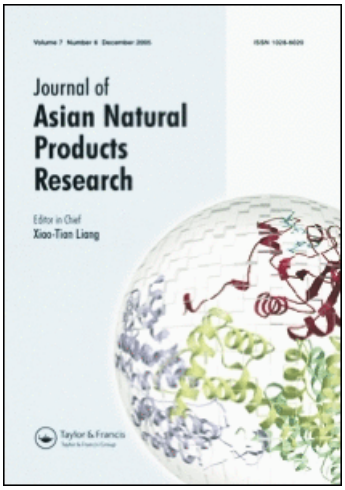

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Isolation, structure determination and biological activity of a new glutarimide antibiotic, S632A$_3$

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A new antibiotic, S632A$_3$, was isolated from a cultured broth of *Streptomyces hygroscopicus* S632. It was purified by column chromatography on silica gel, Sephadex LH-20 and HPLC. Structural studies by analysis of ^{1}H NMR and ^{13}C NMR, MS, UV and IR spectra in comparison with those of S632A$_2$ clarified that S632A$_3$ is an isomer of 9-methylstreptimidone. In addition, this antibiotic showed potent biological activity including differentiation induction effects on HL-60 cell and antitumour activity *in vivo*.

Keywords: Glutarimide antibiotics; Differentiation; Antitumour *in vivo*

1. Introduction

A soil isolate, *Streptomyces hygroscopicus* S632, was found to produce a mixture of homogeneous hydrophobic compounds. It has been reported that antibiotics S632A$_1$, S632A$_2$, S632B$_1$, S632B$_2$ and S632C have been extracted from the filtered culture [1–3]. Interestingly, we obtained an additional component, S632A$_3$, from the same culture and it was determined to be a new member of the glutarimide antibiotics. In this paper, we describe the isolation, physico-chemical properties, structure determination, and biological activities of S632A$_3$.

2. Results and discussion

2.1 Physico-chemical properties and structure identification of S632A$_3$

The ^{1}H NMR and ^{13}C NMR, HRSI-MS, UV and IR spectral data of S632A$_2$ are identical to those of 9-methylstreptimidone [4–6]. The specific rotation of S632A$_2$ is also close to that of 9-methylstreptimidone [4–6], so the structure of S632A$_2$, including stereochemistry, should

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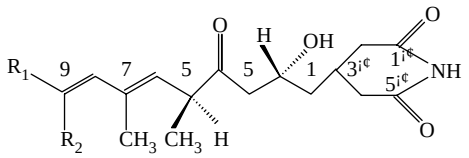

S632A$_2$ R_1=H R_2=CH_3
S632A$_3$ R_1= CH_3 R_2=H

Figure 1. Structures of S632A$_2$ and S632A$_3$.

be identical to that of 9-methylstreptimidone. The structure of S632A$_2$ is thus 3-[(2*R*,5*S*,6*E*,8*Z*)-5,7-dimethyl-4-oxo-2-hydroxy-6,8-decadienyl]-glutarimide, as shown in figure 1. Physico-chemical properties and spectral data of S632A$_3$ in comparison with those of S632A$_2$ are summarized in tables 1 and 2.

Similar to S632A$_2$, the molecular formula of S632A$_3$ was determined to be $C_{17}H_{25}NO_4$ on the basis of the HRESI-MS *m/z* 308.1858 $[M + H]^+$(calcd for $C_{17}H_{25}NO_4$, 308.1856). In the IR spectra of S632A$_2$ and S632A$_3$, absorptions at 3400, 3200 cm^{-1} indicated the presence of hydroxyl and imide groups; a broad absorption around 1700 cm^{-1} showed the presence of carbonyl group. The UV spectra of S632A$_2$ and S632A$_3$ exhibited an absorption maximum at 233 nm, which corresponds to a conjugated diene system in the molecular structure.

Comparison of the ^{1}H NMR and ^{13}C NMR spectra of S632A$_3$ with that of S632A$_2$ indicated that the main differences are due to the substituents in C_9. These are caused by the change of the methyl at C_9, which is *cis*- in S632A$_2$ and *trans*- in S632A$_3$. The main evidence is as follows. In the ^{1}H NMR spectrum of S632A$_3$ the coupling constant between 8-H and 9-H was 15.5 Hz, indicating a *trans* arrangement of the two protons. In comparison with that of S632A$_2$, the chemical shifts of 8-H and 9-H were downfield shifted 0.25 and 0.2 ppm in the ^{1}H NMR of S632A$_3$, which supported that 8-H and 9-H are in the *trans* position, and the δ value of C-7 and 9-CH_3 increased 1.2 and 1.3 ppm, respectively, supporting the above deduction. In the NOESY spectrum, when 6-H was irradiated NOE could be observed in the signal of 8-H; when 8-H was irradiated, NOE could be observed in the signal of 6-H but not in 9-H; when 9-H was irradiated, neither NOE could be observed in the signals of 6-H and 8-H. The data mentioned above suggested that both double bounds at C-6 and C-8 have an E-configuration. In ^{1}H NMR of S632A$_3$, all the coupling constants, except for the *J* value of 8-H and 9-H, are identical to those of S632A$_2$. The optical rotation of S632A$_3$ is also positive, similar to that of 9-methylstreptimidone. Accordingly, the structure of S632A$_3$ was elucidated as 3-[(2*R*,5*S*,6*E*,8E)-5,7-dimethyl-4-oxo-2-hydroxy-6,8-decadienyl]-glutarimide, an isomer of S632A$_2$.

Table 1. Physico-chemical properties of S632A$_2$ and S632A$_3$.

	S632A$_2$	*S632A$_3$*
Appearance	Pale yellowish oil	Pale yellowish oil
$[\alpha]_D^{28}$	+144° (*c* 0.1, $CHCl_3$)	+58.7° (*c* 0.15, $CHCl_3$)
Molecular formula	$C_{17}H_{25}NO_4$	$C_{17}H_{25}NO_4$
HRSI-MS	308.1854 $(M + H)^+$	308.1858 $(M + H)^+$
UV (MeOH), nm	288.0, 233.6	289.4,233.0
IR ($CHCl_3$), cm^{-1}	3420, 3200, 1720–1680 (broad), 1375, 1260, 1143, 720	3420, 3210, 1730–1670 (broad), 1375, 1260, 1143, 750

Table 2. ^{1}H NMR and ^{13}C NMR spectral data of S632A$_2$ and S632A$_3$.

	S632A$_2$		*S632A$_3$*	
	δc	δ_H	δc	δ_H
1	40.8	1.31 (ddd, $J = 13.9, 8.3, 2.5$) 1.57 (ddd, $J = 13.9, 10.5, 5.0$)	40.8	1.31 (ddd, $J = 14.0, 8.3, 2.5$) 1.57 (ddd, $J = 14.0, 10.4, 5.0$)
2	64.7	4.10 (m)	64.7	4.10 (m)
3	47.2	2.56 (dd, $J = 18.0, 3.0$) 2.63 (dd, $J = 18.0, 8.6$)	47.2	2.56 (dd, $J = 18.0, 3.1$) 2.63 (dd, $J = 18.0, 8.4$)
4	212.7		212.7	
5	47.0	3.43 (dq, $J = 9.7, 6.8$)	47.0	3.45 (dq, $J = 9.7, 6.8$)
5-CH$_3$	14.8	1.18 (d, $J = 6.8$)	16.0	1.18 (d, $J = 0.7$)
6	127.9	5.17 (dm, $J = 9.17$)	127.9	5.17 (dm, $J = 9.8$)
7	135.6		136.8	
7-CH$_3$	16.2	1.83 (d, $J = 1.2$)	13.0	1.80 (d, $J = 0.7$)
8	132.7	5.81 (dm, $J = 11.7$)	135.2	6.06 (d, $J = 15.4$)
9	125.3	5.50 (dq, $J = 11.7, 7.2$)	125.6	5.70 (dq, $J = 15.5, 6.7$)
9-CH$_3$	17.2	1.78 (dd, $J = 7.2, 1.8$)	18.5	1.77 (dd, $J = 6.7, 1.4$)
–NH		8.52		8.15
1′	172.5		172.1	
2′	38.4	2.32 (m) 2.76 (m)	38.4	2.32 (m) 2.76 (m)
3′	27.1	2.48 (m)	27.1	2.48 (m)
4′	37.1	2.32 (m) 2.76 (m)	37.1	2.32 (m) 2.76 (m)
5′	172.4		172.0	

2.2 Biological activities

2.2.1 Effect on differentiation of HL-60 cells. Human promyelocytic leukaemia cell line has been used as a model system for studying the differentiation of leukaemic cells [7]. The effects of S632A$_3$ on HL-60 cell differentiation after 5 days of treatment are summarized in table 3. When HL-60 cells were incubated with S632A$_3$ at concentrations of 10^{-8} and 10^{-5} M, approximately 64.7 and 92.1% of HL-60 cells were stained with NBT, respectively, whereas only 10.1% of the untreated cells were positive. In NSAE assay, which reflected the degree of monocyte/macrophage differentiation, the percentage of positive cells was about 4 times higher than that of the control at the dose of 10^{-8} M of S632A$_3$ and 15 times higher than that of the control at the dose of 10^{-5} M S632A$_3$, respectively. Moreover, cells treated with this compound showed apparently phagocytic activity. From all data obtained in the study, it was verified that S632A$_3$ induced granulocyte-like differentiation in human HL-60 cells.

2.2.2 Antitumour experiment in tumour-bearing mice. As shown in table 4, S632A$_3$ inhibited the different xenograft in mice markedly *in vivo* with an inhibitory rate of about 70% at dose of 14 mg/kg, which is 1/20 of LD$_{50}$ in mice by i.p. injection. Although the body weight of mice slightly decreased during the treatment (data not shown), there were no

Table 3. Induction of differentiation markers in HL-60 cells after treatment with S632A$_3$ for 5 d ($n = 3$).

S632A$_3$ (M)	*NBT reduction (%)*	*Phagocytosis (%)*	*NSAE (%)*
0	10.1 ± 1.1	8.6 ± 0.5	4.3 ± 1.2
10^{-8}	64.7 ± 2.3*	53.1 ± 3.8*	15.2 ± 2.7*
10^{-5}	92.1 ± 3.4*	85.0 ± 5.5*	76.4 ± 8.5*

* $P < 0.01$ vs. the untreated group.

Table 4. Antitumour activity of $S632A_3$ in tumour-bearing mice ($n = 10$).

Tumour	*IR (%)**	*D/U*[†]
Sarcoma 180	72.9	0/10
Sarcoma 37	74.7	0/10
Lymphosarcoma Lio-1	69.5	0/10
Harding–Passey melanoma	59.2	0/10
Hepatoma 22	70.1	0/10
Lewis lung cancer	76.3	0/10

* $P < 0.001$ vs. the control group.
† Number of mice that died of toxicity/number of mice used.

deaths due to toxicity. Accordingly, $S632A_3$ displayed potent antitumour activity in mice without causing undesirable effects.

3. Experimental

3.1 Instrumental analysis

HPLC was performed with Shimadzu LC-6A system on Shimpack CLC-ODS C_{18} column (150 × 6 mm i.d.) at UV 230 nm with MeOH/water (60:40) as a mobile phase. Optical rotation was measured in chloroform at 25°C on a Perkin–Elmer 241 polarimeter. UV spectrum was recorded in MeOH on a Shimadzu UV-2201 spectrophotometer and IR spectrum was recorded in chloroform on a Shimadzu IR-435 spectrometer, respectively. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-500 spectrometer. SI-Mass spectrum was obtained on a Bruker APEX II spectrometer.

3.2 Isolation and purification

The filtered broth of *Streptomyces hygroscopicus* S632 was passed through a column of Diaion HP-20. The antibiotic enriched resin column was then washed with water and eluted with 50% acetone. The active eluate was evaporated to remove acetone. The residual solution was adjusted to pH 7.6 and extracted with ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo* to give buffy oily residue.

The isolation of respective components was performed as follows. The residue was applied on a silica gel column eluted with chloroform/acetone (4:1). The active fractions containing $S632A_3$ were concentrated *in vacuo* to produce pale yellow oil, which was isolated by a Sephadex LH-20 column with MeOH as eluent, yielding a crude oil containing $S632A_3$. The active fractions were separated by a reverse middle pressure column (YAMAZEN 1.5 × 52 cm i.d.) with 38% MeOH as developing solution. The active eluates were further purified by preparative HPLC on a Shimpack CLC-ODS C_{18} column (150 × 6 mm i.d.) with MeOH/water (60:40) as mobile phase and 230 nm as a detection monitor to yield fractions of $S632A_2$ and $S632A_3$, and were concentrated *in vacuo* respectively to remove MeOH and extracted with ethyl acetate. The purified $S632A_2$ and $S632A_3$ were obtained after the extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo*. The purity of $S632A_3$ was more than 92% according to the result of HPLC. The spectral data are summarized in tables 1 and 2.

3.3 Biological activities

3.3.1 Differentiation assay [8,9]. (1) NBT reduction test: The percentage of HL-60 cells capable of reducing NBT was determined by counting the number of cells which contained the precipitated formazan particles after cells had been incubated with NBT (1.0 mg/ml) at 37°C for 30 min. TPA was used as a stimulator for the formation of formazan. (2) Phagocytosis test: HL-60 cells (1×10^6 cells/ml) were suspended in serum free RPMI 1640 medium containing 0.2% latex particles (average diameter, 0.81 mM) and incubated at 37°C for 4 h. After incubation, the cells were washed once with phosphate-buffered saline (PBS). Cells containing more than 10 latex particles were scored as phagocytic cells. (3) Non-specific esterase activity (NSAE) test: A smear preparation was chemically stained with α-anaphthyl butyrate as a substrate of this enzyme and was examined with microscope by the standard techniques.

3.3.2 Antitumour activity *in vivo* [10]. The animal use and care protocol was approved by the Institutional Animal Use and Care Committee of the Peking Union Medical College. Suspensions of different lines of tumour cells (3×10^6 cells, 0.1 ml) were injected subcutaneously into the backs (lymphosarcoma lio-1 cells into inguinal area) of Kunming mice ($n = 10$). After 24 h, the animals were treated once daily with S632A$_3$ by i.p. at the dose of 14 mg/kg consecutively. The mice bearing tumours were killed 10 days later and the tumours were cut and weighed. The tumour growth inhibition rate (IR) was calculated by the formula: IR (%) = (1 − the mean tumour weight of a treated group/the control group) × 100%.

4. Conclusions

We have successfully isolated and identified a new glutarimide antibiotic, S632A$_3$, whose structure was elucidated as 3-[(2*R*,5*S*,6*E*,8*E*)-5,7-dimethyl-4-oxo-2-hydroxy-6,8-decadienyl]-glutarimide, an isomer of 9-methylstreptimidone. Encouragingly, it showed potent biological activities in differentiation induction effects and antitumour activity. Moreover, we have reported that S632A$_3$ showed obvious inhibitory effects on enterovirus, herpes simplex type-1 virus (HSV-1) and virus COXB$_{3m}$ [11,12]. Of course, further work is required to elucidate the mechanism of S632A$_3$'s biological activities. However, these results suggest that S632A$_3$ may be a useful drug to be investigated and has potential for the future.

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