The Structure of V214w from an Unidentified Fungus

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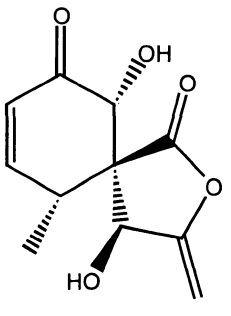
While searching for novel biological activity during a recent reevaluation of samples from our Antibiotic Collection, we isolated the known antibiotic 6-*epi*-5′-hydroxy-mycosporulone (**1**)[1] from culture V214, an unidentified mitosporic fungus (Fig. 1). From this same organism we now report the isolation and structure elucidation of a second metabolite, V214w (**2**).

Strain V214 was isolated from a soil sample collected from a sand dune along the Oregon coast. The organism was identified only as a member of the now obsolete order Sphaeropsidales. Pilot plant scale fermentation (300 liters) was carried out at 22°C for 94.5 hours. The production medium consisted of glucose 2.0%, yeast extract 0.05%, ammonium tartrate 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, KH_2PO_4 0.1%, KCl 0.05%, and $FeSO_4 \cdot 7H_2O$ 0.001%, pH 6.5. Sample extracts were periodically analyzed by TLC to insure that the desired metabolites had been produced.

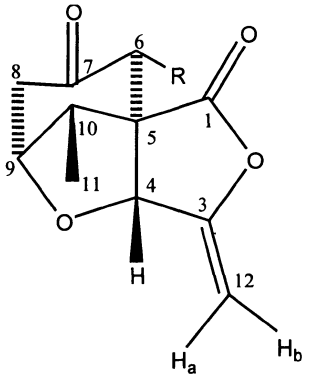
The culture broth (290 liters, pH 5.2) was filtered and the filtrate was extracted with 200 liters of EtOAc. The organic layer (180 liters) was separated and concentrated *in vacuo* to 250 ml of an oily residue. A 50 ml portion weighing ~56 g was subjected to silica gel chromatography (6×40 cm column, 60~200 mesh), and was eluted under slight pressure at 25 ml/minute using an eight liter gradient of 100% benzene to 5% EtOAc/benzene. One liter stepwise gradients of 10%, 25%, and 50% EtOAc/benzene were then applied. A final three liter elution using EtOAc completed the process. Eighty milliliter fractions (170 total) were collected and analyzed by TLC for the desired metabolites. Fractions 126~135 were evaporated to give 7.3 g of V214x, identified as **1**. Fractions 61~110 were concentrated (15 g) and a 200 mg portion was recrystallized to give 120 mg of **2** as colorless rods.

The physicochemical properties of **2** are as follows: UV λ_{max} (MeOH) nm: 204 (end absorption); IR ν_{max} (film) cm^{-1}: 3452, 3418, 2979, 1810 (C=O), 1726 (C=O), 1683 (C=C), and 1134; $[\alpha]_D^{25} = +100.6°$ (*c* 0.4, $CHCl_3$); mp 149~151°C; ^1H and ^{13}C NMR (Table 1); APCI-MS: pos *m/z* 225 $(M+H)^+$, neg *m/z* 223 $(M-H)^-$; HR ESI-MS: $(M+H)^+$ *m/z* 225.07575, calcd 225.07561 for $C_{11}H_{13}O_5$.

Fig. 1. Structures of V214 metabolites.



1



2, R= OH

3, R=

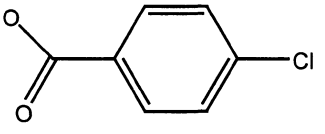


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Table 1. ^{1}H and ^{13}C NMR shifts of **2** and **3**.

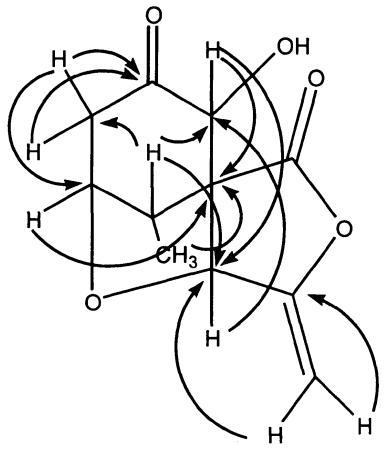
Carbon	**2** δ_C	**2** δ_H (mult, J Hz)	**3** δ_C	**3** δ_H (mult, J Hz)
1	168.8 s		167.8 s	
3	154.8 s		154.4 s	
4	78.0 d	4.96 (1H, t, 1.9)	78.9 d	5.22 (1H, s)
5	61.4 s		59.7 s	
6	76.1 d	4.59 (1H, br s)	76.2 d	6.01 (1H, s)
6-OH		3.77 (1H, br s)		
7	206.3 s		198.9 s	
8_a	47.4 t	2.93 (1H, dd, 17.4, 2.6)	48.3 t	2.95 (1H, d, 17.5)
8_b		2.60 (1H, dt, 17.4, 2.6)		2.64 (1H, d, 17.5)
9	85.0 d	4.48 (1H, t, 2.6)	84.6 d	4.53 (1H, s)
10	45.5 d	2.65 (1H, q, 7.1)	46.5 d	2.81 (1H, q, 6.8)
11	14.0 q	1.32 (3H, d, 7.1)	14.1 q	1.38 (3H, d, 6.8)
12_a	91.1 t	4.90 (1H, t, 3.0)	91.7 t	4.98 (1H, s)
12_b		4.63 (1H, dd, 3.0, 3.0)		4.73 (1H, s)
1'			163.8 s	
2'			127.0 s	
3', 7'			128.9 d	7.42 (2H, d, 8.1)
4', 6'			131.3 d	7.91 (2H, d, 8.1)
5'			140.3 s	

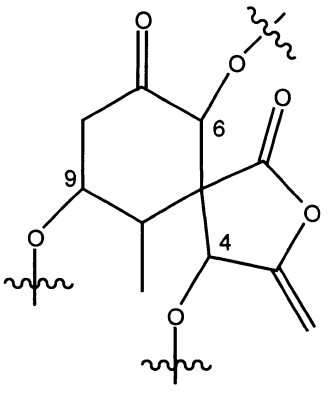
δ from TMS in $CDCl_3$

The molecular formula of **2** was established as $C_{11}H_{12}O_5$ by HR ESI-MS and required six sites of unsaturation. Strong IR absorbances at 1810 and 1726 cm^{-1} indicated the presence of both ester (lactone) and saturated ketone carbonyl groups, while bands at 3452, 3418, and 1134 were indicative of hydroxyl and ether functionalities in the molecule. Eleven signals were well resolved in the ^{13}C NMR spectrum, presented in Table 1. Analysis of DEPT spectra accounted for one methyl, two methylene, four methine, and four quaternary carbons. Comparison with the formula suggested the presence of an additional hydroxyl group, which was seen as a broad singlet at 3.77 ppm in the ^{1}H NMR spectrum. Carbon singlets at δ206.3 and δ168.8 were assigned to ketone and lactone carbonyl groups, respectively. Signals at δ 91.1 (t) and δ 154.8 (s) corresponded to an exomethylene group, as is found in **1** and the related metabolites rosigenin[2] and paecilospirone.[3] Compound **2** lacks the *cis*-olefin however, and to accommodate the degree of unsaturations in the molecular formula, it must be tricyclic.

Detailed analyses of 2D NMR experiments and comparison with data reported in the literature for similar compounds led to the structure of **2**. The similarity between V214w and other fungal metabolites containing a spirolactone skeleton was deduced from chemical shift values and HMBC connectivites, as shown in Fig. 2. Both exomethylene protons on C-12 showed correlations to C-3 at 154.8 ppm. The chemical shift value of C-3 suggested that it was attached to oxygen. H-12_a (δ 4.90) also showed a correlation to C-4, and H-4 in turn could be linked by three bond coupling to the cyclohexanone ring at C-6. H-6 displayed connections to the carbonyl at C-7, quaternary carbon C-5, and C-4, and its chemical shift indicated that it was on a carbon bearing oxygen. Both methylene protons at C-8 showed correlations to the keto carbonyl group at C-7. In addition H_a-8 at δ2.93 displayed cross peaks to an oxygenated carbon at C-9 and a carbon bearing a methyl group at C-10. The proton on C-9 exhibited correlations to C-7, C-5, and C-4, while the hydrogen at C-10 afforded several linkages to C-4, C-5, C-6, C-8 and the C-11 methyl. Connectivites were observed from H-11 to C-5, C-9, and C-10. Very weak correlations were also noted from the hydroxyl proton at δ 3.77 to C-5 and C-7. However, no HMBC cross peaks were seen to the C-1 lactone carbonyl at δ168.8. This problem was resolved by the preparation of a *p*-chlorobenzoate derivative, **3**, which clearly showed a cross peak in the HMBC spectrum between H-6 and C-1. The physicochemical properties of **3** are: UV λ_{max} (CH_3CN/HCOOH) nm: 242; HR ESI-MS: $(M+H)^+$ *m/z* 363.06300, calcd 363.06317 for $C_{18}H_{16}ClO_6$.

Fig. 2. HMBC correlations of **2**.



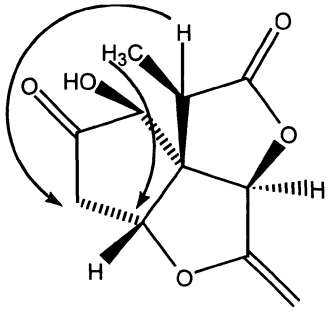


a

Analysis of the HMBC data permitted construction of partial structure **a**. Based on chemical shift values alone, there were potentially three sites for the placement of the hydroxyl group, although the HMBC data suggested that it should be attached at C-6. Evidence for the correct placement of the hydroxyl at C-6 rather than C-4 (as in related metabolites) came from a ^{13}C *beta*-isotope shift experiment, in which a carbon bearing a free hydroxyl that is exchanged with deuterium will show a small isotopic shift. The chemical shift of C-6 measured in $CDCl_3$ at 400 MHz was 75.936 ppm. Addition of a drop of d_4-MeOH to the NMR tube and reacquisition of the carbon data showed an upfield shift for C-6 to 75.695 ppm and broadening of the peak. No appreciable difference in chemical shift (δ 77.886 to δ 77.835) or peak broadening was noted for C-4, supporting the HMBC data and assignment of the hydroxyl group to C-6. Formation of an ether linkage between C-4 and C-9 to give a tetrahydrofuran ring then satisfied the molecular formula and established the structure of **2** as shown.

The relative stereochemistry of V214w is supported by comparisons to related compounds having the spiro-lactone skeleton, correlations observed in the NOESY spectrum of **2**, and ^1H-^1H coupling values. In order to form the tricyclic ring system, the five membered rings are positioned in a horizontal plane but are slightly puckered, while the cyclohexanone ring lies in a semi-chair conformation behind the plane. NOE correlations were noted between H-6 and H-10, and also $H8_b$ and H-9. No other cross peaks were observed. This placed both the methyl and OH in a stable equitorial position, while H-6, H-10, and H-8_b are located in an axial orientation. The lack of vicinal coupling between methines H-9 and H-10 also support this configuration, and from a molecular model, the dihedral angle appears to be 90 degrees.

Fig. 3. Required 4-bond HMBC correlations of coniothyriol.



4

The 5,6 spiro-lactone ring has been reported in at least four other fungi.[1~4] One of these, *Coniothyrium sporulosum*, also produces a compound, coniothyriol (**4**),[5] which has the same molecular formula as **2**. The physicochemical data reported in the literature for **4** is very similar to what we report here. Some differences are apparent in the melting point, optical rotation, and IR values, but the NMR data is nearly identical. If structures **2** and **4** were to represent the same compound, then the HMBC data we observe would require three of the strong

cross peaks (δ $H_{2.65}$ to δ $C_{47.4}$, δ $H_{1.32}$ to δ $C_{85.0}$ in **2**, and δ $H_{6.01}$ to δ $C_{167.8}$ in **3**) to be four bond correlations in coniothyriol (Fig. 3). It seems likely that only a minor biosynthetic modification in 6-*epi*-5′-hydroxy-mycosporulone (**1**) would be needed to produce **2**, and that the proposed structure **2** is a better fit than **4** for the spectroscopic data which we have obtained.

V214w (**2**) has been tested in a number of therapeutic screens, but so far, has shown no interesting biological activity. The original antimicrobial activity noted for this culture has been attributed to **1**.

Acknowledgements

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