JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Identification and Occurrence of the Novel Alkaloid Pentahydroxypentyl-tetrahydro-β-carboline-3-carboxylic Acid as a Tryptophan Glycoconjugate in Fruit Juices and Jams

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The novel carbohydrate-derived β-carboline, 1-pentahydroxypentyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, was identified in fruit- and vegetable-derived products such as juices, jams, and tomato sauces. This compound occurred as two diastereoisomers, a cis isomer (the major compound) and a trans isomer, ranging from undetectable amounts to 6.5 μg/g. Grape, tomato, pineapple, and tropical juices exhibited the highest amount of this alkaloid (up to 3.8 mg/L), whereas apple, banana, and peach juices showed very low or nondetectable levels. This tetrahydro-β-carboline was also found in jams (up to 0.45 μg/g), and a relative high amount was present in tomato concentrate (6.5 μg/g) and sauce (up to 1.8 μg/g). This β-carboline occurred in fruit-derived products as a glycoconjugate from a chemical condensation of D-glucose and L-tryptophan that is highly favored at low pH values and high temperature. Production, processing treatments, and storage of fruit juices and jams can then release this β-carboline. Fruit-derived products and other foods containing this compound might be an exogenous dietary source of this glucose-derived tetrahydro-β-carboline.

KEYWORDS: Tetrahydro-β-carbolines; tetrahydro-β-carboline-3-carboxylic acid; alkaloids; tryptophan; glucose; fruit juices; jams; sauces; Maillard reaction

INTRODUCTION

1,2,3,4-Tetrahydro-β-carbolines are naturally occurring tricyclic indole derivatives produced from indole-ethylamines and aldehydes or α-ketoacids through Pictet−Spengler condensation (*1*). Similarly, 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids arise from a condensation between L-tryptophan and aldehydes. This latter reaction readily occurs in foods and is temperature- and pH-dependent (*2*).

Research in the last two decades has pointed out the occurrence of tetrahydro-β-carbolines and β-carbolines in biological tissues and fluids (*3*−*7*). Those compounds might function as neuromodulators owing to their actions on serotonin uptake, the benzodiazepine receptor, and monoamine oxidase. They have also been increasingly studied in relation to alcoholism (*6*, *8*). Collins and co-workers have reported that *N*-methylated carbolines may act as endogenous neurotoxins (*9*, *10*). 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, the corresponding tryptophan−acetaldehyde condensation product, is a precursor of mutagens upon nitrosation (*11*), and may affect neuronal cell survival in vitro (*12*). However, a full delineation of the biological activity of this family of compounds is still desirable to assign a particular biological effect, if any, to each specific compound.

In the past few years, we have reported the presence of tetrahydro-β-carbolines in fruit and fruit-derived products such as juices and jams (*13*−*16*). These compounds are naturally occurring substances readily produced during fruit production, processing, and storage. Tetrahydro-β-carbolines have been also found in many other foodstuffs (*17*−*20*). Then, dietary sources provide tetrahydro-β-carbolines that may subsequently accumulate in biological tissues and fluids.

In the last two years, the possible formation of tryptophan-derived glycoconjugates, and particularly carbohydrate-derived β-carbolines, in foods following a reaction of tryptophan and carbohydrates has become evident (*21*−*23*). Thus, Herderich and co-workers (*21*) characterized polyol tetrahydro-β-carboline-3-carboxylic acids and studied *N*- and *C*-tryptophan glycoconjugates in human urine, whereas Rönner et al. (*22*) have showed the formation of these compounds in model reactions of tryptophan and glucose. However, so far, the determination and specific identification of these novel polyol tetrahydro-β-carbolines in foods remained to be accomplished. The presence of the carbohydrate-derived β-carboline, 1-pentahydroxypentyl-tetrahydro-β-carboline-3-carboxylic acid as two diastereoisomers, in fruit-derived products, and its quantitative determination in fruit juices and jams has now been established in this study. In addition, the possible chemical formation of this compound is evaluated in terms of the influence of pH and temperature.

MATERIALS AND METHODS

Reference Compounds and Samples. The synthesis of carbohydrate-derived tetrahydro-β-carbolines was achieved by a Pictet−Spengler type reaction. 1-(1,2,3,4,5-Pentahydroxypent-1-yl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid was synthesized from L-tryptophan and D-glucose according to reference *22*. Briefly, L-tryptophanate sodium

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10.1021/jf020090m CCC: $22.00
Published on Web 06/27/2002

Table 1. Concentration (mg/L) of 1,2,3,4,5-Pentahydroxypentyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid Alkaloids (cis and trans isomers) in Commercial Fruit and Vegetable Juices[a]

		trans isomer			cis isomer		
juice	n	X	SD	range	X	SD	range
orange	12	0.037	0.012	0.02–0.063	0.16	0.055	0.1–0.28
pineapple	7	0.17	0.2	0.0–0.48	0.60	0.67	0.036–1.7
grape	3	0.59	0.23	0.43–0.85	2.18	0.76	1.5–3.0
pineapple + grape	3	0.22	0.055	0.16–0.27	0.71	0.16	0.57–0.89
peach nectar	2	-	-	-	-	-	-
peach + grape	4	0.21	0.09	0.11–0.31	0.75	0.3	0.39–1.12
tomato	7	0.24	0.14	0.06–0.43	0.99	0.57	0.27–1.89
banana nectar	2	-	-	-	-	-	-
apple	3	-			-		
tropical	3	0.2	0.15	0.05–0.35	0.64	0.43	0.20–1.07
vegetable	1	0.04	-	-	0.25	-	-
multifruit	2	0.08	0.05	0.045–0.11	0.30	0.15	0.19–0.40

[a] Most of the juices were from concentrate, but some from orange, pineapple, and tomato were from direct fruit (not from concentrate) and others were refrigerated juices. Not detectable or below detection limit (–).

salt was formed from L-tryptophan (1.5 mmol) and NaOH (1.5 mmol) in methanol, and evaporated to dryness. After addition of D-glucose (7.5 mmol) in methanol the reaction was kept under reflux for 2.5 h. The solution was concentrated under vacuum to ca. 10 mL, added dropwise to methanolic 2 N HCl (10 mL) at 0−5 °C, and stirred for 1 h. The mixture was concentrated to dryness. Isolation of glucose-derived tetrahydro-β-carboline was first carried out on a Dowex resin 50W × 8 (15 g) (Fluka) placed on a sintered disk filter funnel, which was eluted with 0.2 M ammonia and 0.2 M ammonia/acetonitrile (1:1) solutions until no further product was detectable by HPLC, and subsequently dried under vacuum. After redissolving in water, a further isolation and purification of the compounds was carried out on a 50 mm × 20 mm i.d. chromatographic column packed with C-18 sorbent by eluting with an increasing gradient of acetonitrile. The fractions containing two compounds, corresponding to the major cis and minor trans diastereoisomers, with the same fluorescence and UV spectra by RPHPLC, were dried under vacuum to give glucose-derived tetrahydro-β-carboline-3-carboxylic acid. The major compound (cis isomer), (1*R*,3*S*)-1-(1,2,3,4,5-pentahydroxypentyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, was characterized by ^{1}H- and ^{13}C-NMR (Varian XL-400 spectrometer) giving signals in agreement with those of previous results (*21*, *22*). ^{1}H NMR (CD_3OD/TFA): δ 3.34 (m, 1H, H-4a), 3.57 (dd, 1H, H-4b), 3.96 (dd, 1H H-4′), 3.96−4.07 (m, 2H, H-5′), 4.13 (d, 1H, H-3′), 4.48 (m, 1H, H-2′), 4.52 (dd, 1H, *J* (3,4a) = 5 Hz, *J* (3,4b) = 12 Hz, H-3), 4.82 (m, 1H, *J* (1′,1) = 1.7 Hz, *J* (1′, 2′) = 4 Hz, H-1′), 5.24 (s, 1H, H-1), 7.25 (t, 1H, *J* = 8 Hz, H-6), 7.35 (t, 1H, *J* = 8 Hz, H-7), 7.56 (d, 1H, *J* = 8 Hz, H-8) 7.66 (d, 1H, *J* = 8 Hz, H-5). ^{13}C NMR (CD_3OD/TFA): δ 22.8 (C-4), 56.9 (C-3), 57.6 (C-1), 64.5 (C-5′), 71.4 (C-1′), 71.9 (C-2′), 72.2 (C-3′), 72.8 (C-4′), 108.6 (C-4a), 112.5 (C-8), 118.9 (C-5), 120.7 (C-6), 123.6 (C-7), 127.4 (C-4b), 128.6 (C-9a), 138.6 (C-8a), 171 (COOH). MS (ESI-positive mode) $[M + H]^+$ *m*/*z* 367; MS (ESI-negative mode) $[M - H]^-$ *m*/*z* 365, 303, 215. Analysis by RP(C18)-HPLC−MS (ESI-positive mode) (see below for chromatographic conditions) confirmed that pentahydroxypentyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid occurs as two diastereoisomers with the same mass spectra eluting at 13.5 min (minor compound) and 15.8 min (major compound cis isomer). MS (fragmentation voltage 50 V): *m*/*z* 367 $[M + H]^+$, and MS (fragmentation voltage 120 V): *m*/*z* 247 $[M + H - C_4H_8O_4]^+$ (100), 230 (96), 367 $[M + H]^+$ (82), 350 (49), 276 (48), 332 (45), and 294 (15). Alternatively, *cis*-(1*R*,3*S*)-1-(1,2-dihydroxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid was synthesized by condensation of L-tryptophan and D-glyceraldehyde in 0.05 N sulfuric acid providing a solid that was filtered off, and its structure was confirmed by NMR and MS: *m*/*z* 277 $[M + H]^+$, 204 $[M + H\text{-}73]^+$. This compound was chosen as an internal standard (IS) for quantitative purposes owing to its structure and good chromatographic properties, and because it afforded recovery percentages similar to those of pentahydroxypentyl tetrahydro-β-carboline-3-carboxylic acid during the isolation procedure.

Different commercial samples of packed fruit and vegetable juices, in both bottles and cartons, including juices made from concentrate (not refrigerated), nectars and squeezed (fresh and refrigerated, not from concentrate) fruit juices, and also commercial jams, tomato sauces, and ketchup (**Tables 1** and **2**), were purchased in local supermarkets and used for analysis of pentahydroxypentyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid.

Table 2. Concentration (μg/g) of 1,2,3,4,5-Pentahydroxypentyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid Alkaloids (cis and trans isomers) in Commercial Jams and Tomato Sauces

		trans isomer	cis isomer
	n	*X*	*X*
strawberry	3	0.08 ± 0.014	0.31 ± 0.02
pineapple	1	0.089	0.19
apricot	1	0.044	0.168
prune	1	0.079	0.37
peach	2	-[a]	-
bitter orange	2	0.019 ± 0.01	0.115 ± 0.002
raspberry	1	0.07	0.21
tomato sauce/ketchup	4	0.35 ± 0.16	1.21 ± 0.62
tomato concentrate	1	1.29	5.19

[a] Not detectable or below detection limit (–).

Isolation of Pentahydroxypentyl-tetrahydro-β-carboline-3-carboxylic Acid. Pentahydroxypentyl-tetrahydro-β-carboline-3-carboxylic acid was isolated using SCX-solid-phase extraction procedures as follows for the differenct food products. (A) Fruit juice (20 mL) was centrifuged (15000*g*, 0 °C) for 10−15 min. An aliquot of supernatant (5 mL) was spiked with 1 mL of *cis*-(1*R*,3*S*)-1-(1,2-dihydroxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid solution (5 mg/L) used as internal standard (IS), 0.5 mL of semicarbazide (Sigma) solution 10 mg/mL, and 1 mL of 0.1 M HCl, and slowly passed through Bond Elut 500 mg/3 mL benzenesulfonic acid SCX columns (Varian, Harbor City, CA) using a vacuum manifold. (B) Jams, concentrates, and sauces (5 g) were homogenized using an UltraTurrax homogenizer with 10 mL of 0.6 M $HClO_4$ containing 1 mg/mL semicarbazide and centrifuged (15000*g*, 10−15 min, 0−5 °C). An aliquot of supernatant (5.5 mL), was added with 1 mL of 1-(1,2-dihydroxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid solution (5 mg/L) and 1 mL of HCl 0.1 M, and loaded onto an SCX column. SCX columns were washed with 6 mL of 0.6 M HCl, 2 mL of methanol, and 6 mL of Milli-Q purified water. Then, elution of pentahydroxypentyl tetrahydro-β-carbolines was carried out with 4 mL of 0.4 M phosphate buffer pH 9.2, and injected onto RPHPLC.

Chromatographic Analysis. Chromatographic analysis of pentahydroxypentyl-tetrahydro-β-carboline was performed by RPHPLC and fluorescence detection. A 150 mm × 3.9 mm, 5 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), gradient programmed from 0% (100% A) to 5% B in 16 min. The

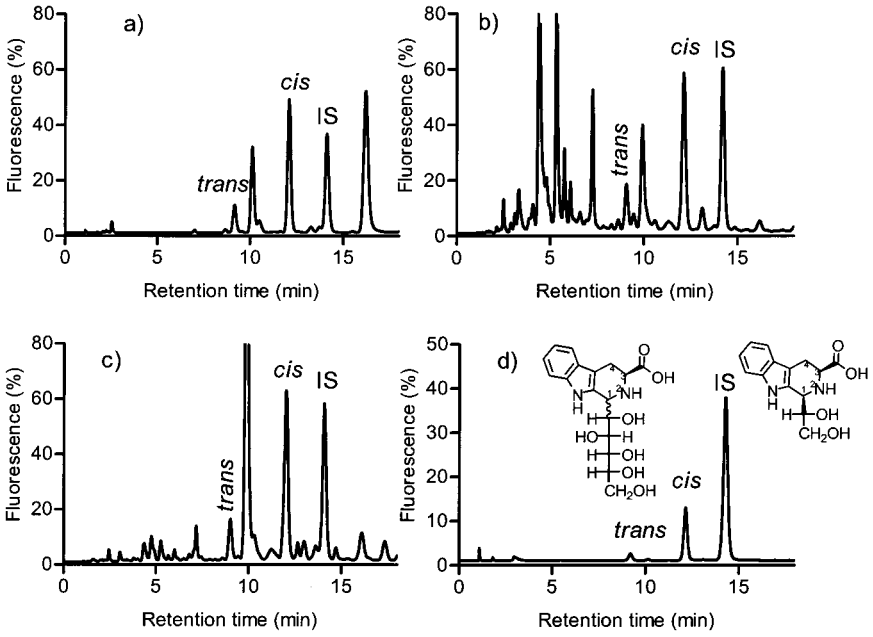

Figure 1. RPHPLC chromatograms of fruit juices: grape juice (a), pineapple juice (b), and tomato juice (c), and pentahydroxypentyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid reference compounds (cis and trans isomers) (d). IS: *cis*-1-(1,2-dihydroxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid.

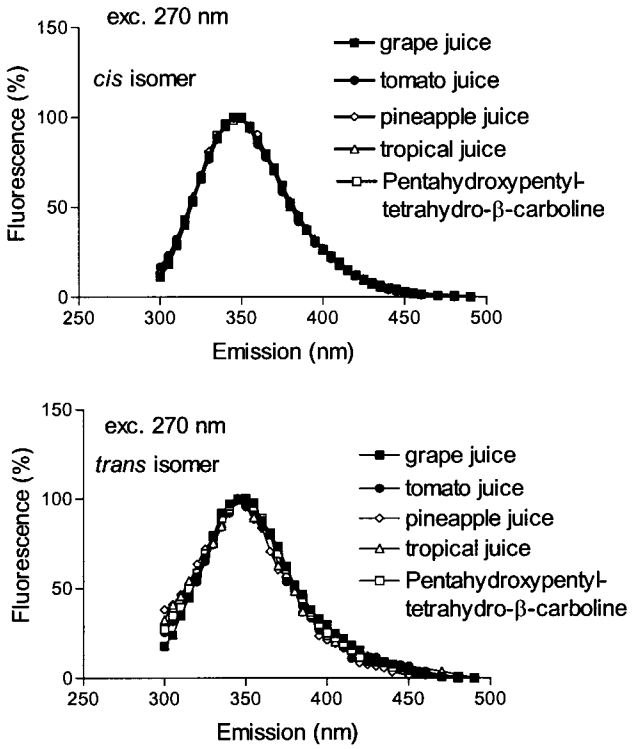

Figure 2. Emission fluorescence (excitation at 270 nm) of pentahydroxypentyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (cis and trans isomers) in fruit juices and standard. Fluorescence data are normalized to 100%.

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 constructed from known standard solutions of pentahydroxypentyl-tetrahydro-β-carboline-3-carboxylic acid reference compound (from 0.05 to 2 mg/L), spiked with dihydroxyethyl-tetrahydro-β-carboline-3-carboxylic acid as IS, and analyzed through the entire procedure as above. The same response factor was used for the two isomers. The recovery of the compound averaged 92% (n = 4, grape juice), whereas the reproducibility was RSD 1.8% (n = 4, grape juice). The detection limit was below 0.01 mg/L. Blanks and control samples (100 mg/L L-tryptophan, 100 mg/L glucose) did not give artifacts during isolation and analysis. Confirmation of the identity of isolated pentahydroxypentyl tetrahydro-β-carboline was established by HPLC retention times, fluorescence spectra, and coelution with authentic standards. Thus, fluorescence spectra of the HPLC peaks were compared with those of reference compounds. For this, eluting peaks corresponding to pentahydroxypentyl tetrahydro-β-carboline in real samples and standards were trapped into the flow cell of the fluorescence detector by stopping the solvent pump, and excitation and emission spectra were monitored. In addition, several samples of juices and tomato concentrate were subjected to HPLC−MS as reported below.

Chemical Identification by HPLC−MS. Samples of fruit juices and tomato concentrate were subjected to SCX-extraction as described above. The eluting fractions corresponding to phosphate buffer pH 9.2 were concentrated under a nitrogen stream and analyzed by 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 consisted of A, formic acid (0.5%); and B, 0.5% formic acid in acetonitrile. The gradient was 0% B to 12.5% B in 25 min. Flow was 0.7 mL/min; cone voltage was 50 or 120 V for higher fragmentation; capillary voltage was 4000 V; drying gas temperature was 350 °C; gas flow was 10 L/min; and the mass range was 50−1000 amu.

Chemical Factors Affecting the Formation of Pentahydroxypentyl Tetrahydro-β-Carboline. According to previous results, reaction to give tetrahydro-β-carbolines is highly affected by chemical factors such as pH and temperature (*2*). To evaluate this effect on the possible formation of glucose-derived tetrahydro-β-carbolines, several test tubes containing L-tryptophan (0.5 g/L) and D-glucose (1 g/L) were incubated in duplicate at different pH values (pH 1 from 0.8% v/v HCl conc + 2.2 g/L NaCl, and 50 mM phosphate buffer solutions of pH values 3, 5, 7, and 9), and temperatures (25, 37, and 70 °C) while following the formation of this carboline by RPHPLC. In addition, whole grapes were crushed in the laboratory and a freshly prepared grape juice was submitted to heating (refluxing for up to 8 h), and subsequently analyzed for pentahydroxypentyl-tetrahydro-β-carboline-3-carboxylic acid.

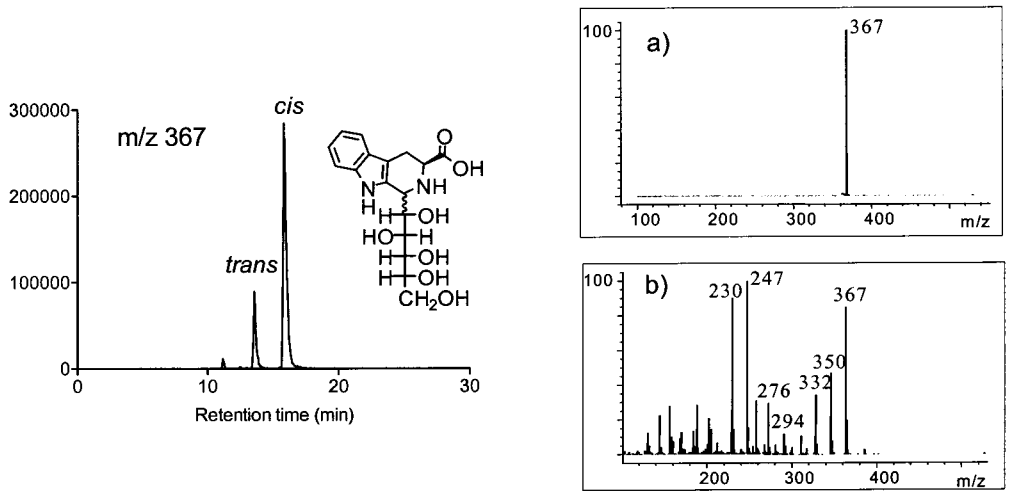

Figure 3. HPLC–MS analysis (electrospray-positive mode) and mass spectra of pentahydroxypentyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid. Fragmentation voltage: 50 V (a) and 120 V (b).

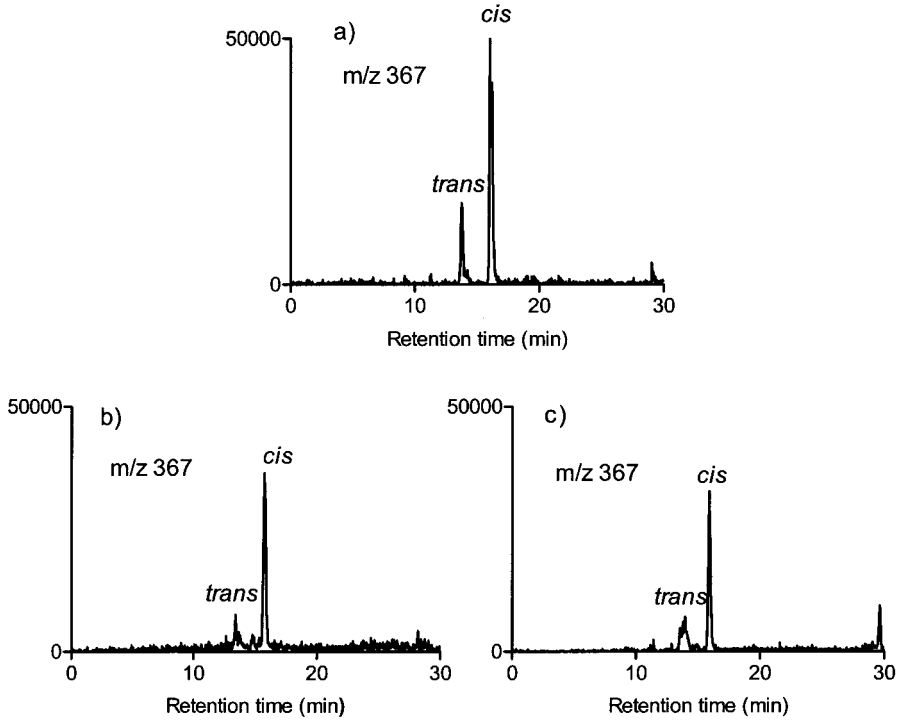

Figure 4. HPLC–MS analysis and reconstructed ion chromatogram at *m/z* 367 $[M + H]^+$ of grape juice (a), pineapple juice (b), and tomato juice (c).

RESULTS AND DISCUSSION

In previous studies we have reported the chemical identification and quantitative analysis of tetrahydro-β-carbolines in fruits and fruit-derived products such as juices and jams (*14*, *15*). During that research we had detected possible unknown tetrahydro-β-carbolines in fruit-derived samples and we have now tried to isolate and identify these possible new compounds. Thus, SCX-extracted fruit-derived products (i.e., juices, jams, tomato sauces, and concentrates) gave chromatographic peaks coeluting with authentic synthetic standards of pentahydroxypentyl-tetrahydro-β-carboline-3-carboxylic acid (cis and trans diastereoisomers) (**Figure 1**). Those HPLC peaks coeluting with standards of this carboline were trapped into the flow cell of the fluorescence detector, and the fluorescence spectra were monitored. Excitation and emission profiles from diastereoisomeric peaks detected in fruit-derived products were very consistent with those from authentic standards exhibiting an emission maximum around 345 nm when excited at 270 nm (**Figure 2**). A further unequivocal proof of the existence of this compound in those products was obtained by HPLC−MS (**Figure 3**). Extracted samples of fruit and vegetable juices and tomato concentrate gave mass spectra corresponding to pentahydroxypentyl tetrahydro-β-carboline-3-carboxylic acid (**Figure 4**). The mass spectra showed the presence of two isomers of this carboline with protonated molecular ions $[M + H]^+$ at *m/z* 367 that coeluted with the corresponding reference standards under the same conditions. As occurs for other 1,3-disubstituted tetrahydro-β-carbolines detected in fruit juices (*14*), the novel polyol tetrahydro-β-carboline appeared as two diastereoisomers corresponding to 1*R*,3*S* (cis) and 1*S*,3*S* (trans) configurations (**Figure 5**). The trans isomer (1*S*,3*S*) eluted as the first peak in RPHPLC and the cis isomer (1*R*,3*S*) eluted as the second peak (*21*). The cis isomer (second chromatographic peak) was the major compound in fruit- and vegetable-derived samples, and the ratio cis/trans generally ranged from 3 to 4.5.

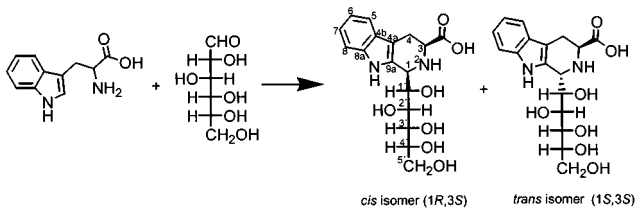

Figure 5. Pictet–Spengler type condensation of L-tryptophan and D-glucose to give pentahydroxypentyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid as two diastereoisomers (cis and trans configuration).

The occurrence of these carbohydrate-derived tetrahydro-β-carbolines (trans and cis diastereoisomers) in juices is summarized in **Table 1**. Its content varied both between individual juices and even more among groups of juices. Grape juices or mixed fruit juices containing concentrate grape juice exhibited the highest level of pentahydroxypentyl-tetrahydro-β-carboline-3-carboxylic acid (up to 3.8 mg/L). Relatively high concentrations of this carboline were also found in tomato and pineapple, as well as in multifruit and tropical fruit juices. In contrast, it was not detected in apple juices or banana and peach nectars, whereas a low amount was detected in orange juices. Most of the fruit juices analyzed here were made from concentrate juice, so that this polyol-tetrahydro-β-carboline might have formed during the processing conditions carried out for juice concentration (i.e., relatively high temperature and concentration of reactants). However, a few commercial juices marked as fresh and refrigerated, and made from direct squeezing of oranges, pineapples and tomatoes, also seemed to contain this compound, although in lower amounts. As shown in **Table 2**, the pentahydroxypentyl tetrahydro-β-carboline was also found in commercial jams and tomato sauces. Most of the jams analyzed, with the exception of those from peach, seemed to contain a low amount of this tetrahydro-β-carboline reaching up to 0.45 μg/g. Tomato concentrates, sauces, and ketchup contained even more of this compound, reaching up to 6.5 μg/g. This polyol-tetrahydro-β-carboline might have formed during the processing conditions carried out for tomato juice concentration and sauce production.

This alkaloid comes from a condensation reaction involving L-tryptophan and D-glucose following by cyclization to give the tetrahydro-β-carboline **(Figure 5)**. As shown in **Figure 6**, the chemical formation of this glucose-derived tetrahydro-β-carboline increased exponentially at low pH and high temperature. This behavior agrees with previous results for other tetrahydro-β-carbolines such as 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (*2*). No reaction to give the polyol tetrahydro-β-carboline took place at the highest pH values (7 and 9) even at high temperature, whereas the formation occurred very slowly at low temperature even in very acidic pH. Pentahydroxypentyl-tetrahydro-β-carboline-3-carboxylic acid needed much more extreme conditions to form readily (i.e., low pH and high temperature) in comparison with the relatively mild conditions needed for formation of other tetrahydro-β-carboline-3-carboxylic acids (*2*, *13*, *14*). Considering these results, it should be expected that food samples containing high levels of glucose and tryptophan, that are processed at low pH and high temperature (as occurs in fruit juices and jams) will provide glucose-derived tetrahydro-β-carboline-3-carboxylic acid in significant amounts. **Figure 7** confirms this assumption for a freshly obtained grape juice. The concentration of pentahydroxypentyl tetrahydro-β-carboline greatly increased during the heating time. The same is expected to occur during processing, concentration, and conservation treatments of fruit-derived products. Thus, differences among samples might reflect distinct concentration of reactants (glucose and tryptophan) and/or processing conditions.

The above results reveal for the first time the occurrence of the carbohydrate-derived tetrahydro-β-carboline pentahydroxypentyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, in fruit and vegetable juices, jams, and tomato concentrate and sauces. This finding extends previous reports on the presence of other tetrahydro-β-carbolines in juices and jams following a Pictet–Spengler condensation between tryptophan and fruit aldehydes (*13*–*16*). It is well recognized that tryptophan may undergo complex transformations giving rise to possible bioactive compounds (*24*). Occurrence of tetrahydro-β-carbolines in commonly ingested foods such as fruit-derived products and others, strongly evidences an exogenous intake of these compounds during food and drink consumption. This may influence the endogenous presence of β-carbolines in tissues,

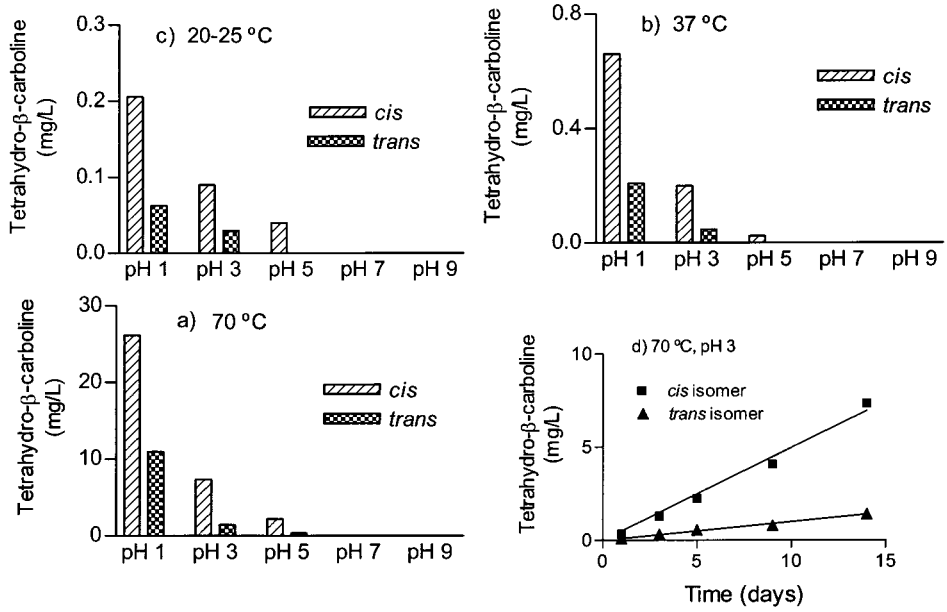

Figure 6. Formation of pentahydroxypentyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (mg/L) from a reaction of D-glucose (1 g/L) and L-tryptophan (0.5 g/L) at different pH values and temperatures (14 days of reaction, a–c).

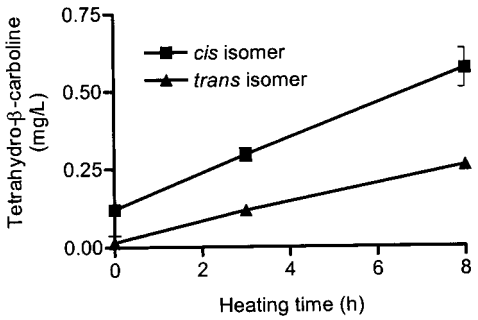

Figure 7. Formation of pentahydroxypentyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (cis and trans isomers) (mg/L) in freshly prepared grape juice when heated at reflux.

organs, and fluids (*2*−*6*) because accumulation in vivo could arise, at least in part, from food-ingested tetrahydro-β-carbolines (*25*). Indeed, pentahydroxypentyl-tetrahydro-β-carboline-3-carboxylic acid and other tryptophan glycoconjugates had been previously detected in human urine (*21*, *26*). In this regard, this alkaloid might form either endogenously or be absorbed from the diet. As chemical formation under physiological conditions (pH 7, 37 °C) is unlikely (**Figure 6**), it should be assumed that any possible presence of this polyol tetrahydro-β-carboline in vivo should surely arise from dietary sources. This is also supported by the extensive presence of this compound in fruit-derived products and probably in other foodstuffs. Although many studies have considered the possible bioactivity of tetrahydro-β-carbolines and β-carbolines, a full delineation of the biological activity of this class of compounds remains to be accomplished.

ACKNOWLEDGMENT

We thank Carmen Fernandez and Maribel Jimenez for assistance in sample processing and mass spectrometry, respectively.

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Received for review January 25, 2002. Revised manuscript received May 8, 2002. Accepted May 8, 2002. This work was supported by the research projects AGL2000-1480 and AGL2000-1569 (CICYT, Spain).

JF020090M