655 cm^{-1} (CH = CH), 1677 cm^{-1} (C = O, conjugated with a double bond), and 1717 cm^{-1} (nonconjugated C = O); for compound B in the 975, 1690, and 1710 cm^{-1} regions; and for compound C in the 975, 1630 and 1675 cm^{-1} regions. The absence from the IR spectra of the absorption bands of hydroxy groups and the presence of a trans-olefinic bond (975 cm^{-1}) shows that the substances obtained are conversion products of vitamin D_2 which retain the aliphatic side chain.

In order to identify these compounds, the products of the oxidative degradation of vitamin D_2 were synthesized – the C_{21} aldehyde (Heilbron) [10], and the C_{19} ketone in its cis [11] and trans [12] forms.

The chromatographic behavior of the C_{21} aldehyde on a plate was identical with that of our compound C. Both these compounds had λ_{max} 245 nm (see Fig. 2). Their IR spectra were also identical.

The facts given above give grounds for identifying compound C formed in the oxidation of vitamin D_2 in an aqueous medium as the C_{21} aldehyde. A confirmation of this is the presence in the NMR spectrum of the carbonyl fraction of a doublet at 9.5 ppm corresponding to aldehydic protons [13a].

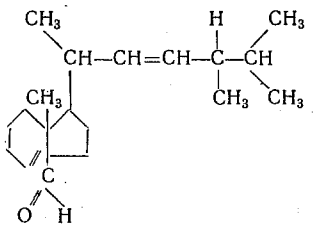
The amount of compound B in the carbonyl fraction was very small, and it was impossible to identify it definitively. It is possible that it is a mixture of two carbonyl compounds in one of which the carbonyl group is conjugated with a double bond while in the other it is not, as is indicated by the IR spectrum.

The main product of the carbonyl fraction (60%) was compound A. The NMR spectrum of this compound (Fig. 3) has a singlet at 9.5 ppm corresponding to aldehyde protons and signals of an olefinic proton at 4.75 ppm [13b].

The integration of the spectrum gave a total intensity of the signals at 4.75 ppm corresponding to two proton units, which shows the absence from the compound of two olefinic protons other than the trans-olefinic protons.

An impurity in compound A is the nonconjugated C_{19} ketone; the vibrations at 1260 and 1717 cm^{-1} characteristic of it also appear in the spectrum of compound A.

What has been said permits the assumption that compound A that we isolated is most probably described by the structure (I)



7a-methyl-1-(1',4',6'-trimethylhex-2'-en-1'-yl)hydrind-3a-ene-3a-carbaldehyde

The structure of the product of the degradation of vitamin D_2 on autoxidation shows that, during this process, isomerization of the system of conjugated double bonds in the direction of the C_{14} carbon atom of the steroid system takes place. The possibility of such an isomerization in the degradation of vitamin D is confirmed by literature data [14].

EXPERIMENTAL

Oxidation of Vitamin D_2 and Extraction of the Oxidation Products. A solution of 1 g of vitamin D_2 in 50 ml of ethanol was poured into 950 ml of distilled water. The suspension was stirred at 50° C for 4 h and was then extracted with a mixture of ethanol and ether (1 : 4) (2 × 270 ml). The extract was dried with calcined sodium sulfate, and after the addition of 5 mg of butylhydroxytoluene (to suppress further oxidation), the solvent was evaporated off in a film evaporator under vacuum. The residue consisted of a light yellow oil (0.5 g).

Thin-layer Chromatography. For chromatography, 30 g of alumina (activity grade II) was mixed with 7% of gypsum and 60 ml of distilled water. The resulting mass was deposited on plates (13 × 18 cm). The thickness of the layer was about 0.5 mm. A plate was activated at 120° C and kept in the air. A solution of 50-100 μg of the substance in benzene was deposited on the plate and then it was placed in a chromatographic chamber containing, in the case of alumina, a mixture of isopropanol and benzene (5 : 95) and in the case of "Silufol" plates petroleum ether–diethyl ether (1 : 1). After the plates had been dried in the air, the substances on the chromatograms were revealed by spraying them with a solution of antimony trichloride in 80% acetic acid (20 g of $SbCl_3$ in 20 ml of CH_3COOH).

Carbonyl compounds were revealed by spraying the plates with a 0.1% solution of 2,4-dinitrophenylhydrazine in ethanol [15].

To reveal peroxides, immediately after removal from the chamber the plates were sprayed with a 0.5% solution of butylhydroxytoluene in ether and then with a freshly prepared saturated solution of KI in 80% CH_3COOH and a 2% solution of starch in distilled water. A characterisitc blue-brown coloration appeared.

For the polarographic analysis of the peroxide (see substance 1, Fig. 1a) and precalciferol (see substance 3, Fig. 1a), the substances were extracted from the thin-layer chromatogram by removing the adsorbent and eluting it with a mixture of ether and methanol.

Column Chromatography. A mixture of the products of the oxidation of 3 g of vitamin D_2 was dissolved in the minimum amount of benzene and deposited on a column (1.5 × 40 cm) containing 90 g of alumina. The alumina for chromatography (activity grade II) was previously treated with dilute hydrochloric acid, washed with water, methanol, and chloroform, dried in the air, and activated at 120° C for 1 h. When

alumina according to Brockmann (activity grade II, neutral, firm of Reanal) was used, before chromatography it was heated at 120° C for 30 min and then 5% of water was added.

The oxidation products were eluted from the column with petroleum ether–ether (4 : 1). By means of a collector, fractions corresponding to compound 4 (see Fig. 1a) were taken (monitoring by thin-layer chromatography in the isopropanol–benzene system). The solvent was distilled off from these fractions, the residue was weighed and dissolved in the minimum amount of petroleum ether, and the solution was deposited on a column of alumina (ratio of the substance to Al_2O_3 1 : 100). The mobile phase used was petroleum ether–benzene (3 : 1). Fractions corresponding to compounds A, B, and C were collected (see Fig. 1) [monitoring by thin-layer chromatography in the petroleum ether–ether (1 : 1) system].

The NMR spectra were obtained on a Hitachi R-20A instrument with a working frequency of 60 MHz. The chemical shifts are given in the δ scale. The internal standard was HMDS (δ 0.05 ppm). The substances were recorded in carbon tetrachloride.

The IR spectra were obtained on a UR-20 instrument in paraffin oil.

SUMMARY

It has been found that in the autooxidation of vitamin D_2 in an aqueous medium substances of peroxide and carbonyl nature are formed. The main nonpolar products are the C_{21} aldehyde (Heilbron) and 7a-methyl-1-(1',4',6'-trimethylhex-2'-en-1'-yl)hydrind-3a-ene-3a-carbaldehyde.

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