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Cyclopeptide alkaloids from the bark of *Waltheria douradinha*

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Abstract

Two peptide alkaloids, waltherine-A and waltherine-B were isolated from *Waltheria douradinha*, together with two known peptide alkaloids, adouetine-Y′ and scutianine-B. Their structures were elucidated on the basis of spectroscopic analyses.

Keywords: *Waltheria douradinha*; Sterculiaceae; Peptide alkaloids; Waltherine

1. Introduction

Chemical studies of some Sterculiaceae species have revealed the co-occurrence of peptide alkaloids compounds (Marchand, Monseur, & Pais, 1968; Pais, Marchand, Jarreau, & Goutarel, 1968; Tschesche, & Reviel, 1968; Servis, Kosak, Tschesche, Frohberg, & Fehlhaber, 1969; Bhakuni, Shukla, & Thakur, 1987). In the course of our current chemical studies on the alkaloidal components of South Brazilian medicinal plants, we have previously reported (Morel, Bravo, Reis, & Rúveda, 1979; Morel, Herzog, & Voelter, 1985; Voelter, & Morel, 1987; Menezes, Mostardeiro, Zanatta, & Morel, 1995; Morel, Machado, & Wessjohann, 1995; Machado, Filho, Morel, & Monache, 1995; Silva et al., 1996; Morel et al., 1998) the isolation of cyclopeptide alkaloids from the bark of *Scutia buxifolia* and of *Discaria febrifuga* (Rhamnaceae). The present paper deals with the isolation and structural elucidation of two new peptide alkaloids, named waltherine-A (**1**) and waltherine-B (**2**), together with the known alkaloids, scutianine-B and adouetine-Y′, which were isolated from the root bark of *Waltheria douradinha* St. Hill (Sterculiaceae), a small shrub of South Brazilian origin, used in traditional medicine for the treatment of various diseases. The elucidation of structures **1** and **2** was achieved through the use of a combination of FAB mass spectrometry and ^{1}H and ^{13}C NMR spectroscopy.

2. Results and discussion

Waltherine-A (**1**) was obtained as a colorless crystalline material. Its FAB-mass spectrum displayed a prominent $[MH]^{+}$ at m/z 535 which, in combination with the ^{13}C-NMR spectroscopy, suggested that **1** had the molecular formula $C_{31}H_{42}N_4O_4$. The base peak appeared at m/z 114 corresponding to $C_7H_{16}N$ suggesting the presence of a *N*,*N*-dimethyl leucine unit, which was confirmed by analysis of the ^{1}H and ^{13}C NMR spectral data (Table 1).

The ^{1}H spectrum ($CDCl_3$, 400 MHz) of **1** showed four methyl doublets, two at δ 1.25 and 0.92 ($J=7.0$ Hz) of the β-hydroxyleucine and two at δ 0.94 and 0.90 ($J=7.0$ Hz) of the *N*,*N*-dimethylated leucine. The C-3 and C-4 methine protons appeared as double doublets at δ 4.96 ($J_{3,17}=2.0$; $J_{3,4}=7.0$ Hz) and δ 4.38 ($J_{4,3}=7.0$; $J_{4,20}=10.0$ Hz), which were due to the α- and β-protons of the β-hydroxyleucine. The C-7 methine proton and the C-29 diastereotopic methylene protons of the phenylalanine appeared as a multiplet at δ

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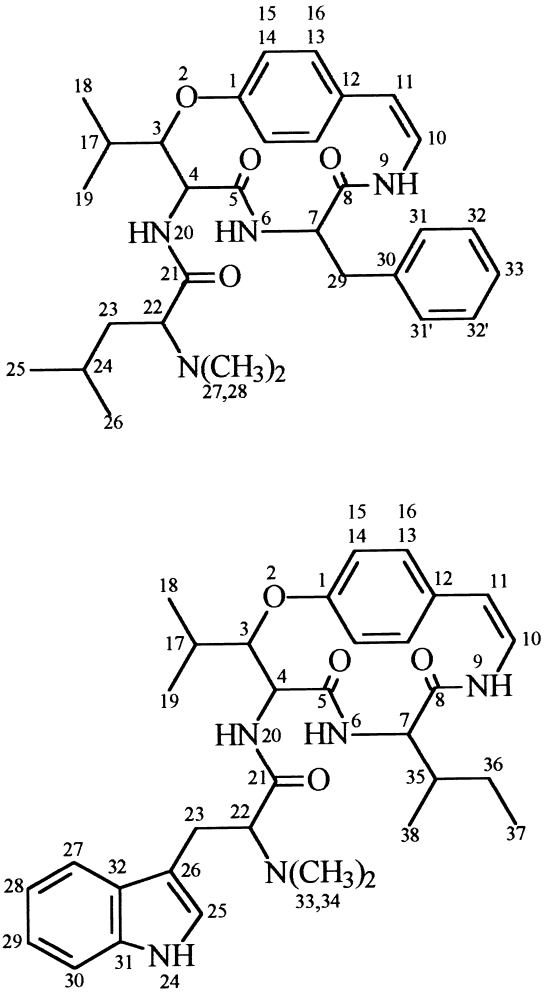

4.46 and two double doublets at δ 3.06 ($J_{29,7}=4.0$; $J_{29,29'}=15.0$ Hz) and δ 2.94 ($J_{29',7}=8.0$; $J_{29',29}=15.0$ Hz), respectively. The C-22 methine proton of the *N*,*N*-dimethylated leucine appeared as a double-doublet at δ 2.48 ($J_{22,23}=6.0$; $J_{22,23'}=8.0$ Hz), whereas the diastereotopics protons at C-23 are identified as multiplets at δ 1.31 and δ 1.49. The C-11 olefinic proton appeared at δ 6.40, overlapping with the signal of the proton at N-9, and the second olefinic proton at C-10 appeared as a double doublet at δ 6.60 ($J_{10,9}=7.5$; $J_{10,11}=7.0$ Hz). The NMR spectrum allowed the assignment of amide protons at δ 6.07 (NH-6), 6.39 (NH-9) and 7.60 (NH-20). Unambiguous assignments of all protons of **1** were made by a series of 2D NMR experiments (COSY and NOESY) and are reported in Table 1. Fig. 1 shows NOE relationships for **1**.

The ^{13}C NMR chemical shifts of **1** were assigned from analysis of the proton noise-decoupled ^{13}C spectroscopy, DEPT 135 and 90° and two dimensional heteronuclear correlated spectroscopy (HMQC and HMBC) and comparison of chemical shifts with those of corresponding data for cyclopeptide alkaloids (Pais et al., 1979; Voelter, & Morel, 1987). In the HMBC spectrum, long-range correlations via ^3J were observed between the following signals: δ_H 6.40 (H-11)-δ_C 131.50 (C-13); δ_H 7.10 (H-13)-δ_C 156.0 (C-1); δ_H 7.16 (H-14)-δ_C 131.60 (C-12); δ_H 7.07 (H-15)-δ_C 131.60 (C-12) and δ_H 6.98 (H-16)-δ_C 156.0(C-1), assigned to the quaternary and methine carbon atoms of the styrylamine unit. Table 1 shows the more relevant connectivities, observed in the HMBC spectra of **1**, that were ascertained by intra- and interresidue heteronuclear correlations.

Compound **2**, designated as waltherine-B, was obtained as a colorless crystalline compound. Its positive ion FAB-mass spectroscopy gave a quasi-molecular ion peak at $m/z=574$, corresponding to $C_{33}H_{43}N_5O_4$. The base peak appeared at $m/z=187$, corresponding to the molecular formula $C_{12}H_{15}N_2$, indicating the presence of *N*,*N*-dimethyl tryptophan unit in waltherine-B. Another peak appeared at $m/z=443$ due to loss of C_9H_8N. The fragment ions at $m/z=130$ (C_9H_8N), 159 ($C_{10}H_{11}N$) and 170 ($C_{11}H_8N$) confirmed the presence of a tryptophan unit in **2**, and the peak at $m/z=135$ (C_8H_9NO) suggested a styrylamine unit.

The ^1H and ^{13}C NMR spectral data and optical rotations of waltherine-B showed that it was a stereoisomer of discarine-A (Mascaretti et al., 1972) and amphybine-A (Tschesche, Kaussmann, & Fehlhaber, 1972).

The ^1H NMR spectrum ($CDCl_3+10\%$ DMSO-d_6, 400 MHz) of **2** showed three sets of doublets, at δ 1.25 ($J_{18,17}=6.8$ Hz), δ 0.93 ($J_{19,17}=6.8$ Hz) and δ 0.52 ($J_{38,35}=7.0$ Hz), which were assigned to C-18, C-19 and C-38 methyl protons respectively. A triplet for the C-37 methyl protons appeared at δ 0.71 ($J_{37,36}=7.4$

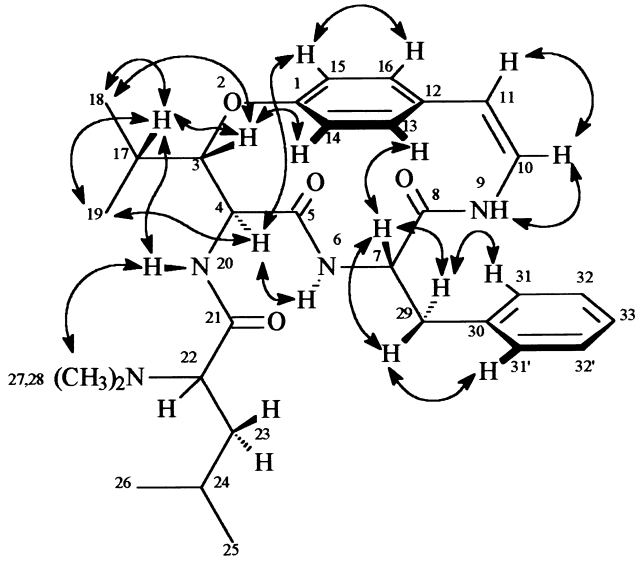

Fig. 1. NOESY correlations of Waltherine-A (**1**).

Table 1
^{1}H and ^{13}C NMR spectral assignments for compound **1** in $CDCl_3$

Position	δ ^{1}H[a] J (Hz)	δ ^{13}C[b]	Significant HMBC (HC)-correlations	
			$^{3}J_{CH}$	$^{2}J_{CH}$
1	–	156.00		
3	4.96 (dd) $J_{3,4}=7$; $J_{3,17}=2$	80.76	5, 18, 19	17
4	4.38 (dd) $J_{4,3}=7$; $J_{4,20}=10$	54.78	21	5, 3
5	–	171.55		
6	6.07 (d) $J_{6,7}=8.0$	–		5
7	4.46 (m)	54.50		8
8	–	167.05		
9	6.39 (d) $J_{9,10}=7.5$	–		
10	6.60 (dd) $J_{10,9}=7.5$; $J_{10,11}=7$	125.53		
11	6.40 (d) $J_{11,10}=7,0$	119.00	13, 16	
12	–	131.60		
13	7.10 (dd) $J_{13,14}=10$; $J_{13,16}=2$	131.50	1	
14	7.16 (dd) $J_{14,13}=10$; $J_{14,15}=2$	122.50	12	
15	7.07 (dd) $J_{15,16}=10$; $J_{15,14}=2$	123.00	12	
16	6.98 (dd) $J_{16,15}=10$; $J_{16,15}=2$	130.00	1	
17	1.88 (m)	29.15		18, 19
18	1.25 (d) $J_{18,17}=7$	20.20	3	
19	0.92 (d) $J_{19,17}=7$	15.00	3	
20	7.60 (d) $J_{20,4}=10$	–		
21	–	173.81		
22	2.48 (dd) $J_{22,23'}=8$; $J_{22,23}=6$	65.97		21, 23
23, 23′	1.31 (m) 1.49 (m)	35.78	25, 26	
24	1.68 (m)	26.16		
25	0.90 (d) $J_{25,24}=7$	23.00		
26	0.94 (d) $J_{26,24}=7$	22.26		
27, 28	2.14 (s)	41.84		
29, 29′	3.06 (dd) $J_{29,7}=4$; $J_{29,29'}=15$ 2.94 (dd) $J_{29',7}=8$; $J_{29',29}=15$	36.44	31, 31′	7, 30
30	7.10–7.30[c]	136.07		
31, 31′	7.10–7.30[c]	128.37		
32, 32′	7.10–7.30[c]	128.77		
33	7.10–7.30[c]	127.66		

[a] Assignments confirmed by 1H–1H COSY and NOESY.
[b] Assignments confirmed by DEPT, HMQC and HMBC.
[c] Peaks occur in the given range, no assignment.

Hz), whereas the C-36 methylene protons were observed as a multiplet at δ 0.85 and δ 1.12. The C-7 methine proton resonated as a double doublet at δ 3.90 ($J_{7,35}=4.0$; $J_{7,6}=8.0$ Hz). The C-23 methylene protons were observed as a two double doublets at δ 3.29 ($J_{23,22}=6.0$; $J_{23,23'}=15.0$ Hz) and δ 3.08 ($J_{23',22}=6.0$; $J_{23',23}=15.0$ Hz), whereas, the C-22 methine proton appeared as a double doublet at δ 3.38 ($J_{22,23}=6.0$; $J_{22,23'}=7.0$ Hz). The C-10 methine proton showed a double doublet at δ 6.55 ($J_{10,11}=8.0$; $J_{10,9}=9.0$ Hz), due to coupling with C-11 and NH-9, while the C-11 methine proton was observed at δ 6.42 ($J_{11,10}=8.0$ Hz). A singlet at δ 9.36 was assigned to the NH-24 of the indole ring. The amide protons NH-6, NH-9 and NH-20 appeared as doublets at δ 6.06 ($J_{6,7}=8.0$ Hz), δ 6.74 ($J_{10,9}=9.0$ Hz) and δ 7.80 ($J_{20,4}=10.0$ Hz), respectively. A singlet at δ 2.48 was assigned to the protons of the *N*,*N*-dimethyl group of the *N*,*N*-dimethyl tryptophan unit. The assignments along with coupling constants (J) of different protons are presented in Table 2 and were further confirmed by the 2D-resolved spectrum and 2D NMR (COSY and NOESY) experiments, which showed prominent cross peaks at the expected positions. Fig. 2 shows the most relevant connectivities, observed in the NOESY spectra of **2**.

The ^{13}C NMR signals of waltherine-B Table 2 revealed similarities with those of discarine-A (Machado et al., 1995) and provided strong evidence for the assigned structure **2**. The spectrum showed 4 methyl signals at δ 10.57, 14.52, 14.93 and 20.19 which were assigned to the C-37, C-38, C-18 and C-19 groups, respectively. Additionally, 15 methine carbons were observed, the upfield signals at δ 30.0 and 34.60 were assigned to C-17 and C-35, whereas the downfield methine signals at δ 54.30, 57.24, 66.87

Table 2
^1H and ^{13}C NMR spectral assignments for compound **2**, in $CDCl_3$ + 10% DMSO-d_6

Position	δ ^1H[a] J (Hz)	δ ^{13}C[b]	Lit; δ ^{13}C[c]	Significant HMBC (HC)-correlations	
				$^3J_{CH}$	$^2J_{CH}$
1	–	155.35	155.6		
3	4.94 (dd) $J_{3,4}=8$; $J_{3,17}=2$	80.65	80.8	5	
4	4.48 (dd) $J_{4,3}=8$; $J_{4,20}=10$	54.30	55.2		5, 3
5	–	170.87	171.7		
6	6.06 (d) $J_{6,7}=8$	–	–	8	5
7	3.90 (dd) $J_{7,6}=8$; $J_{7,35}=4$	57.24	58.5	5	8, 35
8	–	168.74	168.4		
9	6.74 (d) $J_{10,9}=9$	–	–		
10	6.55 (dd) $J_{10,9}=9$; $J_{10,11}=8$	125.39	125.1	12	
11	6.42 (d) $J_{11,10}=8$	115.54	123.7	13, 16	12
12	–	130.50	130.8		
13	6.98	129.68	129.2	1	
14	7.16	119.47	119.5	12	
15	7.12	121.80	121.2	12	
16	7.02	130.20	130.4	1	
17	1.90 (m)	30.0	28.7		18, 19
18	1.25 (d) $J_{18,17}=6.8$	14.93	14.3	3	
19	0.93 (d) $J_{19,17}=6.8$	20.19	19.9	3	
20	7.80 (d) $J_{20,4}=10$	–	–		
21	–	–	172.4		
22	3.38 (dd) $J_{22,23}=6$; $J_{22,23'}=7$	66.87	68.9	26	21
23, 23′	3.29 (dd) $J_{23,22}=6$; $J_{23,23'}=15$ 3.08 (dd) $J_{23',22}=6$; $J_{23',23}=15$	23.50	21.8	25, 32	26
24	9.36 (s)				
25	7.07 (s)	122.93	122.8		
26	–	111.70	111.8		
27	7.55 (d) $J_{27,28}=10$	117.89	117.8	29, 31	
28	7.08	118.55	–		
29	7.12	121.20	121.6		
30	7.35 (d) $J_{30,29}=8$	111.12	111.0	28, 32	
31	–	136.15	136.0		
32	–	126.86	126.7		
33, 34	2.48 (s)	41.12	41.8		
35	1.90 (m)	34.60	35.3		7
36, 36′	0.85 (m) 1.12 (m)	22.50	23.7		35, 37
37	0.71 (t) $J_{37,36}=7.4$	10.57	10.7		
38	0.52 (d) $J_{38,35}=7.0$	14.52	14.9	7	35

[a] Assignments confirmed by 1H–1H COSY and NOESY.
[b] Assignments confirmed by DEPT, HMQC and HMBC.
[c] Data for discarine-A (Pais et al., 1979).

and 80.65 were attributed to C-4, C-7, C-22 and C-3, respectively. The 12 aromatic methine carbons of **2** resonate in the range of δ 110.0–131.0. There were only two methylenic signals appearing in the DEPT experiment at δ 22.50 and 23.50, these being assigned to C-36 and C-23 methylenic carbon atoms. Structure **2** was finally established by detailed analysis of HMQC and HMBC spectra. The result of these analyses revealed long-range correlations via ^2J and ^3J between the following signals: δ_H 6.42(H-11)-δc 129.68 (C-13) and δ_C 130.20 (C-16); δ_H 6.98 (H-13) and δ_H 7.02 (H-16)-δ_C 155.35 (C-1); δ_H 7.16 (H-14) and δ_H 7.12 (H-15)-δ_C 130.50 (C-12); δ_H 3.29 and 3.38 (H-23)-δ_C 111.70 (C-26), δ_C 122.93 (C-25) and δ_C 126.86 (C-32); δ_H 7.55 (H-27)-δ_C 136.15 (C-31) and δ_C 121.20 (C-29); δ_H 7.35 (H-30)-δ_C 126.86 (C-32) and δ_C 118.55 (C-28), assigned to the quaternary and methine carbon atoms of the styrylamine and *N*,*N*-dimethyl tryptophan unit of **2**. These along with all the other connectivities observed in the HMBC spectrum are reported in Table 2.

3. Experimental

3.1. General

Mps are uncorr. MS: 70 eV. FAB-MS were obtained

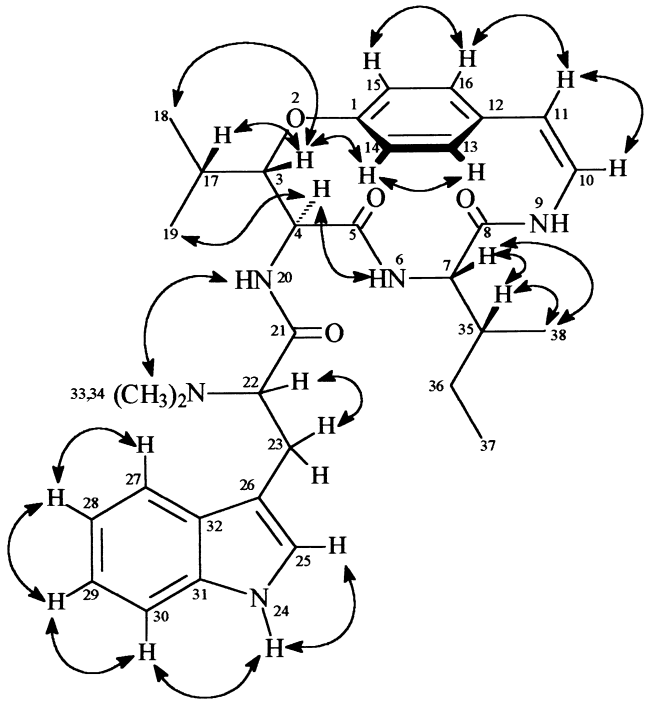

Fig. 2. NOESY correlations of Waltherine-B (**2**).

on a VG analytical 70–150-S mass spectrometer equipped with a FAB ion source from a 3-nitrobenzyl alcohol matrix. The ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker DPX-400 operating at 400 MHz and 100.6 MHz, respectively. Chemical shifts are given in δ (ppm) using TMS as internal standard. Thin layer chromatography (TLC) was performed on pre-coated TLC plates (Merk, silica 60 F-254). The following solvent systems were used: chloroform/methanol (95:5), chloroform/methanol (90:10) and chloroform/methanol/ethyl acetate/ethyl ether (1:0.5:10:20).

3.2. Plant material

Waltheria douradinha was collected in October 1997 in a suburb of Santa Maria, in the state of Rio Grande do Sul, Brazil. A voucher specimen (Gehrke n° 1) is deposited at the Herbarium SMDB of the University of Santa Maria.

3.3. Extraction and isolation

Dried ground root bark (2.8 kg) of *W. douradinha* was extracted exhaustively with hot MeOH as described (Morel et al., 1979), to give, after removal of solvent, 1.7 g of a mixt. of alkaloids. The alkaloids mixt. was fractionated on SiO_2 as described previously (Menezes et al., 1995). Waltherine-A was eluted at $R_f = 0.52$ in $CHCl_3$/MeOH (95:5) and Walterine-B was eluted at $R_f = 0.42$ in $CHCl_3$/MeOH (95:5).

3.4. Waltherine-A (*1*)

Needles from $CHCl_3$–Et_2O, mp 234–235°C, $[\alpha]_D^{20} = -229.8°$ (MeOH, $c = 0.24$). EIMS (m/z) 534 $[M]^+$, 478, 303, 195, 167, 135, 114 (100%), 72. ^{1}H and ^{13}C NMR spectra: Table 1.

3.5. Waltherine-B (*2*)

Needles from $CHCl_3$–MeOH, mp 242–243°C, $[\alpha]_D^{20} = -201.8°$ (MeOH; $c = 0.21$) and $[\alpha]_D^{20} = -356.7°$ ($CHCl_3$; $c = 0.5$). EIMS (m/z): 573 $[M]^+$, 443, 303, 195, 190, 187 [100%], 182, 167, 135,130, 97, 86. ^{1}H and ^{13}C NMR spectra: Table 2.

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