Azafluoranthene Alkaloids from *Cissampelos pareira*

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A novel azafluoranthene alkaloid, norimeluteine, has been isolated as a cytotoxic substance from *Cissampelos pareira* (Menispermaceae), together with an alkaloid having the same skeleton, norruffscine. Carbon-13 nuclear magnetic resonance assignments for the azafluoranthene alkaloids were performed by a combination of two dimentional NMR techniques.

Keywords azafluoranthene alkaloid; *Cissampelos pareira*; norimeluteine; norruffscine; cytotoxic activity; ^{13}C-NMR

Introduction

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

During a 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, pareirubrine, has already been isolated.[7] Further purification, using bioassay and guided by cytotoxicity against P-388 cells, led to the isolation of two azafluoranthene alkaloids, named norimelutein (**1**) and norruffscine (**2**). In the present paper, the structural elucidation of the novel cytotoxic azafluoranthene alkaloid, norimelutein (**1**) by spectroscopic methods and ^{13}C-NMR assignments for azafluoranthene alkaloids are reported.

Results and Discussion

The methylene chloride soluble fraction of the methanol extract was subjected to reversed-phase medium pressure liquid chromatography (MPLC), Sephadex LH-20 and silica gel MPLC to give norimelutein (**1**) and norruffscine (**2**).

Compound **1**, a yellow powder, gives a positive reaction with Dragendorff's reagent and has the molecular formula ($C_{19}H_{17}NO_5$) by high resolution mass spectrometry (HR-MS) (339.1113). In the ^{1}H-NMR spectrum (Table I), the presence of four methoxyl signals (δ 4.04, 4.10, 4.11 and 4.32) and two sets of coupled aromatic protons (δ 7.62 and 8.66, J=5.9 Hz; 7.01 and 7.61, J=8.0 Hz), which exhibit similar spectroscopic data to the tropoloisoquinoline alkaloid, pareirubrine,[7] were observed. However, the *ortho* coupling constant of 5.9 Hz, characteristic of a benzene ring, differs from the larger one (10 to 12 Hz) of one of the olefinic protons, characteristic of a tropolone ring. Other coupled aromatic protons are easy to assign to the H-1 and -15 of the isoquinoline skeleton.

Based on the above spectroscopic properties, **1** was deduced to be an azafluoranthene alkaloid, which is a component of coal tar,[8] cigarette smoke[9] and was also been identified as an air pollutant.[10] Furthermore, three methoxyl groups were assigned to ring A, and a hydroxyl and a methoxyl group to ring D using two dimensional (2D) NMR techniques such as heteronuclear multiple bond correlation (HMBC)[11] and heteronuclear multiple quantum coherence (HMQC)[12] spectra. The position of a hydroxyl and a methoxyl group substituted on ring D was also determined to be at C-10 and -11, respectively, by HMBC correlations between H-8 and C-10, between H-9 and C-11 and between the methoxy signal and C-11, as shown in Fig. 2. Therefore, the structure of **1** was determined to be a novel azafluoranthene alkaloid, named norimelutein, which lacks the methoxy methyl group at C-10 of imelutein isolated from *Abuta imene* and *A. rufescens* (Menispermaceae).[13]

Compound **2**, yellow needles, mp 236—238°C, had the molecular formula ($C_{18}H_{15}NO_4$). One olefinic proton signal (δ 7.41, d, J=2.3 Hz), instead of the methoxy signal in **1**, was observed in the ^{1}H-NMR spectrum. Analysis of 2D NMR spectra indicated that **2** is norruffscine, which was first isolated from *Abuta imene* and *A. rufescens*.[14] It has also been obtained from *Telitoxicum peruvianum* (Menispermaceae).[15]

No ^{13}C-NMR data for azafluoranthene alkaloids have been reported, presumably, because of the long relaxation

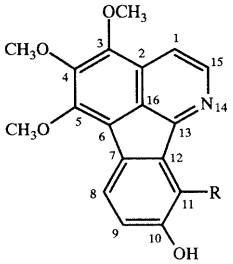

Fig. 1. Norimeluteine (**1**): R=OCH_3. Norruffscine (**2**): R=H

TABLE I. ^{1}H-NMR Assignments of **1** and **2** (500 MHz)

Proton	**1**[a]	**2**[b]
H-1	7.62 (d, J=5.9 Hz)	7.68 (d, J=5.9 Hz)
H-8	7.61 (d, J=8.0 Hz)	7.72 (d, J=8.2 Hz)
H-9	7.01 (d, J=8.0 Hz)	6.88 (dd, J=8.2, 2.3 Hz)
H-11	—	7.41 (d, J=2.3 Hz)
H-15	8.66 (d, J=5.9 Hz)	8.57 (d, J=5.9 Hz)
3-OMe	4.10 (s)	4.04 (s)
4-OMe	4.11 (s)	4.06 (s)
5-OMe	4.04 (s)	3.98 (s)
11-OMe	4.32 (s)	—

a) In $CDCl_3$. *b*) In DMSO-d_6.

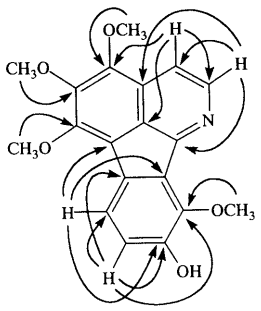

Fig. 2. HMBC correlation of **1** in $CDCl_3$

TABLE II. ^{13}C-NMR Assignments of **1** and **2** (125 MHz)

Carbon	**1**[a]	**2**[b]
C-1	113.1	113.6
C-2	126.1	125.5
C-3	148.4	147.6
C-4	151.1[c]	149.9[d]
C-5	149.8[c]	149.6[d]
C-6	122.4	121.3
C-7	131.8	128.7
C-8	120.4	124.7
C-9	116.0	116.5
C-10	149.2	157.1
C-11	144.1	109.2
C-12	129.2	139.7
C-13	157.9	158.3
C-15	144.9	144.6
C-16	123.9	122.8
3-OMe	61.3	62.0
4-OMe	62.1	61.3
5-OMe	61.4	61.3
11-OMe	61.9	—

a) In $CDCl_3$. *b*) In DMSO-d_6. *c,d*) Assignments may be interchanged.

time of quaternary carbons. A combination of HMBC and HMQC spectra enable us to obtain ^{13}C assignments for compounds **1** and **2** as shown in Table II.

No pharmacological studies have been performed directly on azafluoranthene alkaloids. Norimeluteine (**1**) and norruffscine (**2**) showed cytotoxic activities against P-388 cells (**1**, 3.6 μg/ml; **2**, 5.8 μg/ml).

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 spectra (IR) on a JASCO A-302, ultraviolet spectra (UV) on a Hitachi 557, NMR on a Bruker AM500 and mass spectra (MS) on a VG Auto Spec. Medium-pressure liquid chromatography (MPLC) was carried out on a CIG column (Kusano Scientific Co., Tokyo) packed with 10 μm silica gel and 30 μm octadecyl silica (ODS) as the stationary phase.

Bioassay of Cytotoxic Activity against P-388 Cells See previous paper.[16]

Extraction and Isolation The roots and wood of *Cissampelos pareira* (10.0 kg) were extracted three times with hot methanol 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 (15 g) of which was subjected to reversed-phase column chromatography using methanol as mobile phase. Further chromatographic purification of the active fraction was carried out on Sephadex LH-20 (methylene chloride–methanol solvent system) and silica gel MPLC (*n*-hexane–ethyl acetate solvent system) and led to the isolation of norimeluteine (**1**, 7.8 mg) and norruffscine (**2**, 7.7 mg).

Norimeluteine (**1**): Yellow powder, High-MS: Calcd for $C_{19}H_{17}NO_5$ (M^+): 339.1107. Found: 339.1113. MS *m/z* (%): 339 (M^+, 100), 321 (95), 83 (64). IR ($CHCl_3$) cm^{-1}: 3550, 3050, 1595, 1500, 1475, 1425, 1405, 1300, 1260. UV λ_{MeOH}^{max} nm (ε): 210 (25400), 224 (23400, sh), 240 (22800), 256 (23200), 292 (18200).

Norruffscine (**2**): Yellow needles, mp 236—238 °C, High-MS: Calcd for $C_{18}H_{15}NO_4$ (M^+): 309.1001. Found: 309.0989. MS *m/z* (%): 309 (M^+, 100), 294 (65), 251 (45), 208 (38), 180 (35). IR (KBr) cm^{-1}; 3450, 2950, 1610, 1595, 1470, 1480, 1400, 1380, 1330, 1295, 1260, 1240, 1100, 1010, 890, 830. UV λ_{MeOH}^{max} nm (ε): 208 (25800), 252 (32000), 300 (21300), 308 (19900), 320 (7300, sh), 340 (2400), 358 (4500, sh), 420 (2000, sh).

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