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Isolation of Aristolochic Acids from *Aristolochia Bracteolata* and Studies of their Antioxidant Activities

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إستخلاص أحماض الاريستولوكيك من نبات *أريستولوكيا براكتيولاتا* ودراسة انشطتها المضادة للأكسدة

صالح البوصافي ، منير الحارثي و بشرى الصباحي

خلاصة: إستخلاص وكشف التركيب ألجزيئ لأحما ض ألأريستولوكيك من نبات *أريستولوكيا براكتيولاتا* الأنشطه ألمضاده للأكسده لهذه المركبات الطبيعيه قد تم تقيمها بناء على قدرتها لإختزال Mo(VI) إلى Mo(V). الدراسه أظهرت أن حمض أريستولوكيك-د أكثر نشاطا من فيتامين ج بينما حمض أريستولوكيك-أ مماثل لنشاط فيتامين ج.

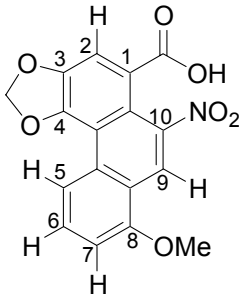
ABSTRACT: The isolation and structural elucidation of aristolochic acid-A and aristolochic acid-D from Omani *Aristolochia bracteolata* plant is reported. Antioxidant activities of these two natural products were evaluated for their capacity to reduce Mo(VI) to Mo(V). The study revealed that aristolochic acid-D is more active than vitamin C while aristolochic acid-A has activity similar to vitamin C.

KEYWORDS: *Aristolochia bracteolata*; aristolochic acid-A; aristolochic acid-D; Antioxidant activities and Omani plants.

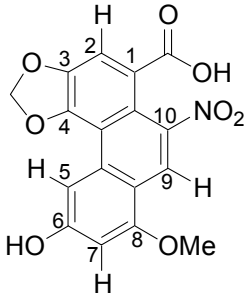
1. Introduction

Aristolochia bracteolata is a climbing perennial plant with cordate leaves and dark-purple tubular flowers with unpleasant smell (Ghazanfar, 1991). Traditionally, the plant has been used to treat wounds and for snakebites. The genus *Aristolochia* is a source of aristolochic acid, which has been evaluated in China for the treatment of wounds and infectious diseases; it was found to be useful for promoting wound healing in ulcers, burns, and scalds (Jiangsu, 1977). Recently, aristolochic acids, present in *Aristolochia* plants, have been shown to be nephrotoxic in rats (Matsuo, *et al.,* 2003). A family of aristolochic acids have been isolated from *Aristolochia argentina* (Priestap, 1987), and from *Aristolochia indica* (Kupchan *et al.,* 1968). Aristolactam alkaloids and amides were isolated from *Aristolochia*

kankauensis (Tian-Shung Wu *et al.,* 1994) and from *Aristolochia auricularia* (Houghton *et al.,* 1990). Aristolochic acid-A was isolated from the root of *Aristolochia bracteata* from Sudan (Mohamed *et al*., 1999). Magnoflorine and aristolochic acid-A were isolated from *A. bracteata* from Saudi Arabia (El Tahir *et al.*, 1991). Both compounds were found to induce contractions in the isolated pregnant rat uterus and stimulated the isolated guinea pig ileum. N-acetylnornuciferine and aristololactam were isolated from *A. bracteata* from India (Chakravarty, *et al.*, 1988). Aristolochic acid-A was characterized as the principal tumer-inhibitory principle (Kupchan *et al.,* 1968). In recent years, the antioxidative action of natural products received much attention, due to their possible role in disease prevention (Aruoma, 1998). In the course of continuing search for natural products with antioxidant properties from Omani plants we report herein the isolation and elucidation of two aristolochic acids (**1, 2**) from *n*-butanol extract of *A. bracteolata* leafs and stems and their antioxidant activities relative to vitamin C. The antioxidant activities were tested using the reduction of phosphomolybdenum complex system (Prieto, *et al*, 1999).



Aristolochic acid-A (**1**)



Aristolochic acid-D (**2**)

2. Results and Discussion

2.1 Isolation and Structural Elucidation of Aristolochic Acids 1 and 2

Isolation of the two compounds was carried out by a standard column chromatography with silica gel (0.13-0.25 mm, 60-120 mesh). The principal product was aristolochic acid-A (**1**), which was eluted with a mixture of chloroform-methanol (95% : 5%). Elution with increasing polar mixtures of chloroform-methanol (88% : 12%) yielded aristolochic acid-D (**2**).

Aristolochic acid-A (**1**) separated from methanol as dark-red solid with melting point of 260-265 ˚C. The mass spectrum ($[M]^+$at *m/z* 341) establishes the molecular formula as $C_{17}H_{11}NO_7$ with fragment ions at 327 $[M\text{-}CH_2]^+$, 281 $[M\text{-}CH_2\text{-}NO_2]^+$, 253 $[M\text{-}CH_2\text{-}NO_2\text{-}CO]^+$, 237 $[M\text{-}CH_2\text{-}NO_2\text{-}CO_2]^+$, 207 $[M\text{-}CH_2\text{-}NO_2\text{-}CO_2\text{-}OCH_2]^+$. Ultraviolet (UV) absorptions at 218, 227, 255, and 312 nm suggest a highly conjugated system. The infrared (IR) spectrum showed the presence of hydroxyl group (strong broad signal at 2850 – 3579 cm^{-1}), carbonyl group (sharp signal at 1714 cm^{-1}), nitro group (signals at 1345 cm^{-1} symmetric stretch and at 1517,1594 cm^{-1} asymmetric stretch). The ^1H nuclear magnetic resonance (^1H NMR) spectrum of **1** showed signals for an aromatic methoxyl group at δ 4.04 (3H, s) and for methylenedioxy group at δ 6.47 (2H, s). Three mutually coupled aromatic protons appeared at δ 7.32 (1H, d, *J*=8.1Hz), 7.79 (1H, t, *J*=8.2 Hz), δ8.66 (1H, d, *J*=8.4 Hz); the signals were assigned to H-7, H-6 and H-5 respectively as revealed by ^1H-^1H correlation spectroscopy (^1H-^1H COSY) and compared to literature values (Tian-Shung Wu *et al.*, 1994). Two singlets at δ 7.75 and 8.47 (each 1H) were assigned to H-2 and H-9 respectively. The position of 8-OMe was deduced from nuclear Overhauser effect (NOESY spectrum) which showed H-7 (δ 7.32) to be within NOE distance from the 8-OMe.

Table 1. NMR spectral data of Aristolochic acids **1** and **2.**

Position	Aristolochic acid-A (**1**)		Aristolochic acid-D (**2**)	
	^{1}H data (ppm)	^{13}C data (ppm)	^{1}H data (ppm)	^{13}C data (ppm)
1		119.4		118.0
2	7.76, s	112.4	7.69, s	112.6
3		146.34		145.7
4		147.06		145.7
4’		117.6		117.0
5	8.62, d, *J* 8.4	119.2	8.06, d, J 2.4	104.1
5’		130.4		132.1
6	7.79,t, *J* 8.2	132.0		158.4
7	7.32, d, *J* 8.1	109.5	6.84, d, J 2.4	100.0
8		156.68		161.5
8’		115.8		112.3
9	8.47, s	119.3	8.36, s	112.6
10		146.34		145.7
10’		117.1		117.0
C=O		168.7		168.9
-OCH_2O-	6.43, s	103.0	6.40, s	102.7
CH_3O-	4.04, s	56.7	3.98, s	56.6

Aristolochic acid-D (**2**) was obtained as brown solid with melting point range of 280-288 ºC. The structure of **2** was inferred from its mass spectrum ($[M-2]^+$ at *m/z* 355) which establishes the molecular formula as $C_{17}H_{11}NO_8$ with fragment ions at 327 $[M-OCH_2]^+$, 281 $[M-OCH_2-NO_2]^+$, 277 $[M-2OH-NO_2]$, 207 $[M-CH_2-NO_2-CO_2-OCH_2O]^+$. UV absorptions at 225, 245, 289, and 364 nm show a close resemblance to that of **1**. The IR spectrum showed the presence of hydroxyl group (strong broad signal at 2720 – 3691 cm^{-1}), carbonyl group (at 1702 cm^{-1}), nitro group (signals at 1517 cm^{-1}). The aromatic region of the ^{1}H NMR spectrum of **2** contained two doublets at δ 6.84 (1H, d, *J*=2.4 Hz) and 8.06 (1H, d, *J*=2.4 Hz) attributed to H-7 and H-5 respectively. The small *J* value suggests a meta coupling system. The low frequency signal of H-7 at δ 6.84 is due to the shielding effect of the ortho hydroxyl and methoxyl groups.

Two singlets at δ 7.69 and 8.36 (each 1H) were assigned to the C-2 and C-9 protons. The ^{1}H NMR spectrum showed the presence of methoxyl group at δ 3.98 (3H, s) and methylenedioxy group at δ 6.40 (2H, s). The position of 8-OMe was deduced from a NOESY spectrum which showed H-7 (δ 7.32) to be within NOE distance from the 8-OMe. The NMR spectral data of aristolochic acid-A (**1**) and aristolochic acid-D (**2**) are summarized in Table 1.

2.2 Antioxidant Activities of Aristolochic Acids 1 and 2

The phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm. The assay was successfully used to quantify vitamin E in seeds (Prieto *et al*., 1999) and measuring antioxidant activity of the sage polyphenols (Yinrong *et al*, 2001). The method is simple and independent of other antioxidant measurements commonly employed. Our value for vitamin C (A_{695} = 1.625) at a concentration of 5 mM, was well comparable with the reported molar absorption coefficient of vitamin C [ε = (3.4 ± 0.1) x 10^3 M^{-1} cm^{-1}] (Prieto *et al*., 1999).

The results in Table 2 show that the antioxidant activity of aristolochic acid-A (**1**) is almost equal to that of vitamin C. On the other hand, aristolochic acid-D (**2**) has better antioxidant activity than vitamin C.

This high antioxidant activity of aristolochic acid-D (**2**) is presumably due to the presence of the hydroxyl group at C-6.

Table 2. Antioxidant activities of Aristolochic acids **1** and **2** relative to vitamin C.

Compound	A_m [a]
Aristolochic acid-A (**1**)	1.03
Aristolochic acid-D (**2**)	1.20
Vitamin C	1

[a] A_m , activity relative to vitamin C on a molar basis.

3. Experimental

3.1 General

The UV spectra were obtained with a Varian model Cary 50 conc spectrophotometer. The IR spectra were obtained as KBr discs with a Nicolet model Magna 560 spectrometer; absorption bands are recorded in wave number (cm^{-1}). The ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz using Bruker spectrometer in DMSO-d_6. Chemical shifts are expressed as δ values (ppm) downfield from TMS.

Thin-layer chromatography (TLC) was carried out on Merck F_{254} silica gel plates (0.2 mm thickness) with mobile phase $CHCl_3$ – MeOH, (80 : 20) and spots were detected under UV 254 nm. Melting points were recorded using Gallen Kamp apparatus. Electron impact mass spectroscopy (EIMS) was performed on a Shimadzu GCMS QP5050 spectrometer.

3.2 Plant Material

Aristolochia bracteolata was collected from Mazara, wilayat of Quraiyat, Sultanate of Oman in 2001 and was authenticated by specialists in the Department of Biology, Sultan Qaboos University.

3.3 Extraction and Isolation

450 g of shade dried powdered leafs and stems of *A. bracteolata* was repeatedly extracted with methanol at room temperature. The combined extract was concentrated under reduced pressure to get 45 g of greenish viscous material which was first partitioned between hexane/H_2O, then EtOAc/H_2O and finally *n*-Butanol/H_2O to give a hexane extract, an ethyl acetate extract and *n*-Butanol extract. The concentrated *n*-Butanol extract (23 g) was chromatographed over a silica gel column and eluted with chloroform then with chloroform-methanol mixture. Aristolochic acid-A (**1**) (40 mg) was isolated from chloroform-methanol mixture (95% : 5%) fractions. Aristolochic acid-D (**2**) (20 mg) was isolated from chloroform-methanol mixture (88% : 12%).

3.4 Evaluation of Antioxidant Activity

The antioxidant activity of aristolochic acids was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*. (1999). An aliquot of 0.1 ml sample solution (5 mM in MeOH) was combined in a 4-ml vial with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95 $^{\circ}C$ for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expressed relative to that of ascorbic acid.

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