PAULOMYCIN-RELATED ANTIBIOTICS: PALDIMYCINS AND ANTIBIOTICS 273a_2

ISOLATION AND CHARACTERIZATION†

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The isolation of paulomycins A and B from fermentations of *Streptomyces paulus* has been reported earlier [J. Antibiotics 35: 285~294, 1982]. Further work on the antibiotics produced by *S. paulus* revealed the production of two paulomycin-related compounds, antibiotics 273a_1 and 273a_2 which were isolated by procedures involving extractions and chromatography over buffered silica gel.

Antibiotic 273a_1 which has been named paldimycin, was found to be a mixture of two materials, paldimycins A and B (antibiotics 273$a_{1\alpha}$ and 273$a_{1\beta}$). Similarly, antibiotic 273a_2 was found to consist of antibiotic 273$a_{2\alpha}$ and antibiotic 273$a_{2\beta}$. Paldimycin and antibiotic 273a_2, which are produced by addition of two or one molecules of *N*-acetyl-L-cysteine, respectively, to paulomycins A and B, are active vs. Gram-positive bacteria.

The production and isolation of paulomycins A and B by *Streptomyces paulus* strain 273 were reported by ARGOUDELIS *et al.*[1] Further studies indicated the presence in fermentations of *S. paulus* of bioactive compounds with chromatographic behavior significantly different from that of paulomycins. This paper describes the isolation and chemical characterization of these compounds.

Experimental

Assay and Testing Procedures

Antibiotic production and purification was measured by a microbiological disc-plate assay procedure with *Micrococcus luteus* (UC 130) as the assay organism.

Thin-layer Chromatographic Procedures

The production and purification of paulomycin, paldimycin and antibiotic 273a_2 were followed by TLC on Silica gel G using chloroform - ethanol - water (25 : 30 : 5) or on cellulose powder using pH 7.0 phosphate buffer as the solvent systems. The antibiotics present in the fermentation or in preparations obtained during purification were detected by bioautography on *M. luteus* seeded trays. Typical TLC of paulomycin, paldimycin and antibiotic 273a_2 are shown in Fig. 1.

Spectroscopic Methods

^{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 dimethyl sulfoxide-d_6 or acetone-d_6 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 reported as ppm relative to tetramethylsilane. MS were obtained on a ZAB-2F high resolution mass spectrometer using a fast atom bombardment (FAB) source.

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High Performance Liquid Chromatography (HPLC)

All HPLC was carried out on a Hewlett-Packard Model 1084B (Hewlett-Packard, Avondale, Calif.) instrument equipped with an HP model 79875A variable wavelength detector and operating in the dual pump mode. A Brownlee 10 cm × 4.6 mm stainless-steel column packed with C_{18} (10 μm) reverse phase was used. Mobile phases were prepared using distilled-in-glass solvents. All samples and aqueous phases were filtered through a 0.45 μm filter prior to use.

Fermentation Conditions

The fermentation conditions used for the production of the paulomycin complex, paldimycin and antibiotic 273a_2 by *S. paulus* strain 273 (UC 5142) were similar to those described by MARSHALL *et al.*[2] A medium consisting of glucose monohydrate (10 g/liter), Pharmamedia (10 g/liter), dextrin (20 g/liter), brewer's yeast (1 g/liter) and UCON (10 g/liter) gave consistently high titers of paldimycin and antibiotic 273a_2.

Isolation of Paulomycin, Paldimycin and Antibiotic 273a_2 from Fermentations of *S. paulus* Strain 273

1. Filtration and Extraction with Ethyl Acetate: The fermentation broth (*ca.* 5,000 liters) was filtered with the aid of diatomaceous earth. The clear filtrate was cooled to 16°C and adjusted to pH 3.0 with sulfuric acid. The acidified solution was extracted with 1,000 liters of Skellysolve B; the extract was found bio-inactive and was discarded. The acidic clear filtrate was then extracted twice with 1,500 liters of ethyl acetate. The ethyl acetate extracts were combined and washed with 1,000 liters of water at pH 5.3 (aqueous-1). The ethyl acetate extract was then concentrated to a volume of *ca.* 40 liters and this concentrate was poured into 800 liters of Skellysolve B with stirring. The precipitated material was isolated by filtration and dried (preparation A, 95 g). The aqueous solution, designated aqueous-1, was adjusted to pH 3.0 with aqueous sulfuric acid and was extracted twice with 300 liters portions of ethyl acetate. The ethyl acetate extracts were concentrated to a volume of *ca.* 24 liters and this solution was mixed with 250 liters of Skellysolve B. The precipitated material was isolated by filtration (preparation B, 176.5 g). Preparation A contained (by TLC) paulomycins and small amounts of antibiotic 273a_2. Preparation B contained some paulomycins, paldimycin and antibiotic 273a_2 as the main components.

2. Separation of Paldimycin from Paulomycins and Antibiotic 273a_2 by Silica Gel Chromatography: Silica gel (Merck-Darmstardt), 1 kg was triturated with 800 ml of a solution containing 38 g of potassium chloride per liter, adjusted to pH 2.0 with 1 N aqueous hydrochloric acid. The KCl-HCl treated silica was activated by heating at 110°C for 20 hours. It was then cooled and packed into a glass column using chloroform - ethanol - water (25 : 30 : 5) as the mobile phase. Preparation B, 10 g, obtained as described earlier, was dissolved in 43 ml of the mobile phase and this solution was added on the top of the column. The column was eluted with the chloroform - ethanol - water (25 : 30 : 5) solvent system at the rate of 20 ml/minute. Collected fractions (20 ml each) were tested for bioactivity and analyzed by TLC. Results are shown below:

Fraction No.	Antibiotics present
80	Paulomycin
100	Antibiotic 273a_2 and a small amount of paulomycin
120~180	Antibiotic 273a_2
200	Traces of antibiotic 273a_2
220~320	No bioactivity or other TLC spots
340~600	Paldimycin only

Fractions 66~140 were concentrated to dryness to yield 10.07 g of a preparation containing antibiotic 273a_2 and paulomycin only. Further purification of this material and separation of paulomycin and antibiotic 273a_2 is described below.

Fractions 340~600 were combined and concentrated to dryness to yeild a preparation containing paldimycin only. This material was distributed between 200 ml of ethyl acetate and 200 ml of water

at pH 3.0. The ethyl acetate phase was separated, dried over sodium sulfate and concentrated to dryness. The residue obtained was dissolved in 10 ml of acetone and this solution was mixed with 400 ml of ether and 400 ml of Skellysolve B. The precipitated paldimycin, 460 mg was isolated by filtration and dried. Characterization of this material is described later in this manuscript.

3. Separation of Antibiotic 273a_2 from Paulomycin by Silica Gel Chromatography: Activated KCl - HCl treated silica gel, prepared as described earlier, was packed into a glass column using methyl ethyl ketone - acetone - water (160 : 50 : 20) as the mobile phase. A purified mixture of antibiotic 273a_2 and paulomycins, 10.07 g, obtained as described above, was dissolved in 55 ml of the mobile phase and added on the top of the column. The column was eluted with the methyl ethyl ketone - acetone - water (160 : 50 : 20) mobile phase at the rate of 20 ml/minute. Collected fractions (20 ml) were tested for bioactivity and analyzed by TLC. Fractions 60~120 contained paulomycins A and B. Fractions 230~250, containing antibiotic 273a_2, were combined and this solution was concentrated to dryness. The residue was distributed between 200 ml ethyl acetate and water at pH 3.0. The ethyl acetate phase was separated, dried over sodium sulfate and concentrated to dryness. The obtained material was dissolved in 20 ml of acetone and this solution was mixed with 200 ml of ether and 300 ml of cyclohexane. The precipitated antibiotic 273a_2 was isolated by filtration and dried; yield 740 mg. Characterization of this material is described later in this manuscript.

Transformation of Paldimycin to Paulomycins A and B

1. By Chromatography Over Amberlite IRA-904: Paldimycin, 1.0 g was dissolved in 20 ml of water at pH 7.3 adjusted with aqueous sodium hydroxide. This solution was passed over a column containing 50 ml of Amberlite IRA-904 anion exchanger in the chloride form. The column was eluted first with methanol - water (1 : 1) followed by 10% aqueous sodium chloride solution. Both eluents contained bioactive material(s) which were identified by TLC and HPLC as paulomycins A and B.

2. By Chromatography over Dowex-1: Paldimycin, 1.0 g, was dissolved as described above and the solution was passed over a column containing 50 ml of Dowex-1 in the chloride form. Elution of the column with methanol - water (1 : 1) or with 10% sodium chloride solution yielded paulomycins A and B only identified by TLC and HPLC chromatograms.

3. By Distribution Between Ethyl Acetate and Water at pH 7.3: Paldimycin, 1.0 g, was stirred for 48 hours in 100 ml of ethyl acetate and 70 ml of water, pH 7.3, adjusted with aqueous sodium hydroxide. The phases were separated and analyzed by TLC and HPLC. The ethyl acetate extract contained paulomycins A and B only identified by UV, IR[1] and by their TLC and HPLC chromatographic behavior.

Transformation of Antibiotic 273a_2 to Paulomycins A and B

By Distribution Between Ethyl Acetate and Water at pH 7.5: Antibiotic 273a_2, 500 mg, was dissolved in 50 ml of ethyl acetate. This solution was mixed with 100 ml of 0.1 M phosphate buffer, pH 7.5, and stirred for 48 hours. The ethyl acetate phase was analyzed at 0, 4, 24 and 48 hours by TLC. Results indicated that antibiotic 273a_2 (present in the 0-hour sample) was transformed to paulomycins A and B. The latter antibiotics were the main antibiotic components of the mixture after 48 hours.

Results and Discussion

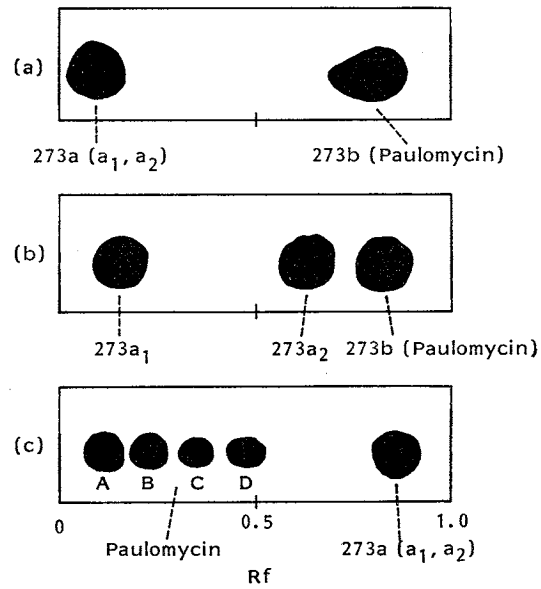
The production of paulomycin, a mixture of related antibiotics by *S. paulus* strain 273, was reported earlier.[1] Two components of the mixture, paulomycins A and B, were isolated, crystallized and characterized, and their structure was reported by WILEY and his co-workers.[3] The biological properties of paulomycins A and B were reported at the 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC).[4]

Very early in our work with *S. paulus* strain 273, we observed the production of other antibiotics more polar than paulomycins. When fermentations were analyzed by TLC on silica gel using chloro-

form - methanol (90 : 10) as the mobile phase and *M. luteus* as the detecting organism, we observed two well-separated bioactive components which we designated as 273a and 273b (Fig. 1a). Antibiotic 273b, which was named paulomycin, was the main component of the fermentation and was later found, by chromatography on cellulose TLC plates using pH 7.0 buffer, to consist of a mixture of several antibiotics designated paulomycins A, B, C and D* (Fig. 1c). When the fermentation broth was analyzed by TLC using silica gel and chloroform - ethanol - water (25 : 30 : 5) as the mobile phase, the 273a component was found to consist of two antibiotics of identical antibacterial spectrum which we designated $273a_1$ and $273a_2$ (Fig. 1b). Antibiotic $273a_1$ has been named paldimycin and this designation is used throughout this paper.

Fig. 1. TLC of fermentation broth of *Streptomyces paulus* strain 273.

(a) Silica gel; chloroform - methanol (90 : 10).
(b) Silica gel; chloroform - ethanol - water (25 : 30 : 5).
(c) Cellulose powder; pH 7.0, 0.1 M phosphate buffer; bioautography on *Micrococcus luteus*-seeded agar trays.



Paldimycin and antibiotic $273a_2$ were originally observed as minor components of the fermentation. However, media studies designed to increase paulomycin production resulted also in increased production of paldimycin and antibiotic $273a_2$. Since paulomycins, paldimycin and antibiotic $273a_2$ have identical antibacterial spectra, the assay system (*M. luteus*) used for the purification work measures total bioactivity due to all paulomycin-related antibiotics. The amount of paulomycins and relative amounts of antibiotics $273a_1$ and $273a_2$ can be determined by HPLC procedures described in this and a previous paper.[1] However, no attempt has been made in the present study to quantitate the production of paldimycin and antibiotic $273a_2$ by *S. paulus* strain 273.

Isolation of Paldimycin and Antibiotic $273a_2$

A crude mixture of paldimycin and antibiotic $273a_2$ containing small amounts of paulomycins is easily obtained by extraction with ethyl acetate at pH 3.0. The extract which contained paulomycin, paldimycin and antibiotic $273a_2$ was washed with water at pH 5.5. Paulomycins, which are weak acids with *pKa'* of 7.5, remained in the ethyl acetate phase. Paldimycin and $273a_2$ being much stronger acids with *pKa'* of 3.8~4.0 were transferred to the aqueous phase from where they were recovered by extraction with ethyl acetate at pH 3.0. Concentration of the extract yielded a residue containing paldimycin and $273a_2$ and small amounts of paulomycins. This material was used for the separation and isolation of paldimycin and $273a_2$ by the silica gel chromatographies outlined below.

Separation of Paldimycin and Antibiotic $273a_2$

Separation and isolation of paldimycin and $273a_2$ was done by chromatographies over silica gel

* The properties of paulomycins C, D, and related materials will be reported in a publication currently under preparation.

triturated with KCl - HCl buffer and reactivated by heating at 120°C for 20 hours. The residue containing paldimycin and $273a_2$ and some paulomycins was used as the starting material. When chloroform - ethanol - water (25 : 30 : 5) was used as the mobile phase, paulomycins were eluted first followed closely by antibiotic $273a_2$. Paldimycin was eluted much later and therefore was easily separated from the other antibacterial components. For separation of $273a_2$ from paulomycin we have used methyl ethyl ketone - acetone - water (160 : 50 : 20) as the mobile phase.

Transformation of Paldimycin and Antibiotic $273a_2$ to Paulomycins A and B

Both paldimycin and antibiotic $273a_2$ were readily converted to mixtures of paulomycins A and B. Passage of solutions of paldimycin (Rf 0.1, silica gel, chloroform - ethanol - water, 25 : 30 : 5) over anion exchange resins in the Cl^- form gave fractions containing paulomycin as indicated by TLC on silica gel plates (Rf 0.8; Fig. 1a). Analysis by TLC on cellulose powder plates or by HPLC[1] showed that the transformed product was a mixture of paulomycins A and B. The same conversion occurred when paldimycin or antibiotic $273a_2$ were distributed between ethyl acetate and water at pH 7.5. A similar analysis of the ethyl acetate phase demonstrated the presence of paulomycins A and B.

These observations suggest that both paldimycin or antibiotic $273a_2$ are not single entities but they are a mixture of two closely related antibiotics which under the conditions described above are transformed to paulomycins A and B, respectively. The two components in paldimycin were designated paldimycins A and B. The compounds present in antibiotic $273a_2$ are known as $273a_{2\alpha}$ and $273a_{2\beta}$. Several attempts have been made to isolate sufficient quantities of the α and β components of paldimycin and antibiotic $273a_2$ for complete chemical and biological evaluation. In all cases (preparative HPLC, silica gel chromatographies, and counter-current distributions) extensive "conversion" of paldimycin and antibiotic $273a_2$ to paulomycin and other unidentified compounds occurred.

Characterization of Paldimycin and Antibiotic $273a_2$

Some of the physical and chemical properties of paldimycin and antibiotic $273a_2$ are tabulated in Table 1. Paldimycin and antibiotic $273a_2$ were isolated as amorphous powders. As we mentioned earlier they are acidic compounds with *pKa'* of *ca.* 3.8~4.0. The free acids are not soluble in water but they are soluble in pH 7.0 aqueous buffers. Potassium, sodium, or ammonium salts are soluble

Table 1. Physical and chemical properties of paldimycin and antibiotic $273a_2$.

	Paldimycin	$273a_2$
Appearance	Amorphous, colorless	Amorphous, colorless
Titration (in 60% aq ethanol)	Acid, *pKa'* 3.8~4.0	Acid, *pKa'* 3.8~4.0
UV λ_{max} nm (*a*) in 95% ethanol	248 (16.2), 274 (sh, 8.51), 321 (8.09)	246 (19.6), 270 (sh, 11.8), 320 (4.35)
IR (Nujol) cm^{-1}	3476, 3350, 3276, 3076, 2637 (sh), 1734, 1658	3479, 3351, 3270, 3070, 1734, 1699, 1660
TLC behavior	Single entity	Single entity
HPLC* (retention time, minutes)	8.88 (paldimycin B), 9.98 (paldimycin A)	11.05 ($273a_{2\beta}$), 12.03 ($273a_{2\alpha}$)
Behavior at pH 7.5 buffer	Yield paulomycins A and B	Yield paulomycins A and B
FAB-MS (*m/z*)	1,098 (paldimycin B), 1,112 (paldimycin A)	935 ($273a_{2\beta}$), 949 ($273a_{2\alpha}$)

* Support: C-18 silica; gradient elution using acetonitrile - 0.05 M phosphate buffer, pH 5.5.

Fig. 2. UV spectra of paldimycin (a) and antibiotic $273a_2$ (b).

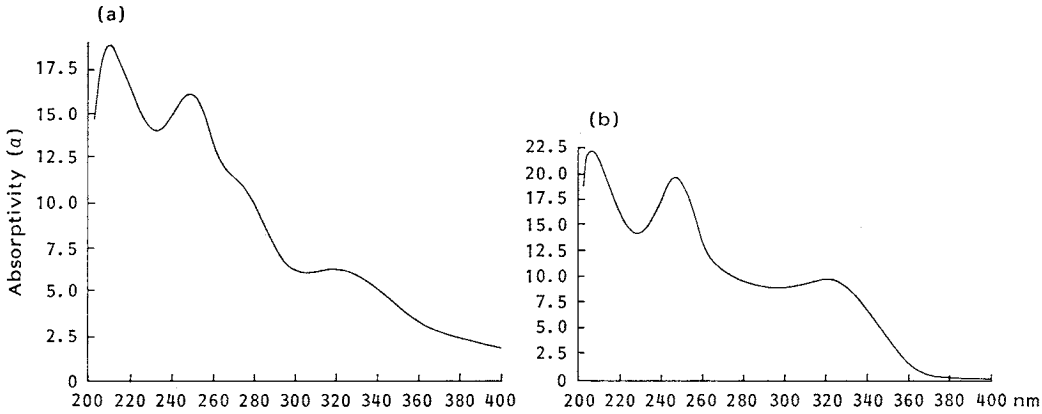


Fig. 3. IR spectra of paldimycin (a) and antibiotic $273a_2$ (b), in Nujol mull.

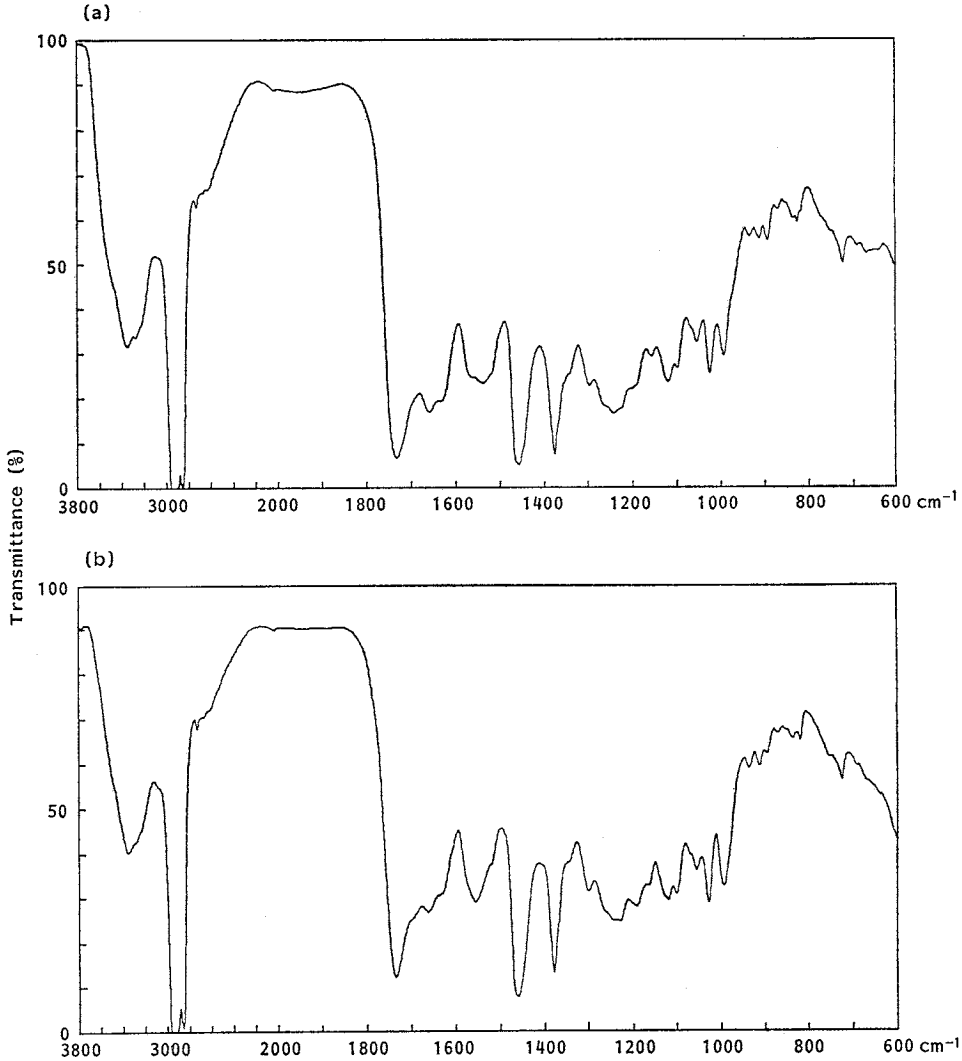
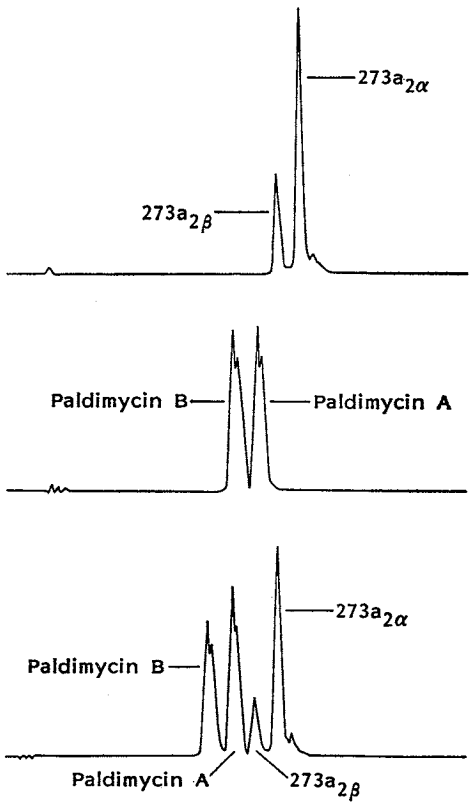


Fig. 4. HPLC chromatography* of paldimycin and antibiotic $273a_2$.

Retention time (minutes): Paldimycin A 9.98, paldimycin B 8.88, $273a_{2\alpha}$ 12.03, $273a_{2\beta}$ 11.05.

* Mobile phase: A=0.05 M phosphate buffer, pH 5.5, B=acetonitrile.

0 minute, % B=15; 5 minutes, % B=15; 15 minutes, % B=40.



in water at concentrations of *ca.* 150~200 mg/ml.

Like paulomycins A and B, paldimycin and antibiotic $273a_2$ have a UV maximum at 320~322 nm (Fig. 2). This maximum has been used to monitor chromatographic separation of these compounds. The IR spectra of paldimycin and antibiotic $273a_2$ (Fig. 3) are quite similar and show ester carbonyl absorptions at *ca.* 1734 cm^{-1}. The strong absorption band at 2050 cm^{-1}, which is the most characteristic feature of the IR spectra of paulomycin A or B,[1] is not present in the spectra of paldimycin and antibiotic $273a_2$ which is indicative of their chemical nature as discussed later in this paper.

Thin-layer and paper chromatograms of paldimycin and antibiotic $273a_2$ indicated the presence of only one bioactive component in each. However, HPLC (Fig. 4) showed that both antibiotics consisted of two closely related compounds designated, as mentioned earlier, paldimycins A and B and antibiotics $273a_{2\alpha}$ and $273a_{2\beta}$, respectively.

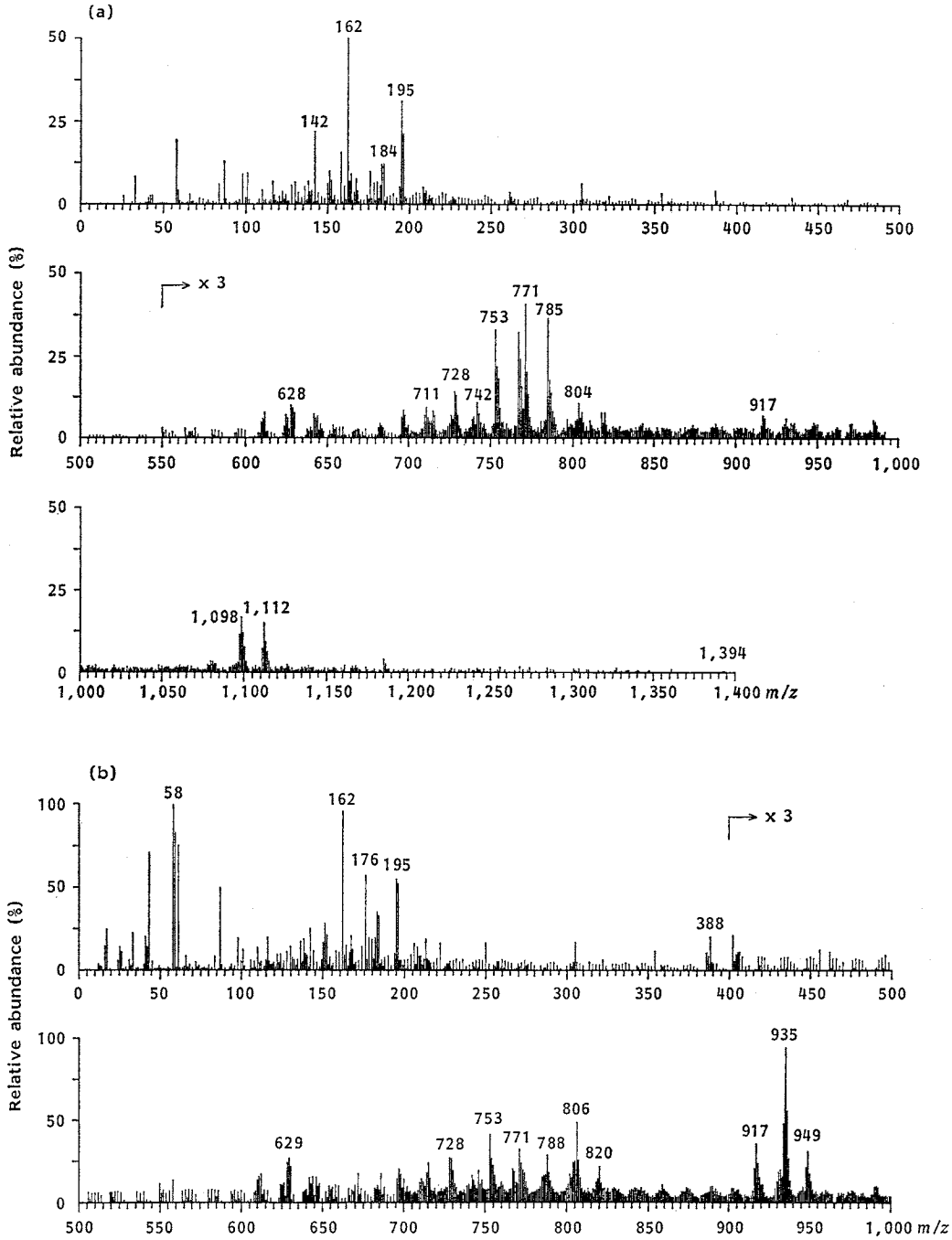
Fast atom bombardment mass spectra (FAB-MS) also show that paldimycin and antibiotic $273a_2$ contain two components. The FAB-MS of paldimycin contain molecular ion peaks at *m/z* 1,112 for paldimycin A and at *m/z* 1,098 for paldimycin B (Fig. 5). Similarly the FAB-MS of antibiotic $273a_2$ has two molecular ion peaks at *m/z* 949 and 935 for antibiotics $273a_{2\alpha}$ and $273a_{2\beta}$, respectively (Fig. 5).

The Structures of Paldimycins and Antibiotics $273a_2$

Table 2 compares the molecular weights and molecular composition of paulomycins A and B, paldimycins A and B and antibiotics $273a_{2\alpha}$ and $273a_{2\beta}$. The molecular weight of paulomycin A is 163 daltons less than that of $273a_{2\alpha}$ which in turn is 163 daltons less than that of paldimycin A. The difference between the molecular composition of the respective antibiotics is $C_5H_9NO_3S$. The same relationship exists among paulomycin B, $273a_{2\beta}$ and paldimycin B. The mass spectral studies suggested that $273a_{2\alpha}$ and $273a_{2\beta}$ are most probably derived from paulomycins A and B, respectively, by addition of a $C_5H_9NO_3S$ fragment and that paldimycins A and B are derived from paulomycins A and B by addition of two $C_5H_9NO_3S$ fragments.

Thus the dissolution of either paldimycin or antibiotic $273a_2$ in pH 7.5 buffer must result in the loss of two $C_5H_9NO_3S$ residues from paldimycins and one from antibiotic $273a_2$ to form paulomycins A and B. The α components in these antibiotics would yield paulomycin A and the β components

Fig. 5. FAB-MS of paldimycin (a) and antibiotic 273a$_2$ (b).



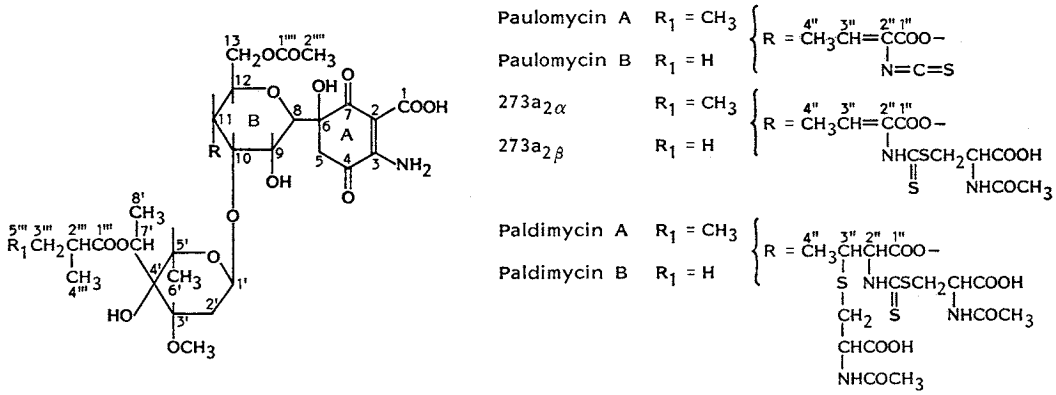
paulomycin B by loss of one or two $C_5H_9NO_3S$ fragments. The structures of paulomycins A and B have been reported by WILEY and his co-workers[3] and are shown in Fig. 6. The nucleus of the paulomycin molecule consists of two ring systems designated A and B. The maximum at 320 nm is the UV spectra of paulomycins must arise from the chromophoric system in ring A. This maximum, as mentioned earlier, is also present in the spectra of paldimycins and antibiotics 273a$_2$. Ring B is a poly-

Table 2. Molecular formulas and molecular weights of paulomycins A and B, paldimycins A and B, and antibiotics 273a$_{2\alpha}$ and 273a$_{2\beta}$.

	MW	MF	Differences from corresponding paulomycins	
			MW	MF
Paulomycin A	786	$C_{34}H_{46}N_2O_{17}S$	—	—
Antibiotic 273a$_{2\alpha}$	949	$C_{39}H_{55}N_3O_{20}S_2$	163	$C_5H_9NO_3S$
Paldimycin A	1,112	$C_{44}H_{64}N_4O_{23}S_3$	163×2	$(C_5H_9NO_3S)_2$
Paulomycin B	772	$C_{33}H_{44}N_2O_{17}S$	—	—
Antibiotic 273a$_{2\beta}$	935	$C_{38}H_{53}N_3O_{20}S_2$	163	$C_5H_9NO_3S$
Paldimycin B	1,098	$C_{43}H_{62}N_4O_{23}S_3$	163×2	$(C_5H_9NO_3S)_2$

MF: Molecular formula.

Fig. 6.



substituted tetrahydropyrane system. An eight carbon branched sugar, named paulomycose, is linked glycosidically to the C-10 hydroxyl group of ring B. This sugar which contains a methoxyl group is esterified to 2-methylbutyric acid in paulomycin A and to isobutyric acid in paulomycin B. A comparison of the ^1H and ^{13}C NMR spectra of paulomycins A and B, paldimycins and antibiotics 273a$_2$* indicated that rings A and B, the 8-carbon esterified sugar and the acetate ester at C-13 of ring B, are present intact in paldimycins A and B and antibiotics 273a$_{2\alpha}$ and 273a$_{2\beta}$.

The differences between the structures of paulomycins and antibiotics 273a are located in the α,β-unsaturated acid moiety which is esterified to the hydroxyl at C-11 of ring B. This acid named paulic acid, contains an isothiocyanate group which is the most characteristic chemical feature of the paulomycins and is responsible for an intense IR absorption at 2050 cm^{-1}. Antibiotics 273a$_{2\alpha}$ and 273a$_{2\beta}$ do not have IR absorptions between 1800~2600 cm^{-1}. This suggested addition of the $C_5H_9NO_3S$ fragment at the isothiocyanate group of the paulomycins. ^1H and ^{13}C NMR studies confirmed this and in addition indicated that the C-5 fragment is *N*-acetylcysteine [^{13}C NMR δ 171.2 (s, COOH), 171.8 (s, NH*C*OCH$_3$), 37.3 (t, SCH$_2$), 53.5 (d, CHNH), 22.6 (q, NHCO*C*H$_3$); ^1H NMR δ 2.03 (3H, s, NHCOC*H*$_3$)] attached to the paulomycin molecule throughout its sulfur [^{13}C NMR δ 199.95

* ^1H and ^{13}C NMR spectra of paldimycins (A and B) and 273a$_2$ (273a$_{2\alpha}$ and 273a$_{2\beta}$) are reported in detail in an accompanying paper[5] related to the synthesis of these antibiotics. Only data from the NMR spectra of paldimycins and antibiotics 273a$_2$ which help to define their relationship to paulomycins A and B are discussed in this paper.

(s, NHCS)]. ^{1}H NMR data also indicated the presence of the C-2′, C-3′ unsaturated system of paulic acid [^{1}H NMR δ 1.97 (3H, d), 6.72 (1H, q, 4″-$CH_3$3″-C*H*=C); ^{13}C NMR δ 136.9 (d, C-3″), 131.7 (s, C-2″)] in the 273a_2 molecules as shown in the middle of Fig. 6.

Paldimycins A and B also do not show the intense IR absorption at 2050 cm^{-1} and therefore they do not contain an isothiocyanate group. The ^{13}C NMR spectra of paldimycin do not contain absorption peaks at *ca.* δ 136.6 (d), 123.4 (s) and 142.5 (s)[3] which are present in the paulomycin ^{13}C NMR spectra and have been assigned to the unsaturated system of paulic acid and the isothiocyanate carbon. This suggested that paldimycins A and B have the structures shown in the bottom of Fig. 6 in which one molecule of *N*-acetylcysteine has been added to the isothiocyanate group of paulomycin A or B as in 273a_2, and a second *N*-acetylcysteine molecule has been attached through a sulfide linkage to the C-3″ of paulic acid.

These structures were confirmed by synthesis of paldimycin A and antibiotic 273$a_{2\alpha}$ from paulomycin A and paldimycin B and antibiotic 273$a_{2\beta}$ from paulomycin B by reaction with *N*-acetylcysteine, as described in the next paper in this series.[5]

The mechanisms of the formation of paldimycin or antibiotic 273a_2 by *S. paulus* is not known. The organism is certainly responsible for the production of *N*-acetylcysteine. However, the subsequent addition of the latter to paulomycin A or B can be either enzymatically controlled or can be a simple chemical addition. Both mechanisms applied to the synthesis of paldimycin give rise to the formation of new asymmetric centers at C-2″ and C-3″ of the paulomycin molecule (Fig. 6). Four stereoisomers are theoretically possible. It appears that at least two stereoisomers are formed and this could explain the presence of shoulders (or two peaks) in the HPLC of natural paldimycins (A or B) as shown in Fig. 4.

Biological Properties of Paldimycin and Antibiotic 273a_2

The biological properties of paldimycin and antibiotic 273a_2 were discussed by FORD and his coworkers.[6] A detailed report on the testing of paldimycin will be reported shortly in a subsequent communication.

Acknowledgment

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