The effect of tertiary amines and their related compounds on the oxidation of unsaturated fatty acids

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Dedicated to Professor RNDr L. Krasnec on his 60th birthday

The relationship between substituent effects on an aromatic nucleus of aryloxyalkylpiperidine, the length of the linking aliphatic chain and substituents on nitrogen on the one hand and the antioxidant and inhibitory activity on the other has been investigated. From the course of autoxidation, oxidation of unsaturated fatty acids (linoleic, linolenic, arachidonic) catalyzed by lipoxygenase and from the examination of degradation of β-carotene it has been found that the greatest stabilizing and inhibitory effects have 1-[4-(4-*tert*-butylphenoxy)butyl]piperidine, 1-[4-(2-*tert*-butylphenoxy)butyl]-piperidine, and 1-[4-(4-hexyloxyphenoxy)butyl]piperidine.

Autoxidation, or enzyme-catalyzed oxidation of the majority of fats is a chain radical reaction, *i.e.* its total rate is dependent on the rate of initiation, propagation, and termination. The reaction rate of this kind of reaction can generally be altered, or the induction period extended by addition of antioxidants. The enzyme-catalyzed oxidation of fats by the enzyme lipoxygenase is encountered with essential unsaturated fatty acids or their esters having a *cis-cis*-1,4-pentadiene configuration in its molecules as is the case with linoleic, linolenic, and arachidonic acids [1]. In both cases, *i.e.* either in autoxidation or in enzyme-catalyzed oxidation, hydroperoxides of unsaturated fatty acids are the primary oxidation products.

Aromatic amines are known to be efficient antioxidants. Substances of this type belong to the group of antioxidants which slowed down the oxidation rate by reacting with free radicals liberated during the chain reaction [2]. Compounds derived from aryloxyalkyl-piperidine form an interesting series revealing various properties. They can be, regarding the nature of functional groups in particular parts of the basic structure of the molecule, pharmacologically active or indifferent and their physicochemical properties can be evaluated [3]. In this paper we would like to report the synthesis of some compounds with predicted antioxidative properties verified both on the autoxidation and enzyme-catalyzed oxidation of unsaturated fatty acids and also on the degradation of β-carotene.

Table 1

Characteristic data of *N*-(ω-aryloxyalkyl)piperidines

No.	R	*n*	Formula	*M*	Calculated/found			Yield	B.p.
					% C	% H	% N	[%]	[°C/torr]
I	4-[$(CH_3)_3C$]C_6H_4	2	$C_{17}H_{27}NO$	261.41	78.05 78.10	10.40 10.50	5.35 5.38	84.2	155—157/0.8
II	4-[$(CH_3)_3C$]C_6H_4	3	$C_{18}H_{29}NO$	275.44	78.49 78.55	10.61 10.70	5.09 5.12	80.2	134—136/0.2
III	4-[$(CH_3)_3C$]C_6H_4	4	$C_{19}H_{31}NO$	289.46	78.84 78.70	10.79 10.82	4.83 4.90	82.3	163—165/0.4
IV	4-[$(CH_3)_3C$]C_6H_4	5	$C_{20}H_{33}NO$	303.49	79.15 79.09	10.96 11.00	4.62 4.68	78.7	186—187/0.8
V	4-[$(CH_3)_3C$]C_6H_4	6	$C_{21}H_{35}NO$	317.52	79.44 79.40	11.11 11.15	4.41 4.50	74.2	172—174/0.2
VI	2-[$(CH_3)_3C$]C_6H_4	4	$C_{19}H_{31}NO$	289.46	78.84 78.75	10.79 10.83	4.83 4.76	80.8	140—142/0.5
VII	3-$(CH_3O)C_6H_4$	4	$C_{16}H_{25}NO_2$	263.38	72.97 73.09	9.57 9.67	5.32 5.33	68.7	156—157/0.4
VIII	2-$(C_6H_5)C_6H_4$	4	$C_{21}H_{27}NO$	309.45	81.51 81.68	8.79 8.82	4.53 4.60	81.4	186—188/0.2
IX	4-$(C_6H_5)C_6H_4$	4	$C_{21}H_{27}NO$	309.45	81.51 81.60	8.79 8.82	4.53 4.56	73.3	202—204/0.1*
X	2-$C_{10}H_7$	4	$C_{19}H_{25}NO$	283.42	80.52 80.56	8.89 8.96	4.94 4.98	73.0	200—203/1.0
XI	4-[$C_6H_{13}O$]C_6H_4	4	$C_{21}H_{35}NO_2$	333.52	75.63 75.73	10.58 10.48	4.20 4.08	82.9	185—187/0.3

* M.p. 49—51°C.

Experimental

Unsaturated fatty acids: linoleic, linolenic (Schuchard, GFR), and arachidonic (Fluka, Switzerland) were distilled under diminished pressure and stored in fused ampoules at −18°C. Enzyme lipoxygenase (Koch-Light, England), crystalline β-carotene (La Roche, Switzerland), butylhydroxyanisole (Merck, GFR). 1-[4-(2-Naphthyloxy)butyl]--1-ethylpiperidinium bromide and α-bromo-ω-aryloxyalkanes were prepared from the corresponding phenols and α,ω-dibromoalkanes according to [4, 5], substituted piperidine--*N*-oxides by oxidation of tertiary amines with hydrogen peroxide according to [6, 7].

Melting points measured on a Kofler micro hot stage are uncorrected, u.v. spectra were taken with a VSU-2 (Zeiss, Jena) spectrophotometer in 1-cm cells.

N-(ω-Aryloxyalkyl)piperidines (I—XI)

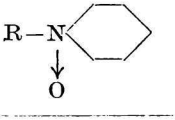
α-Bromo-ω-aryloxyalkane (0.1 mole) was added to a boiling mixture of piperidine (0.2 mole) and dry benzene (200 ml) during 1 hour; the reaction mixture was thereupon stirred and refluxed for 6 hours, cooled and the piperidinium bromide formed was filtered off. The filtrate was extracted with dilute HCl (1 : 1), the acid solution was made alkaline, and the liberated tertiary amine was recovered with benzene. The benzene solution was dried with sodium sulfate, evaporated, and the residue was vacuum-fractionated using Vigreux column. Characteristic data of *N*-(ω-aryloxyalkyl)piperidines thus obtained are listed in Table 1.

Substituted piperidine N-oxides (XII—XIV)

Hydrogen peroxide (35% solution, 0.15 mole) was added during 1 hour into a stirred solution of 1-[4-(2-naphthyloxy)butyl]piperidine (0.1 mole) in methanol (30 ml) at 60—65°C. The reaction mixture was kept at this temperature for additional 4 hours (till the solution became clear), cooled, the excess of peroxide removed catalytically (Pt black), filtered, and the solvent evaporated. The crude product was suspended in light petrol (b.p. 30—60°C), filtered, and crystallized. Characteristic data of the derivatives are given in Table 2.

Table 2

Characteristic data of substituted piperidine-*N*-oxides



No.	R	Formula	*M*	Calculated/found			Yield	M.p. [°C]
				% C	% H	% N	[%]	Solvent
XII	4-(2-Naphthyloxy)-butyl	$C_{19}H_{25}NO_2$	299.42	76.21 76.36	8.42 8.59	4.67 4.58	81.2	130—131 Acetone
XIII	Tetradecyl	$C_{19}H_{39}NO$	297.53	76.70 76.96	13.21 13.46	4.70 5.00	80.8	133—134 Methanol—acetone
XIV	Hexadecyl	$C_{21}H_{43}NO$	325.58	77.47 77.59	13.31 13.23	4.30 4.45	83.1	127—128 Methanol—acetone

Oxidation of unsaturated fatty acids and of β-carotene

Autoxidation and enzymatically catalyzed oxidation of linoleic acid (and similarly also of linolenic and arachidonic acids) was carried out in a model system consisting of the corresponding unsaturated fatty acid Tween 20 (polyoxyethylene(20)sorbitate) and a phosphate buffer solution (pH 7.0). The concentration of fatty acids in substrates varied from 2.5 to 4.7 $\times$ 10^{-4} M, that of the particular tested substances from 0.002 to 0.02% w/v. Preparation of solutions and testing conditions were the same as published in [8].

The oxidation course of substrates incubated at 37°C was examined on the basis of the formation of conjugated dienes by measuring both the absorbance of solutions at 233 nm and the intensity of colour complex of malonic aldehyde with thiobarbituric acid at 535 nm [9—11]. The concentration of conjugated dienes was calculated basing upon the molar absorptivity of linoleic hydroperoxide (ε 26,000) [12].

Catalytic activity of lipoxygenase was studied after addition of the enzyme solution to the reaction mixture by measuring the increase of conjugated dienes within 10 min. of reaction [8, 11, 13].

The degradation of β-carotene was established by measuring the decrease of absorbance of solutions prepared from crystalline β-carotene and Tween 20 in a phosphate buffer solution at pH 7.0 and 460 nm. Solutions were incubated by addition of 0.02% (w/v) of tertiary amines at 37°C for 6 hours [14].

Results and discussion

Solutions or highly disperse suspensions of unsaturated fatty acids in buffer solutions were shown to be a good model system in the entire investigated oxidation course.

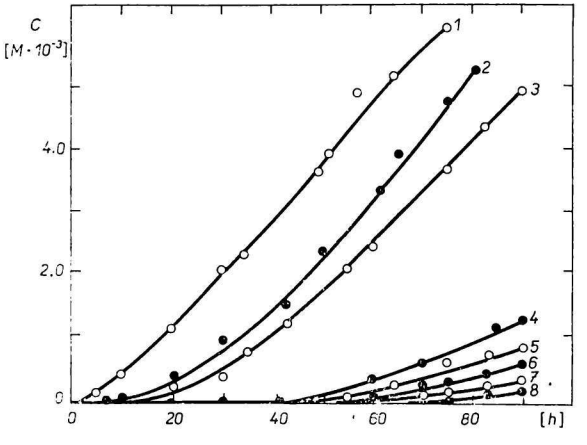


Fig. 1. Content of conjugated dienes (*C*) in solutions of linolic acid in relation to time.
1. reference substance (linoleic acid); *2. IX*; *3. VIII*; *4. III*; *5. VII*; *6. X*; *7.* butylhydroxyanisole; *8. VI.* and *XI.*
Concentration of compounds used 0.02% (w/v).

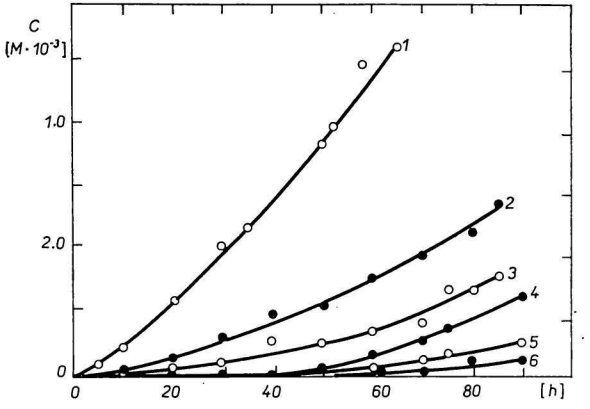


Fig. 2. The dependence of antioxidant effects of compounds with *tert*-butyl group in position 4 on the length of linking chain (*n*).
1. reference substance (linoleic acid); *2. I*; *3. II*; *4. III*; *5. IV*; *6. V*.
Concentration of tertiary amines 0.02% (w/v).

Neither sedimentation nor tendency of layers to become separated has been observed. Reference samples revealed at the end of experiments a slight turbidity which also occurred in systems containing tertiary amines in highest concentrations. To keep the experimental conditions constant and to get reproducible results for comparison of the effect of particular substances, all samples withdrawn were diluted before measurement in a 1 : 10 ratio with 60% ethanol. The thiobarbituric acid test gave, under conditions of model systems of fatty acid solutions, well reproducible results and it was found to be a suitable method especially in a more advanced stage of oxidation.

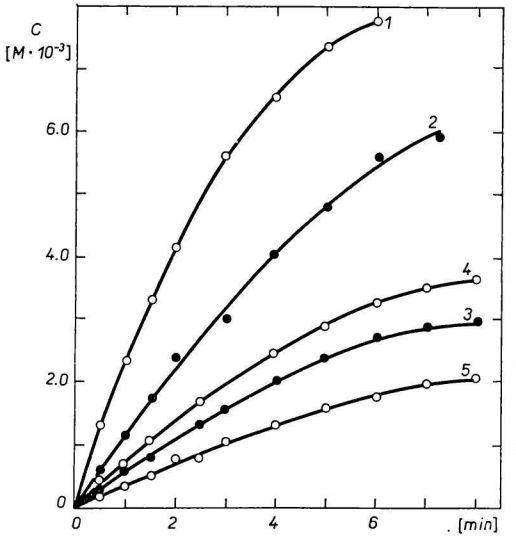


Fig. 3. Oxidation of linolic acid catalyzed by enzyme lipoxigenase.
1. reference substance (linoleic acid); *2.* butylhydroxyanisole; *3. IX*; *4. X*; *5. III*.
Concentration of compounds used 0.002% (w/v).

Fig. 1 shows the autoxidation course of linoleic acid in the presence of selected tertiary amines. Compounds *VIII* and *IX* were found to have a low, *VII* a better antioxidative effect which still increases with *X*, *III*, butylhydroxyanisole (reference antioxidant), *VI*, and *XI*. The last two substances have an equal antioxidative effect. The colour test with thiobarbituric acid offers approximately the same results.

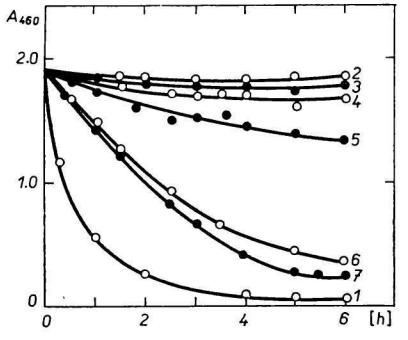


Fig. 4. Degradation of β-carotene in the presence of tertiary amines in relation to time.
1. reference substance (β-carotene); *2. VI*; *3. XI*; *4.* butylhydroxyanisole; *5. X*; *6. VII*; *7. IX*.
Concentration of tested compounds 0.02% (w/v).

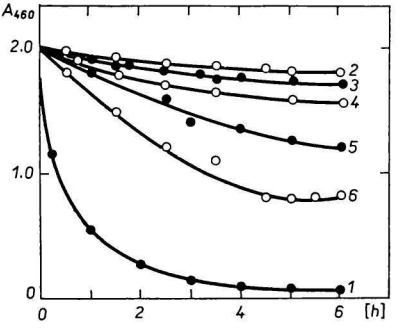


Fig. 5. Comparison of stabilizing effects of compounds having *tert*-butyl group in position 4 on β-carotene in relation to the number of carbon atoms (*n*).
1. reference substance (β-carotene); *2. V*; *3. IV*; *4. III*; *5. I*; *6. II*.
Concentration of tertiary amines 0.02% (w/v).

Since tertiary amines having a *tert*-butyl group in position 4 reveal a marked antioxidative effect on all investigated unsaturated fatty acids, we have further investigated the effect of the length of the linking alkyl chain on the autoxidation course. In the basic structure of 1-[ω-(4-*tert*-butylphenoxy)alkyl]piperidine this chain varied from 2 to 6 carbon atoms. Antioxidative effect of compounds derived from this structure raises with the number of carbon atoms; the top value has been reached with 1-[6-(4-*tert*-butylphenoxy)hexyl]piperidine (Fig. 2). Substances possessing a greater number of carbons are insoluble and could not be applied to the given system.

Common types of antioxidants, as propyl gallate, ascorbyl palmitate, butylhydroxyanisole and others [8] do not inhibit the enzymatically catalyzed oxidation of linoleic acid with lipoxygenase. Fig. 3 shows the increase of conjugated dienes in solutions of linoleic acid in the presence of lipoxygenase. Tertiary amines exhibiting an inhibitory effect in a 0.002% concentration were found to be compounds *III*, *IX*, and *X*, this inhibition being competitive [8]. The inhibition effect of all remaining tertiary amines was worse. In the series with the *tert*-butyl group in position 4 the inhibition effect increases with the number of carbon atoms forming the linkage.

β-Carotene solutions were effectively stabilized with substances *III* and *VI* (Fig. 4), *XI* and *X*. Compounds *VII*, *VIII*, and *IX* are either little or completely inactive. The prolongation of the linking alkyl chain in 1-[ω-(4-*tert*-butylphenoxy)alkyl]piperidine showed positive effect (Fig. 5).

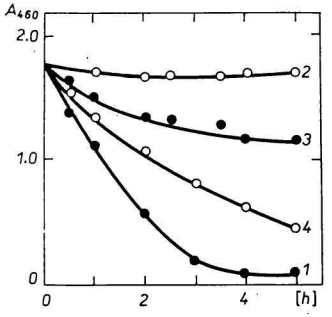


Fig. 6. The effect of structural changes at nitrogen atom on the stabilization of solutions of β-carotene. *1.* reference substance (β-carotene); *2.* butylhydroxyanisole and *X*; *3. XII*; *4.* 1-[4-(2-naphthyloxy)butyl]-1-ethylpiperidinium bromide. Concentration of tested compounds 0.02% (w/v).

The dependence of structural changes at nitrogen atom upon antioxidant and stabilization properties is shown in Figs. 6 and 7. Compound *X* was the model substance. Both ammonium salt and *N*-oxide of 1-[4-(2-naphthyloxy)butyl]-1-ethylpiperidinium bromide reveal a substantial decrease in stabilization properties, as exemplified in Fig. 6 by the decomposition of β-carotene.

Concurrently the disappearance of antioxidant and even inhibitory properties takes place (Fig. 7). Similar is the case with further series of compounds — ammonium salts, where the alkyl chain on ammonium nitrogen consisted of 3 to 18 carbon atoms and with *N*-oxides derived from the examined aryloxyalkylpiperidines. These compounds are well water soluble and indifferent [15], with the increasing number of carbon atoms they exhibit excellent surface-active properties; nevertheless as antioxidants and stabilizers they are ineffective. Toxicity, irritation, and further effects (bactericide effects) of tertiary amines, mainly of those with a *tert*-butyl group in position 4 were tested. Results obtained in this region will be published in the next paper.

The probable action mechanism of tertiary amines derived from aryloxyalkylpiperidines is based on the presence of some functional groups in the molecule of the anti-

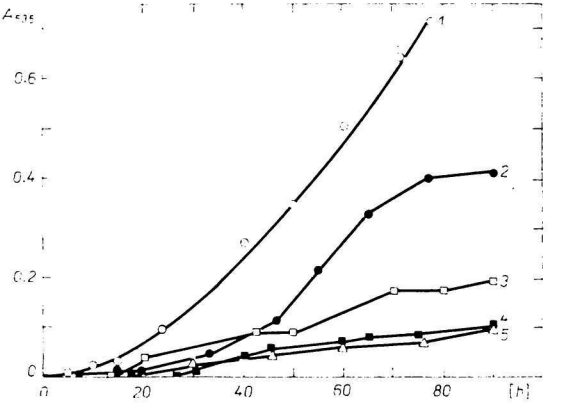


Fig. 7. Comparison of the antioxidant effect of tertiary amines and related substances (colour test with thiobarbituric acid).
1. reference substance (linoleic acid); *2. XIII*; *3. XIV*; *4. III*; *5.* butylhydroxyanisole. Concentration of tested compounds 0.02% (w/v).

oxidant: *tert*-butyl group (in position 2,4) display probably an inhibitory effect in the stage of initiation, whereas the nitrogen of the tertiary amino group with an unshared electron pair has an inhibitory effect during propagation of the radical reaction. Antioxidant effects of both functional groups are combined, the final effect being the stabilization of the hydroperoxide radical and the prevention of further chain reaction.

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