Communications

Subglutinols A and B: Immunosuppressive Compounds from the Endophytic Fungus *Fusarium subglutinans*

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Immunosuppressive drugs are used today to prevent allograft rejection in transplant patients, and in the future they could be used to treat autoimmune diseases such as rheumatoid arthritis and insulin-dependent diabetes.[1] Currently approved immunosuppressive agents such as cyclosporin A and FK506 possess some undesirable side effects, and the search for better immunosuppressive agents continues.[2] We have been investigating endophytic fungi—the fungi that live in the intercellular spaces inside living plants—as a source of novel compounds. This search has emphasized fungi from plants with demonstrated biological activity, and in screening the fungi from the perennial twining vine *Tripterygium wilfordii*, we discovered two novel immunosuppressive compounds that form the basis of this report. *T. wilfordii*, which has been used in Chinese traditional medicine for over two thousand years, is familiar to chemists as the source of cytotoxic diterpene lactones such as triptolide[3] and insecticidal alkaloids[4] such as wilfordine. Recently, several groups[5,6] have reported the isolation of immunosuppressive triterpenes from *T. wilfordii*. An endophytic fungus from *T. wilfordii*, identified as *Fusarium subglutinans*, produces the immunosuppressive but noncytotoxic diterpene pyrones subglutinol A (**1**) and B (**2**).

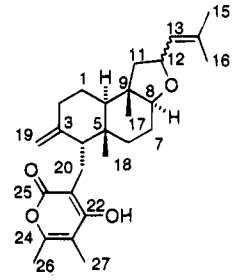
F. subglutinans was cultured in modified M-1-D medium, and immunosuppressive activity, as judged by the mixed lymphocyte reaction, appeared in the ethyl acetate extract of the culture medium. The extract was chromatographed on silica gel using CH_2Cl_2–MeOH mixtures. The active fractions were subjected to reverse phase HPLC on a C-18 column with a solvent gradient of 75% MeOH–H_2O to 100% MeOH to give two active compounds, subglutinol A (**1**) and B (**2**) (3:1). Compounds **1** (mp 185 °C dec; $[\alpha]^{25}_D = -58.7°$ (*c* 0.027, MeOH) and **2** (mp 220 °C dec; $[\alpha]^{25}_D = -75.0°$ (*c* 0.288, MeOH) have identical UV spectra, very similar ^{1}H- and ^{13}C-NMR spectra, and the same molecular formula, $C_{27}H_{38}O_4$, as judged by HRFABMS. The formula requires nine degrees of unsaturation, and since nine signals in the ^{13}C-NMR are sp^2, the compounds contain four rings and a carbonyl (Table 1). A broad IR band at 3400–3100 cm^{-1} and a strong absorption at 1664 cm^{-1} indicate an alcohol, which is consistent with the exchangeable proton in the ^{1}H-NMR, and a conjugated carbonyl.

The planar structure of the more abundant subglutinol A (**1**) can be deduced from analysis of one- and two-dimensional NMR spectra (Table 1). Partial structures

Table 1. NMR Data for Subglutinol A (1) in MeOD

	^{13}C [a]	^{1}H [b,c]	HMBC [d]	ROESY [e]
1	26.2 t	1.56 (qd, 13.0, 5.0), 1.45	2	
2	32.1 t	2.45 (br td, 13.5, 6.0), 2.05 (br dd, 14.0, 3.5)	1, 4, 19	
3	149.9 s		2, 4, 20	
4	56.5 d	2.17 (dd, 11.5, 4.5)	2, 18, 19	0.96, 4.25
5	39.6 s		4, 18	
6	35.9 t	1.90 1.38 (dt, 14.0, 3.5)	7, 18	
7	23.3 t	1.78, 1.74		0.85, 0.96
8	88.6 d	3.17 (dd, 11.5, 4.0)	11, 17	1.69, 4.75
9	45.5 s		11, 17	
10	46.5 d	1.74	2, 4, 6, 17, 18	
11	49.0 t	1.90, 1.45 (dd, 11.5, 4.0)	8, 17	
12	75.9 d	4.75 (td, 9.5, 3.5)	11	1.69, 3.17
13	128.4 d	5.35 (br dt, 8.5, 1.5)	11, 15, 16	1.72, 0.85
14	136.4 s		15, 16	
15	25.8 q	1.72 (d, 0.8)	13, 16	5.35
16	18.2 q	1.69 (d, 1.0)	13, 15	3.17
17	17.5 q	0.85 (s)	8, 11	0.96, 5.35
18	25.1 q	0.96 (s)	4, 6, 10	0.85, 2.17, 4.25
19	110.7 t	4.50 (t, 2.5), 4.25 (t, 2.0)	2, 4	0.96, 2.17
20	22.7 t	2.74 (dd, 13.5, 12.0), 2.58 (dd, 13.5, 5.0)		
21	103.7 s		20	
22*	165.35 s		20, 27	
23	109.0 s		26, 27	
24	156.8 s		26, 27	
25*	165.30 s		20	
26	17.3 q	2.19 (s)		
27	10.4 q	1.91 (s)		

[a] ^{13}C spectrum was recorded at 100 MHz, referenced to d_3-MeOD at 49.0 ppm. Multiplicity was based on the DEPT spectrum. [b] ^{1}H spectrum was recorded at 500 MHz, referenced to CHD_2OD at 3.30 ppm. Assignments to carbons were based on HMQC data. [c] Those signals without indicated splitting patterns are either multiplets or buried within other signals. [d] Numbers correspond to position of the proton. [e] The chemical shifts of the protons are given. Only those that are relevant to stereochemical assignment are shown.



1 Subglutinol A - 12 *S*
2 Subglutinol B - 12 *R*

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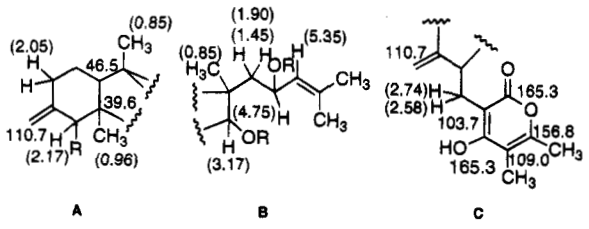

Figure 1. Fragments of **1** derived from COSY and HMBC spectra.

A, **B**, and **C** for **1** (Figure 1) are obtained from analyses of the COSY, HMQC, and HMBC spectra as sketched below. In **A**, the position of the exocyclic double bond is determined from the heteronuclear correlations observed between the protons at δ 2.05 (C-2) and 2.17 (C-4) to the carbons at δ 149.9 (C-3) and 110.7 (C-23). Because the protons of C-1 and C-2 are coupled, the methine signal at δ 46.5 (C-10) must have three-bond correlations to the protons at δ 2.05 (C-2), 2.17 (C-4), 0.85 (C-17), and 0.96 (C-18). The closure of ring **A** is established from the observed cross peaks of the signal at δ 39.6 (C-5) to the δ 2.17 (C-4) and 0.96 (C-18) resonances. In **B**, the olefinic signal at δ 5.35 (C-13) shows a large coupling to δ 4.75 (C-12) and long-range coupling to the terminal methyl groups. Since the quaternary carbon signal at δ 45.5 (C-9) correlates to proton signals at δ 3.17 (C-8), 1.90 (C-11), and 1.45 (C-1), and the carbon signal at δ 35.9 (C-6) correlates to the proton signal at δ 1.78 (C-7), **A** and **B** are connected to form the diterpene skeleton of **1**.

The remaining carbon resonances consist of five sp^2, one methylene, and two methyl signals. Fragment **C** is partially constructed by signals at δ 109.0 and 156.8 having cross peaks to both methyl signals. Further analysis of the HMBC spectra indicate that the three remaining sp^2 signals must be arranged to form a tautomeric α- or γ-pyrone, since the methylene protons at δ 2.74 and 2.58 correlate with all three carbon resonances. Since λ_{max} for **1** (293 nm) is significantly closer to the expected α-pyrone value,[7] and there is no literature precedent for a free 2-hydroxy-4-(γ)-pyrone, **1** is drawn as the α-pyrone tautomer.

Finally, fragment **B** must contain a pentacyclic ether ring. Although no heteronuclear correlation is observed between the C-12 and C-8 positions, a through-space correlation is observed in the ROESY spectrum of **1** (Figure 2). This NOE enhancement determines the relative stereochemistry at C-8 and C-12 and forms the basis of the relative configurations at all of the other chiral centers as illustrated in Figure 2. NOE experiments indicate that subglutinol B (**2**) is the C-12 epimer of subglutinol A (**1**) (Figure 2). A final check on the assigned stereochemistry is provided by a single crystal X-ray diffraction analysis of **2**, which finally crystallized after the NMR experiments on **1** and **2** were completed.[8] The results of this analysis are shown in Figure 3. In the solid state, compound **2** clearly exists as the α-pyrone, not the γ-pyrone, tautomer.

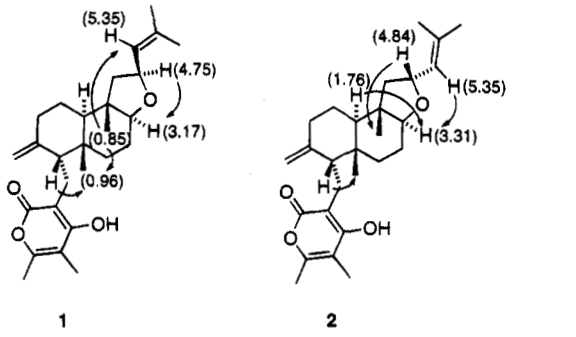

Figure 2. NOE correlations of **1** and **2**.

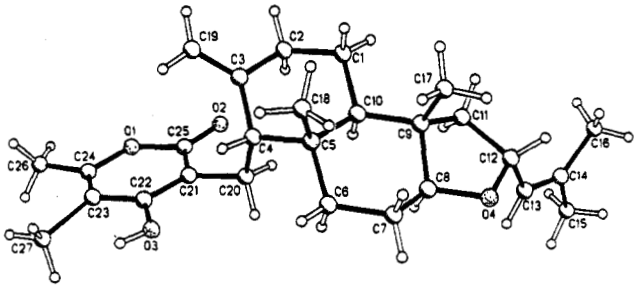

Figure 3. A computer-generated perspective drawing of the final X-ray model of subglutinol B (**2**).

The current analysis defines only the relative stereochemistry of compounds **1** and **2**. The absolute configurations shown are drawn to agree with those established for the related compounds viridoxin A and B, anti-insecticidal metabolites of the entomopathogenic fungus *Metarhizium flavoviride*.[9]

Compounds **1** and **2** are equipotent in the mixed lymphocyte reaction (MLR) assay and thymocyte proliferation (TP) assay (IC_{50} 0.1 μM), and this equipotency suggests that the residue at C-12 does not interact with the biological target. In the same assay systems, cyclosporin A is roughly as potent in the MLR assay and 10^4 more potent in the TP assay, but the lack of toxicity with **1** and **2** suggests that they definitely should be explored in greater detail. Further work on the *in vivo* activity of subglutinol A (**1**) and B (**2**) is in progress and will be reported in due course.

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Supporting Information Available: Spectral and physical data for subglutinol A (**1**) and B (**2**) (8 pages).

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(8) Space group $P2_12_12_1$ with a = 7.187(1), b = 9.685(2), and c = 34.405(7) Å and one molecule in the asymmetric unit. The final R-factor is 0.075 for the 2σ data set.

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