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THE ANTIOXIDANTS OF HIGHER PLANTS

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Abstract—Many enzymes and secondary compounds of higher plants have been demonstrated in *in vitro* experiments to protect against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species. The roles of many other compounds as potential antioxidants can be inferred by their similarity to synthetic antioxidants of related structure. The evidence supports at least a partial antioxidant role *in vivo* for many classes of plant metabolite

OXYGEN TOXICITY AND UV TOXICITY

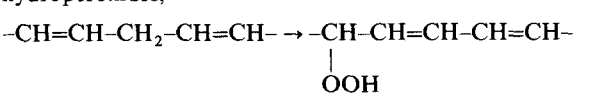
Atmospheric oxygen has been recognized for more than 100 years as the principal agent responsible for the deterioration of organic materials exposed to air The parallel role of oxygen, a molecule essential for many forms of life, as a destructive (toxic) agent for living tissues has been discovered much more recently.

Similarly, light is essential for plants, since photosynthetic pigments efficiently absorb visible wavelengths and use their energy for carbon fixation; but sunlight also contains energetic, short-wavelength ultraviolet (UV) photons which are potentially detrimental because of their destructive interactions with many cellular molecules, such as the amino acids of essential proteins, nucleic acid bases, or membrane lipids [1]. Intense light has long been known to disrupt metabolic processes in plants, including photosynthesis, respiration, glucose assimilation, and phosphorylation [2].

Approximately 4% of the total energy contained in sunlight occurs in the ultraviolet region (wavelengths shorter than 400 nm). The intensity of UV irradiance at the earth's surface varies greatly with season, time of day, latitude, ozone layer thickness, altitude, and cloud cover Distinctions are sometimes made between the 'UV-A' (400–320 nm) and the 'UV-B' (320–290 nm) regions In both cases, however, the fundamental mechanisms of photochemical damage are similar, although different receptor molecules (chromophores) may be involved There have been many reports on deleterious physiological effects on plants exposed to high levels of UV-B, which may increase if stratospheric ozone concentrations decrease. Physiological and biochemical effects of UV-B radiation include effects on enzymes, stomatal resistance, concentrations of chlorophylls, proteins, and lipids, reductions in leaf area, and tissue damage [3]. Wheat plants grown at 3000 m elevation under plastic films which transmit UV-B were significantly shorter than those grown under UV-B-opaque films [4] Some plants, however, appear to be quite resistant to increased UV irradiation. The differential susceptibility of plants to UV stress is clearly an important factor in their competitive relationships in terrestrial ecosystems; experiments with agriculturally important species pairs grown in pots have indicated that significant effects on biomass production took place when UV-B was present either at ambient or artificially increased levels [5].

Photochemically damaging events in cells are initiated by the uptake of the electronic energy of a photon by a UV-absorbing molecule In the UV region of the electromagnetic spectrum, the energy of such photons is sufficient to break covalent bonds, although it is unusual for their energy to be absorbed that efficiently Usually, the absorbed energy converts the target molecule in its ground state to an electronically excited state, whose excess energy manifests itself in a different and often quite unstable electron configuration. The initial excited state, a short-lived singlet having fully paired electrons, may be deactivated by fluorescence (the emission of a photon having a longer wavelength than the exciting radiation) and return to the ground state; it may react with neighboring molecules (although this is not common with singlets since their lifetimes are normally too short for them to diffuse over very many molecular diameters); or it may undergo internal rearrangement to a longer-lived triplet excited state. The triplet state is much more likely to react chemically with surrounding molecules.

A potential route for the formation of a damaging species from a photochemically activated triplet state is the transfer of triplet energy to molecular oxygen The product of the energy transfer reaction is singlet oxygen, $^1O_2$ Several classes of biological molecules are susceptible to attack by $^1O_2$, including several protein amino acids (cysteine, methionine, tryptophan, and histidine) which react with it at quite rapid rates. Polyunsaturated fatty acids also react at much slower rates, increasing with the number of double bonds in the molecule, to form lipid hydroperoxides;



These peroxides are likely contributors to damage and dysfunction in cell and organelle membranes The subject of singlet oxygen and plants has been reviewed by Knox and Dodge [6]

Nonphotochemical routes for oxidative damage in plants usually involve the interaction of molecular oxygen with free radicals to produce new, potentially harmful free radical species containing oxygen This type of reaction may occur directly, or it may be promoted by enzyme catalysts normally present in the plant cell, such as the enzyme lipoxygenase [7]

Atmospheric oxygen is unusual in that its ground state has two unpaired electrons, it is a triplet state with considerable diradical character This permits it to enter into energetically favorable chain reactions with many organic free radicals

$$RH \xrightarrow{\text{init}} R + O\text{–}O \longrightarrow ROO \xrightarrow{R'H} ROOH + R'.$$

The formation of organic (usually carbon-centered) free radicals R from non-radical precursors is called the initiation phase of the autooxidation This process, which is often quite slow, results in the characteristic 'lag period' of a radical chain reaction In the propagation phase of the reaction, there is a buildup of peroxy radicals, ROO , and the subsequent reaction of peroxy radicals with compounds (R'H) having extractable hydrogen atoms The new radicals, R' , are then available for further reaction with molecular oxygen Finally, when all the oxygen or active hydrogen species are used up, the termination phase begins In this phase, the radicals recombine with each other to produce inactive, nonradical products,

$$ROO + R \rightarrow ROOR$$

$$2\ ROO \rightarrow ROOR + O_2$$

Synthetic organic chemists have created many effective inhibitors of oxidative damage for rubber, hydrocarbon fuels, plastics, foodstuffs, and many other materials In principle, free radical chain reactions within a material can be inhibited either by adding chemicals that would retard the formation of free radicals, or by introducing substances that would compete for the existing radicals and remove them from the reaction The first mechanism is exemplified by the addition of carbon black to rubber to prevent the penetration of light into the product Most research in the field of antioxidants, however, is concerned with the second mechanism, designing chemicals which, when added in small quantities to a material, react rapidly with the free radical intermediates of an autooxidation chain and stop it from progressing An excellent example of this type of inhibitor is the synthetic hindered phenol 2,6-di-*tert*-butyl-4-methylphenol, often called 'BHT', which reacts with two mol of peroxy radicals and converts them to much less active products (Fig 1)

It has been recognized for some time that naturally occurring substances, including those found in higher plants, also have antioxidant activity Recently, there has been increasing interest in oxygen-containing free radicals in biological systems and their implied roles as causative agents in the etiology of a variety of chronic disorders Accordingly, attention is being focused on the protective biochemical functions of naturally occurring antioxidants in the cells of the organisms containing them, and on the mechanisms of their action

ENZYMATIC AND PEPTIDE DEFENSE MECHANISMS

Catalase and peroxidases

A long-known metalloenzyme, catalase is one of the most efficient protein catalysts known, it promotes the redox reaction

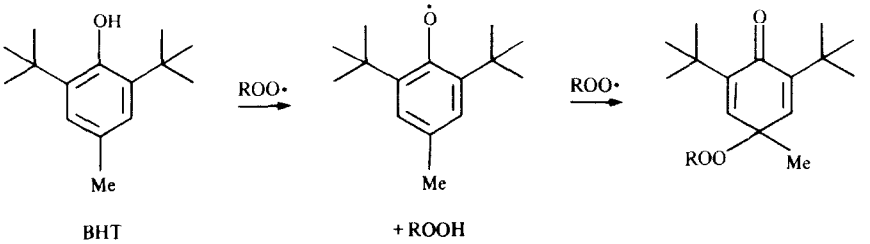
$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but it is probably an intracellular precursor for more reactive oxidants such as HO Although catalase is rather specific for H_2O_2, it reacts with a limited number of organic hydroperoxides, such as MeOOH, using them to carry out oxidative reactions on acceptor molecules while simultaneously reducing the peroxidic substrate [8] Other important plant enzymes, the peroxidases, also function in this mode In addition to defense against active oxygen compounds, plant peroxidases have other important cellular roles [9]

Superoxide dismutase (SOD)

Many one-electron processes have been described that convert O_2 to its radical anion reduction product, O_2^-, superoxide Superoxide dismutases catalyse the conversion of O_2^- to H_2O_2 and oxygen This reaction is quite rapid even without enzymic catalysis at ordinary physiological pHs, although O_2^- is quite stable above pH 11 or so, nevertheless, virtually all aerobic organisms that have been examined contain SOD SOD is a powerful enough catalyst to increase the rate of the reaction by several orders of magnitude at physiological pHs

Superoxide, like H_2O_2, is not directly reactive toward most organic compounds (at least not as an oxidant), but it probably gives rise to more reactive oxygen species of higher potential toxicity



BHT + ROOH

Fig 1 Mechanism of chain-breaking inhibition (peroxy radical destruction) by the synthetic antioxidant BHT

Both superoxide dismutase and catalase activity have been shown to decline in the older leaves of tobacco plants, which revealed signs of membrane damage. There were clear correlations between the activity of these two enzymes and the degree of lipid peroxidation in the leaves. The authors suggested that both enzymes were important agents for protecting leaves from the deleterious effects of membrane lipid destruction [10].

Glutathione

A tripeptide bearing a thiol group, glutathione (GSH) is found in very high concentrations in many cells It reacts with many oxidants such as H_2O_2 to form the oxidized form, a disulphide known as GSSG,

$$ROOH + 2GSH \rightarrow ROH + GSSG + H_2O$$

The above reaction is catalysed in mammalian cells by an important selenium-containing enzyme, glutathione peroxidase. Glutathione also reacts without enzyme catalysis with many other potentially damaging intracellular oxidants such as $^1O_2$, O_2^-, and HO·.

Other proteins

Some soybean proteins have been shown to inhibit lipid oxidation [11] There are many scattered observations, particularly in the food science literature, that peptides or protein hydrolysates protect lipids from oxidation. It is possible that these effects may be due to the metal-complexing capacity of these substances [12].

PHENOLIC DEFENSE COMPOUNDS

Vitamin E

Naturally occurring compounds with vitamin E activity are the tocopherols, a group of closely related phenolic benzochroman derivatives having extensive ring alkylation (**1**). These compounds occur not only in plants but also in mammalian tissues Like other phenolic antioxidants, for example BHT, their normal mechanism of action is the inactivation of two equivalents of chain-carrying peroxy radicals, terminating two potential radical chain reactions per molecule of inhibitor At lower radical concentrations, however, the potential exists for the regeneration of vitamin E through reaction of a reducing agent such as Vitamin C (q.v.)

The most biologically active of the four major tocopherols is α-tocopherol (α-T, **1A**). Burton and Ingold have shown that α-T is one of the most active *in vitro* chain-breaking antioxidants yet tested [13]. It was far superior to the commercial antioxidants, BHA and BHT. The second-order rate constant for the reaction of α-T at 30° with the peroxy radical derived from azobisisobutyronitrile was $2.35 \times 10^6\ M^{-1}\ sec^{-1}$. Burton *et al.* speculate that the long-chain phytyl 'tail' on the tocopherols allows the compound to partition into lipophilic membranes of cells and organelles, where it presumably exerts its antioxidant activity in the prevention of oxidative damage [14]. The high lipid solubility of vitamin E may explain why some *in vivo* antioxidant efficiency measurements for the compound indicate that it is less active than would be predicted from measurements made in solution; relatively water-soluble oxidants may not reach the site of highest vitamin E concentration in time to be destroyed [15]. The peroxy radical derived from α-tocopherol is also stabilized because the unpaired electrons of the chroman ring oxygen are held nearly perpendicular to the plane of the phenyl ring; calculations suggest the stabilization is on the order of 3 kcal/mol [16].

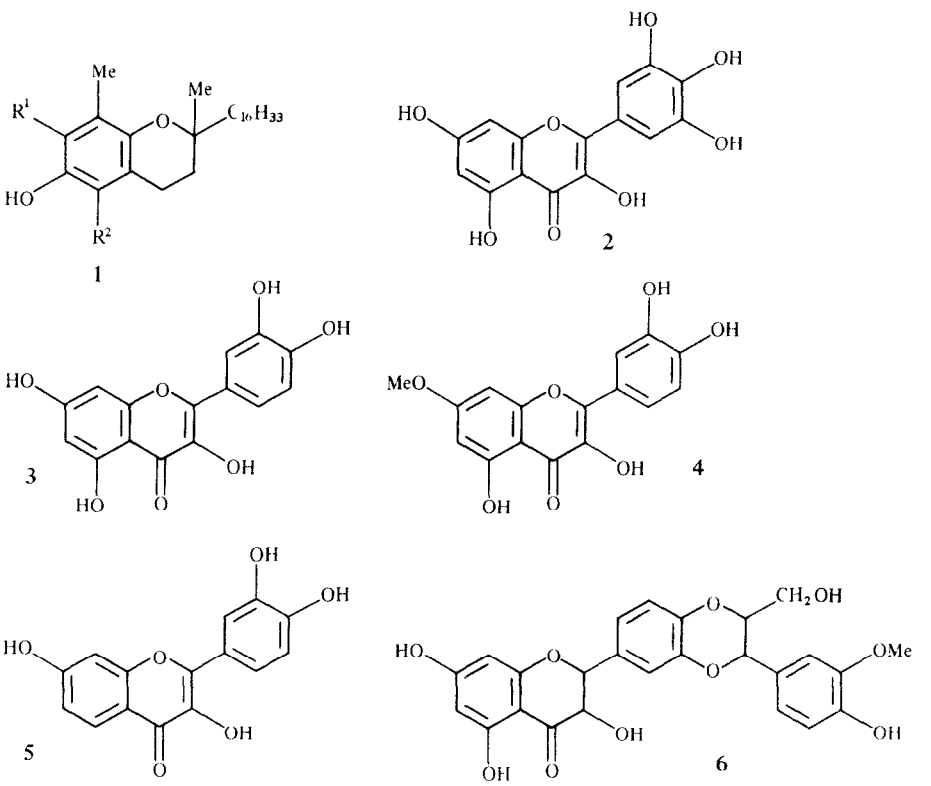
Vitamin E is also one of the best quenchers for $^1O_2$ yet tested, with a quenching rate constant of approximately 6×10^8 (in methanol) [17] and it also appears to react with O_2^- to give a phenoxy radical (although the kinetics of this reaction do not appear to have been determined [18]

Flavonoids

For some time it has been recognized that several classes of flavonoid show antioxidant activity toward a variety of easily oxidizable compounds Flavonoids occur widely in the plant kingdom, and are especially common in leaves, flowering tissues, and pollens They are also abundant in woody parts such as stems and barks The metabolic pool of flavonoids is by no means static, but subject to turnover at widely varying rates [19]. The concentration of flavonoids in plant cells often exceeds 1 mM, with concentrations from 3 to 10 mM being reported in the epidermal cells of *Vicia faba* [20]. The synthesis of many flavonoids and other phenolic compounds is greatly affected by light, for example, tobacco plants grown under supplemental levels of UV contained about twice the concentration of total soluble phenolic compounds compared to control plants [21] Plants grown in full sun have also been shown to contain higher levels of flavonoids than shade-grown plants [22]

Early work by Clemetson and Andersen showed that many flavonoids and other plant phenolics protected against ascorbic acid destruction They noted that flavonoids with either 3′,4′ B ring substituents, or flavonols with their α-hydroxyketone C ring substitution, were active inhibitors. Their work, however, was flawed by the fact that many of the compounds were not soluble in the reaction medium at the levels tested [23]

Pratt measured the antioxidant efficiency of a group of flavonoids in a test system incorporating two easily oxidized lipids, linolenic acid and β-carotene In one set of experiments, activity was demonstrated by the lengthening of the period of time required to bleach the test mixture in the presence of the antioxidant. (This method is similar to a classic kinetic technique, [24] the 'induction-period method', pioneered by Hammond's group and widely used in studies of synthetic autooxidation inhibitors, such as phenols, that act by chain-breaking mechanisms) In suspension tests at 50° with a 5×10^{-4} M flavonoid concentration, the highest activities were shown by free flavonols [myricetin (**2**) and robinetin] having three hydroxyl groups in the B ring with a 3′,4′,5′-substitution pattern Slightly inferior, but still significant, activities were displayed by the pentahydroxy aglycones quercetin (**3**) and dihydroquercetin; the 3-rhamnoside of quercetin; the tetrahydroxymethoxy flavonol rhamnetin (**4**); and the tetrahydroxy flavonol fisetin (**5**). Very little activity was exhibited by rutin (quercetin 3-rhamnoglucoside) or flavonoids having fewer than four hydroxyl groups [25] Dihydroquercetin was isolated from Spanish peanuts (*Arachis hypogea*) and also shown to have high antioxidant activity in a TLC spray test using β-carotene [26] In some studies, quercetin and myricetin have been



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1A, α-tocopherol (R^1 = Me, R^2 = Me)
1B, β-tocopherol (R^1 = H, R^2 = Me)
1A, Γ-tocopherol (R^1 = Me, R^2 = H)
1B, δ-tocopherol (R^1 = H, R^2 = H)

shown to have antioxidant activity at concentrations of less than 10^{-5} M [27, 28]

Cavallini *et al* also showed that quercetin and the complex flavonol-lignan derivative silymarin (**6**) were about equally effective in inhibiting lipid peroxidation in several different microsomal or mitochondrial preparations In a typical experiment, they reported that silymarin at 5×10^{-5} M inhibited 76% of the perchromate-initiated peroxidation of rat liver mitochondria, whereas the identical concentration of quercetin inhibited 67% [29]

Torel *et al* tested a series of flavonoids as inhibitors for the autooxidation of emulsified linoleic acid and methyl linoleate in the dark at room temperature They found that the two compounds with highest activity were morin (**7**) and kaempferol (**8**) They attributed the activity of flavonoids to their ability to donate a hydrogen atom to the peroxy radical derived from the autooxidizing fatty acid derivative [30]

Kaempferol was also reported to undergo photobleaching in illuminated chloroplasts The bleaching response was stimulated by methyl viologen (paraquat), a well-known electron-transfer agent capable of producing O_2^- from molecular oxygen, and it was suppressed by superoxide dismutase These results suggest that flavonoids inhibit O_2^--promoted redox reactions within the chloroplast [31] Further evidence for this postulate was obtained in experiments that showed quercetin and kaempferol, at about 4×10^{-5} M, suppressed lipid photoperoxidation in isolated spinach chloroplasts by 50% The quercetin glycosides, rutin and quercetrin were also effective inhibitors of the peroxidation reaction, but required *ca* 2–3 times higher concentrations to achieve the same level of inhibition [32] Closely related experiments indicated that kaempferol as well as quercetin and its glucosides also inhibited carotenoid photobleaching in chloroplasts at comparable levels to those used in the lipid photoperoxidation experiments [33]

Recent evidence suggests that quercetin, and by implication other flavonoids, are potent quenchers of $^1O_2$ Takahama *et al* demonstrated that 10–100 μM quercetin inhibited the $^1O_2$-induced bleaching of the carotenoid pigment, crocin [34] Larson and Zepp (unpublished) measured the rate constant for reaction of quercetin with $^1O_2$ in water and found it to be comparable to those of the most reactive amino acids

Several flavonoids were shown to be potent inhibitors of the enzymes lipoxygenase and prostaglandin synthetase, which convert polyunsaturated fatty acids to oxygen-containing derivatives. Highest activity against both enzymes was shown by luteolin (5,7,3′,4′-tetrahydroxyflavone) and 3′,4′-dihydroxyflavone, at about 3×10^{-5} M, they inhibited 50% of lipoxygenase activity The authors did not speculate on possible mechanisms for the inhibition of these enzymes by these particular flavonoids [35]

Caldwell *et al* have pioneered a concept that flavonoids, with their strong absorption in the 300–400 nm UV region, are acting as internal light filters for the protection of chloroplasts and other organelles from UV damage

[36]. They have demonstrated that many UV-absorbing compounds occur in high concentrations in the vacuoles of epidermal cells as well as within chloroplasts, and that these compounds can be extracted with polar solvents such as aqueous methanol They have discovered that the leaves of nearly all species in high-UV environments, such as those inhabiting high elevations in arctic and tropical latitudes, have very low epidermal UV transmittance. The light-filtering ability of these compounds may reinforce their powerful antioxidant effects to provide a high level of protection against damaging oxidants generated either thermally or by light

Early work on flavonoid content of alpine plants suggested that their concentrations increased with altitude [37]; it has also been reported that some red-leaved plants such as *Oxyria digyna*, a common species in arctic and alpine ecosystems, are very resistant to UV-B [38]. This may reflect a protective effect of high concentrations of anthocyanin pigments, which are flavonoids. It would be interesting to reexamine the question of flavonoid concentration with altitude using modern techniques. An intriguing paper [39] on the variation of flavonoid content of the red oak, *Quercus rubra*, with elevation in the Appalachian mountains demonstrates that in high-elevation forms, the flavonoid myricetin predominated, whereas in lower-elevation populations, quercetin and kaempferol chemotypes were most abundant It will be recalled that myricetin, with its three adjacent hydroxyl groups, was one of the most active antioxidants among flavonoids [25].

Phenolic acids

Acidic compounds incorporating phenolic groups have been repeatedly implicated as active antioxidants. Caffeic acid (**9**), chlorogenic acid (**10**) and its isomers, including 4-*O*-caffeoylquinic acid (**11**) were isolated from sweet potatoes. Chlorogenic acid was found to be the most abundant phenolic acid in the plant extract and also the most active antioxidant; a 1.2×10^{-5} M solution inhibited over 80% of peroxide formation in a linoleic acid test system [40]. In a different (lard-β-carotene) system, chlorogenic acid (at 5×10^{-5} M) was found to be devoid of antioxidant activity, but caffeic acid at the same concentration had high activity, comparable to that of quercetin [27].

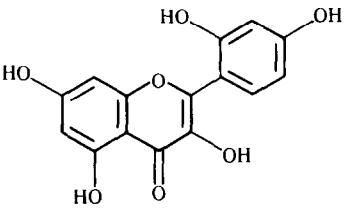
Esters of caffeic acid with sterols and triterpene alcohols have been isolated from the seed of the grass *Phalaris canariensis*. The fatty acids of the seed were predominantly unsaturated, suggesting that the esters were acting to protect them from oxidation. The lipid-soluble esters were effective antioxidants in tests with lard or sardine oil heated at 60° In the tests, the esters were added as mixtures, but at least some components appeared to have activity approaching or exceeding that of BHT [41]

Ferulic acid, at the somewhat high concentration of 10^{-3} M, was shown to delay the photoperoxidation of linoleic acid. Esters of ferulic acid with triterpenes and sterols, which occur naturally in rice bran, were also shown to have some activity [42]

Other phenols

Rosmaridiphenol (**12**), a diterpene derivative with adjacent OH groups, was isolated from *Rosmarinus officinalis* (rosemary). Its antioxidant activity in heated lard exceeded that of BHA and approached that of BHT [43]. Related phenolic diterpenes with antioxidant activity have also been isolated from this plant [44, 45].

A group of potent antioxidants for the air oxidation of linoleic acid was isolated from the methanol extract of the rhizome of *Curcuma longa* (turmeric). The most abundant and most active constituent of the extract was the orange pigment, curcumin (**13**) Its 50% inhibitory concentration



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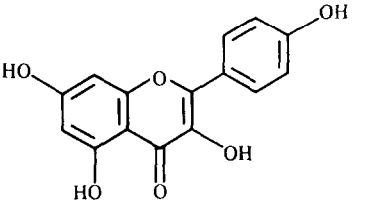

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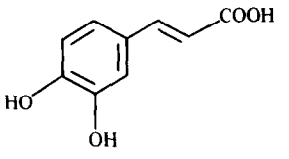

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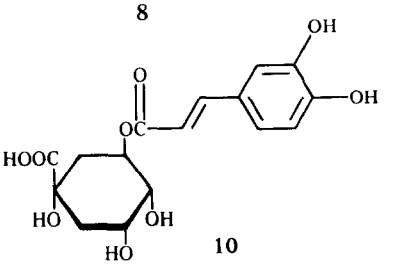

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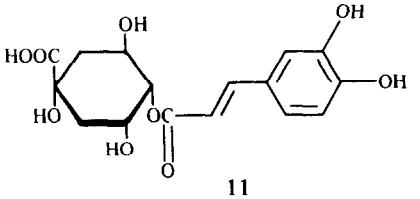

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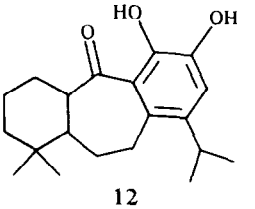

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for the linoleic acid test system was about 5×10^{-4} M, it was more active than vitamin E in the procedure used by the authors, and was roughly an order of magnitude less active than the synthetic antioxidants, BHA and BHT [46]. The mechanism of curcumin activity may include metal ion chelation by the central β-diketone group.

The lignan **14**, isolated from sesame (*Sesamum indicum*) seed, significantly inhibited the autooxidation of linoleic acid at 40° when added at 5.8×10^{-5} M. It was not as active as sesamol or vitamin E [47].

Polyhydroxylated chalcones such as butein (**15**), which are biosynthetic intermediates between cinnamic acids and flavonoids, also show considerable antioxidant activity for lard. In this system, at 100°, butein at about 10^{-3} M was approximately twice as active as the flavonol quercetin or α-tocopherol. It prolonged the induction period for lard autooxidation from 1.3 to 50 hr. Interestingly, chalcones with only two adjacent hydroxyl groups were almost fully effective, introduction of additional hydroxyl groups leading to only slight increases in inhibitory activity [48]. Hydrogenation of the chalcone double bond increased their antioxidant activity to some extent, for example, the pentahydroxydihydrochalcone **16** was *ca* 2–3 times as active as the corresponding unsaturated chalcone [49].

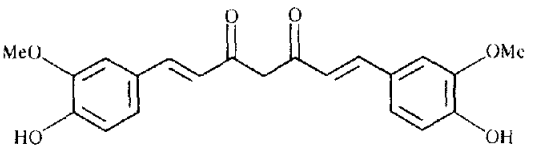
Ubiquinol (**17**), a reduction product of ubiquinone (coenzyme Q), was shown to be a potent *in vivo* antioxidant under conditions of low oxygen concentration, such as would occur in many cellular environments. The compound inhibited lipid peroxidation in emulsions of arachidonic acid containing hemoglobin as initiator, as well as photooxidation of mitochondrial lipids. It was almost as reactive as vitamin E. The observed reactivity of ubiquinol with the stable free radical diphenylpicrylhydrazyl suggested that it was a chain-breaking antioxidant and probably reacted with peroxy radicals *in vivo* [50].

NITROGEN COMPOUNDS

Alkaloids

Increasingly, evidence from a variety of sources is indicating that the basic nitrogen compounds of higher plants include many representatives that are potent inhibitors of various oxidative processes, both *in vivo* and *in vitro*. Lipid peroxidation induced by radioactive cobalt irradiation (in soybean lecithin liposomes) or ferrous ion (in rat liver mitochondria) was inhibited by the bisbenzylisoquinoline alkaloid cepharanthine (**18**). A concentration of 2.5×10^{-5} M prevented 50% of the mitochondrial lipid peroxidation which occurred in its absence in 20 min [51]. Caffeine, from the leaves of tea (*Thea sinensis*) and coffee (*Coffea arabica*), was shown to have antioxidative activity (in a linoleic acid oxidation test) comparable to that of BHA and BHT [52].

A synthetic chemical, 6-hydroxy-1,4-dimethylcarbazole (**19**), which is closely related to the antitumour alkaloid 9-hydroxyellipticine (**20**), was shown to be a remarkably effective inhibitor of several *in vitro* peroxidative systems. The compound completely inhibited peroxidation in rat liver microsomes at less than 1 nmol/mg protein (Fig. 2), a concentration far below that of powerful



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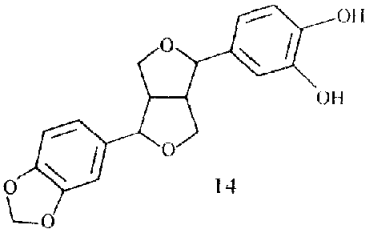

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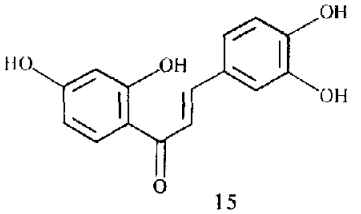

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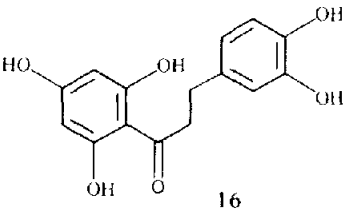

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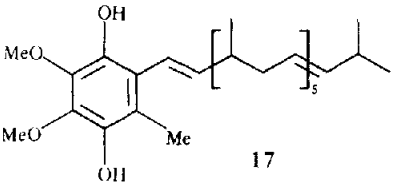

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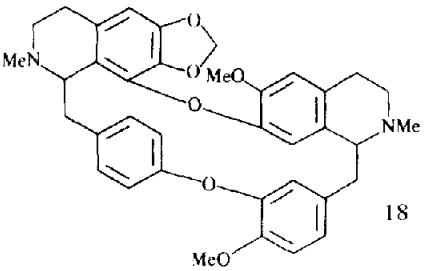

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synthetic antioxidants such as propyl gallate [53]. Similarly, mepacrine (**21**), an antiprotozoal drug closely related to quinine, completely protected (at 50 μM) rat erythrocytes from peroxidative hemolysis and malonaldehyde formation The authors suggested that the hydrophobic, cationic drug was likely to interact strongly with phospholipids and other anionic constituents of cellular membranes [54] This same phenomenon has been clearly demonstrated for ellipticine derivatives with both natural and model lipid membranes [55]

Several alkaloids of various structural types have been found to be potent inhibitors of $^1O_2$. Particularly effective are indole alkaloids such as strychnine (**22a**) and brucine (**22b**) that have a basic nitrogen atom in a rigid, cage-like structure [56] Such alkaloids appear to be strictly physical quenchers, and are not destroyed chemically by the process of quenching [57] Thus, in principle, they could inactivate many molecules of singlet oxygen per molecule of alkaloid

Polyamines such as spermine (**23**) are related to simple acyclic alkaloids found in legumes. This compound was shown in electron spin resonance experiments to scavenge the superoxide radical at rather high spermine concentrations (0.01–0.03 M) This finding may have some bearing on the observed effects of polyamines as membrane-stabilizing substances [58].

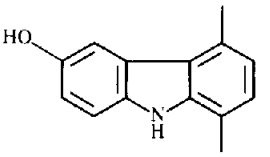
Tertiary amines such as trimethylamine have been shown to be very effective quenchers of peroxy radicals. The radicals derived from the amines have very large termination rate constants, contributing to the efficient cessation of radical chain reactions [59] No studies of this type with alkaloids have been reported to date.

Alkaloids of the quinolizidine type, for example sparteine (**24**), have been found to be stored principally in the epidermal cells of four plants in the genus *Lupinus* Concentrations in these tissues were up to 20 mM [60]. The author suggested that this storage pattern was consistent with a phytochemical role for these substances as antifeedant chemical defense compounds, but it would also be consistent with an antioxidant role It is unlikely that these alkaloids are acting as UV light filtering agents, because their absorption in the solar UV range would be minimal

There has been little work on the variations in alkaloid content with elevation or latitude. Berberine (**25**) concentration in the stems and leaves of two of three Himalayan *Berberis* species increased with elevation [61]. Although berberine, a quaternary alkaloid, is not likely to be a particularly effective antioxidant, it may co-occur with related compounds which are.

Chlorophyll derivatives

Although both chlorophyll (**26**) and pheophytin (**27**) promote the oxidation of lipids in the light, they are inhibitors of autooxidation under dark conditions At 30°,



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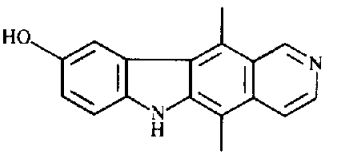

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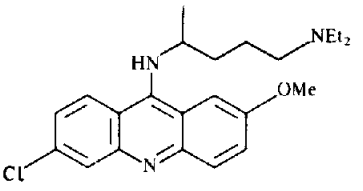

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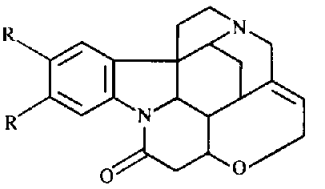

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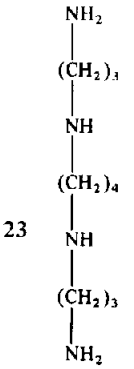

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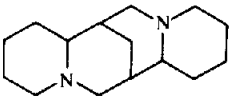
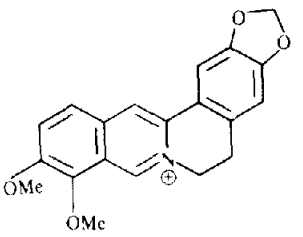


24

22 **22A**, strychnine (R = H)
22B, brucine (R = OMe)



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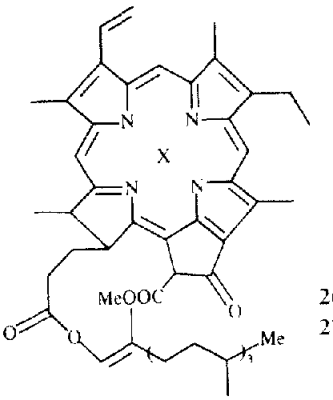

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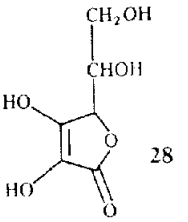

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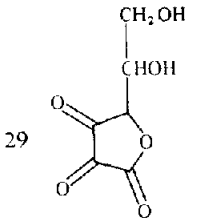

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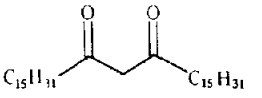


30

26, X = Mg
27, X = 2 H

2×10^{-5} M chlorophyll A was superior by a factor of *ca* two to BHT The compouds appear to be unreactive toward lipid hydroperoxides, but do react with peroxy radicals, electron spin resonance data indicate the presence of a tetrapyrrole radical cation [62]

Amino acids and amines

Many amino acids have been tested for their antioxidant activity, especially in food-based systems. Among the amino acids for which antioxidant activity has been claimed are arginine histidine, cysteine, tryptophan, lysine, methionine, and threonine [63–66] The literature reports are often very confusing, with data suggesting that some amino acids may exhibit antioxidant potential under some conditions of temperature, pH, or oxygen concentration but have no effect or actually promote oxidation in others For example, alanine and histidine were reported to inhibit the oxidation of linoleic acid at pH 9 5 and to promote it at pH 7.5 [67] There is a special need for careful mechanistic work in well-defined systems to clarify the complex behaviour that has been observed

CAROTENOIDS

Although the principal recognized role of carotenoids is to act as photoreceptive 'antenna pigments' for photosynthesis, gathering wavelengths of light that are not absorbed by chlorophylls, it has also been recognized for several decades that they, or at least β-carotene, also have a protective function against oxidative damage.

Studies on the organization of chloroplasts have shown that chlorophyll and enzyme molecules are arranged to allow efficient electronic energy transfers to occur between the excited singlet states of chlorophyll and the acceptor molecules in the photosystems, but under some physiological conditions significant losses from the system take place, with some of the excited state energy from chlorophyll being sidetracked into potentially damaging pathways Both chlorophyll and its tetrapyrrole breakdown products are efficient $^1O_2$ generators Chloroplast membranes are particularly rich in the reactive polyunsaturated fatty acid, linolenate [68]

Singlet oxygen is very powerfully quenched by β-carotene, with a rate constant near that of diffusion control, exceeding 10^{10} M^{-1} sec^{-1} [69] This rate constant exceeds that for the reaction of $^1O_2$ with most biologically important unsaturated fatty acids by four to five orders of magnitude, thus allowing a relatively low concentration of β-carotene to effectively protect membrane lipids from reactions of $^1O_2$ leading to peroxidation

Free radicals are also quite reactive toward β-carotene, at least under certain conditions In a pulse radiolysis study, Packer *et al* showed that the second-order rate constant for the reaction of β-carotene with Cl_3COO was 1.5×10^9 M^{-1} sec^{-1}, about a tenth of the diffusion-controlled rate It was 20–100 times more reactive than

2,5-diphenylfuran and 1,4-diazabicyclooctane [70]. As Burton and Ingold point out, however, a high rate of reaction with free radicals is not a sufficient condition for a good chain-breaking antioxidant. They have shown, however, that β-carotene does behave as a potent antioxidant under lowered oxygen pressures (*ca* 0 1 of atmospheric concentration) At higher oxygen concentrations, β-carotene may be converted to radical species that have some chain-carrying ability and may actually promote oxidation The authors suggest that β-carotene and related compounds may be concentrated in cellular regions that are exposed to low partial pressures of oxygen [71]

OTHER COMPOUNDS

Vitamin C

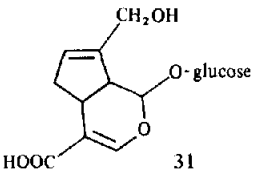
Ascorbic acid (vitamin C, **28**) has been proposed for a long time as a biological antioxidant. It exists in rather high concentrations in many cellular environments, such as the stroma of chloroplasts, where its level is 2–3 $\times 10^{-3}$ M [72]. Ascorbate has been demonstrated in many qualitative studies to possess significant antioxidant activity For example, 10^{-3} M ascorbate inhibited the photooxidation of a kaempferol by illuminated spinach chloroplasts [33] Ascorbate reduces two equivalents of O_2^- (with a second-order rate constant of 3 $\times 10^5$ M^{-1} sec^{-1}) to produce H_2O_2 and the triketo derivative dehydroascorbic acid (**29**) [73]. Ascorbate also reacts with $^1O_2$ at a relatively fast rate (about 10^7 M^{-1} sec^{-1}) [74].

In a well-controlled *in vitro* kinetic study, vitamin C was shown to inhibit the peroxy radical-initiated oxidation of methyl linoleate in methanol-*t*-butanol at 37° Vitamin C was shown to act as a chain-breaking scavenger for peroxy radicals and also to act as a synergist with vitamin E The results suggest that vitamin C can donate a hydrogen atom to the vitamin E-derived phenolate radical, thus regenerating its activity [75].

Miscellaneous compounds

A structurally unusual antioxidant, a long-chain diketone (**30**) has been identified from the leaf wax of *Eucalyptus* species. The compound inhibited the slow autooxidation of linoleic acid heated to 40° in a 1:1 mixture of ethanol and Pi buffer No speculation on the possible mechanism of inhibition was advanced [76]. Similar compounds have been reported to stabilize polymers such as polybutadiene; their activity was attributed to interception of UV light by the enol form of the diketone, followed by intramolecular rearrangement and reconversion to the ground state [77]. The role of light in the antioxidant effectiveness of β-diketones from plants remains to be assessed

A monoterpene glycoside, geniposidic acid (**31**), isolated from *Plantago asiatica*, was as nearly as effective as BHA or BHT at inhibiting the air oxidation of linoleic acid A concentration of about 4×10^{-4} M inhibited 50% of the oxidation as measured by two different tests. Structure-activity experiments with several related glycosides suggested that both the presence of the carboxyl group and the substituent pattern on the five-membered ring were important determinants of the antioxidant activity [78].



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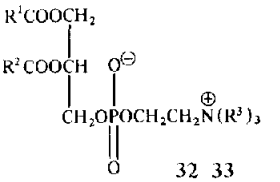

32 33

32 $R^1 = R^2 =$ long-chain fatty acids $R^3 = H$
33 $R^1 = R^2 =$ long-chain fatty acids. $R^3 = Me$

There are several reports that complex lipids, such as phospholipids, have antioxidant activity. It is possible that these compounds act by chelating trace metals that may promote oxidation [79]. Complex lipids may contain some constituents with much higher activity than others As an example, fractionation of a crude soybean lecithin indicated that an alcohol-soluble fraction was far superior to an alcohol-insoluble fraction at preventing the thermal oxidation of lard [80].

SYNERGISTIC EFFECTS

Individual phenolic compounds or mixtures of pure phenolic compounds isolated from an aqueous methanol extract of sweet potatoes had only a small antioxidative effect in a linoleic acid-containing test system, but in combination with a mixture of five amino acids, a significant increase in protective activity was observed The amino acid mixture itself was without activity [40].

The complex lipid, phosphatidylethanolamine (**32**) was shown to be a very effective synergist of flavonoid antioxidation in a test in which the induction period for lard autooxidation was measured [81] It was similarly shown that phosphatidylcholine (**33**) as well as phosphatidylethanolamine synergized the antioxidant effect of α-tocopherol [82]. The presence of nitrogen, together with phosphorus, in these complex lipids presumably increases their metal-complexing ability, but no studies have addressed this problem.

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