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Proposal of a mechanism for the inhibition of all-*trans*-β-carotene autoxidation at elevated temperature by *N*-(2-phenylethyl)-3,4-diphenylpyrrole

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The rate constant for the loss of all-*trans*-β-carotene heated in liquid paraffin at 210°C is reduced by a factor of 2·8 in the presence of phenylalanine. *N*-(2-phenylethyl)-3,4-diphenylpyrrole is the major non-volatile methanol-extractable thermal degradation product of phenylalanine formed at 210°C and a mechanism for its inhibition of all-*trans*-β-carotene autoxidation is proposed.

All-*trans*-β-carotene is extremely prone to free radical-induced autoxidation (Goldman *et al.*, 1983). Phenylalanine thermal degradation products include alkyl-substituted aromatic compounds (Kato *et al.*, 1971), some of which inhibit the autoxidation of heptane, possibly due to the formation of resonance-stabilised benzyl radicals at elevated temperature (Giammaria & Norris, 1962). Hendry and Russell (1964) demonstrated that several polyarylmethanes and polyarylalkenes, which can form stable free radicals, can effectively retard the autoxidation of hydrocarbons at elevated temperatures. The kinetics of the loss of all-*trans*-β-carotene in the presence and absence of L-phenylalanine heated in liquid paraffin at 210°C have recently been reported (Papadopoulou & Ames, 1994*a*) with the rate of loss of all-*trans*-β-carotene being lower by a factor of 2·8 in the presence of phenylalanine. The structure of the major non-volatile methanol-extractable phenylalanine degradation product formed under these heating conditions has been elucidated by infrared, ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR and high resolution mass spectrometry and is *N*-(2-phenylethyl)-3,4-diphenylpyrrole (Papadopoulou & Ames, 1994*b*).

Although β-carotene autoxidation is most likely to proceed by addition of a peroxy radical ROO· to a double bond to give RO_2R· (Mordi *et al.*, 1993), hydrogen abstraction from carbons 4 or 4' to give the carotenyl radical R· may also occur (Ramakrishnan & Francis 1979). The propagation and termination reactions taking place during β-carotene autoxidation are described by eqns (1)–(4).

$$R\cdot + O_2 \xrightarrow{k_O} ROO\cdot \quad (1)$$

$$ROO\cdot + RH \xrightarrow{k_B} ROOH + R\cdot \quad (2)$$

$$ROO\cdot + R \xrightarrow{k_{B'}} ROOR\cdot \quad (3)$$

$$2ROO\cdot \xrightarrow{k_t} \text{non-radical products} + O_2 \quad (4)$$

Benzyl radicals are stable towards oxygen at high temperatures (about 300°C) and tend to react with other radicals to form non-radical products (Giammaria & Norris, 1962). Such radicals formed from toluene, ethylbenzene and *iso*propylbenzene promote termination reactions and have been found to retard the autoxidation of alkanes when added in small amounts in the vapour phase at 250°C (Giammaria & Norris, 1962). Other compounds with the ability to form highly resonance-stabilised radicals may have a similar effect (Hendry & Russell, 1964).

N-(Phenylethyl)-3,4-diphenylpyrrole may form a stable benzyl radical by abstraction of a hydrogen atom from the methylene group adjacent to the benzene ring (Morrison & Boyd, 1992), as shown in Fig. 1. The bond dissociation energy for the abstraction of hydrogen from the methylene group of ethylbenzene is 82 kcal/mol (Korcek *et al.*, 1972) compared to 79·9 kcal/mol for hydrogen abstraction from the hydroxyl group of 2,4,6-tri-*tert*-butyl phenol (Mahoney, 1969) and 82·1 kcal/mol for hydrogen abstraction from the

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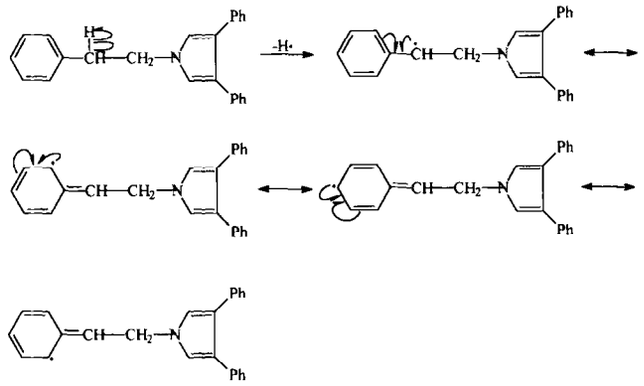

Fig. 1. Resonating structures of the benzyl radical of *N*-(2-phenylethyl)-3,4-diphenylpyrrole, formed by abstraction of hydrogen from *N*-(2-phenylethyl)-3,4-diphenylpyrrole.

hydroxyl group of 4-*tert*-butyl phenol (Mulder *et al.*, 1988), both of which possess antioxidant activity. Therefore, at high temperatures, such as 210°C, hydrogen abstraction from benzyl compounds may be expected to take place as easily as the hydrogen abstraction from a hydroxyl group of a primary antioxidant such as 2,6-di-*tert*-butyl-4-methylphenol (butylated hydroxy toluene, BHT). The benzyl radical of *N*-(2-phenylethyl)-3,4-diphenylpyrrole, A·, would thus be able to take part in the following propagation (eqns 5–7) and termination (eqns 8–11) reactions (Hendry & Russell, 1964), and thereby inhibit the autoxidation of all-*trans*-β-carotene.

$$A\cdot + O_2 \xrightarrow{k_o'} AOO\cdot \quad (5)$$

$$ROO\cdot + AH \xrightarrow{k_p''} ROOH + A\cdot \quad (6)$$

$$AOO\cdot + AH \xrightarrow{k_p'''} AOOH + A\cdot \quad (7)$$

$$2AOO\cdot \xrightarrow{k_t'} \text{non-radical products} \quad (8)$$

$$A\cdot + ROO\cdot \xrightarrow{k_t''} \text{non-radical products} \quad (9)$$

$$A\cdot + AOO\cdot \xrightarrow{k_t'''} \text{non-radical products} \quad (10)$$

$$2A\cdot \xrightarrow{k_t''''} \text{non-radical products} \quad (11)$$

In order for the rate of all-*trans*-β-carotene oxidation to be retarded, $k_{p''}$, $k_{p'''} > k_p$, $k_{p'}$ and $k_o >> k_{o'}$. Thus, an increased concentration of the slow reacting radical, A·, and reduced concentrations of the peroxy radicals, ROO· and AOO·, would result. Also, termination reactions described by eqns (8)–(11) would become important. The more stable a radical is, the more easily it forms (Morrison & Boyd, 1992). Therefore, if A· is more stable than R·, and consequently $k_o >> k_{o'}$, A· would be expected to form more easily than R·, and consequently $k_{p''}$, $k_{p'''} > k_p$.

Apart from the resonance stabilised benzyl radical, A·, *N*-(2-phenylethyl)-3,4-diphenylpyrrole may also form a resonance-stabilised β-peroxy alkyl radical, ROOA· or AOOA·, on addition of a peroxy radical ROO· or AOO· to one of the double bonds of the pyrrole ring (eqns (12) and (13); see Fig. 2).

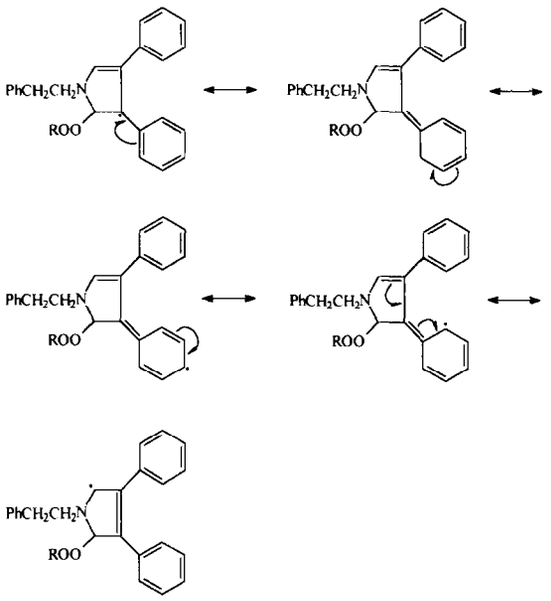

Fig. 2. Resonating structures of the β-peroxy alkyl radical of *N*-(2-phenylethyl)-3,4-diphenylpyrrole, formed by addition of a peroxy radical to *N*-(2-phenylethyl)-3,4-diphenylpyrrole.

$$ROO\cdot + A \rightarrow ROOA\cdot \quad (12)$$

$$AOO\cdot + A \rightarrow AOOA\cdot \quad (13)$$

In order for *N*-(2-phenylethyl)-3,4-diphenylpyrrole to have a protective effect towards β-carotene via the formation of β-peroxy radicals, the β-peroxy carotenyl radicals, ROOR·, formed according to eqn (3), must be relatively short-lived compared to the β-peroxy radicals from *N*-(2-phenylethyl)-3,4-diphenylpyrrole, ROOA· (eqn (12); see Fig. 2).

Accordingly, it is proposed that the inhibition of β-carotene autoxidation by *N*-(2-phenylethyl)-3,4-diphenylpyrrole could take place in the following manner. Heating β-carotene and phenylalanine at 210°C results in the rapid autoxidation of β-carotene and the gradual formation of *N*-(2-phenylethyl)-3,4-diphenylpyrrole. *N*-(2-Phenylethyl)-3,4-diphenylpyrrole is an alternative substrate for reaction of the β-carotene-peroxy radicals, either by abstraction of hydrogen or by addition to a double bond in the pyrrole ring. The involvement of *N*-(2-phenylethyl)-3,4-diphenylpyrrole in the oxidation reactions results in the consumption of the carotene-peroxy radicals in reactions other than those which promote the destruction of β-carotene. The resonance-stabilised radicals formed from *N*-(2-phenylethyl)-3,4-diphenylpyrrole (see Figs 1 and 2) react at lower rates with oxygen than the carotenyl and β-peroxy carotenyl radicals formed from β-carotene, and therefore their concentrations increase. As a consequence, termination reactions, described by eqns (9)–(11) prevail rather than propagation reactions, described by eqns (2), (3), (6), (7), (12) and (13). As degradation of phenylalanine proceeds, *N*-(2-phenylethyl)-3,4-diphenylpyrrole continues to be formed and its antioxidant effect continues, even if a portion of it is consumed in the propagation reactions.

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