Notes

TWO NOVEL PHENOLICS FROM *ACTINOMADURA VERRUCOSOSPORA*

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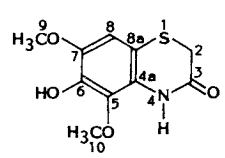
In the course of large-scale fermentation and isolation of esperamicin A_1 from *Actinomadura verrucosospora*[1,2], several unrelated compounds were noted. Compound **1** was found in a fraction which eluted between esperamicins A_1 and A_2 in a $CHCl_3$-MeOH (96:4) silica flash chromatography solvent system. Further flash chromatography in EtOAc-hexane (60:40) gave a reddish product which was homogeneous in three different TLC systems. The reddish pigment was removed by trituration with $CHCl_3$, in which the majority of the mass was insoluble. This gave analytically pure compound **1**.

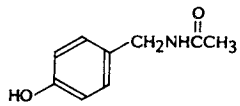
HREI-MS established a molecular formula of $C_{10}H_{11}NO_4S$, MW of 241.0402 (calcd 241.04086). IR disclosed the presence of an amide by a carbonyl band at 1680 cm^{-1} and NH at 3441 cm^{-1}. The UV spectrum in MeOH exhibited absorbance at 295 nm ($\log \varepsilon$ 3.64), as well as 239 (4.23) and 212 nm (4.29). Addition of acid did not change the spectrum, however base addition produced a bathochromic shift to 312 nm (3.81), indicative of a phenolic group.

NMR confirmed the carbon and proton counts. The amide carbonyl was seen at 165 ppm and two exchangeable protons were seen at 10.2 and 8.6 ppm in 100% DMSO-d_6. All proton signals occurred as singlets, with two methoxyl groups and a single aromatic ring proton apparent from the NMR data. The unsaturation number of 6 could be accounted for by a single carbonyl, a pentasubstituted aromatic ring, and a second saturated ring, suggesting a benzothiazine nucleus.

A heteronuclear C-H correlation experiment[3] confirmed the expected one-bond C-H connectivities. Ring substitutions were then worked out largely by selective INEPT experiments[4] which detected 2- and 3-bond couplings from proton to carbon. The proton singlet at 10.2 ppm was coupled to the carbonyl, indicating that this was the amide proton. The proton singlet at 8.6 ppm was coupled only to a ring aromatic carbon at 135 ppm and to the two methoxy-substituted carbons at 144 and 148 ppm, indicating that the phenolic group was located between the two methoxyl groups. The aromatic ring proton at 6.44 ppm was coupled to all other aromatic carbon signals except the methoxy-substituted carbon at 148 ppm, thus defining it as *para* to this methoxyl group. The 3.4 ppm proton coupled strongly to 105 ppm, and weakly to the carbonyl. This led to the substitution pattern illustrated. A detailed analysis of a fully coupled carbon spectrum (Table 1) supports the selective INEPT results.



1



2

Chemical shifts for the ring carbons were compared to those empirically predicted for the structure by the Scientific and Technical Information Network (STN) ^{13}C NMR database††, with only fair agreement with our assignments. A *para*-arrangement of methoxyl groups was also simulated but the fit was poorer.

The *O*-acetate of **1** was formed using acetic anhydride and pyridine (overnight, room temperature). NMR of the product showed clean conversion to a monoacetate. Selective INEPT experiments, EI (HR) and FAB-MS data on the acetate were consistent with the proposed structure. The UV spectrum of the acetate showed a bathochromic shift in base, which we attribute to the amide proton's acidity.

No matches or position isomers of structure **1**

†† STN ^{13}C NMR database: Fachinformationszentrum, Energie, Physik, Mathematik GmbH, D-7513 Eggenstein-Leopoldshafen 2, FRG.

Table 1. Compound **1** NMR assignments, C-H couplings, HETCOR, selective INEPT.

	Assigned shift (J_{CH})		HETCOR to proton	Selective INEPT from proton
C-3	164.74	(0.9, 4.9)	—	3.4, 10.2
C-7	147.76		—	3.73~3.74, 6.44, 8.6
C-5	144.23		—	3.73~3.74, 6.44, 8.6, 10.2
C-6	135.38	(7.9)	—	6.44, 8.6
C-4a	129.14	(1.7, 3.7)	—	6.44, 10.2
C-8a	105.31	(4.5, 7.6)	—	3.4, 6.44, 10.2
C-8	97.72	(2.2, 169.6)	6.44	—
C-9	59.72	(144.7)	3.73~3.74	—
C-10	55.95	(144.6)	3.73~3.74	—
C-2	28.81	(4.0, 143.2)	3.34	—

were found in the Berdy antibiotic database or CAS registry file. Thus the structure would appear to be a novel microbial metabolite.

Compound **2** was isolated in gram quantities from a flash chromatography fraction on silica following esperamicin A_2 in $CHCl_3$-MeOH (96:4). HREI-MS led to the formula $C_9H_{11}NO_2$. ^1H NMR indicated a *para*-disubstituted benzene. A bathochromic UV shift in base assigned one of the substituents as a phenol, while linked-scan MS supported an acetamidomethylene substituent at the other position. All other spectra were consistent with structure **2**, previously synthesized (CAS Reg. No. 34185-04-1, *cf.* ref 5) but novel as a microbial metabolite.

Compound **2** readily oxidized in air, suggesting that it may serve an antioxidant function in the fermentation of esperamicin A_1, which is itself susceptible to air oxidation.

Compounds **1**, **2**, and the acetate of **1** were found to be noncytotoxic in four cell lines (see Experimental section), with the exception of **2** in SW1271 human lung cells ($IC_{50} \sim 10\,\mu g/ml$). Antibiotic activity of **2** was detected vs. several strains of *Streptococcus* and *Staphylococcus*, (Table 2), but no activity was seen against any Gram-negative bacterial strains.

Table 2. Antibacterial activity of **2**.

Organism	MIC ($\mu g/ml$)
Streptococcus pneumoniae (A9585)	0.06
S. pyogenes (A9604)	0.5
S. agalactiae (A22567)	1
Staphylococcus aureus (A9537)	0.13
S. aureus (A9606)	4
S. aureus (A24227)	0.5
17 Gram-negative strains	16 to >125

Experimental

NMR spectra were acquired on Nicolet NT-300 and Varian XL-200 spectrometers in DMSO-d_6. MS data were obtained on a VG ZAB-2F or 70-250. All solvents were of HPLC grade, and $CHCl_3$ was hydrocarbon-stabilized.

Compound **1**

5,7-Dimethoxy-6-hydroxy-2*H*-1,4-benzothiazin-3(4*H*)-one, BMY 40662. Insoluble in $CHCl_3$, moderately soluble in MeOH and DMSO. MP 149~152°C; IR (KBr disk) cm^{-1} 3441, 3200, 3090, 3004, 1680, 1620, 1601, 1500, 1319, 1231, 1100, 1007, 902, 827, 811, 791, 738, 489; UV λ_{max}^{MeOH} nm (ε) 295 (3.64), 239 (4.23), 212 (4.29); $\lambda_{max}^{MeOH+NaOH}$ nm (ε) 312 (3.81), 262 (4.10); $\lambda_{max}^{MeOH+HCl}$ no change from MeOH; EI-MS *m/z* 243 (6%), 242 (13%), 241.0402 (100%), 226.0181 (18%), 198.0217 (12%), 182.9995 (13%), 156.0118 (15%), 68.0139 (16%); ^1H NMR (200 MHz, 100% DMSO) δ 10.25 (1H, s), 8.62 (1H, br s), 3.74 (3H, s), 3.73 (3H, s), 3.34 (2H, s); ^{13}C NMR (50 MHz, 100% DMSO) δ 164.74 (s), 147.76 (s), 144.23 (s), 135.38 (s), 129.14 (s), 105.31 (s), 97.72 (d), 59.72 (q), 55.95 (q), 28.81 (t).

Compound **2**

N-Acetamido-4-hydroxybenzylamine, BMY 40660. Insoluble in $CHCl_3$, soluble in MeOH. MP 113~117°C; UV λ_{max}^{MeOH} nm (ε) 277 (3.68), 310 (sh); $\lambda_{max}^{MeOH+NaOH}$ nm (ε) 292 (3.75), 367 (3.06); FAB-MS (positive ion) *m/z* 331, 165, 107.07349 (C_7H_9N); ^1H NMR δ 6.73~7.11 (4H, m), 4.92 (2H, br s), 4.23 (2H, s), 1.95 (3H, s); ^{13}C NMR δ 172.7 (s), 157.3 (s), 130.2 (s), 129.9 (2C, d), 116.1 (2C, d), 43.7 (t), 22.6 (q).

Biological Testing Protocols: Antibacterial MIC data was determined by broth dilution. Cytotoxicity was measured *in vitro* using the following tumor cell lines: A549 human lung carcinoma; B16-F10 murine melanoma; HCT-116 human colon adenocarci-

noma; and SW1271 human lung squamous cell carcinoma, grade 2.

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