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Thermochimica Acta 331 (1999) 79–86

thermochimica acta

DSC study of antioxidant properties of dihydroxyphenols

G. Litwinienko*, T. Kasprzycka-Guttman, D. Jamanek

Department of Chemistry, Warsaw University, Pasteura 1, 02-093, Warsaw, Poland

Received 14 December 1998; received in revised form 2 March 1999; accepted 9 March 1999

Abstract

The influence of 1,2-, 1,3- and 1,4-dihydroxybenzene and α-tocopherol on thermal-oxidative decomposition of linolenic acid (LNA) initiated by α,α′-azoisobutyronitrile (AIBN) was investigated by DSC in scanning mode. The concentration of the phenols varied from 0.5 to 20 mmol of compound per mol LNA. Values of activation energy calculated from temperatures of the start of LNA thermoxidation (by the Ozawa-Flynn-Wall method) were 74.6±3.4 kJ/mol (initiated but non-inhibited LNA), and for inhibited LNA 93.8±8.7 kJ/mol for hydroquinone, 106.8±5.5 kJ/mol for resorcinol and 116.3±11.0 kJ/mol for pyrocatechol. For a system inhibited by α-tocopherol at 1.94 mmol per mol LNA, the activation energy equals 96.1±13.3 kJ/mol. Arrhenius pre-exponential factors and rate constants of oxidation at 90°C are presented. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Autoxidation; Activation energy; DSC; Phenolic antioxidants; Thermal analysis

1. Introduction

Because autoxidation of fats, fatty acids and lipids is an exothermic process, the methods of thermal analysis are valuable for studying thermostability, thermoxidation and autoxidation. Our previous paper [1] reported several examples of articles in this field.

Naturally occurring antioxidants are chemical compounds of complex structure. Well known among natural antioxidants are polyhydroxyphenols: flavonoids, tocopherols, etc. Although the antioxidants have been studied extensively for 60 years, very little is known for the influence of individual fragments of the antioxidant on its efficiency and activity. For example, most of the polyhydroxyphenols exhibit a chain-breaking effect during radical autoxidation, but the reaction mechanism and the role of hydroxyl group location have not been clearly established. Moreover, the explanations of antioxidant activity by resonance stabilisation of molecules are complicated due to the ability to form intramolecular hydrogen bonds between hydroxyl groups.

It is commonly known that the antioxidant activity of polyhydroxyphenols depends on the presence of hydroxyl groups, but this is not the only reason for their activity. The presence of an alkyl chain in the *para*-position if favourable for electron delocalisation and stabilisation of the phenoxyl radical formed during the chain reaction:

$$\mathrm{PhOH} + \mathrm{LOO}^{\bullet} \rightarrow \mathrm{PhO}^{\bullet} + \mathrm{LOOH} \qquad (1)$$

where LOOH and $\mathrm{LOO}^{\bullet}$ denote lipid hydroperoxide and lipid peroxide radicals, and PhOH and $\mathrm{PhO}^{\bullet}$ are the phenolic antioxidant and phenoxyl radical. Similarly, electron donating groups in the *ortho*-position

*Corresponding author. Fax: +48-22-822-59-96; e-mail: litwin@chem.uw.edu.pl

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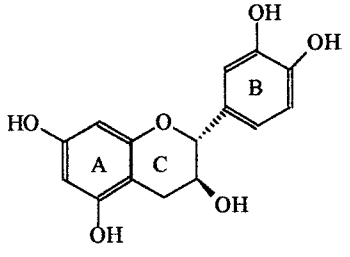
PII: S0040-6031(99)00058-1

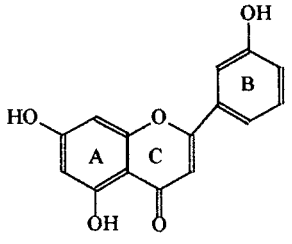
increase the stability of phenoxyl radicals and a better inhibitory effect is observed [2]. The second hydroxyl group in the *ortho-* or *para-* position affects additional resonance stability [3]. Moreover, the products of oxidation are *o*-and *p*-quinones. In the case of *m*-dihydroxybenzene the formation of quinone is not possible. The better antioxidant activity of 3,4-dihydroxycinnamic acid than that of 3-methoxy-4-hydroxycinnamic acid is explained by the presence of two hydroxyl groups [4]. Yamamura et al. [5] investigated the inhibitory effect of polyhydroxyphenols on autoxidation of tetraline by the oxygen consumption method. The measured induction times decreased in the order: catechols>hydroquinones>resorcinols, and were not correlated to the reduction potentials. Hydroquinone has the lowest E_p=0.46 V while the value for pyrocatechol is 0.53 V and for resorcinol 0.81 V.

ESR and electrochemical studies and quantum calculations [6,7] of compounds with both 1,2- and 1,3-dihydroxybenzene rings (for example (±)-catechin):



and other catechol containing flavonoids showed that oxidation takes place in ring B.

When ring B does not possess second hydroxyl group, as in galangin:



the ring A has an antioxidant role [8]. However, data obtained from these experiments strongly depended on the pH of solutions due to protonation–deprotonation equilibria of the hydroxyl groups in rings A and B.

Recently, we reported [1,9,10] that DSC is a good technique for determining the kinetic parameters of non-inhibited and inhibited fatty acid oxidation and that the extrapolated temperatures of the start of the oxidation process are useful points to evaluate antioxidant activity. Our measurements on oxidation of linolenic acid with added mono-, di- and tri-methylphenols allowed assessment of the influence of the alkyl group on the phenolic ring on the antioxidant activity of phenolic derivatives. The present study compares the antioxidant activities of 1,2-dihydroxybenzene (pyrocatechol), 1,3-dihydroxybenzene (resorcinol), 1,4-dihydroxybenzene (hydroquinone) and α-tocopherol in the oxidation of LNA without other solvents.

2. Experimental

2.1. Materials

Commercially purchased linolenic acid (LNA) (purity 98%; Carl Roth, Karlsruhe), α,α′-azoisobutyronitrile (Merck) and α-tocopherol (purity 95%; Sigma) were used without further purification. Dihydroxyphenols were purified by recrystallisation from a mixture of benzene and hexane. Purity of these phenolic compounds as determined by GC was >97%. All compounds were stored under nitrogen at a temperature of about 0°C.

2.2. Apparatus and methods

A Du Pont model 910 differential scanning calorimeter with a Du Pont 9900 thermal analyser and a normal pressure cell were used. The oxygen flow rate was 15 l/h. The apparatus was calibrated with a high purity indium standard.

Samples of LNA with dissolved AIBN and inhibitor were prepared as follows:

Two grams of LNA was placed in 5 ml flask and AIBN was added in the appropriate amount to obtain the initiator concentration 0.04 mol/dm^3. Then, a known volume (2–200 μl) of acetone solution of the phenolic compound was added (finally concentration ranges from 0.5 to 20 mmol of compound per mol of LNA). A homogenous mixture was obtained after addition of 2 ml of acetone. Excess acetone was removed under reduced pressure on a rotary vacuum evaporator in darkness at room temperature. Prepared samples were used immediately in DSC experiments.

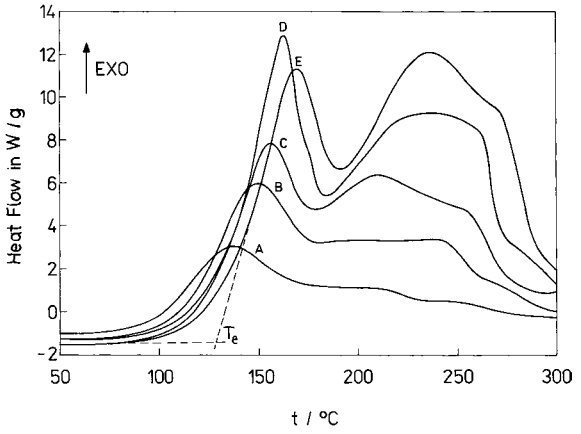


Fig. 1. DSC curves of thermal oxidation of linolenic acid initiated by AIBN. (A) 5; (B) 10; (C) 15; (D) 20; (E) 25 K/min.

The 3–5 mg samples in an aluminium pan were heated at a constant heating rates (β) of 2–20 K/min from 40°C to 350°C in a oxygen flow of 15 dm^3/h. The extrapolated temperatures of the start of the oxidation and temperatures of the maximum heat flow were determined from each DSC scan by using program GENERAL V4.01 (TA Instruments). Measurements and determination of kinetic parameters were the same as described in our previous paper [1].

3. Results

Fig. 1 shows the examples of DSC curves for the oxidation of LNA, initiated using AIBN, at different heating rates. The temperature of the extrapolated start of the exothermic reaction (T_e) and the values of the maximum heat flow (T_p) are listed in Table 1. Every temperature value is an average from at least three runs at the same heating rate β.

Table 1
Temperatures of extrapolated start of oxidation (T_e) and temperatures of the first maximum of heat flow (T_{p1}) for linolenic acid oxidation initiated by AIBN

β (K/min)	T_e (°C)	T_{p1} (°C)
5	98.6	133.6
10	110.3	146.9
15	115.2	157.1
20	119.4	164.0
25	124.6	169.6

Table 2
Kinetic parameters of thermoxidation of LNA+AIBN calculated from temperatures T_e and T_{p1}

	T_e	T_{p1}
Slope (a)	−4.096	−3.467
Standard error of a estim.	0.185	0.087
Constant (b)	11.710	9.234
Standard error of b estim.	0.025	0.014
R^2	0.9939	0.9981
E_a (kJ/mol)	74.6	63.1
ΔE_a (kJ/mol)	3.4	1.6
A (min^{-1})	8.79×10^9	3.05×10^7
k at 90°C		0.025

Arrhenius plots ($\log\beta = aT^{-1} + b$) with the slopes ($a = \mathrm{d}\log\beta/\mathrm{d}T^{-1}$) calculated by means of the least squares method were used to obtain the apparent activation energies of autoxidation from equation: $E_a = -2.19R\,\mathrm{d}\log\beta/\mathrm{d}T^{-1}$, where R is the gas constant. E_a was obtained only for the start of the process and for the first maximum of heat flow. In previous paper [1] we described the calculation procedure and it was explained that the second maximum of heat flow is not connected with the most interesting first stage of the oxidation process. Statistical analysis allowed calculation of the standard deviation, regression coefficient and error of estimation of E_a. The rate constants were calculated for 90°C. All these values are listed in Table 2.

DSC curves obtained for oxidation of LNA inhibited by α-tocopherol and by pyrocatechol are shown in Fig. 2 and Fig. 3. Their shapes are similar to the uninhibited LNA oxidation curves, but kinetic parameters calculated from T_e and T_{p1} are significantly different. The parameters of Arrhenius plots and the activation energies for LNA oxidation are listed in Tables 3 and 4. Among 37 values of activation energy, 23 were calculated with an estimation error less than 6 kJ/mol, in 12 values the error was 6–10 kJ/mol and in two cases the error was 11.0 and 13.3 kJ/mol.

Table 5 presents the pre-exponential factors (A) and rate constants (k) of oxidation calculated for all investigated systems (including non-inhibited LNA) at 90°C. Figs. 4 and 5 show the dependencies of the E_a for systems with addition of, respectively, 1,2-, 1,3- and 1,4-dihydroxybenzene at different concentrations

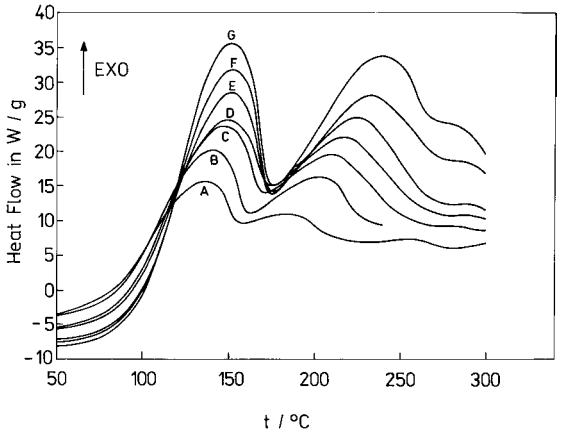


Fig. 2. DSC effects obtained during oxidation of LNA+AIBN system inhibited by 0.65 mmol of tocopherol per mol LNA. (A) 5; (B) 7; (C) 10; (D) 12; (E) 15; (F) 17; (G) 20 K/min.

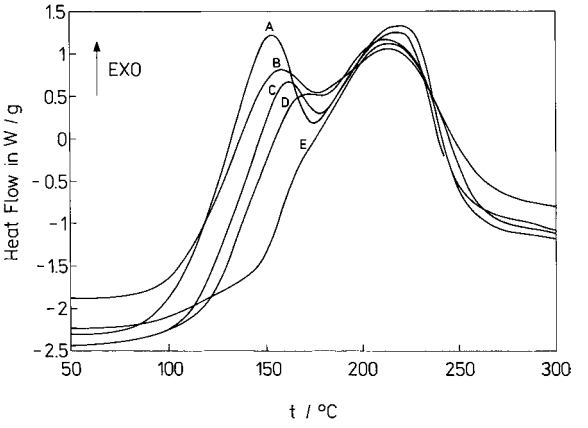


Fig. 3. DSC curves of LNA oxidation obtained for different concentrations of pyrocatechol (mmol/mol LNA). (A) 0.5; (B) 2.5; (C) 7.5; (D) 12.0; (E) 20.0 mmol/mol.

Table 3
Parameters of Arrhenius plots: slope a, intercept b, standard errors (σ), regression R^2 and values of E_a calculated from temperatures of extrapolated onset of oxidation

	a	σ_a	b	σ_b	R^2	E_a (kJ/mol)
1,2-Dihydroxybenzene						
0.5	−4.8621	0.18663	13.9492	0.0295	0.9941	88.5±3.4
2.5	−5.1844	0.25968	14.7971	0.0395	0.9876	94.4±4.7
7.5	−6.3901	0.60496	17.733	0.0808	0.9490	116.3±11.0
12.0	−5.75	0.38569	15.776	0.0580	0.9737	104.7±7.0
20.0	−4.6782	0.31396	12.3458	0.0527	0.9780	85.2±5.7
1,3-Dihydroxybenzene						
0.5	−4.8842	0.18551	14.1687	0.0291	0.9943	88.9±3.4
2.5	−5.0561	0.22231	14.6392	0.0383	0.9885	92.1±4.0
7.5	−5.7602	0.36008	16.535	0.0541	0.9771	104.9±6.6
12.0	−5.8658	0.30286	16.917	0.0414	0.9895	106.8±5.5
20.0	−3.9459	0.2943	11.7721	0.0643	0.9677	71.8±5.4
1,4-Dihydroxybenzene						
0.5	−4.4665	0.40089	12.8244	0.0467	0.9688	81.3±7.3
1.5	−4.7606	0.35064	13.6366	0.0599	0.9736	86.7±6.4
3.5	−4.2405	0.15293	12.5039	0.0312	0.9935	77.2±2.8
5.0	−5.1533	0.47533	14.6894	0.0762	0.959	93.8±8.7
7.5	−4.8627	0.36449	13.7166	0.0587	0.9727	88.5±6.6
12.0	−4.5219	0.52812	13.3368	0.0515	0.9483	82.3±9.6
α-Tocopherol						
0.65	−4.6058	0.24138	13.8037	0.0275	0.9865	83.9±44
1.94	−5.2797	0.72858	15.3671	0.0500	0.9292	96.1±13.3

Table 4
Parameters of Arrhenius plots calculated from the temperatures of first maximum of heat flow T_{p1} (notations are the same as in Table 3)

	a	σ_a	b	σ_b	R^2	E_a (kJ/mol)
1,2-Dihydroxybenzene						
0.5	−3.4025	0.27058	8.90097	0.0605	0.9753	62.0±4.9
2.5	−3.8813	0.31321	10.0192	0.0630	0.9685	70.7±5.7
7.5	−4.5926	0.28919	11.6076	0.0526	0.9806	83.6±5.3
12.0	−3.8612	0.14703	9.75098	0.0332	0.9914	70.3±2.7
20.0	−2.584	0.19733	6.56287	0.0303	0.9942	47.0±3.6
1,3-Dihydroxybenzene						
0.5	−4.0056	0.53846	10.3298	0.1001	0.9326	72.9±9.8
2.5	−3.9595	0.54161	10.2121	0.1136	0.8991	72.1±9.9
7.5	−5.0636	0.495	12.6441	0.0833	0.9458	92.2±9.0
12.0	−4.2348	0.32242	10.6724	0.0606	0.9773	77.1±5.9
20.0	−3.4572	0.2734	8.98871	0.0187	0.9816	62.9±5.0
1,4-Dihydroxybenzene						
0.5	−4.681	0.30105	11.8505	0.0337	0.9837	85.2±5.5
1.5	−3.8801	0.41362	9.97152	0.0855	0.9462	70.6±7.5
3.5	−3.6266	0.29528	9.40878	0.0695	0.9679	66.0±5.4
5.0	−4.1783	0.29774	10.6701	0.0593	0.9752	76.1±54
7.5	−3.6032	0.31033	9.29261	0.0626	0.9712	65.6±5.7
12.0	−4.0974	0.39359	10.5226	0.0427	0.9644	74.6±7.2
α-Tocopherol						
1.94	−3.9329	0.49763	10.117	0.0555	0.9398	71.6±9.1
0.65	−4.8243	0.17087	12.5332	0.0160	0.9950	87.8±3.1

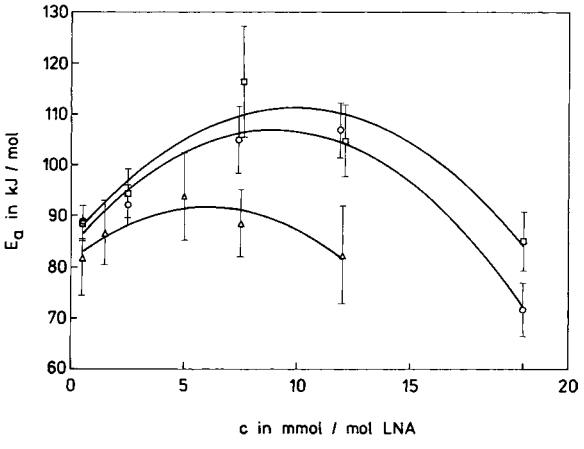


Fig. 4. Activation energies of LNA thermoxidation in presence of various concentration of 1,2-dihydroxybenzene (□), 1,3-dihydroxybenzene (○) and 1,4-dihydroxybenzene (△) calculated from temperatures of extrapolated onset of oxidation (T_e).

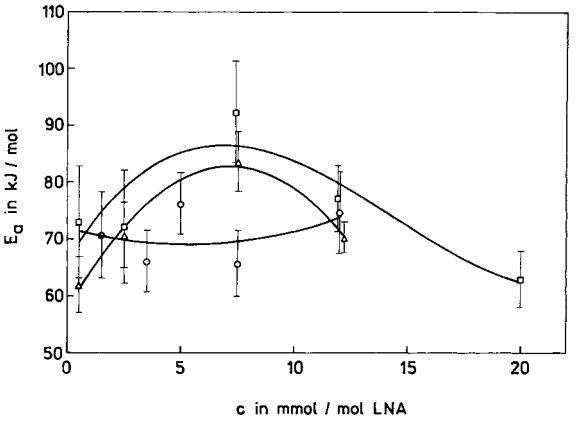


Fig. 5. Activation energies of LNA thermoxidation in presence of various concentration of 1,2-dihydroxybenzene (□), 1,3-dihydroxybenzene (○) and 1,4-dihydroxybenzene (△) calculated from temperatures of first maximum of oxidation (T_{p1}).

Table 5
Pre-exponential factor (A_{p1}) and rate constant k_{p1} of oxidation at 90°C calculated from first maximum of heat flow

	Concentration of inhibitor	A_{p1} (min^{-1})	$K_{p1}\times10^3$ (min^{-1})
Pure LNA+AIBN	0.00	3.05×10^7	25.10
α-Tocopherol	0.65	5.91×10^{10}	13.60
	1.94	1.85×10^8	9.17
1,2-Dihydroxybenzene	0.5	1.52×10^7	18.70
	2.5	1.49×10^8	10.20
	7.50	5.92×10^9	5.57
	12.0	8.67×10^7	6.72
1,3-Dihydroxybenzene	0.5	2.78×10^8	9.01
	2.5	1.97×10^8	8.42
	7.50	5.88×10^{10}	3.23
	12.0	6.26×10^8	5.09
	20.0	1.61×10^7	14.30
1,4-Dihydroxybenzene	0.5	1.01×10^{10}	5.59
	1.5	1.45×10^8	10.00
	3.5	3.41×10^7	10.90
	5.0	6.65×10^8	7.61
	7.5	2.65×10^7	9.71
	12.0	5.02×10^8	9.35

of these compounds. The error bars are given for each value of E_a.

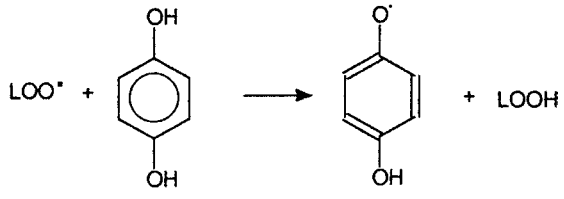
4. Discussion

To obtain constant conditions of the initiation stage of oxidation we decided to use α,α′-azoisobutyronitrile (0.04 mol/dm^3). Since each reaction was initiated by AIBN at the same concentration, the interpretation of the kinetics requires the assumption that the rate of the initial step of autoxidation was the same in each case. DSC curves of initiated oxidation of LNA with 1,2-, 1,3-, and 1,4-dihydroxybenzene and α-tocopherol have similar shapes (Figs. 1–3). The influence of kind, activity and concentration of the inhibitor is the most significant at the first step of oxidation. Therefore, the changes in kinetic parameters calculated from T_e and T_{p1} in comparison with parameters obtained for initiated but not inhibited system (LNA+AIBN) should characterise the antioxidant efficiency of the added compound. Values of T_e and T_{p1} gave good Arrhenius plots, listed in Tables 3 and 4. In all cases, addition of phenolic compound caused an increase in the value of E_a calculated by Ozawa-Flynn-Wall method.

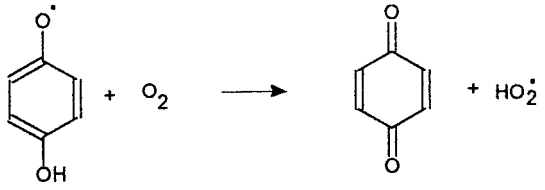
The influence of the phenolic concentration is shown with pyrocatechol (Fig. 3) as example. Increasing concentration of 1,2-dihydroxybenzene leads to higher temperatures of the extrapolated start of the oxidation process, but for large concentrations a small peak appears connected with the oxidation of antioxidant. This small peak overlaps the start of the first oxidation peak and thus T_e is more difficult to determine. In contrast to oxygen consumption methods, the DSC technique shows whether the exothermal effect is caused by oxidation of antioxidant (small peak) or by oxidation of fatty acid (large peak). At low concentrations of inhibitor, this peak disappears.

For all studied compounds, we observed similar activity at low concentrations (0.5–2.0 mmol/mol LNA) and E_a values calculated from T_{p1} were 62–85 kJ/mol. E_a values calculated from T_e were less than 89 kJ/mol (Tables 3 and 4). Measurements for α-tocopherol (used as comparative inhibitor) confirm its good inhibitory effect at low concentrations, 0.94 and 1.65 mmol per mol LNA. Rate constants of oxidation were 13.60×10^{-3} and 9.17×10^{-3} min^{-1}, i.e. about half those for non-inhibited LNA (Table 5).

At the medium concentration range, hydroquinone exhibited rather moderate antioxidant properties. Its best properties were found at 5.0 mmol/mol, 93.8±8.7 kJ/mol (from T_e), 71±5.4 kJ/mol (from T_{p1}), and $k=7.61\times10^{-3}$ min^{-1}. Although these values are advantageous, phenols with two hydroxyl groups in the *para*-position are expected to be better antioxidants than 1,3-dihydroxyphenols at temperatures less than 100°C. This phenomenon can be explained by formation of the hydroperoxyl radical HOO$^{\bullet}$ in reaction of hydroquinone and molecular oxygen [5]:



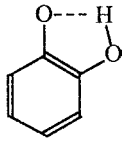
(2)



(3)

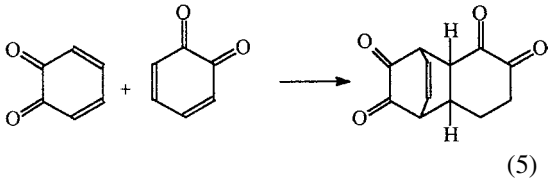
The radical reacts with the neutral molecule of O_2 instead of another radical. Therefore, reaction (3) is a propagation (not termination) process and is accelerated by high concentrations of O_2 (normal pressure, flow 6 l/h) and increasing temperature.

The antioxidant 1,2-dihydroxybenzene exhibits goods activity at concentrations of 2–12 mmol per mol of LNA. The highest E_a values are observed at 7.5 mmol/mol. In 1,2-dihydroxybenzene the electron density on the oxygen atom is increased due to delocalisation of π electrons of the ring. Abstraction of a hydrogen atom from a hydroxyl group is easy and a stable radical is formed. Additionally, the *ortho*-position favours the formation of an intramolecular hydrogen bond:



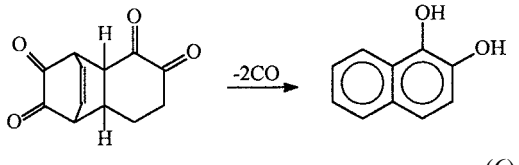
(4)

In comparison with radicals formed from hydroquinone, these radicals are much more stable. It is known [11] that after abstraction of a second hydrogen atom, the *ortho*-benzoquinone can undergo dimerisation.



(5)

The product of this reaction is transformed to 1,2-dihydroxynaphthalene and carbon monoxide during heating:



(6)

1,2-dihydroxynaphthalene is the next compound with two hydroxyl groups at *ortho*-position and can react as an antioxidant.

The kinetic parameters for LNA+1,3-dihydroxybenzene are lower than those for 1,2-dihydroxybenzene. The best activity of resorcinol is observed at 7.5–12.0 mmol/mol. Although the 1,3-substituents cannot create quinone after abstraction of hydrogen atoms, each group may act individually. Probably in this case the mechanism of the inhibitory effect is different than for *o*- and *p*-derivatives.

At concentrations higher than 12 mmol of phenol per mol of LNA, the antioxidant activities decreased; in agreement with our previous experiments with alkylated phenols [1]. Our observations are in good agreement with other papers concerning the pro-oxidative behaviour of tocopherol at higher concentration [12–14]. It was proposed that the hydrogen atom is abstracted from lipids by tocopherol radicals in the reverse of reaction (1), and new lipid free radicals are generated:

$$PhO^{\bullet} + LOOH \rightarrow PhOH + LOO^{\bullet} \quad (7)$$

Another way of the chain propagation is:

$$PhO^{\bullet} + LH \rightarrow PhOH + L^{\bullet} \quad (8)$$

For each investigated compound it is possible to find a concentration at which the autoxidation process is the slowest.

5. Conclusions

Comparison of the inhibitory effect of *o*-, *m*-, *p*-dihydroxybenzene and α-tocopherol on linolenic acid oxidation initiated by AIBN has been made using the Ozawa-Flynn-Wall method. Oxidation of LNA initiated by AIBN is characterised by a low activation energy that increases during inhibited autoxidation, Arrhenius kinetic parameters, E_a, A and k, show that hydroquinone is a moderate antioxidant while pyrocatechol and resorcinol are enhanced antioxidants. The inhibitory effect varies markedly with concentration of added compound and the middle range (5–12 mmol/mol of LNA) gave the best results. Above and below those concentrations the differences between calculated kinetic parameters are insignificant and inhibitory effect is small.

Acknowledgements

This work was supported by grant from the State Committee for Scientific Research (Grant No. GR-976/98).

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