Communications to the Editor

A NEW ANTITUMOR ANTIBIOTIC: DEMETHYLSTREPTONIGRIN

Sir:

In our screening for antitumor antibiotics inhibiting the growth of resistant P388 leukemia cells, a streptomyces strain MG883-12F2 was selected. We found that this strain produced streptonigrin (**2**)[1]. Furthermore we discovered a new analog which has been determined to be 10′-*O*-demethylstreptonigrin (**1**). This strain was classified as *Streptomyces albus*.

It has been reported that streptonigrin has strong antitumor activity against a variety of tumors but extremely strong toxicity[2,3]. Several studies on streptonigrin structure-activity relationships have been reported and the synthesis of its analogs having high antitumor activity and low toxicity has been attempted. In this paper, the isolation and structure determination by means of NMR and physico-chemical properties of demethylstreptonigrin are reported.

A strain of MG883-12F2 was cultured in Erlenmeyer flasks at 27°C for 4 days on a rotary shaker. The production medium contained glucose 1%, beef extract 0.3%, yeast extract 0.5%, Triptose 0.5% and agar 0.15% (pH 7.0). The filtrate of fermentation broth (5 liters) was extracted with 6.5 liters of EtOAc at pH 3.5. The extract was concentrated under reduced pressure to a crude powder (560 mg). The crude powder was chromatographed on silica gel (elution with $CHCl_3$ - MeOH, 100 : 3) (Merck, Kieselgel 60, 22 g) and on Sephadex LH-20 (elution with MeOH, 200 ml) successively. The fractions were analyzed by reversed phase silica gel HPLC (Nucleosil 5C18, 4.5×250 mm, Macherey-Nagel Ltd.) (elution with acetonitrile - 5% potassium acetate buffer, pH 6.0, containing citric acid at 1.0%, 4 : 6). The retention time of **1** and **2** was 4 minutes and 6 minutes, respectively. Further purification was achieved by preparative HPLC on reversed phase silica gel (Nucleosil 5C18, 20×300 mm, Macherey-Nagel Ltd.). The active fraction was extracted with EtOAc at pH 3.5 and again chromatographed on a Sephadex LH-20 column (15×200 mm) to give amorphous brown powder, 5 mg: mp 177~181°C (dec). This preparation showed a single peak on analytical HPLC. It shows UV λ_{max}^{MeOH} nm (ε) 245 (32,800) and 385 (12,300). The IR spectrum of **1** is shown in

Fig. 1. IR spectrum of **1** in KBr.

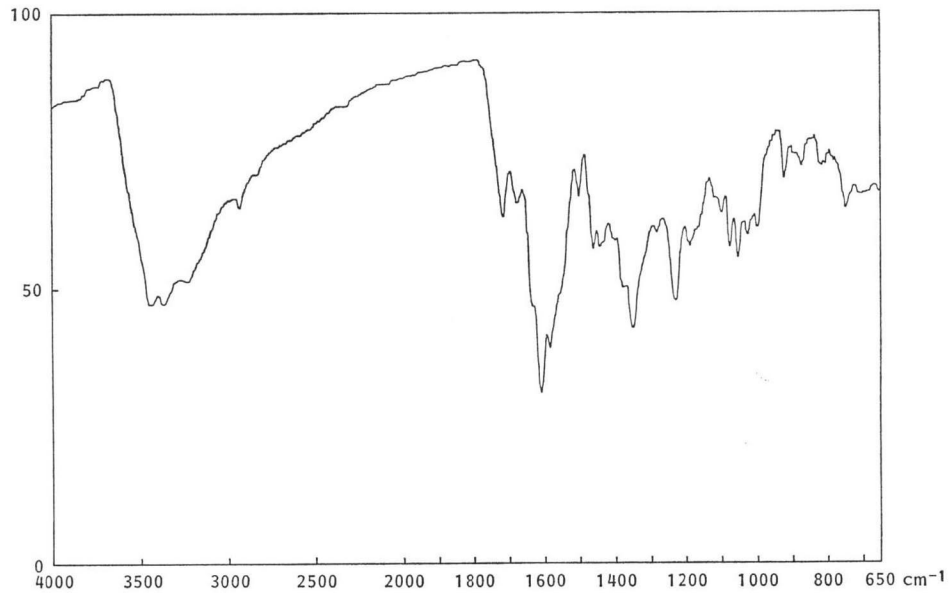
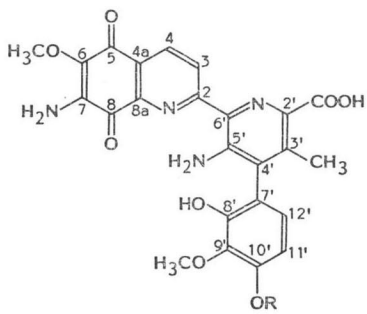


Table 1. ^{1}H NMR spectrum of **1** and **2** in dioxane-d_8 at 400 MHz.

Compound		Multiplicity (J, Hz)	Assignment
1	**2**		
2.35	2.34	s	3′-CH_3
3.83	3.84	s	9′-OCH_3
—	3.88	s	10′-OCH_3
3.97	3.96	s	6-OCH_3
5.89	6.00	br	7-NH_2
6.52	6.70	d (8.5)	11′
6.65	6.77	d (8.5)	12′
7.56	—	br	10′-OH
7.85	7.83	br	8′-OH
8.41	8.38	d (9.0)	4
8.95	8.93	d (9.0)	3
10.9	11.0	br	COOH

Chemical shifts in ppm from internal TMS.

Fig. 2.



Demethylstreptonigrin (**1**) R=H
Streptonigrin (**2**) R=CH_3

Fig. 1. The ^{1}H NMR and ^{13}C NMR of **1** is listed in Tables 1 and 2.

The structure of **1** was deduced from its ^{1}H NMR and ^{13}C NMR spectra in comparison with those of streptonigrin (**2**). The ^{13}C NMR spectrum of **2** was originally reported taken in pyridine-d_5[4] but we studied the spectrum of **1** in dioxane-d_8 by the aid of ^{13}C-^{1}H long range selective proton decoupling (LSPD) experiments. The LSPD experiment irradiating at δ_H 6.00 (C7-NH_2) showed sharp signals at δ_C 181.0 (C-8) and δ_C 137.4. And the chemical shifts of C-9′ and C-10′ were assigned respectively to δ_C 137.9 and δ_C 154.1 by experiments irradiating at δ_H 7.83 (8′-OH), δ_H 6.70 (11′-H) and δ_H 6.77 (12′-H). Furthermore irradiation of OCH_3 groups (δ_H 3.84, 3.88 and 3.96) revealed the carbons (δ_C 137.9, 154.1 and 137.4) connected to them respectively. The ^{1}H NMR and ^{13}C NMR of **1** indicated the lack of a methyl group (δ_C 55.9, δ_H 3.88) in streptonigrin. This missing methyl was now assigned to be the methyl of the C10′-OCH_3. Thus the structure of **1** was deduced to be as shown in Fig. 2.

Table 2. ^{13}C NMR chemical shifts of **1** and **2** in ppm from TMS in dioxane-d_8 at 100 MHz.

Compound		Assignment
1	**2**	
181.0	181.0	8
177.2	177.2	5
165.5	165.5	COOH
160.9	160.8	8a
151.4	154.1	10′
148.9	149.0	8′
147.7	147.5	5′
145.3	145.3	2
141.0	141.0	7
139.2	138.9	3′
137.1	137.9	9′
137.5	137.4	6
134.7	135.0	6′
134.2	134.2	4
133.8	133.6	2′
130.4	130.3	4′
127.7	127.7	4a
126.2	126.2	3
125.9	125.6	12′
114.4	115.7	7′
109.4	105.2	11′
60.6	60.7	9′-OCH_3
60.2	60.2	6-OCH_3
—	55.9	10′-OCH_3
17.3	17.4	3′-CH_3

Streptonigrin (**2**) is known to be active against Gram-positive and Gram-negative bacteria but demethylstreptonigrin (**1**) has only weak antibacterial activity. The cytotoxic activities (50% inhibition concentrations) of **2** and **1** on P388 leukemia cells *in vitro* are 0.025 μg/ml and 0.58 μg/ml, respectively. On P388 resistant to adriamycin, those of **2** and **1** are 0.01 μg/ml and 0.41 μg/ml, respectively.

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