tion of the cations from the phenolic groups to form the zwitterions. hydrogen bonding is not important in this equilibrium. However, the electron-withdrawing enhancement of the carboxamido group by the phenyl group is even stronger in the lowest excited singlet state than in the ground state, as evidenced by the reduction in basicity of arylamines in the lowest excited singlet state (8). Thus, the lower pK_as of salicylanilide relative to salicylamide is attributed entirely to the greater electromeric and field effects of the phenyl-substituted carboxamido group in the lowest excited singlet state.

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Alkaloid Studies VIII: Isolation and Characterization of Alkaloids of *Tabernaemontana heyneana* Wall and Antifertility Properties of Coronaridine

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Abstract □ Extraction of the roots of *Tabernaemontana heyneana* Wall yielded the alkaloids coronaridine, voacangine, ibogamine, 19-oxocoronaridine, and the pseudoindoxyl of voacangine. Coronaridine was demonstrated to prevent pregnancies in adult female rats when administered orally.

Keyphrases □ *Tabernaemontana heyneana* Wall—isolation and characterization of alkaloids, antifertility properties of coronaridine □ Alkaloids—isolation and identification from *Tabernaemontana heyneana*, antifertility properties of coronaridine □ Antifertility agents, potential—isolation, characterization, and screening of coronaridine from *Tabernaemontana heyneana* □ Coronaridine—isolation from *Tabernaemontana heyneana*, screened for antifertility properties in rats

Investigation of the alkaloids of the Tabernaemontanoideae tribe of the Apocynaceae family has received considerable attention because of the interest aroused in the alkaloids isolated from *Tabernanthe iboga* of the same family.

The roots and bark of *Tabernaemontana heyneana* Wall were previously shown to contain the indole alkaloid coronaridine (I) (1) and its 20-hydroxy derivative heyneanine (II) (2). In this study, the roots of this botanical were examined, and the isolation and identification of additional indole alkaloids and some pharmacological properties of coronaridine are described.

DISCUSSION

An aqueous ethanolic extract of the roots of *T. heyneana* Wall was found to prevent fertilization of adult female rats when administered orally. The residue from this extract was treated with aqueous acetic acid, and the soluble components were then extracted into methylene chloride. Removal of the acidic components from the methylene chloride extract by washing with aqueous base gave, after evaporation of the solvent, a brown amorphous crude material (Fraction A), which retained all of the antifertility activity of the original extract. Chromatographic fractionation of a portion of Fraction A on silica gel yielded the alkaloid coronaridine and a closely associated alkaloid which was not obtained pure.

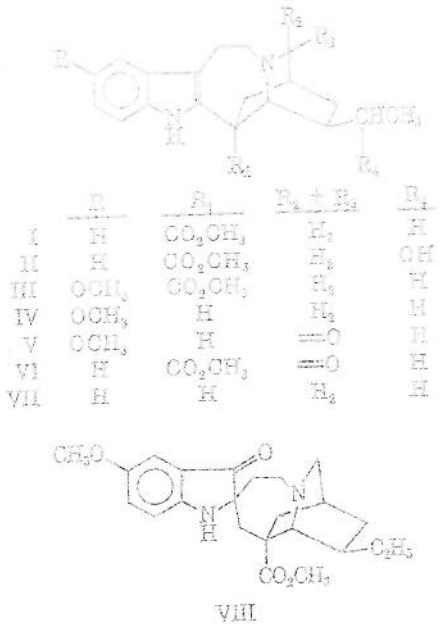
The alkaloids in Fraction A were precipitated as their hydrochloride salts, and the free bases were regenerated to afford a tan amorphous mixture of alkaloids (Fraction B). Sequential chromatography of Fraction B on silica gel, Grade I neutral alumina, and Grade III neutral alumina yielded additional coronaridine and the second alkaloid, which was identified as voacangine (III) by comparison with an authentic sample[1].

The syrup obtained from a methanol wash of the Grade III neutral alumina column was separated further by partition chromatography on diatomaceous earth to give a chromatographically pure, noncrystalline alkaloid. This alkaloid, which appeared on thin-layer chromatograms of solutions of coronaridine that had been exposed to air, was identical with the iodine–sodium bicarbonate oxidation [the procedure for conversion of ibogaine (IV) to 19-oxoibogaine (V) (3)] product of coronaridine. The appearance of a second carbonyl absorption band in the IR spectrum (6.06 μ) and a molecular-ion peak (*m/e* 352.1780) in the mass spectrum, which appeared 14 mass units higher than the molecular-ion peak for coronaridine, indicated this alkaloid to be 19-oxocoronaridine (VI), previously isolated from the bark of *Conopharyngia jollyana* Stapf. (4). [In *Reference 5*, this compound was characterized only by mass spectral analysis (*m/e* 352) and R_f values.]

Column chromatography of Fraction B on Grade II neutral alumina yielded a noncrystalline alkaloid fraction, which was further separated by partition chromatography on diatomaceous earth. A fraction containing 19-oxocoronaridine, contaminated with a highly fluorescent yellow material, was rechromatographed on silica gel to provide a crystalline yellow-green fluorescent alkaloid. The distinctive UV spectrum and mass spectrum indicated this material to be the pseudoindoxyl (VIII) (6) of voacangine (III).

Those fractions collected before the pseudoindoxyl (VIII) was

[1] The authors thank Dr. M. Gorman, Lilly Research Laboratories, for a sample of voacangine.



	R	R_1	$R_2 + R_{2a}$	R_3
I	H	CO_2CH_3	H_2	H
II	H	CO_2CH_3	H_2	OH
III	OCH_3	CO_2CH_3	H_2	H
IV	OCH_3	H	H_2	H
V	OCH_3	H	=O	H
VI	H	CO_2CH_3	=O	H
VII	H	H	H_2	H

VIII

eluted were combined and subjected to partition chromatography utilizing a less polar solvent system. One fraction yielded a crystalline alkaloid which was identified as ibogamine (VII) (7–9).

Although 19-oxocoronaridine and the pseudoindoxyl of voacangine may occur naturally, the susceptibility of the iboga alkaloids to oxidation under mild conditions (10) suggests that these alkaloids may be formed by the autoxidation of the parent alkaloid. Hootele and Pecher (5) suggested the possibility of a similar origin for several 19-oxo iboga alkaloids isolated from *C. jollyana*. The possibility of the pseudoindoxyl derivatives isolated from *T. rupicola* being artifacts was suggested by Niemann and Kessel (11).

EXPERIMENTAL[2]

Extraction Procedure—Dried ground roots of *T. heyneana* Wall[3] (7.5 kg.) were extracted by percolation for 18 hr. with 67.5 l. of 5% 3A ethanol. The percolate was evaporated under reduced pressure at 25°, and the residue was stirred for 18 hr. with 3.3 l. of 10% aqueous acetic acid. The suspension was filtered and the filter cake was washed with 10% acetic acid. The combined extract and wash was extracted three times with 1-l. portions of methylene chloride. The combined extracts were washed two times with 250-ml. portions of 1 *N* sodium hydroxide and then the methylene chloride layer was dried (magnesium sulfate). Evaporation of the solution *in vacuo* yielded 26.4 g. of a brown frothy residue (Fraction A).

A 5.0-g. sample of Fraction A was dissolved in 100 ml. of ether. A solution of dry hydrogen chloride in ether was added, and the pale-yellow precipitate which formed was removed by filtration. The precipitate was distributed between chloroform and 1 *N* sodium hydroxide, and the chloroform layer was dried (magnesium sulfate) and evaporated *in vacuo* to give 3.4 g. of a tan amorphous residue (Fraction B).

Isolation of Coronaridine (I) and Voacangine (III)—Fraction A (2 g.) was chromatographed on a silica gel column (200 g.) by developing with 1 l. of benzene, followed by 200-ml. portions of benzene containing 5, 10, 20, and 40% chloroform. When a yellow band appeared at the column front, collection of 20-ml. cuts was begun. Cuts 75–130 contained 2.1 g. of a mixture of two alkaloids, a 1-g. portion of which was chromatographed on a column[4] using a mixture of chloroform and methanol (200:1); 5-ml. cuts were collected. Pure noncrystalline I (353 mg.) was obtained from the residue of cuts 30–62; $[\alpha]_D^{25}$ −35.2° (c 1.1, $CHCl_3$) [lit. (12) $[\alpha]_D^{25}$ −34° (c 1.0, $CHCl_3$)]; $\lambda_{max}^{CHCl_3}$: 2.89, 5.80, 6.84, and 7.99 nm. The hydrochloride salt was prepared in the usual manner, m.p. 213–214° dec. [lit. (12) m.p. 210–211° dec.].

Fraction B (3.4 g.) was chromatographed on 120 g. of silica gel, and the material eluted with chloroform–methanol (200:1) was purified further on a column of 200 g. of Grade I alumina which was developed with benzene–chloroform (1:1). The first fraction (125 ml.) yielded 395 mg. of coronaridine. The following fractions, which contained a mixture of coronaridine and voacangine, were rechromatographed on a column of 100 g. of Grade III alumina. The column was developed with benzene and, after collecting 17 125-ml. cuts, was washed with chloroform and methanol. The residue (245 mg. Fraction C) from the wash was reserved for further purification. Evaporation of the first cut yielded an additional 429 mg. of coronaridine, whereas the residue from the third cut yielded 122 mg. of voacangine (III), obtained as a pale-yellow syrup. The mass spectrum of this syrup was identical with that of an authentic sample of III[1]. The PMR (13), IR (14), and UV spectra (14) were in accord with published data.

Isolation of 19-Oxocoronaridine (VI)—Fraction C (245 mg.) was subjected to partition chromatography, and the effluent was monitored at 285 nm. 19-Oxocoronaridine (VI) (23 mg.) was obtained in the fourth holdback volume as a pale-yellow syrup, $\lambda_{max}^{C_2H_5OH}$: 222 (28,500), 276 (sh) (6500), 284 (7110), and 292 (6000) nm.; λ_{max}^{KBr}: 5.81, 6.06, 6.92, 8.00, 8.85, and 13.51 μ; PMR ($CDCl_3$): δ 8.20 (s, 1H, exchangeable NH), 7.15 (m, 4H, substituted aromatic), 4.51 (s, 1H, $>C-H$), 3.70 (s, 3H, CO_2CH_3), and 0.97 (t, 3H, CH_2CH_3); mass spectrum, molecular ion at *m/e* 352.1780 ($C_{21}H_{24}N_2O_3$, calc. 352.1786).

Oxidation of Coronaridine (I) to 19-Oxocoronaridine (VI)—Coronaridine hydrochloride (260 mg., 0.7 mmole) was dissolved in 8 ml. of 50% aqueous tetrahydrofuran containing 500 mg. of sodium bicarbonate. A solution of 360 mg. (1.4 mmoles) of iodine in 10 ml. of tetrahydrofuran was added dropwise and, after an additional 30 min. at 25°, the reaction was cooled in an ice bath and 10 ml. of water and 20 ml. of methylene chloride were added. The layers were separated and the aqueous phase was washed with 10 ml. of methylene chloride. The combined methylene chloride solutions were washed consecutively with 20 ml. of 5% sodium thiosulfate, 2 *N* sulfuric acid, and water. After drying (magnesium sulfate), the solution was evaporated to give 240 mg. of a pale-yellow syrup, which was chromatographed on a silica gel column[5] using ethyl acetate for development; 0.75-ml. cuts were collected. Coronaridine (27 mg.) was recovered by evaporation of cuts 2–8, and 19-oxocoronaridine (86 mg.) was obtained after evaporation of cuts 15–30. This material was found to be identical with the 19-oxocoronaridine isolated from *T. heyneana* by comparisons of IR and mass spectra.

Isolation of the Pseudoindoxyl VIII of Voacangine—Fraction B (12.7 g.), obtained from a 16-g. portion of Fraction A, was chromatographed on 400 g. of alumina (Grade II). After elution with 3 l. of benzene, a chloroform–methanol (4:1) wash yielded a fraction (2 l.) which, upon evaporation, gave 6.9 g. of a yellow syrup. Partition chromatography of 6.0 g. of this syrup yielded a yellow syrup (Fraction D) from the first two holdback volumes and 553 mg. of a

Table I—Antifertility Effect in Adult Female Rats

Dose, mg./kg./day	Number Rats Pregnant/ Number Rats Treated
1	3/4
5	0/4
10	0/3
20	0/4
40	0/3

[2] Melting points are uncorrected. IR spectra were determined on a Perkin-Elmer model 21 recording spectrophotometer. UV absorption spectra were determined on a model 11 MS Carey recording spectrophotometer. Mass spectra were obtained on a AEI MS 9 mass spectrometer. Proton magnetic resonance (PMR) spectra were determined on a Varian A 60 spectrometer. Davison silica gel (60–200 mesh) and Woelm neutral alumina were used as indicated. Partition chromatography was performed on Johns Mansville diatomaceous earth using a heptane–ethyl acetate–methanol–water (80:20:17:4) mixture unless otherwise specified.

[3] Collected and shipped through the courtesy of C. N. Correia, Goa, India; a voucher specimen is deposited in the Botanical Collection of Lederle Laboratories, A Division of American Cyanamid Company, Pearl River, NY 10965

[4] Quanta-Gram D-116, Quantum Industries, Fairfield, N. J.

[5] Quanta-Gram D-32.

Table II—Estrogenic Effect in Immature Female Rats

Compound	Dose, mcg./rat/day × 3	Uterine Weight Ratio (Treated/Control)
Estrone	0.37	0.92
	1.1	1.67
	3.3	2.30
	10.0	2.71
Coronaridine hydrochloride	12	0.88
	37	1.00
	111	1.35
	333[a]	1.85
	1000	2.09

[a] Approximate ED_{100} in antifertility assay.

yellow syrup (Fraction E) from the fourth holdback volume. Final purification of Fraction E was effected by chromatography on 15 g. of silica gel and eluting with ethyl acetate, followed by preparative TLC on silica gel plates[6] using ethyl acetate for development. A bright-yellow band (R_f 0.7) was eluted with methanol, and the solution was evaporated *in vacuo* to yield 175 mg. of a yellow froth. Two crystallizations from methanol gave 20 mg. of VIII as yellow-green fluorescent needles, m.p. 201–203° [lit. (6) m.p. 205–208°]; PMR ($CDCl_3$): δ 3.77 (s, 3 H, OCH_3) and 3.55 (s, 3 H, CO_2CH_3). The mass, IR, and UV spectra were in accord with published data (6).

Isolation of Ibogamine (VII)—Fraction D was fractionated by partition chromatography using a 2-methoxyethanol–heptane system. Evaporation *in vacuo* of the third holdback volume and crystallization of the residue from ethanol yielded colorless crystals of VII, m.p. 160–161° [lit. (7) m.p. 162–163°]; $[\alpha]_D^{25}$ −31.3° (c 1.1, $CHCl_3$) [lit. (7) $[\alpha]_D^{26}$ −36.4° ($CHCl_3$)]. The IR (8) and mass (9) spectra were in agreement with published data.

PHARMACOLOGY

Coronaridine was tested previously in a variety of pharmacological systems (12). Although a relatively broad spectrum of biological activity was reported, no antifertility properties were described. In the present study, the oral administration of coronaridine hydrochloride to adult female rats at levels of 5 mg./kg./day or above prevented pregnancies. Voacangine, assayed by the same procedure, did not prevent pregnancies.

Graded doses of coronaridine hydrochloride were administered orally once daily for 10 days to adult female rats (225–250 g.), which were maintained on a standard diet of laboratory rat pellets and water *ad libitum*. The dose was composed of propylene glycol and an appropriate amount of coronaridine hydrochloride, so that the desired dose was administered in a 0.25-ml. volume. Control rats were given propylene glycol without the alkaloid. From the 1st day of treatment, the females were placed with fertile males. Four days after the last dose, the females were sacrificed and uterine fetal implantation sites were counted. Estrone was used as a standard. Under these conditions, simultaneously performed vehicle control groups yielded pregnancy rates of approximately 90%, and the ED_{100} of estrone was consistently 0.5 mg./kg./day.

The results of this study are summarized in Table I.

In the uterotropic assay, which measured estrogenic activity, immature female rats (50–60 g.) received single daily oral doses in 0.25 ml. of propylene glycol for 3 days. The uteri were removed and weighed 24 hr. after the last dose. A minimum of five rats was tested at each dose level and, here also, estrone was used as reference standard. The average uterine weights of estrone- or test compound-treated rats were divided by the average uterine weight of simultaneously performed vehicle controls to estimate the degree of uterine hypertrophy. Thus, for example, a ratio of 1.25 indicated a 25% increase in uterine weight above control values. The results of this assay are summarized in Table II. The data indicated that coronaridine was weakly estrogenic in that it was only about 3×10^{-4} times as potent as estrone in eliciting uterine growth. Nevertheless, doses approximately one-third the minimally effective antifertility dose produced a 35% increase in uterine weight, and the antifertility ED_{100} was associated with an 85% increase. Therefore, the contraceptive action of coronaridine was ostensibly related to its inherent estrogenicity since the two effects were apparently inseparable.

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[6] Quanta-Gram PLQ1-F.