Journal of Natural Products
Vol. 58, No. 8, pp. 1200-1208, August 1995

IDIOSPERMULINE, A TRIMERIC PYRROLIDINOINDOLINE ALKALOID FROM THE SEED OF *IDIOSPERMUM AUSTRALIENSE*

RUJEE K. DUKE,* ROBIN D. ALLAN, GRAHAM A.R. JOHNSTON, KENNETH N. MEWETT, ANN D. MITROVIC,

Department of Pharmacology,

COLIN C. DUKE,

Department of Pharmacy,

and TREVOR W. HAMBLEY

School of Chemistry, University of Sydney, Sydney, New South Wales 2006, Australia

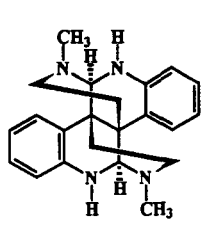
ABSTRACT.—Purification of a methanol extract from the seed of *Idiospermum australiense*, guided by bioactivity on a rat brain cortical wedge preparation has afforded two known dimeric alkaloids, the piperidinoindoline, (+)-calycanthine [**1**] and the pyrrolidinoindoline, (−)-chimonanthine [**2**] along with a new trimeric pyrrolidinoindoline alkaloid, (−)-idiospermuline [**3**]. The structure of idiospermuline [**3**] was determined by spectroscopic methods and the absolute configuration by an X-ray crystallographic study of idiospermuline trimethiodide [**4**].

Idiospermum australiense (Diels) S.T. Blake is the sole member of the Idiospermaceae, a primitive angiosperm family of considerable botanical interest (1). It was once ascribed to the Calycanthaceae (2), a family to which it is closely related. *I. australiense* is a rare tree that occurs only in a few areas near Cairns (North Queensland, Australia), where it inhabits lowland rain forest that receives very high rainfall (1,3,4). Although the seed has been implicated in the poisoning of cattle (D.C. Clague, Queensland Department of Primary Industry, unpublished report), which has been confirmed by feeding tests (T.W.K. Hall, Queensland Department of Primary Industry, unpublished results), the poisonous principle has not been established (5). The seed has been chemically examined and a variety of substances identified; among these, an alkaloid, (+)-calycanthine, was isolated as 0.1% of dry wt (6).

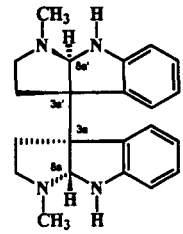
As a part of a search for naturally occurring substances acting on neurochemical transmission, the MeOH extract of *I. australiense* was found to hyperpolarize a rat brain cortical wedge preparation. This paper describes the bioassay-guided isolation of a new alkaloid, (−)-idiospermuline [**3**], a trimeric pyrrolidinoindoline, together with two known dimeric alkaloids, the piperidinoindoline, (+)-calycanthine [**1**], and the pyrrolidinoindoline, (−)-chimonanthine [**2**].

RESULTS AND DISCUSSION

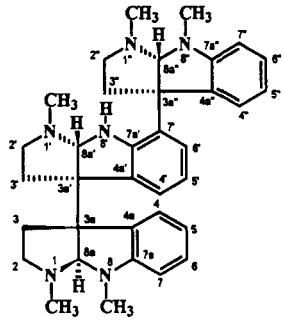
In a rat cortical wedge preparation (7), a depolarizing response (an upward deflection from baseline) was observed for acetylcholine, a cholinergic agonist, and a hyperpolarizing response (a downward deflection from baseline) was observed for calycanthine [**1**] (50–500 μM), chimonanthine [**2**] (10–100 μM), and idiospermuline [**3**] (10–100 μM). Table 1 shows the reduction (expressed in percentage of the control) in depolarizing responses due to 100 μM of acetylcholine, in the presence of **1**–**3**. The results from this assay suggested that these compounds suppressed cholinergic transmission, which was observed as a reduction in the depolarization effect of acetylcholine. Suppression of neurotransmission in the cortical wedge preparation has been extensively studied with glutamate as neurotransmitter where depolarization effects are observed for agonists (7,8) and hyperpolarization effects are observed for some antagonists (7,9). Hyperpolarization has also been observed for $GABA_B$ agonists (10). However, we were unable to find



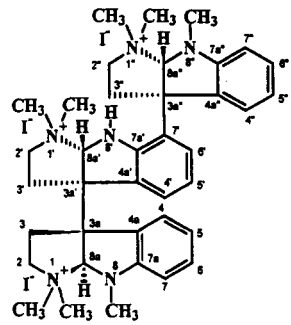
1



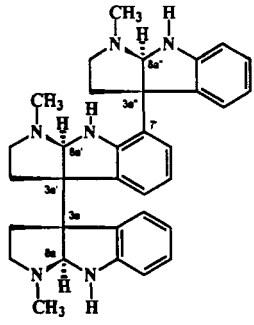
2



3



4



5

any literature precedent for cholinergic modulation of neurotransmission in this cortical wedge preparation. The mechanism of this suppression of cholinergic transmission is unclear and is presently under investigation.

The structure and stereochemistry of compounds **1** and **2** were established by comparison with published physical (11,12) and spectroscopic (13) data. The primary structure of (−)-idiospermuline [**3**] was determined from ^{1}H- and ^{13}C-nmr and ms data,

TABLE 1. Reduction in Depolarizing Responses due to 100 μM Acetylcholine by **1–3**.

Compound	Concentration (μM)	Reduction in acetylcholine depolarizing response (% control)
(+)-Calycanthine [**1**]	50	20.4±7.7 (n=3)
(−)-Chimonanthine [**2**]	10	58.3±0.8 (n=5)
(−)-Idiospermuline [**3**]	10	61.2±1.9 (n=9)

and the absolute stereochemistry was established by an X-ray crystallographic study of idiospermuline trimethiodide [**4**].

Preliminary ms examination of idiospermuline [**3**] indicated a probable relationship to hodgkinsine [**5**], an alkaloid from *Hodgkinsonia frutescens* F. Muell., which belongs to the Rubiaceae (14). Whereas compound **5** is composed of three distinct N_b-methyltryptamine units, it appeared from the mass spectrum that the new base from *I. australiense* had a related structure with two additional methyl groups.

The cims of compound **3** showed a MH^+ peak at *m/z* 547, which suggested a molecular composition of $C_{35}H_{42}N_6$ and which was confirmed by the M^+ ion in the hreims at *m/z* 546 and by eims. The eims of **3** showed two major peaks, indicating that the molecule fragmented into two principal daughter ions. A cims study of parent-daughter ions among the *m/z* 547, 359, and 187 ions confirmed that the ions at *m/z* 359 and 187 were both direct fragments of the *m/z* 547 ion. Hreims of the peak at *m/z* 358 and the base peak at *m/z* 186 gave mol wts consistent with molecular formulas of $C_{23}H_{26}N_4$ (N_b-methyltryptamine-N_aN_b-dimethyltryptamine) and $C_{12}H_{14}N_2$ (N_aN_b-dimethyltryptamine), respectively.

Evidence for the type and sequence of bonds linking three tryptamine units was obtained from the ms of idiospermuline [**3**]. The base peak at *m/z* 186 in the eims spectrum of **3** indicated that the terminal subunit with a C-3a,C-3a′ bond type linkage was N_aN_b-dimethyltryptamine, since rupture of the C-3a–C-3a′ bond in the chimonanthine type-structure has been documented to be facile. Chimonanthine [**2**] and hodgkinsine [**5**] showed base peaks in the eims at *m/z* 172 (15) and 344 (13,16), respectively.

The location of the other extra methyl group in **3** was established by nOe difference nmr experiments, which exhibited nOe interactions between H-7 (δ 6.47) and N_a-CH_3 (Me-8, δ 2.96), and between H-7″ (δ 6.18) and Me-N_a″ (Me-8″, δ 2.89).

The ^{1}H-nmr spectra in $CDCl_3$, CD_3OD, and DMSO-d_6 of **3** all showed unresolved signal sets suggesting the presence of different conformations at 20°. When the temperature was raised ($CDCl_3$, 40°; CD_3OD, 40°, and DMSO-d_6, 80°), the feature of these unresolved signal sets did not alter significantly, however, changes in chemical shifts were observed. When the spectrum was recorded using 15% CD_3OD in $CDCl_3$ solution, changes in chemical shifts were observed and the unresolved signal sets became more definite, which facilitated proton assignment. The ^{13}C-nmr spectra of **3** in $CDCl_3$, CD_3OD, and DMSO-d_6 also showed broadening of some signals supporting the presence of conformers. The presence of two major conformers became most apparent upon inspection of the C-2″ resonances, which appeared as a broad singlet in both DMSO-d_6 (δ 51.0) and CD_3OD (δ 54.1) and two broad singlets of almost equal intensities (δ 52.3 and δ 52.1) in $CDCl_3$. Molecular mechanics methods predicted two stable conformations of **3** (Figure 1) with an energy difference of approximately 2 kcal mol^{-1}, consistent with the observation of the unresolved signal sets in the ^{1}H-nmr spectra and the broadening of the signals in the ^{13}C-nmr spectra. This behavior is similar to that of **5**, for which the ^{1}H-nmr spectrum recorded at room temperature exhibited unresolved signal sets which resolved at −32° into signals consistent with the presence of a major and a minor conformer (17).

Assignment of the ^{13}C-nmr spectra of **3** and **4** was determined by DEPT and 2D ^{13}C-^{1}H correlation (HETCOR) experiments. 2D ^{1}H-^{1}H correlation (COSY, DQCOSY, and NOESY), homonuclear decoupling and nOe difference experiments were used to assign the protons.

The presence of the three pyrrolidinoindoline units in idiospermuline [**3**] was evident by three methine carbons resonating at δ 95.0, 91.8 and 83.6 ppm, which

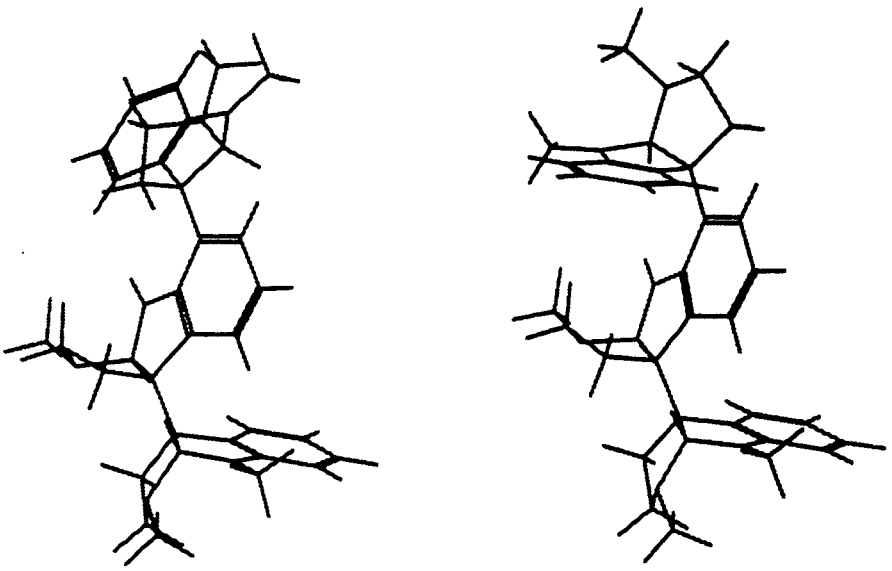


FIGURE 1. Stable conformations of idiospermuline [**3**] predicted by molecular mechanics calculations.

correlated to signals in the ^{1}H-nmr spectrum at δ 4.58, 4.58, and 5.27 ppm, respectively. In idiospermuline trimethiodide [**4**], these methine carbons resonated at lower field [δ 109.0, 104.1, and 94.6 ppm, respectively], consistent with the nitrogens attached to these methine carbons being quaternized.

In the ^{1}H-nmr spectrum of **4**, a characteristic feature was the difference in resonance frequencies of Me-8 (δ 3.36) and Me-8″ (δ 2.72), which suggested that the former was situated within the deshielding plane of an aromatic ring. This conclusion was consistent with the result from the X-ray crystallographic study (Figure 2) that showed that Me-8 was within the deshielding plane of the central aromatic ring and very close to H-4′ (the methyl protons being 2.48, 3.69, and 3.81 Å from H-4′). A strong nOe interaction was observed between Me-8 (δ3.36) and H-4′ (δ 7.65) thereby confirming the spatial arrangement of the methyl group at the 8 position.

The cd spectra of **3** [Δε (271 nm)= −3.2, Δε (322 nm)= −4.9] and **4** [Δε (257 nm)= −14.8, Δε (306 nm)= −12.9] were dissimilar to those of **5** [Δε (248 nm)= −6.6, Δε (314 nm)= +4.4](18), suggesting a different spatial arrangement of pyrridinoindoline subunits in **3** and **4** from that of **5**. X-ray diffraction methods established the absolute configuration of **4** as depicted in Figure 2. The atomic numbering scheme for **4** is shown in Figure 2 and the final atomic coordinates are listed in Table 2. This structure confirms the different configuration of the a′ and a″ subunits compared with that of **5**, the absolute stereochemistry of which has been established by an X-ray crystallographic study of hodgkinsine trimethiodide (19).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The mps were determined on a Reichert hot-stage apparatus and are reported uncorrected. Elemental analyses were carried out by the Australian Microanalytical Service, Melbourne. The hreims determinations were carried out on a Kratos MS9 high-resolution mass spectrometer upgraded to MS50 configuration. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. Cd spectra were measured on a Jasco J-720 cd spectropolarimeter, uv spectra were recorded on a Hitachi U-2000 spectrophotometer, and ir spectra were obtained on a Jasco A 100 double-beam spectrometer.

Cims were obtained with a Finnigan-MAT TSQ46 MS/MS instrument using CH_4 or NH_3 as the reagent gas. 1D ^{1}H- (300 MHz), ^{13}C- (75 MHz), and 2D nmr experiments were performed at 20° using a 300 MHz Varian-Gemini 300 spectrometer. Chemical shift values are given in ppm relative to internal TMS

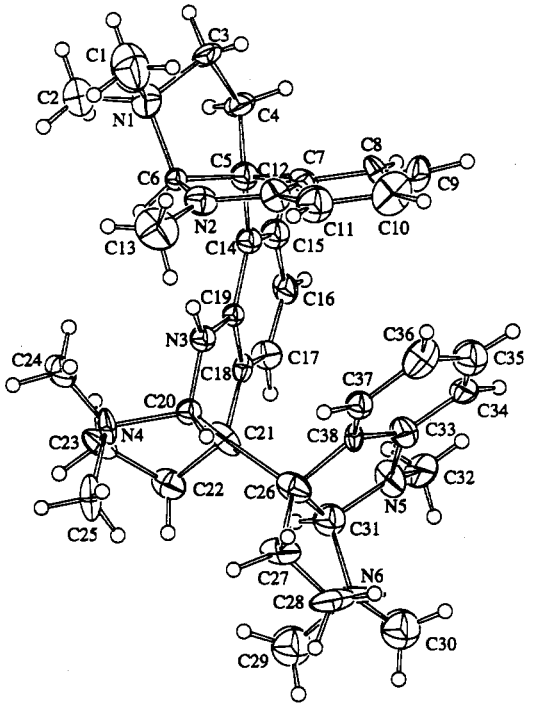


FIGURE 2. ORTEP plot (30% ellipsoids) of idiospermuline trimethiodide [**4**] molecule with atom numbering.

unless stated otherwise. Standard Gemini pulse sequences were used in DEPT, NOEDIF, COSY, DQCOSY, NOESY, and HETCOR experiments.

Molecular mechanics calculations were performed on a 586 Pentium computer with CHEM-X software (PC version), Chemical Design Ltd., Oxford, UK (April, 1994), using MM2 and conformational searching about the two flexible bonds.

PLANT MATERIAL.—The seeds of *Idiospermum australiense* were collected in the Daintree area near Cape Tribulation, North Queensland, Australia, in April 1990, and kept frozen prior to extraction. The seed (average weight 110 g) was approximately 5 cm in diameter with a thin outer brown coating containing 3–4 vertically divided creamy-colored segments (cotyledons), from the center of which the germinating seedling emerges. The bulk of the seed consisted almost entirely of the cotyledons. The plant material was collected and identified by Peter and Anne Radke, and a voucher specimen has been deposited at Yuruga Nursery, Walkamin, Queensland 4872, Australia.

BIOASSAYS.—A rat cortical wedge preparation consisting of slices of neocortex and corpus callosum was prepared from Sprague-Dawley rats weighing between 200–300 g as described by Harrison and Simmonds (7). Slices of forebrain were prepared by cutting coronal sections of 500 μm in thickness using a vibraslice microtome. The slices were placed in Mg^{2+}-free Krebs buffer oxygenated with 95% O_2 and 5% CO_2 and divided at the midline. Further cuts were made on either side of the midline to produce 2–3 mm wide wedge-shaped slices of cortex and corpus callosum.

These cerebral wedges were placed between layers of adsorbent fiber supported on an inclined block at room temperature. The corpus callosum was raised onto a non-depolarizable wick electrode and enclosed with a grease barrier to provide insulation. The cortex was superfused with Mg^{2+}-free Krebs medium at a rate of 1 ml/min. Direct current (dc) potentials between the cortex and corpus callosum were monitored by Ag/AgCl electrodes via agar/saline bridges and a high impedence dc amplifier and were displayed on a linear chart recorder. Alternatively, the dc potentials were passed through a MacLab system via a Bioamp amplifier and the signals were recorded on a MacLab Chart v3.3.2 program on a Macintosh IIcx computer.

Test substances were made up in Mg^{2+}-free Krebs buffer and were applied to the cortex for periods of 1 min when tested for agonist activity, or 4 min when tested for antagonist activity followed by co-

TABLE 2. Position Parameters for Idiospermuline Trimethiodide [4] ($C_{38}H_{51}I_3N_6$).

Atom	x	y	z	B_{eq}
I-1	0.9671 (2)	0.1863	0.3655 (1)	6.6 (1)
I-2	0.4034 (2)	0.4122 (4)	0.1241 (1)	6.1 (1)
I-3	0.1391 (2)	0.0623 (5)	0.9048 (1)	8.8 (2)
N-1	0.895 (2)	0.644 (3)	0.858 (1)	5 (1)
N-2	0.820 (2)	0.379 (3)	0.836 (2)	4 (1)
N-3	0.584 (2)	0.383 (3)	0.809 (1)	3.2 (5)
N-4	0.487 (2)	0.384 (3)	0.900 (1)	4 (1)
N-5	0.253 (2)	0.343 (3)	0.581 (1)	5 (1)
N-6	0.160 (2)	0.173 (5)	0.643 (2)	9 (2)
C-1	1.003 (4)	0.559 (7)	0.901 (2)	10 (3)
C-2	0.879 (3)	0.775 (5)	0.907 (2)	8 (2)
C-3	0.911 (3)	0.703 (5)	0.786 (2)	7 (2)
C-4	0.795 (2)	0.727 (4)	0.735 (2)	4 (2)
C-5	0.739 (2)	0.584 (4)	0.747 (1)	3.7 (7)
C-6	0.796 (2)	0.537 (4)	0.834 (1)	2.3 (6)
C-7	0.767 (2)	0.436 (4)	0.712 (2)	4 (2)
C-8	0.754 (3)	0.409 (5)	0.637 (2)	5 (2)
C-9	0.793 (3)	0.266 (6)	0.618 (2)	6 (2)
C-10	0.829 (3)	0.151 (4)	0.670 (2)	6 (2)
C-11	0.843 (3)	0.181 (6)	0.750 (2)	6.3 (9)
C-12	0.811 (3)	0.326 (4)	0.765 (2)	4 (2)
C-13	0.846 (3)	0.278 (5)	0.902 (2)	7 (2)
C-14	0.618 (2)	0.600 (3)	0.727 (1)	3.1 (6)
C-15	0.564 (2)	0.708 (4)	0.676 (2)	3.8 (7)
C-16	0.455 (2)	0.728 (4)	0.650 (1)	4 (2)
C-17	0.397 (2)	0.632 (4)	0.680 (2)	4 (2)
C-18	0.443 (2)	0.517 (4)	0.729 (1)	3 (1)
C-19	0.548 (2)	0.501 (3)	0.753 (1)	2.3 (6)
C-20	0.495 (2)	0.319 (3)	0.825 (1)	3.1 (6)
C-21	0.393 (2)	0.388 (3)	0.764 (1)	3 (1)
C-22	0.317 (2)	0.458 (4)	0.805 (2)	5 (2)
C-23	0.398 (3)	0.516 (4)	0.877 (2)	5 (2)
C-24	0.588 (3)	0.425 (6)	0.954 (2)	6 (2)
C-25	0.440 (3)	0.248 (3)	0.936 (1)	5 (2)
C-26	0.334 (3)	0.258 (4)	0.706 (2)	4 (2)
C-27	0.306 (3)	0.107 (4)	0.742 (2)	5 (2)
C-28	0.216 (3)	0.055 (6)	0.686 (2)	8 (3)
C-29	0.085 (5)	0.218 (8)	0.692 (3)	7 (2)
C-30	0.073 (5)	0.168 (8)	0.581 (3)	8 (1)
C-31	0.229 (3)	0.322 (4)	0.649 (2)	4.5 (8)
C-32	0.196 (3)	0.451 (5)	0.523 (2)	7 (2)
C-33	0.354 (3)	0.278 (4)	0.588 (2)	5 (2)
C-34	0.397 (2)	0.262 (4)	0.529 (2)	5 (2)
C-35	0.486 (3)	0.186 (5)	0.544 (2)	6.1 (9)
C-36	0.547 (3)	0.118 (4)	0.616 (2)	6 (2)
C-37	0.494 (3)	0.137 (4)	0.673 (2)	3.8 (7)
C-38	0.405 (2)	0.216 (3)	0.657 (1)	2 (1)

application with acetylcholine for 1 min. With this arrangement, recording cortex against corpus callosum, activity was either a depolarizing response measured as an upward deflection from the baseline or as a hyperpolarizing response, a downward deflection from the baseline in which the amplitude was measured in mV. At each step of the extraction and purification, fractions were assayed for activity using the rat brain cortical wedge preparation.

EXTRACTION AND ISOLATION.—The cotyledons from four seeds of *I. australiense* (438.2 g) were finely sliced, then homogenized in MeOH (1 liter). The solid powder was filtered and subjected to re-extraction

in MeOH (2×1 liter). The filtrate and the washings were combined and concentrated under reduced pressure to a viscous reddish brown liquid, the volume of which was adjusted with H_2O to 400 ml. The resulting liquid was exhaustively extracted with *n*-pentane, followed by continuous extraction with Et_2O. The aqueous residue was made alkaline with aqueous NH_3 and subjected to continuous extraction with Et_2O. The second Et_2O extract was concentrated under reduced pressure to give a reddish brown gum.

The concentrated Et_2O extract was subjected to purification by gel filtration chromatography (Sephadex LH-20) using two solvent systems (MeOH and $CHCl_3$-MeOH, 3:2). Repeated gel filtration chromatography gave three fractions containing almost pure alkaloids; the fraction that eluted first contained idiospermuline [**3**], followed by a fraction containing chimonanthine [**2**], while the last fraction contained calycanthine [**1**]. Crystallization of the second fraction (MeOH/MeCN mixture) gave (−)-chimonanthine [**2**] (1.1 g, 0.25% of fresh seeds; mp 189–190°), $[\alpha]^{25}D$ −328° (*c*=1.0, EtOH), cims (CH_4) *m/z* $[M+29]^+$ 375 (8), $[M+1]^+$ 347 (100), 173 (85). Crystallization of the third fraction (MeOH/$CHCl_3$ mixture) gave (+)-calycanthine [**1**] (0.57 g, 0.13% of fresh seeds), mp 234–235° sublimed, $[\alpha]^{25}D$ +675° (*c*=1.0, $CHCl_3$), cims (CH_4) *m/z* $[M+29]^+$ 375 (15), $[M+1]^+$ 347 (100), 173 (15). The mps and optical rotations of **1** and **2** were consistent with published data for (+)-calycanthine (11) and (−)-chimonanthine (12). ^1H- and ^{13}C-nmr spectra of the isolated alkaloids were also in accord with those reported in the literature for racemic chimonanthine and racemic calycanthine (13).

The first fraction, which contained **3**, was further purified by reversed-phase vacuum chromatography (20) (silanized Si gel H-60, 25 g, 4.8×4.0 cm i.d.), eluting with 50% aqueous MeOH (4×50 ml), followed by stepwise elution with aqueous MeOH containing 1% diethylamine in 10% steps from 60% to 100% MeOH (50 ml fractions). Fractions were analyzed by tlc using two solvent systems: (a) 10% diethylamine in Et_2O, and (b) 20% MeOH saturated with NH_3 in $CHCl_3$. The alkaloids were visible under uv (254 nm) light and were positive to ninhydrin spray reagent. Fractions eluted with 80–100% MeOH were combined and concentrated under reduced pressure to 50 ml, then refractionated 3–4 times under the same conditions. The successive reversed-phase short column vacuum chromatography removed a small amount of more polar impurities. The fractions containing idiospermuline [**3**] were combined and evaporated under reduced pressure to give a pale yellow, foamy solid. The solid was resubjected to gel filtration chromatography (Sephadex LH-20, MeOH) to give **3** (1.6 g, 0.39% of fresh seeds) as a colorless powder: mp 95–97°; *anal.*, found C 76.4, H 8.1, N 15.0, calcd for $C_{35}H_{42}N_6$, C 76.9, H 7.7, N 15.4; hreims (70 ev) *m/z* 546.343 (22) (calcd 546.347 for $C_{35}H_{42}N_6$), 358.212 (52) (calcd 358.216 for $C_{23}H_{26}N_4$), 186.117 (100) (calcd 186.116 for $C_{12}H_{14}N_2$); $[\alpha]^{25}D$ −2.5° (*c*=1.0, $CHCl_3$); cims (NH_3) *m/z* $[M+1]^+$ 547 (100); cims (CH_4) *m/z* $[M+1]^+$ 547 (100), 359 (87), 187 (66), eims (100 ev) *m/z* $[M]^+$ 546 (9), 358 (79), 328 (10), 315 (22), 301 (9), 186 (100), 172 (45), 144 (45); cd (MeOH) λ max (Δε) 234 (+0.37), 271 (−3.2), 322 (−4.9) liter $mol^{-1}cm^{-1}$; uv (MeOH) λ max (log ε) 207.0 (5.8), 250.0 (4.4), 304.8 (4.0) nm; ir (KBr) ν max 3330, s, 3260, br (NH), 3050 (CH, aromatic), 1620 (C=C, aromatic), 745 (CH, aromatic) cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.13 (1H, ddd, *J*=7.8, 7.5, and 1.4 Hz, H-6), 7.09–6.75 (4H, m, H-6′, H-6″, H-4′, and H-4″), 6.89 (1H, br d, H-4), 6.72 (1H, dt, *J*=7.4 and 1 Hz, H-5), 6.54–6.38 (2H, m, H-5′ and H-5″), 6.47 (1H, d, *J*=7.7 Hz, H-7), 6.18 (1H, br d, *J*=7.2 Hz, H-7″), 5.27 (1H, br s, H-8a′), 4.58 (2H, br s, H-8a and H-8a″), 3.50 (1H, br s, exchanged in D_2O, NH), 2.96 (3H, s, Me-8), 2.94 (2H, m, CH_2-2), 2.89 (3H, br s, Me-8″), 2.64 (2H, m, CH_2-2′), 2.51 (3H, s, Me-1), 2.40–2.20 (10H, m, Me-1, CH_2-2″, Me-1′, and CH_2-3′), 2.02 (1H, m, CH_2-3″), 1.94 (1H, m, CH_2-3″), 1.92 (2H, m, CH_2-3); ^{13}C nmr ($CDCl_3$) δ 152.8 (C-7a), 152.7 (C-7a″), 149.4 (C-7a′), 133.8 (C-4a′), 132.8 (C-4a″, br), 132.7 (C-4a), 128.1 (C-6), 127.8 (C-6′ or C-4′), 125.3 (C-4), 124.7 (C-4′ or C-6′), 122.7 (C-6″ or C-4″, br), 122.1 (C-4″ or C-6″, br), 117.7 (C-5), 117.0 (C-5″, br), 116.7 (C-5′), 106.6 (C-7), 105.7 (C-7″), 95.0 (C-8a), 91.8 (C-8a″), 83.6 (C-8a′), 62.8 (C-3a′ or C-3a″, br), 62.7 (C-3a″ or C-3a′, br), 59.4 (C-3a), 52.8 (C-2 and C-2′), 52.3 (C-2″, br), 52.1 (C-2″, br), 37.7 (C-3, 1-C and 8″-C), 37.6 (1″-C), 36.8 (8-C), 36.2 (1′-C), 35.3 (C-3′ or C-3″), 35.2 (C-3″ or C-3′).

To a solution of **3** (90 mg, 0.16 mmol) in MeOH (90 ml) and C_6H_6 (10 ml) was added MeI (300 mg, 4 equivalents) and the solution was left standing at room temperature for 18 h. The mixture was concentrated to dryness and redissolved in a minimum volume of MeOH. The solution was saturated with C_6H_6 at 60°, then left to crystallize at room temperature. The crystals were filtered and recrystallized from MeOH to give **4** (100 mg, 63%) as colorless needles: 220–225° (sublimed), mp 230–231° (dec); *anal.*, found C 46.4, H 5.7, I 39.4, N 8.3, calcd for $C_{38}H_{51}I_3N_6$, C 46.9, H 5.3, I 39.1, N 8.4); $[\alpha]^{25}D$ −2.3° (*c*=0.1, H_2O); cd (MeOH) λ max (Δε) 210 (−0.6), 236 (+0.1), 257 (−14.8), 306 (−12.9) liter mol^{-1} cm^{-1}; uv (MeOH) λ max (log ε) 198.1 (4.9), 215 sh (4.9), 223 (4.8), 295 (3.8) nm; ir (KBr) ν max 3680–3150 (NH), 3050 (CH, aromatic), 1620 (C=C, aromatic), 750 (CH, aromatic) cm^{-1}; cims (CH_4) *m/z* 575 (8), 518 (8), 417 (15), 389 (100), 388 (45), 375 (22), 374 (17), 345 (12), 330 (9), 318 (58), 317 (19), 229 (11), 202 (72), 201 (79), 187 (30), 158 (48), 144 (52), 135 (53), 129 (90); fabms (Ar) *m/z* 845 (3), 743 (5), 717 (100), 589 (22), 575 (51), 518 (33), 473 (18), 459 (24); ^1H nmr [D_2O, HOD (δ 4.75)] δ 7.65 (1H, d, *J*=8.1 Hz, H-4′), 7.59 (1H, d, *J*=7.8 Hz, H-6′), 7.43 (1H, d, *J*=7.6 Hz, H-4″), 7.36 (1H, ddd, *J*=7.7, 7.3, and 1.8 Hz, H-6), 7.29 (1H, ddd, *J*=8.1, 7.6, and 1.8 Hz, H-6″), 7.22 (1H, t, *J*=7.8 Hz, H-5′), 7.05 (1H, dd, *J*=7.2 and 1.8 Hz, H-4), 7.03 (1H, t, *J*=7.2 Hz, H-5), 6.90 (1H, d, *J*=6.9 Hz, H-7), 6.89 (1H, t, *J*=7.8 Hz, H-5″), 6.62 (1H,

d, J=7.8 Hz, H-7″), 5.30 (2H, br s, H-8a and H-8a″), 4.42 (1H, br s, H-8a′), 3.61 (1H, dd, J=10.7 and 4.6 Hz, CH_2-2), 3.43 (1H, m, CH_2-2″), 3.39 (1H, m, CH_2-2″), 3.36 (3H, s, Me-8), 3.26 (1H, m, CH_2-2), 3.08 (1H, m, CH_2-3″), 3.05 (3H, s, Me-1*), 3.03 (3H, s, Me-1*), 2.98 (1H, d, J=6.9 Hz, CH_2-3), 2.90 (1H, m, CH_2-2′), 2.87 (1H, m, CH_2-2′), 2.80 (1H, m, CH_2-3′), 2.78 (1H, m, CH_2-3′), 2.74 (3H, s, Me-1*), 2.72 (3H, s, Me-8″), 2.70 (1H, m, CH_2-3), 2.67 (1H, m, CH_2-3″), 2.58 (3H, s, Me-1*), 2.54 (3H, br s, Me-1*), 2.44 (3H, br s, Me-1*); ^{13}C nmr [D_2O, dioxan (δ 67.27)] δ 148.9 (C-7a), 145.1 (C-7a″), 131.9 (C-6″), 131.7 (C-7a′), 131.4 (C-6), 129.7 (C-4a″), 128.4 (C-4′), 126.9 (C-4a′), 125.5 (C-6′), 125.4 (C-4a), 124.8 (C-4″), 124.2 (C-4), 123.8 (C-5′), 122.7 (C-5), 121.8 (C-5″), 109.6 (C-7), 109.0 (C-7″), 104.9 (C-8a″), 104.1 (C-8a), 94.6 (C-8a′), 63.4 (C-2″), 63.2 (C-2′), 61.8 (C-3a″), 61.6 (C-3a′), 61.1 (C-2), 59.2 (C-3a), 50.8, 50.4, 49.9, 47.5 (2 carbons), 46.7 (2×1-C, 2×1′-C, and 2×1″-C), 36.7 (8-C), 35.3 (C-3″), 34.7 (8″-C), 31.0 (C-3′), 30.8 (C-3). *Assignments may be interchanged between subunits.

X-RAY CRYSTALLOGRAPHY.[1]—For diffractometry, the crystal was mounted on a glass fiber with cyanoacrylate resin. Lattice parameters at 21° were determined by least-squares fits to the setting parameters of 25 independent reflections, measured and refined on an AFC-7 four-circle diffractometer employing graphite monochromated CuKα radiation. Intensity data were collected in the range $1<\theta<60°$. Data reduction and application of Lorentz, polarization, absorption (empirical psi scans) and decomposition corrections were carried out using the teXsan system software, Crystal Structure Analysis Package, Molecular Structure Corporation (1985 and 1992).

Formula $C_{38}H_{51}I_3N_6$; M 972.58, monoclinic, space group $P2_1$, a 13.311(8), b 8.530(6), c 18.907(5) Å, β 107.84(3)°; V2044(2) Å^3, Z2, D_c 1.581 g cm^{-3}, μ(CuKα) 63.88 cm^{-1}, λ(CuKα) 1.5418 Å, F(000) 960 electrons, final[2] R=0.066,[3] R_w=0.072, 1653 F with $F>5\sigma(F)$.

The structure was solved by direct methods using SHELXS-86 (21). Hydrogen atoms were included at calculated sites with fixed isotropic thermal parameters. All other atoms were refined anisotropically. Full-matrix least-squares methods were used to refine an overall scale factor, positional and thermal parameters. Neutral atom scattering factors were taken from Cromer and Waber (22). Anomalous dispersion effects were included in F_c (23); the values for $\Delta f'$ and $\Delta f''$ were those of Creagh and McAuley (24). The values for the mass attenuation coefficients are those of Creagh and Hubbel (25). All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation and plots were drawn using ORTEP software, A Thermal Ellipsoid Plotting Program, C.K. Johnson, Oak Ridge National Laboratories, Oak Ridge (1965).

ACKNOWLEDGMENTS

The provision of the plant material by Mr. Peter Radke and Mrs. Anne Radke of Yuruga Nursery is gratefully acknowledged. We thank Mr. Bruce Tattam and Mrs. Helen Elimelakh of the Department of Pharmacy, University of Sydney, for recording mass spectra. We thank Dr. H.W. Tran, Department of Pharmacology, University of Sydney, for technical assistance, and Mr. J.R. Greenwood and Dr. H.R. Capper, Department of Pharmacology, the University of Sydney, for assistance with the molecular mechanics calculations. We also thank Professor W.C. Taylor, School of Chemistry, the University of Sydney, for helpful discussions. Support of this work by a National Health and Medical Research Council grant and an Australian Research Council small grant is gratefully acknowledged.

LITERATURE CITED

1. S.T. Blake, *Cont. Qld. Herb.*, **12**, 1 (1972).
2. L. Diels, *Bot. Jahrb.*, **48**, 107 (1912).
3. K.A.W. Williams, "Native Plants of Queensland." North Ipswich, Queensland, Australia, 1979, Vol. 1, p. 162.
4. K.A.W. Williams, "Native Plants of Queensland," North Ipswich, Queensland, Australia, 1984, Vol. 2, p. 162.
5. S.L. Everist, "Poisonous Plants of Australia," Angus and Robertson, Sydney, 1981, 2nd Ed., p. 371.
6. J. Mock, "The Chemical Constituents of *Idiospermum australiense* (Diels) S.T. Blake," Ph.D. Thesis, University of Sydney, Sydney, 1973, pp. 102–148.
7. N.L. Harrison and M.A. Simmonds, *Br. J. Pharmacol.*, **84**, 381 (1985).

[1]Hydrogen coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

[2]$R=\Sigma(||F_o|-|F_c||)/\Sigma|F_o|$.

[3]$R_w=(\Sigma w(|F_o|-|F_c|)^2/\Sigma wF_o^2)^{1/2}$.

8. B. Ebert, S. Lenz, L. Brehm, P. Bregnedal, J.J. Hansen, K. Frederiksen, K.P. Bogeso, and P. Krogsgaard-Larsen, *J. Med. Chem.*, **37**, 878 (1994).
9. R.H. Evans, in "The NMDA Receptor." Ed. by J.C. Watkins and G.L. Collingridge, Oxford University Press, Oxford, UK, 1989, Chapter 5, pp. 69–74.
10. J. Ong, D.I.B. Kerr, G.A.R. Johnston, and R.G. Hall, *Neuroscience Lett.*, **109**, 169 (1990).
11. "The Merck Index, An Encyclopedia of Chemicals, Drugs, and Biologicals." Ed. by S. Budavari, Merck & Co., Inc., Rahway, N.J., 1989, 11th ed., p. 260.
12. H.F. Hodson, B. Robinson, and G.F. Smith, *Proc. Chem. Soc.*, 465 (1961).
13. F. Libot, N. Kunesch, and J. Poisson, *Heterocycles*, **27**, 2381 (1988).
14. E.F.L. Anet, G.K. Hughes, and E. Ritchie, *Aust. J. Chem.*, **14**, 173 (1961).
15. E.S. Hall, F. McCapra, and A.I. Scott, *Tetrahedron*, **23**, 4131 (1967).
16. N.K. Hart, S.R. Johns, J.A. Lamberton, and R.E. Summons, *Aust. J. Chem.*, **27**, 639 (1982).
17. F. Gueritte-VoeGelein, T. Sevenet, J. Pusset, M.T. Adeline, B. Gillet, J.-C. Beloeil, D. Guenard, and P. Potier, *J. Nat. Prod.*, **55**, 923 (1992).
18. S.F. Mason and G.W. Vane, *J. Chem. Soc.*, 370 (1966).
19. J. Fridrichsons, M.F. MacKay, and A. Mathieson, *Tetrahedron*, **30**, 85 (1974).
20. J.N. Grech, Q. Li, B.D. Roufogalis, and C.C. Duke, *J. Nat. Prod.*, **57**, 1682 (1994).
21. G.M. Sheldrick, SHELXS-86, "Crystallographic Computing 3," Ed. by G.M. Sheldrick, C. Krüger, and R. Goddard, Oxford University Press, Oxford, UK, 1985, pp. 175–189.
22. D.T. Cromer and J.T. Waber, "International Tables for X-Ray Crystallography," Kynoch Press, Birmingham, UK, 1974, Vol. 4.
23. J.A. Ibers and W.C. Hamilton, *Acta Crystallogr.*, **17**, 781 (1964).
24. D.C. Creagh and W.J. McAuley, in: "International Tables for Crystallography," Ed. by A.J.C. Wilson, Kluwer Academic Publishers, Boston, 1992, Vol. C, Table 4.2, 6.8, pp. 219–222.
25. D.C. Creagh and J.H. Hubbell, in: "International Tables for Crystallography." Ed. by A.J.C. Wilson, Kluwer Academic Publishers, Boston, 1992, Vol. C, Table 4.2, 4.3, pp. 200–206.

Received 23 January 1995