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Antioxidative action of the ethanolic extract and some hydroxycoumarins of *Fraxinus ornus* bark

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The antioxidative effectivity and strength of different concentrations of ethanolic extract from *Fraxinus ornus* bark, as well as of esculetin, esculin, fraxetin and fraxin during the autoxidation at 100°C of kinetically pure triacylglycerols of lard (TGL) and triacylglycerols of sunflower oil (TGSO) were determined. The extract exhibited a pronounced antioxidative activity. Esculetin and fraxetin considerably retarded the process in both lipid systems, this being more significant in the less oxidizable lipid substrate (TGL). Fraxetin proved to be a more efficient and stronger inhibitor than esculetin. During the oxidation of TGSO the fraxetin caused a stronger decrease in antioxidative activity. The participation of both hydroxycoumarins in the side reactions of inhibited oxidation, which explains their kinetic behaviour, is discussed. Fraxin and esculin displayed a very weak antioxidative action.

INTRODUCTION

Recently, the need to prepare lipids and lipid-containing products that are stable with respect to the effect of atmospheric oxygen has increased interest in suitable natural sources of harmless antioxidants (Dugan, 1980; Pokorny, 1991; Evans & Reyhout, 1992). The high biological activity of extracts from such sources is often due to the presence of phenolic compounds, many of them exercising a pronounced antioxidative effect on lipids (Herrmann, 1973, 1990; Houlihan *et al.*, 1985; Su *et al.*, 1986; Torel *et al.*, 1986; Marinova *et al.*, 1989).

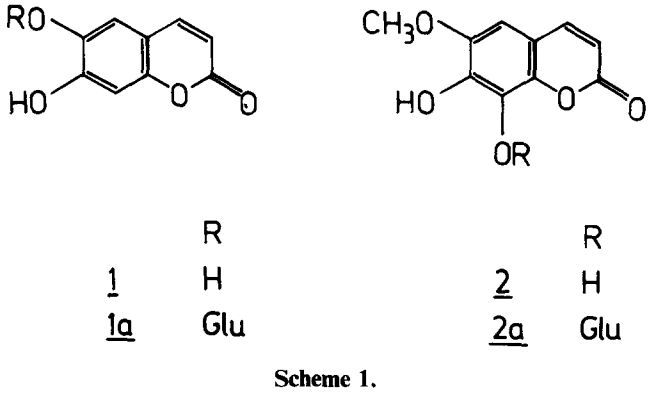
The bark of *Fraxinus ornus* L. (Oleaceae) is used in folk medicine as a remedy for a number of ailments (Stoyanov, 1973; Asenov & Nikolov, 1988). Recently, a correlation between the antimicrobial properties of *Fraxinus ornus* bark preparations and their coumarin contents has been observed (Kostova *et al.*, 1993).

In the literature there is no information on the antioxidative action of extracts from this natural source and the data on coumarins are scarce. It has been established (Dziedzic & Hudson, 1984) that esculetin shows antioxidative properties in lard at 120°C. It inhibits the autoxidation of soya oil and linoleic acid containing β-carotene in an aqueous medium (Barrera-Arellam & Esteves, 1989). Some triterpenoid coumarins inhibit, to a small degree, the oxidation of egg yolk in suspension (Sirov *et al.*, 1987). The antioxidative properties of coumarins control molecular interactions between coumarins and animal organisms (Parfenov & Smirnov, 1988).

In the papers cited above the stabilizing effect is investigated in complex systems which contain a series of components such as water, transition metals, antioxidants, free fatty acids, partial acylglycerols, alcohols, sterols, etc. These components may participate in a complex way in the autoxidation process and affect the inhibiting action of the substance added.

The purpose of the present study is to examine the antioxidative action of the ethanolic extract of *Fraxinus ornus* bark as well as of its main hydroxycoumarin components: esculetin (**1**), fraxetin (**2**), esculin (**1a**) and fraxin (**2a**) (see Scheme 1).

In view of the foregoing, the investigations are performed with kinetically pure triacylglycerols of lard and sunflower oil (TGL and TGSO), which represent models of two types of natural lipid unsaturation.



	R
1	H
1a	Glu

	R
2	H
2a	Glu

Scheme 1.

MATERIALS AND METHODS

Materials

Triacylglycerols of lard (TGL) and sunflower oil (TGSO) were obtained by purifying commercially available samples from antioxidants, peroxides, pro-oxidatively acting metals and other polar components by column chromatography (Popov *et al.*, 1968) and storing the products obtained in an inert atmosphere at −20°C. The fatty acid compositions of TGL and TGSO were as follows: TGL—miristate 2%, palmitate 25%, palmitoleate 2%, stearate 14%, oleate 48%, linoleate 9%; TGSO—palmitate 6%, stearate 4%, oleate 24%, linoleate 66%.

Esculin was isolated from the ethyl alcohol extract (see below) of the bark after concentration to a small volume (200 ml) under reduced pressure, and crystallization (× 3) of the solid from ethanol–water (1 : 1). The 99% purity of esculin (2 g) was confirmed by reverse phase HPLC (Nykolov *et al.*, 1993). Esculetin was prepared by acid hydrolysis of pure esculin, followed by crystallization from ethanol–water (1 : 1). Its 99% purity was determined by reverse phase HPLC (Nykolov *et al.*, 1993). Fraxin and fraxetin were purchased from Aldrich and used as received. All the other chemicals were analytical grade reagents supplied by Merck and used as received.

Preparation of Fraxinus ornus *bark extract*

Stem bark from mature trees of *Fraxinus ornus*, collected from the region of Kresna, southern Bulgaria, was authenticated and a voucher specimen deposited in the herbarium of the Institute of Botany, at the Bulgarian Academy of Science, Sofia. Dried and powered bark (100 g) was extracted (× 3) with 300 ml ethanol under reflux for 90 min. After filtration and evaporation of the solvent to dryness under reduced pressure, powdered residue (14·5 g) was obtained. Quantitative reversed phase HPLC analysis (Nykolov *et al.*, 1993) of the extract revealed the following hydroxycoumarin content: esculin 53·7%, esculetin 0·5%, fraxin 8·7%, fraxetin 0·3%.

Methods

Inhibition of TGL and TGSO was achieved by the addition of aliquots of a solution of the substance in acetone to a weighed lipid sample. Samples containing 0·05 and 0·10% ethanolic extract of *Fraxinus ornus* bark, 0·01, 0·02, 0·05 and 0·10% esculetin, 0·01, 0·02, 0·05 and 0·10% fraxetin, 0·02 and 0·04% esculin and 0·02 and 0·04% fraxin, were prepared.

Oxidation was carried out at 100°C (± 0·2°C) by blowing air through the samples (5 g) in the dark at a rate of 100 ml min^{-1}. The process was followed by withdrawing samples at measured time intervals and subjecting them to iodometric determination of the primary product (peroxide) concentration, i.e. the peroxide value (PV) (Yanishlieva *et al.*, 1978). During the initial stage of the process investigated, the rate of peroxide accumulation was equal to the oxidation rate (Yanishlieva, 1973*a*). Kinetic curves of peroxide accumulation were plotted. The effectivity of the antioxidants was estimated on the basis of the induction period (IP) determined by the method of tangents to the two parts of the kinetic curve (Yanishlieva & Popov, 1971; Le Tutour & Guedon, 1992). The rate of non-inhibited (W_0) and inhibited (W_{inh}) oxidation was found from the tangents to the initial phase of the kinetic curves of peroxide accumulation and was expressed as M s^{-1}. Recalculation of the rate from meq kg^{-1} h^{-1} into M s^{-1} was performed according to the following formula (Marinova & Yanishlieva, 1992*a*):

$$1 \text{ meq kg}^{-1} \text{ h}^{-1} = 1{\cdot}4 \times 10^{-7} \text{ M s}^{-1}.$$

RESULTS AND DISCUSSION

The results obtained are interpreted on the basis of the main regularities of inhibited lipid oxidation.

The introduction of an antioxidant (inhibitor) into the oxidizing system leads to a change in mechanism and kinetics of the process (Schemes 2 and 3) (Denisov & Khudyakov, 1987).

$$2LH + O_2 \rightarrow 2L^{\cdot} + H_2O_2 \quad (0)$$
$$L^{\cdot} + O_2^{\cdot} \rightarrow LO_2^{\cdot} \quad (1)$$
$$LO_2^{\cdot} + LH \rightarrow LOOH + L^{\cdot} \quad (2)$$
$$LOOH \rightarrow LO^{\cdot} + OH \quad (3)$$
$$LOOH + LH \rightarrow LO^{\cdot} + H_2O + L^{\cdot} \quad (3')$$
$$L^{\cdot} + L^{\cdot} \rightarrow L\text{–}L \quad (4)$$
$$L^{\cdot} + LO_2^{\cdot} \rightarrow L\text{–}O\text{–}O\text{–}L \quad (5)$$
$$LO_2^{\cdot} + LO_2^{\cdot} \rightarrow \text{products} \quad (6)$$

Scheme 2. Non-inhibited oxidation

$$LO_2^{\cdot} + InH \rightarrow LOOH + In^{\cdot} \quad (7)$$
$$In^{\cdot} + LOOH \rightarrow InH + LO_2^{\cdot} \quad (-7)$$
$$In^{\cdot} + LO_2^{\cdot} \rightarrow \text{In-OOL} \quad (8)$$
$$In^{\cdot} + In^{\cdot} \rightarrow \text{products} \quad (9)$$
$$In^{\cdot} + LH \rightarrow InH + L^{\cdot} \quad (10)$$
$$InH + LOOH \rightarrow \text{products} \quad (11)$$
$$InH + O_2 \rightarrow In^{\cdot} + HO_2^{\cdot} \quad (12)$$
$$InOOL \rightarrow InO^{\cdot} + LO^{\cdot} \quad (13)$$
$$In^{\cdot} + O_2 \rightarrow InOO^{\cdot} \quad (14)$$

Scheme 3. Non-inhibited oxidation

In the above schemes LH is the oxidizing lipid substrate, $LO_2^{\cdot}$ is the peroxide radical, and InH is the inhibitor.

With a kinetic regime of oxidation (a sufficiently high oxygen concentration), the system being oxidized contains no short-lived radicals $L^{\cdot}$ and the termination proceeds according to reaction (6) and/or reactions (7) and (8). It has been found that the effect of the antioxidant depends on the participation of its molecules and radicals formed from the latter in a series of reactions presented in Scheme 3 (Denisov & Khudyakov, 1987; Roginskii, 1990). The probability of reactions (7)–(14) taking place depends not only on the inhibitor structure but also on the type and degree of lipid unsaturation.

The oxidation of the linoleate is 10 times easier than that of the oleate (Gunston & Hilditch, 1945; Stirton *et al.*, 1945; Silbert, 1962). The linoleate peroxide radicals react several times faster than the oleate peroxide radicals (Yanishlieva *et al.*, 1970). The oleate hydroperoxides are much more stable than the linoleate ones (Yanishlieva, 1973*b*). It is established that both the linoleate and oleate moieties in TGL are oxidized, while in the case of TGSO the oxygen and the peroxide radicals attack the linoleate units alone (Yanishlieva & Popov, 1973). For that reason, LH, $LO_2^{\bullet}$ and LOOH in TGL and TGSO have different compositions and reactivities, which would determine different kinetic behaviours of the antioxidant in these lipid substrates.

The peculiarities of the inhibitor action are described by two kinetic characteristics (Yanishlieva & Marinova, 1992): (1) effectivity, representing the possibility of blocking the radical chain process by interaction with the peroxide radicals (reaction (7)) and (2) strength, expressing the possibility of the inhibitor moieties for participating in other reactions e.g. (–7); (10); (11); (12); (14), which lead to a change in oxidation rate during the IP. A measure of the effectivity is the stabilization factor F:

$$F = IP_{inh}/IP_0$$

where IP_{inh} the induction period in the presence of an inhibitor, and IP_0 is the induction period of the non-inhibited system.

The oxidation rate ratio, ORR, is a measure of the strength:

$$ORR = W_{inh}/W_0$$

where W_{inh} is the rate of formation of hydroperoxides in the presence of an inhibitor, and W_0 is the rate of formation of hydroperoxides in the absence of an inhibitor.

When ORR is larger than 1, the oxidation proceeds faster in the presence of an inhibitor than in its absence; this is observed at high tocopherol concentrations, for example (Marinova & Yanishlieva, 1992*a*). The lower the ORR, the stronger the inhibitor.

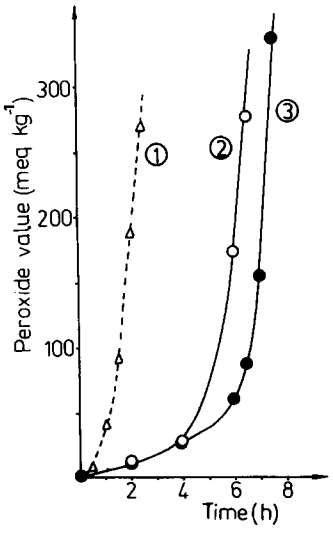


Fig. 1. Kinetic curves of peroxide accumulation during the oxidation of TGL at 100°C in the presence of ethanolic extract from *Fraxinus ornus* bark: 1, Without additive; 2, 0·05%; 3, 0·10%.

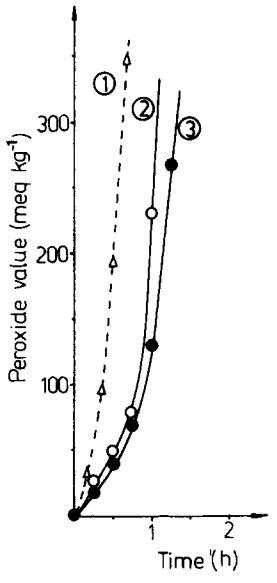


Fig. 2. Kinetic curves of peroxide accumulation during the oxidation of TGSO at 100°C in the presence of ethanolic extract from *Fraxinus ornus* bark. 1, without additive; 2, 0·05%; 3, 0·10%.

Figures 1 and 2 illustrate the kinetic curves of peroxide accumulation during the oxidation at 100°C of TGL and TGSO in the presence of 0·05 and 0·10% ethanolic extracts of *Fraxinus ornus* bark. After processing the kinetic curves, the parameters F and ORR, characterizing the antioxidative action, can be determined (Table 1).

These data indicate that the ethanolic extract of *Fraxinus ornus* bark has a pronounced antioxidative activity during the oxidation of both lipid substrates. This activity is commensurate with the inhibiting effect of the same concentrations of butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) during TGL oxidation (Yanishlieva & Marinova, 1992).

In order to elucidate the contribution of the main phenolic components in the extract (esculetin, fraxetin, esculin and fraxin) in the extract to its stabilizing action, the autoxidation kinetics of the two natural lipid systems (TGL and TGSO) were studied in the presence of different concentrations of esculetin, fraxetin, esculin and fraxin.

Figures 3 and 4 illustrate the kinetic results on esculetin and fraxetin, whereas Fig. 5 shows the accumulation of peroxides during the oxidation of TGL in the

Table 1. Stabilization factor F and oxidation rate ratio ORR for the inhibited oxidation of TGL and TGSO at 100°C in the presence of ethanolic extract from *Fraxinus ornus* bark

Concentration of the extract (%)	TGL		TGSO	
	F	ORR	F	ORR
0·05	4·8	0·28	3·6	0·60
0·10	6·1	0·28	4·0	0·50

TGL, IP_0 = 1·1 h, $W_0 = 2{\cdot}8 \times 10^{-6}$ M s^{-1}; TGSO, IP_0 = 0·25 h, $W_0 = 1{\cdot}8 \times 10^{-5}$ M s^{-1}.[a]

[a] Mean values from three independent experiments.

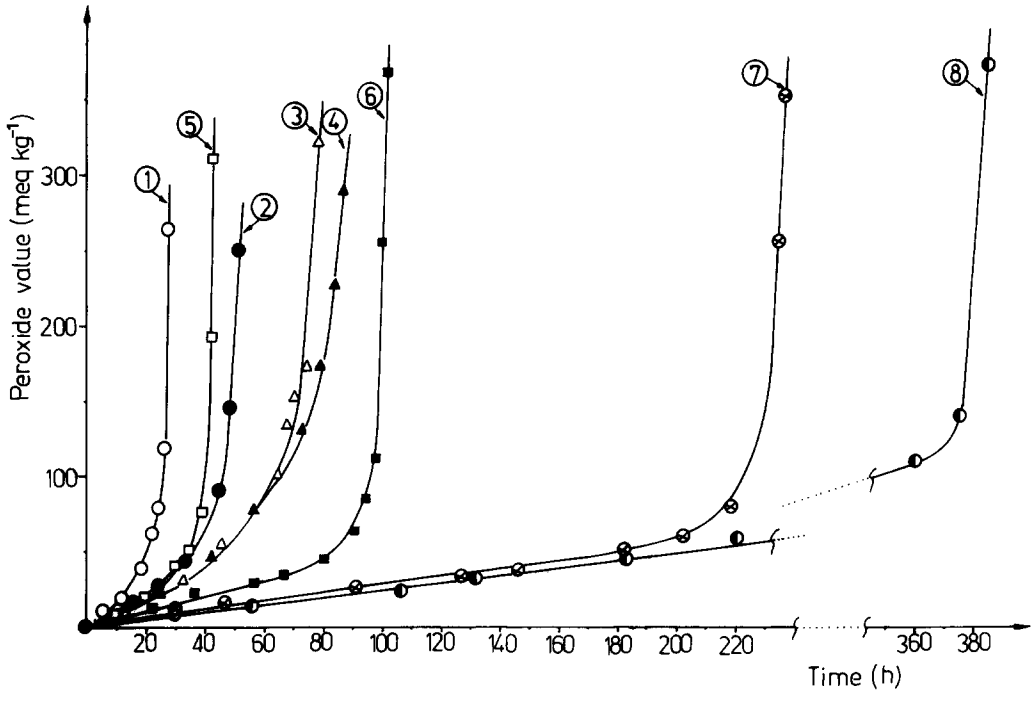


Fig. 3. Kinetic curves of peroxide accumulation during the oxidation of TGL at 100°C in the presence of different concentrations of esculetin (1–4) and fraxetin (5–8): 1 and 5, 0·01%; 2 and 6, 0·02%; 3 and 7, 0·05%; 4 and 8, 0·10%.

presence of 0·04% esculin and 0·04% fraxin. Addition of 0·02% esculin and 0·02% fraxin results in practically no stabilizing action. It has been established that the glucosides in TGSO possess no antioxidative activity.

The kinetic parameters characterizing the inhibitor action of esculetin and fraxetin are presented in Tables 2 and 3. Figure 6 illustrates the dependence of the stabilization factor F on the concentration of esculetin and fraxetin in TGL and TGSO. It is clear that the fraxetin possesses a higher effectivity than does the esculetin. Moreover, both substances are less effective inhibitors in the lipid system with a higher oxidizability (TGSO). The results obtained are in agreement with the fact that the presence of an electron-donating

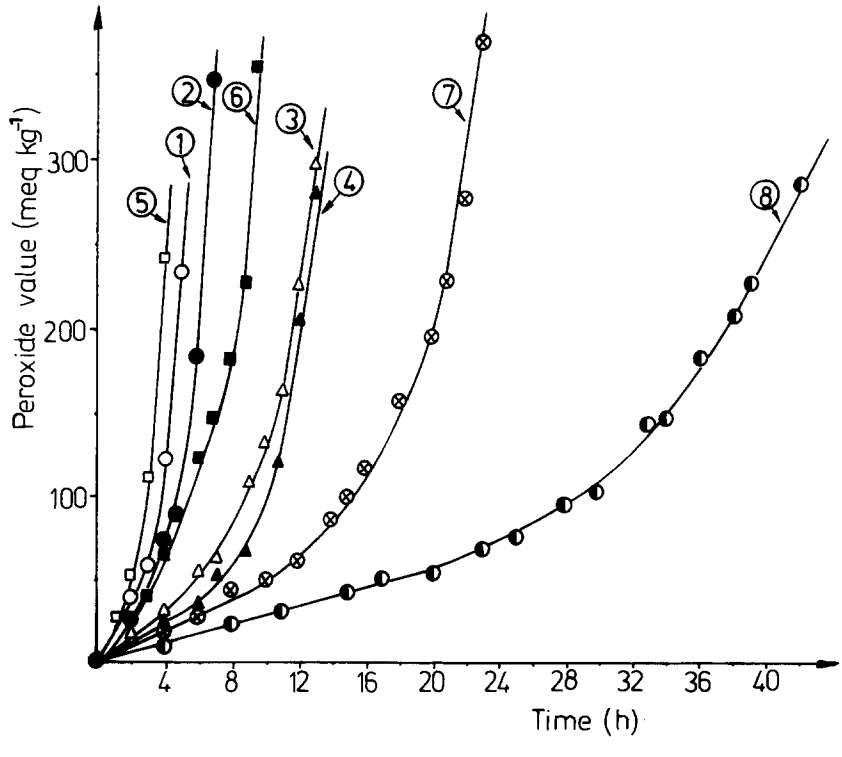


Fig. 4. Kinetic curves of peroxide accumulation during the oxidation of TGSO at 100°C in the presence of different concentrations of esculetin (1–4) and fraxetin (5–8). Symbols as in Fig. 3.

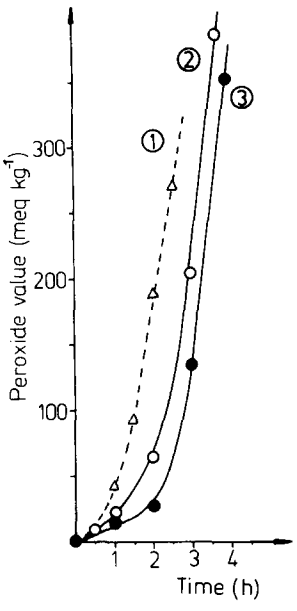


Fig. 5. Kinetic curves of peroxide accumulation during the oxidation of TGL at 100°C in the absence (1) and in the presence of 0·04% fraxin (2) and 0·04% esculin (3).

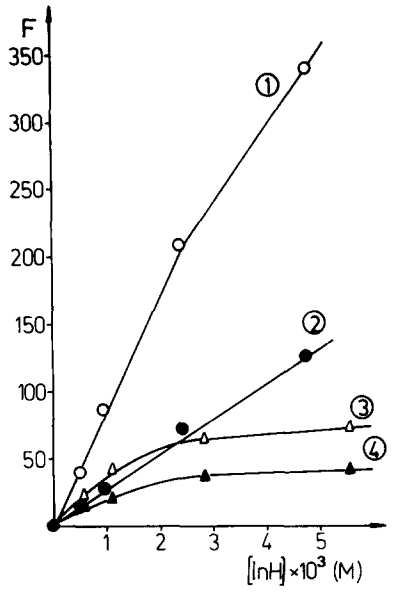


Fig. 6. Dependence of the stabilization factor F on fraxetin (1, 2) and esculetin (3, 4) concentration during oxidation of TGL (1, 3) and TGSO (2, 4) at 100°C.

group as a substituent in the benzene ring leads to increasing activity of the phenolic antioxidants in the chain termination reaction (7) (Roginskii, 1988). On the other hand, it becomes clear that in the system of lower $LO_2^{\cdot}$ activity (in this case TGL), the above effect due to the inhibitor structure, is more pronounced. Moreover, both hydroxycoumarins are antioxidants of a relatively high effectivity because, under the same oxidation conditions for $9{\cdot}1 \times 10^{-4}$ M BHT in TGL $F = 4{\cdot}8$, for $4{\cdot}6 \times 10^{-4}$ M α-tocopherol $F = 6{\cdot}5$ (Yanishlieva & Marinova, 1992), for $1{\cdot}0 \times 10^{-3}$ M ferulic acid $F = 3{\cdot}1$, for $1{\cdot}3 \times 10^{-3}$ M 3,4-dihydroxybenzoic acid $F = 13{\cdot}4$, and for $1{\cdot}1 \times 10^{-3}$ M caffeic acid $F = 62{\cdot}1$ (Marinova & Yanishlieva, 1992*b*). For $1{\cdot}0 \times 10^{-3}$ M ferulic acid in TGSO $F = 2{\cdot}6$, for $1{\cdot}3 \times 10^{-3}$ M 3,4-dihydroxybenzoic acid $F = 3{\cdot}6$, and for $1{\cdot}1 \times 10^{-3}$ M caffeic acid $F = 33{\cdot}6$ (Marinova & Yanishlieva, unpublished data).

If the antioxidant participates in chain termination only, the stabilization factor F will increase linearly with concentration, and $\bar{W}_{InH} = \bar{W}_i/f$ (Emanuel *et al.*, 1965). The absence of linearity in the dependence of stabilization factor F on the concentration of esculetin (Fig. 6, curves 3 and 4) is due to the participation of its molecules in other reactions: (11) and/or (12). In this case there is a relationship between the mean rate of inhibitor consumption $\bar{W}_{InH}$ and the inhibitor concentration [InH] (Emanuel *et al.*, 1965):

$$\bar{W}_{InH} = \bar{W}_i/f + K_{eff}[InH]^n \qquad (15)$$

where $\bar{W}_i$ is the mean rate of initiation during the induction period (M s^{-1}), and f is the stoichiometric coefficient of inhibition. After processing the experimental data (Figs 3 and 4), $\bar{W}_{InH}$ was determined according to the formula:

$$\bar{W}_{InH} = [InH]_o/IP_{inh} \text{ (M s}^{-1}\text{)} \qquad (16)$$

The presentation of the results obtained as dependence (15) shows that, in both lipid systems, the esculetin is consumed in one side reaction, because $n = 1$ (Fig. 7).

The effective rate constants (K_{eff}) of the side reaction in which esculetin participates, are as follows: $2{\cdot}5 \times 10^{-6}$ s^{-1} for TGL and $2{\cdot}0 \times 10^{-5}$ s^{-1} for TGSO. The consumption of the esculetin according to reaction (12) presupposes that K_{eff} should not depend on the character of

Table 2. Kinetic parameters characterizing the inhibited oxidation of TGL at 100°C

Antioxidant	Inhibitor concentration		Stabilization factor F	Inhibited oxidation rate $W_{inh} \times 10^7$ (M s^{-1})	ORR
	[InH] (%)	[InH] × 10^3 (M)			
Esculetin	0·01	0·56	22·7	1·98	0·07
	0·02	1·12	42·7	1·59	0·06
	0·05	2·81	64·5	1·32	0·05
	0·10	5·56	73·1	1·32	0·05
Fraxetin	0·01	0·48	38·2	1·26	0·05
	0·02	0·95	86·3	0·73	0·03
	0·05	2·38	208·0	0·43	0·02
	0·10	4·76	340·0	0·32	0·01

$IP_o = 1{\cdot}1$ h, $W_o = 2{\cdot}8 \times 10^{-6}$ M s^{-1}.

Table 3. Kinetic parameters characterizing the inhibited oxidation of TGSO at 100°C

Antioxidant	Inhibitor concentration		Stabilization factor F	Inhibited oxidation rate $W_{inh} \times 10^7$ (M s^{-1})	ORR
	[InH] (%)	[InH] × 10^3 (M)			
Esculetin	0·01	0·56	14·8	2·78	0·15
	0·02	1·12	20·8	1·73	0·09
	0·05	2·81	37·6	1·11	0·06
	0·10	5·56	41·2	0·84	0·05
Fraxetin	0·01	0·48	13·2	2·78	0·15
	0·02	0·95	27·2	1·73	0·09
	0·05	2·38	72·0	0·68	0·04
	0·10	4·76	125·0	0·37	0·02

IP_o = 0·25 h, $W_o = 1{\cdot}8 \times 10^{-5}$ M s^{-1}.

the medium, which is not the case. Therefore, the molecules of esculetin should take part in a side reaction (11) with the hydroperoxides. This conclusion is motivated by the different composition, and hence, different stabilities of the TGL and TGSO hydroperoxides discussed above. Then, the effective rate constants of esculetin consumption (reaction (11)) would be higher in TGSO, which is confirmed by the K_{eff} values obtained.

In both lipid systems $\bar{W}_{InH}$ for fraxetin is practically independent of its concentration, which means that its molecule is not consumed in side reactions. That is why, in contrast to esculetin, the effectivity of fraxetin grows considerably with increasing concentration (Fig. 6).

Figure 8 presents the dependencies of the oxidation rate ratio ORR on the concentration of esculetin and fraxetin in both lipid systems. It is clear that the fraxetin is a stronger antioxidant than esculetin, especially in the system of lower oxidizability (TGL). Moreover, both hydroxycoumarins are inhibitors of a great strength because under the same oxidation conditions for 9·1 × 10^{-4} M BHT in TGL ORR = 0·33, for 4·6 × 10^{-4} M α-tocopherol ORR = 0·13 (Yanishlieva & Marinova, 1992), for 1·0 × 10^{-3} M ferulic acid ORR = 0·59, and for 1·3 × 10^{-3} M 3,4-dihydroxybenzoic acid ORR = 0·07 (Marinova & Yanishlieva, 1992*b*). For 1·0 × 10^{-3} M ferulic acid in TGSO ORR = 0·60, for 1·3 × 10^{-3} 3,4-dihydroxybenzoic acid ORR = 0·60, and for 1·1 × 10^{-3} M caffeic acid ORR = 0·07 (Marinova & Yanishlieva, unpublished data). As mentioned above, the lower the degree of participation of the inhibitor units in chain propagation and initiation, the greater the inhibitor strength, i.e. the lower ORR.

It has been proved (Denisov & Khudyakov, 1987) that, if the inhibitor radical In· participates in one reaction of chain propagation [reaction (–7) or (10) or (14)], there is a linear correlation between W_{inh} and $[InH]^{0.5}$. Without participation of In·, a linear correlation between W_{inh} and $[InH]^{-1}$ is observed.

The presentation of the results obtained as such correlations (Fig. 9) leads to the conclusion that the radical of esculetin does not participate in chain propagation during oxidation of TGL and TGSO. The same is valid for the radical of fraxetin in TGL. During the

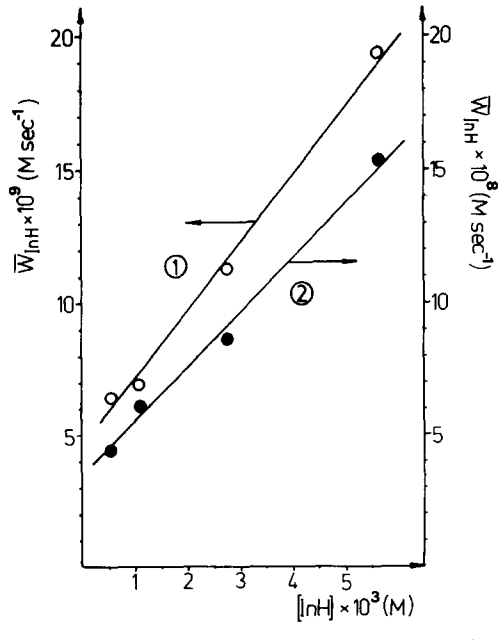


Fig. 7. Dependence of the mean rate of the esculetin consumption $\bar{W}_{InH}$ on its concentration [InH] during oxidation of TGL (1) and TGSO (2) at 100°C.

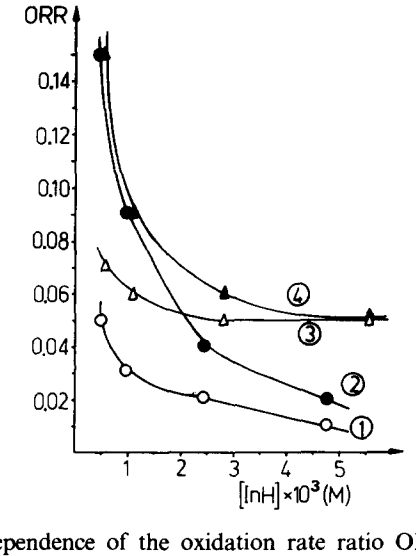


Fig. 8. Dependence of the oxidation rate ratio ORR on the concentration of fraxetin (1, 2) and esculetin (3, 4) [InH] during the oxidation of TGL (1, 3) and TGSO (2, 4) at 100°C.

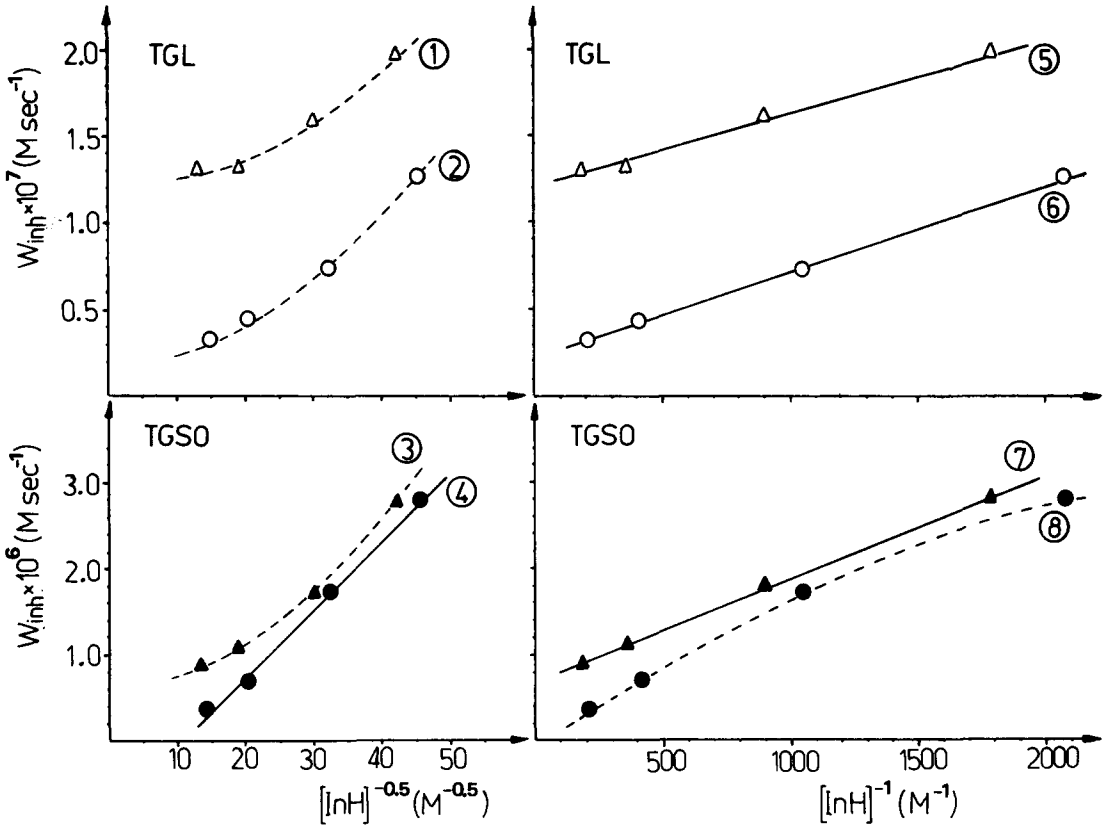


Fig. 9. Dependence of the rate of inhibited oxidation W_{inh} on the concentration $[InH]^{-0.5}$ (1–4) and $[InH]^{-1}$ (5–8) of esculetin (1,3,5,7) and fraxetin (2,4,6,8) during the oxidation of TGL and TGSO at 100°C.

oxidation of TGSO, the radical of fraxetin takes part in chain propagation. Most probably it is reaction (10) that proceeds in the latter case, because during the initial stage of the process, when the hydroperoxide concentration is low, the rate of reaction (–7) is negligibly small in comparison with the rate of reaction (10) (Roginskii, 1990), and reaction (14) does not depend on the character of the substrate being oxidized.

The above results and their interpretation mean that the higher ORR values for esculetin are due to the participation of its molecule in reaction (11) where radical products are formed and chains are initiated.

After processing the kinetic curves of TGL autoxidation in the presence of 0·04% esculin and 0·04% fraxin (Fig. 5), the following data for F and ORR are obtained: esculin, $F = 2{\cdot}5$, ORR = 0·7; fraxin, $F = 2{\cdot}1$, ORR = 1·0. As was mentioned above, in TGSO these glucosides exhibit no antioxidative action.

Comparison of the F and ORR values for the glucosides with the values of the same parameters for aglucones (Tables 2 and 3) at almost the same molar concentrations (0·02% aglucones) means that the glucoside bonding of one of the phenol groups leads to a significant weakening of the inhibiting properties. The results obtained indicate blocking of the more active phenol group in the fraxin, due to which the difference in antioxidative activities of glucosides **1a** and **2a** is, in contrast to aglucones **1** and **2**, negligible.

Proceeding from the antioxidative effect of **1**, **2**, **1a** and **2a** in TGL and TGSO and the content of these compounds in the extract, and eliminating the possibility of antagonism or synergism between them, it was calculated what part of the extract-inhibiting action was due to these substances. It was established that the four compounds under consideration determined two-thirds of the antioxidative effectivity of the extract in TGL and half of the effectivity in TGSO.

Thin-layer chromatographic analysis (Marinova & Yanishlieva, 1986) of the antioxidatively acting components in the ethanolic extract from *Fraxinus ornus* bark revealed the presence of additional antioxidatively acting compounds. Among them, the 2-(3,4-dihydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside, recently isolated from the bark extract (Vassileva & Kostova, unpublished data), demonstrated a significant activity.

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