Journal of Natural Products
Vol. 47, No. 6, pp. 1018-1020, Nov-Dec 1984

ANTINEOPLASTIC AGENTS, 105. *ZEPHYRANTHES GRANDIFLORA*[1]

GEORGE R. PETTIT, VENKATSWAMY GADDAMIDI, and GORDON M. CRAGG

Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, AZ 85287

The history of certain Amaryllidaceous plants used in the primitive treatment of cancer can be traced to the fourth century B.C.[2] In the genus *Zephyranthes, Z. parulla* Killip appears in a history of Peru (by Padre Cobo) dated 1653, for treating tumors, and *Z. rosea* (Spreng.) Lindl. has found use in China for breast cancer (3). The leaves of *Z. candida* (Lindl.) Herb. have been employed in Africa as a treatment for diabetes mellitus (4); and the bulbs from a 1964 Hong Kong collection of this plant gave an extract that displayed a confirmed level of activity (cell line from a human epidermoid carcinoma of the nasopharynx, KB system) in the U. S. National Cancer Institute's (NCI) research programs (5). In this report we have summarized an investigation for antineoplastic constituents in the closely related *Zephyranthes grandiflora* Lindl., employing the NCI murine P-388 lymphocytic leukemia (PS system) for bioassay purposes.

The bulbs of *Z. grandiflora* were extracted with CH_2Cl_2-MeOH-H_2O, essentially, as previously described for the Amaryllidaceous plant *Pancratium littorale* (2). Application of the solvent partitioning sequence 9:1↦1:1 MeOH-H_2O with hexane↦CH_2Cl_2 to the CH_2Cl_2 phase of the original extract led to PS in vitro, but not in vivo, activity in the resulting CH_2Cl_2-MeOH-H_2O fractions (Scheme 1).

Because of our prior experience in locating the major PS in vivo active components of two other Amaryllidaceae species (2) in aqueous fractions, special emphasis was placed on that prospect.

The aqueous MeOH phase of the bulb extract was concentrated, diluted with H_2O, and extracted with *n*-BuOH (6). Separation of the *n*-BuOH fraction by partition chromatography on Sephadex LH-20 resulted in isolation of pancratistatin as the predominant PS in vivo and in vitro active (T/C 135%↦150% at dose levels of 0.78↦3.1 mg/kg, ED_{50} <0.01 μg/ml) constituent of *Z. grandiflora*. In our initial study (2) of pancratistatin (**1**), this promising isocarbostyril exhibited PS in vivo T/C 138%↦165% at dose levels of 0.75↦6.0 mg/kg and T/C 206% at 12.5 mg/kg.

Based on the discovery of pancratistatin as a major antineoplastic biosynthetic product in species from two of three Amaryllidaceous genera we have so far examined, it would seem that this isocarbostyril and related substances (2) may account for some of the potentially valuable medicinal properties attributed to this plant family.

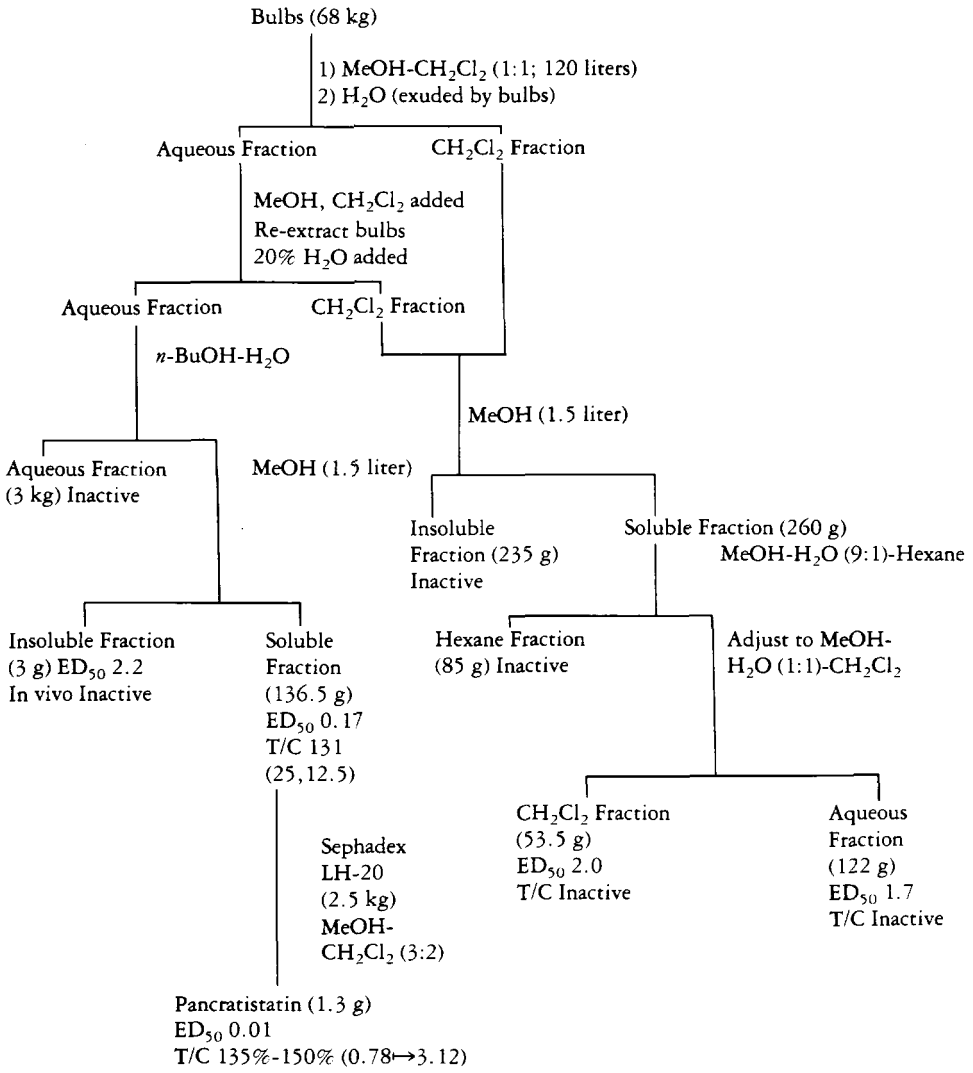
EXPERIMENTAL

GENERAL METHODS.—Chromatographic solvents were redistilled, Tlc was performed on silica gel GHLF Uniplates (0.25 mm layer thickness, Analtech Inc.), with $CHCl_3$-MeOH (3:1) as mobile phase and developed with ceric sulfate spray reagent. Sephadex LH-20 (particle size 25-100 μ) was manufactured by Pharmacia Fine Chemicals AB (Uppsala, Sweden). A Gilson Model FC-200K fraction collector was used for fractionation.

Melting points were determined with a Kofler-type hot-stage apparatus and are uncorrected. Optical rotations were measured with Perkin-Elmer model 241 Automatic Polarimeter. Uv spectra were recorded using a Hewlett-Packard model 8450A UV/VIS spectrophotometer and ir spectra with Perkin Elmer model 299 and Nicolet MX-1 FTIR spectrophotometers. The ^{1}H-nmr and ^{13}C-nmr spectra were obtained by Dr. J. Witschel

[1]The preceding contribution has been recorded (1).

[2]For leading literature citations, refer to Pettit *et al.* (2).



SCHEME 1. Isolation of Pancratistatin

using Varian XL-100 and Bruker HXE-90 (22.63 MHz) spectrometers, respectively. TMS served as internal standard. Mass spectra were obtained by D. Adams employing a Varian MAT 312 spectrometer.

PLANT MATERIAL.—A 68-kg collection of commercial bulbs (B657832) of *Z. grandiflora* was obtained through the Economic Botany Laboratory, Agricultural Research Center-East, USDA, Beltsville, MD, as part of a collaborative NCI-USDA research endeavor directed by Drs. Matthew I. Suffness and James A. Duke. The taxonomic identification (PR55337) was performed by Ms. Sandra M. Saufferer.

EXTRACTION.—Undried, ground, bulbs (68 kg) were parcelled in muslin and extracted with MeOH-CH_2Cl_2 (1:1; 120 liters) for 15 days; H_2O exuded by the bulb caused separation of the CH_2Cl_2 phase. The aqueous phase was adjusted by addition of MeOH and CH_2Cl_2 in the ratio, aqueous phase-MeOH-CH_2Cl_2 (8:3:2), and the bulbs were re-extracted for an additional 15 days. Decantation and addition of 20% by volume of H_2O separated the CH_2Cl_2 phase which was combined with the first CH_2Cl_2 fraction and evaporated to give a combined extract. The latter fraction was treated with MeOH (1.5 liters) and the insoluble material (235 g) was collected by filtration. Evaporation of solvent from the filtrate gave a 260-g residue.

SOLVENT PARTITION.—The above residue (260 g) was dissolved in MeOH-H_2O (9:1; 1.5 liters) and extracted wih hexane (bp 60-65°; 2×1.5 liters). Evaporation of hexane led to an inactive fraction (85 g). The MeOH-H_2O phase was

adjusted to MeOH-H_2O (1:1) and extracted with CH_2Cl_2 (3×1.5 liters). Evaporation of the CH_2Cl_2 and aqueous MeOH phases afforded 53.5 g (PS *in vitro* ED_{50} 2.0 μg/ml) and 122 g (PS *in vitro* ED_{50} 1.7 μg/ml) residues, respectively.

ISOLATION OF PANCRATISTATIN.—The aqueous fraction obtained from the original extraction was concentrated to 8 liters and extracted with *n*-BuOH (6×3 liters). Concentration of the *n*-BuOH extract and treatment of the residue with MeOH (1.5 liters), followed by filtration, yielded a solid (3 g). Evaporation of the filtrate gave a residue (136.5 g), which was chromatographed on Sephadex LH-20 (2.5 kg) using MeOH-CH_2Cl_2 (3:2) as eluent. Fractions (monitored by tlc) containing mainly the component at Rf 0.46 were combined, concentrated, and the solution was filtered to provide a solid (1.3 g; 0.0019% yield) shown to be identical (by ir spectral and tlc comparison) to pancratistatin.

ACKNOWLEDGMENTS

With pleasure we thank for support Mrs. Mary Dell Pritzlaff, the Olin Foundation (Spencer T. and Ann W.), the Fannie E. Rippel Foundation, Mrs. Eleanor W. Libby, the Waddel Foundation (Donald Ware), the Robert B. Dalton Endowment Fund, Mrs. Pearl Spear, Mrs. Virginia L. Bayless, Mr. Elias Romley, Contract NO1-CM-97297 with the Division of Cancer Treatment, NCI, National Institutes of Health, DHW, and Grant No. CA-16049-06-08 awarded by the National Cancer Institute, DHW.

For other assistance we are pleased to thank Drs. M.I. Suffness, J.D. Douros, D.L. Doubek, J.M. Schmidt, and P. Lohavanijaya.

LITERATURE CITED

1. G.R. Pettit, G.M. Cragg, M.I. Suffness, D. Gust, F.E. Boettner, M. Williams, J.A. Saenz-Renauld, P. Brown, J.M. Schmidt, and P.E. Ellis, *J. Org. Chem.* (in press).
2. G.R. Pettit, V. Gaddamidi, A. Goswami, G.M. Cragg, and Y. Sagawa, *J. Chem. Soc. Chem. Commun.* (in press).
3. J.L. Hartwell, *Lloydia*, **30**, 379 (1967).
4. J.W. Watt and M.G. Breyer-Brandwijk, "The Medicinal and Poisonous Plants of Southern and Eastern Africa," London: E. and S. Livingstone Ltd., 1962, p. 42.
5. M.I. Suffness, J.L. Hartwell, and F.C. Cheng, private communication.
6. G.R. Pettit and R.H. Ode, "Biosynthetic Products for Cancer Chemotherapy," vol. 3, New York: Plenum Publishing Corp., 1979.

Received 7 *February 1984*