NEW ANTHRACYCLINE ANTIBIOTICS, 1-HYDROXYAURAMYCINS AND 1-HYDROXYSULFURMYCINS

Sir:

In a previous paper, we reported isolation of new anthracycline antibiotics, auramycins and sulfurmycins, and the structure of their aglycones, auramycinone and sulfurmycinone, respectively[1]. Auramycinone has a methyl group instead of an ethyl group at C-9 in aklavinone and sulfurmycinone has an acetonyl group. The acetonyl side chain has only been found so far in sulfurmycinone and feudomycinone A[2]. We now describe isolation of 1-hydroxyauramycinone and 1-hydroxysulfurmycinone glycosides from the culture of soil isolates.

The strains AC-180 and AC-628, which were isolated from soil samples collected in Atami and Kumamoto, respectively, in Japan, were selected as producers of red anthracycline antibiotics. These strains were identified as *Streptomyces melanogenes* and *Streptomyces galilaeus*, respectively. This is the first time that *Streptomyces melanogenes* were found to produce anthracycline antibiotics. The preliminary characterization of the antibiotics indicated that those produced by the two strains were identical with each other.

S. galilaeus AC-628 was cultivated in 500 ml flasks each containing 100 ml of medium of the following composition (%): Glucose, 2; soluble starch, 2; S-3 meat (Ajinomoto Co., Ltd.), 0.5; yeast extract, 0.25; K_2HPO_4, 0.1; $MgSO_4 \cdot 7H_2O$, 0.1; NaCl, 0.3 and $CaCO_3$, 0.3. Cultivation was conducted at 27°C on a rotary shaker for 3 days. Six hundred ml of the culture thus obtained was transferred to a 50-liter jar fermentor which contained 30 liters of the sterile medium, and fermentation was performed at 27°C for 4 days with an agitation speed of 350 rpm and aeration of 1 v/v medium.

Red pigment antibiotics were recovered into the chloroform layer by extraction with a solvent mixture of chloroform and methanol. The crude antibiotic extract contained many components as detected on tlc plates. Hydrolysis with HCl gave three anthracycline aglycones, the major one of which was identified as ε-pyrromycinone. The other two, aglycone I and aglycone II, were each obtained pure as red powders: Aglycone I: mp. 180.5°C; λ_{max}^{MeOH} ($E^{1\%}$), 234 (960), 256 (540), 292 (200), 493 (310), 511 (260), 526 (220); $\lambda_{max}^{0.1N\ NaOH\text{-}MeOH}$ ($E^{1\%}$), 242 (770), 288 (280), 560 (340), 590 (330); *Anal.* calcd. for $C_{21}H_{18}O_9$, C 60.87, H 4.38, O 34.75; found, C 61.17, H 4.20, O 34.63. Aglycone II: mp. 169.5°C;

Table 1. ^{1}H-NMR spectral data of ε-pyrromycinone, 1-hydroxyauramycinone and 1-hydroxysulfurmycinone.

ε-Pyrromycinone			Aglycone I (1-Hydroxyauramycinone)			Aglycone II (1-Hydroxysulfurmycinone)		
δ (ppm)	H	Assignment	δ (ppm)	H	Assignment	δ (ppm)	H	Assignment
1.10	3H (t)	CH_3	1.43	3H (s)	CH_3	2.28	3H (s)	$CO\text{-}CH_3$
1.60	2H (q)	CH_2				2.27	1H (d, t)	C8<H H
2.26	1H (d, t)	C8<H H	2.22	1H (d, t)	C8<H H	2.42	1H (d, d)	
2.57	1H (d, d)		2.65	1H (d, d)		2.68	1H (d)	C9-CH_2
						2.93	1H (d)	
3.38	1H (q)	C7-OH	3.37	1H (q)	C7-OH			
3.71	3H (s)	$COOCH_3$	3.71	3H (s)	$COOCH_3$	3.70	3H (s)	$COOCH_3$
3.85	1H (s)	C9-OH	4.02	1H (s)	C9-OH	3.85	1H (d)	C7-OH
4.10	1H (d)	C10-H	4.07	1H (d)	C10-H	4.36	1H (s)	C10-H
						4.82	1H (s)	C9-OH
5.38	1H (m)	C7-H	5.38	1H (m)	C7-H	5.23	1H (m)	C7-H
7.31	2H (s)	C2, C3-H	7.31	2H (s)	C2, C3-H	7.30	2H (s)	C2, C3-H
7.74	1H (s)	C11-H	7.72	1H (s)	C11-H	7.68	1H (s)	C11-H
12.16	1H (s)	C1, C4, C6-OH	12.24	1H (s)	C1, C4, C6-OH	12.21	1H (s)	C1, C4, C6-OH
12.84	1H (s)		12.82	1H (s)		12.82	1H (s)	
12.94	1H (s)		12.95	1H (s)		12.95	1H (s)	

λ_{max}^{MeOH} ($E^{1\%}$), 234 (830), 256 (480), 292 (180), 493 (260), 511 (230), 526 (210); $\lambda_{max}^{0.1N\ NaOH\text{-}MeOH}$ ($E^{1\%}$), 242 (580), 290 (330), 570 (310), 590 (300); *Anal.* calcd. for $C_{23}H_{20}O_{10}$, C 60.53, H 4.42, O 35.05; found, C 60.86, H 4.55, O 34.59.

In the ^{1}H-NMR spectrum of aglycone I (Table 1) a signal for CH_3 appeared at δ 1.43 as a singlet, while in that of ε-pyrromycinone a peak for CH_3 was observed at δ 1.10 as a triplet which was accompanied by a quartet (2H) at δ 1.60. This indicated that the side chain of aglycone I at C–9 was a methyl group. Other signals in ^{1}H-NMR spectrum were almost identical to those of ε-pyrromycinone. Furthermore, the signals in the higher field coincided with those in the spectrum of auramycinone[1]. The differences appeared only in the signals for aromatic protons, explained by an extra OH at C–1 in aglycone I. Thus, the structure of aglycone I was determined to be 1-hydroxyauramycinone.

The mass fragments of aglycone II suggested that it differed from ε-pyrromycinone only in the side chain at C–9. In the ^{1}H-NMR spectrum of aglycone II (Table 1), signals for an ethyl side chain of ε-pyrromycinone at δ 1.10 (t) and δ 1.60 (q) were not observed and new signals, a singlet at δ 2.28 (3H) and two doublets at δ 2.68 (1H) and δ 2.93 (1H), appeared. These signals coincided with those for $-CH_2-CO-CH_3$ in sulfurmycinone[1]. The differences between aglycone II and sulfurmycinone in signals for the aromatic protons could be explained by an additional hydroxyl group at C–1 in aglycone II. Thus, the structure of aglycone II was shown to be 1-hydroxysulfurmycinone. ^{13}C-NMR spectra confirmed the structures.

Six anthracycline glycosides in the crude antibiotic complex were purified and isolated by silica gel column chromatography, preparative HPLC and thin-layer chromatography. The major two components were identified as cinerubin A[3] and B[4], respectively. Another two components were 1-hydroxyauramycinone glycosides as detected by mass fragmentation pattern and designated 1-hydroxyauramycin A and B. The other two had 1-hydroxysulfurmycinone as their aglycone and were named 1-hydroxysulfurmycin A and B.

1-Hydroxyauramycin A: mp. 108.5°C; $[\alpha]_D^{20}$ +93.1° (*c* 0.1, $CHCl_3$); λ_{max}^{MeOH} ($E^{1\%}$), 234 (500), 256 (sh) (310), 292 (103), 493 (150), 511 (130), 526 (140), 570 (80); $\lambda_{max}^{0.1N\ NaOH\text{-}MeOH}$ ($E^{1\%}$), 241 (535), 290 (sh) (110), 565 (210), 602 (170); *Anal.* calcd. for $C_{41}H_{51}O_{16}N$, C 60.50, H 6.32, O 31.45, N 1.73; found, C 60.76, H 5.95, O 31.71, N 1.58.

1-Hydroxyauramycin B: mp. 151.5°C; $[\alpha]_D^{20}$ +89.4° (*c* 0.1, $CHCl_3$), λ_{max}^{MeOH} ($E^{1\%}$), 234 (555), 256 (325), 292 (105), 493 (185), 511 (160), 526 (150), 570 (80); $\lambda_{max}^{0.1N\ NaOH\text{-}MeOH}$ ($E^{1\%}$), 241 (595), 290 (sh) (120), 565 (240), 602 (200); *Anal.* calcd. for $C_{41}H_{49}O_{16}N$, C 60.66, H 6.08, O 31.53, N 1.73; found, C 60.25, H 5.93, O 31.97, N 1.85.

1-Hydroxysulfurmycin A: mp. 126.0°C $[\alpha]_D^{20}$ +57.88° (*c* 0.1, $CHCl_3$), λ_{max}^{MeOH} ($E^{1\%}$), 234 (570), 256 (345), 292 (115), 493 (175), 511 (165), 526 (160), 570 (75); $\lambda_{max}^{0.1N\ NaOH\text{-}MeOH}$ ($E^{1\%}$), 241 (530), 292 (170), 307 (sh) (165), 565 (230), 602 (180); *Anal.* calcd. for $C_{43}H_{53}O_{17}N$, C 60.34, H 6.24, O 31.78, N 1.64; found, C 60.85, H 6.05, O 31.37, N 1.73.

1-Hydroxysulfurmycin B: mp. 158.5°C; $[\alpha]_D^{20}$ +60.33° (*c* 0.1, $CHCl_3$); λ_{max}^{MeOH} ($E^{1\%}$), 234 (545), 256 (340), 290 (110), 493 (175), 511 (160), 526 (160), 570 (85); $\lambda_{max}^{0.1N\ NaOH\text{-}MeOH}$ ($E^{1\%}$), 241 (540), 293 (165), 307 (sh) (155), 565 (240), 602 (190); *Anal.* calcd. for $C_{43}H_{51}O_{17}N$, C 60.48, H 6.02, O 31.85, N 1.65; found, C 60.45, H 5.94, O 31.86, N 1.75. Rf values of the above 4 antibiotics on silica gel tlc plates (Kieselgel, F_{254}, Merck) developed by chloroform - methanol (19: 1) (I) and toluene - methanol (10: 1) (II) were: 1-Hydroxyauramycin A; (I) 0.37, (II) 0.24; 1-hydroxyauramycin B; (I) 0.55, (II) 0.52; 1-hydroxysulfurmycin A; (I) 0.31, (II) 0.21; 1-hydroxysulfurmycin B; (I) 0.52, (II) 0.45.

On hydrolysis of 1-hydroxyauramycin A, three sugars were liberated, which were shown to be rhodosamine, 2-deoxyfucose and cinerulose by comparison with authentic samples from aclacinomycin A[5] and auramycin A[6]. Furthermore, the disaccharide acetate and the methylglycoside obtained by partial hydrolysis and methanolysis, respectively, of 1-hydroxyauramycin A were identical with those obtained from auramycin A[6]. Similarly, 1-hydroxysulfurmycin A was concluded to have sugar components identical with those of auramycin A.

By contrast, the sugar moieties of 1-hydroxyauramycin B and 1-hydroxysulfurmycin B were studied in comparison with those of auramycin B and sulfurmycin B[6], as well as aclacinomycin B[5] and cinerubin B[4]. The result indicated that all of these B components had the same sugar moiety.

Fig. 1. Structure of 1-hydroxyauramycin A and B, 1-hydroxysulfurmycin A and B.

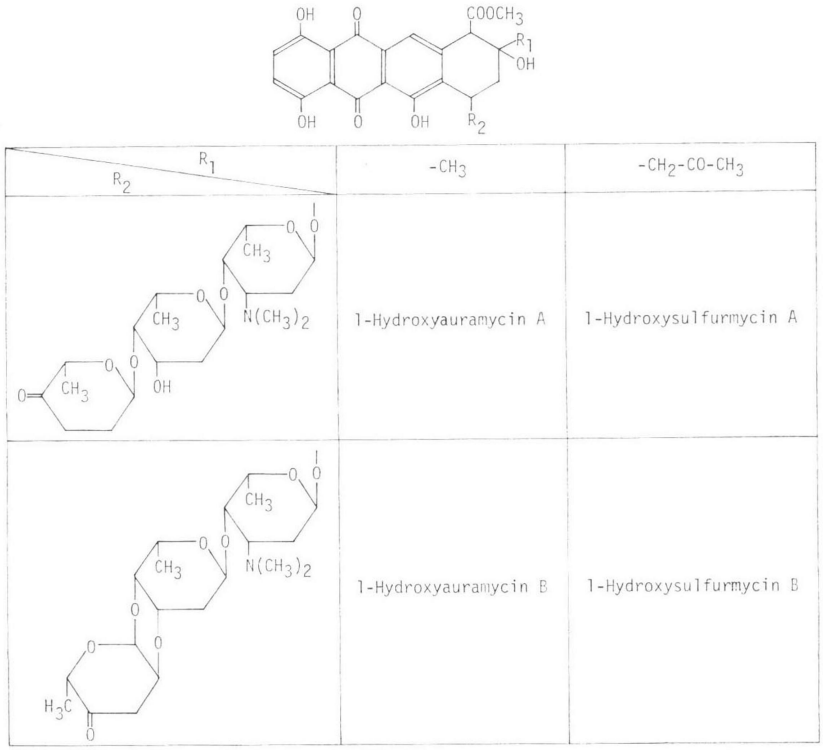

Table 2. Antimicrobial spectrum.

Strain		MIC (μg/ml)			
		1-Hydroxy-auramycin A	1-Hydroxy-auramycin B	1-Hydroxy-sulfurmycin A	1-Hydroxy-sulfurmycin B
Bacillus subtilis IAM 1027	(1)	0.39	0.78	0.78	0.78
Sarcina lutea IAM 1009	(1)	0.19	0.39	0.78	0.78
Staphylococcus aureus 209P IAM 1011	(1)	0.39	0.78	0.78	0.78
Micrococcus flavus ATCC 10240	(1)	0.39	0.78	0.78	0.78
Mycobacterium smegmatis IFO 13167	(1)	3.13	1.56	6.25	3.13
Escherichia coli K-12 IAM 1264	(1)	>50	>50	>50	>50
Escherichia coli NIHJ IFO 12734	(1)	>50	>50	>50	>50
Pseudomonas aeruginosa IFO 12689	(1)	>50	>50	>50	>50
Candida albicans ATCC 10231	(2)	>50	>50	>50	>50
Candida tropicalis ATCC 13803	(2)	>50	>50	>50	>50

(1) Heart infusion agar.
(2) SABOURAUD dextrose agar.

Thus, the structure of 1-hydroxyauramycin A and B and 1-hydroxysulfurmycin A and B were those shown in Fig. 1.

Antimicrobial spectra of these antibiotics are shown in Table 2. They exhibit activity against Gram-positive bacteria and *Mycobacterium smegmatis*. They also show antitumor activity against L1210 leukemia in mice.

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