

AN ARISTOLOCHIC ACID DERIVATIVE OF *ARISTOLOCHIA LIUKIUENSIS*

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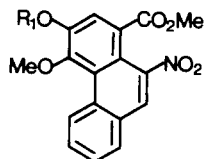
ABSTRACT.—A new aristolochic acid derivative, 3-hydroxy-4-methoxy-10-nitrophenanthrene-1-carboxylic acid methylester (**1**), was isolated from the stems of *Aristolochia liukiuensis* in addition to a cinnamide, another aristolochic acid, aristololactams, dioxaporphine, and isorhamnetin-3-O-robinobioside. This is the first report of this flavonol glycoside in *Aristolochia* species.

The genus *Aristolochia* (Aristolochiaceae) is found in wide areas from the tropics to temperate zones and consists of about 300 species. Some species have been used in the form of crude drugs as anodynes, antiphlogistics, and detoxicants, especially in China. Five species are native to Japan, namely *Aristolochia kaempferi*, *Aristolochia onoei*, *Aristolochia debilis*, *Aristolochia contorta*, and *Aristolochia liukiuensis* (1). *A. liukiuensis* has recently been separated from *A. kaempferi* from a taxonomic standpoint, but these species are morphologically very similar. The chemical constituents of *A. liukiuensis* were investigated. A new aristolochic acid derivative **1** and a flavonol glycoside were isolated from the stems. The present paper deals with the structure elucidation of **1**.

Compound **1**, mp 164–165°, was isolated as pale yellow needles from a CHCl₃ extract after repeated separation with Si gel cc. In the eims, the [M]⁺ was observed at *m/z* 327, which agreed with a molecular formula of C₁₇H₁₃NO₆ by hrms (found 327.0720, calcd 327.0743). The uv [λ max 214, 257, 295, 356, 374 nm], the ir [ν 3470

(OH), 1720 (C=O), 1525 (NO₂)], and the ms [*m/z* 281, a fragment caused by the elimination of a nitro group] suggested that **1** was one of the typical aristolochic acids. In the ¹H-nmr spectrum, two three-proton singlets at δ 3.92 and 3.82 were observed. The former corresponded to a methoxyl group, and the latter was due to a methyl group of a methylester moiety, indicating that **1** was a methylester of an aristolochic acid derivative. With reference to the data described in the literature (2), two one-proton singlets at δ 7.83 and 8.41 were assignable to H-2 and H-9, which implied 3,4-dioxygenation on the A ring. The complicated signal pattern in the aromatic region indicated the C ring to be unsubstituted. The position of the methoxyl group was deduced to be at C-4 because of its low chemical shift (δ 60.6) in the ¹³C-nmr spectrum. Therefore, the structure of **1** was proposed to be 3-hydroxy-4-methoxy-10-nitrophenanthrene-1-carboxylic acid methylester. By usual acetylation or methylation, **1** gave a monoacetate or a monomethylether of **1**. The respective spectral data supported the structure thus proposed.

In addition to **1**, isorhamnetin-3-O- β -L-rhamnopyranosyl (1 \rightarrow 6)- β -D-galactopyranoside (3–8), *N*-(*p*-hydroxyphenyl)- β -ethyl-*p*-hydroxycinnamide (9, 10), aristolochic acid I (2, 11), aristololactams A II, B II, and A III (12, 13), and 2-hydroxy-1-methoxy-4,5-dioxo-6,7-dehydroaporphine (14) were also isolated. These



- 1 R₁=H
- 2 R₁=Ac
- 3 R₁=Me

known structures were characterized by means of their spectral properties.

All aristolochic acid derivatives in *Aristolochia* species have a methylenedioxy group on the A ring; in contrast, in the methyl ester **1** this group is lacking, which suggests that **1** has a possibility to be a species-specific marker useful to characterize the species. To investigate its occurrence in other Aristolochiaceae, the constituents in other species are now being examined.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Büch apparatus and are uncorrected. The uv spectra were recorded on a Hitachi 323 spectrometer, ir spectra on a IR-AI spectrometer, and ms on a JEOL JMS-300 mass spectrometer at 70 eV. ¹H-nmr and ¹³C-nmr spectra were taken on a JEOL JNM-GX270 instrument; chemical shifts are given in ppm with TMS as an internal standard.

PLANT MATERIAL.—The plant material of *A. liukiuensis* used in this study was collected in October 1985, in the Okinawa prefecture, Japan, and a voucher specimen is deposited in the Herbarium of Gifu Pharmaceutical University.

EXTRACTION AND ISOLATION.—The stems (1.0 kg) were air-dried, crushed into pieces, and extracted successively with *n*-hexane, CHCl₃, Me₂CO, and MeOH under reflux. Each fraction was concentrated in vacuo. The CHCl₃ extract (15 g) was subjected to Si gel cc using CHCl₃ as solvent, to give aristololactam A II (25 mg), aristololactam A III (10 mg), and aristololactam B II (15 mg). The Me₂CO extract (25 g) was also chromatographed on Si gel cc (eluent CHCl₃/MeOH system). By recrystallization, 3-hydroxy-4-methoxy-10-nitrophenanthrene-1-carboxylic acid methylester [**1**] (30 mg), aristolochic acid I (50 mg), *N*-(*p*-hydroxyphenyl)-β-ethyl-*p*-hydroxycinnamide (15 mg), and 2-hydroxy-1-methoxy-4,5-dioxo-6,7-dehydroaporphine (35 mg), were obtained in pure forms; the spectral and physical data of the known compounds were completely identical with those in the literature. The MeOH extract (35 g) was also subjected to chromatography on Si gel using CHCl₃-MeOH-H₂O (70:23:7, lower phase) to give isorhamnetin-3-*O*-robino-bioside.

3-HYDROXY-4-METHOXY-10-NITROPHENANTHRENE-1-CARBOXYLIC ACID METHYLESTER [1].—Pale yellow needles (CHCl₃/MeOH): mp 164–165°; uv λ max (MeOH) nm (log ε) 214 (4.5), 257 (4.7), 295 (4.2), 356 (3.8), 374 (3.8);

ir (cm⁻¹, KBr) 3470 (OH), 1720 (C=O), 1525 (NO₂), 1345; ¹H nmr (Me₂CO-*d*₆) δ 3.83 (3H, s, OMe), 3.93 (3H, s, OMe), 7.73 (1H, m, H-7), 7.82 (1H, m, H-6), 7.83 (1H, s, H-2), 8.15 (1H, dd, *J* = 6.2, 1.3 Hz, H-8), 8.41 (1H, s, H-9), 9.60 (1H, br d, *J* = 8.7 Hz, H-5); ¹³C nmr (Me₂CO-*d*₆) δ 52.2 (CH₃OCO), 60.6 (OMe), 167.4 (C=O); eims *m/z* (rel. int.) [M]⁺ 327 (16), [M - OMe]⁺ 296 (7), [M - NO₂]⁺ 281 (100), 166 (47), 251 (42), 223 (27), 51 (24); hrms calcd for C₁₇H₁₃NO₆ *m/z* [M]⁺ 327.0743, found 327.0720.

ACETYLATION OF 1.—A mixture of **1** (5 mg) and Ac₂O (1.0 ml) in dried pyridine (1.0 ml) was allowed to stand at room temperature for 5 days. The reaction mixture was poured into H₂O. The precipitated crystals were washed with H₂O and recrystallized from MeOH to give **2**: mp 205–206°; ¹H nmr (Me₂CO-*d*₆) δ 2.48 (3H, s, Ac), 3.82, 4.02 (3H each, s, OMe), 7.85 (1H, m), 7.191 (1H, m), 7.99, 8.65 (1H each, s, H-2 and -9), 8.27 (1H, dd, *J* = 6.2, 1.4 Hz, H-8), 9.62 (1H, d, *J* = 8.8 Hz, H-5).

METHYLATION OF 1.—A mixture of **1** (10 mg), MeI (1 ml, to excess), and K₂CO₃ (10 mg) in dry Me₂CO was boiled under reflux for 3 h. The reaction mixture was filtered. Evaporation of the filtrate in vacuo afforded a permethyl ether **3** (7 mg): mp 122–123° (MeOH); ¹³C nmr (Me₂CO-*d*₆) δ 52.4, 57.0, 60.4 (OMe).

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