ANTIOXIDANT ACTIVITY AND SYNERGISM OF BUTYLHYDROXYTOLUENE IN PROCESSES OF OXIDATION OF RETINOL ESTERS

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A number of studies have been devoted to the investigation of the oxidation of retinol esters (vitamin A) and the possibility of stabilization of these compounds by additions of various inhibitors [1-6]. In this case, the most varied systems have been used as the medium in which oxidation was conducted – from fish oil [1] to n-decane [3]. Since solutions of vitamin A esters in vegetable oil are among the drug forms of vitamin A, data on the stability precisely of such systems are of special interest. Recently a method was proposed for the accelerated investigation of the stability of oil solutions of retinol esters, and used to study the oxidation of retinyl acetate in soybean oil and the influence of various inhibitors on this process [5]. This method is simple, and its use is especially advisable in the investigation of comparatively dilute solutions (with activity up to 300,000 IU/g). However, for more concentrated solutions (500,000 and 1,000,000 IU/g), the differences between the properties of the investigated system under conditions of accelerated aging and under standard conditions of storage are increased. Moreover, the accelerated method described in [5] is not suitable for evaluating the influence of inhibitors on the stability of solid samples. In view of this, we have developed a new method, a peculiarity of which is the possibility of model study of oxidation processes occurring in systems with a high content of retinol esters (oil solutions with an activity of 500,000 IU/g and higher and pure substances).

In this work we studied the antioxidant activity of two inhibitors – butylhydroxytoluene (BHT; 2,6-di-tert-butyl-4-methylphenol) and butylhydroxyanisole (BHA; a mixture of 2- and 3-tert-butyl-4-methoxyphenol with a predominant content of the 3-isomer [7]) – in processes of oxidation of retinyl palmitate and retinyl acetate.

EXPERIMENTAL

Retinyl acetate with mp 59.0-59.5°, produced by special purification of the preparation [8], was used. Retinyl palmitate was produced by transesterification of retinyl acetate with methyl palmitate in the presence of sodium methylate. It was purified by chromatography on a column with neutral aluminum oxide. The purified retinyl palmitate is characterized by an absorption band in the UV spectrum with λ_{max} = 30,700 cm^{-1} and $E_1^{1\%}{}_{cm}$ = 970 ± 5 (in n-hexane; according to the literature data [9], $E_1^{1\%}{}_{cm}$ = 975).

The samples to be oxidized were films applied on quartz plates, the thickness of which varied from ~0.2 to ~1.0 μ, depending on the concentration of the retinyl ester. The course of the oxidation was followed according to the decrease in the continuously recorded optical density at the maximum of the absorption band. The methods of producing the films and their oxidation were described in [6, 8]. The time from the moment of admission of oxygen to the system until the beginning of oxidation was taken as the value of the induction period. The oxidation of films with a retinyl palmitate concentration 0.525 mole/kg (500,000 IU/g), as well as films with a retinyl palmitate concentration of 1.050 mole/kg and an inhibitor content of more than 10^{-2} mole/kg, was conducted by a somewhat modified method: Plates with films applied on them, fastened in special holders, were exposed to an atmosphere of oxygen in an air-filled constant-temperature chamber, periodically measuring the optical density. In all cases the oxidation was conducted at 25° and an oxygen pressure above 200 mm Hg, since under these conditions the induction period does not depend on P_{O_2}.

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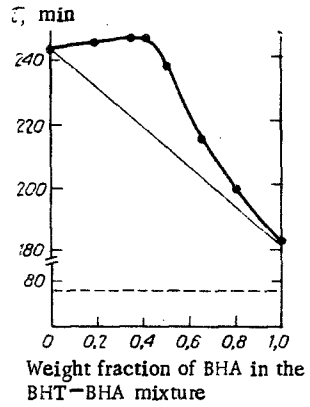


Fig. 1. Dependence of the induction period (τ) of the inhibited oxidation of retinyl palmitate in soybean oil at 25° on the composition of the inhibiting mixture. Concentration of retinyl palmitate 1.050 mole/kg (1,000,000 IU/g); summary concentration of inhibitors 0.02% by weight. Dotted line: duration of the incubation period in the absence of the inhibitor.

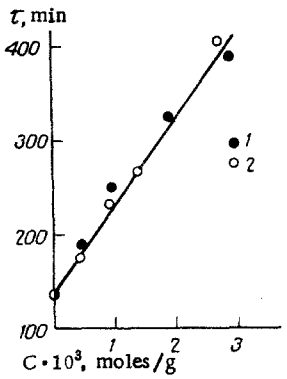


Fig. 2. Influence of the inhibitor concentration (C) on the duration of the induction period (τ) of the oxidation of retinyl palmitate (1.050 mole/kg) in soybeam oil at 25°. 1) BHT; 2) BHT + BHA (weight ratio 65:35).

RESULTS AND DISCUSSION

It is known that BHT and BHA are effective inhibitors of oxidation and are widely used to inhibit the oxidation of various compounds [7], including retinol esters in medicinal preparations. Data on the comparative effectiveness of the inhibition of the oxidation of dilute (0.105 mole/kg) solutions of retinyl acetate in soybean oil by these inhibitors were obtained in [5], in which it was shown that in this system BHT inhibits oxidation more effectively than BHA.

As can be seen from Fig. 1, an analogous curve is observed for concentrated solutions of retinyl palmitate in soybean oil as well. Moreover, when the two inhibitors are used together, a distinct synergistic effect is observed. The deviation of the experimental points from a straight line in Fig. 1, characterizing the deviation from additivity, is a maximum at a mole ratio of BHT:BHA in the mixture of 0.6:0.4 (synergistic mixture) and a summary concentration of them of $(9.0\text{-}9.7)\cdot10^{-4}$ mole/kg (0.20% by weight). Thus, although in this system BHA is a less effective inhibitor than BHT, its use as a component of the inhibiting mixture does not lead to a decrease in its effectiveness in comparison with BHT alone, with a relative weight content of BHA all the way up to 0.55 (see Fig. 1).

The relative effectiveness of the inhibition of processes of oxidation of retinol esters by BHT, BHA, and their mixtures depends on the concentration of all the components of the reaction system. As can be seen from Fig. 2, for an oil solution of retinyl palmitate with concentration 1.050 mole/kg, the effectiveness of BHT and a synergistic mixture is the same when the concentration of the inhibitors is varied from $\sim4.5\cdot10^{-4}$ to $2.8\cdot10^{-3}$ mole/kg. However, for solutions with a lower retinyl palmitate concentration and a higher concentration of the inhibitors, the synergistic effect increases sharply (Fig. 3). Moreover, the relative effectiveness of BHT and BHA varies as their concentration and the concentration of the retinol esters increase. Thus, at concentrations of the order of 10^{-3} mole/kg, BHT is a more effective inhibitor of the oxidation of retinol esters in oil solutions than BHA; at inhibitor concentrations of the order of $2.5\cdot10^{-2}$ mole/kg, their effectiveness is practically the same (see Table 1), while in the oxidation of 100% retinol esters, BHA is a more effective inhibitor (Fig. 4). In an investigation of the oxidation of dilute solutions of retinyl acetate in an inert solvent (n-decane), it was also established that BHA is a more effective inhibitor than BHT [5].

The results obtained can be explained on the basis of the available concepts of the reactivity of phenolic inhibitors used and the phenoxyl radicals corresponding to them in reactions of inhibited oxidation. The general scheme of the process of inhibited oxidation takes the following form:

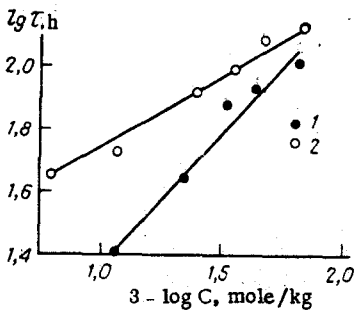


Fig. 3. Dependence of the induction periods (τ) of the inhibited oxidation of retinyl palmitate in soybeam oil on the concentration of the inhibitors (C) at 25° and a retinyl palmitate concentration of 0.525 mole/kg. 1) BHT; 2) BHT + BHA (weight ratio 65:35).

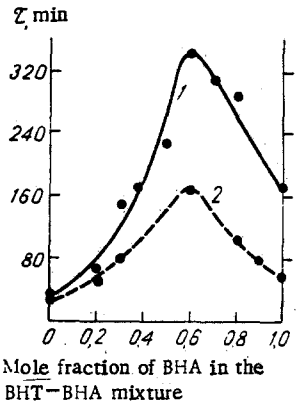


Fig. 4. Dependence of the induction period (τ) of the inhibited oxidation of retinol esters at 25° on the composition of the inhibiting mixture of BHT-BHA. 1) Retinyl acetate, total concentration of inhibitors $\sim 3.1 \cdot 10^{-3}$ mole/kg; 2) retinyl palmitate, total concentration of inhibitors $4.8 \cdot 10^{-3}$ mole/kg.

$$RH \longrightarrow 2R\cdot \quad (1)$$

$$R\cdot + O_2 \xrightarrow{k_2} RO_2\cdot \quad (2)$$

$$RO_2\cdot + RH \xrightarrow{k_3} RO_2H + R\cdot \quad (3)$$
$$\searrow \; RO_2R\cdot$$

$$2RO_2\cdot \xrightarrow{k_4} \text{product} \quad (4)$$

$$RO_2\cdot + InH \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} RO_2H + In\cdot \quad (5, -5)$$

$$In\cdot + RO_2\cdot \xrightarrow{k_6} \text{product} \quad (6)$$

$$2In\cdot \xrightarrow{k_7} \text{product} \quad (7)$$

$$In\cdot + RH \xrightarrow{k_8} InH + R\cdot \quad (8)$$

$$In\cdot + R'H \xrightarrow{k'_8} InH + R'\cdot \quad (8')$$

$$In\cdot + In'H \xrightarrow{k_9} InH + In'\cdot \quad (9)$$

$$RO_2\cdot + In'H \xrightarrow{k_{10}} RO_2H + In'\cdot \quad (10)$$

$$In\cdot + In'\cdot \xrightarrow{k_{11}} InH + \text{product} \quad (11)$$

$$RO_2\cdot + In'\cdot \xrightarrow{k_{12}} \text{product} \quad (12)$$

Here InH = BHA, In'H = BHT, RH is a retinol ester, and R'H represents the components of vegetable oil containing labile hydrogen atoms. BHA, containing primarily 3-tert-butyl-4-methoxyphenol, is a sterically unhindered phenolic inhibitor; BHT is a sterically hindered phenol.

It is known that 2-tert-butyl-4-methoxyphenol [10], like unsubstituted 4-methoxyphenol [11], is a substantially more effective inhibitor of liquid-phase oxidation than 2,6-di-tert-butyl-4-methylphenol. This fact agrees with the data cited above on the inhibition of the oxidation of retinyl acetate and retinyl palmitate by BHT, BHA, and their mixture (see Fig. 4, Table 1). It can be assumed that the unhindred 3-tert-butyl-4-methoxyphenoxyl radicals formed from BHA according to reaction (5) are capable of participating in reactions of chain transfer (−5), (8) and (8') (analogously to the 4-methoxyphenoxyl radicals [11]). As a result, reaction (5) does not always lead to termination of the kinetic chain of oxidation, and the average number of inhibitor molecules corresponding to one event of chain termination increases. But this in turn leads to a shortening of the induction period. The magnitude of this effect should depend on the ratio of the constants k_3/k_8, k_3/k_5, k_3/k'_8 and the ratio [R'H]/[R'H].

TABLE 1. Relative Effectiveness of the Inhibition of Oxidation of Retinol Esters by BHT, BHA, and Their Mixtures

System being oxidized	Retinol concentration, moles/kg	Inhibitor concentration, moles/kg		Relative effectiveness *	Note
		BHT	BHA		
Solution of retinyl palmitate in soybean oil	0,105	$9{,}1\cdot10^{-4}$	—	1,00	According to the data of [5]
The same	1,0105	—	10^{-3}	0,17	According to the data of [5]
Solution of retinyl palmitate in soybean oil	0,525	$1{,}14\cdot10^{-2}$	—	1,0	
The same	0,525	$7{,}39\cdot10^{-3}$	$4{,}86\cdot10^{-3}$	3,1	
" "	0,525	$4{,}43\cdot10^{-2}$	$2{,}92\cdot10^{-2}$	1,3†	
Solution of retinyl palmitate in soybean oil	1,050	$9{,}1\cdot10^{-4}$	—	1,00	
The same	1,050	—	10^{-3}	0,62	
" "	1,050	$5{,}91\cdot10^{-4}$	$3{,}89\cdot10^{-4}$	1,02	
" "	1,050	$2{,}27\cdot10^{-2}$	—	1,0	
" "	1,050	—	$2{,}77\cdot10^{-2}$	1,0	
" "	1,050	$1{,}50\cdot10^{-2}$	$2{,}77\cdot10^{-2}$	2,8	
" "	1,050	$4{,}54\cdot10^{-2}$	$2{,}77\cdot10^{-2}$	2,8	
Retinyl palmitate	1,940	$4{,}78\cdot10^{-3}$	—	1,0	
" "	1,940	—	$4{,}78\cdot10^{-3}$	2,7	
" "	1,940	$1{,}90\cdot10^{-3}$	$2{,}86\cdot10^{-3}$	10,0	Mole ratio BHT:BHA 0,4:0,6
Retinyl acetate	3,070	$3{,}18\cdot10^{-2}$	—	1,0	
" "	3,070	—	$3{,}18\cdot10^{-2}$	3,9	
" "	3,070	$1{,}22\cdot10^{-2}$	$1{,}83\cdot10^{-2}$	8,5	Mole ratio BHT:BHA =0,4:0,6

*The relative increase in the induction period in the presence of the inhibitor, calculated according to the formula $(\tau-\tau_0)/(\tau-\tau_0)_{BHT}$, where τ is the induction period in the presence of the amount of the inhibitor indicated in the table; τ_0 is the induction period in the absence of the inhibitor.

†With respect to the effectiveness of BHT at a concentration of it of $6.82\cdot10^{-2}$ mole/kg.

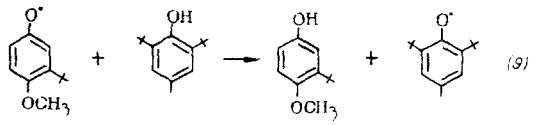
The scheme outlined is confirmed by the results obtained. According to this scheme, with increasing BHA concentration, the reactions of chain transfer (−5), (8), and (8'), in which 2,6-di-tert-butyl-4-methylphenoxy radicals do not participate, should play an ever greater role [12]. Therefore, it might be expected that in the case of inhibition of oxidation by BHA or a mixture of it with BHT, the induction period will increase more slowly with increasing inhibitor concentration than when BHT is used alone. As can be seen from Fig. 3, such a picture is actually observed, and $\tau \approx C_{inh}^{0.45}$ for a mixture of BHT-BHA and $\tau \approx C_{inh}^{0.63}$ for BHT (τ is the induction period; C_{inh} is the inhibitor concentration).

The synergistic effect exhibited by a BHT−BHA mixture is evidently due to regeneration of the more active inhibitor at the expense of the less active inhibitor (just as in the case of other mixtures of unhindered and hindered phenols [10, 12]). Assuming that the reactivity of BHA in reactions (−5, (8), and (8') is no lower than the reactivity of 4-methoxyphenol, on the basis of the data cited in [10, 12, 13] we have:

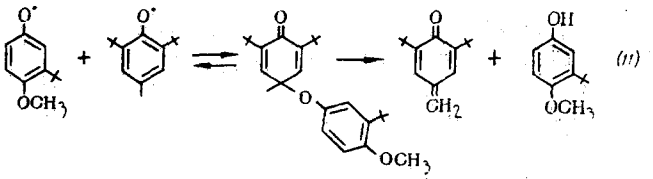
$$k_5 \gg k_{10} \text{ and } V_5 = k_5\,[RO_2^{\cdot}][InH] \gg V_{10} = k_{10}\,[RO_2^{\cdot}][In'H]$$

$$k_{-5} \ll k_9 \quad \text{and} \quad V_{-5} = k_{-5}\,[In^{\cdot}][RO_2H) \ll V_9 = k_9\,[In^{\cdot}][In'H].$$

Thus, when a BHT−BHA mixture is used, the inhibition of the oxidation process is due to the interaction of BHA with peroxide radicals, and BHA is regenerated in reactions (9) and (11) [10, 14]:



(9)



(11)

Since $k_9 \gg k_{-5}$, $k_9 \gg k_8$, and $k_9 \gg k'_8$ at definite ratios of the components of the reaction mixture, BHA is regenerated according to reactions (9) and (11) more rapidly than the phenoxyl radical corresponding to it can enter into reactions (−5), (8) or (8'). Therefore, the value of the synergistic effect, as well as the relative effectiveness of BHT and BHA, is determined both by the absolute values of the concentrations of the retinol esters, oil, and inhibitors and by their ratios. It is interesting that the introduction of a BHT−BHA mixture with a 0.4:0.6 mole ratio of the components into an oil solution of retinyl palmitate leads to the same effect as the use of the mixture with a BHT:BHA ratio equal to ≈ 0.6:0.4, but with three times the amount of BHT (at the same BHA concentration, $2.77 \cdot 10^{-2}$ mole/kg − see Table 1). Thus, the correct selection of the composition of the inhibiting mixture can permit a substantial reduction of the inhibitor concentration in the preparation, while its resistance to oxidation is preserved.

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