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COMMENTARY
REACTION OF PLANT-DERIVED AND SYNTHETIC ANTIOXIDANTS WITH TRICHLOROMETHYLPEROXYL RADICALS

OKEZIE I. ARUOMA[1], JEREMY P.E. SPENCER[1], JOHN BUTLER[2] and BARRY HALLIWELL[1]

[1]*Pharmacology Group, University of London King's College, Manresa Road, London SW3 6LX, UK;* [2]*Paterson Institute for Cancer Research, Christie Hospital and Holt Radium Institute, Wilmslow Road, Manchester, M20 9BX, UK*

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The rate constants for reaction of several lipid-soluble antioxidants with trichloromethylperoxyl radicals are tabulated.

INTRODUCTION

A recent report by Nagababu and Lakshmaiah[1] suggests that eugenol has antioxidant properties that may be of therapeutic importance. The inhibition of microsomal lipid peroxidation was ascribed to the ability of eugenol to quench trichloromethyl- and trichloromethylperoxyl ($\cdot CCl_3/CCl_3O_2\cdot$) radicals.[1]

We have recently investigated the reactions of eugenol, ellagic acid, tannic acid, esculetin, vanillin, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), fisetin, and dl-α-tocopherol with $CCl_3O_2\cdot$, a model peroxyl radical, and thought that it might be useful to tabulate the data for reference purposes. Trichloromethylperoxyl radical ($CCl_3O_2\cdot$), is a reactive organic radical,[2-5] frequently used in studies to assess the ability of a compound to react with peroxyl radicals.[3-6] $CCl_3O_2\cdot$ was generated by radiolysis of an aqueous mixture of propan-2-ol and CCl_4:

$$e^-_{aq} + CCl_4 \rightarrow {}^{\cdot}CCl_3 + Cl^- \quad (1)$$

$$OH\cdot + CH_3CHOHCH_3 \rightarrow H_2O + CH_3\cdot COHCH_3 \quad (2)$$

$$CH_3\cdot COHCH_3 + CCl_4 \rightarrow CH_3COCH_3 + H^+ + \cdot CCl_3 + Cl^- \quad (3)$$

$$\cdot CCl_3 + O_2 \rightarrow CCl_3O_2\cdot \quad (4)$$

Reaction with trichloromethylperoxyl radical was conducted using the Linear Accelerator Facility at the Paterson Institute, Christie Hospital, Manchester, UK. Reaction mixtures contained 1% (v/v) CCl_4 and 50% (v/v) propan-2-ol in 10 mM KH_2PO_4-KOH pH 7.4 and 0.05% (w/v) of the compounds to be tested. The dose

Correspondence to: Dr. O.I. Aruoma, fax + 44 71 333 4949, tel + 44 71 333 4760.

was kept to below 4Gy in order to minimise the mutual decay of the trichloromethylperoxyl radicals. The relative rate constants were calculated from the reaction kinetics[3-6] and were compensated for the small mutual decay of the peroxyl radicals in the absence of compound.

GENERAL DISCUSSION

Previous studies by ourselves and others[4] have shown that the apparent reactions of trichloromethylperoxyl radicals are oxygen and solvent dependent. In our solution mixtures, increases of oxygen concentrations lead to peroxyl radicals being formed from the propan-2-ol radicals at the expense of reaction (3). However, in the above mixture using air saturated solutions, only trichloromethylperoxyl radicals are formed. As some other studies have used different solvent conditions, it is not possible to directly compare all of the rate constants in Table 1. The rates in Table 1 should be considered as an indication of the trends in reactivities. It is for these reasons that all of the reactions were studied at one concentration. This point is further illustrated in Figure 1, which shows the effect of L-dopa concentrations on the rates of reaction with $CCl_3O_2\cdot$. The calculated rate constant $1.3 \times 10^7\ M^{-1}s^{-1}$ is virtually the same as the value we calculate when one concentration of the compound was used.

As expected,[1] eugenol reacted rapidly with $CCl_3O_2\cdot$ (Table 1). dl-α-Tocopherol, several of the other natural compounds tested and the synthetic antioxidant BHA

TABLE 1
Second-order rate constants, for reaction of antioxidants with trichloromethylperoxyl radicals

Compound	Rate Constant $M^{-1}s^{-1}$ ($CCl_3O_2^{\cdot}$ + compound)
Eugenol	5.8×10^6
Esculetin	1.9×10^8
Ellagic acid	2.0×10^8
Tannic acid	3.1×10^8
Butylated hydroxytoluene	see text
Butylated hydroxyanisole	3.0×10^7
dl-α-tocopherol	4.9×10^8
Vanillin	9.5×10^7
Vanillic acid	4.0×10^6
Propyl gallate*	1.7×10^7
Ascorbic acid* (Vitamin C)	1.2×10^8
Hydroxytyrosol*	8.4×10^6
Carnosic acid*	2.7×10^7
Catechin*	6.1×10^6
- Epicatechin*	7.3×10^6
Quercetin*	3.9×10^7
Morin*	3.0×10^8
Fisetin*	4.1×10^8
Myricetin	4.0×10^7
Trolox C*	1.6×10^8
6-Gingerol*	4.7×10^6
Zingerone*	5.6×10^6

Values quoted are the means of 2 or more separate determinations that differed by 5% or less. *Values were abstracted from the literature.[10] 6-Gingerol and zingerone are among the active components in ginger extracts. The catechins are found in tea.

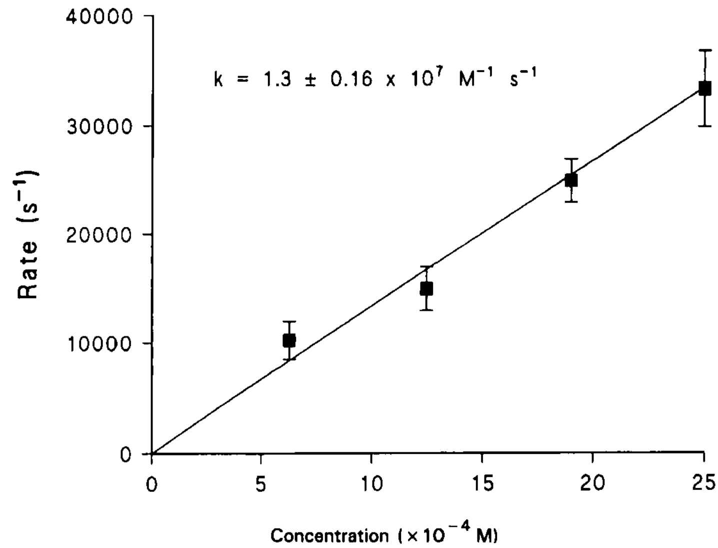

FIGURE 1 Rates of reaction betweeen $CCl_3O_2\cdot$ as a function of L-dopa concentrations. The reaction mixtures were as described in the text. The rates were estimated on two occasions and rate constants are the average of three determinations each. Errors estimated to be about 10–12%. The rates are very temperature dependent, however, the experiment was estimated at a constant temperature of 25°C.

also reacted rapidly with $CCl_3O_2\cdot$. Calculated rate constants are comparable with those for propyl gallate, carnosol, ascorbate and Trolox C. Simic *et al.*[7] quoted rate constants of 3.9×10^7 $M^{-1}s^{-1}$ and 6.1×10^5 $M^{-1}s^{-1}$ for the reactions of peroxyl radicals generated in CCl_4 with BHA and BHT respectively. Unfortunately, we found that BHT was practically insoluble in our reaction mixture even after 3 hrs of bubbling the solution with argon and continuous stirring. Nevertheless, under our assay conditions, the rate constant seemed to be $< 10^5$ $M^{-1}s^{-1}$, in line with the magnitude suggested by Simic *et al.*[7] We calculated a rate constant of $(4.9 \pm 0.2) \times 10^8$ $M^{-1}s^{-1}$ for the reaction of dl-α-tocopherol with $CCl_3O_2\cdot$. This value is in agreement with the one quoted by Packer *et al.*[8] (5.0×10^8 $M^{-1}s^{-1}$). The value of 1.8×10^8 $M^{-1}s^{-1}$ quoted by Simic[7] is in line with the rate constant we calculated (Table 1).

Huie and Neta[9] have also studied the rate constants for one-electron oxidation by methylperoxyl radicals in aqueous solutions. The quoted value of 1.6×10^8 $M^{-1}s^{-1}$ for the reaction of ascorbate with $CCl_3O_2\cdot$ produced by pulse radiolysis of air saturated aqueous solution containing 5 M propan-2-0 and 0.1 M CCl_4 is in agreement with our value (Table 1). However, Packer *et al.*[8] quote a figure of $1.55 \pm 0.2 \times 10^6$ $M^{-1}s^{-1}$ for the reaction of 1.6 mM ascorbate with $CCl_3O_2\cdot$ generated by pulse radiolysis of aerated solution containing 50% (v/v) isopropanol, 10% (v/v) acetone and 40 mM CCl_4.

The well known flavouring agent vanillin reacted rapidly with $CCl_3O_2\cdot$ with a calculated rate constant of $(9.5 \pm 0.5) \times 10^7$. Vanillin possesses an established antioxidant property.[10]

CONCLUSION

We agree with Nagababu and Lakshmaiah that some natural plant phenols may become important therapeutic antioxidants as well as possible replacements for synthetic antioxidants, especially as the role of reactive oxygen species (ROS) in tumour promotion[11] and the involvement of peroxyl radicals as potential mediators of tumour initiation and promotion[12,13] are now widely recognized. However, it is essential that the full profiles of reactions of these compounds with various ROS and interaction with metal ions are established.[10,14,15]

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