Pergamon

Tetrahedron Letters 41 (2000) 7177–7180

TETRAHEDRON LETTERS

Monocillinols A and B, novel fungal metabolites from a *Monocillium* sp.

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Received 28 June 2000; revised 12 July 2000; accepted 18 July 2000

Abstract

Monocillinols A (**1**) and B (**2**), two novel isomeric fungal metabolites, have been isolated from the chloroform extract of the culture filtrate of a *Monocillium* sp. The structures of **1** and **2** were elucidated by interpretation of their spectral data. Published by Elsevier Science Ltd.

Keywords: fungal metabolites; monocillinols; *Monocillium* sp.

Microorganisms have historically provided a rich source of structurally diverse, biologically active secondary metabolites. Previous investigations of fungi in the genus *Monocillium* have led to the isolation of a series of aromatic polyketide derivatives, several of which exhibit antifungal activity.[1] As part of our continuing studies of metabolites produced by microorganisms obtained from soil samples collected throughout Bangladesh,[2–4] we isolated the fungus *Monocillium* sp.[5] from soil collected in the region of Rajshahi. Purification by C_{18} HPLC of the $CHCl_3$ extract

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0040-4039/00/$ - see front matter Published by Elsevier Science Ltd.
PII: S0040-4039(00)01252-1

(80 mg) of the culture filtrate when this organism was grown in a Czapek Dox broth,[6] afforded two novel compounds, monocillinols A (**1**) (3.0 mg)‡ and B (**2**) (2.6 mg).§

The molecular formula of monocillinol A (**1**), established as $C_{11}H_{13}NO_5$ by HR-FABMS measurements, required six double bond equivalents in the molecule. IR absorption bands at ν 1724 and 1667 cm^{-1} suggested the presence of α,β-unsaturated ester and amide carbonyl groups, respectively. The ^{13}C NMR spectrum of **1** displayed 11 carbon resonances, while DEPT and HSQC experiments revealed that seven out of the 11 carbons had attached protons. ^{13}C resonances for two carbonyls (δ 166.3 and 160.4) and four olefin carbons (δ 152.4, 141.1, 127.5, and 98.5) were evident; thus compound **1** was bicyclic. The ^1H NMR spectrum showed proton resonances which could be assigned to an olefinic methine, an exomethylene group, three oxygen or nitrogen bearing methines, and an allylic methylene group. In addition, a methoxyl group and two exchangeable protons were evident. The deshielded nature of both the olefinic (δ 7.09) and exomethylene (δ 4.99 and 5.85) proton signals indicated that they each were β to carbonyl groups. It was possible to trace all of the proton–proton spin systems in **1** with data from a COSY-45 experiment. In the COSY spectrum, the oxymethine proton at δ 4.55 was coupled to the exchangeable OH proton at δ 7.67, to the olefinic proton at δ 7.09, and to a methine resonance at δ 3.83. The latter proton exhibited additional correlations with a methine at δ 4.11, which in turn was coupled to a pair of allylic methylene protons (δ 2.52 and 3.04). Both of these methylene protons also showed long range couplings with the olefinic proton. These data allowed assignment of partial structure **1a** (Fig. 1). Further structural assignments were aided by heteronuclear correlation experiments. HSQC data indicated that the protons at δ 3.83 (δ_c 59.0) and 4.11 (δ_c 72.6) were attached to carbons bearing nitrogen, and oxygen, respectively. In the HMBC spectrum, both of the methylene protons showed correlations to the olefin carbons at δ 127.5 and δ 141.1, while the olefinic proton showed a three bond correlation to the methylene carbon at δ 30.2. This indicated that partial structure **1a** was cyclized to provide substructure **1b**. The methoxyl protons at δ 3.66 revealed a ^3J correlation with the carbonyl group at δ 166.3, which also exhibited HMBC correlations with the olefinic and methylene protons. These correlations defined the location of the methyl ester and helped establish the extended partial structure **1c.**

‡ Compound **1**: amorphous white solid; $[\alpha]_D$ –33.5 (*c* 0.02, MeOH); UV (EtOH) λ_{max} (log ε) 205 (4.08), 218 (4.14) nm; IR ν_{max} (film) 3407, 1724, 1667, 1651, 1616, 1440, 1410, 1387, 1340, 1306, 1258, 1236, 1143, 1102, 1056, 1003, 967, 882, 785, 737 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N) δ 10.05 (1H, bs, NH), 7.67 (1H, d, J = 6.5 Hz, OH-6α), 7.09 (1H, bs, H-7), 5.85 (1H, bs, H-11a), 4.99 (1H, bs, H-11b), 4.55 (1H, m, H-6β), 4.11 (1H, ddd, J = 16.5, 10.0, 6.0 Hz, H-10β), 3.83 (1H, *t*, J = 10.0 Hz, H-5α), 3.66 (3H, s, OCH_3), 3.04 (1H, dd, J = 17.5, 6.0 Hz, H-9β), 2.52 (1H, m, H-9α); ^{13}C NMR (125 MHz, C_5D_5N) δ 166.3 (s, C-12), 160.4 (s, C-3), 152.4 (s, C-2), 141.1 (d, C-7), 127.5 (s, C-8), 98.5 (t, C-11), 72.6 (d, C-10), 70.8 (d, C-6), 59.0 (d, C-5), 51.9 (q, OCH_3), 30.2 (t, C-9); CIMS m/z 240 $[M+H]^+$, 257 $[M+NH_4]^+$; HR-FABMS: m/z 240.0781 $[M+H]^+$; $C_{11}H_{14}NO_5$ requires: 240.0872.

§ Compound **2**: amorphous white solid; $[\alpha]_D$ +53.0 (*c* 0.02, MeOH); UV (EtOH) λ_{max} (log ε) 207 (4.06), 217 (4.01) nm; IR ν_{max} (film) 3515, 3216, 1718, 1679, 1630, 1432, 1374, 1300, 1256, 1238, 1110, 1062, 1049, 1009, 943, 899, 873, 785, 728 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N) δ 9.73 (1H, bs, NH), 7.50 (1H, bs, OH-10α), 6.85 (1H, bs, H-7), 5.82 (1H, d, J = 1.0 Hz, H-11a), 4.97 (1H, bs, H-11b), 4.59 (1H, m, H-6β), 4.02 (1H, m, H-10β), 3.71 (1H, *t*, J = 10.0 Hz, H-5α), 3.61 (3H, s, OCH_3), 3.02 (1H, dd, J = 17.5, 5.0 Hz, H-9β), 2.53 (1H, m, H-9α); ^{13}C NMR (125 MHz, C_5D_5N) δ 166.2 (s, C-12), 161.0 (s, C-3), 153.1 (s, C-2), 134.4 (d, C-7), 130.9 (s, C-8), 98.7 (t, C-11), 75.7 (d, C-6), 67.0 (d, C-10), 58.1 (d, C-5), 52.0 (q, OCH_3), 35.0 (t, C-9); HR-FABMS: m/z 240.0865 $[M+H]^+$; $C_{11}H_{14}NO_5$ requires: 240.0872.

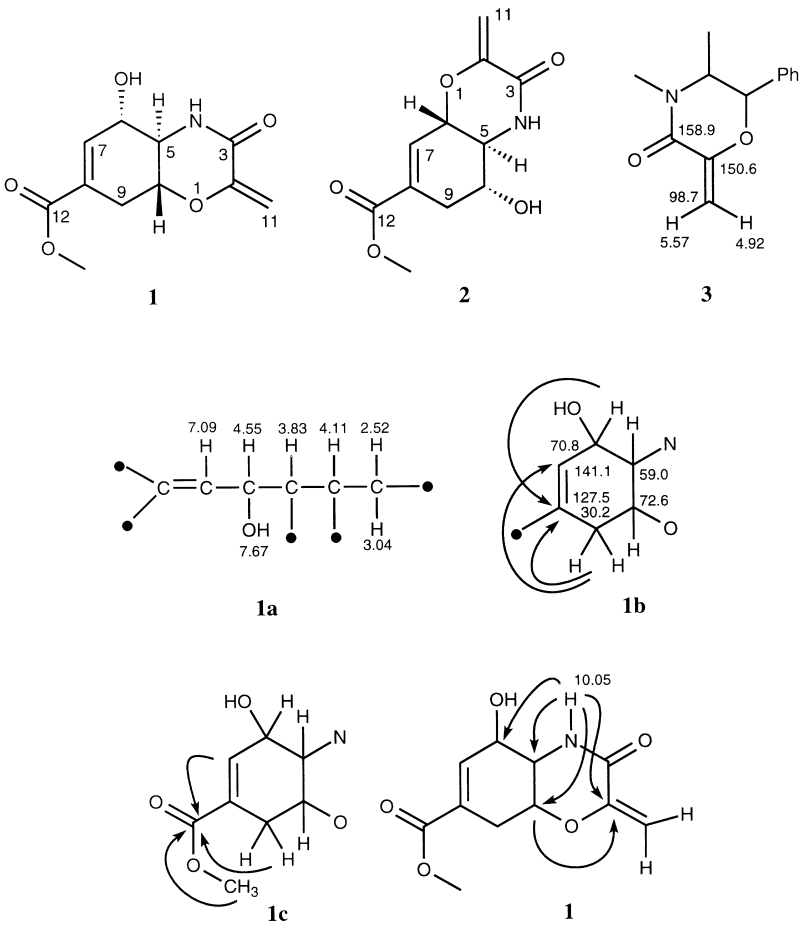
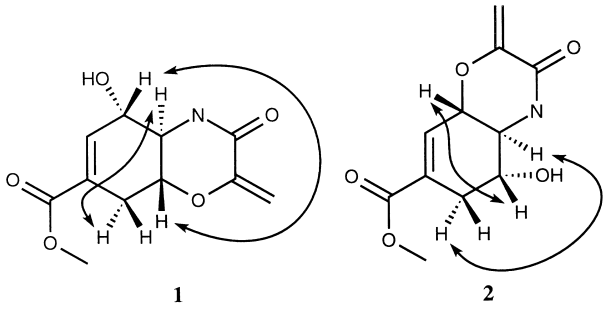


Figure 1. Partial structures of monocillinol A (**1**) and key HMBC correlations

The remaining structural elements of **1** consisted of an α,β-unsaturated amide carbonyl, an exomethylene group and one additional ring. The ^{13}C chemical shifts of the exomethylene group were highly polarized (δ 152.4 and 98.5), which indicated that it was bound to an oxygen. An HMBC correlation from H-10 to C-2 revealed that the C-10 oxygen substituent was linked to the exomethylene group. HMBC correlations from the amide NH to C-2, C-5, C-6, and C-10 (Fig. 1) then allowed assignment of the bicyclic structure shown for **1**. Comparison of the ^{1}H and ^{13}C NMR data reported for the substituted morpholine ring in compound **3**[7] with NMR data from the corresponding ring in monocillinol A (**1**) supported this structure. The relative stereochemistry in **1** was defined by 1,3-diaxial NOE interactions, which were observed in selective 1-D NOESY experiments, between H-5 and H-9α, and between H-6 and the H-10 bridgehead proton (Fig. 2). While the structure of **1** is novel, it shares some features with the bicyclic ring system found in the chromophore of the proteinaceous antibiotic C-1027.[8–10]

The molecular formula of monocillinol B (**2**) was determined by HR-FABMS measurements to be isomeric with **1**. The ^{1}H NMR spectrum of **2** was almost superimposable with that recorded for **1**, and COSY data for **2** defined proton–proton spin systems which were similar to those



1 2

Figure 2. Key NOESY interactions observed in **1** and **2**

observed in **1**. The ^{13}C NMR spectral data of **1** and **2** corresponded closely; however, significant spectral differences were observed for the C-7 (δ 134.4) and C-8 (δ 130.9) olefin carbon resonances as well as the C-6 methine (δ 75.7) in compound 2.¶ In addition, the C-10 oxymethine carbon in **2** was also shifted upfield to δ 67.0. HMBC correlations established that the fused morpholine ring in monocillinol B (**2**) was cyclized via the oxygen substituent on C-6, as opposed to the C-10 oxygen in monocillinol A (**1**). Key data included a modest ^3J correlation between C-2 and H-6, and a ^4J correlation from one of the exomethylene protons (δ 5.82) to C-6. The amide NH (δ 9.73) also revealed correlations to C-2, C-5, C-6, and C-10. All of the other observed HMBC correlations were fully consistent with structure **2**. The relative stereochemistry in **2** was assigned from NOE interactions (Fig. 2) in an analogous manner with **1**.

Monocillinols A (**1**) and B (**2**) did not exhibit anti-HIV activity in an in vitro XTT-based assay[11] or cytotoxicity towards two human cell lines (LOX and OVCAR-3) at concentrations of 50 μg/mL.

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¶ To aid in spectral comparisons, the numbering for monocillinol B (**2**) is based on the same numbering scheme used for monocillinol A (**1**).