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REACTION KINETICS OF α-TOCOPHEROXYL RADICAL WITH BIOLOGICALLY AND PHARMACOLOGICALLY ACTIVE SUBSTANCES

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The α-tocopheroxyl radical (αTR˙) generated in the reaction with 1,1-diphenyl-2-picrylhydrazyl in n-butanol decayed according to second-order kinetics with a rate constant $k_\alpha = 3.10^3$ $M^{-1}s^{-1}$ as determined by EPR spectroscopy. Various biologically and pharmacologically active substances like isoprenaline (ISO), epi nephrine (EPI), histamine (HIS), stobadine (STO), nafazatrom (NAF) and Kampo C medicine (KMC) accelerated the decay rate of αTR˙. The whole process is formally a third-order reaction with the rate constants (in 10^9 $M^{-2}s^{-1}$): k_s(ISO) = 1.28, k_s(NAF) = 1.25, k_s(EPI) = 0.6, k_s(HIS) = 0.4, and k_s(STO) = 0.1. In the kinetics of the reaction mechanism, bimolecular intermediates are assumed and the rate constants of their formation were determined.

KEY WORDS: α-tocopherol, α-tocopheroxyl radical, reduction, kinetics.

INTRODUCTION

α-Tocopherol (αTOC) functions as an important natural anti-oxidant in biological systems by scavenging deleterious radicals in cell membranes such as lipid peroxyl radicals[1,2]. In the process of radical scavenging αTOC is oxidized to the relatively stable α-tocopheroxyl radical (αTR˙). The α-tocopheroxyl radical may be regenerated back to the αTOC by ascorbic acid as suggested by Packer *et al.*[3].

In this work we studied interaction of αTR˙ with variety of biologically and pharmacologically active compounds, known to play a role in free radical mediated damage: catecholamines epi nephrine (EPI) and isoprenaline (ISO) are thought to be involved in myocardial ischemia/reperfusion damage and induce peroxidation in myocardium[4,5]. In the absence of catalytical iron or copper ions, significant antioxidant properties of those compounds were found (K. Ondriaš, M. Hromadová, unpublished results). Nafazatrom (NAF) is an antithrombotic agent which stimulates endothelial release of prostacyclin, inhibits arachidonate metabolism by lipoxygenase enzymes[7], and was found to protect cardiac lipids against oxidative injury[8]. Stobadine (STO) is a new cardio-protective drug with antihypoxic and antiarrhythmic effects[9], which seem to be associated with the antioxidant properties of this drug[10,11]. Histamine (HIS), a naturally occurring

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amine, was shown to be released from granules of mast cells upon attack of free radicals[12]. The Japanese herbal Kampo medicines exert positive effects on cardiovascular circulation[13,14] and in a model of chronic cerebral ischemia. Antioxidant properties of Kampo medicines against oxidation of membrane lipids[15] and low density lipoproteins[16] and their $OH^{\cdot}$ radical scavenging abilities were also demonstrated[17].

MATERIALS AND METHODS

Chemicals were obtained from the following sources: α-tocopherol (αTOC) from Sigma, $DPPH^{\cdot}$ (1,1-diphenyl-2-picryl-hydrazyl) from Fluka, TEMPO (2,2,6,6-tetramethylpiperidinyl-N-oxyl) from Sigma, L-epinephrine bitartate (EPI) from Calbiochem, isoprenaline hydrochloride (ISO), nafazatrom (NAF) from the Institute of Drug Research, Modra (Czechoslovakia), stobadine ((-)-cis-2,8-dimethyl-2,3,4,4a,5,9b-hexahydro-1H-pyrido (4,3b) indole dihydrochloride; STO) was synthesized at the Institute of Organic Chemistry and Biochemistry, Prague (Czechoslovakia), Kampo C medicine (KMC) was from Tsumura & Co. (Japan). All other chemicals were of analytical grade from commercial sources.

α-Tocopherol free radicals were generated in a reaction of αTOC with $DPPH^{\cdot}$ according to Eq. (1) as described by Rousseau-Richard *et al.*[18].

$$\alpha TOC + DPPH^{\cdot} \rightarrow \alpha TR^{\cdot} + DPPHH \qquad (1)$$

The consumption of $DPPH^{\cdot}$ was monitored by visible spectroscopy observing the absorbance of $DPPH^{\cdot}$ at 520 nm. The reaction of $DPPH^{\cdot}$ with αTOC was fast: $DPPH^{\cdot}$ disappeared within 30 seconds after mixing of 0.2 mM $DPPH^{\cdot}$ with 0.3 mM αTOC in n-butanol.

Concentrations of the $\alpha TR^{\cdot}$ were determined using TEMPO radical as a standard. The spectra of TEMPO standard and $\alpha TR^{\cdot}$, both in n-butanol solutions, were compared under identical physical arrangements of the samples and identical instrumental settings, except for receiver gain. The concentration of $\alpha TR^{\cdot}$, 60 seconds after mixing $DPPH^{\cdot}$ with αTOC, was 4 μM. As the line shape did not change during the experiments, the relative integral intensity of EPR spectra, $I^{EPR}_{\alpha TR^{\cdot}}$ was described with the line heights.

RESULTS AND DISCUSSION

Generation of $\alpha TR^{\cdot}$

$DPPH^{\cdot}$ as well as α-tocopherol entering the reaction was characterized by the EPR spectra shown in the inset of Figure 1: $DPPH^{\cdot}$ with a typical quintet (Figure 1.1), and αTOC solution was EPR silent (Figure 1.2). 30 seconds after mixing $DPPH^{\cdot}$ with αTOC, the spectrum of $DPPH^{\cdot}$ disappeared completely and the spectrum of $\alpha TR^{\cdot}$ was observed as shown in the same inset (Figure 1.3). The immediately measured self decay of $\alpha TR^{\cdot}$ is illustrated in Figure 1a. This is described by second order reaction kinetics with a very good reproducibility throughout the measurements. Such self decay measurements are quoted in the corresponding Figures as reference.

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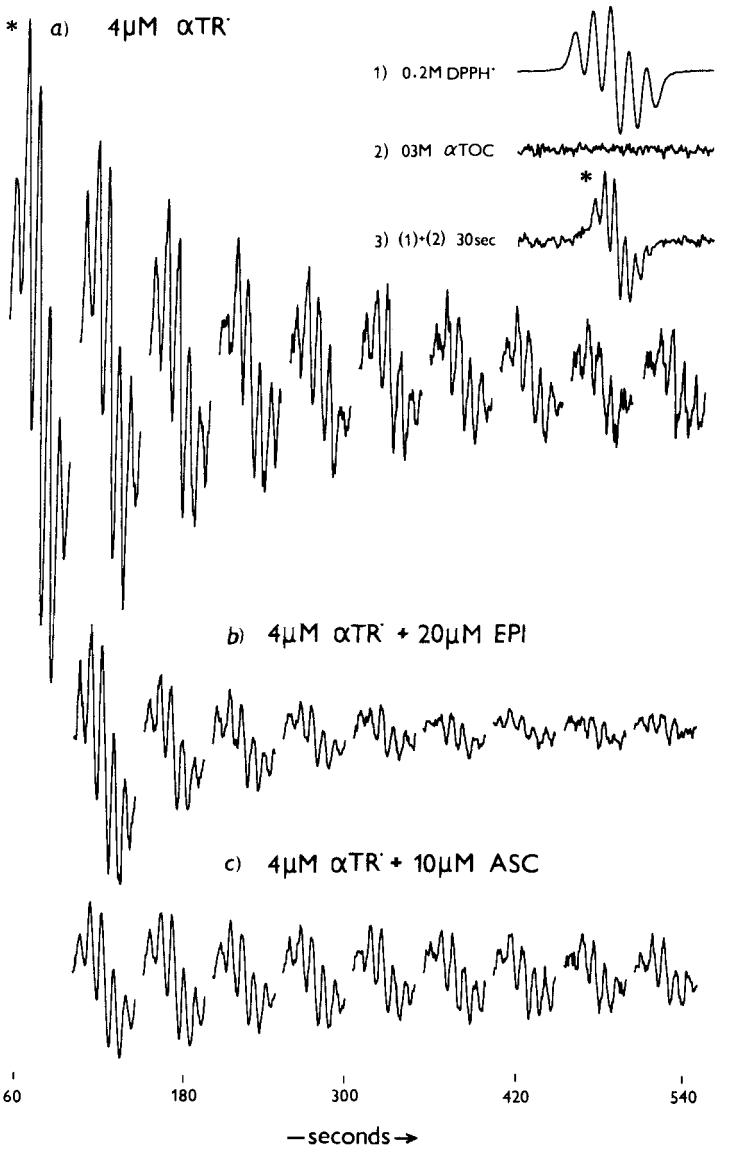

FIGURE 1 The time dependence of EPR spectra of α-tocopherol radical αTR·. Trace a): 4 μM αTR· in n-butanol, trace b): same in the presence of 20 μM epinephrine, trace c): same as a) in the presence of 10 μM of ascorbic acid.

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The time resolution and the kinetics

30 seconds after mixing DPPH$^{\cdot}$ with αTOC, a defined source of αTR$^{\cdot}$ was obtained. This point in time is referred in the kinetic measurements as zero ($t = 0$). Exactly at this time, various substrates were added to the resulting solution of αTR$^{\cdot}$, vortexed, put into the EPR tubes, and spectra were recorded. This took an additional 40 to 60 seconds. What about the kinetics between $t = 0$ and the first EPR measurement? Let us consider two alternatives: i) the reaction with substrate was rapid and finished before the first EPR measurement begun; ii) the reaction was slower and at least a part of its kinetics was monitored by EPR measurements.

Rapid reaction of αTR$^{\cdot}$ with substrates

Ascorbic acid as a substrate may be used as an example for a rapid decay. Figure 1c shows the time decay of αTR$^{\cdot}$ after addition of 10 μM of ascorbic acid. A substantial decrease of αTR$^{\cdot}$ was found in Figure 1c if compared with Figure 1a. Similar experiments were carried out for various concentrations of ascorbic acid. The kinetic evaluation of such measurements is shown in Figure 2. We assume that the reaction of αTR$^{\cdot}$ with ascorbic acid was rapid and was finished before the first EPR measurement. Then actually, only the self decay of αTR$^{\cdot}$ at its various initial concentrations was monitored from the first EPR measurement. The kinetic evaluation of systems with such a rapid reaction between substrate and αTR$^{\cdot}$ shows a characteristic kinetic pattern. Namely, that the time dependence of $1/[\alpha TR^{\cdot}]$ gives straight lines with similar parallel slopes but shifted for various ascorbic acid concentrations and having different intercepts on the $1/[\alpha TR^{\cdot}]$ axis. This is demonstrated in Figure 2. Such a kinetic pattern was not observed when other substrates were investigated.

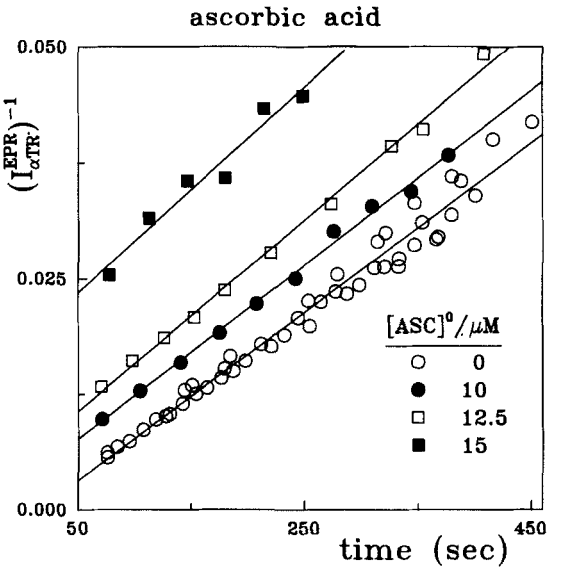

FIGURE 2 The time dependence of reciprocal intensity of EPR spectra $(I^{EPR}_{\alpha TR^{\cdot}})^{-1}$ of α-tocopherol radical αTR$^{\cdot}$ at various initial concentrations of ascorbic acid, [ASC^0].

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The time resolved reactions of αTR· with substrates

If the reactions between αTR· and substrates were considerably longer than 60 seconds, their time profile can be readily followed with the technique described. Mathematical evaluation of the kinetics allows the decay function of αTR· to be reliably approximated from 0 to 60 seconds time. Figure 1b illustrates the αTR· decay after the addition of 20 μM epinephrine. If Figure 1b is compared with those of Figure 1a and Figure 1c, a considerable acceleration of αTR· decay is obvious in Figure 1b. Similar kinetic evaluations as described by the rapid reaction above resulted in a kinetic pattern, where the slopes of the lines are dependent on the substrate concentrations. This holds for all investigated substrates as shown in Figure 3. According to the model suggested below and from the slope dependence, k_{app}, of those line on the initial substrate, $[SX]^0$, ($k_{app} = k_\alpha + k_s[SX]^0$), not only the rate constants k_s for the reaction substrate – αTR· but also k_α for the simultaneous αTR· self decay can be evaluated. A characteristic feature of such kinetic model is that the lines show various slopes and have approximately the same intercept on the $1/I^{EPR}_{\alpha TR\cdot}$ axis. This holds for all investigated substrates shown in Figure 3.

Kinetics of αTR· self decay

The decay of αTR· is a bimolecular reaction and can be described by the second order kinetics according to the Equations (2) and (3):

$$\alpha TR\cdot + \alpha TR\cdot \xrightarrow{k\alpha} \text{non-radical products} \quad (2)$$

$$-d[\alpha TR\cdot]/dt = k_\alpha[\alpha TR\cdot]^2 \quad (3)$$

Solution of the Equation (3) gives Equation (4) for the following boundary conditions: at the time t_0 the concentration of αTR· was $[\alpha TR\cdot]_{0'}$ and at the time t the concentration of αTR· was [αTR·].

$$1/[\alpha TR\cdot] - 1/[\alpha TR\cdot]_0 = k_\alpha(t - t_0) \quad (4)$$

Thus, if the decay of the αTR· were a second-order reaction, a linear dependence of the 1/[αTR·] vs. time should be found. This was confirmed indeed as shown in Figures 3a–f for all control samples (i.e. the samples in the absence of substrate). From the slope of 1/[αTR·] vs. time the rate constant, k_α, of the bimolecular decay of αTR· was determined according to Equation (4) and a value of $k_\alpha = 3.10^3\ M^{-1}s^{-1}$ was found. The magnitude of this rate constant fell within the range reported by others for the bimolecular decay of αTR· in different solvents: $1.4 \times 10^3\ M^{-1}s^{-1}$ in ethanol[18], $560\ M^{-1}s^{-1}$ in heptanol[18], $1.6 \times 10^3\ M^{-1}s^{-1}$ in benzene[19], and $1 \times 10^4\ M^{-1}s^{-1}$ in ethanol[20].

The bimolecular decay of αTR· is a very complex process which may lead to the formation of tocopheryl quinone and regeneration of αTOC[19]. Other reaction pathways include formation of dimers and trimers[6], formed via radical isomerization of αTR·[21].

Kinetics of αTR· decay with various substrates

Further studies were focused on the kinetics of αTR·-decay in the presence of various substrates. Figures 3a–f illustrate the decay of αTR· upon addition of

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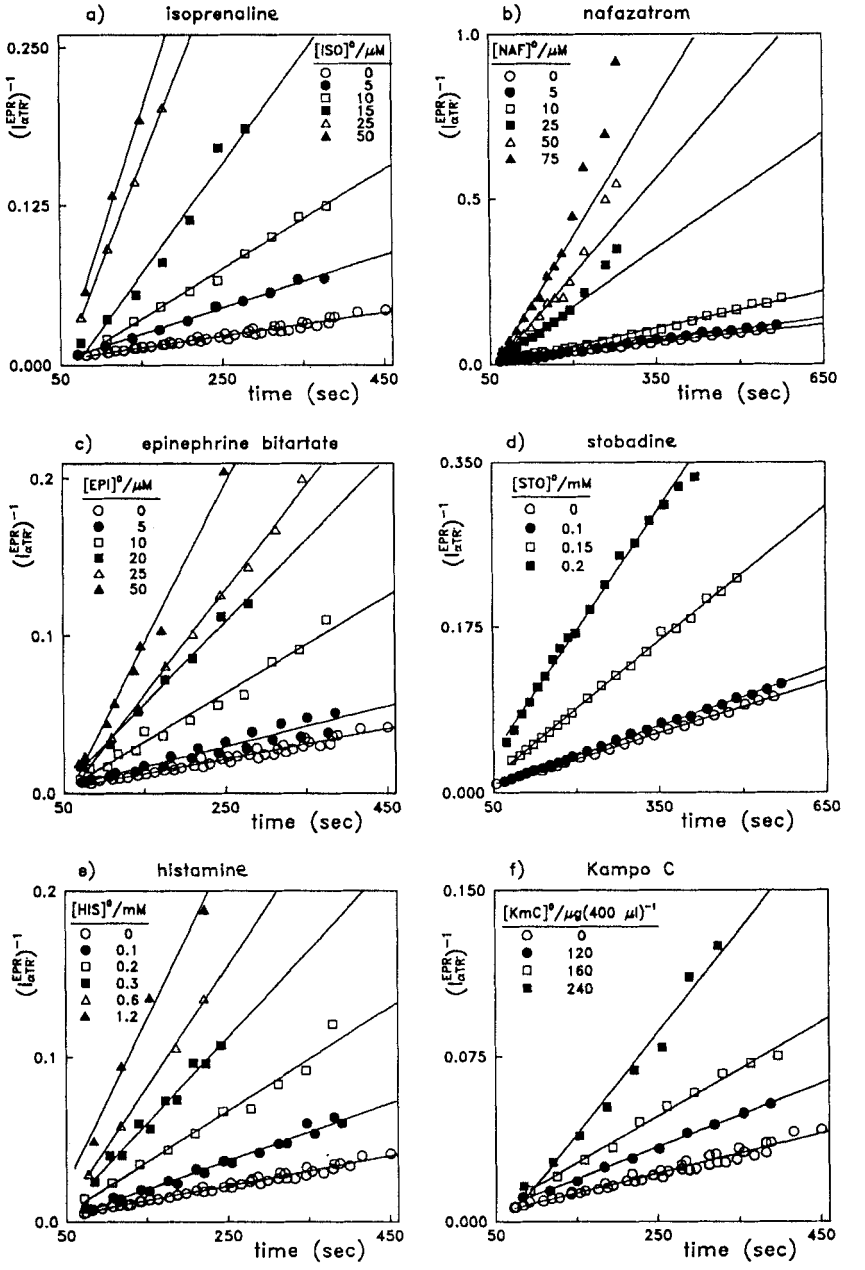

FIGURE 3 Plot of the reciprocal $I_{\alpha TR}^{EPR}$ values (see legend to Fig. 1) against time at various initial concentrations of $[SX]^0$ of the following substrates, SX: a) isoprenaline, b) stobadine, c) epinephrine, nafazatrom, e) histamine, f) Kampo C.

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various concentrations of the test compounds: isoprenaline (Figure 3a), epinephrine (Figure 3c), histamine (Figure 3e), stobadine (Figure 3d), nafazatrom (Figure 3b), Kampo C (Figure 3f) . All substrates accelerated the decay of $\alpha TR^{\cdot}$ which was increased with increasing substrate concentrations. In the presence of these substrates a linear time dependence of $[\alpha TR^{\cdot}]^{-1}$ was confirmed for all investigated compounds as shown in Figure 3; that is, the reaction of $\alpha TR^{\cdot}$ radicals with the test compounds obeys second-order kinetics with regard to $\alpha TR^{\cdot}$. A deviation from linearity was observed only with nafazatrom in advanced stages of the reaction (Figure 3d) which probably was caused by the participation of some secondary reaction products in $\alpha TR^{\cdot}$-decay.

To describe the kinetics in a system containing $\alpha TR^{\cdot}$ and the test substrate, two reactions are to be considered: decay of $\alpha TR^{\cdot}$ by its bimolecular recombination (Equation 2), and the reaction of $\alpha TR^{\cdot}$ with the substrate (Equation 5). During the reaction of $\alpha TR^{\cdot}$ with the test substrates αTOC may be regenerated as suggested for ascorbic acid[3], but formation of other reaction products of αTOC in this reaction cannot be excluded. The whole process is a complex multi-step reaction and can be best described as:

$$A_1 + A_2 \xrightarrow{k_{S,a}} \{A_1 \ldots A_2\}^* \longrightarrow P_a \qquad (5a)_I$$

$$\{A_1 \ldots A_2\}^* + A_3 \xrightarrow{k_{S,b}} P_b \qquad (5b)_I$$

$$A_1 + A_2 + A_3 \xrightarrow{k_S} P_a + P_b \qquad 5(a+b)$$

In the first step (Equation 5a) $\alpha TR^{\cdot}$ interacts with either a second molecule of $\alpha TR^{\cdot}$ ($A_1 = A_2 = \alpha TR^{\cdot}$; model I) or with the substrate SX ($A_1 = \alpha TR^{\cdot}$, A_2 = SX; model II) under the formation of intermediates $\{A_1 \ldots A_2\}^*$. These intermediates may decay in a minor-consecutive reaction to product P_a, but more likely, due to the third order kinetics, the intermediates $\{A_1 \ldots A_2\}^*$ react rapidly with A_3 and form products P_b according to Equation (5b), (A_3 = SX in model I, and $A_3 = \alpha TR^{\cdot}$ in model II). The net process can be described by Equation 5 ($a+b$). For both models I and II Equation 5($a+b$) may be expressed as:

$$2\alpha TR^{\cdot} + SX \xrightarrow{k_S} P_a + P_b \qquad (5)$$

The decay of $\alpha TR^{\cdot}$ in a system containing both $\alpha TR^{\cdot}$ and substrate (SX) is a sum of reactions 2 and 5:

$$-d[\alpha TR^{\cdot}]/dt = k_\alpha[\alpha TR^{\cdot}]^2 + k_S[\alpha TR^{\cdot}]^2[SX] \qquad (6)$$

The concentration of a substrate [SX], can be approximated in the initial stage as $[SX] = [SX]^0 - 2[\alpha TR^{\cdot}]$, where $[SX]^0$ is the initial substrate concentration. Generally $[SX]^0 \gg [\alpha TR^{\cdot}]$ then $[SX] \cong [SX]^0$ and Equation (6) may be simplified to:

$$-\frac{d[\alpha TR^{\cdot}]}{dt} = [\alpha TR^{\cdot}]^2 (k_\alpha + k_S[SX]^0) \qquad (7)$$

The solution of Equation (7) gives:

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$$\frac{1}{[\alpha TR^{\cdot}]} = (k_{\alpha} + k_S[SX]^0)(t - t_0) + \frac{1}{[\alpha TR^{\cdot}]_0} \quad (8)$$

The experimental data shown in Figures 3a–f are well described by Equation (8), where a linear dependence of $1/[\alpha TR^{\cdot}]$ on the time, t, is expected. The slopes k_{app} ($k_{app} = k_{\alpha} + k_S\ [SX]^0$) determined from the data on Figure 3 were plotted against initial substrate concentrations $[SX]^0$ (Figure 4a,b). From the slopes and intercepts of these graphs the rate constants k_S and k_{α} were determined (Table 2). Isoprenaline and nafazatrom exhibited the highest potency to reduce $\alpha TR^{\cdot}$, followed by epinephrine and considerably less potent stobadine and histamine. The relative ratio of their rate constants k_S was:

$$k_{ISO} : k_{NAF} : k_{EPI} : k_{STO} : k_{HIS} = 32 : 31 : 15 : 2.5 : 1$$

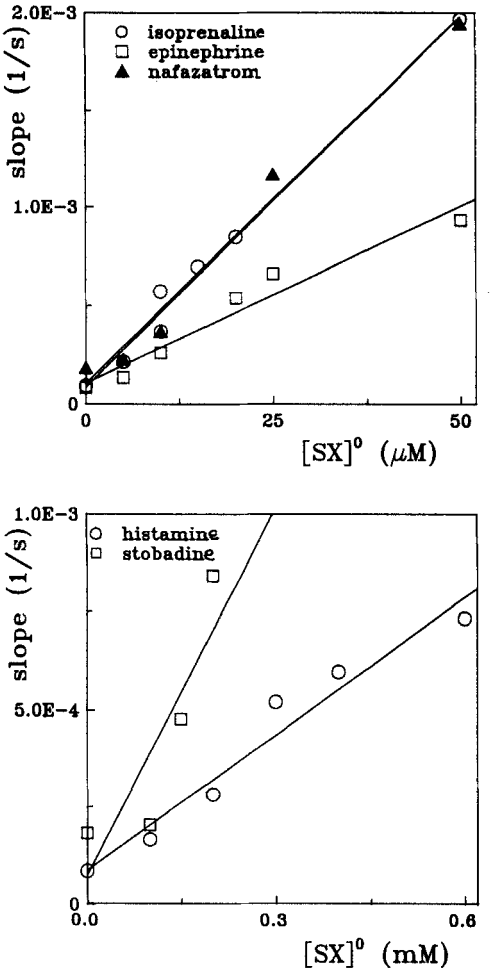

FIGURE 4 The dependence of the slopes k_{app} ($k_{app} = k_{\alpha} + k_S\ [SX]^0$), determined from Figure 3, on the initial substrate concentrations $[SX]^0$ for various substrates: a) isoprenaline, epinephrine and nafazatrom, b) histamine and stobadine.

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Further, the ability of 0.6 mg/ml Kampo C to reduce the $\alpha TR^{\cdot}$ was comparable to a 16 μM solution of epinephrine. Recently Steenken *et al.*[24] showed that stobadine did not reduce vitamin E radical effectively. According to our results the reaction between $\alpha TR^{\cdot}$ and stobadine was taking place.

The k_{α} values obtained from the intercept of the plots are very close to those found in the absence of substrate as stated above and confirm the assumption that Equation (2) and Equation (5) are two independent simultaneous reactions. This implies the preference for the kinetic model II as discussed below.

From the obtained k_S values, the bimolecular rate constants $k_{S,a}$ of the reaction 5a, for both models I and II, can be estimated according to the following procedure:

Model I. According to this model $\alpha TR^{\cdot}$ interacts in the primary step with a second molecule of $\alpha TR^{\cdot}$ (Equation $(5a)_I$) forming intermediate $\{\alpha TR^{\cdot} \ldots \alpha TR^{\cdot}\}^*$. This reacts immediately in a coupled reaction with the substrate SX according to Equation $(5b)_I$. The whole reaction is described by Equation (5).

$$\alpha\text{-}TR^{\cdot} + \alpha\text{-}TR^{\cdot} \xrightarrow{k_{S,a,I}} \{\alpha\text{-}TR^{\cdot} \ldots \alpha\text{-}TR^{\cdot}\}^* \xrightarrow{k_{d,I}} P_{a,I} \tag{5a}$$

$$\{\alpha\text{-}TR^{\cdot} \ldots \alpha\text{-}TR^{\cdot}\}^* SX \xrightarrow{k_{S,b,I}} P_{b,I} \tag{5b}$$

The decay rate $d[\alpha TR^{\cdot}]/dt$ in reaction $(5a)_I$ is at the same time the decay rate of $\alpha TR^{\cdot}$ for the whole reaction (5)

$$k_{S,a,I}[\alpha TR^{\cdot}]^2 = k_S[\alpha TR^{\cdot}]^2[SX] \tag{9}$$

and thereof:

$$k_{S,a,I} = k_S[SX] \tag{10}$$

Model II. In model II the primary $(5a)_{II}$ and the secondary $(5b)_{II}$ steps are assumed:

$$\alpha TR^{\cdot} + SX \xrightarrow{k_{S,a,II}} \{\alpha TR^{\cdot} \ldots SX\}^* \xrightarrow{k_{d,II}} P_{a,II} \tag{5a}_{II}$$

$$\{\alpha TR^{\cdot} \ldots SX\}^* + \alpha TR^{\cdot} \xrightarrow{k_{S,b,II}} P_{b,II} \tag{5b}_{II}$$

Applying the steady-state analysis for intermediate ($d[\{\alpha TR^{\cdot} \ldots SX\}^*]/dt = 0$) Equation 11 was obtained:

$$k_{S,a,II}[\alpha TR^{\cdot}][SX] = [\{\alpha TR^{\cdot} \ldots SX\}^*]\ (k_{d,II} + k_{S,b,II}[\alpha TR^{\cdot}] \tag{11}$$

and

$$[\{\alpha TR^{\cdot} \ldots SX\}]^* = \frac{k_{S,a,II}[\alpha TR^{\cdot}][SX]}{k_{d,II} + k_{S,b,II}[\alpha TR^{\cdot}]} \tag{12}$$

The decay rate $d[\alpha TR^{\cdot}]/dt$ in model II consists of the contributions of reactions $(5a)_{II}$ and $(5b)_{II}$ and is equal to the $\alpha TR^{\cdot}$-decay rate in the whole reaction (5) . Therefore, we can write:

$$k_{S,a,II}[\alpha TR^{\cdot}][SX] + k_{S,b,II}[\alpha TR^{\cdot}][\{\alpha TR^{\cdot} \ldots SX\}^*] = k_S[\alpha TR^{\cdot}]^2\{SX\} \tag{13}$$

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From (12) and (13) Equation 14 is obtained:

$$k_{S,\,a,\,II}\left(1+\frac{k_{S,\,b,\,II}[\alpha TR^{\cdot}]}{k_{d,\,II}+k_{S,\,b,\,II}[\alpha TR^{\cdot}]}\right)=k_S[\alpha TR^{\cdot}] \qquad (14)$$

Since our experiments verified the formal third order of the reaction 5, reaction formalism requires that the formation of product $P_{a,\,II}$ in $(5a)_{II}$ is negligible.

That is, $k_{d,\,II} \ll k_{S,\,b,\,II}\,[\alpha TR^{\cdot}]$ and Equation 14 is simplified to:

$$k_{S,\,a,\,II}=(1/2)k_S[\alpha TR^{\cdot}] \qquad (15)$$

The rate constants $k_{S,\,a,\,II}$ were calculated from Equation 15, taking into account that the concentration of $\alpha TR^{\cdot}$ at the time of mixing with substrates (30 s after the reaction was initiated with $DPPH^{\cdot}$) was $[\alpha TR^{\cdot}]^0 = 6.3\ \mu M$ are summarized in Table 2. The so calculated bimolecular rate constants $k_{S,\,a,\,II}$ enable us to compare the ability of the test compounds to reduce $\alpha TR^{\cdot}$ with that of biologically most important reductant ascorbic acid. As seen from the Table 2, the bimolecular rate constants, $k_{S,\,a,\,II}$ were in the order of $10^3\ M^{-1}s^{-1}$. The rate constants in the order of 10^4–$10^7\ M^{-1}s^{-1}$ were reported for the reduction of $\alpha TR^{\cdot}$ by ascorbic acid in various systems[3, 22, 23].

From the obtained results model II is preferred. Approximately the same values $k\alpha$ were found for bimolecular self-decay of $\alpha TR^{\cdot}$ both in experiments with and without substrates SX (compare Tables 1 and 2). Therefore, it can be assumed that substrate SX does not affect the self-decay reaction of $\alpha TR^{\cdot}$. Model I, however, predicts an effect of the substrate on the bimolecular decay of $\alpha TR^{\cdot}$ (Equation 10). These facts, therefore, support the validity of model II. Thus, in the primary step $\alpha TR^{\cdot}$ probably interacts with substrate SX (reaction $5a_{II}$ model II), rather than with the second molecule of $\alpha TR^{\cdot}$ (reaction $5a_{II}$, model I).

It is important to note, that our results do not bring any evidence whether or not the αTOC was regenerated from $\alpha TR^{\cdot}$ in the reaction with the test compounds, although biological ubiquinones were reported to regenerate vitamin E[25].

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TABLE 1

The decay rate constants of the bimolecular self-decay of $\alpha TR^{\cdot}$. k_α^{EPR} values are slopes of the graphs with control samples in relative EPR units (Figures 2 and 3), k_α values are bimolecular decay rates calculated from the k_α^{EPR} based on the determined concentration of $\alpha TR^{\cdot}$

Fig. No.	$k_\alpha^{EPR} \times 10^5\ (s^{-1})$	$k_\alpha\ (M^{-1}s^{-1})$
3a	9.3 ± 0.3	3139
3c	9.5 ± 0.3	3206
3e	9.1 ± 0.3	3071
3f	9.0 ± 0.2	3038
2	8.8 ± 0.2	2970
average	9.1 ± 0.2	3085 ± 90

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TABLE 2

Rate constants of the $\alpha TR^{\cdot}$ reactions with various substrates SX. k_S^{EPR}- slopes of the graphs (Figure 4) in relative units, k_S- apparent trimolecular rate constants of the reaction 5 (in $M^{-2}s^{-1}$) calculated from the k_S^{EPR} values based on determined concentrations of $\alpha TR^{\cdot}$. $k_{S,a,II}$ - bimolecular rate constants of the reaction 5a, model II, calculated from the k_S values using Equation 15

[SX[	k_S^{EPR} ($M^{-1}s^{-1}$)	k_S (10^9 $M^{-2}s^{-1}$)	$k_{S,a,II}$ (10^3 $M^{-1}s^{-1}$)
isoprenaline	38 ± 1	1.28	3.2
nafazatrom	37 ± 3	1.25	3.13
epinephrine	18 ± 2	0.6	1.5
stobadine	3.1 ± 0.1	0.1	0.25
histamine	1.1 ± 0.1	0.04	0.1

References

1. L.A. Witting (1980) Vitamin E and lipid antioxidants in free radical initiated reactions. In Free Radicals in Biology, Vol 4 (ed. W.A. Pryor), Acad. Press, New York, pp. 295–319.
2. G.W. Burton, A. Joyce and K.U. Ingold (1983) Is vitamin E the only lipid soluble chain breaking antioxidant in human blood plasma and erythrocyte membranes. *Archives of Biochemistry and Biophysics*, **221**, 281–290.
3. J.E. Packer, T.F. Slater and R.L. Willson (1979) Direct observation of free radical interaction between vitamin E and vitamin C. *Nature*, **278**, 737–738.
4. S.L. Jewett, L.J. Eddy and P. Hochstein (1989) Is the autoxidation of catecholamines involved in ischemia-reperfusion injury? *Free Radical Biology and Medicine*, **185**, 185–188.
5. P.K. Singal, R.E. Beamish and N.S. Dhalla (1983) Potential oxidative pathways of catecholamines in the formation of lipid peroxides and genesis of heart disease. In Myocardial Injury (ed. G.G. Spitza), Plenum Press, New York, pp. 391–401.
6. C.K. Chow (1991) Vitamin E and oxidative stress. *Free Radical Biology Medicine*, **11**, 215–232.
7. A.M. Judd, I.S. Login, J.T. Ehreth and R.M. MacLeod (1986) Nafazatrom, an arachidonate metabolism inhibitor, decreases prolactin and GH release. *European Journal of Pharmacology*, **128**, 151–156.
8. D.R. Janero, B. Burghardt, Lopez and M. Cardell (1989) Influence of cardioprotective cyclooxygenase and lipoxygenase inhibitors of peroxidative injury to myocardial-membrane phospholipid. *Biochemical Pharmacology*, **38**, 4381–4387.
9. L. Beneš and S. Štolc (1989) Stobadine. *Drugs of the Future*, **14**, 135–137.
10. K. Ondriaš, V. Mišík, D. Gergel and A. Staško (1989) Lipid peroxidation of phosphatidylcholine liposomes depressed by the calcium channel blockers nifedipine and verapamil and by the antiarrhythmic-antihypoxic drug stobadine. *Biochimica Biophysica Acta*, **1003**, 238–245.
11. A. Staško, K. Ondriaš, V. Mišík, H. Szocsová and D. Gergel' (1990) Stobadine - a novel scavenger of free radicals. *Chemistry Papers*, **44**, 493–500.
12. P.F. Mannaioni and E. Masini (1988) The release of histamine by free radicals. *Free Radical Biology Medicine*, **5**, 177–197.
13. K. Arakawa, J.-C. Cyong and Y. Ohtsuka (1985) Pharmacological Action of components of Orengedoku-to. VI Action of Orengedoku-to in hypertensive MH rats. *Journal of Medical Pharmacological Society Wakan-Yaku*, **2**, 554–555.
14. H. Nagasawa and K. Kogure (1988) Efficacy Oren-gedoku-to (TJ-15) in a model of chronic cerebral ischemia. In Recent Advances in the Pharmacology of Kampo (Japanese Herbal) Medicines (eds. E. Hosoya, Y. Yamamura) , Excerpta Medica, Amsterdam, pp. 213–218.
15. D. Gergel' and V. Mišík (1991) Effect of Kampo medicines on lipid peroxidation of phosphatidylcholine liposomes. *Pharmazie*, **46**, 469–470.
16. K. Ondriaš, A. Staško, D. Gergel', M. Hromadová and L. Beneš (1992) Formation of stable free radicals from Kampo medicines TJ-9, TJ-15, TJ-23, TJ-96, TJ-114 and their antioxidant effect on low density lipoproteins. *Free Radical Research Communications*, **16**, 227–237.
17. V. Mišík and D. Gergel' (1991) Kampo medicines possess OH radical scavenging activity. *Pharmazie*, **46**, 545–546.
18. C. Rousseau-Richard, C. Richard and R. Martin (1988) Kinetics of bimolecular decay of α-tocopheroxyl free radicals studied by ESR. FEBS Letters, **233**, 307–310.

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19. W. Boguth, R. Repqes and I. Pracht (1969) Uber die Reaktion von α-Tocopherol mit 1,1-Diphenyl-2-pikrylhydrazyl: V. Kinetische und termodynamische Daten. *Internat. Z. Vit. Forschung*, **39**, 439–446.
20. M.N. Kaouadji, D. Jore, C. Ferradini and L.K. Patterson (1987) Radiolytic scanning of vitamin E – vitamin C oxidation – reduction mechanisms. *Bioelectrochemistry and Bioenergetics*, **18**, 59–70.
21. W. Boguth and H. Niemann (1971) Electron spin resonance of chromanoxy free radicals from α-, ζ_2-, β-, γ- δ-tocopherol and tocol. *Biochimica Biophysica Acta*, **248**, 121–130.
22. R.H. Bisby and A.W. Parker (1991) Reactions of the alfa-tocopheroxyl radical in micellar solutions studied by nano-second laser flash photolysis. FEBS Letters, **290**, 205–208.
23. M. Scarpa, A. Rigo, M. Maiorino, F. Ursini and C. Gregolin (1984) Formation of α-tocopherol radical and recycling of α-tocopherol by ascorbate during peroxidation of phosphatidylcholine liposomes. *Biochimica Biophysica Acta*, **801**, 215–219.
24. S. Steenken, A.R. Sundquist, S.J. Jovanovic, R. Crockett and H. Sies (1992) Antioxidant Activity of the Pyridoindole Stobadine. Pulse Radiolytic Characterization of One-Electron-Oxidized Stobadine and Quenching of Singlet Molecular Oxygen. *Chemical Research in Toxicology*, **5**, 355–360.
25. K. Mukai, S. Itoh, H. Morimoto (1992) Stopped-flow kinetic study of vitamin-E regeneration reaction with biological hydroquinones (reduced forms of ubiquinone, vitamin-K, and tocopherol-quinone) in solution. *Journal Biological Chemistry*, **267**, 22277–22281.

Accepted by Professor H. Sies

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