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ALKALOIDS FROM *PSYCHOTRIA OLEOIDES* WITH ACTIVITY ON GROWTH HORMONE RELEASE

FRANÇOISE GUÉRITTE-VOEGELEIN, THIERRY SÉVENET,* JACQUES PUSSET, MARIE-THÉRÈSE ADELINE, BRIGITTE GILLET, JEAN-CLAUDE BELOEIL, DANIEL GUÉNARD, PIERRE POTIER,

Centre National de la Recherche Scientifique, Institut de Chimie des Substances Naturelles, 91198 Gif-sur-Yvette, France

RAMAHEFARIZO RASOLONJANAHARY, and CLAUDE KORDON

Institut National de la Santé et de la Recherche Médicale, Unité 159, Centre Paul Broca, 2 ter rue d'Alésia, 75014 Paris, France

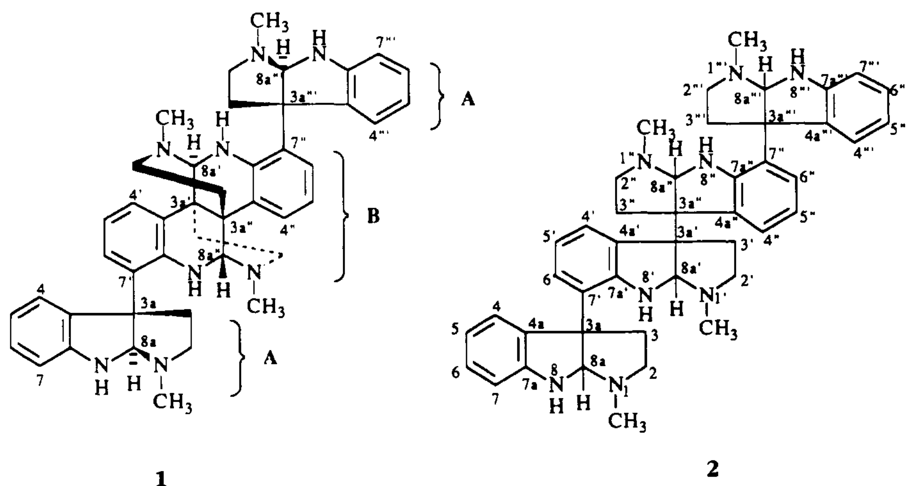
ABSTRACT.—Bioactivity-guided purification of a crude alkaloid extract of *Psychotria oleoides* has afforded a new alkaloid, psycholeine [**1**], together with quadrigemine C [**2**], a tetrameric pyrrolidinoindoline compound of unknown stereochemistry. A comparison study of nmr and cd spectra of quadrigemine C and hodgkinsine [**3**], a trimeric pyrrolidinoindoline substance, led us to suggest the stereochemistry of quadrigemine C. The structure and configuration of psycholeine was determined by spectroscopic means and chemical correlation with quadrigemine C. Psycholeine interacts with somatostatin receptors and exhibits a somatostatin antagonistic activity on GH secretion by pituitary cells in primary culture.

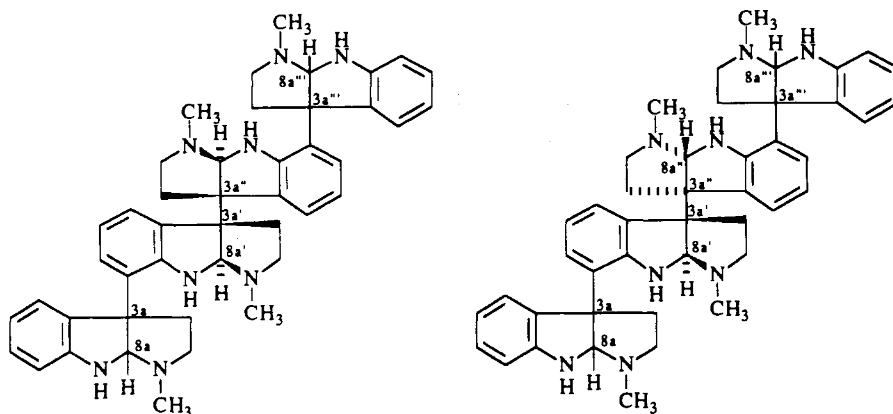
Psychotria oleoides (Baill.) Schltr. (Rubiaceae) is a tropical plant growing in New Caledonia. In a previous communication, the isolation from the leaves of several polyindoline alkaloids has been reported (1). As part of a search for naturally occurring substances acting on the release of the main pituitary hormones, the alkaloid extract of *P. oleoides* was found to exhibit a significant activity on rat pituitary hormone secretion, when tested both in vivo and in vitro according to a screening method already described (2,3). Moreover, the crude extract of *P. oleoides* was shown to contain substance(s) able to bind specifically to the somatostatin receptor(s). The present paper describes the bioassay-guided isolation of a new alkaloid, called psycholeine [**1**], and of quadrigemine C [**2**], a tetrameric pyrrolidinoindoline alkaloid of unknown stereochemistry (1). The structure of psycholeine [**1**], as established by nmr and ms, was confirmed by chemical correlation with quadrigemine C [**2**]. The study of 2D nmr spectra (DQF COSY, ROESY) of quadrigemine C [**2**] and psycholeine [**1**] in comparison to those of hodgkinsine [**3**], also isolated from *P. oleoides* (1), and calycosidine [**4**] derived from hodgkinsine under mild acidic conditions (4), led us to suggest the stereochemistry of quadrigemine C [**2**] and psycholeine [**1**]. Moreover, the comparison of the cd spectra of hodgkinsine and quadrigemine C supported the configurations proposed for **1** and **2**.

RESULTS AND DISCUSSION

Isolation of psycholeine [**1**] from the alkaloid extract of *P. oleoides* followed the steps described (Experimental). Quadrigemine C [**2**] and hodgkinsine [**3**] were also isolated from *P. oleoides* in order to correlate the structure of those compounds with psycholeine [**1**].

The primary structure of quadrigemine C was previously determined by mass and ^{1}H, ^{13}C nmr spectra analysis (1). As it has already been noted by Parry and Smith (5), the eight chiral centers of quadrigemine C could give rise to ten stereoisomers, six of them having an optically active central dimeric unit (**2a**–**2c** and their enantiomers), the four other showing a meso central subunit [**2d** (mesostereoisomer), **2e** (mesostereoisomer), and **2f** and its enantiomer]. Because of the unsymmetrical nmr spectrum and the optical activity of the quadrigemine C isolated from the alkaloid extract of *P. oleoides*, we can conclude that this alkaloid is the optically active **2b** or **2f** diastereoisomer or one of their enantiomers.





2a (3a*R*, 3a′*R*, 3a″*R*, 3a‴*R*)
2b (3a*R*, 3a′*R*, 3a″*R*, 3a‴*R*)
2c (3a*S*, 3a′*R*, 3a″*R*, 3a‴*S*)

2d (3a*R*, 3a′*R*, 3a‴*S*, 3a‴*S*)
2e (3a*S*, 3a′*R*, 3a″*S*, 3a‴*R*)
2f (3a*R*, 3a′*R*, 3a″*S*, 3a‴*R*)

We attempted to assign the configuration at the ring junctions in quadrigemine C by comparing first the nmr spectroscopy results. At room temperature the ^{1}H-nmr spectrum of the tetrameric compound quadrigemine C [**2**] exhibited unresolved signal sets, suggesting the presence of several conformations. The presence of conformers was confirmed by the fact that ^{13}C-nmr signals correspond to one molecule. Low temperature (241°K) enhanced the distinctive ^{1}H-nmr spectra feature mainly in the aromatic region where the presence of a double signal set could result from the presence of two conformers in a 60:40 ratio. The prominent feature of the quadrigemine C spectrum is the presence of four aromatic protons which exhibited upfield shifts at 5.36, 5.76, 6.09, and 6.26 ppm, suggesting the stacking of aromatic rings. Assignment of the aromatic ^{1}H-nmr signals of both conformers has been achieved by DQF COSY (6), and chemical exchanges (7) were determined by a ROESY (Rotating Frame Nuclear Overhauser Effect Spectroscopy) experiment (8). Thus, one conformer exhibited aromatic proton signals of the two central subunits at δ 5.36 (H-4′), 6.09 (H-5′), 6.92 (H-6′), 7.20 (H-4″), 6.65 (H-5″), and 7.12 (H-6″) ppm which are in exchange with an analo-

TABLE 1. Selected Aromatic ^{1}H-nmr Signals[a] for Quadrigemine C [**2**] and Hodgkinsine [**3**].

Proton	Compound			
	2		**3**	
	Conformer		Conformer	
	Predominant	Minor	Predominant	Minor
H-4′	7.18 m	5.36 d	7.25 d	5.56 d
H-5′	6.62 m	6.09 t	6.78 t	6.19 t
H-6′	7.18 m	6.92 d	7.20 d	7.01 d
H-4″	5.76 d	7.20 m	5.38 d	7.41 d
H-5″	6.26 t	6.65 m	6.23 t	6.86 t
H-6″	6.94 d	7.12 m	6.96 t	7.15 t
H-7″			6.58 d	6.49 d

[a]$CDCl_3$, 400 MHz, 241°K.

gous signal set at, respectively, δ 7.18 (H-4′), 6.62 (H-5′), 7.18 (H-6′), 5.76 (H-4″), 6.26 (H-5″), and 6.94 (H-6″) ppm, corresponding to the aromatic protons of the other conformer (Table 1). Studies of molecular models showed that the two most stable conformers possess protons of one aromatic ring (H-4′, H-5′, H-6′, or H-4″, H-5″, H-6″) which are in the plane of another close aromatic ring (distances from 2.5 to 4 Å) (Figure 1). The same situation was observed in the nmr spectrum (ROESY at 241°K) of the trimeric hodgkinsine [**3**] of known stereochemistry (9), isolated first from *Hodgkinsonia frutescens* (10), then from *P. oleoides* (1), *Psychotria forsteriana* (11), and *Calycodendron milnei* (1). Thus two sets of deshielding aromatic protons, corresponding to the central unit of one conformer, are in exchange with those of the other conformer (Table 1). The above results led us to propose structure **2f** for quadrigemine C where the two central pyrrolidinoindoline units are of opposite configuration at C-3a′ and C-3a″ such as in meso-chimonanthine [**5**] (12) and hodgkinsine [**3**] (10). Moreover, the similarity of the cd spectrum of hodgkinsine [**3**] [Δε (248 nm)= −6.6, Δε (314 nm)= +4.4] (13) and quadrigemine C [**2f**] [Δε (245 nm)= −15.8, (284 nm)= +3.0, Δε (310 nm)= +4.0] are in agreement with the proposed configuration for the tetrameric alkaloid.

Psycholeine [**1**] was identified on the basis of its spectral data by comparing these data with those of related substances such as chimonanthine [**6**] (14, 15), calycanthine [**7**] (16, 17), hodgkinsine [**3**] (10), quadrigemine C [**2**] (1) and calycosidine [**4**] (4).

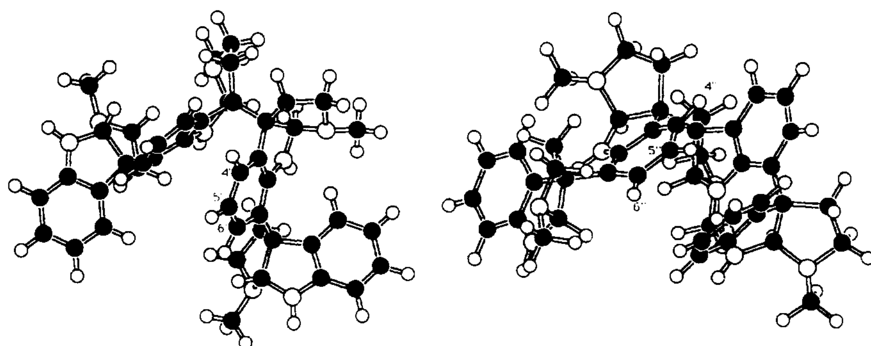
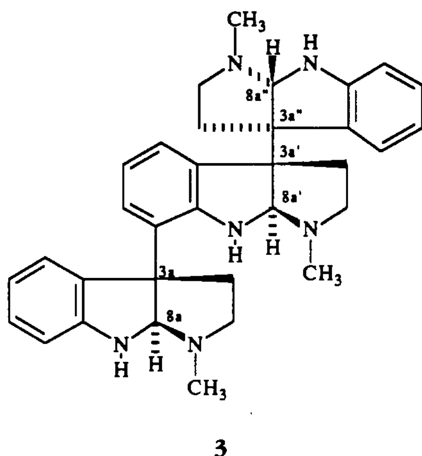


FIGURE 1. Conformations of quadrigemine C [**2**].



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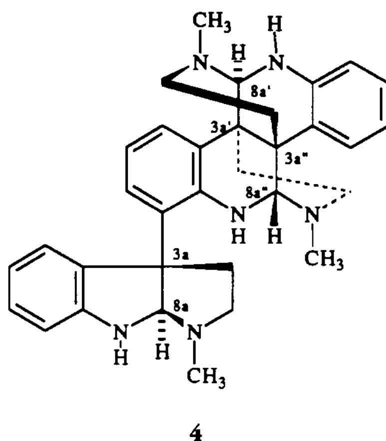

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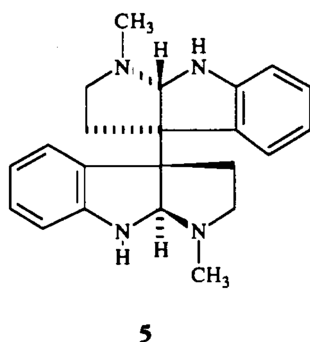

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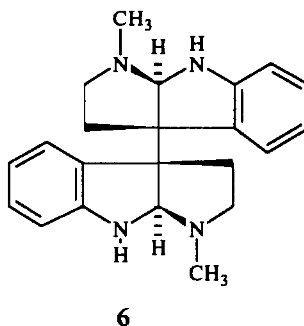

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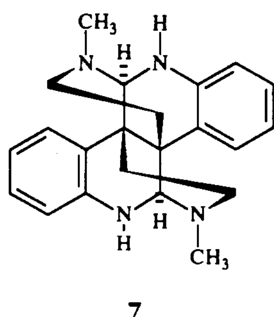

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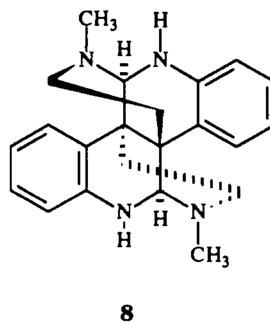

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Hreims of psycholeine [**1**] showed major peaks at 690 ($C_{44}H_{50}N_8$), 633, 575, and 344. The molecular ion base peak at *m/z* 690 (100%) suggested a tetrameric-type structure, but the low abundance of fragments at *m/z* 344 (24%) indicated that psycholeine is not a classical symmetrical quadrigemine-type structure, where the base peak at *m/z* 344 (100%) is typical of a C-3a′–C-3a″ type linkage between the second and third pyrrolidinoindoline subunits. Moreover, no peak at *m/z* 172 was observed (as seen with chimonanthine-type structure), indicating that the terminal Nb-methyltryptamine units are not linked by a C-3a′–C-3a″ bond (18). The other significant features in the ms of psycholeine [**1**] were the presence of ions at *m/z* 633 and 575 showing the loss of ethanamine fragments. The mass spectrum of **1** is, thus, analogous to that of calycosidine [**4**]. The presence of pyrrolidinoindoline and calycanthine [**7**] subunits in

psycholeine was confirmed by 2D ^1H-^{13}C and ^1H-^1H correlation nmr techniques and by a ROESY experiment (Table 2). The presence of the two pyrrolidinoindoline units was evident by the presence of two methine carbons resonating at δ 88.51 and 87.50

TABLE 2. ^1H and ^{13}C Assignments for Psycholeine [1] ($CDCl_3/CD_3OD$).

Position	δ ^1H	δ ^{13}C	Subunit[a]
1 (NMe)	2.33 (s)	36.08	A
	2.40 (s)	35.60	A
	2.40 (s)	43.69	B
	2.16 (s)	42.61	B
2 (NCH_2)	2.20 (m)	47.17	A
	2.66 (m)	48.05	A
	2.20 (m)	45.85	B
	1.74 (m), 1.94 (m)	46.30	B
3 (CH_2)	2.66, 2.45 (m)	37.50	A
	1.04 (d, J = 12), 1.80 (m)	32.80	B
	1.12 (d, J = 12), 1.77 (m)	32.40	B
3a		59.56, 60.65	A
		37.50, 38.05	B
4a		132.40, 133.80	A
		129.00	B
8a	5.57 (s)	88.51	A
	5.95 (s)	87.50	A
	4.21 (s)	74.00	B
	4.46 (s)	72.00	B

[a]Subunit A corresponds to the two terminal N_b-methyltryptamine units; subunit B is the meso-calycanthine unit.

ppm, while the two signals at δ 74.00 and 72.00 ppm accounted for the same carbons of the calycanthine unit. In the ^1H-nmr spectrum, these methine groups showed four singlets at 5.57, 5.95, 4.21, and 4.46 ppm, respectively. The ROESY spectrum showed interaction between the methine protons of the central unit with those of the terminal units and with aromatic protons (pairs H-8a′ and H-8a‴, pairs H-8a″ and H-8a, pairs H-8a′ and H-4′, and pairs H-8a″ and H-4″). Molecular mechanics methods gave the most stable conformation of psycholeine which was consistent with the observed nOe interactions (Figure 2). The ROESY spectra of calycosidine [**4**], whose structure is composed of a pyrrolidinoindoline and a meso-calycanthine [**8**] subunit, showed the same transannular interactions between the pairs H-8a and H-8a″, H-8a′ and H-4′, and H-8a″ and H-4″. The above spectral results led us to suggest that psycholeine [**1**] is composed of a meso-calycanthine central subunit linked at C-7′ and C-7″ by two pyrrolidinoindoline units.

In the same way that acid isomerization of chimonanthine and hodgkinsine [**3**] led, respectively, to calycanthine [**7**] (12, 19) and to calycosidine [**4**] (4), psycholeine was correlated to quadrigemine C [**2f**]. Under acidic conditions, quadrigemine C gave psycholeine in 43% yield. Synthetic psycholeine was identical to natural psycholeine. As it has already been noted in the case of calycosidine, psycholeine extracted from the plant could well be formed from quadrigemine C during the isolation procedure.

Psycholeine [**1**] has been separated from a crude extract of *P. oleoides* following its activity on pituitary rat membranes. The pure alkaloid exhibited a somatostatin (SRIF) antagonistic activity on growth hormone secretion by pituitary cells in primary culture. The activity of psycholeine and of some other pyrrolidinoindoline type alkaloids on

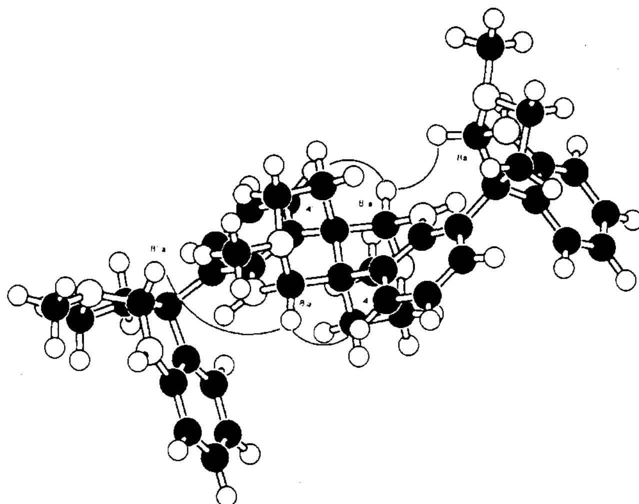

FIGURE 2. Conformation of psycholeine [1] predicted by molecular mechanics calculations. NOe interactions are shown by solid lines.

growth hormone release will be reported elsewhere in details (R. Rasolonjanahary, T. Sévenet, F. Guéritte-Voegelein, and C. Kordon, in preparation). To our knowledge, this is the first example of non-peptidic substances isolated from plants interacting with SRIF receptors, as well as the first described SRIF antagonist.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Preparative hplc was performed using a C-18 reversed-phase column on a Waters Delta Prep 3000 apparatus. Thin (0.2 mm) or thick (2 mm) layer chromatographies were accomplished with Si gel 60F-254 (Merk) plates. Si gel plates were developed with $CHCl_3$-MeOH-NH_4OH (80:20:2) for psycholeine or with $CHCl_3$-MeOH-NH_4OH (80:20:0.5) for quadrigemine C. Si gel or Al_2O_3 (activity II-III) were used for cc under medium pressure (0.3 bar). Spots were visualized by uv light and ceric sulfate spray reagent. Optical rotations were measured on a Perkin-Elmer 241 apparatus.

Uv spectra were measured in EtOH solution on a Roucaire UV-160 spectrophotometer. Ir spectra were taken on a Perkin-Elmer 257 spectrometer. Cd spectra were measured in EtOH solution on a JOBIN YVON Dichrograph Mark V. Hrms was recorded on an AEI SM50. 1D ^{1}H and ^{13}C and 2D nmr experiments were performed at room temperature or at 241°K on a Bruker AM400 spectrometer in $CDCl_3$ or $CDCl_3/CD_3OD$ solution using TMS as internal standard. For DQF COSY experiments the spectral width in F1 and F2 was 4000 Hz. The number of data points in t2 was 2048, and 512 increments were recorded. Before Fourier transformation, zero filling was applied in F1 and the data were multiplied with a square cosine window function. For ROESY experiments the spectral width was 6024.096 Hz in both dimensions. The applied pulse sequence was delay–(π/2)–t1–τm–(FID, t2). The spectra were recorded with a spin-locking time of 0.3 sec (matrix size 512 × 2048). Before Fourier transformation, zero filling and multiplication by a square cosine in both dimensions were performed. Heteronuclear shift correlation spectra were obtained using the pulse sequence delay–(π/2)–t1/2–π(13C)–t1/2–Δ1–π/2(1H),π/2(13C)–Δ2–acquisition with broadband decoupling. A 1-sec recycle delay was used with delay times D1 = 3.8 ms and D2 = 1.9 ms.

Molecular mechanics calculation were performed on a 4D25 work station (Silicon Graphics) with Macromodel as software using MM2 and Monte Carlo method to generate conformers.

PLANT MATERIAL.—The leaves of *P. oleoides* were collected in New Caledonia (Montagne des Sources, altitude 900 m) in January 1978 and identified by J.M. Veillon (ORSTOM). Herbarium specimens are kept in the Centre ORSTOM de Noumea (New Caledonia) under the reference Sévenet-Pusset 1459.

BIOLOGICAL ASSAYS.—The inhibition of GH release and the binding experiments were performed as already described (3).

ISOLATION PROCEDURE.—Extraction of the alkaloids has been described in a previous publication (1). At each step of the purification of the alkaloid extract, fractions were assayed for activity using the binding assay. The crude alkaloid extract (150 g) was fractionated on a column of Al_2O_3 and eluted with a step gradient of 0%, 0.5%, 1%, 2%, 5% . . . . 100% of MeOH in CH_2Cl_2. Crude quadrigemine C [**2**] and hodgkinsine [**3**] (52.7 g) were isolated from the first fractionation using 0.5 to 1% MeOH in CH_2Cl_2. Part of this fraction (1 g was further purified by preparative hplc on a C_{18} Delta-Pak column (30 cm × 47 mm i.d.). The mobile phase of MeOH-H_2O-NEt_3 (80:20:0.75) was delivered at 100 ml/min and monitored at 254 nm. The retention times of **2** (262 mg) and **3** (20 mg) were, respectively, 34 min and 14 min. The major biological activity was found in the MeOH eluate (15.68 g). This fraction was chromatographed on a column of Si gel (70 × 410 mm) using $CHCl_3$-MeOH-NH_4OH (97:7:1) as eluent leading to crude psycholeine [**1**] (1.7 g). Prior to spectral analysis and biological assay, psycholeine was purified by thick layer chromatography on Si gel with $CHCl_3$-MeOH-NH_4OH (80:20:2).

Psycholeine [**1**].—$[\alpha]^{20}D$ −150° (EtOH, *c* = 0.4); uv λ max (EtOH) 240 nm (ε 18600), 310 nm (ε 5300); ir ($CHCl_3$) γ cm^{-1} 3400, 1600; cd λ max (EtOH) 248 (Δε +5.2), 2.66 nm (Δε −11.7), 298 nm (Δε +11.7), 319 nm (Δε −7.8); hreims *m/z* (%) $[M]^+$ 690.4160 (100) (calcd 690.414 for $C_{44}H_{50}N_8$), 688.4014 (15) (calcd 688.3984 for $C_{44}H_{48}N_8$) 634.3629 (11) (calcd 634.3642 for $C_{41}H_{44}N_7$), 633.3561 (20) (calcd 633.3564 for $C_{41}H_{43}N_7$), 632.3489 (14) (calcd 632.3486 for $C_{41}H_{42}N_7$), 589.3030 (15) (calcd 589.3066 for $C_{39}H_{37}N_6$), 578.3123 (16) (calcd 578.3144 for $C_{38}H_{38}N_6$), 577.3114 (33) (calcd 577.3066 for $C_{38}H_{37}N_6$), 576.2959 (21) (calcd 576.2988 for $C_{38}H_{36}N_6$), 575.2909 (38) (calcd 575.2910 for $C_{38}H_{35}N_6$), 4878.1922 (15) (calcd 487.1914 for $C_{34}H_{23}N_4$), 344.1986 (24) (calcd 344.1992 for $C_{22}H_{24}N_4$).

Quadrigemine C [**2f**] (1).—Cd λ max (EtOH) 245 nm (Δε −15.8), 284 nm (Δε +3.0), 310 nm (Δε +4.0); ^1H nmr δ ($CDCl_3$, 241°K) 2.08–2.54 (8 brs, N-Me), 1.80–3.10 (m, H_2-2 and H_2-3), 4.06, 4.13, 4.18, 4.23 (br s, NH), 4.25, 4.42, 4.52, 4.85, 4.98, 5.07, 5.09 (brs, H-8a), 5.36 (d, *J* = 8, H-4′), 5.76 (d, *J* = 8, H-4″), 6.09 (d, *J* = 8, H-5′), 6.26 (d, *J* = 8, H-5″), 6.62 (m, H-5′ + 1H), 6.65 (m, H-5″ + 1H), 6.80 (m, 2H), 6.92 (d, *J* = 8, H-6′), 6.94 (d, *J* = 8, H-6″), 7.05 (m, 1H), 7.12 (m, H-6″ + 1H), 7.18 (m, H-4′ and H-6′ + 1H), 7.20 (m, H-4″ + 1H).

Hodgkinsine [**3**] (9, 13).—^1H nmr δ ($CDCl_3$, 241°K) 1.99–2.44 (5 brs, N-Me), 4.06, 4.22, 4.26, (br s, NH), 4.46, 4.67, 4.99, 5.11, 5.22 (brs, H-8a), 5.38 (d, *J* = 8, H-4″), 5.56 (d, *J* = 8, H-4′), 6.19 (t, *J* = 8, H-5′), 6.23 (t, *J* = 8, H-5″), 6.49 (d, *J* = 8, H-7″), 6.58 (d, *J* = 8, H-7″), 6.65 (m, 1H), 6.70 (d, *J* = 8, 1H), 6.78 (t, *J* = 8, H-5′), 6.86 (t, *J* = 8, H-5″), 6.89 (t, *J* = 8, 1H), 6.96 (t, *J* = 8, H-6″), 7.01 (d, *J* = 8, H-6′), 7.10 (m, 1H), 7.15 (t, d = 8, H-6″), 7.17 (m, 1H), 7.20 (d, *J* = 8, H-6′), 7.25 (d, *J* = 8, H-4′ + 1H), 7.41 (d, *J* = 8, H-4″).

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