Antineoplastic Agents. 553. The Texas Grasshopper *Brachystola magna*[1]

George R. Pettit,* Yanhui Meng, Delbert L. Herald, John C. Knight, and John F. Day

Cancer Research Institute and Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287-2404

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Bioassay (P388 lymphocytic leukemia cell line and human cancer cell lines) guided separation of an extract prepared from the previously chemically uninvestigated Texas grasshopper *Brachystola magna* led to isolation of the cancer cell growth inhibitory pancratistatin (**1**), narciclasine (**2**), and ungeremine (**3**). Pancratistatin (**1**) was first isolated from the bulbs of *Hymenocallis littoralis*), and the original crystal structure was deduced by X-ray analysis of a monomethyl ether derivative. In the present study pancratistatin (**1**) was isolated from an extract of *B. magna*, which led to the X-ray crystal structure of this anticancer drug. Since isoquinoline derivatives **1**−**3** are previously known only as constituents of amaryllidaceous plants, some of the interesting implications of their rediscovery in the grasshopper *B. magna* that does not appear to utilize amaryllis family plants were discussed.

In 1965−1966 we began the first exploratory survey of terrestrial arthropods as potential sources of new and structurally unique anticancer drugs and by 1968 had uncovered a number of promising leads.[2] Over the relatively short four-year period when resources were available, we isolated, for example, antineoplastic constituents from butterfly wings,[3a] an Asian beetle,[3b] and a wasp.[3c] Recently, this relatively unexplored area[4] for discovering medically useful new drugs has begun to be revisited on a much larger scale.[5]

One of the insect leads we began to pursue in 1967 was the lubber grasshopper[6] *Brachystola magna* collected in Texas, but this foraging generalist ranges north to Minnesota and west to Arizona.[7] Most grasshoppers are usually polyphagous and pursue plants from a broad variety of taxa. However, *B. magna* will even consume other insects including grasshoppers alive or dead.[7,8] In this regard, it is believed that insects vary their diet to obtain, for example, plant constituents important for their own chemical defense.[7] When 2-propanol extracts of *B. magna* were found to provide a 40% increase in life span (at 200 mg/kg) against the U.S. National Cancer Institute murine P388 lymphocytic leukemia, we gave increased attention to the lead. By 1970 a combination of negative events occurred, of which the most serious was the untimely passing of our entomologist colleague D. G. Ford. This coincided with, for practical purposes, the disappearance of *B. magna* from the southwest along with the necessary financial resources. Because the extract from the initial collection (less than 1 kg) of *B. magna* amounted to only 11 g, isolation of the antineoplastic constituent had to await the development of improved bioassays (especially with cancer cell lines), isolation, and instrumental techniques. The rather surprising solution to this research objective now follows.

The original Texas collection of *B. magna* provided a 2-propanol extract that upon solvent partitioning between 9:1 → 1:1 CH_3OH-H_2O and hexane → CH_2Cl_2 and the H_2O phase between 3:2 CH_3OH-H_2O and 1-butanol gave CH_2Cl_2 and 1-butanol fractions that were found to significantly inhibit the P388 leukemia cell line and five human cancer cell lines (ED_{50} 3.5 and 1.9 μg/mL, respectively). Bioassay-guided (P388 leukemia cell line) separation of the 1-butanol fraction (2.88 g) by a series of gel permeation and partition separations on Sephadex LH-20 columns, followed by final purification employing HPLC and recrystallization, led to pancratistatin (**1**, 4.1×10^{-2} % yield, P388 ED_{50} 0.048 μg/mL),[9,10] narciclasine (**2**, 1.4×10^{-2} % yield, P388 ED_{50} 0.018 μg/mL),[11] and ungeremine (**3**, 2.3×10^{-2} % yield, P388 ED_{50} 1.2 μg/mL).[12] Pancratistatin (**1**) was recrystallized from CH_3OH-H_2O and small, colorless crystals were obtained. The molecular formula was assigned as $C_{14}H_{15}NO_8$ on the basis of high-resolution $APCI^+$ mass spectroscopy, and the structure was confirmed by an X-ray crystal structure determination. While the X-ray crystal structure of **1** had been solved by our group in 1994, the results had not previously been published. Consequently, when the initial cell parameters of the current data collection were obtained and compared with the 1994 collection, we were surprised to discover that the anticancer compound obtained from the grasshopper extract was identical to the pancratistatin obtained from plant sources.[10,13] Figure 1 shows the stereochemical drawing of **1** obtained from the current X-ray analysis.

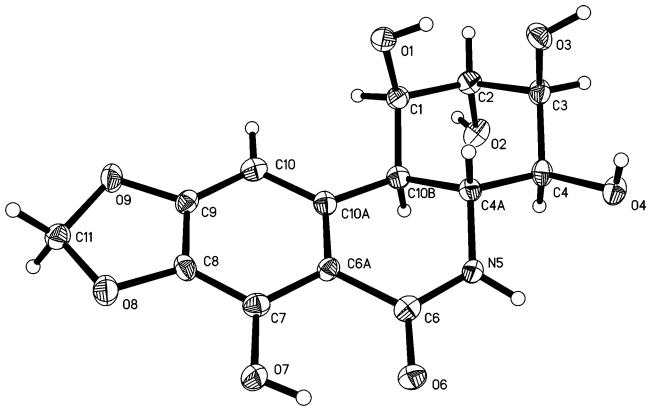

Figure 1. X-ray crystal structure of pancratistatin (**1**) with the atomic thermal ellipsoids being displayed at 50% probabilities.

* To whom correspondence should be addressed. Tel: 480-965-3351. Fax: 480-965-8558. E-mail: bpettit@asu.edu.

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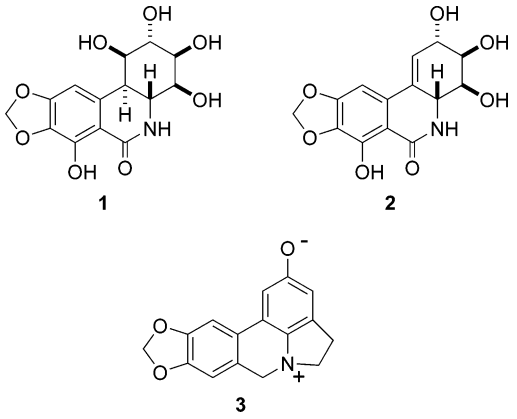

Narciclasine (**2**) was obtained as a colorless amorphous semisolid, and its structure was easily assigned on the basis of high-field 2D-NMR analyses and high-resolution FAB mass spectroscopy, which provided the molecular formula $C_{14}H_{13}NO_7$. Ungeremine (**3**) was isolated as a yellow powder. The molecular formula was assigned as $C_{16}H_{12}NO_3$ on the basis of high-resolution APCI$^+$ mass spectroscopy and combined with results of 2D-NMR analyses confirmed its structure. The isolation of isoquinoline derivatives **1**−**3** from *B. magna* represents, to our knowledge, the first results from a chemical constituent study of this grasshopper.

Presumably, if the serious constraints of the 1970 period had not occurred, we might have discovered the important anticancer/antiviral pancratistatin (**1**), now in preclinical development, some 11−12 years sooner than our first isolation of this promising drug from the spider lilly *Hymenocallis littoralis* (aka *Pancratium littorale*).[9] Furthermore, the amount of pancratistatin (**1**) contained in *B. magna* would suggest a defensive strategy and potential new plant source of isocarbostyril **1**, as amaryllidaceous species have not been recorded among the plants selected by this grasshopper as preferred foods.[7,8] Interestingly, the southern grasshopper mouse, *Onychomy tossidus,* views *B. magna* as quite palatable.[14] Clearly, such biological/chemical relationships will be useful to further investigate.

The cancer cell growth inhibitory properties of *B. magna* constituents **1**−**3** were evaluated using the murine P388 lymphocytic leukemia cell line and a selection of human cancer cell lines (Table 1). As we have consistently found, pancratistatin (**1**) and narciclasine (**2**) both exhibit strong inhibition against growth of the P388 lymphocytic leukemia and the minipanel of human tumor cell lines.[11a,14] Table 1 also includes the first cancer cell line data for ungeremine (**3**).

Experimental Section

General Experimental Procedures. All chromatographic solvents were redistilled. Sephadex LH-20 used for partition column chromatography was obtained from Pharmacia Fine Chemicals. Semipreparative HPLC was conducted with a Gilson model 805 HPLC coupled with a Gilson model 117 U detector. UV spectra were recorded with a Perkin-Elmer lambda 3B UV/vis spectrometer. NMR spectra were obtained with a Varian UNITY INOVA-400 and 500 spectrometers with tetramethylsilane (TMS) as an internal reference. High-resolution mass spectra were obtained using a JEOL LCMate magnetic sector instrument either in the FAB mode, with a glycerol matrix, or by APCI with a poly(ethylene glycol) reference.

Insect Collection. The specimens of *Brachystola magna*, Girard (Acrididea, family Romaleidae, subfamily Pomaleinae)[7,8] were collected in 1967 in Texas, under direction of Ford Labs, Lyford, Texas, and identified by D. G. Ford (deceased May 3, 1970). A voucher specimen of *B. magna* has been retained in our institute. *B. magna*, aka lubber grasshopper, plains lubber, or homesteader[6] is 3−4.5 cm long and ranges east of the U.S. Rocky Mountains from North Dakota south to Texas and Mexico and eastward with isolated populations as far as Minnesota, Iowa, Kansas, and Oklahoma.[7] *B. magna* is known for certain beneficial activities by providing some biological control of weeds in their favored grazing areas containing sunflowers and a selection of other foods. However, large populations of the insect have been very destructive to young cotton plants.[15]

Table 1. Murine P388 Lymphocytic Leukemia Cell Line and Human Cancer Cell Line Inhibition Values (ED_{50} expressed in μg/mL) for Alkaloids **1**, **2**, and **3**[a]

cancer cell line[b]	1	2	3
P388	0.048	0.018	1.2
BXPC-3	0.043	0.024	0.87
MCF-7	0.035	0.014	0.48
SF268	0.032	0.018	0.057
NCI-H460	0.029	0.014	0.65
KM20L2	0.048	0.023	0.50
DU-145	0.037	0.012	0.029

[a] In DMSO. [b] Cancer cell line type (P388, lymphocytic leukemia; BXPC-3, pancreas adenocarcinoma; MCF-7, breast adenocarcinoma; SF268, CNS glioblastoma; NCI-H460, lung large cell; KM20L2, colon adenocarcinoma; DU-145, prostate carcinoma).

Extraction and Initial Separation of *Brachystola magna* Constituents. The 1967 collection of *B. magna* (about 1 kg) preserved in 2-propanol was extracted (2 ×; 10, 5 days) using 1:1 CH_2Cl_2−CH_3OH. After each extraction, 30 vol % of H_2O was added to separate a CH_2Cl_2 fraction. The CH_2Cl_2 phase was separated and solvent evaporated in vacuo, yielding a 0.92 g CH_2Cl_2 fraction (P388 ED_{50} 3.5 μg/mL). The H_2O phase was next partitioned between CH_3OH−H_2O (3:2) and 1-butanol. The 1-butanol layer was separated and solvent evaporated in vacuo, to provide a 2.28 g 1-butanol fraction (P388 ED_{50} 1.9 μg/mL). The solvent partitioning sequence was a modification of the original procedure of Bligh and Dyer.[16]

Isolation Procedures. The 1-butanol fraction (2.88 g) was passed through a Sephadex LH-20 column, using CH_3OH as eluent. Six P388 leukemia cell line inhibitory fractions were obtained. One fraction was recrystallized from CH_3OH (3 ×) to afford 2.5 mg of ungeremine (**3**) as a yellow powder. HRAPCI *m/z* calcd for $[M + H]^+$ $C_{16}H_{12}NO_3$, 266.0817; found 266.0810. The other five P388 cell line bioactive fractions were combined and dissolved in CH_3OH (5 mL). After removing the insoluble residue, the solution components were separated by HPLC using a semipreparative Luna 5 μm C8 (2) column and a gradient mobile phase (20% CH_3OH in H_2O for 42 min, then 100% CH_3OH for 20 min at a flow rate of 2 mL/min) to afford 2.5 mg of pancratistatin (**1**)[9,10] as colorless cubic crystals and 1.5 mg of narciclasine (**2**)[11] as a semisolid. Compounds **1**, **2**, and **3** were identified by direct comparisons with known standards and with literature data.

Crystal Structure of Pancratistatin (1). A low-temperature (−150 °C) data collection was performed on a crystal grown from D_2O/methanol; 30 s frames of data were collected as −0.396° in ω scans on a Bruker AXS SMART 6000 CCD diffractometer using Cu radiation (1.54178 Å). Collection was conducted using the Multirun procedure in the SMART[17] software, such that 96.8% of the theoretically possible reflections available were collected out to a θ limit of 68.43°. Data reduction was subsequently performed with SAINT-PLUS,[18] and an empirical absorption correction was performed with SADABS.[19] Structure solution and refinement were performed with the SHELXTL program package. The positions for all hydrogen atoms were calculated on the basis of atomic geometry. The final structural model obtained for pancratistatin is shown in Figure 1. Crystal data and experimental details follow: $C_{14}H_{15}NO_8$, mol wt = 324.27, crystal size 0.32 × 0.13 × 0.03 mm, orthorhombic, space group $P2_12_12_1$, no. 19, a = 6.7843(4) Å, b = 9.3199(5) Å, c = 20.0409(13) Å, V =

1267.17(13) Å^3, $Z = 4$, D_c 1.705 Mg/m^3, $F(000) = 680$, μ(Cu Kα) = 1.223 mm^{-1}, independent data, 2160 (R_{int} =0.0503), θ range 5.23−68.43°, $R[I > 2\sigma(I)] = 0.0362$, $R_{all} = 0.0560$, ΔF = +0.258 and −0.248 e Å^{-3}. Crystallographic data of **1** including atomic coordinates, bond lengths and angles, thermal parameters, and additional experimental details may be found in the Supporting Information. The material has also been deposited in the Cambridge Crystallographic Data Center (CCDC). Copies can be obtained free of charge on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Cancer Cell Line Bioassay Procedures. The National Cancer Institute's standard sulforhodamine B assay was used to assess inhibition of human cancer cell growth as previously described.[20] The murine P388 lymphocytic leukemia cell line results were obtained as reported previously.[21]

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Supporting Information Available: Crystallographic data of **1** including atomic coordinates, bond lengths and angles, thermal parameters, and additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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