ANTINEOPLASTIC AGENTS, 120. *PANCRATIUM LITTORALE*[1]

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Abstract.—The bulbs of *Pancratium littorale* collected in Hawaii were found to contain a new phenanthridone biosynthetic product designated pancratistatin (**4a**) that proved to be effective (38-106% life extension at 0.75-12.5 mg/kg dose levels) against the murine P-388 lymphocytic leukemia. Pancratistatin also markedly inhibited (ED_{50}, 0.01 μg/ml) growth of the P-388 in vitro cell line and in vivo murine M-5076 ovary sarcoma (53-84% life extension at 0.38-3.0 mg/kg). An X-ray crystal structure determination of pancratistatin monomethyl ether (**4c**) and a detailed high resolution (400 MHz) nmr study of pancratistatin and its pentaacetate (**4b**) completed assignment of structure **4a**. Companion antineoplastic constituents of *P. littorale* were found to be narciclasine (**2c**) and its 7-deoxy derivative (**2a**). The structure of 7-deoxynarciclasine (**2c**) was also confirmed by an X-ray crystallographic analysis.

By the fourth century BC, oil of the well-known daffodil *Narcissus poeticus* L. (Amaryllidaceae) was already known to the Greek physician Hippocrates of Cos (the "Father of Medicine") as a cancer treatment.[2] And by the first century AD, *N. poeticus*[3] was established in the Middle East and Roman Empire (recorded by Pliny the Elder) for this purpose. More than thirty other plants of the Amaryllidaceae representing some eleven genera have also found application in folk medical management of cancer.[2] Six of these are *Hymenocallis amancaes*, *Hymenocallis amoena*, *Hymenocallis caribaea*, *Hymenocallis declinata*, *Hymenocallis ovata*, and *Hymenocallis patens*. Interestingly, it appears that the first chemical investigations of a *Hymenocallis* species (*littoralis*) were described by Gorter (4) in 1920. An alkaloid now known to be lycorine (**1**) and to inhibit growth of the murine P-388 lymphocytic leukemia (PS system),[4] sarcoma 37, Ehrlich ascites carcinoma and lymphoma-NK/LY was isolated from *H. littoralis*. After 1920, no further study of this species appeared. Fortunately, *H. littoralis* was uncovered in the U.S. National Cancer Institute's (NCI) natural products program under the preferred synonym *Pancratium littorale* Jacq. as a plant yielding root extracts with a confirmed level of activity against the PS lymphocytic leukemia.
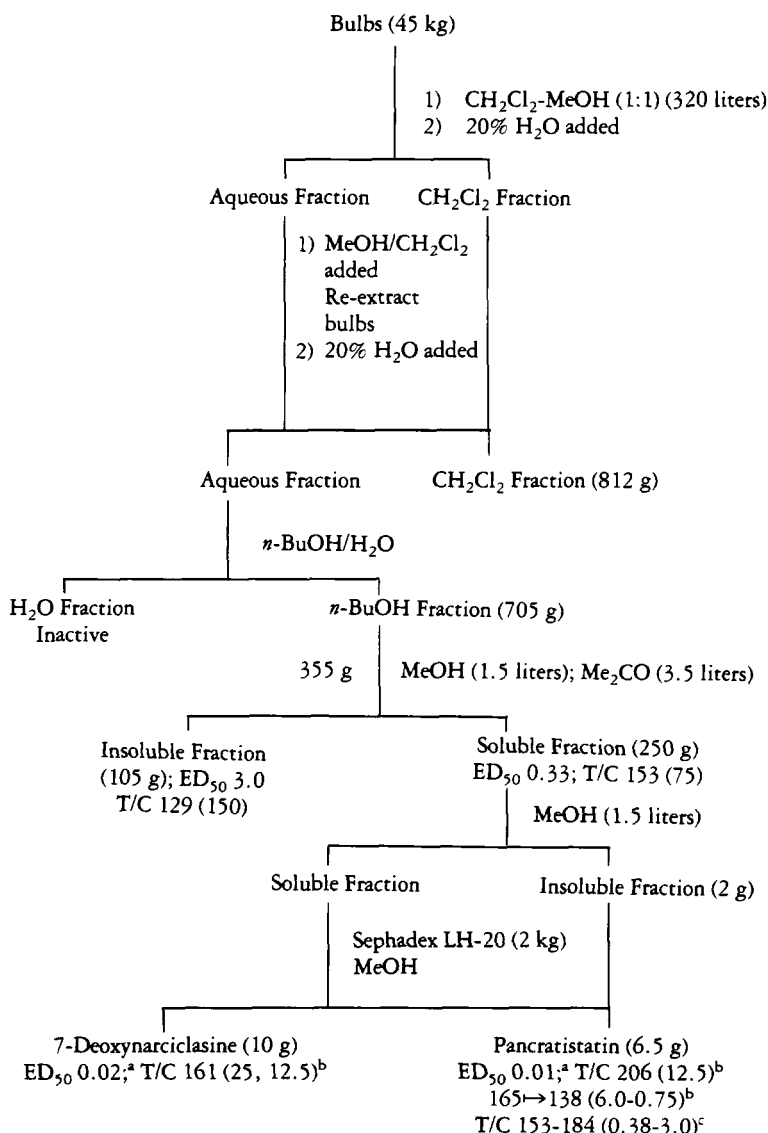
In order to explore the lead presented by *P. littorale*, a 1980 Hawaii re-collection of the bulbs was extracted employing a CH_2Cl_2/MeOH procedure (5) followed by addition of H_2O, and the aqueous phase was extracted with *n*-BuOH (Scheme 1). Bioassay utilizing the PS in vivo and in vitro systems indicated that the antineoplastic activity was concentrated in the *n*-BuOH fraction rather than in the CH_2Cl_2 residue where lycorine (**1**) would be expected. The *n*-BuOH fraction was further separated by gel

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[1]For part 119, consult Pettit *et al.* (1).

[2]The excellent survey of "Plants Used Against Cancer" prepared by Hartwell (2) contains important references to this history.

[3]*N. poeticus* L. is now known to contain some 0.12 g of the antineoplastic agent narciclasine (**2c**) per kg of fresh bulb, Piozzi *et al.* (3).

[4]Private communication from Drs. A.J. Charlson, (Macquarie University, NSW, Australia) and J.L. Hartwell and M.I. Suffness (National Cancer Institute, Bethesda, MD).

Bulbs (45 kg)

1) CH_2Cl_2-MeOH (1:1) (320 liters)
2) 20% H_2O added

Aqueous Fraction CH_2Cl_2 Fraction

1) MeOH/CH_2Cl_2
   added
   Re-extract
   bulbs
2) 20% H_2O added

Aqueous Fraction CH_2Cl_2 Fraction (812 g)

n-BuOH/H_2O

H_2O Fraction n-BuOH Fraction (705 g)
Inactive

355 g   MeOH (1.5 liters); Me_2CO (3.5 liters)

Insoluble Fraction   Soluble Fraction (250 g)
(105 g); ED_{50} 3.0 ED_{50} 0.33; T/C 153 (75)
T/C 129 (150)
                                      MeOH (1.5 liters)

Soluble Fraction   Insoluble Fraction (2 g)

Sephadex LH-20 (2 kg)
MeOH

7-Deoxynarciclasine (10 g)   Pancratistatin (6.5 g)
ED_{50} 0.02;[a] T/C 161 (25, 12.5)[b] ED_{50} 0.01;[a] T/C 206 (12.5)[b]
                                      165$\mapsto$138 (6.0-0.75)[b]
                                      T/C 153-184 (0.38-3.0)[c]

SCHEME. 1.   Isolation of 7-deoxynarciclasine (2a) and pancratistatin (4a)

[a] 9 PS μg/ml.
[b] Dose in mg/kg.
[c] M5076 ovary sarcoma: dose in mg/kg.

permeation chromatography on Sephadex LH-20 using MeOH as eluent. Fraction collection was guided by tlc (3:1, $CHCl_3$-MeOH), and the two principal antineoplastic components were found to correspond to Rf 0.37 and 0.46. Both proved to be very high melting, relatively insoluble, and nitrogen-containing non-basic compounds reminiscent of carbostyrils (6,7) or isocarbostyrils (8).

Purification of the Rf 0.37 product by recrystallization from HOAc/MeOH afforded a lactam (2a) that readily gave a triacetate (2b) derivative. Spectral data indicated that this anticancer constituent (PS, T/C 161 at 12.5 mg/kg, ED_{50}, 0.02 μg/ml) was a 7-deoxy derivative of narciclasine (2c). But physical constants reported for margetine (2a) (3), later renamed lycoricidine (9), indicated otherwise. For those obvi-
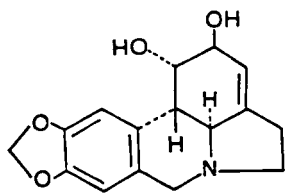
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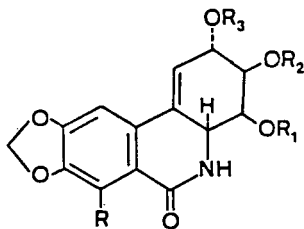
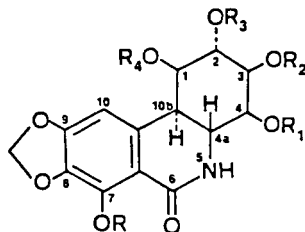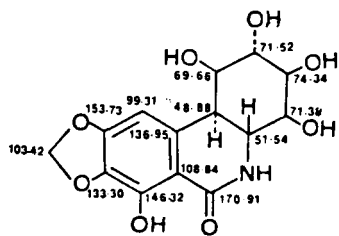2a   R=R$_1$=R$_2$=R$_3$=H
2b   R=H, R$_1$=R$_2$=R$_3$=COCH$_3$
2c   R=OH, R$_1$=R$_2$=R$_3$=H
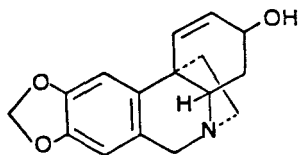
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4a   R=R$_1$=R$_2$=R$_3$=R$_4$=H
4b   R=R$_1$=R$_2$=R$_3$=R$_4$=COCH$_3$
4c   R=CH$_3$, R$_1$=R$_2$=R$_3$=R$_4$=H

pancratistatin (with ^{13}C nmr) (4a)

ous reasons and because initial attempts to obtain an authentic sample of 7-deoxynarcic-lasine (2a)[5] were unsuccessful, we undertook a complete structural determination by X-ray crystallographic methods. The result was definite assignment of structure 2a to the substance with Rf 0.37. The absolute configuration of triol 2a was deduced from its presumed biosynthesis from vittatine (3) (11, 13). Later authentic samples of the triacetate 2b prepared from the natural and (±)-synthetic products were kindly provided by Professor Mondon (11) and Ohta (12), respectively. Interestingly, ir spectral comparison in KBr of the synthetic triacetate with our triacetate 2b derived from *P. littorale* showed substantial differences, but when compared in CHCl$_3$ solution, the spectra were identical.[6] We conclude, on this basis and by results of ir spectral comparison with the natural derivative (2b), that prior assignments (9,10) of structure 2a to 7-deoxynarciclasine are correct.

Recrystallization of the Rf 0.46 anticancer component (T/C 138↦165 at 0.75↦6.0 mg/kg dose levels and 206 at 12.5 mg/kg in the PS system with ED$_{50}$, 0.01 μg/ml) from DMF/MeOH/Et$_2$O gave a pure specimen that displayed elemental analyt-

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[5]In July 1972 (10), the lycoricidine name was suggested as a replacement for margetine. Since the term narciclasine (2c) is now well established, we suggest that margetine/lycoricidine be replaced by the simplification 7-deoxynarciclasine (2a).

[6]In our experience, such changes induced by crystal structure and/or resulting from pressure during formation of the KBr disc are rarely encountered.

ical and spectral data in accordance with pentaol **4a**. The new phenanthridone was designated pancratistatin, and structural elucidation was accomplished by detailed mass and nmr spectral analysis of the pentaol and its peracetate (**4b**) and by an X-ray crystal structure determination of the 7-methyl ether derivative **4c** as outlined in our preliminary communication (14). This report contains a summary of the crystallographic study and ORTEP representation. As with lactam **2a**, unequivocal structure **4a** determined for pancratistatin was completely consistent with the corresponding spectral results.

A high resolution (400 MHz) ^{1}H-nmr spectrum of pancratistatin in DMSO-d_6 was well resolved in terms of individual protons, but due to the solvent, some coupling information was missing. The most downfield signal (not exchangeable with D_2O) at δ 6.55 (singlet) was assigned to the phenyl H-10, and two lines at δ 6.11 and 6.09 ppm were assigned to the methylenedioxy protons. Since the methylenedioxy protons are diastereotopic and therefore magnetically nonequivalent, they should give an AB system pattern. In DMSO-d_6 we did not see the predicted two outer signals even by enlarging the requisite spectral region. However, with peracetate **4b** the diastereotopic methylenedioxy protons were well resolved and gave an AB quartet at δ 6.079 ($J = 1.16$ Hz) (see Experimental section). Assignment of the other pancratistatin (**4a**) ^{1}H-nmr signals was based on two-dimensional-homonuclear shift correlated spectroscopy (COSY) using a D_2O-exchanged sample. A broad doublet at δ 3.04 showed two major (intense) cross peaks (coupled) at δ 4.35 and 3.76 and long range coupling with the phenyl H-10 proton at δ 6.55. Therefore, the 3.04 signal was assigned to H-10b, and the signals at 4.35 (br s) and δ 3.76 (br d) were assigned to H-1 and H-4a, respectively. The 4.35 (H-1) signal was also coupled to a broad singlet at 4.04 (H-2) which, in turn, was coupled to a broad singlet at δ 3.92 (H-3). The broad doublet at 3.76 (H-4a) was coupled to a broad singlet at δ 3.80 (H-4) which was, as predicted, coupled to the H-3 proton at δ 3.92. The broad singlet at δ 4.35 (H-1) showed a minor long range coupling (5-bond) with the phenyl H-10 proton. The ^{13}C-nmr spectrum of pancratistatin furnished fourteen lines corresponding to the necessary fourteen carbons (cf., structure **5**). Unequivocal assignment of the carbon signals corresponding to the cyclohexane ring was achieved by two-dimensional-heteronuclear (^{1}H-^{13}C) shift correlations. All the hydrogen-bearing carbons were cross correlated to their respective proton signals.

The assignments of other carbon signals were made on the basis of chemical shift additivity rules in substituted benzenes (15) and relative peak heights. The quaternary signal at 170.91 ppm was assigned to the carbonyl carbon. Calculated and observed values of the chemical shift were in reasonable agreement for most of the carbon assignments. At this point the structures assigned pancratistatin (**4a**) and 7-deoxynarciclasine (**2a**) were clearly established and should serve as useful reference points for related biosynthetic products.

Because of the need for large quantities of pancratistatin to meet detailed evaluation requirements of the U.S. National Cancer Institute (NCI), a modified isolation procedure which eliminated the use of Sephadex LH-20 was developed. Percolation of 70% aqueous EtOH through the dried, ground bulbs followed by addition of H_2O and sequential extraction with CH_2Cl_2 and EtOAc, concentrated the pancratistatin and 7-deoxynarciclasine in the EtOAc extract. Column chromatography of the EtOAc fraction on silica gel and elution with CH_2Cl_2-EtOH (9:1) further concentrated both isocarbostyrils (**2a** and **4a**). Final purification was achieved by reversed phase chromatography on C-18 silica gel and elution with a DMSO/H_2O gradient. The pancratistatin obtained was contaminated by a small amount of narciclasine (**2c**) which was separated by reversed phase chromatography (C-18 silica gel) by elution with acetonitrile-1% HOAc (1:4).

Both 7-deoxynarciclasine (**2a**) and narciclasine (**2c**) have been shown to inhibit growth of *Avena coleoptile* (rice seedling test), tobacco plant tissue cultures, and the murine Ehrlich carcinoma (9). Narciclasine has been found to show other types of antineoplastic activity (11) and has been undergoing evaluation by the NCI. These observations combined with the promising antineoplastic properties so far uncovered with lactam **2a** and pancratistatin (**4a**) suggest that one (or more) of these hydroxylated isocarbostyrils may become a useful cancer chemotherapeutic drug and/or find application in other medical areas (16,17). Presently, both lactam **2a** and pancratistatin (**4a**) are being examined in the NCI programs.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All solvents employed for chromatography were redistilled. Tlc was performed on silica gel GHLF Uniplates (0.25 mm layer thickness) supplied by Analtech Inc., using $CHCl_3$-MeOH (3:1) as mobile phase and ceric sulfate spray reagent for development. Sephadex LH-20 (particle size 25-100 μ) was supplied by Sigma Chemical Co. A Gilson Model FC-200K fraction collector was used for fractionations.

Melting points (uncorrected) were determined on a Kofler-type hot-stage apparatus, and optical rotations were measured with a Perkin-Elmer Model 241 Automatic Polarimeter. Uv spectra were recorded with a Hewlett-Packard Model 8450A UV/VIS spectrophotometer and ir spectra with Perkin Elmer Model 299 and Nicolet MX-1 FTIR spectrophotometers. The ^1H- and ^{13}C-nmr spectra were obtained with Varian XL-100 and Brucker HXE-90 (22.63 MHz) and Bruker AM-400 (400 MHz) spectrometers using TMS as internal standard. Mass spectra were determined employing a MAT 312 spectrometer.

PLANT COLLECTION.—Plant material (collection No. K and I 3915; B844009) was provided under the auspices of a joint National Cancer Institute-University of Hawaii collaborative research program directed by Dr. Matthew Suffness. A voucher specimen is deposited at the Harold L. Lyon Arboretum, University of Hawaii.

EXTRACTION.—Chopped bulbs (45 kg) were extracted with $MeOH$-CH_2Cl_2 (1:1, 320 liters) at ambient temperature for 20 days. The extract was decanted and the CH_2Cl_2 phase separated by the addition of 20% (by volume) H_2O. The aqueous phase was adjusted by addition of further MeOH and CH_2Cl_2 to give $MeOH$-CH_2Cl_2-aqueous phase (2:1:1) and the bulbs were re-extracted for 20 days. Decantation and addition of 20% (by volume) of H_2O separated the CH_2Cl_2 phase. The combined CH_2Cl_2 fraction was concentrated to give a fraction (812 g) with 9PS ED_{50} 7.5 μg/ml and in vivo PS T/C (mg/kg), toxic (200) and 109 (100,50).

SOLVENT PARTITION.—The aqueous phase from the above extraction procedure was concentrated to ca. 16 liters and centrifuged to remove insoluble material. The clear solution was extracted with *n*-BuOH (3×10 liters) and the alcohol extract concentrated to a 705 g fraction. An aliquot (355 g) was dissolved in MeOH (1.5 liters) and Me_2CO (3.5 liters) added. The insoluble material (105 g) was collected and the filtrate evaporated to give a residue (250 g).

ISOLATION OF 7-DEOXYNARCICLASINE (**2a**), NARCICLASINE (**2c**), AND PANCRATISTATIN (**4a**).—*Method A.*—Treatment of the preceding residue (250 g) with MeOH (1.5 liters) caused pancratistatin (**4a**) (2 g) to separate. The filtrate was chromatographed on a column of Sephadex LH-20 (2 kg; 105×10 cm) using MeOH as eluent and monitoring the fractions by tlc. Fractions containing the component Rf 0.37 were combined, concentrated, and filtered to give 7-deoxynarciclasine (**2a**) (10 g) which crystallized from HOAc/MeOH as fine needles, mp 251-252° [lit. 214.5-215.5° (9); 230° (dec) (12)]; $[\alpha]^{33}D$ +157.3° (c 0.96, DMSO); eims *m/z* 291 (M^+, $C_{14}H_{13}NO_6$); uv λ max (MeOH) (log ε) 233 (4.14), 248 (4.15), and 302 (3.75) nm; ir (KBr) ν max 3450, 3250, 1672, 1632, 1620, 1602, 1505, 1473, 1415, 1400, 1340, 1320, 1270, 1250, 1080, 1045, 1015, 976, 940, 890, 860, 785, 700, 670, 623 cm^{-1}; ^1H nmr 100 MHz (pyridine-d_5) δ 4.81-4.92 (2H), 5.0-5.35 (2H), 6.05 (2H), 6.72 (1H, br s), 7.33 (1H, s), 8.06 (1H, s), 8.57 (1H, br s, removed by D_2O exchange) and 7.0-8.6 (br hump, removed by D_2O) ppm; ^{13}C nmr (DMSO-d_6) δ 163.12, 150.58, 147.72, 131.70, 130.01, 123.61, 121.95, 106.19, 103.24, 101.81, 72.56, 69.21, 52.74 ppm (one carbon masked by DMSO; 42.77-36.72).

Anal. calcd for $C_{14}H_{13}NO_6$: C, 57.73; H, 4.47; N, 4.81. Found: C, 57.79; H, 4.49, N, 4.79.

Further elution gave fractions containing mainly the component Rf 0.46 which, on concentration and filtration, led to pancratistatin (**4a**) (4.5 g). Crystallization from DMF/MeOH/Et_2O gave a colorless solid, mp 322-324° (dec); $[\alpha]^{34}D$ +48° (c 1.0, DMSO); eims *m/z* 325 (M^+, $C_{14}H_{15}NO_8$); uv λ max (MeOH) (log ε) 209 (sh), 219 (sh), 233 (4.32), 278 (3.91), and 308 (br, sh) nm; ir ν 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, 610 cm^{-1}; ^{1}H nmr (400 MHz, DMSO-d_6) 3.04 (1H, brd, J = 10.52 Hz, H-10b), 3.76 (1H, brd, J = 10.5, H-4a), 3.80 (1H, brd, J = 9.5 Hz, H-4), 3.92 (1H, brs, H-3), 4.04 (1H, brs, H-2), 4.35 (1H, brs, H-1), 6.09, 6.11 (2H, -OCH$_2$O-), 6.55 (1H, brs, H-10), 4.93, 5.14, 5.47, 7.56, 13.05, (1H, each, brs, D$_2$O exchangeable).

Anal. calcd for C$_{14}$H$_{15}$NO$_8$: C, 51.69; H, 4.61; N, 4.31. Found: C, 51.65; H, 4.55; N, 4.24.

Method B^7.—Aqueous EtOH (3:7; 300 liters) was percolated through the dried and ground roots (31.5 kg) for 48 h. The process was repeated twice with fresh 70% aqueous EtOH, and the combined extracts were concentrated to half volume (340 liters). The concentrate was treated with deionized H$_2$O (80 liters) and extracted three times with CH$_2$Cl$_2$ (1 × 220 liters and 2 × 108 liters). The combined CH$_2$Cl$_2$ extracts were concentrated to 40 liters and extracted with aqueous MeOH (9:1). After saturation with NaCl, the aqueous MeOH phase was extracted with EtOAc (2 × 8 liters). The aqueous EtOH extract remaining after CH$_2$Cl$_2$ treatment was extracted with EtOAc (2 × 120 liters). After addition of NaCl (6.8 kg), the aqueous solution was again extracted with EtOAc (120 liters). Additional NaCl (4.5 kg) was added and the extraction with EtOAc (120 liters) repeated three more times. The combined EtOAc extracts were concentrated to approximately 7 liters weighing 6.25 kg (containing about 1.19 kg of solid material). The EtOAc solution was applied to a column of silica gel (Davisil 633; 12.5 kg). Initial development with CH$_2$Cl$_2$-EtOH (95:5, 40 liters) followed by elution with CH$_2$Cl$_2$-EtOH (9:1) gave ten fractions (2.5 liters each). On standing, a solid precipitated. After approximately 60 h, each fraction was filtered and concentrated to 250 ml. Standing for another 16 h gave more solid which was isolated by filtration. In this manner, crude pancratistatin (**4a**) (16 g) was obtained from the first five fractions and crude 7-deoxynarciclasine (**2a**) (13.8 g) from the remaining five fractions. Combination and evaporation of the filtrates gave a red-brown, tar-like fraction (A, 83 g) which was shown by tlc on silica gel (CH$_2$Cl$_2$-EtOH, 95:5) to contain additional pancratistatin and 7-deoxynorciclasine.

The crude pancratistatin (16 g) was recrystallized from DMF/MeOH/Et$_2$O to give 9.8 g needing further purification. Recrystallization of the crude 7-deoxynarciclasine (13.8 g) from boiling glacial HOAc/MeOH gave pancratistatin (1.5 g; removed by filtering the hot solution) and 7-deoxynarciclasine (9.6 g) requiring further purification. The mother liquors from both recrystallizations were combined and concentrated to give a tan solid (9 g) which was treated with boiling HOAc. Filtration of the hot solution gave additional crude pancratistatin (2.2 g), and, on cooling, the filtrate yielded more crude 7-deoxynarciclasine (2.2 g). The combined yield of crude pancratistatin was dissolved in DMSO, treated with carbon black, and the solution filtered and diluted with a threefold excess of H$_2$O to give a further concentration of pancratistatin (11.4 g). Similar treatment of the combined amounts of crude 7-deoxynarciclasine gave 9.8 g. Purity of the products was monitored in each instance by hplc on a reversed phase C-18 column using DMSO-H$_2$O (1:4) as eluent.

Final purification of pancratistatin (11.4 g) was achieved by dissolution in DMSO (100 ml), filtering, and injecting the filtrate (in two 50 ml aliquots) into a Waters Prep LC/500 unit containing two Waters reverse phase C-18 cartridges. Elution with DMSO/H$_2$O (1:4) and monitoring fractions by tlc gave fractions that recrystallized from DMSO/H$_2$O to afford pancratistatin (**4a**) (9.6 g) containing a small amount of narciclasine (**2c**). The latter was separated by reversed phase chromatography using acetonitrile-1% HOAc (1:4) as eluent. The narciclasine (0.22 g) was identical with an authentic specimen by tlc, supported by ir and nmr spectral comparisons.

The 7-deoxynarciclasine was also purified by reversed phase chromatography employing DMSO-H$_2$O (35:65) as eluent. Fractions containing isocarbostyril **2a** were concentrated to 100 ml, treated with carbon black, the solution filtered and poured into a large excess of H$_2$O. Filtration provided pure 7-deoxynarciclasine (**2a**) (6.8 g). The mother liquors from the preceding crystallizations were concentrated to dryness and the residues combined with the corresponding fractions from the initial silica gel column (fraction A; 83 g) separation. Reversed phase chromatography (see above) yielded more pancratistatin (3 g, total yield 0.039%) and 7-deoxynarciclasine (4.5 g), together with a mixture (20.4 g) of the two compounds.

7-DEOXYNARCICLASINE TRIACETATE (**2b**).—A 0.5 g sample of 7-deoxynarciclasine (**2a**) was treated with Ac$_2$O (2 ml) and pyridine (2 ml) at room temperature for 48 h. The mixture was added to ice H$_2$O and the solution filtered to give a product (0.5 g) which was chromatographed on a column of silica gel-60 (Merck; 70-230 mesh). Elution with CH$_2$Cl$_2$-MeOH (99:1) afforded triacetate **2b** (0.35 g) which crystallized from CH$_2$Cl$_2$/MeOH as colorless needles, mp 244-246° [lit. 201° (9,11), 233-235° (12)]; [α]^{27}D +219° (c 1.0, CHCl$_3$) [lit. [α]^{20}D +195 (c 0.45, CHCl$_3$) (11)]; eims m/z 417 (M$^+$, C$_{20}$H$_{19}$NO$_9$); uv λ max (MeOH) (log ε), 231 (4.20), 251 (4.21), and 305 (3.93) nm; ir (KBr) ν max 3392, 1760, 1748, 1733, 1663, 1640, 1615, 1500, 1485, 1470, 1400, 1373, 1364, 1263, 1245, 1225, 1076, 1040, 1015, 966, 940, 828, 663 cm^{-1}; ^{1}H nmr (CDCl$_3$) δ 2.12 (3H, s), 2.14 (3H, s), 2.19 (3H, s), 4.71 (1H, dd,

J=9 and 2 Hz), 5.32 (1H, dd, J=9 and 2 Hz), 5.38 (1H, m), 5.55 (1H, m), 6.12 (1H, m), 6.12 (2H, s), 7.06 (1H, s), 7.24 (1H, br, s, removed by D_2O) and 7.59 (1H, s) ppm; ^{13}C nmr (CDCl$_3$) δ 170.4, 169.79, 169.53, 164.52, 151.79, 149.25, 134.24, 130.43, 122.56, 117.12, 107.52, 103.43, 102.07, 71.23, 68.59, 68.30, 50.27, 21.02, 20.86, 20.73 ppm.

Anal. calcd for $C_{20}H_{19}NO_9$: C, 57.55; H, 4.55. Found: C, 57.37; H, 4.63.

The structure was solved by direct methods (18-21) and atomic coordinates refined by full matrix least-squares programs provided in a structure solution package (22). Hydrogen atom coordinates were either calculated and/or located via difference maps and were included in the final refinement. The final standard crystallographic residuals (weighted and unweighted R factors) for the model, which contained anisotropic temperature factors for all heavy atoms and fixed (B=4.0) isotropic factors for all hydrogens, were 0.063 and 0.044, respectively. The maximum shift to error ratio in the last cycle of refinement was 0.55, with all bond distances and angles having nominal values.

Crystal data: compound (**2b**), $C_{20}H_{19}NO_9$, monoclinic, space group P2$_1$, with a=8.325(2), b=8.013(2), c=14.551 (2)Å, β=102.87(2)Å, V=946 4Å^3, DM=1.45, Dc=1.46 g cm^{-3} for Z=2. One quadrant of data on a crystal of dimensions ca. 0.10×0.15×0.75 mm was collected[8] to a maximum of 2θ of 150° using the w/2θ scan technique and graphite monochromated Cu Kα radiation (λ 1.5418Å), and after Lorentz and polarization corrections, 1581 of the reflections with /F/≧3σ (F) were used in the structure determination; absorption corrections were deemed unnecessary (μ=9.5 cm^{-1}).[9]

PANCRATISTATIN PENTAACETATE (**4b**).—Pancratistatin (**4a**) (0.5 g) was acetylated as described above for 7-deoxynarciclasine (**2a**). The product (0.5 g) was chromatographed on a column of Sephadex LH-20 (100 g) using CH$_2$Cl$_2$-MeOH (2:3) as eluent to yield the amorphous pentaacetate: mp 162-166°; [α]^{29}D +85° (c 1.0, CHCl$_3$); eims *m/z* 535 (M$^+$, $C_{24}H_{25}NO_{13}$); uv λ max (MeOH) (log ε) 227 (4.31), 247 (sh), 271 (sh) and 299 (3.75) nm; ir (KBr) ν max 3370, 1760, 1680, 1635, 1510, 1490, 1375, 1340, 1295, 1250, 1220, 1180, 1080, 1045, 950, 930, 860, 815, 758, 640 cm^{-1}; ^1H nmr (400 MHz, CDCl$_3$) 2.056, 2.059, 2.083, 217 (3H each, S, OCCH$_3$), 2.377 (3H, s, ArOCCH$_3$), 3.437 (1H, dd, J_1=13.0, J_2=2.7 Hz, H-10b), 4.258 (1H, dd, J_1=13.0, J_2=10.80 Hz, H-4a), 5.133 (1H, dd, J_1=10.80, J_2=3.52 Hz, H-4), 5.22 (1H, m, H-3), 5.453 (1H, m, H-2), 5.555 (1H, m, H-1), 5.734 (1H, brs, NH, D_2O exchanged), 6.079 (2H, ABq, J=1.16 Hz, -OCH$_2$O), 6.473 (1H, d, J=0.84 Hz, H-10); ^{13}C nmr (CDCl$_3$) δ 170.08, 169.69, 169.07, 169.01, 168.29, 162.96, 152.60, 139.86, 134.53, 132.90, 116.20, 102.94, 101.84, 71.68, 67.69, 66.84, 66.42, 47.86, 40.00, 20.86, 20.70 (last two signals 5C) ppm.

Anal. calcd for $C_{24}H_{25}NO_{13}$: C, 53.83; H, 4.67; N, 2.62. Found: C, 53.75; H, 4.68; N, 2.61.

PANCRATISTATIN METHYL ETHER (**4c**).—Pancratistatin (**4a**) (0.33 g) in MeOH (100 ml) was treated with excess CH$_2$N$_2$ in Et$_2$O. After stirring for 8 h at room temperature, an additional quantity of CH$_2$N$_2$ was added and stirring continued for another 8 h. Evaporation of the solvent gave a residue (0.34 g) that was chromatographed on Sephadex LH-20 using MeOH as eluent to give methyl ether **4c** (0.1 g). Crystallization from MeOH produced colorless plates: mp 294-298° (dec); [α]^{25}D +289.9° (c 0.69, DMSO); ir (KBr) ν 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, 620 cm^{-1}; ^1H nmr (100 MHz, DMSO-d_6) δ 3.58-4.44 (6H), 3.88 (3H, s), 4.70-5.50 (4H, removed by D_2O), 6.13 (2H), 6.74 (1H, s), and 6.92 (1H, removed by D_2O) ppm.

Anal. calcd for $C_{15}H_{17}NO_8$: C, 53.10; H, 5.01; N, 4.13. Found: C, 53.44; H, 5.06; N, 4.08.

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[8]These data were collected on an Enraf-Nonius CAD-4 diffractometer at ambient temperature. Cell constants were determined by least squares refinement of 25 carefully centered reflections in the region 6°≦2θ≦70°.

[9]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Center and can be obtained on request by citing reference (14) from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 IEW, UK.

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