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ISOLATION OF THE ANTIMICROBIAL ALKALOID STEMMADENINE FROM IRAQI *RHAZYA STRICTA*

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The plant *Rhazya stricta* Decsn. (Apocynaceae) is indigenous to India and Pakistan (1) but is the only *Rhazya* species found in the southwestern region of Iraq (2). In folkloric medicine the plant is used for the treatment of a number of illnesses such as chronic rheumatism, syphilis, sore throat, fever, cancer, and general debility (1-3). Previous investigators have isolated many pharmacologically active monomeric and dimeric indole alkaloids from this plant (4,5). Although stemmadenine is found in many members of the Apocynaceae (6,7), the presence of this alkaloid in the genus *Rhazya* has not previously been reported. We now report the isolation of stemmadenine from *R. stricta* and present a limited account of its antimicrobial activity.

EXPERIMENTAL

GENERAL PROCEDURES.—Uv and ir spectra were determined using Pye-Unicam SP-200 and SP 3-300 spectrometers. Nmr and mass spectral data were obtained at the Morgan Schaffer Corp. (Montreal) using (¹H nmr with 400.13 MHZ) and Hitachi/Perkin-Elmer RM4-6D instruments.

PLANT MATERIAL.—Leaves of *R. stricta* were collected from Shabicha (220 km southwest of Baghdad) and authenticated by the Directorate of Botany, Ministry of Agriculture, Baghdad, Iraq. A herbarium sample is deposited at the National Herbarium of Iraq.

ISOLATION OF STEMMADENINE.—Powdered leaves (4 kg) of *R. stricta* were defatted with 7.5 liters of petroleum ether (bp 40-60°) and then extracted successively with 8 liters MeOH, MeOH containing 2% NH₃, and 0.25 N methanolic NaOH. The extracts were evaporated to dryness in vacuo, and the three residues were combined and mixed with 1 N HCl. The mixture was filtered, and the filtrate was extracted six times with 1 liter CHCl₃ to yield a weakly basic fraction and a remaining aqueous acidic layer (A-1). The residue from the CHCl₃ extract was dissolved in 1 N HCl, and the resulting solution was filtered and extracted six times with 1 liter CHCl₃. The CHCl₃ extracts were combined, concentrated, and washed with H₂O. The yellow solid that appeared at the interface was crystallized from EtOAc-MeOH (3:1) to yield 300 mg of stemmadenine, mp. 214-215°; uv, ir, ¹H-nmr, and ms data agree with the literature (6,7). An additional 17 mg of alkaloid was obtained by alumina chromatography, followed by preparative tlc using Si gel GF254, of the combined residues obtained from CHCl₃ extracts of A-1 after adjustment to pH 6 and then after adjustment to pH 11. Co-tlc agreed with an authentic sample of stemmadenine.

ANTIMICROBIAL ACTIVITY.—Minimum inhibitory concentration (MIC) values of stemmadenine and thiomersal (B.D.H. Limited, London), benzalkonium chloride, U.S.P., and chlorhexidine diacetate ("hibitane", I.C.I., Pharmaceutical Division, Cheshire, England) were determined using the microorganisms listed in Table 1. The inoculum size was ca. 10⁶ organisms/ml in each case. Antimicrobial activity was determined in two stages: an initial ranging test employing doubling dilutions of the test agents and a second experiment using ten concentration increments spaced equally about the MIC values found in the first experiment.

TABLE 1. Minimum Inhibitory Concentrations (MIC $\mu\text{g/ml}$) of Stemmadenine (St), Benzalkonium Chloride (BAC), Thiomersal (Th), and Chlorhexidine Diacetate (Ch) Towards Representative Microorganisms

| Test Microorganisms | MIC ($\mu\text{g/ml}$) | | | |
|--|--------------------------|-----|------|-----|
| | St | Th | BAC | Ch |
| <i>Pseudomonas aeruginosa</i> ATCC 9027 | 7.5 | 1.5 | 8.0 | 1.5 |
| <i>Escherichia coli</i> ATCC 8739 | 1.25 | 0.6 | 2.8 | 0.4 |
| <i>Staphylococcus aureus</i> ATCC 6538 | 5.0 | 0.2 | 0.32 | 0.2 |
| <i>Candida albicans</i> ATCC 10231 | 37.5 | 0.1 | 1.8 | 4.5 |

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