Biochemical Pharmacology, Vol. 42, No. 3, pp. 459–464, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

ANTIOXIDATIVE PROPERTIES OF HARMANE AND β-CARBOLINE ALKALOIDS

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(*Received* 1 *August* 1990; *accepted* 31 *January* 1991)

Abstract—β-Carboline alkaloids are derived as a result of condensation between indoleamine (e.g. tryptamine) and short-chain carboxylic acid (e.g. pyruvic acid) or aldehyde (e.g. acetaldehyde), a reaction that occurs readily at room temperature. These compounds have been found endogenously in human and animal tissues and may be formed as a byproduct of secondary metabolism; their endogenous functions however, are not well understood. Indoles and tryptophan derivatives exhibit antioxidative actions by scavenging free radicals and forming resonance stabilized indolyl radicals. Harmane and related compounds exhibited concentration-dependent inhibition of lipid peroxidation (measured as thiobarbiturate reactive products) in a hepatic microsomal preparation incubated with either enzymatic dependent (Fe^{3+}ADP/NADPH) or non-enzymatic dependent (Fe^{3+}ADP/dihydroxyfumarate) oxygen radical producing systems. Alkaloids with hydroxyl substitution and a partially desaturated pyridyl ring were found to have the highest antioxidative potencies. Substitution of a hydroxyl group by a methoxyl group at the 6-position resulted in a decrease of greater than 10-fold in the antioxidative activities. Harmane showed high efficacy in an enzymatic system but low efficacy in a non-enzymatic system. The antioxidative effects of harmane in the former system may be attributed to its ability to inhibit oxidative enzymes in the microsomal system. These results suggest that β-carbolines may also serve as endogenous antioxidants.

Harmane (1-methyl-β-carboline), first isolated in *Peganum harmala*, and related alkaloids are distributed widely in medicinal plants. Harmala alkaloids have been used in hallucinogenic preparations of South American and African tribes [1]. These alkaloids have a wide spectrum of pharmacological actions, including monoamine oxidase inhibition [2], binding to benzodiazepine receptors [3], convulsive or anticonvulsive actions [4], tremorigenesis [5], and anxiolytic and behavioral effects [6]. β-Carbolines structurally related to harmala alkaloids have also been found endogenously in mammalian tissues, including the central nervous system, liver, and platelets, and in plasma and urine [7, 8]. The metabolic pathway leading to the formation of β-carbolines is via Pictet–Spengler condensation between an indolamine, e.g. tryptamine, and aldehydes, e.g. acetaldehyde. Such reaction between tryptamine and acetaldehyde occurs non-enzymatically at physiological pH and forms 1-methyl-1-carboxyl-tetrahydro-β-carboline. Once formed, 1-methyl-1-carboxyl-tetrahydro-β-carboline is further metabolized, via ring hydroxylation (at the 5-, 6-, and 7-positions) [9], decarboxylation [10], methylation and dehydrogenation [11], to form a series of β-carboline alkaloids.

Indole precursors of β-carbolines, e.g. tryptophan and tryptamines, are known to have antioxidative activities [12, 13], possibly by scavenging reactive oxygen radicals and forming a stable indolyl radical at the pyrrole ring [14]. The indole nucleus in β-carboline may possess similar antioxidant properties. In this study, we have investigated the anti-oxidative potential of a series of tryptamine and β-carboline derivatives. Structural modification (hydroxyl or methoxyl substitution) and degrees of saturation at the pyridyl ring were found to be important determinants in the antioxidative efficacy.

METHODS

Rat hepatic microsomes. Male Sprague–Dawley rats (250–300 g body weight, Charles Rivers, MA) were killed by decapitation, and their livers were excised immediately. The tissue was homogenized in 9 vol. of pH 7.2 buffer (0.25 M sucrose, 0.01 M MOPS† and 0.001 M EDTA). The homogenate was centrifuged at 4° (10,000 *g*, 10 min). The supernatant was separated and centrifuged again at 4° (100,000 *g*, 60 min), and then the pellet was washed and suspended in potassium phosphate–sucrose buffer (KSP buffer, pH 7.2, 0.12 M KCl, 0.01 M K_2HPO_4/KH_2PO_4 and 0.05 M sucrose). Next the suspension was centrifuged (4°, 100,000 *g*, 60 min). The supernatant was discarded and the pellet was suspended in 10 mL of KSP buffer and kept at −70°.

Lipid peroxidation. Lipid peroxidation was estimated using the thiobarbiturate method. Liver

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† Abbreviations: MOPS, 4-morpholinepropanesulfonic acid; BHT, butylated hydroxytoluene; NADPH, reduced nicotinamide adenine dinucleotide phosphate; DHF, dihydroxyfumarate; TBA, thiobarbituric acid; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; and MPP^+, 1-methyl-4-phenyl-pyridinium ion.

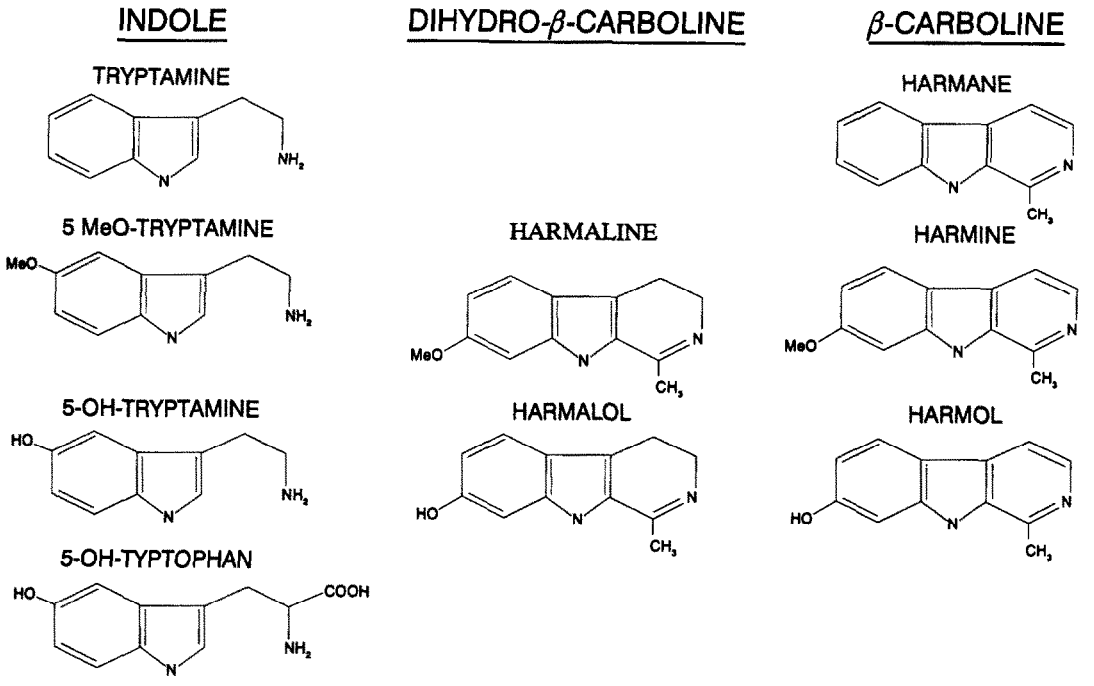

Fig. 1. Structural derivatives of tryptamines and β-carbolines investigated for antioxidative actions.

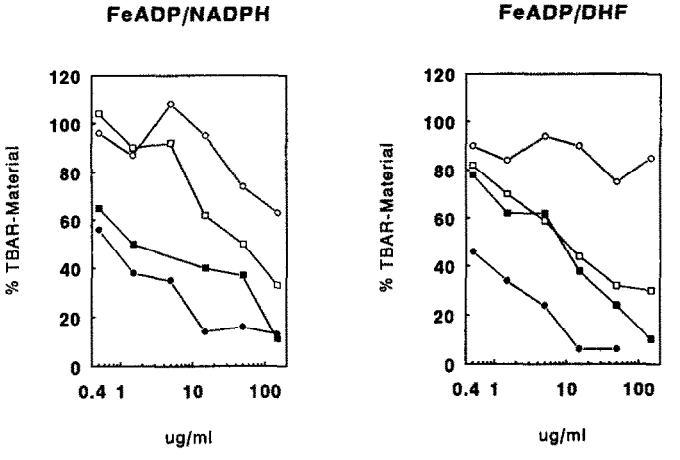

Fig. 2. β-Carboline inhibition of lipid peroxidation in hepatic microsomes. (Left panel) enzymatic system with FeADP-NADPH; and (right panel) non-enzymatic system with FeADP-dihydroxyfumarate. TBAR material = thiobarbituric acid reactive material. Each point represents three separate concentration–response experiments with duplicates. Key: (●) harmalol; (■) harmaline; (□) harmol; and (○) harmine. Rank order of potency: Harmalol > harmaline > harmol >>> harmine.

microsomes were resuspended in 500 μL of KSP buffer (final concentration, 1 mg protein/mL). Lipid peroxidation was initiated by either one of the following systems:

(i) FeADP-NADPH enzymatic dependent system: a stock solution of Fe^{3+}ADP was made up with 100 μM $FeCl_3$, chelated by 1 mM ADP, and diluted to reach a nominal concentration of 6.25 μM $FeCl_3$. NADPH was added as a cofactor to the incubation mixture (final concentration, 0.2 mM).

(ii) FeADP-dihydroxyfumarate (DHF) non-enzymatic dependent system; dihydroxyfumaric acid undergoes spontaneous auto-oxidation to generate oxygen free radicals. The rate of oxygen radical production is enhanced in the presence of Fe^{3+}. The incubation mixture consisted of 50 μM dihydroxyfumarate and FeADP (6.25 μM Fe^{3+}).

Table 1. Antioxidative potencies of tryptamines and β-carbolines

Compound	IC_{50} (μM) FeADP/NADPH	FeADP/DHF
5-OH-Tryptophan	145	159
5-OH-Tryptamine	5.6	2.8
5-MeO-Tryptamine	10.1	4.0
Tryptamine	17.8	4.0
Harmalol	3.2	1.7
Harmaline	10.4	23.9
Harmol	213	38
Harmine	>600	>600
Harmane	11.7	145
BHT	0.54	0.51

The IC_{50} values (concentration that inhibited lipid peroxidation by 50%) were calculated from the concentration–response curves.

Table 2. Relative potencies and 95% confidence intervals of the potency ratios in the carboline group

	(A) FeADP-NADPH System Harmaline	Harmane	Harmol
Harmalol *vs*	5.1* (1.4–28.1)	5.7* (1.2–50.2)	75.6* (19.5–597.7)
Harmaline *vs*		1.4 (0.3–7.2)	20.1* (6.3–20.6)
Harmane *vs*			8.6* (2.7–40.0)

Potency rank order:
Harmalol > harmaline ≥ harmane > harmol > harmine

	(B) FeADP-DHF System Harmaline	Harmol	Harmane
Harmolol *vs*	11.0* (4.1–39.4)	34.1* (13.4–115.0)	129.7* (29–1451)
Harmaline *vs*		2.3* (1.1–5.2)	4.9* (2.0–14.0)
Harmol *vs*			2.8* (1.2–7.4)

Potency rank order:
Harmalol > harmaline > harmol > harmane > harmine

The potency ratios and the confidence intervals between two compounds were computed from the concentration–response curves, as described in Ref. 15. The potencies of two compounds were not significantly different if the 95% confidence intervals overlapped with unity. Compounds are ordered and compared in descending order of potencies. In the carboline group, harmine was not included in the calculation as the IC_{50} was above 600 μM, and its potency was much smaller than all the other compounds in the carboline group.
* $P < 0.05$.

Tryptamines or β-carbolines (hydrochloric salt) were dissolved in KSP buffer. Each compound was added to incubation mixtures to reach final concentrations of 0.5 to 150 μg/mL. All the chemicals and cofactors were added at the beginning of the experiment. The microsomal suspension was incubated for 30 min at 37°. At the end of the incubation, the mixture was acidified by 50 μL of 5% trichloroacetic acid. Five hundred microliters of 0.5% thiobarbituric acid (TBA) was added, and the mixture was heated at 80° for 20 min. After rapid cooling in ice, 500 μL of ice-cold trichloroacetic acid (70%) was added to stop further color development. The mixture was centrifuged (10 min, 1000 *g*) and the absorbance of the supernatant was measured at 535 nm (Beckmann DU-8 Spectrophotometer). Control incubation mixtures contained only the microsomal suspension and free radical producing systems. Results were expressed as percent inhibition of lipid peroxidation (absorbance) compared to the control incubation. The IC_{50} values (concentration producing 50% inhibition) were measured from the concentration–response curves and converted to micromolar concentrations.

All reagents, tryptamine and β-carboline analogs were obtained from the Sigma Chemical Co. (St. Louis, MO). Structural correlations in the tryptamine and β-carboline series studied are illustrated in Fig. 1.

RESULTS

Harmalol, harmaline, harmol and harmine (0.5 to 150 μg/mL) inhibited lipid peroxidation in both the enzymatic (FeADP-NADPH) (Fig. 2, left panel) and non-enzymatic (FeADP-DHF) (Fig. 2, right panel) systems. Structural modification by substituting the hydroxyl group with a methoxyl group (harmalol to harmaline; harmol to harmine) resulted in a 3- to 15-fold decrease in antioxidant efficacies, particularly in the FeADP-DHF system. Dehydrogenation of the pyridyl ring (e.g. harmalol to harmol; harmaline to harmine) also drastically reduced the antioxidant effects. Of all the β-carbolines studied, harmalol was found to have the highest antioxidant efficacy, whereas harmine showed little if any antioxidative effects in the concentration range tested.

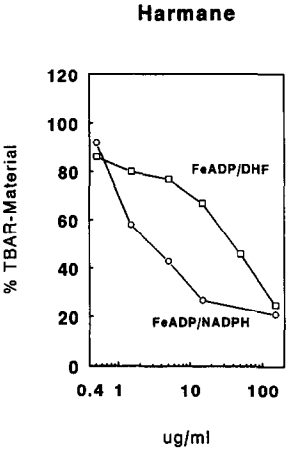

Fig. 3. Antioxidative effects of harmane.

Table 3. Relative potencies and 95% confidence intervals of the potency ratios in the tryptamine group

	(A) FeADP-NADPH System		
	5-Methoxytryptamine	Tryptamine	5-Hydroxytryptamine
5-Hydroxytryptamine *vs*	2.4 (0.7–9.1)	5.7* (2.0–21.4)	24.9* (6.1–216.1)
5-Methoxytryptamine *vs*		3.6* (1.8–7.5)	9.9* (5.4–20.3)
Tryptamine *vs*			2.4* (1.1–5.3)
Potency rank order:			
5-Hydroxytryptamine ≥ 5-methoxytryptamine > tryptamine > 5-hydroxytryptophan			

	(B) FeADP-DHF System		
	Tryptamine	5-Methoxytryptamine	5-Hydroxytryptophan
5-Hydroxytryptamine *vs*	1.7 (0.4–7.6)	4.1* (1.5–13.3)	97.4* (21–1174)
Tryptamine *vs*		2.4 (0.8–8.3)	49.7* (11.4–530.6)
5-Methoxytryptamine *vs*			29.1* (10.2–119.8)
Potency rank order:			
5-Hydroxytryptamine ≥ tryptamine ≥ 5-methoxytryptamine > 5-hydroxytryptophan			

The potency ratios and confidence limits between two compounds were computed from the concentration–response curves, as described in Ref. 15. The potencies between two compounds were not significantly different if the 95% confidence intervals overlapped with unity.

* $P < 0.05$.

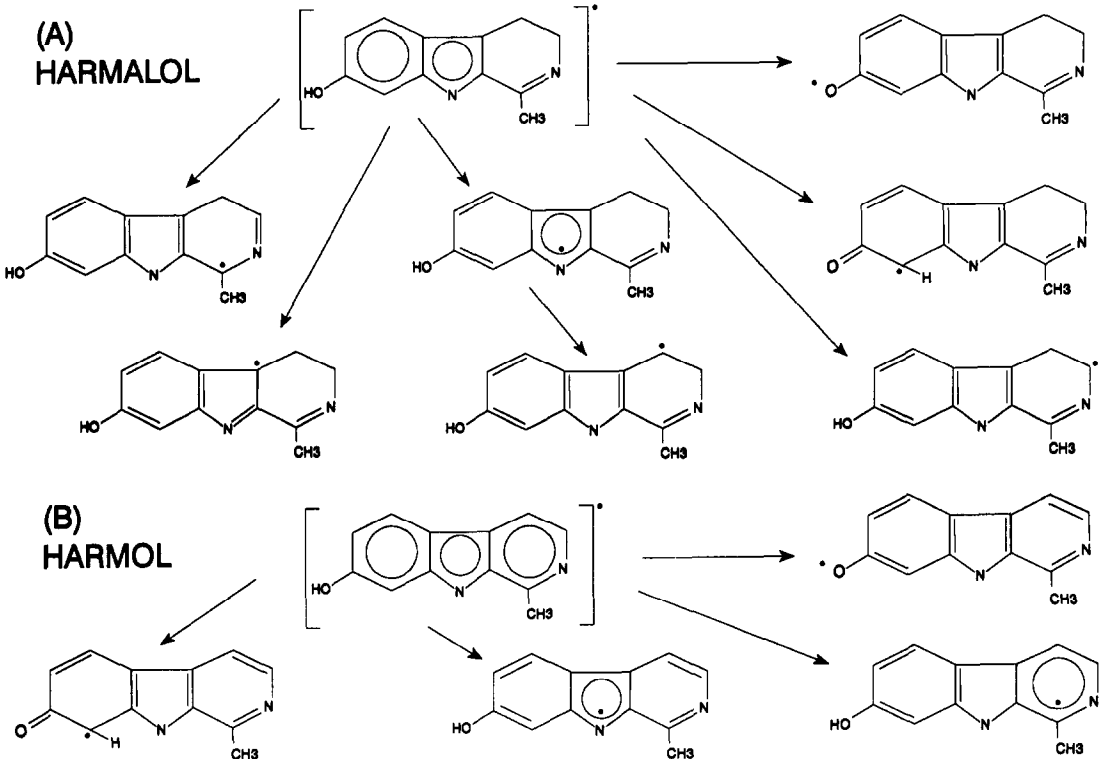

Fig. 4. Possible resonance structures of (A) harmalol and (B) harmol radicals. In harmalol, the radical may be stabilized by delocalization over the pyrrole ring, as well as by resonation among the various allylic sites. Harmol can form a radical that may be stabilized by delocalization over the pyrrole and the pyridyl ring. However, the allylic sites are not available. Both harmalol and harmol can form the semi-quinone radical at the 7-hydroxyl position, whereas this structure is not possible in harmane and the methoxy derivatives.

The antioxidative potency of each compound tested was expressed as IC_{50} (concentration at which lipid peroxidation is inhibited 50% in the control samples). The IC_{50} values of all the compounds tested are listed in Table 1. Butylated hydroxytoluene (BHT), a phenolic antioxidant, was used as a positive control. Most β-carbolines and tryptamines showed similar relative potencies in both the enzymatic and the non-enzymatic systems, with the exception of harmol and harmane. Harmane was a weak antioxidant in the FeADP-DHF system, whereas it had high efficacy in the FeADP-NADPH system (Fig. 3). The inhibitory effects of harmane in the FeADP-NADPH system may be related to inhibition of oxidative enzymes rather than direct free radical scavenging. The potency ratios and rank order of potencies in the carboline group are listed in Table 2. In the FeADP-NADPH system, harmane was similar in potency to harmaline (potency ratio of harmaline to harmane 1.4, 95% confidence limits, 0.3–7.2), whereas in the FeADP-DHF system, harmane was an ineffective antioxidant and was below harmaline and harmol in the potency rank order.

Tryptamine, 5-hydroxytryptamine and 5 methoxytryptamine, similar to other tryptophan derivatives, were all found to be potent inhibitors of lipid peroxidation in both the enzymatic (FeADP-NADPH) and the non-enzymatic (FeADP-DHF) systems. 5-Hydroxytryptophan was a relatively weak antioxidant in both systems. Similarly but to a smaller extent, substitution of the hydroxyl group by a methoxyl group increased the antioxidative efficacy. The potency ratios and the rank order of potencies in the tryptamine group are listed in Table 3. In the FeADP-NADPH system, 5-hydroxytryptamine and 5-methoxytryptamine were similar in potencies (potency ratio: 2.4, 95% confidence intervals 0.7–9.1). Nonetheless, both compounds were better antioxidants than tryptamine and 5-hydroxytryptophan. In the FeADP-DHF system, distinctions in the antioxidant potencies among 5-hydroxytryptamine, 5-methoxytryptamine and tryptamine were less clear, although there were small differences in the potencies. These differences were not significant since the 95% confidence intervals of the potency ratios overlapped with unity.

DISCUSSION

The presence of β-carboline alkaloids in mammalian tissues raises speculations regarding their functions as endogenous benzodiazepine receptor ligands, as mediators of ethanol intoxication, or as regulators of monoamine oxidases. However, whether these alkaloids serve any physiological functions or whether they are simply byproducts of secondary metabolism is still unknown. We have shown in this study that β-carbolines have antioxidative actions, and the relative efficacies are highly dependent on structural modification of the β-carboline ring. β-Carbolines derived from secondary metabolism and Pictet–Spengler condensation may serve as endogenous antioxidants *in vivo*.

Recently, it was suggested that endogenous β-carbolines may be metabolized to intermediates that resemble 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [16]. MPTP is metabolized to a proximal toxin 1-methyl-4-phenyl-pyridinium ion (MPP^+) via free radical intermediates. In analogy, free radical intermediates may also be involved in the metabolism of β-carbolines, as the indolyl nucleus in tryptophan and tryptamine is known to form stable free radicals [17]. Oxidation of tryptophan and tryptamine, for instance, may form the indolyl radicals which are resonance stabilized. Resonance-stabilized free radical species may also be formed during the oxidation of β-carbolines, and the formation and stability of such resonating radical species may explain the relative antioxidative potencies. Similarly, scavenging of oxygen free radicals by β-carbolines and tryptamines may also lead to the formation of secondary radical species which are resonance stabilized. Our results indicated that hydroxylation of the ring greatly enhances the antioxidative efficacies, whereas dehydrogenation of the pyridyl ring greatly decreases the efficacies. Phenolic compounds are known to be effective antioxidants [18], due to the formation of stable phenoxy radicals [19]. In the case of harmalol, the phenoxy radical formed may be stabilized by dislocation of the lone electron over the β-carboline ring, which may be further stabilized by the many possible resonance structures (Fig. 4A). In the case of harmaline, formation of the phenoxy radical would be impossible due to methoxy substitution, and further, resonation to the semiquinone forms (as in harmalol) is hindered. Harmaline may still have antioxidatant properties due to the stability of the radical formed at the allylic sites of the pyridyl ring. However, in the cases of harmol and harmine, the number of resonance structures at the positions allylic to both the pyrrole and pyridyl nitrogen is not possible after dehydrogenation. Harmol is found to be a more effective antioxidant compared to harmine, probably due to the hydroxyl group substitution and the formation of the phenoxy radical (Fig. 4B).

Interestingly, tryptamine, 5-hydroxytryptamine and 5-methoxytryptamine have similar antioxidative potencies. It is probable that the stable indolyl radical formed is the major contributing factor in stabilizing the radical intermediates. This may also explain our finding that 5-hydroxytryptophan was much less effective as an antioxidant than 5-hydroxytryptamine. Substitution of the carboxyl group and its inductive effects may have destabilized the indolyl radical.

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