Journal of Natural Products
Vol. 58, No. 5, pp. 756-759, May 1995

ANTINEOPLASTIC AGENTS, 301. AN INVESTIGATION OF THE AMARYLLIDACEAE GENUS *HYMENOCALLIS*[1]

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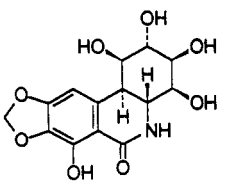
ABSTRACT.—Seven species (and one cultivated variety) of *Hymenocallis* (Amaryllidaceae) and the related *Pancratium maritima*, representing a broad geographical selection, were investigated as sources of pancratistatin [**1**] now undergoing preclinical development as an anticancer agent. Pancratistatin [**1**] was found to be a constituent of *H. speciosa* (Singapore), *H.* variegated (Singapore), *H. pedalis* (Seychelles), *H. expansa* (Bermuda), *H. sonoranensis* (Mexico), and *P. maritimum* (Israel). Only two species of *Hymenocallis* failed to yield one or more of the related cell-growth inhibitory isocarbostyrils such as narciclasine [**3a**], 7-deoxynarciclasine [**3b**], and 7-deoxy-*trans*-dihydronarciclasine [**2**].

Over the past 2,400 years, primitive medical records have revealed the use of extracts from many species of the plant family Amaryllidaceae for a variety of human medical problems, and cancer treatment represents a prominent application (2,3). Our long-term study (2–10) of Amaryllidaceae species as sources of potential anticancer and antiviral drugs has led to the discovery of antineoplastic and antiviral isocarbostyrils such as pancratistatin [**1**][3] and 7-deoxy-*trans*-dihydronarciclasine [**2**] in *Hymenocallis* Salisb. (2,3,9,10) and *Zephyranthes* Herb.



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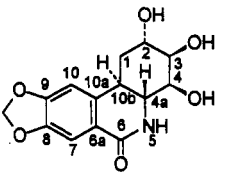

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(7,8) species. Because pancratistatin [**1**] has been undergoing preclinical development and the discovery of additional sources of this potentially useful substance has become necessary, we have evaluated seven *Hymenocallis* species (and one cultivated variety of unknown derivation) from geographically widely separated regions. In addition, *Pancratium maritimum* L. was studied while applying considerable effort to cloning (2,3) the original source of pancratistatin (9,10), *Hymenocallis littoralis* (Jacq.) Salisb.

Both *H. speciosa* (Salisb.) Salisb. and *H.* variegated obtained in Singapore, *H. pedalis* Herb. collected in the Republic of Seychelles, *H. expansa* (Herb.) Herb. from Bermuda, *H. sonoranensis* Standley, common in tropical locations of Mexico, as well as *P. maritimum* from Israel were found to contain pancratistatin [**1**] in 10^{-3} to 10^{-4}% yields. Interestingly, only the Singapore *H.*×'Tropical Giant' and

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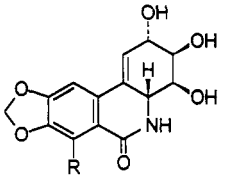
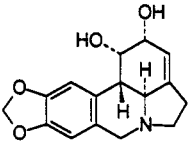
[3]We initially discovered pancratistatin (9,10) in a Hawaiian species originally designated *Pancratium littorale* and recently reidentified as *Hymenocallis littoralis* (2,3).

Ismene ×'Advance' from the Netherlands failed to provide any member of the isocarbostyril series [**1–3**] while *Ismene* ×'Sulphur Queen' yielded 7-deoxynarciclasine [**3b**]. The first isolation of pancratistatin [**1**] from *P. maritimum*, reported here, also requires comment. Previous studies of this plant collected in



3a R=OH
3b R=H



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Egypt (11) and Turkey (12) failed to uncover pancratistatin, but did afford a common alkaloid constituent of this family, lycorine [**4**].

While the present investigation did not lead to a more productive source of pancratistatin than our cloned *H. littoralis*, it did provide an interim supply. A scale-up collection (390 kg) of the Seychelles *H. pedalis* was used to isolate 11.3 g (2.9×10^{-3}% yield) of pancratistatin [**1**] and 4.54 g (0.001% yield) of 7-deoxynarciclasine [**3b**]. Flowers from our greenhouse cultivation of *H. pedalis* were utilized for taxonomic identification. For the final purification of pancratistatin [**1**] a procedure was developed based on acetylation→Si gel cc→CH_3OH/NH_3 deacetylation to afford colorless pancratistatin. Research directed at the discovery of more productive natural sources of pancratistatin [**1**] will be continued.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All solvents used were redistilled. Tlc was performed on Si gel GHLF uniplates (0.25-mm layer thickness) supplied by Analtech, Inc., using CH_2Cl_2-MeOH (3:1) as mobile phase and ceric sulfate spray reagent for development. Sephadex LH-20 (particle size 25–100 μm) was supplied by Pharmacia. For other general methods refer to Pettit *et al.* (2). The ^{1}H-nmr spectra were obtained using a Varian Gemini 300 MHz spectrometer with DMSO-d_6 as solvent and TMS as internal standard.

PLANT MATERIAL.—Bulbs (wet wt) from each of the following specimens were utilized: *Hymenocallis caribaea* (L. emend Gawler) Herbert (8.6 kg), *H. latifolia* (Mill.) Roemer (2.5 kg), *H.* variegated (1.02 kg), *H. speciosa* (1.7 kg), *H.* ×'Tropical Giant' (1.5 kg), all obtained in Singapore from commercial growers; *Ismene*×'Sulphur Queen' (1.5 kg) and *Ismene*×'Advance' (1.5 kg) were obtained from commercial sources in the Netherlands; *Hymenocallis sonorensis* (2.5 kg) was obtained from the Desert Sonoran Museum in Tucson, Arizona; *H. expansa* (1.5 kg) was collected in Bermuda; *Hymenocallis pedalis* (390 kg wet wt bulbs) was collected on La Digue Island, Republic of the Seychelles; and *Pancratium maritimum* (1.5 kg) was collected in Israel. Voucher specimens are maintained in our University greenhouses and their flowers were used for taxonomic identification by one of us (A.M.).

ISOLATION OF PANCRATISTATIN [**1**] AND RELATED ISOCARBOSTYRILS.—In a typical experiment, the chopped bulbs (1.0 kg) of each species were extracted with MeOH-CH_2Cl_2 (1:1, 3 liters) at room temperature for 15 days. Pancratistatin [**1**] and/or the related isocarbostyrils were isolated as summarized in Pettit *et al.* (2,3). The samples of pancratistatin and other previously known natural products isolated from each species were found to be identical by Si gel tlc using CH_2Cl_2-MeOH (3:1) and by high-resolution ^{1}H-nmr spectroscopy. The following results were obtained.

Hymenocallis speciosa.—A 1.76-g *n*-BuOH fraction yielded 3.0 mg of pancratistatin (**1**, 3.0×10^{-4}%) and 2.0 mg of narciclasine (**3a**, 2.0×10^{-4}% yield).

Hymenocallis expansa.—From 950 mg of a *n*-BuOH fraction, 4.0 mg of pancratistatin (**1**, 4.0×10^{-4}%) and 3.0 mg of narciclasine (**3a**, 3.0×10^{-4}%) were obtained.

Hymenocallis sonorensis.—A 1.2-g *n*-BuOH fraction afforded 3.0 mg of pancratistatin (**1**, 3.0×10^{-4}%).

Ismene ×'Sulphur Queen'.—A 970-mg *n*-BuOH fraction provided 32 mg of 7-deoxynarciclasine (**3b**, 3.2×10^{-3}%).

Ismene ×'Advance'.—From 212 mg of a *n*-

BuOH fraction, the analagous separation procedure did not yield any of the antineoplastic isocarbostyrils.

Hymenocallis ×'Tropical Giant'.—Again 1.14 g of a *n*-BuOH fraction did not yield any of the antineoplastic isocarbostyrils.

Hymenocallis variegated.—Here a 3.15-g *n*-BuOH fraction led to 6.0 mg of pancratistatin being isolated (**1**, 6.0×10^{-4}%).

The wet bulbs of *Hymenocallis pedalis* (390 kg) were shipped via air, chopped (Tomahawk Chipper), and placed in five 55-gallon steel containers with 120 liters of MeOH per container. The alcohol level was adjusted to ca. 12 cm above the tissue level. The plant was extracted (2×) for periods of 11 to 45 days. After each extraction, the CH_3OH layer was decanted and concentrated to an aqueous phase. The H_2O fraction was partitioned (5×) against *n*-BuOH (equal volume). The *n*-BuOH was evaporated and the residue was redissolved in CH_3OH (1.2 liters). Me_2CO was added (2.9 liters) and the resulting milky tan precipitate was collected. The solid was washed (3×) with CH_3OH/H_2O. The dark combined filtrate was evaporated to dryness to afford a 1.65-kg residue that was active against the P-388 lymphocytic leukemia cell line. An aliquot (80 g) of the residue was dissolved in 250 ml of CH_3OH and the solution filtered. The 58 g (after recrystallization from DMF-Et_2O) of lycorine [**4**], which crystallized, was collected, and the solution chromatographed on a column of Sephadex LH-20 using CH_3OH as eluent. The remaining 1,570 g of a CH_3OH-soluble active (P-388) residue was partitioned between CH_2Cl_2 and CH_3OH-H_2O (3:2). Roughly 75% remained in the CH_3OH-H_2O solution. The remaining CH_2Cl_2 extract (P-388 inactive) was discarded. The CH_3OH/H_2O fraction was again evaporated to dryness, leaving a residue weighing 1,077 g. The residue was divided into eleven portions and each was chromatographed on a column (15-cm diameter) of Sephadex LH-20 with CH_3OH as eluent. Pancratistatin-rich fractions totaled 13.8 g, and were divided into 6.8 g and 7.0 g amounts. Each aliquot was acetylated by addition of pyridine (200 ml) and Ac_2O (70 ml), maintained at room temperature for 16 h and monitored by tlc ($CHCl_3$-MeOH, 5:1). Upon addition of ice H_2O, precipitation of the product occurred. The light-brown crystals were collected (filtration), washed with H_2O, and dried (Na_2SO_4) to give two acetate fractions (8.7 g and 5.3 g). The filtrate was extracted with $CHCl_3$ (2×2.0 liters) and washed with 5% HCl (1×), $KHCO_3$ (1×), and brine (4×), dried (Na_2SO_4), and the solvent removed to give a brown oil (8.9 g). Both crystalline fractions and the oil were separately dissolved in a small amount of $CHCl_3$ and each was passed through a SiO_2 gel column using $CHCl_3$ as eluent. Fractions were monitored by tlc and similar fractions combined to give four new fractions. Each fraction was analyzed by 300 MHz nmr spectroscopy. Results revealed fraction I to be less polar impurities, fraction II (colorless solid) a pancratistatin tetraacetate derivative, fraction III (colorless solid) the pancratistatin peracetate derivative, and fraction IV proved to be a mixture of the components found in fractions I, II, and III. Fraction IV was rechromatographed in $CHCl_3$ on a column of SiO_2 to give more of fractions II and III. The tetraacetate and peracetate derivatives of pancratistatin [**1**] from fractions II and III were dissolved in a small volume of $CHCl_3$, followed by addition of CH_3OH and 1–3 ml of H_2O. The reaction mixture was saturated with gaseous NH_3 for 30 min at −78° in a dry ice/Me_2CO bath. At room temperature (several hours) colorless crystals began forming. Completion of the ammonolysis deprotection reaction was monitored by tlc ($CHCl_3$-MeOH 3:1). The crystalline product was collected, washed with excess MeOH, and dried to provide 11.3 g of pure (by 300 MHz nmr) pancratistatin (**1**, 2.9×10^{-3}% overall yield).

Pancratium maritimum.—A 1.4-g *n*-BuOH fraction led to 6.0 mg of pancratistatin (**1**, 1.0×10^{-3}% yield).

ACKNOWLEDGMENTS

Financial assistance was provided by Outstanding Investigator Grant CA-44344-01A1-06 and Grant CA-16049-05-12, Division of Cancer Treatment, National Cancer Institute, DHHS; the Fannie E. Rippel Foundation; Eleanor W. Libby; the Arizona Disease Control Research Commission; Herbert and Diane Cummings; the Nathan Cummings Foundation, Inc.; and the Robert B. Dalton Endowment Fund. We are also pleased to thank the Government of the Republic of Seychelles (Dr. Selwyn Genron, Mr. Victorin Laboudallon, Mr. Cedras Aterville, and Mr. Radley Webber), Dr. Roberta L. Dow, Dr. Fiona Hogan and Larry P. Tackett, Denise Nielsen-Tackett, David A. Webb, Joy A. Bell, Joe Tyler, Fernando Reyna, and Dr. Mark Dimmitt of the Arizona Sonora Desert Museum, Tucson, Arizona.

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Received 6 September 1994