NEW ANSAMYCIN ANTIBIOTICS, DIASTOVARICINS I AND II

Sir:

During the course of our screening program for new inducers of Friend mouse erythroleukemia cells[1,2], two active substances were isolated from the broth filtrate of a streptomycete. These antibiotics belonging to the ansamycin group were named diastovaricins I and II.

The diastovaricin producing organism was identified as *Streptomyces diastochromogenes* subsp. *variabilicolor* n. subsp. A detailed description of taxonomical studies will be reported elsewhere. This organism was cultivated at 27°C for 2 days in a 50-liter jar fermentor containing 20 liters of a medium consisting of soluble starch 2.5%, soybean meal 1.5%, dry yeast 0.2% and calcium carbonate 0.4%. Diastovaricins were isolated and purified as shown in Scheme 1.

The physico-chemical properties of diastovaricins I and II are as follows:

Diastovaricin I: red needles; mp 237~238°C; $[\alpha]_D^{21}$ +351° (*c* 0.1, MeOH); UV λ_{max}^{MeOH} nm (log ε) 232 (4.41), 285 (4.37), 350 (3.70), 440 (2.84); IR ν_{max}^{KBr} cm^{-1} 3450 (NH, OH), 1630 (amide); *Anal* Calcd for $C_{39}H_{45}NO_{10}$: C 68.11, H 6.59, N 2.04, O 23.26. Found: C 67.05, H 6.55, N 1.95, O 24.47; FD-MS *m/z* 688 $(M+H)^+$.

Diastovaricin II: orange powder; mp 160~164°C; $[\alpha]_D^{21}$ +281° (*c* 0.1, MeOH); UV λ_{max}^{MeOH} nm (log ε) 210 (4.40), 229 (4.43), 290 (4.32), 470 (2.92); IR ν_{max}^{KBr} cm^{-1} 3450 (NH, OH), 1630 (amide); *Anal* Calcd for $C_{44}H_{52}N_2O_{12}S$: C 63.46, H 6.25, N 3.37, O 23.08, S 3.85. Found: C 62.92, H 6.61, N 2.97, O 23.16, S 3.89; FD-MS *m/z* 833 $(M+H)^+$.

The ^1H and ^{13}C NMR spectral assignments of diastovaricins I and II were performed by 2-D COSY and 2-D C-H correlation spectral analysis and LSPD (long range selective proton decoupling) experiments[3]; the ^1H NMR data are summarized in Table 1.

The ^1H NMR spectral data of diastovaricin I are quite similar to those of naphthomycin B[4]. Comparison of the molecular formula of these two compounds indicates that diastovaricin I is a dechloro-hydroxyl derivative of naphthomycin B. From the close UV and visible absorption spectral similarity of diastovaricin I and actamycin[5], it is concluded that diastovaricin I is 30-dechloro-30-hydroxynaphthomycin B.

Based on the established structure of diastovaricin I, the stereochemistry of the olefinic bonds still unresolved in actamycin, was ana-

Scheme 1. Isolation of diastovaricins I and II.

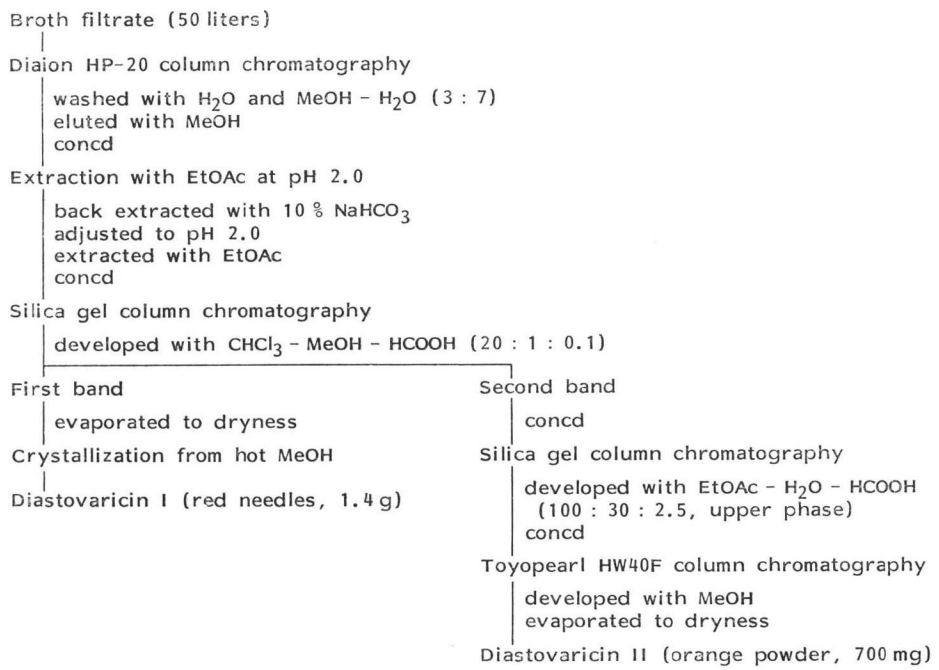
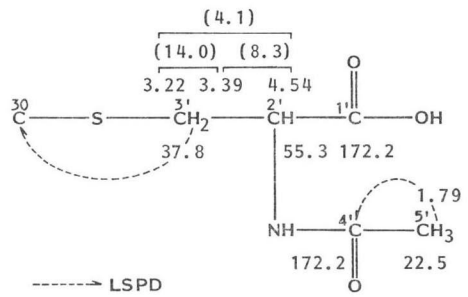


Table 1. ^{1}H NMR data of diastovaricins I and II.

Assignment	Diastovaricin I*		Diastovaricin II**	
	(ppm)	J (Hz)	(ppm)	J (Hz)
CH_3C (20)	0.85 d	6.5	0.89 d	6.5
CH_3C (18)	0.99 d	6.5	0.97 d	6.8
CH_3C (8)	1.20 d	6.5	1.10 d	6.5
CH_3C (12)	1.69 s		1.72 s	
CH_3C (5′)			1.79 s	
CH_3C (22)	2.04 s		2.05 s	
HC (18)	2.10~2.20		2.0~2.04	
CH_3C (26)	2.26 s		2.37 s	
H_2C (14)	2.31~2.40		2.36~2.44	
HC (8), HC (20)	2.73 m		2.75 m	
HaC (10)	2.80 dd	17.0, 6.5	2.67 dd	17.0, 6.0
HbC (10)	2.99 dd	17.0, 3.5	2.98 dd	17.0, 5.0
HC (19)	3.16 dd	10.0, 2.0	3.01 dd	6.0, 6.0
HaC (3′)			3.22 dd	14.0, 4.1
HbC (3′)			3.37 dd	14.0, 8.3
HC (9)	3.66 m		3.91 m	
HC (15)	4.13 m		4.0 m	
HC (2′)			4.54 dd	8.3, 4.1
HC (7)	5.47 dd	10.0, 10.0	5.38 dd	10.5, 10.5
HC (17)	5.52 dd	15.0, 9.5	5.50 dd	15.0, 8.0
HC (16)	5.65 dd	15.0, 7.0	5.57 dd	15.0, 7.2
HC (2)	5.93 d	11.0	6.05 d	11.6
HC (21)	6.0 dd	10.0, 1.0	5.97 dd	10.0, 1.0
HC (6)	6.28 dd	11.0, 10.0	6.04 dd	10.5, 10.5
HC (13)	6.75 t	5.5	6.64 t	5.5
HC (3)	6.79 dd	11.0, 11.0	6.66 dd	11.6, 11.6
HC (5)	6.81 dd	15.0, 11.0	6.70 dd	15.0, 10.5
HC (4)	7.34 dd	15.0, 11.0	7.42 dd	15.0, 11.6
HC (27)	7.84 s		7.94 s	
NH	8.51			

* in $CDCl_3$. ** in CD_3OD.

Fig. 1. *S*-Substituted *N*-acetylcysteine with the relevant ^{1}H and ^{13}C chemical shifts, and coupling constants (in brackets), as revealed by LSPD experiments.



lyzed by ^{1}H NMR. In the ^{1}H NMR spectrum of diastovaricin I the following coupling constants were observed between the olefinic protons: $J_{2,3}$=11.0 Hz, $J_{4,5}$=15.0 Hz, $J_{6,7}$=10.0 Hz, and nuclear Overhauser effects (NOE) were proved between 12-CH_3 (δ_H 1.69) and H-14b (2.31) and between 22-CH_3 (2.04) and H-20 (2.73), thereby showing the 2*Z*,4*E*,6*Z*,12*E*,21*E*-configurations for diastovaricin I. On the other hand, the 2*Z*,4*Z*,6*E*,12*E*,16*E*,21*E*-configurations for actamycin were established by the NMR spectral similarity between the two substances and the coupling constants as follows: $J_{2,3}$= 11.0 Hz, $J_{4,5}$=11.5 Hz and $J_{6,7}$=15.0 Hz. On the basis of spectral similarity it is concluded that diastovaricin I is a geometrical isomer of actamycin.

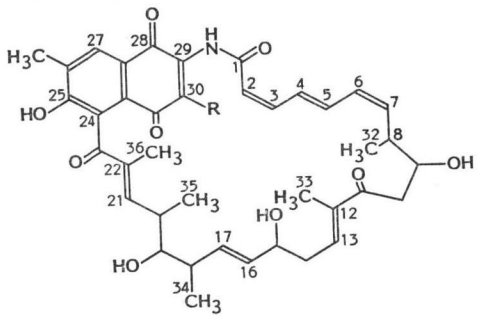
Based on the ^{13}C NMR spectral data (not shown), diastovaricin II is assumed to be an analog of diastovaricin I with the hydroxyl group at the C-30 position being replaced by

Table 2. Effect of diastovaricins I and II on the induction of differentiation of Friend cells.

Concentration (μg/ml)	Diastovaricin I		Diastovaricin II	
	Cell number (cells/ml)	Benzidine reactive cells (%)	Cell number (cells/ml)	Benzidine reactive cells (%)
15.6	1.8×10^6	<1	1.7×10^6	2
31.2	1.6×10^6	3	1.8×10^6	2
62.5	1.7×10^6	20	9.3×10^5	20
125	5.2×10^5	36	9.1×10^5	16
250	0	—	5.4×10^5	2
None	1.7×10^6	<1		
DMSO (2%)	8.1×10^5	41		

Friend cells at 1×10^5 cells/ml were incubated with various concentrations of diastovaricins for 5 days and then benzidine reactive cells were examined.

Fig. 2. Structures of diastovaricins I and II and naphthomycin B.



Diastovaricin I R=OH
Diastovaricin II R=$SCH_2CHCOOH$ (NHCOCH$_3$)
Naphthomycin B R=Cl

another substituent (C-30, in diastovaricin I 146.4 ppm, in diastovaricin II 135.7 ppm). The NMR spectral similarity of these antibiotics indicates that the remaining moieties possess the same structures. The modified moiety in diastovaricin II has been elucidated to be *S*-substituted *N*-acetylcysteine by NMR spectral analysis and LSPD experiments as shown in Fig. 1. Thus, it is concluded that the structures of diastovaricins I and II are as shown in Fig. 2.

The effects of diastovaricins I and II on the induction of differentiation of Friend cells are summarized in Table 2. They showed no antimicrobial activity at 100 μg/ml nor toxicity after a single intraperitoneal administration of 200 mg/kg in mice.

Acknowledgments

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Mitsuyuki Hotta
Yoichi Hayakawa
Kazuo Furihata
Akira Shimazu
Haruo Seto
Noboru Ōtake*

Institute of Applied Microbiology,
The University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan

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