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QUINOLINE ALKALOIDS FROM *PSYCHOTRIA GLOMERULATA*

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Key Word Index—*Psychotria glomerulata*; Rubiaceae; quinoline alkaloids; calycanthine; glomerulatine.

Abstract—Three new quinoline alkaloids have been isolated from the aerial parts of *Psychotria glomerulata* from Panama. The major alkaloid, glomerulatine A, was identified as 8-8a,8′-8′a tetradehydro(−)-calycanthine on the basis of spectral data including ^{1}H, ^{13}C, COSY-45, HMBC, HMQC and ROESY techniques. Computerized ^{1}H NMR analysis was used to establish that glomerulatine A possessed the calycanthine- and not the iso-calycanthine-type structure. Two minor alkaloids, glomerulatines B and C, were also isolated and on the basis of spectroscopic evidence it is proposed that they are 8-8a,8′-8′a-tetrahydro-N′-demethyl(−)-calycanthine and 8-8a-didehydro-(−)-calycanthine, respectively.

INTRODUCTION

Cephaëlis species have been regarded as a promising source of emetine and related alkaloids. Emetine, which is commercially obtained from *C. ipecacuanha*, is effective in the treatment of amoebic dysentery but, because of its adverse reactions, it is not the drug of choice [1]. Because of our interest in antiprotozoal natural products and the possibility of obtaining emetine-related alkaloids, we decided to investigate some Panamanian *Cephaëlis* species. Our knowledge of the chemistry of *Cephaëlis* species is limited to the ancient drug, *C. ipecacuanha*, known to contain monoterpenoid isoquinoline alkaloids [2, 3].

Recently, we reported the isolation and characterization of a series of indole alkaloids from *C. dichroa* (*Psychotria dichroa*) [4]. 2-Aza-anthraquinone and a new 1-hydroxybenzoisochromanquinone were isolated from *C. camponutans* (*P. camponutans*) and the taxonomic position of the *Cephaëlis* was discussed [5]

The present investigation is concerned with *P. glomerulata* (Don. Smith.). Steyermark, previously known as *Cephaëlis glomerulata* J.D. Sm., a shrub occurring from Guatemala to Panama [6]; extracts show strong positive reaction to Dragendorff's reagent.

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RESULTS AND DISCUSSION

The alkaloids **1**–**3**, namely, glomerulatines A–C, respectively, were isolated from the aerial parts of *P. glomerulata*. The UV spectrum of **1** showed a band at 274 nm indicative of a conjugated quinoline moiety , whilst the IR spectrum showed absorption for C=N (1625 cm^{-1}) and C=C (1590 cm^{-1}). The HR mass spectrum of **1** showed a strong $[M]^+$ peak at *m/z* 342.18565 corresponding to the molecular formula $C_{22}H_{22}N_4$, while the EI-mass spectrum showed a strong $[M]^+$ with 100% relative abundance, indicative of calycanthine-type alkaloids, as opposed to the chimonanthine type [7]. However, the peak at *m/z* 231 was of weak intensity suggesting the iso-calycanthine-form to be the more likely for this compound [7].

The ^{1}H NMR (400 MHz) spectrum of **1** showed only 11 proton signals, whereas the ^{13}C NMR showed 11 carbon signals, indicating the dimeric nature of the alkaloid. There were signals for four aromatic protons from δ 7.48 to 6.62, characteristic of a 1,2-disubstituted aromatic ring. The four proton signals from δ 3.09 to 1.35, were clearly separated from each other and were assigned to protons on C-2 and C-3 using COSY 45 and ^{1}H–^{13}C heterocorrelation NMR experiments. Also, the ^{1}H NMR showed a singlet integrating for three protons at δ 2.92 for a *N*-methyl; ^{1}H–^{13}C long-range heterocorrelation NMR experiments showed a correlation between the signal of the *N*-methyl and the carbon signals at δ 165.1 (C-8a) and 48.2, (C-2), establishing its position on N-1. C-3a

showed a signal at δ 49.1 shifted to lower field because it is adjacent to an additional conjugated carbon (C-8a), rather than to a methine group, as in calycanthine, which displays a signal at δ 30–40 characteristic of a piperidinoquinoline ring [8]. A ROESY effect was shown between the *N*-methyl and the proton on C-4′, and the α-proton on C-2 and C-4′, which are more feasible in the iso-calycanthine form than in the calycanthine one, where the methyl and the protons α on C-2 are farther away from the protons on C-4′. However, the NMR data did not establish unequivocally whether **1** was a calycanthine or iso-calycanthine-type of alkaloid.

The [α]D value showed that **1** is laevorotatory with a value of −466, close to that reported for (−)-calycanthine (−489) [7], whereas for iso(−)-calycanthine a value of −150 has been reported [7]. The CD spectrum indicated the *S*-configuration for **1**. Hence, the isolated compound may have a configuration similar to calycanthine with a *cis* ring-junction, since the *trans* ring-junction leads to an achiral compound. However, none of the spectroscopic techniques used in the structural elucidation, which include EI-mass spectrometry, ^{1}H and ^{13}C NMR, 2D NMR experiments, [α]D and CD could distinguish between the calycanthine, or iso-calycanthine type. Hence, compound **1** is either the 8-8a,8′-8′a-tetradehydro-(−)-calycanthine or the 8-8a,8′-8′a-tetradehydro-(−)-iso-calycanthine.

Table 1 shows a comparison between the spectroscopic data of calycanthine (**4**) and iso-calycanthine (**5**) published previously and alkaloid **1**. It is important to note that the chemical structure of calycanthine has been established by X-ray diffraction [9]. In a more recently published work, Adjibade *et al.* [7] characterized the first five-membered ring quinoline alkaloid as iso-calycanthine. Their arguments were based mainly on slight differences in ^{1}H and ^{13}C NMR, differences in fragment relative abundance in the EI-mass spectrum and the major difference being the [α]D value, when comparing iso-calycanthine with calycanthine.

Furthermore, Adjibade *et al.* [7] did not find any differences in the displacements of the signals in the ^{13}C NMR for C-2 and C-3, although the signals in ^{13}C NMR for a five-membered ring are shifted downfield when compared with a six-membered ring. For example, C-2 and C-3 in chimonanthine, with a five-member ring shows signals at δ 52.6 and 35.7, respectively, whereas, in calycanthine, as part of a six-membered ring, they were signals at δ 46.6 and 31.7, respectively [8]. The structure of iso-calycanthine has not been confirmed by X-ray diffraction analysis.

In order to differentiate between the two proposed dimeric structures a molecular mechanics study was carried out in order to determine the expected coupling constants for the hydrogens on the ethyl bridge (—CH_2—CH_2) and to compare them with those obtained experimentally. The structures were minimized using the Macromodel program (Version 4.0) [10] in an IBM RS/6000 running on AIX 3.2. With the Monte-Carlo method, four conformers for the calycanthine-type and three for the iso-calycanthine-type were found, but the coupling constants calculated by the equation of Hassnot, DeLeeuw and Altona [11] were unclear due to the unusual structure and highly stretched molecule. Then the semi-empiric Hamiltonian calculation AM1 [12] from the MOPAC 6.0 program [13] was used showing three conformers for the iso-calycanthine-type due to the flipping of the five-membered ring. The coupling constants obtained for each conformer were very close and only slight variations were observed, whilst the calycanthine-type showed only one conformer with a ΔH 200.53 kCal. In order to obtain an average of the coupling constants for the iso-calycanthine-type, the difference in

Table 1. Comparison of spectral data of calycanthine-type alkaloids

	Calycanthine*	iso-Calycanthine*	Compound **1**
[α]D	−489	−150	−466
UV	250, 310	247, 305	274, 304
IR	1600, 1570	1500	1625, 1590
EIMS	346 (100)	346 (100)	342 (100)
	302 (30)	—	298 (11)
	231 (77)	231 (20)	230 (7)
^{1}H NMR			
N-Me	2.41	2.32	3.29†
8a	4.31	4.30	4.2‡
aromatic protons	6.25 to 7.03	6.57, 6.73, 7.02, 7.05	6.63–6.74, 7.02–7.05†
^{13}C NMR			
N-Me	43.39	42.95	30.87 (39.57)‡
7a	146.20	145.35	147.32
8a	71.82	71.66	165.11 (76.51)§

* Taken from ref. [7].
† Measured in $CDCl_3$.
‡ Value for glomerulatine C in $CDCl_3$.
§ Value for glomerulatine C in C_6D_6.

Table 2. Computerized coupling constants calculated for the two structural isomers of calycanthine*

Compounds	$J_{2a\text{-}3a}$	$J_{2a\text{-}3b}$	$J_{2b\text{-}3a}$	$J_{2b\text{-}3b}$	ΔH_f (kcal)
Calycanthine form	8.48	0.45	9.59	7.51	200.35
iso-Calycanthine form†	10.85	2.48	5.91	10.58	133.75‡

* Values in Hz.
† Values calculated at 300 K.
‡ Value obtained for two of the conformers.

Table 3. Chemical shifts used to simulate spectra, showing the only four possibilities*

Protons	Simulated spectra A	B	C	D
2a	3.45	3.45	3.58	3.58
2b	3.58	3.58	3.45	3.45
3a	1.70	2.45	1.70	2.45
3b	2.45	1.70	2.45	1.70

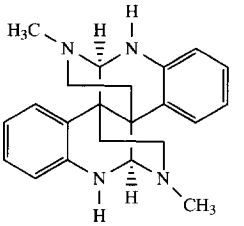
* Values in δ.

energy of the conformer at the temperature of observation (300 K) was used. Table 2 shows the coupling constants for both proposed dimeric structures. Since, it was not possible to depict the coupling constant values from the ^{1}H NMR spectrum, it was decided to simulate the spectra.

The ^{1}H NMR experimental spectrum determined from a $CDCl_3$ solution was preferred since it shows no interference with the methyl group signal and the four signals for the four protons on the ethyl bridge. Although, the signals at δ 1.70 and 2.45 were assigned to the protons on the C-3 farther from the nitrogen and those at δ 3.45 and 3.58 were assigned to the protons on C-2 adjacent to the nitrogen, it is not possible to differentiate between the α and β protons on these carbons; therefore, there are four possible spectra. Table 3 shows the resultant combination depicted from the chemical shifts, named A, B and C; Fig. 1 shows the simulated spectra for the iso-calycanthine-type using the experimental chemical shift and the geminal coupling constants. Figure 2 shows the simulated spectra for the calycanthine-type. Only the spectrum B for the calycanthine-type is identical to the experimental ^{1}H NMR ($CDCl_3$), while all the others are different. Hence, these computerized simulated spectra confirm that **1** is the 8-8a,8′-8′a-tetradehydro-(−)-calycanthine, named as glomerulatine A.

Compound **2**, named as glomerulatine B, showed similar UV and IR spectra to alkaloid **1**. The HR-mass spectrum showed a $[M]^+$ peak at m/z 328.16957 corresponding to the molecular formula $C_{21}H_{20}N_4$. The EI-mass spectrum showed a strong $[M]^+$ with 100% relative abundance, 14 mu less than **1**, suggesting that this alkaloid is the structural *N*-demethyl isomer.

The ^{1}H NMR showed an unsymmetrical molecule and only one signal integrating for three protons at δ 2.90 for a *N*-methyl group similar to **1**. The signal for



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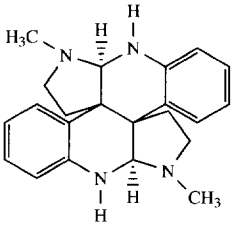

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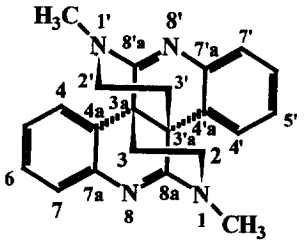
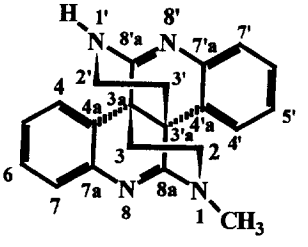


1



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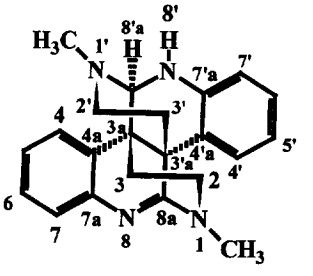

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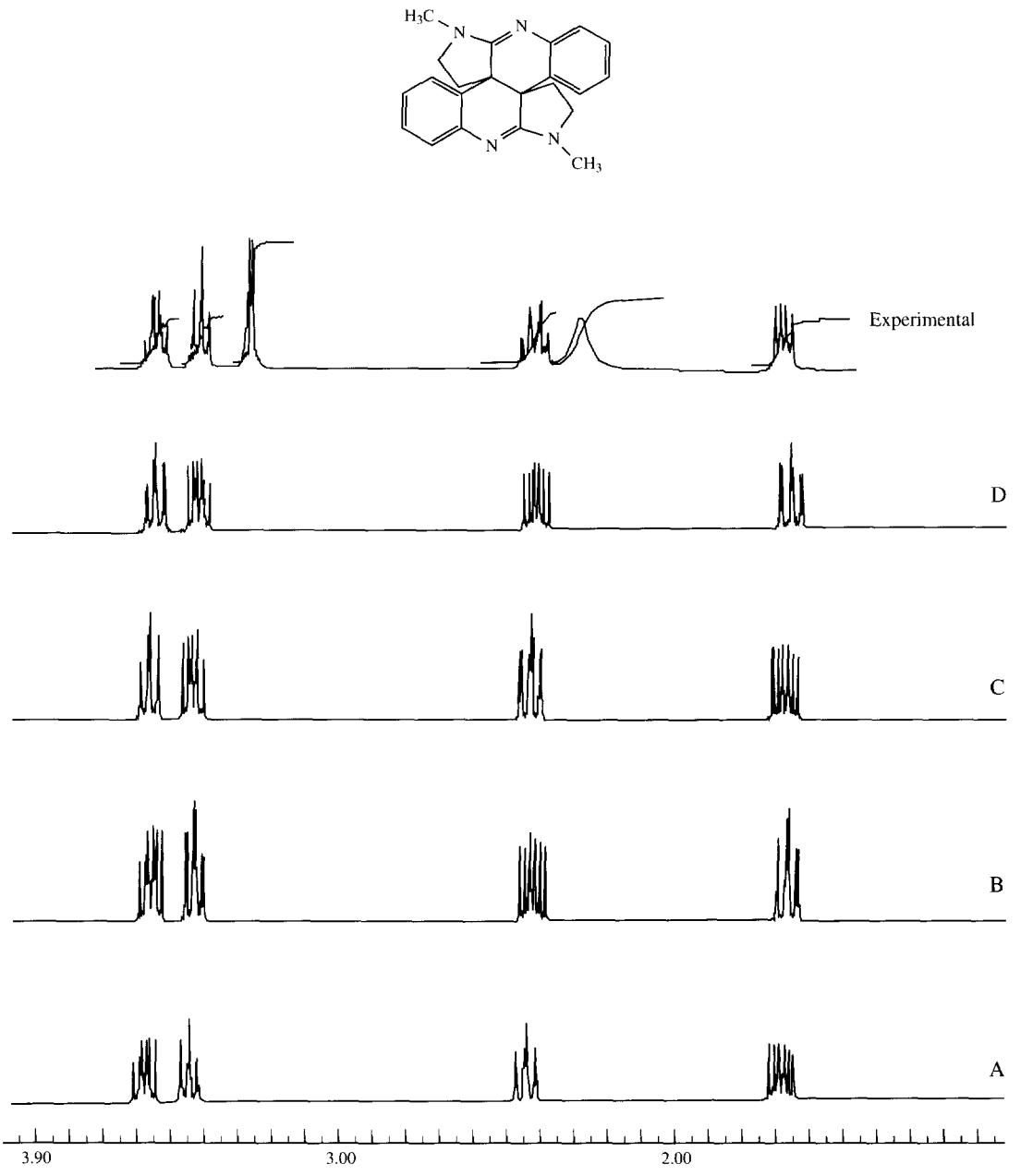

Fig. 1. Simulated spectra for iso-calycanthine-type.

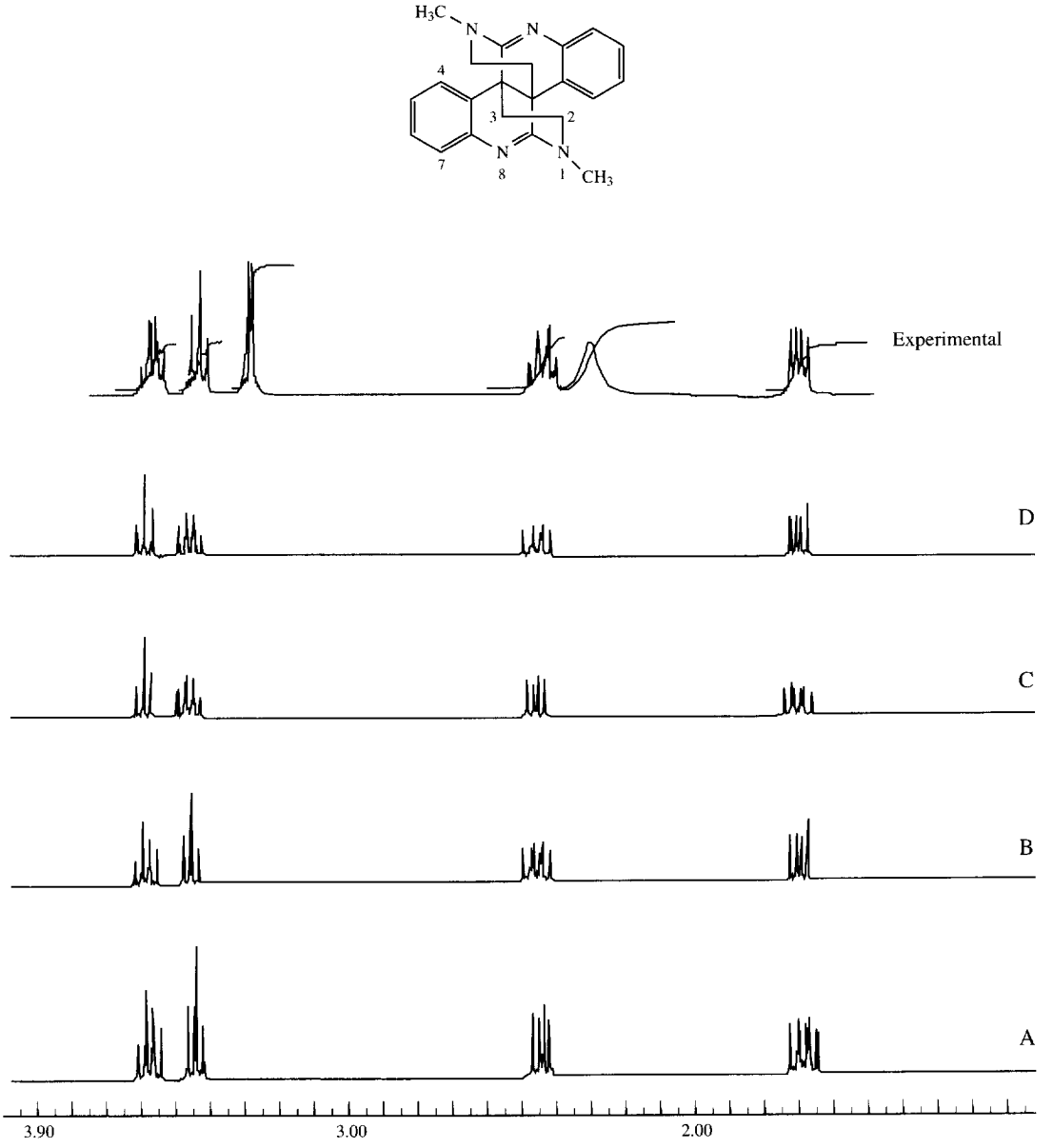

Fig. 2. Simulated spectra for calycanthine-type.

the protons on C-2 and C-3 were spread from δ 1.30 to 3.33, whereas the aromatic part of the spectrum showed eight protons indicating two *ortho*-disubstituted aromatic rings. Furthermore, ^{1}H NMR in $CDCl_3$ showed a very broad singlet at δ 2.55 that disappeared after the addition of D_2O, indicating the NH at position 1′. The assignment of this spectrum was achieved by comparison with **1**, since one monomeric moiety is identical, and also from 2D NMR experiments, such as COSY 45,^{1}H–^{13}C heterocorrelation and ^{1}H–^{13}C long-range heterocorrelation.

The ^{13}C NMR showed 16 signals indicating that some of them were overlapped. In the upper field, the signals for C-8a and C-7a were quite similar to those for compound **1**. While, the aromatic CH and the C-4a were split showing 10 separated signals, they resembled the values observed for **1**.

Compound **3** also showed UV and IR spectra similar to compound **1** and the HR-mass spectrum showed a $[M-1]^+$ at *m/z* 343.19227 corresponding to the molecular formula $C_{22}H_{23}N_4$. The EI-mass spectrum showed a relatively weak $[M]^+$ with only 23% relative abundance, but a stronger $[M-1]^+$ of 68% relative abundance, two mu less than alkaloid **1**, suggesting that this alkaloid has one less double bond. The ^{1}H NMR , as in compound **2**, showed an unsymmetrical molecule including two singlets integrating for three protons each, one of them at δ 2.97, as in **1** and **2**, and the other at δ 2.14 (δ 2.40 in $CDCl_3$) as reported for calycanthine [8]. Also, a doublet at δ 3.78 and a broad singlet at δ 3.86 for a proton on the carbon between the two nitrogens and for N—H, respectively, was observed. These data suggested that one of the monomers is similar to **1**, and the other being similar to calycanthine.

Furthermore, the ^{13}C NMR of **3** showed 22 carbons, a CH signal at δ 76.5 for the methine (C-8) and a quaternary carbon at δ 166.5, which are in favour of the first hypothesis. A ^{1}H–^{13}C long-range heterocorrelation NMR experiment showed a correlation between the singlet at δ 2.4 (in $CDCl_3$) and the carbon at δ 76.5 and 50.5, establishing that this methyl group is on the N-1′. In addition, the methyl, displaying a signal at δ 2.97, showed the same correlation as observed in compound **1**. ROESY NMR experiment showed, apart from the signals characteristic of the monomer unit similar to **1**, a correlation between the doublet at δ 3.78, for the proton on C-8′a, and the multiplet at δ 7.01–7.04, for the proton on C-4, indicating that this proton is in a position α to the six-membered ring. Therefore it can be concluded that the isolated compound is 8′-8′a-dehydro-(−)-calycanthine, named as glomerulatine C.

The evidence presented herein indicated that glomerulatine A (**1**) possesses the calycanthine-type structure and is not of the iso-calycanthine type. Glomerulatines B and C (**2** and **3**) possess very similar ^{1}H and ^{13}C NMR spectra to **1** and the implication is that they too are of calycanthine-type. However, further evidence is required for unequivocal structural assignments. Attempts to relate **2** and **3** to **1** by chemical modifications were not successful, since compound **1** decomposed on reaction and compounds **2** and **3** were isolated only in very small amounts. The chiral nature of **1**–**3** is evident from their CD spectra, and $[\alpha]_D$ values. Compound **1** possesses a *cis*-configuration at the 'dimer' junction, since the *trans*-isomer would be achiral. The CD spectra of **1** and **2** are virtually superimposable and, therefore, if **2** is also a calycanthine-type, as postulated, then it would possess the same absolute configuration as **1**. Compound **3** possesses an additional chiral centre adjacent to *N*-1′ and the similarity of its CD spectrum to that of **1** again implies the same absolute stereochemistry if it also, as postulated, is of calycanthine-type. Simulated spectra of the *trans*-isomers of the calycanthine-type and *cis*- and *trans*-isomers of the iso-calycanthine-type are not superimposable on the experimental spectrum, as is the *cis*-isomer of the calycanthine-type (data not shown).

EXPERIMENTAL

General. UV spectra were measured in EtOH. IR spectra were measured on NaCl cells. ^{1}H NMR were recorded at 400 MHz, ^{13}C NMR at 100 MHz, with TMS as int. standard. COSY 45, HMQC, HMBC and ROESY were also, measured on a Bruker AMX-400. TLC was performed on Al-backed precoated silica gel GF-254 plate (Merck). CC was carried out on silica gel 60 (70–230 mesh) (Merck).

Plant material. Psychotria glomerulata was collected in Comarca de San Blas (PEMASKY) Panama, in January 1991 and identified by Prof. Mireya Correa, Director of the Herbarium of the University of Panama, where voucher specimens have been deposited (FLORPAN 599 PMA).

Extraction. Dried powdered aerial parts (792 g) were extracted with MeOH by percolation. The extract was filtered and concd to a gum (49.4 g) under vacuum and then partitioned between 2 M HCl and $CHCl_3$. The acidic fr. was filtered, basified to pH 9 using NH_4OH and re-extracted with $CHCl_3$. The $CHCl_3$ extract was dried (Na_2SO_4) and concd under vacuum. The residue obtained (1.08 g) was submitted to CC using $CHCl_3$–MeOH + NH_4OH (49 : 1 + 0.1%). A total of 50 frs were obtained; 47 mg of compound **1** were obtained from frs 9–11. Frs 13–27 containing compounds **1** and **3** were purified by prep. TLC using EtOAc–Me_2CO + NH_4OH (4 : 1 + 0.1%) system; 5 mg of compound **3** were obtained. Frs 32–38 were also purified by prep. TLC developed using the same conditions, yielding 4 mg of **2**.

Compound **1**. (47 mg). UV λ_{max}^{EtOH} nm: 274, 304 shoulder. IR ν_{max}^{NaCl} cm^{-1}: 2919, 2860, 1625 (C=N), 1590 (C=C), 1466, 1407, 1272, 1219, 755. $[\alpha]_D^{20}$ −466.1 (*c* 0.36, $CHCl_3$). CD (MeCN, *c* 105 nM) λ nm ($\Delta\varepsilon$): 310.3 (−13.49), 296.0 (−13.11), 280.6 (+0.04), 268.8 (+12.66), 246.6 (+2.99), 214.7 (+12.17). EIMS *m/z*

(rel. int.): 342 (100), 327 (3), 313 (10), 298 (11), 284 (20), 270 (14), 256 (16), 255 (16), 242 (12), 230 (7), 209 (14), 128 (10), 115 (12). HRMS *m/z* calculated: 342.18445 ($C_{22}H_{22}N_4$) found: 342.18565. ^{1}H NMR (C_6D_6, 400 MHz): δ 1.35–1.39 (*dd*, J = 6.2 Hz, 1H, H-3β), 1.90–1.98 (*m*, 1H, H-3α), 2.61 (*t*, J = 8.98 Hz, 1H, H-2β), 2.92 (*s*, 3H, N-1 Me), 2.969–3.09 (*m*, 1H, H-2α), 6.62–6.67 (*m*, 1H, H-5), 6.76–6.78 (*dd*, J = 7.7, 1.5 Hz, 1H, H-4), 6.93–6.98 (*m*, 1H, H-6), 7.46–7.48 (*dd*, 1H, H-7). ^{13}C NMR (C_6D_6, 100 MHz): δ 30.3 (C-3), 30.9 (N-1 Me), 48.2 (C-2), 49.1 (C-3a), 122.3 (C-5), 123.7 (C-4), 125.0 (C-7), 126.4 (C-4a), 128.9 (C-6), 147.3 (C-7a), 165.1 (C-8a). COSY 45, HMQC, HMBC and ROESY NMR expts were used to confirm assignments.

Compound **2**. (4 mg). UV λ_{max}^{MeCN} nm: 279. CD (MeCN, *c* 116 nM) λ nm ($\Delta\varepsilon$): 305.5 (−12.97), 219.9 (−13.11), 274.3 (+0.098), 262.9 (+12.19), 242.2 (+8.09), 213.9 (+12.27). EIMS *m/z* (rel. int.): 328 (100), 313 (6), 299 (12), 284 (9), 270 (8), 243 (5), 229 (4), 132 (17), 113 (11), 50 (27). HRMS *m/z* calculated: 328.16880 ($C_{21}H_{20}N_4$) found 328.16957. ^{1}H NMR (C_6D_6, 400 MHz): δ 1.30–1.34 (*m*, 1H, H-3β), 1.46–1.49 (*m*, 1H, H-3′β), 1.92–2.03 (*m*, 2H, 3α and 3′α), 2.58 (*t*, J = 8.98 Hz, 1H, 2β), 2.90 (*s*, 3H, N-1 Me), 2.93–2.95 (*m*, 1H, 2α), 3.17–3.19 (*m*, 1H, 2′β), 3.29–3.33 (*m*, 1H, 2′α), 6.58–6.62 (*m*, 1H, 5′), 6.64–6.68 (*dd*, J = 7.51, 1H, H-5), 6.77 (*d*, J = 7.51 Hz, 1H, H-4), 6.88–6.92 (*dd*, J = 7.63, 7.39 Hz, 1H, H-6′), 6.94–6.98 (*dd*, J = 7.51 Hz, 1H, H-6), 7.10–7.12 (*m*, J = 7.63, 6.15 Hz, 2H, H-7′ and 4′), 7.47 (*d*, J = 7.63 Hz, 1H, H-7). ^{13}C NMR (C_6D_6, 100 MHz): δ 30.3 (C-3 and 3′), 30.8 (N-1 Me), 48.1 (C-2 and 2′), 48.6 (C-3a and 3′a), 120.9 (C-4), 122.2[a] (C-5), 122.6[a] (C-5′), 124.0[b] (C-4′), 124.4[b] (C-7′), 125.2[b] (C-7), 125.4[c] (C-4′a), 126.1[c] (C-4a), 128.8[d] (C-6′), 129.0[d] (C-6), 147.1 (C-7a and 7′a), 164.7 (C-8a and 8′a); superscript indicates interchangeable assignment within the same letter. COSY 45, HMQC, HMBC and ^{13}C DEPT NMR expts were used to confirm assignments.

Compound **3**. (5 mg). UV λ_{max}^{EtOH} nm: 274, 304 shoulder. IR ν_{max}^{NaCl} cm^{-1}: 2952, 2857, 2352, 1629 (C=N), 1588 (C=C), 1470, 1407. CD (MeCN, *c* 65 nM) λ nm ($\Delta\varepsilon$): 305.1 (−12.08), 287.2 (−12.32), 258.6 (+0.1), 247.9 (+12.47), 228.4 (+9.40), 216.4 (+13.35). EIMS *m/z* (rel. int.): 344 (23), 343 (68), 273 (23), 256 (57), 219 (20), 197 (23), 169 (25), 153 (22), 135 (19), 130 (41), 127 (22), 115 (30). HRMS *m/z* $[M-1]^+$ calculated 343.19227 ($C_{22}H_{23}N_4$) found 343.19248. ^{1}H NMR (C_6D_6, 400 MHz): δ 1.52–1.58 (*m*, 1H, H-3′β), 1.83–1.87 (*m*, 1H, H-3β), 1.91–1.98 (*m*, 1H, H-3′α), 2.14 (*s*, 3H, N-1′Me), 2.17–2.20 (*m*, 1H, 2′β), 2.30–2.34 (*m*, 1H, 3α), 2.63–2.70 (*m*, 2H, H-2β and 2′α), 2.97 (*s*, 3H, N-1 Me), 2.95–3.02 (*m*, 1H, 2α), 3.78 (*d*, J = 4.52 Hz, 1H, H-8′a), 3.86 (*s* broad, 1H, H-8′) 6.19–6.21 (*dd*, J = 0.88, 7.48 Hz, 1H, H-7′), 6.45–6.49 (*ddd*, J = 1.12, 7.36, 7.52 Hz, 1H, H-5′), 6.74–6.86 (*m*, 3H, H-5, 4′ and 6′), 6.96–7.00 (*dd*, J = 7.68, 7.44 Hz, 1H, H-6) 7.01–7.04 (*dd*, J = 1.2, 7.72 Hz, 1H, H-4), 7.49–7.51 (*dd*, J = 1.24, 7.76 Hz, 1H, H-7). ^{13}C NMR (C_6D_6, 100 MHz): δ 30.7 (N-1 Me), 31.7 (C-3), 34.0 (C-3′), 39.6 (N-1′ Me), 45.3 (3′a), 48.5 (C-2), 49.2 (C-3a), 50.5 (2′), 76.5 (C-8′a), 114.4 (C-7′), 117.5 (C-4′), 122.1 (C-5), 122.3 (C-4′a), 123.0 (C-4), 124.9 (C-5′), 125.2 (C-7), 127.1 (C-6′), 128.8 (C-6), 129.5 (C-4a), 142.1 (C-7′a), 148.6 (C-7a), 166.5 (C-8a). COSY 45, ^{13}C DEPT, HMBC and ROESY NMR expts were used to confirm assignments.

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