O-DEMETHYLPAULOMYCINS A AND B, U-77,802 AND U-77,803, PAULOMENOLS A AND B, NEW METABOLITES PRODUCED BY *STREPTOMYCES PAULUS*

A. D. ARGOUDELIS,† L. BACZYNSKYJ, S. A. MIZSAK and F. B. SHILLIDAY

Research Laboratories, The Upjohn Company,
Kalamazoo, Michigan 49001, U.S.A.

(Received for publication March 28, 1988)

O-Demethylpaulomycin A ($C_{33}H_{44}N_2O_{17}S$), *O*-demethylpaulomycin B ($C_{32}H_{42}N_2O_{17}S$), paulomenol A ($C_{29}H_{43}NO_{16}$), paulomenol B ($C_{28}H_{41}NO_{16}$), and the hydrogen sulfide adducts of paulomycin A (U-77,802, $C_{34}H_{48}N_2O_{17}S_2$), and paulomycin B (U-77,803, $C_{33}H_{46}N_2O_{17}S_2$) have been isolated from fermentations of *Streptomyces paulus* strain 273. The structure of these compounds was determined by ^{1}H and ^{13}C NMR and fast atom bombardment mass spectrum spectroscopic techniques and degradative studies. The antibacterial properties of these new metabolites, which are related to paulomycins A and B (J. Antibiotics 35: 285~294, 1982), are briefly discussed.

Over the last 5 years, we have reported the production of the paulomycin complex by *Streptomyces paulus* strain 273 and the isolation of paulomycins A and B,[1] the production and isolation of paldimycins A and B, and antibiotics 273a$_{2\alpha}$ and 273a$_{2\beta}$,[2] and the isolation and characterization of paulomycins A$_2$, C, D, E, and F.[3] This paper describes the isolation, characterization, and structure of six additional metabolites produced by *S. paulus*. These compounds, which are related to paulomycins A and B, have been designated *O*-demethylpaulomycin A, *O*-demethylpaulomycin B, paulomenol A, paulomenol B, U-77,802, and U-77,803. The biological properties of these new paulomycin-like materials are also briefly discussed.

Experimental

Assay and Testing Procedures

Antibiotic production and purification was measured by a microbiological disc-plate assay procedure with *Micrococcus luteus* as the assay organism.

TLC Procedures

The production of paulomycins and related metabolites was followed by TLC on Silica gel G or Silica gel GF plates (Analtech Inc.) using chloroform - ethanol - water (25 : 30 : 5) or chloroform - methanol (90 : 10) as the solvent system. Paulomycins were separated by TLC using Brinkman's cellulose-coated plates and pH 7.0 phosphate buffer as the solvent system. Bioactive materials were detected by bioautography on *M. luteus*-seeded trays. Bioinactive compounds were detected by a short wavelength UV lamp.

Spectroscopic Methods

UV spectra were obtained in methanol using a Perkin-Elmer Lambda 7 spectrophotometer. IR spectra were run in Nujol mull using a Digilab FTS-ISE spectrometer. ^{1}H NMR spectra were recorded on a Varian XL-200 spectrometer operating at 200 MHz. Solutions (*ca.* 0.4 ml, *ca.* 0.25 M) of the compounds in deuterated chloroform or acetone were used. ^{13}C NMR spectra were recorded on a Varian CFT-20 spectrometer operating at 20.0 MHz. ^{1}H and ^{13}C NMR chemical shifts are

† Dr. A. D. ARGOUDELIS died on July 19, 1988.

reported as ppm relative to TMS. Negative and positive ion mass spectra were obtained on a V.G. Analytical ZAB-2F high resolution mass spectrometer using a fast atom bombardment (FAB) source. Bombarding gas, xenon, accelerating voltage 7.0 KeV, matrix, tetraethylene glycol for negative ion mass spectra and polyethylene glycol (PEG-400) (Aldrich Chemical Co., Milwaukee, Wisconsin, U.S.A.) for the positive ion mass spectra.

Analytical HPLC

All HPLC chromatography was carried out with a Varian Model 5560 (Varian Instruments, Sugar Land, Texas) instrument equipped with a LKB Rapid Spectral Detector (LKB, Broma, Sweden). A Zorbax C-8 25 cm×4.6 mm stainless-steel column packed with C_8 (6 μ) reverse-phase silica was used. In most cases, the mobile phase consisted of a gradient of solvent A to solvent B; flow rate 1.5 ml/minute. Solvent A consisted of 0.05 M phosphate buffer, pH 5.5 - acetonitrile - THF (80 : 20 : 20). Solvent B consisted of 0.05 M phosphate buffer pH 5.5 - acetonitrile - THF (40 : 30 : 30). A 10-μl sample was usually injected.

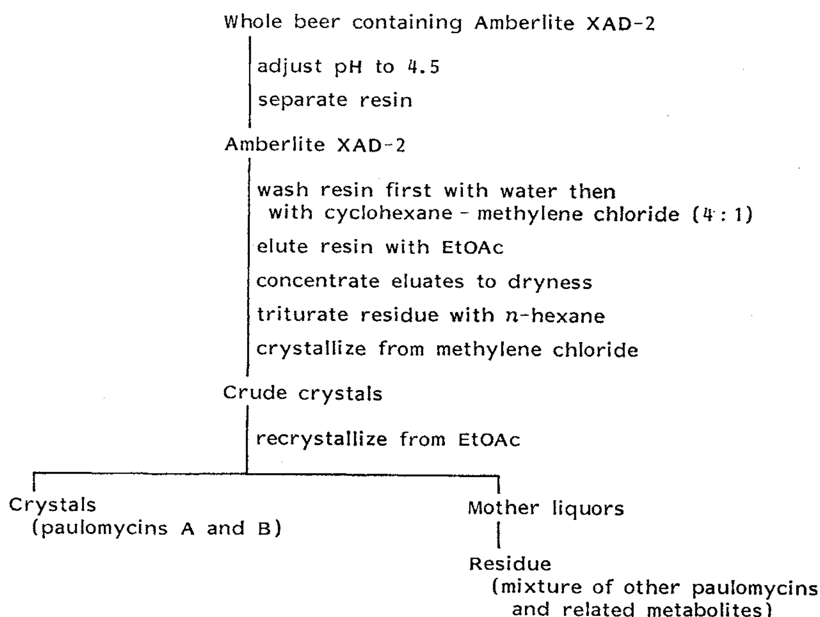
Fermentation Conditions

The fermentation conditions, used for the production of the paulomycin complex by *S. paulus* strain 273 (UC 5142), were identical to those described by MARSHALL *et al.*[4] Amberlite XAD-2 resin (150 liters per 5,000-liter fermentation) was added into the fermentor. Bioassays indicated that at harvest time (120 hours after inoculation) most of the bioactive components produced by *S. paulus* were adsorbed on the resin.

Isolation of Paulomycin-related Metabolites Produced by *S. paulus*

Crude crystals of paulomycin (mixture of all paulomycin-related metabolites) were isolated from a 5,000-liter fermentation by the procedure described by ARGOUDELIS *et al.*,[3] outlined in Scheme 1. The mother liquors concentrated to dryness yielded a residue containing, in addition to paulomycins A and B, paulomycins A_2, C, D, E, F, *O*-demethylpaulomycins A and B, U-77,802 and U-77,803, and paulomenols A and B. This residue was used as the starting material for the isolation of the six metabolites which are the subject of this paper.

Scheme 1. Isolation of paulomycin-related metabolites from fermentations of *Streptomyces paulus.*



Separation of "Polar Paulomycins"† from Paulomycins A, A_2 and B – Preparative HPLC

The procedure described by ARGOUDELIS *et al.*[3] was followed. The residue, obtained as described above, was purified by HPLC over Waters C-18 reverse-phase silica gel-packed columns using a Waters Prep 500A instrument and acetonitrile - pH 5.5, 0.01 M phosphate buffer (1 : 1). A total of 108 g of a mixture of "polar paulomycins" was obtained from purification of 480 g of the crude paulomycin mixture.

Separations of "Polar Paulomycins" – Preparative HPLC

The procedure reported by ARGOUDELIS *et al.*[3] was followed. The "polar paulomycin" mixture was purified by preparative HPLC over Waters C-18 reverse phase silica gel-packed columns using a Waters Prep 500A instrument and acetonitrile - pH 5.5, 0.1 M phosphate buffer (45 : 55). Four pools were made which, after extraction with methylene chloride and evaporation to dryness, gave four preparations designated purified paulomycin C (13.5 g), purified paulomycin D (38.1 g), purified paulomycin E (28.8 g), and purified paulomycin F (4.98 g). Analytical HPLC and TLC indicated that the purified C fraction contained *ca.* 80% of *O*-demethylpaulomycin A, the D fraction contained both *O*-demethylpaulomycin B and U-77,802, the E contained U-77,803, and the F fraction contained paulomenols A and B.

Isolation of Pure *O*-Demethylpaulomycin A, *O*-Demethylpaulomycin B, U-77,802, U-77,803, Paulomenol A, and Paulomenol B

Pure *O*-demethylpaulomycins A and B, U-77,802 and U-77,803 and paulomenols A and B were obtained by repeated HPLC's using conditions identical to those described by ARGOUDELIS *et al.*[3] with the exception that the following starting materials and mobile phases were used.

1) For Isolation of Pure *O*-Demethylpaulomycin A: Starting material, preparation designated purified paulomycin C; mobile phase, acetonitrile - pH 5.5, 0.1 M phosphate buffer (40 : 60).

2) For Isolation of Pure *O*-Demethylpaulomycin B: Starting material, purified preparation D (see above); mobile phase THF - acetonitrile - pH 5.5, 0.1 M phosphate buffer (3 : 1 : 1).

3) For Isolation of Pure U-77,802: Starting material, purified preparation D; mobile phase acetonitrile - pH 5.5, 0.1 M phosphate buffer (40 : 60).

4) For Isolation of Pure U-77,803: Starting material, purified preparation E; mobile phase, gradient from acetonitrile - pH 7.0, 0.02 M ammonium acetate (30 : 70) to acetonitrile - pH 7.0, 0.02 M ammonium acetate (70 : 30).

5) For Isolation of Pure Paulomenols A and B: Starting material, preparation designated purified F (see above); mobile phase, THF - pH 5.5, 0.05 M phosphate buffer (35 : 65).

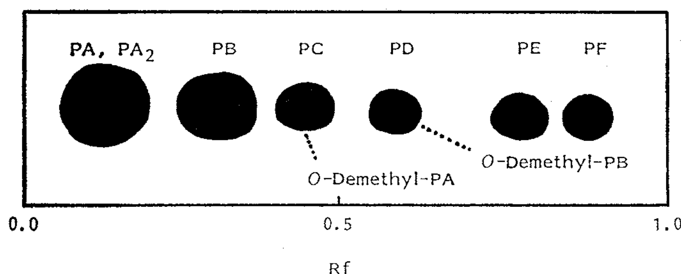
Fractions obtained during the chromatographies were examined by analytical HPLC, UV, and bioactivity. Appropriate pools were made and these solutions were extracted with 15~20% of their volume of methylene chloride. The extracts were dried over sodium sulfate and concentrated to dryness. The residues were dissolved in acetone and the solutions were mixed with cyclohexane. Precipitated pure *O*-demethylpaulomycins A and B, U-77,802, U-77,803, and paulomenols A and B were isolated by filtration and dried. Characterization of these materials is described in the following section (Results and Discussion).

Results and Discussion

As reported earlier,[1,3] *S. paulus* strain 273 produces paulomycin, a mixture of chemically-related metabolites, when grown either on agar or in a submerged culture. The isolation of the paulomycin mixture was followed with bioassays and TLC on silica gel plates using chloroform - ethanol - water (25 : 30 : 5) as the mobile phase. The paulomycin complex appeared as one zone of bioactivity (Rf ~0.8) when TLC's were developed by bioautography on *M. luteus*. However, the paulomycin com-

† The term "polar paulomycins" has been used to designate collectively all the new paulomycins and paulomycin-related metabolites with HPLC retention times (RT) lower than the RT of paulomycin B. Paulomycin A_2, which has RT higher than that of paulomycin B, is not included in this group.

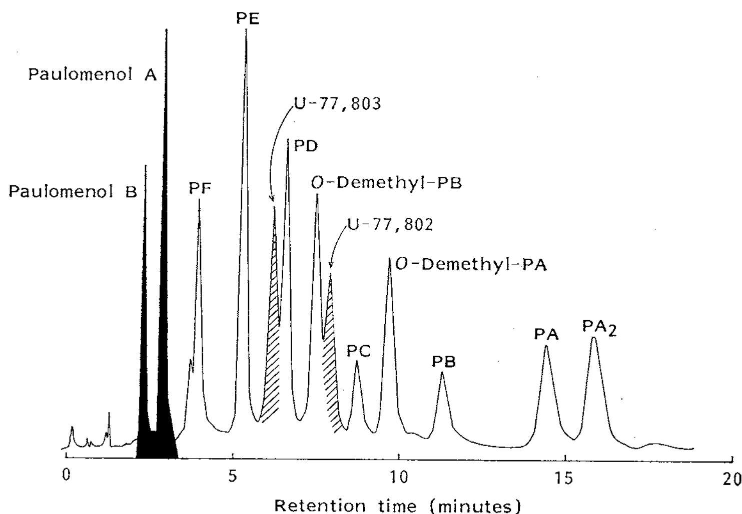
Fig. 1. TLC[a] of paulomycins A, A_2, B, C, D, E, F,[b] and *O*-demethylpaulomycins A and B.



[a] Cellulose (Polygram CEL 300) pre-coated sheets; pH 7.0, 0.1 M phosphate buffer. Antibiotics were detected by bioautography on *Micrococcus luteus*-seeded agar.

[b] PA, PA_2, PB, PC, PD, PE, and PF refer to paulomycins A, A_2, B, C, D, E, and F, respectively.

Fig. 2. HPLC of metabolites produced by *Streptomyces paulus*.



Conditions: Zorbax C-18, 4.1×25 cm reverse-phase silica column. Mobile phase, water - THF - acetonitrile (3 : 1 : 1) containing 0.2% glacial acetic acid. Detection at 320 nm.

PA, PA_2, PB, PC, PD, PE, and PF refer to paulomycins A, A_2, B, C, D, E, and F, respectively.

ponents were separated by TLC on cellulose sheets. Six zones of bioactivity were detected and designated as paulomycin A (Rf 0.14), paulomycin B (Rf 0.32), and paulomycin C (Rf 0.43), paulomycin D (Rf 0.58), paulomycin E (Rf 0.77), and paulomycin F (Rf 0.88) (Fig. 1).

HPLC analysis of preparations containing, by TLC, paulomycin C as the only bioactive component indicated that the C-preparations contained an additional bioactive material which was later designated *O*-demethylpaulomycin A. Similarly, preparations containing paulomycin D as the only antibiotic, also contained another bioactive component designated *O*-demethylpaulomycin B.

TLC using a short wave UV lamp for detection of the spots indicated the presence of additional metabolites in the different paulomycin preparations; four of which: Paulomenols A and B, U-77,802, and U-77,803 are discussed later in this report.

Isolation of *O*-Demethylpaulomycins A and B, Paulomenols A and B, U-77,802, and U-77,803

The fermentations, run according to procedures developed by MARSHALL and his coworkers,[4] contained Amberlite XAD-2 (*ca.* 150 liters per 5,000-liter fermentor). Under these conditions, most of the bioactivities produced by the culture were absorbed on the resin. At harvest time, 120 hours after inoculation, the whole beer was adjusted to pH 4.5 and passed over a large vibrating screen in order to isolate the resin free of mycelia. The resin was washed first with water then with cyclohexane - methylene chloride (4 : 1) and was eluted with ethyl acetate. The eluates were concentrated to dryness to yield a residue, which after trituration with *n*-hexane, crystallized from methylene chloride. The crude crystals obtained, recrystallized from ethyl acetate, yielded colorless crystals containing mainly paulomycins A and B. The mother liquors gave a residue, which as shown in Fig. 2 was enriched in the minor components of the paulomycin complex and also contained several paulomycin-related metabolites.

Fig. 3. UV spectra of *O*-demethylpaulomycins (A), U-77,802 or U-77,803 (B), and paulomenol A or B (C).

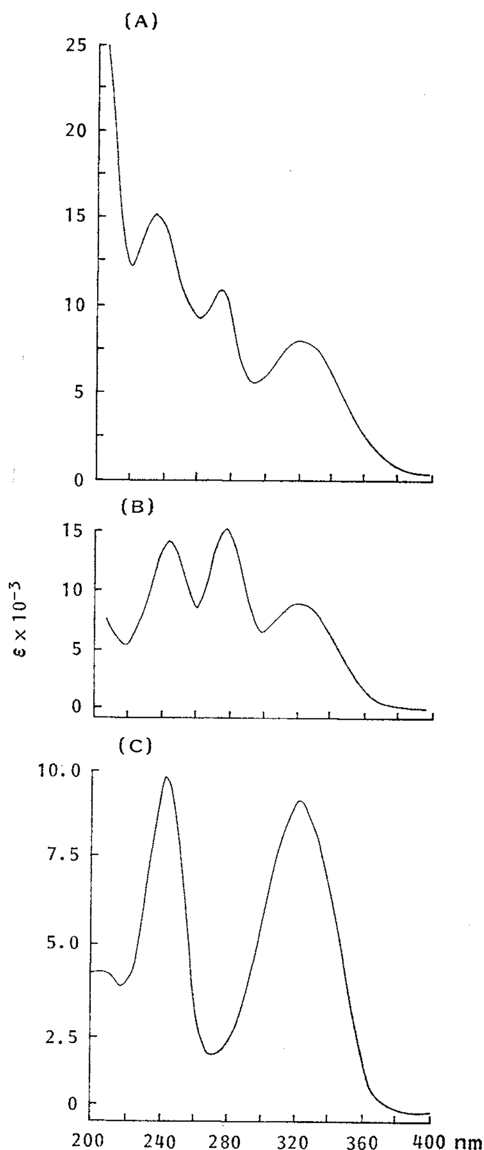


Fig. 2 presents an analytical HPLC of the residue obtained from the mother liquors. The chromatogram was run isocratically using water - THF - acetonitrile (3 : 1 : 1) containing 0.25% acetic acid, as the mobile phase. Of the thirteen peaks observed in the chromatogram, nine had UV spectra identical to those of paulomycins A and B. Seven of these peaks were identified as due to paulomycins A_2, A, B, C, D, E, and F.[3] The remaining two peaks were later designated as *O*-demethylpaulomycins A and B. The compounds responsible for the shaded peaks have identical UV spectra, different from those of the paulomycins and are known as U-77,802 and U-77,803. The two solid peaks are due to two paulomycin-related metabolites, named paulomenols A and B, which also have identical UV spectra, different from the rest of the eleven compounds shown in the chromatogram. Representative UV spectra of the three classes of compounds are presented in Fig. 3.

All these compounds were isolated pure by repeated preparative HPLC using systems containing acetonitrile - THF - water - acetic acid or acetonitrile - THF - pH 5.5, phosphate buffer in ratios

depending on the retention times of the components to be separated.

Characterization and Structures of *O*-Demethylpaulomycins A and B, U-77,802, U-77,803, and Paulomenols A and B

O-Demethylpaulomycins A and B

O-Demethylpaulomycins A and B were isolated as colorless crystalline compounds readily soluble in chloroform, methylene chloride, lower alcohols, ethyl and butyl acetates, acetone, and most other organic solvents. The antibiotics are less soluble in ether and water and quite insoluble in saturated hydrocarbon solvents.

The physical and chemical properties of all six compounds under consideration are listed in Table 1. All of them met with decomposition over a broad range of temperature; they, like paulomycins A, A_2, B, C, D, E, and F,[3] are dextrorotatory when the specific rotation was determined in chloroform and levorotatory when methanol was used as the solvent. The molecular formulas and molecular weights were determined by negative ion high resolution mass spectrometry (HR-MS). The IR spectra of *O*-demethylpaulomycins A and B, like those of paulomycins A and B, are nearly identical and are characterized by a strong absorption band at 2040 cm^{-1} attributable to an isothiocyanate group.[1] The IR spectra (Fig. 4) which show the presence of carbonyl groups at *ca.* 1735 and 1700 cm^{-1} can not be used for differentiation of these two antibiotics from paulomycins A, A_2, B, C, D, E, and F. The UV spectra of *O*-demethylpaulomycins A and B are identical to the UV spectra of paulomycins A and B[1] and contain three peaks at *ca.* 236, 276, and 321 nm. The maximum at 276 and 321 nm indicate the presence of the same chromophoric systems in *O*-demethylpaulomycins as those in paulomycins A and B, *i.e.* ring A and the isothiocyanate-containing acid known as paulic acid[3] (Fig. 5). This conclusion agrees with the data obtained by FAB-MS. The negative ion FAB-MS of *O*-demethylpaulomycins A and B have fragmentation patterns in the low mass area (m/z <360) identical to that of paulomycins A and B[3] indicating that moiety **9** is a common feature in *O*-demethylpaulomycins and paulomycins.

Table 1. Physical and chemical properties of *O*-demethylpaulomycins A and B, U-77,802, U-77,803 and paulomenols A and B.

	O-Demethyl-paulomycin A	*O*-Demethyl-paulomycin B	U-77,802	U-77,803	Paulomenol A	Paulomenol B
Molecular formula[a]	$C_{33}H_{44}N_2O_{17}S$	$C_{32}H_{42}N_2O_{17}S$	$C_{34}H_{48}N_2O_{17}S_2$	$C_{33}H_{46}N_2O_{17}S_2$	$C_{29}H_{43}NO_{16}$	$C_{28}H_{41}NO_{16}$
MW[a]						
Found:	772.2367	758.2188	820.2394	806.2238	661.2595	647.2418
Calcd:	772.2360	758.2204	820.2385	806.2231	661.2582	647.2425
MP	Melt over broad range whith decomposition	Melt over broad range whith decomposition	Melt over broad range whith decomposition	Melt over broad range whith decomposition	Melt over broad range whith decomposition	Melt over broad range whith decomposition
$[\alpha]_D^{25}$ ($CHCl_3$)	+4° (*c* 0.42)	+2° (*c* 0.97)	+5° (*c* 0.74)	+6° (*c* 0.86)	+2° (*c* 0.67)	+3° (*c* 0.20)
$[\alpha]_D^{25}$ (CH_3OH)	−28° (*c* 0.41)	−25° (*c* 0.91)	−12° (*c* 1.04)	−22° (*c* 0.69)	−29° (*c* 0.59)	−26° (*c* 0.15)
UV λ_{max}^{MeOH} (ε)	236 (14,500), 276 (10,200), 322 (8,200)	237 (15,000), 276 (10,800), 320 (8,730)	245 (11,545), 278 (12,382), 322 (7,384)	244 (13,744), 278 (13,827), 322 (8,424)	242 (9,850), 321 (9,254)	243 (10,157), 322 (9,446)
IR (Nujol) cm^{-1}	3463, 3236, 2921, 2040, 1735, 1701	3465, 3242, 2922, 2040, 1735, 1700	3354, 3233, 2870, 1735, 1700, 1625	3354, 3233, 2870, 1736, 1700, 1626	3348, 3240, 1735, 1700, 1625	3340, 3234, 1740, 1700, 1625

[a] By high resolution negative ion FAB-MS.

Fig. 4. IR spectra of *O*-demethylpaulomycin A (A), U-77,802 (B), and paulomenol A (C) (in Nujol mull).

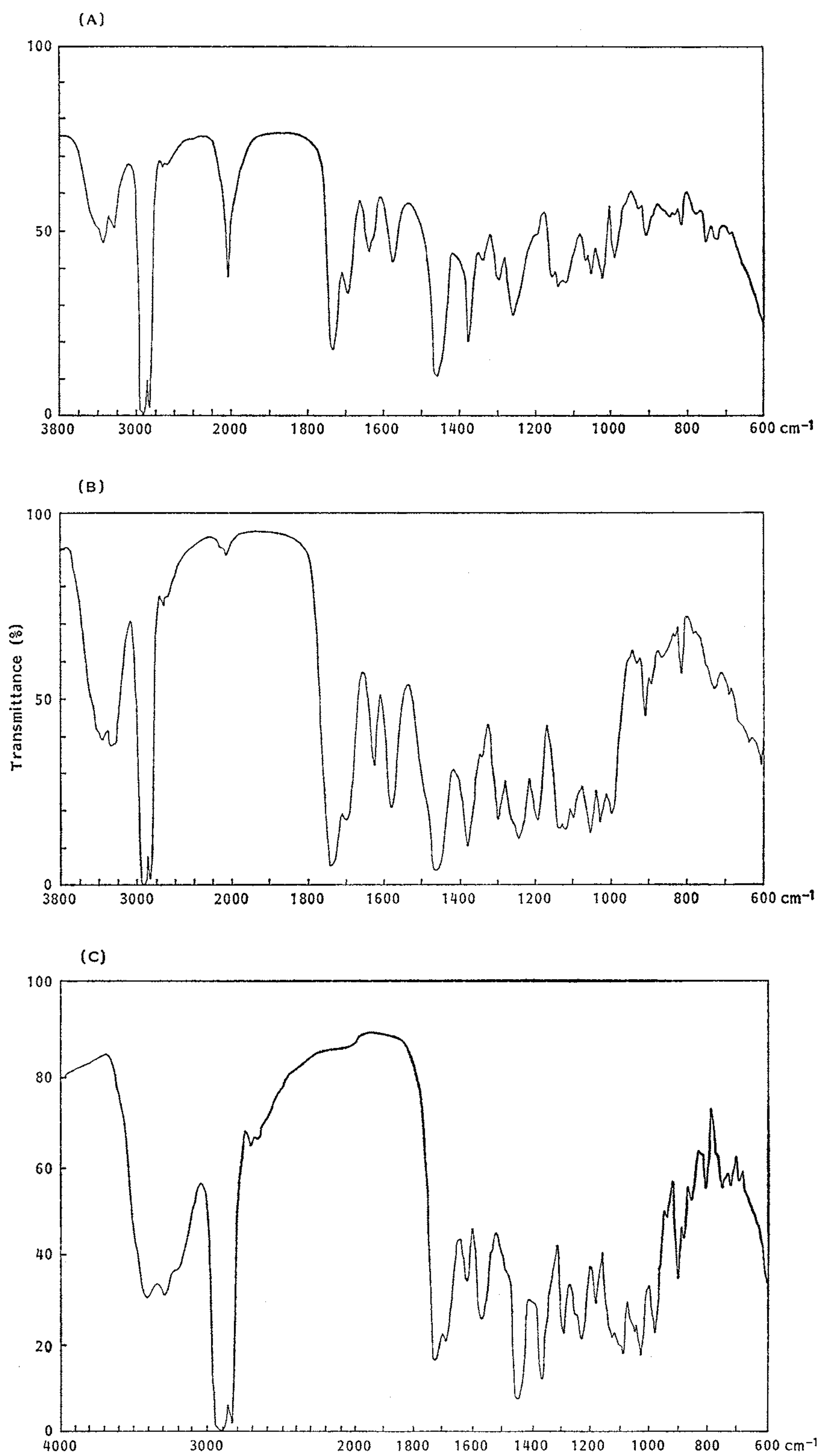
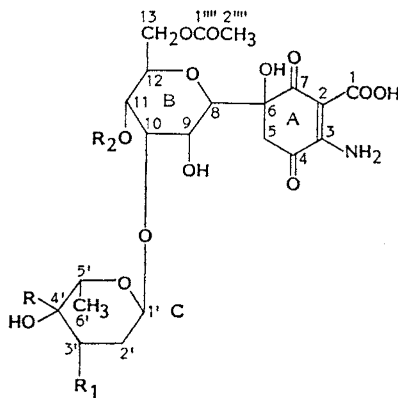


Fig. 5. Structures of **1**~**8**.



Compound	R	R_1	R_2
Paulomycin A (**1**)	$R = CH_3CH_2CH(CH_3)COOCH(CH_3)-$ (4''', 3''', 2''', 1''', 5''', 7', 8')	$R_1 = OCH_3$	$R_2 = CH_3CH{=}C(N{=}C{=}S)-CO-$ (4'', 3'', 2'', 1'', 5'')
Paulomycin B (**2**)	$R = CH_3CH(CH_3)COOCH(CH_3)-$ (3''', 2''', 1''', 4''', 7', 8')	$R_1 = OCH_3$	
O-Demethylpaulomycin A (**3**)	$R = CH_3CH_2CH(CH_3)COOCH(CH_3)-$ (4''', 3''', 2''', 1''', 5''', 7', 8')	$R_1 = OH$	
O-Demethylpaulomycin B (**4**)	$R = CH_3CH(CH_3)COOCH(CH_3)-$ (3''', 2''', 1''', 4''', 7', 8')	$R_1 = OH$	
U-77,802 (**5**)	$R = CH_3CH_2CH(CH_3)COOCH(CH_3)-$ (4''', 3''', 2''', 1''', 5''', 7', 8')	$R_1 = OCH_3$	$R_2 =$ 4" CH_3–3"CH–2"CH–1"CO– (ring: 3"–S–5"C(=S)–NH–2")
U-77,803 (**6**)	$R = CH_3CH(CH_3)COOCH(CH_3)-$ (3''', 2''', 1''', 4''', 7', 8')	$R_1 = OCH_3$	
Paulomenol A (**7**)	$R = CH_3CH_2CH(CH_3)COOCH(CH_3)-$ (4''', 3''', 2''', 1''', 5''', 7', 8')	$R_1 = OCH_3$	$R_2 = H$
Paulomenol B (**8**)	$R = CH_3CH(CH_3)COOCH(CH_3)-$ (3''', 2''', 1''', 4''', 7', 8')	$R_1 = OCH_3$	

The structural differences between paulomycins and *O*-demethylpaulomycins are located in the esterified paulomycose part of the paulomycin molecule. ^{1}H NMR spectra of *O*-demethylpaulomycins A and B were identical to those of paulomycins A and B with the exception that the singlet at δ 3.33 (3H) assigned to the OCH_3 group of paulomycose in paulomycins A and B[1] is not present in the ^{1}H NMR spectra of *O*-demethylpaulomycins A and B. Furthermore, ^{13}C NMR spectra of *O*-demethylpaulomycins A and B (Table 2) are also characterized by the absence of the absorption at δ 56.62 (q)[1] which has been assigned to the OCH_3 present in paulomycose.

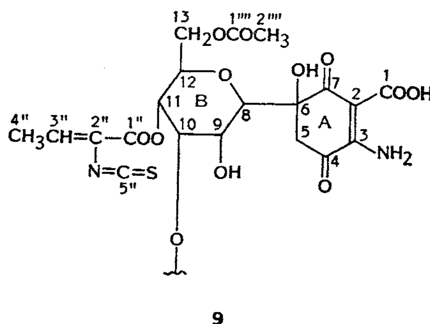


Fig. 6. Positive ion FAB-MS of paulomycin A.

Table 2. Chemical shifts observed in the ^{13}C NMR spectra of paulomycins A and B, *O*-demethylpaulomycins A and B, U-77,802, U-77,803, and paulomenols A and B.

Carbon No.[a]	Chemical shift, δ[b] (multiplicity)							
	Paulomycin A	*O*-Demethyl-paulomycin A	Paulomycin B	*O*-Demethyl-paulomycin B	U-77,802	U-77,803	Paulomenol A	Paulomenol B
1	169.35 (s)	169.83 (s)	169.35 (s)	169.06 (s)	169.10 (s)	169.69 (s)	170.01 (s)	170.06 (s)
2	100.72 (s)	100.42 (s)	100.14 (s)	99.85 (s)	100.42 (s)	100.33 (s)	100.53 (s)	100.56 (s)
3	159.37 (s)	159.89 (s)	159.36 (s)	158.01 (s)	159.76 (s)	159.71 (s)	160.01 (s)	160.05 (s)
4	198.50 (s)	198.82 (s)	198.37 (s)	197.51 (s)	198.81 (s)	198.74 (s)	197.60 (s)	197.45 (s)
5	48.01 (t)	48.45 (t)	47.95 (t)	47.64 (t)	48.47 (t)	48.32 (t)	48.76 (t)	48.76 (t)
6	78.20 (s)	78.63 (s)	78.15 (s)	78.35 (s)	78.72 (s)	78.63 (s)	78.98 (s)	78.94 (s)
7	188.39 (s)	188.96 (s)	188.40 (s)	187.51 (s)	188.82 (s)	188.81 (s)	188.66 (s)	188.72 (s)
8	78.26 (d)	78.71 (d)	78.14 (d)	77.67 (d)	78.51 (d)	78.54 (d)	78.98 (d)	78.94 (s)
9	69.20 (d)	69.86 (d)	69.28 (d)	68.97 (d)	70.49 (d)	70.46 (d)	68.58 (d)	68.45 (d)
10	76.18 (d)	75.92 (d)	75.91 (d)	76.25 (d)	76.58 (d)	76.27 (d)	81.37 (d)	81.07 (d)
11	70.73 (d)	71.40 (d)	70.71 (d)	69.69 (d)	71.06 (d)	71.04 (d)	70.34 (d)	70.37 (d)
12	72.29 (d)	72.12 (d)	72.21 (d)	72.27 (d)	72.74 (d)	72.58 (d)	75.72 (d)	75.68 (d)
13	62.30 (t)	62.76 (t)	62.23 (t)	61.68 (t)	62.80 (t)	62.71 (t)	64.04 (t)	64.07 (t)
1′	99.04 (d)	99.24 (d)	98.94 (d)	99.07 (d)	99.74 (d)	99.65 (d)	100.30 (d)	100.19 (d)
2′	30.56 (t)	31.58 (t)	30.33 (t)	33.30 (t)	31.18 (t)	31.06 (t)	30.91 (t)	30.92 (t)
3′	74.43 (d)	72.73 (d)	74.38 (d)	72.93 (d)	74.89 (d)	74.85 (d)	75.02 (d)	75.08 (d)
4′	73.62 (s)	74.32 (s)	73.66 (s)	73.49 (s)	74.03 (s)	74.06 (s)	74.23 (s)	74.31 (s)
5′	67.18 (d)	65.25 (d)	67.65 (d)	64.53 (d)	68.05 (d)	67.88 (d)	68.24 (d)	68.31 (d)
6′	15.28 (q)[c]	15.23 (q)[c]	15.23 (q)[c]	15.54 (q)[c]	15.76 (q)[c]	15.63 (q)[c]	15.99 (q)[c]	15.92 (q)[c]
7′	69.93 (d)	67.70 (d)	69.96 (d)	67.40 (d)	70.82 (d)	70.63 (d)	70.59 (d)	70.71 (d)
8′	15.39 (q)[c]	15.87 (q)[c]	15.44 (q)[c]	14.63 (q)[c]	15.94 (q)[c]	15.90 (q)[c]	15.98 (q)[c]	15.93 (q)[c]
OCH_3	56.62 (q)	—	56.59 (q)	—	57.10 (q)	56.95 (q)	57.26 (q)	57.24 (q)
1″	160.25 (s)	160.68 (s)	160.25 (s)	160.23 (s)	171.08 (s)	170.92 (s)	—	—
2″	123.36 (s)	123.85 (s)	123.32 (s)	122.91 (s)	69.77 (d)	69.67 (d)	—	—
3″	136.64 (d)	137.19 (d)	136.66 (d)	136.32 (d)	48.27 (d)	48.14 (d)	—	—
4″	14.11 (q)	14.74 (q)	14.13 (q)	14.31 (q)	22.41 (q)	22.39 (q)	—	—
5″	142.64 (s)	143.29 (s)	142.54 (s)	143.31 (s)	200.41 (s)	200.27 (s)	—	—
1‴	175.15 (s)	175.85 (s)	175.71 (s)	176.16 (s)	175.81 (s)	176.30 (s)	175.67 (s)	175.70 (s)
2‴	41.51 (d)	41.67 (d)	34.15 (d)	34.12 (d)	41.98 (d)	34.54 (d)	42.15 (d)	34.85 (d)
3‴	26.65 (t)	27.18 (t)	18.77 (q)[d]	18.79 (q)[d]	26.91 (t)	19.20 (q)[d]	27.32 (t)	19.39 (q)[d]
4‴	11.39 (q)	11.82 (q)	18.93 (q)[d]	18.91 (q)[d]	11.92 (q)	19.35 (q)[d]	11.87 (q)	19.59 (q)[d]
5‴	16.73 (q)	17.29 (q)	—	—	17.29 (q)	—	15.99 (q)	—
1⁗	170.18 (s)	170.96 (s)	170.18 (s)	170.74 (s)	169.89 (s)	169.80 (s)	171.15 (s)	171.19 (s)
2⁗	19.99 (q)	20.56 (q)	19.98 (q)	20.61 (q)	20.60 (q)	20.45 (q)	20.73 (q)	20.75 (q)

[a] For numbering of carbons, see Fig. 5.

[b] Relative to TMS (solvents used; acetone-d_6 for paulomycin A, *O*-demethylpaulomycin A, paulomycin B, U-77,802, U-77,803, paulomenol A, and paulomenol B. $CDCl_3$ was used for *O*-demethylpaulomycin B).

[c] or [d] Assignments are interchangeable.

Table 3. Positive ion FAB HR data of paulomycin A (I)[a].

m/z	Measured mass	Theory	For	Δ (mmu)	Assignments[a]
197	197.1161	197.1177	$C_{11}H_{17}O_3$	−1.6	Part of paulomycose (ring C)
241	241.1440	241.1440	$C_{13}H_{21}O_4$	0.0	II
273	273.1695	273.1702	$C_{14}H_{25}O_5$	−0.7	III
354	354.0820	354.0825	$C_{15}H_{16}NO_9$	−0.5	IV−H_2O
372	372.0923	372.0931	$C_{15}H_{18}NO_{10}$	−0.8	IV
473	473.0866	473.0866	$C_{18}H_{21}N_2O_{11}S$	−1.5	V−CH_2=C=O
497	497.0862	497.0866	$C_{20}H_{21}N_2O_{11}S$	−0.4	V−H_2O
515	515.0977	515.0972	$C_{20}H_{23}N_2O_{12}S$	+0.5	V

[a] For structural assignments, see Fig. 7.

The structure of the two new paulomycins is, therefore, represented by **3** and **4**.

The data presented are sufficient to establish the structures of *O*-demethylpaulomycins A and B. However, we would like to discuss briefly the positive ion FAB-MS of paulomycin A and *O*-demethylpaulomycins A and B. These spectra, in contrast to the negative ion FAB-MS discussed in detail in a previous publication,[3] are of no help in obtaining molecular weights and molecular formulas. However, the positive ion FAB-MS gives valuable information about the substituents in the paulomycose part of paulomycins A, A_2, B, C, D, E, and F[3] or *O*-demethylpaulomycins A and B.

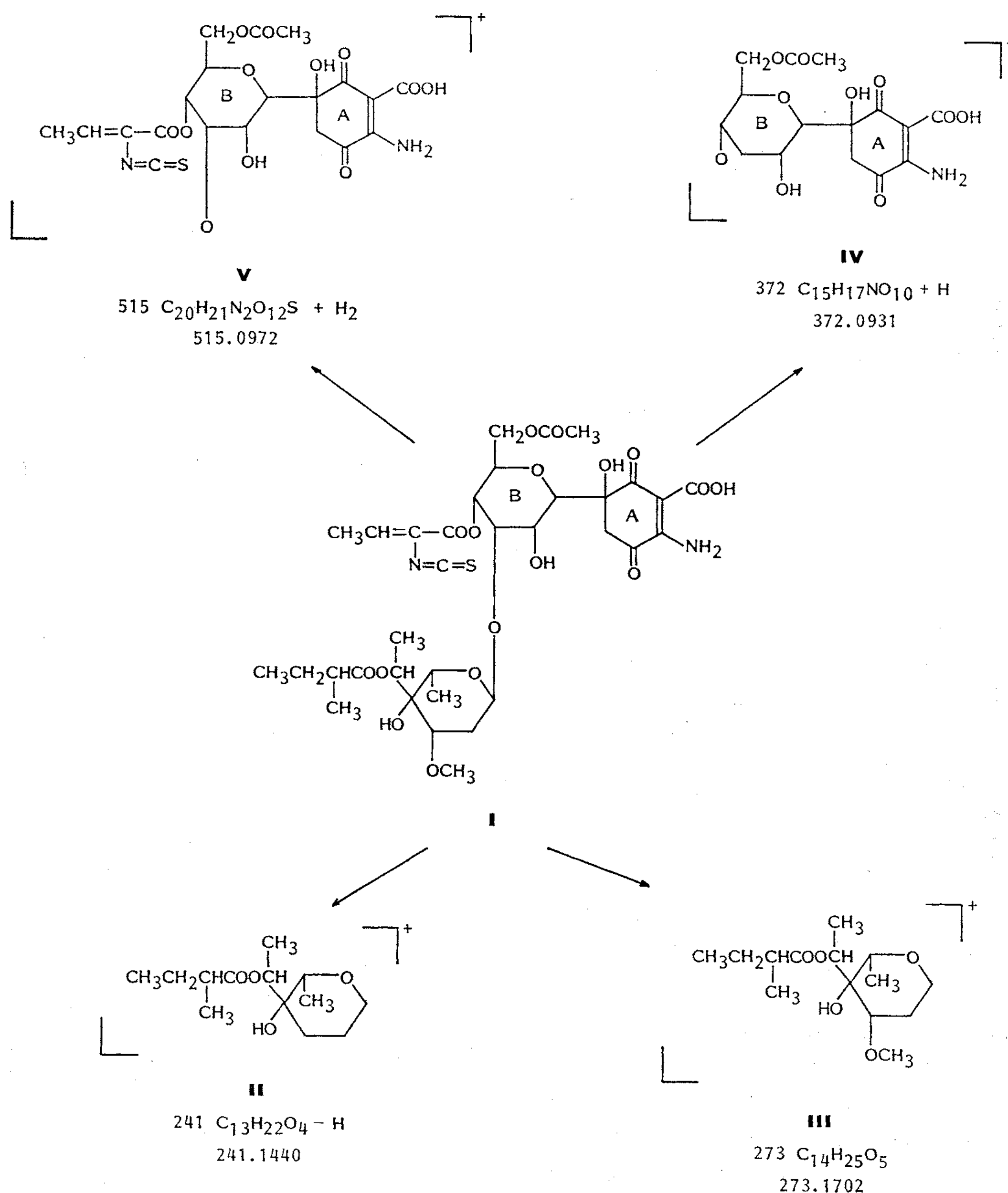
The positive ion FAB-MS spectra of paulomycin A is presented in Fig. 6 and positive ion FAB HR data of paulomycin A are presented in Table 3 and Fig. 7. The ions resulting from the acylpaulomycose moiety in paulomycin A appear at *m/z* 273 ($C_{14}H_{25}O_5$) and 241 (273−CH_3OH) (Fig. 7, **II** and **III**) in paulomycin B, these ions appear at *m/z* 259 ($C_{13}H_{23}O_5$) and 227 (259−CH_3OH) indicating that the two antibiotics, which differ by a CH_2 group,[1] have different acyl substituents esterified at C-7′ of paulomycose since the loss of 42 mass units, indicative of loss of CH_3OH, is observed in the positive ion FAB-MS of both paulomycins A and B.

Positive ion FAB-MS of *O*-demethylpaulomycins A and B are presented in Fig. 8. The fragments due to the acylpaulomycose of these antibiotics appear at *m/z* 259 ($C_{13}H_{23}O_5$) and 241 (259−H_2O) in *O*-demethylpaulomycin A and at 245 ($C_{12}H_{21}O_5$) and 227 (245−H_2O) in *O*-demethylpaulomycin B. These results indicate that there is no OCH_3 group in both antibiotics and the relationship of the acyl substituents esterified at C-7′ of *O*-demethylpaulomycose is the same as that of paulomycins A and B (see Fig. 5). The use of both negative ion[3] and positive ion FAB-MS has permitted the early recognition of the components of paulomycin mixtures and, thus, facilitated greatly the isolation of these compounds.

U-77,802 and U-77,803

U-77,802 and U-77,803 were also isolated as colorless crystalline compounds with solubilities similar to those of *O*-demethylpaulomycins A and B. Inspection of the molecular formulas listed in Table 1 indicate that U-77,802 and U-77,803 appear to be derived from paulomycins A ($C_{34}H_{46}N_2O_{17}S$) and B ($C_{33}H_{44}N_2O_{17}S$) by addition of H_2S in their molecules, respectively. This agrees with IR spectral data (Table 1 and Fig. 4). The IR spectra of U-77,802 and U-77,803, identical to each other, are characterized by the absence of the characteristic band of 2040 cm^{-1} due to the isothiocyanate group of the paulomycins.[1] This suggests addition of the H_2S on the N=C=S system of paulomycin A or B with the subsequent formation of the chromophoric system **11** different from that

Fig. 7. Selected ion fragments observed in the positive ion HRFAB-MS of paulomycin A.

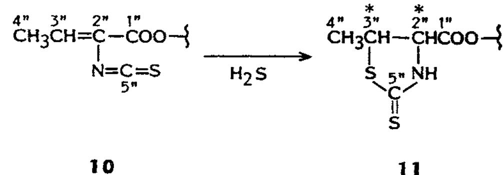


of paulic acid ester **10** which is present in paulomycins and is responsible for the UV maximum at 276 nm. The new chromophoric system has UV maximum at 278 nm; however, the molecular extinction coefficients at 278 nm is much greater than that of paulomycins (Fig. 3). The maxima at *ca.* 242 and 321 nm which are due to the ring A of paulomycins are also present in the UV spectra of U-77,802 and U-77,803 excluding the addition of H_2S in this part of the paulomycins molecule.

The postulation of a five-membered ring in structures **5** and **6** for U-77,802 and U-77,803 (Fig. 5) is based on ^{13}C NMR data. The ^{13}C NMR spectra of both U-77,802 and U-77,803 (Table 2) are char-

Fig. 8. Positive ion FAB-MS spectra of *O*-demethylpaulomycins A (A) and B (B).

acterized by the absence of absorptions at *ca.* δ 123.36 (s), 136.64 (d), and 142.64 (s) assigned to the unsaturated system and the isothiocyanate carbon of paulic acid (**10**).[5] Instead C-2″, C-3″, and C-5″ appear at *ca.* δ 69.77 (d), 48.27 (d), and 200.41 (s) as expected for the cyclic structures **5** and **6** of Fig. 5. It should be noted that positive ion FAB-MS of U-77,802 contain fragment ions at *m/z* 273 and 241 (273−CH_3OH) assigned to fragments **III** and **II** (Fig. 7) indicating substitution in the acylpaulomycose moiety of U-77,802 identical to that of paulomycin A. Similarly, the positive ion FAB-MS of U-77,803 contain fragments at 259 and 227 (259−CH_3OH) mass units as expected from the acylpaulomycose moiety present in both U-77,803 and paulomycin B. The addition of H_2S to the paulic acid moiety of paulomycins A and B results in the formation of two new asymmetric centers at C-2″ and C-3″ of **5**, **6**, or **11**. No information on the stereochemistry of these asymmetric



carbons is available at present.

Paulomenols A and B

Both compounds have been isolated as colorless amorphous materials. Paulomenol A has been reported earlier by WILEY *et al.*[5] as a product of alkaline degradation of paulomycin A. The solubility of both paulomenols in several solvents is similar to that of paulomycins; however, HPLC data (Fig. 2) indicate that paulomenols A and B are the most polar metabolites isolated so far from fermentations of *S. paulus*. The molecular formulas of paulomenols A and B indicate that they differ from the respective paulomycins by a $C_5H_3NO_2S$ moiety. This is consistent with the assumption that paulic acid (**10**) is not part of the paulomenol molecules. UV, IR, and ^{13}C NMR data support this assumption. The IR spectra (Fig. 4) of paulomenols A and B, identical to each other, do not contain the isothiocyanate absorption at 2040 cm^{-1}. The UV spectra (Fig. 3) have maxima at 242 and 342 nm indicating the presence of ring A of paulomycins. However, the UV maximum at 276 which is present in paulomycins is absent in the UV spectra of paulomenols A and B. This maximum has been associated with the paulic acid (**10**) moiety of paulomycins.[3] ^{13}C NMR (Table 2) indicate the absence of absorptions at *ca.* δ 160.25 (s), 123.36 (s), 136.64 (d), 14.11 (q), and 142.64 (s) which are assigned to C-1″, C-2″, C-3″, C-4″, and C-5″ of the paulic acid fragment present in paulomycins A and B. Positive ion FAB-MS showed the presence of fragment ions at *m/z* 273 and 241 (273 − CH_3OH) (Fig. 7, II and III) in the spectra of paulomycin A and paulomenol A and 259 and 227 (259 − CH_3OH) in the spectra of paulomycin B and paulomenol B indicating identical acylpaulomycose moieties respectively. The structures of paulomenols A and B are, therefore, represented by **7** and **8** as shown in Fig. 5.

As reported earlier,[5] paulomenol A was isolated by alkaline degradation of paulomycin A. Under acidic conditions, the paulic-ester bond is very stable. Dehydration of the C-6 tertially-hydroxyl group and hydrolysis of the acylpaulomycose moiety of paulomycins occur before any hydrolysis of the paulic acid ester takes place. The fact that paulomenols A and B appear early in the acidic fermentation of *S. paulus* (pH 5.0～6.0) suggest that they are true metabolites produced by *S. paulus* and not products of degradation of paulomycins; whether paulomenols A and B are intermediates is the production of paulomycins A and B is not known at present.

Biological Properties of *O*-Demethylpaulomycins A and B, U-77,802, U-77,803, and Paulomenols A and B

The *in vitro* antibacterial spectrum of paulomycins A and B, *O*-demethylpaulomycins A and B and U-77,802 and U-77,803 is presented in Table 4. All compounds are mainly active against Gram-positive organisms especially *Staphylococcus aureus*, including strains resistant to penicillin, streptomycin, neomycin, macrolide, and lincosaminide antibiotics. *O*-Demethylpaulomycin A had about half of the paulomycin A activity while U-77,802 and U-77,803 in which the isothiocyanate group participates in a rather stable five-member cyclic system are much less active. However, paldimycins A and B[6] which do not have a free isothiocyanate group are as active as paulomycins A and B. The work of LABORDE *et al.*[7] suggests that the activity of paldimycin is due to paulomycin which is formed under the conditions of the testing system used. This finding would suggest that the isothiocyanate group of paulomycins is needed for bioactivity and that the degree of bioactivity would depend on the rate of transformation of the "prodrug" (paldimycins, U-77,802, U-77,803) to paulomycin. Paulomenols A and B which do not contain the paulic acid moiety did not show any activity against the

Table 4. Antimicrobial *in vitro* testing of paulomycins A and B, *O*-demethylpaulomycins A and B, and U-77,802 and U-77,803.

Organism	MIC (μg/ml)[a]					
	Paulomycin		*O*-Demethyl-paulomycin		U-77,802	U-77,803
	A	B	A	B		
Staphylococcus aureus UC 9218	0.06	0.06	1.00	2.00	16.00	16.00
S. aureus UC 3665	0.125	0.125	0.50	4.00	8.00	32.00
S. aureus UC 6685	0.125	0.125	1.00	4.00	16.00	16.00
S. aureus UC 9213	0.125	0.125	0.50	8.00	16.00	32.00
S. epidermidis UC 30031	0.06	0.06	1.00	2.00	16.00	8.00
Streptococcus faecalis UC 694	0.50	0.50	0.50	64.00	8.00	—
S. pneumoniae UC 41	0.06	0.06	0.125	0.50	4.00	4.00
S. pyogenes UC 152	0.06	—	0.06	0.25	2.00	2.00

[a] Test method: Agar dilution, pH 6.0.

In vitro antibacterial data were provided by GARY E. ZURENKO and BETTY H. YAGI of The Upjohn Company.

organisms listed in Table 4.

Acknowledgments

The authors express their appreciation to Mr. K. J. GEIPEL for technical assistance, Mr. G. E. ZURENKO for biological testing, Mr. J. A. BUEGE and Mr. W. J. HAAK for large-scale extractions, and to Dr. P. F. WILEY for valuable comments and suggestions.

References

1) ARGOUDELIS, A. D.; T. A. BRINKLEY, T. F. BRODASKY, J. A. BUEGE, H. F. MEYER & S. A. MIZSAK: Paulomycins A and B. Isolation and characterization. J. Antibiotics 35: 285~294, 1982
2) ARGOUDELIS, A. D.; L. BACZYNSKYJ, J. A. BUEGE, V. P. MARSHALL, S. A. MIZSAK & P. F. WILEY: Paulomycin-related antibiotics: Paldimycins and antibiotics 273a_2. Isolation and characterization. J. Antibiotics 40: 408~418, 1987
3) ARGOUDELIS, A. D.; L. BACZYNSKYJ, W. T. HAAK, W. M. KNOLL, S. A. MIZSAK & F. B. SHILLIDAY: New paulomycins produced by *Streptomyces paulus*. J. Antibiotics 41: 157~169, 1988
4) MARSHALL, V. P.; M. S. LITTLE & L. E. JOHNSON: A new process and organism for the fermentation production of volonomycin. J. Antibiotics 34: 902~904, 1981
5) WILEY, P. F.; S. A. MIZSAK, L. BACZYNSKYJ, A. D. ARGOUDELIS, D. J. DUCHAMP & W. WATT: The structure and chemistry of paulomycins. J. Org. Chem. 51: 2493~2499, 1986
6) ARGOUDELIS, A. D.; L. BACZYNSKYJ, S. A. MIZSAK, F. B. SHILLIDAY, P. A. SPINELLI & J. DEZWAAN: Paldimycins A and B and antibiotics 273$a_{2\alpha}$ and 273$a_{2\beta}$. Synthesis and characterization. J. Antibiotics 40: 419~436, 1987
7) LABORDE, A. L.; R. J. MOUREY, S. C. LINN & G. H. CHAMBLISS: Mechanism of action studies on and paulomycin. J. Antibiotics, in preparation