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Identification and Occurrence of β-Carboline Alkaloids in Raisins and Inhibition of Monoamine Oxidase (MAO)

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Monoamine oxidase (MAO) is a mitochondrial enzyme involved in the oxidative catabolism of neurotransmitters and xenobiotic amines, including vasopressor and neurotoxic amines, and a current target for antidepressant and neuroprotective drugs. Raisin extracts and homogenates exhibited reversible in vitro inhibition of MAO isozymes, particularly MAO-A, suggesting the presence of MAO-inhibiting substances. Chromatographic and spectrometric studies showed the occurrence of aromatic β-carboline alkaloids in raisins, and norharman and harman were identified as the key contributors to MAO inhibition. On average, harman ranged from 6 to 644 ng/g and norharman from 2 to 120 ng/g. Several technological variables appeared to determine the presence of these compounds in raisins. Dark-brown raisins (i.e., sun-dried) contained much higher levels than golden raisins. Tetrahydro-β-carboline-3-carboxylic acid compounds that are direct precursors of aromatic β-carbolines were also identified in raisins and appeared in a higher amount, reaching up to 50 μg/g. β-Carbolines were isolated from raisins and acted as good competitive inhibitors of MAO-A (harman) and MAO-B (norharman) isozymes. These results suggest that β-carboline alkaloids and perhaps raisins containing a high level of β-carbolines might exhibit potential activity as MAO inhibitors. The results also show that some raisins can be a source of dietary exposure to bioactive β-carbolines.

KEYWORDS: Monoamine oxidase; MAO inhibition; raisins; β-carbolines; norharman; norharmane; harman; harmane; tetrahydro-β-carbolines; alkaloids; heterocyclic amines

INTRODUCTION

Monoamine oxidase (MAO) is a flavin-adenosine-dinucleotide (FAD)-containing enzyme located at the outer membranes of mitochondria in the brain, liver, intestinal mucosa, and other organs. It catalyzes the oxidative deamination of biogenic amines, such as brain neurotransmitters, vasoactive dietary amines, and xenobiotic amines, including dopamine, serotonin, norephinephrine, tyramine, tryptamine, and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) neurotoxin. MAO appears as two isozymes, MAO-A and B, distinguished by substrate and inhibitor selectivities (*1, 2*). MAO-A preferentially catalyzes the oxidation of serotonin and norephinephrine and is inhibited by clorgyline, whereas MAO-B catalyzes the oxidation of phenylethylamine and benzylamine and is inhibited by (*R*)-deprenyl. Tyramine, dopamine, and tryptamine appear to be substrates for both enzymes. MAO plays a significant role in the central nervous system and peripheral organs (*3*). Abnormal activity of MAO-B has been implicated in neurological disorders and diseases, and MAO-A plays an important role in psychiatric conditions and depression (*4*).

Oxidation of biogenic amines and neurotransmitters by MAO results in the production of hydrogen peroxide (H_2O_2), ammonia, and aldehydes, which represent risk factors for cell oxidative injury (*5, 6*). MAO bioactivates amines such as MPTP to toxic metabolites (*7–9*). In this regard, protection offered by MAO inhibitors may result from a diminished activation of toxins and a reduced production of reactive oxygen species. On the other hand, inhibition of peripheral MAO (e.g., intestinal and hepatic) by MAO-inhibiting drugs has been linked with hypertensive crisis caused by a reduced metabolism of vasopressor dietary amines (e.g., tyramine) (*10*). In this regard, inhibition of MAO might potentiate the physiological effects of dietary vasoactive amines and exert food–drug interactions.

The biological implications of MAO isozymes and the identification of MAO inhibitors in drug discovery are subjects of current interest (*1, 2, 11, 12*). Inhibitors of MAO have been used as antidepressants and neuroprotectants (*2, 4*) Moreover, environmental and dietary factors might affect the activity of this enzyme. MAO is reduced in smokers compared to nonsmokers (*13, 14*), and cigarette smoke appears to inhibit MAO isozymes because it contains inhibitory substances (*15, 16*). Recently, two β-carbolines, norharman and harman, were identified as MAO inhibitors in smoke (*15, 17*). Inhibition of MAO in smokers may be linked with the addictive properties

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of cigarettes and depression and with a lowest incidence of Parkinson's disease (*13, 15, 16*). Coffee also appears to inhibit MAO activity and the same two β-carbolines were isolated from coffee (*18*). Interestingly, regular coffee drinking has been also correlated with a lower incidence of Parkinson's disease (*19*). Further research is currently needed to study the influence of the environment and diet on the activity of MAO and its inhibition.

β-Carboline alkaloids are naturally occurring bioactive substances that exhibit pharmacological effects owing to their binding to benzodiazepine, imidazoline, serotonin, and opiate receptors as well as MAO inhibition (*8, 20–27*). Some of these compounds may also trigger toxicological effects (*8, 9*). β-Carbolines occur endogenously in humans where they might exert biological actions (*28–30*). These substances have been also found in foods and cigarette smoke (*17, 31, 32*), suggesting that the diet and smoking may contribute to their presence in biological systems. Continuing with our research interest in β-carbolines in foods and their biological activity, the present research was aimed to study the occurrence of β-carbolines in raisins and to investigate the MAO-inhibiting properties of raisin extracts as well as of β-carbolines isolated from raisins. It reports the occurrence of β-carbolines in raisins and their contribution to MAO inhibition, suggesting that β-carbolines and raisins containing high level of β-carbolines might inhibit monoamine oxidase (MAO).

MATERIALS AND METHODS

Recombinant human monoamine oxidase A and B were obtained from Gentest BD Biosciences (Woburn, MA). Enzymes were expressed in insect cells from MAO-A and MAO-B cDNA using a baculovirus expression system and were prepared as membrane protein fractions. Kynuramine, 4-hydroxyquinoline, norharman (9*H*-pyrido-(3,4-*b*)-indole) and harman (1-methyl-9*H*-pyrido-(3,4-*b*)-indole) were purchased from Sigma. HPLC-grade acetonitrile, methanol, and dimethylsulfoxide (DMSO) were from Scharlau (Spain), and dichloromethane was from Merck.

Commercial samples of raisins from different producers and origins belonging to different varieties such as Thompson seedless, sultana, and Muscat, and subjected to different processing conditions, i.e., sun-dried raisins (dark-brown raisins) and golden (oven-dried raisins) dehydrated raisins, were purchased in supermarkets. Sample preparation of raisins for analysis and enzyme inhibition was carried out in several ways: (a) Raisins (10 g) were homogenized in 0.6 M $HClO_4$ (15 mL) using an Ultraturrax homogenizer and centrifuged at 10,000 rpm for 10 min at 5 °C. An aliquot of this supernatant was neutralized with aqueous NaOH and used for MAO inhibition. (b) Raisins (10 g) were homogenized in 100 mM phosphate buffer (pH 7)/10% DMSO (15 mL) as above, and a fraction of the supernatant was used for MAO inhibition. (c) Raisins (10 g) were homogenized in 0.6 M $HClO_4$ (15 mL); a fraction of the supernatant (5.5 mL) was used for the isolation of β-carbolines by solid phase extraction (SPE), and eluates were used for studying MAO inhibition. (d) Raisins (5–10 g) were homogenized in 0.6 M $HClO_4$ (15–20 mL) and centrifuged, and fractions (5.5 mL) were used for isolation and analysis of tetrahydro-β-carboline-3-carboxylic acids and β-carbolines by SPE. Aromatic β-carbolines were isolated and purified by HPLC and then used to study MAO inhibition.

Isolation and Analysis of Tetrahydro-β-carboline-3-carboxylic Acids and β-Carbolines from Raisins. To isolate tetrahydro-β-carboline-3-carboxylic acids from raisins, an aliquot of 0.6 M $HClO_4$ (5.5 mL) was added to semicarbazide (1 mg/mL) and 1-ethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (0.45 mg/L) used as an internal standard and passed through benzenesulfonic acid (SCX) columns following a previously described procedure (*33, 34*). The elution fraction of phosphate buffer/methanol (1:1) pH 9, containing the tetrahydro-β-carbolines, was analyzed by reverse-phase HPLC with fluorescence detection (excitation, 270 nm; emission, 343 nm) for quantitative analysis and by HPLC-MS for identification. Quantitation was carried out from calibration curves constructed with standards of tetrahydro-β-carbolines isolated under the same procedure.

Aromatic β-carbolines were isolated for subsequent chromatographic analysis, identification, and MAO studies by using a previous SPE procedure (*17, 35*). Briefly, an aliquot (5.5 mL) of 0.6 M $HClO_4$ from raisin homogenate was added to ascorbic acid (5 mg/mL) and 125 μL of a 0.2 mg/L solution of 1-ethyl-β-carboline as internal standard and passed through a propylsulfonic acid (PRS)-derivatized silica column (Varian, USA) that was eluted with water (6 mL), 0.4 M phosphate buffer pH 9 (3 mL), and 0.2 M phosphate buffer/methanol (1:1) pH 9 (3 mL), containing ascorbic acid. The eluates of 0.2 M phosphate buffer/methanol (1:1) pH 9 (3 mL), containing the β-carbolines, were analyzed by HPLC, and norharman and harman were detected by fluorescence (excitation 300 nm and emission 433 nm). Quantitation was obtained from calibration curves of standards isolated under the same procedure. The same PRS SPE procedure (*17, 35*) but lacking ascorbic acid and internal standard was used to isolate the fractions containing β-carboline for subsequent MAO inhibition studies. In this case, the eluting fractions of 0.4 M phosphate buffer pH 9 (3 mL) and 0.2 M phosphate buffer/methanol (1:1) pH 9 (3 mL) were mixed and used for MAO inhibition. To further isolate and purify β-carbolines for subsequent MAO inhibition studies, several pooled PRS-eluates of 0.2 M phosphate buffer/methanol (1:1) pH 9 (3 mL) were evaporated under vacuum and extracted with dichloromethane (Merck) in alkaline pH. The organic phase was evaporated and added with 100 mM phosphate buffer pH 7 containing 30% DMSO (600 μL), and successive injections (20 μL) were carried out into HPLC with peaks corresponding to each β-carboline (norharman or harman) separately collected. After removing acetonitrile in a rotoevaporator, β-carbolines isolated from raisins were extracted with dichloromethane in alkaline pH, concentrated and resuspended in 100 mM phosphate buffer pH 7 containing 30% DMSO (100 μL). Aliquots of these extracts were conveniently diluted and used for MAO inhibition studies of β-carbolines isolated from raisins. Blank extracts were obtained under the same procedure and used as control in the same studies.

Monoamine Oxidase (MAO-A and -B) Assay and Inhibition. MAO enzyme assays were performed as elsewhere (*15, 36*). Briefly, membrane protein fractions containing MAO-A or MAO-B were diluted to the desired concentrations in 100 mM potassium phosphate buffer (pH 7.4). A 0.2-mL reaction mixture containing 0.01 mg/mL protein and 0.25 mM kynuramine in 100 mM potassium phosphate (pH 7.4) was incubated at 37 °C for 40 min. After incubation the reaction was stopped by the addition of 2 N NaOH (75 μL), followed by the addition of 70% $HClO_4$ (25 μL), and the sample was centrifuged (10,000*g*) for 5 min. The supernatant (20 μL) was injected into the HPLC, and the deamination product of kynuramine (i.e., 4-hydroxyquinoline) formed during the enzymatic reaction (*36*) was determined by RP-HPLC-diode array detector at 320 nm. A response curve of area versus concentration was constructed to calculate the concentration of 4-hydroxyquinoline.

To perform inhibition assays, aliquots of raisin extracts and homogenates prepared as above (i.e., neutralized perchloric homogenates or buffer phosphate/DMSO) or, instead, isolated fractions containing β-carbolines (i.e., buffer/methanol PRS fractions) or conveniently isolated and purified β-carbolines from raisins were added to reaction mixtures containing kynuramine (0.25 mM) and MAO enzyme (A or B) (0.01 mg/mL membrane protein) in 100 mM potassium phosphate buffer (pH 7.4), as above. MAO kinetics and mechanism of inhibition were assessed by analyzing the corresponding Michaelis–Menten curves and double reciprocal Lineweaver–Burk plots obtained at different concentrations of kynuramine. To determine MAO-binding reversibility, membrane proteins of MAO-A (0.12 mg/mL) in 100 mM phosphate buffer (pH 7.4) were preincubated (37 °C, 40 min) with raisin homogenates (neutralized perchloric homogenate or buffer phosphate/methanol from PRS extracts, 60 μL). The mixtures were centrifuged (15,000*g*) for 15 min to pellet membrane proteins and washed twice with 100 mM phosphate buffer, and finally the pellet was resuspended in 100 mM phosphate buffer pH 7 + 10% DMSO (0.1 mL). An aliquot (40 μL) was used to measure MAO activity and compared with corresponding controls.

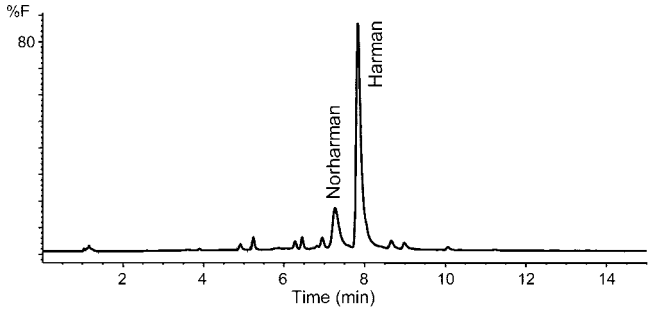

Figure 1. RP-HPLC of a PRS extract from dark-brown (sun-dried) raisins. Fluorescence detection (excitation 300 nm, emission 433 nm).

Reverse Phase HPLC Chromatographic Analysis. The analysis of kynuramine deamination product, 4-hydroxyquinoline, as well as β-carbolines and tetrahydro-β-carbolines was performed by RP-HPLC with UV diode array and fluorescence detection using a HPLC 1050 (Hewlett Packard) with a 1100 diode array detector (DAD) and a 1046A fluorescence detector. A 150 mm × 3.9 mm i.d., 4 μm, Nova-pak C18 column (Waters, Milford, MA) was used for chromatographic separation. Chromatographic conditions were 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 90% B at 15 min. The flow rate was 1 mL/min, the column temperature was 40 °C, and the injection volume was 20 μL. Absorbance detection was set at 320 nm (analysis of 4-hydroxyquinoline), whereas fluorescence detection was used for tetrahydro-β-carbolines (excitation 270 nm and emission 343 nm) and norharman and harman (excitation 300 nm and emission 433 nm). Identification of compounds was done by UV, fluorescence, and mass spectrometry.

Identification by HPLC-ESI-Mass Spectrometry. Mass spectrometric identification of 4-hydroxyquinoline in MAO assays was carried out as previously (*36*). Identification of the aromatic β-carbolines norharman and harman from raisins was made from extracts obtained from PRS SPE columns and solvent extraction as mentioned above, which were analyzed on a 150 mm × 2.1 mm i.d. Zorbax SB-C18, 5 μm, column (Agilent Technologies) by using a series 1100 HPLC-MSD (Hewlett-Packard) (electrospray-positive ion mode). Eluents: A, formic acid (0.5%); B, formic acid (0.5%) in acetonitrile; 80% B in 30 min, flow rate 0.25 mL/min; column temperature 40 °C; mass range 50–700 amu and cone voltage 100 V. On the other hand, raisin SCX extracts containing tetrahydro-β-carboline-3-carboxylic acids were analyzed with the same apparatus by using a 150 mm × 3.9 mm i.d., 4 μm Nova-Pak C18 column (Waters) with the same elutents and a gradient of 30% B in 30 min; flow rate 0.6 mL/min; column temperature 40 °C and cone voltage 50 V.

RESULTS AND DISCUSSION

Some raisin samples appeared to contain a relative high level of aromatic β-carbolines as suggested by HPLC-fluorescence detection (**Figure 1**). After sample preparation by solid phase and solvent extraction, identification was performed by HPLC-ESI, confirming the presence of β-carbolines. Two main compounds within the extracts were identified by co-injection with authentic standards, UV–vis spectra, and HPLC-mass spectrometry (electrospray) as the pyridoindoles norharman (*m/z* 169 $(M + H)^+$, UV λ_{max} 248, 302, and 370 nm) and harman (*m/z* 183 $(M + H)^+$, λ_{max} at 248, 302, and 365 nm) (**Figure 2**). A number of different samples of raisins were subsequently analyzed (**Table 1**). Harman was the major β-carboline within the samples, although its concentration showed a large variation between raisins, ranging on average from 5.5 to 643.5 ng/g. Norharman ranged from 2.1 to 120 ng/g. Raisins containing the highest level of β-carbolines (norharman and harman) generally belonged to dark-brown raisins, which were prepared by a traditional sun-drying process. In contrast, golden raisins contained much lower levels of β-carbolines within the samples analyzed (less than 35.6 ng/g, as the sum of both β-carbolines).

It has been previously reported that norharman and harman in foods arise from the oxidation of the corresponding tetrahydro-β-carboline-3-carboxylic acid (*32*, *37*–*39*). Raisins were analyzed for the presence of these precursors, and they contained high levels of them, as proven by chromatographic and mass spectrometric analysis (i.e., *m*/z 217 $(M + H)^+$ for 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and *m/z* 231 $(M + H)^+$ for 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid) (**Figure 3** and **Table 2**). The main tetrahydro-β-carboline was 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, which appeared as two diastereoisomers (1*S*,3*S* and 1*R*,3*S*) with the same mass spectra and ranged from 1.88 to 49.6 μg/g, as the sum of both isomers (**Table 2**). This pattern agrees well with the highest presence of harman in raisins, since 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid is a direct precursor of harman. Tetrahydro-β-carboline-3-carboxylic acids and, particularly, 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid appeared in higher proportion in dark-brown raisins than in golden raisins, and in general high levels of these precursors in raisins were usually accompanied with higher amount of aromatic β-carbolines, as well.

As seen in **Table 1**, raisins contained β-carbolines, and these alkaloids have been previously reported to be inhibitors of monoamine oxidase (MAO) (*15*, *18*, *36*). The inhibitory effects

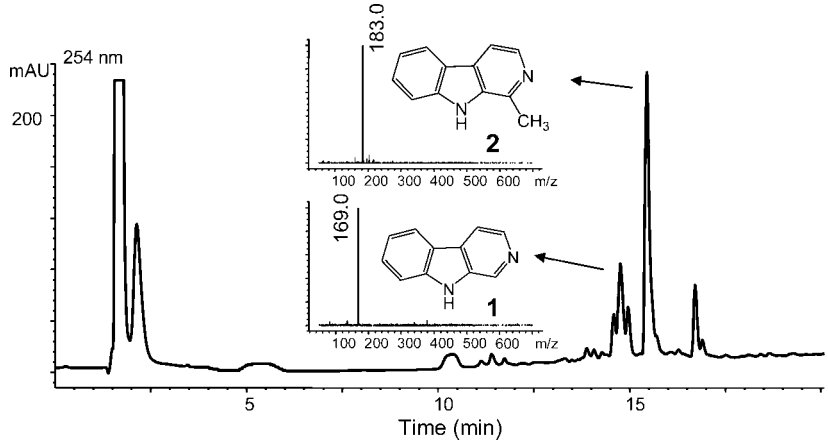

Figure 2. RP-HPLC (DAD)-MS (ESI) of aromatic β-carbolines isolated by PRS and solvent extraction from dark-brown (sun-dried) raisins with the structures and mass spectra of identified norharman (**1**) and harman (**2**).

Table 1. Aromatic β-Carbolines Norharman and Harman in Raisins

raisin samples		X ± SD	
	N	norharman (ng/g)	harman (ng/g)
dark-brown[a]			
1	4	120 ± 48	491.7 ± 123
2	2	76.4 ± 16.5	643.5 ± 45
3	2	22.0 ± 9.6	207.4 ± 41
4	2	30.1 ± 9.7	121 ± 4.1
5	2	38.2 ± 2.2	99.15 ± 39.2
6	1	62.6	291.3
7	1	16.0	194.5
8	1	59	193.7
9	1	24.1	33.4
golden[b]			
10	2	2.1 ± 2.9	5.5 ± 3.7
11	2	10.6 ± 3.5	25.0 ± 8

[a] Brown to dark-brown or black raisins. [b] Yellow and golden raisins.

of raisin extracts on MAO activity, measured as kynuramine deamination, were then investigated. Raisins containing a relative high level of β-carbolines were homogenized, and their extracts were tested for MAO inhibition (**Figure 4**). MAO-A isozyme was inhibited by raisin extracts and homogenates. A similar degree of inhibition was observed both with direct homogenates and with PRS extracts containing the isolated β-carbolines, suggesting that the latter compounds could be remarkable contributors to this inhibition. Raisin extracts inhibited MAO-B to a much lower degree than MAO-A (**Figure 4**). Inhibition of MAO was reversible since enzyme activity was recovered following incubation of MAO-A with extracts compared with controls under the same conditions (100% and 70% recovered for acidic and PRS extracts, respectively).

Raisin extracts inhibiting MAO should contain specific compounds acting as MAO inhibitors. Results in **Figure 4** suggest that a high proportion of MAO inhibition mainly arises from compounds extracted on PRS solid phase extraction such as β-carbolines or others. To further isolate these compounds, β-carbolines (norharman and harman) present in PRS extracts from MAO-inhibiting raisins were isolated following successive HPLC injections by collecting their chromatographic peaks and subsequent solvent extraction, and the purified compounds were used to study inhibition kinetics under varying concentration of kynuramine (Michaelis–Menten curves). Inhibition of MAO-A by harman isolated from raisins exhibited a competitive type of inhibition as illustrated with double reciprocal curves (**Figure 5**). It provided a 39.4 ± 4% and 50.5 ± 3% inhibition over MAO-A (kynuramine 250 μM) with 0.25 and 0.38 μM, respectively, of calculated concentrations of harman isolated from raisins that were introduced into assays. On the other hand, norharman isolated from raisins inhibited MAO-B, giving a 20% and 36% inhibition with 2 and 4 μM, respectively, of calculated concentration into the assays. These values of inhibition afforded by β-carbolines isolated from raisins are in general agreement with previous results obtained for these compounds arising from other sources and also with their IC_{50} values (*15, 18*). Finally, when the calculated amount of harman in raisin homogenates and extracts and their corresponding MAO-A inhibition (**Figure 4**) were considered and subsequently compared with the inhibition degree provided by isolated and purified harman, it appeared that a high percent of inhibition in raisin homogenates and extracts (ca. 50–80%) belonged to this β-carboline. β-Carbolines are reversible inhibitors of MAO (*15*), which agrees well with the type of inhibition observed from raisin homogenates containing these compounds.

The above results show the occurrence of aromatic β-carbolines and tetrahydro-β-carboline-3-carboxylic acids in raisins. Two β-carbolines, norharman and harman, were identified and analyzed in raisins, with harman being the major compound. By comparison with other foodstuffs (*17*), the amount of β-carbolines in some raisins was relatively high, although it was also highly variable among raisins, suggesting that processing conditions and technological factors may affect the concentration of these compounds to a high degree. Raisins also contained a high level of tetrahydro-β-carboline-3-carboxylic acids, which are the direct precursors of aromatic β-carbolines (*37*). In general, raisins with a high level of tetrahydro-β-carboline-3-carboxylic acids, particularly 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, also exhibited a high level of aromatic β-carbolines, particularly harman. This is in agreement with previous results reporting the oxidation of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid to give harman and norharman, respectively. This oxidative conversion is accelerated by heating and in presence of oxidants and free radicals (*32, 37, 39, 40*).

Several technological factors may influence the content of these compounds in raisins. Among them are grape variety, drying process (i.e., sun-drying or mechanical dehydratation),

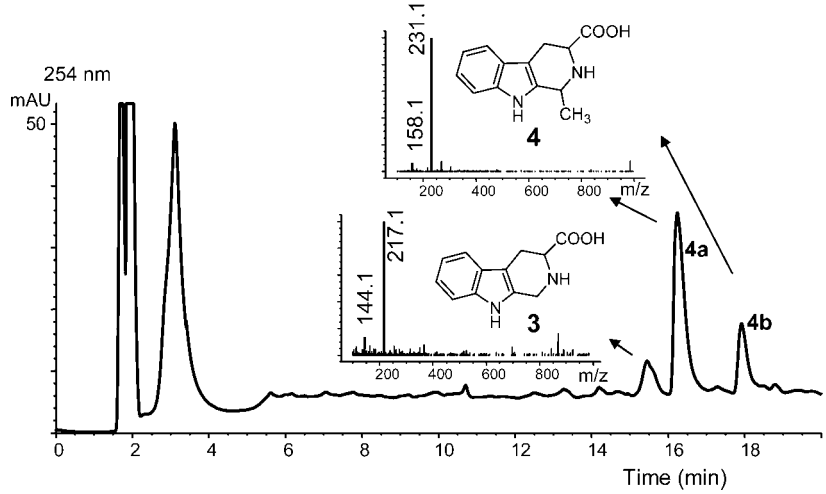

Figure 3. RP-HPLC (DAD)-MS (ESI) of tetrahydro-β-carboline-3-carboxylic acid isolated by SCX SPE from dark-brown (sun-dried) raisins with the structures and mass spectra of identified 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**3**) and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**4**) as two diastereoisomers, 1*S*,3*S* (**4a**) and 1*R*,3*S* (**4b**).

Table 2. Tetrahydro-β-carboline-3-carboxylic Acids (μg/g) Determined in Raisins[a]

raisin samples		$X \pm$ SD		
	N	**3**	**4a**	**4b**
dark-brown				
1	3	2.4 ± 0.2	20.8 ± 4.8	7.1 ± 1.3
2	2	0.5 ± 0.13	36.9 ± 3.2	12.7 ± 1.6
3	2	0.44 ± 0.4	3.8 ± 0.17	1.17 ± 0.01
4	2	0.68 ± 0.14	11.3 ± 2.5	4.0 ± 0.77
5	1	0.91	10.1	3.8
6	1	1.18	15.1	4.29
7	1	0.70	9.6	2.67
8	1	0.41	10.2	3.64
9	1	0.26	4.2	1.54
golden				
10	2	0.11 ± 0.04	1.3 ± 0.5	0.58 ± 0.33
11	2	0.19 ± 0.10	1.5 ± 0.7	0.57 ± 0.29

[a] Raisins and samples are as in **Table 1**. Compound numbers: 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**3**); (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**4a**); (1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic (**4b**).

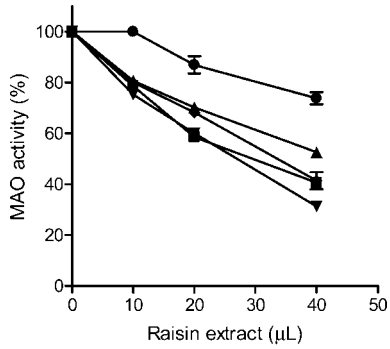

Figure 4. Inhibition (%) of monoamine oxidase (MAO) enzyme activity by homogenates and extracts from dark-brown (sun-dried) raisins. MAO-A assays: neutralized $HClO_4$ homogenate (sample 1, ♦; sample 2, ■), phosphate buffer (pH 7)/10% DMSO homogenates (▼), and extracts from PRS extraction corresponding to combined eluting fractions of phosphate buffer (3 mL) plus phosphate buffer/methanol 1:1 (3 mL) (▲). MAO-B assays: neutralized $HClO_4$ homogenate (●). Convenient blanks without raisin extract were used as control for absence of inhibition. Results are average of duplicate determinations.

sulfur dioxide, drying time, temperature, and moisture. Raisins are made by dehydrating grapes following a drying process that reduces moisture to roughly 15%, while increasing sugar. Dark-brown raisins are generally produced by allowing grapes to naturally sun-dry on paper trays for 2 or 3 weeks, whereas golden raisins (yellow color) are obtained by treating the grapes with sulfur dioxide and usually drying by mechanical dehydration processes (oven-drying), taking several hours. During the grape-drying process, tryptophan may slowly react in acidic conditions with released aldehydes, mainly acetaldehyde, producing tetrahydro-β-carboline-3-carboxylic acid alkaloids (**Figure 6**). In fact, a higher amount of these compounds are found in raisins (**Table 2**) than previously found in grapes (*41*). The same reaction occurs during alcoholic fermentation of grapes and in other foods, including fruits and juices (*33*, *34*, *41*, *42*). It is expected that this initial reaction will be favored in sun-dried raisins. Tetrahydro-β-carboline-3-carboxylic acids could be subsequently oxidized to afford β-carbolines (**Figure 6**). In this regard, the drying process from grapes to raisins, which involves relative high temperatures and time, at least in sun-dried raisins, might favor the formation of β-carbolines from the corresponding tetrahydro-β-carboline-3-carboxylic acid.

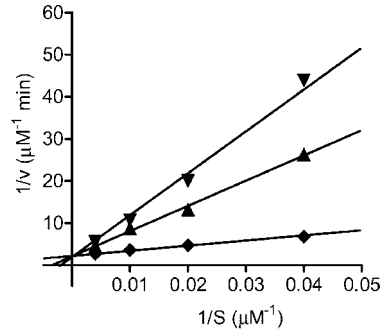

Figure 5. Lineweaver–Burk plot of MAO-A activity and inhibition with harman isolated from dark-brown raisins (sun-dried). Calculated concentrations of harman from raisins introduced into the assays: 0 (♦), 0.25 (▲), and 0.38 μM (▼). MAO activity was determined as the concentration of 4-hydroxyquinoline (μM/min) produced from kynuramine. Results are the average of duplicate determinations.

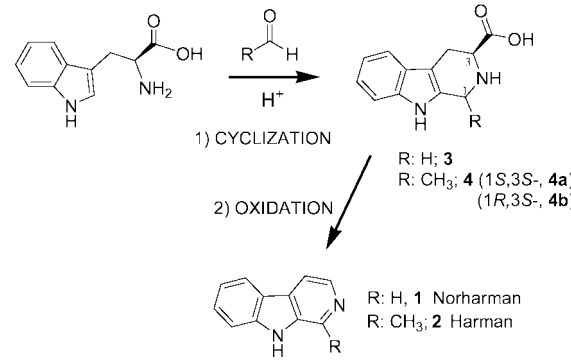

Figure 6. Scheme proposed for the formation of tetrahydro-β-carboline-3-carboxylic acids (**3** and **4**) from tryptophan during the drying of grapes to produce raisins and their subsequent oxidation to give the fully aromatic β-carbolines **1** and **2**, which act as MAO inhibitors.

Dark-brown raisins and raisins obtained by sun-drying processes should contain much higher levels of aromatic β-carbolines and tetrahydro-β-carboline-3-carboxylic acids compared to golden raisins or raisins dried following technological dehydration processes and also treated with sulfur dioxide. Sulfur dioxide may also reduce the level of tetrahydro-β-carboline-3-carboxylic acids (*41*). The results obtained here follow this pattern.

β-Carbolines are pyridoindole alkaloids that occur and accumulate in biological tissues and fluids (*25*, *29*, *30*, *43*–*45*) and that are also environmental and/or dietary xenobiotics (*17*, *32*, *35*, *38*, *46*, *47*). As tetrahydro-β-carbolines and β-carbolines occur in many foods and drinks, their absorption may contribute to their physiological occurrence in the body where they can become bioactive compounds (*8*, *15*, *20*–*23*, *25*, *26*, *35*, *48*). Some of the biological effects attributed to β-carbolines could be produced by interaction with MAO enzymes. The primary role of MAO isozymes lies in the metabolism of amines, regulating neurotransmitter levels and intracellular amine stores. In the gastrointestinal tract, circulatory system, and the liver, MAO may serve a protective function by regulating the levels of dietary amines exerting vasopressor effects. These biological implications are of pharmacological interest (*2*, *4*, *49*), and MAO-A inhibitors are useful as antidepressants, although their current use is somehow restricted because they may produce hypertensive crisis when the patients consume tyramine-containing foods (the so-called "cheese effect") (*10*, *49*). The oxidation of biogenic amines by MAO results in the production of potentially toxic hydrogen peroxide, ammonia, and aldehydes that represent risk factors for cell oxidative injury (*6*). MAO

also bioactivates toxic xenobiotic amines such as MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) (*7*, *8*). The use of MAO-inhibiting substances may result in biological protection against toxicants and oxidative stress (*5*, *50*). Alternatively, peripheral inhibition of MAO could eventually affect the metabolism of exogenous and dietary amines such as tyramine or tryptamine, potentiating their vasopressor effects (*4*).

Results obtained in this research have shown that homogenates and extracts of raisins inhibited MAO, suggesting that raisins might somehow affect this enzyme. MAO inhibition was observed in vitro, and therefore further studies are required in this regard. However, results clearly show that some raisins contained MAO inhibitors. Two β-carboline alkaloids (i.e., norharman and harman) were isolated from MAO-inhibiting raisins and accounted for a high percentage of this inhibition. Furthermore, raisins also contained high levels of tetrahydro-β-carboline-3-carboxylic acids, which are immediate precursors of MAO-inhibiting β-carbolines. Results in **Table 1** suggest that some raisins could be a dietary source of exposure to these alkaloids in addition to others already known (*17*, *35*). As mentioned above, aromatic β-carbolines are absorbed and distributed in tissues including the brain, in which they may accumulate, reaching higher local concentrations than plasma (*23*, *28*, *30*, *45*, *51*). Endogenous β-carbolines might locally affect the metabolism of exogenous amines and neuroamines and exert potential actions on serotonin, opioid, and imidazoline receptors. Alternatively, a very different perspective currently focuses on β-carbolines as potential endogenous and/or environmental proneurotoxins (*8*, *9*, *45*, *52*, *53*). Further studies are needed to fully delineate the implications of dietary, environmental and endogenous β-carbolines in human health.

In conclusion, raisin extracts showed in vitro inhibition of monoamine oxidase (MAO) activity under a competitive and reversible type of inhibition, suggesting that raisins contain MAO inhibitors. Two aromatic β-carbolines (i.e., norharman and harman) were quantified and isolated from raisins, and they were accounted to be the main contributors to MAO inhibition observed in raisin extracts. Raisins also contained tetrahydro-β-carboline-3-carboxylic acids that are immediate precursors of MAO-inhibiting aromatic β-carbolines. β-Carboline alkaloids occurring in raisins are bioactive compounds that might inhibit MAO and/or also exert other biological activities.

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