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A Novel Antileukemic Tropoloisoquinoline Alkaloid, Pareirubrine, from *Cissampelos pareira*

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Pareirubrine, a novel tropoloisoquinoline alkaloid with antileukemic activity was isolated from *Cissampelos pareira* and the structure was determined on the basis of its spectroscopic data and X-ray diffraction analysis.

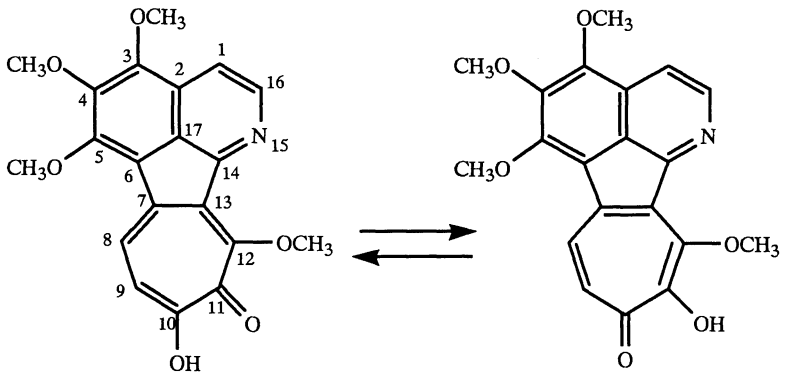
Cissampelos pareira (Menispermaceae), a perennial climbing shrub found in many parts of the tropical world, is a rich source of 1-benzylisoquinoline derived alkaloids.[1-3] It's antispasmodic action makes it influential in treating cramps, painful menstruation and pre and postnatal pain.[4] In tropical countries, the roots are used to prevent a threatened miscarriage and the herb is also used to stop uterine hemorrhages.[5]

During the survey of novel antileukemic compounds from South American medicinal plants,[6] the crude extract of *Cissampelos pareira* showed antileukemic activity and a novel tropoloisoquinoline alkaloid, which is condensed tropolone and isoquinoline alkaloid, was isolated as an active principle. In this communication, we described the isolation, structural elucidation and antileukemic activity of a novel tropoloisoquinoline alkaloid, named pareirubrine.

The methanolic extract of *C. pareira* was partitioned with methylene chloride and water. The methylene chloride extract was subjected to reversed phase MPLC and HPLC, followed by recrystallization from methanol, to give reddish-brown needles of pareirubrine (**1**), mp 168 - 170 °C.

The molecular formula of **1** was established to be $C_{20}H_{17}O_6N$ from high-resolution mass spectrometry (HR-FABMS) [Found: *m/z* 368.1116. Calcd for $C_{20}H_{18}O_6N$: M+1, 368.1134].

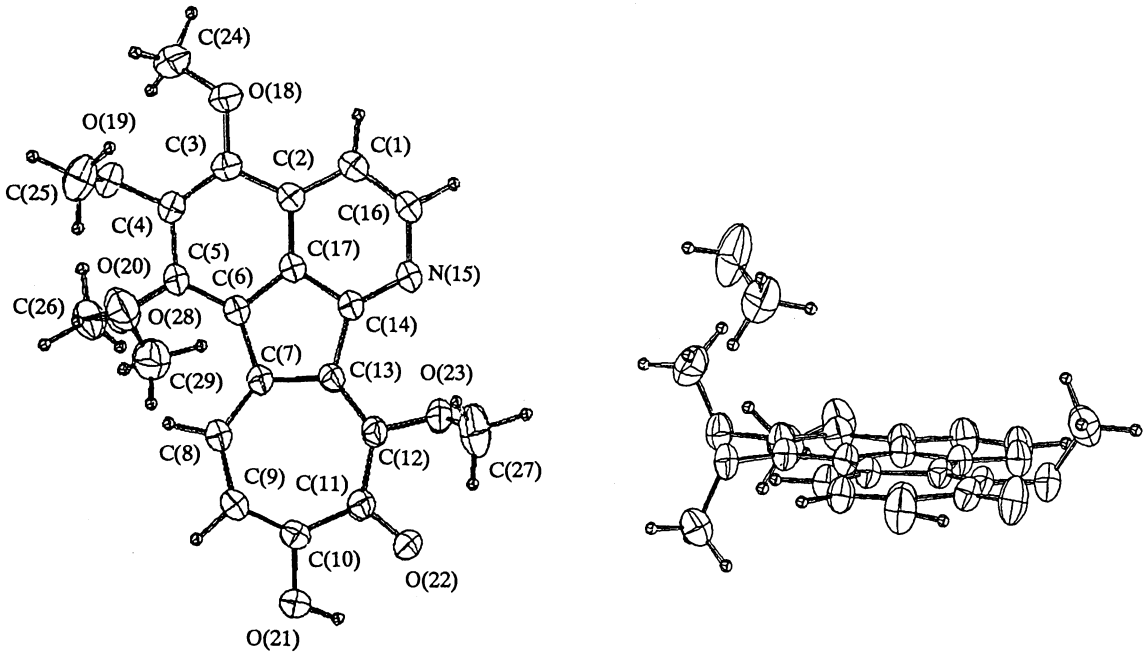
The ^1H NMR spectrum (400 MHz, $CDCl_3$) indicated four set of methoxy methyl signals at δ_H 4.05, 4.16, 4.18 and 4.24 and two set of each coupled olefinic proton signals at δ_H 7.79 and 8.88 (each 1H, d, J 5.8; H-1 and 16, respectively), and 7.46 and 8.37 (each 1H, d, J 10.3; H-9 and 8, respectively).



1a **1b**

Fig. 1. Molecular structure of pareirubrine **1**.

The UV spectrum in EtOH, showing maxima (ε) at 472 (7700), 420 (3800), 364 (20200), 294 (21000), 274 (25200) nm, suggested feature of tropoloisoquinoline-type alkaloids such as imeruburine[7] and gradirubrine[8] isolated from the tropical American genus *Abuta* (Menispermaceae). ^{13}C NMR assignments[9] supporting the above assumption could be obtained from either HMQC[10] and HMBC[11] experiments.



(a) (b)

Fig. 2. Two views of the molecule of pareirubrine with methanol solvate molecule. Drawn by ORTEP with 50% probability thermal ellipsoid for C, N, and O atoms. (a) Viewed perpendicular to the molecular plane (b) Rotated 70° about the horizontal line in (a)

The structure of pareirubrine (**1**) has been determined, unambiguously by X-ray crystallographic analysis.[12] Dark orange single crystals of **1** methanol solvate was grown from methanol-methylene chloride. An ORTEP[13] drawing of the molecule is given in Fig. 2. As is clear in Fig. 2 (b) and in Table 1 showing planarity of the molecule, the molecule is almost completely planar and the configuration of the double bond extends throughout the molecule except for the four substituted methoxyl groups (Fig. 1). An intramolecular H bond is formed between the hydroxyl O21 - HO21 and the carbonyl O22 atom. The distance O21...O22 is 2.562(2)Å and HO21 is 2.03(3)Å. O21-H also forms an intermolecular H bond to O22 situated at 1-x, -y, -1-z of distances O21...O22 2.704(3)Å and HO21...O22 2.02(4)Å. It seems to be that HO21 is shared among the two H bonds forming a bifurcated H bond. HO21 was assumed to belong to O21 rather than to O22 by considering the difference in the C-O lengths C10-O21 of 1.343(4)Å versus C11-O22 of 1.248(3)Å, which was further confirmed by the difference electron density map.

The tropoloisoquinoline alkaloids so far known are derivatives of the tautomers, **1a** and **1b**. In crystalline state, pareirubrine exists in only **1a**. In the ^{1}H NMR, however, pareirubrine showed a much closer resemblance

to the **1b** than to **1a** by comparing of chemical shifts of H-8 and 9 with those of imeruburine[7] and gradirubrine.[8]

Pharmacological activities about tropoloisoquinolines have not been known. Pareirubrine exhibited antileukemic activity against P-388 cell line (IC_{50} 0.33 μg/ml). Further evaluation and structure activity relationship of derived tropoloisoquinolines are now under investigation.

Table 1. Planarity of the molecule of pareirubrine

Plane formed[a]	D.from plane(Å)[b]	Coeffi-cient[c]	Plane formed	D.from plane(Å)	Coeffi-cient	Atom	D.from plane(Å)
Group I			Group IV				
C1	-0.004(2)	A 0.8443	C1	0.009(2)	A 0.8378	O18	-0.029(2)
C2	0.007(2)	B -0.2890	C2	-0.010(2)	B -0.3091	O19	-0.064(2)
C17	-0.004(2)	C 0.4513	C3	-0.009(2)	C 0.4501	O20	0.058(2)
C14	-0.002(2)	D 1.8503	C4	-0.030(2)	D 1.8537	O21	-0.008(2)
N15	0.005(2)		C5	0.023(2)		O22	-0.060(2)
C16	-0.002(2)		C6	-0.005(2)		O23	0.007(2)
			C7	0.014(2)		C24	0.588(2)
Group II			C8	0.022(2)		C25	-1.375(2)
C2	0.012(2)	A 0.8340	C9	0.031(2)		C26	1.370(2)
C3	0.008(2)	B -0.3160	C10	-0.005(2)		C27	-1.179(2)
C4	-0.024(2)	C 0.4522	C11	-0.034(2)			
C5	0.022(2)	D 1.8278	C12	-0.025(2)			
C6	-0.002(2)		C13	0.001(2)			
C17	-0.015(2)		C14	-0.009(2)			
			N15	0.027(2)			
Group III			C16	0.029(2)			
C6	0.002(2)	A 0.8365	C17	-0.029(2)			
C7	0.002(2)	B -0.3250					
C13	-0.005(2)	C 0.4411					
C14	0.007(2)	D 1.8613					
C17	-0.006(2)						

a) Plane formed: The least-squares plane formed by the atoms listed below. b) D. from plane: The perpendicular distance of the individual atom from the least-squares plane. c) 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 ∥ c, X in ac plane, Y⊥to X and Z. Interplanar angles between the plane group IV and group I is 1.10°, group II is 0.65° and group III is 1.10°.

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9) δc (100MHz, $CDCl_3$) 114.8 (C-1), 126.1 (C-2), 149.6 (C-3), 152.1 (C-4), 149.8 (C-5), 121.8 (C-6), 136.4 (C-7), 127.6 (C-8), 117.3 (C-9), 162.4 (C-10), 173.0 (C-11), 157.5 (C-12), 138.4 (C-13), 158.5 (C-14), 146.3 (C-16), 125.7 (C-17), 62.1 (CH_3O- at C-3), 61.4 (CH_3O- at C-4), 61.6 (CH_3O- at C-5), 60.7 (CH_3O- at C-12).

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12) Crystal data: $C_{20}H_{17}NO_6 \cdot CH_3OH$, M_r=399.4, triclinic, space group P1, z=2, a=11.708(6), b=9.769(5), c=9.192(5) Å, α=86.13(5), β=112.17(6), γ=104.87(5)°, V=940.5Å^3, Dx=1.410 g cm^{-3}, F(000)=420. A needle crystal of approximately 0.18 X 0.31 X 0.48 mm in length was mounted on a Philips PW1100 diffractometer with the graphite-monochromated CuKα radiation (μ=8.5 cm^{-1}) at 25 °C. A total of 4911 reflections were observed above the 2σ(I) level, with the 2θ range from 6° through 146°. The structure was determined by the direct method using the SHELXS-86 program[14] and the refinement was carried out by the block-diagonal-matrix least-squared method. The final R value was 0.049 ($R_W=\Sigma w(|Fo|-|Fc|)^2 / \Sigma w |Fo|^2$=0.002, where, $\sqrt{w}$=0.1 when $|Fo| \leq 1.0$, $\sqrt{w}$=1 when $1.0<|Fo| \leq 50$, $\sqrt{w}=50/|Fo|$ when $|Fo|>50$). The number of atoms refined were 29 C, N, and O atoms with anisotropic thermal parameters and 21 H 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 were ranged in 0.13-0.57. The maximum residual electron densities were 0.21 e/Å^3 and the average values 0.14 e/Å^3. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.

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