MYROCIN C, A NEW DITERPENE ANTITUMOR ANTIBIOTIC FROM *MYROTHECIUM VERRUCARIA*

II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE DETERMINATION

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The structure of a new antitumor antibiotic, myrocin C, from a strain of *Myrothecium verrucaria* was characterized as a pentacyclic pimarane diterpene composed of a γ-lactol group and a unique cyclopropane ring on the basis of its physico-chemical properties and spectroscopic data as well as a single-crystal X-ray diffraction analysis of its monoacetyl derivative.

In the previous paper,[1] we reported the isolation procedure and biological activities of a new diterpene antitumor antibiotic, myrocin C (**1**), which was produced by a culture of *Myrothecium verrucaria* strain No. 55 and was characterized by broad spectrum antimicrobial activity against various Gram-positive bacteria, fungi and yeasts, and a therapeutic effect in mice infected with Ehrlich ascites carcinoma. The structure of **1** has been described in our preliminary communication.[2] This paper deals with a full account of our physico-chemical properties and structure determination of **1**.

1 was isolated as colorless needles, mp 178~180°C, $[\alpha]_D^{20}$ +43.5° (*c* 0.35, MeOH), soluble in methanol, chloroform, ethyl acetate and acetone, while insoluble in *n*-hexane, benzene and water. **1** showed positive color reactions to potassium permanganate (decolorized), 2,4-dinitrophenylhydrazine (brown) and iodine vapor, but negative to ferric chloride, 2,6-dichloroindophenol, ninhydrin and Dragnedorff reagents. It gave Rf values of 0.47 (benzene - EtOAc - MeOH, 85 : 10 : 5), 0.32 (*n*-hexane - acetone, 7 : 3) and 0.27 ($CHCl_3$ - acetone, 9 : 1) on silica gel TLC.

The IR spectrum (Fig. 1) of **1** had bands characteristic of hydroxyl groups (3350 cm^{-1}), γ-lactone (1760 cm^{-1}) and α,β-unsaturated ketone (1680 and 1610 cm^{-1}). The UV spectrum of **1** in methanol showed an absorption maximum at 251 nm (ε 11,400), supporting the presence of an α,β-unsaturated ketone in its molecule. The molecular formula of **1** was established as $C_{20}H_{24}O_6$ by using fast atom bombardment (FAB)-MS (*m/z* 327 $(MH-H_2O)^+$), high-resolution electron impact (HREI)-MS (*m/z* 326.1496 $(M-H_2O)^+$, *Anal* Calcd for $C_{20}H_{22}O_4$: 326.1517) and elemental analysis (*Anal* Calcd for $C_{20}H_{24}O_5$: C 69.77, H 6.98, Found: C 69.65, H 7.00).

The ^{13}C NMR spectrum††† (25 MHz, $CDCl_3$) (Fig. 2), the insensitive nuclei enhanced by polari-

††† In the ^{13}C NMR spectrum (Fig. 2) only 17 peaks were observed because two peaks at δ_C 23.9 and 24.6, and three peaks at δ_C 28.8, 29.1 and 29.2 overlapped, respectively.

Fig. 1. IR spectrum of myrocin C (1) in KBr.

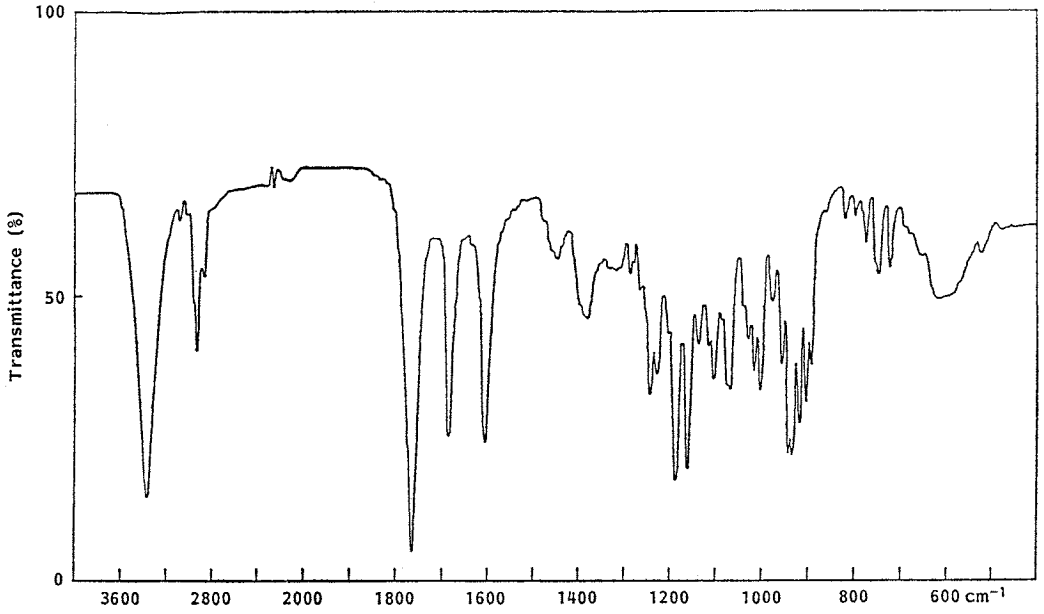
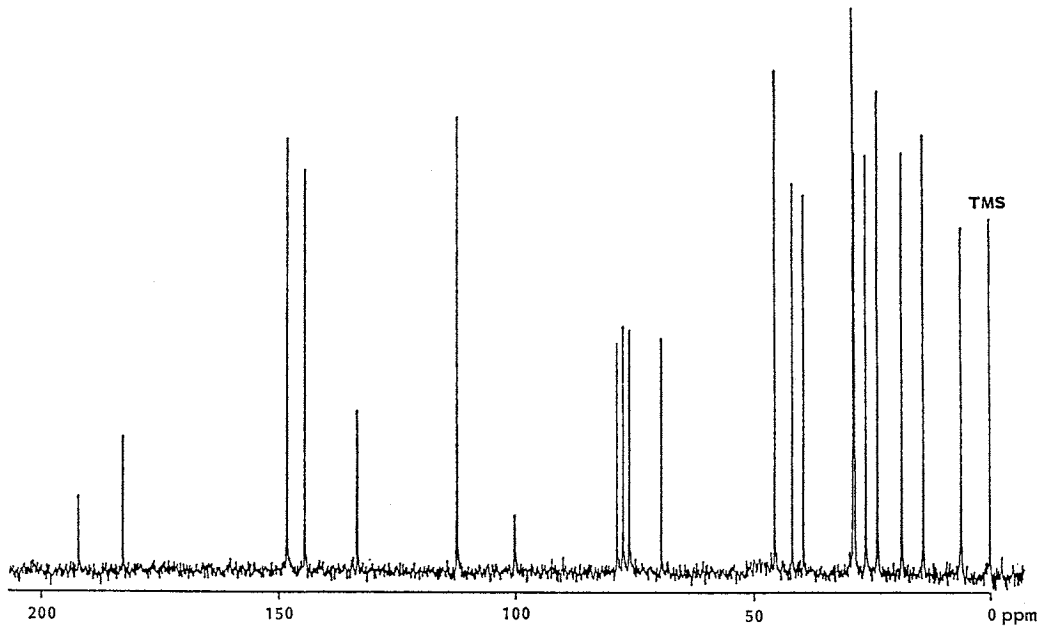


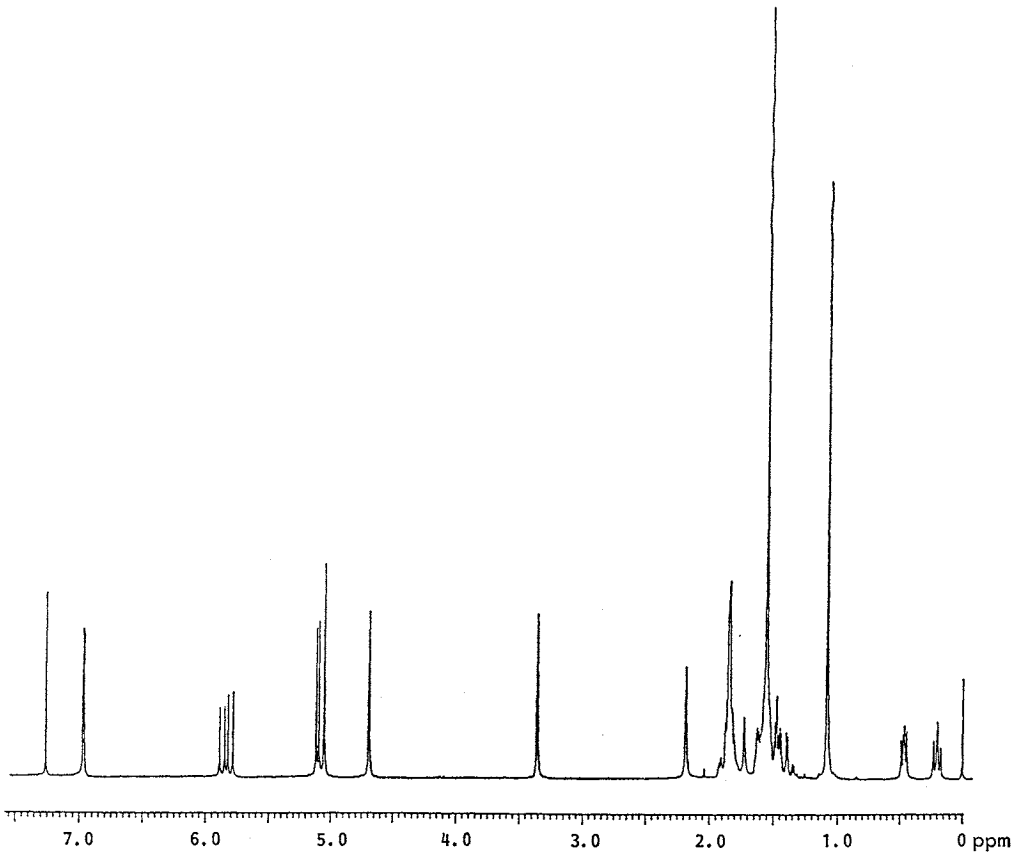
Fig. 2. ^{13}C NMR spectrum (25 MHz, $CDCl_3$) of myrocin C (1).



zation transfer (INEPT) experiment and ^{1}H-^{13}C shift correlation 2D NMR spectrum (COSY) of **1** indicated the presence of two carbonyl carbons, four olefinic carbons, two methyl carbons, five methylene carbons, two methine carbons and five quaternary carbons including two oxygen-bearing atoms (δ_C 69.4 and 100.6).

The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) (Fig. 3) of **1** was quite consistent with the ^{13}C NMR assignments, suggesting the presence of two tertiary methyl groups (δ_H 1.09 and 1.56), a cyclopropyl

Fig. 3. ^1H NMR spectrum (400 MHz, $CDCl_3$) of myrocin C (**1**).



group (δ_H 0.21 and 0.48), an isolated vinyl group (δ_H 5.01, 5.15 and 6.98). The ^1H and ^{13}C NMR spectral data of **1** are listed in Tables 1 and 2, respectively. Two partial structures A and B, as shown in Fig. 4, were revealed by ^1H-^1H and ^1H-^{13}C shift correlation 2D NMR spectra in connection with the detailed proton spin decoupling experiments, where two sets of long-range couplings (W_1 and W_2) were observed between the methine proton at δ_H 3.45 (5-H) and one of the cyclopropyl methylene protons at δ_H 0.21 (20-Ha), and between the olefinic proton at δ_H 6.98 (14-H) and one of the methylene protons at δ_H 1.58 (12-Ha). Two deuterium exchangeable signals in D_2O at δ_H 2.20 and 4.75 (each 1H, s), and two fragment ion peaks at *m/z* 327 ($(MH-H_2O)^+$) and *m/z* 309 ($(MH-2H_2O)^+$) in the FAB-MS indicated the presence of two tertiary hydroxyl groups.[3]

Acetylation of **1** (Ac_2O - pyridine) gave a monoacetate (**2**) with hydroxyl absorptions (3480 and 3375 cm^{-1}) in the IR spectrum. The ^1H (270 MHz) and ^{13}C NMR (25 MHz) spectra of **2** in $CDCl_3$, as recorded in Tables 1 and 2, revealed the signal due to the acetyl group (δ_H 2.16 (3H, s) and δ_C 20.9 (q), 171.1 (s)). The IR spectrum of **2** showed a characteristic acetoxy-γ-lactone absorption band at ν 1800 cm^{-1}, which was 40 cm^{-1} higher than that of **1**, suggesting the presence of a γ-lactol moiety in the original molecule.[4,5] Since there were five oxygen atoms in **1** and three of them existed in an ester group (δ_C 183.5) and a ketone group (δ_C 193.0), the quaternary carbon at δ_C 100.6 (C-6), which is attributable to an acetal function, must have been bonded to a hydroxyl group to form a γ-lactol

Table 1. ^1H NMR data of myrocin C (**1**) and its monoacetate (**2**).

Proton	δ (J, Hz)	
	1[a]	**2**[b]
1-H	1.62 m	1.61~1.65 m
2-Ha	1.76~1.83 m	1.54~1.57 m
2-Hb	1.76~1.83 m	1.91~1.93 m
3-Ha	1.46 m	1.91~1.93 m
3-Hb	1.76~1.83 m	1.91~1.93 m
5-H	3.45 br s	3.50 br s
6-CH_3		2.16 s
11-Ha	1.39 ddt (14.2, 13.6, 3.6)	1.24 m
11-Hb	1.48 ddd (13.6, 3.6, 3.6)	1.61~1.65 m
12-Ha	1.58 dddd (13.6, 3.6, 3.6, 1.5)	1.61~1.65 m
12-Hb	2.05 ddd (14.2, 13.6, 3.6)	2.07 m
14-H	6.98 d (1.5)	6.99 d (1.7)
15-H	5.85 dd (17.5, 10.7)	5.84 dd (17.5, 10.4)
16-Ha	5.01 dd (10.7, 1.0)	5.08 d (17.5)
16-Hb	5.15 dd (17.5, 1.0)	5.07 d (10.4)
17-CH_3	1.09 s	1.08 s
18-CH_3	1.56 s	1.50 s
20-Ha	0.21 ddd (8.5, 6.5, 1.0)	0.26 dd (7.9, 6.8)
20-Hb	0.48 dd (6.5, 4.7)	0.39 dd (6.8, 4.9)
	2.20 br s (OH)[c]	4.33 br s (OH)[c]
	4.75 br s (OH)[c]	

[a] 400 MHz; in $CDCl_3$ with TMS as an internal standard.
[b] 270 MHz; in $CDCl_3$ with TMS as an internal standard.
[c] Exchangeable with D_2O.

system as shown in partial structure C. The remaining carbons were a methyl (C-18) and two sp^3 quaternary carbons (C-13 and C-9) as shown in partial structures D~F.

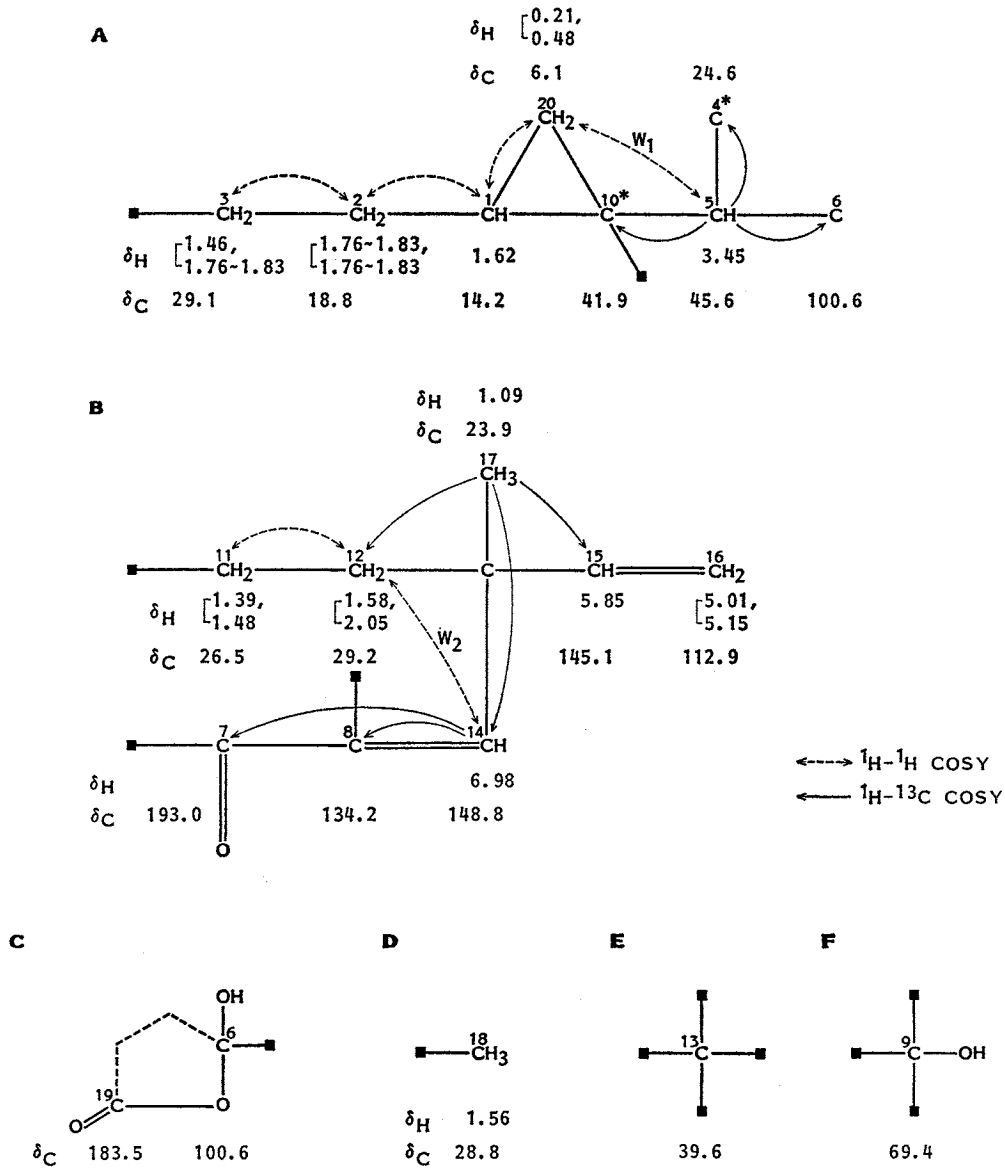
Judging from the connections of partial structures A~F, and combining with the index of unsaturation (nine degrees) derived from the molecular formula, **1** must be pentacyclic with a pimarane skeleton. In order to clarify the complete structure, a single-crystal X-ray analysis was carried out using the crystal of **2** obtained from a *n*-hexane - ethyl acetate (1 : 4) solution. Fig. 5 shows a stereoscopic view of the molecular structure of **2**. The X-ray experiment defined only the relative, not the absolute stereochemistry. Therefore, the structure of myrocin C (**1**) was finally determined to be 1,20-cyclo-6,6,9-trihydroxy-19-oic-7-oxo-8,15-pimaradiene-19,6-γ-lactone as shown in Fig. 6, except for the absolute configuration at the asymmetric centers of C-1, C-4, C-5, C-6, C-9, C-10 and C-13 is now in progress.

Table 2. ^{13}C NMR data of myrocin C (**1**) and its monoacetate (**2**).

Carbon	δ	
	1[a]	**2**[a]
C-1	14.2 (d)	13.8 (d)
C-2	18.8 (t)	18.6 (t)
C-3	29.1 (t)	29.1 (t)[b]
C-4	24.6 (s)[b]	24.3 (s)[b]
C-5	45.6 (d)	45.6 (d)
C-6	100.6 (s)	101.6 (s)
C-7	193.0 (s)	186.4 (s)
C-8	134.2 (s)	134.1 (s)
C-9	69.4 (s)	69.0 (s)
C-10	41.9 (s)[b]	40.4 (s)[b]
C-11	26.5 (t)	25.6 (t)
C-12	29.2 (t)	29.2 (t)[b]
C-13	39.6 (s)	39.6 (s)
C-14	148.8 (d)	149.5 (d)
C-15	145.1 (d)	144.6 (d)
C-16	112.9 (t)	112.9 (t)
C-17	23.9 (q)	24.1 (q)
C-18	28.8 (q)	28.4 (q)
C-19	183.5 (s)	180.0 (s)
C-20	6.1 (t)	6.6 (t)
6-O*C*OCH$_3$		171.1 (s)
6-OCO*C*H$_3$		20.9 (q)

[a] 25 MHz; in $CDCl_3$.
[b] These assignments may be interchangeable.
( ): Multiplicity.

Fig. 4. Partial structures of myrocin C (1).



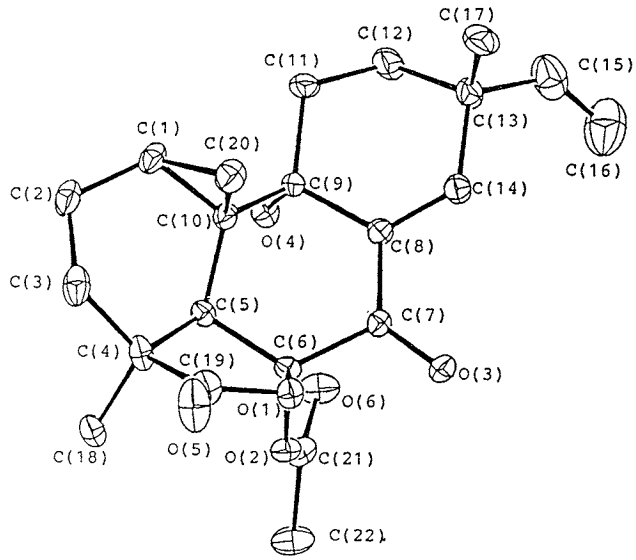
* Assignments may be interchanged.

Experimental

General

MP's were taken with a Yanagimoto Co. melting point apparatus and are uncorrected. The optical rotation was measured with a Jasco DIP-SL polarimeter. IR spectra were obtained using a Jasco IRA-2 infrared spectrophotometer. UV spectra were taken with a Shimadzu UV-180 double beam spectrometer. The HR-MS and FAB-MS were obtained with a Jeol JMS D-300 mass spectrometer, and the LR-MS, with a Hitachi RMU-6M mass spectrometer at 20 eV. The ^{1}H and ^{13}C NMR spectra were recorded on Jeol FX-400, Jeol JNM-GX 270 and Jeol FX-100 spectrometers with TMS as the internal standard. TLC was performed on pre-coated plates of Merck Kieselgel

Fig. 5. A perspective view of myrocin C monoactate (**2**).



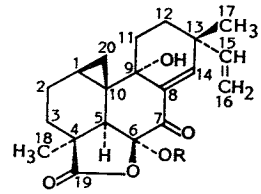
Hydrogens are omitted for clarity and no absolute configuration is implied.

60 GF_{254} and detected with iodine vapor and UV lamp at 254 nm.

Acetylation of **1**

To a solution of **1** (30 mg) in pyridine (0.5 ml), acetic anhydride (0.5 ml) was added and stirred for 12 hours at room temp. The reaction mixture was poured into ice water and extracted with ethyl acetate. The extract was washed with diluted HCl followed by 10% $NaHCO_3$ and dried over anhydrous Na_2SO_4. The solution was evaporated and recrystallized from *n*-hexane - EtOAc (2:3) to give **2** as colorless needles in 90% yield.

Fig. 6. Structures of myrocin C (**1**) and its monoacetate (**2**) with relative configuration.



1 R=H
2 R=$COCH_3$

2: MP 234~235°C (dec); $[\alpha]_D^{20}$ +120° (*c* 0.2, $CHCl_3$); its molecular formula was determined to be $C_{22}H_{26}O_6$ by elemental analysis (*Anal* Calcd for $C_{22}H_{26}O_6$: C 68.39, H 6.74, Found: C 68.46, H 6.71) and EI-MS (*m/z* 386 $(M)^+$); IR ν_{max}^{KBr} cm^{-1} 3480, 3375, 2930, 2875, 1800, 1770, 1725, 1690, 1610, 1450, 1380, 1310, 1250, 1195, 1165, 1110, 1075, 1020, 1000, 970, 940, 920, 875, 800, 745, 720, 630, 530.

X-Ray Diffraction Analysis of **2**

A crystal of approximate dimensions 0.3×0.3×0.5 mm of **2** was selected for the analysis. Crystal data: $C_{22}H_{26}O_6$, orthorhombic, space group $P2_12_12_1$, a=10.091(3), b=28.863(9), c=6.785(2) Å D_{calcd}=1.299 gcm^{-3}, Z=4. All diffraction maxima with $2° \leq 2\theta \leq 55°$ were collected on a computer-controlled four-circle diffractometer using graphite monochromated Mo-Kα radiation (0.71068 Å) and ω-scan mode. A total of 2739 reflections was collected and after correction for Lorentz polarization and background effects, 1830 (66.8%) were judged observed [$|F_o| \geq 3\sigma(F_o)$]. The structure was solved by direct method using MULTAN 80. Block-diagonal least-squares refinements with anisotropic temperature factors for nonhydrogen and isotropic ones for hydrogen atoms have finally converged to a final R value of 0.068. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.

Acknowledgments

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