Free Rad. Res. Comms., Vol. 2, No. 4–6, pp. 289–294
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Printed in Great Britain

RADICAL SCAVENGING BY FLAVONOID ANTIOXIDANTS

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(Received July 21st 1986)

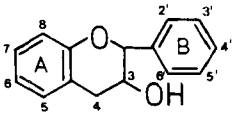
Aroxyl radicals of fifteen structurally distinct flavonoids were generated by attack of azide radicals ($N_3^\cdot$) on the parent compounds dissolved in aqueous solution at pH 11.5. Generation rate constants were all found to be very high ($2.4 - 8.8 \times 10^9\,dm^3\,mol^{-1}\,s^{-1}$), whereas the decay rates differed considerably, ranging from 10^5 to $10^8\,dm^3\,mol^{-1}\,s^{-1}$. In most cases the spectral characteristics of the transient aroxyl radicals relate to structural features of the parent compounds and according to spectral similarities they can be classed in three distinct groups (with only two exceptions).

Although the data do not conclusively prove that the biological function of flavonoids might be the scavenging of radicals, the very high rate constants of formation and the relative stability of some of the aroxyl radicals, are in support of such a hypothesis.

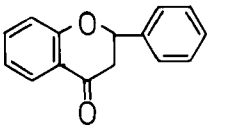
KEY WORDS: Aroxyl radicals, azide radicals, flavonoids, pulse radiolysis, rate constants, transient spectra.

INTRODUCTION

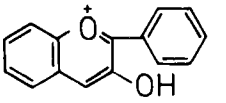
Flavonoids are representatives of a multitude of phenolic compounds, exclusively present in plants. More than 2000 individual substances are known and in general they are hydroxylated, methoxylated and/or glycosylated derivatives of but a few variations of the chromane ring.[1] The structural features of the flavonoid sub-groups which were investigated are depicted in Scheme I.



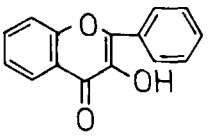
Flavan-3-ol



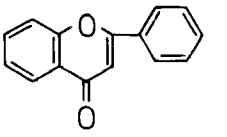
Flavanone



Anthocyanidin



Flavon-3-ol



Flavone

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Flavonoids have – almost from the time of their discovery – been suggested to act as antioxidants,[2] and both their radical-scavenging and metal-chelating capabilities are thought to account for this function.[3–6] The protection of vitamin C from autoxidation[7] might be an example for a metal-chelating effect but very little is known about the radical-scavenging properties.[8,9] Takahama proposes that the two flavonols kaempferol and quercetin are scavengers of superoxide anions (O_2^-) in plant systems.[10,11]

In a recent study on the reactions of fatty acid peroxyl radicals,[12] we found that both kaempferol and quercetin were exceptionally good scavengers of linoleic acid peroxyl radicals (LOO·). We therefore decided to screen various flavonoids in view of the formation and decay of their respective aroxyl radicals. We limited the studies to flavonoid aglycones because they have more consistently been invoked in antioxidative processes (c.f. references [3–6]).

Radical reactions of peroxyl radicals are kinetically very complex[12] and reactions of ·OH radicals are extremely rapid and rather unspecific. Reactions with more weakly oxidizing radicals should exhibit a higher selectivity for the site of attack. Unfortunately, $Br_2^{\cdot -}$ cannot be employed at pH 11.5 owing to complex kinetic processes[13] and $(SCN)_2^{\cdot -}$ interferes with the determination of the aroxyl radical spectrum because of optical overlap.[14] O_2^- has been shown to react with a number of aromatic polyols by oxidizing them to the respective semiquinones[15,16] and it has also been suggested to react with flavonoids.[8,10,11] Yet, in preliminary experiments we found, at pH 11.5, no reaction at all with kaempferol and quercetin and two anthocyanidins with the same substitution pattern (pelargonidin chloride and cyanidin chloride), while at pH 8 O_2^- turned out to be a *reducing* species (as was CO_2^-; unpublished results).

Since other moderately reactive oxidizing radicals do not easily lend themselves for investigation, we selected azide radicals as the primarily oxidizing species and decided on trying to identify possible sites of radical attack at the flavonoid structures by comparing the spectral features of the aroxyl radicals produced.

MATERIALS AND METHODS

The flavonoids – as listed in Table I – were obtained from Roth/Karlsruhe (# **2**, **4**, **6–9**, **12–15**), Serva/Heidelberg (# **3**, **5**, **10**) or Fluka/Neu-Ulm (# **1**, **11**) and were used as supplied. Solutions of up to 0.5 mM were prepared by adding the substances to N_2O-saturated aqueous solutions of 10 mM NaN_3 (Merck/Darmstadt) at pH 11.5. Alkaline pH was chosen to enhance solubility and N_2O-saturation to prevent autoxidation and to double the yield of the precursor radical ·OH – an ancillary benefit was the much higher absorbance of the aroxyl radical anions at pH 11.5.

Azide radicals were produced by reaction of radiolytically generated ·OH radicals with azide anions,[17]

$$N_3^- + OH \longrightarrow N_3^{\cdot} + OH^-$$

the aroxyl radicals in turn by univalent oxidation of the parent flavonoids by the azide radicals.[18,19] All transient spectra obtained were normalized to a dose of 10 Gy.

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RESULTS AND DISCUSSION

Table I lists the compounds investigated – grouped according to their structural relationship – together with the generation rate of the aroxyl radicals by $N_3^{\cdot}$, their respective bimolecular decay rates and spectral parameters.

Generation rates of aroxyl radicals by $N_3^{\cdot}$ at several substrate concentrations were determined by kinetic analysis of the decrease of the absorption of the parent compounds (bleaching kinetics) or increase of the aroxyl absorption (transient build-up kinetics). Similar values were obtained by these two procedures for all substances with the exception of cyanidin chloride (**7**), where kinetics at 315 nm were almost twice as fast as at 490 nm. This might be evidence for concurrent radical attack at different sites of a flavonoid molecule.

Azide radicals, owing to their strong electrophilicity,[19] attack phenolic hydroxy groups rather indiscriminately without adding to the aromatic ring. This is shown by

TABLE I
Spectral and kinetic parameters of flavonoids and their aroxyl radicals

# Trivial name	Substituent position OH	OCH_3	$k_{N_3^{\cdot}}$ [a] $dm^3\,mol^{-1}\,s^{-1}$ ($\times 10^{-9}$)	$2k_d$ [b] ($\times 10^{-6}$)	ε $dm^3\,mol^{-1}\,cm^{-1}$	λ_{max} nm
flavanol:						
1 (+)-catechin	3,5,7,3′,4′	–	5.0	0.6	10.000	310
flavanone:						
2 dihydrofisetin	3,7,3′,4′	–	5.6	c)	5.400	315
3 dihydroquercetin (taxifolin)	3,5,7,3′,4′	–	2.4	0.1	7.600 4.300	315 360
4 dihydroluteolin (eriodictyol)	5,7,3′,4′	–	3.1	0.4	8.900 4.500	315 360
5 hesperitin	5,7,3′	4′	5.8	d)	5.800	270
anthocyanidine (flavylium salt):						
6 pelargonidin chloride	3,5,7,4′	–	6.2	210	19.500	685
7 cyanidin chloride	3,5,7,3′,4′	–	3.0 (315) 1.6 (490)	e)	4.500 1.800	315 490
flavonol:						
8 fisetin	3,7,3′,4′	–	5.2	0.3	4.100	600
9 kaempferol	3,5,7,4′	–	8.8	140	24.000	550
10 quercetin	3,5,7,3′,4′	–	6.6	3.4	15.600	530
11 morin	3,5,7,2′,4′	–	7.3	63	17.600	525
12 kaempferid	3,5,7	4′	6.5	370	8.000	480
flavone:						
13 apigenin	5,7,4′	–	4.8	170	8.100	360
14 luteolin	5,7,3′,4′	–	4.1	0.2	8.100	475
15 acacetin	5,7	4′	2.8	500	6.600	325

[a] Formation rate constant.
[b] Decay rate constant.
[c] First-order decay: $1.45 \times 10^0\,s^{-1}$.
[d] First-order decay: $2.8 \times 10^3\,s^{-1}$.
[e] Pseudo-first order decay (reaction with parent compound): $7.5 \times 10^5\,dm^3\,mol^{-1}\,s^{-1}$ at 315 nm (signal at 490 nm too weak for decay evaluation).

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the close similarity of the generation rates, ranging from 2.4–8.8 $\times$ 10^9 $dm^3 mol^{-1} s^{-1}$. In contrast, the wide range (10^5 to 10^8 $dm^3 mol^{-1} s^{-1}$) of the decay rates reflects the potential of some of the aroxyl radicals to undergo secondary reactions, e.g. forming adducts with peroxyl radicals.[12,20]

Figure 1(a)–(c) depicts the transient spectra of all $N_3^{\cdot}$-generated flavonoid aroxyl radicals, divided into three groups. A clear distinction can be made between the substances in group I (**1–5**) and group II (**8–12**); the fewer representatives of the other groups show a less consistent behavior. Group I consists of compounds with a saturated heterocyclic ring and thus no resonance overlap exists between the two aromatic rings. As a common feature most of them have hydroxy groups in position 3′ and 4′ and we might attribute the absorption peak at 310–320 nm (with shoulders at 360 nm) to the *o*-semiquinone anions.[21] While this is also the case for cyanidin chloride (**7**), the 4′ position in hesperitin (**5**) is blocked by a methoxy-substituent and this compound consequently shows a different radical spectrum. Referring to the table, we find that the semiquinones are consistently rather stable or decay by slow first-order reactions.

Group II consists of flavonols, which contain a 2,3-double bond and a 3-hydroxy substituent. The close similarity of the transient spectra for the substances **9–11** (initial transient absorption at 525–550 nm, final absorption at various decay times between 300–350 nm) suggest an identical site of attack. From the rather small signal of fisetin (**8**) and the bathochromic shift of λ_{max} to 600 nm, we deduce that attack at the 3- *and* 5-hydroxy moieties is kinetically equivalent due to resonance overlap and/or hydrogen bonds with the 4-keto group. This is in line with the transient spectra of the flavones apigenin (**13**) and acacetin (**15**), which lack the 3-OH substituent. However, inconsistent with this view is the transient spectrum of another flavone, luteolin (**14**), which shows an *initial* absorption similar to the 3-hydroxyflavonol kaempferid (**12**, λ_{max} 475 nm and 480 nm, respectively) but a much higher radical stability. We have, at present, no explanation for this deviation.

The transient spectra of the two 4′-methoxylated substances kaempferid (**12**) and acacetin (**15**) resemble, aside from a hypsochromic shift and smaller absorbance, very closely the spectra of the hydroxylated analogs kaempferol (**9**) and apigenin (**13**), which argues for a contribution of the B-ring via resonance overlap in the chromophore. However, only the 4′-OH group seems to participate, since the substances **9–11** which differ only with respect to the fifth hydroxy substituent (**9** – none, **10** – at 3′ and **11** at 2′ positions) exhibit almost identical spectra. While the stabilities of these three aroxyl radicals differ widely, it is obvious that the methoxylated derivatives (**12**, **15**) decay 2.5–3 times faster than their hydroxylated analogs (**9**, **13**).

The final substances to be discussed are the two anthocyanidins (flavylium salts) which exhibit striking discrepancies, although they differ only by one 3′-OH substituent. Flavylium salts have been reported to form weakly absorbing hydrate complexes in aqueous solution (pseudo-bases as opposed to reduced leuko-anthocyanidins) which may account for the spectral behavior of cyanidin chloride (**7**). Since the oxonium moiety is reduced after complexation with water, we find the 3′,4′-dihydroxy group as preferred site of radical attack. Because of the completely different spectral behavior of pelargonidin chloride (**6**) – its strong absorption bands shift both with pH and the redox state of the molecule – we may assume that it does not form such aquo complexes.

Summarizing the results on aroxyl radicals of structurally divergent flavonoids, we can distinguish several cases:

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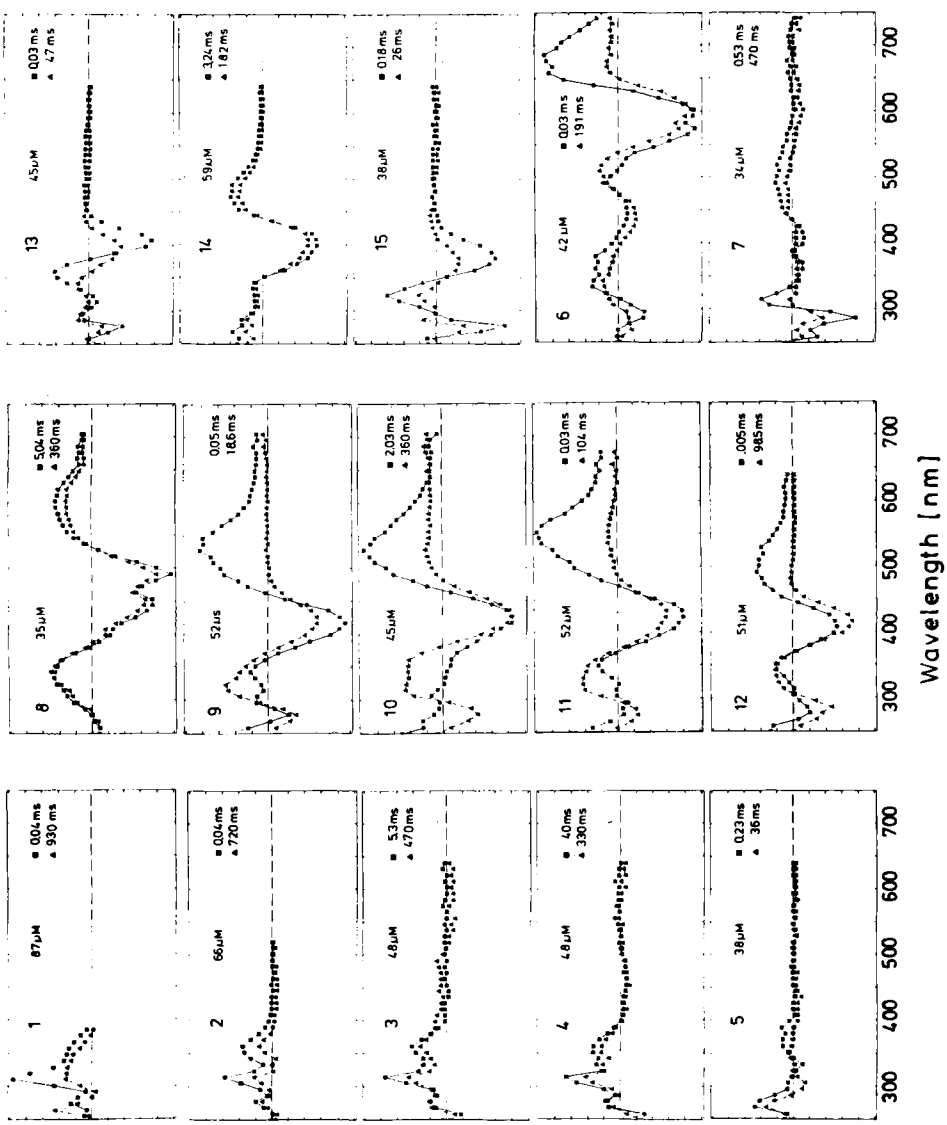

FIGURE 1 Transient spectra of aroxyl radicals of different flavonoids, generated by azide radicals in aqueous solution (pH 11.5). (a) flavanol and flavanones, (b) flavonols, (c) flavones and anthocyanidins. [All solutions contained 10 mM NaN_3 and were saturated with N_2O, the respective solute concentrations are given in the figure; the transient spectra are dose-normalized and were recorded at time of maximal transient absorption (■) and at final observation time (▲); absorbance scale: ± 50 mAUFS (**1–5**, **7**, **8**, **10**, **15**), ± 100 mAUFS (**9**, **11–14**); wavelength scale: 250–750 nm].

i) substances with a saturated heterocyclic ring are predominantly attacked at the *o*-dihydroxy site in the B-ring; the semiquinones formed are quite stable (compounds **1–5**, **7**).

ii) Substances with a 2,3-double bond and both 3- and 5-hydroxy substituents show the strongest transient absorption. Yet, the extensive resonance, extending to the 4′-OH group of the B-ring does not translate into a higher stability of the radicals (compounds **8–12**), nor is there any apparent correlation of structure with decay rate constants.

iii) Substances lacking a 3-OH group (flavones) are, from the structure of their transient spectra, distinct from flavonols (compounds **13–15**).

iv) The unique behavior of the anthocyanidin pelargonidin chloride (**6**) merits further studies.

In a previous study[12] we presented data, that linoleic acid peroxyl radicals react very efficiently with the flavonoids kaempferol (**9**) and quercetin (**10**). In this study we show that the reaction of $N_3^{\cdot}$ radicals with different classes of flavonoids is also very efficient and that, by observing the spectral features of the aroxyl radicals produced, conclusions can be drawn about the primary site of radical attack leading to univalent oxidation of the flavonoid compound.

References

1. Wollenweber, E. and Dietz, V.H. *Phytochemistry*, **20**, 869 (1981).
2. Kühnau, J. *Wld. Rev. Nutr. Diet.*, **24**, 117 (1976).
3. Heimann, W. and Reifl, F. *Fette, Seifen, Anstrichm.*, **55**, 451 (1953).
4. Simpson, T.H. and Uri, N. *Chemistry & Industry*, 956 (1956).
5. Mehta, A.C. and Seshadri, T.R. *J. Sci. Ind. Res.*, **18B**, 24 (1959).
6. Letan, A. *J. Food Sci.*, **31**, 518 (1966).
7. Harper, K.A., Morton, A.D. and Rolfe, E.J. *J. Fd. Technol.*, **4**, 255 (1969).
8. Baumann, J., Wurm, G. and Bruchhausen, F.v. *Arch. Pharm.*, **313**, 330 (1980).
9. Mavelli, I., Rossi, L., Autuori, F., Braquet, P. and Rotilio, G. in *Oxy Radicals and Their Scavenger Systems. II. Cellular and Medical Aspects*, eds. R.A. Greenwald and G. Cohen (Elsevier, New York, 1983), p. 326.
10. Takahama, U. *Photochem. Photobiol.*, **38**, 363 (1983).
11. Takahama, U. *Plant Physiol.*, **71**, 598 (1983).
12. Erben-Russ, M., Bors, W. and Saran, M., manuscript in preparation.
13. Behar, D. *J. Phys. Chem.*, **76**, 1815 (1972).
14. Behar, D., Bevan, P.L.T. and Scholes, G. *J. Phys. Chem.*, **76**, 1537 (1972).
15. Vartanyan, L.S. *Russ. Chem. Rev.*, **44**, 859 (1975).
16. Bors, W., Saran, M. and Michel, C. *J. Phys. Chem.* **83**, 3084 (1979).
17. Treinin, A. and Hayon, E. *J. Chem. Phys.*, **50**, 538 (1969).
18. Bors, W. *Life Chem. Rep.*, **3**, 16 (1985).
19. Alfassi, Z.B. and Schuler, R.H. *J. Phys. Chem.*, **89**, 3359 (1985).
20. Boozer, C.E., Hammond, G.S., Hamilton, C.E. and Sen, J.N. *J. Am. Chem. Soc.*, **77**, 3233 (1955).
21. Gohn, M. and Getoff, N. *JCS, Faraday Trans. I*, **73**, 1207 (1977).

Accepted by Prof. H. Sies

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