STUDIES ON *ARISTOLOCHIA*, I. CONVERSION OF ARISTOLOCHIC ACID-I TO ARISTOLIC ACID-ONE-STEP REMOVAL OF A NITRO GROUP

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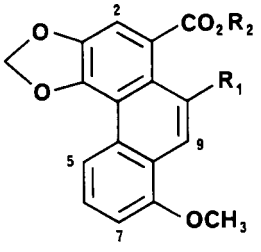
ABSTRACT.—A high-yield, one-step procedure for the reduction of aristolochic acid (**3**) to aristolic acid (**1**) is described.

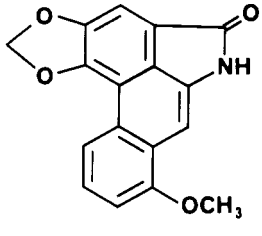
As part of our continuing studies of plants and their constituents possessing fertility-regulating activity (1), we became interested in the plant *Aristolochia indica* L. (2). It has been reported that aristolic acid (**1**), a compound isolated from *A. indica*, produces an 80% fetal loss in rabbits at a dose of 90 mg/kg (3) and that at 60 mg/kg in mice, in the early stages of gestation, prevents implantation (4). The methyl ester of aristolic acid, **2**, has also been reported to be active in mice as an interceptive (5) and antiimplantation (6) agent[1], and an evaluation of its toxicity in mice has been made (7).

We, therefore, sought compounds **1** and **2** in extracts of *A. indica*.[2] Although **1** could be detected, an insufficient amount was present to consider isolation for the purpose of biological testing. In order to make available material for biological studies, the transformation of aristolochic acid (**3**) to aristolic acid (**1**) was examined. In essence, this involves the removal of a nitro group from **3**, and one could envisage a number of standard techniques to carry out this transformation in which an amino acid could be a key intermediate.

We report here our experiments in this area, which have led to a facile one-step conversion of **3** to **1**. Our biological results on these compounds will be reported subsequently.



	R_1	R_2
1	H	H
2	H	CH_3
3	NO_2	H



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[1]The term "interceptive" has been used by Pakrashi *et al.* to describe the antifertility effects of samples administered to animals shortly after the time of implantation. "Antiimplantation" has been used when animals were dosed with the drug under evaluation prior to implantation.

[2]The phytochemical work was conducted by Dr. N. Bunyapraphatsara and Dr. C.T. Che, and some details of this were presented as partial fulfillment of the degree of Doctor of Philosophy by Che to the Graduate College, University of Illinois at the Medical Center, March 1982.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot-stage instrument and are uncorrected. The uv spectra were measured on a Beckman model DB-G grating spectrophotometer, and the ir spectra were obtained on a Beckman model 18-A spectrophotometer, with polystyrene calibration at 1601 cm^{-1}. Proton-nmr spectra were recorded at 60 MHz on a Varian model T-60A instrument, equipped with a Nicolet model TT-7 Fourier Transform attachment. Tetramethylsilane was used as an internal standard, and chemical shifts are reported on the (ppm) scale. Low-resolution ms were obtained with a Varian MAT 112S double-focusing spectrometer operating at 70 ev. Microanalyses were carried out by Micro-Tech Laboratories, Inc., Skokie, IL.

ISOLATION OF ARISTOLOCHIC ACID.—Aristolochic acid (**3**) was isolated from the roots of *A. indica* and crystallized from methanol to afford pure **3**; mp, 277-281°. Details of the spectral properties of **3** may be obtained from the authors.

ATTEMPTED REDUCTION OF ARISTOLOCHIC ACID (**3**).—A summary of the unsuccessful reactions that were attempted is given in table 1. If any reaction occurred, the product was typically aristolactam (**4**), indicating that rapid dehydration of the intermediate amino acid occurs. Aristolactam (**4**) was identified through comparison with a sample isolated from natural sources.[2]

TABLE 1. Reduction of aristolochic acid (**3**).

Reagent(s)	Solvent(s)	Temperature	Time	Product
PtO_2/H_2	$CHCl_3$	25°	24 h	complex mixture[a,b]
PtO_2/H_2	EtOH/$CHCl_3$	25°	27 h	complex mixture[a]
PtO_2/H_2	$CHCl_3$/HOAc (3 drops)	25°	24 h	complex mixture[a,b]
PtO_2/H_2	THF	25°	24 h	complex mixture[a,b]
PtO_2/H_2	EtOAc	25°	3 h	complex mixture[a,b]
Pd/C/H_2	THF	25°	24 h	complex mixture[a]
Zn/25% NH_4OH	THF	reflux	100 min	complex mixture[a]
$LiAlH_4$	THF	reflux	2 h	aristolactam and starting material
NH_2NH_2/10% Pd/C	THF	reflux	4 h	starting material
$NaBH_4$	THF	reflux	24 h	starting material
$NaBH_4$/10% Pd/C	THF	25°	24 h	starting material
$NaBH_4$/10% Pd/C	1% Aqueous NH_3	100°	4 h	aristolactam and two unidentified products

[a]Some aristolactam produced (tlc comparison, Si gel, $CHCl_3$-MeOH (97:3), Rf 0.43).
[b]Trace of aristolic acid produced (tlc comparison, Si gel, $CHCl_3$-MeOH (97:3), Rf 0.14).

CONVERSION OF ARISTOLOCHIC ACID (**3**) TO ARISTOLIC ACID (**1**).—To a solution of aristolochic acid (**3**, 104.9 mg) in 1% aqueous ammonium hydroxide solution (10 ml, pH 12) was added sodium borohydride (77 mg); the mixture was stirred at 25° for 4 h. The reaction mixture was poured onto crushed ice, acidified with hydrochloric acid to pH 4, and extracted with ethyl acetate (5 x 25 ml). The combined ethyl acetate phases were washed with water, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to afford a homogenous residue of aristolic acid (**1**, 80.2 mg, 90% yield), which was crystallized from DMF-EtOH to afford fine needles of **1**; mp, 255-256°; pmr δ (DMSO-d_6) 4.01 (3H, s, 8-OCH_3), 6.42 (2H, s, -OCH_2O-), 7.20 (1H, d, J=7.8 Hz, 7-H), 7.62 (1H, t, J=7.2 Hz, 6-H), 7.89 (1H, s, 2-H), 8.06 (1H, d, J=9.9 Hz, 9-H), 8.65 (1H, d, J=8.0 Hz, 5-H), and 8.81 (1H, d, J=9.8 Hz, 10-H); ms, *m/z* 296 (M^+, 100%), 282 (8.9), 281 (49.2), 253 (20.7), 251 (8.3), 223 (9.5), 193 (7.2), 163 (7.2), 153 (9.4), 151 (13.2), 150 (21.0), 148 (7.3), 139 (28.5), 138 (6.2), 87 (7.0), 76 (11.0), 75 (17.8), 74 (8.0), 69 (26.7), 62 (9.6), and 43 (25.7).

Anal. calcd for $C_{17}H_{12}O_5$: C, 68.91; H, 4.08; O, 27.01. Found: C, 68.33; H, 4.19, O, 27.34.

These physical data are in agreement with those of aristolic acid (**1**) (8), and identity was confirmed by comparison (mmp, tlc, ms) with an authentic sample.

METHYLATION OF ARISTOLIC ACID (**1**).—A solution of aristolic acid (**1**, 50 mg) in ether (20 ml) was

added dropwise to an ether solution of diazomethane[3] (100 ml), and the reaction mixture was kept at 4° overnight. Evaporation of the ether *in vacuo* afforded methyl aristolate (51.0 mg); mp, 160°; pmr δ ($CDCl_3$) 3.97 (3H, s, $-CO_2CH_3$), 4.01 (3H, s, 8-OCH_3), 6.28 (2H, s, $-OCH_2O-$), 7.01 (1H, d, J=7.5 Hz, 7-H), 7.58 (1H, t, J=8.1 Hz, 6-H), 7.74 (1H, s, 2-H), 8.20 (1H, d, J=9.8 Hz, 9-H), 8.70 (1H, d, J=8.2 Hz, 5-H), and 8.80 (1H, d, J=9.7 Hz, 10-H); ms, *m/z* 310 (M^+, 100%), 296 (14.8), 295 (78.0, 280 (15.1), 279 (59.4), 267 (15.5), 252 (5.2), 251 (14.7), 236 (8.9), 221 (13.0), 208 (6.9), 193 (12.0), 179 (6.4), 178 (12.4), 155 (34.2), 152 (14.7), 151 (13.3), 150 (30.1), 147 (16.9), 140 (14.9), 139 (20.0), 118 (14.1), 110 (26.0), 104 (6.9), 103 (5.5), 96 (9.0), 89 (8.4), 87 (5.4), 81 (8.5), 76 (22.2), 75 (26.2), and 58 (9.0). These physical data are in agreement with those of methyl aristolate (**2**) (8), and identity was confirmed by comparison (mmp, tlc, ms) with an authentic sample.

DISCUSSION

Reduction of aristolochic acid (**3**) was initially attempted with a variety of catalytic and chemical agents, but either no reaction was observed or cyclization of the intermediate amino acid occurred to afford aristolactam. Previous work in this field (8) had reported the conversion of **3** to aristolic acid (**1**) in 75-80% yield by heating **3** in diglyme at 90-100° in the presence of sodium borohydride. Our attempts to reproduce this reaction afforded somewhat lower yields than those claimed, and this led us to use a method (9) described for the reduction of a nitro acid in the anthraquinone series to the corresponding amino acid.

In our experience, neither the amino acid or aristolactam (**4**) was observed, rather, aristolic acid (**1**) was, reproducibly, the sole product, formed in high yield when **3** was treated with sodium borohydride in 1% aqueous ammonia solution at room temperature for 4 h. We believe this method to be a particularly effective and facile removal of an aromatic nitro group in this series of compounds. Attempts to extend this reaction to the benzenoid series have thus far failed.

ACKNOWLEDGMENTS

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[3]Generated from DIAZALD obtained from the Aldrich Chemical Co., Milwaukee, WI.