been detected so far only in *Ficus macrophylla* (7) and *Celtis laevigata* (8). The occurrence of 24-methylene-24-dihydrolanosterol (8) in higher plants also is rare, and only some plants belonging to the families Cruciferae (9) and Solanaceae (10) have been known to contain this compound. The great majority of higher plants are known to contain a Δ^5-sterol bearing a saturated 24-ethyl side chain, i.e., sitosterol [24α-ethylcholesterol, (24α)-**17**] as the most predominant sterol component (5,11). From this point of view, it is worth noting here that the two ginseng seed oils contain Δ^5-sterols with monounsaturated 24-ethyl side chains, i.e., 28-isofucosterol (**18**) as for *P. ginseng* seed oil, while 24-ethyl-22E-dehydrocholesterol (**16**) as for *P. quiquefolium* seed oil, is the most predominant sterol component. The content of squalene (**1**) in the unsaponifiable lipid and the compositions of sterol fraction could be used for differentiation of the seed oils of *P. ginseng* and *P. quiquefolium*.

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Radiolytic Resistance of DL-α-Tocopherol in Lipid Systems with Different Degrees of Unsaturation

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The radiolytic resistance of the DL-α-tocopherol irradiated by low (10^5rad), medium (10^6rad) and high (10^7rad) doses of gamma rays at a molar ratio of 1:1, 1:1 $\times$ 10^{-2} and 1:1 $\times$ 10^{-3} mole in methyl laurate, methyl oleate, methyl linoleate, methyl linolenate and benzene (chosen as solvent media) has been studied.

Under the experimental conditions stated, it has been established that, contrary to ordinary autoxidation, the unsaturated lipid systems exert a progressive, protective effect on DL-α-tocopherol as the number of double bonds increases.

When the DL-α-tocopherol was in a pure state, for example in benzene and in methyl esters of the fatty acids at a molar ratio 1:1, no effect of ionizing radiation was detected.

It has been proved that during the oxidation of lipids the stability of α-tocopherol and its efficiency as an antioxidant decrease as the degree of unsaturation of the solvent medium (1,2) increases.

The behavior of tocopherols under autoxidation and thermal-initiated oxidation of the different lipid systems has been investigated extensively (1-6). Data about the resistance of these important natural antioxidants and bioregulators against the ionizing radiation do not have a systematic character; they concern various lipid systems treated under different conditions and in a theoretically undefined tocopherol concentration range (7-12). As a result they cannot easily be compared and interpreted in a uniform fashion. Because of this no general theory about the radiolytic behavior of tocopherols as a function of the degree of unsaturation of the lipid system has been formulated so far.

This paper deals with the radiolytic resistance of the tocopherols at a concentration range established at a molar level with chemically defined lipid systems as a function of the degree and type of unsaturation of the solvent medium, namely:

- Establishing the rate of destruction of the DL-α-tocopherol (chosen as a most characteristic representative of the tocopherol homologs) as well as the character of the radiolytic alterations at fixed molar ratios with a given solvent medium as a function of the irradiation dose applied.
- Investigation of the influence of the molar ratio level (solvent medium/tocopherol) on the degree of radiolytic degradation of the tocopherol at a fixed degree of unsaturation and irradiation dose.
- Investigation of the influence of the degree and type of unsaturation of the solvent medium on the radiolytic destruction of the tocopherol at given molar ratios and irradiation doses.

For the accomplishment of these tasks, methyl esters of fatty acids with a progressively increasing number of double bonds and a benzene (as a model of a polyunsaturated type of resonance stabilized system) have been chosen as solvent media.

The treatment has been carried out in liquid phase by low, medium and high doses of gamma rays under conditions designed to closely approximate natural surroundings.

EXPERIMENTAL PROCEDURES

Mixtures of methyl esters of lauric acid, oleic acid, linoleic acid, linolenic acid and a benzene (Merck, purity 99% and A.R. grade) with DL-α-tocopherol (Fluka; purity 98%) in molar ratios 1:1, 1:1 × 10^{-2} and 1:1 × 10^{-3} mole have been used for these investigations.

For irradiation, 5 g of these materials were sealed in 10-ml glass ampoules in an air atmosphere and exposed to gamma rays from the ^{60}Co source (dose rate: 5000 Rö/min). The specimens received doses of 10^5, 10^6 and 10^7 rad (the doses absorbed were controlled by dosimeter) at 20 C.

To establish the irradiation effect, all samples were kept under dry ice refrigeration after exposure and were analyzed immediately after the return shipment to our laboratory. Nonirradiated duplicates of all samples were treated absolutely identically and analyzed in parallel with the specimens irradiated.

After preparative isolation by means of TLC on Silica gel G (Fluka) and mobile phase-n-hexane/diethyl ether (7:3, v/v), the contents of DL-α-tocopherol were spectrophotometrically determined (13). The results (the average from three parallel measurements) were calculated from the standard curve (obtained by performing the same procedure as described above) of known concentrations of the DL-α-tocopherol. The relative standard deviation (S_r, %) of the method varied at concentrations of the tocopherol of 1 to 8 μg/ml (n = 9) within the range of 2.6 to 1.4%.

The effect of irradiation (the degree of radiolytic degradation) on the tocopherol in the samples given was expressed in relative percents compared to its concentration in the respective, nonirradiated control duplicates.

Peroxide contents were determined iodometrically (14).

The determination of the carbonyl compounds was carried out according to the methods of Henick et al. (15) and Chipault et al. (16).

The electronic spectra of the samples (220–350 nm) were recorded on a Specord UV-VIS (Karl Zeiss) spectrophotometer in isooctane.

The infrared spectra (1000–900 cm^{-1}) were recorded on a Perkin Elmer Model 337 spectrophotometer in carbon disulfide solutions of the specimens.

RESULTS AND DISCUSSION

Under the experimental conditions stated, no peroxides and only traces of carbonyl compounds were detected in the irradiated samples. No change in the quantitative ultraviolet and infrared characteristics of the specimens was registered after treatment. According to the information thus summarized, the effect of the oxidative attack evidently was negligible. Thus, it could be assumed that predominantly radiolytic types of reactions were taking place in the systems investigated.

The experimental data indicate that the irradiation with gamma rays provokes radiolytic destruction of the tocopherol only in the samples (excluding the benzene) where the molar ratio is 1:1 × 10^{-2} and 1:1 × 10^{-3} mole (Table 1). The effect of irradiation increases with the dose and is expressed most strongly in methyl oleate, decreasing progressively from methyl linoleate toward methyl linolenate (Figs. 1 and 2). In any case, the effect of irradiation in methyl laurate is lower than that in methyl oleate, but it is more strongly expressed than that in methyl linoleate. The only exception was noted in the specimen of methyl linolenate/tocopherol = 1:1 × 10^{-2} mole at 10^6 rad, where the effect of irradiation exceeds that proven for the methyl laurate at the same dose and the molar ratio with the tocopherol.

A comparison of these data with the results shown in Table 2, however, shows that under the influence of the unit energy absorbed (100 eV), the degradation of tocopherol (as absolute value) regularly decreases on increasing the unsaturation of the solvent medium. The case where the methyl laurate was at a molar ratio 1:1 × 10^{-2} mole with the tocopherol should be pointed out as the only exception to this tendency. Thus, while for the molar ratio 1:1 × 10^{-3} mole, at 10^5, 10^6 and 10^7 rad, the degradation of tocopherol in the methyl laurate is respec-

TABLE 1

Radiolytic Degradation of the DL-α-Tocopherol

Dose (rad)	Molar ratio slv/tph	DL-α-tocopherol concentration (mg/g) in				
		Methyl laurate	Methyl oleate	Methyl linoleate	Methyl linolenate	Benzene
0	1:1	2.01 × 10^3	1.45 × 10^3	1.46 × 10^3	1.47 × 10^3	5.52 × 10^3
	1:1 × 10^{-2}	20.12	14.50	14.63	14.72	55.22
	1:1 × 10^{-3}	2.01	1.43	1.44	1.45	5.52
10^5	1:1	2.01 × 10^3	1.45 × 10^3	1.46 × 10^3	1.47 × 10^3	5.52 × 10^3
	1:1 × 10^{-2}	19.46	13.58	13.82	14.34	55.22
	1:1 × 10^{-3}	1.84	1.28	1.33	1.35	5.52
10^6	1:1	2.01 × 10^3	1.45 × 10^3	1.46 × 10^3	1.47 × 10^3	5.52 × 10^3
	1:1 × 10^{-2}	18.60	11.40	12.01	12.75	55.22
	1:1 × 10^{-3}	1.38	0.96	1.02	1.15	5.52
10^7	1:1	2.01 × 10^3	1.45 × 10^3	1.46 × 10^3	1.47 × 10^3	5.52 × 10^3
	1:1 × 10^{-2}	14.42	8.24	9.18	11.16	55.22
	1:1 × 10^{-3}	0.00	0.00	0.08	0.43	5.52

tively 13.1%, 26.7% and 28.6% higher than that in the methyl oleate, for the molar ratio 1:1 × 10^{-2} mole the radiolytic processes in the same medium seem to approach some critical levels on increasing the energy absorbed. At 10^7 rad the latter effects in methyl laurate and methyl oleate are approximately balanced. The difference is only 11.5%. (This needs further, extensive investigation and cannot be discussed only on the basis of the data presented here.)

Under the specifically chosen conditions, at molar ratio 1:1 mole with methyl esters of the fatty acids as well as in all cases where the benzene was the solvent medium, the tocopherol does not undergo substantial radiolytic destruction.

Within the range of sensitivity of the analytical method, no radiolytic changes in the pure state of tocopherol (regardless of the dose applied) were proven.

The calculated irradiation yields (−G values) demonstrate the presence of a concentration effect and prove that the radiolytic processes do not have a chain character (Table 2). Considering this data, it is possible to explain

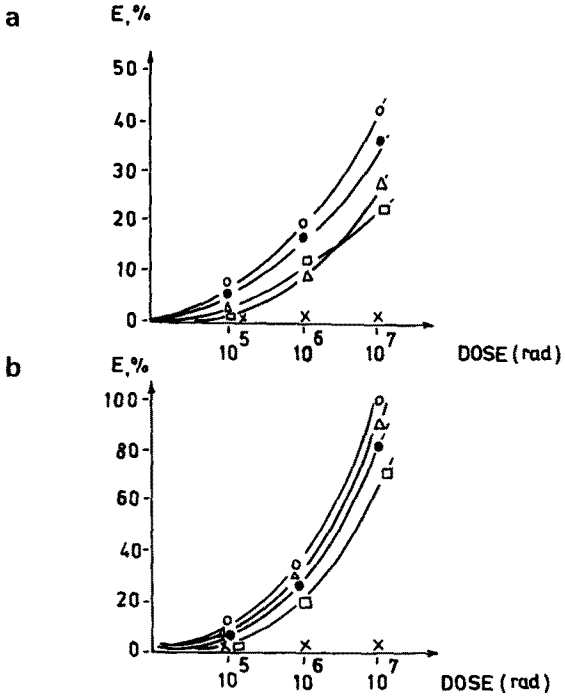

FIG. 1. Irradiation effect on DL-α-tocopherol as a function of the dose applied in methyl laurate (△); methyl oleate (○); methyl linoleate (●); methyl linolenate (□) and benzene (×) at (a) molar ratio 1:1 × 10^{-2} mole and (b) molar ratio 1:1 × 10^{-3} mole.

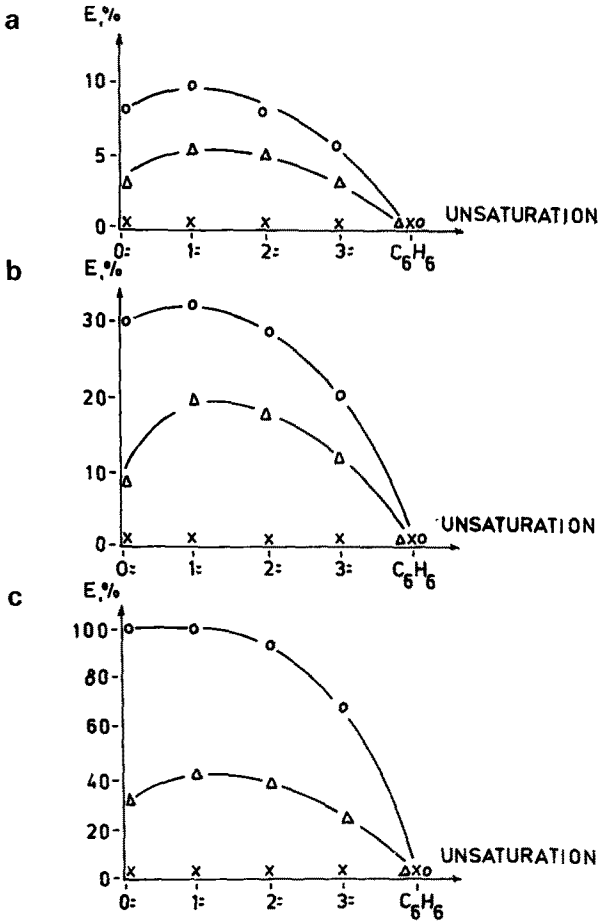

FIG. 2. Irradiation effect on DL-α-tocopherol as a function of the unsaturation of the solvent medium at molar ratio 1:1 mole (×); 1:1 × 10^{-2} mole (△), and 1:1 × 10^{-3} mole (○) at (a) 10^5 rad; (b) 10^6 rad and (c) 10^7 rad.

TABLE 2

Irradiation Yields (−G Values) of the DL-α-Tocopherol

Dose (rad)	Molar ratio slv/tph	Number of molecules DL-α-tocopherol destroyed/100 eV in				
		Methyl laurate	Methyl oleate	Methyl linoleate	Methyl linolenate	Benzene
10^5	1:1	0.00	0.00	0.00	0.00	0.00
	1:1 × 10^{-2}	13.47	19.15	16.53	7.72	0.00
	1:1 × 10^{-3}	3.52	3.06	2.23	1.96	0.00
10^6	1:1	0.00	0.00	0.00	0.00	0.00
	1:1 × 10^{-2}	3.10	6.46	5.46	4.11	0.00
	1:1 × 10^{-3}	1.31	0.96	1.08	0.64	0.00
10^7	1:1	0.00	0.00	0.00	0.00	0.00
	1:1 × 10^{-2}	1.16	1.31	1.14	0.74	0.00
	1:1 × 10^{-3}	0.42	0.30	0.28	0.22	0.00

the destruction of tocopherol only partially by the possibility of a direct gamma quantum radiolyses, which in this case should have been proportional to the tocopherol contents in a unit volume of the respective medium. A similar deduction could be correct only in the case where no interaction reactions between the tocopherol molecules and the radiation activated molecules of the solvent medium take place. If there is no specific reflective influence by the solvent medium, the G values must be constant regardless of the dose, i.e. the number of radiolyzed tocopherol molecules in a unit volume will increase linearly with an increase in the quantity of absorbed energy. But it is the dependency of G on the dose (under other equal conditions) that indicates the tocopherol radiolysis has been provoked mainly by interaction reactions with the activated solvent molecules. The high radiolytic resistance of the tocopherol in a pure state (or dissolved in benzene), as well as the increase of the irradiation effect (at a given dose) on decreasing the tocopherol relative contents (Fig. 3) in the solvent systems (excluding the benzene) support the above statement. Whereas the degree of the postulated interactions for the medium value concentrations passes through a maximum at the methyl oleate, for the molar ratio 1:1 × 10^{-3} mole (as stated above), it decreases progressively from the methyl laurate toward the methyl linolenate, reaching at 10^7 rad the minimum value of $-G = 0.22$ for this medium (Table 2). Thus, the effect noted is both concentration and energy dependent. Such a regularity could be interpreted by the proceeding of competitive (to the tocopherol radicals) processes of recombination between the solvent medium radicals. Moreover, the formation of resonance-stabilized structures which could indirectly protect the tocopherol through redistribution of the absorbed energy (as a function of the unsaturation degree) is quite probable. The experimentally proven fact that the initially established contents of *trans* isomers in the methyl oleate (0.9%), of conjugated dienes in the methyl linoleate (0.3%, as a *trans-trans* form) and conjugated dienes (0.5%, as a *trans-trans* form) as well as trienes (0.1%, as a *trans-trans-trans* form) in methyl linolenate do not undergo any changes at molar ratio 1:1 × 10^{-2} and 1:1 × 10^{-3} mole with the tocopherol, regardless of the dose, supports the latter assumption. The full absence of irradiation effect in benzene, which is in itself a resonance-stabilized system of double bonds, serves as another proof as well.

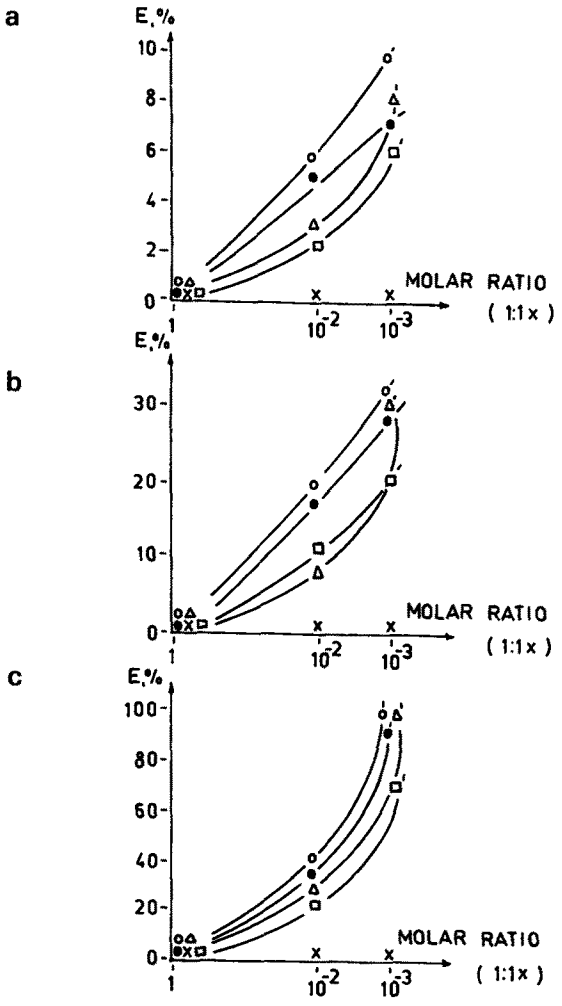

FIG. 3. Irradiation effect on DL-α-tocopherol as a function of its concentration in methyl laurate (△); methyl oleate (○); methyl linoleate (●); methyl linolenate (□), and benzene (×) at (a) 10^5 rad; (b) 10^6 rad, and (c) 10^7 rad.

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