Pergamon

PII: S0031-9422(98)00028-4

Phytochemistry, Vol. 48, No. 4, pp. 743–745, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

SODIUM ARISTOLOCHATE DERIVATIVES FROM LEAVES OF *ARISTOLOCHIA FOVEOLATA*

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(*Received in revised form* 24 *November* 1997)

Key Word Index—*Aristolochia foveolata*; *A. kaoi*; Aristolochiaceae; leaves; sodium aristolochates; flavonoids; aristolactams.

Abstract—Three new sodium aristolochate derivatives, sodium aristolochate-I, -C and sodium 7-hydroxy-aristolochate-A, together with 11 known compounds, were isolated from fresh leaves of *Aristolochia foveolata*. Their structures were determined by spectral and chemical methods. IR and ^{1}H NMR methods to distinguish aristolochic acid from its salt are discussed. This is the first reported isolation of sodium aristolochates from natural sources. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Aristolochia foveolata is a herbaceous perennial, which is distributed in Malaysia, Indonesia, the Philippines and the southern part of Taiwan [1]. Its constituents have not been reported previously. Interest in the constituents of this species was stimulated by the pharmacological action of related species of the genus [2]. In the present paper, the isolation and structural elucidation of three new compounds, sodium aristolochate-I (**1**), sodium 7-hydroxy-aristolochate-A (**2**) and sodium aristolochate-C (**3**) are described, together with 11 known compounds, from leaves of *A. foveolata*. IR and ^{1}H NMR methods were established to distinguish aristolochic acid from its salt.

RESULTS AND DISCUSSION

Sodium aristolochate-I (**1**) was isolated as a yellow powder. UV absorptions of **1** at 221, 250, 295, 318 and 388 nm suggested an aristolochic acid derivative [2, 3]. The IR spectrum showed the presence of a carboxylic salt and a nitro group at 1560 and 1345 cm^{-1}. In the ^{1}H NMR spectrum there were methoxyl and methylenedioxy signals at δ 4.03 (3H, *s*) and 6.35 (2H, *s*). In the aromatic region, two singlet signals at δ 8.30 and 7.62 were ascribed to H-9 and H-2. A pair of 1,2,3-trisubstituted ABC-type aromatic signals at δ 8.63 (1H, *d*, J = 8.2 Hz), 7.74 (1H, *t*, J = 8.2 Hz) and 7.27 (1H, *d*, J = 8.2 Hz) were assigned to H-5, 6 and 7, respectively. Based on these data, compound **1** was proposed to be aristolochic acid-1 (**1a**). Comparison of the spectral of **1** and **1a** [2] revealed that the carbonyl groups in the IR and H-9 in the ^{1}H NMR appeared at different positions (Table 1). Structure **1** was assigned to the salt of **1a**. Compound **1** was dissolved in 5% HCl, applied to a Sephadex LH-20 column and eluted with H_2O, and then MeOH. The residue obtained from the H_2O fractions was confirmed as sodium ion by atomic absorption spectroscopy. Compound **1a** was obtained from the MeOH eluted fractions. Thus, compound **1** was assigned as sodium aristolochate-I.

Sodium 7-hydroxyaristolochate-A (**2**) exhibited a UV spectrum characteristic of a phenanthrene chromophore [2, 3]. The ^{1}H NMR spectrum showed the presence of one methylenedioxy group at δ 6.37, a methoxyl group at δ 3.95 and a hydroxyl signal at δ 10.50. The aromatic region of the ^{1}H NMR spectrum showed a close resemblance to that of a 2,5,6,9-unsub-

Table 1. H-9 (^{1}H NMR) and carbonyl (IR) of Compounds **1**, **1a**, **2**, **2a**, **3** and **3a**

	Carbonyl (IR, cm^{-1})*	H-9 (^{1}N NMR, δ)†
1	1560	8.30
1a	1701	8.59
2	1574	8.26
2a	1679	8.35
3	1550	8.18
3a	1668	8.45

* Recorded in KBr.
† Recorded in DMSO-d_6.

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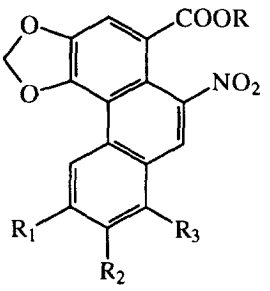

1: R=Na, R1=H, R2=H, R3=OMe
1a: R=H, R1=H, R2=H, R3=OMe
2: R=Na, R1=H, R2=OH, R3=OMe
2a: R=H, R1=H, R2=OH, R3=OMe
3: R=Na, R1=OH, R2=H, R3=H
3a: R=H, R1=OH, R2=H, R3=H

stituted aristolochic acid. Thus, H-5 appeared as the characteristic most downfield signal at δ 8.65 and was coupled with H-6 at δ 7.48. The NOESY spectrum showed H-9 (δ 8.26) to be proximate to the 8-OMe (δ 3.95). On the basis of the above results, structure **2** was similar to 7-hydroxyaristolochic acid-A (**2a**). Comparison of the IR spectra of **2** and **2a** [4], indicated that their carbonyl groups appeared at different positions (**2**: 1574 cm^{-1};**2a**:1679 cm^{-1}). Therefore, **2** was assigned to be a salt of 7-hydroxyaristolochic acid. Compound **2** was treated with the same acidification procedure used for compound **1**, to afford sodium ion and 7-hydroxyaristolochic acid-A (**2a**), in which the carbonyl group appeared at 1683 cm^{-1} in the IR spectrum. On the basis of the above data, structure **2** was assigned to sodium 7-hydroxyaristolochate-A.

Sodium aristolochate-C (**3**) was obtained as orange needles. The UV spectrum showed a close resemblance to that of **1**. In the IR spectrum, the carbonyl group appeared at 1550 cm^{-1}. Thus, **3** was also a salt of aristolochic acid. The aromatic region of the ^1H NMR spectrum revealed an ABX-pattern at δ 8.43, 7.98 and 7.21 attributable to H-5, 8 and 7, respectively. Three signals at δ 8.18 (1H), 7.69 (1H) and 6.36 (2H) were assigned to the H-9, H-2 and methylenedioxy protons, respectively. Therefore, the structure of **3** was proposed to be a salt of aristolochic acid-C (**3a**). By acidification, **3** gave **3a** [2], in which the carbonyl absorption band had shifted to 1668 cm^{-1} in the IR spectrum; the sodium salt was confirmed by atomic absorption spectroscopy. This combined evidence supported structure **3** for sodium aristolochate-C.

To conclude the spectral data of the sodium aristolochates and aristolochic acids (Table 1), it was found that H-9 of the sodium aristolochates appeared from δ 8.35 to 8.15 and that of H-9 of the aristolochic acids appeared in the range from δ 8.60 to 8.45 in the ^1H NMR spectra. In the IR spectra, the carbonyl group of the sodium aristolochates appeared in the range 1580 to 1540 cm^{-1}, whereas the carbonyl group of the aristolochic acids absorbed in the range 1710 to 1660 cm^{-1}.

The known compounds aristolactam-C-*N*-β-D-glucoside (**4**) [2], aristolactam (**5**) [2], aristolactam-AII (**6**) [2], cepharanone-A-*N*-β-D-glucoside (**7**) [5] cepharadione-A (**8**) [2], kaempferol-7-*O*-glucoside (**9**) [6], quercetin-3,7-*O*-diglucoside (**10**) [6], isorhamnetin-3-*O*-rutinoside (**11**) [6, 7], 4-β-D-glucopyranosylferulic acid (**12**) [8], methyl vanillate (**13**) [2] and methyl 4-hydroxy cinnamate (**14**) [2] were also isolated from the leaves of *A. foveolata*. Their structures were characterized by comparison of their spectroscopic data (UV, IR, NMR and mass spectrometry) with literature data.

EXPERIMENTAL

Mps: uncorr. ^1H NMR: DMSO-d_6, TMS as int. standard except where noted. UV: MeOH, IR: KBr, unless otherwise stated.

Plant material

Aristolochia foveolata (*A. kaoi* Liu & Lai) was collected from Ping-Tong Hsien, Taiwan, and identified by Prof. I. S. Chen (Kaohsiung Medical College). A voucher specimen (NCKU-WU-920701) is deposited in the Herbarium of Cheng Kung University, Taiwan.

Extraction and separation

Fresh leaves (152 g) were extracted with MeOH (× 5) at room temp. and concd to give a deep brown syrup. The MeOH extract was partitioned successively between H_2O and $CHCl_3$. The $CHCl_3$ layer was concd under red. pres. to leave a brown syrup which was subjected to chromatography on silica gel and eluted with a gradient of $CHCl_3$ and MeOH to afford 10 fr. Fr 3 was separated by CC on silica gel and eluted with $CHCl_3$ and EtOAc (9:1) to give **5** (1 mg), **6** (2 mg) and **8** (1 mg), respectively. Fr. 8 was treated in the same way to afford **7** (1.5 mg) and **13** (1 mg). Fr. 9 was filtered to obtain **1** (5 mg). The H_2O layer was chromatographed on Sephadex LH-20 and eluted with a gradient of H_2O and MeOH to afford 14 fr. Fr. 3 was filtered to afford **11** (1.5 mg). Frs 4–6 were combined and treated in a similar manner to fr. 8 of the $CHCl_3$ layer to give **1** (2 mg) and **14** (4 mg). Frs 7 and 8 were combined and chromatographed on silica gel to yield **10** (5 mg) and **12** (6 mg). Fr. 10 was treated in the same manner as frs 7 and 8 to give **2** (2 mg), **3** (4 mg), **4** (1 mg) and **9** (3 mg), respectively.

Sodium aristolochate-I (**1**). Orange needles ($CHCl_3$–MeOH). $NaC_{17}H_{10}NO_7$.Mp > 280°. UV λ_{max} nm: 221,

250, 318, 385. IR ν_{max} cm^{-1}: 1560, 1470, 1420, 1345, 1275, 1045, 950. ^1H NMR: δ 8.63 (1H, *d*, $J = 8.2$ Hz, H-5), 8.30 (1H, *s*, H-9), 7.74 (1H, *t*, $J = 8.2$ Hz, H-6), 7.62 (1H, *s*, H-2), 7.27 (1H, *d*, $J = 8.2$ Hz, H-7), 6.35 (2H, *s*, OCH_2O), 4.03 (3H, *s*, OMe).

Acidification of **1–3**. Isolated **1–3** were dissolved in 5% aq. HCl (1 ml), respectively. The soln was eluted from a Sephadex LH-20 column with H_2O, then MeOH, to afford NaCl and the acids **1a**, **2a** and **3a**, successively.

Aristolochic acid-I (**1a**). Orange powder ($CHCl_3$–MeOH). $C_{17}H_{11}NO_7$. Mp 283–285°. UV λ_{max} nm: 220, 246, 248, 310, 385. IR ν_{max} cm^{-1}: 1701, 1593, 1521, 1467, 1269, 1150, 1041, 945. FAB-MS *m/z* (rel. int.): 342 ($[M+1]^+$, 14), 341 (26), 295 (33), 280 (10). ^1H NMR: δ 8.66 (1H, *d*, $J = 8.0$ Hz, H-5), 8.59 (1H, *s*, H-9), 7.86 (1H, *t*, $J = 8.0$ Hz, H-6), 7.82 (1H, *s*, H-2), 7.38 (1H, *d*, $J = 8.0$ Hz, H-7), 6.50 (2H, *s*, OCH_2O), 4.07 (3H, *s*, OMe).

Sodium 7-hydroxyaristolochate-A (**2**). Orange needles ($CHCl_3$–MeOH). $NaC_{17}H_{10}NO_8$ Mp > 280°. UV λ_{max} nm: 219, 263, 310, 372. IR ν_{max} cm^{-1}: 1574, 1526, 1508, 1350, 1242, 1045, 980. ^1H NMR: δ 10.50 (1H, *br s*, OH), 8.65 (1H, *d*, $J = 9.0$ Hz, H-5), 8.26 (1H, *s*, H-9), 7.70 (1H, *s*, H-2), 7.48 (1H, *d*, $J = 9.0$ Hz, H-6), 6.37 (2H, *s*, OCH_2O), 3.95 (3H, *s*, OMe).

7-Hydroxyaristolochic acid-A (**2a**). Orange powder ($CHCl_3$–MeOH). $C_{17}H_{11}NO_8$.Mp 267–269°. UV λ_{max} nm: 226, 265, 313, 371. IR ν_{max} cm^{-1}: 1679, 1527, 1510, 1458, 1350, 1261. FAB-MS *m/z* (rel. int.): 358 ($[M+1]^+$, 3), 357 ($[M]^+$, 6), 307 (17), 155 (32), 154 (100), 139 (25), 138 (44), 137 (76), 136 (80), 123 (20), 109 (20). ^1H NMR: δ 8.60 (1H, *d* $J = 9.2$ Hz, H-5), 8.35 (1H, *s*, H-9), 7.68 (1H, *s*, H-2), 7.48 (1H, *d*, $J = 9.2$ Hz, H-6), 6.41 (2H, *s*, OCH_2O), 3.95 (3H, *s*, OMe).

Sodium aristolochate-C (**3**). Orange needles ($CHCl_3$–MeOH). $NaC_{16}H_8NO_7$.Mp > 280°. UV λ_{max} nm: 250 (sh), 257, 304, 375. IR ν_{max} cm^{-1} 1550, 1423, 1332, 1232. ^1H NMR: δ 8.43 (1H, *d*, $J = 2.2$ Hz, H-5), 8.18 (1H, *s*, H-9), 7.98 (1H, *d*, $J = 8.4$ Hz, H-8), 7.69 (1H, *s*, H-2), 7.21 (1H, *dd*, $J = 8.4$, 2.2 Hz, H-7), 6.36 (2H, *s* OCH_2O).

Aristolochic acid-C (**3a**). Orange needles ($CHCl_3$–MeOH). $C_{16}H_9NO_7$.Mp 279–281°, UV λ_{max} nm: 248 (sh), 257, 278, 300, 371. IR ν_{max} cm^{-1}: 3300, 1668, 1600, 1529, 1350, 1232, 1043, 945. EI-MS *m/z* (rel. int.): 327 ($[M]^+$, 18), 282 (46), 281 (62), 280 (28), 279 (100), 252 (8), 166 (11), 139 (18). ^1H NMR: δ 10.60 (1H, *br s*, OH), 8.45 (2H, *br s*, H-5, 9), 8.07 (1H, *d*, $J = 8.8$ Hz, H-8), 7.72 (1H, *s*, H-2), 7.26 (1H, *dd*, $J = 8.8$, 2.4 Hz, H-7), 6.46 (2H, *s*, OCH_2O).

Acknowledgements—The authors are grateful for financial support from a National Science Council of the Republic of China grant (NSC-87-2732-M-006-001 and 87-2113-M-006-012).

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