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NEW CYTOTOXIC ARISTOLACTAMS FROM *PARARISTOLOCHIA FLOS-AVIS*

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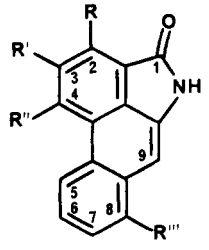
ABSTRACT.—Two new aristolactams, aristolactam-FI [**1**] and -FII [**2**], were isolated from active extracts of *Pararistolochia flos-avis*. Their structures were elucidated on the basis of nmr, ms, uv, and ir spectral data. Aristolactam-I [**3**] and -AII [**4**] were also isolated from this plant. Aristolactam-AII showed cytotoxicity against PS and KB cells in culture.

Several members of the genus *Aristolochia* have been used in folk medicine (1-5). Aristolochic acid, a constituent of many *Aristolochia* species, was reported by Kupchan and Doskotch to have antitumor activity (6). As part of a program to isolate antineoplastic agents from higher plants, a member of the genus *Pararistolochia* (separated from *Aristolochia*) (7) has been studied. No chemical or biological studies on this plant, *Pararistolochia flos-avis* (A. Cheval.) Hutch. and Dalz. (Aristolochiaceae), have been reported in the literature. An ethanolic extract of *P. flos-avis* was separated into neutral, acidic, and basic fractions by treatment with 3% $NaHCO_3$ solution and 3% citric acid, respectively. The neutral and acidic fractions were found to be cytotoxic and active in the P-388 mouse leukemia system (8). Two novel aristolactams, namely aristolactam-FI [**1**] and -FII [**2**], were isolated from the neutral fraction. Aristolactam-I [**3**] and -AII [**4**] were also isolated from the same fraction (see Figure 1).



	R	R′	R″	R‴
1	H	OMe	OH	H
2	OMe	OH	OMe	H
3	H	O—CH_2—O		OMe
4	H	OH	OMe	H

RESULTS AND DISCUSSION

Compound **1** was identified as a 4-hydroxyaristolactam based on the ir spectrum, which showed absorptions at 3440, 3250, and 1675 cm^{-1} indicating the presence of OH, NH, and C=O functions, respectively, and the uv absorptions at 315 and 303 nm, shifted to 325 and 315 nm upon adding NaOAc. The molecular weight of **1** was found by cims to be 265, and a molecular formula of $C_{16}H_{11}NO_3$ could be assigned based on the hrms of its diacetyl derivative ($C_{20}H_{15}NO_5$). The 470 MHz ^{1}H-nmr spectrum of **1** showed one methoxyl group at δ 4.05 (3H, s). Two uncoupled, aromatic protons at δ 7.48 (1H,s) and 8.09 (1H, s) could be assigned to the C-9 and C-2 protons, respectively. Signals at δ 9.45 (1H, dd, J=7.1, 2.3), 7.65 (2H, td, J=7.1, 2.3), and 7.92 (1H, dd, J=7.1, 2.3) suggested four adjacent aromatic protons at C-5, 6, 7, and 8, respectively. Comparison of chemical shifts among **1** and other aristolactams (9)

showed that the hydroxyl group is located at C-4, and the methoxyl group is located at C-3, as indicated by the downfield shift of the C-2 proton (δ 8.09). The replacement of the hydroxyl group at C-3 in aristolactams by a methoxyl group causes a significant deshielding of the C-2 proton. On the basis of these results, the structure of **1** was established as 10-amino-4-hydroxy-3-methoxyphenanthrene-1-carboxylic acid lactam.

High resolution mass analysis of **2** established the molecular formula $C_{17}H_{13}NO_4$, and the typical ir absorption bands at 3480, 3160, and 1680 cm^{-1} indicated that **2** was a close analog of **1**. The nmr spectrum of **2** (Table 1) showed two methoxyl signals one at δ 4.16 and the other downfield at δ 4.61 suggesting that this methoxyl group was peri to the carbonyl group. The absence of the C-2 proton signal in **2** when compared to **1** confirms this suggestion. The singlet centered at δ 7.14 could be assigned to the C-9 proton by comparison with chemical shift values of other aristolactams (9). The 5, 6, 7, and 8 protons appeared as a coupled pattern at 9.21 (1H, dd, J=7.1, 2.3), 7.56 (2H, td, J=7.1, 2.3), and 7.83 (1H, dd, J=7.1, 2.3), respectively. The absence of the downfield signal for the phenolic proton eliminates the possibility of hydroxy substitution at C-2 (*peri* to the carbonyl group), while the absence of a bathochromic uv shift in the presence of NaOAc eliminated the possibility (e.g., the one observed for **1**) of a C-4 hydroxyl isomer. Furthermore, irradiation of the acetoxyl methyl protons (δ 2.48) of the diacetyl derivative of **2** did not result in nOe on the C-5 proton but produced significant nOe on both methoxyl protons. Irradiation of the *N*-acetyl methyl protons (δ 2.83) produced a nOe on the C-9 proton. Thus, the structure of **2** was established as 10-amino-3-hydroxy-2,4-dimethoxyphenanthrene-1-carboxylic acid lactam.

Two additional aristolactams were isolated and identified as aristolactam-I [**3**] and -AII [**4**] by comparison with literature data (9-12). Aristolactam-AII exhibited cytotoxicity against PS and KB cells in culture at ED_{50} 3.2 and 2.1 μg/ml, respectively. The other compounds were not cytotoxic. Further fractionation aimed at detection of the minor in vivo actives is underway.

EXPERIMENTAL

PLANT MATERIALS.—Root and stem of *P. flos-avis* were obtained from Ghana in March 1977, and authenticated by the Medicinal Plant Resources Laboratory, Plant Genetics and Germplasm Institute, Agricultural Research Center East, Beltsville, MD 20705 (PR #48973).

GENERAL EXPERIMENTAL PROCEDURES.—All melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. The uv spectra were obtained in MeOH on a Beckman DU-7 spectrometer. The ir spectra were taken as KBr pellets on a Beckman-33 spectrometer. High resolution (470 MHz) ^{1}H-nmr spectra were recorded in $CDCl_3$ or DMSO-d_6 (δ in ppm, J in Hz), using TMS as internal standard at the Purdue University Biological Magnetic Resonance Laboratory on a Nicolet NTC-470 NMR spectrometer. Low and high resolution mass spectra were measured on a Finnigan 4023 gc/ms with Incos 2000 data system and a Kratos MS50S, respectively, and recorded at 70 eV. Si gel (230-400 mesh size) was used for flash column chromatography. Fractions were combined on the basis of their tlc patterns as detected by uv light (250 and 360 nm).

EXTRACTION AND ISOLATION.—The dried, ground root and stem (1.66 kg) were exhaustively percolated with 95% EtOH. The extract was concentrated to dryness in vacuo at 40° and yielded the EtOH extract (84.3 g) which was then partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ fraction was washed with 3% $NaHCO_3$ solution and then with 3% citric acid. The $CHCl_3$ fraction was further partitioned between 10% aqueous MeOH and hexane. The neutral, aqueous, MeOH extract weighed 20 g. The aqueous bicarbonate phase was acidified and extracted with $CHCl_3$ to give an acidic fraction (10.2 g). The neutral and acidic fractions exhibited antileukemic activity (3PS, T/C tx/400, 158/200, 118/100, 128/50 and 200/400, 126/200, 113/100, 106/50 mg/kg, respectively) in the P-388 mouse leukemia system in vivo (8).

The neutral fraction (20 g) was chromatographed on a Si gel flash column with $CHCl_3$ and increasing concentrations of MeOH (2-5%) in $CHCl_3$ to yield 7 fractions A-G. On repeated separations, two fractions equivalent to F and G (below) showed borderline activity (130-140/50-75) in the P-388 mouse assay.

Fraction D (0.36 g) on trituration with Me_2CO yielded compound **3** (37 mg). This was the major component of this fraction and **3** was not cytotoxic. Fraction E (0.26 g) was crystallized from Me_2CO and

recrystallized from a mixture of Me_2CO and Et_2O to give major component **2** (16 mg), which was also not active. Fraction F (1.71 g) was subjected to chromatography on a Si gel flash column, eluted with $CHCl_3$ and increasing concentrations of MeOH (1-5%) in $CHCl_3$ to give 5 fractions, H-L. Fraction J was crystallized from Me_2CO to give compound **4** (30.8 mg), which was cytotoxic but inactive in P-388 at 20 mg/kg. Fraction G (0.3 g) was chromatographed on a chromatotron (Si gel plate) eluted with $CHCl_3$-Me_2CO-MeOH (6:3:1). A major band was separated (25.5 mg) that crystallized from EtOAc to give compound **1** (10.6 mg), which was inactive.

ARISTOLACTAM-FI [**1**].—Mp 271-273° (EtOAc); uv λ max nm (log ε) 210 (4.57), 243 (4.60), 303 (4.17), 315 (4.19); uv λ max (+NaOAc) 208 (5.00), 221 (5.02), 315 (4.13), 325 (4.10); ir ν (KBr) cm^{-1} 3440, 3250, 1675, 1295; ^1H nmr (DMSO-d_6, see text); cims *m/z* 265 (M+1, 46%), 250 (86), 222 (44), 166 (100), 139 (66).

ACETYLATION OF ARISTOLACTAM-FI [**1**].—A mixture of compound **1** (5 mg), Ac_2O (0.2 ml) and pyridine (0.2 ml) was kept at room temperature for 24 h; excess Ac_2O was decomposed with MeOH, and the solvents were removed under reduced pressure with the aid of toluene and at a temperature not exceeding 50°. The diacetyl derivative was purified by preparative tlc to give 4.5 mg of crystals. Hrms *m/z* 349.097 (calcd. for $C_{20}H_{15}NO_5$, 349.094); eims *m/z* 349 (M^+), 307 (M-Ac), 265 (M-2Ac).

ARISTOLACTAM-FII [**2**].—Mp 225-227° (Me_2CO/Et_2O); hrms *m/z* 295.084 (calcd. for $C_{17}H_{13}NO_4$, 295.084); eims *m/z* (M^+ 100%), 280 (18), 252 (34), 234 (28), 221 (12), 209 (16), 153 (29); uv λ max nm (log ε) 204 (4.45), 247 (4.59), 281 (4.45), 289 (4.45), 388 (4.05); uv λ max (+NaOAc) 216 (5.03), 219 (5.02), 254 (4.60), 282 (4.38), 290 (4.43), 389 (3.95); ir ν (KBr) cm^{-1} 3480, 3160, 1680, 1270; ^1H nmr ($CDCl_3$, see text).

ACETYLATION OF ARISTOLACTAM-FII [**2**].—Compound **2** (5.7 mg) was acetylated with Ac_2O (0.3 ml) and pyridine (0.3 ml) at room temperature for 96 h; excess Ac_2O was decomposed with MeOH, and the solvents were removed under reduced pressure with the aid of toluene and at a temperature not exceeding 50°. The residue was triturated with absolute EtOH to give the diacetyl derivative of **2** (6 mg). Eims *m/z* 379 (M^+, 29%), 337 (85), 295 (100), 280 (19), 277 (33), 252 (19), 236 (26), 209 (17); ^1H nmr ($CDCl_3$) δ 9.11 (1H, dd, *J*=8, 2.5), 8.58 (1H, s), 7.98 (1H, dd, *J*=8, 2.5), 7.62 (2H, td, *J*=8, 2.5), 4.39 (3H, s), 4.10 (3H, s), 2.82 (3H, s), 2.48 (3H, s).

ARISTOLACTAM-I [**3**].—Ei-hrms *m/z*, obsd. 293.0671 (M^+), calcd. for $C_{17}H_{11}NO_4$, 293.068. Compound **3** was identified as aristolactam-I by comparison with literature data (9-11).

ARISTOLACTAM-AII [**4**].—Ei-hrms *m/z*, obsd. 265.0738 (M^+), calcd. for $C_{16}H_{11}NO_3$, 265.0735. Compound **4** was identified as aristolactam-AII by comparison with literature data (9,12).

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