814. *The Chemistry of the* Aristolochia *Species. Part III.* Aristolochic Acids and Related Substances from* Aristolochia reticulata *and* A. Indica.

By R. T. COUTTS, J. B. STENLAKE, and W. D. WILLIAMS.

Aristolochic acid from *A. reticulata* and *A. indica* is shown to be identical with that from *A. sipho* and *A. clematitis*. A new acidic substance, aristored, has been isolated from *A. reticulata* and identified as 9-amino-1 : *x* : *x*-trimethoxy-5 : 6-methylenedioxy-8-phenanthroic lactam.

RECENT publications of Pailer, Belohlav, and Simonitsch,[1,2] which established the structure (I) for aristolochic acid from *Aristolochia clematitis*, Linn., prompt us to record our own study of the acids of *A. reticulata* and *A. indica*.

Cold percolation of the powdered and defatted rhizomes and roots of *A. reticulata* with ethanol gave an oil which deposited β-sitosteryl β-D-glucoside, identified as its tetraacetate and by conversion into β-sitosterol. Both the sterol and the glucoside have been identified in the unsaponifiable matter of *A. serpentaria*.[3] After separation of bases, acidic substances were obtained from the residual oil by treatment of the ether-soluble material with dilute aqueous potassium hydrogen carbonate and subsequent precipitation. Fractional crystallisation of the crude product from glacial acetic acid gave two acidic substances. Aristolochic acid, only, has previously been reported in *A. reticulata* although three acids are said to be present in *A. Argentina*,[4] and two in *A. clematitis*.[1]

The less-soluble acid, $C_{17}H_{11}O_7N$, m. p. 275—277° (decomp.), from dioxan or glacial acetic acid, was yellow and non-fluorescent and gave a green colour with concentrated sulphuric acid in agreement with the descriptions of aristolochic acid from *A. sipho* [5] and *A. Argentina*.[4] It has since been reported [2] that aristolochic acid (I) from *A. clematitis*, also $C_{17}H_{11}O_7N$, formed orange-red threads, m. p. 281—286° (decomp) (dependent on

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* Part II, *J.*, 1955, 2114.

[1] Pailer, Belohlav, and Simonitsch, *Monatsh.*, 1955, **86**, 676.

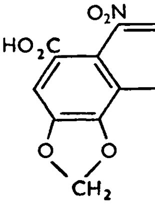
[2] *Idem, ibid.*, 1956, **87**, 249.

[3] Kind and Celentano, *J. Org. Chem.*, 1953, **18**, 1473.

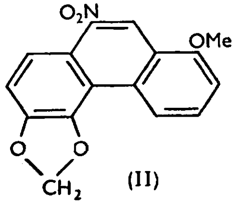
[4] Hesse, *Arch. Pharm.*, 1895, **233**, 684.

[5] Rosenmund and Reichstein, *Pharm. Acta Helv.*, 1943, **18**, 243.

rate of heating). Our own acid, also, readily crystallises from *NN*-dimethylformamide–ethanol as orange-yellow needles as described by the Austrian workers. Both forms, however, melted at 275—277° (decomp.) in a tube, but at 284° (decomp.) on a microblock



(I)



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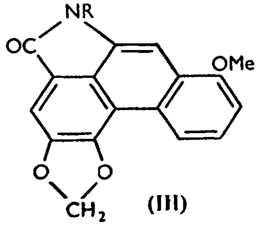
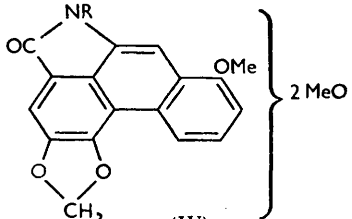


(III)

The identity of aristolochic acid from *A. reticulata* with that from other sources has been confirmed by comparison of its properties and reactions with those reported in the literature.[1,2,5] The ultraviolet absorption spectrum showed peaks identical with those recorded [5] for methyl aristolochate (from *A. sipho*), and decarboxylation with copper powder in quinoline gave the neutral compound,[5] $C_{16}H_{11}O_5N$ recently identified [2] as 1-methoxy-5 : 6-methylenedioxy-9-nitrophenanthrene (II).

Reduction of aristolochic acid (from *A. sipho*) with zinc and acetic acid [6] is said to give a neutral yellow substance, $C_{17}H_{13}O_4N$. Rosenmund and Reichstein [5] obtained a similar compound, formulated as $C_{17}H_{13}O_4N,\frac{1}{2}H_2O$, by catalytic reduction of methyl aristolochate. This gave a crystalline " diacetate," $C_{22}H_{15-17}O_6N$, also obtained directly by reduction of methyl aristolochate with zinc, acetic anhydride, and pyridine. The product $C_{17}H_{11}O_4N$ obtained by hydrogenation of both acid and ester (from *A. clematitis*) has since been identified [2] as 9-amino-1-methoxy-5 : 6-methylenedioxy-8-phenanthroic lactam (III; R = H). Hydrogenation of our aristolochic acid with both platinum and palladium catalysts in dioxan and acetic acid was extremely slow and always incomplete. Reduction with zinc and acetic acid, however, gave the lactam (III; R = H) in excellent yield. This was readily acetylated giving a monoacetate, $C_{19}H_{13}O_6N$ (III; R = Ac), identical with the so-called "diacetate" (from *A. sipho*) as shown by m. p., ultraviolet absorption,[5] and analysis (analytical data [5] for the " diacetate " agree more closely with that for a monoacetate).

The more-soluble acidic fraction was contaminated with aristolochic acid, but repeated crystallisation gave a small amount of a deep red crystalline substance, m. p. 286·5°,



(IV)

designated aristo-red. Elementary analysis gave the formula as $C_{19}H_{15}O_6N$, and a modified Zeisel determination [1] showed the presence of three methoxyl groups (contrast noraristolochic acid, $C_{16}H_9O_6N$, m. p. 209°, isolated from the more-soluble acid fractions of *A. clematitis* [1]). Aristo-red showed an intense fluorescence in ultraviolet light both in the solid state and in solution, and in this respect resembled the lactam (III; R = H) rather than aristolochic acid. This behaviour and the molecular formula suggested that the compound could be formulated as the dimethoxy-derivative (IV; R = H) of the lactam (III; R = H).

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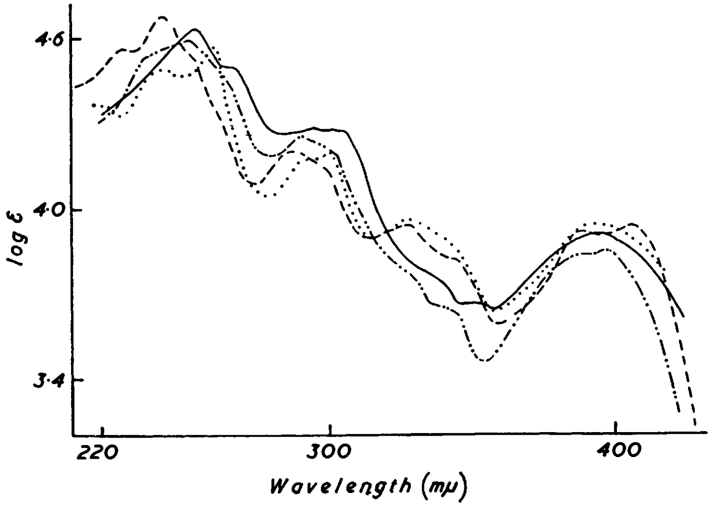
[6] Castille, *J. Pharm. Belg.*, 1922, **4**, 125, 141, 569.

Alkali solubility, in the absence of a carboxyl group (confirmed by the infrared absorption) is explicable in terms of the lactam grouping,[5] which also explains the formation of a monoacetate (IV; R = Ac). Strong confirmatory evidence comes from a comparison of the ultraviolet and infrared spectra of aristo-red (IV; R = H) and its acetate with those of the lactam (III; R = H) and its acetate. The ultraviolet absorptions are very similar in wavelengths and relative intensities (see Figure); and aristo-red and the lactam (III; R = H) show almost identical infrared bands, and in particular a broad band in the region 3000—3300 cm.$^{-1}$ (lactam -NH-) which is absent from the spectra of the acetates; further in the 1650—1700 region (lactam-carbonyl) aristo-red shows a broad band and peak at 1704 cm.$^{-1}$ and a peak at 1652 cm.$^{-1}$ which move to 1728 cm.$^{-1}$ and 1702 cm.$^{-1}$ respectively on acetylation; the lactam (III) similarly shows broad absorption in this region with a peak at 1691 cm.$^{-1}$, and a second peak at 1655 cm.$^{-1}$ which move to 1724 cm.$^{-1}$ and 1702 cm.$^{-1}$ respectively on acetylation. The two bands in the region of 1724 and 1702 cm.$^{-1}$ shown by both lactam acetates are typical of *NN*-diacylarylamines.[7, 8, 9]

Ultraviolet absorption of aristo-red (IV; R = H) (——), *its acetate* (— · · —), *and the lactam* (III; R = H) (. . . .), *and its acetate* (— — —).



Extraction of *A. reticulata* with hot ethanol gave a further yield of aristolochic acid and a small amount of allantoin. A similar examination of *A. indica* has confirmed that aristolochic acid (I), characterised as the lactam (III), is the only acidic substance present.[10]

A neutralised solution of aristolochic acid (1 : 1000) was kindly tested by Dr. E. O. Morris of this College against eleven bacteria, four yeasts, and fourteen moulds. Restriction of growth was marked only with two moulds, *Rhizopus nigricum* and a *Mucor* sp.

EXPERIMENTAL

Ultraviolet absorption spectra were determined in absolute ethanol on a Hilger Uvispek photoelectric spectrophotometer. Rotations were determined in $CHCl_3$ in a 1 dcm. tube. R_F values were determined on Whatman No. 1 paper with 4 : 1 ethanol–5% formic acid as solvent. We thank Mr. W. McCorkindale, Dr. A. C. Syme, and Mr. W. Gardiner for the microanalyses, and Mr. S. G. E. Stevens and Mr. A. J. Cross for the infrared spectra.

Extraction of A. reticulata.—(*a*) The dried root (31 kg.; No. 60 powder), previously defatted with light petroleum (b. p. 40—60°), was extracted in batches of 6 kg. with ethanol by cold

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[7] Grove, Jeffs, and Rustidge, *J.*, 1956, 1956.

[8] Witkop and Patrick, *J. Amer. Chem. Soc.*, 1952, **74**, 3861.

[9] Abramovitch, *J.*, 1956, 1413.

[10] Krishnaswamy, Manjunath, and Rao, *J. Indian Chem. Soc.*, 1935, **12**, 476.

percolation. Concentration of the percolate under reduced pressure gave an almost black thick oil which still contained some solvent.

The product from one such batch, after further concentration slowly deposited β-sitosteryl β-D-glucoside (0·6 g.), m. p. 295—296° (block) (from ethanol), λ 210 mμ (ε 1530, end absorption) (Found: C, 72·3; H, 10·5. Calc. for $C_{35}H_{60}O_6$: C, 72·9; H, 10·5%). Kind and Celentano [3] gave m. p. 295—297°. The tetra-acetate had m. p. 166·5—167·5° (block), $[\alpha]_D^{17}$ −23·91° (*c* 0·92) (Found: C, 69·1; H, 9·2. Calc. for $C_{43}H_{68}O_{10}$: C, 69·35; H, 9·2%). Kind and Celentano [3] gave m. p. 167·5—168·5°, $[\alpha]_D$ −23·7°, −24·2°) The glucoside (165 mg.), refluxed for 9 hr. in ethanol (15 ml.) and concentrated hydrochloric acid (0·3 ml.), gave needles of β-sitosterol, m. p. 139° (tube), 140·5—141° (block), mixed m. p. 139—140·5°, $[\alpha]_D^{19}$ −37·75° (*c* 0·5) (Kind and Celentano [3] gave m. p. 140°, $[\alpha]_D$ −37° and −38°) (Found: C, 82·4; H, 12·1. Calc. for $C_{29}H_{50}O,\frac{1}{2}CH_3{\cdot}OH$: C, 82·2; H, 12·2%).

The bulk of the oily residue was dissolved in ether, and the solution extracted with dilute hydrochloric acid (treatment of acid extract will be reported elsewhere). Extraction of the ethereal solution with 2% aqueous potassium hydrogen carbonate followed by acidification of the aqueous layer with dilute hydrochloric acid gave a yellowish-brown precipitate of crude acids (25 g.). Recrystallisation gave aristolochic acid, m. p. 275—277° (decomp.) (tube), 284° (decomp.) (block), as yellow microcrystals (from dioxan or glacial acetic acid) or as orange-yellow needles from *NN*-dimethylformamide–ethanol (1 : 6) (yield, 6 g.), R_F 0·91—0·94, $\lambda_{max.}$ 223 (ε 30,000), 250 (ε 29,400), 318 (ε 13,100), 390 mμ (ε 7300) [Rosenmund and Reichstein [5] give m. p. 274—278° (decomp.) and Pailer, Belohlav, and Simonitsch [2] give m. p. 281—286° (decomp.)] (Found: C, 60·2, 59·6; H, 3·3, 3·2; N, 4·1; OMe, 9·15. Calc. for $C_{17}H_{11}O_7N$: C, 59·8; H, 3·2; N, 4·1; OMe, 9·1%).

Concentration of the mother-liquors remaining after the isolation of aristolochic acid yielded an orange-red solid, which on repeated fractional recrystallisation gave red needles (from ethanol) of *aristo-red* (50 mg.), m. p. 286·5° (block), R_F 0·77—0·80, $\lambda_{max.}$ 253 (ε 42,400), 265 (ε 31,500), 294 (ε 19,350), 300 (ε 19,100), 305 (ε 18,800), 395 mμ (ε 8,200) with inflections at 335 (ε 5850) and 352 mμ (ε 5000) [Found: C, 64·6; H, 4·2; N, 3·8; OMe, 25·5, 26·1. $C_{16}H_6O_3N(OMe)_3$ requires C, 64·6; H, 4·3; N, 3·95; OMe, 26·3%].

(*b*) A single batch (6 kg.) of the marc remaining from the cold percolation was continuously extracted with hot ethanol (14 l.) until the percolate was pale yellow. Concentration of the percolate to 2 l. and cooling gave a bulky resinous precipitate, which was rejected before further concentration of the liquid to a dark thick oil. The latter deposited allantoin (1·1 g.) as colourless needles (from 80% aqueous ethanol; charcoal), m. p. and mixed m. p. 232° (decomp.) (Found: C, 30·8; H, 4·1; N, 35·2. Calc. for $C_4H_6O_3N_4$: C, 30·4; H, 3·8; N, 35·4%). Treatment of the residual oil as described above, gave aristolochic acid (5 g.).

1-*Methoxy*-5 : 6-*methylenedioxy*-9-*nitrophenanthrene*.—Aristolochic acid (102 mg.) was refluxed for 10 min. with copper powder (120 mg.) and quinoline (16 ml.). The mixture was cooled and extracted with ether, the ethereal solution washed with dilute hydrochloric acid, water, 5% aqueous sodium hydrogen carbonate, and water, dried (Na_2SO_4), and evaporated and the residue chromatographed in benzene on alumina. The eluate was evaporated, and the residue crystallised from chloroform–ethanol and sublimed at 200°/0·1 mm., to yield orange needles (61 mg.) of 1-methoxy-5 : 6-methylenedioxy-9-nitrophenanthrene, m. p. 213° (block), $\lambda_{max.}$ 247·5 (ε 39,930), 286 (ε 12,510), 310 (ε 9970), 395 mμ (ε 4460) [Pailer, Belohlav, and Simonitsch [2] gave m. p. 212° (block)] (Found: C, 64·9; H, 4·1; N, 4·5. Calc. for $C_{16}H_{11}O_5N$: C, 64·65; H, 3·7; N, 4·7%).

9-*Amino*-1-*methoxy*-5 : 6-*methylenedioxy*-8-*phenanthroic Lactam*.—Aristolochic acid (360 mg.) was refluxed for 45 min. with zinc powder (1·04 g.) and glacial acetic acid (20 ml.). The fluorescent solution was filtered hot, and on cooling deposited the bulk of the crude product (225 mg.). The mother-liquors, treated with water (50 ml.), yielded a further precipitate, which was dissolved in chloroform, washed repeatedly with water, dried (Na_2SO_4), and evaporated, to give a further 40 mg. of crude product. Sublimation at 240—250°/0·1 mm. gave greenish-yellow crystals of lactam, m. p. 320° (block; inserted at 315°), $\lambda_{max.}$ 222 (ε 22,900), 242 (ε 30,840), 250 (ε 29,740), 260 (ε 36,130), 291 (ε 15,050), 301 (ε 15,450), 327 (ε 9220), 346 (ε 7190), 395 mμ (ε 8470) (Pailer, Belohlav, and Simonitch [1] gave m. p. 319°) (Found: C, 69·8; H, 4·2; N, 4·8. Calc. for $C_{17}H_{11}O_4N$: C, 69·6; H, 3·8; N, 4·8%).

9-*Acetamido*-1-*methoxy*-5 : 6-*methylenedioxy*-8-*phenanthroic Lactam*.—The lactam (100 mg.) was refluxed for 30 min. with acetic anhydride (0·5 ml.) and pyridine (1 ml.). The yellow precipitate, which separated during the reaction and on cooling (52 mg.), sublimed at 250—260°/0·1 mm., yielding the greenish-yellow N-*acetyl-lactam* solid, which fluoresced under ultraviolet light both in the solid state and in solution and had m. p. 283—286° (decomp.; sealed tube), 295° (decomp.; block), $\lambda_{max.}$ 227 (ε 36,200) 242 (ε 47,750), 252 (ε 34,700), 288 (ε 15,800), 300 (ε 13,500), 328 (ε 8630), 344 (ε 6600), 388 (ε 8130), 406 mμ (ε 8630) [Rosenmund and Reichstein[5] gave m. p. 292—296° (decomp.) for the so-called "diacetate"] (Found: C, 68·15; H, 3·6; N, 4·4; OMe, 8·5. $C_{19}H_{13}O_5N$ requires C, 68·1; H, 3·8; N, 4·2; OMe, 9·25%).

9-*Acetamido*-1 : x : x-*trimethoxy*-5 : 6-*methylenedioxy*-8-*phenanthroic Lactam*.—Aristo-red (15 mg.) was refluxed for 30 min. with acetic anhydride (0·25 ml.) and pyridine (0·5 ml.). The cooled mixture was extracted with ether, which slowly deposited pale orange needles of 9-*acetamido*-1 : x : x-*trimethoxy*-5 : 6-*methylenedioxy*-8-*phenanthroic lactam* (6 mg.). This fluoresced under ultraviolet light both in the solid state and in solution, and had m. p. 276—278° (block), $\lambda_{max.}$ 240 (ε 35,730), 250·5 (ε 38,860), 291 (ε 17,960), 302 (ε 15,690, shoulder), 330 (ε 5580, infl.), 343 (ε 4410), 385 (ε 6680), 400 mμ (ε 6740) (Found: N, 4·2. $C_{21}H_{17}O_7N$ requires N, 3·55%).

Extraction of A. indica.—The dried root (3 kg.), previously defatted with light petroleum (b. p. 40—60°), was extracted with ethanol by cold percolation. The percolate was concentrated to 200 ml., acidified with dilute hydrochloric acid, and extracted with ether. The ethereal solution was extracted with 2% aqueous potassium hydrogen carbonate, and the latter solution acidified, to yield aristolochic acid (2·5 g.) as yellow needles (from dioxan), m. p. 284° (decomp.; block) (identical with the acid from *A. reticulata*), $\lambda_{max.}$ 223 (ε 29,300), 250 (ε 32,300), 317·5 (ε 12,900), 391 mμ (ε 6000). Reduction with zinc and glacial acetic acid gave the lactam, m. p. and mixed m. p. 318° (block).

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