

SULFUR-CONTAINING ALKALOIDS FROM *CASSIPOUREA GUIANENSIS*

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ABSTRACT.—New sulfur-containing alkaloids, **1** and **2**, were isolated from the bark of *Cassipourea guianensis* and identified as 1-methyl-2-(4'-hydroxy-1',2'-dithiolan-1'-oxo-3'-yl)-5-(4'-hydroxy-1'',2''-dithiolan-3''-yl)pyrrolidine and 1-methyl-2,5-bis-[4'(4'')-hydroxy-1'(1''),2'(2'')-dithiolan-1'(1'')-oxo-3'(3'')-yl]pyrrolidine, respectively.

The sulfur-containing pyrrolidine alkaloid, gerrardine (**3**), from the bark of *Cassipourea gerrardii* and *Cassipourea guianensis* Aubl. (Rhizophoraceae) has already been reported in previous papers (1-3). In the present paper, the isolation and structural elucidation of two new minor alkaloids (**1** and **2**) from *C. guianensis* is described. Evidence for the structures was obtained by spectral studies and comparison with derivatives from an authentic sample of gerrardine (**3**).

RESULTS AND DISCUSSION

The dried bark of *C. guianensis* (2 kg) was extracted with petroleum ether and 80% MeOH. The concentrate from 80% MeOH was extracted with 2% aqueous citric acid and partitioned with C₆H₆. The aqueous extract was basified with 10% Na₂CO₃ and extracted with CHCl₃; the CHCl₃ extract was dried over anhydrous Na₂SO₄, and the solvent was removed to give the crude alkaloids (4.5 g). First, a major component, gerrardine (**3**), in the crude alkaloidal extract was separated by chromatography on a silica gel column with C₆H₆-Et₂O-CHCl₃-MeOH (10:10:3:3). On further treatment with a second eluant, Me₂O-EtOAc (1:1), the column furnished compound **1**, which was crystallized from CHCl₃-MeOH as colorless crystals. Continued elution with the same solvent system yielded compound **2** which was crystallized from CHCl₃-MeOH as white needles.

In order to elucidate the structures of both compounds, **1** and **2**, a comparative study of spectral data among **1**, **2**, and a known compound, gerrardine (**3**), was carried out as follows. The molecular formulas C₁₁H₁₉O₃NS₄ and C₁₁H₁₉O₄NS₄ were advanced for **1** and **2**, respectively, on the basis of fdms. Their fdms gave (M⁺ + H) signals at *m/z* 342 and 358 which are in accordance with each formula (C₁₁H₁₉O₃NS₄ + H, C₁₁H₁₉O₄NS₄ + H) corresponding to mono- and di-oxidation products of gerrardine (C₁₁H₁₉O₂NS₄). It is assumed that **1** and **2** are biogenetically related to gerrardine (**3**) by differences in their oxidation levels.¹ In their fdms, both **1** and **2** exhibited significant strong peaks at *m/z* 220, which are probably due to the 1-methyl-5-(4-hydroxy-1,2-dithiolan-1-oxo-3-yl)dehydropyrrolidinium cation (C₈H₁₄NO₂S₂)⁺ formed from the cleavage shown in Figure 1. The ir spectra of **1** and **2** showed the presence of both hydroxyl groups [*ν* max (KBr) 3400-3450 cm⁻¹] and sulfoxide groups (1035, 1045, 1050 cm⁻¹), respectively. The uv spectrum of **1** showed absorptions characteristic of 1,2-dithiolane [*λ* max (MeOH) 330 nm, log *ε* 2.17] (4) and 1,2-dithiolan-oxide [*λ* max (CHCl₃) 247 nm, log *ε* 3.57] (5, 6), while the uv of **2** revealed only the absorption

¹The possibility that the alkaloids **1** and **2** are artifacts of the isolation process was excluded by an experiment in which O₂ was bubbled into a solution of 3 mg of gerrardine (**3**) in MeOH-CHCl₃ (1:1) at room temperature for 8 h. The alkaloid was not oxidized to **1** or **2** under these conditions. After the solution had been made acidic with 3% aqueous citric or basified with 5% aqueous NH₃, the supply of O₂ to the solution was continued overnight, but **3** remained unchanged.

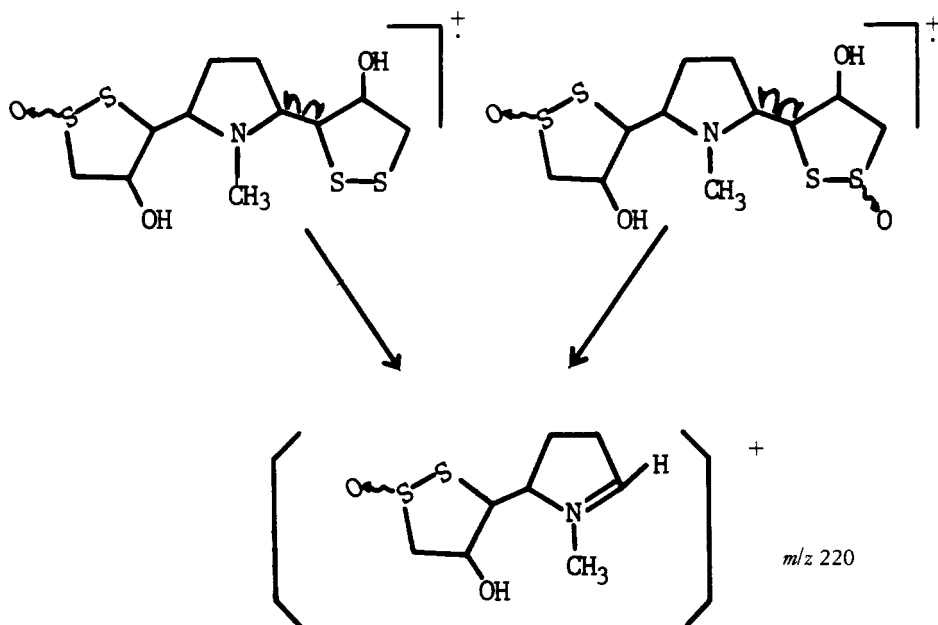


FIGURE 1. Fdms of compounds **1** and **2**.

of 1,2-dithiolan-1-oxide [λ max (CHCl₃) 247 nm, log ϵ 3.83]. The nmr spectra of the two 1,2-dithiolane rings in **2** showed magnetic equivalence from their signal patterns, while those of **1** exhibited magnetic nonequivalence. From these properties of ms, ir, uv, and nmr spectra, it was evident that **2** and **1**, as well as **3**, have in common 2,5-bis-(1,2-dithiolan-3-yl)pyrrolidine as a parent skeleton for their structure. Direct proof of this assignment was achieved by converting gerrardine (**3**) with NaIO₄ to compounds **1** and **2**, identified by comparison of tlc and ms data with the natural products.

In compound **2**, the possibility that both additional oxygen atoms were bonded to the same dithiolane ring is excluded because the nmr spectra of the 1,2-dithiolane rings exhibited magnetic equivalence with each other as shown in Figure 2. Thus, both dithiolane rings of **2** contain the same sulfinyl group (-S-SO-). The sulfoxide of the sulfinyl group must be either an S₁-oxide or an S₂-oxide, and assignment of the S-position was carried out by analysis of the coupling constants of the CH₂-protons adjacent to the sulfur atoms. The geminal coupling constant (J_{gem}) of CH₂-protons adjacent to sulfoxide generally is slightly larger than those of CH₂-protons adjacent to an unoxidized sulfur atom, that is, in both **1** and **2**, the inductive removal of the electrons from the CH₂ by the S=O group should make the J -value more positive (7). In the ¹H-nmr spectrum of **1**, the geminal coupling constant ($J_{5'A,B} = 13.1$) of the CH₂-protons [δ , 3.05(H-5'A); 4.04(H-5'B)] linked to sulfoxide was larger by 1.6 Hz than the geminal coupling constant ($J_{5''A,B} = 11.5$) of the CH₂-protons [δ , 3.24(H-5''A); 3.31(H-5''B)] adjacent to the unoxidized sulfur atom. The appearance of the H-5'B' signal at an extraordinary low field was readily understandable in terms of the anisotropic deshielding effect of the sulfoxide group. The anisotropic effect appears equally in the ¹³C nmr of these compounds, in which the signal for C-5' was shifted to a lower field (ca. 21 ppm) than that for C-5''.

From the coupling constant and chemical shift arguments described above, it was determined that all of the oxidized sulfur atoms of **1** and **2** are the outside atoms on the molecular structure, viz. S_{1'}, for both **1** and **2**. These results thus support the assignment of the structures of **1** and **2** as 1-methyl-2-(4'-hydroxy-1',2'-dithiolan-1'-oxo-

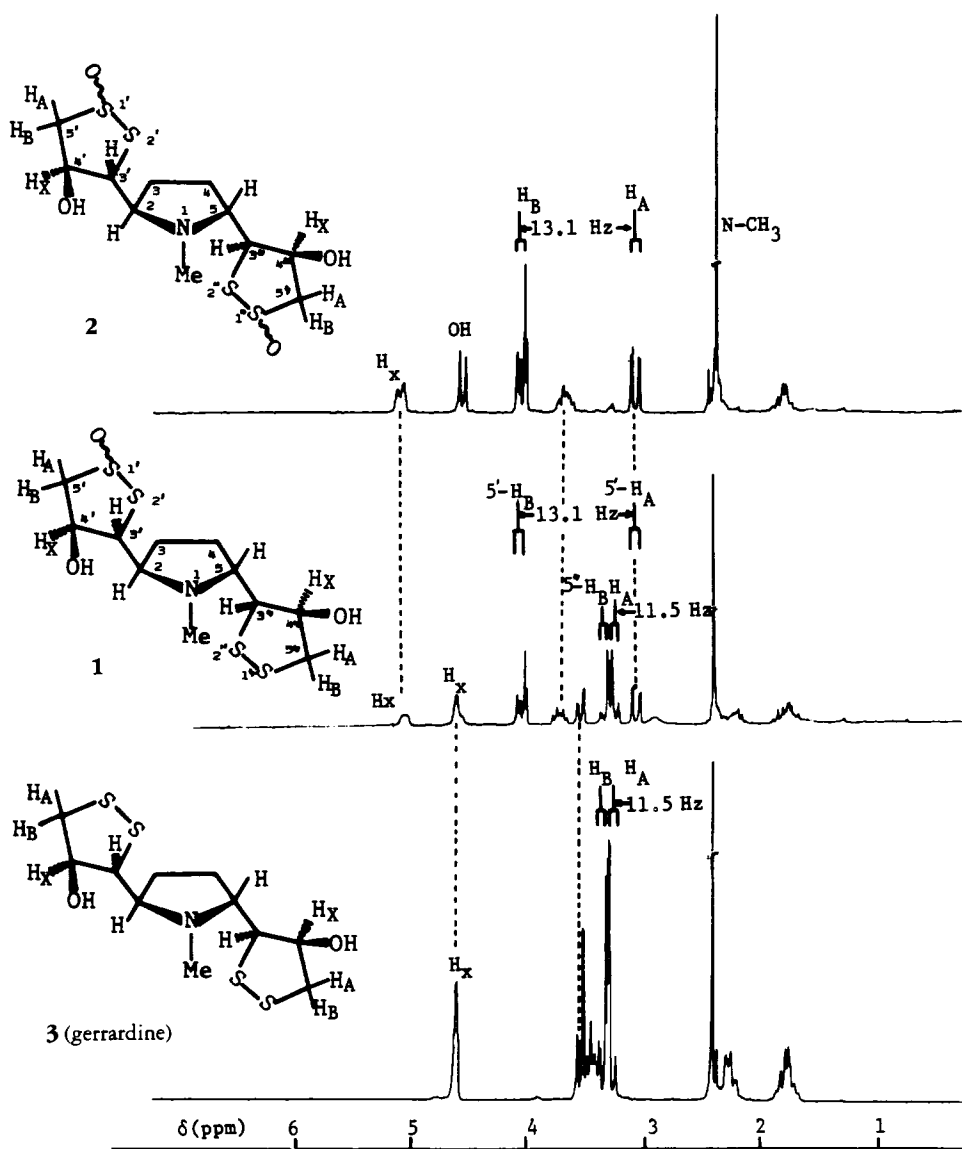


FIGURE 2. 200 MHz ^1H -nmr spectra of **1**, **2**, and **3** in CDCl_3 .

3'-yl)-5-(4''-hydroxy-1'',2''-dithiolan-3''-yl)pyrrolidine and 1-methyl-2,5-bis-[4'(4''-hydroxy-1'(1''),2'(2'')-dithiolan-1'(1'')-oxo-3'(3'')-yl)pyrrolidine, respectively.

Further studies on configurations of sulfoxides of these compounds are in progress.

TABLE 1. The Influence of an Adjacent S Atom on J_{gem} (Hz) in CH_2 -Groups

Compound	Methylene proton	Chemical Shift (δ)	J_{gem} (Hz)
1	-S- CH_2 -	3.24 (H-A), 3.31 (H-B)	11.5
	-SO- CH_2 -	3.05 (H-A), 4.04 (H-B)	
2	(-SO- CH_2) ₂	3.07 (H-A), 4.05 (H-B)	13.1
3	(-S- CH_2) ₂	3.26 (H-A), 3.31 (H-B)	11.5

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Decomposition points were determined in a Yanagimoto apparatus and are uncorrected. Spectra were recorded with the following instruments: uv, Hitachi 124; ir: Hitachi 215; nmr, Varian XL-200 (200 MHz and 50 MHz for ^1H nmr and ^{13}C nmr, respectively); ms, Hitachi M-80 operating at 70 eV. Optical rotation was measured on a Model DIP 181 digital polarimeter for Japan Spectroscopic Co., Ltd.

PLANT MATERIAL.—*C. guianensis* was collected at Belém Pará in Brazil. The plant was authenticated as previously described (3). The bark of the tree was dried for the investigation.

EXTRACTION AND ISOLATION.—The plant material was extracted and the alkaloids isolated as described in the previous section. The quantities of alkaloids obtained from 2 kg of the dried bark were 32 mg (0.0016%) of **1**, 20 mg (0.001%) of **2**, and 200 mg (0.01%) of **3**, respectively.

IDENTIFICATION OF 1-METHYL-2-(4'-HYDROXY-1',2'-DITHIOLAN-1'-OXO-3'-YL)-5-(4"-HYDROXY-1",2"-DITHIOLAN-3"-YL)PYRROLIDINE (**1**).—The compound has colorless crystals, decomp. 183-185°; $[\alpha]^{24\text{D}} + 140.66$ ($c=0.39$, in CHCl_3); fdms 70 eV [m/z 342 ($\text{M}^+ + \text{H}$, $\text{C}_{11}\text{H}_{19}\text{O}_3\text{NS}_4 + \text{H}$), 220, ($\text{C}_8\text{H}_{18}\text{NO}_2\text{S}_2$) $^+$]; ir ν max (KBr) cm^{-1} 340 (OH), 1035-1050 (-S-S-O); uv λ max (CHCl_3) nm (log ϵ) 330 (2.17) (1,2-dithiolane), 247 (3.56) (1,2-dithiolan-1-oxide); ^1H nmr (200 MHz, CDCl_3) δ 1.74 (2H, m, H-3A, -4A), 2.26 (2H, m, H-3B, -4B), 2.38 (3H, s, H-6), 3.05 (1H, dd, H-5'A), 3.24 (1H, dd, H-5''A), 3.31 (1H, dd, H-5''B), 3.52 (1H, dd, H-3'', $J_{3'',5} = 10.5$, $J_{3'',4''\text{X}} = 2.6$), 3.70 (1H, m, H-5), 4.02 (1H, dd, H-3', $J_{3',2} = 11.0$, $J_{3',4'\text{X}} = 3.5$), 4.04 (1H, dd, H-5'B), 4.60 (1H, bs, H-4''X), 5.05 (1H, bs, H-4'X), $J_{\text{gem } 5''\text{A,B}} = 11.5$, $J_{5''\text{A,B}} = 13.1$, $J_{5''\text{A},4''\text{X}} = 1.70$, $J_{5''\text{B},4''\text{X}} = 3.10$, $J_{5''\text{A},4'\text{X}} = 3.0$, $J_{5''\text{B},4'\text{X}} = 1.84$; ^{13}C nmr (50 MHz, d_5 -pyridine, assignment by off-resonance) δ 28.56 (t, C-3), 28.62 (t, C-4), 38.91 (q, C-6), 47.05 (t, C-5''), 64.65 (d, C-3''), 66.00 (d, C-2), 67.68 (d, C-5), 68.17 (t, C-5'), 68.70 (d, C-3'), 76.04 (d, C-4''), 79.23 (d, C-4').

IDENTIFICATION OF 1-METHYL-2,5-BIS-[4'(4"-HYDROXY-1'(1''),2'(2''))-DITHIOLAN-1'(1'')-OXO-3'(3'')-YL]PYRROLIDINE (**2**).—The compound has white crystals, decomp. 190-193°; fdms [m/z 358 ($\text{M}^+ + \text{H}$, $\text{C}_{11}\text{H}_{19}\text{O}_4\text{NS}_4 + \text{H}$), 220 ($\text{C}_8\text{H}_{14}\text{NO}_2\text{S}_2$) $^+$]; ir ν max (KBr) cm^{-1} 3400 (OH), 1045 (-S-S-O); uv λ max (CHCl_3) nm (log ϵ) 247 (3.83) (1,2-dithiolan-1-oxide); ^1H nmr (200 MHz, CHCl_3) δ 1.78-2.38 (4H, m, H-3A, -3B, -4A, and -4B), 2.37 (3H, s, H-6), 3.07 (2H, dd, H-5', -5''A), 3.66 (2H, m, H-2, -5), 4.04 (2H, dd, H-3', -3''), $J_{3',2} = 11.0$, $J_{3',4'\text{X}} = 3.6$, 4.05 (2H, dd, H-5'B, -5''B), 4.55 (2H, d, OH), 5.10 (2H, m, H-4', -4''), $J_{\text{gem } 5'(5'')\text{A,B}} = 13.1$, $J_{5'(5'')\text{A},4'(4'')\text{X}} = 3.0$, $J_{5'(5'')\text{B},4'(4'')\text{X}} = 1.6$ Hz; ^{13}C nmr (50 MHz, d_5 -pyridine, assignment by inept.) δ 28.66 (t, C-3, -4), 39.34 (q, C-6), 67.17 (d, C-2, -5), 67.86 (d, C-3', -3''), 68.07 (t, C-5', -5''), 79.74 (d, C-4', -4'').

OXIDATION OF GERRARDINE (**3**) WITH SODIUM METAPERIODATE.—A solution of 42.8 mg (0.2 mmol) of sodium metaperiodate in 3 ml of H_2O was added dropwise to a solution of 20.6 mg (0.1 mmol) of gerrardine (**3**) in 4 ml of $\text{MeOH}-\text{CHCl}_3-\text{CH}_3\text{CN}$ (6:4:2) at -2° to 0° . The resulting white slurry was stirred at 0° to 2° for 4 h and then was allowed to stand at 3° for 1 h. The precipitated sodium iodate was removed by filtration, the filtrate was removed in vacuum, and then the residue was extracted with CHCl_3 . The CHCl_3 solution was dried over anhydrous Na_2SO_4 and the solvent removed to give pale yellow solid (18.0 mg). Tlc analysis on silica gel with $\text{Me}_2\text{CO}-\text{EtOAc}-\text{H}_2\text{O}$ (5:5:1) of the material showed it to contain six oxidation products, and two alkaloidal spots ($\text{Rf}=0.48$ and 0.30) had identical Rf values as **1** and **2**, respectively. Mass spectra data [m/z 342 ($\text{M}^+ = \text{H}$ for $\text{C}_{11}\text{H}_{19}\text{O}_3\text{NS}_4 + \text{H}$), 220 ($\text{C}_8\text{H}_{14}\text{NO}_2\text{S}_2$) $^+$], and m/z 358 ($\text{M}^+ + \text{H}$ for $\text{C}_{11}\text{H}_{19}\text{O}_4\text{NS}_4 + \text{H}$), 220 ($\text{C}_8\text{H}_{14}\text{NO}_2\text{S}_2$) $^+$] of the compounds from these two spots isolated by silica gel preparative tlc with $\text{Me}_2\text{CO}-\text{EtOAc}-\text{H}_2\text{O}$ (5:5:1) were also identical with those of **1** and **2**, respectively.

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