Sch 47554 AND Sch 47555, TWO NOVEL ANTIFUNGAL ANTIBIOTICS PRODUCED FROM A *Streptomyces* sp.

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In the course of screening for new antifungal agents from fermentation broths, we have discovered two novel antifungal compounds, Sch 47554 (**1**) and Sch 47555 (**2**), which are structurally related to the angucycline group of antibiotics[1] which includes aquayamycin and saquayamycins. Both compounds were produced by a strain of *Streptomyces* sp. SCC-2136 (ATCC 55186) isolated from a soil sample collected in Edmonton, Alberta, Canada. In this paper we wish to report the production, isolation, and structure elucidation of **1** and **2** using spectroscopic methods as well as chemical degradations.

Preparation of the first stage germination consisted of inoculating 3.5 ml of a frozen whole broth sample of the producing strain in a 250-ml Erlenmeyer flask containing 70 ml of a medium consisting of (g/liter) beef extract, 0.3%; Tryptone (Bacto), 0.5%; D-glucose, 0.1%; soluble starch, 2.4%; yeast extract, 0.5%; and calcium carbonate, 0.2%. The pH of this medium was adjusted to 7.5 prior to sterilization. The seed culture was incubated at 30°C for 48 hours on a rotary shaker at 300 rpm. For the second stage inocula, 25 ml of the first seed was transferred to a 2-liter Erlenmeyer flask containing 500 ml of the same germination media, and incubated under the same conditions as described above. Fermentation was initiated by transferring 500 ml the second stage germination to a 14-liter fermenter containing 10 liters of media consisting of (g/liter) PD-650 dextrin, 3%; pea flour, 1.5%; maltose, 0.5%; fructose, 0.5%; molasses (dark), 0.5%; sea salts, 0.01%; yeast extract, 0.3%; NZ-Amine, 0.3%; and Antifoam (AF-1 Dow Corning), 0.05%. The fermentation was carried out at 30°C for 90 hours with an air flow of 0.35 v/v/m and 35 rpm agitation and the antimicrobial activity was monitored by disc agar diffusion assay against *Staphylococcus aureus*, ATCC 209P (pH 8.0), *Escherichia coli*, ATCC10536 (pH 8.0), and *Candida albicans* Wisconsin.

The fermentation broth (10 liters) was filtered, absorbed on Amberlite XAD-16 resin (1 liter), then eluted with a MeOH-H_2O gradient. The active fractions of XAD-16 eluant (70~100% MeOH combined fractions, 1.8 g) were concentrated, and then chromatographed on a silica gel column eluting with chloroform. After further purification by a Sephadex LH-20 gel column eluting with $CHCl_3$-MeOH (1 : 1, v/v), a mixture of **1** and **2** was isolated. The mixture was finally separated by silica gel column chromatography with a 0~5% MeOH gradient in $CHCl_3$ (v/v) to obtain 50 mg of **1** and 15 mg of **2** from the 10 liters of fermentation broth.

Compounds **1** and **2** are reddish-orange and red powders, respectively. Both compounds are soluble in dichloromethane, chloroform, and dimethyl sulfoxide, partially soluble in methanol, diethyl ether and ethyl acetate, insoluble in hexane and water. The compounds are negative to ninhydrin and Rydon tests. Other physico-chemical properties are summarized in Table 1.

Fig. 1. Structures of Sch 47554 (**1**) and Sch 47555 (**2**).

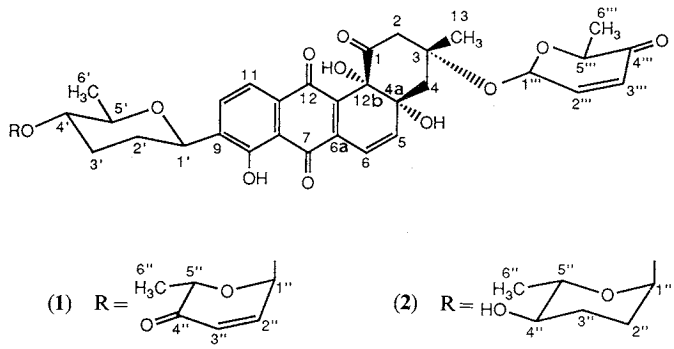
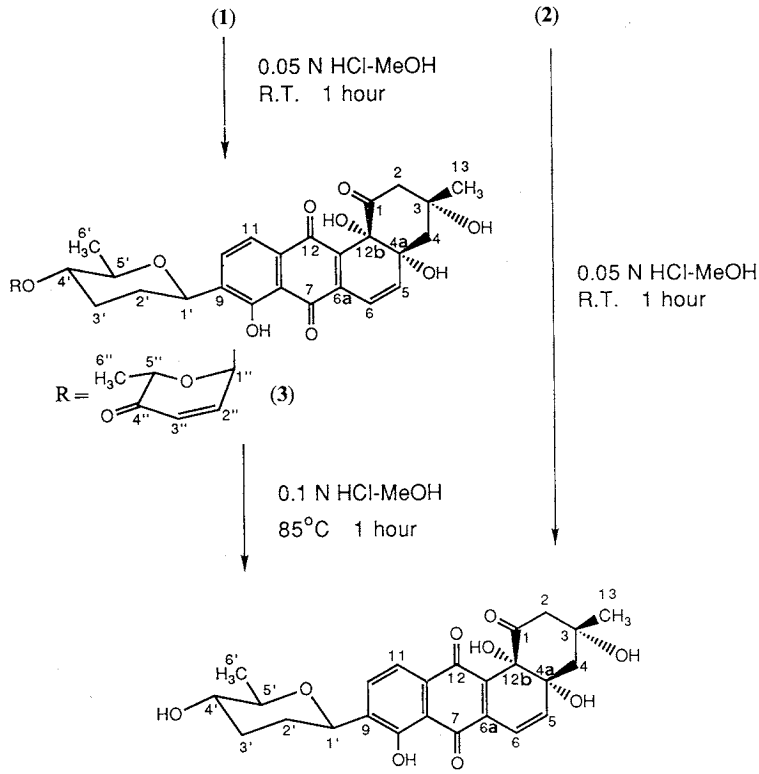


Table 1. Physico-chemical properties of **1**, **2**, **3** and **4**.

	1	**2**	**3**	**4**
Appearance	Reddish-orange powder	Red power	Red powder	Red powder
FAB-MS (−eV)	690 $(M)^-$	694 $(M)^-$	580 $(M)^-$	469 $(M-H)^-$
Molecular formula	$C_{37}H_{38}O_{13}$	$C_{37}H_{42}O_{13}$	$C_{31}H_{32}O_{11}$	$C_{25}H_{26}O_9$
MP °C (dec.)	168～169	151～153	162～163	165～167
IR (KBr) ν_{max} cm^{-1}	3440 (br), 2936, 1728, 1700, 1640, 1276, 1261, 1087, 1041	3450 (br), 2934, 1728, 1699, 1641, 1276, 1261, 1086, 1052	3430 (br), 2931, 1721, 1639, 1621, 1434, 1295, 1279, 1263, 1090, 1052	3425 (br), 2930, 1723, 1639, 1621, 1435, 1295, 1279, 1262, 1090, 1053
UV (MeOH) λ_{max} nm	218, 320, 436	218, 321, 438	219, 317, 436	219, 318, 440

Fig. 2. Degradation of Sch 47554 (**1**) and Sch 47555 (**2**).



3′-Deoxyaquayamycin (**4**)

The molecular weights of **1** and **2** were determined to be 690 and 694 based on FAB-MS data that showed their negative molecular ion *m/z* 690 and 694 $(M)^-$, respectively. The molecular formulas were deduced as $C_{37}H_{38}O_{13}$ and $C_{37}H_{42}O_{13}$, respectively, by means of elemental analysis (Calcd: C 64.35, H 5.51. Found: C 64.39; H 5.48 for $C_{37}H_{38}O_{13}$; Calcd: C 63.98, H 6.05. Found: C 63.92, H 6.02 for $C_{37}H_{42}O_{13}$), as well as ^1H and ^{13}C NMR spectroscopy. The IR and UV spectra of **1** and **2** were very similar to that of aquayamycin.[2～5] The ^1H and ^{13}C NMR spectral data (Tables 2 and 3) exhibited that both compounds possess an aglycone resemble to aquayamycin which connected to two hexose moieties. Further evaluation of NMR spectra suggested the presence of two L-aculose sugar units in **1**, one L-amicetose and one L-aculose in **2** by comparison with the literature data.[6] To confirm the NMR assignments of **1** and **2** and to further characterize the aglycone, chemical de-

Table 2. ^{1}H NMR data for **1**, **2**, **3** and **4**[a].

Proton	1	2	3	4
2-CH_2 ax	2.55 d[b] (13 Hz)[c]	2.53 d (13.2 Hz)	2.63 d (13.0 Hz)	2.62 d (13 Hz)
eq	3.23 dd (3, 13 Hz)	3.21 dd (13, 13.2 Hz)	2.96 dd (3, 13 Hz)	2.96 dd (3, 13 Hz)
4-CH_2 ax	1.83 d (15.7 Hz)	1.81 d (15.4 Hz)	7.83 d (15.0 Hz)	1.83 d (15, 13 Hz)
eq	2.48 dd (3, 15.7 Hz)	2.46 dd (13, 15.4 Hz)	2.28 dd (3, 15 Hz)	2.26 dd (13, 15 Hz)
5-H	6.42 d (9.8 Hz)	6.40 d (9.8 Hz)	6.41 d (9.8 Hz)	6.41 d (9.8 Hz)
6-H	6.92 d (9.8 Hz)	6.90 d (9.8 Hz)	6.91 d (9.8 Hz)	6.91 d (9.8 Hz)
8-OH	12.31 s	12.28 s	12.30 s	12.29 s
10-H	7.89 d (7.9 Hz)	7.87 d (7.8 Hz)	7.90 d (7.8 Hz)	7.89 d (7.8 Hz)
11-H	7.63 d (7.9 Hz)	7.60 d (7.8 Hz)	7.63 d (7.8 Hz)	7.61 d (7.8 Hz)
13-CH_3	1.48 s	1.47 s	1.30 s	1.30 s
1′-H	4.83 br d (9.8 Hz)	4.80 br d (9.8 Hz)	4.83 br d (9.8 Hz)	4.80 br d (9.8 Hz)
2′-CH_2 ax	1.82 m	1.80~2.20 m	1.82 m	1.40 m
eq	2.25 m		2.23 m	2.20 m
3′-CH_2	2.28 m	~2.25 m	2.29 m	1.66 m
4′-H	3.42 m	3.46 m	3.41 m	3.42 m
5′-H	3.58 dq (6.1, 9.1 Hz)	3.54 dq (6, 9 Hz)	3.58 dq (6, 9 Hz)	3.55 dq (6, 9 Hz)
6′-CH_3	1.35 d (6.1 Hz)	1.31 d (6.2 Hz)	1.37 d (6.7 Hz)	1.38 d (6.2 Hz)
1″-H	5.33 d (3.5 Hz)	4.70 br s	5.33 d (3.5 Hz)	
2″-H	6.85 dd (3.5, 10.1 Hz)	1.80 m	6.84 dd (3.5, 10.2 Hz)	
3″-H	6.12 d (10.2 Hz)	~2.20 m	6.11 d (10.2 Hz)	
4″-H		3.43 m		
5″-H	4.61 q (6.7 Hz)	3.73 dq (6, 9 Hz)	4.61 q (6.8 Hz)	
6″-CH_3	1.38 d (6.7 Hz)	1.23 d (6.2 Hz)	1.39 d (6.7 Hz)	
1‴-H	5.59 d (3.5 Hz)	5.58 d (3.5 Hz)		
2‴-H	6.70 dd (3.5, 10.1 Hz)	6.69 dd (3.5, 10.1 Hz)		
3‴-H	6.08 d (10.2 Hz)	6.06 d (10.2 Hz)		
5‴-H	4.75 q (6.7 Hz)	4.73 q (6.7 Hz)		
6‴-CH_3	1.44 d (6.7 Hz)	1.43 d (6.7 Hz)		

[a] Recorded at 300 MHz in $CDCl_3$, chemical shifts in ppm from TMS.
[b] Multiplicity.
[c] Coupling constant.

gradations were carried out as shown in Fig. 2. Hydrolysis of **1** with 0.05 N HCl-MeOH at room temperature for 1 hour yielded a product **3**. After a purification on silica gel column chromatography with 1% MeOH in $CHCl_3$, pure **3** was obtained. Physico-chemical and spectral data were recorded, and are listed in Tables 1, 2 and 3. The molecular weight of **3** was found to be 580 based on FAB-MS data that showed a negative molecular ion *m/z* 580 $(M)^-$. The loss of 110 mu (mass unit) indicated the absence of L-aculose unit ($C_6H_6O_2$). By a comparison of the ^{13}C NMR spectra between **1** and **3**, a signal of C-3 was shifted up-field from δ 82.7 to 75.8, while signals of carbons at C-4a and C-12b remained unchanged. This evidence suggested that a L-aculose attached to C-3 in **1** was hydrolyzed in the reaction. The further hydrolysis of **3** with 0.1 N HCl-MeOH at 85°C for 1 hour eliminated the second L-aculose residue to yield the aglycone **4**. The FAB-MS data of **4** showed strong peaks at *m/z* 469 $(M-H)^-$ and 470 $(M)^-$. The IR and UV spectra of **4** appeared to be closely related to that of aquayamycin. The ^{1}H and ^{13}C NMR spectra of **4** were quite similar to those of aquayamycin with the exception of the chemical shift at C-3′. The methine carbon resonance of C-3′ at δ 74.4 in aquayamycin was found to be replaced by the methylene signal at δ 33.1 in **4**,[†] while the proton of C-3′ in aquayamycin at δ 3.67 (m) was not observed in the ^{1}H NMR spectrum of **4**. Furthermore, the molecular weight of **4** was 16 mu lower than that of aquayamycin (*m/z* 486, M^+). Based on these data the structure of the aglycone **4** was determined as 3′-deoxyaquayamycin.

[†] For a comparison purpose, the ^{1}H & ^{13}C NMR spectra of compound (**4**) was also recorded using CD_3OD. ^{13}C NMR data: δ 206.9 (C-1), 53.2 (C-2), 77.7 (C-3), 44.7 (C-4), 146.2 (C-5), 118.2 (C-6), 189.8 (C-7), 158.9 (C-8), 139.9 (C-9), 134.3 (C-10), 120.0 (C-11), 183.6 (C-12), 30.2 (C-13), 78.5 (C-4a), 140.4 (C-6a), 115.5 (C-7a), 132.1 (C-11a), 140.1 (C-12a), 82.1 (C-12b), 72.6 (C-1′), 33.8 (C-2′), 33.1 (C-3′), 80.2 (C-4′), 74.3 (C-5′), 18.8 (C-6′).

Table 3. ^{13}C NMR data for **1**, **2**, **3** and **4**[a].

Carbon	1	2	3	4
Aglycone				
1	204.1	204.2	204.6	205.1
2	50.2	50.2	51.9	52.1
3	82.7	82.8	75.8	76.1
4	42.6	42.6	43.0	43.2
4a	79.2	79.3	80.3	80.6
5	145.2	145.2	144.0	144.3
6	117.5	117.4	117.4	117.5
6a	138.8	138.7	138.6	138.7
7	188.0	188.1	187.8	187.9
7a	113.8	113.8	113.7	113.8
8	158.0	158.3	158.1	158.3
9	138.4	138.4	137.9	138.1
10	133.6	133.7	133.5	133.7
11	119.8	119.8	119.7	119.8
11a	130.2	130.1	130.0	130.0
12	182.3	182.3	181.9	182.1
12a	139.3	139.8	199.3	139.6
12b	77.1	77.4	77.1	76.0
13	26.5	26.5	30.1	30.2
1′	70.7	69.8	70.3	71.9
2′	31.5	31.6	31.4	31.9
3′	31.8	31.9	31.6	33.0
4′	80.8	79.1	80.6	78.8
5′	76.7	77.4	76.0	73.0
6′	18.5	18.5	18.3	18.3
Sugar 1				
1″	94.9	98.1	94.8	
2″	142.8	27.6	142.7	
3″	127.7	30.0	127.2	
4″	196.8	72.0	196.0	
5″	73.1	73.0	72.9	
6″	15.2	17.8	15.0	
Sugar 2				
1‴	88.7	88.7		
2‴	142.9	142.9		
3‴	127.4	127.6		
4‴	196.8	196.9		
5‴	70.4	70.6		
6‴	15.1	15.1		

[a] Recorded at 75 MHz in $CDCl_3$, chemical shifts in ppm from TMS.

Hydrolysis of **2** with 0.05 N HCl-MeOH at room temperature for 1 hour led to a product which was identical to the aglycone **4** by comparison of their ^1H and ^{13}C NMR spectra. The only difference between **2** and **1** was found to be a sugar unit connected to C-4′ based on the analysis of NMR data (see Tables 2 and 3). This saturated sugar in **2** was identified as an L-amicetose since the NMR data totally agreed with the data of L-amicetose reported in literature.[6]

Sch 47554 (**1**) and Sch 47555 (**2**) exhibit antifungal activity against various yeasts and dermatophytes. As shown in Table 4, compound **1** was more active than **2**. Structurally related compounds, the angucycline group metabolites, are often reported as antitumor agents with considerable cytotoxicity.[1,5] Besides interesting antitumor activity, some of the angucyclines act as enzyme inhibitors (inhibitions of tryptophan 5-monooxygenase[7] and tyrosine hydroxylase[8]), some display the inhibition of blood platelet aggregation[9] and some exhibit antiviral activity.[10,11] Compounds **1** and **2** represent two novel members of this interesting group of compounds.

Table 4. *In vitro* activity of Sch 47554 (**1**) and Sch 47555 (**2**) against various fungi.

	Geometric mean MICs (μg/ml)	
	Sch 47554 (**1**)	Sch 47555 (**2**)
Y (SDB) (10)	≥43	≥128
D (SDB) (7)	≥ 5	≥132
Y (EMEM) (10)	78	95

Y: Yeast (SDB); 6 strains *Candida albicans*, 2 each *C. tropicalis*, *C. stellatoidea*.

D: Dermatophytes (SDB); 2 strains each *Trichophyton mentagrophytes*, *T. rubrum*, *T. tonsurans*, 1 *Microsporum canis*.

Y: Yeast (EMEM); 6 strains *Candida albicans*, 2 each *C. tropicalis*, *C. stellatoidea*.

SDB: Sabouraud dextrose broth, pH 5.7.

EMEM: EAGLE's minimum essential medium, pH 7.0.

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