NOTES

5-Hydroxy-9-methylstreptimidone, a New Glutarimide from a *Streptomyces* sp. HIL Y-9065403

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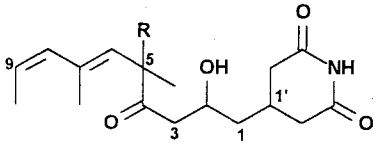
During the course of our screening for microbial secondary metabolites possessing herbicidal activity, we isolated a new compound 5-hydroxy-9-methylstreptimidone (**1**), along with the known antibiotic 9-methylstreptimidone (**2**)[1], from the culture filtrate of a fermented *Streptomyces* sp. Y-9065403. In this paper, we report their production, isolation, structure elucidation and biological properties.

Strain HIL Y-9065403 was isolated from a soil sample collected in Nainital, India. The strain was identified as belonging to the genus *Streptomyces* using the methods described by SHIRLING and GOTTLIEB[2]. A loopful of mature slant culture of *Streptomyces* Y-9065403 was inoculated into Erlenmeyer flasks (500 ml capacity) containing 100 ml of seed medium consisting of glucose 1.5%, soyabean meal 1.5%, corn steep liquor 0.5%, $CaCO_3$ 0.2% and NaCl 0.5%, pH 7.0 before autoclaving.



5-Hydroxy-9-methylstreptimidone (**1**) R=OH
9-Methylstreptimidone (**2**) R=H

The flasks were cultivated at 29°C on a rotary shaker at 180 rpm for 96 hours. The seed culture (4%) was inoculated into Erlenmeyer flasks (1 liter capacity) containing 200 ml each of production medium consisting of glucose 2%, soyabean meal 1%, $CaCO_3$ 0.02% and $CoCl_2$ 0.0001%, pH 7.2 before autoclaving. The flasks were cultivated at 29°C on a rotary shaker at 180 rpm for 96 hours. The culture filtrate exhibited antibacterial activity against *Staphylococcus* species and also the herbicidal activity in *Lemna gibba* and *Avena sativa* models.

The culture filtrate (17 liters) was passed through a column of Diaion HP-20 (1 liter). The column was washed with water (15 liters) followed by 50% MeOH in water (5 liters) and then eluted with MeOH (8 liters). The active MeOH eluates were concentrated to dryness under reduced pressure to a dark brown solid (7 g) which was subjected to medium pressure liquid chromatography (MPLC) over silica gel (200~300 mesh) using a 5% step gradient of ethyl acetate in petroleum ether (60~80°) as the eluant. Pure 9-methylstreptimidone (**2**) eluted out in 30% ethyl acetate in petroleum ether which on concentration afforded 1.44 g of **2** as an oil. 5-Hydroxy-9-methylstreptimidone (**1**) eluted out in 75% ethyl acetate in petroleum ether which on concentration afforded 770 mg of semi-pure **1**. This was finally purified by preparative TLC on silica gel (Article no. 13794, E. Merck) using CH_2Cl_2 - MeOH - H_2O (10 : 0.5 : 0.1) as the developing solvent to give 27 mg of pure **1** as a colourless solid.

The physico-chemical properties of **1** and **2** are listed in Table 1. The ^1H and ^{13}C NMR spectral data are summarized in Table 2. The proton assignments were made by the analysis of the phase-sensitive DQF HH shift correlated COSY spectrum. The multiplicities and assignments of the carbons were determined by DEPT-135 and HMQC spectra respectively. Both **1** and **2** showed one keto carbonyl, two amide carbonyls and

Table 1. Physico-chemical characteristics of 5-hydroxy-9-methylstreptimidone (**1**) and 9-methylstreptimidone (**2**).

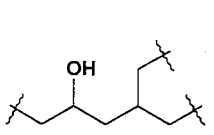
	1	**2**
Appearance	White solid	Pale yellow oil
Solubility	CH_2Cl_2, $CHCl_3$, EtOAc, MeOH	CH_2Cl_2, $CHCl_3$, EtOAc, MeOH
$[\alpha]_D$	−52.27° (*c* 0.44, $CHCl_3$)	+109.59° (*c* 14.6, $CHCl_3$)
DCI-MS $(M+H)^+$	324	308
Molecular formula	$C_{17}H_{25}NO_5$	$C_{17}H_{25}NO_4$
Elemental analysis		
Found:	C 63.01; H 7.90; N 4.21	C 65.14; H 8.84; N 5.24
Calcd:	C 63.15; H 7.74; N 4.33	C 66.40; H 8.14; N 4.56
TLC (SiO_2) Rf	0.36[a], 0.35[b]	0.57[a], 0.56[b]
UV (MeOH) nm	238, 290	252, 295
IR (KBr) cm^{-1}	3450 (broad), 1710~1650 (broad)	3450, 3200, 1720~1670 (broad)

[a] CH_2Cl_2 - EtOAc (50 : 50).
[b] CH_2Cl_2 - MeOH - H_2O (10 : 0.5 : 0.1).

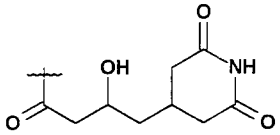
Table 2. ^1H and ^{13}C NMR spectral data of 5-hydroxy-9-methylstreptimidone (**1**) and 9-methylstreptimidone (**2**).

Position	δ_H (300 MHz, $CDCl_3$)		δ_C (75 MHz, $CDCl_3$)	
	1	**2**	**1**	**2**
1	1.50, 1.32 (2×m)	1.57, 1.30 (2×m)	41.91	41.51
2	4.18 (m)	4.08 (m)	65.23	65.42
3	2.72 (m)	2.58 (m)	45.40	48.01
4	—	—	212.71	213.36
5	—	3.43 (m)	78.66	47.64
6	5.47 (s)	5.13 (d, 10.37 Hz)	130.26	128.65
7	—	—	140.03	136.43
8	5.79 (d, 11.60 Hz)	5.77 (d, 11.60 Hz)	133.73	133.43
9	5.55 (dq, 11.60, 6.70 Hz)	5.46 (dq, 11.60, 6.70 Hz)	126.54	125.96
1′	2.49 (m)	2.44 (m)	27.63	27.76
2′	2.75, 2.34 (2×m)	2.68, 2.27 (2×m)	38.95[a]	39.07[a]
3′	—	—	174.05[b]	173.50[b]
5′	—	—	173.97[b]	173.40[b]
6′	2.75, 2.34 (2×m)	2.68, 2.27 (2×m)	37.66[a]	37.80[a]
5-CH_3	1.48 (s)	1.14 (d, 7.0 Hz)	16.32	16.90
7-CH_3	1.72 (s)	1.84 (s)	27.81	17.98
9-CH_3	1.81 (dd, 6.71, 1.20 Hz)	1.75 (dd, 6.70, 1.22 Hz)	15.19	15.45
2-OH	3.13 (s)[c]	3.41 (s)[c]	—	—
5-OH	4.72 (s)[c]	—	—	—
4′-NH	7.99 (s)[c]	8.95 (s)[c]	—	—

[a,b] Interchangeable.
[c] D_2O exchangeable.



(A)



(B)

a D_2O exchangeable amide NH signal. The COSY spectrum showed a methine proton coupled to three methylene groups, two of which had chemical shifts typical of protons α to carbonyl. The third methylene group was relatively upfield and further coupled to an oxymethine proton which, in turn, showed interaction with another methylene adjacent to a carbonyl group. The $^1J_{CH}$ correlated HMQC spectrum facilitated the resolving of the overlapping methylene protons. Isolation of the extracted spin network (**A**) when effected by an optimum arrangement of the three carbonyls led to the glutarimide structure (**B**) shared by both **1** and **2**.

A comparison of the physico-chemical and spectral data of **2** with known glutarimides revealed that **2** was identical to the known antiviral antibiotic 9-methylstreptimidone[3,4].

Compound **1** has an additional oxygen over 9-methylstreptimidone (**2**) (see Table 1). A daughter ion at m/z 288 $(M-2H_2O+H)^+$ in the mass spectrum of **1** indicated the presence of two hydroxyls in **1**. Compound **1** differed in its NMR spectra from 9-methylstreptimidone (**2**) only at C-5. Thus, C-5 which appeared as a CH signal at δ_C 47.64 in **2**, appeared as an oxygenated quaternary carbon at δ_C 78.66 in **1**. Further, the disappearance of H-5 and the appearance of the olefinic proton H-6 as a singlet at δ 5.47 strongly suggested that a hydroxyl group was present at C-5 in **1**. In the proton-detected long-range CH shift-correlated multiple bond correlation (HMBC) NMR spectrum of **1**, the 5-CH_3 protons showed cross peaks with C-4, C-5 and C-6, while H-6 showed a cross peak with C-5. Thus, the structure of **1** was established to be 5-hydroxy-9-methylstreptimidone. The *Z* geometry of the disubstituted 8,9-double bond was established by the observed coupling constant of 11.70 Hz between H-8 and H-9. The absolute configurations at C-2 and C-5 in **1** were not established.

Biological Properties

Some members of the glutarimide class of antibiotics are known to display herbicidal activity, *e.g.*, cycloheximide[5], naramycin B[6]. Although 9-methylstreptimidone (**2**) exhibited powerful herbicidal activity in primary screening models (100% inhibition at 2 ppm concentration in *Lemna gibba* and *Avena sativa* models), activity in *in vivo* studies against phytopathogens was weak. 5-Hydroxy-9-methylstreptimidone (**1**) showed only poor activity as a herbicide in the primary screening models. Both **1** and **2** did not show any antibacterial activity.

Acknowledgments

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References

1) SAITO, N.; F. KITAME, M. KIKUCHI & N. ISHIDA: Studies on a new antiviral antibiotic, 9-methylstreptimidone. I. Physicochemical and biological properties. J. Antibiotics 27: 206~214, 1974
2) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
3) BECKER, A. M. & R. W. RICKARDS: The absolute configuration of the glutarimide antibiotics streptimidone and 9-methylstreptimidone. Helv. Chim. Acta 59: 2393~2401, 1976
4) SAITO, N.; F. SUZUKI, K. SASAKI & N. ISHIDA: Antiviral and interferon-inducing activity of a new glutarimide antibiotic, 9-methylstreptimidone. Antimicrob. Agents Chemother. 10: 14~19, 1976.
5) DEMAIN, A. L.; G. A. SOMKUTI, J. C. HUNTER-CEVERA & H. W. ROSSMOORE (*Ed.*): Novel Microbial Products for Medicine and Agriculture. Chapter 22, pp. 195~202, 1989
6) BYCROFT, B. W. (*Ed.*): Dictionary of Antibiotics and Related Substances. Chapman and Hall, p 291, 1988.