The Isolation and Structural Elucidation of Novel Derivatives of Aristolochic Acid from *Aristolochia indica*[1]

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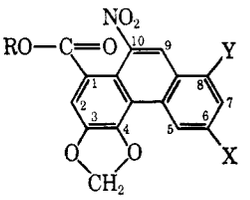
The isolation and structural elucidation are reported of three new companion aristolochic acid derivatives, aristolochic acid-D (**4**), aristolochic acid-D methyl ether lactam (**6**), and aristololactam β-D-glucoside (**8**). Aristolochic acid-D was assigned the molecular formula $C_{17}H_{11}NO_8$ on the basis of elemental analysis and nmr spectroscopy. Methylation with diazomethane yielded the dimethyl derivative **5**, and hydrogenation of **5** yielded aristolochic acid-D methyl ether lactam (**6**), identical with the material of natural origin. The structure of **5** was initially deduced on the basis of spectral evidence, and confirmed by direct comparison with a sample prepared by total synthesis. Spectral arguments are presented which favor structure **4** for aristolochic acid-D. Aristololactam β-D-glucoside (**8**) was characterized by elemental and nmr, ir, and uv spectral analysis. Acetylation gave the tetraacetate, **9**. Lithium aluminum hydride reduction of **8**, followed by mild acid hydrolysis, gave α-D-glucose and the aglycone **11**, characterized by comparison with the product obtained by lithium aluminum hydride reduction of aristololactam.

In the course of a continuing search for tumor inhibitors from plant sources, an extract of *Aristolochia indica* L. was found to show reproducible tumor inhibitory activity against the adenocarcinoma 755 test system.[2] Aristolochic acid (**1**) was characterized as the principal tumor-inhibitory principle.[2] We report herein the isolation and structural elucidation of three new companion aristolochic acid derivatives, aristolochic acid-D (**4**), aristolochic acid-D methyl ether lactam (**6**), and aristololactam β-D-glucoside (**8**). In addition, we report the isolation of aristololactam (**7**), previously isolated from other species of *Aristolochia*.[3,4]

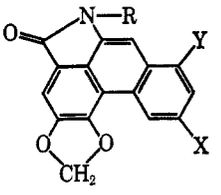
The total crude acids were isolated from *A. indica* roots by the procedure described earlier.[2a] Fractionation of the acid fraction was effected by careful chromatography on silicic acid–Celite 545. The principal product was aristolochic acid (**1**), which was eluted with chloroform. Elution with increasingly polar mixtures of chloroform–methanol yielded aristolochic acid-D (**4**), aristololactam (**7**), aristololactam β-D-glucoside (**8**), and aristolochic acid-D methyl ether lactam (**6**).

Aristolochic acid-D (**4**), separated from methanol as deep wine-red crystals, mp 269–272°, and the molecular formula $C_{17}H_{11}NO_8$ was assigned on the basis of elemental analysis. The nmr spectrum supported the empirical formula and indicated the presence of methoxyl, methylenedioxy, aromatic, and two D_2O-exchangeable protons (see Table I). The ir spectrum showed bands indicative of the presence of phenolic hydroxyl, carboxyl, and nitro functions, and the uv spectrum indicated the probable presence of a phenanthrene chromophore.

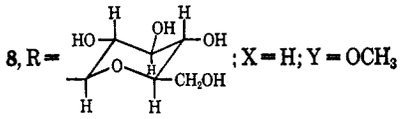
Methylation of aristolochic acid-D with excess diazomethane in ether yielded the dimethyl derivative **5**, $C_{19}H_{15}NO_8$, mp 238–240°. The ir spectrum indicated the presence of an ester carbonyl function in place of the carboxyl carbonyl of the precursor. The nmr spectrum indicated the presence of two additional methoxy groups and the absence of exchangeable protons. Hydrogenation of **5** with 10% palladium–charcoal yielded the lactam **6**, $C_{18}H_{13}NO_5$, mp 350–355°. The ir spectrum indicated the presence of a lactam carbonyl function in place of the ester carbonyl of the precursor, and the nmr spectrum indicated the presence of two methoxy groups (one less than in the precursor) and one exchangeable proton.



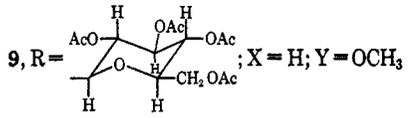
1, R = H; X = H; Y = OCH_3
2, R = CH_3; X = H; Y = OCH_3
3, R = CH_3; X = OCH_3; Y = H
4, R = H; X = OH; Y = OCH_3
5, R = CH_3; X = OCH_3; Y = OCH_3



6, R = H; X = OCH_3; Y = OCH_3
7, R = H; X = H; Y = OCH_3



8, R = ; X = H; Y = OCH_3



9, R = ; X = H; Y = OCH_3

The structure of **5** was deduced largely on the basis of spectral evidence. The similarity of the uv, ir, and nmr spectra to those of aristolochic acid (**1**) strongly supported assignment of a 3,4-methylenedioxydimethoxy-10-nitrophenanthroic acid methyl ester structure. Furthermore, a detailed analysis of the nmr data in Table I supported assignment of the two methoxy groups to C-6 and C-8. The nmr spectra of **1**

(1) (a) Tumor Inhibitors. XXXII. Part XXXI: S. M. Kupchan, R. J. Hemingway, D. Werner, A. Karim, A. T. McPhail and G. A. Sim, *J. Amer. Chem. Soc.*, **90**, 3596 (1968). (b) Supported by grants from the National Cancer Institute (CA-04500) and the American Cancer Society (T-275). (c) Abstracted in part from the dissertation submitted by J. J. Merianos to the University of Wisconsin Graduate School, Aug 1966, in partial fulfillment of the requirements of the Ph.D. Degree.

(2) (a) S. M. Kupchan and R. W. Doskotch, *J. Med. Pharm. Chem.*, **5**, 657 (1962). (b) *Cf.* L. N. Filitis and P. S. Massagetov, *Vopr. Onkol.*, **7**, 97 (1961); *Chem. Abstr.*, **56**, 4060 (1962); Charles Pfizer & Co., Inc., British Patent 895,037 (1962).

(3) M. Tomita and S. Sasagawa, *J. Pharm. Soc. Jap.*, **79**, 973 (1959).

(4) S. Sasagawa, *ibid.*, **82**, 921 (1962).

TABLE I

NUCLEAR MAGNETIC RESONANCE DATA[a]

	OCH_3	$-O>CH_2$	C-2 H	C-5 H	C-6 H	C-7 H	C-9 H	O-H or N-H
1	5.96(s)	3.54(s)	2.20(s)	1.52(d, J = 8 cps)	2.30(t, J = 8 cps)	2.78(d, J = 8 cps)	1.48(s)	−0.63(s)
2	5.93(s) 6.20(s)	3.49(s)	2.15(s)	1.36(d, J = 8 cps)	2.15(t, J = 8 cps)	2.62(d, J = 8 cps)	1.33(s)	
3[b]	6.05(s) 6.24(s)	3.47(s)	2.20(s)	1.42(s)		2.48(2d, J = 9, 2 cps)	1.42(s)	
4	5.92(s)	3.45(s)	2.15(s)	1.89(d, J = 2 cps)		3.12(d, J = 2 cps)	1.45(s)	−0.66(br. s, 1 H) 1.50(br. s, 1 H)
5	6.00(s) 6.10(s) 6.24(s)	3.58(s)	2.31(s)	2.13(d, J = 2 cps)		3.16(d, J = 2 cps)	1.60(s)	
6	6.02(s) 6.09(s)	3.60(s)	2.48(s)	2.40(d, J = 2 cps)		3.23(d, J = 2 cps)	2.73(s)	−0.68(s)
7	6.00(s)	3.57(s)	2.47(s)	2.01(d, J = 8 cps)	2.58(t, J = 8 cps)	2.89(d, J = 8 cps)	2.73(s)	−0.67(s)
8	5.93(s)	3.48(s)	2.20(s)	1.77(d, J = 8 cps)	2.38(t, J = 8 cps)	2.70(d, J = 8 cps)	2.20(s)	
9	5.96(s)	3.72(s)	2.18(s)	1.83(d, J = 8 cps)	2.56(t, J = 8 cps)	2.98(d, J = 8 cps)	2.46(s)	

[a] All chemical shifts are reported in τ values (parts per million). [b] C-8 H, 1.97 (d, J = 9 cps).

and the methyl ester **2** were very similar except for a new signal at τ 6.20 for the ester methoxy protons in place of the acidic proton signal of **1**. The signals for the protons at C-5, C-6, and C-7 appeared as an AMX pattern. The nmr spectrum of aristolochic acid-C methyl ester methyl ether (**3**)[5] showed a low field singlet (τ 1.42, 2 H) attributable to the protons at C-5 and C-9.[6] The signal at 1.97 (1 H, d, $J_{7,8}$ = 9 cps) was assigned to the C-8H, and that at 2.48 (1 H, 2d, $J_{7,8}$ = 9, $J_{5,7}$ = 2) to the C-7H. The nmr spectrum of aristolochic acid-D methyl ester methyl ether (**5**) showed the presence of two methoxyl groups as well as the ester methoxyl function. Two doublets at 2.13 (1 H, $J_{5,7}$ = 2) and 3.16 (1 H, $J_{5,7}$ = 2) were assigned to the C-5 and C-7 protons in **5**.

The structure of aristolochic acid-D methyl ester methyl ether (**5**) was confirmed by direct comparison with a sample prepared by total synthesis. Pailer and coworkers[7] recently reported the total synthesis of **5** ("aristolochic acid-IV-methyl ester") by photocyclization of the appropriate 2-iodostilbene. The melting point of aristolochic acid-D methyl ester methyl ether was not depressed by admixture with the synthetic sample, and the ir spectra were essentially superimposable.

The structural problem which remained at this point was the choice between C-6 and C-8 of aristolochic acid-D as the location of the methoxy group. The data in Table I indicate that C-8 methoxy protons resonate at *ca.* τ 0.1 unit lower than C-6 methoxy protons (*cf.* **2** *vs.* **3**). The location of the methoxy proton signal at τ 5.92 in the nmr spectrum of aristolochic acid-D favors assignment of the 3,4-methylenedioxy-6-hydroxy-8-methoxy-10-nitro-1-phenanthroic acid structure (**4**).

Aristololactam β-D-glucoside (**8**) separated from absolute ethanol as light yellow crystals, mp 330–333°, and the empirical formula $C_{23}H_{21}NO_9$ was assigned on the basis of elemental analysis. The uv spectrum resembled that of aristololactam (**7**),[3,4] but the intensities of the peaks were consistently lower than those in the uv spectrum of **7**. The latter observation suggested that **8** contained an aristolochic acid lactam system in addition to a nonabsorbing moiety. This view was supported by the results upon attempted sublimation, whereupon extensive decomposition to a charred black residue resulted, and a small yield of aristololactam was obtained as sublimate. The ir spectrum of **8** showed broad peaks at 2.82, 2.95 (hydroxyl), and 5.92 μ (carbonyl). The peak at 3.10 in the ir spectrum of **7**, attributable to N–H stretching, was absent. Hence, the nonabsorbing moiety of **8** could be considered to be attached at the lactam nitrogen. This view was supported by the nmr spectrum of **8**, which showed no signal for an N–H proton. In addition to the signals attributable to the aristololactam moiety (see Table I), signals for eleven additional protons appeared [τ 2.20 (1 H, s), 4.40–4.90 (5 H, m), 5.32 (2 H, m), and 5.93 (3 H, br)]. The addition of D_2O to the solution of **8** in hexadeuterated dimethylsulfoxide resulted in exchange of four protons and made apparent a signal at τ 4.43 (1 H, d, J = 9 cps, anomeric sugar proton). Earlier workers[8–10] have reported that the nmr spectra of sugars in d_6-DMSO exhibit a strong hydrogen-bonding effect and shifted hydroxyl resonance downfield. A similar effect was observed in the nmr spectrum of **8** and supported the view that a sugar moiety was attached at nitrogen, as an N-glycoside.

Acetylation of **8** with acetic anhydride–pyridine gave the tetraacetate **9**, which was crystallized from methanol–chloroform as light yellow crystals, mp 287–288°. The nmr spectrum of **9** in $CDCl_3$ showed four singlets assignable to acetates (τ 7.90, 7.92, 7.98, and 8.22, 3 H each). The 60-Mc spectrum of **9** showed a complex multiplet at τ 4.02–4.74 (4H). At 100 Mc the multiplet was resolved into a doublet (J = 10 cps) assignable to the anomeric proton, and three triplets (J = 10 cps) assignable to the C-2′, -3′, and -4′ protons. The large first-order coupling constants observed for the C-2′, -3′, and -4′ protons were indicative of the diaxial relationships in the molecule. In agreement with this view, all four acetoxy methyl signals were grouped in

(5) S. M. Kupchan, H. C. Wormser, and M. Sesso, *J. Org. Chem.*, **30**, 3935 (1965).

(6) *Cf.* M. Pailer and P. Bergthaller, *Monatsh. Chem.*, **97**, 484 (1966).

(7) M. Pailer, H. Berner, and S. Makleit, *ibid.*, **98**, 102 (1967). We thank Professor Pailer cordially for a sample of "aristolochic acid-IV-methyl ester."

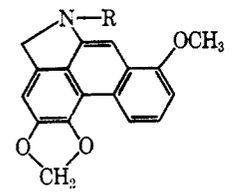
(8) A. S. Perlin, *Can. J. Chem.*, **44**, 539 (1966).

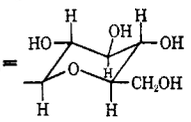
(9) O. L. Chapman and R. W. King, *J. Amer. Chem. Soc.*, **86**, 1256 (1964).

(10) J. G. Trayham and G. A. Kuesel, *ibid.*, **87**, 4220 (1965).

the region characteristic of primary and equatorial secondary acetoxy groups.[11] A broadened singlet at τ 5.96 (4 H) was assigned to the C-8 methoxy group and the C-5′ proton. A doublet at τ 5.79 (2 H, J = 3 cps) was assigned to the C-6′ protons. The nmr signals attributable to the sugar moiety of the glycoside tetraacetate were suggestive of the presence in the glycoside of glucose, in a glucopyranosyl form.

Aristololactam β-D-glucoside was exceedingly resistant to mineral acid hydrolysis, and this property paralleled the behavior of the N-glycosides of the pyrimidine nucleoside group.[12] A route to the isolation of the unaltered sugar component was suggested by the observation of Levene and LaForge,[13] who reported that catalytic hydrogenation of the pyrimidine ring of nucleosides renders the linkage between the sugar residue and the nitrogenous aglycone susceptible to subsequent mild acid hydrolysis. Accordingly, reduction of **8** with lithium aluminum hydride in tetrahydrofuran yielded **10**, which was readily hydrolyzed to yield glucose and **11**. Characterization of the



10, R = 

11, R = H

glucose was effected by parallel paper chromatography with authentic hexose samples in four chromatographic systems. The aglycone moiety **11** was characterized by direct comparison with the product obtained by lithium aluminum hydride reduction of aristololactam.

The specific rotation of aristololactam β-D-glucoside (**8**) in aqueous solution was −14°, compared with the specific rotation of −9°[14] for 9-β-D-glucopyranosyladenine. Furthermore, the rotation of the tetraacetate **9** was similar in sign and magnitude to those reported for several 9-(tetra-*O*-acetyl-β-D-glucopyranosyl)-purine derivatives.[15] Hence, the D configuration is indicated for the glucose moiety in **8**. The large coupling constant (J = 9 cps) of the C-1′ anomeric proton doublet at τ 4.43 in the nmr spectrum of **8** establishes the *trans* diaxial relationship between the C-1′ and C-2′ protons.[16-19] Hence, the anomeric linkage in **8** is β, for the only glucopyranoside conformation and configuration with *trans* diaxial C-1′ and C-2′ protons in the β anomer in the stable *CI* conformation.[16-19] The combined evidence supports assignment of the aristololactam β-D-glucoside structure (**8**) for the glycoside.

(11) L. D. Hall, *Advan. Carbohyd. Chem.*, **19**, 51 (1954).
(12) *Cf.* J. J. Fox and I. Wempen, *ibid.*, **14**, 283 (1959).
(13) P. A. Levene and F. B. LaForge, *Ber.*, **45**, 608 (1912).
(14) K. Onodera, S. Hirano, N. Kashimura, F. Masuda, T. Yajima, and N. Miyazaki, *J. Org. Chem.*, **31**, 1291 (1966).
(15) G. M. Blackburn and A. W. Johnson, *J. Chem. Soc.*, 4347 (1960).
(16) R. U. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Amer. Chem. Soc.*, **80**, 6098 (1958).
(17) P. W. K. Woo, H. W. Dion, and L. F. Johnson, *ibid.*, **84**, 1066 (1962).
(18) H. Iwamura and T. Hashizume, *J. Org. Chem.*, **33**, 1796 (1968).
(19) R. U. Lemieux and J. W. Lown, *Can. J. Chem.*, **41**, 889 (1963).

Experimental Section

Melting points were determined on a Fisher-Johns melting point stage which had been calibrated with standard samples and a Thomas-Hoover melting point apparatus. Ultraviolet absorption spectra were determined in 95% ethanol on Beckman (Model DK2A) and Cary (Model 11-MS) recording spectrophotometers. Infrared absorption spectra were recorded in chloroform (unless otherwise specified) on a Beckman (Model 5A) recording spectrophotometer. Microanalyses were carried out by J. F. Alicino, Metuchen, N. J., and Spang Microanalytical Laboratory, Ann Arbor, Mich. Nmr spectra were determined on Varian spectrometers (A-60 and A-60A for 60 Mc, and HA-100 for 100 Mc) in hexadeuterated dimethyl sulfoxide (unless otherwise specified), with tetramethylsilane as the internal standard. Skellysolve B refers to petroleum ether, fraction boiling at 60–68°. Thin layer chromatography (tlc) was carried out with silica gel G and silica gel $HF_{254+366}$ (Brinkmann Instruments). Silicic acid (Mallinckrodt) (analytical reagent) and Celite 545 (Johns Manville) were used. Paper chromatography was conducted by the descending technique on Whatman No. 1 paper.

Isolation of Total Acids from *Aristolochia indica* L.—Dried ground roots of *Aristolochia indica* L. (1 kg) were extracted in a Soxhlet extractor with 95% ethanol. The ethanolic extracts were concentrated to a thick black-brown syrup under reduced pressure and at a temperature no higher than 45°, to yield about 110 g of residue. The residue was treated with chloroform (0.5 l.) and 2% sodium bicarbonate solution (3 l.) and the suspension was subjected to vigorous stirring for 24 hr. The wine-red alkaline solution was decanted and filtered. The extraction with 2% sodium bicarbonate was repeated several times, until no further precipitation resulted upon acidification of the bicarbonate extract. The combined filtered alkaline solution was acidified with 5% hydrochloric acid to yield a yellow-brown precipitate, which was filtered and dried to yield 10–12 g of total acid fraction.

Isolation and Purification of Components of the Total Acid Fraction.—The crude total acid fraction (1.5 g) was extracted with chloroform (2 l.) in a Soxhlet apparatus for 10 hr (residue, 0.41 g). The chloroform solution was added to a column (4.8 × 49 cm) of silicic acid–Celite 545 (4:1, 500 g). In 4–5 hr, passage of 2 l. of chloroform through the column resulted in resolution of four major colored segments: (A) highly fluorescent reddish yellow, (B) yellow-green nonfluorescent (the largest), (C) reddish brown mixture of bands, and (D) brown-black fluorescent mixture of bands. Chloroform (5 l.) was used to elute band A (15 mg), a noncrystalline unidentified substance, and band B (875 mg), identified below as aristolochic acid (**1**). The polarity of the eluting solvent was gradually increased to 1, 2, and 5% methanol in chloroform (2 l. each). The two bands, C and D, were moved slowly and separated into several bands. One deep wine-red band yielded crystalline product from methanol (compound **4**, 35 mg), mp 269–271°. Elution with 5–10% methanol in chloroform (2 l. each) yielded two highly fluorescent compounds, aristololactam (**7**, 30 mg) and aristololactam β-D-glucoside (**8**, 25 mg), in addition to some noncrystalline solid (20 mg). With further increase of the polarity to 15 and 20% methanol in chloroform (3 l. each), a greenish highly fluorescent band (compound **6**, 2 mg) was collected among the other minor bands. Finally, the column was washed with methanol and yielded tarry black residue (250 mg).

The purification of aristolochic acid (**1**) was effected by crystallization of the crude acid from a mixture of dimethylformamide and ethanol; yellow-orange crystals, mp 275–278°, resulted.

Compound **4** was crystallized from methanol to yield bright wine red crystals: mp 269–271°; λ_{max}^{Nujol} 2.95, 5.92, 6.58, 7.40, 7.98, 8.50, 9.55, 9.90, 10.70, 10.85 μ; λ_{max}^{EtOH} 325 mμ (ε 11,300), 292 (13,850), 252 (37,800), 242 (37,790), 220 (29,800).

Anal. Calcd for $C_{17}H_{11}NO_8$: C, 57.15; H, 3.10; N, 3.92. Found: C, 57.14; H, 3.24; N, 3.87.

Aristololactam (**7**) was purified by sublimation at 300° to yield yellow crystals, mp 315–317°.

Purification of aristololactam β-D-glucoside (**8**) was accomplished by dissolving (50 mg) in absolute ethanol (0.5 l.), evaporating on a hot plate to 50 ml, and allowing the solution to stand at room temperature, which afforded yellow threadlike crystals, mp 330–333° (inserted at 300°). The melting point was very much dependent upon the rate of heating. Crystallization of compound **8** from methanol and water afforded light yellow needles, mp 331–333° (inserted at 320°). Tlc with 5% methano

in chloroform of compound **8** showed R_f 0.42, whereas aristololactam (**7**) showed R_f 0.76. The melting point of **8** was considerably depressed upon admixture with **7**. Compound **8** showed $[\alpha]^{25}D$ −14° (*c* 0.02, H_2O); $[\alpha]^{25}D$ −18° (*c* 0.11, dimethylformamide); λ_{max}^{Nujol} 2.82, 2.95 (hydroxyl), 5.92 μ (carbonyl); λ_{max}^{EtOH} 328 mμ (ε 9521), 298 (15,197), 290 (15,278), 258 (36,482), 249 (31,435), 243 (33,210), 238 (30,892).

Anal. Calcd for $C_{23}H_{21}NO_9$: C, 60.66; H, 4.65; N, 3.08; CH_3O, 6.82. Found: C, 61.25; H, 4.89; N, 3.28; CH_3O, 7.03.

3,4-Methylenedioxy-6,8-dimethoxy-10-nitrophenanthroic Acid Methyl Ester (5).—To a magnetically stirred suspension of 3,4-methylenedioxy-6-hydroxy-8-methoxy-10-nitro-1-phenanthroic acid (**4**, 50 mg, 0.00014 mol, mp 269–271°) in anhydrous ether (100 ml) was added excess diazomethane in ether (250 ml). The reaction mixture was stirred at 0° for 2 hr and then at room temperature for 24 hr. The ether was evaporated and replaced with chloroform (200 ml), and the solution was extracted with 5% sodium hydroxide. The chloroform solution was dried over sodium sulfate and evaporated under reduced pressure to yield **4** (45 mg, 88%), mp 238–240°. Recrystallization from ethyl acetate gave orange rods: mp 241–243°; λ_{max}^{Nujol} 5.82, 6.58, 7.44 μ; λ_{max}^{EtOH} 326 mμ (ε 11,250), 255 (36,900), 242 (37,200), 220 (32,000).

Anal. Calcd for $C_{19}H_{15}O_8N$: C, 59.22; H, 3.92; N, 3.64. Found: C, 59.43; H, 3.92; N, 3.78.

Sublimation at 200° and 1.7 mm afforded rods, mp 240–241°, shown to be identical with an authentic sample of "aristolochic acid-IV-methyl ester"[7] by mixture melting point, mixed tlc, and ir spectral comparison.

3,4-Methylenedioxy-6,8-dimethoxy-10-amino-1-phenanthroic Acid Lactam (6).—Atmospheric hydrogenation of 3,4-methylenedioxy-6,8-dimethoxy-10-nitro-1-phenanthroic acid methyl ester (**5**, 50 mg, 0.00013 mol) with 10% palladium on charcoal (50 mg) in ethyl acetate (100 ml) resulted in consumption of the required volume of hydrogen in 30 min. The reaction mixture was filtered, and the catalyst was washed with ethyl acetate (250 ml). Evaporation of the solvent under reduced pressure afforded 3,4-methylenedioxy-6,8-dimethoxy-10-aminophenanthroic acid lactam (37 mg, 88%), mp 350–355° (inserted at 330°). Sublimation at 300° under reduced pressure yielded yellow crystals: mp 353–355° (inserted at 340°); λ_{max}^{Nujol} 3.16 (N–H stretching), 5.94 (carbonyl), 7.00, 7.50, 8.56, 8.85, 9.50, 9.90, 10.55, 10.69 μ (methoxyl and methylenedioxy); λ_{max}^{EtOH} 334 mμ (ε 7050), 330 (11,200), 290 (11,800), 2.62 (23,300), 240 (27,950).

Anal. Calcd for $C_{18}H_{13}NO_5$: C, 66.87; H, 4.05; N, 4.35. Found: C, 66.59; H, 3.92; N, 4.02.

Acetylation of Aristololactam β-D-Glucoside (8 → 9).—A mixture of acetic anhydride (3 ml), dried pyridine (3 ml), and aristololactam β-D-glucoside (**8**, 50 mg, 0.00011 mol, mp 331–333°) was stirred at room temperature for 22 hr. Excess acetic anhydride was decomposed with methanol (5 ml), and the solvents were removed under reduced pressure with the aid of toluene (20 ml) (to distil an azeotrope with pyridine and water) and at a temperature not exceeding 50°. The crude yellow residue (**9**, 68 mg, mp 284–286°) was crystallized from methanol with a few drops of chloroform, to yield light yellow crystals (51 mg): mp 287–289°; $[\alpha]^{25}D$ −97° (*c* 0.12, chloroform); λ_{max}^{Nujol} 5.68 (acetate carbonyl), 5.85 (lactam carbonyl), 8.10 (acetate), 9.60 and 9.80 (methoxy), 10.70, 10.90, and 13.80 μ (methylenedioxy group); λ_{max}^{EtOH} 330 mμ (ε 9556), 300 (14,568), 290 (14,812), 258 (36,200), 249 (30,987), 243 (37,550), 239 (37,987).

Anal. Calcd for $C_{31}H_{29}NO_{13}$: C, 59.61; H, 4.58; N, 2.46. Found: C, 58.82; H, 4.87; N, 2.62.

Reduction and Hydrolysis of Aristololactam β-D-Glucoside (8).—To a stirred suspension of lithium aluminum hydride (20 mg) in tetrahydrofuran (10 ml), a solution of aristololactam β-D-glucoside (**8**, 10 mg, mp 330–333°) in tetrahydrofuran (10 ml) was added slowly. The reaction mixture was stirred for 6 hr at room temperature and was monitored by tlc for the disappearance of starting material. The excess lithium aluminum hydride was decomposed with wet ethyl acetate (30 ml). The precipitate was filtered and washed several times with ethyl acetate (50 ml). The filtrate and washings were combined, and the solvent was removed under reduced pressure to yield a yellowish brown residue (8 mg). The residue was dissolved in methanol (2 ml) and refluxed on a steam bath with 0.5 *N* hydrochloric acid (3 ml) for 6 hr. The methanol was evaporated under reduced pressure and the aqueous concentrate was extracted repeatedly with chloroform (total volume, 100 ml). Evaporation of the chloroform solution yielded a brown oil which showed an ir spectrum essentially identical with that of product of lithium aluminum hydride reduction of aristololactam (**7**). Characterization of the hexose residue as glucose was effected by paper chromatography of the aqueous layer on Whatman No. 1 filter paper, in parallel with authentic samples of D-galactose, D-mannose, and D-glucose.[20] Four different solvent systems were used: ethyl acetate–acetic acid–water (3:3:1); 1-butanol–ethanol–water (40:11:19); 1-butanol–water–acetic acid (4:5:1); and ethyl acetate–pyridine–water (8:2:1). Spray reagents for the chromatographic studies were 5% silver nitrate and 5% *p*-anisidine hydrochloride in 1-butanol–ethanol–water (4:1:1).

Reduction of Aristololactam (7).—To a stirred suspension of lithium aluminum hydride (50 mg) in tetrahydrofuran (50 ml) in a Soxhlet extractor, aristololactam (**7**, 58 mg, 0.0002 mol, mp 315–317°) was introduced by extraction from the thimble by the tetrahydrofuran. The reaction mixture was heated under reflux for 12 additional hr, and the excess lithium aluminum hydride was decomposed with wet ethyl acetate (100 ml). The filtrate and washings were combined and evaporated to dryness under reduced pressure. The ir spectrum (Nujol) of the brown oily residue showed the absence of 5.92 μ (lactam carbonyl) absorption and was essentially identical with that of the product obtained by reduction and hydrolysis of **9**.

Registry No.—**1**, 313-67-7; **2**, 1169-60-4; **3**, 4849-91-6; **4**, 17413-38-6; **5**, 17448-02-1; **6**, 17413-39-7; **7**, 13395-02-3; **8**, 17413-41-1; **9**, 17413-42-2.

(20) L. Hough and J. K. N. Jones, *Methods Carbohyd. Chem.*, **I**, 21 (1962).