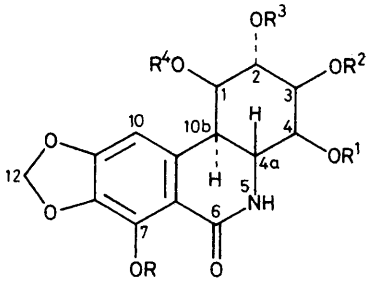
Isolation and Structure of Pancratistatin[1]

George R. Pettit,* Venkatswamy Gaddamidi, Gordon M. Cragg, Delbert L. Herald, and Yoneo Sagawa
Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, Arizona 85287, U.S.A.

Pancratium littorale Jacq. collected in Hawaii has been found to produce a new phenanthridone, pancratistatin, that significantly inhibits growth of the murine P388 lymphocytic leukaemia; an *X*-ray crystal structure determination of pancratistatin monomethyl ether (**1c**) was employed to assign structure (**1a**) to pancratistatin.

Because of early[2] interest in certain medicinal and/or poisonous plant species of the relatively large Amaryllidaceae family[3] about 10% have been investigated for alkaloid constituents, and over 70 such basic metabolites have been isolated.[4] Some 30 Amaryllidaceae species have found use in primitive treatment of cancer.[5] Most significantly, in the U.S. National Cancer Institute's (N.C.I.) exploratory plant evaluation programme some Amaryllidaceae species have yielded extracts with confirmed levels of anticancer activity. We now report that roots of the Hawaiian *Pancratium littorale* Jacq.† contain a new anticancer (38—106% life extension at 0.75—12.5 mg dose levels using the murine P388 lymphocytic leukaemia, PS system, and 53—84% life extension at 0.38—3.0 mg/kg against the murine M5076 ovary sarcoma) biosynthetic product herein named pancratistatin (**1a**).

The bulb section (45 kg) of *P. littorale* was extracted with methylene chloride–methanol–water and pancratistatin was concentrated (separation was guided primarily by bioassay using the PS *in vivo* system) in a butyl alcohol extract of the aqueous phase. Purification of half of the crude product employing selective solubility properties and gel permeation chromatography (Sephadex LH-20) afforded 6.5 g (0.028% yield) of pancratistatin (**1a**) that separated from dimethylformamide–methanol–ether as a colourless solid, m.p. 322—324 °C, electron impact mass spectrum *m/z* 325 (M^+, $C_{14}H_{15}NO_8$); $[\alpha]_D^{34}$ + 48° (*c* 1.0, Me_2SO); $\lambda_{max.}$ (MeOH) (log ε) 209 (sh), 219 (sh), 233 (4.32), 278 (3.91), and 308 (br. sh) nm; i.r. (KBr) $\nu_{max.}$ 3500—3200, 1675, 1615, 1600, 1500, 1465, 1445, 1420, 1375, 1350, 1300, 1230, 1200, 1160, 1118, 1085, 1070, 1040, 1030, 930, 912, 880, 840, 720, 655, 640, and 610 cm^{-1}; and ^1H n.m.r. [100 MHz, $(CD_3)_2SO$] δ 3.6—4.4



(1)

a, R = R^1 = R^2 = R^3 = R^4 = H
b, R = R^1 = R^2 = R^3 = R^4 = Ac
c, R = Me, R^1 = R^2 = R^3 = R^4 = H

† The more usual range is tropical Africa to Asia; also known as *Hymenocallis littoralis* (Jacq.) Salisb.

(6H), 4.72—5.70 (5H, removed by D_2O), 6.11 (2H, br. s), 6.56 (1H, s), and 13.15 (1H, removed by D_2O). Reaction of pancratistatin (**1a**) with acetic anhydride–pyridine provided the penta-acetate (**1b**) (m.p. 162—166 °C) and with diazomethane in methanol yielded the monomethyl ether (**1c**); m.p. 294—298 °C (decomp.); $[\alpha]_D^{25}$ + 289.9°, (*c* 0.69, Me_2SO); i.r. (KBr) $\nu_{max.}$ 3500, 3400, 3300, 1635, 1600, 1485, 1450, 1390, 1343, 1298, 1226, 1207, 1152, 1122, 1090, 1060, 1035, 967, 937, 920, 880, 840, 795, 724, 660, and 620 cm^{-1}; ^1H n.m.r. [$(CD_3)_2SO$] δ 3.58—4.44 (6H), 3.88 (3H, s), 4.70—5.50 (4H, removed by D_2O), 6.13 (2H, d, *J* 5 Hz), 6.74 (1H, s), and 6.92 (1H, removed by D_2O).

The remarkable insolubility of pancratistatin in a variety of organic solvents, very high decomposition point, non-basic character, and i.r. spectrum suggested a carbostyril or isocarbostyril system. The most plausible interpretation of the elemental analyses and spectral data for pancratistatin (**1a**), its penta-acetate (**1b**), and its monomethyl ether (**1c**) pointed to a new phenanthridone. An *X*-ray crystal structure determination for (**1c**), recrystallized from 95% aqueous ethanol, was utilized to make the stereochemical assignments and confirm the overall structure of pancratistatin as (**1a**).

Crystal data. (**1c**), $C_{15}H_{17}NO_8.H_2O$ (crystal dimensions 0.125 × 0.25 × 0.37 mm), monoclinic, space group $P2_1$ (from systematic absences 0*k*0, *k* = 2*n* + 1), *a* = 9.040(1), *b* = 8.317(1), *c* = 10.187(2) Å, β = 99.78(2)°, *U* = 754.8 Å^3, *Z* = 2, D_c = 1.572, D_m = 1.565 g cm^{-3}, *F*(000) = 376, λ(Cu-K_α) = 1.541 84 Å, μ(Cu-K_α) = 10.038 cm^{-1}.

Intensities of all unique reflections with 2θ <75° were measured at 25 °C using a variable speed ω/2θ scan technique on an Enraf-Nonius (Delft) CAD4 diffractometer employing graphite-monochromated Cu-K_α radiation. The intensities of three monitor reflections, recorded every 250 min, varied by <0.5% during the data collection. 1647 Unique reflections were required for subsequent processing [$I > \sigma(I)$]. All intensity data were corrected for anisotropic decay using the monitor intensities of the standards. The data were corrected for Lorentz–polarization.[6] Extinction or absorption adjustments were not applied. The structure was determined by using the program MULTAN.[7] Full-matrix least-squares refinement using anisotropic thermal parameters for the non-hydrogen atoms and isotropic for the hydrogen atoms led to a final *R* value of 0.0426 {R_w = 0.0622; $w = 1/\sigma(F^2) = 4F^2/[\sigma(F)^2]^2$; $\sigma(F^2) = \sqrt{[\sigma(I)^2 + (0.05\ F^2)^2]}$} (maximum shift to error ratio 0.84, most in the range 0.1—0.2), for 1596 reflections with $I > 3\sigma(I)$. A difference map showed negligible electron densities (<0.22 e Å^{-3}).‡

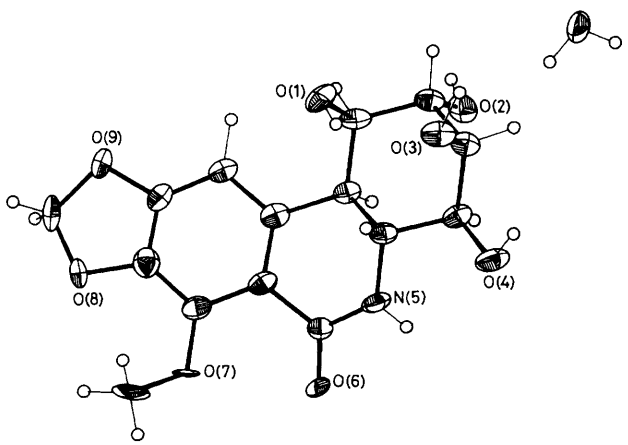


Figure 1. An ORTEP[11] illustration of the pancratistatin monomethyl ether (**1c**) molecular structure.

The *R* factor for the enantiomer of (**1c**) was almost identical, thus excluding the possibility of assigning the absolute configuration by application of Hamilton's method.[8] Consequently, the absolute stereochemistry shown (Figure 1) for pancratistatin derivative (**1c**), although preferred, is tentative. Support for this absolute configuration assignment is based on biosynthetic evidence and previous *X*-ray crystal structure studies of narciclasine,[9,10] a 1-dehydrophenanthridone derivative of pancratistatin (**1a**). We are currently attempting to determine the absolute configuration of pancratistatin and other natural antitumour agents *via* the method of Engel,[12] involving the careful measurement of Bijvoet differences for light atom structures, and the results will be published elsewhere.

Pancratistatin (**1a**) is presently undergoing evaluation against the key N.C.I. experimental cancer systems, and these results will also be published elsewhere.

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‡ The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Rd., Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.