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ANTINEOPLASTIC AGENTS, 294[1]. VARIATIONS IN THE FORMATION OF PANCRATISTATIN AND RELATED ISOCARBOSTYRILS IN *HYMENOCALLIS LITTORALIS*

GEORGE R. PETTIT,* GEORGE R. PETTIT III, RALPH A. BACKHAUS, and FRED E. BOETTNER

Cancer Research Institute, Department of Chemistry and Department of Botany, Arizona State University, Tempe, Arizona 85287-1604

ABSTRACT.—By cloning *Hymenocallis littoralis*, a practical biosynthetic procedure was developed for producing pancratistatin [**1**]. The plant tissue culture→greenhouse→field production sequence was successively utilized for increasing an original 1.5 kg of wild *H. littoralis* bulbs to some 60,000 bulbs at present. In the central Arizona Sonoran Desert, the tropical *H. littoralis* was found to reach a maximum pancratistatin content in October and a minimum in May. Generally pancratistatin [**1**] was accompanied by lesser yields of narciclasine [**2**], 7-deoxynarciclasine [**3**], and 7-deoxy-*trans*-dihydronarcicasine [**4**]. Improved laboratory and pilot-plant scale techniques were also developed for the isolation of pancratistatin [**1**] from difficult-to-separate mixtures of narciclasine [**2**] and 7-deoxynarciclasine [**3**] occurring in *H. littoralis*.

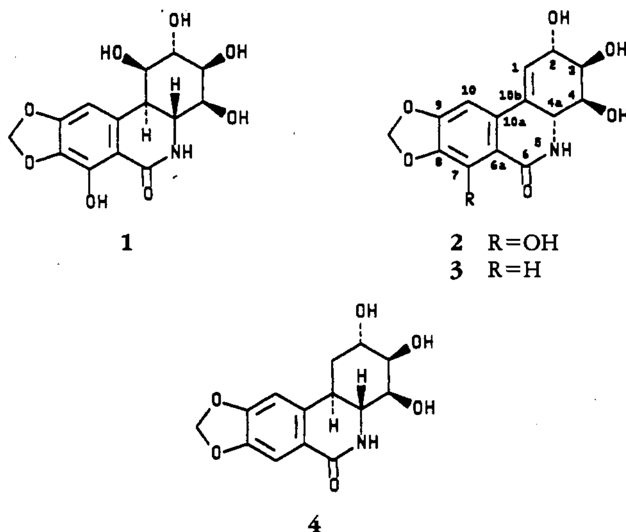
Plants of the family Amaryllidaceae comprise about 860–1100 species in 85 genera, which are often bulbous and are usually found growing in tropical and subtropical regions. More than 30 species in 11 genera have been used in the primitive treatment of human cancer (2). Six species in the genus *Hymenocallis* (New World, closely related or synonymous with the genus *Pancratium*, Old World) have found application in the folk medicinal management of cancer. The first chemical investigation (3, in 1920) of a *Hymenocallis* (4) species (*littoralis*) (*Pancratium littorale*) led to the isolation of lycorine (5) which subsequently proved to have antineoplastic and antiviral (herpes simplex virus) activities (6). No further study of *H. littoralis* appeared until we reported (5) that the bulbs contained a new phenanthridone biosynthetic product designated as pancratistatin [**1**], which proved to be very effective in vivo against the murine P-388 lymphocytic leukemia and M-5076 ovary sarcoma.

More recently, pancratistatin [**1**] was found to exhibit a highly characteristic differential cytotoxicity profile against the U.S. National Cancer Institute's (NCI) panel of sixty human cancer cell lines. The melanoma subpanel lines were most sensitive followed by certain brain, colon, lung, and renal cancer lines (7). Pancratistatin has also been found to exhibit strong RNA antiviral activity. For example, it increased survival by 100% when used to treat mice infected with Japanese encephalitis. Furthermore, it showed activity against two other RNA-containing flaviviruses (yellow fever and dengue) and against the bunyaviruses Punta Tora and Rift Valley fever (8,9). During the original 1985 isolation of pancratistatin, companion antineoplastic constituents of *H. littoralis* were found to be narciclasine [**2**] and 7-deoxynarciclasine [**3**]. For the preceding compelling reasons and others (10), pancratistatin has been undergoing development toward human clinical trials. Because this superficially rather straightforward target for total synthesis has so far resisted solution by an efficient route, in our hands and others (11,12), a practical source of pancratistatin became a serious problem.

RESULTS AND DISCUSSION

By 1987, plant biosynthesis seemed to be the most economical and efficient way to produce pancratistatin [**1**], so a biotechnological approach was developed, which involved a large tissue culture cloning operation of *H. littoralis* bulbs. These "seedling"

[1]For part 293, see Pettit *et al.* (1).



replicas were transplanted to potting soil under greenhouse conditions and then final planting in native soil, exposing the plant to light and temperature conditions typical of arid Tempe, Arizona (4). Fortunately, *H. littoralis* produces an average of ten asexually produced offsets per year. After two years at that rate, cloning was terminated. Overgrown or root-bound plants in our shade cloth-covered greenhouses were removed from their 5-gallon containers, washed free of soil, and their offsets separated. The mother bulb was returned to its 5-gallon container. Fresh soil was added along with two or three offsets, and the remaining offsets planted in furrows of an open field using flood irrigation techniques for watering. During the growing season, the soil was always kept damp in both greenhouse and field environments.

Because *Hymenocallis littoralis* normally grows in tropical to subtropical regions, day length and temperature do not fluctuate much during the year and precipitation is plentiful. The growing season is year-round, with several flowering periods. However, in the arid Sonoran Desert of Arizona, the growing season for this organism proved to be only six months (early April–late October) with one flowering period (July–August) and then a dormancy of six months (early November to late March). Temperatures in our experimental field plot fluctuated from 0°–50° (shade readings) and precipitation was some 15 cm/year.

Because of the uncertain effects of growth in the Sonoran desert region, a two-year experiment was developed to study concentrations of pancratistatin [**1**] in greenhouse and field-grown bulbs. The results obtained would then enable a decision about whether or not *H. littoralis* could be grown economically under such conditions, hence, reducing development and shipping costs and determining if this biosynthetic route would still be the best source for clinical supplies of pancratistatin. Bulbs of *H. littoralis* were grown and analyzed to determine whether or not the pancratistatin content would strongly fluctuate or maintain a steady level when grown in non-environmentally controlled greenhouses or open fields of the Central Arizona Salt River flood plain. Greenhouse plants were examined for an entire two-year period (1991–1992), whereas field bulbs were studied from March 1992–January 1993. Mature field-grown plants were not available until March of 1992. A 1.0-kg bulb sample was collected each month for extraction and analysis.

The results clearly indicated that pancratistatin [**1**] levels do markedly fluctuate over the year and that these levels reflect the growth pattern of *H. littoralis*. During October,

the pancratistatin content was found to reach its highest levels (22 mg/kg wet wt). At this point, in Arizona, the plant had undergone six months of growth to produce the necessary primary and secondary metabolic products. Also noted during the growing period was a drop in pancratistatin yields from mid-June–mid-August while the plant produced flowers. During this period the plant probably puts less emphasis on secondary metabolites and more on reproduction. Once the flowering stage lapsed, pancratistatin yields increased rapidly. Over the yearly growth period, yields of the closely related narciclasine [**2**], 7-deoxynarciclasine [**3**], and 7-deoxy-*trans*-dihydronarciclasine [**4**] also varied. After October, when the plant went into dormancy, levels of pancratistatin were found to drop off drastically to only 4.0 mg/kg (wet wt) by May. Even though the plant ends its dormancy in late April, presumably there is a lag period in May when the plant concentrates mainly on food production (see Figure 1).

Field-grown bulbs, which also showed monthly changes in pancratistatin [**1**] content, generated somewhat smaller amounts of the desired product (2.0–5.0 mg/kg) when compared to greenhouse biosynthesis for the same time period (see Figure 2). Such changes could be directly related to differences in environmental conditions. Soils used for growing were quite different. Greenhouse bulbs received a forest mulch high in organic nutrients and were fertilized. The plant presumably acquired most of these artificial nutrients directly affecting secondary plant product production. Field bulbs were grown in flood plain clay-type soil low in essential nutrients. Fertilizer granules were placed around leaf bases. Because flood irrigation methods were required for mass cultivation, the amount of dissolved nutrients actually reaching *H. littoralis* roots was probably minimal. Temperature, light, and humidity also act as important external factors. Field plants were exposed to hot, dry, summer air with intense direct light, resulting in faster rates of evapotranspiration. The net results were shorter leaves and less growth. Greenhouse-grown plants received reduced amounts of light through an overhead shade cloth which brought temperatures and the rate of evapotranspiration down somewhat. Because the greenhouse was a closed unit with moderate ventilation

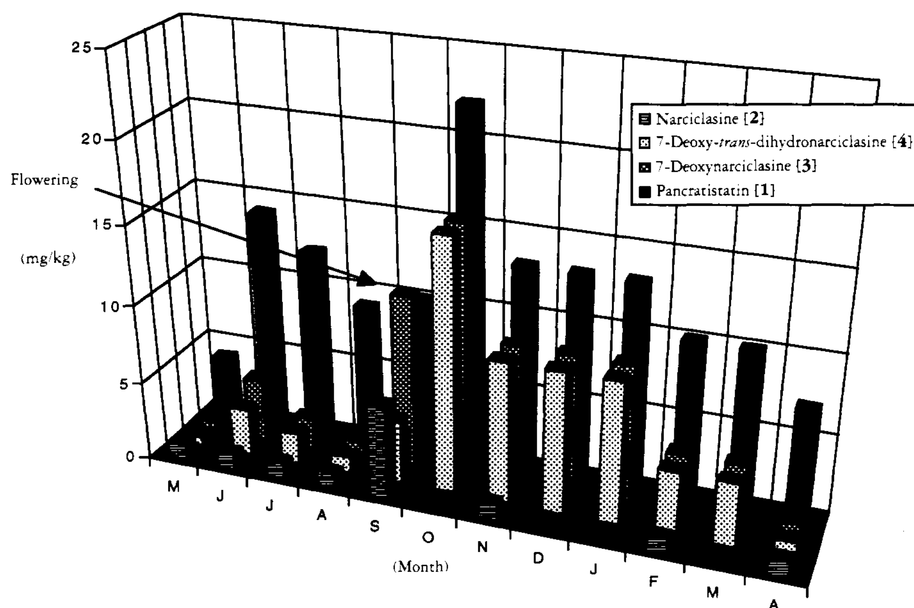


FIGURE 1. Fluctuations of antineoplastic constituents found in greenhouse-grown bulbs of *Hymenocallis littoralis* during a one-year growth period.

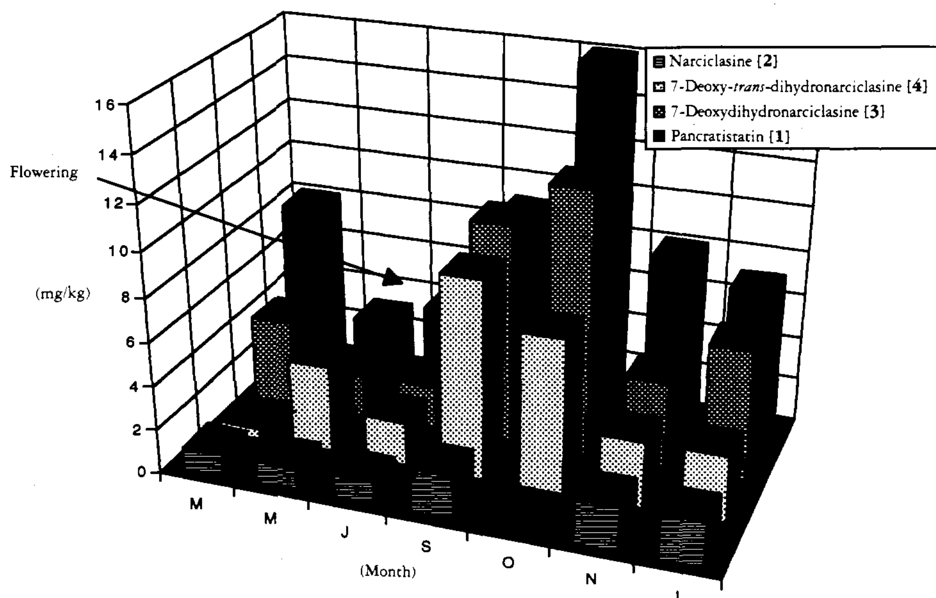


FIGURE 2. Fluctuations of antineoplastic constituents found in field-grown bulbs of *Hymenocallis littoralis* during a one-year growth period.

and an overhead sprinkler watering system, the humidity levels were much higher compared to outdoors even during the months of July and August when summer monsoon storms cross the Salt River Valley. The result of this enclosed environment was an apparent increase in growth and pancratistatin biosynthesis over that experienced with the open field cultivation.

While the preceding biosynthethic route to pancratistatin [**1**] production was in its initial stage, a final large-scale (100 kg) recollection (Hawaii) of wild *H. littoralis* was undertaken and used to isolate 15 g (0.015% yield) of pure pancratistatin. Here a pilot plant-type procedure was developed that eliminated use of the *n*-BuOH extraction and Sephadex LH-20 chromatography steps that are very effective for laboratory-scale isolation of pancratistatin (3), but costly on a large scale. Again the isolation of pancratistatin was complicated by the closely related isocarbostyrils **2** and **3**. In general, the improved laboratory- and large-scale isolations of pancratistatin described in the Experimental proved efficient and rapid. The techniques described herein will now be applied to isolating pancratistatin from the current large-scale cultivation of *H. littoralis* amounting to some 60,000 bulbs. If not for the monthly evaluation of pancratistatin content summarized above, the maximum content (October) could have easily been missed. Clearly the foundation for future production of pancratistatin from plant material has been established.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Tlc was performed on Si gel GHLF uniplates (0.25 mm layer thickness) supplied by Analtech, Inc., using CH_2Cl_2-CH_3OH (3:1) as mobile phase and ceric sulfate spray reagent for development. In the large-scale isolation experiments involving pancratistatin [**1**], tlc analyses were conducted with Polygram SilG/μv 254 plastic plates (Brinkman). The eluent was EtOH-CH_2Cl_2 (1:4) and either uv or anisaldehyde spray (2.0 g anisaldehyde, 194 g glacial HOAc and 4 g of H_2SO_4) was employed for development. With this solvent system, pancratistatin [**1**] was found at R_f 0.46 and 7-deoxynarciclasine [**3**] at R_f 0.38. Pancratistatin and narciclasine [**2**] were found at the same R_f using the EtOH-CH_2Cl_2 (1:4) mobile phase. For the latter problem, use of a Whatman C_{18} reversed-phase plate eluted with 15% THF in

saline gave a separation with R_f values of 0.15 and 0.09, respectively, for pancratistatin [**1**] and narciclasine [**2**]. The identity of each compound was established by comparison tlc, ^{1}H nmr (300 MHz), and ir. Sephadex LH-20 (particle size 25–100 μm) was supplied by Pharmacia Co. For the small-scale isolation, a Gilson Microfractionator was used for fractionations. The ^{1}H-nmr spectra were recorded with a Varian-Gemini 300 MHz spectrometer using DMSO as internal standard. Other procedures as previously described (7).

PLANT MATERIAL.—Specimens of *Hymenocallis littoralis* Salisb. were grown in our Institute by tissue culture methods (4). Asexually produced offsets were separated and replanted. The initial 1.5 kg (wet wt) of *H. littoralis* bulbs employed in the present study came from Hawaii and a voucher specimen has been deposited in the Harold L. Lyon Arboretum, University of Hawaii. Originally, this plant was identified as *Pancratium littorale* Jacq. (3) but was taxonomically reidentified recently by Prof. Alan W. Meerow (University of Florida) (7). Flowers from our greenhouse plants were utilized for the definitive taxonomic studies.

REPRODUCTION OF *HYMENOCALLIS LITTORALIS*.—For simplification, a description of the general procedures used for cultivating *H. littoralis* in the Arizona State University greenhouses and Salt River flood plain farm have been outlined in the Results and Discussion section.

ISOLATION OF PANCRATISTATIN [**1**].—In a typical experiment, a 1.0-kg (wet wt) bulb sample was removed from the greenhouse on October 15, 1991, chopped into small pieces and extracted with CH_2Cl_2-MeOH (1:1) (3 liters) for 15 days. The viscous solution was filtered through muslin, to remove large particles, and the filtrate transferred to a 5.0-liter separatory funnel. Phase separation occurred due to H_2O extracted from the bulb tissue. The lower orange-brown CH_2Cl_2 layer was separated and discarded. The pale yellow MeOH/H_2O solution was filtered through cotton to remove a very fine silty sediment and the filtrate evaporated to 250 ml. The solution was transferred to a 1.0-liter separatory funnel and diluted to twice the volume with distilled H_2O. The aqueous mixture was extracted (3×40 ml) with *n*-BuOH. The *n*-BuOH extract was combined and evaporated to an orange-brown residue weighing 0.38 g. The residue was redissolved in 3.0 ml of MeOH and Me_2CO (50 ml) added whereupon a silky white precipitate formed. The solution was placed overnight in a refrigerator to ensure complete precipitation and then filtered. The orange-brown filtrate was rotoevaporated to an orange-brown resin weighing 0.15 g. The resin was dissolved in 2.0 ml of CH_3OH and chromatographed (fractions were monitored by tlc) on a Sephadex LH-20 column (80 g 106×3 cm) using CH_3OH as eluent. Fractions containing the components with tlc R_f 0.37 and R_f 0.48 were allowed to evaporate slowly over several days whereupon sharp needles (straw colored) appeared. The crop with R_f 0.37 weighed 31 mg (3.1×10^{-3}% yield), and the product with R_f 0.48 weighed 22 mg (2.2×10^{-3}% yield). The tlc R_f 0.48 component corresponded to pure pancratistatin [**1**] while the two-constituent mixture (1:1) at R_f 0.37 contained 7-deoxynarciclasine [**3**] and 7-deoxy-*trans*-dihydronarciclasine [**4**].

Over a two-year period the following 1.0-kg (wet wt) bulb samples were harvested (on the fifteenth of each month noted) from our greenhouses (see Figure 1). The first year (1991) included the months of February (10 mg of pancratistatin [**1**]; 1×10^{-3}%; 2 mg of narciclasine [**2**], 2×10^{-4}%; 4 mg of 7-deoxynarciclasine [**3**], 4×10^{-4}%; and 4 mg of 7-deoxy-*trans*-dihydronarciclasine [**4**], 4×10^{-4}% yields), April: **1** (7 mg, 7×10^{-4}%), **2** (2 mg, 2×10^{-4}%), **3** (1 mg, 1×10^{-4}), and **4** (1 mg, 1×10^{-4}% yields), June: **1** (14 mg, 1.4×10^{-3}%), **2** (1 mg, 1×10^{-4}%), **3** (4 mg, 4×10^{-4}%), and **4** (3 mg, 3×10^{-4}% yields), August: **1** (9 mg, 9×10^{-4}%), **2** (1 mg, 1×10^{-4}%), **3** (1 mg, 1×10^{-4}%), and **4** (1 mg, 1×10^{-4}%), October: **1** (22 mg, 2.2×10^{-3}%), **2** was not detected, **3** (16 mg, 1.6×10^{-3}%), and **4** (16 mg, 1.6×10^{-3}%), and December: **1** (13 mg, 1.3×10^{-3}%), **2** was not detected, **3** (9 mg, 9×10^{-4}%), and **4** (9 mg, 9×10^{-4}% yields). Year two (1992) covered the months of January: **1** (13 mg, 1.3×10^{-3}%), **2** was not detected, **3** (9 mg, 9×10^{-4}%), and **4** (9 mg, 9×10^{-4}% yields), March: **1** (10 mg, 1×10^{-3}%), **2** was not detected, **3** (4 mg, 4×10^{-4}%), and **4** (4 mg, 4×10^{-4}% yields). May: **1** (4 mg, 4×10^{-4}%), **2** (1 mg, 1×10^{-4}%), **3** (0.5 mg, 5×10^{-5}%), and **4** (0.5 mg, 5×10^{-5}% yields), July: **1** (12 mg, 1.2×10^{-3}%), **2** (1 mg, 1×10^{-4}%), **3** (2 mg, 2×10^{-4}%), and **4** (2 mg, 2×10^{-4}% yields), September: **1** (10 mg, 1×10^{-3}%), **2** (6 mg, 6×10^{-4}%), **3** (11 mg, 1.1×10^{-3}%), and **4** (4 mg, 4×10^{-4}% yields), and November: **1** (13 mg, 1.3×10^{-3}%), **2** (1 mg, 1×10^{-4}%), **3** (9 mg, 9×10^{-4}%), and **4** (9 mg, 9×10^{-4}% yields). Bulb samples (1 kg wet wt) were taken from the open field crop (1992–93 cf., Figure 2) during the months of March: **1** (8 mg, 8×10^{-4}%), **2** (1 mg, 1×10^{-4}%), **3** (4 mg, 4×10^{-4}%), and **4** (0.5 mg, 5×10^{-5}% yields), May: **1** (3 mg, 3×10^{-4}%), **2** (1 mg, 1×10^{-4}%), **3** (2 mg, 2×10^{-4}%), and **4** (4 mg, 4×10^{-4}% yields), July: **1** (4 mg, 4×10^{-4}%), **2** (1 mg, 1×10^{-4}%), **3** (2 mg, 2×10^{-4}%), and **4** (2 mg, 2×10^{-4}% yields), September: **1** (9 mg, 9×10^{-4}%), **2** (2 mg, 2×10^{-4}%), **3** (10 mg, 1×10^{-3}%), and **4** (9 mg, 9×10^{-4}% yields), November: **1** (8 mg, 8×10^{-4}%), **2** (2 mg, 2×10^{-4}%), **3** (4 mg, 4×10^{-4}%), and **4** (3 mg, 3×10^{-4}% yields), and January: **1** (7 mg, 7×10^{-4}%), **2** (2 mg, 2×10^{-4}%), **3** (6 mg, 6×10^{-4}%), and **4** (3 mg, 3×10^{-4}% yields).

LARGE-SCALE ISOLATION OF PANCRATISTATIN [**1**] FROM WILD *HYMENOCALLIS LITTORALIS*.—The dried and

cut bulbs of *Hymenocallis littoralis* (100 kg), collected in Hawaii in 1988, were ground in a Wiley Mill using a 4-mm screen yielding 99 kg of fine powder. The total amount was placed in a stainless steel, open head, 125-gallon vessel having a conical bottom with a 2-cm center drain. The center drain orifice was covered with a piece of 1-cm stainless steel wire mesh followed by 8 layers of freshly washed burlap. The burlap was covered with a 1-m circle of 80 mesh stainless steel screen wire held down with two circles of stainless steel. The burlap assembly acted as a very efficient filter. The powdered bulb was covered with 220 liters of 70% EtOH, and the solution percolated (drained and readded) through the plant material at a rate of 60–64 liters/h. During initial percolation, it was necessary (because of plant material swelling) to add another 100 liters of 70% EtOH. After percolation for 5 h, the mixture was covered with EtOH and extraction continued overnight. The dark red extract (136 liters) was drained and the plant material covered with 144 liters of 70% EtOH, percolation continued for 3 h and overall extraction for 2 days. Before tlc analysis showed essentially all pancratistatin had been removed, three more extractions were performed to provide 768 liters of EtOH extract (from a total of 912 liters of EtOH) which was concentrated to 320 liters. Pancratistatin was not detected in the fifth extract upon tlc analysis; however, a trace of narciclasine [**2**] was noticed.

The 320 liters of EtOH extract were placed in a 900-liter stainless steel tank with a conical base leading to a 5-cm drain connected to a glass concentric reducer and a 2.5-cm glass valve to provide a very effective separatory funnel. The extract was diluted with 200 liters of H_2O and 220 liters of CH_2Cl_2. The mixture was stirred vigorously for 30 min and allowed to separate overnight. The dark red CH_2Cl_2 layer was removed and concentrated (Rodney Hunt evaporator). Solvent partitioning was repeated twice more with 144-liter and 140-liter portions of CH_2Cl_2, and the combined CH_2Cl_2 extract concentrated to 72 liters. The chlorocarbon extract was diluted with 40 liters of H_2O and 20 liters of 95% EtOH. After separation (overnight), the aqueous layer was added to the previous aqueous alcohol phase.

To the combined aqueous EtOH solution from CH_2Cl_2 partitioning was added 66 kg of NaCl and the mixture stirred vigorously until all salt dissolved. The solution was stirred (30 min) with 166 liters of EtOAc and the phases were allowed to separate over 2 days. The EtOAc extraction was repeated (4× with a total of 733 liters of EtOAc) and solvent removed to yield 926 g of solid residue.

Two columns (12 cm×3.3 m each) in series were prepared with 63 kg of Davisil 633 Si gel (200–325 mesh), suspended in CH_2Cl_2-MeOH (95:5) and washed to equilibrium with CH_2Cl_2-EtOH (95:5). The 926-g EtOAc fraction was diluted with 5% EtOH in CH_2Cl_2 and the solution filtered to remove 30 g of NaCl. The filtrate was pumped onto the columns and chromatographed with a stepwise gradient of EtOH in CH_2Cl_2 from 5% to 25% EtOH. Following elution with 360 liters, 16.7 g of pancratistatin [**1**] were obtained from the next 100 liters of eluent. Another 80-liter elution gave a mixture (31.6 g) of pancratistatin [**1**] and 7-deoxynarciclasine [**3**] and the following 120 liters afforded 16.9 g of 7-deoxynarciclasine [**3**]. The carbostyril mixture of **1** and **3** (31.6 g) was heated in refluxing glacial HOAc (300 ml) and the hot solution filtered (sintered glass). The insoluble residue was heated again with glacial HOAc (300 ml) and filtered. The remaining residue was washed well with CH_2Cl_2, and dried (vacuum desiccator over P_2O_5, $CaCl_2$, and NaOH) to provide 15.9 g of pancratistatin [**1**]. The glacial HOAc filtrate was cooled, and light tan 7-deoxynarciclasine [**3**] collected, washed with CH_2Cl_2 and dried (as above) to afford 12.0 g. The 32.6 g total amount of pancratistatin was dissolved in 265 ml of hot DMF, treated with 5 g of carbon black, cooled to room temperature, the solution filtered through a 1-cm bed of Celite 545 and the filtrate diluted with 350 ml of MeOH. During crystallization, the suspension was diluted with 350 ml of Et_2O and cooled to yield a 22-g light tan first crop (overnight). The mother liquors were concentrated to 18.2 g of red oily residue which crystallized on cooling. This red solid was mixed with 200 ml of CH_2Cl_2-EtOH (95:5) and cooled (overnight) to give 10.2 g (2nd crop) of pancratistatin [**1**]. Both crops appeared to be pure when examined by tlc; however, by hplc, using two Waters C_{18} RP columns in series with 5% THF in H_2O as solvent, they proved to be mixtures of two compounds (**1** and **2**). The second crop was a little richer in pancratistatin than the first crop. A partial (considerable overlap) separation was achieved using a Waters Prep LC/500 unit and two 2″×2′ columns, containing Waters reversed-phase (C_{18}) packing, placed between a compression chamber and a pump. Two Waters RP cartridges were placed in the compression chamber and elution conducted with 15% THF in H_2O. All of the nearly pure pancratistatin (23.6 g) was dissolved in 150 ml of hot DMF treated with 1 g of carbon black, cooled to room temperature, and the solution filtered through a 1-cm bed of Celite 545. The filtrate was diluted with 100 ml of MeOH and 100 ml of Et_2O to yield 14.3 g of pancratistatin [**1**]. The mother liquors were concentrated, diluted with MeOH and the solid collected to yield 6 g of a second crop. On examination by analytical hplc, the first crop material was 90–96% pancratistatin and two other substances. Narciclasine [**2**] was found to be the main impurity. Mother liquor material appeared to be about 40% pancratistatin with the remainder being narciclasine and two other compounds. First crop material (14.3 g) was again recrystallized from DMF (diluted with MeOH) to provide 13.4 g of 98–99% pure pancratistatin [**1**].

Second crop material (6.0 g) and impure pancratistatin from previous Prep LC/500 attempts were combined and dissolved in 250 ml of hot DMF, treated (twice) with carbon black and the solution filtered. The light amber filtrate was diluted to 1200 ml with H_2O. Crystallization started upon scratching and

cooling (refrigerator for several hours) to yield nearly colorless crystals (9.0 g). By analytical hplc the 9.0 g proved to be a 60/40 mixture of pancratistatin and narciclasine [**2**]. Another 4 g of pure pancratistatin was obtained from this 9.0 g using a Waters analytical hplc instrument and a Waters µBondapak C_{18} column in series with a Whatman Partisil M9-10/25 ODS-2 column and THF-H_2O (95:5) at 4 ml/min as solvent. The sample volume was 500–600 µl per injection (ri detector) of a solution containing 1.3 g dissolved in 15 ml of DMF. A total of 70 injections was carried out resulting in 4 g of pure pancratistatin [**1**] and 0.26 g of narciclasine [**2**].

The pure combined pancratistatin samples, weighing 17.9 g, were dissolved in DMF (180 ml) by heating. The solution was filtered (sintered glass), cooled to room temperature, and diluted with 300 ml of Me_2CO, upon which crystallization started immediately. After cooling overnight, the crystals were collected, washed with 500 ml of H_2O (to remove residual DMF), and dried, affording 15 g of pure pancratistatin [**1**].

Isocarbostyril **3** from the large Si gel column amounted to 31 g by combining less pure fractions and recrystallizing from HOAc. Recrystallization from HOAc and from 95% EtOAc/EtOH followed by dilution with CH_2Cl_2 gave 11.8 g of 7-deoxynarciclasine [**3**, mp 216–218°, lit. (3) mp 214.5–215.5°, dec 230°, 251–252°] and 4.2 g from a second crop (mp 216–218°).

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