NOTES

A NEW OLIVANIC ACID DERIVATIVE PRODUCED BY *STREPTOMYCES OLIVACEUS*: ISOLATION AND STRUCTURAL STUDIES

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During recent years, members of a novel class of β-lactam antibiotics containing a carbapenem nucleus have been reported[1,2,3]. Studies with a culture of *Streptomyces olivaceus* CBS 349.80 capable of producing members of the olivanic acid series of carbapenem antibiotics, have demonstrated the presence of a new, but related antibiotic as a minor component which has been designated MM 27696.

Culture filtrate for extraction studies was produced by growing *S. olivaceus* CBS 349.80 in a fermentation medium composed of glucose 4%, soybean flour 2%, $CaCO_3$ 0.04%, $CoCl_2 \cdot 6H_2O$ 0.0002%, Na_2SO_4 0.1%, Pluronic L81 antifoam (10% suspension in soybean oil) 0.2% (v/v) in stainless steel fermenters. The procedure used for the extraction of MM 27696 is shown in Fig. 1. MM 27696 was assayed by C_{18} reversed phase HPLC, using 5% acetonitrile in 0.05 M ammonium phosphate buffer (pH 4.7) as eluant, and monitoring UV absorption at 300 nm. As with previous compounds in this series ion pair extraction proved a valuable first stage in the isolation[4]. Using this procedure 3,800 liters of culture filtrate yielded approximately 15 mg of MM 27696 in substantially pure form as its disodium salt.

The UV spectrum of MM 27696, having maxima at 306 nm and 228 nm, was very similar to that of MM 13902. The results of thin-layer and paper chromatographic studies on both compounds are listed in Table 1, and show their similar ionic properties whilst demonstrating the greater lipophilicity of MM 27696. The results shown in Tables 2 and 3 demonstrate that MM 27696 has broad spectrum antibacterial activity and potent β-lactamase inhibitory activity.

Fig. 1. Isolation procedure for MM 27696.

Culture filtrate
↓ extract with Aliquat 336 in dichloromethane
Dichloromethane extract
↓ back extract with sodium nitrate solution
Sodium nitrate extract
↓ adsorb impurities onto freshly precipitated $Al(OH)_3$ and remove precipitate
Aqueous phase
↓ adsorb on Diaion HP20 elute with deionized water
Combine fractions containing MM 27696
↓ chromatograph on QAE Sephadex A25 elute with gradient 0.1 ~ 0.7 M NaCl in 0.05 M sodium phosphate buffer (pH 7.0)
Combine fractions containing MM 27696
↓ adsorb on Diaion HP20 elute with deionized water
Combine fractions containing MM 27696
↓ concentrate by reverse osmosis
Concentrate
↓ chromatograph on Diaion HP20 elute with deionized water
Combine fractions containing MM 27696
↓ chromatograph on Biogel P2 elute with deionized water
Combine fractions containing MM 27696
↓ chromatograph on Diaion HP20 elute with deionized water
Combine fractions containing MM 27696
↓ chromatograph on reversed phase HPLC with 0.01 M potassium phosphate buffer (pH 7.0)
Combine fractions containing MM 27696
↓ desalt on Diaion HP20 elute with deionized water
MM 27696

The structure of MM 27696 was determined by conversion to its *p*-nitrobenzyl ester. A freeze-dried sample of MM 27696 disodium salt was treated with *p*-nitrobenzyl bromide in *N*,*N*-dimethylformamide and the resulting monoester was purified by column chromatography on silica gel. The IR spectrum ($\nu_{max.}$ (KBr) 1760, 1690, 1627, 1250 and 1210 cm^{-1}) and UV spectrum ($\lambda_{max.}$ (H_2O) 325, 266 and 220 nm) were characteristic of an olivanic acid ester with the

Table 1. Paper and thin-layer chromatographic properties of MM 27696 and MM 13902.

System	Rf	
	MM 27696	MM 13902
1. TLC cellulose (Eastman-Kodak) *n*-Propanol - water, 4: 1	0.83	0.74
2. TLC DEAE cellulose 0.1 M NaCl in 0.05 M pH 7.0 phosphate buffer	0.18	0.17
3. Paper (Whatman No. 1) Butanol - pyridine - water, 1: 1: 1	0.43	0.31

Table 2. Antibacterial activity of MM 27696 disodium salt.

Organism	MIC (μg/ml)
Enterobacter cloacae N1	0.2
Escherichia coli 0111	0.8
Klebsiella aerogenes A	0.4
Proteus mirabilis C977	<0.1
Pseudomonas aeruginosa A	50
Staphylococcus aureus Oxford	0.4
Streptococcus faecalis I	12.5
Streptococcus pneumoniae CN33	<0.1

Tests were carried out by serial dilution in nutrient broth by microtitre. Inoculum was prepared by dilution of an overnight broth culture to give the equivalent of 10^6 cells/ml.

Table 3. β-Lactamase inhibitory activity of MM 27696 disodium salt.

β-Lactamase	MM 27696 (I_{50} μg/ml)
Enterobacter cloacae P99	0.002
Klebsiella aerogenes E70	0.003
E. coli JT4	0.01
S. aureus Russell	0.03

I_{50} values were determined with preincubation (5 minutes) of inhibitor with enzyme using nitrocefin (250 μg/ml) as substrate.

amidoethenylthio-substituent at C-3[5]. The NMR spectra of the *p*-nitrobenzyl ester revealed the structural difference between the new metabolite and MM 13902. The ^{1}H NMR spectrum (Table 4) was very similar to that of MM 13902, the most important difference being that the three-proton singlet due to the acetyl moiety in MM 13902 was absent and was replaced by a three-proton triplet at δ 1.07 coupled to a two-proton quartet at δ 2.31 (J=7.5 Hz). This was consistent with the presence of a propionamido function in the C-3 side-chain of MM 27696 in place of the acetamido moiety possessed by MM 13902 and the other olivanic acids. The ^{13}C NMR spectrum (Table 5) was fully in accord with these conclusions, showing the presence of 21 carbon atoms and confirming the structure of the ester

Table 4. ^{1}H NMR spectrum of the mono-*p*-nitrobenzyl ester of MM 27696.

δ (DMF-d_7)	No. of H	Multiplicity	J (Hz)	Assignment
1.07	3	t	7.5	CH_3CH_2
1.45	3	d	6	CH_3CH
2.31	2	q	7.5	CH_2CH_3
3.03	1	dd	19.5, 9.5	4-CH_a
3.73	1	dd	5.5, 11	6-CH
3.86	1	dd	19.5, 8.5	4-CH_b
4.29	1	m		5-CH
4.56	1	m		CH-CH_3
5.33	1	d	13.5	$CH_aC_6H_4$-NO_2
5.57	1	d	13.5	$CH_bC_6H_4$-NO_2
5.95	1	d	14	=CHS
7.23	1	dd	11, 14	=CHN
7.81	2	d	9	aromatic protons
8.28	2	d	9	
10.53	1	d	11	NH

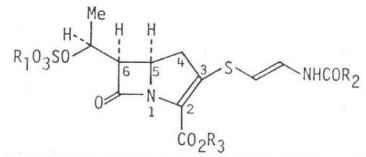
Tetramethylsilane was employed as the internal standard.

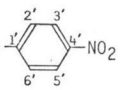
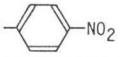
Table 5. ^{13}C NMR spectrum of the mono-*p*-nitrobenzyl ester of MM 27696.

δ (DMF-d_7)	Assignment	δ (DMF-d_t)	Assignment
9.45	CH_3CH_2	124.14	Aromatic C-3′, 5′
20.35	CH_3CH	129.03	Aromatic C-2′, 6′
29.27	CH_2CH_3	133.66	$=CHN$
37.78	4-C	144.99	Aromatic C-1′
54.53	6-C	148.21	Aromatic C-4′
59.91	5-C	154.04	2-C
65.30	$CH_2C_6H_4$–NO_2	161.30	CO_2
69.21	$CHCH_3$	171.97	$COCH_2$
98.01	$SCH=$	177.76	β-lactam CO
121.96	3-C		

Tetramethylsilane was employed as the internal standard.

Fig. 2. Structure of MM 27696, MM 13902 and their esters.



Compound	R_1	R_2	R_3
1 MM 27696	H	CH_2CH_3	H
2	Na	CH_2CH_3	
3 MM 13902	H	CH_3	H
4	Na	CH_3	

as that shown in Fig. 2, and hence the structure of MM 27696 shown in the same Fig. 2.

The discovery of MM 27696, an olivanic acid derivative with the acetyl function in the C-3 side-chain replaced by a propionyl group, suggests the possibility of the natural occurrence of a range of such compounds with altered acyl side-chains.

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