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N-Methylated Tryptamine Derivatives in Citrus Genus Plants: Identification of *N,N,N*-Trimethyltryptamine in Bergamot

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ABSTRACT: The occurrence of N-methylated tryptamine derivatives in bergamot plant (*Citrus bergamia* Risso et Poit) is reported for the first time. Interestingly, the most abundant of these substances is *N,N,N*-trimethyltryptamine, which has not been previously identified in any citrus plant. The N-methylated tryptamine derivatives were identified and quantitated in leaves, peel, juice, and seeds by high-performance liquid chromatography−electrospray ionization−tandem mass spectrometry. *N,N,N*-Trimethyltryptamine was confirmed by MS^3 and comparison with the synthesized authentic standard. In addition, the study of the distribution of tryptophan, tryptamine, *N*-methyltryptamine, *N,N*-dimethyltryptamine, and *N,N,N*-trimethyltryptamine indicated that these compounds are differently expressed in the various tissues of the bergamot plant. Intriguingly, chemically synthesized *N,N,N*-trimethyltryptamine was reported to possess nicotine-like activity being a stimulant of parasympathetic ganglia by exerting its action on acetylcholine receptors. On this basis, the identification of *N,N,N*-trimethyltryptamine at a relatively high level in leaves suggests a possible role in a physiological mechanism of plant defense.

KEYWORDS: *N,N,N-trimethyltryptamine, tryptamine derivatives, quaternary ammonium compounds, bergamot fruit, citrus fruits*

INTRODUCTION

Tryptophan decarboxylation by tryptophan decarboxylase produces tryptamine and represents the starting point of the metabolic processes leading to the formation of the tryptophan-derived secondary metabolites.[1,2] In these phytochemicals, which can also have complex structures, the bicyclic indole structure is usually preserved. For this reason, they are referred to as indole derivatives and are separated in two classes of substances, that is, the monoterpene alkaloids[3] and monoamine alkaloids, also known as tryptamine derivatives or simply tryptamines, which are structurally simpler.[4,5] Monoterpene alkaloids originate from the action of the enzyme strictosidine synthase[6] on two substrates, tryptamine and secologanin,[7] forming strictosidine,[8] which is the precursor of numerous alkaloid classes with many biological and pharmacological functions.[9] The second class of compounds, the tryptamine derivatives, originates from methylation and hydroxylation reactions of tryptamine that produce important bioactive compounds with a wide range of properties on the plant or animals, including neurotransmitters such as serotonin (5-hydroxytryptamine) or substances with hormonal activity such as melatonin (*N*-acetyl-5-methoxytryptamine). Interestingly, the *N,N*-dimethylation of tryptamine results in a large number of derivatives with psychoactive and/or hallucinogenic properties. The simplest representative of this class of compounds is *N,N*-dimethyltryptamine. The psychotropic activity is greatly affected by substitution at position 4 or 5 of the indole ring. Psilocin (4-hydroxy-*N,N*-dimethyltryptamine), psilocybin (4-phosporyloxy-*N,N*-dimethyltryptamine), and bufotenine (5-hydroxy-*N,N*-dimethyltryptamine) are well-known psychoactive drugs occurring in many mushroom species and plants.[10,11]

We examined various parts of bergamot (*Citrus bergamia* Risso et Poit), a plant of great commercial importance in South Italy, where it is cultivated mainly for the production of the essential oil widely used in the perfume industry. Here, we report for the first time the identification and distribution of N-methylated tryptamine derivatives in a *Citrus* genus plant.

MATERIALS AND METHODS

Reagents. Tryptophan, tryptamine, *N*-methyltryptamine, methyl iodide, and 0.1% solution of formic acid in water used for liquid chromatography−electrospry ionization−tandem mass spectrometry (LC-ESI-MS) analyses were obtained from Sigma-Aldrich (Milan, Italy). *N,N*-Dimethyltryptamine was from LGC Standards (Milan, Italy). The AG50WX8-H+ resins were purchased from BioRad (Milan, Italy). SPE-C18 columns for flash chromatography (particle size 33 μm) were obtained from Phenomenex (Anzola Emilia, Italy). All other solvents and reagents used were of analytical grade.

Standard and Plant Sample Preparations. The standard stock solutions of tryptophan, tryptamine, *N*-methyltryptamine, *N,N*-dimethyltryptamine, and *N,N,N*-trimethyltryptamine were prepared at a concentration of 2000 ng/mL and stored at 4 °C. Prior to injection, stock solutions were appropriately diluted with water containing 0.1% formic acid before being used as working solutions.

The plant materials (samples of bergamot fruits and leaves) were collected in the "Pellaro" area near Reggio Calabria (Calabria, Italy). Six lots of 1 kg of fruits and 100 g of leaves were used for the successive analyses. Three lots were collected on January and three on February

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2012 from three different bergamot plants. Fruits were previously washed with water to remove dust and pollutants from the exocarp.

Bergamot Peel Extracts. The preparations started with water washing of the bergamot fruits followed by the manual scraping of the exocarp to remove the essential oils from the utricles on the fruit surface. Then, after the fruits were washed again with water, the peel and seed were separated manually. The peel (flavedo and albedo) was homogenized in a mixer with 0.2% formic acid in water in a 1:1 (w/w) ratio. The homogenate was kept for 2 h under constant agitation and then centrifuged at 18000*g* for 30 min at 4 °C. The supernatant was finally frozen at −20 °C until used for the successive determinations.

Bergamot Endocarp Extracts. The endocarp (edible part of the fruit constituted by juice and pulp) deprived of seeds was homogenized in a mixer and then centrifuged at 18000*g* for 30 min at 4 °C, and the supernatant was frozen at −20 °C until used for the successive determinations.

Bergamot Seed Extracts. The seeds recovered from each lot were initially washed with water, drained, and dried on filter paper. Successively, 2−4 g of the seeds was homogenized in a mixer with 20 mL of 0.2% formic acid in water. The homogenate was allowed to stand 3 h under constant agitation and then centrifuged at 18000*g* for 30 min at 4 °C, and the supernatant was frozen at −20 °C until used for the successive determinations.

Bergamot Leaf Extracts. The leaves were washed with distilled water and dried with filter paper. Then, 25 g of product, finely chopped, was homogenized in a blender with 100 mL of 0.2% formic acid in Milli Q grade water and then kept under stirring for 3 h. The homogenate was finally centrifuged at 18000*g* for 30 min, and the supernatant was stored in 20 mL vials at −20 °C.

High-Performance Liquid Chromatography−Electrospray Ionization−Tandem Mass Spectrometry (HPLC-ESI-MS/MS) Instrumental Conditions. The HPLC-ESI-MS analyses were performed with an HPLC Agilent 1100 series equipped with on line degasser and automatic injector coupled online with an Agilent LC-MSD SL quadrupole ion trap. The MS acquisition was performed by using ESI in positive ion mode, with nitrogen as the nebulizing and drying gas under the following conditions: nebulizer pressure, 30 psi; drying temperature, 350 °C; and drying gas, 7 L/min. The ion charge control (ICC) was applied with a target set at 30000 and maximum accumulation time at 20 ms. The measurements were performed from the peak area of the extracted ion chromatogram (EIC). The quantitation was achieved by comparison with the calibration curves obtained with standard solutions. The retention time (min) and peak areas of the monitored fragment ions were determined by the Agilent software Chemstation version 4.2.

HPLC-ESI-MS/MS and FIA-ESI-MS/MS Analyses. The optimization of the instrumental parameters for the analyses of tryptophan, tryptamine, and its methylated derivatives was performed by continuous infusion of 5 μM standard solution in 0.1% formic acid. The mass cutoff and amplitude were optimized to obtain the most efficient MS/MS transitions from the positively charged precursor ion $[M + H^+]$ to the fragment ions. The transitions utilized for MS/MS quantitation were 205.2 → 188 for tryptophan, 161.2 → 144 for tryptamine, 175.2 → 144 for *N*-methyltryptamine, 189.2 → 144 for *N*,*N*-dimethyltryptamine, and 203.2 → 144 for *N*,*N*,*N*-trimethyltryptamine. Subsequently, the substances were analyzed by HPLC-ESI-MS/MS, as described by Servillo et al.[12,13] for the betaine analyses. Briefly, the chromatography, isocratically conducted with 0.1% formic acid in water, was performed with a 100 mm × 3.0 mm i.d., 5 μm, Discovery-C8 column (Supelco, PA) at flow rate of 100 μL/min. Volumes of 10−20 μL of standard solutions or samples were injected.

Synthesis and Purification of *N*,*N*,*N*-Trimethyltryptamine. For the conversion of tryptamine into its ammonium quaternary compound, *N*,*N*,*N*-trimethyltryptamine, we used a modified procedure proposed by Chen and Benoiton,[14,15] which is based on a heterogeneous phase reaction employing methyl iodide as the methylating agent in the presence of $KHCO_3$. Briefly, 200 mg of tryptamine was dissolved in 20 mL of methanol, and 1 g of $KHCO_3$ was added and, subsequently, 10 mL of methyl iodide (CH_3I). The mixture was stirred for 12 h at room temperature. The addition of methyl iodide (10 mL) and $KHCO_3$ (1 g) was repeated twice more. Finally, the mixture was centrifuged, and the supernatant was collected and evaporated to dryness at 40 °C in a rotavapor. The residue, containing the *N*,*N*,*N*-trimethyltryptamine, was dissolved in 10 mL of Milli Q grade water and purified by flash chromatography on SepPac C_{18} cartridge (Phenomenex, Anzola Emilia, Italy). The sample-loaded column was washed with 100 mL of Milli Q water, and *N*,*N*,*N*-trimethyltryptamine was eluted from the column by applying 50 mL of a solution of H_2O/acetonitrile (80:20) and evaporated to dryness under a stream of air and dried overnight in vacuum over P_2O_5. The yield was 64%.

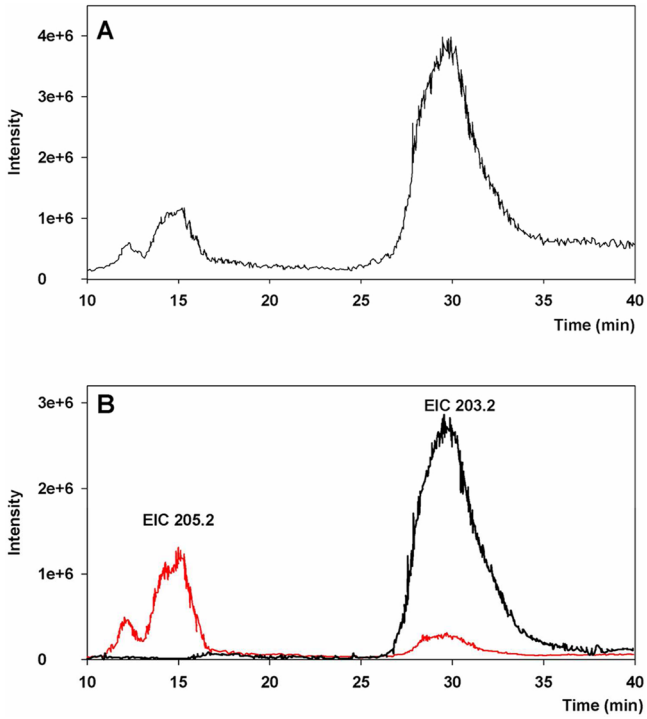

Figure 1. (A) LC-ESI-MS chromatogram conducted in zoom scan mode on a bergamot leaf extract in the *m*/*z* range 201−209 amu. (B) LC-ESI-MS extracted ion chromatograms (EIC) at *m*/*z* 203.2 (black line) and *m*/*z* 205.2 (red line) obtained from the bergamot leaf extract chromatography reported in panel A.

Tryptophan quantitation in the samples was performed by reverse-phase HPLC employing a model 2690 Waters instrument equipped with a model 474 fluorescence detector. The juice or extract samples of about 10 mL were centrifuged at 12000*g* at 4 °C for 10 min. Then, 1 mL of supernatant, after filtration, was loaded on a column (5 cm × 1 cm) filled with AG 50WX8-(H^+) resin [Bio-Rad, Segrate (MI), Italy]. After loading, the column was washed with five volumes of milli Q water, and then, the amino acids were one step eluted with 10 mL of ammonia solution (1:1 v/v water) and 5 mL of water. The eluted solution was dried with a rotavapor, dissolved in 5 mL of 0.01 M HCl, and passed through 0.45 μm filter. The chromatography was performed as for the HPLC-ESI-MS/MS analyses by employing a longer Discovery C8 column (150 mm × 3.0 mm), conditioned at 32 °C. The elution was isocratically conducted with 0.1% formic acid in water at flow rate of 400 μL/min. The injection volumes were 5−10 μL.

Tryptophan was fluorimetrically detected by excitation at 280 nm and emission at 340 nm. Tryptophan was identified on the basis of the retention time and quantitated by comparison of the sample peak area with the calibration curve.

RESULTS AND DISCUSSION

Preliminary Study. With the aim to detect possible tryptophan-derived osmolytes, we first sought to quantitate this amino acid in the fruit and other parts of the bergamot plant by

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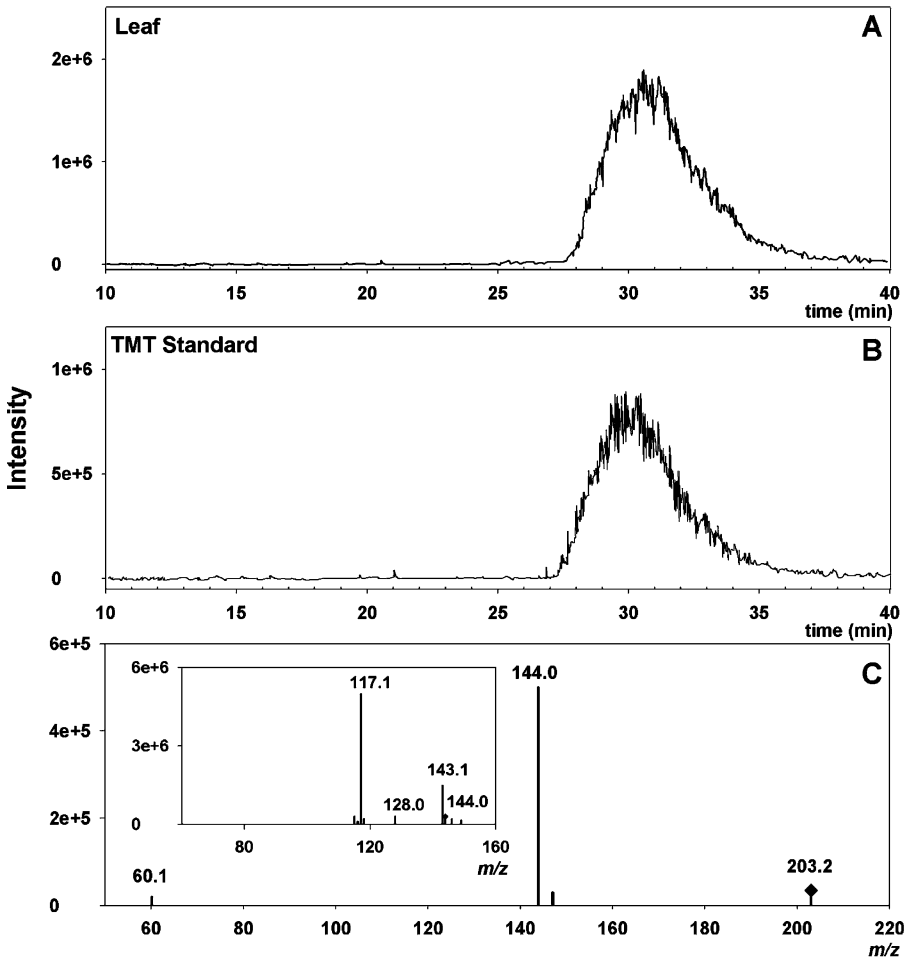

Figure 2. (A) Representative chromatogram of a bergamot leaf extract by HPLC-ESI-MS/MS following the MS^2 transitions 203.2 → 144. (B) Chromatography of the synthesized *N,N,N*-trimethyltryptamine (TMT) standard solution performed in the same experimental conditions. (C) MS^2 fragmentation pattern of the peak reported in panel A. Inset of C: MS^3 fragmentation pattern of the peak of panel A by isolating at m/z 144. The instrumental conditions for CID were as follows: mass cutoff, 50 amu, and amplitude, 0.80 V, for MS^2; and mass cutoff, 60 amu, and amplitude, 1.40 V, for MS^3 measurements. The MS^2 and MS^3 fragmentation patterns of the peak of B (standard TMT) being identical to those of the peak of A were omitted.

employing the same chromatographic approach previously used for determination of betaines in several citrus plants. For these analyses, a 10 cm C8 column was used and isocratic elution with 0.1% formic acid in water, which allows rather short analysis time and no re-equilibration time after each determination. One of these analyses conducted on a bergamot leaf extract is reported in Figure 1A. Tryptophan was abundant in the leaves and, as expected, more consistently retained on the C8 column than the more polar betaines that, in the same condition, are eluted within 10 min.[12] It is worth noting that in the preliminary survey analyses, as the tryptophan levels were not yet known, MS zoom scan on the mass range 201−209 was performed to gain more sensitivity. For leaf extracts, total ion current chromatograms like that of Figure 1A were obtained, in which three peaks could be observed. The mass spectrum analysis showed that the first and the second peak corresponded to compounds with m/z 205.2 (Figure 1B). The chromatographic analysis of leaf extracts executed in MS/MS mode by isolating at m/z 205.2 showed that the peak at retention time of 15 min presented the typical fragmentation pattern of tryptophan with a major fragment at m/z 188. The comparison of retention time and fragmentation pattern with the authentic standard easily confirmed that it was tryptophan. As for the first peak at m/z 205.2, with retention time of about 13 min, it is presently unknown, and its characterization is currently under investigation.

***N,N,N*-Trimethyltryptamine Identification.** The third intense peak at retention time of 30 min (Figure 1B), corresponding to m/z 203.2, aroused our interest as it showed in successive MS/MS experiments an intense signal at m/z 144 (Figure 2), which is typical of many indole-containing compounds. Further evidence of the presence of indole in the unknown compound was its UV spectrum obtained by performing the chromatography with the use of a diode array detector. The peak showed the same UV spectrum as the peak of the tryptophan standard solution, utilized for comparison, which in the same conditions eluted at 15 min (data not shown). Notwithstanding the simple MS/MS fragmentation of the unknown substance, composed only of an intense fragment at m/z 144 and another less intense fragment at m/z 60 (Figure 2), we were not able to find in various mass spectrometric database a substance with such a fragmentation pattern. To have insight into the structure of this unknown substance, we first increased the amplitude potential from 0.80 to 1.00 V. This resulted in a fragment at m/z 117 and a proportional reduction of the intensity of the fragment at m/z 144. A successive MS^3 experiment, in which the

MS^2 fragment at m/z 144 was isolated and fragmented, showed that the ion at m/z 144 generated a high intensity MS^3 fragment at m/z 117 (Figure 2C inset), indicating that the MS/MS fragment at m/z 117 was indeed the product of the ion at m/z 144. As it is well-known that many tryptamine derivatives show a MS/MS fragment at m/z 144 (corresponding to protonated vinylindole), which generates a MS^3 fragment at m/z 117 believed to be produced by the HCN neutral loss from the parent ion at m/z 144,[4,5] our results gave a strong indication that the unknown substance was a tryptamine derivative.

On the other hand, in the same conditions, the chromatography of a standard solution of tryptamine, *N*-methyltryptamine, and *N*,*N*-dimethyltryptamine resolved three peaks at retention times of 16.0 (tryptamine, m/z 161.2), 22.0 (*N*-methyltryptamine, m/z 175.2), and 27.5 min (*N*,*N*-dimethyltryptamine, m/z 189.2). All three substances showed a MS/MS fragmentation pattern with a major fragment at m/z 144, which gave a MS^3 fragment at m/z 117. Moreover, the increase of the chromatographic retention times from tryptamine to *N*,*N*-dimethyltryptamine gave a clue on the nature of the MS^2 fragment at m/z 60. In fact, in our previous studies,[12,13] it was observed that betaines, notwithstanding the permanent positive charge on nitrogen atom, show higher retention time on the C8 column than their less methylated analogues. As an example, the retention time order for proline derivatives is *N*,*N*-dimethylproline > *N*-methylproline > proline. Therefore, considering that the unknown compound showed a retention time higher than *N*,*N*-dimethyltryptamine, we hypothesized that it was the trimethylated form of tryptamine and that the MS^2 fragment at m/z 60 was the trimethylammonium ion, which is almost invariably present in the MS^2 fragmentation pattern of substances containing the trimethylammonium moiety. As *N*,*N*,*N*-trimethyltryptamine was not commercially available, the compound was synthesized according to the Chen and Benoiton method[14,15] to confirm that hypothesis.

Pure *N*,*N*,*N*-trimethyltryptamine was subjected to mass spectrometric analysis to find the optimal instrumental conditions for detection and quantitation. In particular, amplitude and cutoff, which are the main parameters for a ion trap mass spectrometer to achieve the most effective collision-induced dissociation (CID) of the parent ion toward its ion fragments, were optimized. This study was performed in positive ion mode by direct infusion of 5 μM *N*,*N*,*N*-trimethyltryptamine solution in 0.1% formic acid.

The chromatographic behavior and MS^2 and MS^3 fragmentation patterns of the synthesized *N*,*N*,*N*-trimethyltryptamine were compared with those of the unknown compound from the leaf extract (Figure 2). Both compounds showed the same parent ion mass at m/z 203.2, the same MS^2 fragmentation pattern, the same intensity ratio between the two main fragments at m/z 144 and 60, and the same MS^3 fragmentation pattern of the isolated MS^2 fragment at m/z 144 (Figure 2C). Moreover, they eluted at the same retention time. These observations strongly suggest that the unknown compound is *N*,*N*,*N*-trimethyltryptamine. However, to get further evidence of the identity of the compound, bergamot leaf extracts were treated with *N*,*N*,*N*-trimethyltryptamine standard solutions and subjected to chromatography by utilizing two C8 columns of 10 and 15 cm and isocratic elution conditions of either 100% water with 0.1% formic acid or a mixture of 75% water with 0.1% formic acid containing 25% methanol. In all cases, only one symmetrical peak was seen in the EIC chromatograms obtained by isolating the ion at m/z 203.2, thus confirming the identity of the leaf unknown compound with *N*,*N*,*N*-trimethyltryptamine (data not shown).

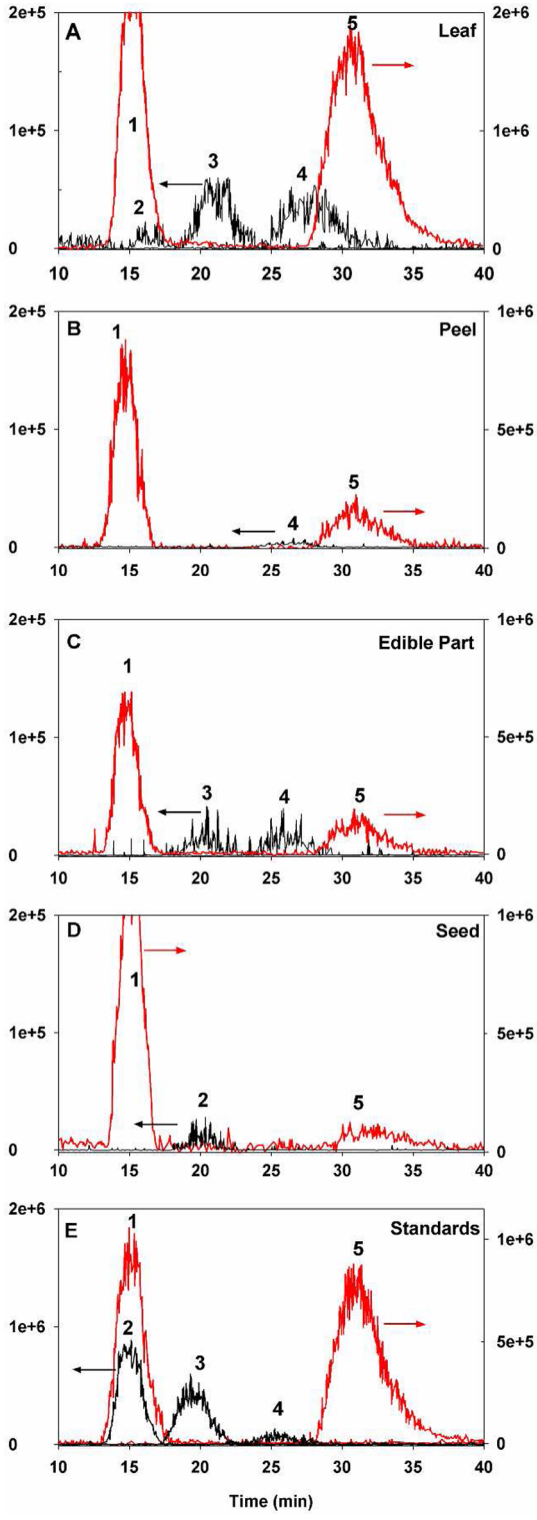

Figure 3. Distribution of tryptophan (**1**), tryptamine (**2**), *N*-methyltryptamine (**3**), *N*,*N*-dimethyltryptamine (**4**), and *N*,*N*,*N*-trimethyltryptamine (**5**) in plant tissues. (A) Leaves, (B) peel, (C) edible part, (D) seeds, and (E) standard solution of the compounds. The left scale refers to the black lines (as indicated by a black arrow). The right scale refers to the red lines (as indicated by a red arrow).

Distribution of Methylated Tryptamine Derivatives in the Various Parts of the Bergamot Plant. The occurrence

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Table 1. Distribution of Tryptophan and Tryptamine-Derived Compounds in Various Parts of Bergamot Plants by LC-ESI-MS/MS

		range (mg/kg)			
compd		peel	fruit edible part	seed	leaf
TRP	min−max	8−10	2−5	30−50	21−60
TRP[a]	min−max	9−12	2−6	30−50	19−62
TPA	min−max	absent	absent	trace	0.5−2.0
MMT	min−max	trace	0.02−0.10	0.05−0.2	0.2−0.5
DMT	min−max	0.01−0.10	0.03−0.05	trace-0.1	0.2−0.4
TMT	min−max	0.4−0.8	0.15−0.30	0.8−2.5	6.0−12.0

[a]Analyses performed by HPLC with fluorescence detection.

of *N*,*N*,*N*-trimethyltryptamine in bergamot leaves led us to look for the possible presence of less methylated forms of tryptamine, likely arising by the action on tryptamine of the same or more *N*-methyltransferases. It is well-known that, differently from those of primary metabolite biosynthesis, most of the enzymes involved in the biosynthesis of secondary metabolites, such as *N*-methyltransferases and hydroxylases, have a rather low substrate specificity.[16] Therefore, after optimization of the instrumental conditions for the LC-ESI-MS/MS analysis of tryptamine, *N*-methyltryptamine, *N*,*N*-dimethyltryptamine, and tryptophan using commercial standards, the occurrence of such compounds was investigated in various parts of the bergamot plant. The tryptophan determinations were also conducted by HPLC analysis with fluorescence detection. The results showed the different distribution of tryptophan, tryptamine, and its N-methylated derivatives in leaves (Figure 3A), peel (Figure 3B), edible part (Figure 3C), and seeds (Figure 3D). It is worth noting that the simple isocratic chromatographic method that we utilized for the betaine analyses in citrus plants[13,17] also worked well with the methylated tryptamine derivatives, being able to separate these compounds in about 30 min with good resolution. This chromatographic approach does not utilize gradients and uses the moderately hydrophobic C8 stationary phase, generally employed for apolar compounds, to separate polar substances that normally are analyzed by ion exchange chromatography or hydrophilic interaction chromatography. However, in these cases, it is the required use of gradients[18] that generally causes background drift during analysis.

The distribution of compounds shows that tryptamine and its methylated derivatives as well as tryptophan are all present in leaves at higher levels than those found in other plant tissues (Figure 3A). This is not surprising because in many species of plants leaves have specialized roles in the biosynthesis and accumulation of secondary metabolites with toxic properties, including alkaloids, flavonoids, and terpenes, as reported in localization studies by Kutchan.[19] Instead, besides leaves, tryptamine only was present in seeds although at trace levels (Figure 3B−D). *N*-Methyltryptamine is essentially absent in the peel while it is present at low levels in both seeds and edible parts. As for *N*,*N*-dimethyltryptamine, which is the compound of major concern due to its well-known hallucinogenic properties, it is present at very low levels also in peel, edible parts, and seeds. The quantitative data also showed the discrete levels of tryptophan in all parts of the plant with the highest content in leaves and seeds (Table 1). This amino acid was quantitated by both LC-ESI-MS/MS and LC with fluorescence detection, with similar results.

The occurrence of tryptamine and its N-methylated derivatives had never been described before in a plant of *Citrus* genus. To the best of our knowledge, the occurrence of the most represented of them, that is, the quaternary ammonium compound *N*,*N*,*N*-trimethyltryptamine, has never been reported so far in other plants. Among the various parts of the bergamot plant that we examined, leaves had the highest levels of those substances and also contained the highest levels of tryptophan, which is their most likely metabolic precursor. In fact, it is well-known that tryptophan decarboxylation by the enzyme tryptophan decarboxylase produces tryptamine and represents the starting point of the metabolic processes, leading to the formation of the tryptophan-derived secondary metabolites.[1,2] Among these metabolites, the monoamine alkaloids, also known as tryptamine derivatives, are those structurally simpler.[4,5] In bergamot, the N-methylated tryptamines can represent the simplest forms of monoamine alkaloids.

As for the presence of *N*,*N*-dimethyltryptamine in the fruit juice of bergamot, it must be said that, although the bergamot fruit is not largely utilized in the human diet, the occurrence of a substance with well-known psychotropic properties also could be cause of concern for the presumption that it may occur in fruit juices of citrus plants more widely consumed. Actually, our data do indicate that *N*,*N*-dimethyltryptamine occurs also in orange and lemon juices. However, also in these juices, it is present at the low levels found in the bergamot juice (unpublished results). On the other hand, one must consider that *N*,*N*-dimethyltryptamine is not a substance entirely foreign to the human organism. *N*,*N*-Dimethyltryptamine is considered a trace amine; that is, it belongs to a group of structurally related amines synthesized in mammalian brain and peripheral nervous tissues, generally present in body fluids at trace concentrations.[20] *N*,*N*-Dimethyltryptamine has been found to be an endogenous regulator of the Sigma-1 receptor that is widely distributed in the central nervous system and periphery[21] and has been detected in human blood and urine.[22] However, there are no conclusive quantitative studies measuring the abundance of endogenous *N*,*N*-dimethyltryptamine because of its rapid metabolism.[20] Therefore, it is conceivable that the innocuousness of citrus juices relies on both their low contents of *N*,*N*-dimethyltryptamine and the rapid biotransformation of the substance after the juice intake.

The occurrence of all of the N-methylated tryptamine derivatives in the bergamot plant leads lead us to hypothesize the involvement of tryptophan decarboxylase in a new metabolic pathway (Figure 4). In this proposed pathway, the formation of *N*,*N*,*N*-trimethyltryptamine from tryptamine, as a consequence of successive methylation reactions, catalyzed by the same or different *N*-methyltranferases, could represent the ultimate function in a plant defense mechanism against herbivores, as its accumulation mainly occurs in leaves. This possibility is supported by the pharmacological study on *N*,*N*,*N*-trimethyltryptamine conducted on sections of frog and guinea pig intestines.[23] In that study, it was shown that *N*,*N*,*N*-trimethyltryptamine is a stimulant of parasympathetic ganglia, possessing nicotine-like activity.[23]

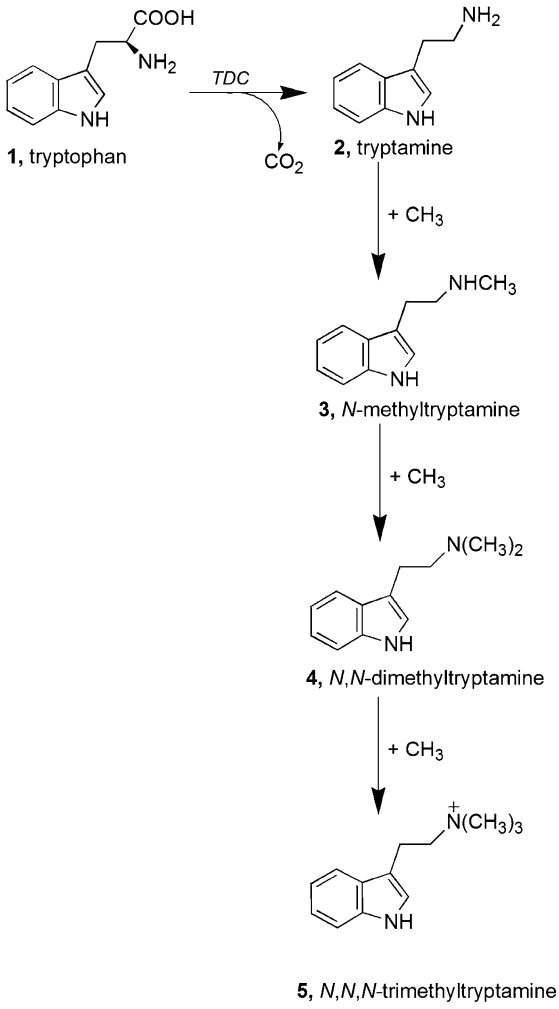

Figure 4. Hypothesized pathway for the biosynthesis of the methylated tryptamine derivatives. The first step is catalyzed by tryptophan decarboxylase (TDC) followed by the action of the same or different methyltransferases methylating ($+CH_3$) the tryptamine (**2**) in a consecutive manner. The methylated compounds produced are *N*-methyltryptamine (**3**), *N*,*N*-dimethyltryptamine (**4**), and *N*,*N*,*N*-trimethyltryptamine (**5**).

Therefore, it could play a toxic role against herbivores by exerting its action on acetylcholine receptors. Interestingly, the authors also showed that a hydroxylated form of *N*,*N*,*N*-trymethyltryptamine is an even more potent stimulator of parasympathetic ganglia (about 10-fold) than *N*,*N*,*N*-trimethyltryptamine. As matter of fact, our ongoing studies also seem to indicate the occurrence in the bergamot plant of hydroxylated forms of tryptamine-derived compounds, which led us to hypothesize that the N-methylated tryptamines could themselves be precursors of compounds with more powerful physiological actions against the plant aggressors.

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Notes

The authors declare no competing financial interest.

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