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Aristolochic Acid-1 and Aristoloside from the Rhizome of *Aristolochia albida* Duch

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The methanolic extract of the rhizome of *Aristolochia albida* afford aristolochic acid-1 and aristoloside (aristolochic acid–O–glucoside), along with other already isolated compounds. Their structures were characterised by spectroscopic analysis.

Aristolochia albida Duch (Aristolochiaceae) is a perennial climbing shrub commonly found in the Savannah.[1] The plant is widely used and is held in high repute in Hausa folkloric medicine in the treatment of abdominal colic and other ailments. The rhizome of this plant was reported to be beneficial in abdominal spasms and snakebites[2, 3] The family of this plant is known to contain aristolochic acids and aristolactams (aporphinoids), which are considered to be the chemo taxonomic markers of the family: Aristolochiaceae. Previous phytochemical investigations on the plant have shown the presence of lignans[4] and an earlier report revealed that the rhizome of the plant contained aristolactactam, aristolic acid, aristolone (-), aristolochic acid and its 6-hydroxy derivative.[5] Also from this plant the occurrence of phytosterols and fatty acids in addition to a diterpenoidal compound were reported.[6, 7] This communication reports the isolation of aristolochic acid-1 and aristoloside from the anti-phospholipase-A2 active fraction of the rhizome and their structural elucidation, as a result of our efforts to isolate compounds of biological interest.

EXPERIMENTAL

The plant was collected from a forest reserve in Katsina, Nigeria, and authenticated at the herbarium, Ahmadu Bello University. Column chromatography was carried out using silica gel (BDH, 120 mesh). TLC using pre-coated Silica gel F_{254}, 0.25 mm (thickness) plates (Polygram SIL G/UV 254, Camlab.). Melting points were uncorrected and the UV spectra were recorded on Pye Unicam SP 8-100. IR spectra were recorded on a Perkin-Elmer and 1710 FT IR. The ^{1}H-NMR, COSY, and the ^{13}C-NMR spectra were run on a Bruker WM 250 and AM 400 in DMSO-d_6.

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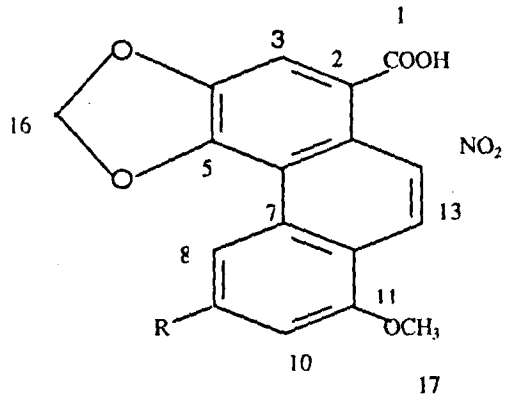
Extraction:

The air-dried powdered rhizome (840 g) of *A. albida* was exhaustively defatted with light petroleum (60-80°) using soxhlet apparatus. The air-dried marc was extracted exhaustively with methanol. The methanol was concentrated *in vacuo* to a brown gummy mass (23 g), which was suspended in water and then extracted with several portions of dichloromethane. The aqueous portion was concentrated to dryness *in vacuo* and the dried mass was investigated further. The combined dichloromethane portion was reduced to about 200 ml and then basified with dilute ammonium hydroxide to pH 9. This was agitated and subsequently extracted with chloroform. The aqueous ammonical layer was then acidified to pH 3 with dilute hydrochloric acid under cold condition (ice bath), and then extracted with chloroform. The combined chloroform portion was concentrated and then evaporated to dryness *in vacuo* to afford a yellow residue (2.9 g). This fraction revealed several spots on TLC. The fraction was subjected to column chromatography on silica gel column. Elution was carried out with light petrol (60-80°) - Chloroform - Methanol, which resulted in a mixture of

compounds showing yellow fluorescence on exposure to U.V. The mixture was repeatedly chromatographed on Silica gel (chloroform – methanol gradient), yielding, after final purification (prep. TLC), compound **1** (68 mg) and compound **2** (43 mg).

Aristolochic acid-1 (compound 1):

Crystallized from chloroform-methanol as Compound **1**, which occurred as pale yellow micro needle-like crystals, m.p. 280-283° (lit. m.p. 283-285°)[8] slightly soluble in Methanol, molecular formula $C_{17}H_{11}O_7N$, $[M]^+$ m/z 341. U.V. $\lambda_{max}^{(EtOH)}$ nm: 228(4.10), 261(4.50), 315(4.01), 410(3.63). I.R. $\nu_{max}^{(KBr)}$ cm^{-1}: 1692 (C = O), 1517, 1351, 1250, 1044, 940 (methylenedioxy). ^{1}H-NMR and ^{13}C-NMR (DMSO-d_6) Table 1 and 2. EI-MS [positive] m/z (rel.int.): 341(39), 296(41), 295(100), 75(54), 74(32), 53(25). The fragmentation is consistent for aristolochic acid-1.9.



Compound **1**, R = H
Compound **2**, R = glucose

Aristoloside (compound 2):

Molecular formula $C_{23}H_{21}O_{13}N$ (EI-MS). $[M]^+$ m/z 519, single fluorescent spot (U.V. lamp at 254 and 366 nm) on TLC. Compound 2 was obtained as pale yellow amorphous flakes from Methanol, mp. 191-194° (lit. m.p.193-196°).[10] U.V. $\lambda_{max}^{(EtOH)}$ nm: 226(4.20), 248(4.58), 256(4.23), 399(2.61). ^{1}H-NMR and ^{13}C-NMR (DMSO-d_6) Table 2. I.R. $\nu_{max}^{(KBr)}$ cm^{-1}: 3380 (hydroxyl), 1694, 1517, 1355, 1010. EI-MS, [positive] m/z (rel.int.): 519(100), 489(52), 357(49). The fragmentation is consistent for aristoloside.[10]

Acid hydrolysis:

Compound **2** was refluxed with 2 M HCl in aqueous methanol on water bath for 2 h. The filtrate from the hydrolysate was neutralized with silver carbonate and then filtered. The filtrate was concentrated *in vacuo*. The concentrate (positive test for sugars) was co-chromatographed with reference sugars by paper chromatography (Whatman no.1). The sample gave a single spot corresponding to glucose.

RESULTS AND DISCUSSION

When the methanol percolate of the rhizome was handled in the usual manner and then treated with a base, the basified aqueous layer (when *worked-up)* afforded compounds **1** and **2**, identified as aristolochic acid-1 and aristoloside respectively, in addition to some minor compounds (not identified). Compound **1**, which occurred as pale yellow micro needle-like crystals, m.p. 280-283° (lit.m.p.283-285°),[8] slightly soluble in methanol, molecular formula $C_{17}H_{11}O_7N$, $[M]^+$ m/z 341. The U.V. $\lambda_{max}^{(EtOH)}$, 228 nm (4.10), 261nm (4.50), 315 nm (4.01), 410nm (3.63) are typical absorption maxima for polycyclic aromatic hydrocarbons. The presence of the carboxylic acid group was suggested by the I.R. ν_{max} at 1692 cm^{-1} indicative of C=O stretching and a strong absorption at 1517cm^{-1} corresponding to aryl nitro- function. The presence of methylenedioxy ring absorption at 940cm^{-1} was supported by ^{1}H-NMR signal at δ = 6.28 (br s, 2H). These signals were characterised as methylenedioxy group protons.[11] Single proton signal at δ = 10.13 ppm confirmed the I.R. absorption for the carboxylic group. The relative up-field resonance observed by this proton may plausibly be due to the methylenedioxy ring slightly opposing and distorting the electromeric effect on the –C=O function of the -COOH making this proton to appear at slightly lower frequency than predicted. The methoxy group attached to C-11 ensures the assignment of ^{1}H-NMR signal at δ = 7.80 (t, 1 H; J=8 Hz) to a proton at C-9, coupling with proton at C-10 δ = 7.23 (d, 1H; J= 8 Hz) and another proton at C-8 δ = 8.23 (d, 1H; J= 8Hz). The latter proton appears at a higher frequency, because of the influence of negative inductive effect by the electronegative atoms of the methylenedioxy ring. All the protons were successfully assigned from the ^{1}H - ^{1}H COSY experiments. This was aided by the ^{13}C-NMR DEPT spectrum (HETCOR) as summarized Table 2.

The EI–MS of compound **1** showed $[M]^+$ at m/z 341 and m/z 295 (100) corresponding to [M - methylenedioxy]$^+$. An intense peak observed was m/z 75 (54) corresponding to a fairly stable carbonium-ion, $[H_3C\text{-}O\text{-}CH_2\text{-}O\text{-}CH_2]^+$. Also another diagnostic peak was $[M\text{-}30]^+$, characteristic

TABLE 1 : ^{1}H-NMR SPECTRAL DATA OF COMPOUNDS **1** AND **2** δ (PPM) (DMSO-d_6)

Compound 1			Compound 2		
H	δ (ppm)	H-H cosy Correlated with: H	H	δ (ppm)	H-H cosy Correlated with: H
1	10.13 (br s, 1H)	-	1	10.21 (s, 1H)	-
2	-	-	2	-	-
3	6.84 (s, 1H)	-	3	7.24 (s, 1H)	-
4	-	-	4	-	-
5	-	-	5	-	-
6	-	-	6	-	-
7	-	-	7	-	-
8	8.23 (d, 1H, J=8Hz)	9	8	8.44 (d, 1H, J=2.4Hz)	10
9	7.80 (t, 1H, J=8Hz)	8,10	9	-	-
10	7.23 (d, 1H, J=8Hz)	9	10	7.60 (d, 1H, J=2.4Hz)	8
11	-	-	11	-	-
12	-	-	12	-	-
13	8.42 (s, 1H)	-	13	9.61 (s, 1H)	-
14	-	-	14	-	-
15	-	-	15	-	-
16	6.28 (br s, 2H)	-	16	6.47 (s, 2H)	-
17	3.98 (s, 3H)	-	17	4.11 (s, 3H)	-
			1/	5.11 (d, J=8Hz)	
			2/	3.90 (dd, J=8,9Hz)	
			3/	4.16 (dd, J=9,9Hz)	
			4/	4.01 (dd, J=9,9.5Hz)	
			5/	3.79 (ddd, J=2,5,9.5Hz)	
			6/α	4.45 (dd, J=2, 11Hz)	
			6/β	4.29 (dd, J=5, 11Hz)	

of loss of formaldehyde ion by compounds having methylenedioxy moiety.[12] These peaks were prominently observed in the mass spectrum of aristolochic acid-1.[9] The structure of compound 1 was then established and it was found to be identical with aristolochic acid-1 (co-TLC and mmp).

Compound **2** was assigned molecular formula $C_{23}H_{21}O_{13}N$ (EI-MS). $[M]^+$ m/z 519, single fluorescent spot (U.V. lamp at 254 and 366 nm) on TLC. Compound **2** was obtained as pale yellow amorphous flakes from methanol, m.p. 191-194° (lit.m.p.193-196°)[10]. The I.R. (KBr) spectrum of this compound showed a hydroxyl group absorption at 3380cm^{-1}, and –C=O absorption at 1694 cm^{-1}, aryl-nitro absorption at 1517cm^{-1}, and 1010 cm^{-1} for methylenedioxy group.

Compound 2 on EI-MS, showed $[M]^+$ at m/z 519 and other discernible peaks at m/z 489 and m/z 357 consistent with $[M - CH_2O]^+$ and $[M$ – glucosyl$]^+$ respectively.

TABLE 2 : ^{13}C-NMR SPECTRAL DATA OF COMPOUNDS **1** AND **2** δ (PPM) (DMSO-d_6)

Compound 1				Compound 2			
C	δ (ppm)	DEPT	HETCOR Correlated with:H	C	δ (ppm)	DEPT	HETCOR Correlated with: H
1	183.97	C	-	1	179.12	C	-
2	150.22	C	-	2	142.10	C	-
3	127.28	CH	3	3	126.45	CH	3
4	140.71	C	-	4	144.16	C	-
5	138.66	C	-	5	126.52	C	-
6	119.92	C	-	6	118.66	C	-
7	112.67	C	-	7	114.22	C	-
8	108.26	CH	8	8	121.89	CH	8
9	102.34	CH	9	9	137.23	C	-
10	111.39	CH	10	10	124.36	CH	10
11	133.81	C	-	11	129.55	C	-
12	101.22	C	-	12	99.87	C	-
13	120.44	C	-	13	116.79	C	-
14	132.91	C	-	14	133.29	C	-
15	125.52	C	-	15	120.56	C	-
16	128.12	CH_2	16 α, β	16	131.22	CH_2	16 α, β
17	68.81	Me	17	17	59.88	Me	17
				1/	97.20		1/
				2/	74.12		2/
				3/	79.40		3/
				4/	70.99		4/
				5/	78.15		5/
				6/	62.64		6/

Acid hydrolysis of compound **2** afforded (after the usual treatments) a sugar identified on paper chromatography (Whatman no.1), as glucose. The ^{1}H-NMR data of compound **2** Table 1 when compared with compound **1** showed the disappearance of H-9 (C – 9 quarternary), also indicated by the HETCOR analysis Table 2. This suggests that the glycosidic linkage was at C - 9. Although C - 1 was also available for such linkage, but ^{1}H-NMR proton signal at δ = 10.21 (br s, 1H) (carboxylic proton) in the ^{1}H-NMR spectrum of **2** eliminated that possibility. All the anomeric protons were also assigned.

Unlike aristoloside which was reported to be isolated from *Aristolochia argentina* (trace quantity),[13] *A. cinnabarina* (0.000413%),[14] and *A. manshuriensis* (0.0203%),[10] aristolochic acid–1 has a much wider occurrence than its glycoside (aristoloside), and it was found to be present in species such as *A. acuminata* (trace)[15], *A. argentina* (0.00316%),[13] *A. auricularia (0.00562%),*[16] *A. bracteolate* (0.06-0.16%),[17,18] *A. chilensis,(0.00771%),*[19] *A. cinnabarina (0.0048%),*[14] *A. clematitis (0.259%),*[20,21] *A. debilis (0.61%),*[22] *A. fangchi (0.05%)*[23] *A. indica(0.0186%),*[24] *A. kaempferi (0.1%),*[23] *A.*

manshuriensis (0.00114%),[25] *A. maurorum (0.02%)*,[26] *A. tuberosa (0.27%)*,[27] and many others.[15] Due to their legendary roles in eliciting various pharmacological responses, glycosides, where ever they occur, are subject of extensive study. The biological actions of glycosides are chiefly due to the genin (aglycone) portion of the molecules. The sugar component by and large modifies the solubility and distribution profile of the molecule. Aristoloside was reported to inhibit carcinogenesis,[28] whereas, aristolochic acid-1 was reported to possess various biological actions and the most prominent is the inhibition of phospholipase A-2 (PLA-2). This compound was observed to decrease haemolytic actions and oedema provoked by snake envenomation. It was also reported to inhibit inflammation induced by immune complexes and non-immunological agents and its anti-inflammatory actions were reported to be by directly blocking the PLA-2-catalysed release of arachidonic acid and other steps involved in eicosanoids (cyclooxiginase and lipooxiginase pathways) release.[29, 30, 31] The presence of aristolochic acid-1 and its glycoside, isolated from this plant could explain the anti-phospholipase A-2 activity of the said fraction.

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