Notes

ISOLATION AND CHARACTERIZATION OF A NEW ANTIBIOTIC, DIOXAPYRROLOMYCIN, RELATED TO PYRROLOMYCINS

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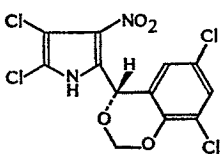
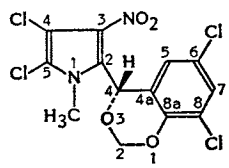
In the course of screening for new antibiotics, we have isolated dioxapyrrolomycin (**1**), which inhibits the growth of Gram-positive bacteria, some Gram-negative bacteria and some fungi, from cultured broth of *Streptomyces* sp. MG796-AF7. The structure of the *N*-methyl derivative (**2**) was determined by X-ray crystallographic analysis to be (4*S*)-6,8-dichloro-4-(4,5-dichloro-1-methyl-3-nitro-2-pyrrolyl)-1,3-benzodioxane (Fig. 1). In this report, the production, isolation, physico-chemical properties, structure and biological properties of **1** are reported.

Fig. 1. Structures of dioxapyrrolomycin and its *N*-methyl derivative.



Dioxapyrrolomycin (**1**)



N-Methyl-dioxapyrrolomycin (**2**)

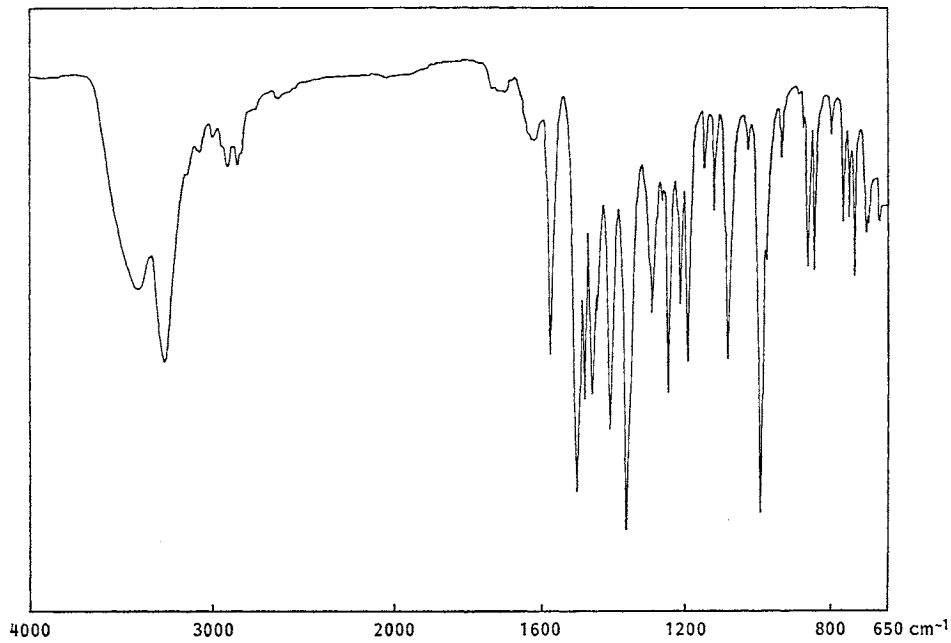
A strain which produced **1** was isolated from a garden soil sample collected in our institute and classified as *Streptomyces* sp. MG796-AF7 (strain number in the Institute of Microbial Chemistry, MG796-AF7). The strain was precultured in a 500-ml Erlenmeyer flask containing 110 ml of a medium consisting of Bacto-Soytone (Difco) 1.0%, galactose 2.0%, corn steep liquor (Nippon Shokuhin Kako Co.) 0.5%, dextrin 2.0%, $(NH_4)_2SO_4$ 0.2%, $CaCO_3$ 0.2%, silicone oil (Shin-Etsu Chemical Industry, KM-70) 0.03%, pH 7.4 before sterilization. Incubation was on a rotary shaker at 27°C for 5 days. Two ml of the precultured broth was inoculated into each 500-ml Erlenmeyer flask containing 110 ml of the same medium; the flasks were then shaken at 27°C for 4 days.

The culture filtrate (5.8 liters) thus obtained was adjusted to pH 8.0 and extracted with BuOAc. The mycelium was extracted with MeOH, the extract concd and the concentrate transferred into BuOAc. The BuOAc extracts of the culture filtrate and the mycelium were combined and concd under reduced pressure to give a brownish oil (1.22 g). The residual oil was dissolved in $CHCl_3$ and charged on a silica gel column (Merck, Art 7734, 70 g). The column was developed with $CHCl_3$ and the eluate concd under reduced pressure to give a yellow powder (157 mg). This was dissolved in hot EtOH and kept in the refrigerator to yield 59 mg of pale yellow crystals of **1**, mp 200~207°C, $[\alpha]_D^{25}$ −110° (*c* 0.5, EtOH).

Antibiotic **1** is soluble in MeOH, Me_2CO, EtOAc and ethyl ether but hardly soluble in hexane and water. It gives negative reaction with ninhydrin, but positive reaction with $KMnO_4$ and Rydon-Smith reagent.

The molecular formula of **1** was established to be $C_{12}H_6N_2O_4Cl_4$ (MW 384.00) by electron impact mass spectra (EI-MS) (M^+ *m/z* 382, 384, 386, 388 and 390). Elemental *Anal* calcd: C 37.53, H 1.57, N 7.30, Cl 36.93; found: C 37.75, H 1.77, N 6.94, Cl 42.88. UV λ_{max}^{MeOH} nm (log ε) 211 (4.44), 278 (3.78) and 320 (3.65). UV $\lambda_{max}^{0.01N\,HCl}$ nm (log ε) 211 (4.44), 273 (3.85) and 321 (3.54). UV $\lambda_{max}^{0.01N\,NaOH}$ nm (log ε) 212 (4.48), 296

Fig. 2. IR spectrum of dioxapyrrolomycin (KBr).



(3.84) and 319 (3.95). The IR spectrum is shown in Fig. 2. After structure determination, the signals of the ^{1}H NMR (400 MHz, MeOH-d_4) spectrum were assigned as follows (all protons connect to benzodioxane ring): δ 7.39 (1H, d, $J_{5,7}$=2.8 Hz, 7-H), 6.85 (1H, d, $J_{5,7}$=2.8 Hz, 5-H), 6.83 (1H, s, 4-H), 5.52 (1H, d, $J_{a,b}$=6.4 Hz and 2-H_2) and 5.44 (1H, d, $J_{a,b}$=6.4 Hz, 2-H_2). The ^{13}C NMR (100 MHz, MeOH-d_4) signals were as follows: δ 149.2 (s), 132.4 (s), 131.1 (s), 130.5 (d), 127.4 (s), 126.1 (d), 125.5 (s), 124.0 (s), 117.4 (s), 106.3 (s), 92.3 (t) and 70.4 (d).

Treatment of **1** with diazomethane gave the *N*-methylated derivative (**2**): MP 142~143°C; $[\alpha]_D^{25}$ −20° (*c* 1.0, benzene); EI-MS M^+ *m/z* 396, 398, 400, 402 and 404. The ^{1}H and ^{13}C NMR spectra are shown in Table 1. The structure of **2** was determined by X-ray crystallographic analysis as follows. Compound **2** was recrystallized in ethyl ether to give well developed pyramidal crystals. An X-ray specimen (0.1×0.4×0.15 mm in size) was mounted on a Philips PW1100 diffractometer and the intensities were measured using CuKα radiation monochromated by a graphite plate.

Crystal data: *N*-Methyl derivative of dioxapyrrolomycin, $C_{13}H_8N_2O_4Cl_4$, MW (398.0). Orthorhombic, space group $P2_12_12$, Z=4, D_{calc}=

Table 1. ^{13}C and ^{1}H NMR chemical shifts (ppm) of *N*-methyldioxapyrrolomycin.

	Carbon	Proton
Benzodioxane moiety		
C-2	92.3 t	5.38 d, 5.63 d (J=6.0)
C-4	68.9 d	7.10 br s
C-4a	123.2 s	—
C-5	124.2 d	6.72 d (J=2.4)
C-6	127.3 s	—
C-7	130.0 d	7.35 d (J=2.4)
C-8	123.1 s	—
C-8a	147.6 s	—
Nitropyrrole moiety		
N-CH_3	33.7 q	3.45 s
C-2	127.5 s	—
C-3	132.6 s	—
C-4	106.1 s	—
C-5	119.6 s	—

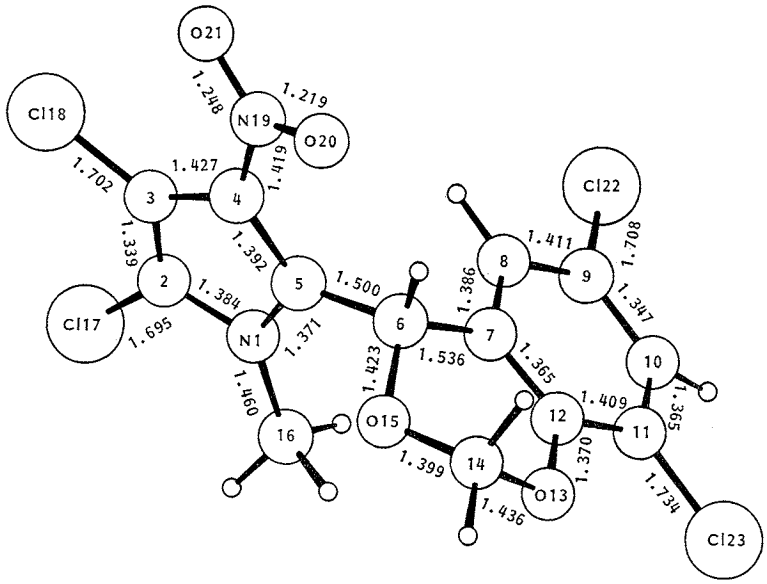
The sample was dissolved in $CDCl_3$ and recorded at 400 MHz and 100 MHz for proton and carbon, respectively (internal reference TMS). Coupling constants (Hz) are in parentheses. Signals were assigned by the aid of ^{1}H-^{13}C shift correlation spectrum and long range ^{1}H-^{13}C shift correlation spectrum.

Table 2. Antimicrobial activities of dioxapyrrolomycin.

Test organisms	Medium*	MIC (μg/ml)	Test organisms	Medium*	MIC (μg/ml)
Staphylococcus aureus FDA 209P	a	1.56	*Pseudomonas aeruginosa* A3	a	>50
S. aureus Smith	a	1.56	*Klebsiella pneumoniae* PCI 602	a	>100
S. aureus MS8710	a	1.56	*Mycobacterium smegmatis* ATCC 607	a	3.12
S. aureus MS9610	a	1.56	*Aeromonas punctata* IAM 1646	b	25
Micrococcus luteus FDA 16	a	<0.2	*A. salmonicida* ATCC 14174	b	6.25
M. luteus IFO 3333	a	0.39	*Aeromonas* sp. (KT-444)	b	6.25
M. luteus PCI 1001	a	1.56	*Vibrio anguillarum* NCMB6	b	3.12
Bacillus anthracis	a	0.78	*Pseudomonas fluorescens*	b	25
B. subtilis NRRL B-558	a	0.78	*P. lachrymans*	b	>50
B. subtilis PCI 219	a	1.56	*Erwinia aroideae*	b	>50
B. cereus ATCC 10702	a	1.56	*Candida tropicalis* F-1	c	>100
Corynebacterium bovis 1810	a	0.39	*C. pseudotropicalis* F-2	c	12.5
Escherichia coli NIHJ	a	6.25	*C. albicans* 3147	c	>100
E. coli K-12	a	25	*Candida* Yu-1200	c	>100
E. coli ML1629	a	>50	*C. krusei* F-5	c	>100
Shigella dysenteriae JS11910	a	>50	*Saccharomyces cerevisiae* F-7	c	>100
S. flexneri 4b JS11811	a	>50	*Cryptococcus neoformans* F-10	c	6.25
S. sonnei JS11746	a	>50	*Helminthosporium oryzae*	c	50
Salmonella typhi T-63	a	>50	*Piricularia oryzae*	c	50
S. enteritidis 1891	a	>100	*Pellicularia sasakii*	c	50
Proteus vulgaris OX19	a	>50	*Xanthomonas citri*	c	100
P. mirabilis IFM OM-9	a	>50	*X. oryzae*	c	3.12
P. rettgeri GN311	a	>50	*Aspergillus niger* F-16	c	>100
P. rettgeri GN466	a	25	*Trichophyton asteroides* 429	c	25
Serratia marcescens	a	>50	*T. mentagrophytes*	c	12.5

* a: Mueller-Hinton agar 37°C, b: Mueller-Hinton agar 27°C, c: nutrient agar + glucose 1%, 27°C.

Fig. 3. Molecular structure of *N*-methyldioxapyrrolomycin.



1.714 gcm^{-3}, μ for CuKα=73.5 cm^{-1}. Lattice constants, a=8.595(5), b=20.556(11), c=8.728(5) Å, U=1542 Å^3. The 1569 reflections were measured as above the 2σ (I) level, within the 2θ range of 6°~156°, out of 1918 theoretically possible ones. The 322 $\bar{h}$kl Friedel reflections were also measured immediately after the measurement of hkl reflections.

The crystal structure was determined by the direct method and refined by the block-diagonal-matrix least-squares method. All the hydrogen atoms were located on the difference electron-density map and the structure was refined to an R value of 0.092. Absolute configuration was determined by the anomalous dispersion method. The dispersion corrections for CuKα radiation were applied to Cl, O, N and C atoms. Seventy-two Friedel pairs out of 77 clearly indicated the absolute configuration shown in Fig. 3. Final refinement including dispersion corrections yielded the R value of 0.070.

The molecular structure, denoting the bond distances, is shown in Fig. 3†.

† Atomic coordinates have been deposited with the Cambridge Crystallographic Data-base and the list of Fo and Fc and other data may be obtained from one of the authors (HIKARU NAKAMURA) upon request.

Antibiotic **1** is closely related structurally to pyrrolomycins[1,2], which have chlorinated nitropyrrole and the dichlorophenol ring. The former is different from the latter in its dichlorobenzodioxane moiety instead of the dichlorophenol of the latter.

The antimicrobial activities of **1** are shown in Table 2. It inhibited the growth of Gram-positive bacteria, some Gram-negative bacteria and some fungi. The LD_{50} (ip) of **1** in mice was in the range of 125~250 mg/kg.

Addendum in Proof

As already reported, this antibiotic is identical with antibiotic Al-R2081 [RENGARAJU, S.; S. NARAYANAN, P. L. GANJU, M. A. AMIN, M. R. S. IYENGAR, J. ITOH, Y. TAKEUCHI, K. FUJITA, S. MIYADOH, T. SHOMURA, M. SEZAKI & M. KOJIMA: A new antibiotic Al-R2081 related to pyrrolomycin B. Meiji Seika Kenkyu Nempo (Scientific Reports of Meiji Seika Kaisha) 24: 48~51, 1985]. Recently, CARTER *et al.* (CARTER, G. T.; J. A. NIETSCHE, J. J. GOODMAN, M. J. TORREY, T. S. DUNNE, D. B. BORDERS & R. T. TESTA: LL-F42248α, a novel chlorinated pyrrole antibiotic. J. Antibiotics 40: 233~236, 1987) reported that antibiotic LL-F42248α is structurally identical with dioxapyrrolomycin.

References

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2) KOYAMA, M.; N. EZAKI, T. TSURUOKA & S. INOUYE: Structural studies on pyrrolomycins C, D and E. J. Antibiotics 36: 1483~1489, 1983