Antioxidant Evaluations of Novel *N*-H and *N*-Substituted Indole Esters

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The anti-lipid peroxidation (LP) activity and anti-superoxide formation (SOD) of new *N*-H and *N*-substituted indole derivatives were evaluated to determine their antioxidant activity. The results showed that compounds 1, 2, 5, 9 and 12 demonstrate considerable inhibition of lipid peroxidation of mouse liver homogenate. On the other hand, compounds 5 and 12 showed anti-superoxide formation activity at a concentration of 10^{-4} M. Consequently, it can be concluded that these compounds exhibit important activity compared with reference compound α -tocopherol (vitamin E).

Key words anti-oxidant activity; *N*-substituted indole derivatives; superoxide dismutase; lipid peroxidation

The serious consequences of free radical action on biological systems, and the multiple complex aspects of their intervention in a series of inflammatory¹⁾ and nutritional diseases,^{2–4)} explain the increasing interest being given to antioxidant medication and to obtaining compounds which can reduce the effects of these radicals. Many anti-inflammatory agents are known to act through the scavenging of oxygen radicals.^{5,6)} Studies on the stearyl ketons have shown a good correlation between oxygen radical scavenging and anti-inflammatory activities.⁷⁾ A number of studies have implicated oxygen-free radicals in the process of inflammation.⁸⁾ Superoxide dismutase, which scavenges superoxide, has shown good anti-inflammatory activity in several models.⁹⁾ It has been shown that the oxidation of arachidonic acid to prostaglandins requires superoxide anions, and that the prostaglandin phase of rat carrageen paw edema was completely suppressed by repeated injections of superoxide dismutases.^{10,11)} Furthermore, it has been shown that there is a good correlation between superoxide scavenging and anti-inflammatory activity of substituted phenyl butenons.¹²⁾

Recent data from our laboratories showed important antioxidant effects of *N*-substituted indole amides.¹³⁾ The relationships between inflammatory and free radical action have been suggested to indicate the antioxidant capacity of our indole esters, which were previously synthesized and reported to show COX-2 enzyme inhibition (Table 1).¹⁴⁾

MATERIALS AND METHODS

General Experimental Procedures Superoxide dismutase (SOD), cytochrome c, xanthine, thiobarbituric acid (TBA) and malondialdehyde (MDA) were purchased from Sigma, and potassium chloride, *n*-butanol and *o*-phosphorous acid were purchased from Aldrich.

Cytochrome c Test of Synthesized Compounds Superoxide anion ($O_2^{\cdot-}$) was generated as a mixture of the enzyme xanthine oxidase and its substrate xanthine.¹⁴⁾ The activity of the enzyme was detected by its ability to reduce cytochrome c, which causes an increase in absorbance at 550 nm. Enzymatic formation of superoxide anion was detected on the basis of cytochrome c reduction by xanthine oxidase plus xanthine. The incubation mixture (1.0 ml, total volume) consisted of phosphate buffer (pH=7.8, 0.05 M), xanthine (50 μ M), cytochrome c (60 mM), xanthine oxidase (0.32

units/ml) and different concentrations of synthesized compounds at 100 μ l. The reaction was started by the addition of xanthine oxidase to this mixture, then the reaction was conducted. The cytochrome c reduction was monitored at 550 nm for 3 min. For any given set of conditions, a calibration curve was obtained using purified SOD from Sigma as a standard.

Assay of Lipid Peroxidation (LP) LP was measured by spectrophotometric estimation of thiobarbituric acid reactants substances (TBARS).¹⁵⁾ The amount of TBARS was expressed in terms of nmol malondialdehyde (MDA/g tissue). Rat liver tissue was homogenized with 1.15% KCl solution to make a 5% homogenate. 0.5 ml of homogenate, 3 ml of 1% *o*-phosphorous acid, 1 ml of 0.6% TBA in aqueous solution, and various concentrations of different test compounds were added into a 10 ml centrifuge tube. The mixture was heated for 45 min in a boiling water bath. After cooling the mixture to room temperature, 4 ml of *n*-butanol was added and mixed vigorously. The butanol phase was separated by centrifugation, and the absorbance was measured at 532 and 520 nm. The difference of absorbance was determined as the TBA value. Amounts of TBARS were expressed in terms of nmol MDA/g tissue.

RESULTS AND DISCUSSION

The antioxidative effects of the synthesized compounds in rat liver microsomes were investigated using two different assays.^{15,16)} α -Tocopherol, a natural antioxidant, is an effective anti-oxidant for lipid-containing foods.¹⁷⁾ Because of its anti-oxidant capacity, it has been chosen as a reference compound to evaluate the anti-oxidant properties of our test compounds.

The inhibition of superoxide anion ($O_2^{\cdot-}$) formation assay can thus be used to determine whether a compound is a general free radical scavenger or a scavenger specific for the superoxide anion. A substance with no free radical scavenging activity has no effect on the assay.¹⁸⁾ The occurrence of carbon and nitrogen-centered radicals can explain the possible antioxidant mechanism of indole esters, which was reported in Fig. 1.¹⁹⁾

The effects of different concentrations of indole esters on lipid peroxidation and superoxide anion inhibition are shown in Table 2.

The activity results of both suggests that compounds **5** and **12** inhibit lipid peroxidation and superoxide anion produc-

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Table 1. The Structural Formula of Ester Derivatives 1–12

1-7			7-12		
Comp. No	R ₁	R ₂	Comp. No	R ₃	R ₄
1		H	7		H
2		H	8		H
3		H	9		H
4		H	10		H
5			11		
6			12

Fig. 1. Mechanism of Carbon and Nitrogen-Centered Radicals Induced by Indole Esters

tion, and can thus be evaluated as antioxidant compounds.

On the other hand, while compounds **1**, **2** and **9** have considerable effects on lipid peroxidation, they have no effects on superoxide anion production. The observation of this difference is not surprising because the mechanisms of production of oxidative stress (or reactive oxygen species) in these assays are different.

From the chemical point of view, the ester moiety of compounds **9** and **12** was constituted of *o*-cresol. However, compound **5** does not have the same constitution, since this compound was found active. Consequently, it can be concluded that the *o*-cresol substitution of indole esters is not important for the anti-oxidant activity.

Table 2. Effects of Compounds 1–12 on Liver LP Levels and Liver Superoxide Anion Production

Compound	Concentration in the incubation medium (M)	Superoxide anion production percent of control	LP (nmol MDA/g tissue)	Percent of control
1	10 ⁻⁴	100±0.7	17.61±1.8	63
	10 ⁻⁵	106±4.5	25.15±0.9	93
2	10 ⁻⁴	102±2.8	18.86±1.7	70
	10 ⁻⁵	102±0.7	20.12±2.7	93
3	10 ⁻⁴	103±1.8	27.78±0.8	103
	10 ⁻⁵	113±7.0	28.67±0.7	106
4	10 ⁻⁴	89±5.6	18.86±0.9	93
	10 ⁻⁵	92±1.4	26.41±1.8	100
5	10 ⁻⁴	23±7.0	22.64±1.7	79
	10 ⁻⁵	102±6.3	25.15±1.5	98
6	10 ⁻⁴	91±5.6	20.12±0.9	98
	10 ⁻⁵	116±1.4	25.15±1.8	100
7	10 ⁻⁴	89±4.2	23.89±2.7	88
	10 ⁻⁵	92±1.4	25.15±0.0	93
8	10 ⁻⁴	103±2.1	22.64±0.9	84
	10 ⁻⁵	101±1.4	27.04±0.9	100
9	10 ⁻⁴	98±2.1	17.61±2.7	65
	10 ⁻⁵	100±5.6	25.16±0.9	93
10	10 ⁻⁴	103±3.3	22.64±0.0	84
	10 ⁻⁵	102±8.5	27.04±1.8	100
11	10 ⁻⁴	101±5.6	27.89±1.4	103
	10 ⁻⁵	109±3.5	27.22±0.9	101
12	10 ⁻⁴	14±1.4	21.38±0.7	72
	10 ⁻⁵	100±1.4	23.90±1.6	111
Control ^{b)}		100	27.04±1.8	100
α -Tocopherol	10 ⁻⁴	5±2.3	20.50±2.4	63
	10 ⁻⁵	69±5.1	33.14±1.9	103
Control ^{c)}		100		
SOD	30 IU	28±2.7		
	45 IU	13±1.2		

a) Each value represents the mean±S.D. of 2–4 independent experiments. b) Methanol plus dimethyl sulfoxide (DMSO); control solvent for compounds 1–12. c) Water; control for SOD.

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