Evolving Trends in the Dereplication of Natural Product Extracts. 2. The Isolation of Chrysaibol, an Antibiotic Peptaibol from a New Zealand Sample of the Mycoparasitic Fungus *Sepedonium chrysospermum*

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By the application of an HPLC bioactivity profiling/microtiter technique in conjunction with capillary NMR instrumentation and access to the AntiMarin database the conventional evaluation/isolation dereplication/characterization procedures can be dramatically truncated. This approach is illustrated using the isolation of a new peptaibol, chrysaibol (**1**), from a New Zealand isolate of the mycoparasitic fungus *Sepedonium chrysospermum*. The unique nature of chrysaibol was recognized by bioactivity-guided fractionation using HPLC bioactivity profiling/microtiter plate analysis in conjunction with capillary NMR instrumentation and the AntiMarin database. 2D NMR techniques, in combination with MS fragmentation experiments, determined the planar structure of chrysaibol (**1**), while the absolute configurations of the amino acid residues were defined by Marfey's method. Chrysaibol (**1**) was cytotoxic against the P388 murine leukemia cell line (IC_{50} 6.61 μM) and showed notable activity against *Bacillus subtilis* (IC_{50} 1.54 μM).

In an attempt to reduce the scale of effort spent on isolation processes and to determine much earlier in an investigation whether the bioactive components of a particular extract are known or new compounds, we have developed methodologies based on small-scale HPLC separations of extracts, early use of ^{1}H NMR spectroscopy on microgram quantities of compounds, and reference to databases of known compounds. The combination of extract evaluation using HPLC profiling with biological evaluation[1] followed by capillary probe NMR spectroscopy/ESIMS/UV/AntiMarin database evaluation reduces the crude extract requirement for dereplication to submilligram quantities. Furthermore, for compounds of molecular mass less than ~600 Da, it is possible to acquire high-quality 1D and 2D NMR data over a reasonable time frame to allow full characterization from the material collected into a single microtiter plate well (20−40 μg). This effectively telescopes crude extract evaluation, isolation, dereplication, and characterization into just one process.[2,3]

These methods have been applied as a key strategy in our continuing search for new, bioactive metabolites from New Zealand fungi. A particular strain of *Sepedonium chrysospermum* (CANU E609) (Hypocreaceae) attracted our attention. *S. chrysospermum* is a yellow pigmented anamorphic fungus living parasitically on basidiomycetes.[4] Mycoparasitic fungi are a diverse and prolific source of compounds with potential therapeutic value.[5] The polyketides sepedonin and derivatives,[6] along with chrysodin,[7] have been isolated previously from this species, while the New Zealand strain CANU E609 has been the source of the cyclic pentapeptide chrysosporide, two aromatic polyketides, and phomalactone.[8] Previously, it had been observed that strain CANU E609 gave an altered metabolic profile under different culture conditions;[8] however even when regrown under identical conditions to those in a previous study the crude extract showed a different metabolite pattern. The crude extract was cytotoxic against the P388 murine leukemia cell line (IC_{50} 5.1 μg/mL). HPLC analysis on an analytical RP-18 HPLC column revealed just two peaks: one major and the other minor. A larger aliquot (700 μg) of the crude extract was then chromatographed with collection of fractions (88 × 250 μL) into a 96-well microtiter plate. After biological evaluation of a daughter plate and evaporation of the solvent, the two isolated bioactive compounds (located in wells E8−E9 and E11−E12) were analyzed using capillary probe NMR spectroscopy and ESIMS. The minor compound (E11−E12) was readily identified as chrysosporide, the cyclic pentapeptide previously isolated from the same strain.[8] The ^{1}H NMR spectra of the major compound (E8−E9: 33 μg) acquired using a CapNMR probe (Figure 1) contained readily recognizable structural features such as multiple amide NH groupings and an indolic moiety (δ_H 8.37−6.99), resonances for α-protons of α-amino acids, alcohols, and amines (δ_H 3.0−4.6), an acetyl group (δ_H 2.00), and up to 20 methyl singlets and doublets. In addition it could be noted that the compound contained no *O*-Me or *N*-Me groupings. When these data, or combinations of them, were searched against the AntiMarin database,[9] a database of some 47 000 natural products, just 19 compounds matched these data. These compounds were all peptidic and in the main belonged to the peptaibol class of compounds. If the molecular weight data (*m/z* 1619.9) were added to the search profile, no hits resulted, confirming that this compound, named chrysaibol (**1**), was a new bioactive peptidic molecule.

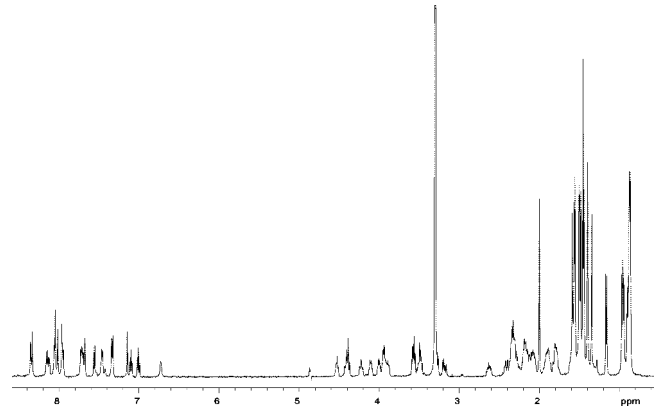

Figure 1. ^{1}H NMR spectrum of chrysaibol (**1**) acquired from 33 μg of material in 6 μL of CD_3OD using a capillary NMR probe. Total acquisition time 2 min.

Results and Discussion

The 33 μg of chrysaibol (**1**) recovered from wells E8−E9 following the chromatography of 700 μg of crude extract had given

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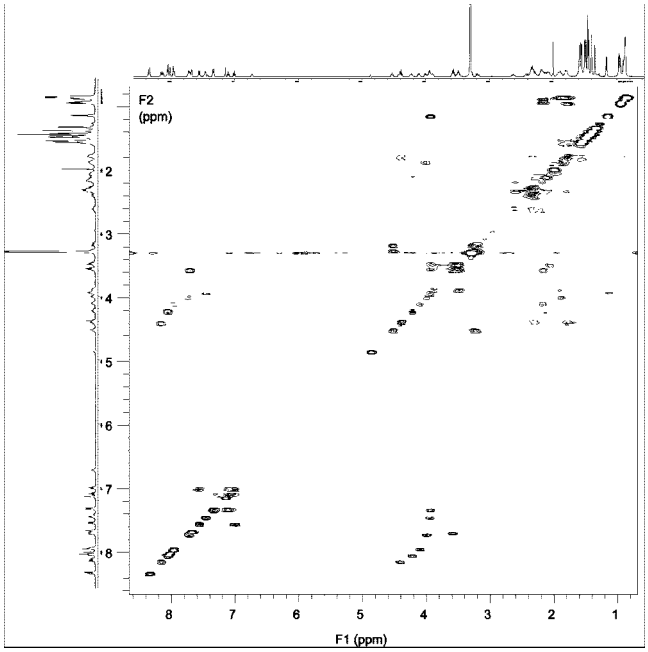
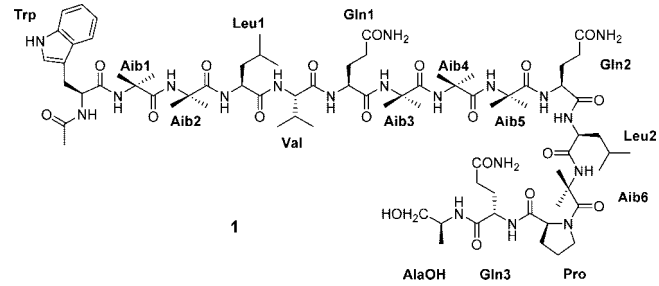


Figure 2. COSY NMR spectrum of chrysaibol (**1**) acquired on 33 μg of material in 6 μL of CD_3OD using a capillary NMR probe. Total acquisition time 16 min.

very acceptable 1D (Figure 1) and 2D (Figure 2) homonuclear ^{1}H NMR spectra that had allowed an initial assessment of structural features. Application of various MS techniques, using this sample, established a working structure for chrysaibol (**1**) based on the sequential fragmentation of the peptide, but this structure was not fully definitive (*vide infra*). The mass of sample, in this instance 33 μg, was insufficient to allow the acquisition of sufficient heteronuclear 2D NMR data on a molecule of this size in a reasonable period of time (overnight run).

By semipreparative HPLC a larger sample of chrysaibol (**1**) (~30 mg) was isolated as an optically active solid and a full NMR data set obtained (see Supporting Information). Using HRESIMS, in combination with the ^{13}C NMR data, the molecular formula $C_{77}H_{125}N_{19}O_{19}$ was established. Although the presence of overlapping signals significantly complicated the structural elucidation of **1**, a detailed analysis of the 1D and 2D NMR data (Supporting Information, Table 1) led to the identification of the following five proteinogenic amino acids: an acetylated tryptophan, three glutamines, two leucines, a proline, and a valine. Six of the seven remaining unidentified residues had identical structural features. In the ^{1}H NMR spectrum each of these residues had two singlet methyl groups, which each showed long-range H,C-correlations in the CIGAR-HMBC experiment to quaternary α-carbons and carbonyl carbons, thus unambiguously identifying six aminoisobutyric residues (Aib). The final residue was identified as the amino alcohol alaninol. The TOCSY and HSQC-DEPT experiments were used to confirm that the chemical shift for the α-proton of alaninol was δ 3.94 and that it was correlated with a methine carbon at δ 48.8. This was necessary due to overlap of this resonance with the α-proton of Gln1 and partially with one of the δ-protons of Pro. In the COSY spectrum, the α-proton of alaninol at δ 3.94 showed couplings to a doublet methyl group (δ 1.16) and to two diastereotopic methylene protons (δ_H 3.57 and 3.49; δ_C 66.6 (HSQC-DEPT)) to define the spin system for alaninol.

Taking into account the molecular formula and the amino acid composition of **1**, it was concluded that chrysaibol was a linear peptide. Furthermore, the presence of multiple aminoisobutyric acid residues and an amino alcohol in the structure of chrysaibol (**1**) suggested that this compound was a member of the peptaibol family of peptides.



The amino acid sequence of **1** was deduced from a detailed interpretation of the IMPRESS,[10] ESIMS/MS, and ESIMS data. In the IMPRESS NMR experiment the higher resolution possible in the F1 dimension allowed the $^2J_{CH}$ correlations between the amide protons and the carbonyl carbons of the preceding amino acid to be resolved, establishing a partial sequence for **1** as (Ac-Trp-Aib1-Aib2-Leu1-Val-Gln1-Aib3-Aib4-Aib5-Gln2-Leu2-Aib6) (see Figure 3; Supporting Information, Table 1). This partial structure was confirmed by the ESIMS/MS experiment (Figure 3; Supporting Information, Figure S1). The fragmentation of the protonated molecular ion gave only an intense b_{12} ion (*m/z* 1320.7). The subsequent fragmentation of this daughter ion gave, in turn, intense b_{11}, b_9, b_8, b_7, b_6, b_5, b_4, and b_3 ions, confirming the amino acid sequence proposed from the IMPRESS experiment (Supporting Information, Figure S1). Additional information clarifying the structure of peptaibol **1** was extracted from the ESIMS experiment, where y-type as well as b-type fragment ions were detected (see Experimental Section). In the ESIMS spectrum of **1**, b_{13} together with intense b_{12} and y_3 ions were observed. This served to define the connection of a proline residue to Aib6, as the strong b_{12} and y_3 fragment ions are most logically explained by the preferential cleavage of the peptide bond at the proline residue.

No data were available from the IMPRESS, CIGAR-HMBC, or MS experiments that could be used to establish the position of the two remaining subunits, glutamine-3 and alaninol. Two alternative connectivities were possible: -Pro-Gln3-AlaOH, with two amide bonds connecting glutamine-3 with proline and alaninol, or -Pro-AlaOH-Gln3, with an ester bond between alaninol and glutamine-3. Distinction between these two possibilities came from an evaluation of the chemical shifts of the β-protons of alaninol. As these were both less than 4 ppm (δ_H 3. 57 and δ_H 3. 49), it could be concluded that alaninol was not involved in an ester bond and was therefore the *C*-terminal group in chrysaibol (**1**), establishing the planar structure of chrysaibol (**1**) as (Ac-Trp-Aib1-Aib2-Leu1-Val-Gln1-Aib3-Aib4-Aib5-Gln2-Leu2-Aib6-Pro-Gln3-AlaOH).

Using Marfey's method[11] all amino acids as well as alaninol in chrysaibol (**1**) were found to be of *S*-configuration.

Chrysaibol (**1**) exhibited moderate cytotoxic activity against the P388 murine leukemia cell line (IC_{50} 6.61 μM), while in the antimicrobial assays **1** showed notable activity against *Bacillus subtilis* (IC_{50} 1.54 μM), but was inactive against *Pseudomonas aeruginosa* and *Candida albicans*.

Chrysaibol (**1**) is a rare natural peptaibol containing alaninol on the *C*-terminus. The only other peptaibol with this end grouping was found recently by LC/ESIMS/MS study on *Trichoderma* cf. *brevicompactum*.[12] The closest relative to **1**, which also contains the fragment (Gln-Aib-Aib-Aib-Gln-Leu-Aib), is ampullosporin, an antibiotic 15-membered peptaibol isolated from *Sepedonium ampullosporum*.[13] Chrysaibol shows structural features typical for the peptaibol SubFamily6 (SF6),[14] which includes ampullosporin, e.g., an oligopeptide chain composed of 15 amino acid residues, a conserved tryptophan at position 1, and a conserved glutamine at positions 6 (6 or 7 in other members) and 14, but unlike the other members of the SF6 subfamily, it has the characteristic leucinol on the *C*-terminus replaced by alaninol.

Many peptaibols have antimicrobial properties,[15] particularly ampullosporin, structurally related to chrysaibol, which was active

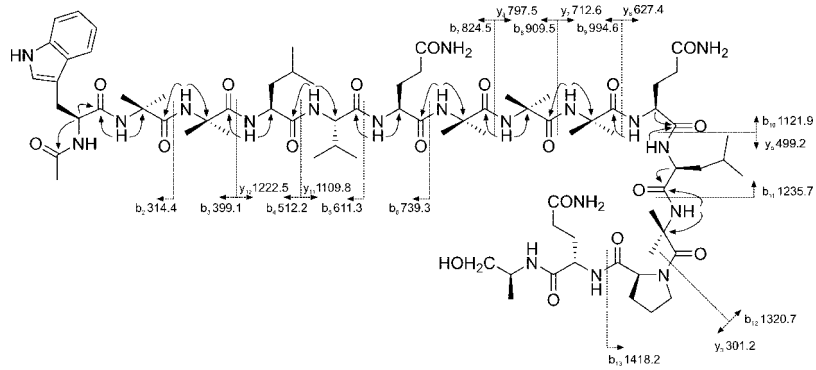


Figure 3. Fragmentation pattern and long-range H,C-correlations for chrysaibol (**1**). Fragment ions of b and y type were observed in the ESIMS spectrum. Only b-type fragment ions were observed in the ESIMS/MS spectrum. Long-range H,C-correlations (arrows) establishing the amino acid sequence of **1** were extracted from the IMPRESS experiment.

at 150 μg per well in an agar diffusion assay against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* but exhibited no activity against *Candida albicans*.[13]

In this example, this approach to dereplication allowed the rapid recognition of a new peptaibol using just 700 μg of crude *Sepedonium chrysospermum* extract. The high-quality 1D and 2D homonuclear ^{1}H NMR and ESIMS/MS data (Figures 1 and 2; Supporting Information, Figure S1) obtained on the isolated sample of chrysaibol (**1**) (33 μg) fully defined the structural type, but not the final structural details, which needed further work.

This technique is now being applied routinely in our laboratory and being successfully applied to the full characterization of known and new fungal metabolites, including peptides, in the mass range 300−700 Da. From acquisition of the ^{1}H NMR spectra to establishment of the "uniqueness" of a component is a matter of minutes and depends only on how long it takes to complete a definitive database search. As the sensitivity of the capillary NMR probes is improved, the scale on which the dereplication of natural product extracts can be performed will drop, allowing more ready access to smaller extract samples or to the lower concentration components in a crude extract.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. UV spectra were registered on a Hewlett-Packard 8452A spectrophotometer. NMR spectra were recorded on a Varian INOVA AS-500 spectrometer (500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively), using the signals of the residual solvent protons and the solvent carbons as internal references (δ_H 3.3 and δ_C 49.3 ppm for CD_3OD). A Protasis CapNMR capillary probe was used for the microplate dereplication studies. HRESIMS were acquired using a Micromass LCT TOF mass spectrometer. MS/MS experiments were performed on a Bruker Daltonics Esquire 4000 system. Solvents used for extraction and isolation were distilled prior to use. Bioactivity assays were made using standard protocols.[1] Melting points were measured using an Electrothermal melting point apparatus and are uncorrected.

Fungus. The collection site, morphological characteristics, and culture conditions for *Sepedonium chrysospermum* CANU E609 were reported previously.[8] After 28 days of growth, the mycelium was separated from the culture medium, macerated, and extracted with EtOAc (3 × 100 mL). The culture broth (500 mL) was extracted with EtOAc (3 × 500 mL). The combined extracts were dried to yield the crude extract (172 mg).

Evaluation of Extract. An aliquot of the extract (700 μg) was analyzed by HPLC (RP-18, solvents: (A) H_2O + 0.05% TFA, (B) MeCN; gradient: 0 min 10% B, 2 min 10% B, 14 min 75% B, 24 min 75% B, 26 min 100% B; flow: 1 mL/min; 40 °C). The eluent from the DAD was split in a 1:10 ratio between the ELSD and the fraction collector configured to collect into a 96-well microtiter plate (15 s/well). A total of 88 wells were collected (2.5−24.5 min). A daughter plate was prepared by transferring an aliquot (5 μL) from each well of the master plate. After complete evaporation of the solvent, the wells in the daughter plate were analyzed for activity against P388 murine leukemia cells as described previously.[1] The assay established that cytotoxicity was correlated with each of the two peaks observed by HPLC/ELSD/UV. The wells E8−E9 and E11−E12 of the dried master plate, containing the bioactive compounds chrysaibol (**1**) and chrysosporide,[8] respectively, were analyzed using capillary probe NMR spectroscopy. The contents of wells E8−E9 and E11−E12 were each dissolved in CD_3OD (7 μL) and transferred into the Protasis CapNMR capillary probe. Calibrations have shown that this effectively transfers 6 μL of sample into the probe. Standard operating conditions were used to acquire 1D and 2D homonuclear NMR experiments. The quantity of the compounds was estimated according to the formula

$$(MW/^{\#}H) \times (\text{total integral for } ^{\#}H)/(\text{integral for } CHD_2OD) \times CF$$

where MW is the actual molecular weight of the compound (ESMS), or an estimated value, $^{\#}$H is the number of protons included in the integration of the ^{1}H NMR spectrum, and CF is the calibration factor that had previously been determined from a standard solution containing quinine (30 μg in 6 μL) in the same CD_3OD solvent.

Purification of Chrysaibol (1). The crude extract (172 mg) was chromatographed on a Sephadex LH-20 column (25 × 1000 mm) using MeOH as eluent (7 mL fractions). Fractions 1 and 2 were combined and further purified by semipreparative HPLC (Phenomenex Luna C18, 10 × 250 mm, 5 μm; eluents: H_2O containing 0.05% (v/v) TFA (A), MeCN (B); gradient: 0 min 50% B, 14 min 75% B; flow: 5 mL min^{-1}; UV detection at 210 nm) to yield **1** (6.68−7.90 min, 30.3 mg) and chrysosporide[8] (8.50−9.01 min, 1.3 mg).

Chrysaibol (1): white crystals, 17.6% of dry wt of the crude extract; mp 252−254 °C; $[\alpha]^{20}_D$ −21 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 222 (4.65), 274 (sh) (3.87), 282 (3.88), 290 (sh) (3.84); for ^{1}H and ^{13}C NMR data, see Supporting Information, Table 1; ESIMS *m/z* (%) 1642.4 $[M + Na]^+$ (6.0), 1620.9 $[M + H]^+$ (6.5), b_{13}: 1418.2 [MH − (Gln-AlaOH)]$^+$ (4.0), b_{12}: 1320.7 [1418.2 − (Pro)]$^+$ (100), y_{12}: 1222.5 [MH − (Ac-Trp-Aib-Aib) + H]$^+$ (0.7), b_{10}: 1121.9 [1320.7 − (Leu-Aib)]$^+$ (2.5), y_{11}: 1109.8 [1222.5 − Leu + H]$^+$ (3.7), b_9: 994.6 [1121.9 − Gln]$^+$ (4.4), b_8: 909.5 [994.6 − Aib)]$^+$ (1.7), y_8: 797.5 [1109.8 − (Val-Gln-Aib) + H]$^+$ (1.1), y_7: 712.6 [797.5 − Aib + H]$^+$ (1.1), y_6: 627.4 [712.6 − Aib + H]$^+$ (3.5), b_4: 512.2 [909.5 − (Val-Gln-Aib-Aib)]$^+$ (0.1), y_5: 499.2 [627.4 − Gln + H]$^+$ (2.1), b_2: 314.4 [512.2 − (Aib-Leu)]$^+$ (3.0), y_3: 301.2 [499.2 − (Leu-Aib) + H]$^+$ (15.4); HRESIMS *m/z* 832.95723 ± 3.36 ppm $[M + 2Na]^{2+}$ (calcd for $C_{77}H_{125}N_{19}O_{19}Na_2$, 832.95973).

Alaninol. L-Alaninol and D-alaninol were prepared by standard literature procedures from the ethyl esters of the corresponding stereochemically pure alanines using $LiBH_4$ in THF.[16] The physical properties and spectroscopic data for L-alaninol and D-alaninol matched published data.[17]

Preparation and Analysis of Marfey Derivatives. Chrysaibol (**1**) (0.5 mg) was hydrolyzed by heating in HCl (6 M; 0.5 mL) at 110 °C for 24 h. For analysis of tryptophan a second hydrolysis in the presence of an antioxidant (1% phenol in 6 M HCl) and heating at 130 °C for 1 h was performed. The Marfey analysis on each hydrolysis sample was carried out using the procedures described previously.[8] Retention times (min) of the FDAA amino acid derivatives used as standards were as follows: L-AlaOH (6.2), D-AlaOH (7.6), L-Glu (9.6), D-Glu

(5.9), L-Trp (15.5), D-Trp (18.4), L-Pro (7.5), D-Pro (8.1), L-Val (11.5), D-Val (16.2), L-Leu (17.2), and D-Leu (23.7). Retention times (min) of the observed peaks in the HPLC trace of the FDAA-derivatized hydrolysates of **1** were as follows: L-AlaOH (6.2), L-Glu (9.6), L-Trp (15.2), L-Pro (7.4), L-Val (11.5), L-Leu (17.2).

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Supporting Information Available: Table with 1D and 2D NMR data for **1** and 1D, 2D NMR and ESI MS/MS spectra for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Lang, G.; Mitova, M.; Ellis, G.; van der Sar, S.; Phipps, R. K.; Blunt, J. W.; Cummings, N. J.; Cole, A. L. J.; Munro, M. H. G. *J. Nat. Prod.* **2006**, *69*, 621–624.

(2) Lang, G.; Mahyudin; Mitova, M.; Lin Sun; van der Sar, S.; Blunt, J. W.; Cole, A. L. J.; Ellis, G.; Laatsch, H.; Munro, M. H. G. *J. Nat. Prod.* **2008**, *71*, 1595–1599.

(3) (a) Blunt, J. W.; Cole, A. L. J.; de Silva, D.; Lang, G.; Mitova, M.; MacLean, W.; van der Sar, S. 4th European Conference on Marine Natural Products, Paris, September 12−16, 2005. (b) Blunt, J. W.; Cole, A. L. J.; de Silva, D.; Lang, G.; Mitova, M.; MacLean, W.; van der Sar, S. Pacifichem 2005, International Chemical Congress of Pacific Basin Societies, Honolulu, HI, December 14−19, 2005. (c) Lang, G.; Mitova, M.; van der Sar, S.; Mahyu din, N. A.; Cummings, N. J.; Abdullah, N.; Blunt, J. W.; Cole, A. L. J.; Din, L. B.; Vikneswary, S.; Munro, M. H. G. ICOB-5 & ISCNP-25 IUPAC International Conference on Biodiversity and Natural Products, Kyoto, Japan, July 23−28, 2006. (d) Blunt, J. W. 47th Annual Meeting of the American Society of Pharmacognosy, Arlington, VA, August 5−9, 2006. (e) Blunt, J. W.; Munro, M H. G. 5th European Conference on Marine Natural Products. Ischia, September 16−21, 2007. (f) Blunt, J. W.; Munro, M H. G. 2nd CMDD Symposium on Marine Natural Products, Seoul, Korea, November 1−4, 2007. (g) Blunt, J. W.; Chamyuang, S.; Cole, A. L J.; Hickford, S.; Lang, G.; Mahyudin, N. A.; Mitova, M.; Munro, M H. G.; van der Sar, S.; Sun, Lin.; Yusof, M. T. Asian Mycology Congress (2007) and Xth International Marine and Freshwater Symposium, Penang, Malaysia, December 2−6, 2007.

(4) Sahr, T.; Ammer, H.; Besl, H.; Fischer, M. *Mycologia* **1999**, *91*, 935–943.

(5) Mitova, M. I.; Lang, G.; Blunt, J. W.; Cummings, N. J.; Cole, A. L. J.; Robinson, W. T.; Munro, M. H. G. *J. Org. Chem.* **2006**, *71*, 492–497.

(6) Wright, J. L. C.; McInnes, A. G.; Smith, D. G.; Vining, L. C. *Can. J. Chem.* **1970**, *48*, 2702–2708.

(7) Closse, A.; Hauser, D. *Helv. Chem. Acta* **1973**, *56*, 2694–2698.

(8) Mitova, M. I.; Stuart, B. G.; Cao, G. H.; Blunt, J. W.; Cole, A. L. J.; Munro, M. H. G. *J. Nat. Prod.* **2006**, *69*, 1481–1484.

(9) *AntiMarin Database*; Blunt, J. W.; Munro, M. H. G.; Laatsch, H., Eds.; University of Canterbury: Christchurch, New Zealand, and University of Göttingen; Göttingen, Germany, 2006.

(10) Crouch, R.; Boyer, R. D.; Johnson, R.; Krishnamurthy, K. *Magn. Reson. Chem.* **2004**, *42*, 301–307.

(11) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.

(12) Degenkolb, T.; Graefenhan, T.; Nirenberg, H. I.; Gams, W.; Brueckner, H. *J. Agric. Food Chem.* **2006**, *54*, 7047–7061.

(13) Ritzau, M.; Heinze, S.; Dornberger, K.; Berg, A.; Fleck, W.; Schlegel, B.; Hartl, A.; Grafe, U. *J. Antibiot.* **1997**, *50*, 722–728.

(14) Chugh, J. K.; Wallace, B. A. *Biochem. Soc. Trans.* **2001**, *29*, 565–570.

(15) Duclohier, H. *Chem. Biodiversity* **2007**, *4*, 1023–1026.

(16) Baker, G. L.; Fritschel, S. J.; Stille, J. K. *J. Org. Chem.* **1981**, *46*, 2954–2960.

(17) Sigma Aldrich home page: http://www.sigmaaldrich.com/spectra/fnmr/FNMR009323.PDF and FNMR002590.PDF. Accessed August 2006.

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