Constituents of the Leaves of *Aristolochia kaempferi*

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Two new phenanthrene derivatives, aristoliukine-A, -B, and a benzenoid, sodium (2*R*)-(*p*-hydroxyphenyl)-lactate, together with thirty-three known compounds were isolated from the fresh leaves of *Aristolochia kaempferi*. Structures were elucidated by spectral analysis. Among the new compounds, sodium (2*R*)-(*p*-hydroxyphenyl)lactate was determined to be in the salt form by IR and ^{1}H-NMR methods.

Key words *Aristolochia kaempferi*; Aristolochiaceae; aristolactam; aristoliukine-A; aristoliukine-B

Plants of the genus *Aristolochia* (Aristolochiaceae) are known to number about 400 different species, and are found from the tropics to temperate zones. Five species are native to Taiwan, namely *Aristolochia cucurbitifolia*, *A. foveolata*, *A. heterophylla*, *A. zollingeriana* and *A. kaempferi*, and certain species have been used in folk medicine as anodynes, antiphlogistics and detoxicants.[1] *A. kaempferi* (*A. liukiunesis*) is distributed in the southern Ryukyu islands and also in Taiwan,[1] and several aristolochic acids and aristolactams have been isolated from this species.[2—7] In this paper, we describe the isolation and structural elucidation of three new compounds, aristoliukine-A (**1**), aristoliukine-B (**6**), and sodium (2*R*)-(*p*-hydroxyphenyl)lactate (**17**), together with thirty-three known compounds from the leaves of *A. kaempferi*.

Aristoliukine-A (**1**) was isolated as yellowish needles, and exhibited an UV spectrum characteristic of a phenanthrene chromophore.[8] The IR bands at 3380, 3300, 3170 and 1668 cm^{-1} revealed the presence of hydroxyl OH, amido NH and lactam carbonyl groups. The ^{1}H-NMR spectrum of **1** showed the presence of an amide NH proton at δ 9.70 (1H, br s, exchangeable with D_2O) and one methoxyl at δ 3.98 (3H, s). In the aromatic region, three mutually coupled signals at δ 8.46 (1H, d, J=2.8 Hz), 7.76 (1H, d, J=8.4 Hz) and 7.07 (1H, dd, J=8.4, 2.8 Hz) were attributed to H-5, H-8 and H-7, respectively. A singlet at δ 7.04 (1H, s) was assigned to H-9 and an oxygenated benzylic methylene signal appeared at δ 5.07 as a 2-proton singlet. This data resembled that of the 5,7,8,9-unsubstituted aristolactam. The methoxyl group was located at C-4 on the basis of a nuclear Overhauser effect (NOE) spectroscopy (NOESY) experiment, which showed NOE correlations between H-5 (δ 8.46) and the methoxyl signal (δ 3.98). The methylene group at C-2 was determined by heteronuclear multiple-bond correlation spectroscopy (HMBC) from correlations of the methylene protons at δ 5.07 with δ 119.3 (s, C-2), 131.4 (s, C-1a) and 149.6 (s, C-3). Based on these data, the structure **1** was assigned for aristoliukine-A.

Aristoliukine-B (**6**) was obtained as red needles. FAB-MS displayed a *pseudo*-molecular ion peak at *m/z* 310 (M^++H). The UV spectrum showed absorptions characteristic of a 4,5-dioxoaporphine derivative[8] at 219, 235, 246, 275, 321, 332, 373 and 480 nm. IR revealed the presence of hydroxyl and amino groups between 3400 to 3100 cm^{-1} and a strong carbonyl group absorption at 1676 cm^{-1}. The ^{1}H-NMR spectrum of **6** showed three exchangeable proton signals at δ 11.93 (1H, s), 10.62 (1H, s) and 9.94 (1H, s) attributable to amide NH and two phenolic hydroxyl protons. An ABX system for the aromatic protons was observed at δ 8.89 (1H, d, J=2.0 Hz) for H-11 and 7.76 (1H, d, J=8.8 Hz), 7.15 (1H, dd, J=8.8, 2.0 Hz) for H-8 and H-9, respectively. The remaining three singlet signals at δ 8.06 (1H), 7.41 (1H) and 4.08 (3H) could be assigned to H-3, H-7 and a methoxyl group. From this data, compound **6** was determined to be triangularine-A (1,10-dihydroxy-2-methoxy-4,5-dioxoaporphine).[7] By comparison of their ^{1}H-NMR spectra, the positions of substituents were revealed. To confirm the position of the methoxyl, a NOESY experiment was conducted and the hydroxyl (δ 9.94) was found to be within NOE distance of H-11 (δ 8.89) and H-9 (δ 7.15), and the methoxyl group (δ 4.08) had NOE correlation with H-11 (δ 8.89), which indicated that the methoxyl must be located at C-1. Thus, structure **6** was proposed for aristoliukine-B.

Sodium (2*R*)-(*p*-hydroxyphenyl)lactate (**17**) was isolated as an optically active colorless powder. The UV spectrum showed absorptions at 225, 279 and 285 nm, which suggested it was a benzenoid. In the ^{1}H-NMR spectrum, a set of *ortho*-coupled protons appeared at δ 7.13 (2H, d, J=8.6 Hz) and 6.83 (2H, d, J=8.6 Hz) attributable to *para*-substituted aromatic protons. Three mutually coupled aliphatic protons at δ 3.82 (1H, dd, J=7.6, 5.2 Hz), 3.13 (1H, dd, J=14.4, 5.2 Hz)

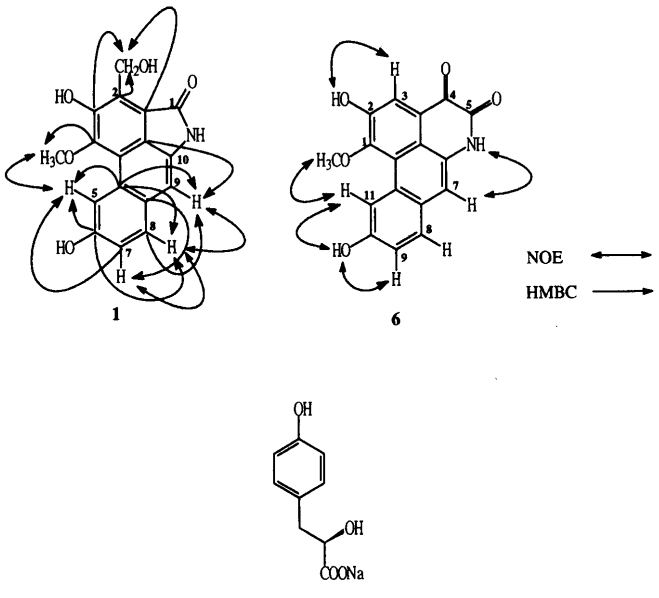

Chart 1

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and 2.93 (1H, dd, J=14.4, 7.6 Hz) were assigned to the α and β-protons of a β-hydroxyarylpropanoic acid moiety. The above data for compound **17** was similar to (*p*-hydroxyphenyl)lactic acid.[9] However, the IR spectrum of **17** displayed a carboxyl absorption at 1590 cm^{-1}, indicative of a salt form. Acidification of **17** with HCl afforded sodium chloride, which was determined with an atomic absorption spectrometer and (2*R*)-(*p*-hydroxyphenyl)lactic acid ($[\alpha]_D$ −13°, c= 0.15, MeOH). On the basis of the above data, compound **17** was assigned to be sodium (2*R*)-(*p*-hydroxyphenyl)lactate.

In addition to **1**, **6** and **17**, cepharanone-A (**2**),[10] aristolactam-IIIa (**3**),[11] -AII (**4**),[10] -AIIIa (**5**),[11] 4,5-dioxodehydroasimilobine (**7**),[12] cepharadione-A (**8**),[10] aristolochic acid-I (**9**),[10] -II (**10**),[10] -IIIa (**11**),[10] -IVa (**12**),[10] sodium aristolochate-I (**13**),[13] aristolic acid (**14**),[14] aristofolin-A (**15**),[7] aristofolin-B (**16**),[15] *p*-hydroxybenzaldehyde (**18**),[16] benzoic acid (**19**),[17] *p*-hydroxybenzoic acid (**20**),[18] methylparaben (**21**),[10] vanillic acid (**22**),[19] methyl vanillate (**23**),[10] cinnamamide (**24**),[20] *p*-hydroxy cinnamic acid (**25**),[10] methyl *p*-hydroxy cinnamate (**26**),[10] ferulic acid (**27**),[10] methyl 3,4-dihydroxy cinnamate (**28**),[21] kaempferol (**29**),[22] kaempferol-3-*O*-glucoside (**30**),[22] tiliroside (**31**),[23] isorhamnetin 3-*p*-coumaroylglucoside (**32**),[23] 3-carboxy pyridine (**33**),[24] *N*-*p*-coumaroyltyramine (**34**),[10] pheophytin-a (**35**)[25] and β-sitosterol-3-*O*-glucoside (**36**)[26] were also isolated from the leaves of *A. kaempferi.* These known compounds were characterized by their spectral properties.

Experimental

UV spectra were recorded in MeOH, and IR spectra were determined as KBr discs. ^{1}H-NMR spectra were obtained on a Bruker NMR spectrometer, with tetramethylsilane (TMS) as internal standard. EI-MS was measured with a 70 eV direct inlet system on a VG70-250AS spectrometer. Melting points were uncorrected. Optical rotations were recorded on a Jasco DIP-370 digital polarimeter.

Plant Material *Aristolochia kaempferi* was collected at Nantou, Taiwan, in April, 1994 and verified by Prof. C.S. Kuoh. A voucher specimen is deposited in the Herbarium of Cheng Kung University, Taiwan.

Extraction and Separation The fresh leaves (1.3 kg) were extracted with MeOH at room temperature and concentrated to give a deep brown syrup (190 g). The MeOH extract was partitioned between H_2O and $CHCl_3$, and the H_2O layer was filtered to give an H_2O insoluble fraction and a H_2O soluble layer. The $CHCl_3$ layer was directly chromatographed on silica gel column and eluted with $CHCl_3$ containing increasing proportions of MeOH (0→100%, stepwise elution with a 10% increase at each step) to give 9 fractions. Fraction 3 was rechromatographed on silica gel and eluted with *n*-C_6H_{14} : EtOAc (14 : 1) to obtain **19** (1 mg) and **35** (1 mg). Fraction 4 was also rechromatographed on silica gel with $CHCl_3$: EtOAc (14 : 1) to give **2** (15 mg) and **8** (10 mg). Fraction 5 was treated in a similar way to fraction 4 to give **4** (3 mg), **5** (6 mg) and **24** (1 mg). Fraction 6 was also treated in the same manner as fraction 4 to obtain **7** (2 mg), **16** (0.5 mg), **21** (2 mg) and **36** (100 mg), respectively. Fraction 8 was filtered to give **9** (450 mg). The H_2O soluble layer was chromatographed on Diaion HP-20 and eluted with H_2O containing increasing proportions of MeOH (0→100%, stepwise elution with a 10% increase at each step) to give 9 fractions. Fraction 3 of the H_2O soluble layer was filtered to give **17** (200 mg). Fraction 4 was chromatographed on silica gel with $CHCl_3$: MeOH (9 : 1) to give **20** (4 mg), **22** (2 mg), **27** (3 mg) and **33** (5 mg). Fractions 5 and 7 were combined and rechromatographed on silica gel with $CHCl_3$: EtOAc (7 : 1) to give **25** (10 mg) and **30** (30 mg). Fraction 6 was treated similarly to obtain **5** (2 mg), **15** (7 mg), **18** (1 mg), **21** (1 mg), **28** (1 mg), **31** (5 mg), **32** (2 mg) and **34** (2 mg). Fraction 8 was treated in a similar way as fraction 4 to give **3** (3 mg), **6** (1 mg), **10** (1.5 mg), **14** (7 mg), **26** (3 mg), **29** (1 mg) and **31** (1 mg), respectively.

The H_2O insoluble portion was chromatographed on a Rp-18 column to give 13 fractions. Fraction 5 was rechromatographed on silica gel with $CHCl_3$: MeOH (5 : 1) to obtain **11** (1.5 mg) and **23** (5 mg). Fraction 10 was treated in the same manner as fraction 5 to give **1** (7 mg), **5** (2 mg), **6** (1 mg) and **12** (10 mg). Fractions 11 and 12 were combined and filtered to give **13** (5 mg).

Aristoliukine-A (**1**): Yellowish needles (MeOH), mp 274—276 °C. HR-FAB-MS (Pos.): Calcd for $C_{17}H_{14}NO_5$, m/z 312.0872 $[M+H]^+$, Found 312.0876. UV λ_{max} nm: 239, 256, 280(sh), 321, 345(sh), 404. IR ν_{max} cm^{-1}: 3380, 3300, 3170, 1668, 1651, 1377, 1238, 1224, 1147, 1043, 1001, 667. FAB-MS (Pos.) m/z (rel. int.): 312 (M^++1), 307 (24), 289 (11), 155 (29), 154 (100), 137 (70), 107 (23). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 10.88 (1H, s, OH), 9.75 (1H, s, OH), 9.70 (1H, br s, NH), 8.46 (1H, d, J=2.8 Hz, H-5), 7.76 (1H, d, J=8.8 Hz, H-8), 7.07 (1H, dd, J=8.8, 2.8 Hz, H-7), 7.04 (1H, s, H-9), 5.80 (1H, br s, OH), 5.07 (2H, s, CH_2), 3.98 (3H, s, OCH_3). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 169.5 (C-1), 155.5 (C-2), 149.6 (C-3), 148.3 (C-4), 132.4 (C-10), 131.4 (C-1a), 130.2 (C-8), 127.4 (C-8a), 127.2 (C-9), 122.3 (C-10a), 119.3 (C-2), 118.9 (C-4a), 117.1 (C-7), 111.5 (C-5), 104.9 (C-9), 60.0 (OCH_3), 56.1 (C-11).

Aristoliukine-B (**6**): Red needles; mp 249—250 °C (dec.); HR-FAB-MS (Pos.) Calcd for $C_{17}H_{12}NO_5$ m/z 310.0715 $[M+H]^+$, Found 310.0723. UV λ_{max} nm: 219, 235, 246, 275(sh), 321(sh), 332, 373(sh) and 480 nm. IR ν_{max} cm^{-1}: 3400-3100, 1676, 1569, 1524, 1400 and 1211. FAB-MS m/z (*rel. int.*): 310 (M^++1, 3), 289 (12), 219 (8), 154 (100), 137 (70), 124 (12), 107 (22). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.93 (1H, s, NH), 10.62 (1H, s, OH), 9.94 (1H, s, OH), 8.89 (1H, d, J=2.0 Hz, H-11), 8.06 (1H, s, H-3), 7.76 (1H, d, J=8.8 Hz, H-8), 7.41 (1H, s, H-7), 7.15 (1H, dd, J=8.8, 2.0 Hz, H-9), 4.08 (3H, s, OCH_3).

Sodium (2*R*)-3-(*p*-Hydroxyphenyl)lactate (**17**): Colorless powder (MeOH), mp>300 °C. $[\alpha]_D$ −33° (c=0.01, MeOH). UV λ_{max} nm: 225, 279, 285. IR ν_{max} cm^{-1}: 3200, 1590, 1520, 1470, 1455, 1420, 1360, 1335, 1250. ^{1}H-NMR (D_2O, 200 MHz) δ: 7.13 (2H, d, J=8.6 Hz, H-5,9), 6.83 (2H, d, J=8.6 Hz, H-6,8), 3.82 (1H, dd, J=7.6, 5.2 Hz, H-2), 3.13 (1H, dd, J=14.4, 5.2 Hz, H-3), 2.93 (1H, dd, J=14.4, 7.6 Hz, H-3).

Acidification of **17**: Compound **17** (2 mg) was dissolved in 5% HCl (aq) (1 ml). The solution was eluted in a Sephadex LH-20 column with H_2O, then MeOH, to afford NaCl (0.5 mg) and (2*R*)-3-(*p*-hydroxyphenyl)lactic acid (1.6 mg) ($[\alpha]_D$ −13°, c=0.15, MeOH).

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