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Penicillazine, a Unique Quinolone Derivative with 4*H*-5,6-Dihydro-1,2-oxazine Ring System from the Marine Fungus *Penicillium* sp. (Strain #386) from the South China Sea

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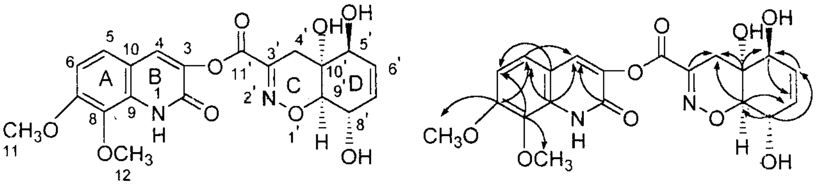
Abstract—Penicillazine (**1**) is a new compound with both quinolone and 4*H*-5,6-dihydro-1,2-oxazine ring systems that was isolated from a culture of the marine fungus *Penicillium* sp. (strain #386). Its structure was elucidated using spectroscopic methods, primarily 2D NMR techniques, and was confirmed by an X-ray diffraction analysis. Variations in the ^{1}H NMR spectrum of penicillazine (**1**) were observed over a 65°C temperature range. © 2000 Elsevier Science Ltd. All rights reserved.

Recent years have seen the discovery of many new compounds from marine organisms, especially marine fungi. As a source of new organisms to screen, marine fungi offer great potential, as most are unique to their marine habitat.[1–3] We have embarked on a study of the metabolites of marine fungi from the South China Sea and this has yielded a number of interesting compounds. In this paper, we report a new compound, penicillazine (**1**), that was isolated from a *Penicillum* sp. collected from sand in a marine habitat.

A 20 L fermentation broth was concentrated and extracted with ethyl acetate. The extract was repeatedly chromatographed on silica gel columns. Penicillazine (**1**) was obtained as colorless needles, mp 216–218°C, that reacted with $KBiI_4$ forming a brownish-red precipitate(the test for alkaliods). Penicillazine (**1**) has the molecular formula $C_{20}H_{20}N_2O_9{\cdot}H_2O$ as determined by FABMS and elemental analysis. The numbers of hydrogen and carbon atoms in the ^{1}H and ^{13}C NMR spectra were in agreement with the molecular formula.

The IR spectrum of **1** showed the presence of two carbonyl groups with bands at 1720 and 1675 cm^{-1}. In the ^{13}C NMR spectrum, there were two carbonyl signals and only thirteen aromatic signals, therefore it was deduced that there were six double bonds, one of which should be carbon–nitrogen double bond. There were four signals assigned to carbons bearing oxygen at δ 85.2 (CH), 75.2 (CH), 69.4 (C) and 68.4 (CH). The twelve unsaturation equivalents required by the molecular formula indicated that the compound has four rings. The partial structure of ring D in **1** was revealed by the COSY spectrum that showed a contiguous sequence of coupled signals from H-5′ to H-9′, and a long-range



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H-C correlations from HMBC of 1

Figure 1. Compound **1** and its H–C correlations from HMBC.

Keywords: penicillazine; quinolone; *Penicillium* sp.

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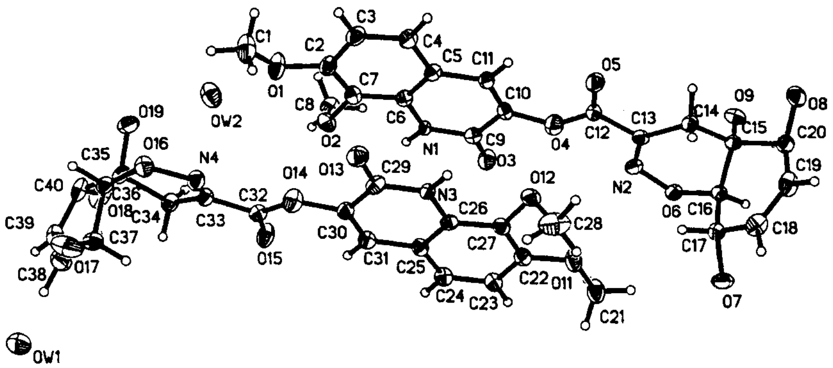

Figure 2. The view of two molecules of **1**.

coupling between H-4′ and H-9′. The HMBC data established the overall structure of **1** (Fig. 1), especially the multiple correlations from H-4, H-5 and H-6 that were used to define the quinolone ring system. The HMBC spectrum clearly showed the correlations from H-4 to C-2, C-5 and C-9, from H-5 to C-4, C-7and C-9, and from H-6 to C-8, C-10 and C-11, respectively.

The *trans* relationship of H-8′ and H-9′ was assigned from the coupling constant ($J_{8',9'}$=8 Hz). A correlation in the ROESY spectrum indicated that H-5′ and H-9′ were *cis* to one another. The structure of penicillazine (**1**) was finally confirmed by X-ray diffraction analysis that showed the relative configuration of the compound to be (5′*S**,8′*S**,9′*R**,10′*R**). The dihydro-oxazine and cyclohexene rings were *cis*-fused. The crystal structure showed that **1** crystallized as a monohydrate (Fig. 2.).

Variable temperature ^{1}H NMR spectra of **1** showed an interesting phenomenon (Table 1). With the temperature raised, the chemical shifts of signals due to protons on the C and D rings moved upfield, while those of protons on the A and B rings moved downfield. In striking contrast, the hydroxy signals associated with the C and D rings moved downfield, while the methoxy signals on the A and B rings moved upfield. There were no changes in the coupling constants. We propose that these chemical shift changes are due to intermolecular hydrogen bonding, and that the C and D rings were rigid over the temperature range. Further analysis is in progress.

Penicillazine (**1**) is a structurally unique compound. To our knowledge, this is the first report of a natural product with a (4H)-5,6-dihydro-1,2-oxazine ring system.

Experimental

General

NMR data were recorded on a Varian Inova 500 NMR spectrometer, mass spectra on a VG-ZAB mass spectrometer, IR spectra on Nicolet 5DX-FTIR spectrophotometer, UV spectra on a Shimadzu UV-240 spectrophotometer, optical rotations on a Horiba High Sensitivity Polarimeter SEPA-300, elemental analyses on a Perkin–Elmer 240C elemental analyzer and X-ray data were generated on a Bruker Smart 1000 CCD system diffractometer.

Fungal strain

A strain of the fungus *Penicillum* sp. (#386) was isolated from sand in a marine habitat from the South China Sea, and was stored in Department of Biology and Chemistry, City University of Hong Kong, Hong Kong, P. R. China.

Table 1. Data from variable temperature ^{1}H NMR of **1** (solvent DMSO)

	25°C	50°C	90°C	To upfield
1-NH	9.33 (s)	9.31(s)	9.27(s)	upfield
H-4	8.55(s)	8.53(s)	8. 50(s)	upfield
H-5	7.50(d, 9)	7.46(d, 9)	7.41(d, 9)	upfield
H-6	7.14(d, 9)	7.12(d, 9)	7.10(d, 9)	upfield
OCH_3-11	3.91(s)	3.91(s)	3.91(s)	downfield
OCH_3-12	3.85(s)	3.86(s)	3.88(s)	downfield
H-4′a	2.04(d, 19)	2.05(d, 19)	2.09(d, 19)	downfield
H-4′b	2.48(d, 19, 3)	2.50(d, 19, 3)	2.52(d, 19, 3)	downfield
H-5′	4.23(ddd,10,2,2)	4.24 (ddd,10,2,2)	4.26 (ddd,10,2,2)	downfield
H-6′	5.45 (ddd,10,2,2)	5.46 (ddd,10,2,2)	5.47 (ddd,10,2,2)	downfield
H-7′	5.38 (ddd,10,2,2)	5.40 (ddd,10,2,2)	5.41 (ddd,10,2,2)	downfield
H-8′	3.98(m)	3.98(m)	3.99(m)	downfield
H-9′	4.00(dd,8, 2.5)	4.01(dd,8,2.5)	4.02(dd, 8, 2.5)	downfield
OH-5′or8′	5.40(d,5.5)	5.28(d, 5.5)	5.08(d, 5.5)	upfield
OH-5′or8′	5.28(d, 5.5)	5.14(d, 5.5)	4.91(d, 5.5)	upfield
OH-10′	5.24(s)	5.13(s)	4.94(s)	upfield

Table 2. NMR data of **1**

	^{13}C	^{1}H	^{1}H–^{1}HCOSY	HMBC	ROESY
1					
2	159.8(C)			H-4	
3	122.4 (C)				
4	125.7(CH)	8.59(s)	H-5	H-5	H-5
5	123.9 (CH)	7.30(d, *J*=9.0 Hz)	H-4, 6	H-4	H-4
6	111.4(CH)	7.08(d, *J*=9.0 Hz)	H-5, 11		H-11
7	155.8(C)			H-5, 11	
8	137.5(C)			H-6, 12	
9	145.4(C)			H-4, 5	
10	115.5(C)			H-6	
11	57.1(CH_3)	3.97(s)	H-6		H-6
12	61.8(CH_3)	3.92(s)			
1′					
2′					
3′	151.7(C)			H-4′	
4′	24.5(CH_2)	a.2.28(d, 19.5 Hz)	H-4′b, 9′		
		b. 2.74(dd, 19.5, 2.5 Hz)	H-4′a, 9′		
5′	75.2(CH)	4.39(dd,5.5, 2.0 Hz)	H-6′, 7′, 8′	H-7′	H-9′
6′	131.1 (CH)	5.62(ddd, 10.0, 2.0, 2.0 Hz)	H-7′, 5′, 8′	H-5′	H-8′
7′	128.8 (CH)	5.55(ddd, 10.0, 2.0, 2.0 Hz)	H-6′, 5′, 8′		H-8′, 5′
8′	68.4(CH)	4.15(ddd, 5.5, 7.5, 2.0 Hz)	H-9′,5′, 7′, 6′	H-6′, 9′	H-7′, 6′
9′	85.2(CH)	4.12(dd, 7.5, 2.5 Hz)	H-4′, 8′	H-7′, 4′b	H-5′
10′	69.4(C)			H-5′, 4′	
11′	162.9(C)				
	OH	4.48(s,2H), 4.67(s, ^{1}H)			
	NH	9.40(brs)			

Culture conditions

Starter cultures (from Professor E. B. G. Jones and Dr L. L. P. Vrijmoed) were maintained on cornmeal seawater agar. Plugs of agar supporting mycelial growth were cut and transferred aseptically to a 250 mL Erlenmeyer flask containing 100 mL liquid medium (glucose 10 g/L, peptone 2 g/L, yeast extract 1 g/L, NaCl 30 g/L). The flask was incubated at 30°C on a rotary shaker for 5–7 days. The mycelium was aseptically transferred to 500 mL Erlenmeyer flasks containing culture liquid (200 mL). The flasks were incubated at 30°C for two months.

Extraction and separation of metabolites

The cultures (20 L) were filtered through cheesecloth. The filtrate was concentrated to 2 L below 50°C, and extracted three times by shaking with an equal volume of ethyl acetate. The combined extracts were chromatographed on silica gel using a gradient elution from petroleum to ethyl acetate to obtain penicillazine (**1**, 12 mg) from the 10% methanol/ethyl acetate fraction as colorless crystals: mp 216–218°C, $[\alpha]_D^{25}=-11.91$ (*c* 2.2, $CHCl_3$); IR (KBr) 3550, 3375, 1720, 1675, 1610, 1525, 1460, 1375, 1090 cm^{-1}; UV: λ_{max} ($CHCl_3$) 205 nm (log ϵ 4.6), 266 nm (log ϵ 4.0), 343 nm (log ϵ 4.0); ^{1}H NMR ($CDCl_3$,TMS), ^{13}C NMR ($CDCl_3$) and 2D NMR see Table 2; MS (EI) *m/z* 433 $(M+1)^+$, 290, 247, 232, 206, 192, 176, 178, 167, 161, 149, 132, 122, 97, 69, 55; MS (FAB) *m/z* 433$(M+1)^+$, 248, 221, 192, 176, 165, 149, 120, 95, 69, 65, 57; Anal. Found: C 53.18, H 4.86, N 5.76. Calcd (for $C_{20}H_{20}N_2O_9\cdot H_2O$): C 53.33, H 4.89, N 6.22.

X-Ray crystallography

Crystal system, space group Triclinic, P1, Unit cell dimensions a=7.5361(7) Å, α=80.533(2)°, **b**=7.9805(7) Å, β=80.121(2)°, c=17.2201(15) Å, γ=83.599(2)°. Volume=1002.70(16) Å^3, Z=2, D_{calcd}= 1.495 mg/m^^{3}, m=0.121 mm^{-1}, F_{000}=474. All single-crystal data were collected using the hemisphere technique on a Bruker SMART 1000 CCD system diffractometer with graphite monochromated Mo Kα radiation λ=0.71069 at 173 (2) K. The structures were solved by direct methods using SHELXTLV5.0 (Siemens IndustriaI Automation lnc, Madison.WI) and refined using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Absorption corrections were applied with the Siemens Area Detector ABSorption program (SADABS). The final value of R was 0.0602, wR_2=0.1712 [$I>2\sigma(I)$].

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