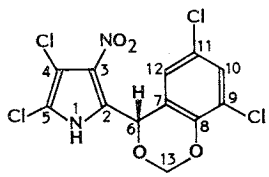
Communications to the Editor

LL-F42248α, A NOVEL CHLORINATED PYRROLE ANTIBIOTIC

Sir:

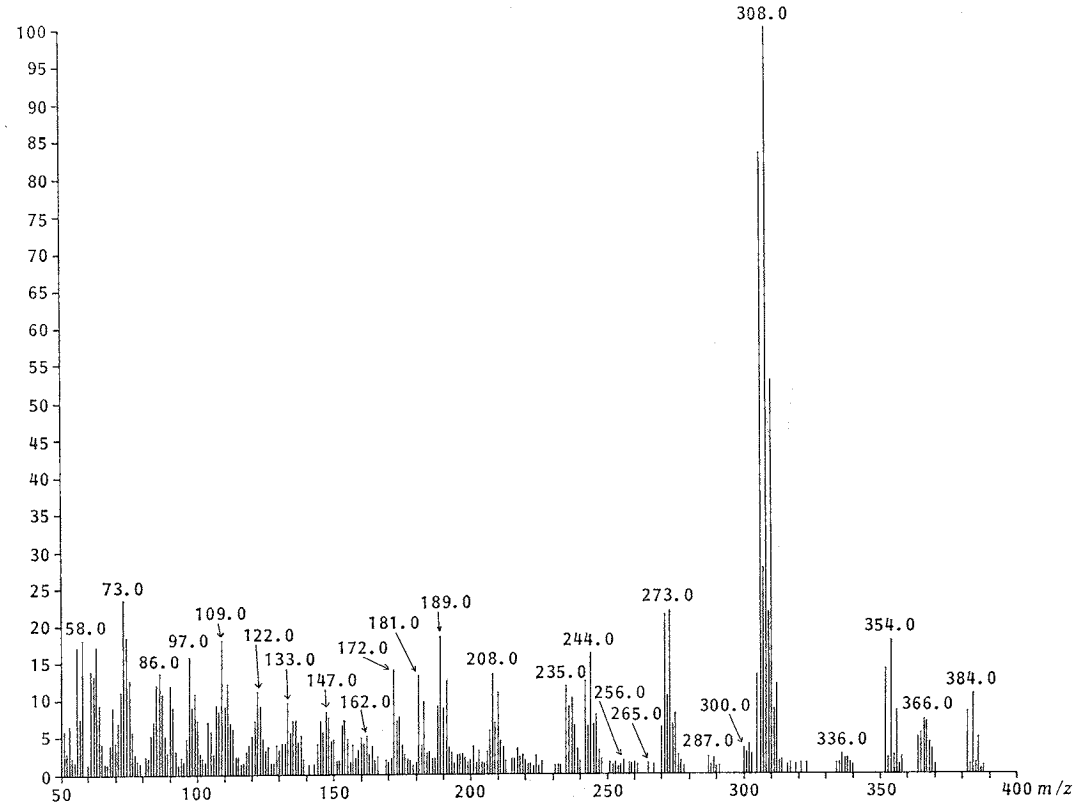
Culture LL-F42248, preliminarily identified as an unusual *Streptomyces* species, was found to produce the novel antibiotic **1**, designated LL-F42248α. This compound, which is related to the coproduced pyrrolomycin C (**2**)[1] is the first tricyclic member of this family and is the only chiral pyrrolomycin reported to date.[2]

The culture was grown in a 30-liter tank fermentor. Inoculum for the tank was prepared in stages using a seed medium consisting of glucose 1.0%, dextrin 2.0%, yeast extract 0.5%, N-Z Amine A 0.5% and $CaCO_3$ 0.1%. The inoculum was cultivated at 28°C for 3 days (two stages) and used at the rate of 5% to inoculate the fermentation tank. The medium employed in the tank consisted of molasses



1 **2**

Fig. 1. Electron impact mass spectrum of LL-F42248α.

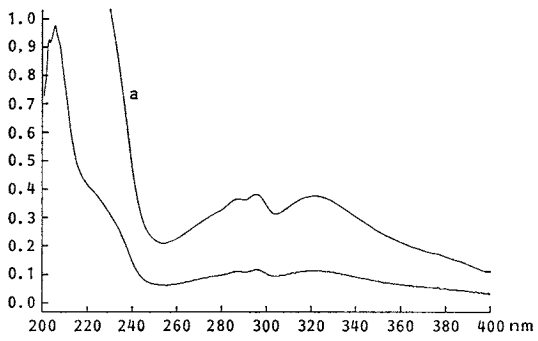


2.0%, dextrin 1.0%, soy peptone 1.0% and $CaCO_3$ 0.1%. The tank was stirred at 500 rpm aerated at 1 vol/vol/minute, and silicone antifoam agent (Hodag FD82, 25 ml) was added. At harvest (89 hours) the pH had risen to 8.4. The antibiotics were recovered by extraction of the whole broth (29 liters) with one volume of ethyl acetate. The crude extract was purified by two stages of chromatography on silica gel to yield LL-F42248α (92 mg) and pyrrolomycin C (9 mg).

LL-F42248α was readily obtained as fine yellow needles [mp 204~206°C, $[\alpha]_D^{26}$ −88° (c 0.5, MeOH)] from various solvents, including hexane-ethyl acetate mixtures. The compound is soluble in common organic solvents and practically insoluble in water.

Fig. 2. UV absorption spectrum (MeOH) of LL-F42248α.

(a) Off-scale expansion to enhance long wavelength maxima.



The electron impact mass spectrum (EI-MS) of LL-F42248α (Fig. 1) contains a molecular ion cluster between m/z 382~388. The isotopic distribution is characteristic of four chlorine atoms. High resolution measurements of the $^{35}Cl_4$ species indicated a molecular formula of $C_{12}H_6N_2O_4Cl_4$ (calcd: C 37.53, H 1.57, N 7.30, Cl 36.93 and M^+ ($^{35}Cl_4$)=m/z 381.9081; found: C 37.30, H 1.56, N 7.14, Cl 37.02 and m/z 381.9065). Evidence for the structure **1** was obtained by spectroscopic analysis. The UV spectrum (Fig. 2) contains maxima at 207 nm (ε=41,100), 286 nm (ε=5,380), 296 nm (ε=5,040) and 323 nm (ε=4,150) which correlates well with the spectra of other 3-nitropyrroles.[3] Nitro substitution is supported by strong IR bands at 1358 and 1502 cm^{-1} (Fig. 3), as well as EI-MS fragment ions at m/z 336 $(M-NO_2)^+$ and 306 $[M-(NO_2+CH_2O)]^+$. The methylenedioxy functional group was identified by the facile loss of formaldehyde in the EI-MS (m/z 351.8981) and its characteristic NMR signals (Table 1). In the ^{13}C NMR spectrum, the methylene carbon resonates at δ 91.4 while in the ^1H NMR spectrum the CH_2 protons give rise to geminally coupled doublets (J=6.2 Hz) at δ 5.37 and 5.58 ppm. The magnitude of the coupling requires the six-membered ring geometry.[4] Signals for the *meta*-coupled protons (δ 6.89 and δ 7.33, J=1.6 Hz) and N-H (δ 9.07) were also readily discerned in the ^1H NMR

Fig. 3. IR absorption spectrum (KBr) of LL-F42248α.

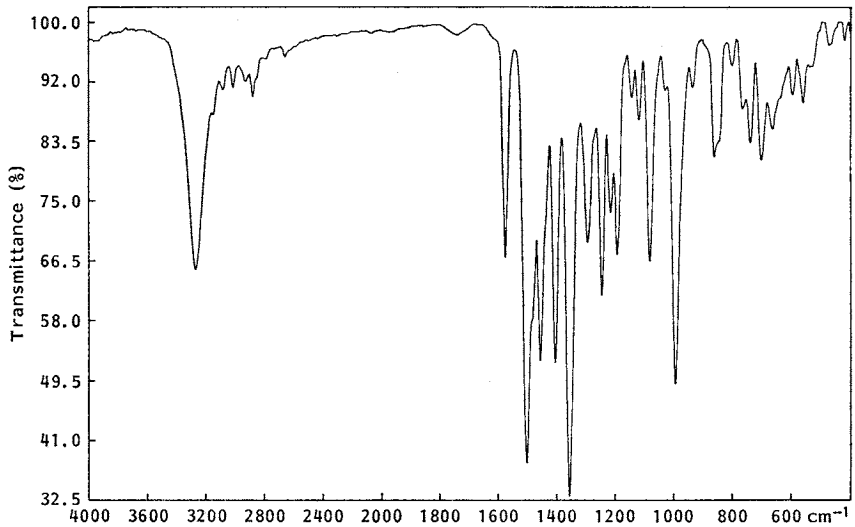
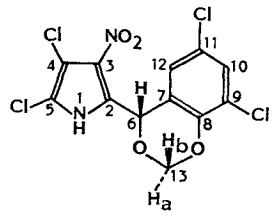


Table 1. Selected NMR data for LL-F42248α (**1**) and derivative **3**.



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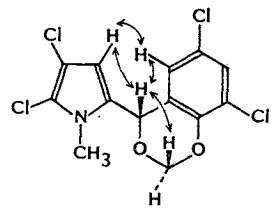

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Position	**1**				**3**				
	^{1}H NMR[a]			^{13}C NMR[b]	^{1}H NMR[a]				
	δ	M[c]	J (Hz)	δ		δ	M[c]	J(Hz)	NOE
1	9.07	br s	—		CH_3	3.65	s	—	
2				130.0					
3				131.9		5.98	s	—	H-6, H-12
4				106.0					
5				116.4					
6	6.85	s	—	69.8		6.25	s	—	H-3, H-12, H-13b
7				123.0					
8				148.7					
9				126.5					
10	7.33	d	1.6	129.9		7.44	d	2.4	
11				124.5					
12	6.89	d	1.6	126.2		7.07	d	2.4	H-3, H-6
13a	5.37	d	6.2	91.4		5.27	d	6.1	
13b	5.58	d	6.2			5.37	d	6.1	H-6

[a] $CDCl_3$, δ in ppm downfield from TMS.
[b] Acetone-d_6, δ in ppm referenced to acetone-d_6 at 29.8 ppm.
[c] Multiplicity.

Table 2. Antimicrobial spectrum of LL-F42248α (agar dilution method).

Organism	No. strains tested	MIC (μg/ml) range	Organism	No. strains tested	MIC (μg/ml) range
Bacteria[a]			*Citrobacter diversus*	1	>128
Staphylococcus aureus	4	0.03~0.25	*Pseudomonas aeruginosa*	3	>128
Streptococcus faecalis	3	0.25	Fungi[b]		
Escherichia coli	4	>128	*Trichophyton* spp.	5	8
Klebsiella pneumoniae	3	>128	*Mucor fragilis*	1	4
Enterobacter cloacae	3	>128	*Aspergillus* spp.	6	>512
Serratia marcescens	3	>128	*Candida albicans*	8	>512
Morganella morganii	3	>128	*C. parapsilosis*	5	>512
Providencia rettgeri	1	>128	*C. tropicalis*	5	>512
P. stuartii	1	>128			

[a] Bacteria tested in Mueller-Hinton agar.
[b] Fungi tested in yeast-nitrogen base agar in pH 7.0 buffer supplemented with 1% glucose and 0.15% asparagine.

spectrum. The proton attached to the bridging carbon (C-6) has a very low field chemical shift (δ 6.85) due to the deshielding influence of the two aromatic rings and attached oxygen.

The substitution patterns of the two aromatic rings were established by determining nuclear

Overhauser effects (NOE) in the denitro derivative **3**. Reaction of **1** with diazomethane yielded the N-1-methyl derivative which when treated with $NaBH_4$ in dimethyl sulfoxide[5] afforded the denitro analog **3**. NOE difference measurements on compound **3** showed the interactions listed in Table 1. The regiochemistry of the benzene ring was established by the observed NOE between H-6 and one of the *meta*-coupled benzene protons, which must therefore be bonded to C-12. It follows that the chlorine substituents are at positions 9 and 11. NMR and UV data for **1** fail to distinguish between the 3-nitro-4-chloro and 3-chloro-4-nitro pyrrole isomers, however, the NOE interaction between the proton introduced in the $NaBH_4$ reaction with both H-6 and H-12 requires placement of the nitro group at position 3. The observed NOE interaction between H-6 and H-13b indicates these are in a 1,3-diaxial orientation. The absolute configuration at position 6 has not yet been determined.

LL-F42248α is primarily active against Gram-positive bacteria with some limited antifungal activity as demonstrated by the MIC values in Table 2. The compound also is quite toxic to mammals giving an oral LD_{50} at 13 mg/kg in the mouse. The chirality and novel 1,3-dioxane ring may confer additional unique biological properties to the molecule in comparison to other members of the pyrrolomycin class.

Acknowledgments

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G. T. CARTER
J. A. NIETSCHE
J. J. GOODMAN
M. J. TORREY
T. S. DUNNE
D. B. BORDERS
R. T. TESTA

Medical Research Division,
American Cyanamid Co.,
Lederle Laboratories,
Pearl River, NY 10965, U.S.A.

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