Communications to the Editor

PLURACIDOMYCIN A_2, A NEW CARBAPENEM BEARING A SULFINIC ACID, AND OTHER MINOR PLURACIDOMYCINS

Sir:

Streptomyces pluracidomyceticus PA-41746 (FERM BP-174) was found to produce additional β-lactam components besides the previously reported pluracidomycins (PLM) A, B and C[1]. In particular, fermentation at 38°C resulted in an increase of the minor components with a decrease of PLM C, while the yields of PLM A and PLM B remained almost unchanged. Reported here are the isolation and structure elucidation of these minor pluracidomycins.

The pluracidomycins were isolated from the fermentation filtrate as previously reported using phase-transfer extraction, ion-exchange chromatography on QAE-Sephadex and gel filtration of the respective fractions to obtain fractions A, B, C and D (in the order of decreasing acidity).

Fraction A containing the main component PLM A_1 (formerly PLM A), a new minor component PLM A_2, and a considerable amount of PLM B, was separated by column chromatography on MCI GEL CHP20P (Mitsubishi Chem. Ind.) using 15% NaCl solution. Fraction B contained a small amount of PLM A_2 and the second major component PLM B and was separated with a CHP20P column, as was fraction A. The PLM A_2 portions were combined, desalted by gel filtration on Biogel P-2 and freeze-dried.

Fraction C was further fractionated on CHP20P using 10%, 5% and 2% NaCl solutions (stepwise) into fractions C_1, C_2, C_3 and C_4. Fraction C_1 gave PLM C_1 (formerly PLM C). The active principles of fractions C_2 and C_3 were assumed to be new compounds according to HPLC data but those of fraction C_4 were identified as MM 4550 and MM 17880[2]. Fraction C_2 was rechromatographed on CHP20P using 5% NaCl, desalted and freeze-dried. When the minor component was purified by HPLC on Nucleosil ${}_8C_8$ (Macherey-Nagel) with 0.1 M phosphate buffer (pH 7.0), desalted and freeze-dried, a new component PLM C_2 was obtained in extremely poor yield. Further purification of fraction C_3 on CHP20P column using 3% NaCl solution, desalting and freeze-drying gave a new component PLM C_3.

Fraction D had three active components, MM 4550, MM 17880 and a new substance according to the HPLC data. In chromatography with a CHP20P column, the new substance, PLM D, was eluted by 10% NaCl solution, while the two known antibiotics were eluted by water. The PLM D fractions were combined, desalted and freeze-dried.

The yields of the new components, isolated from about 500 liters of filtrate as respective sodium salts, were approximately 40 mg of PLM A_2, 3 mg of PLM C_2, 15 mg of PLM C_3 and 100 mg of PLM D. The ^1H NMR spectra in D_2O of these pluracidomycins are shown in Fig. 1, and the relative mobilities on paper electrophoresis are listed in Table 1.

The ^1H NMR spectrum of PLM A_2 is closely related to that of PLM A_1; some differences were observed in the chemical shifts of the protons at C-4 and C-5, but not in the total proton number and the proton-proton coupling constants. The ^{13}C NMR data (Table 2), in accordance with ^1H NMR, clearly indicated that PLM A_2 has nine carbon atoms as has PLM A_1 and differs from PLM A_1 only in the substituent at C-3. As shown in Table 1, PLM A_2 revealed a mobility equal to that of PLM A_1 and therefore the substituent at C-3 should be an acidic function lacking the carbon atom, like that of PLM A_1. Since the UV absorption ($\lambda_{max}^{H_2O}$ 273 nm) is different from those of PLM A_1, B and C_1[1], the substituent should be a new type of carbapenem antibiotic. Conjecture about its function was based on comparison of the IR spectra (Fig. 2). The spectrum of PLM A_1 has a strong band (1200~1270 cm^{-1}) attributable to the S=O stretching of the $-SO_3^-$ and the $-O-SO_3^-$ functions. In the spectrum of PLM A_2, however, the corresponding band is narrowed due to lack of the absorption at 1200 cm^{-1}, and two new bands appear at 1020~1060 cm^{-1} and at 965 cm^{-1}, which may be assignable to the S=O and S–O stretching of a sulfinate[3]. The molecular formula of PLM A_2 was determined to be

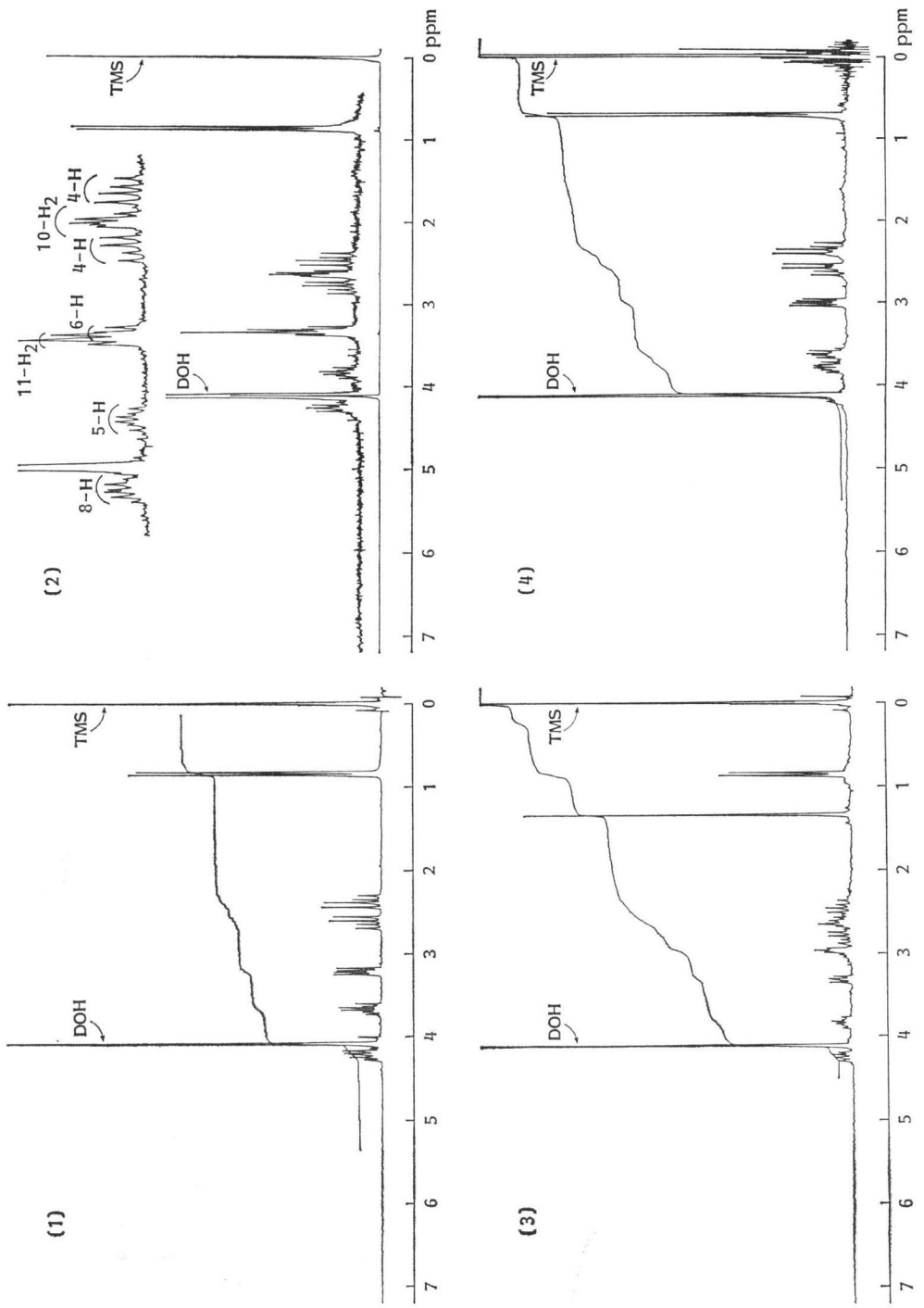


Fig. 1. ^{1}H NMR spectra of pluracidomycins A_2 (1), C_2 (2), C_3 (3) and D (4) at 200 MHz. Chemical shifts are shown in δ (ppm) from external TMS (in D_2O).

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

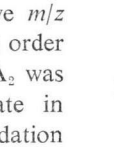
Antibiotic	Relative mobiility
PLM A_2	3.0
PLM C_2	1.9
PLM C_3	1.9
PLM D	2.3
PLM A_1	3.0
Epithienamycin A	1.0

$C_9H_8O_9NS_2Na_3$ by mass spectrometry (SIMS); the MS gave *m/z* 408 $[M+H]^+$ and *m/z* 430 $[M+Na]^+$ whereas that of PLM A_1 gave *m/z* 424 $[M+H]^+$ and *m/z* 446 $[M+Na]^+$. In order to confirm the 3-sulfino structure, PLM A_2 was treated with sodium *m*-chloroperbenzoate in phosphate buffer (pH 7.0). The oxidation proceeded smoothly at room temperature and, as expected, gave PLM A_1 in almost quantitative yield.

A few naturally occurring sulfinic acids related to hypotaurine have been isolated from animal sources; for example, hypotaurocyamine

Table 2. ^{13}C NMR of pluracidomycins in D_2O (δ ppm).

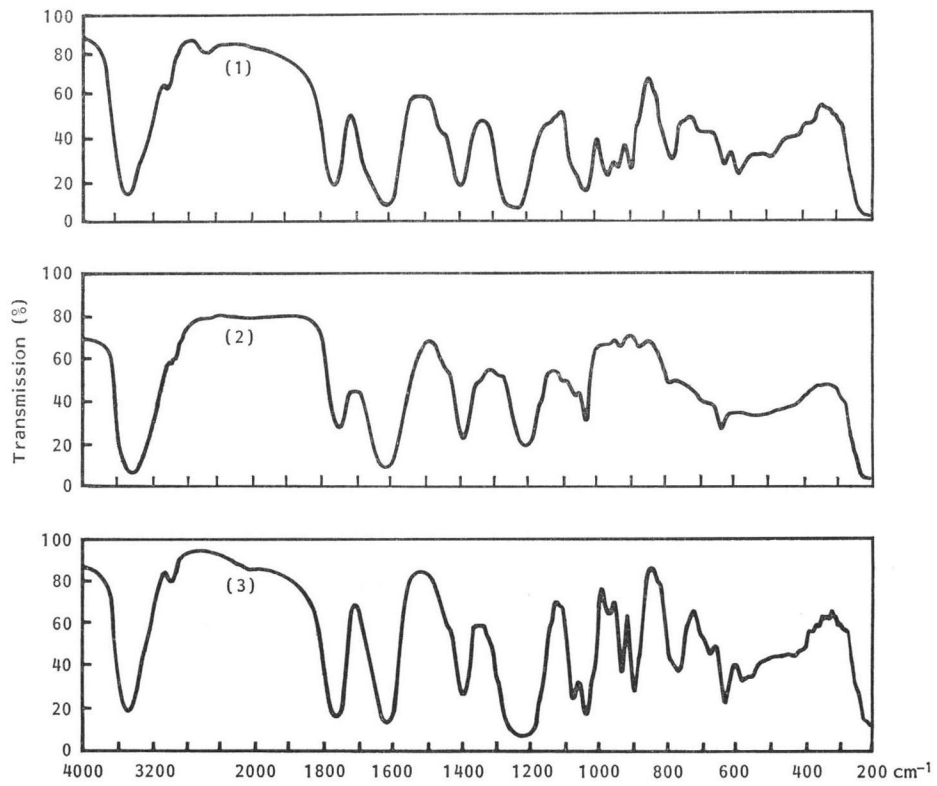
C	PLM A_1	PLM A_2	PLM D
2	139.2 (s)	129 (s)	139.8 (s)
3	129.0 (s)	152.0 (s)	127.7 (s)
4	32.8 (t)	27.0 (t)	32.4 (t)
5	55.1 (d)	54.5 (d)	55.0 (d)
6	58.7 (d)	57.8 (d)	60.4 (d)
7	168.7 (s)	167.4 (s)	169.0 (s)
8	73.7 (d)	73.9 (d)	64.4 (d)
2-COONa	177.9 (s)	178.4 (s)	179.4 (s)
8-CH_3	19.4 (q)	19.4 (q)	21.7 (q)



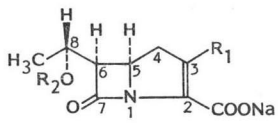
$H_2NCNHCH_2CH_2SOH$ has been isolated from marine annelids[4]. PLM A_2 seems to be the first sulfinic acid identified as a microbial metabolite.

PLM C_2 is presumably a member of the sulfated olivanic acid group bearing a sulfinyl function at C-3 based on its UV ($\lambda_{max}^{H_2O}$ 287 nm) and ^1H

Fig. 2. IR spectra of pluracidomycins A_2(1), D(2) and A_1(3). (KBr).



Pluracidomycin (PLM)



	R_1	R_2
PLM A_2	$S(=O)-ONa$	SO_3Na
PLM C_2	$S(=O)-CH_2CH_2OH$	SO_3Na
PLM C_3	$S(=O)-CH_2CH_2NHAc$	SO_3Na
PLM D	SO_3Na	H
PLM A_1	SO_3Na	SO_3Na
PLM B	$S(=O)-CH_2COONa$	SO_3Na
PLM C_1	$S(=O)-CH(OH)(OH)$	SO_3Na
MM4550	$S(=O)-CH=CHNHAc$	SO_3Na
MM17880	$S-CH_2CH_2NHAc$	SO_3Na

NMR spectra. Its ^1H NMR showed two significant methylene proton signals near 3.26 ppm (2 H, m) and near 3.95 ppm (2 H, m) attributable to $-S(=O)CH_2CH_2X$. The X group should be a hydroxyl group, because of the downfield shift of the signals of one methylene group compared with that of asparenomycin B[5] and the absence of the CH signals due to the X group. Though the sequences of CH were confirmed by spin-spin decoupling experiments, further evidence to confirm the structure could not be obtained due to the limited amount of this component. PLM C_2 seems to be a precursor of PLM B.

PLM C_3 was easily deduced to be the sulfoxide of MM 17880 by comparison of its ^1H NMR with those of MM 17880 and asparenomycin B. The downfield shift of the proton at C-5 (slightly) and of SCH_2 (remarkably) clearly shows the sulfinyl structure of the side-chain[5]. The molecular formula was confirmed to be $C_{13}H_{16}O_9N_2S_2Na_2$ by SIMS: m/z 455 $[M+H]^+$, 477 $[M+Na]^+$. Also, the sulfinyl function was easily reduced by titanium trichloride in acetate buffer[5], and the product was identified as MM 17880 by HPLC.

PLM D has a chromophore (UV $\lambda_{max}^{H_2O}$ 232, 267 nm) similar to that of PLM A_1 but its paper electrophoresis suggested that presence of only two acidic functions (Table 1). Comparison of the ^1H and ^{13}C NMR of PLM D and PLM A_1 indicated that PLM D is a compound corresponding to the desulfated PLM A_1 at C-8. The IR spectrum of PLM D, in agreement with the above assumption, lacked the absorption bands at 1250 cm^{-1}, 935 cm^{-1} and 895 cm^{-1} which are commonly observed in the spectra of sulfated olivanic acids. The mass spectrum (SIMS) supported the molecular formula, $C_9H_9O_7NSNa_2$; m/z 322 $[M+H]^+$, 344 $[M+Na]^+$.

The carbapenem antibiotics are generally labile in aqueous solutions, however, PLM D is fairly stable against hydrolysis at neutral condition. The stability seems to be comparable to that of C-19393 H_2[6] (carpetimycin B).

These minor pluracidomycins also showed broad antibacterial and β-lactamase inhibitory activities, as will be reported elsewhere.

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