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BIOACTIVE COMPONENTS OF *ALLAMANDA SCHOTTII*

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In our search for natural antitumor compounds, we have extracted the previously uninvestigated *Allamanda schottii* Pohl (Apocynaceae). The genus *Allamanda* is known to produce bioactive (antitumor, algicidal, and antimicrobial) iridoids (1–3) and in a recent report has been shown to contain coumarins (4). Interest in the bioactive iridoids has led to papers outlining synthetic methodology (5,6) and their distribution in the Apocynaceae (7,8) as well as the Clionidae (the boring sponges) (9).

We report here the isolation from this plant of seven previously known compounds. Brine shrimp (BS) lethality was used as a monitor in the bioactivity-directed fractionation (10). The active components, in order of elution on Si gel, are: isoplumericin, plumericin, scoparone, allamandin, scopoletin, pinoresinol, and allamcin. The brine shrimp assay is a simple indicator of cytotoxicity and expedites the isolation of a wide range of bioactive compounds (in this case iridoids, coumarins, and a lignan). This assay correlates well with 9KB (human nasopharyngeal carcinoma) results. Antitumor activity was detected by the potato disc assay, which measures the inhibition of crown gall tumors on potato discs inoculated with *Agrobacterium tumefaciens* (11). This assay shows excellent agreement with 3PS (P-388 murine leukemia) results.

The brine shrimp and potato disc assays are suggested as convenient supplements to the traditional 9KB and 3PS antitumor assays (12), sparing the need for higher animals or their serum (Table 1).

EXPERIMENTAL

PLANT MATERIAL.—*A. schottii* was collected in Brazil (B-811602, PR-47094) under the auspices of Dr. Robert E. Perdue, Medicinal Plant Laboratory, USDA, Beltsville, Maryland, where voucher specimens are maintained.

BIOLOGICAL EVALUATIONS.—The brine shrimp lethality assay and the potato disc assay were performed in our laboratory as previously described (10,11). 9KB cytotoxicities were determined in the Purdue Cell Culture Laboratory following protocols established by the National Cancer Institute (12).

EXTRACTION AND PARTITION.—The ground stems (11 kg) of *A. schottii* were exhaustively extracted with 95% EtOH and condensed in vacuo. The EtOH residue (1 kg) was partitioned between H_2O and $CHCl_3$ to yield a nontoxic (BS LC_{50} >1000 ppm) interface (600 g), a nontoxic aqueous residue (264 g), and a toxic (BS

TABLE 1. Biological Activity.

Compound	Brine shrimp (BS) LC_{50} (ppm)	9KB ED_{50} (μg/ml)	3PS % T/C	Potato Disc % Inhibition (two determinations)
Isoplumericin	1.7 (0.5/3.5)[a]	2.7 (2)	145 (13)	44 (44/44)[b]
Plumericin	1.7 (0.9/3.6)	2.6 (2)	—	44 (43/45)
Allamandin	21.3 (15/28.3)	2.1 (2)	145 (13)	51 (49/53)
Allamcin	>1000	—	—	Inactive
Scoparone	349 (256/462)	61	—	Inactive
Scopoletin	55 (33/81)	16	133 (13)	20 (18/22)
Pinoresinol	377 (269/500)	100	—	Inactive

[a]95% confidence levels in parentheses.
[b]Values of two determinations in parentheses.

LC_{50} 49 ppm) $CHCl_3$ residue. The $CHCl_3$ residue was partitioned between 90% MeOH and hexane to give two layers and an interface. The interface was collected, triturated with $CHCl_3$, and the clear crystalline plates collected (30 mg) to give allamandin; allamcin (58 mg) was also collected. The hexane layer was condensed, and in the process a precipitate formed. The collected precipitate (0.5 g) proved to be plumericin and isoplumericin. H_2O was added to the 90% MeOH layer to give an 80% MeOH layer, which was partitioned with hexane. Concentration of the hexane layer again formed a precipitate of plumericin and isoplumericin. The filtrate residue (10 g) was nontoxic. The 80% MeOH layer was evaporated to dryness (92 g) (BS LC_{50} 72 ppm).

The 80% MeOH residue (80 g) was subjected to flash chromatography on Si gel (2 kg) with increasing MeOH in $CHCl_3$ as the solvent. Fractions were pooled based on brine shrimp lethality and tlc similarities. Early fractions deposited crude crystals of plumericin and isoplumericin (11 g) that were resolved on the Chromatotron with a 2-mm Si gel rotor using 1 % MeOH in $CHCl_3$ as the solvent. Low pressure chromatography using a Michel-Miller column (200 g Si gel) with 5% EtOAc in $CHCl_3$ yielded scoparone (1.7 g), allamandin (374 mg), scopoletin (205 mg), pinoresinol (298 mg), and allamcin (186 mg).

All of the compounds were identified by mp, ir, ^{1}H-nmr, and ms data (2,6,8,9,14–17). Reference samples of allamandin and allamcin were synthesized from plumericin according to the procedures developed by Trost and co-workers (5,6). Details of the isolation and identification are available from the major author.

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LITERATURE CITED

1. J.E. Little and D.B. Johnstone, *Arch. Biochem.*, **30**, 445 (1951).
2. S.M. Kupchan, A.L. Dessertine, B.T. Blaylock, and R.T. Bryan, *J. Org. Chem.*, **39**, 2477 (1974).
3. J.J.W. Coppen, *Phytochemistry*, **22**, 179 (1983).
4. J. Bhattacharyya and M. De Socorro Q. De Morais, *J. Nat. Prod.*, **49**, 354 (1986).
5. B.M. Trost, M.K.-T. Mao, J.M. Balkovec, and P. Buhlmayer, *J. Am. Chem. Soc.*, **108**, 4965 (1986).
6. B.M. Trost, J.M. Balkovec, and M.K.-T. Mao, *J. Am. Chem. Soc.*, **108**, 4974 (1986).
7. J.J.W. Coppen and A.L. Cobb, *Phytochemistry*, **22**, 125 (1983).
8. F. Abe, T. Mori, and T. Yamauchi, *Chem. Pharm. Bull.*, **32**, 2947 (1984).
9. G.E. Martin, R. Sanduja, and M. Alam, *J. Org. Chem.*, **50**, 2383 (1985).
10. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, and J.L. McLaughlin, *Planta Med.*, **45**, 31 (1982).
11. N.R. Ferrigni, J.E. Putnam, B. Anderson, L.B. Jacobsen, D.E. Nichols, D.S. Moore, and J.L. McLaughlin, *J. Nat. Prod.*, **45**, 679 (1982).
12. R.I. Geran, N.H. Greenberg, N.N. McDonald, A.A. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972).
13. J.L. Hartwell, *Cancer Treat. Rep.*, **60**, 1031 (1976).
14. G. Albers-Schonberg and H. Schmid, *Helv. Chim. Acta*, **44**, 1447 (1961).
15. P. Joseph-Nathan, M. Dominguez, and D.A. Ortega, *J. Heterocycl. Chem.*, **21**, 1141 (1984).
16. M. Windholz and S. Budavari, "The Merck Index," 10th ed., Merck & Co., New Jersey, 1983, pp. 1086, 1208, 1209.
17. C.H. Ludwig, B.J. Nist, and J.L. McCarthy, *J. Am. Chem. Soc.*, **86**, 1186 (1964).

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