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Tetrahydro-β-carboline Alkaloids Occur in Fruits and Fruit Juices. Activity as Antioxidants and Radical Scavengers

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Tetrahydro-β-carbolines are biologically active alkaloids that occur and accumulate in mammalian tissues, fluids, and brain, but their ultimate origin or biological role is still uncertain. Four tetrahydro-β-carboline alkaloids: 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, 1-methyl-1,2,3,4-tetrahydro-β-carboline, and 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline, are found as naturally occurring substances in some fruit and fruit juices. These compounds occur in the μg/g level in those products, and a characteristic and distinct profile appears to exist depending on the type of fruit and juice involved. Thus, 1-methyl-1,2,3,4-tetrahydro-β-carboline may appear in tomato, tomato juice, and kiwi; 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline in bananas, pineapple, tomato, and their corresponding juices; and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid in oranges and grapefruits, although it also occurred in most juices. Fruit-occurring tetrahydro-β-carboline alkaloids acted as antioxidants and free radical scavengers in the ABTS assay when compared with ascorbic acid and Trolox. This suggests that tetrahydro-β-carboline alkaloids might act as antioxidants when absorbed and accumulated in the body, contributing to the antioxidant effect of fruit products containing these compounds.

KEYWORDS: Tetrahydro-β-carboline; β-carbolines; alkaloids; antioxidants; radical scavengers; ABTS; fruit; fruit juices

INTRODUCTION

Tetrahydro-β-carbolines (THβCs) (tetrahydro-pyrido(3,4-b)-indole) and β-carboline (βCs) (pyrido(3,4-b)indole) are naturally occurring indole alkaloids that exhibit a broad range of pharmacological and biological activity (*1*−*3*). Biological interest on THβCs and βCs has grown from reports showing their occurrence under physiological conditions in biological tissues and fluids (*1*, *4*−*8*). These alkaloids have attracted the attention of neurochemists who have speculated on their putative role in the central nervous system, where they might function as mild neuromodulators. They inhibit monoamine oxidase (MAO), monoamine uptake, and bind to benzodiazepine-GABA receptors and imidazoline binding sites (*1*, *2*, *7*−*11*). THβCs and βCs have also been studied in relation to alcoholism or in pathological states (*12*−*14*). They may act as co-mutagens, precursors of mutagens, or *N*-nitroso compounds (*15*−*17*) or be bioactivated to give endogenous neurotoxins (*18*, *19*). Therefore, β-carboline alkaloids exhibit a broad range of biological actions and may play an as yet unknown physiological role. Whether their origin in biological systems is endogenous, environmental, or both, remains controversial. Tetrahydro-β-carbolines and β-carbolines may form in dietary sources by a nonenzymatic Pictet−Spengler reaction (*20*−*23*). The availability of β-carbolines during food consumption is an interesting matter, because they may accumulate in tissues and fluids (*24*). An exogenous intake from the diet might contribute to the ultimate presence of these alkaloids in the human biological tissues and fluids, where they might exhibit further biological effects (*25*).

It is currently accepted that the consumption of fruits and vegetables offers a preventive value against cancer and cardiovascular diseases. This is attributed to antioxidants such as vitamins C and E, carotenoids, and polyphenols or flavonoids. These compounds have the ability to protect against reactive oxygen species and reactive nitrogen species involved in pathological states (*26*−*30*). On the other hand, the existence of many other unknown phytochemicals in fruits and fruit products that may exhibit significant biological actions is also assumed. This study was designed to identify several tetrahydro-β-carboline alkaloids in fruits and juices and assess the occurrence of these biologically active substances in such products. Moreover, it shows that these alkaloids detected in fruit products may act as antioxidants. A role of these compounds as protective agents against free radicals is suggested.

MATERIALS AND METHODS

Reference Compounds and Samples. 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid was purchased from Sigma (St. Louis, MO) and synthesized from L-tryptophan and acetaldehyde to give a

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Table 1. Concentration of Tetrahydro-β-carboline Alkaloids Found in Several Fruits (μg/g)[a]

		1	3a	3b	4
	N	X (SD)	X (SD)	X (SD)	X (SD)
tomato	11	0.71 (0.54)	*b*	*b*	0.17 (0.16)
peach	3	*b*	0.01 (0.014)	0.003 (0.003)	*b*
pear	4	*b*	0.02 (0.002)	0.006 (0.006)	*b*
apple	3	*b*	*b*	*b*	*b*
orange	3	*b*	1.11 (0.65)	0.37 (0.18)	*b*
grapefruit	3	*b*	2.71 (1.37)	0.78 (0.37)	*b*
kiwi	5	0.31 (0.23)	*b*	*b*	1.02 (0.91)
grape	3	*b*	0.03 (0.04)	0.01 (0.01)	*b*
pineapple	3	0.62 (1.02)	0.05 (0.09)	0.02 (0.02)	0.03 (0.06)
prune	3	0.11 (0.04)	*b*	*b*	*b*
banana	10	1.87 (2.05)	0.26 (0.31)	0.09 (0.12)	*b*

[a] Tetrahydro-β-carbolines **1**, **3a,b** and **4** are as in Figure 1. [b] Not detected.

diastereoisomeric mixture (1*S*,3*S*-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, major compound, and 1*R*,3*S*-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, minor compound). 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid and 1-ethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid were obtained according to Brossi et al. (*31*). 6-Hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline was synthesized from serotonin oxalate (Sigma) and acetaldehyde, whereas 1-methyl-1,2,3,4-tetrahydro-β-carboline was synthesized from tryptamine and acetaldehyde by a Pictet−Spengler condensation. NMR, MS, and GC-MS (*N*-methoxycarbonyl methyl ester derivatives) data were consistent with the structures of the synthesized compounds (*32*, *33*).

Commercial samples of fruits and fruit juices (**Tables 1** and **2**) from different origins (local and imported) were purchased locally. Fruits were washed, and peeled (except for grape, tomato, and peach) before analysis.

Isolation of Tetrahydro-β-carbolines. Tetrahydro-β-carbolines (THβCs) were isolated using solid-phase extraction (*20*, *21*). (a) Fruit sample (5−8 g) was mixed with 10 mL of 0.6M $HClO_4$ containing 1 mg/mL semicarbazide (Sigma), homogenized in an Ultra-Turrax and centrifuged (5100 g, 0−5 °C) for 10−15 min. (b) Fruit juices were centrifuged at 5100 g, 0−5 °C for 10−15 min. An aliquot of supernatant (5.5 mL) was adjusted to acidic pH (pH 2) with 0.1M HCl if needed (fruit juices) and then spiked with 0.5 mL of 1-ethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid solution (5 mg/L) used as an internal standard (IS). The sample was slowly passed through Bond Elut, 500 mg/3 mL size benzenesulfonic acid-SCX columns (Varian, Harbor City, CA) using a vacuum manifold. After washing with 6 mL of 0.1 N HCl, 2 mL of methanol, 6 mL of HPLC water, and rinsing with 2 mL of 0.4M phosphate buffer (pH 9.1), the THβCs were eluted with 5 mL of methanol + 0.4 M phosphate buffer, pH 9.1 (1:1). The eluates were injected into the HPLC.

Chromatographic Analysis. The analysis of tetrahydro-β-carbolines by RP-HPLC and fluorescence detection was carried out as previously described (*21*). A 150 mm × 3.9 mm, 4 μm, Nova-pak C18 column (Waters, Milford, MA) 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). The gradient was programmed from 0% (100% A) to 32% B in 8 min and then 90% B at 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 for excitation and 343 nm for emission.

Quantitation was obtained from calibration curves (area ratio vs concentration) constructed from solutions of reference compounds and analyzed through the entire procedure. Confirmation of the identity of isolated tetrahydro-β-carbolines was established by HPLC retention times and coelution with authentic standards. Also, fluorescence spectra of the HPLC peaks were compared with those of reference compounds to ensure that quantified peaks correspond to those expected. For this, eluting peaks corresponding to tetrahydro-β-carbolines were trapped into the flow cell of the fluorescence detector by stopping the solvent pump, and excitation and emission spectra monitored.

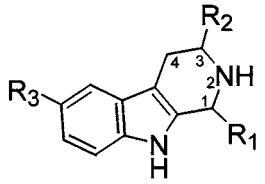
RP-HPLC-Mass Spectrometry Analysis. Samples of fruit and juices were isolated for tetrahydro-β-carbolines by SCX-extraction as above. The eluting fractions corresponding to phosphate buffer/methanol (1:1) containing the tetrahydro-β-carbolines were evaporated under a stream of nitrogen and subsequently injected into the HPLC-MS. Chemical identification was accomplished by HPLC-MS on a 3.9 mm × 150 mm Novapak C18 column (Waters), by using an HPLC-MSD series 1100 (Hewlett-Packard) (electrospray-positive ion mode). Eluents: A, formic acid (0.5%); B, 0.5% formic acid in acetonitrile; linear gradient from 0 to 60% B in 60 min. Flow was 0.7 mL/min. Cone voltage was 50 V. Mass range was 50−600 amu. Identification of tetrahydro-β-carbolines was based on the protonated molecular ions $(M + H)^+$ and small fragments corresponding to the loss of 29 amu (CH_2NH) for tetrahydro-β-carbolines and 73 amu (CHNHCOOH) for tetrahydro-β-carboline-3-carboxylic acid due to the retro-Diels−Alder fragmentation (*22*, *32*, *33*). Also, a sample of authentic standards was analyzed under the same conditions and used for comparison.

Antioxidant and Radical Scavenger Activity. Radical scavenger activity of tetrahydro-β-carbolines was measured with the ABTS assay introduced by Re et al. (*34*) that has proved to work well to measure total antioxidant activity (*29*, *35*). 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) was dissolved in water to 7 mM concentration, and the long-lived ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with potassium persulfate (2.45 mM final concentration) 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. Tetrahydro-β-carbolines detected in fruit products, Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) and ascorbic acid were dissolved in deionized water at 1 mM and then used for radical scavenger assay (from 0 to 20 μM, final concentration) by measuring the decrease of absorbance at 734

Table 2. Concentration of Tetrahydro-β-carboline Alkaloids Found in Several Fruit Juices (mg/L)[a]

		1	2	3a	3b	4
	N	X (SD)	X (SD)	X (SD)	X (SD)	X (SD)
tomato	5	2.03 (1.69)	0.76 (0.26)	1.25 (0.57)	0.39 (0.11)	0.13 (0.07)
peach	2	*b*	0.02 (0.01)	0.10 (0.01)	0.03 (0.01)	*b*
pear	2	*b*	0.04 (0.04)	0.04 (0.03)	0.015 (0.01)	*b*
apple	7	*b*	0.05 (0.04)	0.19 (0.22)	0.06 (0.07)	*b*
orange	9	*b*	0.097 (0.05)	2.2 (1.44)	0.66 (0.35)	*b*
grapefruit	3	*b*	0.10 (0.07)	2.0 (1.5)	0.64 (0.39)	*b*
kiwi	1	0.64	0.05	0.36	0.11	0.39
pineapple	8	1.69 (1.40)	0.16 (0.09)	0.26 (0.14)	0.11 (0.04)	0.03 (0.03)
banana	3	1.37 (0.62)	0.068 (0.03)	0.25 (0.07)	0.10 (0.02)	*b*
tropical	3	0.79 (0.03)	0.036 (0.02)	0.47 (0.09)	0.15 (0.02)	0.01 (0.001)
multifruit	4	1.61 (1.14)	0.08 (0.02)	0.54 (0.33)	0.19 (0.09)	0.01 (0.02)
grape	1	*b*	0.03	0.7	0.22	*b*
peach + grape	3	*b*	0.04 (0.02)	0.37 (0.36)	0.12 (0.11)	*b*

[a] Tetrahydro-β-carbolines **1**, **2**, **3a,b**, and **4**, are as in Figure 1. [b] Not detected.



R_1	R_2	R_3	Comp.
CH_3	H	OH	6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (**1**)
H	COOH	H	1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**2**)
CH_3	COOH	H	1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (1*S*,3*S* isomer: **3a**; 1*R*,3*S* isomer: **3b**)
CH_3	H	H	1-methyl-1,2,3,4-tetrahydro-β-carboline (**4**)

Figure 1. Structure of biologically active tetrahydro-β-carboline alkaloids.

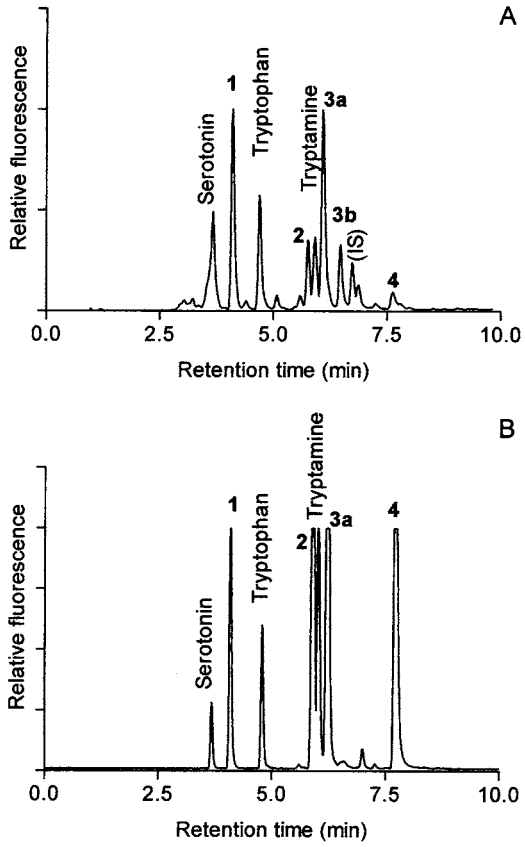


Figure 2. RP–HPLC chromatograms of tetrahydro-β-carboline alkaloids extracted from (A) tomato juice and (B) standards. Compounds **1**, **2**, **3**, and **4** are as in Figure 1.

nm as a function of time. The antioxidant capacity was measured in comparison with Trolox, the water soluble vitamin E analogue, as standard.

RESULTS AND DISCUSSION

Tetrahydro-β-carboline bioactive alkaloids are shown in **Figure 1**. After isolation by solid-phase extraction, fruit and juices gave HPLC chromatographic peaks coeluting with authentic standards of tetrahydro-β-carboline alkaloids (**Figure 2**). Besides coelution with standards, the fluorescence spectra of these chromatographic peaks measured into the flow cell of the fluorescence detector were also in agreement with those of standards as shown for 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline and 1-methyl-1,2,3,4-tetrahydro-β-carboline (**Figure 3**). The presence of β-carboline alkaloids in various fruits and juices was confirmed by RP-HPLC-MS under electrospray ionization (ESI) that afforded the needed specificity for chemical

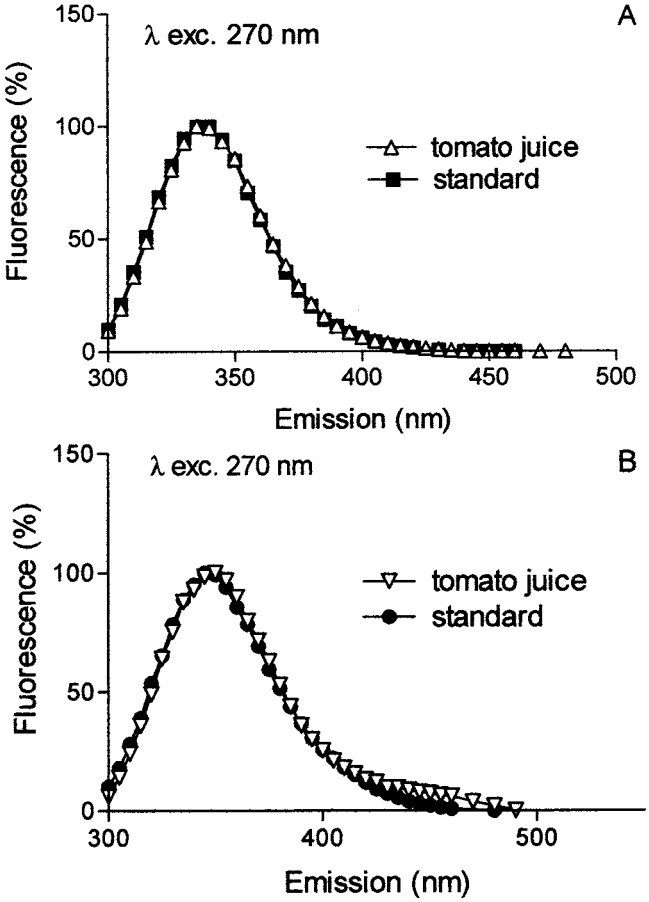


Figure 3. Fluorescence pattern of emission (excitation at 270 nm) of (A) 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline **1** and (B) 1-methyl-1,2,3,4-tetrahydro-β-carboline **4** from SCX-extracted tomato juice and standards.

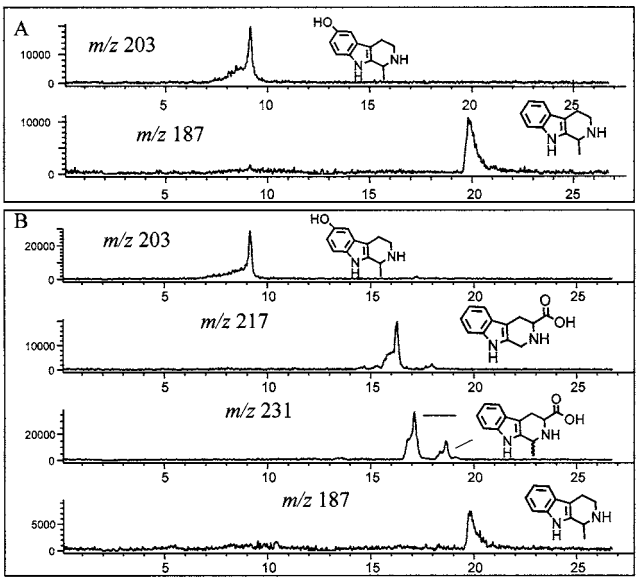


Figure 4. Ion trace chromatograms obtained by RP-HPLC-ESI of SCX-extracted A. tomato and B. tomato juice with the corresponding identified tetrahydro-β-carboline alkaloids.

identification (**Figure 4**). Ion trace and mass spectra of tetrahydro-β-carbolines showed the occurrence of these compounds. The mass spectra afforded the $(M + H)^+$ ions and small fragments corresponding to the retro-Diels–Alder loss of 73 amu ($M + H - C_2H_3NO_2$) (tetrahydro-β-carboline-3-carboxylic

acid) and 29 amu (M + H−CH_3N) (1-methyl-tetrahydro-β-carbolines). A summary of the HPLC-MS results is as follows: 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline **1** was positively identified in banana, kiwi, and tomato, as well as pineapple juice, banana nectar, multifruit juice, and tomato juice; 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **2** was detected in tomato juice and multifruit juice; 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (1*S*,3*S* and 1*R*,3*S* diastereoisomers) **3a,b** was positively identified in ripened banana, banana, pineapple juice, banana nectar, tomato juice, orange, orange juice, and multifruit juice, whereas 1-methyl-1,2,3,4-tetrahydro-β-carboline **4** was positively identified in kiwi, tomato, and tomato juice, as well as multifruit and pineapple juice.

The presence of these alkaloids in fruits indicates their exogenous intake via the diet. Tetrahydro-β-carboline alkaloids were then quantified in various commercial samples of fruits and fruit juices (**Tables 1** and **2**). The content of these compounds varied both between different groups and within samples of the same group and ranged from undetectable amount to various μg/g levels. In fruits, 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline **1** averaged 1.87 μg/g in bananas, with relative high concentrations also in tomato, kiwi, and pineapple; 1-methyl-1,2,3,4-tetrahydro-β-carboline **4** averaged 1 μg/g in kiwi, and 0.17 μg/g in tomato; 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **3** mainly occurred in citrus fruits such as oranges (1.5 μg/g) and grapefruits (3.5 μg/g). A similar pattern was observed in fruit juices, although in those samples, tetrahydro-β-carboline-3-carboxylic acids (compounds **2** and **3a,b**) were widely distributed among most of the samples. Interestingly, the profile of tetrahydro-β-carboline alkaloids was characteristic for each particular fruit or fruit product. Thus, 1-methyl-1,2,3,4-tetrahydro-β-carboline mainly appeared in tomato, tomato juice, and kiwi; 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline was more abundant in bananas, pineapple, tomato, and their corresponding juices; and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid was the representative carboline in oranges and grapefruits, although it occurred also in most juices. The occurrence of tetrahydro-β-carbolines in fruits and juices seemed to correlate with the presence or absence of their respective amine (serotonin or tryptamine) or amino acid precursors, suggesting a link between them. Thus, an estimation of serotonin from the chromatograms gave 9.4, 15.3, 6.0, and 27 μg/g on average, in banana, tomato, kiwi, and pineapple, all of which may give 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline. In contrast, no serotonin appeared in citrus fruits (orange and grapefruit), in agreement with the absence of 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline. The estimated average concentration of tryptamine was 2.8, 9, and 1.5 μg/g in tomato, kiwi, and pineapple, all of which may afford 1-methyl-1,2,3,4-tetrahydro-β-carboline. In contrast, no tryptamine was detected in citrus and banana that did not exhibit 1-methyl-1,2,3,4-tetrahydro-β-carboline, either. The estimated average concentration of tryptophan was 20, 9, 9.5, 7, and 5.2 μg/g in banana, tomato, citrus, kiwi, and pinneapple, respectively. 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid isomers mainly appeared in citrus and banana, and increased in most juices.

Currently, there is much scientific evidence correlating the consumption of fruits and fruit products with a better health and disease prevention. Fruits contain antioxidants such as ascorbic acid, vitamin E, and polyphenols or flavonoids that protect against free radicals damaging biomolecules such as lipids, proteins, and DNA within cells. Following the confirmation of the occurrence of tetrahydro-β-carboline alkaloids in fruits and fruit products, we tested their activity as antioxidant and radical scavengers. For this purpose, the elimination of the cation radical $ABTS^{\bullet+}$ by antioxidants was measured, a test that has already proved to work well to determine the total antioxidant activity (*35*). **Figure 5** shows that tetrahydro-β-carboline alkaloids occurring in fruit products behave as antioxidants in the elimination of the cation radical $ABTS^{\bullet+}$. The elimination caused by the alkaloids (TEAC value) ranged from 1.6 to 1.9-fold that of Trolox, a water soluble analogue of Vitamin E, or ascorbic acid at the same concentration (6.6 μM) in this assay. During the reaction with $ABTS^{\bullet+}$, these classes of compounds are consumed, and some are converted to aromatic β-carbolines (*42*). Therefore, tetrahydro-β-carbolines might scavenge free radicals that could cause damage to biomolecules and suggests that these alkaloids may act as another type of antioxidants occurring in fruit products. However, further studies will be needed under other conditions and assays, mainly based on biological radicals.

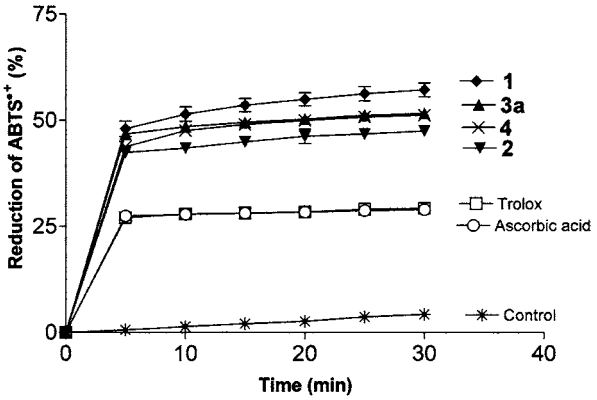


Figure 5. Elimination (decolorization at 734 nm) of radical cation $ABTS^{\bullet+}$ by tetrahydro-β-carbolines, ascorbic acid and Trolox (soluble form of vitamin E). Concentration of the compounds in the assay: 6.6 μM. Control has no added compounds. Data are averages of quadruplicate experiments. Compounds **1**, **2**, **3**, and **4** are as in Figure 1.

The above results reveal the occurrence and formation of several tetrahydro-β-carboline alkaloids in fruits and their juices, extending previous results (*36*−*38*). Concerning the origin of tetrahydro-β-carbolines in fruits, it is known that acetaldehyde and formaldehyde are natural constituents of fruits and vegetables (*39*). Both aldehydes may condense with L-tryptophan or amines such as serotonin and tryptamine to provide tetrahydro-β-carbolines (*20*, *21*). It is then likely that aldehydes (mainly acetaldehyde) released metabolically in the whole fruit or fruit juice may react with precursors such as tryptamine, tryptophan, and serotonin to provide tetrahydro-β-carbolines through a Pictet−Spengler chemical condensation as illustrated in **Figure 6**. This condensation will further progress during time in fruit juices affording tetrahydro-β-carbolines from the corresponding precursors. This likely formation was supported here as the occurrence of a particular tetrahydro-β-carboline correlated with the presence of the corresponding amine or amino acid precursor. Consequently, a distinct and characteristic profile of tetrahydro-β-carbolines occurred for each particular fruit as seen in **Tables 1** and **2**. Nevertheless, it should be noticed that the final concentration of these alkaloids in each juice or fruit may depend on variables such as fruit ripeness, harvest and storage, season time, fruit type, and origin, or processing conditions. This may explain the fact that the content was variable, ranging from undetectable amounts of alkaloid to a relatively high content.

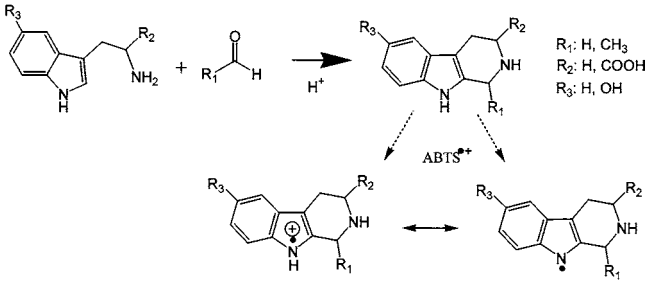


Figure 6. Formation of tetrahydro-β-carboline alkaloids based on a Pictet–Spengler reaction of the amine and amino acid precursors with aldehydes. Tetrahydro-β-carbolines may afford an indolyl cation or neutral radical by a single electron transfer to a free radical ($ABTS^{\bullet+}$) as the first step in their action as antioxidants.

The occurrence of tetrahydro-β-carbolines in fruits and fruit juices suggest an exogenous intake of these substances in the diet. Following absorption, these alkaloids may accumulate in tissues, becoming biologically active. So far, much research dealing with the biological significance of tetrahydro-β-carbolines or β-carbolines has studied their effects on the CNS such as inhibition of serotonin uptake, MAO inhibition, binding to benzodiazepine-GABA or imidazoline receptors (*1*, *2*, *9*, *11*) or possible toxicological effects (*18*). This work has focused on the possible action of tetrahydro-β-carbolines as antioxidants and free radical scavengers. As shown in **Figure 5**, those tetrahydro-β-carbolines occurring in fruit and juices acted as radical scavengers. Their antioxidant capacity in the test used was stronger than ascorbic acid and Trolox. Tetrahydro-β-carbolines contain an indole ring that may afford the indolyl cation or neutral radical through single electron transfer while acting as radical scavengers (**Figure 6**). A similar mechanism has been proposed for other indole antioxidants such as melatonin (*40*, *41*). The indolyl radical might be further oxidized to the fully aromatic β-carbolines such as harman and norharman, in the case of tetrahydro-β-carboline-3-carboxylic acids (compounds **2** and **3a,b)**, or breakdown to unknown compounds (*42*). These environmental (dietary) or endogenously formed alkaloids after being absorbed and/or accumulated in tissues and fluids could then play a role as potential antioxidants by protecting against radicals formed during oxidative stress. In this regard, it has been recently suggested that endogenous tetrahydro-β-carbolines may serve as antioxidants (*42*−*44*). However, the expected contribution of these compounds to the measured total antioxidant activity of fruit and fruit juices should be small, given the low relative concentration of these compounds compared with vitamins, carotenoids and phenols.

Finally, although tetrahydro-β-carbolines are known biologically active compounds and might act as free radical scavengers as shown here, there nevertheless still exists the possibility that both dietary and endogenous β-carbolines may act as precursors of mutagens or endogenous toxins after accumulation in tissues (*19*), and in this regard, more research is needed to assign a comprehensive biological activity or role to these mammalian and dietary alkaloids.

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