Cassipoureamide-A and -B: New Sulfur-Containing Amides from Stem Wood of *Cassipourea guianensis*

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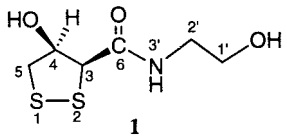
New sulfur-containing amides, cassipoureamide-A (**1**) and -B (**2**), have been isolated from the stem wood of *Cassipourea guianensis*, and their structures were determined to be *cis*-*N*-(2-hydroxyethyl)-4-hydroxy-1,2-dithiolane-3-carboxamide and *cis*-*N*-(2-hydroxyethyl)-5-hydroxy-1,2,3-trithiane-4-carboxamide, respectively, by MS and NMR studies.

As part of our studies to isolate new biologically active compounds from natural resources, we have paid particular attention to the sulfur-containing compounds in Rhizophoraceae plants. Previously, we reported on sulfur-containing alkaloids isolated from the bark of *Cassipourea guianensis* (Rhizophoraceae) collected in Belen, Para, Brazil. These compounds displayed antimicrobial activity or insecticidal activities.[1−3] In this study, an extract of the stem wood of this plant was examined. We report here the isolation and the structural elucidation of two new sulfur-containing compounds.

The stem wood was finely cut and continuously extracted with hot 1-butanol. The 1-butanol extract was concentrated in vacuo and chromatographed on sephadex LH-20, and a fraction rich in sulfur-containing compounds was obtained. This fraction was further subjected to silica column chromatography to yield two compounds.

We named the first new compound "cassipoureamide-A" (**1**). It was isolated as an amorphous powder, mp 105−109° ($CHCl_3$−MeOH). Compound **1** was soluble in pyridine, slightly soluble in MeOH and Me_2CO, and nearly insoluble in $CHCl_3$, AcOEt, and H_2O. HREIMS of **1** revealed a molecular formula of $C_6H_{11}NO_3S_2$. The presence of an amide moiety in **1** was suggested by an IR band at 1648 cm^{-1} and a ^1H NMR signal at δ 7.60 (br s, in $(CD_3)_2CO$). Its ^1H NMR spectrum showed ABXM type signals indicating a 3-substituted 4-hydroxy-1,2-dithiolane ring similar to that of guinesines.[3] HMBC correlations from H-3 and H-4 to the carbonyl carbon (C-6) were observed. The ^1H−^1H COSY, HMQC, HMBC, and the NOESY correlations are shown in Figure 1, and the planar structure of **1** was determined to be *N*-(2-hydroxyethyl)-4-hydroxy-1,2-dithiolane-3-carboxamide. On the basis of linked scan experiments, the mass fragmentation of **1** was explained as shown in the Supporting Information.

To determine the relative configuration of the C(3)−C(4) bond of **1**, ^1H NMR spectra with the NOE of **1**-diacetate in $CDCl_3$ were examined. When H-4 and H-3 were each irradiated, 10.9% and 8.9% of NOE appeared in H-3 and H-4, respectively (Figure 2). On guinesine-A and -B, which have a *cis* relationship at the corresponding bond of the 1,2-dithiolane ring, a 10% NOE appeared between H-3 and H-4.[3] The NOE of guinesine-C,[3] which has a *trans* relationship, was only 3%. Consequently, it was concluded that the C(3)−C(4) bond of **1** must have the *cis* configuration.



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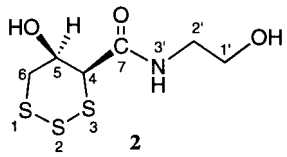

2

We named the second new compound "cassipoureamide-B" (**2**). It was isolated as colorless needles with slight odor, $[\alpha]_D$ −97°(MeOH), mp 109.5−113° ($CHCl_3$−MeOH). The molecular formula was determined to be $C_6H_{11}NO_3S_3$ by HRSIMS. Compound **2** differed from **1** in degree of solubility, being more soluble than **1** in MeOH and Me_2CO. The UV spectrum of **2** exhibited a maximum at 267 nm (log ϵ = 3.18), suggesting the presence of a 1,2,3-trithiane ring.[4] The IR spectrum of **2** was similar to that of **1**, and it showed hydroxyl and amide bands at 3328 and 1634 cm^{-1}. The ^1H NMR spectrum of **2** resembled that of **1**, except for slightly different chemical shifts. On the basis of the above experimental results, the structure of **2** was assigned to be *N*-(2-hydroxyethyl)-5-hydroxy-1,2,3-trithiane-4-carboxamide. All the NMR experimental data of **2** (Figure 3) supported the proposed structure. The ^{13}C NMR signals for the carbons belonging to the trithiane ring were very broadened because of slow flapping inversion of the ring.

When the ^1H NMR of **2** was measured in pyridine-d_5, small signals attributable to **1** were recognized. Therefore, we speculate that **2** is unstable in pyridine solvent and loses a sulfur atom to form **1**. This observation also indicates a *cis* relationship with respect to the C(4)−C(5) bond of cassipoureamide-B (**2**).

From the stem wood of *Cassipourea guianensis*, the other sulfur-containing alkaloids isolated previously from the bark of this plant [1−3] were not detected.

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanaco MP-500D micro melting point apparatus and are uncorrected. IR spectra were obtained on a Hitachi 270-30 IR spectrometer, and UV spectra were measured with a Shimazu UV-240 spectrophotometer. EIMS, CIMS, and linked scan experiments were taken with a Hitachi

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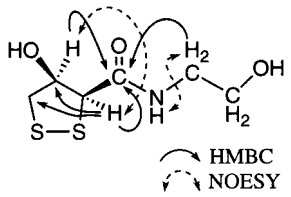

Figure 1. Significant correlations observed in the HMBC and NOESY spectra of cassipoureamide-A (**1**).

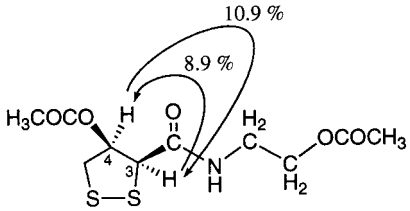

Figure 2. Observed NOEs of **1** diacetate.

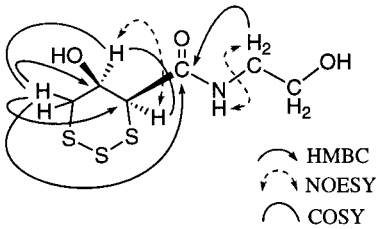

Figure 3. Significant correlations observed in the COSY, HMBC, and NOESY spectra of cassipoureamide-B (**2**).

M-80 or M-4100 spectrometer. SIMS was performed on a Hitachi M-4100 spectrometer. Optical rotations were measured with a Jasco DIP-181 digital polarimeter. All NMR experiments, except NOE, were performed on a Varian VXR-500 spectrometer equipped with 5 mm ^{1}H and ^{13}C probes operating at 499.84 and 125.7 MHz, respectively. Chemical shifts were referenced to internal TMS. NOE difference studies were performed on a Varian XL-200 spectrometer. The following adsorbents were used for purification: column chromatography, Sephadex LH-20; Merck Art 7733 Kieselgel 60 Si-gel (60−230 mesh); TLC plate (for analytical), Merck Art 5715 Kieselgel 60 F_{254} Si-plate.

Plant Material. Stem wood of *Cassipourea guianensis* was collected at Belém Pará in Brazil. The botanical identification was determined by comparing with an authenticated specimen in the herbarium at Musea Goldi Belém Pará in Brazil.

Extraction and Isolation. Stem wood of *Cassipourea guianensis* (3.5 kg) was finely cut and continuously extracted with hot 1-butanol to give 84 g of crude extract. The crude extract dissolved in MeOH was applied to a Sephadex LH-20 column and eluted with MeOH, and five fractions were collected (I−V). A fraction (III) rich in sulfur compounds (9.5 g) was further submitted to silica gel column chromatography ($CHCl_3$−MeOH). Eluate containing 8−10% MeOH gave **2** (25 mg) and then **1** (60 mg).

Cassipoureamide-A (1): white amorphous powder ($CHCl_3$−MeOH); mp 105−109 °C; IR (KBr) ν_{max} 3304 (br), 1648, 1550, 1060 cm^{-1}; ^{1}H NMR ($(CD_3)_2CO$, 499.8 MHz) δ 7.60 (1H, br s, N*H*), 5.06 (1H, m, H-4), 4.09 (1H, d, J = 3.0 Hz, H-3), 3.59 (2H, t like, H-1′), 3.40 (1H, dd, J = 4.6, 11.5 Hz, H-5), 3.31 (2H, m, H-2′), 3.19 (1H, dd, J = 3.5, 11.5 Hz, H-5); ^{1}H NMR (pyridine-d_5, 499.8 MHz) δ 9.23 (1H, br s, N*H*), 5.75 (1H, m, H-4), 4.75 (1H, d, J = 3.3 Hz, H-3), 4.01 (2H, t like, H-1′), 3.77 (2H, m, H-2′), 3.76 (1H, dd, J = 4.5, 11.5 Hz, H-5), 3.52 (1H, dd, J = 4.0, 11.5 Hz, H-5); ^{13}C NMR (pyridine-d_5, 125.7 MHz) δ 171.0 (s, C-6), 80.0 (d, C-4), 63.0 (d, C-3), 60.8 (t, C-1′), 47.5 (t, C-5), 43.1 (t, C-2′); EIMS *m*/*z* 209 [M]$^+$ (43), 210 (7), 211 (5), 134 (100); HREIMS *m*/*z* 209.0191 (calcd for $C_6H_{11}NO_3S_2$, 209.0180), 134.0281 (calcd for $C_4H_8NO_2S_1$, 134.0275), 116.0170 (calcd for C_4H_6NOS, 116.0170), 103.9726 (calcd for $C_3H_4S_2$, 103.9753), 102.9655 (calcd for $C_3H_3S_2$, 102.9675).

Cassipoureamide-A (1) Diacetate. Compound **1** was acetylated in the usual manner[5] by treatment with Ac_2O and pyridine: colorless crystals ($CHCl_3$); mp 42−46.5 °C; $[\alpha]^{23}_D$ +173.9° (*c* 0.89, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3420, 1750, 1685, 1530, 1385, 1250, 1065, 1038 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ϵ) 244 (2.87), 280 (sh) (2.45), 320 (2.28) nm; ^{1}H NMR ($CDCl_3$, 499.8 MHz) δ 7.08 (1H, br s, N*H*), 6.06 (1H, m, H-4), 4.21 (1H, d, J = 2.0 Hz, H-3), 4.16 (2H, m, H-1′), 3.60 (2H, m, H-2′), 3.49 (1H, dd, J = 5.0, 12.5 Hz, H-5), 3.23 (1H, dd, J = 3.0, 12.5 Hz, H-5), 2.08 (3H, s, 4-OCOC*H*$_3$), 2.07 (3H, s, 2′-OCOC*H*$_3$); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 171.1 (s, 2′-O*C*OCH$_3$), 170.2 (s, 4-O*C*OCH$_3$), 167.4 (s, C-6), 80.9 (d, C-4), 62.7 (t, C-1′), 62.7 (d, C-3), 44.2 (t, C-5), 39.3 (t, C-2′), 21.0 (q, 4-OCO*C*H$_3$), 20.9 (q, 2′-OCO*C*H$_3$); CIMS (isobutane) *m*/*z* 294 [M + H]$^+$ (100); HRCIMS *m*/*z* 294.0488 (calcd for $C_{10}H_{16}NO_5S_2$, 294.0470).

Cassipoureamide-B (**2**)**:** colorless crystals ($CHCl_3$−MeOH); mp 109.5−113 °C; $[\alpha]^{23}_D$ −97° (*c* 0.71, MeOH); IR (KBr) ν_{max} 3328 (br), 1634, 1556, 1426, 1358, 1060, 1032, 640 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 206 (4.03), 223 (sh) (3.78), 267 (3.18); ^{1}H NMR ($(CD_3)_2CO$, 499.8 MHz) δ 7.56 (1H, br s, N*H*), 4.11 (1H, br s, H-5), 3.83 (2H, d, J = 9.5 Hz, H-6), 3.58 (2H, br s, H-1′), 3.31 (2H, m, H-2′), 3.10 (1H, br t like, H-4); ^{13}C NMR ($(CD_3)_2CO$, 125.7 MHz) δ 168.8 (s, C-7), 68.9 (d, br, C-5), 61.4 (t, C-1′), 60.4 (t, br, C-6), 43.0 (t, C-2′), 42.1 (d, br, C-4); positive SIMS (matrix: 3-NOBA) *m*/*z* 242 [M + H]$^+$; HRSIMS *m*/*z* 241.9989 (calcd for $C_6H_{12}NO_3S_3$, 241.9979).

Supporting Information Available: Scheme for MS fragmentation of cassipoureamide-A (**1**) confirmed by linked scan experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Kato, A.; Okada, M.; Hashimoto, Y. *J. Nat. Prod.* **1984**, *47*, 706−707.
(2) Kato, A.; Okada, M.; Hashimoto, Y. *J. Nat. Prod.* **1985**, *48*, 289−292.
(3) Kato, A.; Ichimaru, M.; Hashimoto, Y.; Mitsudera, H. *Tetrahedron Lett.* **1989**, *30*, 3671−3674.
(4) Kato, A.; Hashimoto, Y.; Otsuka, I.; Nakatsu, K. *Chem. Lett.* **1978**, 1219.
(5) Bianco, A.; Passacantilli, P.; Polidori, G. *Planta Med.* **1982**, *46*, 38−41.

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