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Goniomedines A and B: Unprecedented Bisindole Alkaloids Formed through Fusion of Two Indole Moieties via a Dihydropyran Unit

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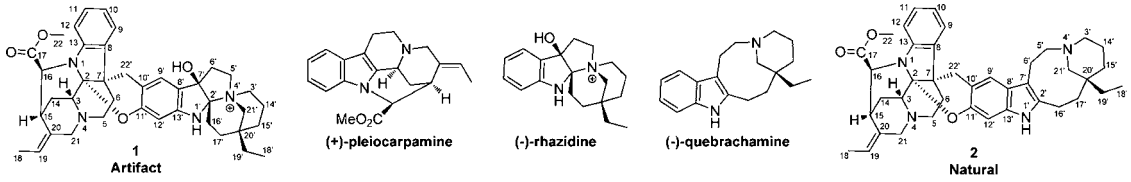
ABSTRACT



Two novel bisindole alkaloids, goniomedines A (1) and B (2), possessing an unprecedented quebrachamine–pleioarpamine-type skeleton, in which indole moieties are fused via a dihydropyran unit, were isolated from the stem bark of *Gonioma malagasy*. The structures were elucidated by comprehensive analysis of MS and NMR spectroscopic data. Their absolute configurations were deduced following the comparison of experimental and theoretically calculated ECD spectra and through biogenetic considerations. Goniomedine B (2) exhibited moderate activity against *Plasmodium falciparum*.

In continuation of our efforts to identify new antiprotozoal compounds from tropical plants,[1] we investigated chemically the stem bark of *Gonioma malagasy* Mgf and P.Bt[2] (Apocynaceae), a tree collected in the forest of Toliara in the southwest of Madagascar. The genus *Gonioma* comprises only two species, the second one, *G. kamassi*, being limited to South Africa.[3] Previous phytochemical investigations of *G. malagasy* led to the isolation of three structurally interesting compounds such as pleiomutinine,[4] goniomine,[5] and goniomitine.[6] The latter possesses a unique ring system in natural products. Herein, we report the isolation and structure elucidation of goniomedines A (**1**) and B (**2**), representing the first example of an unprecedented class of quebrachamine-pleiocarpamine bisindole alkaloids.

Air-dried and powdered stem bark of *G. malagasy* afforded a mixture of alkaloids using a traditional solid–liquid extraction procedure. The alkaloidic extract, which exhibited a significant antiplasmodial activity (100% at 10 μg/mL) with no cytotoxcity, was subjected to repeated chromatography to give goniomedine A (**1**)[7] as the major constituent (0.04% w/w). The UV spectrum

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(7) Goniomedine A (**1**): red amorphous powder; $[\alpha]^{25}_{D}$ + 114 (*c* 0.1, MeOH); IR ν_{max} 3400, 1750, 1130; UV (MeOH) λ_{max} (log ε) 209 (4.56) 254 (3.5) 296 (3.80) nm; ^{1}H and ^{13}C NMR data: see table 1; HRESIMS (pos) *m*/*z* 649.3756 $[M]^{+}$ (calcd for $C_{40}H_{49}N_4O_4$, 649.3754).

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was characteristic of a dihydroindole chromophore with absorption maxima at 254 and 296 nm. The IR spectrum of **1** showed absorptions bands at ν_{max} 3400, for OH and NH elongation, and at 1750 cm^{-1}, for an aliphatic ester. The HR-ESIMS of **1** showed a $[M]^+$ ion peak at m/z 649.3756 suggesting a molecular formula of $C_{40}H_{49}N_4O_4$ (corresponding to a DBE value of 19). The ^1H and ^{13}C NMR data (Table 1), and HSQC spectrum suggested the presence of five sp^3 quaternary carbons, 13 sp^3 methylenes, three sp^3 methines, three methyls, seven sp^2 methines, and eight sp^2 quaternary carbons.

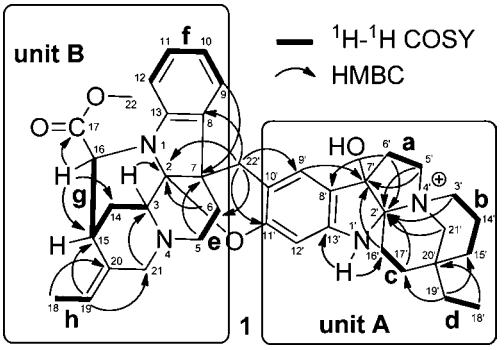

Figure 1. Selected 2D NMR correlations for goniomedine A (**1**).

The planar structure of **1** was deduced from extensive analysis of the two-dimensional NMR data, including the $^1H-^1H$ COSY, HSQC, TOCSY, and HMBC spectra in DMF-d_7. The $^1H-^1H$ COSY and HSQC revealed connectivities of eight partial structures (**a**–**h**) as shown in Figure 1. These partial structures were classified into two units A and B.

In unit A, the connectivity of partial structure **a** and dihydroindole ring (C-2′, C-7′–C-13′ and N-1′) was revealed by the HMBC correlations from H_2-5′ to C-7′ and C-2′, and from H_2-6′ to C-2′, whereas HMBC correlations from H_2-21′ to C-2′, C-5′, C-15′, C-17′, and C-20′ established the connections between partial structures **a**, **b**, and **c**. In addition, cross peak from H_3-18′ to C-20′ indicated that the ethyl group **d** is attached at C-20′, and from H-3′ to C-2′ suggested the linkage between C-3′ and C-2′ through a nitrogen atom, resulting in a pyrrole ring formation. The ^1H and ^{13}C NMR signals of the unit A were consistent with a rhazidine moiety,[8] a quebrachamine-type alkaloid that was previously isolated from *G. kamassi*.[9]

In unit B, the presence of a dihydroindole ring (C-2, C-7–C-13) was established by HMBC correlations from H_2-6 to C-2 and from H-9 to C-7, whereas the connectivity of C-2 and C-3 was deduced from correlation for H-3 to C-2. The presence of an ethylidene group at C-20 was indicated by HMBC correlations from H_3-18 to C-20, and from H-19 to C-15 and C-21. In the ^1H NMR spectrum of **1**, a well resolved methine doublet at δ_H 4.66 (J = 3.8 Hz) is reminiscent of the H-16 signal of pleiocarpamine and of bisindoles incorporating pleiocarpamine unit.[10] The downfield shift of C-2 indicated that it is linked to both a nitrogen and an oxygen atom, while the chemical shift of C-7 suggested connection to a carbon atom. The bridging of C-2 by an oxygen and C-7 by a carbon atom is common and can be exemplified by the bisindoles villalstonine[11] and pleiocorine.[12]

Rhazidine and pleiocarpamine units accounted for 18 DBE, and the remaining degree of unsaturation required the presence of an additional ring in goniomedine A (**1**). A close examination of 1 and 2D NMR spectra led to identify signals at δ_C/δ_H (25.7/3.53 and 3.49) for an additional methylene group (CH_2-22′). HMBC correlations from H_2-22′ to C-2, C-6, C-8, C-9′ and C-11′ suggested that this methylene group belongs to a dihydropyran ring formed by one oxygen atom and C-2, C-7, C-10′, C-11′, and C-22′ and that this ring is fused to two monomers. The gross structure of **1** was assigned to be a new bisindole alkaloid possessing an unprecedented linkage between the two dihydroindole moieties. The relative stereochemistry of each monoterpeneindole unit was assigned by ROESY correlations as shown in Figure 2.

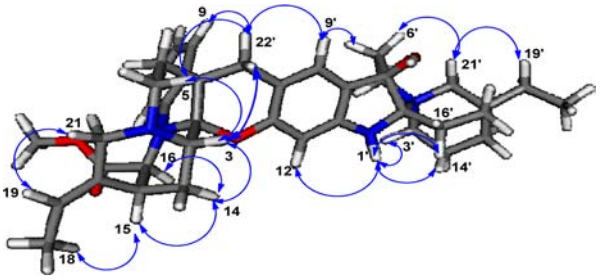

Figure 2. Key ROESY correlations of **1a**.

In the dihydropleiocarpamine unit (left part of the molecule), correlations H-3/H-5a, H-14a and H-22′, and H-14a/H-15 and H-16 indicated that they were all cofacial, while the correlation H-19/H-21 and H_3-18/H-15 established the *E*-configuration of the ethylidene side chain. The relative stereochemistry of this unit was consequently assigned as 2*R**,3*S**,7*S**,15*S**,16*S**. In the rhazidine unit (right part of the molecule), correlations H-21′/H-6′ and H-19′ suggested that they were all cofacial and randomly assigned as β oriented. The strong intensity of correlations between NH-1′ and H-3′, H-14′, and H-16′ indicated that they were α oriented. Thus, the relative configuration of this unit was established as 2′*S**,4′*S**,7′*R**,20′*R**. Because of the large distance between the two alkaloidic monomers,

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Table 1. ^{1}H and ^{13}C NMR Data of **1** and **2** (in DMF-d_7)

	1		**2**	
position	δ_H (mult, *J*, Hz)[a]	δ_C[b]	δ_H (mult, *J*, Hz)[a]	δ_C[b]
2		93.3		94.3
3	4.15 (1H, br s)	50.4	4.32 (1H, br s)	51.6
5	3.38 (1H, br d, 13); 3.86 (1H, m)	46.2	3.39 (1H, m); 3.91 (1H, m)	47.0
6	2.22 (2H, m)	30.7	2.24 (2H, m)	31.5
7		43.1		44.6
8		134.3		135.4
9	7.33 (1H, d, 7.2)	121.9	7.23 (1H, d, 7.2)	123.0
10	6.84 (1H, dd, 7.2, 7.8)	120.6	6.72 (1H, dd, 7.2, 7.6)	121.2
11	7.10 (1H, t, 7.8)	127.8	7.01 (1H, dd, 7.6, 7.9)	128.4
12	6.42 (1H, d, 7.8)	110.8	6.37 (1H, d, 7.9)	111.5
13		145.5		146.6
14	2.14 (1H, m); 2.93 (1H, m)	24.9	2.12 (1H, br d, 13.9); 3.00 (1H,br d, 13.9)	25.8
15	3.59 (1H, m)	30.2	3.61 (1H, m)	31.3
16	4.66 (1H, d, 3.8)	57.1	4.75 (1H, d, 3.5)	58.1
17		169.6		170.7
18	1.65 (3H, dd, 6.7, 1.9)	11.9	1.65 (3H, dd, 6.7, 1.6)	12.9
19	5.65 (1H, qd, 6.7, 1.9)	124.2	5.64 (1H, q, 6.7)	125.1
20		130.2		131.4
21	4.74 (1H, br d, 13.4); 3.82 (1H, m)	52.0	3.81 (1H, m); 4.76 (1H, m)	52.8
22	3.83 (3H, s)	52.2	3.82 (3H, s)	53.2
2′		100.8		139.0
3′	3.44 (1H, td, 13.5, 5.1); 3.65 (1H, dd, 13.5, 4.0)	56.2	3.33 (1H, m)	56.32
4′			6.48 (1H, m)	
5′	3.31 (1H, m); 3.59 (1H, m)	60.3	3.08 (1H, m); 3.40 (1H, m)	52.5
6′	2.41 (1H, m); 2.91 (1H, m)	37.8	3.24 (1H, dd, 15, 3.5); 3.55 (1H, m)	19.8
7′		88.9		102.9
8′		125.0		125.1
9′	7.12 (1H, s)	124.0	7.31 (1H, s)	118.0
10′		110.6		113.1
11′		154.6		150.8
12′	6.05 (1H, s)	96.7	6.58 (1H, s)	97.2
13′		146.3		136.0
14′	1.84 (1H, m); 2.46 (1H, m)	19.2	1.76 (1H, d, 14.6); 2.31 (1H, m)	20.7
15′	1.55 (1H, td, 13.2, 6.3); 1.74 (1H, m)	30.3	1.45 (1H, m); 1.59 (1H, td, 13, 4.5)	31.1
16′	2.17 (1H, m); 2.47 (1H, m)	28.3	2.88 (2H, m)	20.8
17′	1.73 (2H, m)	30.0	1.94 (2H, m)	32.0
18′	0.86 (3H, t, 7.6)	6.4	0.87 (3H, t, 7.4)	7.7
19′	1.31 (2H, q, 7.6)	33.9	1.36 (1H, m); 1.47 (1H, m)	30.6
20′		31.6		38.1
21′	3.29 (1H, d, 12) ; 3.69 (1H, d, 12)	61.9	2.89 (1H, m); 4.46 (1H, d, 12.5)	56.38
22′	3.49 (1H, d, 17.2); 3.53 (1H, d, 17.2)	25.7	3.58 (1H, d, 16.5); 3.76 (1H, d, 16.5)	27.8
1′NH	7.16 (1H, s)		10.90 (1H, s)	

[a] Data recorded at 600 MHz. [b] Data recorded at 150 MHz.

it was not possible to assign a relative configuration for **1** at that stage.

In order to determine the absolute configuration of **1**, experimental ECD spectrum was compared with that of theoretically calculated ECD curves of the four possible stereoisomers **1a-1d** [formed with (+)- or (−)-rhazidine and (+)- or (−)-pleiocarpamine units] (see the Supporting Information, Figure 3). Although the calculated ECD curves of diastereoisomers **1a** and **1c**, sharing the same stereochemistry for the dihydropleiocarpamine unit, matched well with the experimental CD spectrum, an unambiguous assignment of the absolute configuration of **1** cannot be confirmed.

The synthesis[8] of (+)-rhazidine by oxidation of (+)-quebrachamine caught our attention on the fact that **1** might be an artifact formed during the alkaloid extraction. This hypothesis was strongly supported since the ethanolic extract of *G. malagasy* stem bark afforded another unknown bisindole alkaloid named goniomedine B (**2**), whereas goniomedine A (**1**) was absent.

The N4′-protonated form of compound **2**[13] was isolated as an amorphous powder. The HR-ESIMS of the

(13) Goniomedine B (**2**): red amorphous powder; $[\alpha]^{25}_{D}$ + 62 (*c* 0.05, MeOH); IR ν_{max} 3400, 1750, 1130; UV (MeOH) λ_{max} (log ε) 206 (4.66) 237 (4.4) 293 (4.04) nm; ^{1}H and ^{13}C NMR data: see table 1; HRESIMS (pos) *m*/*z* 633.3813 $[M]^+$ (calcd for $C_{40}H_{49}N_4O_3$, 633.3805).

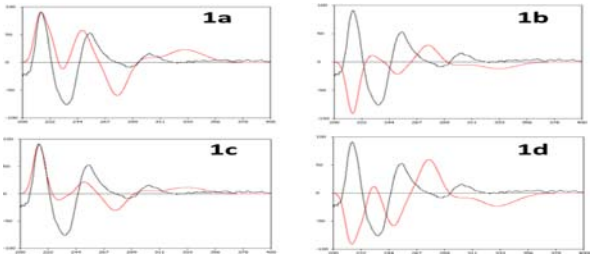

Figure 3. Calculated ECD spectra of **1a**–**d**: experimental ECD (black); calculated ECD (red).

quasi-molecular positive ion peak $[M]^+$ at m/z 633.3813 established the molecular formula as $C_{40}H_{49}N_4O_3$, indicating a molecular weight of 16 mass units lower than **1**. In its ^1H and ^{13}C NMR spectra, signals closely matched those of goniomedine A. The main differences between **1** and **2** was the presence of an additional signal for an exchanged proton at δ_H 6.48 in **2**, which was located on the nitrogen atom N-4′ by TOCSY correlations (see S21, Supporting Information), and the replacement of the two sp^3 quaternary carbons by two sp^2 quaternary carbons C-7′ and C-2′ at δ_C 102.9 and δ_C 139.0, respectively. These observations strongly suggested that compound **2** differed from **1** in that the rhazidine unit was replaced by a quebrachamine unit. A thorough analysis of COSY, HSQC and HMBC data confirmed this assignment. To test the hypothesis that goniomedine A (**1**) might be an artifact formed during the alkaloid-extraction process, goniomedine B (**2**) was subjected to an acid/base treatment at room temperature. Compound **1** was afforded with reasonable yield (20%). The relative stereochemistry of the ″quebrachamine″ part of **2** was assigned by analysis of ROESY data. The strong correlation between H-4′ and H_2-17′ implied the *anti* orientation of the ethyl side chain with respect to H-4′ (see the Supporting Information), suggesting the relative configuration 4′*R**,20′*R**. Finally, we proposed that the absolute configuration of both **1** and **2** was based on the absolute stereochemistry of the quebrachamine unit of **2**. Indeed, the chemical investigation of the stem bark and leaves alkaloid extracts afforded (+)-pleiocarpamine[14] and (−)-quebrachamine,[15] according to NMR data and $[\alpha]_D$ values. Thus, on the basis of comparison of experimental and theoretically calculated ECD spectra, and through biogenetic considerations, the absolute configuration of goniomedines A (**1**) and B (**2**) can be assigned as 2*R*,3*S*,7*S*,15*S*,16*S*,2′*S*,4′*S*,7′*R*,20′*R* and 2*R*,3*S*,7*S*,15*S*,16*S*,4′*R*,20′*R*, respectively.

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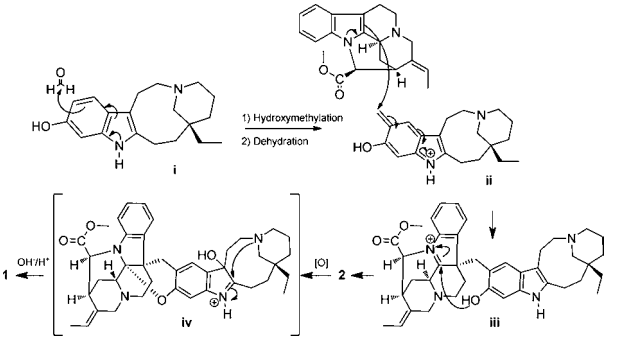
Scheme 1. Plausible Biosynthetic Pathway for **1** and **2**



A possible biogenetic pathway for **1** and **2** is presented in Scheme 1. The sequence is initiated by an hydroxymethylation of (−)-11-hydroxyquebrachamine **i** with formaldehyde (as was the case for pycnanthinine[16]),[17] whose presence may result from microbial degradation reactions.[18] Then, a conjugate addition of (+)-pleiocarpamine onto the intermediate **ii** should lead to dimer **iii**, which undergoes a nucleophilic attack by the phenolic OH at C-2 to give **2**, which should lead to **1** during extraction, presumably through the intermediate **iv**.[8]

Goniomedine B (**2**) showed moderate antiplasmodial activity toward the chloroquine-resistant strain FcB1 of *P. falciparum*, with IC_{50} of 2.8 μM, whereas goniomedine A (**1**) was not active.

Compounds **1** and **2** represent the first example of bisindole alkaloids constituted from the bridging of one (+)-pleiocarpamine and a (−)-rhazidine or a (−)-quebrachamine moiety via a dihydropyrane unit.

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Supporting Information Available. Experimental procedures, computational methods, DFT-optimized geometry, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.