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SEVEN ARISTOLOLACTAMS FROM *ARISTOLOCHIA ARGENTINA*

HORACIO A. PRIESTAP

Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junin 956, Buenos Aires, Argentina

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Key Word Index—*Aristolochia argentina*; Aristolochiaceae; aristololactam alkaloids.

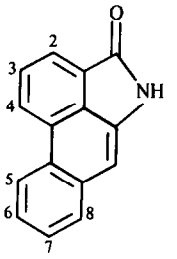
Abstract—Seven aristololactam alkaloids were isolated from *Aristolochia argentina*. Five are reported for the first time.

INTRODUCTION

A previous investigation [1] established the occurrence in the rhizomes of *Aristolochia argentina* of aristololactams I, AII, AIII, BII and BIII. The present paper deals with the isolation of seven new lactams, named as aristololactams II, III, IV, Ia, IIIa, AIa and AIIIa, from the same source. Five of them, aristololactams III, Ia, IIIa, AIa and AIIIa, are reported for the first time. Aristololactams II (cepharanone A) and IV (aristolochic acid-D methyl ether lactam) were previously isolated from the callus tissue of *Stephania cepharantha* (Menispermaceae) by Akasu *et al.* [2] and from *Aristolochia indica* by Kupchan and Merianos [3], respectively. Aristololactam IIIa as the *N*-β-D-glucoside has been also found by Pakrashi *et al.* [4] to occur in *A. indica*.

RESULTS AND DISCUSSION

The structure of aristololactams II, $C_{16}H_9NO_3$ ($[M]^+$ 263), III, $C_{17}H_{11}NO_4$ ($[M]^+$ 293), IV, $C_{18}H_{13}NO_5$ ($[M]^+$ 323), Ia, $C_{16}H_9NO_4$ ($[M]^+$ 279) and IIIa, $C_{16}H_9NO_4$ ($[M]^+$ 279) was inferred from their mass spectra and from the signal multiplicities of their hydrogen resonances. ^{1}H NMR data of these and other aristololactams of *A. argentina* are compiled in Table 1. A comparison of shift values indicates certain correlations which may be of diagnostic value. Apart from known shielding effects, the effects of substituents on C-2, C-5 and C-9 protons are noteworthy. The signal for the C-2 proton is differentiated in an aristololactam substituted in ring A with two methoxyl groups or a CH_2O_2 group and replacement of a methoxyl substituent at C-3 by a



Aristololactam	3	4	6	8
I		OCH_2O		OMe
II		OCH_2O		
III		OCH_2O	OMe	
IV		OCH_2O	OMe	OMe
Ia		OCH_2O		OH
IIIa		OCH_2O	OH	
AII	OH	OMe		
AIII	OH	OMe	OMe	
AIa	OH	OMe		OH
AIIIa	OH	OMe	OH	
BI	OMe	OMe		OMe
BII	OMe	OMe		
BIII	OMe	OMe	OMe	

Table 1. ^{1}H chemical shifts of aristololactams

	H-2	H-5	H-6	H-7	H-8	H-9	CH_2O_2	MeO-3	MeO-4	MeO-6	MeO-8	NH
I	7.52	7.99 *dd*	7.41 *t*	7.09 *dd*	—	7.24	6.43	—	—	—	4.01	10.65
II	7.64	8.48 *m*	7.55 *m*	7.55 *m*	7.91 *m*	7.11	6.48	—	—	—	—	10.78
III	7.52	7.86 *d*	—	7.19 *dd*	7.79 *d*	7.00	6.40	—	—	3.88	—	10.59
IV	7.62	7.65 *d*	—	6.82 *d*	—	7.28	6.48	—	—	3.94	4.01	10.64
Ia	7.62	8.04 *dd*	7.38 *t*	7.06 *dd*	—	7.38	6.46	—	—	—	—	10.69
IIIa	7.60	7.96 *d*	—	7.14 *dd*	7.78 *d*	7.05	6.47	—	—	—	—	10.68
AII	7.62	9.13 *m*	7.54 *m*	7.54 *m*	7.93 *m*	7.08	—	—	4.03	—	—	10.77
AIII	7.65	8.67 *d*	—	7.25 *dd*	7.89 *d*	7.08	—	—	4.08	3.95	—	10.67
AIIIa	7.63	8.57 *d*	—	7.09 *dd*	7.76 *d*	7.03	—	—	4.02	—	—	10.62
BI	7.85	8.72 *dd*	7.50 *t*	7.18 *dd*	—	7.43	—	4.04	4.04	—	4.04	10.73
BII	7.85	9.12 *m*	7.55 *m*	7.55 *m*	7.93 *m*	7.13	—	4.06	4.06	—	—	10.78
BIII	7.81	8.63 *d*	—	7.20 *dd*	7.84 *d*	7.07	—	4.05	4.05	3.92	—	10.70

δ values (ppm) from TMS. DMSO-d_6 solutions. J (Hz): 5, 6 = 6, 7 = 7, 8 = 8.5; 5, 7 = 2.5. Singlets not denoted.

hydroxyl group causes a significant shielding of this proton. The signal for C-5 proton which can be recognized by its low-field position, moves further downfield in aristololactams substituted at C-4 with a methoxyl. It is also to be noted that the presence of a hydroxyl or methoxyl group at C-8 results in a downfield shift of the C-9 proton due to *peri* interaction.

The more polar fractions of the neutral extract yielded the aristololactams AIa and AIIIa, both $C_{16}H_{11}NO_4$ ($[M]^+$ 281). Their dimethyl ethers were characterized as taliscanine (aristololactam BI), a component of *Aristolochia taliscana* [5], and aristololactam BIII, respectively. Evidence for the location of the methoxyl group in aristololactams AIa and AIIIa was obtained from their ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum of these showed resonance values for the C-2 protons (δ7.64 and 7.63) and methoxyl protons (δ4.00 and 4.02) which strongly suggest a 3-hydroxy-4-methoxy substitution pattern. ^{13}C NMR analysis of the aromatic hydroxyl carbons and methoxyl carbons in aristololactams AIa and AIIIa supported this view. Carbon shift assignment was based on aristololactams AII, AIII, BII and BIII as models. The data in Table 2 indicate that C-3 and C-4 resonate at *ca* δ152 and 149 in aristololactams AII and AIII, and at *ca* 154 and 150.5 in aristololactams BII and BIII. The signal for C-6 is differentiated by its low-field position. Assignment of C-3 and C-4 in aristololactams AII, AIII, BII and BIII as shown in Table 2 was inferred from the similarity of the shifts with those of guaiacol and veratrole [6] (comparison of guaiacol and veratrole shows that replacement of the hydroxyl substituent by a methoxyl group causes deshielding of the *ipso* carbon (3.2 ppm) and, in a minor extent (2.0 ppm), of the *ortho* methoxylated carbon). The methoxyl carbon shifts, because of their spreading and shift invariance, allow an unequivocal assignment and constitute another useful diagnosis. The carbon shifts of aristololactams AIa and AIIIa are presented in Table 2. Resonances for C-3, C-4 and methoxyl groups are in close agreement with those observed for aristololactams AII and AIII and support the assignment of the methoxyl group to C-4. Thus, aristololactams AIa and AIIIa can be formulated as 3,8-dihydroxy-4-methoxy and 3,6-dihydroxy-4-methoxyphenanthrene derivatives, respectively.

With the isolation of the new aristololactams described herein, this limited group of compounds is enlarged to 13 members. Aristololactam-type alkaloids are not confined to Aristolochiaceae. Aristololactams II and BII (cepharanones A and B, respectively) occur in *Stephania cepharantha* (Menispermaceae) [2] and the latter was also found in *Schefferomitra subaequalis* (Annonaceae) [9]. Occurrence of aristololactams in these three families is of taxonomic significance and supports the view that Aristolochiales is related to Magnoliales and Ranunculales [10]. A total of 12 aristololactams have been isolated from *A. argentina* and they all show a similar substitution pattern to that of the accompanying aristolochic acids [7], i.e. none possess substituents on C-2, C-5 and C-7. The structural relationship between both these two groups suggests that aristolochic acids are derived from aristolo-

Table 2. ^{13}C chemical shifts of aromatic oxy carbons and OMe groups in aristololactams AII, AIII, AIa, AIIIa, BII and BIII

	C-3	C-4	C-6	C-8	MeO-3	MeO-4	MeO-6
AII	152.2	148.9	—	—	—	59.5	—
AIII	151.9	148.9	157.0	—	—	59.6	55.2
AIa	152.1	148.9	—	153.7	—	59.4	—
AIIIa	151.7	149.0	155.2	—	—	59.5	—
BII	154.2	150.5	—	—	57.0	59.9	—
BIII	154.0	150.6	157.1	—	57.1	60.0	55.2

δ-values (ppm) from TMS. Solutions in DMSO-d_6.

lactams rather than directly from quaternary aporphine alkaloids as proposed earlier [8].

Little is known about the biosynthesis of aristololactams. However, aristololactams are biogenetically related to aporphine alkaloids from which they probably originate. The isolation of 4-hydroxy, 5-oxo and 4,5-dioxoaporphines from *Stephania cepharantha* (Menispermaceae) [2], *Fusea longifolia* (Papaveraceae) [11], *Piper sanctum* (Piperaceae) [12] and *Glaucium flavum* (Papaveraceae) [13] has led to the idea that 4,5-dioxoaporphines might arise by oxidation of aporphines and function as intermediates in the biosynthesis of aristololactams [2, 11, 13]. Indeed, conversion of pontevedrine, a 4,5-dioxoaporphine, into an aristololactam takes place *in vitro* and can be regarded as a benzilic acid rearrangement followed by loss of carbon [13].

EXPERIMENTAL

Mps are uncorr. UV spectra were recorded in 95% EtOH and IR spectra in KBr discs. ^{1}H NMR and ^{13}C NMR spectra were determined in DMSO-d_6 solns. Chemical shifts are expressed as δ-values (ppm) downfield from TMS using central resonance of DMSO-d_6 as internal reference. TLC was carried out as follows: (1) silica gel, C_6H_6–MeCOEt (17:3), two developments (aristololactam, R_f: I, 0.67; II, 0.65; BI, 0.60; BII, 0.58; IV, 0.56; III, 0.53; BIII, 0.45); (2) Al_2O_3, $CHCl_3$–EtOH (100:1), two developments (aristololactam, R_f: BI, 0.83; BII, 0.76; BIII, 0.71; I, 0.66; II, 0.63; IV, 0.57; III, 0.55); (3) Woelm Mg silicate, C_6H_6–MeCOEt (3:1) (aristololactam, R_f: BII, 0.60; II, 0.51; BI, 0.51; BIII, 0.41; I, 0.32; III, 0.25; IV, 0.12); (4) Al_2O_3, $CHCl_3$–EtOH (95:5) (aristololactam, R_f: IIIa, 0.55; AII, 0.44; AIII, 0.43; Ia, 0.29); (5) silica gel, $CHCl_3$–EtOH (9:1) (aristololactam, R_f: AII, 0.70; AIII, 0.68; IIIa, 0.64; Ia, 0.54; AIIIa, 0.28; AIa, 0.24); (6) silica gel, C_6H_6–MeCOEt (7:3) (aristololactam, R_f: Ia, 0.69; IIIa, 0.67; AII, 0.65; AIII, 0.56; AIa, 0.39; AIIIa, 0.34). Fluorescence on silica gel at 360 nm: aristololactam I, blue; II, dark blue; III, dark green; IV, orange; Ia, dark green; IIIa, green; AII, blue; AIII, yellow; AIa, blue–green; AIIIa, yellow–green; BI, green; BII, light blue; BIII, yellow–green.

Extraction of Aristolochia argentina. Rhizomes of *A. argentina* (24.08 kg, dry wt) were extracted as described previously [1]. The residue from the ethereal extracts containing the neutral components were submitted to repeated column chromatography on silica gel and Al_2O_3 (C_6H_6–$CHCl_3$–MeOH) to give several fractions. By prep. TLC (systems 1, 2 and 3) the nonphenolic fraction afforded 3 mg aristololactam II, 3.5 mg aristololactam III and 4 mg aristololactam IV. The phenolic fraction, after the removal of a mixture of aristololactams AII and AIII by crystallization, was fractionated by column (Al_2O_3, $CHCl_3$–EtOH) and prep. TLC (systems 4 and 5) yielding 44 mg aristololactam IIIa and 3 mg aristololactam Ia. Only a small portion of the latter was obtained as pure crystals. TLC showed that aristololactams Ia and IIIa are contained in the rhizomes in equal amounts. After prep. TLC (system 6, two developments), a fraction corresponding to 13.77 kg (dry wt) of rhizomes afforded 3 mg of aristololactam AIa and 13.8 mg of aristololactam AIIIa.

Benzo[f]-1,3-benzodioxolo [6,5,4-cd]indol-5(6H)-one, aristololactam II, cepharanone A. Mp 297–298° (*n*-PrOH); UV λ_{max} nm (log ε): 264 (4.38), 276 (4.46), 287 (4.43), 327 (3.87), 339 (3.86), 374 (3.79), 391 (3.79); IR ν_{max} cm^{-1}: 3145, 1706, 1686, 1377, 1266, 1166, 1044; EIMS *m/z* (rel. int.): 263 $[M]^+$ (100), 262 (11), 235 (6), 207 (8), 179 (11), 178 (8), 177 (14), 152 (12), 151 (6), 150 (17), 131.5 (9), 131 (13).

10-Methoxy-benzo[f]-1,3-benzodioxolo[6,5,4-cd]indol-5(6H)-one, aristololactam III. Mp 297–304° (HOAc); UV λ_{max} nm (log ε): 237 (4.49), 254 sh (4.35), 267 (4.45), 281 (4.48), 294 (4.33), 334 (4.06), 348 (4.07), 396 (3.92); IR ν_{max} cm^{-1}: 3195, 1702, 1376, 1047, 852; EIMS *m/z* (rel. int.): 293 $[M]^+$ (100), 278 (73), 250 (22).

8,10-Dimethoxy-benzo[f]-1,3-benzodioxolo[6,5,4-cd]indol-5(6H)-one, aristololactam IV. Mp 345° (HOAc); UV λ_{max} nm (log ε): 240 (4.45), 263 (4.39), 291 (4.06), 302 (4.04), 336 (3.84), 346 (3.85), 410 (3.80); IR ν_{max} cm^{-1}: 3226, 1689, 1626, 1377, 1366, 1337, 1284, 1264, 1047; EIMS *m/z* (rel. int.): 323 $[M]^+$ (100), 308 (58), 293 (10), 280 (22), 278 (9), 265 (41), 250 (6), 237 (14), 161.5 (17).

8-Hydroxy-benzo[f]-1,3-benzodioxolo[6,5,4-cd]indol-5(6H)-one, aristololactam Ia. Mp > 350° (HOAc); UV λ_{max} nm (log ε): 212 (4.38), 242 (4.54), 249 sh (4.51), 258 (4.55), 291 (4.18), 301 sh (4.15), 330 (4.00), 400 (3.98); IR ν_{max} cm^{-1}: 3175, 1642, 1276, 1053, 933, 806, 740; EIMS *m/z* (rel. int.): 279 $[M]^+$ (100), 278 (21.4), 251 (16.5), 250 (13.4), 223 (24.5), 222 (10.5), 221 (9.2), 195 (23.1), 193 (15.3), 168 (12.6), 167 (9.8), 166 (14.9), 165 (8.3), 164 (21.9), 140 (8.8), 139 (53.7), 138 (14.4), 137 (11.8), 111 (10.7). Treatment with CH_2N_2 afforded aristololactam I (TLC).

10-Hydroxy-benzo[f]-1,3-benzodioxolo[6,5,4-cd]indol-5(6H)-one, aristololactam IIIa. Mp > 350° (HOAc); UV λ_{max} nm (log ε): 212 (4.46), 236 (4.47), 255 sh (4.37), 266 (4.45), 280 (4.41), 296 (4.26), 333 (4.04), 348 (4.04), 398 (3.93); IR ν_{max} cm^{-1}: 3311, 1653, 1366, 1314, 1270, 1044, 870, 789; EIMS *m/z* (rel. int.): 279 $[M]^+$ (100), 223 (13.6), 195 (9.6), 193 (8.0), 168 (8.9), 166 (8.7), 164 (11.4), 149 (14.7), 139 (28.4), Methylation with CH_2N_2 gave aristololactam III (TLC).

2,7-Dihydroxy-1-methoxy-dibenz[cd, f]indol-4(5H)-one, aristololactam AIa. Mp > 350° (HOAc); EIMS *m/z* (rel. int.): 281 $[M]^+$ (100), 266 (58.1), 238 (33.6), 182 (22.2). Methylation of aristololactam AIa with CH_2H_2 gave a product identical to aristololactam BI (mp, TLC, IR).

2,9-Dihydroxy-1-methoxy-dibenz [cd, f]indol-4(5H)-one, aristololactam AIIIa. Mp > 350° (HOAc); UV λ_{max} nm (log ε): 212 (4.39), 237 (4.53), 252 (4.38), 260 sh (4.37), 279 (4.27), 292 (4.28), 321 (4.08), 402 (3.87); IR ν_{max} cm^{-1}: 3106, 1689, 1292, 1209, 984; EIMS *m/z* (rel. int.): 281 $[M]^+$ (100), 267 (14.5), 266 (88.6), 238 (41.3), 182 (40.8), 155 (13.5), 153 (11.0), 126 (12.4). Treatment with CH_2N_2 afforded aristololactam BIII (mp, TLC, IR).

1,2,7-Trimethoxy-dibenz [cd,f]indol-4(5H)-one, aristololactam BI, taliscanine. This compound was obtained in low yield from aristololactam I by acid hydrolysis [14], methylation with CH_2N_2 and purification by prep. TLC (system 2), mp 260° (*n*-PrOH); UV λ_{max} nm (log ε): 242 (4.60), 247 (4.58), 253 (4.59), 259 (4.47), 297 (3.95), 336 (4.06), 397 (3.39); IR ν_{max} cm^{-1}: 3260, 1715, 1658, 1471, 1261, 1046, 1028; EIMS *m/z* (rel. int.): 310 (43.2), 309 $[M]^+$ (100), 308 (10.2), 295 (22.9), 294 (54.9), 279 (29.4), 266 (21.7), 251 (41.3), 250 (15.8), 236 (17.6), 223 (16.1), 180 (15.2), 164 (10.3), 152 (12.0).

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