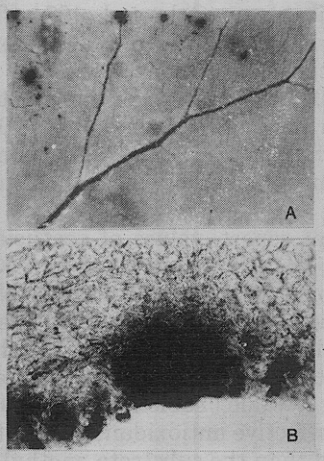

Figure 1. Localization of terpenoid aldehydes (black areas) in cotyledons of germinating glandless cotton seedlings. (A) Terpenoids in lesions and vascular tissue of cotyledons inoculated with *Verticillium dahliae* (×50). (B) Terpenoids in lesion on abaxial surface of cotyledon inoculated with *Colletotrichum dematium* (×300).

ration. Pfleger and Harman (1975) reported failure of deteriorating pea seeds to synthesize phytoalexins, but moisture levels in their seeds never exceeded 20%.

Cotyledons of glandless cotton have the capacity to synthesize terpenoid aldehydes (Table I, Figure 1) when inoculated with either the cotton wilt pathogen *V. dahliae* or a nonpathogenic isolate of *C. dematium*. Induced synthesis of terpenoid aldehydes occurred in localized lesions and in vascular tissues. Histochemical staining of noninoculated cotyledons revealed terpenoids in their vascular tissues as well, thereby accounting for the traces of terpenoid aldehydes extracted from them.

In summary, cottonseed and seedling cotyledons have the capacity to synthesize nonglandular terpenoid aldehydes, but do so only at moisture levels conducive to germination or rapid deterioration. Thus, synthesis of phytoalexins is not a determining factor in resistance of seed embryo tissues to infection by fungi, but may contribute to disease resistance in seedlings. Additionally, gossypol found in preparations of glandless seed is more likely due to contamination by glanded seed than to production of nonglandular terpenoids. Microbial-induced terpenoids should present no problem in production of gossypol-free or low-gossypol food products from glandless and mechanically deglanded seed preparations.

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Antioxidative and Quantum Chemical Properties of Some Hydroxy N-Heterocyclic Compounds

The inhibiting effects of some N-heterocyclic compounds on autoxidation of methyl linoleate increased with decreasing the highest molecular orbital energies (E_{ho}) of these compounds. 5-Hydroxy-2,3-dimethylindole ($E_{ho} = \alpha + 0.3\beta$) was found to have a marked antioxidant activity.

Amino acids are well known to affect the course of lipid oxidation (Kawashima et al., 1977a,b). Their antioxidative effects have been generally recognized to be of synergistic nature. Certain amino acids such as tryptophan and histidine may, however, occasionally function as primary antioxidants themselves. Mitsuda et al. (1965) reported that tryptophan and tryptamine were effective for preventing the development of oxidized flavor in raw and dried milk preparations. The antioxidant activities of tryptophan and the related indole compounds were shown to correlate to some extent with the highest occupied molecular orbital (HOMO) energies of the molecules (Mitsuda et al., 1966, 1967).

In the present communication, HOMO energies of some heterocyclic compounds such as indole and quinoline derivatives were calculated by the simple Hückel molecular

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Table I. Highest Occupied Molecular Orbital Energy and Antioxidant Effect of N-Heterocyclic Compounds

heterocyclic compds	K^a	IP,[b] h
indol	0.532	39
2-hydroxy-4-methylquinoline	0.491	45
2-hydroxypyridine	0.477	51
3-hydroxypyridine	0.442	60
4-hydroxy-2-methylquinoline	0.435	63
6-hydroxyindazole	0.371	131
8-hydroxyquinoline	0.357	109
8-hydroxy-2-methylquinoline	0.355	127
5-hydroxyisoquinoline	0.350	436
5-hydroxyindole	0.337	629
5-hydroxy-2,3-dimethylindole	0.300	731
2,3-dihydroxypyridine	0.278	587

[a] K was calculated from the following equation: $E_{ho} = \alpha + K\beta$, where α and β are Coulomb integrals of a carbon atom and resonance integral of a carbon–carbon double bond, respectively. [b] Induction period (IP) was the time required for methyl linoleate added with 5×10^{-6} mol of the compound to gain 10 mg/g in weight. IP of control without addition and that of methyl linoleate with 5×10^{-6} mol of butylated hydroxyanisol were 41 and 642 h, respectively.

orbital (HMO) method in order to predict antioxidant activities, and the antioxidant activities were practically evaluated.

EXPERIMENTAL SECTION

Quantum Chemical Calculation. HOMO energies ($E_{ho} = \alpha + K\beta$) of heterocyclic molecules listed in Table I were calculated by the HMO method (Streitwieser, 1961). Parameter values of Coulomb and resonance integrals for hetero atoms were as a set of "recommended" parameters described by Yonezawa et al. (1964).

Materials. 2-Hydroxy-4-methylquinoline, 4-hydroxy-2-methylquinoline, 6-hydroxyindazole, 5-hydroxyisoquinoline, 5-hydroxyindole, and 2,3-dihydroxypyridine were purchased from Aldrich Chemical Co., Milwaukee, WI; 8-hydroxyquinoline was obtained from Nakarai Chemical Co., Kyoto, Japan. 5-Hydroxy-2,3-dimethylindole was synthesized by the method of Harley-Mason and Jackson (1955). Methyl linoleate was obtained from safflower oil by methanolysis and the following urea adduct formation. Methyl linoleate thus obtained was found to have a purity of ca. 98% by gas–liquid chromatography.

Evaluation of Antioxidant Activity. Antioxidant activity was evaluated by the weighing method of Olcott and Einset (1958) with a slight modification. Methyl linoleate ($1.0000g \pm 0.0050g$) and antioxidant (5×10^{-6} mol) were placed in a beaker of 2.8 cm in diameter. Each beaker was stored in an oven maintained at a constant temperature of 37.0 ± 0.5 °C and accurately weighed at appropriate intervals in order to estimate weight increase. Induction period was arbitrarily taken as the time required to gain 10 mg/g of substrate. Controls without any additive and with BHA were also run for comparison.

RESULTS

Table I shows the K values of N-heterocyclic molecules together with their antioxidative effect evaluated by the weighing method. It is apparent that the smaller the K value of compounds, the more effective as antioxidants they were, although there were a few exceptions. The compounds having K values less than 0.35 showed excellent antioxidant activities. In particular, 5-hydroxy-2,3-dimethylindole exhibited a marked antioxidant effect.

DISCUSSION

In recent years, N-heterocyclic compounds having hydroxyl groups have been attracting considerable research interests as antioxidants (Cole et al., 1974; Shmulovich et al., 1978; Kawashima et al., 1979). Antioxidant properties of N-heterocyclic compounds have been reviewed by Schulz et al. (1977). On the other hand, activities of antioxidants such as phenols (Fueno et al., 1959) and aromatic amines (Samukawa, 1969) have been interpreted with some quantum chemical indices calculated by the HMO method.

In the present report, values of coefficient K in the equation of the HOMO energy ($E_{ho} = \alpha + K\beta$) were first calculated on some commercially available N-heterocyclic compounds. The antioxidant activities of these compounds were also practically measured by the weighing method. As expected, the antioxidant activities generally increased with decreasing K value (Table I). One possible explanation is that an effective antioxidant would itself be more readily oxidized than the substrate, methyl linoleate. If so, the lower the energy of the HOMO, the more effective the antioxidant. Thus, the HMO method seemed useful to predict antioxidant effects of N-heterocyclic compounds. The K value of 5-hydroxy-2,3-dimethylindole was calculated as 0.3, suggesting that the compound would presumably have an excellent antioxidant activity. In fact, the synthesized 5-hydroxy-2,3-dimethylindole was more effective than a conventional antioxidant, BHA.

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