

MAGDALENA TEICHERT

**RADIOLYSIS OF VITAMIN D<sub>3</sub>, 7-DEHYDROCHOLESTEROL AND ERGOSTEROL IN ALCOHOL-WATER SOLUTIONS**

Department of Food Technology, Warsaw Agricultural University

Key words: vitamin D<sub>3</sub>, 7-dehydrocholesterol, ergosterol, gamma <sup>60</sup>Co, toxisterol.

The GLC-MS method was used to analyze products of radiation degradation of vitamin D<sub>3</sub>, 7-dehydrocholesterol and ergosterol in 90% ethanol (gamma radiation doses — <sup>60</sup>Co 0.05-5 Mrad). Comparison of elution spectra of compounds determined as free and as TMS ethers, analysis of molecular weight, general formulas of some of the products, and considerations of diverse ways of formation of the latter — all these made it possible to observe that toxisterols analogous to those resulting from high doses of UV radiation, do not develop in effect of radiolysis.

The interest in changes induced by ionizing radiation in provitamins and vitamins of D group began with very extensive investigations of photochemical reactions of these compounds. All the analysis [11, 20, 9, 13, 2] reported that in effect of prolonged exposure of solutions of vitamin D<sub>2</sub> and ergosterol to UV a mixture of products with toxic properties develops. They were called toxisterols (toxisterins).

On account of growing application of radiation methods in preservation of foods of animal origin the interest in the mechanism and identification of radiolysis products of vitamins soluble in fats, such as provitamins and vitamins of D group, has been expanding. Even though Hummels [8], following a series of long-term biological studies on mice, did not observe cancerous properties in cholesterol irradiated with X-rays (1650 r), yet, according to Mead [12], the same kind of radiation leads to degradation of vitamin D accompanied by production of small amounts of toxisterol. Arnaud and Balestic [1] noticed that in effect of irradiation of crystalline ergosterol at 20°C with gamma rays precalciferol begins to form, while at 60°C the process gives calciferol. Fazakerley [4] proved that as a result of absorption of 18 Mrad gamma rays by crystalline ergosterol six unidentified products take form. The only clue so far is

that they have more groups containing oxygen than the initial compound. Irradiation of crystalline vitamin D<sub>2</sub> with 18 Mrad gamma rays results in formation of peroxides and other substances with properties approximating those of toxisterols.

Teichert and Horubała [18], analyzing effects of gamma rays of Co-60 on vitamin D and some sterols, observed an analogy between radiosensitivity of vitamin D<sub>3</sub> in 90% ethanol and in butter. Summing up the review of literature it should be pointed out that it has not been ascertained whether at all and under what conditions radiation degradation products of provitamins and vitamins of D group develop potential toxic effects. No studies were found concerning products of radiolysis of vitamin D<sub>3</sub>, 7-dehydrocholesterol and ergosterol.

The aim of the present study was to determine whether low and moderate doses of gamma radiation lead to formation of compounds with toxisterol properties. This question is of considerable practical and theoretical significance since introduction of irradiation methods for preservation of foods containing sterols and vitamins D group depends on it.

#### MATERIALS AND METHODS

The investigations were carried out with the following compounds: vitamin D<sub>3</sub> — Philips Duphar; 7-dehydrocholesterol — Fluka — two to three times crystallized from methanol, and ergosterol — Loba Chemie — also two or three times crystallized from a mixture of methanol and ethanol.

Solutions in 90% ethanol (vit. D<sub>3</sub> and 7-DCH concentrations:  $6.5 \times 10^{-5}$  mol/l; ergosterol -  $2.5 \times 10^{-3}$  mol/l) were irradiated with Co-60 gamma rays (0.05 to 5 Mrad dose power: 1 Mrad/hr). Solvents were removed from the control and the irradiated samples. The analyzed compounds and products of their degradation were determined with the GLC method as TMS ethers on a chromatograph (Gide, Model G, C. M. F. — 18.3; column filled with 1.5% FS-12713 with Gas Chrome Q carrier 100-200 mesh; column temp. — 225°C; flow velocity — Ar 20 ml/min; the inner standard method and appropriate correction coefficients were also used), or as free forms on Pye-Unicam chromatograph, Series 104, Model 84 (column filled with 5% and 1% FS-12713 on Gas Chrome Q 100-200 mesh; column temp. — 237°C and 204°C, respectively; flow velocity — Ar 40 ml/min.). In the latter case the percentage of degradation products and of the undegraded initial compounds were computed with the inner normalization method, giving a very significant discrepancy of results on account of the temperature difference of 33°C and 4% concentration of the liquid phase. Uniform numbering of peaks was used. The peaks corresponded to products of degradation of vitamin D<sub>3</sub>, 7-de-

hydrocholesterol and ergosterol. The relative retention times/RRT/ were calculated in ratio of the compound subjected to irradiation. Mass spectra of some of the products of radiolysis of the investigated compounds forming in 90% ethanol in effect of a dose of 3 Mrad were obtained with type 9000 LKB gaschromatograph/mass spectrometer.

Samples were fed into a column filled with 3% QF-1, Chromosorb G, temperatures ranging from 220° to 245°C. Helium was used as a gas carrier (flow velocity 20 ml/min). Temperature of the molecular separator and of the ion source 250°C; ion accelerating voltage 3.5 kV; energy of the bombarding electrons 70 eV; trap current 60 μA.

Molecular weights of some of the products of radiolysis were determined with their mass spectra [10, 15, 16] after identification of the molecular ion band and calculation of differences of mass units between adjacent groups of peaks. Conclusions were drawn as to kinds of fragments separated from the molecular ion. A general formula for a given compound corresponding to a specified molecular weight was produced on the basis of intensity of isotope bands and tabulated data [3].

## RESULTS AND DISCUSSION

Table 1 includes percentage of degradation products of vitamin D<sub>3</sub> in 90% ethanol and their corresponding RRT. Starting with 0.25 Mrad peaks I, II and III appear on the chromatograms. They correspond to degradation products of irradiation with short RRT: 0.15, 0.21, 0.33. The compounds have the following molecular weights: 264, 276, 290; general formulas: C<sub>19</sub>H<sub>36</sub>, C<sub>19</sub>H<sub>32</sub>, C<sub>21</sub>H<sub>38</sub>, (Table 2, No. 1, 2, 3). These are the products of the so-called "shallow" radiation degradation of vitamin D<sub>3</sub>. The compounds with peaks IV (RRT=0.44) and V (RRT = 0.68) have molecular weights of 366 and 364, respectively, and general formulas C<sub>27</sub>H<sub>42</sub> and C<sub>27</sub>H<sub>40</sub> (Table 1 and Table 2, No. 5, 8). Analysis of chromatograms of vitamin D<sub>3</sub>, irradiated in 90% ethanol and determined as TMS ethers (Table 3) showed that only the compound with RRT = 0.23 retained a 3-hydroxyl group and seems to be identical with the compound whose molecular weight is 276 and its general formula C<sub>19</sub>H<sub>32</sub> (Table 2, No. 2). Compounds with RRT = 1.00, 1.12 and 1.35 (Table 2, No. 10, 12 and 14) have molecular weight of 384 and are isomers of vit. D<sub>3</sub>. Their peaks also appear in the chromatogram of the control sample.

Summing up, it can be said that radiolysis of vitamin D<sub>3</sub> does not form products of hydroxylation. The compounds of 276 molecular weight and C<sub>19</sub>H<sub>32</sub>O general formula (Table 2, No. 2) develops probably in effect of separation from a molecule of vitamin D<sub>3</sub> of the side chain R = C<sub>8</sub>H<sub>16</sub> with simultaneous hydrogenation of binary bonds with the original hydroxyl group being retained. The hypothesis is confirmed by the fact

Table 1. Products of radiation decomposition of vit. D<sub>3</sub> irradiated in 90% ethanol; solution concentration —  $6.5 \times 10^{-3}$  m, dose — 1 Mrad/hr

Peak No.	Relative retention time RRT	Dose (Mrad)								
		0	0.05	0.10	0.15	0.25	0.50	1.0	3.0	
		%								
I	0.13(0.15) <sup>a</sup> (0.15) <sup>b</sup>	—	—	—	on the 1% FS-12713 column these were not determined	—	1(—)	1 (traces)	3(4)	4(1)
II	0.21(0.21) <sup>a</sup> (0.21) <sup>b</sup>	—	—	—		—	1(—)	2 (traces)	3(4)	4(1)
III	0.32(0.32) <sup>a</sup> (0.33) <sup>b</sup>	—(5) <sup>c</sup>	—(6)	—(7)		(4)	1(2)	1(4)	1(4)	3(5)
IV	(0.44) <sup>a</sup> (0.43) <sup>b</sup>	—(4)	—(5)	—(5)		(5)	—(2)	—(4)	—(4)	—(4)
V	0.68(0.67) <sup>a</sup> (0.68) <sup>b</sup>	—(—)	—(—)	—(1)		(1)	—(1)	—(2)	1(3)	3(3)
VI	1.00	67(57)	60(43)	69(43)		(54)	64(57)	65(53)	62(50)	54(46)
VII	1.12(1.10) <sup>a</sup> (1.10) <sup>b</sup>	29(34)	37(26)	29(24)		(31)	27(33)	27(31)	24(27)	23(27)
VIII	1.35(1.24) <sup>a</sup> (1.35) <sup>b</sup>	4(—)	3(20)	2(20)		(5)	3(5)	4(6)	4(5)	7(13)

Notes: a) in slanted brackets: values of RRT obtained by separation on a column with 5% FS-12713.

b) in slanted brackets: values of RRT obtained with LBK Type 9000

c) in slanted brackets: proportion (%) of products obtained by separation on a column with 5% FS-12713 at 237°C

The other values were calculated on the basis of chromatograms from a column filled with 1% FS-12713 at 204°C

Table 2. Comparison of radiolysis products of alcohol-water solutions of vit. D<sub>3</sub>, 7-dehydrocholesterol (7-DCh) and ergosterol (E)

No.	Retention distance (mm)		RRT of Vit. D <sub>3</sub>		RRT of 7-DCh		Presence of compound in elution spectrum			Molecular weight	Notes (possible general formula)
	1% FS-12713	5% FS-12713	1% FS-12713	5% FS-12713	1% FS-12713	5% FS-12713	D <sub>3</sub>	7-DCh	E		
1	5	6.5	0.13	0.15	0.10	0.12	+	—	—	264	C <sub>19</sub> H <sub>36</sub>
2	8	9	0.21	0.21	0.15	0.17	+	+	—	276	C <sub>19</sub> H <sub>32</sub> O
3	13	13.5	0.33	0.32	0.25	0.26	+	+	+	290	C <sub>21</sub> H <sub>38</sub>
4	15	—	0.38	—	0.29	—	—	+	—	366	C <sub>27</sub> H <sub>42</sub>
5	—	18.5	—	0.44	—	0.35	+	—	+	366	C <sub>27</sub> H <sub>42</sub>
6	21	23	0.54	—	0.40	0.44	—	+	—	364	C <sub>27</sub> H <sub>40</sub>
7	—	25	—	0.59	—	—	—	—	+		
8	26.5	28.5	0.68	0.67	0.50	0.54	+	—	—	364	C <sub>27</sub> H <sub>40</sub>
9	—	31	—	0.73	—	0.59	—	—	+		
10	39	42.5	1.00	1.00	0.74	0.81	+	+( <sup>1</sup> )	—	384	pyrowit. D <sub>3</sub>
11	40.5	44	1.04	1.04	0.77	0.84	—	+	+	302	C <sub>19</sub> H <sub>26</sub> O <sub>3</sub>
12	44	46.5	1.12	1.10	0.83	0.88	+	+( <sup>1</sup> )	—	384	izopyrowit. D <sub>3</sub>
13	48	48.5	1.23	1.14	0.91	0.92	—	+	—	386	C <sub>27</sub> H <sub>46</sub>
14	52.5	52.5	1.35	1.24	1.00	1.00	+	+	+	384	7-dehydrocholesterol
15	—	58	—	1.36	—	1.10	—	—	+		
16	—	62	—	1.46	—	1.18	—	—	+		
17	—	69	—	1.62	—	1.31	—	—	+		
18	77.5	76	1.99	1.79	1.48	1.95	—	+	—	382	ergosterol
19	—	93	—	2.19	—	1.77	—	—	+		
20	111	—	—	—	2.11	—	—	+	—		
21	130	—	—	—	2.48	—	—	+	—		
22	146	—	—	—	2.78	—	—	+	—		
23	198	—	—	—	3.77	—	—	+	—		

<sup>1</sup> The compound develops within dose range from 0.10 to 0.15 Mrad

Table 3. Qualitative presentation of elution spectra of TMS ethers and radiolysis products of vit. D<sub>3</sub>, 7-DCh in 90% ethanol

Vitamin D <sub>3</sub>	7-DCh	Ergosterol
Values of RRT of Radiolysis Products		
0.23	0.67*)	0.51
1.00	0.90	0.70
1.44	1.00	1.00

\*) The compound develops only within doses between 0.05 and 0.25 Mrad

that an analogous product of radiolysis develops as a result of irradiation of 7-dehydrocholesterol in 90% ethanol. Elution spectra of degradation products of 7-dehydrocholesterol determined in its free form as well as the analysis of the products with the GLC-MS method (Table 4 and 2) indicate that the first two compounds (peak I, RRT = 0.15 and peak II, RRT = 0.25) whose molecular weights were not determined because of high background of the solvent, begin to form only after the solution is irradiated with 3 Mrad. The quantity is ca 0.5%. For this reason the first one of the compounds, despite the fact that it contains the hydroxyl group, was not registered in the elution spectrum of 7-dehydrocholesterol determined as TMS ethers (Table 3). The two compounds appear also in the elution spectrum of the vitamin D<sub>3</sub> degradation products, and molecular weights 276 and 290, respectively, can be ascribed to them (Table 2, No. 2 and 3). Peak III (Table 4); RRT = 0.29 — 0.32 (depending on the concentration of the stationary phase) represents a compound of general formula C<sub>27</sub>H<sub>42</sub>; and molecular weights 366 (Table 2, No. 4), which is a radiolysis product specific for 7-dehydrocholesterol. It forms probably in effect of elimination of water molecule from 7-dehydrocholesterol (loss of the hydroxyl group). This is confirmed by lack of the compound in the elution spectrum of TMS ethers of radiolysis products of 7-dehydrocholesterol (Table 3). The compound represented by peak IV (Table 4, and 2, No. 6) develops, in all likelihood, in effect of simultaneous dehydration and hydrogenation of the 7-dehydrocholesterol molecule. Its molecular weight is 364. It should be pointed out that products of dehydration of vitamin D<sub>3</sub> and 7-dehydrocholesterol are compounds with identical molecular weight (366) but they differ significantly in their retention distances. The same pertains to the compound of mol weight 364 (Table 2, No. 4 and 5 as well as 6 and 8). This fact indicates that the retention distance is a correct measure of identification of compounds of closely similar structures.

The compound marked by peak V; RRT = 0.77-0.84 (Table 4 and 2, No. 11) is a radiolysis product characteristic for provitamine D. It can be represented by a general formula: C<sub>19</sub>H<sub>26</sub>O<sub>3</sub>, and mol. weight 302. It

Table 4. Products of radiation decomposition of 7-DCh irradiated in 90% ethanol; Solution concentration —  $6.5 \times 10^{-3}$  m, dose — 1 Mrad/hr

Peak No.	Relative Retention Time RRT	Dose (Mrad)							
		0	0.05	0.10	0.15	0.25	0.50	1.0	3.0
		%							
I	0.15(0.17) <sup>a</sup> (0.16) <sup>b</sup>	—	—	—	—	—	—	—	0.5(—)
II	0.25(0.26) <sup>a</sup> (0.24) <sup>b</sup>	—	—	—	—	—	—	—	0.5(—)
III	0.29(0.32) <sup>b</sup>	traces	traces	traces	traces	traces	traces	traces	1(—)
IV	0.40(0.44) <sup>a</sup> (0.44) <sup>b</sup>	2(2) <sup>a</sup>	2(2)	2(2)	3(2)	4(3)	4(3)	6(5)	9(16)
IV'	0.74(0.81) <sup>a</sup>	—	—	—(2)	—(3)	—	—	—	—
V	0.77(0.84) <sup>a</sup> (0.79) <sup>b</sup>	—	—	—	2(—)	2(—)	2(—)	2(6)	2(—)
VI	0.91(0.92) <sup>a</sup> (0.92) <sup>b</sup>	—	—	—(1)	—(4)	5(10)	5(13)	12(15)	9(36)
VII	1.00	98(96)	98(95)	98(92)	93(87)	86(83)	79(77)	32(67)	19(35)
VIII	1.48(1.43) <sup>b</sup>	—(2)	—(3)	—(3)	2(4)	3(4)	5(7)	5(6)	2(13)
IX	2.11	—	—	—	—	—	2(—)	2(—)	3(—)
X	2.48	—	—	—	—	—	2(—)	4(—)	5(—)
XI	2.78	—	—	—	—	—	7(—)	29(—)	36(—)
XII	3.77	—	—	—	—	—	3(—)	10(—)	13(—)

## Notes:

a) in slanted brackets: values of RRT obtained by separation in a column with 5% FS-12713

b) in slanted brackets: values of RRT obtained with LBK, type 9000 chromatograph-spectrometer

c) in slanted brackets: proportions (%) of compounds obtained by separation in a column with 5% FS-12713 on Gas Chrome Q 100/120 mesh at 237°C

The other values were calculated on the basis of chromatograms from a column filled with 1% FS-12713 on Gas Chrome Q at 204°C.

contains no hydroxyl groups since it was not found in the elution spectrum of TMS ethers, products of radiolysis of 7-dehydrocholesterol (Table 3). Considering the reports in the literature [14, 5, 6, 7, 19], a possibility of formation of compounds containing carbonyl groups can not be excluded. Thus, it should be assumed that the above compound develops in effect of oxidation of the 3-hydroxyl group into carbonyl group and formation of two additional new carbonyl groups.

The compound with peak VI; RRT = 0.88—0.92 (Table 4 and 2, No. 13) a characteristic product of radiolysis of 7-dehydrocholesterol, is defined as C<sub>27</sub>H<sub>46</sub>O, and mol weight 386. It may be surmised that it forms in effect of hydrogenation of 7-dehydrocholesterol and that it retains the 3-hydroxyl group. This is confirmed by the presence of the compound in the elution spectrum of degradation products of 7-dehydrocholesterol determined as TMS ethers (Table 3, peak with RRT = 0.90). Peak VIII; RRT = 1.43-1.48 depending on the concentration of the stationary phase (Table 3 and 2, No. 18) corresponds to a compound with molecular weight of 382. Probably it develops in effect of dehydrogenation of 7-dehydrocholesterol. It is a radiolysis product specific for provitamin D<sub>3</sub>. In the elution

spectrum of 7-dehydrocholesterol four peaks appear, starting at a dose of 0.5 Mrad, which have long RRT: 2.11; 2.48; 2.78; and 3.77 (Table 4 and 2, No. 20-23), whose m. weights were not determined. According to the published data [17], compounds approximating the above figures of RRT are products of radiation oxidation.

It was experimentally determined that they do not contain hydroxyl groups since they do not form TMS ethers (Table 3). Products of ergosterol radiolysis were not identified with the GLC-MS method. Conclusions concerning possible molecular weights and structures of ergosterol radiation degradation products were drawn only on the basis of comparison of retention indices of the products with products of radiolysis of vitamin D<sub>3</sub> and 7-dehydrocholesterol (Table 2.) The compound which represents of peak I, RRT = 0.19 (Table 5) is probably identical with the compound appearing in the elution spectrum of vitamin D<sub>3</sub> and 7-dehydrocholesterol general formula C<sub>21</sub>H<sub>38</sub> and mol. weight 290 (Table 2, No. 3). Peak II (Table 5 and 2, No. 5) corresponds to compound of general formula of C<sub>27</sub>H<sub>42</sub>; and mol weight 366.

Table 5. Products of radiation decomposition of ergosterol irradiated in 90% ethanol; Solution concentration —  $2,5 \times 10^{-3}$ , dose — 1 Mrad/hr

Peak No.	RRT	Dose (Mrad)							
		0	0.05	0.10	0.15	0.25	0.50	1.0	3.0
		%							
I	0.19(0.31) <sup>a</sup>	—	—	—	—	—	—	2	4
II	0.27(0.43)	—	—	—	—	—	—	2	4
III	0.36(0.60)	—	—	—	—	—	—	traces	2
IV	0.45(0.73)	5	5	5	5	6	6	8	8
V	0.64(1.03)	—	—	2	2	6	6	5	6
VI	0.75(1.22)	—	—	3	3	8	8	9	14
VII	0.84(1.36)	—	—	—	—	—	—	9	14
VIII	0.90(1.46)	—	—	—	—	—	—	13	19
IX	1.00(1.62)	87	86	80	80	69	69	40	18
X	1.33(2.18)	8	9	10	10	12	11	12	10

Note: <sup>a</sup>) In slanted brackets: values of RRT for ergosterol decomposition products calculated in relation vitamin D<sub>3</sub>

Peak III and IV (Table 5), corresponding to specific products of radiolysis (Table 2, No. 7, 9) similarly to those discussed earlier, do not contain hydroxyl groups since they do not form TMS ethers. Peak V (Table 5 and 2, No. 11) corresponds to the compound of mol. weight 302, which develops also in effect of radiation oxidation of 7-dehydrocholesterol already discussed. Peak VI (Table 5 and 2, No. 14) was identified as 7-dehydrocholesterol with m. weight 384 on the basis of identical retention distance (at the same parameters of chromatographic analysis). It can



form in effect of separation from ergosterol molecule of methylene group as well as hydrogenation of the binary bond in the side chain. Peaks VII, VIII and X (Table 5 and 2, No. 15, 16 and 19) do not contain hydroxyl groups either since they were not found in the elution spectrum of TMS ethers of ergosterol radiolysis products (Table 3). They are specific products of radiolysis of this compound.

### CONCLUSIONS

1. Irradiation of vitamin D<sub>3</sub> in 90% ethanol resulted in formation of six radiation degradation products for which molecular weights were established and the following general formulas proposed:

$C_{19}H_{36}$	; 264
$C_{19}H_{32}O$	; 276
$C_{21}H_{38}$	; 290
$C_{27}H_{42}$	; 366
$C_{27}H_{40}$	; 364
$C_{27}H_{44}O$	; 384 (7-dehydrocholesterol)

The compound of mol. weight 290 is a joint product of radiolysis of vitamin D<sub>3</sub>, 7-dehydrocholesterol and ergosterol.

2. In effect of irradiation of 7-dehydrocholesterol in 90% ethanol twelve products of degradation were identified as present. For six of the products of radiolysis of the compound molecular weights were determined and general formulas were proposed as follows:

$C_{19}H_{32}O$	; 276	} compounds analogous to those discussed above
$C_{21}H_{38}$	; 290	
$C_{27}H_{42}$	; 366	} isomers of vitamin D <sub>3</sub> degradation products
$C_{27}H_{40}$	; 364	
$C_{19}H_{26}O_3$	; 302	
$C_{27}H_{46}O$	; 386	

The compound of mol weight 302 forms also in effect of radiolysis of ergosterol.

3. Irradiation of ergosterol in 90% ethanol resulted in formation of ten products of degradation, one of which has possibly the mol. weight of 290 and the general formula  $C_{21}H_{38}$ , while another  $C_{19}H_{26}O_3$  and mol weight 302 respectively.

4. Radiolysis of vit. D<sub>3</sub>, 7-dehydrocholesterol and ergosterol in an alcohol-water solution does not render products of hydroxylation, which may be an evidence that the toxisterol-type compounds, analogous to those found after irradiation with UV (with its absorbed energy of ca 2 to 3 Mrad), do not develop.

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*Author address: Rakowiecka 26/30, 02-528 Warszawa*

*M. Teichert*

## RADIOLIZA WITAMINY D<sub>3</sub>, 7-DEHYDROCHOLESTEROLU I ERGOSTEROLU W ALKOHOLOWO-WODNYCH ROZTWORACH

Instytut Technologii Żywności, SGGW-AR, Warszawa

### Streszczenie

Celem niniejszej pracy było wyjaśnienie czy stosowanie niskich i średnich dawek promieniowania gamma <sup>60</sup>Co nie powoduje tworzenia się związków typu toksysteroli analogicznych do tych, jakie znajdowano w wyniku przedłużonego napromieniowania UV.

Witaminę D<sub>3</sub> 7-dehydrocholesterol i ergosterol w roztworze 90% etanolu poddawano działaniu promieniowania gamma <sup>60</sup>Co w zakresie dawek od 0.05-5 Mrad. Analizując produkty radiolizy stosowano metodę GLC-MS. Badane związki i produkty ich radiolizy oznaczano w postaci eterów TMSi i w formie wolnej. Wprowadzono jednolitą numerację pików odpowiadających produktom radiolizy witaminy D<sub>3</sub>, 7-dehydrocholesterolu i ergosterolu. Ciężary cząsteczkowe i odpowiadające im wzory sumaryczne niektórych produktów radiolizy badanych związków, tworzących się w 90% etanolu pod wpływem dawki 3 Mrad, ustalano na podstawie ich widm masowych. Taki sposób postępowania pozwolił na określenie, które produkty radiolizy posiadają grupy wodorotlenowe i mogłyby być toksysterolami. Analiza chromatogramów produk-

tów radiolizy witaminy D<sub>3</sub> oznaczanych w postaci eterów TMSi (tabela 3) wykazała, że jedynie związek o RRT = 0,23 posiada zachowaną grupę 3-hydroksylową i wydaje się być identyczny ze związkiem o m.c. = 276 i wzorze sumarycznym C<sub>19</sub>H<sub>32</sub>O (tabela 2, lp. 2). Tworzy się on w wyniku oderwania od cząsteczki witaminy D<sub>3</sub> łańcucha bocznego R = C<sub>8</sub>H<sub>16</sub> z jednoczesnym uwodornieniem wiązań podwójnych, przy czym zachowana zostaje pierwotna grupa hydroksylowa. Potwierdza to fakt, że analogiczny produkt tworzy się w wyniku napromieniowania 7-dehydrocholesterolu (tabela 4, pik I). Powstaje on dopiero pod wpływem dawki 3 Mrad i to w ilościach około 0,5%. Z tego powodu pomimo, że posiada grupę hydroksylową nie został zarejestrowany na widmie elucyjnym 7-dehydrocholesterolu oznaczanego w postaci eterów TMSi (tabela 3). Związek, któremu odpowiada pik V o RRT = 0,77–0,84 (tabela 4 i 2 lp. 11) jest produktem radiolizy charakterystycznym dla prowitamin D. Można mu przypisać m.c. = 302 i wzór sumaryczny C<sub>18</sub>H<sub>26</sub>O<sub>3</sub>. Nie posiada on grup hydroksylowych, gdyż nie wykryto go w widmie elucyjnym eterów TMSi produktów radiolizy 7-dehydrocholesterolu (tabela 3). Związek, któremu odpowiada pik VI o RRT = 0,88–0,92 (tabela 4 i 2 lp. 13), będący specyficznym produktem radiolizy 7-dehydrocholesterolu posiada m.c. 386 i wzór sumaryczny C<sub>27</sub>H<sub>46</sub>O. Należy przypuszczać, że tworzy się on w wyniku uwodornienia 7-dehydrocholesterolu i ma zachowaną grupę 3-hydroksylową (tabela 3, pik o RRT = 0,90). Pozostałe produkty radiolizy nie zawierają grup hydroksylowych, gdyż nie tworzą eterów TMSi. Produkty radiolizy ergosterolu nie były zidentyfikowane metodą GLC-MS. Wnioski o przypuszczalnych masach cząsteczkowych oraz o budowie produktów radiolizy ergosterolu wyciągnięto na podstawie porównania wskaźników retencji tych związków z produktami radiolizy witaminy D<sub>3</sub> i 7-dehydrocholesterolu (tabela 2).

Reasumując, należy stwierdzić, że w wyniku radiolizy witaminy D<sub>3</sub>, 7-dehydrocholesterolu i ergosterolu w alkoholowo-wodnym roztworze nie tworzą się produkty hydroksylowania, co świadczyć może, że nie powstają związki typu toksysteroli analogiczne do tych, jakie znajdowano w wyniku napromieniowania UV o energii pochłoniętej równoważnej 2-3 Mrad.