Journal of Natural Products
Vol. 47, No. 2, pp. 331-341, Mar-Apr 1984

STUDIES ON *ARISTOLOCHIA* III. ISOLATION AND BIOLOGICAL EVALUATION OF CONSTITUENTS OF *ARISTOLOCHIA INDICA* ROOTS FOR FERTILITY-REGULATING ACTIVITY[1]

CHUN-TAO CHE, MOHAMED S. AHMED,[2] SAM S. KANG,[3] DONALD P. WALLER, A.S. BINGEL, ANNAMARIE MARTIN, PATHMANAYAKI RAJAMAHENDRAN,[4] NUNTAVAN BUNYAPRAPHATSARA,[5] DAVID C. LANKIN, GEOFFREY A. CORDELL, DJAJA D. SOEJARTO, R.O.B. WIJESEKERA,[6] and HARRY H.S. FONG*

Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612

ABSTRACT.—An ethanol extract of *Aristolochia indica* roots decreased fertility in both rats and hamsters when administered postcoitally (days 1-10 and 1-6, respectively). Petroleum ether (A), $CHCl_3$ (B), and aqueous (C) fractions, tested similarly in rats, were inactive and/or toxic. Partition of fraction B afforded non-acidic (D) and acidic (E) fractions. Savinin (**1**), isolated from fraction D and not previously reported from the Aristolochiaceae, was inactive when administered postcoitally to rats. Aristolochic acid-I (**2**), reported previously from *A. indica* and isolated from fraction E, was inactive when administered postcoitally to rats and toxic when administered postcoitally to hamsters.

(12*S*)-7,12-Secoishwaran-12-ol (**3**), previously reported from *A. indica* and isolated from fraction A, did not interrupt pregnancy when administered to mice on day 6 of pregnancy. Four additional compounds, aristolic acid (**4**) [prepared from aristolochic acid-I (**2**)], methyl aristolate (**5**) [prepared by methylating aristolic acid (**4**)], and *cis*- and *trans-p*-coumaric acid (both obtained commercially), were similarly tested in mice and found to be inactive. Aristolic acid (**4**), and the *cis*- and *trans-p*-coumaric acids also were inactive when administered postcoitally (days 1-10) to rats.

Seven compounds reported previously from *A. indica* were also isolated, as were a new naphthoquinone, aristolindiquinone (**6**) (fraction E), and magnoflorine (fraction C).

The roots of *Aristolochia indica* L. (Aristolochiaceae), commonly known as "Indian birthwort," are reputedly used in Indian folk medicine as an emmenagogue (1-5) and as an abortifacient (1-4, 6). Petroleum ether, C_6H_6, $CHCl_3$, and EtOH extracts (7) of the roots of this plant, solvent partition and column chromatographic fractions (8, 9), as well as the isolates, (12*S*)-7,12-secoishwaran-12-ol (**3**) (10, 11), aristolic acid (**4**) (12, 13), methyl aristolate (**5**) (14, 15), *p*-coumaric acid (16-18), and aristolactam N-β-D-glucoside (**7**) (17), have been evaluated previously for antifertility effects in mice, rats, hamsters, and/or rabbits.

As part of our continuing program on the investigation of fertility-regulating agents from plants, we have evaluated the 95% EtOH extract of *A. indica* roots for postcoital antifertility activity in rats and hamsters according to previously discussed bioassay protocols (19), and have similarly tested fractions and/or isolates in rats and/or hamsters. We have also tested aristolic acid (**4**) (prepared in our laboratory) and *cis*- and *trans-p*-coumaric acids (obtained commercially), in rats by the same bioassay, as well as in mice according to a dosing protocol reported in the literature (9, 16); methyl aristolate (**5**) (prepared in our laboratory) has similarly been tested in mice.

RESULTS AND DISCUSSION

As shown in Table 1, postcoital administration of the 95% EtOH extract of *A. in-*

[1]For paper II in this series, see: reference (47).

[2]Faculty of Pharmacy, Cairo University, Cairo, Egypt.

[3]Natural Products Research Institute, Seoul National University, Seoul, Korea.

[4]University of Sri Lanka, Peradeniya Campus, Peradeniya, Sri Lanka.

[5]Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

[6]United Nations Industrial Development Organization, Vienna International Center, Vienna, Austria.

TABLE 1. Postcoital Antifertility Effects of *Aristolochia indica* Root Extracts and Fractions

Species	Test Group				Vehicle Group			
	Days of treatment postcoitum	Test substance administered[a]	Dose/kg (po)	No. pregnant / No. treated	Days of treatment postcoitum	Vehicle administered	Dose/kg (po)	No. pregnant / No. treated
Rat	1-10	EtOH extract (ga)	1.0 g	3[b]/10	1-10	5% gum acacia	4 ml	9/10
Hamster . . .	1-6	EtOH extract (ga)	0.5 g[c]	3[b]/9[d]	1-8	H_2O	8 ml	10/10
Rat	1-8	MeOH/petroleum ether interphase (PVP)	0.5 g	0[b]/4[e]	1-10	20% PVP	5 ml	8-10

[a]Test substances were administered, either suspended in 5% gum acacia (ga), or as their polyvinylpyrrolidone (PVP) co-precipitates (4 parts PVP:1 part test substance)(55).

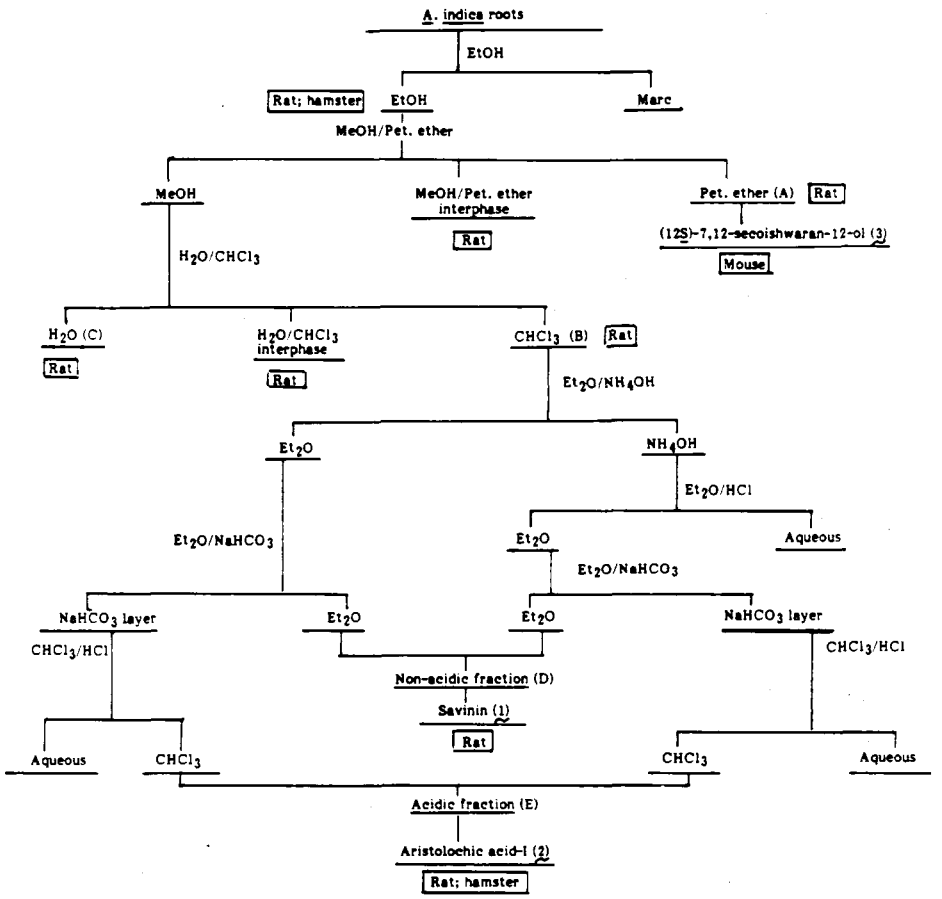
[b]$p<0.05$ compared with respective vehicle treated group.

[c]Doses of 1.4 and 1.0 g/kg were lethal in this species, death occurring before the dosage regimen could be completed.

[d]A tenth animal (non-pregnant) died on day 9.

[e]Six other animals (one pregnant) died between days 8 and 14.

dica roots significantly decreased the number of pregnancies in groups of rats and hamsters, relative to the number of pregnancies in their respective vehicle-treated groups. In an attempt to obtain the compound(s) responsible for the antifertility activ-



SCHEME 1. Fractionation scheme for ethanol extract of *A. indica* roots.
☐ indicates species in which extract/fraction/isolate tested.

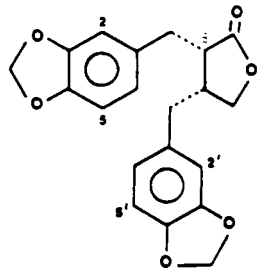
ity, the EtOH extract was fractionated, and the fractions and/or isolates tested, as indicated in Scheme 1. However, except for the MeOH-petroleum ether interphase material (see Table 2), the content of which presumably resembled that of the original EtOH extract, the various materials tested were inactive and/or toxic at the doses employed.

TABLE 2. Inactive Fractions, Isolates, and Pure Compounds from *Aristolochia indica* Roots

Species	Test Group				Vehicle Group			
	Days of treatment postcoitum	Test substance administered[a]	Dose/kg (po)	No. pregnant / No. treated	Days of treatment postcoitum	Vehicle administered	Dose/kg (po)	No. pregnant / No. treated
Rat	1-10	Fraction A (ga)	116 mg	10/10	1-10	5% gum acacia	4 ml	10/10
		Fraction B (ga)	122 mg	10/10				
		$H_2O/CHCl_3$ interphase (ga)	94 mg	9/9[b]				
	1-10	Fraction A (PVP)	940 mg	9/10	1-10	60% PVP	5 ml	10/10
		Fraction B (PVP)	940 mg	[c]				
		Aristolochic acid-I (PVP)	10 mg	9/10				
	1-10	Aristolochic acid-I (PVP)	40 mg	0/1[d]	1-10	40% PVP	4 ml	10/10
Hamster	1-6	Aristolochic acid-I (PVP)	12.5 mg[e]	3/6[f]	1-8	H_2O	8 ml	10/10
Rat	1-10	Fraction C (PVP)	500 mg	8/9[b]	1-10	20% PVP	5 ml	8/10
	1-6	Savinin (PVP)	58 mg	8/8	1-6	H_2O	5 ml	8/9
		Aristolic acid (PVP)	40 mg	8/8				
Mouse	6	Aristolic acid (ga)	100 mg	14/15	6	H_2O	10 ml	16/16
	6	Methyl aristolate (PVP)	50 mg	10/10	6	H_2O	10 ml	9/10
	6	(12*S*)-7,12-Secoishwaran-12-ol (ga)	100 mg	10/10	6	5% gum acacia	4 ml	11/11
		cis-p-coumaric acid (ga)	50 mg	10/11				
	6	*trans-p*-coumaric acid (ga)	50 mg	9/9	6	5% gum acacia	4 ml	9/9
Rat	1-10	*cis-p*-coumaric acid (ga)	2 g	6/8[g]	1-10	5% gum acacia	4 ml	9/10
		trans-p-coumaric acid (ga)	2 g	6/7[g]				

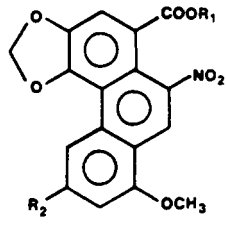
[a]See footnote to Table 1.
[b]A tenth animal died before the dosing regimen could be completed.
[c]All animals died before day 16, most before the dosing regimen could be completed.
[d]Nine other animals died before day 16, most before the dosing regimen could be completed.
[e]A dose of 25 mg/kg was lethal in this species.
[f]Four other animals died between days 5 and 8.
[g]Five additional animals were dosed, but most died before the dosing regimen could be completed.

Chromatography of the petroleum ether fraction (A) afforded (+)-ledol and (12*S*)-7,12-secoishwaran-12-ol (**3**), both previously reported from this plant (20), and a mixture of phytosterols. This fraction was inactive in the rat at doses of 116 and 940 mg/kg administered on days 1-10. (12*S*)-7,12-Secoishwaran-12-ol (**3**) likewise failed to interrupt pregnancy in the mouse when administered at a dose of 100 mg/kg on day 6. The latter result contrasts with that reported in the literature (10); however, whether more prolonged dosing in the strain of mouse we used might have proven effective could not be determined due to paucity of the compound.



12
1 $\Delta^{\alpha,\beta}$

The $CHCl_3$ fraction (B) was inactive, postcoitally, in the rat at a dose of 122 mg/kg and lethal at 940 mg/kg. The H_2O-$CHCl_3$ interphase, tested only at 94 mg/kg, also was inactive in the rat.

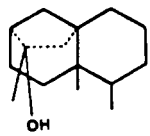


2 $R_1=R_2=H$
11 $R_1=H$, $R_2=OH$
10 $R_1=CH_3$, $R_2=H$

The aqueous fraction (C) afforded aristolactam N-β-D-glucoside (**7**), also previously reported from this plant (21), as well as magnoflorine, a compound isolated for the first time from *A. indica,* although previously reported from 13 other *Aristolochia* species (22-30). This fraction was inactive postcoitally in the rat at a dose of 500 mg/kg.

The non-acidic fraction (D) afforded aristolactam (**8**), aristolactam A-II (**9**), and methyl aristolochate (**10**), all previously reported from *A. indica* (21, 31, 32), and the lignan savinin (**1**), representing a class of compound that hitherto had not been found in the Aristolochiaceae. Savinin (**1**) has been found in such unrelated families as Rutaceae (33-36), Cupressaceae (37-40), and Taxodiaceae (41).

Analysis of the ^1H-coupled cmr spectrum of savinin (**1**) led to almost complete chemical shift assignments principally through comparison with the data reported for the related compounds, hinokinin (42) and kaerophyllin (43). In the region upfield of



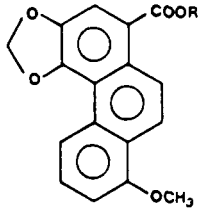
3

$CDCl_3$, three resonances were observed which, in the ^1H-coupled spectrum, appeared as two triplets and a doublet (J=99 Hz). The latter signal, at δ39.8, was assigned to C-β′ and the most upfield triplet (J=99 Hz), at δ37.6, to C-α′, leaving the lactone methylene carbon as a triplet (J=158 Hz) at δ69.5. Four doublets at δ108.5 (J=164 Hz), 108.7 (J=161 Hz), 108.8 (J=167 Hz), and 109.2 (J=161 Hz) are due to C-2, C-2′, C-5, and C-5′, and because of the absence of long-range ($^3J_{CH}$) coupling, the signals at δ108.5 and 108.8 were assigned to C-5 and C-5′. The signal at δ108.7 exhibited long-range coupling to two protons (J=6 Hz) and could be assigned to C-2, whereas the δ109.2 resonance was clearly coupled to three protons at long range and could be assigned to C-2′. In a similar manner, the doublet of quartets (J=6, 159 Hz) centered at δ122.1 was assigned to C-6′, and a doublet of triplets (J=6, 162 Hz) at δ126.0 to C-6. A distinction based on observed long-range coupling also permitted the assignment of the broad triplet (J=7 Hz) at δ128.2 to C-1, and the broad multiplet at δ125.9 to C-1′. The two methylenedioxy carbons resonated at δ101.0 and 101.7 and could not be assigned unambiguously. Two olefinic carbons appeared as a doublet (J=160 Hz) centered at δ137.2 (C-α) and as a broadened singlet at δ131.5 (C-β), and the lactone carbonyl carbon was observed as a broadened singlet at δ172.4. No attempt was made to assign unambiguously the four signals in the region δ146.6-149.2 for C-3, C-3′, C-4, and C-4′.

Savinin (**1**) was inactive in rats when administered at a dose of 58 mg/kg on days 1-6 postcoitum; insufficient material precluded testing at higher dosages and/or for a more prolonged period of time.

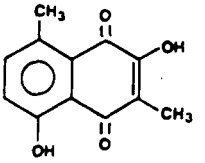
The acidic fraction (E) afforded aristolochic acid-I (**2**), aristolochic acid-D (**11**), and cepharadione A, all previously reported from *A. indica* (21, 31, 32, 44-46), and aristolindiquinone (**6**), a new naphthoquinone, the structure elucidation of which has been described elsewhere (47), and more recently confirmed through single-crystal X-ray analysis (48).

Aristolochic acid-I (**2**) was lethal to rats at a dose of 40 mg/kg and to hamsters at a dose of 25 mg/kg. A dose of 10 mg/kg was nonlethal to rats, but also inactive, while 12.5 mg/kg was still somewhat lethal to hamsters (4/10 died), but tended to decrease fertility in the survivors.



4 R=H
5 R=CH_3

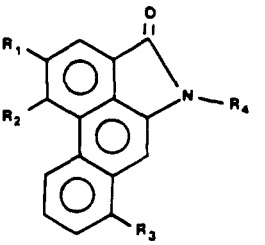
The presence of aristolic acid (**4**) had been detected in the MeOH phase, by tlc, after removal of the petroleum ether fraction (A). However, the amount detected was too small to justify its isolation for bioassay. Consequently, a method was developed for producing aristolic acid (**4**) from aristolochic acid-I (**2**) in one step and in high yield (49). Methyl aristolate (**5**), for bioassay, was produced by the methylation of aristolic acid (**4**) with CH_2N_2. Two additional compounds, *cis*- and *trans-p*-coumaric acids, were obtained commercially and purified by recrystallization, prior to bioassay; *trans-p*-coumaric acid had been isolated previously from this plant (32), but literature reports did not specify which isomer(s) was(were) evaluated biologically.



6

All four of the above compounds were ineffective in interrupting pregnancy when administered to mice on day 6 postcoitum, in contrast to literature reports (7, 9, 14, 16, 17) indicating antifertility effectiveness with similar dosage regimens. Whether or not more prolonged dosing with these compounds would prove effective in the strain of mouse we used remains to be determined. When tested on a more prolonged basis in the rat, however, aristolic acid (**4**) (40 mg/kg administered on days 1-6), and *cis*- and *trans*-*p*-coumaric acids (2 g/kg administered on days 1-10) were inactive.

It remains to be explained why activity could not be demonstrated following fractionation of the original extract. Perhaps a combination of compounds having different solubilities is necessary for the antifertility effects to be manifested. That inappropriate dosages were selected is possible, although in at least two instances, toxic levels were employed. The antifertility effects seen in the surviving animals may have been the result of nonspecific toxic effects of the test substances.



8 R_1-R_2=O-CH_2-O
R_3=OCH_3, R_4=H
9 R_1=OH, R_2=OCH_3
R_3=R_4=H
7 R_1-R_2=O-CH_2-O
R_3=OCH_3, R_4=glucose

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot plate and are uncorrected. High pressure liquid chromatography was conducted on a J-Y Instrument, Chromatospac apparatus. The uv spectra were obtained with a Beckman model DB-G spectrophotometer. The ir spectra were obtained with a Beckman model 18-A spectrophotometer with polystyrene calibration at 1601 cm^{-1}; absorption bands are recorded in wave numbers (cm^{-1}). Pmr spectra were recorded in deuterated solvents with a Varian T-60A instrument operating at 60 MHz with a Nicolet Model TT-7 Fourier Transform attachment or with a JEOL FX-100 instrument operating at 100 MHz; TMS was used as an internal standard, and chemical shifts are reported in δ (ppm). Cmr spectra were recorded on a JEOL FX-100 instrument operating at 25.04 MHz or with a JEOL FX-270 instrument operating at 67.83 MHz. Electron impact mass spectra were obtained with a Varian MAT-112S double focusing spectrometer operating at 70 eV.

Known isolates were compared with authentic samples by mixture melting point, tlc pattern in at least two solvent systems, ir, uv, pmr, and ms. In the case of previously known isolates, only those physical and spectral data not found in the literature are presented. Detailed data on all isolates are available on request.

PLANT MATERIAL.—Roots of *A. indica* were collected in India in 1980. A voucher specimen has been deposited at the Herbarium of the Field Museum of Natural History, Chicago, IL.

EXTRACTION AND FRACTIONATION.—An EtOH extract of the dried roots (100 kg) was prepared and furnished by Hoechst Pharmaceuticals Ltd., Bombay, India. The pulverized plant material was extracted five times (24 h each) with 400 liters of 95% EtOH with stirring, at reflux temperature, and the solvent was removed from the combined extracts under reduced pressure to yield 7.4 kg of concentrated extract.

An aliquot (2.3 kg) of the EtOH extract was suspended in 90% MeOH (10 liters) and partitioned against petroleum ether (boiling point 40-60°) (8×10 liters). The petroleum ether layers were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to yield fraction A (861.9 g).

The MeOH portion was brought to dryness *in vacuo*, resuspended in H_2O (10 liters), and extracted with $CHCl_3$ (5×10 liters). After drying (Na_2SO_4), filtration, and removal of solvent, this $CHCl_3$-soluble fraction yielded fraction B (470.5 g). After separation from $CHCl_3$, the aqueous phase was frozen and lyophilized to produce fraction C (515.6 g).

A portion of fraction B (250 g) was suspended in aqueous NH_4OH solution (pH 9.5, 2 liters) and extracted with Et_2O (10×2 liters) to separate the acidic and non-acidic components. The Et_2O phase, after drying (Na_2SO_4), filtering, and removal of solvent *in vacuo*, gave "Et_2O fraction I" (57.2 g). Acidification of the alkaline aqueous phase with HCl to pH 3, followed by extraction with Et_2O (5×2 liters) produced "Et_2O fraction II" (100.4 g). Both of these Et_2O fractions were subjected to acid and base extractions to separate further the acidic components from non-acidic materials. Non-acidic portions were combined to produce fraction D (51.7 g), following removal of excess solvent, while the acid-containing portions yielded a pooled fraction E (25.4 g).

CHROMATOGRAPHY OF PETROLEUM ETHER SOLUBLE FRACTION A.—Fraction A (35 g) was chromatographed over a column of silica gel 60[7] (1 kg) packed in petroleum ether. Elution was initiated with the same solvent and progressed through a solvent series of Et_2O-petroleum ether (3:97, 5:95, 10:90), $CHCl_3$-petroleum ether (10:3), and $CHCl_3$. A total of 82 fractions (100 ml each) were collected from the column and combined on the basis of the similarity of their tlc patterns.

ISOLATION OF (+)-LEDOL.—Fractions 26-28 (0.8 g), on crystallization from petroleum ether, yielded (+)-ledol (166 mg). The physical and spectral properties were in accord with those reported for (+)-ledol (20). Direct comparison with an authentic sample confirmed its identity.

ISOLATION OF (12*S*)-7,12-SECOISHWARAN-12-OL (**3**).—Combined column fractions 38-47 (1.5 g), on crystallization from hot MeOH, afforded a crystalline compound, which, on repeated recrystallization from acetonitrile, afforded (12*S*)-7,12-secoishwaran-12-ol (**3**, 65 mg). The physical and spectral properties were in agreement with those reported (20). Identity was confirmed by direct comparison with an authentic sample.

ISOLATION AND IDENTIFICATION OF PHYTOSTEROLS.—Fractions 55-64 (2.7 g) from the column were combined, concentrated *in vacuo*, and taken up in hot MeOH. Colorless crystals (1.9 g), mp 134-137°, resulted, which gave a positive Liebermann-Burchard reaction; their mass spectrum exhibited a base peak at *m/z* 414, together with ions at *m/z* 412 (11%) and 400 (11%). Gc over 3% OV-17 [on Gas Chrom Q (100-120 mesh)], indicated the presence of constituents with retention times corresponding to β-sitosterol (57.4%), stigmasterol (25.4%), and campesterol (17.2%).

ISOLATION OF SAVININ (**1**) FROM THE NON-ACIDIC FRACTION D.—Fraction D was dissolved in Et_2O and allowed to stand overnight. A pale yellow precipitate formed which was recrystallized from hot MeOH to yield savinin (**1**, 352 mg), mp 146-147°; $[\alpha]^{24}D$ −85.6° (c 0.8, $CHCl_3$); uv (MeOH) λmax 334 (log ε 4.48), 293 (4.35), 237 (4.45) and 215 (4.55) nm; ir (KBr) νmax 2920, 1740, 1640, and 1610 cm^{-1}; pmr (60 MHz, $CDCl_3$) δ2.77 (1H, dd, J=14.1, 9.6 Hz, H-α′$_a$), 2.86 (1H, dd, J=14.1, 4.6 Hz, H-α′$_b$), 3.54-4.05 (1H, m, H-β′), 4.23 (2H, d, J=4.2 Hz, H-γ′), 5.91 (2H, s, O-CH_2-O), 6.03 (2H, s, O-CH_2-O), 6.54-7.22 (4H, m, H-5, H-6, H-5′, H-6′), 6.73 (1H, d, J=1.3 Hz, H-2), 7.02 (1H, s, H-2′), and 7.47 (1H, d, J=1.4 Hz, H-α); cmr (25.04 MHz, $CDCl_3$) 37.6 (bd t, J=99 Hz, C-α′), 39.8 (bd d, J=99 Hz, C-β′), 69.5 (bd t, J=158 Hz, C-γ′), 101.0 (t, J=173 Hz, O-CH_2-O), 101.7 (t, J=175 Hz, O-CH_2-O), 108.5 (d, J=164 Hz, C-5), 108.7 (t d, J=6, 161 Hz, C-2), 108.8 (d, J=167 Hz, C-5′), 109.2 (q d, J=5, 161 Hz, C-2′), 122.1 (q d, J=6, 159 Hz, C-6′), 125.9 (bd s, C-1′), 126.0 (t d, J=6, 162 Hz, C-6), 128.2 (d, J=7 Hz, C-1), 131.5 (bd s, C-β), 137.2 (d, J=160 Hz, C-α), 146.6 (bd s, C-4 or C-4′), 147.9 (bd s, C-4′ or C-4), 148.4 (bd s, C-3 or C-3′), 149.2 (bd s, C-3′ or C-3), and 172.4 (bd s, C-γ); ms *m/z* (rel. int.) 352 (M^+, 12%), 217 (25), 189 (3), 159 (11), 135 (100), 131 (12), 77 (27), and 51 (24). These physical properties were in agreement with those of a sample of savinin (**1**) available in our own laboratory.

[7]E. Merck, Darmstadt, West Germany.

HYDROGENATION OF SAVININ (**1**).—Savinin (**1**, 20 mg) was dissolved in HOAc (5 ml) containing 10% palladium-charcoal (2 mg). The mixture was stirred under H_2 at room temperature for 2 h. After removal of the catalyst and excess solvent, the residue was chromatographed on silica gel 60 to yield (+)-isohinokinin (**12**, 11 mg) (50), mp 114-116°; $[\alpha]_D^{25}$ +104.7° (c 1.0, $CHCl_3$); pmr (60 MHz, $CDCl_3$), δ2.24-3.37 (6H, m, CH_2-α′, H-β, H-β′), 4.02 (2H, d, J=1.5 Hz, CH_2-γ′), 5.92 (2H, s, O-CH_2-O), 5.96 (2H, s, O-CH_2-O), 6.54-6.83 (4H, m, H-5, H-5′, H-6, H-6′), 6.76 (2H, s, H-2, H-2′); ms *m/z* (rel. int.) 354 (M^+, 20%), 218 (6), 192 (4), 162 (11, 135 (100), 131 (12), 77 (26), and 51 (14).

SEPARATION OF POST-SAVININ FRACTION D.—The post-savinin mother liquor of fraction D (48 g) was chromatographed on a preparative hplc column of silica gel 60 (1.6 kg). Elution was initiated with 5% EtOAc in $CHCl_3$, and progressed through $CHCl_3$-MeOH mixtures of increasing polarity. A total of 54 fractions (1 liter each) were collected and were combined based on the similarity of their tlc patterns.

ISOLATION OF METHYL ARISTOLOCHATE (**10**).—Column fraction 5 (2.9 g) afforded a yellow precipitate from $CHCl_3$. Crystallization from EtOH afforded methyl aristolochate (**10**, 24 mg), mp 172-175°; uv (EtOH) λmax 222 (log ε 4.56), 255 (4.30) and 315 (4.01) nm; ir (KBr) νmax 1725, 1600, 1530, and 1350 cm^{-1}; pmr (60 MHz, DMSO-d_6), δ3.81 (3H, s, $COOCH_3$), 4.05 (3H, s, OCH_3), 6.43 (2H, s, O-CH_2-O), 7.28 (1H, d, J=8.1 Hz, H-7), 7.68 (1H, t, J=7.7 Hz, H-6), 7.80 (1H, s, H-2), 8.36 (1H, s, H-9) and 8.66 (1H, d, J=8.1 Hz, H-5); ms *m/z* (rel. int.) 355 (M^+, 18%), 309 (100), 294 (62), 279 (50), 266 (15), 251 (20), 151 (12), 139 (14), 137 (15), and 75 (10). The identity of the compound was confirmed by direct comparison (uv, ir, ms, nmr, mmp) with a synthetic sample of the methyl ester of aristolochic acid-I.

ISOLATION OF ARISTOLACTAM (**8**).—Pooled fractions 15-16 (3.5 g) from the hplc yielded a yellow precipitate from $CHCl_3$. Rechromatography on a low-pressure column of silica gel 60 eluted with $CHCl_3$-MeOH (99:1), yielded a yellow compound which, after repeated recrystallization from EtOAc, afforded aristolactam (**8**, 46 mg), mp>300°; uv (EtOH) λmax 210 (log ε 4.38), 238 (4.47), 243 (4.47), 250 (4.41), 260 (4.55), 292 (4.15), and 298 (4.12) nm; ir (KBr) νmax 1690 and 1650 cm^{-1}; pmr (60 MHz, DMSO-d_6), δ4,01 (3H, s, OCH_3), 6.50 (2H, s, O-CH_2-O), 7.18 (1H, d, J=7.8 Hz, H-7), 7.38 (1H, s, H-9), 7.51 (1H, t, J=8.1 Hz, H-6), 7.63 (1H, s, H-2), 8.15 (1H, d, J=7.7 Hz, H-5), and 10.7 (1H, bs, NH); ms *m/z* (rel. int.) 293 (M^+, 100%), 278 (93), 250 (26), 166 (10), 164 (16), 147 (14), and 125 (16). Identity was confirmed by direct comparison with an authentic sample.

ISOLATION OF ARISTOLACTAM A-II (**9**).—Fractions 38-39 (8.6 g) from the hplc yielded a yellow precipitate from hot $CHCl_3$. Crystallization from CH_2Cl_2 afforded aristolactam A-II (**9**, 70 mg). The physical properties were in accord with those published for aristolactam A-II (**9**) (28).

DIRECT ISOLATION OF ARISTOLOCHIC ACID-I (**2**) FROM ACIDIC FRACTION E.—Fraction E (25.4 g), when dried *in vacuo* and taken up in $CHCl_3$, yielded a yellow precipitate which, on recrystallization (three times) from MeOH, afforded aristolochic acid-I (**2**, 2.2 g). The physical properties were in agreement with those reported for aristolochic acid-I (**2**) (21). The identity of the isolate was further confirmed by direct comparison with an authentic sample.

SEPARATION OF ACIDIC FRACTION E.—A second portion of fraction E (23 g) was chromatographed on a preparative hplc column of silica gel 60 and celite (1.6 kg:200 g). A total of 34 fractions (1 liter each) were collected as the solvent was progressively changed from $CHCl_3$ to $CHCl_3$-MeOH (8:2).

ISOLATION OF CEPHARADIONE A.—Fractions 21-25 (5.6 g) were taken up in hot MeOH, resulting in a yellow precipitate, which was further purified by chromatography on silica gel 60. Elution with $CHCl_3$-MeOH (95:5) afforded cepharadione-A (6 mg). The physical properties were in accord with those reported for cepharadione-A (51).

ISOLATION OF ARISTOLOCHIC ACID-I (**2**).—Pooled fractions 26-27 on crystallization from $CHCl_3$ afforded a yellow precipitate, which, on recrystallization from DMF-MeOH, gave aristolochic acid-I (**2**, 76 mg). Identification was established as described above.

ISOLATION OF ARISTOLOCHIC ACID-D (**11**).—Pooled fractions 29-30 (0.9 g) were taken to dryness *in vacuo* and redissolved in MeOH. A yellow precipitate formed, which was further purified by preparative tlc on silica gel. The major band was removed and crystallized from MeOH to yield aristolochic acid-D (**11**, 12 mg). The physical properties were in agreement with those reported for aristolochic acid-D (**11**) (21).

ISOLATION OF ARISTOLINDIQUINONE (**6**).—Combined hplc fractions 11-15 (4.8 g) were rechromatographed over a column of silica gel 60 using CH_2Cl_2-MeOH (99:1) as the eluent. Work-up of pooled fractions 8-14 (200 ml each) resulted in the formation of orange crystals. Recrystallization from

MeOH afforded aristolindiquinone (**6**, 42 mg); mp 176-178°: $[\alpha]^{25}D$ ±0° (c 0.9, EtOH); uv (EtOH) λmax 208 (log ε 4.62), 236 (4.59), 284 (4.52), and 433 (3.91) nm; uv (EtOH+KOH) λmax 220 (log ε 4.60), 238 (4.58), 270 (4.59), 394 (3.91), and 495 (3.89) nm; ir (KBr) νmax 3340, 1645, and 1615 cm^{-1}; pmr (60 MHz, $CDCl_3$), δ2.07 (3H, s, 3-CH_3), 2.64 (3H, bd s, 8-CH_3), 7.10 (1H, d, J=8.6 Hz, H-6), 7.40 (1H, d, J=8.9 Hz, H-7), 7.67 (1H, s, 2-OH), and 13.03 (1H, s, 5-OH); (100 MHz, DMSO-d_6), δ1.89 (3H, s, 3-CH_3), 2.54 (3H, bd s, 8-CH_3), 7.14 (1H, d, J=8.6 Hz, H-6), 7.42 (1H, d, H=8.6 Hz, H-7), and 13.02 (1H, s, 5-OH); cmr (25.04 MHz, $CDCl_3$) δ7.9 (3-CH_3), 22.3 (8-CH_3), 114.8 (C-4a), 118.5 (C-3), 125.9 (C-8a), 126.3 (C-6), 135.2 (C-8), 139.3 (C-7), 154.0 (C-2), 160.8 (C-5), 181.5 (C-1), and 191.2 (C-4); (67.83 MHz, DMSO-d_6) 7.4 (3-CH_3), 21.4 (8-CH_3), 114.0 (t, J=4.7 Hz, C-4a), 117.5 (q, J=6.6 Hz, C-3), 124.5 (bd dd, J=6.8 and 165 Hz, C-6), 126.3 (bd dq, J=3.7 and 6.8 Hz, C-8a), 133.2 (qt, J=6.8 Hz, C-8), 139.2 (bd dq, J=2.9 and 162 Hz, C-7), 156.3 (q, J=4.4 Hz, C-2), 159.2 (ddd, J=1.9, 4.4 and 9.7 Hz, C-5), 181.0 (s, C-1), and 190.9 (q, J=3.5 Hz, C-4); ms *m/z* (rel. int.) 218 (M^+, 100%), 190 (19), 175 (16), 162 (10), 161 (16), 147 (22), and 135 (9).

SEPARATION OF AQUEOUS FRACTION C.—A portion (240.5 g) of the lyophilized aqueous fraction C was taken up in H_2O (700 ml) and filtered. To the filtrate was added Mayer's reagent (1 liter) in aliquots. The resultant precipitate was collected by filtration, washed with H_2O, and dried in a vacuum desiccator to yield a powder (45.7 g). A portion of the powder (32.7 g) was dissolved in MeOH-Me_2CO (1:1) and stirred with IRA-410 ion exchange (Cl^-) resin (150 g) for 20 h. After removal of the resin by filtration, the filtrate was evaporated to dryness *in vacuo* to afford the quaternary alkaloid chloride fraction. The aqueous filtrate separated from the Mayer's precipitate was extracted with *n*-BuOH (6×600 ml). After drying over Na_2SO_4, the *n*-BuOH layer was brought to dryness *in vacuo* yielding a dark-brown syrup (105.7 g).

ISOLATION OF MAGNOFLORINE.—The quaternary alkaloid chloride fraction (19.5 g) was chromatographed over silica gel 60 (500 g) eluting with $CHCl_3$-MeOH (1:1). A total of 40 fractions (50 ml) was collected. Fractions 12-18 were pooled, dried *in vacuo*, and taken up in EtOH. To the EtOH solution, a hot, saturated aqueous solution of KI was added dropwise until solution became slightly cloudy. After refrigeration for 10 h, filtration, and washing, 42 mg of a crude crystalline alkaloid iodide was obtained. Recrystallization from EtOH afforded magnoflorine iodide (33 mg). The physical properties were in accord with published data for magnoflorine iodide (52). Its identify was further confirmed by direct comparison with an authentic sample available in our laboratory.

ISOLATION OF ARISTOLACTAM N-β-D-GLUCOSIDE (**7**).—A portion of the *n*-BuOH extract (30.4 g) was chromatographed over a reversed-phase C_{18}-bonded silica gel column (300 g) and eluted with MeOH-H_2O (1:1); 31 fractions (100 ml) were collected. Fractions 14-16 (0.6 g) were pooled and taken up in MeOH to afford aristolactam N-β-D-glucoside (**7**, 21 mg). The identity of the isolate was confirmed by comparison with an authentic sample of aristolactam N-β-D-glucoside (**7**). The spectral properties were in agreement with those reported previously (21).

PREPARATION OF ARISTOLIC ACID (**4**) AND METHYL ARISTOLATE (**5**).—The preparation of these compounds has been described previously, and their physical and spectral properties presented (49).

PURIFICATION OF *p*-COUMARIC ACIDS.—For confirmatory bioassays, samples of *cis*- and *trans*-*p*-coumaric acids were obtained commercially[8] and recrystallized from MeOH. The identity of these compounds and their purity were established through analysis of their physical and spectroscopic properties.

BIOASSAYS FOR POSTCOITAL ANTIFERTILITY ACTIVITY.—Animals were obtained from commercial sources[9] and housed in environmentally controlled quarters that provided 14 h of light and 10 h of darkness per 24-h period, a temperature of 22±2°, and a relative humidity of 45±5%. Food and H_2O were available *ad libitum*. The animals were allowed a period of acclimatization and observed appropriately for each species, prior to pairing. Males (proven breeder) and females were paired individually (males were placed into the females' cages in the case of the rats and mice, females into the males' cages in the case of the hamsters), and the first morning on which evidence of a positive mating was found was designated day 1 of pregnancy. Following mating, females were alternately assigned to the vehicle or one of the treatment groups, weighed daily, and dosed as indicated in Tables 1 and 2, by oral gavage with a stainless steel feeding needle. At autopsy (day 16 for rats and mice; day 14 for hamsters), the number of pregnant (defined as the presence of at least one implantation site) animals within each treatment and vehicle group was determined; for each pregnant animal, the number of implantation sites, normal fetuses, dead/degenerating fetuses, and corpora lutea of pregnancy were counted.

[8]Aldrich Chemical Co., Milwaukee, WI.

[9]Sprague-Dawley rats were obtained from either the Harlan Sprague-Dawley, Inc., Indianapolis, IN, or the King Animal Labs, Inc., Oregon, WI. Golden Syrian hamsters were obtained from the Engle Laboratory Animals, Inc., Farmersburg, IN.

The estrous cycles of the rats (eight-week-old Sprague-Dawley) were monitored by daily examination of their vaginal smears obtained by saline lavage. The first morning on which sperm were found in the smear was designated day 1 of pregnancy. The estrous cycles of the hamsters (eight-week-old Golden Syrian) were monitored by the method of Orsini (53) in which the external vaginal phenomena are examined daily; a vaginal smear, however, was obtained the morning after pairing to obtain evidence (sperm in the smear) of a positive mating (day 1 of pregnancy). Mice (six-week-old CD-1[10]) were first housed in groups to suppress their estrous cycles, to increase the likelihood of synchrony of mating after their individual pairing with males (54). Mice were examined for copulation plugs both in the afternoons and in the mornings; only those mice mating overnight were used in the experiments, the first morning on which a copulation plug was found in the vagina being designated as day 1 of pregnancy.

ACKNOWLEDGMENTS

This investigation was supported, in part, by the Special Programme of Research, Development and Research Training in Human Reproduction, World Health Organization (Project WHO 77918C).

The authors wish to thank Dr. S.C. Pakrashi, Indian Institute of Experimental Medicine, Calcutta, India, for supplying reference samples of (+)-ledol, (12*S*)-7,12-secoishwaran-12-ol, aristolochic acid-I, aristolochic acid-D, aristolactam, and aristoactam-N-β-D-glucosode. The authors are also indebted to Dr. C.A. Evans, JEOL (USA) Inc., Cranford, NJ, for providing the 67.83 MHz cmr spectrum of aristolindiquinone and Dr. N. Zaki, Amideast Peace Fellow, Department of Pharmacognosy and Pharmacology, UIMC, for purification of the coumaric acid derivatives.

One of us (C.-T. Che) wishes to thank the Graduate College, University of Illinois at the Medical Center, for the award of a fellowship.

LITERATURE CITED

1. K.R. Kirtikar and B.D. Basu, "Indian Medicinal Plants, Part II," Allahabad, India: S.N. Basu, 1918, p. 1088.
2. K.R. Kirtikar and B.D. Basu, "Indian Medicinal Plants," 2nd ed., Vol. III, Allahabad, India: L.M. Basu, 1935, p. 2123.
3. B. Mukerji, "The Indian Pharmaceutical Codex Vol. 1 - Indigenous Drugs," New Delhi, India: Council of Scientific and Industrial Research, 1953, p. 21.
4. J.C. Saha, E.C. Savini, and S. Kasinathan, *Indian J. Med. Res.*, **49**, 130 (1961).
5. "A Note on the Plants of Medicinal Value Found in Pakistan," Karachi, Pakistan: The Manager of Publications, Medicinal Plant Branch, Pakistan Forest Research Insitute, 1957, p. 7.
6. B.S. Malhi and V.P. Trivedi, *Quart. J. Crude Drug Res.*, **12**, 1922 (1972).
7. A. Pakrashi, B. Chakrabarty, and A. Dasgupta, *Experientia*, **32**, 394 (1976).
8. A. Pakrashi and P.L. Pakrasi, *Indian J. Exp. Biol.*, **15**, 428 (1977).
9. A. Pakrashi and B. Chakrabarty, *Indian J. Med. Res.*, **66**, 991 (1977).
10. A. Pakrashi and C. Shaha, *Experientia*, **33**, 1498 (1977).
11. A. Pakrashi and C. Shaha, *Indian J. Exp. Biol.*, **15**, 1197 (1977).
12. A. Pakrashi and B. Chakrabarty, *Indian J. Exp. Biol.*, **16**, 1283 (1978).
13. A. Pakrashi and B. Chakrabarty, *Experientia*, **34**, 1377 (1978).
14. A. Pakrashi and C. Shaha, *Experientia*, **34**, 1192 (1978).
15. A. Pakrashi and C. Shaha, *IRCS Med. Sci.*, **7**, 78 (1979).
16. A. Pakrashi and P. Pakrasi, *Indian J. Exp. Biol.*, **16**, 1285 (1978).
17. A. Pakrashi and P. Pakrasi, *Contraception*, **20**, 49 (1979).
18. A. Pakrashi, P.L. Pakrasi, and S.N. Kabir, *Experientia*, **35**, 843 (1979).
19. N.R. Farnsworth, A.S. Bingel, D.D. Soejarto, R.O.B. Wijisekera, and J. Perea-Sasiain, in: "Recent Advances in Fertility Regulation." Ed. by C.-C. Fen, D. Griffin, and A. Woolman, Geneva: Atar S.A., 1981, p. 330.
20. S.C. Pakrashi, P.P. Ghosh-Dastidar, S. Chakrabarty, and B. Achari, *J. Org. Chem.*, **45**, 4765 (1980).
21. S.M. Kupchan and J.J. Merianos, *J. Org. Chem.*, **33**, 3735 (1968).
22. E.A. Rúveda, H.A. Priestap, and V. Deulofeu, *An. Asoc. Quim. Argent.*, **54**, 237 (1966); *Chem. Abstr.* **69**, 61518w (1968).
23. H.R. Schütte, U. Orban, and K. Mothes, *Eur. J. Biochem.*, **1**, 70 (1967).
24. L.M. Carreras, *An. Inst. Bot. A.J. Cavanilles*, **30**, 253 (1973); *Chem. Abstr.* **80**, 24780g (1974).
25. F.T. Hussein, *Planta Med.*, **32**, 29 (1970).
26. M. Tomita and S. Kura, *Yakugaku Zasshi*, **77**, 812 (1957).
27. M. Tomita and S. Sasagawa, *Yakugaku Zasshi*, **79**, 1470 (1959).

[10]CD-1 mice were obtained from the Charles River Laboratories, Inc., Wilmington, MA.

28. R. Crohare, H.A. Priestap, M. Farina, M. Cedola, and E.A. Rúveda, *Phytochemistry*, **13**, 1957 (1974).
29. M. Tomita and K. Fukagawa, *Yakugaku Zasshi*, **82**, 1673 (1962).
30. M. Pailer and G. Pruckmayr, *Monatsh. Chem.*, **90**, 145 (1959).
31. B. Achari, S. Chakrabarty, S. Bandyopadhyay, and S. C. Pakrashi, *Heterocycles*, **19**, 1203 (1982).
32. S.C. Pakrashi, P. Ghosh-Dastidar, S. Basu, and B. Achari, *Phytochemistry*, **16**, 1103 (1977).
33. J. Reisch, I. Novák, K. Szendrei, and E. Minker, *Pharmazie*, **22**, 220 (1967).
34. J.E.T. Corrie, G.H. Green, E. Ritchie, and W. C. Taylor, *Aust. J. Chem.*, **23**, 133 (1970).
35. M.M. Badawi, A.A. Seida, A.D. Kinghorn, G.A. Cordell, and N.R. Farnsworth, *J. Nat. Prod.*, **44**, 331 (1981).
36. E. Agullo Martinez, J.L. Breton Funes, A. Gonzalez Gonzalez, and F. Rodriguez Luis, *An. Quim.*, **65**, 809 (1969).
37. M. Masumura, *Nippon Kagaku Zasshi*, **76**, 423 (1955).
38. J.B. Braedenberg and J. Runeberg, *Acta Chem. Scand.*, **15**, 455 (1961).
39. N. Narasimhachari and E. von Rudloff, *Can. J. Chem.*, **39**, 2572 (1961).
40. J.L. Hartwell, J.M. Johnson, D.B. Fitzgerald, and M. Belkin, *J. Am. Chem. Soc.*, **75**, 235 (1953).
41. Y.T. Lin., K.T. Wang, and B. Weinstein, *Chem. Commun.*, 592 (1965).
42. E. Wenkert, H.E. Gottlieb, O.R. Gottlieb, M.O. Pereira, and M.D. Formiga, *Phytochemistry*, **15**, 1547 (1976).
43. G.A. Mikaya, D.G. Turabelidze, E.P. Kemertelidze, and N.S. Wulfson, *Planta Med.*, **43**, 378 (1981).
44. R.T. Coutts, J.B. Stenlake, and W.D. Williams, *J. Chem. Soc.*, **1957**, 4120 (1957).
45. R.T. Coutts, J.B. Stenlake, and W.D. Williams, *J. Pharm. Pharmacol.*, **11**, 607 (1959).
46. S.M. Kupchan and R.W. Doskotch, *J. Med. Pharm. Chem.*, **5**, 657 (1962).
47. C.-T. Che, G.A. Cordell, H.H.S. Fong, and C.A. Evans, *Tetrahedron Lett.*, **24**, 1333 (1983).
48. P.J. Cox, C.-T. Che, G.A. Cordell, and H.H.S. Fong, unpublished results.
49. S. Mukhopadhyay, S. Funayama, G. A. Cordell, and H.H.S. Fong, *J. Nat. Prod.*, **46**, 507, (1983).
50. A.W. Schrecker and J.L. Hartwell, *J. Am. Chem. Soc.*, **76**, 4896 (1954).
51. M. Akasu, H. Itokawa, and M. Fujita, *Tetrahedron Lett.*, **1974**, 3609 (1974).
52. S. R. Hemingway, J.D. Phillipson, and R. Verpoorte, *J. Nat. Prod.*, **44**, 67 (1981).
53. M.W. Orsini, *Proc. Animal Care Panel*, **11**, 193 (1961).
54. W.K. Whitten, *Adv. Reprod. Physiol.* **1**, 155 (1966).
55. A.P. Simonelli, S.C. Mehta, and W.I. Higuchi, *J. Pharm. Sci.*, **58**, 538 (1969).

Received 22 April 1983