11. V. A. Moroshkin, E. V. Kartasheva, É. V. Frisman, E. I. Ramm, V. N. Nikun, V. I. Vorob'ev, R. R. Kamilova, N. I. Koryakina, and V. K. Burichenko, Mol. Biol. (Moscow), 14, No. 4, 795 (1980).
12. L. I. Mar'yash, L. V. Abaturov, and V. A. Shibnev, Stud. Biophys., 43, No. 1, 41 (1974).
13. N. G. Esipova, V. M. Lobachev, V. N. Rogulenkova, A. A. Makarov, V. A. Shibner, and M. Finogenova, Mol. Biol. (Moscow), 18, No. 3, 725 (1984).
14. A. J. Adler, N. G. Greenfield, and G. D. Fasman, Meth. Enzymol., 28, 675 (1975).
15. E. R. Blout, in: Fundamental Aspects and Recent Developments in ORD and CD (ed. E. Ciardelli), Heyden, New York (1973), p. 352.
16. V. Madison and J. Schellman, Biopolymers, 11, 1041 (1972).
17. D. Aebersold and E. S. Pysh, J. Chem. Phys., 11, 1919 (1970).
18. V. A. Zubkov, T. M. Birshtein, J. S. Milevskaya, and M. V. Volkenshtein, Biopolymers, 10, 2051 (1971).
19. P. J. Flory, The Statistical Mechanics of Chain Molecules, Interscience, New York (1969).
20. E. I. Ramm, V. I. Vorob'ev, R. R. Kamilova, V. K. Burichenko, and N. G. Esipova, in: IVth All-Union Symposium on the Chemistry of Proteins and Peptides [in Russian], Minsk (1977), p. 58.
21. E. I. Ramm, G. S. Ivanov, V. I. Vorob'ev, N. G. Esipova, M. V. Grigolava, V. S. Grechishko, and V. K. Burichenko, Biofizika, 28, No. 1, 35 (1983).
22. B. Jirgensons and L. S. Hnilica, Biochem. Biophys. Acta, 109, 241 (1965).
23. A. Shin and G. L. Eichhorn, Biopolymers, 16, 225 (1977).
24. G. D. Fasman, M. S. Valenzula, and A. J. Adler, Biochemistry, 10, No. 20, 3795 (1971).
25. G. N. Ramachandran, G. M. Venkatachalam, and S. Krimm, Biophys. J., 6, 849 (1966).

PRODUCTS OF PHOTOTRANSFORMATION OF PROVITAMIN D_4 OBTAINED FROM A MUTANT *Saccharomyces cerevisiae* YEAST.

II. IRRADIATION IN HEPTANE

A. V. Andreev, T. V. Ekhvalova, N. A. Klyuev, N. P. Mikhailova, and K. A. V'yunov

UDC 547.92

The composition of the photolytic mixture formed on the irradiation of provitamin D_4 in heptane has been studied. In addition to the main reaction products – vitamin D_4 and previtamin D_4 – a number of by-products were formed the structures of which have been determined by spectral methods. In contrast to that formed in ethanol, the photolytic mixture obtained in hexane contained only small amounts of by-products. The solvent therefore has an influence on the occurrence of phototransformation in the preparation of vitamins of the D group.

The phototransformation of the provitamins D is a complex process depending on many factors such as the wavelength of the source of irradiation, the initial concentration, stirring, the presence of dissolved oxygen, and a number of others [1]. One of the determining factors is the nature of the solvent, which affects the quantitative and qualitative composition of the photolytic mixture [2]. The photolysis of provitamins D is most frequently carried out in ethanol, which is due to its availability, cheapness, and low toxicity. However, because of its high reactivity, ethanol interacts with the excited reaction intermediates increasing the total number of by-products [3]. It is obvious that it is possible considerably to increase the selectivity of the process and the yield of final product by performing photolysis in a medium less aggressive towards the components of the reaction mixture.

Lensovet Leningrad Technological Institute. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 398-404, May-June, 1989. Original article submitted August 15, 1988.

 0009-3130/89/2503-0344 $12.50

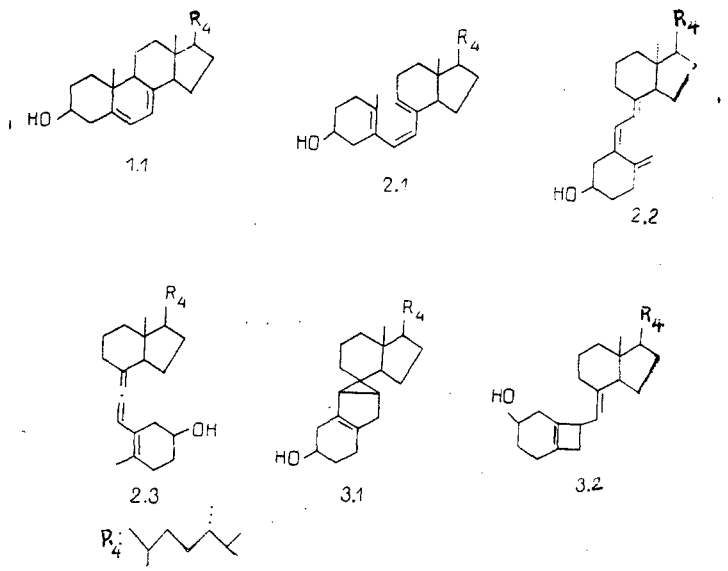


Fig. 1. Products of the photolysis of provitamin D_4 in heptane.

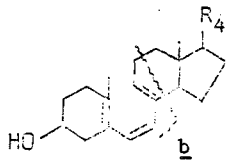
Continuing a study of the phototransformation of provitamin D_4, we have investigated the influence on this process of a nonpolar aprotic solvent, as which we selected heptane.

The isolation of the initial provitamin D_4 has been described previously [4, 5]. The results of the separation of the photolytic mixture and of a physicochemical analysis of its components are given in Table 1, and the structural formulas of the compounds in Fig. 1.

According to the results of high-performance liquid chromatography (HPLC) and gas-liquid chromatography (GLC), fraction 1, obtained on the separation of the mixture with the aid of thin-layer chromatography (TLC) contained a single substance. Analysis of the results of chromatography, mass spectrometry, and UV spectrophotometry and comparison with the analogous results obtained for standards enabled this compound to be identified as provitamin D_4 – ergosta-5,7-dien-3β-ol (1.1) – that had not undergone photolysis [4, 5].

HPLC permitted fraction 2 (TLC) to be separated into three subfractions, which were collected and analyzed separately. On the gas-chromatographic separation of fractions (2.1) and (2.2) doublet peaks issued with relative retention times R_t = 112.5 and 125 in a ratio of 2:1. A comparison of the retention times with those of a standard sample of vitamin D permitted the assumption that fraction (2.1) contained provitamin D_4 and (2.2) vitamin D_4. The appearance of doublet peaks on the chromatogram is a consequence of the thermal cyclization of the provitamin and the vitamin in the injector of the chromatograph with the formation of the corresponding pyro and isopyro components [6].

The UV spectrum of fraction (2.1) contained a single absorption maximum at 256 nm, which is characteristic for the previtamins D [7]. Analysis of the mass spectrum with high-intensity peaks of ions with m/z 176 and 158 showed that the main type of fragmentation of compound (2.1) was the cleavage $M^+ \rightarrow$ b:



Fragmentation of this type is predominant for endocyclic trienes [8]. The extremely low intensity of the peaks of ions with m/z 136 and 118 indicates the practically complete suppression of fragmentation of the $M^+ \rightarrow$ a type (see below), probably because of the high stability of the $\Delta^{5(10),6,8}$ triene system. From the facts given above, compound (2.1) was determined as 9,10-secoergosta-5(10),cis-6,8-trien-3β-ol (previtamin D_4).

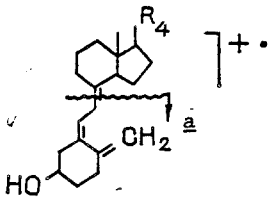
TABLE 1. Products of the Photolysis of Provitamin D_4 in Heptane

TLC		HPLC		GLC	UV spectrum, λ, nm	Amount,** %	Main ions in the mass spectrum, m/z (rel. intensity)
fraction	$R_f \times 100$	fraction	$R_t \times 100$*	$R_t \times 100$*			
1	77	1,1	100	100	271,5; 282; 293,5	8,55	M^+ 398 (100), 383 (18), 365 (95), 339 (20), 271 (31), 253 (25)
2	80	2,1	113	112,5 125	256	38,44	M^+ 398 (56), 383 (10), 380 (12), 365 (22), 271 (42), 253 (28), 176 (58), 158 (46), 136 (20), 118 (8), 109 (100)
		2,2	116	112,5 125	265	20,80	M^+ 398 (65), 383 (11), 380 (10), 365 (25), 271 (39), 253 (30), 176 (15), 158 (24), 136 (68), 11 (100)
		2,3	118	54; 112,5; 125	230	2,73	M^+ 398 (52), 383 (18), 380 (11 , 365 (23), 271 (48), 253 (32), 176 (57), 158 (42), 136 (12), 118 (8), 109 (100)
3	86	3,1	120	110	261	28,67	M^+ 398 (32), 383 (6), 380 (9), 365 (22), 339 (1 271 (28), 253 (15), 176 (9), 158 (18), 136 (100), 118 (79)
		3,2	122	112,5; 125	—	0,81	M^+ 398 (80), 383 (8), 380 (15), 365 (10), 339 (8), 271 (26), 253 (18), 176 (5), 158 (10), 136 (100), 118 (76)

*In relation to ergosterol.
**According to GLC.

The UV spectrophotometric analysis of compound (2.2) showed that its spectrum was typical for the vitamins D, with a single absorption maximum in the 265 nm region [9]. Intense absorption in this region is characteristic for the triene system of the vitamins D.

The mass spectrum of compound (2.2) had features characteristic of the spectra of the vitamins D. The most intense peaks in the spectrum were those of ions with m/z 136 and 118, which are characteristic for the cleavage of the vitamins D and the corresponding fragmentation $M^+ \rightarrow \underline{a}$:



This type of dissociation of the molecular ion is possible when a triene system exocyclic with respect to a 6-membered ring is present [10]. The peak of an ion with m/z 118 had a high intensity, which was probably connected with the difficulty of its dissociation as

a consequence of aromaticity (the $[\underline{a} - H_2O]^+$) ion [8]. The intensities of the peaks of the ions with m/z 176 and 158 in the spectrum of compound (2.2) were considerably lower than in the spectrum of previtamin D_4, which indicates a predominance in the breakdown of the molecule ion of cleavage of the $M^+ \rightarrow \underline{a}$ type over that of the $M^+ \rightarrow \underline{b}$ type.

Two characteristic features of the mass spectrum of compound (2.2) may be assigned the lower intensity of the peak of the molecular ion in comparison with that of provitamin D_4 because of its low stability on electron impact. The intensity of the peak of the $[M - H_2O]^+$ ion is low because of competition on the part of the process $M^+ \rightarrow \underline{a}$. It is also interesting to note the low intensity of the peak of the $[M - H_2O - CH_3]^+$ ion, which is a consequence of the predominance of fragmentation with the formation of the ion a and the considerable expenditure of energy in the elimination of the 19-methyl group [10].

On the basis of these facts, compound (2.2) was identified as 9,10-secoergosta-5,7,10-(19)-trien-3β-ol (vitamin D_4).

On the performance of TLC, compound (2.3) formed a single fraction with vitamin and previtamin D_4. This indicated their structural closeness and identical numbers of double bonds. In the course of separation by the HPLC method, substance (2.3) issued as a single peak with a retention time differing from those of vitamin and previtamin D_4 (Table 1). When compound (2.3) was analyzed by the GLC method, three peaks were revealed, two of which presumably belonged to pyro- and isopyrocalciferols (2.3a and 2.3b, respectively, Fig. 2). The UV spectrum of compound (2.3) contained a single maximum in the region of 230 nm. This coincided with the spectra of vinylallene derivatives of the vitamins D [3]. A distinguishing feature of these compounds is their low thermal stability, which is due to isomerization into (2.3a) and (2.3b) and the conversion of (2.3) into Grundmann's ketone (2.3c) because of ozonolysis and the action of a high temperature in the evaporator of the gas chromatograph (Fig. 2) [11].

The mass spectrum of compound (2.3) proved to be similar to the spectrum of previtamin D_4. Dissociation of the molecular ions took place predominantly through the cleavage of ring C at the C_{11}–C_{12} and C_8–C_{14} bonds with the formation of an intense peak of an ion with m/z 176 (b), and its subsequent dehydration $[\underline{b} - H_2O]^+$, m/z 158. This type of dissociation is characteristic of endocyclic trienes the system of double bonds of which is, as mentioned above, resistant to electron impact [7]. The high intensity of the peak of the $[\underline{b} - H_2O]^+$ ion showed the stability of the cation formed, which was a consequence of the conjugation in ring A. Attention is attracted by the increase in the intensity of the peak of the $[M - CH_3]^+$ ion with m/z 383 in comparison with the spectrum of the previtamin D. Since this process leads to the formation of ions with identical structures in the two cases, a possible explanation is connected with the easier elimination of the methyl group in (2.3) because of the allyl activation of the C_{13}–C_{18} and/or C_{10}–C_{19} bonds. The increase in the intensity of the peaks of the ions corresponding to the elimination of the side chain $[M - R]^+$ with m/z 271 and $[M - R - H_2O]^+$ with m/z 253 indicates the presence of a double bond in the 8(14) position. This feature of the mass spectrum is characteristic for isotachysterol (2.3d) (Fig. 2.) [10]. However, it is known that, in contrast to (2.3), isotachysterol does not give the peaks of pyro- and isopyrocalciferols [12]. The similarity of the mass spectrum of substance (2.3) to that of isotachysterol is probably explained by the migration of the double bond from the 7-8 position to the 8-14 position on electron impact. The available facts permitted compound (2.3) to be identified as 9,10-secoergosta-5(10),6,7-trien-3β-ol.

Fraction 3 (TLC) was separated by HPLC into two components. The UV spectrum of compound (3.1) contained an absorption maximum in the region of 261 nm, which coincides with that of known suprasterols [3, 11] and permitted the assumption that substance (3.1) belonged to this group.

Analysis of the mass spectrum of (3.1) revealed its similarity to the spectrum of vitamin D_4. As in the case of the vitamin, the dissociation of the molecular ion took place with the formation of a fragmentary ion with m/z 136 (a), which subsequently underwent dehydration with the formation of an ion having m/z 118. The intensities of these peaks coincided with those given in a number of papers for the suprasterols [13]. Although in the case of the vitamins D the formation of the ion a takes place with the cleavage of the C_7–C_8 bond, which is accompanied by the migration of the double bonds under the action of electron impact, and in a suprasterol it takes place as a consequence of C_7–C_8 and C_6–C_8 cleavage, it has been shown that this process includes a transition through common intermediate

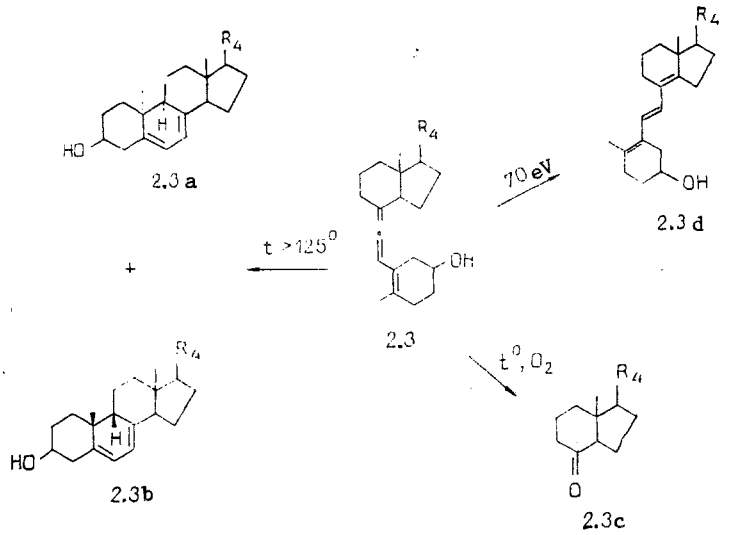


Fig. 2. Transformations of compound (2.3) as a consequence of the action of high temperatures, ozonolysis, and electron impact.

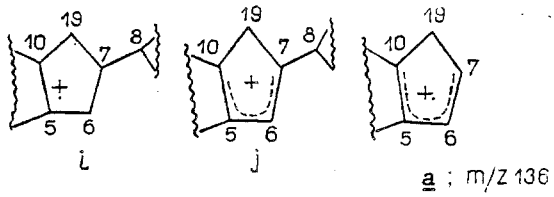


Fig. 3. Intermediate structures formed on the dissociation of vitamin D_4 and of suprasterol$_4$.

structures (i and/or j) to the same fragmentary ion a with m/z 136 (Fig. 3) [13]. In the region of the mass spectrum containing ions with m/z > 140 an appreciable fall in the intensities of the peaks of the characteristic ions relative to the spectrum of vitamin D_4 was observed, which is also in harmony with [13]. It is interesting to note the high intensity of the peak of an ion with m/z 339, which, in the spectrum of vitamin D_4, has a low value. This is obviously connected with the presence of a double bond in the 5-10 position, activating the cleavage of ring A, which is also a feature of the mass-spectrometric dissociation of a suprasterol [13]. These features confirm the hypothesis that compound (3.1) is 7,19:8,19-dicyclo-9,10-secoergosta-5(10)-en-3β-ol (suprasterol$_4$). The similarity of the mass spectra of vitamin D_4 and suprasterol$_4$ which are isomers and are closely connected photogenically suggests the existence of parallelism between the pathways of the mass-spectrometric and photochemical reactions.

Substance (3.2) was a minor impurity in the fraction containing the suprasterol$_4$. The coincidence of the R_f value (TLC) indicated its closeness with respect to structure and degree of saturation to the suprasterol. The m/z value of its molecular ion and the nature of its fragmentation (Table 1) showed its isomeric nature in relation to vitamin D_4 and suprasterol$_4$. Since compound (3.2) was isolated in insignificant amounts, it did not appear possible to identify it reliably. On the basis of the available facts and their comparison with the literature [3, 14] the assumption was made that substance (3.2) was a vinylcyclobutene derivative of vitamin D_4 – 6,19-cyclo-9,10-secoergosta-5(10),7-dien-3β-ol.

The biological properties of the compounds formed in the process of obtaining vitamin D_4 by irradiation in heptane remain little studied. It has been shown that suprasterol$_4$, which is the main impurity, although it does not possess antirachitic activity nevertheless exhibits a toxic action on the organism [14].

EXPERIMENTAL

The initial provitamin was obtained from the biomass of the phototrophic strain of the yeast Saccharomyces cerevisiae 78-PT21 [15]. The cultivation of the strain and the conditions of isolating the sterols has been described previously [4, 5].

The provitamin D_4 was irradiated in a quartz test-tube in an atmosphere of argon with the bubbling of this gas through the solution at room temperature. Heptane was used as the solvent and the concentration of the solution was 1.5 mg/ml. The source of radiation was a RL-250 high-pressure mercury lamp with a linear emission spectrum [16] and the distance of the lamp to the irradiated surface was 10 mm. The course of the reaction was monitored with the aid of GLC and UV spectrophotometry.

TLC was performed on Silufol plates impregnated with silver nitrate in the solvent system chloroform–acetone [95:5 (by volume)].

UV spectrophotometric analysis was conducted on a specord M 40 spectrophotometer. GLC was performed on a Tsvet 100 chromatograph with a 0.2 × 500 cm column containing 3% of OV-17 on Chromaton N Super at 280°C. The carrier gas was helium at a rate of flow of 35 ml/min. Flame-ionization detector.

HPLC was performed on a Du Pont chromatograph with a 4.6 × 250 mm Zorbax Sil column, the mobile phase being chloroform–acetone [200:2 (by volume)] at a rate of flow of 1.25 ml/min. Ultraviolet detector.

Mass spectrometric analysis was carried out on a Varian MAT 311 A instrument, and samples obtained as the result of separation with the aid of HPLC were introduced into the ion source directly. The evaporation temperature was varied at the rate of 1°C/min in the interval from 50 to 300°C. The mass spectra were scanned every 1 s. The ionizing energy was 70 eV.

SUMMARY

The composition of the photolytic mixture formed on the irradiation of provitamin D_4 in heptane has been studied. In addition to the main reaction products – vitamin D_4 and previtamin D_4 – a series of by-products were formed the structures of which were determined from spectral characteristics. In contrast to that obtained in ethanol, the photolytic mixture obtained in hexane contained very small amounts of the by-products. Thus, the solvent exerts an influence on the occurrence of phototransformation in the preparation of the vitamins of the D group.

LITERATURE CITED

1. H. J. C. Jacobs, J. W. J. Giela, and E. Havinga, Tetrahedron Lett., 22, No. 40, 4013 (1981).
2. H. J. C. Jacobs and E. Havinga, Adv. Photochem., 11, 305 (1979).
3. A. G. M. Barrett, D. H. R. Barton, R. A. Russell, D. A. Widdowson, J. Chem. Soc. Perkin Trans. 1, No. 6, 631 (1977).
4. A. V. Andreev, A. V. Moseichuk, K. A. V'yunov, et al., Khim. Prir. Soedin., 841 (1985).
5. A. V. Andreev, A. V. Moseichuk, K. A. V'yunov, and N. P. Mikhailova, Khim. Prir. Soedin., 196 (1986).
6. H. Ziffer, W. J. A. Van der Heuvel, E. O. A. Haahti, and E. C. Horning, J. Am. Chem. Soc., 82, No. 24, 6411 (1960).
7. S. A. Holick, M. St. Lezin, D. Young, et al., J. Biol. Chem., 269, No. 22, 12181 (1985).
8. Z. V. I. Zaretskii, Adv. Mass Spectrom., 8, 590 (1980).
9. G. Jonas, K. Kano, S. Yamada, et al., Biochemistry, 23, No. 16, 3749 (1984).
10. Z. V. I. Zaretskii, Rec. Dev. Mass Spectrom. Biochem. Med. Environ. Res., 7, 224 (1980).
11. S. A. Bakker, J. Lugtenburg, and E. Havinga, Rec. Trav. Chim., 91, No. 15, 1459 (1972).
12. H. J. Andersson and J. J. Sjövall, Steroid Biochem., 23, No. 4, 465 (1985).
13. W. H. Okamura and M. L. Hammond, Tetrahedron Lett., No. 52, 4807 (1976).
14. H. J. C. Jacobs, F. Boomsma, E. Havinga, and A. Van der Gen., Rec. Trav. Chim., 96, No. 4, 113 (1977).
15. N. P. Mikhailova, Z. A. Zhakovskaya, A. V. Andreev, and K. A. V'yunov, Khim.-farm. Zh., No. 2, 1490 (1987).
16. Handbook on Photochemistry [in Russian], Moscow (1983), p. 78.