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CYTOTOXICITY OF *HYMENOCALLIS EXPANSA* ALKALOIDS

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ABSTRACT.—From the bulbs and leaves of *Hymenocallis expansa* (Amaryllidaceae), three alkaloid constituents were identified: (+)-tazettine, (+)-hippeastrine, and (−)-haemanthidine. These alkaloids demonstrated significant cytotoxicity when tested against a panel of human and murine tumor cell lines.

Hymenocallis expansa (Herb.) Herb. is one of nine species belonging to five genera of the Amaryllidaceae that grow in Puerto Rico (1). The family is well-known for its alkaloid constituents which possess antitumor, antiviral, and a variety of other biological activities, such as analgesic, hypotensive, insecticidal, and insect antifeedant effects (2–4). A phenanthridone derivative, pancratistatin, isolated from *Pancratium littorale* Jacq., is now under preclinical development by the U.S. National Cancer Institute (2,5).

In a screening program to investigate the flora of Puerto Rico for potential anticancer constituents, the crude alkaloidal extracts of *H. expansa* leaves and bulbs demonstrated significant cytotoxicity when tested against a panel of human and murine tumor cell lines. A literature search indicated that this plant had not been investigated previously. The present study has led to the identification of the cytotoxic alkaloids (+)-tazettine, (+)-hippeastrine, and (−)-haemanthidine.

Two of the three alkaloids isolated in this study, i.e., (+)-tazettine and (−)-haemanthidine, belong to the crinine group of alkaloids, whereas (+)-hippeastrine is a lycorenine-type base. Reinvestigation by others of the alkaloid extracts of *Lycoris radiata* Herb. (6) and *Narcissus tazetta* L. (7) has revealed that (+)-tazettine, which was earlier isolated from these plants, was an artifact of the isolation procedure. The naturally occurring alkaloid was actually (+)-pretazettine, which was found to inhibit HeLa cell growth as well as protein synthesis in eukaryotic cells (8,9). Additionally, it inhibits avian myeloblastosis virus reverse transcriptase by binding to the polymerase enzyme itself rather than the template (10). (+)-Pretazettine also exhibited antiviral activity against the Rauscher leukemia virus in mouse embryo cells (11). But other studies at the National Cancer Institute suggest that this activity does not carry over to other tumor models (4). (+)-Hippeastrine, on the other hand, has been reported to possess weak insect antifeedant activity (4). In the current study, the three alkaloids isolated from *H. expansa* demonstrated varying degrees of selective cytotoxicity in a tumor panel cell line (Table 1). Using an ED_{50} of $\leqq 5$ μg/ml as the criterion for significant activity, all three alkaloids were inactive against the BC1 and the vinblastine-resistant KB cell lines. However, (+)-tazettine was active against the Col2 cell line, whereas (−)-haemanthidine demonstrated activity

TABLE 1. Cytotoxic Activity of (+)-Tazettine, (+)-Hippeastrine, and (−)-Haemanthidine.

Compound	Cell line[a]										
	A-431	BC1	Co12	HT	KB	KB-V1	LNCaP	Lu1	Me12	ZR-75-1	P-388
(+)-Tazettine	11.1	>20	5.0	11.8	7.3	>20	5.9	11.1	12.9	6.9	1.0
(+)-Hippeastrine	6.3	>20	7.8	4.7	>20	>20	1.8	5.2	>20	7.3	4.0
(−)-Haemanthidine ..	3.0	>20	6.0	1.6	3.6	8.4	0.7	2.1	1.9	2.6	0.4

[a]A-431, epidermoid carcinoma; BC1, breast; Co12, colon; HT, sarcoma; KB, nasopharyngeal carcinoma; KB-V1, drug resistant KB; LNCaP, prostate; Lu1, lung; Me12, melanoma; ZR-75-1, breast; P-388, murine lymphocytic leukemia. Results are expressed as ED_{50} values (μg/ml).

against the A-431, KB, Lu1, Me12 and ZR-75-1 cell lines. Both (+)-hippeastrine and (−)-haemanthidine were significantly active against the LNCaP and HT cell lines. This work extends the available information on the cytotoxicity profile of the Amaryllidaceae alkaloids.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's (uncorrected) were determined on a Kofler micro hot-stage apparatus. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Uv spectra were taken on a Beckman DU-7 spectrometer, and ir spectra on a Midac Collegian Ft-ir spectrophotometer. Nmr spectra (δ ppm, *J* Hz) were obtained with TMS as internal standard, using Varian XL-300 (300 MHz ^{1}H-nmr, 75.6 MHz ^{13}C-nmr spectra) and Nicolet NT-360 instruments. Eims was measured on a Varian MAT 112S double-focusing mass spectrometer (70 eV). Cims and fabms were recorded on a Finnegan MAT 90 instrument.

PLANT MATERIAL.—*H. expansa* was collected in April 1990 from the sandy beaches west of the mouth of Río Grande de Loíza in Puerto Rico. Their plant was identified by one of us (GRP). A voucher specimen was deposited in the herbarium of the Department of Natural Resources.

EXTRACTION AND ISOLATION OF ALKALOIDS.—The alkaloids from the bulbs (1 kg) and leaves (1 kg) were isolated by standard acid-base partition of the 95% EtOH extracts with 1% aqueous HCl, followed by basification with NH_4OH (pH 9.5) and extraction with $CHCl_3$ to give 5 g (bulbs) and 1.6 g (leaves) of extracts. The crude alkaloidal extract of *H. expansa* leaves exhibited significant cytotoxicity against the HT, LNCaP, Lu1, and ZR-75-1 cell lines (ED_{50} 2.2, 2.3, 2.1, and 2.5 μg/ml respectively). Both extracts were active against the Co12 cell line (ED_{50} 5.0 and 2.2 μg/ml for bulb and leaves extracts, respectively).

Separation of each extract, by radial centrifugal chromatography (Chromatotron, Harrison Research, Palo Alto, California) on Si gel, gave bands from which (+)-tazettine (20 mg, 0.002%, 6% MeOH/$CHCl_3$, bulbs), (+)-hippeastrine (20 mg, 0.002%, 40% MeOH/$CHCl_3$, leaves), and (−)-haemanthidine (24 mg, 0.002%, 10% MeOH/$CHCl_3$, leaves) were crystallized.

(+)-Tazettine.—Pale yellow needles: mp 202–203°; $[\alpha]^{25}D$ +138° (*c*=0.23, $CHCl_3$) [lit. (12) mp 203–204°, $[\alpha]^{25}D$ +160° ($CHCl_3$)]. The physical and spectral data (uv, ^{1}H and ^{13}C nmr, ms) obtained were consistent with those reported for (+)-tazettine (12–14).

(+)-Hippeastrine.—Colorless cubes from $CHCl_3$: mp 211–212°; $[\alpha]^{25}D$ +143° (*c*=0.43, MeOH) [lit. (12) mp 210–212°, $[\alpha]^{25}D$ +160° ($CHCl_3$)]. The identity of the compound was confirmed by spectral comparison (uv, ir, ^{1}H and ^{13}C nmr, ms) with reported data for (+)-hippeastrine (12,15,16).

(−)-Haemanthidine.—Colorless needles from Me_2CO: mp 176–179°; $[\alpha]^{25}D$ −14° (*c*=0.28, $CHCl_3$) [lit. (12) mp 189–190°, $[\alpha]^{25}D$ −41° ($CHCl_3$)]. ^{13}C nmr (pyridine-d_5) δ 129.5 (C-1), 130.1 (C-2), 73.6 (C-3), 73.4 (C-3), 29.1 (C-4), 29.3 (C-4), 56.1 (C-4a), 62.9 (C-4a), 86.8 (C-6), 89.2 (C-6), 130.4 (C-6a), 131.8 (C-6a), 109.0 (C-7), 110.2 (C-7), 146.2 (C-8), 147.6 (C-9), 103.3 (C-10), 137.1 (C-10a), 138.2 (C-10a), 51.2 (C-10b), 51.7 (C-10b), 79.5 (C-11), 80.6 (C-11), 54.3 (C-12), 59.9 (C-12), 101.2 (OCH_2O), 56.0 (OMe), 57.7 (OMe). Comparison of physical and spectral data (mp, $[\alpha]^{25}D$, uv, ir, ^{1}H nmr, ms) with those reported for (−)-haemanthidine confirmed the compound identity (12,13,17). The ^{13}C-nmr data has not appeared in the literature previously and the data are reflective of the tendency of this molecule to exist as different epimers.

CYTOTOXIC ASSAYS.—The isolated alkaloids were tested for cytotoxic activity against 11 cell lines (Table 1). These evaluations were performed as described in the literature (18).

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