*Free Rad. Res.*, Vol. 30, pp. 233–240
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# Antioxidant Activities of Carotenoids: Quantitative Relationships Between Theoretical Calculations and Experimental Literature Data

ANS E.M.F. SOFFERS, MARJON J.H. VAN HAANDEL, MARELLE G. BOERSMA,
BOZENA TYRAKOWSKA, COLJA LAANE and IVONNE M.C.M. RIETJENS*

*Department of Biomolecular Sciences, Laboratory of Biochemistry, Agricultural University,
Dreijenlaan 3, 6703 HA Wageningen, The Netherlands*

Accepted by Prof. H. Sies

*(Received 25 June 1998; In revised form 24 September 1998)*

Quantitative structure activity relationships (QSARs) are described for the antioxidant activity of series of all-*trans* carotenoids. The antioxidant activity of the carotenoids is characterised by literature data for (i) their relative ability to scavenge the $ABTS^{\bullet+}$ radical cation, reflected by the so-called trolox equivalent antioxidant capacity (TEAC) value, (ii) their relative rate of oxidation by a range of free radicals, or (iii) their capacity to inhibit lipid peroxidation in multilamellar liposomes, leading to a decrease in formation of thiobarbituric acid reactive substances (TBARS). All these antioxidant values for radical scavenging action correlate quantitatively with computer-calculated ionisation potentials of the carotenoids. These correlations are observed both when the ionisation potential is calculated as the negative of the energy of the highest occupied molecular orbital ($-E$(HOMO)) of the molecule, or as the relative change in heat of formation ($\Delta\Delta$HF) upon the one-electron oxidation of the carotenoids.

The calculations provide a theoretical assay able to characterise the intrinsic electron donating capacity of an antioxidant, in hydrophilic, hydrophobic or artificial membrane environment.

*Keywords:* Carotenoids, antioxidant activity, lipid peroxidation, calculated ionisation potential, quantitative structure activity relationship

*Abbrevations:* QSAR, quantitative structure activity relationship; PM3, Parametrization Method 3; $E$(HOMO), energy of the highest occupied molecular orbital; HF, heat of formation; IP, ionisation potential; TEAC, trolox equivalent antioxidant capacity; ABTS, 2,2′-azinobis(3-ethylbenzothiozoline-6-sulphonic acid) diammonium salt; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TBARS, thiobarbituric acid reactive substances

## INTRODUCTION

Carotenoids are antioxidants present in food and are suggested to be involved in the protective effects of fruits and vegetables against cardiovascular disease, certain forms of cancer and/or photosensitivity diseases.[1–7] The radical scavenging capacity of carotenoids, and especially of $\beta$-carotene, has been documented in *in vitro*

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*Corresponding author. Tel.: 31 031 7 482868. Fax: 31 0317484801. E-mail: ivonne.rietjens@P450.BC.WAU.NL.

studies.[8–11] Based on studies on the reactivity of carotenoids with nitrogen dioxide, thiyl, sulphonyl, alkyl, alkoxyl and peroxyl radicals, mechanisms for the radical scavenging activity of carotenoids have been suggested.[12–15] These mechanisms include electron donation resulting in formation of a relatively stable carotenoid cation radical, the formation of a relatively stable carotenoid radical cation adduct, and/or scavenging of singlet $O_2$ ($^1O_2$).[7–10]

For mechanisms including radical scavenging antioxidant action, resonance stabilisation of the radical species formed is provided by the extended conjugated $\pi$-system of the carotenoid and provides the basis for the protective radical scavenging effect. This implies that the radical scavenging antioxidant activity of the carotenoids will be related to their electron donating capacity, which is influenced by the extent of resonance stabilisation of their radical cation. Therefore, a direct correlation between the structure of the carotenoid and its radical scavenging antioxidant capacity can be expected. Qualitative structure activity relationships for the radical scavenging or antioxidant activity of carotenoids have been reported.[5,11,16–19]

Quantum mechanical calculations should allow the description of quantitative structure activity relationships (QSARs) for the antioxidant activity of carotenoids. Here various radical scavenging antioxidant values, reported previously for series of carotenoids,[11,17–19] are taken as the experimental values describing the antioxidant radical scavenging activity, and are quantitatively correlated to quantum mechanically calculated parameters.

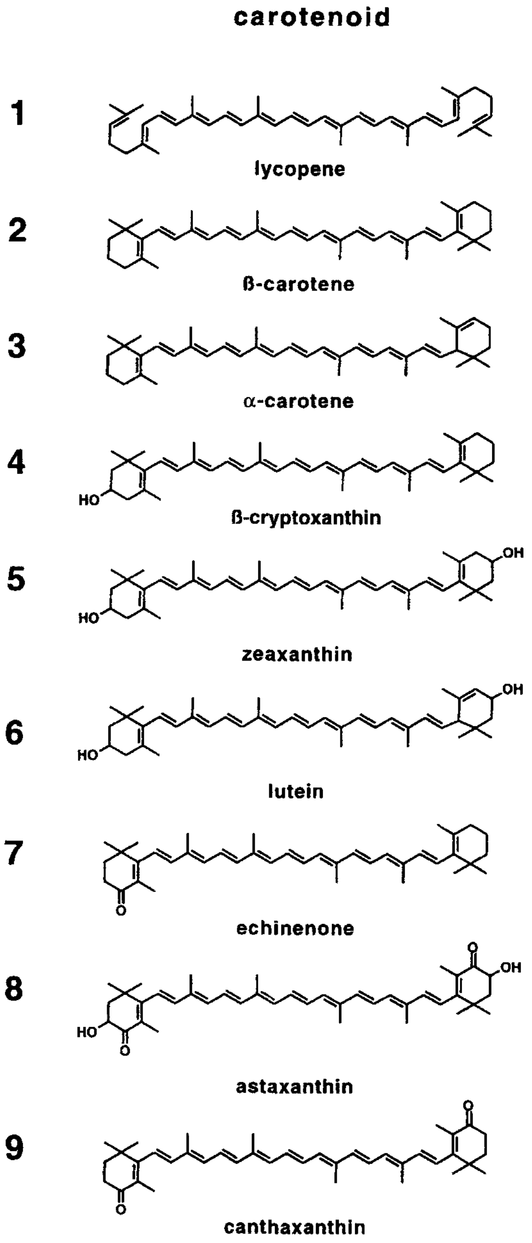

FIGURE 1 Structural formulas, and numbering of the all-*trans* carotenoids of the present study.

## MATERIALS AND METHODS

### Carotenoid Model Compounds

Figure 1 presents the structural formulas of the carotenoids included in the present study. These are the all-*trans* carotenoid structures, for which various antioxidant parameter values are reported in the literature.[11,17–19] With respect to literature data selection, it is important to point out that some data sets from the literature are not included in this study, because (i) they did not contain enough data points,[20] (ii) the mechanism of antioxidant action by the carotenoids has been defined to be different from radical scavenging,[21,22] or (iii) they result from experimental procedures of low sensitivity, leading to data sets in which the difference between the highest and lowest value is less than a factor 3, being thus too much influenced by experimental errors.[17,20,21]

### Definition of Starting Geometries for the Calculations

The three-dimensional structure of all-*trans* β-carotene was taken from the Cambridge Structural Database at the CAOS/CAM Centre in Nijmegen (The Netherlands). Structures of the other all-*trans* carotenoids were built from the crystal structure of all-*trans* β-carotene using the builder option in Insight II version 97.0 (Molecular Simulations Inc. Cambridge, United Kingdom).

### Quantum Mechanical Calculations

Quantum mechanical calculations were carried out on a Silicon Graphics Indigo$^2$ using Spartan 5.0 (Wavefunction Inc., CA, USA). Semi-empirical molecular orbital calculations were performed using the PM3[23] Hamiltonian.

In this study, the outcomes of quantum mechanical calculations on carotenoids in vacuum are related to the electronic characteristics and experimental data of the carotenoids in solution or embedded in a liposomal membrane. Due to solvation effects and a different dielectric constant, the intrinsic properties of the compounds might be influenced upon solvation and/or membrane incorporation. When these influences are assumed to be similar over the whole series, this phenomenon will not have a substantial influence on the relative differences between parameters for a series of closely related compounds, because it will affect the outcomes in an absolute way, without disturbing the relative order within the series.[24–28] The outcomes of the computer calculations on molecules in vacuum can thus be used as an approach to study relative differences within a series of related compounds[24–27] or within one molecule.[28] When the assumption of similar influence of solvation and/or membrane incorporation is not valid in a specific assay, the correlation will be lost, but this will provide mechanistic insight.

## RESULTS

### Quantum Chemical Calculation of the Ionisation Potentials

Two strategies are used to obtain the computer-calculated parameter representing the ease of electron donation by the carotenoids. Following Koopmans theorem[29] the ease of electron donation by a molecule, i.e. its ionisation potential (IP), equals the negative of the energy of the so-called highest occupied molecular orbital, *E*(HOMO). Table I presents the −*E*(HOMO) values of the different carotenoids of the present study calculated using the semi-empirical PM3 Hamiltonian.

In a second approach, the relative differences in heat of formation (ΔΔHF) for conversion of the parent carotenoid into its one electron oxidised radical cation is calculated as the parameter reflecting the relative ease of electron donation by the antioxidant molecule, i.e. its ionisation

TABLE I Ionisation potential (IP) of the carotenoids calculated by the semi-empirical PM3 method as −*E*(HOMO) and as the relative ΔHF between the parent compound and its radical cation, $\Delta HF = HF(\text{carotenoid}^{\bullet +}) - HF(\text{carotenoid})$. ΔΔHF values are calculated relative to the lowest ΔHF value which was set at 0

| Carotenoid | −*E*(HOMO) (eV) | ΔΔHF (kcal/mol) |
|---|---|---|
| **1** Lycopene | 7.776 | 0.000 |
| **2** β-carotene | 7.833 | 5.928 |
| **3** α-carotene | 7.815 | 6.739 |
| **4** β-cryptoxanthin | 7.824 | 5.768 |
| **5** Zeaxanthin | 7.855 | 6.500 |
| **6** Lutein | 7.871 | 6.820 |
| **7** Echinenone | 7.886 | 6.115 |
| **8** Astaxanthin | 8.028 | 10.930 |
| **9** Canthaxanthin | 7.985 | 10.087 |

potential. In this case, the relative differences in free energy for the reaction ($\Delta G$) are represented by differences in the calculated reaction enthalpy in vacuum ($\Delta$HF). This implies that it is assumed that relative changes in the reaction entropy ($\Delta S$) are similar for the different carotenoids and that effects of solvent on the $\Delta$HF values are also similar for the series of compounds compared. As a result, the calculated $\Delta$HF values do not represent the actual energy requirements of the reaction, but can be assumed to reflect the differences between the reactions with different carotenoids on a relative scale. The advantage of this second approach over the approach using $-E$(HOMO) is that now the radical delocalisation and its effect on energy stabilisation of the carotenoid radical cation are also taken into account. Table I presents the relative $\Delta$HF (=$\Delta\Delta$HF) values calculated for the one-electron oxidation of the different carotenoid molecules.

## Quantitative Structure Activity Relationships for Antioxidant Activity

First, the experimental antioxidant activity of the various carotenoids can be quantified by the so-called TEAC values, taken from the literature[11] (Table II). The TEAC value is based on the ability of the carotenoid to scavenge the blue coloured ABTS$^{\bullet+}$ (2,2′-azinobis(3-ethylbenzothiozoline-6-sulphonic acid) diammonium salt) radical cation. The TEAC value represents the ratio between the extent of scavenging of ABTS$^{\bullet+}$ by the antioxidant under investigation, compared to the extent of ABTS$^{\bullet+}$ scavenging by the water soluble vitamin E analogue Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The TEAC values reported for the carotenoids were obtained in a so-called decolourisation assay.[11] TEAC values thus obtained are reported to include the extent and not the rate of reduction of the ABTS$^{\bullet+}$ radical cation.[30] This implies that, from a theoretical point of view, the TEAC value and not its natural logarithm should be plotted against the calculated ionisation potential. Figure 2(a) presents the TEAC values plotted against the ionisation potential calculated as $-E$(HOMO) of the carotenoids. A quantitative correlation ($r = 0.914$) is obtained. The plot presented in Figure 2(a) illustrates that when the calculated ionisation potential of the carotenoid increases, its TEAC value decreases. The TEAC values also correlate with the calculated relative differences in heats of formation for one-electron oxidation of the carotenoids ($\Delta\Delta$HF) ($r = 0.914$) (figure not shown).

Besides the TEAC assay, performed in hydrophilic environment, there are many other assays described in the literature used to define the experimental antioxidant efficiency of series of carotenoids. Thus, once obtaining the quantitative

TABLE II (Antioxidant parameters for carotenoids taken from the literature (nd = not determined)

| Carotenoid | TEAC[11] mM | Rate of carotenoid degradation by | | | TBARS[19] % |
|---|---|---|---|---|---|
| | | Fenton radicals[17] µM min$^{-1}$ | Peroxyl radicals[17] µM min$^{-1}$ | Phenoxyl radicals[18] $k_{\text{relative}}$ | |
| 1 Lycopene | 2.90 | 12.95 | 0.582 | 1.66 | 75 |
| 2 $\beta$-Carotene | 1.90 | 3.346 | 0.421 | 1.00 | 27 |
| 3 $\alpha$-Carotene | 1.30 | nd | nd | nd | 49 |
| 4 $\beta$-cryptoxanthin | 2.00 | nd | nd | nd | 45 |
| 5 Zeaxanthin | 1.40 | 4.070 | 0.310 | 0.79 | 27 |
| 6 Lutein | 1.50 | 2.571 | nd | 0.70 | 23 |
| 7 Echinenone | 0.70 | 2.257 | 0.294 | 0.68 | nd |
| 8 Astaxanthin | 0.03 | 0.813 | 0.177 | nd | nd |
| 9 Canthaxanthin | 0.02 | 0.581 | 0.152 | nd | nd |

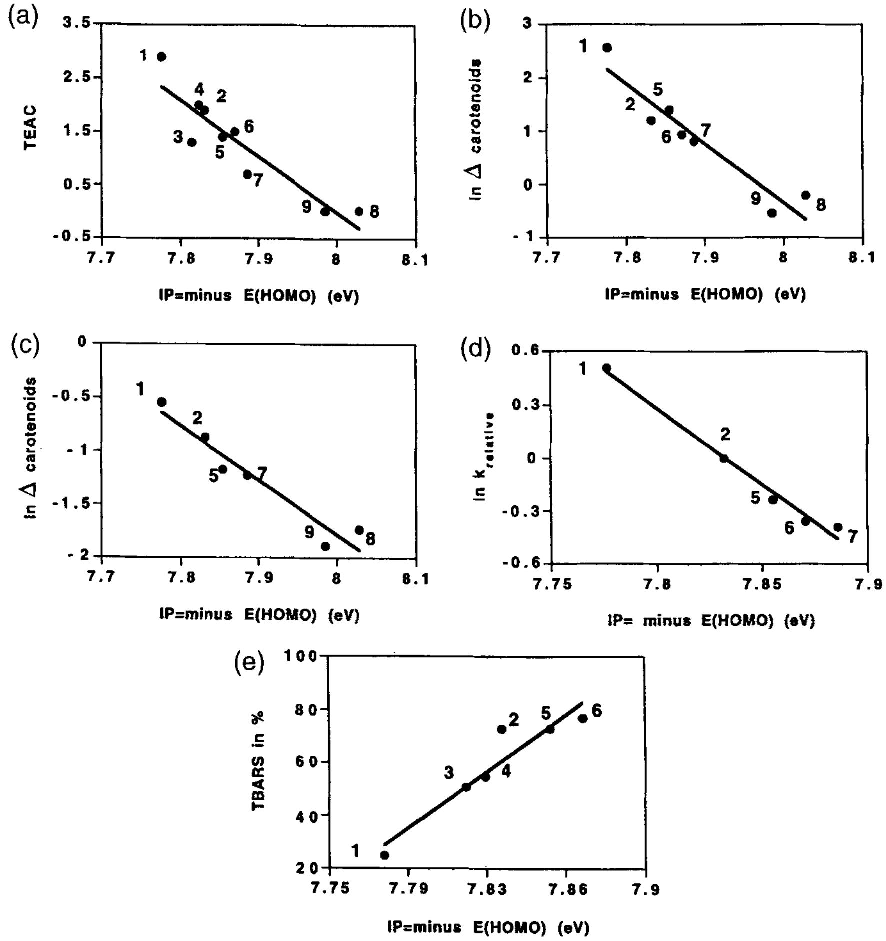

FIGURE 2 Relationships between the antioxidant activity of the carotenoids and their ionisation potentials calculated by the PM3 method as −*E*(HOMO). Experimental parameters used are: (a) TEAC value[11] ($r = 0.914$), the natural logarithm of carotenoid degradation by (b) Fenton radicals[17] ($r = 0.947$), (c) peroxyl radicals[17] ($r = 0.960$), (d) phenoxyl radicals[18] ($r = 0.992$) and (e) the % of TBARS formed upon lipid peroxidation[19] ($r = 0.947$).

structure activity relationships for the data derived from the TEAC assay (Figure 2(a)) it is also of interest to investigate whether similar quantitative correlations can be obtained when antioxidant activity of the carotenoids is quantified in lipophilic systems or even in artificial membranes.

To this end data reported for the relative rates of carotenoid oxidation in organic solvents upon their exposure to different types of radicals are available[17,18] (Table II). Figure 2(b)–(d) presents the natural logarithm of the initial rate of carotenoid oxidation by respectively a mixture of Fenton-type radicals, peroxyl radicals and phenoxyl radicals, plotted against the calculated ionisation potential −*E*(HOMO). In all cases excellent correlations are obtained ($r = 0.947$, $r = 0.960$ and $r = 0.992$ respectively). When ΔΔHF is used as the *x*-axis descriptor similar quantitative structure activity relationships are obtained ($r = 0.955$, $r = 0.940$ and $r = 0.933$ respectively) (figures not shown). In line with theory, the plots show that an increased ionisation potential decreases the rate of radical scavenging by the carotenoids.

Finally, data published recently for the antioxidant activity of a series of carotenoids in an artificial membrane system were used[19] (Table II). In this assay carotenoids are embedded within liposomal membranes and their ability to prevent lipid peroxidation is quantified by measuring their inhibition on formation of thiobarbituric acid reactive substances (TBARS). When the percentage of TBARS formation is plotted against the calculated $-E$(HOMO) (Figure 2(e)) also for this membrane type assay a quantitative correlation between experimental and calculated data is obtained ($r = 0.947$). Using the calculated $\Delta\Delta$HF values the correlation obtained equals 0.847 (figure not shown). The plot presented in Figure 2(e) shows that the amount of TBARS formed decreases when the carotenoid becomes a better antioxidant, reflected by a lower ionisation potential.

## DISCUSSION

Quantitative structure activity relationships (QSARs) for the radical scavenging antioxidant activity of series of all-*trans* carotenoids are described. Most carotenoids occur in nature predominantly or entirely in the all-*trans* form,[5] although 9-*cis*-, 13-*cis*- and 15-*cis*-carotenoids have been described and purified from specific biological samples.[5,31] However, the synthetic analogues for which the various antioxidant reactivities are reported[11,17–19] are in the all-*trans* form. Although the antioxidant activity of carotenoids can include their effect as scavengers of $^1O_2$,[7–10] the experimental literature values used were based on their activity as radical scavengers.[11,17–19]

The QSARs presented here are based on quantum mechanically calculated ionisation potentials and experimental antioxidant activities of the carotenoids reported in the literature on the basis of different types of antioxidant assays.[11,17–19] Thus, instead of giving a qualitative description of the effect of functional groups and the number of conjugated double bonds on the capacity of the carotenoid molecule to donate electrons,[5,11,16–19] computer-calculation-based quantitative structure activity relationships are defined.

The correlations obtained refer to different type of experimental assays to describe the radical scavenging antioxidant activity of the carotenoids. These include the TEAC value obtained in an assay performed in water surroundings, for which the carotenoids are difficult substrates due to their low solubility. However, the results of the present study show that antioxidant values obtained in assays performed in organic solvents, using the rate and not the extent of radical scavenging by the carotenoids,[17,18] provide quantitative structure activity relationships of the same quality as obtained for the TEAC values. Finally, the fact that correlations are still observed when the carotenoids are embedded within a multilamellar liposomal membrane supports the conclusion that in this artificial membrane the relative effectiveness of the carotenoids is still mainly determined by their chemical radical scavenging ability and not much affected by their local environment.

Generally, the more related to an *in vivo* situation, the more complicated and mechanistically difficult it will become to describe the system used for quantification of the antioxidant activity. As a result, parameters additional to the ease of electron donation and stabilisation of the antioxidant radical cation may become of influence, leading to changes in the order of antioxidant activity, and, as a result loss of the correlation with calculated ionisation potentials as observed in the present study. Nevertheless, the theoretical approach of the present study may be especially of value to characterise the intrinsic chemical characteristics of the antioxidants and to quantify the effect of structural features on the electron-donating capacity of the molecules. Once this intrinsic electron-donating capacity has been established and validated with experimental data, deviations from linear relationships between experimental antioxidant parameters and the calculated reactivity parameter may point at a mechanism of antioxidant action in which parameters additional to the intrinsic chemical reactivity of the antioxidant for electron donation, determine its biological effect. Woodall *et al.*[22] illustrate this approach providing data which suggest that in their test system the chemical reactivity of a carotenoid is no longer the only factor determining its ability to protect membranes against oxidation. In these studies the position and orientation of the carotenoid in the membrane was reported to be an important additional factor.[22]

## Acknowledgements

This work was supported by EC grant ERBIC 15 CT961 004.

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