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Tetrahydro-β-carboline Alkaloids that Occur in Foods and Biological Systems Act as Radical Scavengers and Antioxidants in the ABTS Assay

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Tetrahydro-β-carboline alkaloids that occur in foods such as wine, seasonings, vinegar and fruit products (juices, jams) acted as good radical scavengers (hydrogen- or electron donating) in the ABTS (2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) assay, and therefore, they could contribute to the beneficial antioxidant capacity attributed to foods. In contrast, the fully aromatic β-carbolines norharman and harman did not show any radical scavenger activity in the same assay. During the reaction with $ABTS^{\bullet+}$ radical cation, tetrahydro-β-carboline-3-carboxylic acid such as 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) and 1-methyl-1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acid (MTCA-COOH) were converted to harman, whereas 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (THCA) and 1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acid (THCA-COOH) afforded norharman. These results suggest that food and naturally-occurring tetrahydro-β-carboline alkaloids if accumulated in tissues, as reported elsewhere, might exhibit antioxidant activity.

Keywords: Tetrahydro-β-carbolines; β-Carbolines; Tryptoline; Harman; Alkaloids; Foods

INTRODUCTION

Tetrahydro-β-carbolines (THβCs) and β-carbolines (βCs) are naturally occurring alkaloids with a common tricyclic pyrido (3,4-b)indole ring structure (Fig. 1). THβCs are produced from indoleamines and aldehydes or α-ketoacids through a non-enzymatic Pictet–Spengler chemical condensation that is pH and temperature dependent.[1,2] Tryptophan gives rise to tetrahydro-β-carboline-3-carboxylic acid (MTCA or THCA) or tetrahydro-β-carboline-1,3-dicarboxylic acid (MTCA-COOH or THCA-COOH), tryptamine provides 1,2,3,4-tetrahydro-β-carboline (THβC, tryptoline) or 1-methyl-1,2,3,4-tetrahydro-β-carboline (MTHβC), whereas serotonin (5-hydroxy-tryptamine) affords 6-hydroxy-1-methyl-tetrahydro-β-carboline (OHMTHβC). The oxidation of the tetrahydropyrido ring of THβCs accompanied with decarboxylation produces the fully aromatic βCs, which belong to a subclass of those alkaloids with singular biological properties.

Biological interest on THβCs and βCs has grown from studies reporting their occurrence under physiological conditions in biological tissues and fluids.[2–7] THβCs and βCs exhibit a broad range of pharmacological and biological activity. They might function as neuromodulators through inhibition of the monoamine oxidase (MAO), monoamine uptake and benzodizepine receptor,[3–6] and also as protective agents against lipid peroxidation.[8–11] Furthermore, *N*-methylated THβCS and βCs may act as endogenous neurotoxins,[12] and oxidized βCs may be co-mutagenic substances.[13]

Currently, there is considerable and growing interest in the beneficial health effects of certain foods and beverages.[14] Many of these proposed health benefits are attributed to antioxidants present in these foods and beverages, usually the focus has

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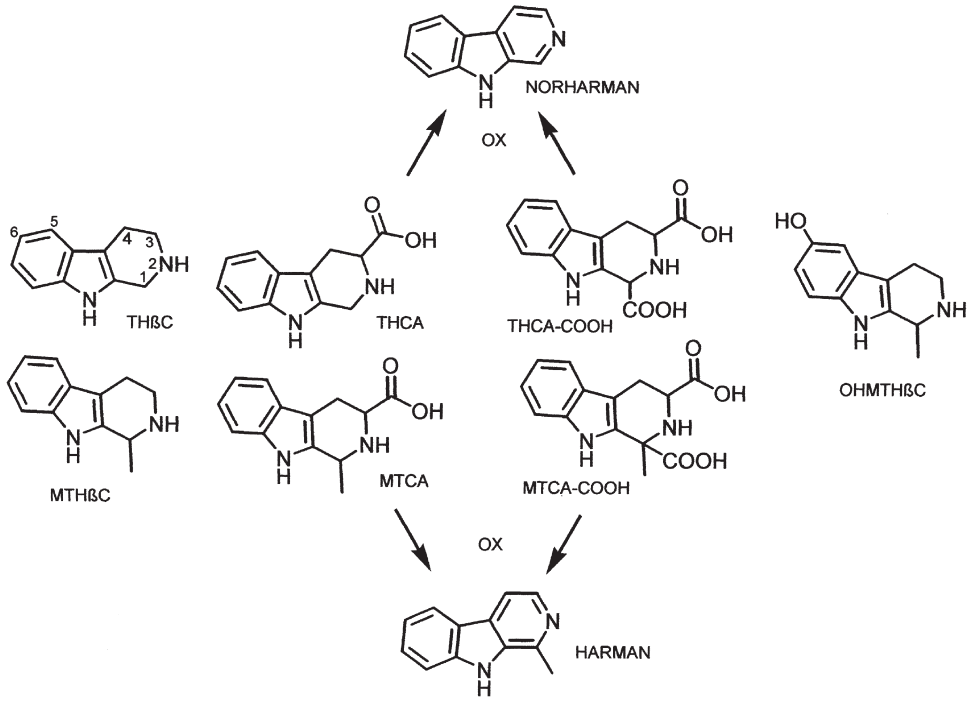

FIGURE 1 Tetrahydro-β-carboline alkaloids in foods and biological systems. Upon oxidative tetrahydro-β-carboline-3-carboxylic acid afford norharman and harman.

been on phenolic compounds, such as tocopherols, catechins and other flavonoids.[14–17] In the last few years, we have investigated the presence of THβC and βC alkaloids in many foodstuffs,[2,18–22] and have proved the ubiquitous occurrence of this class of compounds in many of them. In this regard, tetrahydro-β-carboline alkaloids should be considered as naturally occurring substances readily produced during food production, processing and storage. This finding further suggests that the diet will surely contribute to the ultimate presence of THβC and βC alkaloids in the human biological tissues and fluids. Interestingly, in this paper we report that food and naturally-occurring tetrahydro-β-carboline alkaloids act as radical scavengers in the ABTS test and therefore, might exhibit potential bioactive antioxidant actions. Their TEAC capacity is, at least, comparable to many other commonly considered antioxidants such as ascorbic acid, vitamin E and some phenols. A possible contribution of these compounds as antioxidants is discussed.

MATERIALS AND METHODS

Reference Compounds and Standards

2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and potassium persulfate were obtained from Sigma (St. Louis MO, USA), ascorbic acid was obtained from Merck and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) from Aldrich. Tetrahydro-β-carboline alkaloids were synthesized as previously.[23] 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) was purchased from Sigma (St. Louis, MO, USA), and also synthesized from L-tryptophan and acetaldehyde through a Pictet–Spengler condensation. 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid (THCA) was obtained from L-tryptophan ad formaldehyde through a Pictet–Spengler chemical condensation. Similarly, 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (OHMTHβC) was synthesized from serotonin oxalate (Sigma) and acetaldehyde, whereas 1,2,3,4-tetrahydro-β-carboline (THβC or tryptoline) and 1-methyl-1,2,3,4-tetrahydro-β-carboline (MTHβC or methyltryptoline) were synthesized from tryptamine and formaldehyde or acetaldehyde, respectively. 1-Methyl-1-carboxy-1,2,3,4-tetrahydro-β-carboline-3-carboxylic and (MTCA-COOH) was synthesized from tryptophan and pyruvate, whereas 1-carboxy-1,2,3,4-tetrahydro-β-carboline-3-dicarboxylic acid (THCA-COOH) was obtained from tryptophan and glyoxylic acid. Data of NMR, MS and GC-MS (trifluoroacetyl and methoxycarbonyl methyl ester derivatives) were consistent with the structures of the synthesized compounds.[23] The fully aromatic βCs norharman and harman were purchased from Sigma. All the THβCs and βCs were analyzed by RP-HPLC showing a purity higher than 97%.

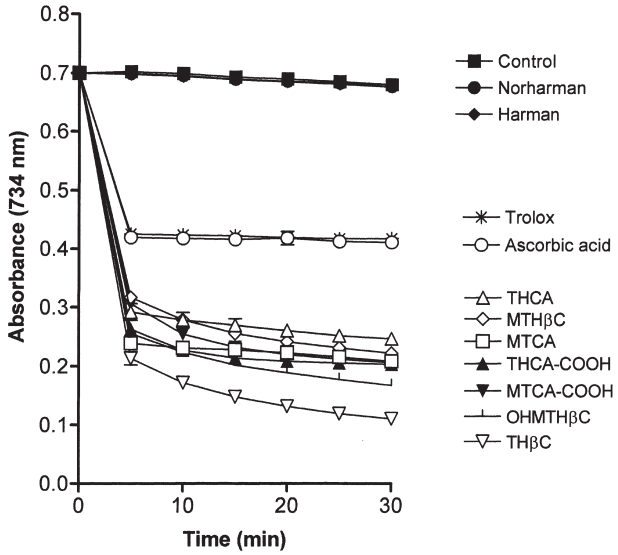

FIGURE 2 Suppression of the absorbance of the $ABTS^{\bullet+}$ radical cation as a function of time for the control $ABTS^{\bullet+}$ radical cation, and tetrahydro-β-carbolines, β-carbolines, Trolox and ascorbic acid in concentration of 10 μM.

RP-HPLC and GC-MS Analysis of the Reaction Products of THβCs and βCs with $ABTS^{\bullet+}$

The analysis of THβCs and βCs by RP-HPLC and UV and fluorescence detection was carried out as previously,[18–20] using an HPLC 1050 (Hewlett Packard) provided with a Diode Array Detector (DAD) and a 1046A fluorescence detector. A 150 × 3.9 mm, 5 μm, Nova-pak C18 column (Waters, Milford, MA, USA) was used for separation. Chromatographic conditions were as follows: 50 mM ammonium phosphate buffer (pH 3) (buffer A) and 20% of A in acetonitrile (buffer B). Gradient programmed from 0 (100% A) to 32% B in 8 min and then to 90% B in 18 min. The flow rate was 1 ml/min, the column temperature was 40°C and the injection volume was 20 μl. Fluorescent detection was set at 270 nm (excitation) and 343 nm (emission) for THβCs, and 300 nm (excitation) and 433 nm (emission) for βCs. The identity for compounds was established by spectral data and by HPLC coelution with authentic standards. Also, fluorescence spectra of the HPLC peaks were compared with those of reference compounds. For this, eluting peaks corresponding to THβCs or βCs were trapped into the flow cell of the fluorescence detector by stopping the solvent pump, and excitation and emission spectra were monitored.

Analysis by Gas Chromatography-Mass Spectrometry (GC-MS) of the reaction products of THCA, THCA-COOH, MTCA and MTCA-COOH with $ABTS^{\bullet+}$ was performed following extraction with ethyl acetate at pH 9–10. The organic solvent was concentrated under a stream of nitrogen and subsequently injected into a 6890-5973N GC-MS (Agilent Technologies). A 30 m × 0.25 mm i.d. HP 5MS 5% phenyl–methyl siloxane capillary column (Agilent Technologies) was used for separation. The oven temperature was 80°C (2 min), then 5°C/min to 260°C; the carrier gas was helium (1 ml/min); the injector temperature and the transfer line were 250 and 280°C, respectively, and the MS ion source was operated in electron ionization mode (70 eV).

ABTS Radical Scavenger Test for Tetrahydro-β-carbolines and β-carbolines

To measure the radical scavenger activity, we have used the ABTS assay introduced by Re *et al.*[17] that has proved to work well to measure total antioxidant activity.[15] 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was dissolved in Milli-Q water to 7 mM concentration and the ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with potassium persulfate (2.45 mM final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. $ABTS^{\bullet+}$ radical cation was diluted with 5 mM phosphate buffered saline (PBS), pH 7.2 to give an absorbance value of 0.7 at 734 nm. THβCs, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and ascorbic acid were dissolved in Milli-Q water at 1 mM, and then used for radical scavenger assay in the stated concentrations (between 1.67 and 20 μM, final concentration) (total volume 3 ml) by measuring the percentage of the inhibition of the absorbance at 734 nm as a function of time. The antioxidant capacity was measured in comparison with Trolox, the water soluble vitamin E analog, as standard.

RESULTS AND DISCUSSION

Figure 1 shows the molecular structure of several THβCs and βCs that occur in foods and biological samples. The activity of THβCs and βCs as radical scavengers and antioxidants was measured with the $ABTS^{\bullet+}$ radical cation test.[17] This test measures the inhibition of the absorbance at 734 nm of the radical cation $ABTS^{\bullet+}$ produced by an antioxidant (hydrogen or electron donor). THβC compounds dissolved in water were added to the test in concentration from 0 to 20 μM, and the suppression of the absorbance of the radical cation $ABTS^{\bullet+}$ was determined during the time (Fig. 2). The decrease in absorbance was rapid and reached a high percentage of inhibition within 5 min (minimum time taken for reading absorbance in our case), which is in good agreement with the previous results for other antioxidants using this method.[17] The reaction was still going on slowly for tryptoline and OHMTHβC, which might indicate different rates of reaction for these compounds, or perhaps

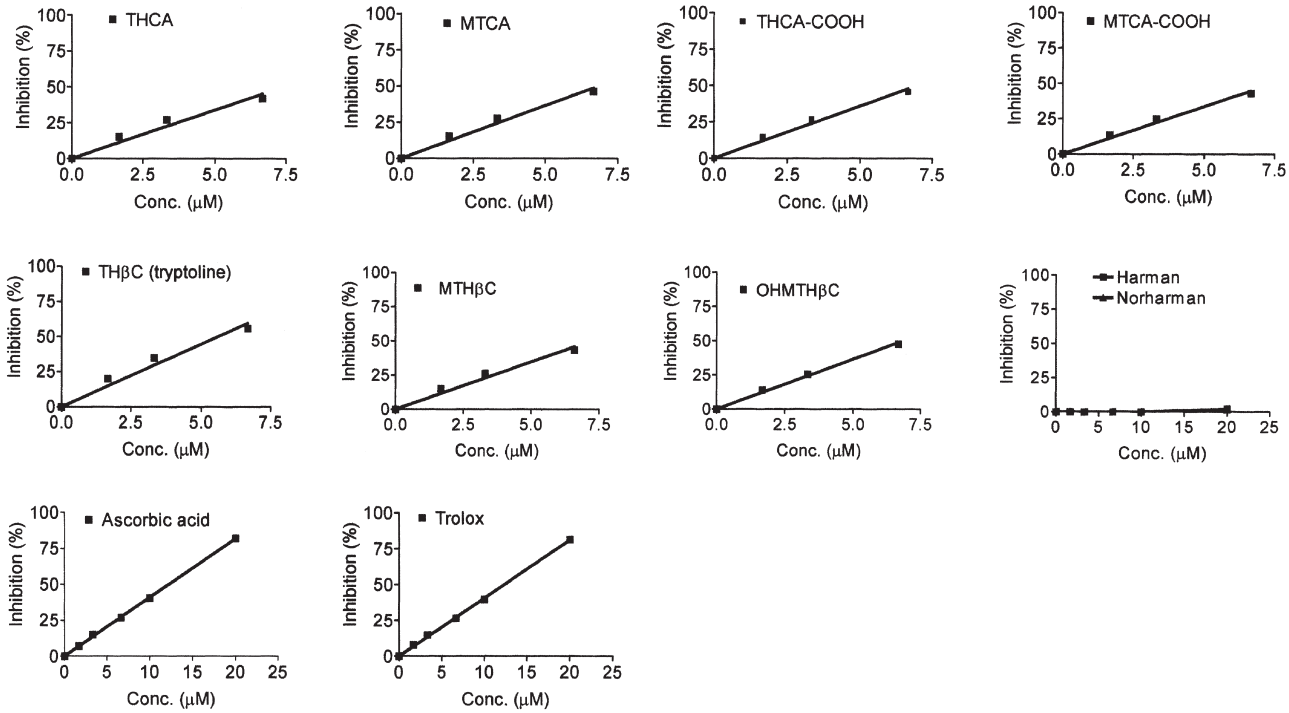

FIGURE 3 Concentration–response curves for the inhibition of the absorbance at 734 nm of $ABTS^{+\bullet}$ as a function of the concentration of carbolines and standards. The assay was performed at least in quadruplicate.

the formation of secondary products still reacting with $ABTS^{\bullet+}$. THβCs exhibited greater reactivity with the ABTS radical cation than the classical antioxidant ascorbic acid and Trolox. In contrast, no decolorization was observed for the fully aromatic norharman and harman. The extent of inhibition of the absorbance of $ABTS^{\bullet+}$ by THβCs was determined as a function of the concentration as shown in Fig. 3. Linear curves were generally obtained with concentrations of the compounds lower than 10 μM, suggesting that the reaction is not significantly affected by possible side reactions and reflecting the scavenging antioxidant activity of THβCs. All the THβCs alkaloids acted as good radical scavengers of $ABTS^{\bullet+}$ radical cation, whereas the fully aromatic βCs norharman and harman did not show any inhibition of this radical.

Table I lists the antioxidant capacity of the alkaloids measured as Trolox Equivalent Capacity (TEAC) (mM) at a fixed time point of 5 min. The concentration of antioxidant giving the same percentage inhibition of absorbance of the radical cation at 734 nm as 1 mM Trolox was calculated in terms of the TEAC. Similar antioxidant values of TEAC ranging from 1.67 to 2.2 mM were obtained for THβCs and were higher than those for ascorbic acid and trolox (1.01 mM). In contrast, no radical scavenger capacity measured as TEAC was shown for the βCs norharman and harman. The TEAC (mM) value for THβCs seems to be comparable to known phenolics and flavonoids.[24]

The ability of THβCs to act as antioxidants in the reaction with $ABTS^{\bullet+}$ might occur through an electron transfer mechanism (SET). In this regard, the reaction would initially occur at the indole ring affording an indolyl radical by a single electron transfer mechanism that might occur in different isoforms (cation radical or neutral radical). A similar mechanism has been proposed for melatonin, an indole working as a powerful antioxidant.[25,26] THβCs could be oxidized further with the donation of electrons and possible radical scavenging activity.

It should be mentioned that while working as radical scavengers, THβCs might be further converted into potentially bioactive or perhaps toxic

TABLE I Antioxidant capacities as TEAC (mM) of tetrahydro-β-carboline alkaloids in the $ABTS^{\bullet+}$ decolorization assay

Compounds	TEAC (mM) at 5 min*
MTCA	1.80 ± 0.04
THCA	1.67 ± 0.06
Harman	0
Norharman	0
MTCA-COOH	1.66 ± 0.03
THCA-COOH	1.77 ± 0.04
MTHβC	1.71 ± 0.05
THβC (tryptoline)	2.20 ± 0.07
OHMTHβC	1.80 ± 0.02
Ascorbic Acid	1.01 ± 0.01

* Results expressed as Trolox Equivalent Antioxidant Capacity (TEAC) were determined at 5 min. Data shown are mean values ± SD of four complete sets of experiments performed at different concentrations (1.67, 3.33, and 6.67 μM).

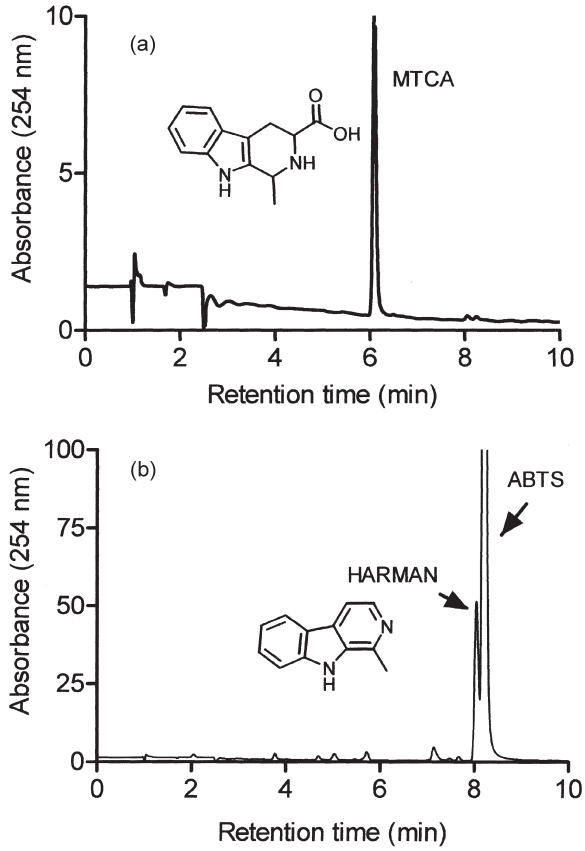

FIGURE 4 (a) RP-HPLC chromatogram of control MTCA (10 μM) (before the addition of $ABTS^{\bullet+}$), and (b) MTCA (10 μM) at 5 min after reaction with $ABTS^{+\bullet}$.

compounds. Then, we have studied the formation of possible new compounds following the reaction with the radical cation of ABTS. The reaction products were analyzed by RP-HPLC-diode array and fluorescence detection and by GC-MS. The βCs norharman and harman did not suffer any change during the assay (up to 40 min of reaction) as expected, given its absence of activity. However, the THβCs MTCA and MTCA-COOH were oxidized and converted into harman (Fig. 4), whereas THCA and THCA-COOH were converted into norharman. The identity of the products was assessed by HPLC retention time, uv, fluorescence and mass spectra (GC-MS). Norharman (HPLC peak at 7.4 min) gave UV max. at 247, 300 and 370 nm; fluorescence max. at 445 nm (excitation 245 nm) and mass spectra: m/z at 168 ($M^{+\bullet}$) and 140. Harman (HPLC peak at 8.0 min) gave UV max. at 247, 300 and 370 nm, fluorescence max. at 435 nm (excitation 245 nm) and mass spectra: m/z at 182 ($M^{+\bullet}$), and 154. Previous electrochemical studies have shown that oxidation of tetrahydro-β-carboline-1- and 3-carboxylic acids proceeds with the loss of electrons probably starting at the indole nucleus accompanied with oxidative decarboxylation–dehydrogenation affording the fully aromatic compound harman.[27] The same products were obtained here after reaction with the radical $ABTS^{\bullet+}$ (see Fig. 1). On the other hand, tryptoline (THβC), 1-methyl-tetrahydro-β-carboline (MTHβC) and 6-OH-1-methyl-tetrahydro-β-carboline (OHMTHβC) quickly disappeared after reacting with ABTS radical cation although no further products were identified.

With these results, tetrahydro-β-carboline alkaloids found in foods or that are present in biological fluids and tissues might act as good radical scavengers. Table II summarizes the concentration of some of these alkaloids detected in foods. Given the relatively low amount of THβCs in comparison with other well-established antioxidants in foods, the total contribution to its whole antioxidant capacity must be relatively low perhaps with exception of wines and seasonings. In a recent study, Long *et al.*[30] have reported a powerful antioxidant activity in dark soy sauces. THβCs present in soy sauce might account for some of this activity (up to 5 mM). However, a major part of it, should still be attributed to unknown compounds. Nevertheless, it has been reported that THβCs might be absorbed and accumulated in tissues.[31] Then, dietary sources rich in THβCs likely contribute to increase the amount of these compounds occurring in biological tissues and fluids,[2] where they might have a significant contribution by acting as radical scavengers. Foods containing high level of these compounds such as soy sauces and seasoning, wines, vinegars and fruit products (juices, jams, etc.), might provide these potentially acting antioxidant or radical scavengers.

TABLE II Tetrahydro-β-carbolines detected in foodstuffs (mg/l or mg/kg)

Foodstuffs	MTCA*	THCA*	MTHβC†	OHMTHβC†	THCA-COOH‡	MTCA-COOH‡
Wine	0.5–18	0–0.7	<0.2	–	>0.01	>0.01
Liquors	nd–7.5	0–0.23	nd	–	–	–
Wine vinegar	3.9–9.6	0–0.12	nd	–	>0.01	>0.01
Fruit juices	0–11	0–1.2	0–0.4	<2.0	–	–
Fruits	0–8.7	0–1.2	0–2	<0.7	–	–
Soy sauce	92–447	2.1–70	51.7¶	–	>10	>10
Seasoning	1–30	0–3.1	0.83¶	–	>10	>10
Beer	0–17	0–0.8	0.09	<0.5	–	–

*From Refs. [2,18–21]. †Herraiz, unpublished results. ‡From Ref. [29]. ¶From Ref. [28].

CONCLUSIONS

Tetrahydro-β-carboline alkaloids that occur in many foods such as wine, seasonings, vinegar and fruit juices, and which may occur in biological samples acted as radical scavengers and antioxidants in the ABTS test. These alkaloids could contribute to the beneficial antioxidant properties attributed to foods. In contrast, βCs norharman and harman did not show any radical scavenger activity in the same assay. After being oxidized during the ABTS test (reaction with $ABTS^{\bullet+}$), tetrahydro-β-carboline-3-carboxylic acid and tetrahydro-β-carboline-1,3-dicarboxylic acid afforded the fully aromatic β-carboline harman (from MTCA and MTCA-COOH) or norharman (from THCA and THCA-COOH).

Acknowledgements

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References

[1] Herraiz, T. and Ough, C.S. (1993) "Chemical and technological factors determining tetrahydro-β-carboline-3-carboxylic acid content in fermented alcoholic beverages", *J. Agric. Food Chem.* **41**, 959–964.
[2] Herraiz, T. (2000) "Analysis of the bioactive alkaloids tetrahydro-β-carboline and β-carboline in food", *J. Chromatogr. A* **881**, 483–499.
[3] Buckholtz, N.S. (1980) "Neurobiology of tetrahydro-β-carbolines", *Life Sci.* **27**, 893–903.
[4] Airaksinen, M.M. and Kari, I. (1981) "β-Carbolines, psychoactive compounds in the mammalian body", *Med. Biol.* **59**, 21–34.
[5] Melchior, C. and Collins, M.A. (1982) "The route and significance of endogenous synthesis of alkaloids in animals", *CRC Crit. Rev. Toxicol.* **10**, 313–356.
[6] Rommelspacher, H., May, T. and Susilo, R. (1991) "β-Carbolines and tetrahydroisoquinolines: detection and function in mammals", *Planta Med.* **57**(Suppl.), S85–S92.
[7] Peura, P., Johnson, J.V., Yost, R.A. and Faull, K.F. (1989) "Concentrations of tryptoline and methryptoline in rat brain", *J. Neurochem.* **52**, 847–852.
[8] Tse, S.Y.H., Mak, I-T. and Dickens, B.F. (1991) "Antioxidative properties of harmane and β-carboline alkaloids", *Biochem. Pharmacol.* **42**, 459–464.
[9] Garcia, J.J., Martinez-Ballarín, E., Robinson, M., Allué, J.L., Reiter, R.J., Osuna, C. and Acuña-Castroviejo, D. (2000) "Protective effect of β-carbolines and other antioxidants on lipid peroxidation due to hydrogen peroxide in rat brain homogenates", *Neurosci. Lett.* **294**, 1–4.
[10] Pari, K., Sundari, C.S., Chandani, S. and Balasubramanian, D. (2000) "β-Carbolines that accumulate in human tissues may serve a protective role against oxidative stress", *J. Biol. Chem.* **275**, 2455–2462.
[11] Kawashima, Y., Horiguchi, A., Taguchi, M., Tuyuki, Y., Karasawa, Y., Araki, H. and Hatamaya, K. (1995) "Synthesis and pharmacological evaluation of 1,2,3,4-tetrahydro-β-carboline derivatives", *Chem. Pharm. Bull.* **53**, 783–788.
[12] Collins, M.A. and Neafsey, E.J. (1985) "β-Carboline analogs of *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): endogenous factors underlying idiophathic parkinsonism?", *Neurosci. Lett.* **55**, 179–183.
[13] Totsuka, Y., Ushiyama, H., Ishihara, J., Sinha, R., Goto, S., Sugimura, T. and Wakabayashi, K. (1999) "Quantification of the co-mutagenic β-carbolines, norharman and harman in cigarette smoke condensates and cooked foods", *Cancer Lett.* **143**, 139–143.
[14] Halliwell, B. (1999) "Food-derived antioxidants. Evaluating their importance in food and *in vivo*", *Food Sci. Agric. Chem.* **1**, 67–109.
[15] Long, L.H. and Halliwell, B. (2001) "Antioxidant and prooxidant abilities of foods and beverages", *Methods Enzymol.* **335**, 181–190.
[16] Rice-Evans, C.A. and Miller, N.J. (1996) "Antioxidant activities of flavonoids as bioactive components of food", *Biochem. Soc. Trans.* **24**, 790–795.
[17] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999) "Antioxidant activity applying an improved ABTS radical cation decolorization assay", *Free Radic. Biol. Med.* **26**, 1231–1237.
[18] Herraiz, T., Huang, Z. and Ough, C.S. (1993) "1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid in wines", *J. Agric. Food Chem.* **41**, 455–459.
[19] Herraiz, T. (1996) "Occurrence of tetrahydro-β-carboline-3-carboxylic acids in commercial foodstuffs", *J. Agric. Food Chem.* **44**, 3057–3065.
[20] Herraiz, T. (1998) "Occurrence of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid in fruit juices, purees and jams", *J. Agric. Food Chem.* **46**, 3484–3490.
[21] Herraiz, T. (1999) "1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid in fruits", *J. Agric. Food Chem.* **47**, 4883–4887.
[22] Herraiz, T. (2000) "Tetrahydro-β-carboline-3-carboxylic acid compounds in fish and meat: possible precursors of co-mutagenic β-carbolines norharman and harman in cooked foods", *Food Addit. Contam.* **17**, 859–866.
[23] Herraiz, T. (1997) "Analysis of tetrahydro-β-carbolines and their precursors by electron ionization mass spectrometry. Identification in foodstuffs by gas chromatography/mass spectrometry", *Rapid Commun. Mass Spectrom.* **11**, 762–768.
[24] Pannala, A.S. and Rice-Evans, C. (2001) "Rapid screening method for relative antioxidant activities of flavonoids and phenolics", *Methods Enzimol.* **335**, 266–272.
[25] Poeggeler, B., Saarela, S., Reiter, R.J., Tan, D-X., Chen, L-D., Manchester, L.C. and Barlow-Walden, L.R. (1994) "Melatonin—a highly potent endogenous radical scavenger and electron donor: New aspects of the oxidation chemistry of this indole accessed *in vitro*", *Ann. NY Acad. Sci.* **738**, 419–420.
[26] Turjanski, A.G., Rosenstein, R.E. and Estrin, D.A. (1998) "Reactions of melatonin and related indoles with free radicals: a computational study", *J. Med. Chem.* **41**, 3684–3689.
[27] Bobbitt, J.M. and Willis, J.P. (1980) "Electrochemistry of natural products. 7. Oxidative decarboxylation of some tetrahydro-β-carbolinecarboxylic acids", *J. Org. Chem.* **45**, 1978–1984.
[28] Suchiya, T., Sato, M., Hayashi, H., Kato, H., Kureshiro, H. and Hayashi, T. (1996) "Simultaneous determination of tetrahydro-β-carbolines and β-carbolines", *Chromatographia* **43**, 419–425.
[29] Gutsche, B. and Herderich, M. (1997) "HPLC-MS/MS profiling of tryptophan-derived alkaloids in food: identification of tetrahydro-β-carbolinedicarboxylic acids", *J. Agric. Food Chem.* **45**, 2458–2462.
[30] Long, L.H., Kwee, D.C.T. and Halliwell, B. (2000) "The antioxidative activities of seasonings used in Asian cooking. Powerful antioxidant activity of dark soy sauce revealed using the ABTS assay", *Free Radic. Res.* **32**, 181–186.
[31] Ogawa, Y., Adachi, J. and Tatsuno, Y. (1993) "Accumulation of 1-methyl-tetrahydro-β-carboline-3-carboxylic acid in blood and organs of rat. A possible causative substance of eosinophilia-myalgia syndrome associated with ingestion of L-tryptophan", *Arch. Toxicol.* **67**, 290–293.