Structures and Solid State Tautomeric Forms of Two Novel Antileukemic Tropoloisoquinoline Alkaloids, Pareirubrine A and B, from *Cissampelos pareira*

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Two novel tropoloisoquinoline alkaloids, Pareirubrine A and B, have been isolated as antileukemic substances from *Cissampelos pareira* (Menispermaceae), together with the same skeleton alkaloids, grandirubrine and isoimerubrine. Their structures were elucidated by nuclear magnetic resonance (NMR) studies, and their solid state tautomeric forms were examined by X-ray crystallographic analysis.

Keywords tropoloisoquinoline alkaloid; pareirubrine; *Cissampelos pareira*; Menispermaceae; antileukemic activity; X-ray analysis

To date, several tropoloisoquinoline alkaloids, such as imerubrine¹⁾ and grandirubrine,²⁾ a condensed tropolone and isoquinoline alkaloid, have been isolated from the tropical American genus *Abuta* (Menispermaceae). However, the pharmacological activities of these tropoloisoquinolines have not been elucidated.

During our survey of novel antileukemic compounds from South American medicinal plants,³⁾ the crude extract of *Cissampelos pareira* showed antileukemic activity, and a novel tropoloisoquinoline alkaloid named pareirubrine A, one of the active principles, has been reported in a preliminary form.⁴⁾ Further bioassay-directed purification, guided by cytotoxicity against P-388 cells, led to the isolation of a novel tropoloisoquinoline alkaloid named pareirubrine B, together with grandirubrine and isoimerubrine. Their structures were elucidated by spectroscopic methods, and X-ray analysis of these pareirubrines revealed to us their solid state conformations.

In the present paper, a full account of structural elucidation and the solid state conformation of pareirubrines A and B are described.

Isolation and Structure Determination The roots of *Cissampelos pareira* (Menispermaceae) are used to prevent a threatened miscarriage, and the herb is also used to stop

uterine hemorrhaging.⁵⁾ It is a perennial climbing shrub found in many parts of the tropical world. The methanol extract showing cytotoxicity against P-388 cells was partitioned between methylene chloride and water. The methylene chloride soluble fraction was further separated by chromatographic purification using silica gel and reversed-phase medium pressure liquid chromatography (MPLC), high pressure liquid chromatography (HPLC) and Sephadex LH-20, conducted in conjunction with a bioassay against P-388 cells, to give four antileukemic compounds (1—4), which are positive to Dragendorff reagent.

Compound 1, named pareirubrine A, was obtained as reddish-brown needles, mp 168—170 °C, possessing the molecular formula, C₂₀H₁₇NO₆, according to high-resolution fast atom bombardment mass spectrometry (HR-FAB-MS). In the ¹H-NMR spectrum (Table I), only four sets of methoxy methyl signals, at δ_{H} 4.05, 4.16, 4.18 and 4.24, and two sets of each coupled olefinic proton signals at δ_{H} 7.79 and 8.88 (each 1H, d, $J=5.8$ Hz; H-1 and 16, respectively), and 7.46 and 8.37 (each 1H, d, $J=10.3$ Hz; H-9 and 8, respectively) were observed. The ultraviolet (UV) spectrum in EtOH, showing maxima (ϵ) at 472 (7700), 420 (3800), 364 (20200), 294 (21000), 274 (25200) nm, suggested features of tropoloisoquinoline alkaloids, which are

Fig. 1. Molecular Structures of Tropoloisoquinoline Alkaloids (1—4)

Compounds 1—3 exist in two states (a and b) in solution.

TABLE I. ^1H -NMR Assignments of Compounds **1**–**4** (500 MHz)

Protons	1 ^{a)}	2 ^{b)}	3 ^{a)}	4 ^{a)}
H-1	7.79 (d, $J=5.8$ Hz)	7.77 (d, $J=5.7$ Hz)	7.80 (d, $J=5.7$ Hz)	7.79 (d, $J=5.8$ Hz)
H-3	—	7.43 (s)	—	—
H-8	8.37 (d, $J=10.3$ Hz)	8.24 (d, $J=10.5$ Hz)	8.33 (d, $J=10.6$ Hz)	8.25 (d, $J=12.2$ Hz)
H-9	7.46 (d, $J=10.3$ Hz)	7.31 (d, $J=10.5$ Hz)	7.41 (d, $J=10.6$ Hz)	7.38 (d, $J=12.2$ Hz)
H-12	—	7.96 (s)	8.41 (s)	7.91 (s)
H-16	8.88 (d, $J=5.8$ Hz)	8.68 (d, $J=5.7$ Hz)	8.73 (d, $J=5.7$ Hz)	8.73 (d, $J=5.8$ Hz)
3-OMe	4.16 (s)	—	4.16 (s)	4.18 (s)
4-OMe	4.18 (s) ^{c)}	4.06 (s) ^{d)}	4.19 (s) ^{e)}	4.20 (s) ^{f)}
5-OMe	4.05 (s) ^{c)}	4.04 (s) ^{d)}	4.06 (s) ^{e)}	4.03 (s) ^{f)}
11-OMe	—	—	—	4.17 (s)
12-OMe	4.24 (s)	—	—	—

a) In CDCl_3 . b) In $\text{DMSO}-d_6$. c–f) Assignments may be interchanged.

Fig. 2. HMBC Correlations of Pareirubrine A (**1**) and B (**2**)

condensed tropolone and isoquinoline alkaloids. In the ^{13}C -NMR spectrum, under normal conditions using a 45-degree pulse width, the signals were not easily detectable, presumably because of many consecutive quaternary carbons. However, by use of a short pulse width, such as 25 degrees, the signals of many quaternary carbons grew (Table II). The correlation between the above ^1H and ^{13}C signals was revealed by two-dimensional (2-D) NMR techniques such as heteronuclear multiple bond correlation (HMBC)⁶⁾ and heteronuclear multiple quantum coherence (HMQC)⁷⁾ spectra. From the results of the ^1H – ^{13}C long range correlation shown in Fig. 2, the structure of **1** was determined to be as shown in Fig. 1.

Compound **2**, named pareirubrine B, was obtained as reddish-brown needles, mp 290 °C (dec.), possessing the molecular formula $\text{C}_{18}\text{H}_{13}\text{NO}_4$ as determined by HR-MS spectrum. In the ^1H NMR spectrum of **2**, only two methoxy methyl signals at δ_{H} 4.04 and 4.06 and two singlet signals at δ_{H} 7.43 and 7.96 in place of the four methoxyl groups of **1** were observed. One of these singlet signals, at δ_{H} 7.96, and a set of olefinic protons at δ_{H} 7.31 and 8.24 (each 1H, d, $J=10.5$ Hz) bore a close resemblance to those based on the tropolone part of grandirubrine.²⁾ Further, another set of olefinic protons, at δ_{H} 7.77 and 8.68 (each 1H, d, $J=5.7$ Hz), was assignable to H-1 and H-16 of the isoquinoline section. Another singlet signal at δ_{H} 7.43 was ascribable to H-3 from HMBC correlation between H-1 and C-3 as shown in Fig. 2. Therefore, the structure of pareirubrine B (**2**) was determined to be as shown in Fig. 1. The tropoloisoquinoline alkaloids detected so far are derivatives of the tautomers **a** and **b** in Fig. 1. In the ^1H -NMR spectra, pareirubrine A and B showed a much closer resemblance to the **b** than **a** form by comparing the

TABLE II. ^{13}C -NMR Assignments of Compounds **1**–**4** (125 MHz)

Carbon	1 ^{a)}	2 ^{b)}	3 ^{a)}	4 ^{a)}
C-1	114.8	119.2	115.7	115.8
C-2	126.1	130.5	126.2	125.9
C-3	149.6	105.6	149.9	151.3
C-4	152.1 ^{c)}	147.2 ^{e)}	152.4 ^{g)}	154.4 ^{h)}
C-5	149.8 ^{c)}	159.1 ^{e)}	150.1 ^{g)}	149.5 ^{h)}
C-6	121.8	125.3	121.8	121.3
C-7	136.4	140.4	137.1	136.5
C-8	127.6	131.4	131.3	132.5
C-9	117.3	119.0	119.9	136.9
C-10	162.4	167.7	167.8	180.2
C-11	173.0	175.2	173.5	164.4
C-12	157.5	120.2	120.1	106.8
C-13	138.4	145.3	146.3	139.0
C-14	158.5	156.4	157.9	158.5
C-16	146.3	146.2	145.7	145.2
C-17	125.7	123.6	124.5	121.0
3-OMe	62.1	—	62.0	62.2
4-OMe	61.4 ^{d)}	56.7 ^{f)}	61.5	62.0 ⁱ⁾
5-OMe	61.6 ^{d)}	61.0 ^{f)}	61.5	61.5 ⁱ⁾
11-OMe	—	—	—	56.9
12-OMe	60.7	—	—	—

a) In CDCl_3 . b) In $\text{DMSO}-d_6$. c–i) Assignments may be interchanged.

chemical shifts of H-8 and 9 with those of imerubrine¹⁾ and isomerubrine (**4**).²⁾

Compounds **3** and **4** were identical to grandirubrine and isomerubrine, which was derived from grandirubrine by methylation,²⁾ respectively, of the three tropoloisoquinoline alkaloids determined to date.

Solid State Tautomeric Form and Planarity In the ^1H -NMR spectrum, the signal of one methoxyl group substituted at C-11 of isoimerubrine (**4**) was broadened more than the other methoxyl group substituted at the isoquinoline skeleton. It is known that tropolones are derivatives of two tautomers such as **a** and **b** in Fig. 1. However, this broadened signal cannot be explained by these tautomers. X-Ray analysis of pareirubrine A has already been reported.⁴⁾ Further, to analyze the conformation of a tropolone ring and to confirm the structure of pareirubrine B, X-ray crystallographic analysis of **2** was conducted.

A dark orange single crystal of **2** was grown from methanol–methylene chloride. PLUTO⁸⁾ drawings of the molecules are given in Fig. 3. The bond lengths and bond angles found in the crystal are given in Table III. The

TABLE III. Bond Lengths and Bond Angles Found in the Crystal of Pareirubrine B (2)

Bond lengths in Å between heavier atoms			Bond angles in degrees			
Atom 1	Atom 2	Length (S.T.D.)	Atom 1	Atom 2	Atom 3	Angle (S.T.D.)
Bond lengths in Å between heavier atoms						
C1	— C2	1.418 (5)	C2	— C1	— C16	119.4 (3)
C1	— C16	1.386 (5)	C3	— C2	— C1	130.1 (3)
C2	— C3	1.423 (4)	C3	— C2	— C17	116.2 (3)
C2	— C17	1.386 (4)	C1	— C2	— C17	113.8 (3)
C3	— C4	1.379 (5)	C4	— C3	— C2	119.0 (3)
C4	— C5	1.445 (4)	C5	— C4	— C3	123.2 (3)
C4	— O18	1.358 (4)	C5	— C4	— O18	112.7 (3)
C5	— C6	1.372 (4)	C3	— C4	— O18	124.1 (3)
C5	— O19	1.355 (4)	C6	— C5	— C4	117.9 (3)
C6	— C7	1.476 (3)	C6	— C5	— O19	119.7 (3)
C6	— C17	1.406 (4)	C4	— C5	— O19	122.2 (3)
C7	— C8	1.366 (4)	C7	— C6	— C5	134.8 (3)
C7	— C13	1.454 (4)	C7	— C6	— C17	107.4 (3)
C8	— C9	1.407 (4)	C5	— C6	— C17	117.8 (3)
C9	— C10	1.373 (4)	C8	— C7	— C6	126.1 (3)
C10	— C11	1.455 (5)	C8	— C7	— C13	126.6 (3)
C10	— O20	1.345 (3)	C6	— C7	— C13	107.3 (3)
C11	— C12	1.427 (4)	C9	— C8	— C7	128.3 (3)
C11	— O21	1.258 (3)	C10	— C9	— C8	130.7 (3)
C12	— C13	1.363 (4)	C11	— C10	— C9	129.8 (3)
C13	— C14	1.483 (4)	C11	— C10	— O20	114.4 (3)
C14	— C17	1.403 (4)	C9	— C10	— O20	115.8 (3)
C14	— N15	1.320 (4)	C12	— C11	— C10	123.9 (3)
C16	— N15	1.368 (4)	C12	— C11	— O21	120.3 (3)
C22	— O18	1.435 (4)	C10	— C11	— O21	115.9 (3)
C23	— O19	1.447 (6)	C13	— C12	— C11	129.9 (3)
Bond lengths including hydrogen atoms						
C1	— HC1	1.06 (3)	C14	— C13	— C12	122.6 (3)
C3	— HC3	1.05 (3)	C7	— C13	— C12	130.7 (3)
C8	— HC8	1.02 (3)	C17	— C14	— C13	107.6 (3)
C9	— HC9	1.07 (3)	C17	— C14	— N15	123.7 (3)
C12	— HC12	1.04 (4)	C13	— C14	— N15	128.7 (3)
C16	— HC16	1.07 (3)	N15	— C16	— C1	126.0 (3)
C22	— HC22	1.06 (5)	C2	— C17	— C6	125.9 (3)
C22	— H'C22	1.01 (4)	C2	— C17	— C14	123.0 (3)
C22	— H''C22	1.04 (3)	C6	— C17	— C14	111.1 (3)
C23	— HC23	1.03 (4)	C4	— O18	— C22	118.9 (3)
C23	— H'C23	1.02 (4)	C5	— O19	— C23	118.4 (3)
C23	— H''C23	1.03 (5)	C14	— N15	— C16	114.1 (3)
O20	— HO20	1.00 (4)

Fig. 3. (a) Molecular Structure Viewed Perpendicular to the Molecular Plane. (b) Molecular Structure Viewed upwards along the Vertical Line on the Molecular Plane Shown in (a)

TABLE IV. Planarity of the Molecule of Pareirubrine B (2)

Plane formed	D. from plane (Å)	Coefficient	Plane formed	D. from plane (Å)	Coefficient	Atom	D. from plane (Å)
Group I			Group IV				
C1	0.002 (3)	A 0.0157	C1	0.004 (3)	A 0.0151	O18	0.006 (3)
C2	-0.000 (3)	B -0.1625	C2	0.007 (3)	B -0.1583	O19	0.010 (3)
C17	-0.001 (3)	C 0.9866	C3	0.002 (3)	C 0.9873	O20	0.051 (3)
C14	0.001 (3)	D 2.5785	C4	-0.015 (3)	D 2.5605	O21	-0.127 (3)
N15	-0.001 (3)		C5	-0.036 (3)		C22	0.013 (4)
C16	-0.002 (3)		C6	-0.002 (3)		C23	-1.014 (3)
Group II			C7	0.003 (3)			
C2	-0.008 (3)	A 0.0025	C8	0.018 (3)			
C3	0.004 (3)	B -0.1512	C9	0.036 (3)			
C4	0.005 (3)	C 0.9885	C10	0.012 (3)			
C5	-0.011 (3)	D 2.5046	C11	-0.052 (3)			
C6	-0.007 (3)		C12	-0.019 (3)			
C17	-0.002 (3)		C13	0.003 (3)			
Group III			C14	0.014 (3)			
C6	-0.003 (3)	A 0.0112	N15	0.010 (3)			
C7	0.004 (3)	B -0.1537	C16	0.002 (3)			
C13	-0.003 (3)	C 0.9881	C17	0.012 (3)			
C14	0.001 (3)	D 2.5483					
C17	0.002 (3)						

Plane formed: The least-squares plane formed by the atoms listed below. D. from plane: The perpendicular distance of the individual atom from the least-squares plane. Coefficient: Coefficient of the equation of the least-squares plane. $AX+BY+CZ=D$, where X, Y, Z are the orthogonal axes measured in Å unit and taken as $Z \parallel c$, X in ac plane, $Y \perp$ to X and Z . Interplanar angles between the plane group IV and group I is 0.00° , group II is 0.73° and group III is 0.00° .

Fig. 4. The Crystal Structure of 2 Viewed along the c Axis Showing the Stacking of the Molecules Related by a Center of SymmetryTABLE V. Fractional Atomic Co-ordinates ($\times 10^4$) with Estimated Standard Deviations in Parentheses for Compound 2

No.	Atom	$x \ 10^4$	$y \ 10^4$	$z \ 10^4$
1	C1	4380 (3)	-3425 (3)	-11 (5)
2	C2	3570 (3)	-2514 (3)	903 (5)
3	C3	2146 (3)	-2784 (3)	1045 (5)
4	C4	1593 (3)	-1703 (3)	1998 (5)
5	C5	2390 (3)	-295 (3)	2861 (5)
6	C6	3762 (3)	-38 (3)	2765 (4)
7	C7	4902 (3)	1207 (3)	3453 (4)
8	C8	4812 (3)	2510 (3)	4509 (5)
9	C9	5863 (3)	3723 (3)	5213 (5)
10	C10	7232 (3)	3942 (3)	5006 (5)
11	C11	8018 (3)	2977 (3)	3965 (5)
12	C12	7445 (3)	1533 (3)	3036 (5)
13	C13	6129 (3)	783 (3)	2816 (4)
14	C14	5711 (3)	-727 (3)	1764 (4)
15	C16	5767 (3)	-2911 (3)	36 (5)
16	C17	4301 (3)	-1157 (3)	1781 (4)
17	C22	-667 (4)	-3184 (4)	1455 (7)
18	C23	894 (3)	988 (4)	2804 (6)
19	N15	6473 (2)	-1566 (3)	916 (4)
20	O18	250 (2)	-1843 (2)	2256 (4)
21	O19	1825 (2)	742 (2)	3874 (3)
22	O20	7971 (2)	5243 (2)	5888 (4)
23	O21	9275 (2)	3487 (2)	3936 (4)
24	HC1	3932 (29)	-4488 (31)	-755 (46)
25	HC3	1540 (30)	-3792 (31)	425 (47)
26	HC8	3842 (31)	2527 (32)	4934 (48)
27	HC9	5520 (28)	4622 (30)	5971 (45)
28	HC12	8109 (32)	934 (34)	2388 (50)
29	HC16	6389 (30)	-3580 (31)	-705 (47)
30	HC22	-523 (37)	-3883 (38)	2112 (57)
31	H'C22	-1621 (39)	-3115 (40)	1779 (61)
32	H''C22	-514 (35)	-3595 (37)	-130 (55)
33	HC23	50 (41)	165 (43)	2456 (64)
34	H'C23	641 (41)	1855 (43)	3748 (64)
35	H''C23	1350 (47)	1185 (49)	1480 (74)
36	HO20	8932 (39)	5331 (42)	5398 (63)

planarity of the skeleton of the molecule, as well as that of the various parts of the molecule, are shown in Table IV. As is clear in Fig. 3 and in Tables III and IV, the molecule is almost completely planar, similarly to pareirubrine A,⁴⁾ and the conjugation of the double bond extends throughout the molecule except for the two substituted methoxyl groups. An intramolecular H bond is formed between the hydroxyl HO20 and carbonyl O21 atoms. The distance from O20...O21 is $2.561(3)$ Å and from HO20...O21 is $2.01(4)$ Å. O20-H also forms an intermolecular H bond to O21 situated at $2-x, 1-y, 1-z$ of distances O20...O21 $2.782(3)$ Å and HO20...O21 $1.93(4)$ Å. It seems that HO20 is shared among the two H bonds, forming a bifurcated H bond. HO20 was assumed to belong to O20 rather than to O21 by considering that the difference in the C-O lengths

of C10–O20 is 1.345(3) Å versus C11–O21, which is 1.258(3) Å. This was further confirmed by the difference electron density map. The molecules are stacked antiparallel to each other as shown in Fig. 4. The interplanar distances are 3.403(3) Å between the molecules at x, y, z and $1-x, -y, -z$ and 3.439(3) Å between those at x, y, z and $1-x, -y, 1-z$. As indicated above, the planarity of the isoquinoline and tropolone rings in pareirubrine B was very good, like those of pareirubrine A.

Pareirubrines A (1) and B (2) exhibited antileukemic activities against the P-388 cell line (IC₅₀ 1, 0.33 µg/ml; 2, 0.17 µg/ml). The solution conformation of the tropolone ring in tropoloisoquinoline alkaloids and the structure activity relationship of derived tropoloisoquinolines are now under investigation.

Experimental

All melting points were recorded on a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. The spectral data were obtained on the following instruments: infrared spectrum (IR) on a JASCO A-302, ultraviolet spectrum (UV) on a Hitachi 557, NMR on a Bruker AM500 and mass spectrum (MS) on a VG AutoSpec. MPLC was carried out on a CIG column system (Kusano Scientific Co., Tokyo) packed with 10 µm silica gel and 30 µm octadecyl silica (ODS) as the stationary phase. Reversed-phase HPLC was carried out on an Inertsil PREP-ODS column packed with 10 µm ODS.

Extraction and Isolation The roots and woods of *Cissampelos pareira* (10.0 kg) were extracted with hot methanol three times and concentrated to give a methanolic extract (235 g). This extract was successively partitioned between methylene chloride and water. The cytotoxic activity was concentrated in the methylene chloride soluble fraction (47 g), a portion (5 g) of which was subjected to reversed phase MPLC, using a methanol, to give four fractions. Chromatographic purification of fr. 2 by silica gel MPLC using a methylene chloride–methanol (9:1) solvent system led to the isolation of pareirubrine B (2: 7.8 mg). Further purification of fr. 3 by ODS HPLC using a methanol–acetonitrile–pH 3.5 potassium phosphate buffer (2:2:1) solvent system led to the isolation of pareirubrine A (1: 15.5 mg), grandirubrine (3: 67.5 mg) and isoimerubrine (4: 11.6 mg).

Pareirubrine A (1): Reddish-brown needles, mp 168.0–170.0 °C. HR-FAB-MS: Calcd 368.1134 for C₂₀H₁₈NO₆ (M+1), Found 368.1116. EI-MS m/z (%): 367 (100), 354 (75), 326 (34), 268 (30), 240 (30), 197 (20), 169 (20), 135 (27). IR (CHCl₃) cm⁻¹: 3625, 1610, 1580, 1460, 1420. UV λ_{EtOH}^{max} nm (ε): 217 (2900), 274 (25200), 294 (21000), 364 (20200), 420 (3800, sh), 472 (7700).

Pareirubrine B (2): Reddish-brown needles, mp 290 °C (dec.). HR-MS: Calcd 307.0845 for C₁₈H₁₃NO₄ (M), Found 307.0851. Electron impact (EI)-MS m/z (%): 307 (100), 279 (40), 264 (13), 236 (36), 193 (20), 164 (11). IR (CHCl₃) cm⁻¹: 3610, 1620, 1590, 1480, 1290. UV λ_{MeOH}^{max} nm (ε): 252 (22900), 268 (19950, sh), 300 (13200), 364 (24560), 394 (9300), 416 (6900), 460 (3580, sh), 520 (1500, sh).

Grandirubrine (3): Reddish-brown needles, mp 201–203 °C. EI-MS

m/z (%): 337 (100), 322 (19), 309 (10), 294 (26), 279 (13), 251 (20), 236 (21), 208 (16). IR (CHCl₃) cm⁻¹: 3650, 3500, 1620, 1590, 1460, 1420. UV λ_{EtOH}^{max} nm (ε): 218 (14600), 250 (16600), 262 (15700), 264 (15500), 298 (9800), 300 (9700), 363 (16900), 399 (7500, sh), 420 (4200, sh), 570 (3400).

Isoimerubrine (4): Reddish-brown needles, mp 183–185 °C. EI-MS m/z (%): 351 (M⁺, 100), 336 (20), 323 (21), 308 (51). IR (CHCl₃) cm⁻¹: 1610, 1590, 1460, 1420, 1130. UV λ_{EtOH}^{max} nm (ε): 218 (9200), 257 (11200), 304 (3500), 362 (17700), 414 (3400), 480 (1200, sh).

Bioassay of Cytotoxic Activity against P-388 Cells See previous paper.⁹⁾

X-Ray Crystallographic Analysis of Pareirubrine B (2) Crystal Data: C₁₈H₁₃NO₄, M_r = 307.3, triclinic, space group $P\bar{1}$, z = 2, a = 10.478(6), b = 10.800(7), c = 6.931(4) Å, α = 110.50(6), β = 81.32(4), γ = 108.22(6)°, V = 697.1 Å³, D_x = 1.464 g cm⁻³, $F(000)$ = 320. A needle crystal of approximately 0.05 × 0.08 × 0.90 mm in length was mounted on a Philips PW1100 diffractometer with graphite-monochromated CuK α radiation (μ = 8.2 cm⁻¹) at 22 °C. A total of 1630 reflections were observed above the 2 $\sigma(I)$ level, with a 2 θ range of 6° through 120°. The structure was determined by the direct method using the SHELXS-86 program¹⁰⁾ and the refinement was carried out by the block-diagonal-matrix least-squares method. The final R value was 0.047 ($R_w = \sum w(|F_o| - |F_c|)^2 / \sum w|F_o|^2 = 0.002$, where $\sqrt{w} = 0.1$ when $|F_o| \leq 2.0$, $\sqrt{w} = 1$ when $2.0 < |F_o| \leq 50$, $\sqrt{w} = 50/|F_o|$ when $|F_o| > 50$). The number of atoms refined were 23C, N, and O atoms with anisotropic thermal parameters; 13H atoms were found on the difference electron-density map and located at the calculated positions. These H atoms were refined with isotropic parameters. Final shift/esd values of the coordinates and the temperature factors for C, N, and O atoms ranged from 0.25–0.54. The maximum residual electron densities were 0.23 e/Å³ and the average value was 0.13 e/Å³. The refined fractional atomic coordinates are shown in Table V.

References

- 1) J. V. Silverton, C. Kabutol, K. T. Buck, M. P. Cava, *J. Am. Chem. Soc.*, **99**, 6708 (1977).
- 2) M. D. Menachery, M. P. Cava, *Heterocycles*, **14**, 943 (1980).
- 3) H. Itokawa, K. Takeya, K. Watanabe, H. Morita, Y. Ichihara, N. Totsuka, O. Shirota, H. Izumi, M. Satake, I. Yasuda, U. Sankawa, M. Motidome, F. A. Flores, *J. Pharmacobio-Dyn.*, **15**, s-2 (1992).
- 4) H. Morita, K. Matsumoto, K. Takeya, H. Itokawa, Y. Iitaka, *Chem. Lett.*, **1993**, 339; The name of pareirubrine for compound 1 in the preliminary form was changed to pareirubrine A.
- 5) W. H. Lewis, M. Lewis, "Medical Botany," John Wiley and Sons, Inc., New York, 1977, p. 324.
- 6) A. Bax, M. F. Summers, *J. Am. Chem. Soc.*, **108**, 2094 (1986).
- 7) A. Bax, S. Subramanian, *J. Magn. Reson.*, **67**, 565 (1986).
- 8) W. D. S. Motherwell, PLUTO. A program for drawing crystal and molecular structures, University Chemical Laboratory, Cambridge, England, 1972.
- 9) H. Itokawa, N. Totsuka, K. Nakahara, M. Maezuru, K. Takeya, M. Kondo, M. Inamatsu, H. Morita, *Chem. Pharm. Bull.*, **37**, 1619 (1989).
- 10) G. M. Sheldrick, "SHELXS-86," in "Crystallographic Computing," ed. by G. M. Sheldrick, C. Kruger, R. Goddard, Oxford University Press, 1985, pp. 175–189.