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GUINESINE-A, -B AND -C: NEW SULFUR CONTAINING INSECTICIDAL ALKALOIDS FROM *CASSIPOUREA GUIANENSIS*

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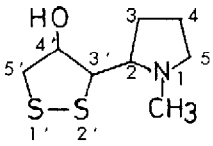
Abstract: New sulfur containing insecticidal alkaloids, guinesine-A (1a), -B (1b) and -C (1c) have been isolated from the bark of *Cassipourea guianensis* and their structures determined by spectroscopic studies and X-ray analyses.

In the course of our experiments to isolate new biologically active compounds from tropical plants, we investigated extracts of the bark of *Cassipourea guianensis* (Rhizophoraceae) collected in Belém, Pará, Brazil and discovered a series of new alkaloids which had similar molecular structures. We discuss here the determination of structures of these alkaloids mainly by ^{1}H- and ^{13}C-NMR spectra with decoupling, NOE techniques and X-ray analyses.

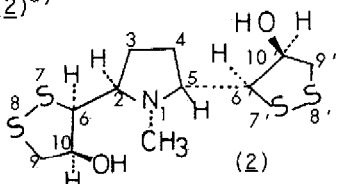
The bark was extracted with 80% methanol continuously at 60°C, and the extract was treated for alkaloids in a conventional way. The tertiary alkaloidal fraction on silica gel column chromatography and preparative TLC gave three known alkaloids, viz. gerrardine (2)[1] and gerrardine sulfoxides[2], and three new alkaloids which we named guinesine-A (g-A), guinesine-B (g-B) and guinesine-C (g-C).

G-A, -B and -C were isolated in ca. a 6:3:1 ratio with a 0.0015% combined yield. G-A was isolated as a yellowish oil, $[\alpha]_D^{24}$ +80.5°(c=0.71, $CHCl_3$), g-B as needles (mp. 61-2°C), $[\alpha]_D^{24}$ -36.5°(c=0.34, $CHCl_3$) and g-C as needles (mp. 76-7°C), $[\alpha]_D^{24}$ -4.8°(c=0.48, $CHCl_3$). High resolution mass spectra revealed that these compounds all had the same molecular formula of $C_8H_{15}NOS_2$ [HR-MS (CI, isobutane); Calcd. for QM$^+$ $C_8H_{15}NOS_2$+H 206.0672: Found 206.0662 for g-A, 206.0679 for g-B and 206.0681 for g-C] with very similar UV, IR and NMR spectra. IR spectra showed that the guinesines had a hydroxy group [$\nu_{max}^{CHCl_3}$ cm^{-1};3600-3000]. Their UV spectra had the same chromophores, 330 nm (1,2-dithiolane ring) and 242 nm (N-methylpyrrolidine ring), as gerrardine (2). Their ^{1}H-NMR spectra showed that they are structurally similar to the gerrardine (Table 1). The presence of typical ABXM type signals based on the partial structure -C(5')H_2-C(4')H(OH)-C(3')H- indicated that the guinesines have a 3-substituted-4-hydroxy-1,2-dithiolane ring. Furthermore, they had one N-methyl signal assumed to represent an N-methylpyrrolidine ring.

Table 1. ^{1}H-NMR assignments for guinesines (1) and gerrardine (2)[a)]



(1)



(2)

	guinesine-A	guinesine-B	guinesine-C		gerrardine
3 -H_2	1.96(2H,m)	2.00(m),2.16(m)	1.80(4H,m)	3 -H_2	1.73,2.23(each 2H,m)
4 -H_2	1.84(2H,m)	1.83(2H.m)		4 -H_2	
5 -H_a	2.30(m)	2.39(m)	2.35(m)	5 -H	3.43(2H.m)
5 -H_b	3.15(m)*	3.14(m)	3.08(m)	2 -H	
2 -H	2.86(m)	2.93(m)	2.85(m)		
N-CH_3	2.44(s)	2.47(s)	2.44(s)	N-CH_3	2.83(3H,s)
5'-H_a	3.13(dd,$J_{5',5}$=11, $J_{5'a,4'}$=7.5)	3.21(dd,$J_{5',5}$=11, $J_{5'a,4'}$=3)	3.19(dd,$J_{5',5}$=11.5, $J_{5'a,4'}$=5)	9&9' -Ha	3.26 [2H,dd, $J_{9,9(9',9')}$=11.5, $J_{9a,10(9'a.10')}$=2]
5'-H_b	3.39(dd,$J_{5',5}$=11, $J_{5'b,4'}$=6.2)	3.31(dd,$J_{5',5}$=11, $J_{5'b,4'}$=4)	3.29(dd,$J_{5',5}$=11.5, $J_{5'b,4'}$=4.8)	9&9' -Hb	3.31 [2H.dd, $J_{9,9(9',9')}$=11.5, $J_{9b,10(9'b,10')}$=3]
3'-H	3.58(dd,$J_{3',4'}$=7.8, $J_{3',2}$=4.5)	3.64(dd,$J_{3',4'}$=4.2, $J_{3',2}$=7.4)	3.52(dd,$J_{3',4'}$=5, $J_{3',2}$=7.2)	6&6' -H	3.52 [2H,dd, $J_{6,2(6'.5)}$=10. $J_{6,10(6',10')}$=2.5]
4'-H	4.40(q-like)	4.77(m)	4.46(q-like)	10&10' -H	4.60(2H,m)
OH	4.10(bs)				

* overlapped with 5'-H_a

a) Chemical shifts ($CDCl_3$) in ppm(δ), relative to internal TMS, coupling constants in Hz on Varian XL-200 spectrometer. The assignments are on the basis of ^{1}H-^{1}H selective decoupling experiments.

The ratio of the signals belonging to the 4-hydroxy-1,2-dithiolane ring and the N-methylpyrrolidine ring was 2:1 in gerrardine but was 1:1 in the guinesines. The two C(5)-protons have appeared in the guinesines, due to the lack of a C(5)-substituent. The CI-MS(isobutane) [m/z; 206($M+H)^+$, 84 (100%) for g-A, -B and -C] and EI-MS spectra [m/z; 205(M^+, less than 1%), 84 (100%) for g-A, -B and -C] showed a common base peak m/z 84 whose composition (HR-MS; Calcd. for $C_5H_{10}N$ 84.0812: Found 84.0808 for g-A, 84.0819 for g-B and 84.0786 for g-C) corresponded to the 1-methyl-2-dehydropyrrolidium cation.

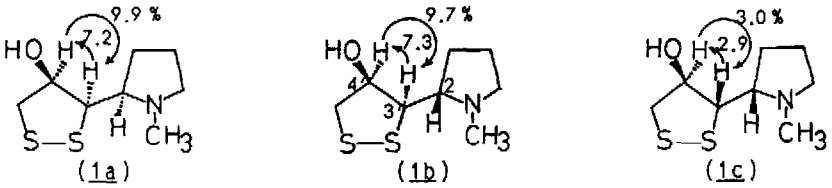
Their ^{13}C-NMR spectra each exhibited eight signals [g-A: 24.53(4-C), 26.44 (3-C), 41.41(N-Me), 42.56(5'-C), 57.04(3'-C), 57.50(5-C), 65.79(2-C), 78.27 (4'-C). g-B: 22.86(4-C), 30.41(3-C), 42.49(N-Me), 46.86(5'-C), 62.78(3'-C), 57.20(5-C), 67.05(2-C), 77.43(4'-C). g-C: 23.52(4-C), 28.73(3-C), 42.68 (N-Me), 45.52(5'-C), 63.68(3'-C), 56.61(5-C), 68.02(2-C), 79.71(4'-C).].

On the basis of the spectral data described above, we determined the planar structure of the guinesines to be 1-methyl-2-(4'-hydroxy-1',2'-dithiolan-3'-yl)-pyrrolidine (1).

1-Methyl-2-(4'-hydroxy-1',2'-dithiolan-3'-yl)pyrrolidine has four possible isomers, i.e. 2R3'R4'R/SSS (3',4'-*cis*-2,3'-*erythro*), RRS/SSR (3',4'-*trans*-2,3'-*erythro*), RSS/SRR (3',4'-*cis*-2,3'-*threo*) and RSR/SRS (3',4'-*trans*-2,3'-*threo*).

To determine the stereo-structures of the guinesines, ^{1}H-NMR with NOE of natural guinesines and X-ray analyses of synthetic (±)-guinesines were examined. It is noted that NOE data give only information to determine the relative configurations of the hydroxy group of the 1,2-dithiolane ring, and the N-methylpyrrolidine group of each isomer. When C(4')-H of g-A and g-B were irradiated, 9.9% and 9.7% of NOE appeared in C(3')-H of both isomers (1a, 1b), respectively. On the other hand, irradiation of the frequency at C(4')-H of g-C was effected by only 3% of NOE in C(3')-H of g-C (1c). These observation therefore prove a *cis* relationship between the C(4')-hydroxy group and the C(3')-pyrrolidine group in g-A and g-B, and a *trans* relationship in g-C. X-Ray analyses of synthetic (±)-guinesines were executed to establish the relative configurations of C(2)-C(3') which remain unsolved with NMR experiments because of the free rotation of the C(2)-C(3') single bond. Syntheses of guinesines were accomplished by condensation of 1,3-bis-(benzylthio)-2-propanone with 1-methyl-2,2-diethoxypyrrolidine, affording (±)-g-A, -B and -C in the ratio of 12:65:23 in a good yield[3]. These synthetic (±)-g-A, -B and -C were identified using physical, chromatographical and spectral data of their respective natural guinesines, except for optical rotation.

The molecular structures based on X-ray single-crystal analyses in crystals of *p*-bromophenylcarbamates (1b' and 1c') of (±)-g-B and (±)-g-C are shown in Fig. 2. These reveal that the relative configurations of C(2)-C(3')



(1a) (1b) (1c)

Fig. 1 NOE of C(3')-H and (4')-H

of g-B (1b) and g-C (1c) are *threo* and *erythro*, respectively, and that those of C(3')-C(4') of g-B and -C, are *cis* and *trans*, respectively, the same as the NOE results[4].

Thus, g-B and -C are unequivocally shown to be 3',4'-*cis*-2,3'-*threo* and 3',4'-*trans*-2,3'-*erythro*, respectively, and the 2,3'-configuration of g-A (1a), assumed to possess a 3',4'-*cis* relationship, should be assigned to be *erythro*, because g-A is one of the four relative configurational compounds.

We, therefore, conclude that guinesine-A, -B and -C are 3',4'-*cis*-2,3'-*erythro*, 3',4'-*cis*-2,3'-*threo* and 3',4'-*trans*-2,3'-*erythro* isomers of 1-methyl-2-(4'-hydroxy-1',2'-dithiolan-3'-yl)pyrrolidine, respectively.

Further studies on the absolute configuration of these alkaloids are now in progress.

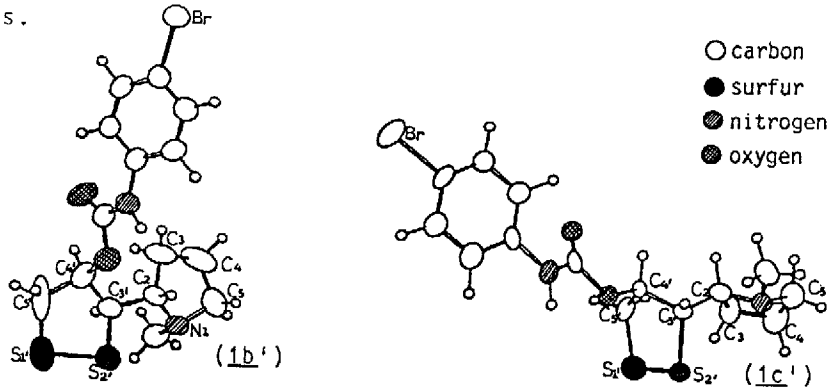


Fig. 2 Perspective views of *p*-bromophenylcarbamates (1b' and 1c') of synthetic g-B and -C

G-A and g-B showed insecticidal activities against larvae of rice stem borer with LD_{50} values of 5.12 μg/insect (g-A) and 1.10 μg/insect (g-B) on topical administration. Their mode of action was similar to that of nereistoxin[5] as judged by the symptoms. Gerrardine showed no activity against the larvae.

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References and Notes

1. Atsushi Kato, Momoyo Okada and Yohei Hashimoto, *J.Nat.Prod.*, 47, 706(1984).
2. Atsushi kato, Momoyo Okada and Yohei Hashimoto, *J.Nat.Prod.*, 48, 289(1985).
3. The detailed synthetic method of (±)-guinesines will be reported elsewhere.
4. Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre. *Crystal data* for (1b'): monoclinic; space group $P2_1/a$; a=11.031(3), b=16.813(4), c=9.482(3) Å; β=100.24(3)°; V=1730.7(8) Å^3; Z=4; D_x=1.50 g.cm^{-3}. The intensity mesurements were performed for $3° \leq 2\theta \leq 50°$ with MoKα radiation. The structure was solved by direct methods (MULTAN) and refined to give R=0.076 for 2141 observed reflections $F_o \geq 2\sigma(F_o)$. For (1c'): triclinic; space group $P\bar{1}$; a=13.351(4), b=6.441(2), c=10.382(5) Å; α=89.43(4), β=105.76(3), γ=92.75(3)°; V=858.2(6) Å^3; Z=2; D_x=1.56 g.cm^{-3} $3° \leq 2\theta \leq 50°$(MoKα); R=0.096; 1735 [$F_o \geq 2\sigma(F_o)$].
5. K.Konishi, *Agric. Biol. Chem.*, 34, 935(1970).

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