Tumor Inhibitors II

Alkaloids of *Ervatamia dichotoma*. Isolation, Crystallization, and Pharmacological Properties of Coronaridine

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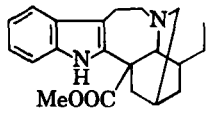
Extraction of the fruit, root bark, and stem bark of *Ervatamia dichotoma* (Roxb.) Blatter from Mangalore, Mysore State, afforded substantial yields of alkaloids. Column chromatography of the petroleum ether-extractable alkaloids yielded coronaridine, which was obtained in crystalline form for the first time. Preliminary pharmacological testing in a variety of pharmacologic procedures revealed that the alkaloid mixtures and coronaridine possess interesting biologic activities.

ERVATAMIA DICHOTOMA (Roxb.) Blatter (syn. *Tabernaemontana dichotoma* Roxb.) is a small apocynaceous tree common in India and Ceylon. The seeds have been known to be poisonous and to possess narcotic and purgative properties; the leaves, bark, and milky sap have also been shown to be purgatives (1). The bark of *Ervatamia dichotoma* was studied by Subbaratnam, who reported the presence of alkaloids (0.01%) and sterols (0.2%) (2).

The work reported herein concerns the alkaloids of *Ervatamia dichotoma* from Mangalore, Mysore State. Initial petroleum ether extraction of the fruit yielded 0.7% of crude alkaloid; the root bark, 0.18%; and the stem bark, 0.09%. Subsequent methanol extraction of the fruit yielded 0.39% of crude alkaloids; the root bark, 0.49%; and the stem bark, 0.32%. The petroleum ether-extractable alkaloids of the fruit showed CNS depressant and hypotensive activities; the methanol-extractable fraction showed reproducible antitumor activity against 9KB cell culture.[1] Fractionation studies of the methanol-extractable alkaloids are in progress and will be described in a later communication.

Column chromatography on neutral alumina of the petroleum ether-extractable alkaloids of the fruits gave a crystalline material, m.p. 92–93°, $[\alpha]_D^{25}$ −34°, identified as coronaridine (I) (3).



I

Coronaridine does not appear to have been obtained in a crystalline form previously. The hydrochloride of the alkaloid showed m.p. 210–211° (decompn.) $[\alpha]_D^{25}$ −8°. The hydrochloride was found to be identical with coronaridine hydrochloride (3) by comparisons of infrared and ultraviolet spectra (4) and of X-ray diffraction patterns.

EXPERIMENTAL

Melting points, determined on a Hershberg apparatus, were corrected for stem exposure. Infrared spectra were determined on a Baird model B double beam infrared recording spectrophotometer. Ultraviolet absorption spectra were determined on a model 11 MS Cary recording spectrophotometer. All solutions were reduced in volume by heating at a temperature not exceeding 50–55° under reduced pressure. Chromatographic fractions were combined where indicated by paper chromatography and infrared spectroscopy. Petroleum ether of b.p. 40–60° was used throughout unless otherwise specified.

Extraction Procedure.—Dried powdered fruit of *Ervatamia dichotoma* (gathered in Mangalore, Mysore State, in April 1958)[2] (460 Gm.) was extracted with petroleum ether (2.5 L.) in a Soxhlet apparatus for 14 hours. The alkaloids were extracted with 4% hydrochloric acid and the acid solution was neutralized with ammonium carbonate and extracted with ether. Evaporation left an amorphous powder (3.0 Gm., fraction A). The dried marc remaining from the petroleum ether extraction was next extracted continuously with hot methanol (3 L.) with a fresh charge of solvent at the end of 2 days. The methanolic extract was evaporated to dryness under reduced pressure and the residue was triturated with 4% hydrochloric acid solution for 3 days. The process was repeated twice more with fresh charges of 4% hydrochloric acid. The suspension was filtered, and the acid solution was neutralized with ammonium carbonate and extracted with ether to yield a crude alkaloidal residue (1.78 Gm., fraction B).

Dried, powdered root bark (2.7 Kg.) was extracted as described above for the fruit. The petroleum

Received May 17, 1962, from the Department of Pharmaceutical Chemistry, University of Wisconsin, Madison, and Smith Kline and French Laboratories, Philadelphia, Pa.
Accepted for publication May 31, 1962.

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The investigation at the University of Wisconsin was supported in part by research grants from the National Institutes of Health (H-2952 and CY-4500).

Part I in the series: S. M. Kupchan and R. W. Doskotch, *J. Med. Pharm. Chem.*, **5**, 657(1962).

[1] The evaluation of the 9KB assay results by the Cancer Chemotherapy National Service Center in sequential testing is such that a material is considered active if the ED50 ≦ 20 mcg./ml. Assays were performed by CCNSC by the procedures described in Cancer Chemotherapy Reports, **25**, 1 (1962).

[2] We thank Dr. C. B. Sulochana, University Botany Laboratory, Madras, India, for confirming the identity of the plant, and Rajaranga and Co., Madras, India, for gathering and forwarding the dried plant material to us.

ether extract yielded 5.0 Gm. of crude alkaloidal residue (fraction C); the methanol extract yielded 13.30 Gm. (fraction D).

Dried, powdered stem bark (2.08 Kg.) yielded 1.9 Gm. of petroleum ether-extractable alkaloids (fraction E) and 6.67 Gm. of methanol-extractable alkaloids (fraction F).

Isolation of Coronaridine: Fraction A.—The crude mixture (3.0 Gm.) was dissolved in benzene and chromatographed on "neutral" alumina (Woelm. grade 1, 90 Gm.). The fractions eluted with benzene alone were combined. The chromatography on alumina was repeated as above, and the fractions eluted with benzene yielded crystalline residues upon evaporation. Repeated recrystallization from petroleum ether (b.p. 60–68°) gave colorless needles, 560 mg., m.p. 92–93°, $[\alpha]_D^{25} -34°$ (c 1.00, $CHCl_3$); $\lambda_{max.}^{alc.}$ 226 mμ (34,800), 286 mμ (8300), and 294 mμ (7800), $\lambda_{max.}^{CS_2}$ 2.82, 2.86, 3.33, 3.45, 5.82, 6.17, 7.04, 8.00, 8.51, 8.77, 9.26, and 9.80 μ.

Treatment of the crystalline alkaloid with a saturated ethereal solution of hydrogen chloride afforded an amorphous hydrochloride salt. Recrystallization from acetone yielded a microcrystalline solid, m.p. 210–211° (decompn.), $[\alpha]_D^{25} -8°$ (c 1.00, MeOH). The ultraviolet and infrared spectra were identical to those recorded for coronaridine hydrochloride (4). The X-ray diffraction pattern was found to be identical with an authentic sample of the hydrochloride of m.p. 235° (decompn.).[3]

PHARMACOLOGICAL RESULTS

Coronaridine was tested in a variety of pharmacologic procedures and found to possess biological activity. Thus, ptosis was produced in the mouse after doses as low as 5 mg./Kg. intraperitoneally. Higher doses, up to 100 mg./Kg., caused hypotonia, intermittent periods of increased and decreased activity, lacrimation, salivation, bradypnea, and finally tremors at the high dose level. Orally, coronaridine produced slight depression of spontaneous motor activity, lacrimation, bradypnea, and slight ptosis after doses as low as 20 mg./Kg. Lethal effects were apparent at 500 mg./Kg.

In the larger unanesthetized animal, coronaridine exhibited a variety of actions which were not consistent with different species. For instance, doses of 20 mg./Kg. intraperitoneally in the cat caused emesis, vocalization, ataxia, and death. A dose of 10 mg./Kg. intravenously in the dog produced excitation, ataxia, fear, ptosis, and restlessness, and yet in the monkey no overt effects were observed after doses of 10 mg./Kg. intravenously or 100 mg./Kg. orally. This phenomenon of species sensitivity to drugs is not uncommon. Many potentially interesting drugs have been very active in the lower animal but less active or inactive in the higher animal.

Coronaridine was found to possess analgetic activity in the rat using both the D'Amour Smith and Randall-Selitto test procedures over a range of doses from 15 to 100 mg./Kg. orally. In addition, this compound demonstrated weak but significant activity against foot shock induced rage in mice. This test can be used to uncover compounds which may possess mid-range tranquilizing activity.

Other tests were carried out in an effort to determine the possible central nervous system action of the compound. It was found that coronaridine did not potentiate hexobarbital anesthesia, that it failed to antagonize the lethal effect of amphetamine in aggregate mice, and did not produce an anticonvulsant action against maximal electroshock in mice.

The principal action of coronaridine on the blood pressure was depressor. In the anesthetized cat, this compound produced falls in pressure and a concomitant depression of respiration after intravenous doses as low as 0.5 mg./Kg. Lethality occurred at a dose of 10 mg./Kg.

In summary, coronaridine produced signs of autonomic as well as central nervous system activity when tested for biological action in animals. It produced analgesia and was effective in suppressing foot shock induced rage in mice. Toxicity appeared to be associated with respiratory depression in the anesthetized cat. Coronaridine was inactive against 9KB cell culture.

[3] We thank Dr. Norbert Neuss of the Lilly Research Laboratories, Indianapolis, Ind., for the X-ray pattern comparison of our material with authentic coronaridine hydrochloride.

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