ORGANIC LETTERS

2002
Vol. 4, No. 12
2095−2096

A Novel Cyclodepsipeptide, HA23, from a *Fusarium* sp.

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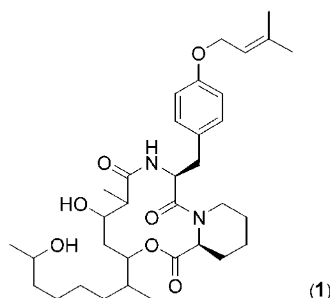
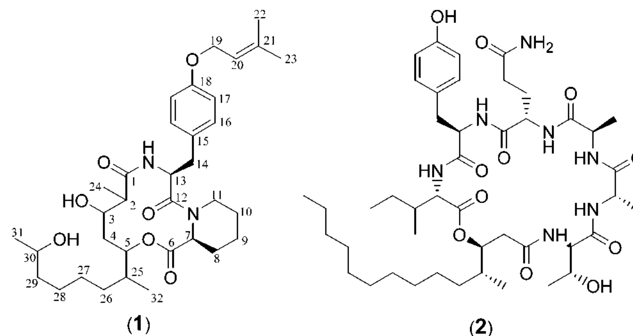
Received April 15, 2002

ABSTRACT



HA 23, a novel cyclodepsipeptide (1) of mixed peptide–polyketide origins, was isolated from a fungal isolate of a *Fusarium* sp. The structure was determined from 1D and 2D NMR and mass spectral data.

During an investigation of the biologically active metabolites produced by fungi, the extract from a *Fusarium* sp. showed strong antifungal activity. Bioassay-guided fractionation and purification of the culture extract led to the isolation of a novel cyclodepsipeptide HA 23 (**1**) and the known antifungal secondary metabolite W 493-B (**2**).[1] We report here the isolation and structural elucidation of (**1**).[2]



Fusarium sp. (CANU-HA 23) was fermented in half strength Sabouraud dextrose yeast broth under static conditions at 26 °C for 28 days. The ethyl acetate extract (273 mg) from both the mycelium and culture filtrate (1 L) was chromatographed on a flash reverse phase (rp) column using

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(2) Compound **1** was obtained as a colorless oil: $[\alpha]^{20}_{D}$ −46° (*c* 0.001, MeOH); UV (MeOH) λ_{max} (log ϵ) 232 (3.23), 278 (2.80); IR (chloroform) γ_{max} 3692, 3638, 3414, 2943, 2839, 2367, 2336, 1730, 1676, 1634, 1603, 1510, 1464, 1335, 1267, 1182, 1126 cm^{-1}; HRESIMS 601.3858 (M + H$^+$) (calcd for $C_{34}H_{53}N_2O_7$, 601.3853); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.12 (1H, d, 9.0, H16), 6.83 (1H, d, 9.0, H17), 6.56 (1H, brs, NH), 5.47 (1H, t, 5.0, H20), 5.40 (1H, d, 5.0, H7), 5.32 (1H, m, H13), 4.77 (1H, dt, 10.0, 3.0, H5), 4.47 (2H, d, 6.5, H19), 4.14 (1H, m, H3), 3.98 (1H, brd, 14.0, H11a), 3.77 (1H, m, H30), 3.16 (1H, dd, 13.0, 9.5, H14a), 2.88 (1H, dd, 13.0, 5.5, H14b), 2.69 (1H, t, 12.0, H11b), 2.56 (1H, m, H2), 2.10 (1H, brd, 14.0, H8a), 1.96 (1H, m, H25), 1.78 (3H, brs, H22), 1.73 (3H, brs, H23), 1.69 (2H, m, H4), 1.63 (1H, m, H10b), 1.57 (1H, m, H26a), 1.55 (1H, m, H9a), 1.54 (1H, m, H10a), 1.47 (1H, t, 7.5, H29a), 1.36 (2H, m, H28), 1.33 (1H, m, H8b), 1.32 (1H, m, H29b), 1.30 (2H, m, H27), 1.18 (3H, d, 6.0, H31), 1.08 (1H, m, H26b), 1.07 (3H, d, 7.0, H24), 0.86 (1H, m, H9b), 0.80 (3H, d, 6.0, H32); ^{13}C APT NMR ($CDCl_3$, 125 MHz) δ 173.7 (C1), 171.5 (C12), 169.7 (C6), 157.7 (C18), 138.1 (C21), 129.9 (C16), 128.7 (C15), 119.6 (C20), 114.8 (C17), 77.0 (C5), 68.9 (C3), 68.1 (C30), 64.8 (C19), 51.1 (C7), 48.8 (C13), 47.8 (C2), 45.1 (C11), 38.8 (C29), 36.0 (C14), 34.4 (C25), 33.1 (C4), 31.8 (C26), 26.3 (C27), 25.8 (C22), 25.7 (C10), 25.4 (C28), 24.6 (C8), 23.6 (C31), 19.9 (C9), 18.2 (C23), 15.8 (C32), 7.3 (C24). NOE correlations: H2/NH, H2/H3, H2/H24, H3/NH, H3/H4, H3/H25, H4/H5, H4/H24, H5/H26b, H5/H32, H7/H8a, H7/H8b, H8a/H8b, H9a/H9b, H11a/H11b, H11a/H13, H13/H14a, H13/H14b, H13/H16, H14a/H14b, H14a/H16, H14b/H16, H17/H19, H19/H20, H19/H23, H20/H22, H25/H32, H28/H30, H30/H31.

10.1021/ol0260167 CCC: $22.00 © 2002 American Chemical Society
Published on Web 05/21/2002

a sharp, stepped gradient from water through methanol to dichloromethane. The fraction that eluted off the rp column with methanol/water (9:1) was partitioned between petroleum ether and methanol. The methanol fraction was repeatedly chromatographed on diol eluting with a petroleum ether/ethyl acetate gradient (4:1 to 3:2 to 1:1) to yield **1** (1.8 mg) and **2** (2.2 mg).

The cyclodepsipeptide (**1**) was obtained as a colorless oil with $[\alpha]_D = -46°$. The UV spectrum of **1** displayed absorption maxima at 232 and 278 nm, suggesting the presence of an oxygen-substituted aromatic system. IR peaks at 3692, 3414, 1730, 1676, and 1634 cm^{-1} indicated the presence of alcohol, amine, and amide (or ester) functionalities. The ES positive ion mass spectrum of **1** showed strong $M + H^+$ and $M + Na^+$ peaks at *m/z* 601 and 623. High-resolution mass measurement on the $M + H^+$ (*m/z* 601.3858) in the ESI mass spectrum, in combination with ^1H and ^{13}C NMR data[2] supported the molecular formula $C_{34}H_{52}N_2O_7$ (10 double bond equivalents).

The ^1H NMR spectrum of **1**[2] contained signals assignable to a 1,4-disubstituted phenyl ring system, an exchangeable proton at 6.56 ppm, six methines (five were heteroatom-substituted), two heteroatom-substituted methylenes, and five methyl groups. The APT NMR experiment[2] exhibited 32 carbon signals, comprising 5 CH_3, 11 CH_2, 10 CH, and 6 quaternary carbon signals. In addition to the four aromatic carbon signals for a 1,4-disubstituted phenyl group, two other sp^2 carbon signals accounted for a further double bond. Three quaternary carbon signals at 169.7, 171.5, and 173.7 ppm were assigned as amide (or ester) carbonyl groups.

A series of COSY, HSQC, and CIGAR[3] experiments established several sequences of partial connectivities and defined the subunits **b**−**f** (Figure 1 in bold). The assignments of two hydroxyls at C3 and C30 and one ester (or lactone) substituent at C5 in subunits **e** and **f** were based on the ^1H and ^{13}C chemical shifts at the three positions[2] and supported by the molecular formula. Subunit **a** was established by the COSY correlations from two broad methyl singlets to the methine group, and the CIGAR correlations from the protons at the two methyl groups to the two sp^2 carbons (Figure 1). A series of 1D TOCSY experiments confirmed the spin systems in subunits **a**−**d**. The connectivity of subunits **e** and **f** through two methylene groups was also established by 1D and 2D TOCSY experiments.

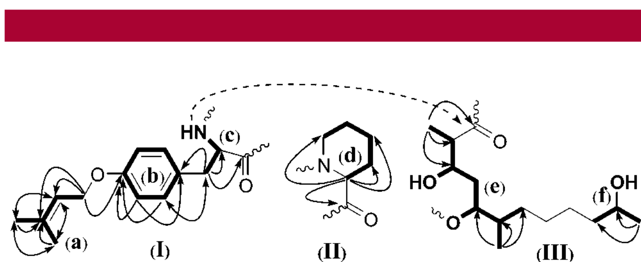

Figure 1. CIGAR correlations in subunits **I**, **II**, and **III**: (solid line) in chloroform-*d*; (dash line) in pyridine-d_5.

A detailed analysis of the CIGAR experimental data allowed the assembly of the subunits into an *O*-prenyl-substituted tyrosine residue (**I**), a pipecolinic acid residue (**II**), and the polyketide residue (**III**) (Figure 1).

The residues **I**−**III** accounted for all but one degree of unsaturation required by the molecular formula, suggesting that **1** is a cyclic depsipeptide. The assembly of **I**−**III** was determined by a second CIGAR experiment using a different solvent, pyridine-d_5. In this experiment an additional correlation from the amide NH in **I** to the carbonyl group in **III** was observed (Figure 1), allowing the assignment of HA 23 as **1**.

The absolute stereochemistry of the amino acid residues in the molecule was determined. Acid hydrolysis of **1** with 6 N HCl at 110° C provided tyrosine and pipecolinic acid. HPLC analysis of the FDAA-derivatized amino acids confirmed that each amino acid had an *S*-configuration. NOESY experiments on **1** were illuminating but not conclusive. Although a range of NOE correlations were observed,[2] uncertainty on the conformations of the 12-membered depsipeptide reduced the reliability of the possibilities of any stereochemical conclusions to speculation only. Because of the limited amount of sample available, no other chemical reactions were attempted to determine the configurations of the remaining stereogenic centers in the polyketide portion.

The second metabolite, W 493-B (**2**), had been previously reported by a Japanese group in 1998. The NMR spectral data were identical to those reported in the literature.[1]

Compound **1** is a unique depsipeptide containing a 14-carbon polyketide unit, a substituted tyrosine, and pipecolinic acid. This metabolite of mixed peptide−polyketide origins possesses a structurally novel polyketide moiety and is composed of just three residues, whereas depsipeptides (and cyclic peptides) typically incorporate either four or more than five residues.[4]

Biological studies on natural products with similar structural features indicated that they possess a variety of biological activities.[5] In this present study, W 493-B showed antifungal activity against *Cladosporium resinae*, whereas HA 23 was not active in either antimicrobial or cytotoxicity bioassays.

Acknowledgment. Financial support from the University of Canterbury for a Postdoctoral fellowship (Y.F.) is acknowledged and from Instituto BioMar SA for technical support. We thank Mr. Bruce Clark for mass spectrometric analyses, Ms. Gill Ellis for bioactivity assays, and Mr. Nick Cummings for fungal isolation and fermentation.

Supporting Information Available: Detailed description of experimental procedures and ^1H and ^{13}C APT NMR spectra for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0260167

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