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Guanidine Alkaloids and Pictet-Spengler Adducts from Black Cohosh (*Cimicifuga racemosa*)[†]

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Abstract

As an extension of work on the recently discovered nitrogenous metabolites from *Cimicifuga/Actaea* species, three new guanidine alkaloids have been isolated and characterized from *C. racemosa* (syn. *A. racemosa*) roots. Of these, cyclo-cimipronidine (**1**) and cimipronidine methyl ester (**2**) are congeners of cimipronidine (**3**), whereas dopargine (**5**) is a derivative of dopamine. By employing NMR- and MS-guided chemodiversity profiling of a polar serotonergic (5-HT₇) fraction, the guanidine alkaloids were initially detected in a clinical extract of black cohosh and were isolated along with a congener of salsolinol **4**, **5**, and 3-hydroxytyrosol 3-*O*-glucoside (**7**). The structures of **1**, **2**, and **5** were confirmed by 1D and 2D NMR spectroscopy as well as LC-MS and HRMS spectroscopy. A plausible biosynthetic relationship may be inferred between the homoproline-analogue cimipronidines and the dopamine-derived *Cimicifuga* alkaloids. These strongly basic and frequently zwitterionic nitrogenous metabolites contribute considerable chemical diversity to the polar serotonergic fraction of black cohosh.

Black cohosh (*Cimicifuga racemosa* (L.) Nutt., syn. *Actaea racemosa* L.), historically referred to as “black snakeroot”, is a Native American botanical, for which a monograph was included in the first U.S. Pharmacopoeia in 1820. During the 19th century, black cohosh emerged as an important treatment for a variety of female-related health conditions. Recent clinical trials have evaluated its efficacy for the alleviation of menopausal symptoms, such as hot flashes, night sweats, and various psychological complaints.^{1–5} In spite of the known beneficial effects of *C. racemosa* root extract, the bioactive constituent(s) still need to be conclusively identified, especially for the development of a meaningful standardization procedure that is based on both the biological activity of the active principal(s) and the currently practiced chemical standardization protocol related to the content of certain abundant triterpenes. Competitive binding to the dopamine D₂ receptor by an ethanolic (70% v/v) extract was observed⁶ as well as binding to the serotonin 5-HT_{1A}, 5-HT_{1D}, and 5-HT₇ receptors by isopropanolic (40% v/v), ethanolic (75% v/v), and methanolic (100%) extracts.⁷ The 5-HT receptor subtypes, 5-HT_{1A} and 5-HT₇, of the hypothalamus have been shown to be associated with the generation of hot flashes,^{7,8} and in several clinical trials the efficacy of selective serotonin reuptake inhibitors (SSRIs) was demonstrated for the treatment of hot flashes (for reviews see ref 9–11). In addition, scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals has been reported for a methanolic root extract of *C. racemosa*.¹²

[†]Dedicated to Dr. David G. I. Kingston of Virginia Polytechnic Institute and State University for his pioneering work on natural products.

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Upon initial detection by LC-MS analysis, cyclo-cimipronidine (**1**) was isolated and, along with cimipronidine methyl ester (**2**), characterized as a congener of the previously described low-molecular weight guanidine alkaloid cimipronidine (**3**).¹³ Additionally, the presence of salsolinol (**4**)¹⁴ and a new compound, **5**, which are both derived from dopamine, was substantiated by LC-MS analysis and point to shared in situ or biosynthetic pathways that involve Pictet-Spengler reactions.¹⁵⁻²³ Compound **5** constitutes a new natural product for which the trivial name dopargine is proposed, in accordance with the naming of the well-studied tryptamine analogue tryptargine.^{24,25} Dopargine (**5**) is the presumed Pictet-Spengler reaction product of dopamine with γ -guanidinobutyraldehyde (**6**). The latter is a catabolism product of arginine and may be considered a key intermediate in the possible subsequent biosynthetic pathways that eventually lead to guanidine derivatives. Figure 1 presents the proposed origin of **5** and **3**. The presumed presence of **6** in extracts of black cohosh was detected by LC-MS experiments.

In addition to the isolated alkaloids, a glycosidic nitrogen-free derivative of dopamine, 3-hydroxytyrosol 3-*O*-glucoside (**7**),²⁶⁻²⁸ which is structurally related to the antioxidant hydroxytyrosol, was obtained from the same fraction. The present study links previous efforts to characterize constituents of the polar fractions of *C. racemosa*, which has recently led to the identification of the serotonergic active principle *N*_ω-methylserotonin (**8**).²⁹ This represented the first report of a derivative of a biogenic amine from black cohosh, and its concentrations in root extracts have been found to be consistent with the observed 5-HT₇ activity. The polar partition of the serotonergic extract, which is also rich in phenolic compounds such as cimicifugic and caffeic acids, was also the source of the structurally diverse alkaloids reported here.

Results and Discussion

Cimipronidine-Type Alkaloids

Compound **1** was obtained as a white, amorphous powder with a mass of 154.0986 amu (calcd 154.0980), consistent with the molecular formula C₇H₁₂N₃O. The initial evidence for the structure of **1** came from the ¹³C NMR spectrum, which exhibited carbon resonances with chemical shifts and relative intensities very similar to those of **3**. Three of the signals, namely, C-9 (δ 170.65), the guanidine C-6 (δ 149.83), and C-8 (δ 34.80), were shifted to higher field compared to the corresponding resonances in **3**. This suggested a structural variation of **3**, not involving the pyrrolidine ring moiety. The molecular weight obtained for the protonated **1** (m/z = 154.0986) 18 units lower than that of **3** (m/z = 171.1078, C₇H₁₃N₃O₂),¹³ suggested intramolecular lactam formation of **3**, with expulsion of one molecule of H₂O during a ring-closure reaction leading to **1**. Further detailed analysis of the ¹H NMR, COSY, and HSQC experiments was carried out (Table 1; Figures S1-S4, Supporting Information) and complemented by a 3D structural simulation of **1**. The HMBC experiment was optimized for 4 Hz, which displayed most of the expected long-range coupling information (Figures S5 and S6, Supporting Information). The results of the NOESY analysis helped establish a relative configuration for **1** (Figure S7, Supporting Information). In addition to cross-peaks arising between geminal protons, NOESY correlations occurred between protons H-5, H-8a, H-4a, and H-3b and also between H-8b, H-4b, H-3a, and H-2b, respectively, indicating that these groups of protons are located on opposite sides of the molecule. By analogy to lactone-ring-opening reactions of natural products (e.g., coumarins), a sample of **1** was dissolved in NaOD at a pH >10 to monitor whether or not a similar lactam-ring-opening reaction could be observed using ¹H NMR spectroscopy. The study showed that approximately 50% of **1** was converted to **3** during the first week of the experiment (Figure S26, Supporting Information) by slow chemical reaction. Upon addition of TFA (pH <2) to the sample, the reverse reaction toward ring-closure was observed (Figure S26, Supporting Information). The study also indicates that

3 and **1** are chemically interconvertible depending on the pH to which they are exposed. Cyclo-cimipronidine (**1**) is structurally related to the natural product arenain (**9**), which has been previously reported,³⁰ and while both possess the same central ring system (**1** having the “parent” ring system, i.e., unsubstituted), the origins of their respective biosyntheses, however, are probably different.

A mixed fraction (fr 14) showed, in addition to the peaks of **7** and minor amounts of **1**, three signals indicative of the presence of a cimipronidine derivative: one multiplet at δ 4.187 (H-5) as well as two double doublets at δ 2.741 and 2.492 for the methylene protons, H-8a and H-8b. An additional peak accounting for three protons at δ 3.601 indicated the presence of a methoxy group. Further 1D and 2D NMR analysis (Figures S8-S12, Supporting Information) helped assign all the signals, confirming the structure of **2** (Table 1). The HMBC cross-peak of the methoxy ¹H NMR signal with C-9 established its connection to the carboxyl group, fully consistent with the structure of **2** being the methyl ester of **3**. Accurate mass measurements for **2** gave a protonated molecular ion at m/z = 186.1243, consistent with a molecular formula of C₈H₁₆N₃O₂ (calcd 186.1234). Even though the spin systems of compounds **1-3** displayed very characteristic ¹H NMR patterns that made them easily distinguishable (Table 1) and despite the fact that they consist of only a relatively small number of protons per spin system, most proton signals appeared as highly coupled multiplets due to higher order virtual coupling effects.^{31,32} An iterative, full spin analysis of the three derivatives, using a method communicated recently,³³ is necessary in order to extract the coupling and in some cases the exact chemical shift information.

Dopamine-Type Derivatives

The guanidine **5** was obtained as a light yellow, amorphous powder, exhibiting an exact mass for the protonated molecular ion at 265.1673 amu (calcd 265.1665), corresponding to C₁₃H₂₁N₄O₂, consistent with the proposed structure. The ¹H NMR spectrum showed signals for 13 protons. Seven protons with chemical shifts and characteristic splitting patterns similar to that of arginine were observed at δ 4.386 (H-1), 1.985 (H-1'a), 1.895 (H-1'b), 1.637 (H-2'), and 3.151 (H-3'), indicating an arginine-like partial structure of the molecule. Two singlets accounting for one proton each at δ 6.678 and 6.664 suggested the presence of a substituted aromatic ring system, and three additional multiplet signals, accounting for four protons at δ 3.457 (H-3a), 3.287 (H-3b), and 2.768-2.970 (H-4a/b), could be attributed to a C₂-bridge, as found, for example, in phenylethane derivatives. The DEPTq NMR spectrum exhibited 13 carbon signals. On the basis of detailed analysis of the ¹H NMR, DEPTq, COSY, and HSQC spectra (Figures S13-S17, Supporting Information), the ¹H and ¹³C NMR data for **5** could be assigned to three parts of the molecule: an arginine moiety, a substituted aromatic system, and a chain of two aliphatic carbons as linker (Table 2). The HMBC spectrum exhibited strong correlations between the proton signal at δ 4.386 (H-1) and both carbon signals at δ 30.23 (C-1') and 24.05 (C-2'), of the arginine substructure, and also with carbons C-4a (δ 124.12), C-8 (δ 113.83), and C-8a (δ 123.58). This established the connection of the pendant arginine moiety to the aromatic ring substructure of **5**. Correlations between H-3a and carbons appearing at δ 54.70 (C-1), 24.03 (C-4), and 124.12 (C-4a) helped determine the connectivity to the remainder of the molecule, with the aliphatic carbon chain forming a tetrahydroisoquinoline structure with the CH (C-1) derived from the arginine portion (Table 2). Comparison of the recorded NMR data for **5** (D₂O) with the reported data (DMSO-*d*₆) for the 6,7-dimethoxylated derivative crispine E (**5a**, Table 2)³⁴ exhibited good agreement with the structural assignment for **5**.

Besides **1** and **2**, the mixed fraction, fr 14, contained quantities of **7**. From another fraction, 3.3 mg of **7** could be obtained, which was used for initial identification and dereplication. Accurate mass measurement provided a molecular formula of C₁₄H₂₀O₈Na based on a mass of 339.1049

amu (calcd 339.1056). Analysis of ^1H NMR, gated decoupled ^{13}C NMR, gCOSY, gHSQC, and gHMBC spectra (Figures S18-S23, Supporting Information) was in agreement with the proposed structure of **7** as a 3-hydroxytyrosol derivative possessing a β -glucose moiety (Figure S25, Supporting Information). An HMBC cross-peak between the anomeric H'-1 of the glucose moiety and the C-3 of the aromatic aglycone established the glycosidic connectivity. While observations are in general agreement with ^1H and ^{13}C NMR literature reports,³⁵ the present work presents specific proof of the glycosidic linkage (regioisomerism, 3-*O* vs 4-*O*) and will support future dereplication efforts by providing ^1H NMR spin simulation data for both moieties of the molecule (Figures S23 and S25, Supporting Information).

Chemical and Biosynthetic Relationship of Polar Constituents of Black Cohosh Roots

The alkaloid **5** represents a new compound from *C. racemosa* and can be understood as arising from the Pictet-Spengler product of dopamine with the aldehyde **6**.¹⁵ It is established that the Pictet-Spengler reaction leads to the formation of substituted 1,2,3,4-tetrahydroisoquinolines (THIQs). This reaction is known to take place spontaneously both in solution (acid or base catalysis) and in the solid state. Thus, the Pictet-Spengler reaction can affect compounds in solution, as well as in dried extracts or even plant materials. The resulting alkaloids are condensation products of a structurally equivalent amine and aldehyde.¹⁶⁻²¹ A similar reaction reportedly enzyme catalyzed has also been described in plants.³⁶ Three of these enzymes that are involved in plant THIQ alkaloid biosynthesis have been previously identified to reveal the reaction conditions, substrate specificity, and mechanism of the condensation reaction,^{36,37} which is analogous to an asymmetric Pictet-Spengler reaction pathway. Both spontaneous and enzyme catalyzed Pictet-Spengler reactions can potentially occur in a stereoselective manner. In the case of **5**, the condensation partner of dopamine is γ -guanidinobutyraldehyde (**6**, γ -GBAlD), which is formed as a byproduct during arginine catabolism (Figure S27, Supplementary Information) and studied primarily in microorganisms. While four major pathways have been delineated,³⁸⁻⁴¹ not all have been shown to occur in plants. However, a key enzyme, γ -GBAlD dehydrogenase, which uses preferably **6** as a substrate, has been found in plant leaves,⁴² suggesting that the corresponding arginine catabolism could take place in plants. Therefore, the intermediate **6** could be viewed as a key building block for Pictet-Spengler adducts. Figure 1 summarizes a proposed biosynthetic pathway for **5**.

While the isolation and structural characterization of **3** was first reported in 2005, its biosynthetic origin is still unclear. However, since **3** contains a guanidine moiety, it may be perceived as a biosynthetic link to the γ -GBAlD dehydrogenase pathway of arginine catabolism (Figure S27, Supplementary Information). The two compounds **1** and **2** may be viewed as "chemical artifacts" of **3**. The cyclic **1** results from intramolecular lactam formation of **3**, catalyzed in acidic conditions, e.g., caused by cimicifugic and/or other organic acids contained in the extract (Figure S25, Supporting Information). The methyl ester **2** is more likely an isolation artifact produced from **3** by esterification during the extraction with MeOH.

The aglycone of **7**, hydroxytyrosol, is the nitrogen-free dopamine analogue biosynthesized from tyrosine.⁴³ This deamination pathway has been reported in bacterial systems, yeast, and from plant sources.⁴⁴ Hydroxytyrosol has been frequently reported in the literature^{44,45} and is one of the major constituents in olive oil, and contributes to the antioxidant behavior of olive oil products;²⁶⁻²⁸ its bioavailability was also demonstrated in vitro.⁴⁶ Compound **7** has previously been isolated from *C. racemosa*⁴⁷ but NMR data sufficient for the dereplication of **7**^{35,48} and distinction of the 4-*O*- vs the 8-*O*-glycosylated regioisomers^{49,50} are unavailable. NMR data for the aglycone⁵¹ show significant differences in chemical shift values as compared with the NMR data for the glycoside. Thus, to unequivocally establish the position of attachment of the glucose unit, it was necessary to obtain a full complement of NMR data (^1H , ^{13}C , COSY, HSQC, and HMBC) for **7** (Figures S17-S23, Supporting Information) and

perform a detailed structural analysis (summarized in Figures S24 and S25, Supporting Information).

The initial discovery of **3** as the first strongly basic, N-containing, polar constituent of the serotonergic partition of black cohosh,¹³ which also contains cimicifugic and caffeic acid derivatives, led to the hypothesis of complex formation (molecular recognition) between the acids and bases present in the extract.⁵² On the basis of this hypothesis, novel workup procedures were developed to facilitate the isolation and identification of a series of nitrogenous compounds. Subsequently, the presence of **8** was discovered in one of the fractions, and this compound was shown to account for almost all of the observed 5-HT₇ binding activity.²⁹ The discovery of the new alkaloids **1**, **2**, and **5**, as well as the known **3**, provides additional evidence for a biosynthetic link to the γ -GBAld dehydrogenase pathway of arginine catabolism, which may serve to supply the guanidyl moiety that is common to all of these compounds. The identification of the alkaloids described here from the serotonergic polar fraction of *C. racemosa* and their potentially common biosynthetic origin contribute significantly to a better understanding of the structural diversity of the plant. The discovery of derivatives of the biogenic amines serotonin and dopamine make the nitrogenous fraction an interesting target for further investigation and is being actively investigated in our laboratories for possible CNS indications. Additionally, the presence of the nitrogen-free dopamine derivative **7**, which can be considered a pro-drug of hydroxytyrosol, may be a contributor to the reported antioxidant activity of the black cohosh root extract.¹²

The nitrogenous constituents found in black cohosh suggest that secondary metabolism of this plant involves two previously undiscovered major elements: (i) the incorporation of guanidyl moieties into alkaloids and (ii) the likely involvement of the Pictet-Spengler reaction as a biosynthetic pathway. Compound **5** may therefore be viewed as “a link” between the cimipronidine-type alkaloids containing a guanidino group and the class of alkaloids formed through a Pictet-Spengler ring-closure reactions. Both structural classes have been previously described from other plants.^{13,14} The absolute configuration of **5** and other cimipronidines, in comparison with homoproline derivatives will be reported in due course.

Experimental Section

General Experimental Procedures

UV data were acquired using a Shimadzu UV-2401 PC UV-vis recording spectrometer. One-dimensional (1D) ¹H, gated decoupled ¹³C, and DEPTq⁵³ spectra, as well as homo- and heteronuclear 2D NMR experiments (gCOSY, gHSQC, and gHMBC) were acquired on Bruker AVANCE-400 and AVANCE-600 spectrometers (Karlsruhe, Germany), in either 3 or 5 mm NMR tubes. The chemical shifts are expressed relative to the residual solvent signal (δ_{H} 4.700 for D₂O; δ_{H} 3.210 and δ_{C} 49.00 for MeOH-*d*₄). NMR data were processed and simulated (NS mode) with the NUTS system (Acorn NMR Inc.; acornnmr.com). Line resolution of experimental data was enhanced by Lorentz-Gauss (LG) transformation. Digital resolution was better than 0.0004 ppm and 0.008 ppm for ¹H and ¹³C, respectively, and chemical shifts are reported with three and two decimal places on the ppm scale, respectively, to appropriately reflect relative chemical shifts of signals.

LC-MS data and exact mass measurements were carried out using negative-or positive-ion electrospray with a Micromass (Manchester, UK) Q-TOF-2 hybrid mass spectrometer. To monitor separations, analytical thin-layer chromatography (TLC) was performed at room temperature on precoated silica gel 60 F254 glass plates (20 × 20 cm; Merck, Darmstadt, Germany). Fractions were monitored under UV light at 254 and 365 nm and after spraying with ninhydrin reagent (30 mg of ninhydrin in 10 mL of *n*-BuOH and 0.3 mL of 98% acetic acid) and 10% H₂SO₄ in MeOH both after heating to fully develop colors in daylight.

Plant Material

Authentic *C. racemosa* (syn. *Actaea racemosa*) roots and rhizomes were obtained through Naturex (formerly Pure World Botanicals), South Hackensack, NJ, and voucher specimens are deposited in the UIC/PCRPS repository (#BC 066) and at the supplier's facility.

Extraction and Isolation

The dried roots (1 kg) of *C. racemosa* were milled, homogenized, and exhaustively extracted with MeOH at rt (percolation with 11 L of fresh MeOH). The crude organic extract was concentrated in vacuo (<40 °C) to yield 164 g of a syrupy residue. The residue was reconstituted in deionized water (250 mL) and partitioned with EtOAc (20 × 250 mL, 52 g) and *n*-BuOH (19 × 250 mL, 32 g). The *n*-BuOH partition (30 g) was subjected to column chromatography (CC) consisting of Amberlite XAD-2 (Sigma, St. Louis, MO; 1.7 kg, 110 × 6 cm, 6 L) equilibrated with H₂O. Elution with H₂O (8 L), H₂O-MeOH (1:1) (5 L), then MeOH (12 L) yielded three fractions of 18, 3 and 4.5 g, respectively. A second batch of 1 kg of the same plant material was treated in the same way, and the resulting 173 g of dry extract reconstituted in 250 mL deionized water and partitioned with EtOAc (20 × 400 mL, 42 g). The aqueous residue was concentrated in vacuo (<40 °C) to remove the organic solvent and subjected to CC on Amberlite XAD-2 material, equilibrated with H₂O. After washing the column with H₂O (19 L), elution with MeOH (9 L) yielded 6 g of the MeOH-soluble portion.

Isolation of Cyclo-cimipronidine (1)

A 4 g sample of the MeOH-soluble fraction (first extraction) was further subjected to CC (MCI gel CHP20P, Mitsubishi, Tokyo, Japan; 400 g, 28 × 3.5 cm, 540 mL) equilibrated with 0.1% TFA in H₂O (fraction size 10 mL). The combined fractions 16-70 (with 1-15 containing sugars) were subjected to CC on Sephadex LH-20 (Amersham Biosciences, Piscataway, NJ; 100 g, 20 mm × 2 m plus precolumn, 200 mL), equilibrated with 25% MeOH in water. Elution was carried out with the same solvent (fraction size 5 mL), and the combined fractions 41-50 yielded 15 mg of **1**.

Characterization of Cimipronidine Methyl Ester (2)

A 1 g sample of the MeOH-soluble fraction (second extraction) was further subjected to CC on MCI gel, equilibrated with 0.1% TFA in H₂O. Elution was carried out with 0.1% TFA in water (fraction size 100 mL), and after fraction 5, 10% MeOH was added. After fraction 8, an acid-free gradient of H₂O-MeOH (100:0 to 0:100) was applied. Fraction 14 (6.6 mg) contained a mixture of mainly **2** and **7**, with minor amounts of **1**. Compound **2** was identified from this mixture.

Isolation of Dopargine (5)

As described for **1**, the amount of 4 g of the MeOH-soluble fraction (first extraction) was subjected to CC on MCI gel, equilibrated with 0.1% TFA in water. Elution was carried out with the same solvent (fraction size 10 mL), and the combined fractions 71-150 rechromatographed on Sephadex LH-20 with 25% MeOH in water (fraction size 5 mL). The combined fractions 51-55 yielded 4.9 mg of **5**.

Isolation of 3-Hydroxytyrosol 3-O-glucoside (7)

In addition to the presence of **7** in the previously mentioned mixed fraction (fr 14), **7** was obtained as a pure compound as follows. An amount of 1 g of the H₂O-MeOH (1:1) fraction (first extraction) was subjected to solvent-solvent partitioning using a 1 L fast centrifugal partition chromatography (FCPC) rotor (FCPC, CPC Kromaton), solvent pump (LabAlliance, Series III), dual-wavelength UV detector (Shimadzu, SPD-10A VP, UV-vis detector), and a

pH meter (Navi F-51, Horiba, Japan) connected to a fraction collector. The solvent system was H₂O-EtOAc-BuOH (5:1:4), which showed a volume ratio upper phase to lower phase of 54/46. After phase separation, 2 mg of (NH₄)₂CO₃ per mL of solvent was added to the water phase (LP), which was used as the mobile phase, and 0.1% TFA was added to the lighter organic phase (UP), used as the stationary phase. The sample was dissolved (2 mL, 1:2 (v/v); LP/UP) and injected immediately after loading the column with the stationary phase, the mobile phase was then pumped through the column, and the separation was run in a tail-to-head mode. The column was spun at 1100 rpm, and the flow rate was 4 mL/min (fraction size 4 mL). The stationary phase retention volume ratio was 0.75. The UV (345 and 254 nm) and pH profiles as well as TLC analysis were used to combine fractions. Combined fractions 81-140 were subsequently subjected to CC on Sephadex LH-20 in 25% MeOH in water (fraction size 5 mL). Fraction 55 yielded 3.3 mg of **7**.

Cyclo-cimipronidine (1)

white powder; ¹H and ¹³C data for **1** in D₂O, see Table 1; MW calcd for C₇H₁₁N₃O 154.0980; HR electrospray MS/MS2 *m/z* 154.0986 (M + H)⁺ (100), 112.0816 (28), 95.0557 (46), 94.0670 (40), 70.0661 (40), 67.0573 (15).

Cimipronidine methyl ester (2)

yellow powder of mixture; ¹H and ¹³C data for **2** in MeOH-*d*₄, see Table 1; MW calcd for C₈H₁₆N₃O₂ 186.1234; HR electrospray MS/MS2 *m/z* 186.1243 (M + H)⁺ (60), 154.0996 (20), 144.1032 (60), 137.0715 (30), 112.0787 (20), 95.0529 (35), 70.0656 (100).

Cimipronidine (3)

data as reported earlier;¹³ for ¹H and ¹³C data for **3** in D₂O, see Table 1. The original assignments¹³ were reversed; ¹³C NMR (D₂O, 400 MHz) δ[ppm] (carbon position) 31.13 (4), 22.61 (3).

Dopargine (5)

light yellow powder; UV (H₂O) λ_{max} (log ε) 286 (3.2) nm; ¹H and ¹³C data for **5** in D₂O, see Table 2; MW calcd for C₁₃H₂₁N₄O₂ 265.1665; HR electrospray MS/MS2 *m/z* 265.1673 [M⁺] 206.1178 (100), 137.0613 (18), 70.0560 (10), 60.0448 (5).

3-Hydroxytyrosol 3-O-glucoside (7)

light yellow powder; UV (H₂O) λ_{max} (log ε) 285 (3.3), 300_{sh} (3.1) nm; ¹H and ¹³C data for **7** in D₂O and MeOH-*d*₄, see Figure S25, Supplementary Information; MW calcd for C₁₄H₂₀O₈Na 339.1056; HR electrospray MS/MS2 *m/z* 339.1049 (M + Na)⁺, 137.0603 (100), 85.0476 (8).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

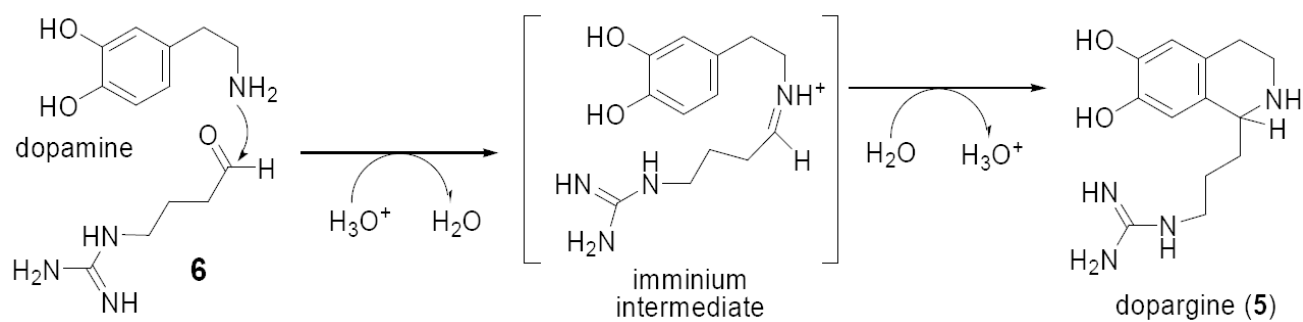
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**Figure 1.**

Proposed asymmetric Pictet-Spengler reaction pathway leading to the biosynthesis of **5** that uses dopamine as precursor and involves the incorporation of γ -guanidinobutyraldehyde (**6**) via an iminium intermediate.

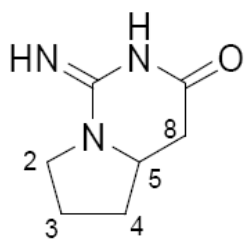
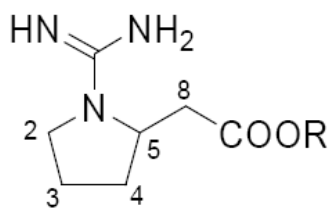
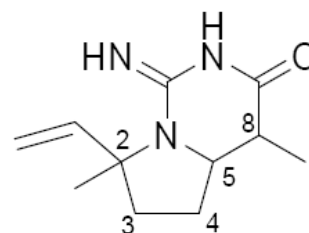
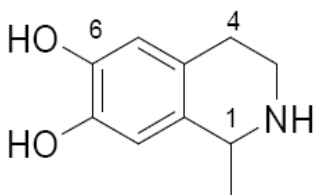
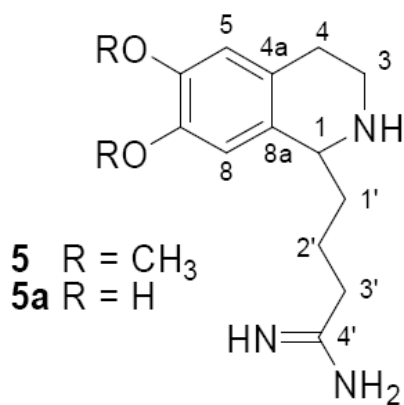
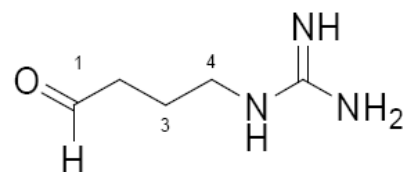
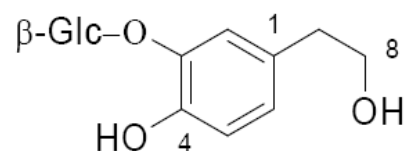
**1****2** R = CH₃
3 R = H**8****4****5** R = CH₃
5a R = H**6****7**

Table 1
NMR Spectroscopic Data of **1** and **3**¹³ (400 MHz ¹H), **2** (600 MHz ¹H).

	1 (D ₂ O)	2 (MeOH- <i>d</i> ₄)	3 (D ₂ O)	^a δ _H , mult. (J)	^b δ _C , mult. (J)	^a δ _H , mult. (J)	^b δ _C , mult. (J)	^b δ _C
2a	3.580 m ^d	45.76 ^c 3.405 m ^d	48.30 ^c 3.594 m ^d					47.31 ^c
2b	3.459 m ^d	3.290 m ^d	3.467 m ^d					
3a	2.128 m ^d	22.38 ^c 1.959 m ^d	23.33 ^c 2.17 m ^d					22.61 ^{c,e}
3b	1.953 m ^d		2.13 m ^d					
4a	2.279 m ^d	30.78 ^c 2.075 m ^d	31.82 ^c 2.20 m ^d					31.13 ^{c,e}
4b	1.624 m ^d	1.880 m ^d	1.97 m ^d					
5	3.952 m ^d	54.89 ^c 4.187 m ^d	56.19 ^c 4.331 m ^d					56.99 ^c
6		149.83	155.85					154.57
8a	2.831 dd (16.9, 4.2)	34.80 ^c 2.741 dd (16.5, 4.2)	37.14 ^c 2.692 dd (15.1, 5.4)					41.02 ^c
8b	2.599 dd (16.9, 13.6)	2.492 dd (16.5, 9.1)	2.410 dd (15.1, 8.4)					179.83
9		170.65	172.90					
		3.601 s	52.17 ^c					

^aReferenced to the residual solvent signals at δ_H 4.700 for D₂O and δ_H 3.210 for MeOH-*d*₄.

^bThe ¹³C NMR signals were referenced through HSQC and HMBC cross-peaks of the analytes in D₂O solutions and to the residual solvent signal at δ 49.20 for MeOH-*d*₄ solutions.

^cSignals showed cross-peaks in HSQC experiments.

^dSignals exhibit multiplet characteristics due to higher order virtual coupling effects; full spin analysis of these complex spin systems is beyond the scope of the present work and will be communicated separately.

^eOriginal assignments¹³ are reversed.

Table 2
NMR Spectroscopic Data of **5** in D₂O (400 MHz ¹H) in Comparison to Reported Data for Crispine E (**5a**) in DMSO-*d*₆.^{3,4}

position	5					5a ³⁴	
	δ _H ^a mult. (J)	δ _C ^b mult.	HMBC ^b (8 Hz) (H → C)	NOESY (H → H) δ _H ^a mult. (DMSO- <i>d</i> ₆)	δ _C (DMSO- <i>d</i> ₆)		
1	4.386 bt (6.4)	54.70 ^c CH	1', 2', 4a, 8, 8a	1'a, b	53.40		
3a	3.457 m	39.43 ^c CH ₂	1, 4, 4a	3b, 4	38.71		
3b	3.287 m			3a, 4			
4	2.768-2.970 m	24.03 ^c CH ₂	3, 4a, 5, 8a	1, 3a, 4	24.46		
4a		124.12qC		5, 3a, 3b	124.17		
5	6.678 s	116.03 ^c CH	4, 4a, 6, 7, 8a		111.67		
6		143.18 ^d qC			148.06		
7		144.20 ^d qC			147.63		
8	6.664 s	113.83 ^c CH	1, 4a, 6, 7, 8a		109.96		
8a		123.58qC			124.39		
1'a	1.985 m	30.23 ^c CH ₂	1, 2', 3', 8a	1, 8	30.57		
1'b	1.895 m			1'a			
2'	1.637 m	24.05 ^c CH ₂	1', 3'	1, 8	24.57		
3'	3.151 t (6.9)	40.64 ^c CH ₂	1', 2', 4'	1, 3', 8	40.21		
4'		157.23			157.01		
OMe					55.49		
OMe					55.91		

^aReferenced to residual water signal (HDO) at δ_H 4.700 ppm.

^bThe ¹³C signals were referenced to δ 54.70 ppm for C-1.

^cSignals showed cross-peaks in HSQC.

^dAssignments are interchangeable.