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QUENCHING OF SINGLET OXYGEN BY ALKALOIDS AND RELATED NITROGEN HETEROCYCLES

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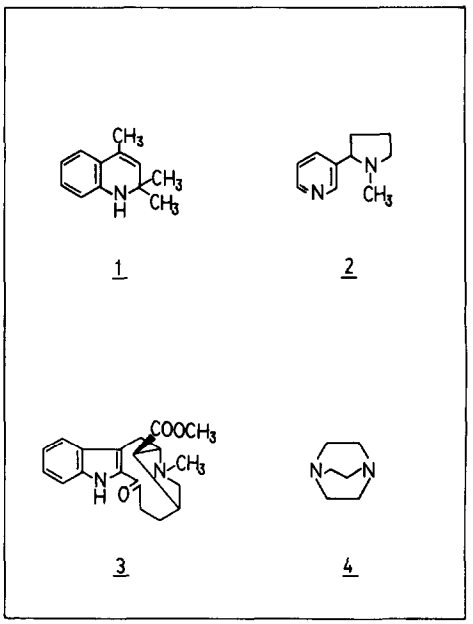
Abstract—Fifteen plant alkaloids and related heterocyclic compounds were tested for their ability to quench singlet oxygen Most of the compounds showed high activity, brucine and strychnine were especially efficient quenchers Brucine, at a concentration of *ca* $2\,6 \times 10^{-5}$ M, is capable of inactivating half the singlet oxygen molecules it encounters This quenching may serve in nature to protect plants from the deleterious effects of singlet oxygen or other reactive oxidants

INTRODUCTION

Many physiological functions for the plant alkaloids have been proposed, including roles as growth regulators, agents for ionic balance, nitrogen storage compounds, and feeding inhibitors or toxicants for herbivores [1] However, despite the widespread use of nitrogen-containing antioxidants such as ethoxyquin (**1**) in industrial processes, the suggestion that alkaloids could play a role in protecting plant tissues from damage due to reactive oxygen species such as singlet oxygen ($^1O_2$) or other agents has received little attention A number of naturally occurring photosensitizers, such as chlorophyll degradation products, flavins and *N*-formylkynurenine (a metabolic product of tryptophan) may be involved in $^1O_2$ generation in plant tissues [2]

Unsaturated membrane lipids react rapidly with $^1O_2$, affording peroxides and other oxidized derivatives Recently, several alkaloid-related drugs have been shown to be potent inhibitors of lipid peroxidation in *in vitro* systems [3–5] but the mechanisms of their inhibition are as yet undescribed Some amino acids, such as histidine, tryptophan and methionine, are also rapidly destroyed by $^1O_2$, either in solution or as constituents of proteins [6] Accordingly, $^1O_2$ quenchers may also be needed to protect enzymes from oxidative damage Unsaturated lipids and amino acids may be oxidized by free radical processes as well as by $^1O_2$ mechanisms, but there is a high correlation between radical and $^1O_2$ quenching activity [7], thus an agent showing strong activity toward one oxidant is also likely to protect against other related forms

High concentrations of alkaloids are very commonly found in plant epidermal cells, where light intensities are highest [1] Because many alkaloids absorb UV-B light strongly, it has been suggested that they, as well as other compounds with similar absorbance characteristics located in the superficial cell layers, such as flavonoids, may be acting as light filters, protecting the mesophyll from UV-B [10]



Nicotine (**2**) was demonstrated many years ago [8] to be a relatively efficient quencher of $^1O_2$, but only one alkaloid, dregamine (**3**), has since been tested as a $^1O_2$ quencher [9] We report that several alkaloids of various structural types are efficient quenchers of $^1O_2$ at concentrations comparable to those in plant tissues

RESULTS AND DISCUSSION

Quenching rate constants and *beta*-values for the alkaloids and related compounds tested are listed in Table 1 Several of these compounds were much better quenchers than the tertiary amine 1,4-diaza[2 2 2]bicyclooctane (DABCO, **4**), which has been widely used in $^1O_2$ quenching studies and is known to be highly active A number of other alkaloids were rather close to the activity of DABCO

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Table 1 Singlet oxygen quenching activity of alkaloids and nitrogen heterocycles

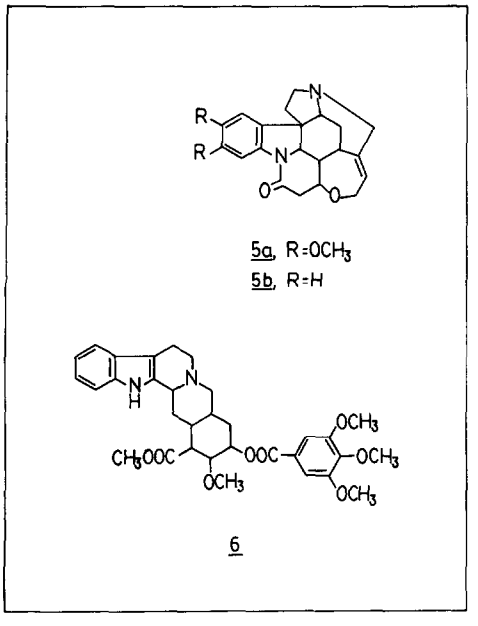
Compounds	Rate constant*	β (M)†
Brucine (**5a**)	$(3.85 \pm 0.62) \times 10^8$	2.6×10^{-5}
Strychnine (**5b**)	$(2.35 \pm 0.28) \times 10^8$	4.3×10^{-5}
8-Hydroxyquinoline (**12a**)	$(1.05 \pm 0.02) \times 10^8$	9.5×10^{-5}
DABCO (**4**)	$(5.84 \pm 0.85) \times 10^7$	1.7×10^{-4}
Atropine (**9**)	$(4.32 \pm 0.85) \times 10^7$	2.3×10^{-4}
5,6-Dimethoxyindole (**8c**)	$(4.29 \pm 0.57) \times 10^7$	2.3×10^{-4}
Vincamine (**7**)	$(4.27 \pm 0.31) \times 10^7$	2.3×10^{-4}
Nicotine (**2**)	$(3.82 \pm 0.18) \times 10^7$	2.6×10^{-4}
Glaucine (**11b**)	$(3.08 \pm 0.30) \times 10^7$	3.2×10^{-4}
Reserpine (**6**)	$(2.66 \pm 0.31) \times 10^7$	3.8×10^{-4}
Boldine (**11a**)	$(2.39 \pm 0.21) \times 10^7$	4.2×10^{-4}
Eucatropine (**10**)	$(1.90 \pm 0.43) \times 10^6$	5.3×10^{-3}
5-Hydroxyindole (**8a**)	$< 2 \times 10^5$	$> 5 \times 10^{-2}$
5-Methoxyindole (**8b**)	" "	" "
6-Methoxyquinoline (**12b**)	" "	" "

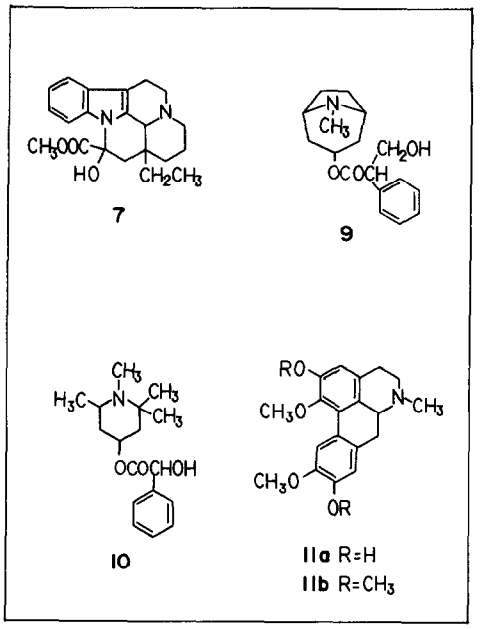
*Including standard deviation

†β is the ratio of the decay of $^1O_2$ due to solvent quenching to its decay due to reaction with the quencher It represents the concentration of quencher necessary to inactivate half the molecules of $^1O_2$ in a given solvent

Indoles

Brucine and strychnine (**5a** and **5b**) were particularly effective quenchers These complex indole alkaloids have two nitrogen atoms, but that of the dihydroindole portion of the structure is in an amide linkage and is only very weakly basic Amides in general are unreactive toward $^1O_2$, so it is a reasonable assumption that the quenching activity of brucine and strychnine is associated with the other (basic) nitrogen atom Two other indole alkaloids, reserpine (**6**) and vincamine (**7**), with tertiary basic nitrogen atoms and fully conjugated indole nuclei, quenched $^1O_2$ efficiently The reactivity data for a related indole alkaloid, dregamine (**3**), also fall into this range (2.7×10^{-7}) [9]





Simple monosubstituted indoles reacted much more slowly with $^1O_2$, as would be expected for a weakly basic heterocycle (Indoles with 2,3-double bond substitution, however, are much more reactive [11]) The difference between the simple heterocycles 5-methoxyindole (**8b**) and 5,6-dimethoxyindole (**8c**), however, illustrates the importance of electron-donating groups to $^1O_2$ quenching activity Electron-rich phenols and phenol ethers quench $^1O_2$ with high efficiency [12] It is also likely that the dimethoxy substituents of brucine help to make it a somewhat better quencher than strychnine, which lacks such substitution, by providing an alternative site for $^1O_2$ attack

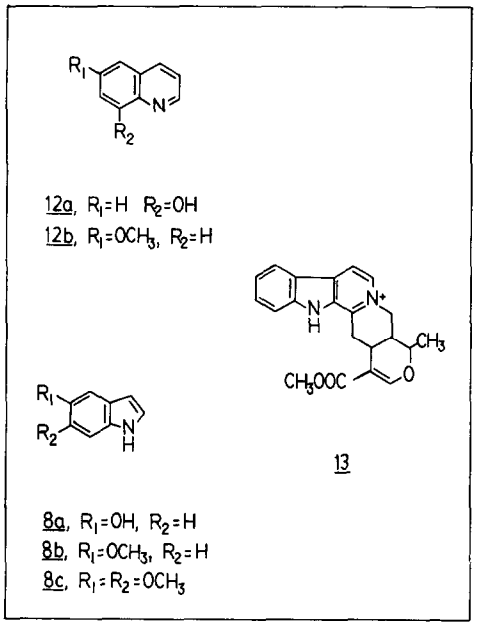
Other alkaloids

The large (*ca* $\times 20$) difference in quenching activity between atropine (**9**) and eucatropine (**10**) may be explained by the extremely hindered steric environment of the basic nitrogen atom of the latter compound Similar effects were noted in 2,6- and 2,2,6,6-methylpiperidines by Monroe [13]

Two aporphine alkaloids, boldine (**11a**) and glaucine (**11b**), showed comparable activities, in the range of the other tertiary amines tested

Our quenching value for nicotine in chloroform was far higher than that obtained 25 years ago for this compound in methanol (4.4×10^5) [8] More recently, however, Smith recalculated the quenching constant relative to that of DABCO (**4**) in pyridine and obtained an approximate value 4.3×10^6 [14] We are uncertain whether solvent effects, experimental design, or other factors are responsible for the wide variability of the data relative to this compound

The differences in quenching activity between 8-hydroxyquinoline (**12a**) and 6-methoxyquinoline (**12b**) are not easy to interpret It is possible that the steric proximity of the phenolic hydroxyl group with the lone pair on nitrogen helps to promote $^1O_2$ deactivation by facilitating orbital overlap in the transition state



Quenching mechanisms

Amines quench $^1O_2$ by both physical and chemical mechanisms [7] In the former, a collisional deactivation of $^1O_2$ back to the triplet state has been suggested, perhaps via a charge-transfer complex

$$^1O_2 + NR_3 \rightarrow [^1O_2 - + NR_3] \rightarrow {}^3O_2 + NR_3$$

However, many instances of quenching of $^1O_2$ with accompanying reactions are also known For example, some tertiary amines may be demethylated upon attack by $^1O_2$, with the production of formaldehyde and secondary amines [9]

We have not examined the quenching reaction mixtures for the possible presence of reaction products However, the geometry about the tertiary basic nitrogen atom of brucine and strychnine (cf Fig 1) is quite suggestive of a site where both physical quenching (due to the rigid, cage-like environment of the nitrogen atom) and chemical quenching by reaction at the nearby electron-rich double bond could occur This steric environment may help to explain the unusually efficient destruction of $^1O_2$ by these alkaloids

Ecophysiological significance

Many authors have noted that alkaloids are rapidly metabolized, or at least turned over, in plant tissues For example, morphine concentrations are highest in the morning and fall during the day [15] A conceivable explanation may be that alkaloids are destroyed by oxidative reactions, either abiotic or enzyme-promoted, and converted to other products For example, *Catharanthus roseus* tissue cultures, when exposed to light, accumulate the oxidized alkaloid serpentine (**13**) at the expense of the reduced alkaloid ajmalicine [16]

If the prevention of photochemical oxidative damage is one possible rationale for the occurrence of alkaloids, they should be common in plant species inhabiting regions of high UV light intensities The short-wave 'UV-B' region (290–320 nm) contains especially energetic and biologically damaging wavelengths UV light does appear to play a role in the biosynthesis of some alkaloids, in potato plants, formation of the steroidal alkaloid solanine is stimulated by UV [17] Alkaloids occur with greatest frequency in species of tropical origin, where UV and UV-B intensities are much higher than they are in temperate regions [18] UV and UV-B light intensities also vary with altitude, at 3000 m, UV-B intensities are *ca* 30% higher than they are at sea level [19] There are fewer data on alkaloid concentrations as a function of altitude In two out of three Himalayan *Berberis* species, leaf berberine concentrations were significantly higher in populations from higher elevations [20] In addition, *Lycopersicon* species growing at high elevations in Peru contained much higher concentrations of tomatine (a steroidal alkaloid) than related species from lower elevations [G Cooper-Driver, personal communication] Although not many North American alpine plants have been investigated for their alkaloid content, 30 (26%) of the 113 genera which occur in the southern Rocky Mountain alpine tundra region [21] include alkaloid-bearing species, compared to *ca* 9% for higher plant genera as a whole [22]

Although alkaloids are often stored in cell vacuoles, they are actively translocated, and it is conceivable that small equilibrium concentrations exist in other parts of the cell, such as cell or organelle membranes, where they would be effective in inhibiting oxidative damage This possibility requires further research

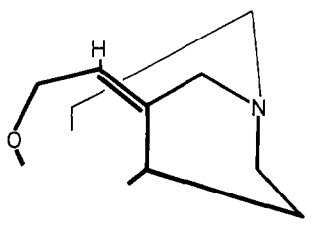


Fig 1 Geometry around the tertiary basic nitrogen atom of brucine and strychnine

EXPERIMENTAL

Chemicals Alkaloids and other heterocycles were obtained commercially and were purified by recrystallization Their structures include examples of indole, aporphine, quinoline, pyridine and tropane nuclei

Determination of quenching rate constants The method of ref [13] was used In this technique, the ability of an added substrate to inhibit the self-sensitized photo-oxidation by visible light of a colored polycyclic hydrocarbon sensitizer, rubrene, is determined Rubrene is advantageous in that the wavelengths which are effective in generating $^1O_2$ occur well into the visible (> 500 nm), so that photochemical processes due to absorption of light by the alkaloids do not interfere

In these experiments, typical alkaloid concns were 1 mM (The mean concn for alkaloids in the leaves of alkaloid-bearing plants is 0 62% dry wt [23], or *ca* 4 mM assuming on average MW of 300 and 80% H_2O content) The solvent used in the quenching studies was $CHCl_3$, in which the decay constant for $^1O_2$ is *ca* 1×10^4/sec [24]

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NOTE ADDED IN PROOF

A A Gorman *et al* [*Tetrahedron Letters* **25**, 581 (1984)] have independently shown that strychnine is a very fast physical quencher of $^1O_2$ Their quenching constants were determined in different solvents, but are in good agreement with ours