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SINGLET OXYGEN AND PLANTS

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Key Word Index—Singlet oxygen, chlorophyll, carotenoids, herbicides, photodynamic reactions, photosensitizers

Abstract—The generation, occurrence and action of singlet oxygen in plant tissue is reviewed Particular emphasis is placed upon its formation from triplet sensitizers and its reactivity with molecules of biological importance such as lipids and amino acids The possibility of singlet oxygen generation in chloroplasts is discussed in relation to potential quenching systems such as carotenoid pigments, ascorbate and α-tocopherol The problems associated with carotenoid diminution and some stress and herbicide treatment conditions are related to the possibility of damage by singlet oxygen The action of a number of secondary plant substances, including quinones, furanocoumarins, polyacetylenes and thiophenes, as plant defence agents is discussed in relation to the photodynamic generation of singlet oxygen

INTRODUCTION

The immobility of plants, in diverse and changing physical environments, along with the possibility of predation by animals and attack by pathogens, has necessitated the development of numerous chemical and biochemical adaptations for protection and offence [1] In recent years considerable attention has been paid to the specific ecological roles of secondary products, often formerly regarded as waste [2]

Singlet oxygen ($^1O_2$), a potential product of photochemical reactions of many compounds, is a damaging agent to all living organisms The chemistry and biochemistry of $^1O_2$ has been covered in several recent reviews [3–7] In this paper particular attention is paid to the formation and control of $^1O_2$ within plants, as well as to its possible role in plant defence

Chemistry of singlet oxygen

Although oxygen is essential for the functioning of most organisms it is a potential toxicant to all forms of life This dichotomous nature arises from certain unique aspects of its chemistry The ground state of molecular oxygen has two unpaired electrons with parallel spin Such a triplet state is rare in ground state molecules and thus, because electrons occupying the same orbital must have opposed spin (the Pauli exclusion principle), the reaction of ground state oxygen with most substances is restricted The activation of oxygen involves overcoming this spin restriction to reaction Reduction leads to the potentially toxic superoxide anion, hydrogen peroxide and the hydroxyl radical Electronic excitation of molecular oxygen, involving spin inversion, results in excited states with no unopposed spins, designated as singlet states

Two excited singlet states of oxygen occur The first singlet ($^1\Delta g$) and second singlet ($^1\Sigma g$) states are of 0 98 and 1 63 eV excitation energy respectively The second singlet state is extremely short lived, being rapidly deactivated by collisional quenching to the first singlet state, which has a lifetime long enough to allow chemical reaction It is the first excited singlet state of molecular oxygen that is involved in certain photooxidative, photodynamic and biological processes

Although $^1O_2$ can be produced from various sources, including enzymes, the major mechanism of formation in biological systems is by energy transfer from photoexcited compounds The absorption of a photon by such sensitizers results in an excited singlet state, with a very short lifetime (10^{-6}–10^{-8} sec) (reaction 1), which by intersystem crossing, involving spin inversion, may be relaxed to a longer lived triplet state (*ca* 10^{-3} sec) (reaction 2) Molecular oxygen, in a non spin restricted reaction, can quench such a triplet state by energy transfer resulting in $^1O_2$ and the regeneration of the ground state sensitizer (reaction 3)

$$S \rightarrow {}^1S \quad (1)$$

$$^1S \rightarrow {}^3S \quad (2)$$

$$^3S + {}^3O_2 \rightarrow S + {}^1O_2 \quad (3)$$

The relatively low excitation energy of its first excited singlet state allows oxygen to quench the triplet states of a variety of compounds Photosensitizing compounds, capable of the efficient population of triplet states, are of diverse origin and structure and are active in regions of the electromagnetic spectrum ranging from the near UV, through visible to the near infra-red [8, 9] Singlet oxygen is responsible for type II photodynamic reactions of exogenous and endogenous sensitizers in biological systems, although direct reaction of the excited sensitizer with a substrate (a type I reaction) can occur [3, 9]

Singlet oxygen is a metastable entity Its lifetime is phase and solvent dependent, being much reduced by collisional quenching in condensed phases The lifetime of

Abbreviations $^1O_2$, singlet oxygen, ^{1}Chl, singlet excited chlorophyll, ^{3}Chl, triplet chlorophyll, PS I, photosystem I, PS II, photosystem II, DABCO, 1,4-diazabicyclo[2,2,2]octane

$^1O_2$ varies from 2 to 4 μsec in H_2O to 25–100 μsec in non-polar solvents [10, 11] Evidence has been presented indicating the increased lifetime of $^1O_2$ in the hydrophobic interior of membranes relative to the aqueous environment of the cell [12] The lifetime of $^1O_2$ appears to be long enough to allow diffusion between aqueous and non-polar phases [13]

Although $^1O_2$ has no spin restriction to reaction, it is not indiscriminantly reactive Its electrophilic nature results in chemical reaction with compounds with heavily substituted double bonds or an electron-rich functionality [3] In addition $^1O_2$ may be physically quenched to its ground state by a variety of substances As a consequence of this selectivity there are certain biomolecules that are cellular targets for the action of $^1O_2$ Specific amino acids, most notably histidine, methionine and tryptophan, are susceptible to oxidation by $^1O_2$ with the possible consequence of enzyme inactivation Membrane disruption, a common feature of photodynamic action, is due to lipid peroxidation initiated by the formation of hydroperoxides from the reaction of $^1O_2$ with unsaturated fatty acids Of the nucleic acids, guanine is particularly sensitive to attack by $^1O_2$ Other classes of compounds may quench as well as react with $^1O_2$, examples being the phenol α-tocopherol and certain amines The most efficient naturally occurring physical quenchers of $^1O_2$ are the carotenoids which are discussed in more detail below

SINGLET OXYGEN GENERATION AND QUENCHING IN CHLOROPLASTS

The potential for $^1O_2$ production and its damaging action

The conditions which favour $^1O_2$ production, i e triplet excited molecules and ubiquitous oxygen, are likely to be found within the active chloroplast The close proximity of excited chlorophyll and an oxygen evolving system is thus potentially hazardous as is also an active electron transport system with the potential for electron donation to oxygen to yield the superoxide anion Superoxide is probably dismutated via a linked series of enzymes involving superoxide dismutase, ascorbate peroxidase and glutathione reductase [14]

The organisation of the pigments into the lipo-protein membranes of the chloroplast is now generally resolved into a series of pigment–protein complexes which cooperate together to promote electron flow [15] When a chlorophyll molecule absorbs a quantum of light energy it enters an excited singlet state Much of the excitation energy is passed via resonance transfer to the energy traps P680 and P700, thus promoting electron flow [16] Some energy may be passed from PS II to PS I as spillover, and some lost as fluorescence In the presence of photosynthetic electron transport inhibitor herbicides, such as diuron, fluorescence emission increases [17] If the excitation energy from ^{1}Chl is not dissipated by any of these mechanisms intersystem crossing may occur to form the longer lived (10^{-3} sec) triplet state [16] Singlet chlorophyll has a lifetime of approximately 10^{-8} sec The triplet state is not only potentially damaging itself in type I reactions [3], but also by virtue of triplet–triplet interaction with ground state oxygen it may generate $^1O_2$ [3, 9]

The potential damaging action of $^1O_2$ has already been discussed The chloroplast thylakoid membranes are particularly susceptible to $^1O_2$ induced lipid peroxidation as approximately 90% of the fatty acid component of the thylakoid glycolipids, phospholipids and sulpholipids is the 18 3 unsaturated fatty acid α-linolenate [18] Isolated chloroplast thylakoid membranes when incubated in the absence of electron acceptors and thus likely to favour $^1O_2$ generation, have been shown to undergo pigment and lipid breakdown [19–21] These reactions were enhanced by conditions which promoted $^1O_2$ generation and lifetime, such as high light, oxygen, ^2H_2O, and limited by $^1O_2$ quenchers such as DABCO and carotenoids [20–22] Chloroplast membranes were also disrupted by the action of exogenous generators of $^1O_2$ such as rose bengal [20, 21, 23]

$^1O_2$ quenching systems

As $^1O_2$ is potentially damaging to chloroplast membrane lipids, as well as to proteins and nucleic acids (see Introduction), it is to be expected that there are protective systems to quench this species The primary means of defence within the chloroplast are the carotenoid pigments of which there is approximately one molecule to five of chlorophyll [18] Quantitatively lutein and β-carotene are the most important and to a lesser extent violoxanthin and α-carotene The carotene pigments appear to be associated with PS I and PS II reaction centres, where in addition to a photoprotective role they are associated with light harvesting and prevent the transfer of excitation energy from the centres [24] The xanthophyll pigments are located in the light harvesting pigment–protein complexes [25] In dark grown plants a small amount of carotenoid pigment is present in the total absence of chlorophyll [26, 27], thus upon illumination the early formed chlorophyll pigment and membrane systems are protected from destruction The ratio of carotenoid to chlorophyll is favourable for protection in the early stages of greening prior to the inception of full photosynthetic activity [Gillham, D, personal communication]

The carotenoid pigments appear to have a dual protective role quenching both ^{3}Chl and $^1O_2$ [28] The de-excitation of $^1O_2$ to $^3O_2$ by β-carotene has been estimated to proceed at a diffusion controlled rate of 2–3 $\times 10^{10}$ M^{-1} sec^{-1} [6], and the triplet carotenoid so produced is deactivated by heat transfer All carotenoid pigments with nine or more conjugated double bonds are almost equally effective, but below nine, quenching becomes increasingly ineffective [29]

The $^1O_2$ quencher α-tocopherol (vitamin E) is located within the thylakoid membranes in a ratio of around one molecule to 24 chlorophylls [18, 30] Although this may have a structural role within the chloroplast [31], it quenches $^1O_2$ at a rate constant of around 1 $\times 10^8$ M^{-1} sec^{-1} [32] Bishop and Wong [33] demonstrated that an α-tocopherol deficient mutant of *Scenedesmus obliquus*, was particularly susceptible to photodynamic injury

Thus far quenching of $^1O_2$ within the chloroplast has been discussed in relation to membrane bound components Within the chloroplast stroma ascorbate is generally present at a concentration of 2–3 mM [34] Although ascorbate acts as an effective quencher of superoxide [35], and the hydroxyl radical [36], it has also been shown to scavenge $^1O_2$ [37] It is of interest that molybdenum deficient plants show a considerably reduced level of ascorbate and also display major ultrastructural damage [38, 39]

Problems of carotenoid limitation

It is not possible at present to establish the quantitative role of $^1O_2$ quenchers in chloroplasts under relatively normal conditions However some indications of their importance is gained by studies with carotenoidless mutants and carotenoid inhibited plants Although a suggestion that carotenoids protect chlorophyll was made as early as 1902 [40], it was not until nearly sixty years later that conclusive evidence for this role was accumulated from higher plant [41], algal [42] and bacterial systems [43, 44] In the experiments of Anderson and Robertson [41] with an albino mutant of maize, chlorophyll was formed normally but further metabolism of phytoene to cyclized carotenoids was prevented After a period of chlorophyll formation in low light, the exposure to high light and air resulted in a rapid chlorophyll breakdown This was not the case when air was replaced by nitrogen

More recently a number of herbicides have been developed which appear to have a specific action by blocking carotenoid biosynthesis [45] There is good evidence for an effect on the desaturase reactions between phytoene and lycopene for the herbicides metflurazon (SAN 6706) [46–49], norflurazon (SAN 9789) [50, 51], fluridone [50], dichlormate [52], pyridinol [52], difunon [53] and fluorometuron [54] The triazole herbicide amino triazole has been suggested to inhibit the cyclization reactions between lycopene and the carotenes [55] In all instances these herbicides promoted chlorosis, which was enhanced by increased light intensity, as shown for metflurazon in wheat seedlings [56] and fluridone in *Potamogeton nodosus* [57]. Kunert and Boger [53] demonstrated that chlorophyll breakdown in *Scenedesmus acutus* cultures was delayed by nitrogen gassing after treatment with norflurazon A number of investigators have shown that bleaching was paralled by a major disruption of membrane integrity and cell structure [50, 54, 56, 58, 59]

Stress conditions and the formation of $^1O_2$

A number of other treatment conditions also promote photodestruction in plants and these could involve the generation and action of $^1O_2$ This has been shown for example during chlorophyll formation under high light conditions [60], during the incubation of leaves in the absence of carbon dioxide [61], under chilling conditions [62–64] and in the presence of photosynthetic electron transport inhibitor herbicides [65–69] Chlorophyll breakdown in chilled leaves proceeded more slowly than carotenoid breakdown and was diminished by incubation under nitrogen [62] In the case of electron transport inhibitor herbicides, damage to leaves was diminished if incubated under argon [68], and reduced to a limited extent by incubation with the $^1O_2$ quencher, DABCO [70] Approximately half of all currently utilized herbicides operate by inhibiting photosynthetic electron flow and these include the phenylureas, triazines and uracils [71] There appears to be one primary site of action, the so-called Q_B protein, located between PS II and plastoquinone [72, 73] Non-covalent binding at this site by these herbicides renders electron flow thermodynamically unfavourable Pallett and Dodge [68] have proposed that the inhibition of electron flow promotes the generation of ^{3}Chl and hence $^1O_2$ Experiments with flax cotyledons and a number of herbicides including monuron and ioxynil showed that carotenoid pigments were destroyed prior to the destruction of chlorophyll Damage to chloroplast and cellular membranes could be promoted by both type I and type II reactions and lead to massive cellular disorganization, clearly observed by electron microscopy [58, 74]

If cellular destruction is promoted *in vivo* by conditions which limit $^1O_2$ quenching or de-excitation of ^{1}Chl, it should be possible to destroy plants with exogenous $^1O_2$ generators This was first suggested by Zweig and Nachtigall [75] who used the hydrocarbon compound fluoranthene which is photoexcited by UV radiation The xanthene dye and $^1O_2$ generator rose bengal has been shown to have some potential as an insecticide [76], bacteriocide [77] and also to promote damage to plants [78–80] The related dye eosin, also known to photogenerate $^1O_2$ inhibited photosynthesis in pea leaves by inactivating certain photosynthetic enzymes as well as promoting the disorganization of chloroplast thylakoids [81] The primary site of damage appeared to be associated with PS II [82]

SINGLET OXYGEN AND SECONDARY PLANT SUBSTANCES

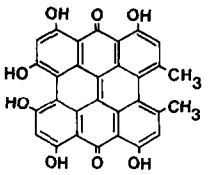
It is now generally accepted that certain secondary plant substances have a defensive role, offering protection against predators, pathogens and competitors [2, 83, 84] It is increasingly recognised that certain of these defensive chemicals are capable of photosensitizing reactions that involve the transfer of light energy to oxygen [85] It is thus apparent that plants may utilize these activated forms of oxygen, such as $^1O_2$, in their own defence Other secondary plant products may have a physiological role in that they protect the plant against damaging photodynamic reactions by quenching the excited singlet state of oxygen

A role for the activation of oxygen in the interaction between species is suggested by the fact that secondary plant metabolites, capable of the photodynamic generation of $^1O_2$, are proposed in the diverse biological roles of insect deterrents, phytoalexins, fungal toxins and allelopathic agents Although toxicity may also occur independently of any photodynamic reactions, the enhanced toxicity due to the transfer of light energy to oxygen may be sufficient to confer an evolutionary advantage

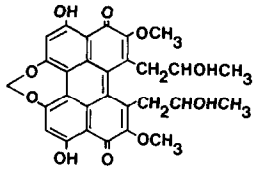
Although photosensitizers could be classified in relation to their putative biological roles, we will discuss such compounds according to their chemistry

Quinones

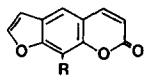
The extended quinones provide important examples of photodynamic secondary substances and were some of the first to be isolated, a procedure aided by their intense colouration The hypericins, occurring in the genus *Hypericum* (St Johns Worts) are responsible for the photosensitization when these plants are ingested by grazing animals [86, 87] The condition of intense skin irritation is known as hypericism [88] Hypericin (**1**) is the most highly condensed quinone known and often occurs with a range of related pigments [87] Hypericin is contained in specialized glands, presumably as a protection against autotoxicity, located on flowers, stems and leaves [88, 89] Evidence has recently been presented that



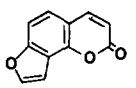
1 Hypericin



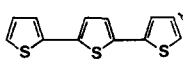
2 Cercosporin



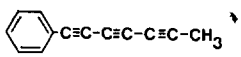
3 R=H, Psoralen
4 R=OCH_3, Xanthotoxin



5 Angelicin



6 alpha-Terthienyl



7 Phenylhepatriyne

hypericin, isolated from the glandular trichomes of the calyx of *Hypericum hirsutum*, is capable of the generation of $^1O_2$ and hence lipid peroxidation [89] The photodynamic reactions of hypericin are promoted by visible light, predominantly in the region 500–600 nm [88, 89] Hypericin has been shown to be phototoxic to the larvae of *Aedes atropalpus* (mosquito) [90] and *Manduca sexta* (tobacco hornworm) [Knox, J P, unpublished result] As hypericin-containing plants are generally free of insect predators, hypericin has been proposed as a deterrent to phytophagous insects [88] However, at least one insect species, *Chrysolina brunsvicensis*, has the ability to detoxify or tolerate hypericin, and indeed uses it as a chemical signal to locate its host plant, *H hirsutum* [91] The adaptation of this beetle thus allows it to feed on a host that is free from other insect predators, a striking instance of the dynamic nature of evolution Interestingly, hypericin has been found to be the chromophore in the photoreceptor of a blue–green ciliate (*Stentor coeruleus*) [92], a factor which predisposes this organism to photodynamic injury [93]

A hypericin derivative, fagopyrin, occurs in the flowers of buckwheat (*Fagopyrum esculentum*), and gives rise to a photogenic condition in animal herbivores that is comparable with hypericism [87] This compound has received no attention as regards its function within the plant, its mechanism of photodynamic action and as yet has not been precisely characterized [87, 88]

A range of photodynamic condensed quinones are of fungal origin [87] The most thoroughly studied of these is cercosporin (2) This fungal toxin is produced by members of the genus *Cercospora* [94, 95], which includes fungi that are responsible for leaf spot diseases of a wide range of plants of economic importance [95] A series of investigations of the effect of cercosporin on plant cells have provided evidence that cercosporin may be involved in producing some of the symptoms of the leaf spot disease [96–99] The photodynamic action of this toxin results in lipid peroxidation and membrane damage that resembles that of the pathogen [97, 99–102] The pigment is photoinduced in many species [103, 104] and the establishment of the disease has been observed to be promoted by light [105] Evidence has accumulated indicating that cercosporin is capable of the photochemical generation of $^1O_2$ [106, 107] The recent detection of $^1O_2$ luminescence in the presence of irradiated cercosporin provides the first direct and unequivocal evidence for the production of $^1O_2$ by a secondary plant metabolite [108] All organisms tested have proven susceptible to the photodynamic action of cercosporin with the exception of the producing species and other fungi [109] The mechanism of fungal tolerance of this action is unknown

Other related photodynamic fungal toxins include the elsinochromes produced by *Elsinoe* species [87], phleichrome produced by *Cladosporium phlei* which is responsible for a leaf spot disease of timothy grass (*Phleum pratense*) [110] and cladochromes associated with *Cladosporium cucumerinum* [111] The latter are remarkable in that they are only observed when etiolated cucumber seedlings are infected by this fungus and not in an attack upon normal green seedlings or in culture [111] The phototoxicity properties of these compounds have not been studied.

Furanocoumarins

The furanocoumarins (psoralens), characteristic of the Rutaceae and Apiaceae but also occurring in several other families, have received considerable pharmacological interest due to their photosensitizing activity in mammalian skin [112] This photosensitizing activity arises from their ability to photobind to pyrimidine bases of DNA, resulting in crosslinks, when irradiated by long wavelength UV light (320–400 nm), an activity that does not involve molecular oxygen [112, 113] However it is increasingly apparent that furanocoumarins are also capable of photodynamic reactions involving the activation of oxygen The photogeneration of $^1O_2$ by furanocoumarins has now been thoroughly documented and indicates that they may be responsible for the direct disruption of membranes and enzyme inactivation [114–118]

Relatively little attention has been directed at the role of furanocoumarins within plants They are found in all parts of the plants and are generally located in oil glands and ducts, seed coats and leaf surface wax [119, 120] Psoralen (3) is found in large quantities in the seed of *Psoralea subacaulis* and may act as an inhibitor of seed germination [121] However the ability of these compounds to be toxic towards various organisms is suggestive of a defensive role [85] Xanthotoxin (4) has been observed to increase twenty-fold in parsnip root tissue when inoculated with non-pathogenic fungi, supporting a role as a phytoalexin [122] Xanthotoxin along with bergapten is also found in celery tissue infected with *Sclerotinia sclerotiorum* but not in the uninfected tissue [123] Cultured parsley cells produce a range of furanocoumarins in the dark in response to treatment with fungal elicitors, the particular furanocoumarins being dependent upon the nature of the elicitor [124] Although furanocoumarins have been demonstrated to be toxic to a range of fungi [122, 124–126], remarkably the role of light in this action does not appear to have been investigated and thus the involvement of activated forms of oxygen in their role as phytoalexins is uncertain

A defensive role for the photochemical reactions of

furanocoumarins in the protection of plants against phytophagous insects has been thoroughly investigated by Berenbaum [119, 127–131] The toxicity of xanthotoxin, when fed at levels found in plant tissue, to armyworm larvae (*Spodoptera eridania*), a generalist herbivore, was greatly enhanced in the presence of UV light [127] A detailed analysis of the furanocoumarins produced by a range of plant species, in a variety of habitats, along with an assessment of insect predators revealed increased production of furanocoumarins in habitats receiving more UV radiation and a strong correlation between the pattern of insect herbivores and furanocoumarin chemistry [131] Evidence is presented of a striking progressive coevolution of the furanocoumarins in species of the Umbelliferae and their insect predators [119, 129, 131] The proposed latest evolutionary chemical advance are the angular furanocoumarins such as angelicin (**5**), which exhibits toxicity to the specialist insect herbivore, *Papilio polyxenes*, which is able to tolerate xanthotoxin [128, 130] The mechanism of phototoxicity of furanocoumarins towards insects, whether involving DNA or the reactions of $^1O_2$, is unknown Interestingly, angular furanocoumarins although capable of binding to DNA are unable to induce crosslinks but do appear to be more efficient generators of $^1O_2$ than linear furanocoumarins [118] However the role of light in the toxicity of angular furanocoumarins towards insects does not appear to have been thoroughly assessed

Polyacetylenes and thiophenes

The polyacetylenes and their thiophene derivatives are a diverse class of compounds occurring predominantly in the Compositae [132] Certain of these substances display a phototoxic action against a wide range of organisms [85] These compounds, like furanocoumarins, are activated by near UV light (320–400 nm), but differ in that they are not capable of interacting with DNA [85] Polyacetylenes are found in all parts of the plants including the cuticle but are frequently restricted to specific organs such as the roots [85, 133]

Fifteen years after the initial isolation of α-terthienyl (**6**) from the roots of the common marigold (*Tagetes*), its nematocidal activity was demonstrated to be greatly enhanced by light [134] This thiophene has received considerable attention and its phototoxicity to microorganisms [135, 136], insects [90, 137], human erythrocytes [138, 140] and even plants [141] demonstrated The phototoxic action of this compound against a range of plant species has led to its proposal as an allelopathic agent and also to the recognition of a herbicidal potential [141] The mechanism whereby autotoxicity is prevented is unknown

Phenylhepatriyne (**7**) found in the leaves of *Bidens pilosa*, is the most studied polyacetylene, and also displays phototoxic action against a wide range of organisms [85, 90, 140–142] This compound also displays an anti-feedant activity, apparently unrelated to phototoxic reactions [143]

However, polyacetylenes and thiophenes do not appear to have a common mechanism of action Evidence has accumulated that thiophene derivatives including α-terthienyl are efficient generators of $^1O_2$ [144–147] and that the primary mechanism of action is the photodynamic disruption of membranes and does not involve DNA [148–150], although reports of phototoxic action of α-terthienyl in the absence of oxygen have appeared [135, 151] In contrast, photosensitization involving straight chain and ring stabilized polyacetylenes such as phenylheptatriyne appears to be predominantly non-photodynamic occurring under anaerobic conditions [147] The rates of $^1O_2$ production by polyacetylenes is considerably less than for thiophenes [147] The mechanism of this non-photodynamic action is not yet understood but may involve photoaddition reactions of the photolabile polyacetylenes [147]

The biological activity of these secondary plant metabolites against a wide range of organisms suggests that they may have a protective role within the plant, especially against insect predators [90] As yet, no ecological survey of their occurrence in relation to habitat, competitors, pathogens or predators has been attempted

Other compounds

Certain other phototoxic plant products have been isolated Furanoquinoline alkaloids such as dictamine, occurring in the Rutaceae are phototoxic against microorganisms [152] and mosquito larvae [90] They structurally resemble the furanocoumarins and have been demonstrated to photobind to DNA [153, 154] Although biogenetically distinct from furanocoumarins both classes of compounds occur in *Skimmia japonica* [153] Several β-carboline alkaloids such as harmaline and isoquinoline alkaloids such as bereberine are also phototoxic [90, 155] Other secondary plant products capable of phototoxic reactions include benzofurans and chromenes, both from species of the genus *Encelia* [156] These latter compounds do not disrupt membranes and reactions with DNA are proposed [156] These and other compounds with a structural resemblance to the tricyclic furanocoumarins and acridines are activated by near UV light and are thought to react with DNA [154, 157] Their capacity to generate $^1O_2$ is unknown

A recent report has described the photoactivation by UV light of a range of isoflavonoid phytoalexins, resulting in enzyme inactivation [158] The mechanism is unclear, but appears to involve free radicals with $^1O_2$ being responsible for only a small component of the activity [158] The production of $^1O_2$ during the autooxidation of tannins has been proposed to have a protective role by its fungistatic and deterrent effects [159]

Another aspect of the relationship between $^1O_2$ and secondary plant substances is the possibility that they act as quenchers of $^1O_2$ Compounds such as the carotenoids are of primary importance in chloroplast metabolism as already discussed The reactions of quercetin, a flavonol, with $^1O_2$ have been studied in relation to its possible physiological role as an anti-oxidant [160, 161] The role of certain alkaloids, such as strychnine and brucine, shown to be efficient physical quenchers of $^1O_2$, is less clear [162]

It is thus apparent that the potential for photosensitizing reactions among secondary plant products is a widespread phenomenon involving UV as well as visible light The role of light in influencing ecological interaction in this way does not seem to have been generally realized Secondary plant substances of diverse biogenetic origin are capable of the photogeneration of $^1O_2$ suggesting the widespread use of this potent toxicant as a protective and defensive agent An analogous situation occurs in mammalian tissues in which the enzymic activation of oxygen,

usually to the superoxide anion, by polymorphonuclear leukocytes and other phagocytes is an important defence against foreign organisms and material [163, 164] A recent report of the enzymic generation of superoxide anions by plant cells in response to attack by the pathogen *Phytophthora infestans*, and its involvement in the hypersensitive reaction [165, 166] further increases our awareness of the diverse ways in which the activation of oxygen can be utilized to gain an evolutionary advantage

MISCELLANY

In addition to damage or offence posed by $^1O_2$ generated from chloroplast membranes or secondary products, a number of other molecules are potential sensitizers of $^1O_2$ formation The inactivation of the enzyme citrate synthase and the oxidation of certain amino acids has been demonstrated *in vitro* with the sensitizer rose bengal [167] The susceptibility of enzymes to $^1O_2$ has been further demonstrated with nitrate reductase from spinach In this instance the inactivation was mediated by $^1O_2$ resulting from the irradiation of FMN with blue light [168] Experiments with K^+ stimulated ATPase from the plasma membrane of suspension-cultured cells of *Rosa damascena*, showed that inactivation was promoted by UV irradiation [169] This was mediated by tryptophan, which upon absorbing light of 290 nm, transferred energy to oxygen from its excited triplet state

A number of investigations have demonstrated the generation of $^1O_2$ in both fresh water [170–173] and seawater [174] In these instances the photodynamic generation of $^1O_2$ appeared to be mediated by humic compounds or other organic matter Barltrop and Martin [175] have shown that $^1O_2$ generated in water from certain lakes in Florida inhibited the growth of the aquatic plant *Hydrilla verticillata* It is possible that this mechanism is a basis for the control of this problem weed In further experiments the inhibition of growth of lettuce seeds has been promoted by light in association with lake sediments Singlet oxygen, generated in aqueous conditions, has been shown to induce changes in the fatty acid composition of, and inactivate, pine pollen [176] Experiments have indicated that $^1O_2$ could be generated on soil surfaces by a number of photosensitizing compounds including certain inorganic oxides as well as organic components [177] It is possible that $^1O_2$ thus generated could be involved in the degradation or transformation of pesticides or biological materials The systemic fungicides ethirimol and dimethirimol have been shown to react with $^1O_2$ [178]

CONCLUSION

Since the discovery of oxygen over 200 years ago, its potential toxic action, particularly at elevated levels, has been known for all biological systems In recent years some actual mechanisms of toxicity have become apparent with particular emphasis placed upon superoxide and its control by dismutating enzymes This brief review has attempted for the first time to collate the various actions of $^1O_2$ in plants The study of the chemistry of $^1O_2$ has advanced in recent years [10] However the use of certain techniques and assay systems, although applicable in chemical systems, is considerably more difficult in complex, structured biological systems Thus with the chloroplast for example it is difficult to assess the quantitative importance of $^1O_2$ within the thylakoid membrane *in vitro* and much more so *in vivo*

It is possible that $^1O_2$ damage is involved in many plant stress conditions such as high light, limited water or carbon dioxide and after the application of certain herbicides Likewise some previous work on photodynamic damage to cells, organelles or enzymes might be re-investigated in the light of what is now known about the action of $^1O_2$ Much remains to be discovered about the presence and action of this transient yet pernicious molecule

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