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Constituents of the roots of *Melochia chamaedrys*

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Abstract

The cyclic peptide alkaloid, chamaedrine, was isolated from the roots of *Melochia chamaedris* (Sterculiaceae), along with four known cyclic Peptide alkaloids (adouetine X, frangulaline, scutianine B and scutianine C), and waltherione A, parasorbic acid, propacine, and (−)-epicatequine. Their structures were elucidated on the basis of spectroscopic analysis, especially by 2D NMR (^{1}H–^{1}H-COSY, NOESY, HMQC, HMBC).

Keywords: *Melochia chamaedrys*; Sterculiaceae; Cyclic peptide alkaloid; Chamaedrine

1. Introduction

The genus *Melochia* (Sterculiaceae), comprising nearly 65 species, is widespread in the tropics (Goldberg, 1967). Of these, *Melochia corchorifolia* Linn (Bhakuni et al., 1987, 1991) and *Melochia tomentosa* Linn (Goldberg, 1967; Kapadia et al., 1975, 1978, 1980, 1993; Shukla et al., 1976) are the most widely studied. Plants of this genus are used for relief of throat inflammation (Morton, 1974), as tumorogenic agents (O'Gara et al., 1974), and as a cure for abdominal swelling, dysentery and water snakebite (Chopra et al., 1956). Plants belonging to this family are recognized to be rich in alkaloids, particularly the cyclopeptide alkaloids, quinolinone and isatin alkaloids (Goldberg, 1967; Shukla et al., 1976; Bhakuni et al., 1987, 1991; Kapadia et al., 1975, 1978, 1993).

Melochia chamaedris A. St.Hil, locally called "douradinha", is a plant that grows in South America (Shouther Brazil, Argentine and Uruguay). In the Rio Grande do Sul (Brazil), *M. chamaedris* is used by the local population for the treatment of various diseases such as cancer and as an anti-hypertensive agent. However, nothing is known to date about its chemical constituents. In the course of our chemical studies on the alkaloidal components of South Brazilian medicinal plants, we have previously reported the isolation of cyclopeptide alkaloids (Morel et al., 1999a,b) and one quinolone (Hoelzel et al., 2005) from the bark of *Waltheria douradinha*, another genus of the Sterculiaceae family. In a continuation of this work, we now report the isolation and structural elucidation of chamaedrine (**1**), a cyclic peptide alkaloid from the roots of *M. chamaedris*. In addition, four known cyclic peptide alkaloids, adouetine X (**2**), frangufoline (**3**), scutianine B (**4**) and scutianine C (**5**), one quinolinone alkaloid, waltherione A (**6**), one lactone, parasorbic acid (**7**), one coumarinolignoid, propacine (**8**) and one flavonoid, (−)-*epi*-catechin (**9**) were also isolated.

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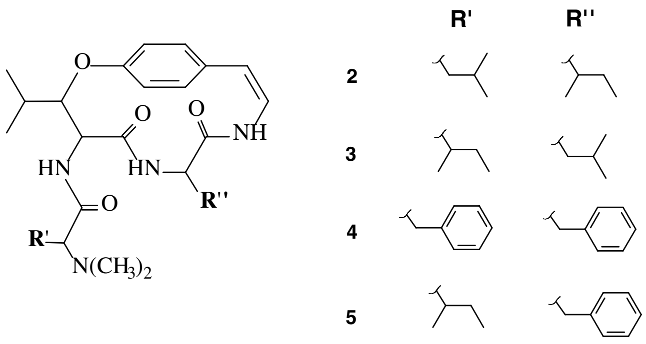

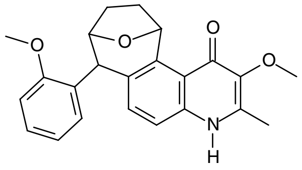

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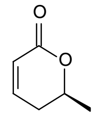

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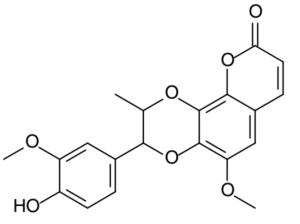

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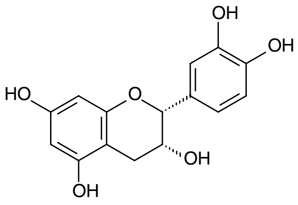

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2. Results and discussion

Chamaedrine (**1**) was obtained as a white powder. Its molecular formula was determined as $C_{36}H_{41}N_5O_4$ by HRESIME ($[M+H]^+ = 608.32308$ Da; $[M-H]^- = 606.30996$ Da), in combination with analysis of the ^{13}C NMR spectrum that showed 36 carbon resonances, including 21 methine, two methylenes, four methyls, and nine non-hydrogenated carbons, three of which had carbonyl groups. In the EIMS, the base peak appeared at m/z 148, corresponding to $C_{10}H_{14}N$, suggesting the presence of an *N*,*N*-dimethyl phenylalanine unit, which was confirmed by analysis of the ^1H and ^{13}C NMR spectroscopic data, and by enantioselective GC analyses.

The ^1H NMR spectrum (400 MHz, $CDCl_3$,) of **1** showed two sets of doublets at δ 0.87 ($J = 6.7$ Hz) and 1.15 ($J = 6.7$ Hz), which were assigned to the C-18 and C-19 methyl group hydrogens, respectively. The C-3 and C-4 methine hydrogens appeared at δ 4.84 (*dd*, $J = 8.0$; 2.0 Hz) and 4.33 (*dd*, $J = 10.5$; 8.0 Hz), respectively. In the ^1H–^1H-COSY spectrum, the two doublets of 18-Me and 19-Me have a cross-peak with the signal at δ 1.85 (1H, *m*), which corresponds to H-17. In turn, the H-17 resonance shows a cross-peak with H-3 and this with H-4. H-4 also exhibits another cross-peak with H-20 (NH) at δ 7.38 (d, $J = 10.5$ Hz). This spin system confirms β-hydroxyleucine as the hydroxylated amino acid of the macrocycle ring. The vicinal coupling constant of ca. 8.0 Hz of the methine hydrogens (H-3/H-4) indicates an *erythro* configuration for this residue (Sierra et al., 1972). In addition, the NOESY spectrum of **1** (see Fig. 1) exhibits a NOE cross-peak between H-3 and H-14, suggesting that H-3 is located in the β-position. However, H-3 shows no cross-peak with H-4 indicating that they are in the *anti*-position. This evidence suggests that the β-hydroxyleucine moiety in **1** has a L-*erythro* configuration. In addition, from the ^{13}C NMR spectra, the C-3 resonance observed at δ 81.9 confirms that the β-hydroxyleucine moiety possesses a L-*erythro* ($3S^*,4S^*$) configuration. The side-chain amino acid *N*,*N*-dimethyl phenylalanine was characterized by the occurrence of a double doublet at δ 2.60 (*dd*, $J = 4.5$; 8.0 Hz), which was assigned to the H-22 methine hydrogen. It showed cross-peaks with H_2-23 at δ 2.70 (*dd*, $J = 4.5$; 14.0 Hz) and 2.81 (*dd*, $J = 8.0$; 14.0 Hz). The absolute stereochemistry of the *N*,*N*-dimethyl phenylalanine side-chain was determined as (*S*) by chiral phase gas chromatography using 3-pentyl-2,6-dimethyl-β-cyclodextrin (3-Pe-2,6-Me-β-CD) (König et al., 1990) as stationary phase.

Tryptophan, which is the α-amino acid of the macrocycle, was identified by the cross-peaks between H_2-30, H-7 and NH-6. The H_2-30 at δ 2.90 (*dd*, $J = 8.0$; 15.0 Hz) and 2.83 (*dd*, $J = 4.0$; 15.0 Hz) exhibits a cross-peak with H-7 at δ 4.32 (*m*) and H-7 shows another cross-peak with H-6 (NH) at δ 5.75 (d, $J = 8.0$). In the NOESY spectrum, the H-4 signal exhibits a cross-peak with H-6 (NH), but does not show a cross-peak with H-7. This suggests that the α-amino acid tryptophan has an L (S^*) configuration.

The ^{13}C NMR spectrum (100.6 MHZ, $CDCl_3$) of **1** indicated the presence of 36 carbons. DEPT, HMQC and HMBC experiments showed the presence of two methylene, four methyls, 21 methine and nine non-hydrogenated carbons. The signals at δ_C 172.6, 171.5 and 167.2 permitted the assignment of three carbonyl groups in **1**. In the HMBC spectrum, cross-peaks between δ_H 2.60 (H-22)

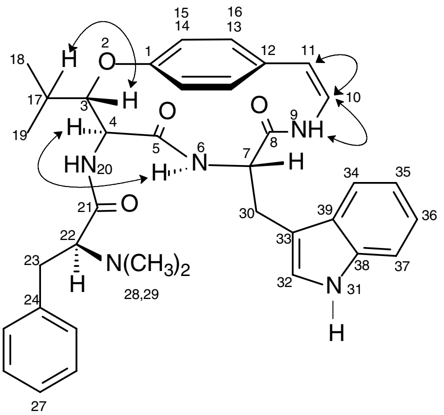

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Fig. 1. Cyclopeptide alkaloid **1** and important ^1H–^1H-NOESY correlations.

and δ_H 2.70/2.81 (H_2-23) to δ_C 172.6 permitted the assignment of a carbonyl group at C-21, while cross-peaks between δ_H 4.84 (H-3) and δ_H 4.33 (H-4) to δ_C 171.5, and δ_H 4.32 (H-7) to δ_C 167.2, were indicative of carbonyl groups at C-5 and C-8, respectively.

The ^{1}H and ^{13}C NMR spectra of **1**, along with the coupling constants of the hydrogen's and the most important connectivities observed in the HMBC spectrum, are summarized in Table 1.

A number of known compounds were also isolated from this source for the first time. Alkaloids **2**–**6** were identified by comparison with authentic samples of adouetine X (**2**), frangufoline (**3**), scutianine B (**4**), scutianine C (**5**), and waltherione A (**6**), whereas compounds **7**–**9** were identified by comparing their spectroscopic data (^{1}H NMR and ^{13}C NMR) with the literature (Cardellina and Meinwald, 1980; Zoghbi et al., 1981; Baldé et al., 1991).

Our results on *M. chamaedris* confirm previous findings that members of the genus *Melochia* contain cyclic peptide alkaloids and quinolinone alkaloids (Goldberg, 1967; Shukla et al., 1976; Bhakuni et al., 1987, 1991; Kapadia et al., 1975, 1978, 1993). The finding of parasorbic acid (**7**) and propacine (**8**) in *M. chamaedris* was unexpected since such compounds have not been recorded from the family Sterculiaceae before.

3. Experimental

3.1. General

General experimental procedures: Melting points were determined with an "MQAPF-301" apparatus and are uncorrected. Optical rotations were taken on a Perkin–Elmer 341 digital polarimeter. High-resolution ESI mass spectra were recorded on a Bruker Bio Apex 70 eV FT-ICR (Bruker Daldonis, USA) instrument. Low resolution MS were recorded at an ionization potential of 70 eV. ^{1}H and ^{13}C NMR spectra were recorded at 400.1/100.6 MHz, on a Bruker DPX-400 spectrometer using $CDCl_3$ as solvent and TMS as internal standard. Thin layer chromatography was performed on a pre-coated TLC plates (Merk, silica 60 F-254), by spraying with Dragendorff reagent, and by spraying with 10% H_2SO_4 in EtOH, followed by heating.

3.2. Plant material

The plant material (roots) was collected in February–May 2001 in the state Rio Grande do Sul, Brazil. Prof. Thais S.C. Dorow identified the plant and a voucher specimen (SMDB 9262) is deposited at the herbarium of the University of Santa Maria.

Table 1
^{1}H and ^{13}C NMR spectroscopic data for chamaedrine (**1**) (in $CDCl_3$, 400/100.6 MHz)[a]

H/C	δ ^{1}H (J, Hz)	δ ^{13}C (ppm)	HMBC correlations	
			$^2J_{CH}$	$^3J_{CH}$
01	–	156.0	–	–
03	4.84 *dd* (2.0, 8.0)	81.8	C-17	C-1, C-5
04	4.33 *dd* (8.0, 10.5)	54.9	C-5	–
05	–	171.5	–	
06	5.75 *d* (8.0)	–	–	–
07	4.32 *m*	54.0	C-8	C-5
08	–	167.2	–	–
09	6.40 *d* (7.5)	–	–	–
10	5.75 (signal overlapping)	131.4	C-11	C-12
11	5.76 (signal overlapping)	126.9	C-10, C-12	–
12	–	131.6	–	–
13–16	7.0–7.5	120.0–129.4	–	C-1
17	1.85 *m*		–	
18	0.87 *d* (6.7)	14.0	C-17	
19	1.15 *d* (6.7)	20.3	C-17	
20	7.38 *d* (10.5)		–	–
21		172.6		
22	2.60 *dd* (4.5, 8.0)	69.3	C-21, C-23	C-24
23	2.70 *dd* (4.5, 14.0)/2.81 *dd* (8.0, 14.0)		C-22	
24		141.0	–	
25–27		119.0–130.0	–	–
28–29	2.12 *m*	41.5		
30	2.83 *dd* (4.5, 15.0) /2.90 *dd* (8.0, 15.0)	39.2	C-7	
31	9.55 (*br s*)	–	–	–
34–37				
38		136.0	–	–
39		126.6	–	–

[a] Assignments were obtained by analyses 2D ^{1}H–^{1}H-COSY, NOESY, DEPT and 2D ^{1}H–^{13}C COSY (HMQC, HMBC) experiments.

3.3. Extraction and isolation

Air-dried roots of *M. chamaedris* (780 g) were pulverized and extracted with MeOH (3 l × 5, 5 days each time), at room temperature. Removal of the solvent from the combined extracts was performed under reduced pressure in a rotary evaporator to obtain a dark crude extract (200 g). This extract was next suspended in H_2O (0.3 l) and successively partitioned with *n*-hexane (HF, yield 10 g), $CHCl_3$ (CF, yield 4.5 g), EtOAc (AcF, yield 11 g) and *n*-BuOH (BuF, 28 g). The $CHCl_3$ residue (CF) containing six Dragendorff-positive spots in TLC, was subjected to silica gel CC eluted with a gradient of $CHCl_3$ containing increasing amounts of MeOH (up to 10% to give 15 fractions). Fraction 2 ($CHCl_3$:MeOH, 99.5:0.5) consisting of one compound, was evaporated to give **7** (210 mg). Fraction 3 ($CHCl_3$:MeOH, 99.5:0.5), consisting of two compounds, was evaporated (55 mg) and submitted to preparative TLC ($CHCl_3$:MeOH, 99.5:0.5, two elutions) to yield **7** (10 mg) and **8** (35 mg). Fraction 5 ($CHCl_3$:MeOH, 99:1), containing two Dragendorff-positive spots by TLC, was evaporated (79 mg) and submitted to preparative TLC ($CHCl_3$:MeOH, 99:1, two elutions) to yield **1** (9 mg) and **2** (25 mg). Fractions 6 and 7 ($CHCl_3$:MeOH, 98:2), containing three Dragendorff-positive spots in TLC, were combined (160 mg) and resubmitted to silica gel CC (19 g) with $CHCl_3$ containing increasing amounts of MeOH (up to 4%) to yield **3** (20 mg), **4** (75 mg) and **5** (45 mg). Fraction 8 ($CHCl_3$:MeOH, 98:2) consisting of two alkaloids, was evaporated (55 mg) and submitted to preparative TLC ($CHCl_3$ MeOH, 98:2, two elutions) to yield **5** (20 mg) and **6** (25 mg). The EtOAc fraction (1 g) was applied to a silica gel column (80 g) and eluted with toluene containing increasing amounts of EtOAc (up to 50%) to give 12 fractions. Fractions 5–8 (toluene:EtOAc, 4:1) containing only one $FeCl_3$-positive spots in TLC (120 mg) was submitted to preparative TLC (toluene:EtOAc, 2:1) to yield **9** (70 mg).

3.4. Chamaedrine (*1*)

White powder; m.p. 222–223 °C, $[\alpha]_D^{25}$: −122.5 (*c* 0.12 $CHCl_3$). IR (KBr): ν_{max} 3437, 3272, 1640 cm^{-}. HRESIME $([M+H]^+$ at *m*/*z* 608. 32308 ($C_{36}H_{42}N_5O_4$, requires 608.32331). EIMS: *m*/*z* = 607 $[M]^+$, 190, 148 (100%), 135, 130. For ^1H and ^{13}C NMR spectroscopic data (400 MHz, $CDCl_3$ and 100 MHz, $CDCl_3$), and HMBC, see Table 1.

3.5. Hydrolysis and amino acid derivatization

Hydrolysis of chamaedrine (**1**) (3 mg) was performed in a sealed tube at 110 °C with 6 N HCl for 24 h as previously described (Silva et al., 1996). The acidic solution was concentrated and the residue was used to identify the absolute stereochemistry of *N*,*N*-dimethyl phenylalanine. Acid-catalyzed esterification of the resulting amino acids (2 mg) was carried out by the addition of a 1.6 N anhydrous solution of HCl gas in MeOH for 30 min at room temperature (Bayer and Konig, 1969). After removal of the reagents under N_2, the sample was taken up in CH_2Cl_2 (200 μl) and trifluoroacetic anhydride (50 μl). The mixture was kept at room temperature for 30 min and the excess reagent was removed under a dry N_2 stream.

3.6. GC analysis of *N*,*N*-dimethyl phenylalanine

The derivatized amino acid was analyzed by enantioselective capillary GC by employing modified cyclodextrin as chiral stationary phase and by coinjection with standard L- and D, L-amino acids (Bayer and Konig, 1969). *N*,*N*-dimethyl phenylalanine was obtained as previously described (Mcdermott and Benoiton, 1973).

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