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Amides from the fungus *Streptomyces hygroscopicus* and their antimicrobial activity

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Abstract

Three amides, *N*-salicyloyl-2-aminopropan-1,3-diol (**1**) and 1-acetyl-*N*-salicyloyl-2-aminopropan-3-ol (**2**) including a natural product, *N*-salicyloyl-2-aminopropan-1-ol (**3**) were isolated from an ethyl acetate extract of the culture filtrate of a fungus, *Streptomyces hygroscopicus*. The structures of these compounds were unambiguously established by interpretation of their spectral data including, a series of 1D and 2D-NMR and MS analyses. Compounds **1**–**3** showed significant antibacterial activity against a wide range of Gram positive and Gram negative bacteria.

Keywords: *Streptomyces hygroscopicus*; Amides; *N*-salicyloyl-2-aminopropan-1,3-diol; 1-acetyl-*N*-salicyloyl-2-aminopropan-3-ol; *N*-salicyloyl-2-aminopropan-1-ol; Antimicrobial

1. Introduction

Microorganisms have been the sources of numerous structurally diverse and biologically active compounds. Studies of *Streptomyces* led to the discovery of many antibiotics (Dictionary of Natural Products (DNP) CD-ROM, 2001; ISIC database, 2003) including, streptomycin, neomycin, tetracycline and chloramphenicol (Harvey, 1993). As part of our ongoing research of microbial metabolites (Jabbar et al., 1998, 1999; Biswas et al., 2000), we isolated an actinomycetes, *Streptomyces hygroscopicus*, from a soil sample collected in the region of Rajshahi. We, herein, report the isolation of three new amides (**1**–**3**) including a new natural product (**3**) along with their antimicrobial activities.

2. Results and discussion

Preparative TLC of an ethyl acetate extract of the culture filtrate of the fungus, *S. hygroscopicus*, when grown in a Czapek Dox broth (acidic) medium at 30 °C, afforded three amides (**1**–**3**). The structures of these compounds were elucidated by spectral studies.

The high resolution EIMS of **1** showed a molecular ion peak at *m/z* 211.0848 which analyzed for $C_{10}H_{13}NO_4$. It also displayed a base peak at *m/z* 121 due to a ketene ion, $[C_7H_5O_2]^+$. The ^1H NMR spectrum (Table 1) of compound **1** revealed the presence of four aromatic protons at δ 8.33, 6.86, 7.38 and 7.17 and a chelated hydroxyl group signal as a broad singlet at δ

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Table 1
NMR data of **1**–**3** in C_5D_5N; *J* in Hz, in parentheses

Position	^{1}H NMR			^{13}C NMR		
	1	**2**	**3**	**1**	**2**	**3**
1	–	–	–	161.4	160.9	161.8
2	–	–	–	118.0	117.6	117.4
3	8.33 *dd* (8.0, 1.6)	8.38 *dd* (8.0, 1.6)	8.25 *dd* (8.1, 1.4)	129.7	129.6	129.0
4	6.86 *td* (8.0, 0.9)	6.86 *t* (7.7)	6.84 *t* (8.1)	119.5	119.4	119.2
5	7.38 *td* (8.0, 1.6)	7.39 *td* (7.7, 1.6)	7.38 *td* (8.1, 1.4)	134.3	134.1	134.2
6	7.17 *br d* (8.0)	7.19 *br d* (7.8)	7.18 *br d* (8.1)	118.6	118.3	118.5
1′	4.39 *dd* (10.8, 4.9)	4.73 *m*	3.97 *m*	62.0	64.1	65.7
2′	4.93 *dtt* (7.8, 5.8, 4.9)	5.02 *m*	4.72 *m*	55.2	51.7	48.7
3′	4.30 *dd* (10.8, 5.8)	4.19 *m*	1.42 *d* (6.8)	62.0	61.4	17.9
HO-1	13.55 *br s*	NS	13.66 *br s*	–	–	–
NH	9.31 *d* (7.8)	9.49 *d* (8.0)	9.12 *d* (7.8)	–	–	–
*CH$_3$*CO	–	1.96 *s*	–	–	20.9	–
CH$_3$*C*O	–	–	–	–	169.8	–
CO	–	–	–	170.2	171.2	170.1

NS = not seen

13.55. A downfield doublet at δ 9.31 (1H, $J = 7.8$ Hz), a doublet of a triplet of a triplet at δ 4.93 ($J = 7.8$, 5.8, 4.9 Hz) and two sets (each integrating for two protons) of doublets of doublet centred at δ 4.39 ($J = 10.8$, 4.9 Hz) and 4.30 ($J = 10.8$, 5.8 Hz) in the ^{1}H NMR spectrum were consistent with an *N*-aminoglycerol moiety in **1**. The *J*-modulated ^{13}C NMR spectrum (Table 1) exhibited the presence of a carbonyl (δ 170.2), oxymethylene (δ 62.0), aliphatic methine (δ 55.2), four aromatic methines (δ 129.7, δ 119.5, δ 134.3, δ 118.6), a quaternary carbon (δ 118.0) and an oxygenated quaternary carbon (δ 161.4). The assignments of all protons and carbons were achieved by two dimensional HMBC experiment (Table 2). In the HMBC spectrum both H-3 (δ 8.33) and H-5 (δ 7.38) showed ^3J correlations to a common oxygen bearing quaternary carbon at δ 161.4. Thus, this was assigned as C-1 and the hydrogen bonded hydroxyl group was placed at this position. The quaternary carbon at δ 118.0 was connected via three bond correlations to H-4 (δ_H 6.86) and H-6 (δ_H 7.17). So this carbon was assigned as C-2. The downfield shift of H-3 could be explained by its β-position to the carbonyl group. The HMBC experiment also revealed ^3J correlations in the ABCD system methine protons and carbons (H-3 to C-5; H-5 to C-3; H-6 to C-4). Although the oxymethylene protons in the ^{1}H NMR spectrum of **1** were non-equivalent (δ 4.30, 4.39, each 2H), the ^{13}C NMR showed an intense peak for two oxymethylene carbons at δ 62.0 ppm. In the HMBC experiment, the oxymethylene protons (H-1′ and H-3′ showed both direct and long range correlation to δ_C 62.0 ppm. This revealed that this signal was for two oxymethylene (C-1′ and C-3′) carbons. Both H-1′ and H-3′ also showed ^2J correlations to a methine carbon at δ 52.2. The methine proton at δ 4.93 showed ^2J connectivity with C-1′/C-3′ and ^3J correlation to the carbonyl group and thus was assigned as H-2′. The NH proton appeared as a doublet at δ 9.73 and showed a connectivity over ^2J with the carbonyl group. In the NOESY experiment, the NH showed strong interaction with H-2′, H-1′/3′ and H-3

Table 2
HMBC correlations observed for **1**–**3** in C_5D_5N

Protons	HMBC (H→C)					
	1		**2**		**3**	
	^2J	^3J	^2J	^3J	^2J	^3J
H-3	–	C-1, C-5, CO	–	C-1, C-5, CO	–	C-1, C-5, CO
H-4	–	C-2, C-6	–	C-2, C-6	–	C-2, C-6
H-5	–	C-1, C-3	–	C-1, C-3	–	C-1, C-3
H-6	–	C-2, C-4	–	C-2, C-4	–	C-2, C-4
H-1′	C-2′	C-3′	C-2′	C-3′, CH$_3$*CO*	C-2′	C-3′
H-2′	C-1′, C-3′	CO	–	–	–	–
H-3′	C-2′	C-1′	C-2′	C-1′	C-2′	C-1′
NH	CO	–	CO	–	–	–
C*H$_3$*CO	–	–	CH$_3$*CO*	–	–	–

suggesting their close proximity. On this basis, the compound was identified as *N*-salicyloyl-2-aminopropan-1,3-diol (**1**), which appears to be new.

The HREIMS of compound **2** provided the molecular ion peak at *m/z* 253.0964 consistent with the molecular formula $C_{12}H_{15}NO_5$. The presence of the same ketene ion, $[C_7H_5O_2]^+$, in the EIMS of **2** and similar ^1H and ^{13}C NMR spectral data of compounds **1**–**2** suggested very close structural relationship between these compounds. In fact, the ^1H and ^{13}C NMR spectra of **2** were almost identical to those observed for compound **1**. However, the NMR data of **2** indicated the presence of an acetyl group (δ_H 1.96, 3H, *s*; δ_C 20.9 and 169.8) suggesting that compound **2** was an acetylated analogue of **1**. The placement of this acetyl group in the molecule was determined by the heteronuclear 2D experiment. In the HMBC spectrum, the methyl protons showed ^2J correlation to the carbonyl at δ 169.8. The latter carbon was also connected by a ^3J interaction with the methylene protons at δ 4.73 (δ_C 64.4). This allowed placement of the acetyl group at C-1′. Although C-1′ and C-3′ appeared at δ 62.0 in the ^{13}C NMR spectrum of **1**, they were observed at δ 64.4 and 61.8 due to their non-equivalence in **2**. Thus, compound **2** was identified as 1-acetyl-*N*-salicyloyl-2-aminopropan-3-ol, which also appears to be new.

The molecular formula of **3** was established as $C_{10}H_{13}NO_3$ from the HREIMS data. Both the ^1H NMR and ^{13}C NMR data of this compound were in close agreement of those of **1** except some peaks assignable to the glyceryl part. Thus, the ^1H NMR spectrum showed a methine proton as a multiplet at δ 4.72, a three proton doublet for methyl at 1.42 ($J = 6.8$ Hz) and a methylene signal at 3.97. In the HMBC experiment, the methyl protons showed ^2J and ^3J correlations to the methine (δ_C 48.7) and the oxymethylene (δ_C 65.7) carbons, respectively. On the other hand, the oxymethylene protons (H-1′) demonstrated HMBC correlations to the above methine (δ_C 48.7) and the methyl (δ_C 17.9) carbons. Although the NH and H-2′ did not show the expected HMBC correlations, they displayed coupling in the COSY spectrum. So this part of the molecule consisted a 2-aminopropan-1-ol moiety and thereby, **3** was identified as *N*-salicyloyl-2-aminopropan-1-ol which was further substantiated by comparison of these spectral data with published values (Huneck and Porzel, 1994). Though compound **3** has been synthesized once (Huneck and Porzel, 1994), this is the first report of its occurrence from a natural source.

The compounds isoneoantimycin (Takeda et al., 1998), antimycin A_7 (Barrow et al., 1997) and antimycin A_8 (Barrow et al., 1997) have previously been isolated from *Streptomyces* species which have the amide linkage connecting an aromatic part with an aliphatic moiety. The isolation of amides **1**–**3** from *S. hygroscopicus* supports their chemical profile.

Compounds **1**–**3** were screened for their antibacterial and antifungal activities by the disc diffusion method (Bauer et al., 1966; Barry, 1976). Although only **1** showed antifungal activity against *Candida albicans* (12 mm), *Aspergillus flavus* (9 mm), *A. niger* (9 mm) and *A. fumigatus* (11 mm) at a concentration of 200 μg/disc, all three compounds showed significant antibacterial activity against a wide range of Gram positive and Gram negative organisms (Table 3). The minimum inhibitory concentrations (MIC), observed by serial dilution technique (Reiner, 1982), for **1** were found to be 128 μg/ml against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi*, while 64 μg/ml against *Sarcina lutea* and *Shigella boydii*. The MIC values of **2** were 256 μg/ml against *S. typhi* and 128 μg/ml against *S. lutea*, *Stap. aureus* and *E. coli* while 64 μg/ml against

Table 3
Antibacterial activity of compounds **1**–**3** in comparison with kanamycin

Bacteria	Diameter of zone of inhibition (mm)			
	1 200 (μg/disc)	**2** 200 (μg/disc)	**3** 200 (μg/disc)	Kanamycin 30 (μg/disc)
Gram positive				
Bacillus subltilis	18	20	20	25
Bacillus megatarium	16	21	19	25
Staphylococcus aureus	19	23	18	26
Streptococcus β-*haemolyticus*	20	21	19	25
Sarcina lutea	15	22	17	24
Gram negative				
Escherichia coli	20	19	21	27
Shigella dysenteriae	18	19	18	25
Shigella shiga	18	18	20	26
Shigella flexneri	23	20	21	25
Shigella sonnei	19	20	19	24
Pseudomonas aeruginosa	17	22	19	22
Salmonella typhi	20	20	28	26

B. subtilis and *Sh. boydii*. The MIC values of **3** were 128 μg/ml against *Stap. aureus*, *B. subtilis*, *Sh. boydii* and *S. typhi* whereas 64 μg/ml against *S. lutea* and *E. coli*.

3. Experimental

3.1. General experiment procedures

Optical rotations were measured on a Perkin–Elmer Polarimeter 341. IR spectra were recorded as KBr disc on a Mattson Galaxy 5000 FT-IR spectrometer. UV spectra were obtained on a Unicam UV 4-100 UV/visible spectrophotometer in MeOH. HREIMS were recorded on a JEOL JMS-AX505HA double-focusing instrument at 70 eV. NMR spectra (both 1D and 2D) were obtained on a Bruker AMX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer, using the residual solvent peaks as internal standard. *J*-modulated ^{13}C spectra were acquired with relaxation time (d_1) of 6 s. HMBC spectra were optimized for a long range J_{H-C} of 7 Hz ($d_6 = 0.07$ s). NOESY experiment was carried out with a mixing time of 0.6 s. PTLC was carried out using Merck Si gel 60 PF_{254} on glass plates (20 cm × 20 cm) at a thickness of 0.5 mm. TLC was conducted on normal-phase Merck Si gel 60 PF_{254} on plates. Spots on TLC and PTLC plates were visualized under UV light (254 and 366 nm) and spraying with 1% vanillin-H_2SO_4 followed by heating at 110 °C for 5–10 min.

3.2. Organisms

Streptomyces hygroscopicus was isolated from a soil sample collected from Rajshahi, Bangladesh and identified at the Department of Molecular Biology and Biotechnology, Institute for Biomedical Research, National University of Mexico, Mexico, DF, Mexico, where a voucher specimen is maintained under the accession number X79853.

3.3. Extraction and isolation

The culture broth (50 × 200 ml) of *S. hygroscopicus* was partitioned with ethyl acetate (50 × 60 ml) and concentrated to dryness by using a rotary evaporator under vacuum at 40 °C. Preparative TLC of the ethyl acetate soluble part (1.5 gm) over Si gel using mobile phase, petroleum ether, EtOAc (1:1), yielded compounds **1** (35 mg), **2** (10 mg), and **3** (10 mg).

3.4. Antibacterial and antifungal screening

Both antibacterial and antifungal activities of compounds **1**–**3** were observed by disc diffusion assay (Bauer et al., 1966; Barry, 1976). The minimum inhibitory concentrations (MIC) were determined by serial dilution technique (Reiner, 1982).

3.5. N-salicyloyl-2-aminopropan-1,3-diol (*1*)

White amorphous solid; UV (MeOH) λ_{max} (log ϵ) 225 (4.32), 283 (sh) (3.54), 302 (sh) (3.50) nm; IR (KBr) ν_{max} cm^{-1} 3397, 2952, 2849, 1721, 1639, 1550, 1494, 1369, 1255, 1118, 1025, 758; ^{1}H and ^{13}C NMR (Table 1); HR-EIMS: *m/z* 211.0848 (calcd. for $C_{10}H_{13}NO_4$, 211.0845); EIMS (rel. int.) 211 $[M]^+$ (33), 193 $[M–H_2O]^+$ (8), 162 (7), 138 (12), 121 $[C_7H_5O_2]^+$ (100), 93 (18), 65 (24).

3.6. 1-Acetyl-N-salicyloyl-2-aminopropan-3-ol (*2*)

Yellow amorphous solid; $[\alpha]_D^{25}$ –29.04° (MeOH, *c* 0.138); UV (MeOH) λ_{max} (log ϵ): 228 (4.56), 282 (sh) (4.17), 301 (sh) (4.13) nm; IR (KBr) ν_{max} cm^{-1} 3367, 2932, 2859, 1726, 1640, 1595, 1545, 1492, 1453, 1366, 1256, 1135, 1042, 811, 754; ^{1}H and ^{13}C NMR (Table 1); HRMS *m/z* 253.0964 (calcd. for $C_{12}H_{15}NO_5$, 253.0950); EIMS 253 $[M]^+$ (25), 180 (13), 162 (31), 121 $[C_7H_5O_2]^+$ (100), 65 (16).

3.7. N-salicyloyl-2-aminopropan-1-ol (*3*)

White amorphous solid; $[\alpha]_D^{25}$ −2.5° (MeOH, *c* 0.080) (Lit. –16.4°; Huneck and Porzel, 1994); ^{1}H, ^{13}C NMR and EIMS were identified to reported values (Huneck and Porzel, 1994).

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