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# ISOLATION AND STRUCTURE OF THE MARINE SPONGE CELL GROWTH INHIBITORY CYCLIC PEPTIDE PHAKELLISTATIN 1[1]

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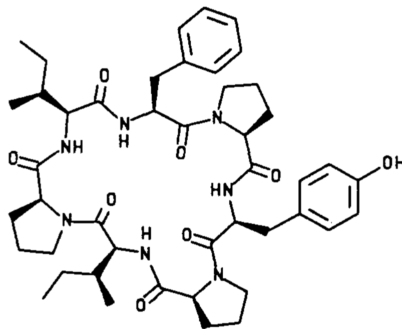
ABSTRACT.—A new cell growth inhibitory (P-388 murine leukemia $ED_{50}$ 7.5 μg/ml) cycloheptapeptide designated phakellistatin 1 was isolated from two Indo-Pacific sponges, *Phakellia costata* and *Stylotella aurantium*. Structural elucidation was accomplished utilizing high field nmr, amino acid analyses, and mass spectral techniques (fab, tandem ms/ms), followed by chiral gas chromatographic procedures for absolute configuration assignments (all *S*-amino acid units). By these methods phakellistatin 1 [**1**] was found to be cyclo (Pro-Ile-Pro-Ile-Phe-Pro-Tyr), and this assignment was finally confirmed by an X-ray crystal structure determination.

Porifera of the class Demospongia have been of long-term (2,3) interest to us and are commonly found (about 10,000 species) in all oceans and at least to −6000 m. These organisms are prominent, diverse, and frequently brightly colored due to carotenoproteins (4) and other pigments. However, taxonomy in this class is difficult and is based predominantly on the microscopic characteristics of spicules and skeletal organization. Our earlier murine-PS-leukemia-guided chemical studies of Porifera containing antineoplastic constituents (5) led to isolation of the geodiastatin proteins (6) and later to the PS cell line growth inhibitors axinastatin 1 (7), a cycloheptapeptide from an *Axinella* sp., and hymenistatin 1 (8), a cyclooctapeptide, from a sponge originally identified as a *Hymeniacidon* sp. and more recently reidentified as *Stylotella aurantium* Kelly-

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[1]Contribution 249 of "Antineoplastic Agents." For part 248, see Pettit *et al.* (1).

Borges & Bergquist (class Demospongiae, order Halichondrida). The number of bioactive metabolites isolated from marine sponges has been rapidly increasing, but only a relatively small number of such amino acids (9) and peptides (10–16) have been discovered. In the large (51 nominal genera) family Axinellidae several of the genera such as *Axinella* have continued to receive our attention as sources of new cell growth inhibitory and antineoplastic substances.

Upon a return (17) expedition (1985) to Palau in the Western Caroline Islands, we located specimens of the sponge *S. aurantium*. As part of a 1987 exploratory survey of the Truk Archipelago (Federated States of Micronesia) Porifera, we found a *Phakellia* species, *Phakellia costata* Kieschnick (class Demospongiae, order Axinellida), that, like the *S. aurantium*, afforded aqueous iPrOH extracts which significantly inhibited growth of the PS leukemia in vivo. Both sponges were found to contain the same new PS (cell line) growth inhibitory cycloheptapeptide herein termed phakellistatin 1 [**1**]. The discovery of phakellistatin 1 in these two species (previously thought to be distantly related) raises questions such as: Is this new peptide actually common in sponges? Is it produced by a commensal microorganism? Would a taxonomic reinvestigation of these sponges be useful? In fact, the presence of phakellistatin 1 in both *Phakellia* (family Axinellidae) and *Stylotella* (family Hymeniacidonidae) is further chemotaxonomic evidence (18) in support of their monophyletic origin, as recently suggested (19) in a proposal to combine the halichondrids and many of the axinellids in a single redefined order Halichondrida.

After extraction and preliminary fractionation based on solvent partition procedures (20), both sponges gave PS cell line active $CH_2Cl_2$ extracts. Initial gel permeation chromatographic separation of the $CH_2Cl_2$ fraction from *P. costata* followed by successive separation based on high speed countercurrent distribution (hsccd) (21) and Si gel Lobar-type cc gave a further increase in PS cell line activity. Final isolation of peptide **1** was accomplished by hplc on a Partisil 10 SIL column. Two major fractions were obtained. The first was a very complex, PS-active mixture currently under investigation. The second fraction afforded pure phakellistatin 1 [**1**] as a colorless amorphous solid.

Phakellistatin 1 was isolated from *S. aurantium* in a somewhat similar manner. Here the active $CH_2Cl_2$ fraction was subjected to three sequences of gel permeation and partition chromatography on Sephadex LH-20. The nearly pure cytostatic component obtained by this route was purified by successive chromatographies using a Si gel Lobar column followed by a Phenomenex reversed-phase hplc column to yield phakelliastatin 1 [**1**].

The structural elucidation of phakellistatin 1 was achieved as follows. The $^{13}C$-nmr spectrum exhibited seven amide carbonyls and seven α-methine carbons. These results and the hrms measurements, which indicated the presence of seven nitrogen atoms, suggested a heptapeptide. The relatively high intensity of the molecular ion (base peak) and the lack of terminal amino group protons in the $^1H$-nmr and ir spectra suggested a cyclic heptapeptide. This hypothesis was supported by lack of reaction between the cycloheptapeptide and $Ac_2O$/pyridine.

The seven amino acids were readily identified by means of 2D nmr techniques. The 500 MHz $^1H$-nmr spectrum clearly showed the presence of four amide hydrogen atoms. By following the spin systems of these hydrogen atoms using $^1H,^1H$-COSY, these amino acids were found to be phenylalanine, tyrosine, and two isoleucine units. Utilization of $^1H,^1H$-COSY, HMQC, and HMBC (22) showed that the remaining nmr resonances consisted of three independent spin systems of the type X-CH-$CH_2$-$CH_2$-$CH_2$-X typical of proline (23). The seven amino acid units accounted for the observed accurate-mass mol wt. Amino acid analyses of the hydrolysate prepared from phakellis-

tatin 1 with 6 N HCl also confirmed the presence of seven amino acids. Evidence for the linkage of the amino acid units was provided by HMBC correlations to give rise to the structure of phakellistatin 1 as (cyclo)-Pro-Ile-Pro-Ile-Phe-Pro-Tyr [**1**]. The proposed structure was confirmed by tandem ms/ms (24). Loss of amino acid residues from the C terminus was observed as the major fragmentation process. As shown in Figure 1, protonation was favored at each proline nitrogen, which afforded three different linear peptide ions in the mass spectrum. All of the ions in each series were observed, thereby confirming the structure assigned to phakellistatin 1 [**1**].

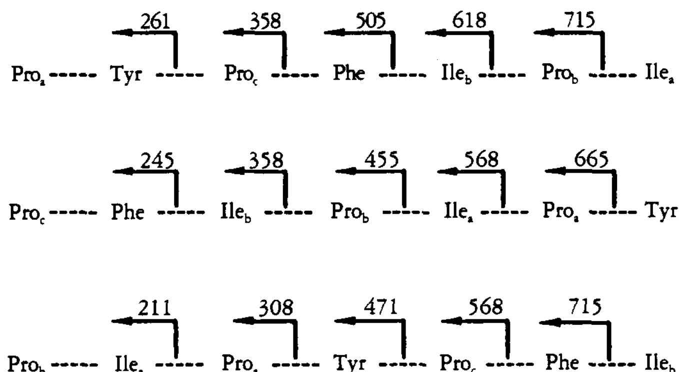


FIGURE 1. Phakellistatin 1 [**1**] peptide sequence determination by ms/ms.

Each of the three PS cell growth inhibitory sponge cyclic peptides we have isolated has been derived completely from *S*-amino acids. Even the strongly antineoplastic sea hare peptides dolastatins 10 (27) and 15 (28) are composed of extensively modified *S* amino acids. Intensive investigations of marine animal antineoplastic and/or cell growth inhibitory peptides currently proceeding in our laboratories should further illuminate the significance of these results.

After all of the preceding structural studies were completed we succeeded in solving the crystal structure (Figure 2) of phakellistatin 1. Very reassuringly the X-ray crystal structure determination gave exactly the same amino acid sequence and absolute configuration originally assigned phakellistatin 1.

## EXPERIMENTAL

GENERAL PROCEDURES.—All solvents were redistilled prior to use. Gilson FC-202 and F-80 fraction collectors connected to Gilson HM uv-vis Holochrome detectors at 230 nm were used for chromatographic fractionation experiments. Tlc analyses were performed on Analtech "uniplate" type 5 × 10 cm hptlc plates using the solvent system $CH_2Cl_2$-MeOH (9:1). Component positions were visualized either by uv detection, a 0.2% ninhydrin solution in EtOH (developed at 120°), or by 3% ceric sulfate in 3 N $H_2SO_4$ spray (heating to approximately 150° for 10 min). The hsccd separations were made using the horizontal coil planet centrifuge P.C. Inc. Model #1 with the planet gear drive at 450 rpm, $\beta = 0.5$–$0.8$. The hsccd column #10 (consisting of 60 m 2.6 mm i.d. PTFE tubing) with a volume of 350 ml was employed. The hplc analyses were performed on a Partisil 5 Silica (250 × 4.6 mm) column with *n*-heptane–$CH_2Cl_2$–MeOH (4:9:1) controlled by an analytical Gilson hplc (Model 802B, 811, 302 × 2). The hplc instrument was equipped with a uv detector (set for 220 nm from the range 210–400 nm) and data station (Hewlett-Packard 1040A). The preparative hplc separations were performed with another Gilson apparatus (811B).

The melting points were observed using a Köfler-type melting point apparatus and are uncorrected. Optical rotation and ir spectral data were obtained using a Perkin-Elmer 241 polarimeter and a Nicolet MX-1 Ft-ir spectrophotometer, respectively. The amino acid analyses were performed with the Hewlett-Packard Amino Quant amino acid analyzer. The low resolution eims mass spectra were recorded with a FINNIGAN-MAT 312 instrument (70 eV) and the ms/ms studies with a Kratos MS-50. The nmr experi-

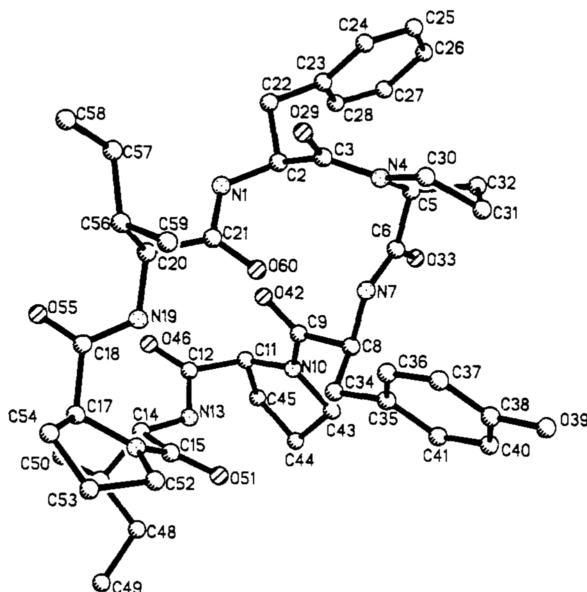


FIGURE 2. Computer-generated X-ray crystal structure of phakellistatin 1 [**1**], prepared using SHELXTL-PLUS (41).

ments were conducted with a Bruker AM 400 and Varian unity 500 instrument using $CD_2Cl_2$ as solvent (TMS internal standard).

Voucher specimens of the two Porifera species employed in this study are maintained in the Queensland Museum (J.N.A.H.) and in our Institute.

*P. COSTATA* COLLECTION AND PRELIMINARY EXPERIMENTS.—One kilogram (wet wt) of the sponge was collected (at − 15 m) by scuba on the southeast side of North Pass on the reef surrounding Pis Island in the Truk Archipelago (Federation of Micronesia) during May 1985, and preserved in MeOH. A specimen of this sponge from the re-collection (see below) was determined to be *Phakellia costata*. Removal of solvent and evaluation of the extract against the murine P-388 lymphocytic leukemia cell line showed an $ED_{50}$ of 17 μg/ml.

*P. COSTATA* RE-COLLECTION, EXTRACTION AND SOLVENT PARTITIONING.—From December 1986 through February 1987, a 220-gallon (about 500 kg wet wt) re-collection was made in the Truk Archipelago and preserved in MeOH. In late May 1987, the shipping solution was decanted. The 55-gallon sponge containers were refilled with $CH_2Cl_2$-MeOH (1:1). After extraction for about 3 weeks the solution was removed and enough $H_2O$ (about 15%) added to complete separation of the $CH_2Cl_2$ layer. The $CH_2Cl_2$ phase was evaporated (in vacuo) and the recovered $CH_2Cl_2$ was remixed with the upper MeOH/$H_2O$ layer. MeOH was added (2.4:1) to reform a single phase. Then the mixture was returned to the sponge containers. After a second extraction of 3 weeks' duration, the barrels were again drained and the solution diluted with $H_2O$ (15%). The $CH_2Cl_2$ layer (second extraction) was evaporated (in vacuo) to give a very dark brown solid (857.8 g, PS $ED_{50}$ 3.7 μg/ml). The brown residue was dissolved in MeOH-$H_2O$ (9:1) and hexane. The hexane layer was separated and concentrated (three times) to a pale brown amorphous solid (562 g, PS $ED_{50}$ 77 μg/ml). The MeOH/$H_2O$ phase was diluted to 3:2 with $H_2O$ and extracted with $CH_2Cl_2$. The $CH_2Cl_2$ extract was concentrated, and the residue (56.3 g) showed significant PS cytostatic activity (PS $ED_{50}$ 0.42 μg/ml). The remaining MeOH/$H_2O$ extract was found to be PS-inactive.

ISOLATION OF PHAKELLISTATIN 1 [**1**].—*Procedure A. From* P. costata.—The PS active $CH_2Cl_2$ fraction was first separated by gel permeation chromatography employing Sephadex LH-20 (2.7 kg, 9 × 130 cm) with MeOH as solvent. Eluted fractions were concentrated and tested. A 0.6-g aliquot of the fractions (6.257 g) that showed the strongest activity (PS $ED_{50}$ 0.32 μg/ml) was separated by high speed countercurrent distribution (22) using hexane-EtOAc-MeOH-$H_2O$ (3:7:5:5), and this resulted in a significant increase in activity. This fraction (121 mg, PS $ED_{50}$ 0.03 μg/ml) still contained an inactive brown material which was removed utilizing a medium pressure (to 50 psi; 1 psi = 6.9 kPa) liquid chromatography unit

with a prepacked Si gel 60 column (2.5 × 30 cm; Lobar B) and elution with hexane-$CH_2Cl_2$-MeOH (2:9:1). The active fraction (29.2 mg, PS $ED_{50}$ 0.03 μg/ml) was finally separated on a Partisil 10SIL hplc column (500 × 10 mm) using heptane-$CH_2Cl_2$-MeOH (4:9:1). Two major fractions were obtained. The first fraction (14.0 mg, PS $ED_{50}$ 0.02 μg/ml) was a mixture of highly active compounds, and this isolation is in progress. The second fraction (7.5 mg, PS $ED_{50}$ 8.0 μg/ml) corresponded to pure phakellistatin 1 [**1**] isolated as a colorless amorphous solid.

*Procedure B. From* S. aurantium.—In March 1985, 250 kg (wet wt) of *S. aurantium* was collected (by scuba) at −10 and −20 m near the south side of Long Island in the Palau Archipelago, Western Caroline Islands, and preserved in iPrOH. The iPrOH solution was decanted and the sponge extracted again with iPrOH. The combined extracts were evaporated (in vacuo) to a 50-liter $H_2O$ concentrate. The brown solution and suspension were decanted and centrifuged to remove solid material (1.2 kg). The cream-colored aqueous phase was partitioned successively between $CH_2Cl_2$ (90 liters) and *n*-BuOH (90 liters). The combined chlorocarbon extract showed the best biological activity. The $CH_2Cl_2$ fraction (180 g, PS $ED_{50}$ 1.8 μg/ml) was dissolved in MeOH-$H_2O$ (9:1), and extracted with hexane (3 × 1 liter). After separation, $H_2O$ (500 ml) was added to the MeOH/$H_2O$ phase to reach MeOH-$H_2O$ (3:2), and the resulting solution was extracted with $CH_2Cl_2$. Only the $CH_2Cl_2$ residue (20 g, PS $ED_{50}$ 0.26 μg/ml) was found to be significantly cytostatic.

The PS-active $CH_2Cl_2$ fraction (20 g) was chromatographed on a Sephadex LH-20 column (2.5 kg, 9 × 130 cm) using $CH_2Cl_2$-MeOH (3:2) as eluent. The fractions were concentrated and bioassayed. The PS-active fraction (18 g, PS $ED_{50}$ 1.6 μg/ml) was placed on a Sephadex LH-20 column (2.5 kg, 9 × 130 cm) and eluted with hexane-toluene-MeOH (3:1:1). The active fractions were combined to give 2.1 g (PS $ED_{50}$ 2.0 μg/ml) that was further separated on a Sephadex LH-20 column (500 g, 3.5 × 120 cm) using MeOH as eluent. The resulting active fraction was concentrated to give 0.75 g (PS $ED_{50}$ 3.1 μg/ml). For further purification 0.3 g of this fraction was placed on a prepacked Si gel column (2.5 cm × 30 cm, Lobar B, 40-63 μm) using medium pressure (to 50 psi) and eluted with $CH_2Cl_2$-MeOH (97:3). The next important fraction (180 mg, PS $ED_{50}$ 8.0 μg/ml) was purified on a Phenomenex reversed-phase hplc column (type $C_{18}$, prepax 25–40 μm, size 250 × 10 mm) using MeOH-MeCN-$H_2O$ (1:1:0.56) as solvent. Phakellistatin 1 [**1**] was again isolated as a colorless, amorphous solid (43 mg): mp 247–249°; $[\alpha]^{25}D$ −50.5 ($c$ = 0.333, $CHCl_3$); uv (MeOH) λ max 228, 278 nm ($\xi_1$ = 3063.1, $\xi_2$ = 1631); ir (KBr) 3420, 2962, 2876, 1656, 1620, 1518, 1450, 1278 $cm^{-1}$; hr fabms $[M+H]^+$ 828.4660 for $C_{45}H_{62}N_7O_8$ (calcd 828.46598); nmr ($CD_2Cl_2$) as follows.

Pro-a: $^1H$ nmr δ 4.15 (t, $J$ = 7.8 Hz, H-2), 3.86 (m, 2H, H-5), 2.33 (m, $H_a$-3), 2.15 (m, $H_a$-4), 2.07 (m, $H_b$-4); 1.90 (m, $H_b$-3); $^{13}C$ *nmr* δ 171.96 (CO), 62.89 (C-2), 47.45 (C-5), 29.88 (C-3), 26.19 (C-4). Ile-a: $^1H$ nmr δ 6.40 (d, $J$ = 6.7 Hz, NH), 4.26 (t, $J$ = 6.1 Hz, H-2), 1.68 (m, H-3), 1.62 (m, $H_a$-4), 1.49 (m, $H_b$-4), 1.00 (t, $J$ = 7.4 Hz, 3H, H-5), 0.91 (d, $J$ = 6.9 Hz, 3H, H-6); $^{13}C$ nmr δ 171.28 (CO), 55.55 (C-2), 40.33 (C-3), 26.43 (C-4), 15.91 (C-6), 12.38 (C-5). Pro-b: $^1H$ nmr δ 4.46 (d, $J$ = 8.3 Hz, H-2), 3.56 (m, 2H, H-5), 2.25 (m, $H_a$-3), 2.11 (m, $H_b$-3), 1.96 (m, $H_a$-4), 1.87 (m, $H_b$-4); $^{13}C$ nmr δ 170.08 (CO), 61.79 (C-2), 46.95 (C-5), 32.23 (C-3), 22.09 (C-4). Ile-b: $^1H$ nmr δ 6.60 (d, $J$ = 6.8 Hz, NH), 4.38 (dd, $J$ = 6.9, 4.1 Hz, H-2), 1.91, H-3), 1.47 (m, $H_a$-4), 1.05 (m, $H_b$-4), 0.93 (d, $J$ = 7.0 Hz, 3H, H-6), 0.86 (t, $J$ = 7.3 Hz, 3H, H-5); $^{13}C$ nmr δ 169.30 (CO), 57.31 (C-2), 9.11 (C-3), 24.90 (C-4), 14.09 (C-6), 11.85 (C-5). Phe: $^1H$ nmr δ 7.29 (m, 3H, H-6, H-7, H-8), 7.17 (d, $J$ = 7.0 Hz, 2H, H-5, H-9), 5.93 (br, NH), 4.43 (m, H-2), 3.02 (m, 2H, H-3); $^{13}C$ nmr δ 169.79 (CO), 135.31 (C-4), 129.83 (C-5, C-9), 129.43 (C-6, C-8), 128.19 (C-7), 54.95 (C-2), 38.67 (C-3). Pro-c: $^1H$ nmr δ 3.30 (m, $H_a$-5), 3.22 (m, $H_b$-5), 3.09 (m, H-2), 1.84 (m, $H_a$-3), 1.46 (m, $H_a$-4), 1.13 (m, $H_b$-3), 0.84 (m, $H_b$-4); $^{13}C$ nmr δ 171.07 (CO), 60.77 (C-2), 47.32 (C-5), 31.32 (C-3), 21.90 (C-4). Tyr: $^1H$ nmr δ 8.38 (d, $J$ = 8.9 Hz, NH), 7.06 (d, $J$ = 8.5 Hz, 2H, H-5, H-9), 6.70 (d, $J$ = 8.5 Hz, 2H, H-6, H-8), 4.65 (m, H-2), 3.08 (m, $H_a$-3); 2.73 (t, $J$ = 13.4 Hz, $H_b$-3); $^{13}C$ nmr δ 171.19 (CO), 155.54 (C-7), 130.42 (C-5), 129.7 (C-4), 115.55 (C-6), 54.33 (C-2), 36.71 (C-3).

TREATMENT OF PHAKELLISTATIN 1 WITH $Ac_2O$/PYRIDINE.—A solution of phakellistatin 1 (250 μg) in $Ac_2O$-pyridine (1:1) (4 drops) was stirred at room temperature overnight. According to the $^1H$-nmr spectrum no reaction occurred.

AMINO ACID ANALYSIS OF PHAKELLISTATIN 1.—A specimen of phakellistatin 1 (10 μg) in 6 N HCl containing 1% phenol was heated to 105° for 24 h. After evaporation the residue was dissolved in 3 M borate buffer (ph = 10.4), and all amino acids except Pro were converted to fluorescent derivatives by reaction with *o*-phthalaldehyde (OPA) in the presence of 2-mercaptopropionic acid. The proline was allowed to react with 9-fluorenylmethylchloroformate (FMOC). The mixture was injected onto a Hewlett-Packard reversed-phase hplc column (ODS Hypersil C18, 2.1 × 200 mm) using a gradient system of solvents: A (20 mM NaOAc, pH 7.2, containing 0.1 mM $Na_2EDTA$, 0.0015% triethylamine, and 0.5% THF); B (20 mM NaOAc, pH 7.2 in 80% MeCN, 20% $H_2O$ containing 0.02 mM $Na_2EDTA$); gradient t = 0, 0% B;

t = 9 min, 30% B; t = 13 min, 50% B; t = 14 min, 100% B; flow rate 0.45 ml/min. All amino acids were eluted within 13 min. The OPA derivatives were detected by illuminating at 340 nm and measuring fluorescence at 450 nm, while the FMOC derivative of Pro was detected by illuminating at 266 nm and measuring fluorescence at 305 nm. The hydrolysis of cyclic peptide **1** resulted in the following amino acids: Pro (3 mol), Ile (2 mol), Phe (1 mol), Tyr (1 mol).

ASSIGNMENT OF THE PHAKELLISTATIN 1 CHIRAL CENTERS.—Cycloheptapeptide **1** (250 μg) was hydrolyzed with propionic acid-concentrated HCl (1:1) at 160° for 15 min (25). The corresponding amino acids were converted to *N*-pentafluoropropionyl-2-propyl ester derivatives and configurations established by chiral capillary chromatography (26) using a Chirasil Val III column and comparison with standard amino acids. Each amino acid component of phakellistatin was found to belong to the *S* (L) series.

PHAKELLISTATIN 1 [**1**] X-RAY CRYSTAL STRUCTURE DETERMINATION[2].—Recrystallization of phakellistatin 1 [**1**] from MeCN/MeOH/$H_2O$ provided colorless crystals (marginal quality) for X-ray crystallography. The best specimen (0.30 × 0.46 × 0.56 mm) was used in the data collection. Crystal data: $C_{45}H_{61}N_7O_8$·MeCN·$H_2O$, monoclinic space group $P2_1$, with $a$ = 9.289 (1), $b$ = 20.560 (4), $c$ = 13.113 (9) Å, β = 109.04 (3)°, V = 2367.33 $Å^3$, $\rho_o$ (variable) = 1.213 g·$cm^{-3}$, $\rho_c$ = 1.244 g·$cm^{-3}$ for Z = 2, and F.W. = 887.09, (000) = 952. One quadrant of data was collected to a maximum of 2θ = 150° on an Enraf-Nonius CAD-4 diffractometer at low temperature (−128°). After measurement of each reflection, the Friedel equivalent was also collected whenever possible. The ω/2θ scan technique was used with graphite monochromated CuKα radiation (1.5418 Å). After Lorentz and polarization corrections, merging of equivalent reflections and rejection of systematic absences, a total of 8676 unique reflections remained, of which 8326 [Fo>3σ(Fo)] were considered observed and used in the subsequent structure determination and refinement.

Linear and anisotropic decay corrections were applied to the intensity data was well as an empirical absorption correction based on a series of psi-scans (29). Near the conclusion of the refinement process, an additional least-squares absorption correction was made using the program DIFABS (30-32) contained in the CRYSTALS (33) suite of programs. A number of attempts at a structure solution for peptide **1** were made using a variety of direct-methods programs, among these being MULTAN (34), SHELXS-86 (35), and MITHRIL (36–38). A vector search solution method was also attempted utilizing PATSEE (39). In each case, the absence of recognizable fragments precluded further structure expansion. As a result, X-ray crystal structure solution efforts were discontinued for several years until we were able to apply a new software direct methods program, SIR88 (40). All but two non-hydrogen atoms were revealed with SIR88 when the PTEN (application of p-10 formula) and MESS (Maximally Entroprical Starting Sets) options were applied. The remaining two non-hydrogen atoms of the parent molecule, as well as atoms attributed to solvation with 1 mole each of MeCN and $H_2O$, were located in subsequent difference Fourier maps. Refinement was performed with CRYSTALS using $1/\delta_F{}^2$ weighting. The hydrogen atom coordinates were calculated at optimum positions and were included but not refined in the final cycle of least-squares refinement. Full matrix least-squares anisotropic refinement on all non-hydrogen atoms (except for the solvate atoms, which were refined isotropically), and isotropic temperature factors (U = 0.08) for hydrogens yielded standard crystallographic residuals of R = 0.108, $R_\omega$ = 0.134. The rather high R values (particularly for a low temperature data collection) are attributed primarily to the poor quality of the crystal, although the absorption corrections are also suspected of being less than adequate. The X-ray structure determined agreed in all respects with the structure assignment based upon nmr/mass spectral results. A computer-generated drawing of phakellistatin 1 is shown in Figure 2. The stereochemical designations for the 9 chiral centers (using the numbering system in Figure 2) are as follows: 2(*S*), 5(*S*), 8(*S*), 11(*S*), 17(*S*), 20(*S*), 47(*S*), 56(*S*).

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[2]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained from Dr. Olga Kennard, 12 Union Road, Cambridge, CB2 1EZ, UK.

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