THE STRUCTURES OF PLURACIDOMYCINS, NEW CARBAPENEM ANTIBIOTICS

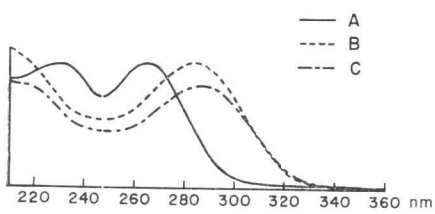
Sir:

New β-lactam antibiotics which have broad antibacterial and strong β-lactamase inhibitory activities were detected among the metabolites of a new strain of streptomycete named *Streptomyces pluracidomyceticus* nov. sp. (PA-41746). On paper electrophoresis at pH 7.0, certain of these components migrated toward the anode with greater mobility than that of olivanic acids[1,2] (Table 1). From these preliminary experiments we assumed that these components should be new carbapenem compounds presumably having two or three acidic functions, and named them pluracidomycins (PLM).

Pluracidomycins were extracted from the fermentation filtrate using a phase-transfer reagent, separated by ion-exchange resin to factors A, B and C, and purified by reverse-phase chromatography and gel filtration in the forms of sodium salts. In a typical batch, 200 liters of the filtrate gave about 450 mg of factor A, 100 mg of factor B and 150 mg of factor C. Pluracidomycins are fairly stable in the pH range of 6.0~6.5. Their UV and ^{1}H NMR spectra are shown in Figs. 1 and 2, respectively. Besides pluracidomycins, penicillin N, cephamycin C, deacetoxycephalosporin C, epithienamycin A and MM17880 were detected in the broth, and also the sulfoxides of epithienamycin B and D which were characterized as new compounds.

From the ^{1}H NMR spectra it was easily pointed out that the pluracidomycins all have the sulfated olivanic acid structure[1~3]. However, they lack the *N*-acetyl proton signal found in olivanic acids such as MM4550 (4), MM13902 (5) and MM17880 (6) and have neither –CH=CH– nor $-CH_2-CH_2-$ functions. Therefore, pluracidomycins can be distinguished from the sulfated olivanic acids in the substituents at C-3. This conclusion was also confirmed by the ^{13}C NMR data listed in Table 2.

Fig. 1. UV spectra of pluracidomycins in H_2O.



The main component, pluracidomycin A (PLM A) shows the highest mobility on paper electrophoresis (Table 1) and has no ^{1}H and ^{13}C signals attributable to the C-3 substituent, which may be an acidic function. The IR spectrum of PLM A exhibited broader and stronger absorption bands at 1200~1260 cm^{-1} and 1030~1080 cm^{-1} than those of MM4550, therefore, the substituent at C-3 was concluded to be a sulfonate as shown in the structure (1), which was also agreeable with the elemental analysis. PLM A is probably identical with antibiotic SF 2103A found by the Meiji-Seika group[4].

Pluracidomycin B (PLM B) as well as pluracidomycin C (PLM C) has ^{1}H signals essentially

Table 1. Paper electrophoresis (1/30 M phosphate buffer pH 7.0; 12 V/cm; 1.5 hours).

Antibiotics	Relative mobility
Epithienamycin A	1.0
MM4550	1.9
MM17880	1.9
PLM A	3.0
PLM B	2.7
PLM C	1.9

Table 2. ^{13}C NMR in D_2O[a].

Carbon No.	PLM A[b]	PLM B[c]	PLM C[c]
2	139.2 (s)	140.0 (s)	140.0 (s)
3	129.0 (s)	140.6 (s)	140.5 (s)
4	32.8 (t)	30.5 (t)	30.4 (t)
5	55.1 (d)	54.7 (d)	54.7 (d)
6	58.7 (d)	59.1 (d)	59.2 (d)
7	168.7 (s)	166.5 (s)	166.4 (s)
8	73.7 (d)	74.0 (d)	73.9 (d)
2-COONa	177.9 (s)	177.5 (s)	177.5 (s)
8-CH_3	19.4 (q)	19.3 (q)	19.3 (q)
S(O)–CH_2–	—	60.2 (t)	—
S(O)–CH– | OH	—	—	86.5 (d)
–COONa	—	172.3 (s)	—

[a] δ ppm from DSS (internal CH_3CN, δ=1.7).
[b] Recorded with a Varian XL-100-12A spectrometer.
[c] Recorded with a Varian XL-200 spectrometer.

Fig. 2. ^1H NMR spectra of pluracidomycins A, B and C in D_2O at 100 MHz (δ ppm from external TMS).

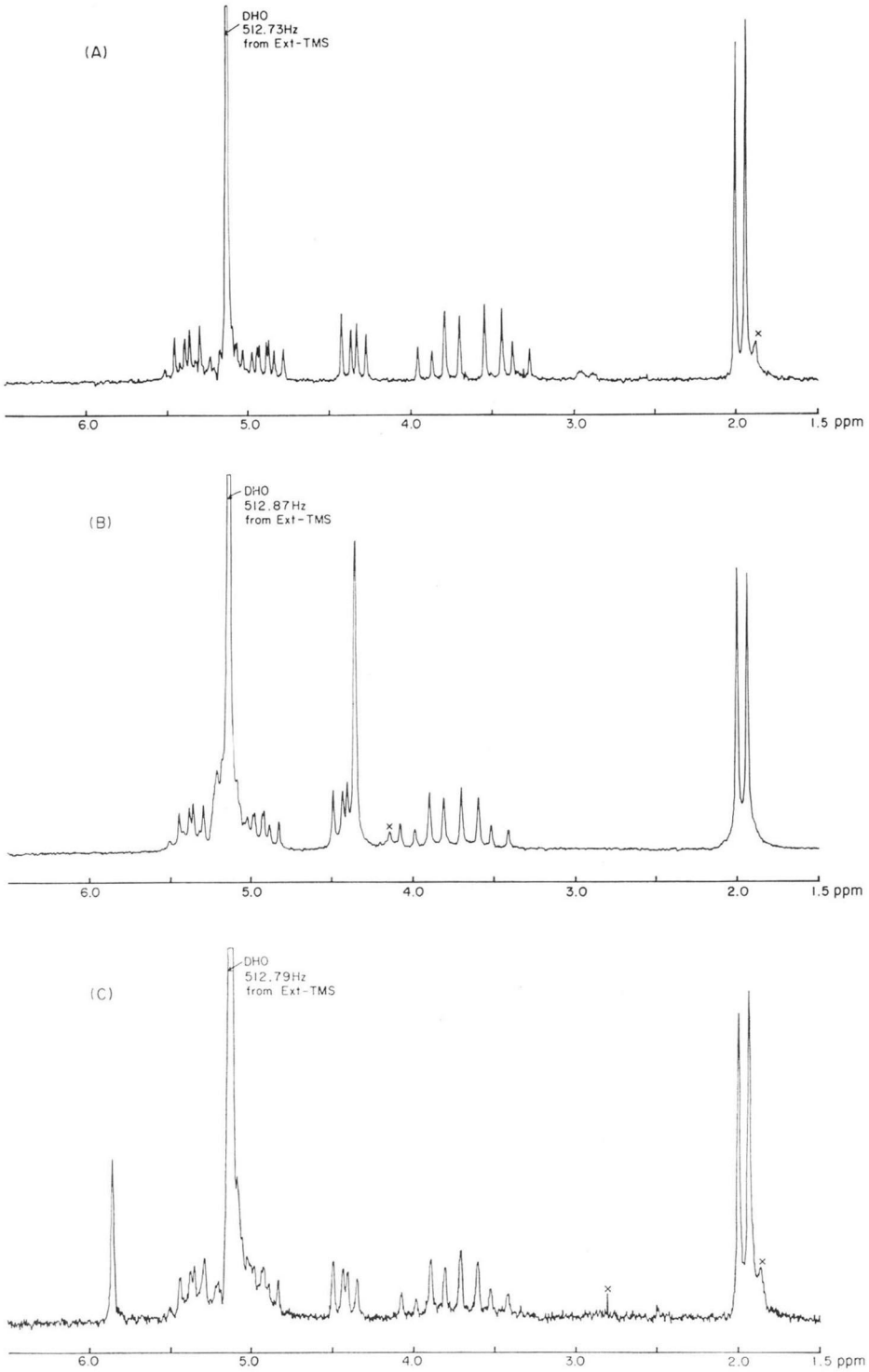
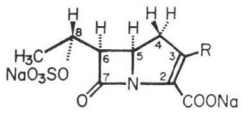


Fig. 3. Structures of pluracidomycins and related compounds.



(1) R = SO_3Na PLM A

(2) R = $S(=O)-CH_2COONa$ PLM B

(3) R = $S(=O)-CH(OH)(OH)$ PLM C

(3') R = $S(=O)-CHO$

(4) R = $S(=O)-CH=CHNHAc$ MM 4550

(5) R = $S-CH=CHNHAc$ MM 13902

(6) R = $S-CH_2CH_2NHAc$ MM 17880

(7) R = $S(=O)-CH_2OH$ dihydro PLM C

(8) R = $S-CH_2COONa$ deoxy PLM B

identical with those of MM4550[1~3] except the signals due to the substituents at C-3. Moreover, since PLM B and C exhibit the ^{13}C chemical shifts of C-3 almost identical to that of MM-4550[1,3], both compounds should also have the sulfoxide function adjacent to the C-3 substituent as in MM4550.

Different from MM4550, PLM B has a methylene group (δ ^1H; 4.31, 2H, s., δ ^{13}C; 60.2 t.) and a carbonyl function (δ ^{13}C; 172.3 s.) in the C-3 substituent. Taking account of the paper electrophoresis and the elemental analysis the carbonyl group is probably a constituent of a carboxyl function. PLM B has much stronger IR bands assignable as COO^- at 1600 cm^{-1} and 1400 cm^{-1} in comparison with those of PLM A and C or MM4550. This evidence suggests that PLM B has the structure (2) as shown in Fig. 3.

PLM C has a methine group (δ ^1H; 5.86, 1H, s., δ ^{13}C; 86.5, d.) next to the sulfoxide on C-3, and no additional ^{13}C signals could be observed. From the ^1H and ^{13}C chemical shifts the presence of at least one hydroxyl group, on this methine is likely. The ^1H NMR of PLM C in DMSO-d_6 dramatically changed this methine signal from 5.86 ppm to 9.62 ppm (1 H, broad singlet. inextinguishable by D_2O-addition), but the ^1H NMR of the recovered sample in D_2O regenerated the original methine signal at 5.86 ppm. This change suggests the conversion, $-S(=O)-CH(OH)(OH) \rightleftharpoons -S(=O)-CHO$,

and the structure of PLM C is concluded to be (3) or (3'). Among pluracidomycins, only PLM C was positive to 2,4-dinitrophenyl hydrazine and the formation of hydrazone or semicarbazone was confirmed by HPLC. Further, PLM C was reduced by sodium borohydride to the alcohol (7), which also has strong β-lactamase inhibitory activity.

The relative configurations of the C-5, C-6, C-8 and the sulfoxide of PLM B and C should be identical with those of MM4550 because of the good correlation of ^1H chemical shifts and coupling constants[1~3]. MM4550 has been related biosynthetically to MM22380[5] (epithienamycin A), the stereochemistry of which was elucidated as 5-*R*, 6-*R*, 8-*S*[6]. With respect to the sulfoxide of MM4550, the configuration is inferred to be *R* from the CD spectrum[1] which closely resembles that of C-19393[7] (carpetimycin) whose structure has been established by X-ray analysis[8,9].

As the chromophores of pluracidomycins and MM4550 are different, the direct comparison of

Table 3. Antibacterial activity of pluracidomycins A, B and C.

Organism	MIC (μg/ml)[a]		
	PLM A	PLM B	PLM C
Staphylococcus aureus 209P JC-1	25	50	25
Staphylococcus aureus C-14[b]	50	50	50
Streptococcus pyogenes C-203	>50	50	50
Escherichia coli NIHJ JC-2	6.3	6.3	12.5
Escherichia coli 377[c]	6.3	6.3	6.3
Klebsiella pneumoniae SRL-1	6.3	12.5	25
Klebsiella sp. 363[b]	6.3	6.3	6.3
Proteus mirabilis PR-4	12.5	25	12.5
Proteus vulgaris CN-329	25	50	50
Enterobacter cloacae 233	12.5	100	>100
Serratia marcescens ATCC 13880	12.5	25	100
Pseudomonas aeruginosa ATCC 25619	>100	>100	>100

[a] Determined by agar dilution method in sensitivity-disc agar and inoculated by one loopful of *ca.* 10^6 cells per ml.

[b] Penicillinase producing strain.

[c] Cephalosporinase producing strain.

Table 4. Inhibition of β-lactamases produced by Gram-negative bacteria by pluracidomycins.

Source of β-lactamase[a]	Class[b]	Minimum effective concentration (μg/ml)[c]		
		PLM A	PLM B	PLM C
Escherichia coli 6	Ib	0.008	0.008	0.004
Enterobacter cloacae 92	Ia	0.001	0.008	0.008
Proteus vulgaris 31	Ic	<0.001	<0.001	<0.001
Escherichia coli W3110 RTEM	IIIa	0.25	0.002	0.001
Klebsiella sp.363	IV	0.008	0.008	0.004
Enterobacter cloacae 53	IVa	0.125	<0.001	<0.001

[a] Enzyme preparations used were partially purified.

[b] Classification of RICHMOND and SYKES.

[c] Inhibitor was incubated with enzyme at room temperature for 10 minutes prior to adding nitrocefin (50 μg/ml) and minimum concentration to inhibit color change was determined.

their COTTON-effects is of no use for the determination of the absolute configuration. Therefore, PLM B was reduced to the corresponding sulfide (8) by $TiCl_3$[10], and the CD spectrum was compared with that of epithienamycin A. Both spectra showed the same COTTON-effects, accordingly, pluracidomycins should have the configuration identical with that of MM4550 as shown in Fig. 3. The sulfoxides of PLM B and C are arranged spatially in the same direction as those of MM4550, carpetimycins and asparenomycins[10], but in the case of PLM C only the sulfoxide should be designated by the *S*-configuration according to the sequence rule.

The biological properties of pluracidomycins are summarized in Tables 3 and 4*. The details will be reported separately.

Acknowledgements

The authors wish to thank to Dr. K. TANAKA for his interest. Thanks are also due to Messrs. S. KOHZUKI, Y. TAKAHASHI, N. UOTANI and T. TANIMOTO for technical assistance.

* The *in vitro* data were obtained by Dr. T. YOSHIDA and his colleague.

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(Received January 14, 1982)

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