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Isolation and Structure Elucidation of Autolytimycin, A New Compound Produced by *Streptomyces Autolyticus* JX-47

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Abstract: Autolytimycin **1** was isolated from the culture filtrate of *Streptomyces autolyticus* JX-47, together with two known compounds, lebstatin **2** and 17-O-demethyl-geldanamycin **3**. These compounds showed the activities of anti-HSV-I. The structure of **1** was determined by spectral analysis.

Keywords:*Streptomyces autolyticus*, autolytimycin, lebstatin, 17-O-demethyl-geldanamycin.

In our previous studies on the actinomycete resources in Yunnan Province, we found some strains with potential bioactivities. *Streptomyces autolyticus* JX-47, a strain producing broad-spectrum antiviral metabolites was studied. A new compound autolytimycin **1**, together with the other two known compounds, lebstatin **2**[1] and 17-O-demethyl-geldanamycin **3**[2,3], were isolated from the culture broth. The structure of autolytimycin was determined by spectral evidence, and shown to be similar to geldanamycin[4] and herbimycin[5,6]. Geldanamycin is a well-known antibiotic obtained from *Streptomyces hygroscopicus* var. *geldanus*[7,8]. In this paper, we report the production, isolation, and the structural elucidation of autolytimycin.

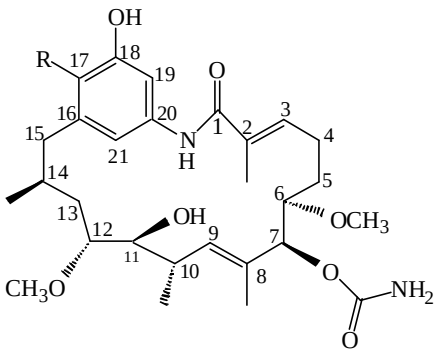
A mature slant of the strain was inoculated into a 500 mL flask for seed culture containing 100 mL medium. The seed medium consisted of (wt/vol) 2% soya bean powder, 4% starch, 0.4% NaCl and 0.5%$CaCO_3$ without pH adjustment. The seed culture was shaken at 600 rpm, 28°C for 48 hours. Then approximately 10 mL of seed culture was transferred to a 500 mL flask containing 100 mL of producing medium consisting of (wt/vol) 5% sucrose, 0.5% corn steep liquor, 1.5% soya bean powder, 1.0% yeast extract, 1.0%NaCl and 0.3% $CaCO_3$, adjusted pH to 7.0-7.2 with 0.1 mol/L aq. NaOH. The flask was incubated on the shaker at 28°C, 550 rpm. The culture was harvested after 6 days incubation in the producing medium. The volume of total cultured broth was about 60 L, which was extracted with EtOAc. The EtOAc extract was chromatographed over silica gel column, eluting with petroleum ether-acetone 4:1, 3:1, 2:1 and acetone to get compounds **1**, **2** and **3** respectively.

Compound **1** was obtained as colorless needles. The molecular formula of **1** was determined to be $C_{28}H_{42}N_2O_7$ by its HRESI-MS at *m/z* 541.2881 $[M+Na]^+$, together with the ^{13}C NMR and DEPT spectra. The ^{13}C DEPT spectrum revealed the presence of four methyls, four methylenes, eleven methines, seven quaternary carbons and two methoxy groups. The presence of the following functional groups in **1** was deduced from the ^{13}C-NMR (DMSO), IR (KBr) and ^1H NMR spectra: $OCONH_2$ [δ_C 157.3 (s), 1711cm^{-1}], CONH (δc 171.1(s), 1655 cm^{-1}), two methyl protons (δ 1.71 and 1.35).

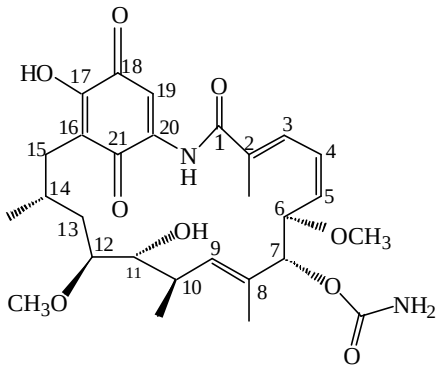
The ^1H NMR and ^{13}C NMR spectra of **1** were similar to those of **2** except for the difference of the protons and carbons chemical shift of the benzene ring and absence of a methoxy group in **1**. Long rang couplings were observed for 6-OCH_3 [δ_H 3.31 (s, 3H)] to C-6 [δ 79.3 (CH)] and 12-OCH_3 [δ_H 3.21 (s, 3H)] to C-12 [δ 80.5 (CH)] in the HMBC spectrum of **1**. In the down field of ^1H NMR spectrum, an additional signal appeared at δ 6.26 (s, 1H) due to H-17 in contrast with **2**. The stereochemistry at the other chiral centers in **1** was identical to that of lebstatin **2**, as supported by its ^1H-^1H COSY, HMBC and ROESY spectra. Thus, the structure of **1** was determined to be autolytimycin.

The other two known compounds were identified as **2** and **3** by comparison of their spectral data with the reported values[1,2,3].

Figure 1 The structures of **1**, **2** and **3**



1. R= H
2. R= OCH_3



3

Compound **1**, colorless needles (MeOH); mp 226-228°C; UV (MeOH) λ_{max} (logε) 208 (1.41), 283, 287 nm; IR (KBr) ν 3389，3282，3202，2943，2928，2827，1711，1657，1616，1594，1457，1399，1383，1314，1109，1039，871 cm^{-1}; The data of ^1H and ^{13}C NMR, see **Table 1**; FAB (negative)-MS *m/z* 517 $[M-1]^+$ (83), 474 $[M-CONH_2]^+$ (100); HRESI-MS *m/z* 541.2881 $[M+Na]^+$ (calcd for $C_{28}H_{42}N_2O_7$, 541.2890).

Figure 2 ^{1}H-^{1}H COSY and HMBC correlations of autolytimycin

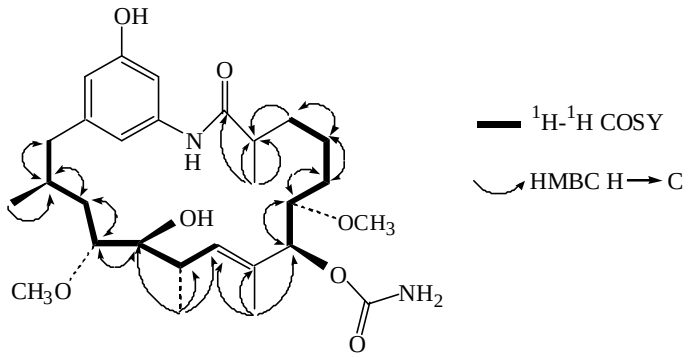


Table 1 ^{1}H and ^{13}C NMR spectral data of autolytimycin **1** and lebstatin **2** (400M Hz)

No.	Autolytimycin [a]		Lebstatin [b]	
	δ_C	δ_H	δ_C	δ_H
-NHCO-	171.1s	9.36 (1H, s)	174.6s	8.61 (1H, s)
2	131.7s		135.7s	
2-CH_3	13.2q	1.71 (3H,s)	13.7q	1.76 (3H, s)
3	134.1d	5.66 (1H, brs)	136.6d	5.84 (1H, brs)
4	23.2t	2.03, 2.16 (2H, m)	24.9t	2.15, 2.28 (2H, m)
5	29.6t	1.06, 1.23 (2H,m)	31.4t	1.10, 1.30 (2H, m)
6	79.3d	3.19 (1H, m)	81.4d	3.30 (1H, m)
6-OCH_3	58.3q	3.31 (3H, s)	59.7q	3.43 (3H, s)
7	80.8d	4.83 (1H,d J=7.5Hz)	83.7d	4.90 (1H, d, J=6.3Hz)
7-$OCONH_2$	157.3s		159.1s	
8	129.8s		131.5s	
8-CH_3	11.6q	1.35 (3H, s)	12.2q	1.47 (3H, s)
9	133.1d	5.20 (1H, d J=9.6Hz)	134.8d	5.26 (1H, d, J=9.6Hz)
10	34.1d	2.30 (1H, m)	35.8d	2.45 (1H, m)
10-CH_3	11.6q	0.91 (3H, d J=6.5Hz)	17.2q	1.01 (3H, d J=6.6Hz)
11	73.2d	3.39 (1H, m)	75.5d	3.52 (1H, m)
12	80.5d	2.95 (1H, d J=9.5Hz)	83.1d	3.08 (1H, m)
12-OCH_3	56.3q	3.21 (3H, s)	57.3q	3.33 (3H, s)
13	33.2t	1.06, 1.51 (2H, m)	35.1t	1.15,1.69 (2H, m)
14	30.4d	1.80 (1H, m)	32.8d	1.92 (1H, m)
14-CH_3	18.5q	0.71 (3H, d J=6.4Hz)	19.8q	0.78 (3H, d, J=6.6Hz)
15	42.6t	2.16, 2.55 (2H, m)	37.2t	2.73,2.50 (2H,m)
16	141.1s	--	135.4s	
17	112.8d	6.26 (1H, s)	145.3s	
17-OCH_3	--	--	60.9q	3.70 (3H, s)
18	156.2s		133.2s	
19	105.7d	6.58 (1H, s, -OH)	109.6d	6.70 (1H, s)
20	140.0s		151.7s	
21	115.2d	6.18 (1H, s)	118.3d	6.35 (1H, s)

[a] obtained in DMSO d_6.
[b] obtained in CD_3OD.

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