Amaiouine, a Cyclopeptide Alkaloid from the Leaves of *Amaioua guianensis*[#]

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Amaiouine, a cyclopeptide alkaloid, was isolated from the ethanolic extract of the leaves of *Amaioua guianensis.* The structure was elucidated by NMR spectroscopy and confirmed by X-ray crystallography.

As part of our program to assess the chemical and biological diversity of native plants of the Brazilian Cerrado, we have examined promising active extracts of Rubiaceae species concerning their antioxidant potential. Among the assayed extracts, the crude ethanolic extract of *Amaioua guianensis* Aubl., which exhibited a moderate antioxidant activity (IC_{50} 70 μg/mL), was subjected to solvent partitioning and chromatographic separation to provide a new cyclopeptide alkaloid (**1**) and a mixture of two known biflavonoids (**2** and **3**). The structures were assigned by a combination of one- and two-dimensional NMR methods. The cyclopeptide alkaloid (**1**) was crystallized from EtOAc as colorless needles, and this structure has now been confirmed by X-ray analysis. The IR absorptions at 3280, 1660, and 1600 cm^{-1} implied the existence of amine, carbonyl, and aromatic groups. The ^1H NMR spectrum ($CDCl_3$, Table 1) exhibited signals for double bonds, aromatic protons, methine protons due the proton linked to a heteroatom-bearing carbon, and methylene protons. In addition, the ^1H NMR spectrum featured NH signals at δ 5.89 (d), 6.59 (d), and 7.48 (s).

A comparison of ^{13}C NMR, DEPT, and HMQC spectra revealed the presence of 40 carbon resonances, which were attributable to nine quaternary carbons, including four carbonyl groups at δ 171.1, 170.6, 167.1, and 166.2; 27 methine groups with four saturated carbons at δ 82.2, 59.0, 56.1, and 54.3; and four methylene groups at δ 24.7, 26.0, 36.3, and 47.0. The combined use of the 1D and 2D spectra (COSY and HMQC) with literature data[1a−e] allowed us to identify the typical spin systems of the amino acid units and the presence of a *p*-oxygenated *Z*-styrylamine group, as shown in Figure 1.

An HMBC experiment was used to assemble the skeletal fragments through quaternary carbons and heteroatoms. HMBC correlations were observed for H-3 and H-4 to C-5 (δ 171.1) and for H-3 to aromatic carbons (δ 137.5 and 128.4), confirming the presence of the β-substituted phenylalanine unit. The connectivity of C-24 (δ 170.6) to C-25 (δ 59.0) and C-25 to C-26 (δ 26.0) was implied by HMBC correlations for H-25 to C-24 and H-26 to C-24, reaffirming the presence of a proline moiety. HMBC cross-peaks for methylene hydrogens (H-39) to C-7 (δ 54.2) indicated the connectivity between these two carbons, suggesting the presence of an α-phenylalanine unit. The NOESY spectrum identified the presence of a cinnamoyl moiety bearing the proline unit through correlations between H-31 (δ 6.27) and H-28 (δ 3.02) and was also used to confirm the presence of the α-phenylalanine unit by a correlation between H-39 and aromatic signals at δ 7.07. The β-substituted phenylalanine moiety was characterized as *erythro*

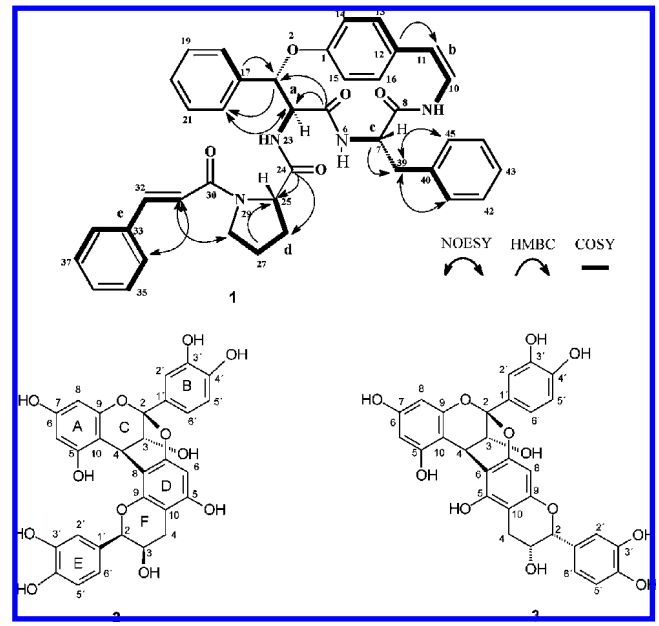

Figure 1. Important HMBC, NOESY, and COSY correlations of amaiouine **1** and structures of the proanthocyanidins **2** and **3**.

by the coupling constant (J = 7.2 Hz) between H-3 and H-4 and the ^{13}C NMR resonance of C-3 (δ 82.2).[1a−e]

Since the ^1H and ^{13}C NMR signals, mainly the aromatic systems, were broadened, further correlations could not be done by the use of 1D and 2D NMR data. Support for the structure and relative configuration of compound **1** was provided by a single-crystal X-ray analysis using direct methods. The ORTEP diagram of the crystal structure of this compound (Supporting Information) confirmed that the cyclopeptide alkaloid skeleton was made up of two units of phenylalanine, a unit of proline bearing a cinnamoyl group, and a styrylamine moiety (Figure 1). At this point, the ^1H and ^{13}C NMR assignments of all carbons and hydrogens were further confirmed by detailed analysis of COSY, HMQC, and HMBC as shown in Table 1 and Figure 1. The X-ray analysis also confirmed the *trans* configuration (3*S**, 4*S**) between H-3 and H-4 and was used to decisively assign a β-orientation (7*S**, 25*S**) to the hydrogens at H-7 and H-25.

Compounds **2** and **3** were isolated as an approximate 1:1 mixture as shown by paired peaks in the NMR spectra. The ^1H NMR spectrum showed the isolated AB coupling systems at δ 4.20 and 4.32 (each d, J = 3.3 Hz)/4.02 and 4.27 (each d, J = 4.0 Hz), oxymethines at δ 4.98/4.70 (brs), and methylenes at δ 2.59 (dd, J = 16.9 and 8.7 Hz) and 2.81 (dd, J = 16.9 and 1.8 Hz), which were attributed to the C/F rings of the flavan-3-ol units. The ketal carbon at δ 100.0/100.1(C−C2) in the ^{13}C NMR spectrum and the

[#] This paper is dedicated to our colleague and friend Clara M. A. Tanaka *in memoriam*.

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Table 1. NMR Spectroscopic Data (300 MHz, $CDCl_3$) for Amaiouine (**1**)

position	δ ^{13}C, mult.	δ ^{1}H (J in Hz)	HMBC	COSY	NOESY
1	155.3, C		H14, H15		
3	82.2, CH	5.92, d (7.2)	H4, H22	H4	H4, H6, H22
4	56.1, CH	4.76, dd (7.2, 2.7)		H23, H3	H3, H22
5	171.1, C		H3, H4		
7	54.3, CH	4.58, m	H39	H39, H6	
8	167.1, C		H9		
10	125.8, CH	6.74, dd (10.0, 7.6)	H11	H9, H11	H9, H11
11	116.3, CH	6.40, d (7.6)		H10	
12	132.6, C		H11		
13	123.7, CH	7.24, m	H11	H11	
14	128.6, CH	7.18, m	H13	H13	
15	130.5, CH	7.08, m		H16	
16	123.7, CH	7.24, m	H11	H15	
17	137.5, C		H3		
18	128.1, CH	7.54, m	H22		
19	126.9, CH	7.07, m			
20	128.9, CH	7.09, m			
21	126.9, CH	7.07, m			
22	128.4, CH	7.37, m	H3		H4, H3
24	170.6, C		H25, H26		
25	59.0, CH	3.90, d (6.9)		H26	H23
26	26.0, CH_2	1.42, m and 2.20, dd (11.5, 5.1)	H25, H28	H25	
27	24.7, CH_2	1.46, m and 1.82, dd (11.5, 5.1)	H25, H28	H28, H26	
28	47.0, CH_2	3.02, t (8.4) and 3.21, m	H25	H27	
30	166.2, C		H31, H32		H28
31	117.5, CH	6.27, d (15.6)		H32	H32, H34
32	143.6, CH	7.50, d (15.6)	H34	H31	H31, H38
33	135.0, C		H31, H32		
34	129.3, CH	7.44, m	H35	H32	H31
35	128.6, CH	7.20, m	H34	H34	
36	130.4, CH	7.09, m		H35	
37	128.6, CH	7.20, m	H38	H38	
38	129.3, CH	7.44, m	H37	H32	H32
39	36.3, CH_2	2.78, dd (15.0, 10.2) 3.37, dd (15.0, 3.6)	H41	H39, H7	H41, H45
40	136.6, C				
41	128.8, CH	7.07, m			H39
42	128.7, CH	7.20, m			
43	132.1, CH	7.15, m			
44	128.7, CH	7.20, m			
45	128.8, CH	7.07, m			H39, H7
NH (6)		5.89, d (10.5)			H4
NH (9)		6.59, d (10.0)		H10	H10, H45
NH (23)		7.48, s		H4	H25
N (29)					

combined use of 1D and 2D NMR with literature data[2] allowed us to identify compounds **2** and **3** as proanthocyanidins A-2 and A-6, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. IR was recorded with an FTIR Bomem MB100 using KBr pellets. NMR spectra were recorded with a Varian Mercury plus BB spectrometer operating at 300 MHz for ^{1}H and at 75.457 MHz for ^{13}C. $CDCl_3$ or acetone-d_6 was used as the solvent, with TMS used as the internal standard. Elemental analysis was carried out using a Perkin-Elmer Series II 2400.

Plant Material. The leaves of *A. guianensis* were collected in Pirenopolis/GO and identified by Dr. Piero G. Delprete. A voucher specimen has been deposited in the herbarium at the Instituto de Ciencias Biológicas/Universidade Federal de Goiás under no. 9312.

Extraction and Isolation. The air-dried and powdered leaves were extracted with EtOH by percolation for 48 h. The resulting extract was concentrated under reduced pressure to give the ethanolic crude extract. This crude extract was dissolved in a mixture of MeOH/H_2O and partitioned with *n*-hexane, CH_2Cl_2, and EtOAc. The CH_2Cl_2 fraction was subjected to successive CC on silica gel 60 using $CHCl_3$/EtOAc mixtures of increasing polarity to give compound **1** (12.8 mg) as a yellow solid. The proanthocyanidins **2** and **3** were isolated as a mixture from the EtOAc fraction after CC on silica gel 60 using EtOAc/MeOH as a solvent.

Scavenging Activity of DPPH Radicals. The potential antioxidant activity of the crude ethanolic extract was determined using 2,2-diphenyl-1-picrylhydrazil (DPPH) free radicals.[3] The reaction mixture (3 mL) contained 2850 μL of the freshly prepared DPPH solution (final concentration 100 ppm) and 150 μL of various concentrations of tested extract or of standard reference (BHT) dissolved in EtOH. After 30 min in the dark at room temperature, the absorbance was recorded at 515 nm. The percentage disappearance (% disp DPPH) DPPH was calculated as follows: % inhibition $= [(A_{DPPH} - A_{Extr})/A_{DPPH}] \times 100$, where A_{DPPH} is the absorbance value of the DPPH blank sample, and A_{Extr} is the absorbance value of the test solution. The antioxidant activity of the tested sample was compared in terms of IC_{50} (concentration of sample required to scavenge 50% DPPH free radicals).

Crystallography Data. Crystals suitable for X-ray analysis were obtained by recrystallization of compound **1** from EtOAc. A colorless needle of approximate dimensions of 0.5 × 0.15 × 0.15 mm^3 was used for analysis. X-ray data were measured on an Enraf-Nonius CAD-4 diffractometer employing graphite-monochromated Cu Kα radiation ($\lambda = 1.5418$ Å) at 293 K and operating in the $\varphi-\omega$ scan mode. Crystal data: $C_{40}H_{36}N_4O_5$, $M = 654.74$, monoclinic, space group $P2_1$, $a = 12.827(2)$ Å, $b = 20.944(4)$ Å, $c = 13.013(2)$ Å, $\beta = 90.26(2)^o$, $V = 3495.9(10)$ Å^3, $Z = 2$, $D_c = 1.244$ g/cm^3, $F(000) = 1384$, and μ(Cu Kα) $= 0.67$ mm^{-1}. All tested crystal samples showed twinning; the collected sample was visually homogeneous but still presented a small degree of pseudomerohedral twinning, causing a reduction in the point group symmetry from 4-fold to 2-fold symmetry (giving two molecules in the asymmetric unit), leading to a worse internal consistency parameter R_{int}. A total of 19 232 reflections were collected (6561 unique, $R_{int} = 0.116$) in the range of 3.4° to 68.31° of θ and index ranges of h = from −15 to 15, k from −24 to 25, and l from −15 to 15. Cell

refinement and data reduction: XCAD4 software suite. The program used to refine structure was SHELXL-97, using refinement on F^2 by full-matrix least-squares calculations. Non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized coordinates and refined as riding atoms with the relative isotropic parameters to their parent atoms. The final refinement gave $R_1 = 0.091$ ($wR_2 = 0.22$) for 4273 observed reflections [with $I > 2\sigma(I)$] and 884 variable parameters, and $R_1 = 0.13$ ($wR_2 = 0.26$) for all unique reflections with GoF = 1.09. Due to the lack of any heavy atom, the absolute configuration is not reliably determined from the X-ray data. The Friedel pairs were merged before the last refinement cycle, and the relative configuration has been identified with respect to the known L-amino acids.

Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 698769. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: C44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

Amaiouine (1): colorless needles (EtOAc); mp 248−249 °C; $[\alpha]^{25}_{D}$ −87.7 (*c* 1.27, CH_3OH); IR (KBr) ν_{max} 3280, 1660, 1600 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) (Table 1); *anal*. C 73.42%, H 5.80%, N 8.48%, calcd for $C_{40}H_{38}N_4O_5$, 73.38%, H 5.85%, N 8.56%.

Mixture of the proanthocyanidins **2** and **3**: brown, amorphous solid; IR (KBr) λ_{max} 3300, 1660 cm^{-1}; ^1H NMR (acetone-d_6, 300 MHz) δ 4.98/4.71 (1H, brs, H-F2), 4.32 (1H, d, $J = 3.3$ Hz, H-C3)/4.02 (1H, d, $J = 4.0$ Hz, H-C3), 4.27 (1H, d, $J = 4.0$ Hz, H-C4)/ 4.20 (1H, d, $J = 3.3$ Hz, H-C4), 4.15 (2H, m, H-F3), 2.81 (2H, dd, $J = 16.9$, 1.8 Hz, H-F4a), 2.59 (2H, dd, $J = 16.9$, 8.7 Hz, H-F4b); ^{13}C NMR (acetone-d_6; 75 MHz) δ 158.0 (C, C-A7), 157.0 (C, C-D5), 156.7/156.2 (C, C-A5), 155.7 (C, C-D5), 154.0/154.1 (C, C-A9), 151.9 (C, C-D9), 151.3/151.7 (C, C-D7), 146.5/146.6 (C, C-B3′), 146.3 (C, C-E4′), 146.1/146.0 (C, C-E3′), 145.7/145.1 (C, C-B4′), 132.4/132.3 (C, C-B1′), 130.9/130.7 (C, C-E1′), 120.8/121.0 (CH, C-B6′), 119.8 (CH, C-E6′), 116.3/116.1 (CH, C-B2′), 116.1 (CH, C-E5′), 115.7 (CH, C-E2′), 115.3 (CH, C-B5′), 106.9 (C, C-D6), 106.7 (C, C-D8), 104.1/103.9 (C, C-A10), 103.3 (C, C-D10), 102.4 (C, C-D10), 100.0/100.1 (C, C-C2), 98.2/98.0 (CH, C-A6), 96.5 (CH, C-D6), 96.7 (CH, C-D8), 96.3 (CH, C-A8), 84.8/81.7 (CH, C-F2), 67.6/67.4 (CH, C-C3), 67.5/66.0 (CH, C-F3), 30.5/29.9 (CH_2, C-F4), 28.9/29.9 (CH, C-C4); EIMS *m/z* 470 (40), 313 (90), 287 (20), 141 (100).

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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