11#-Hydroxy-D: A-Friedoolean-1-en-3-one from the Stem Bark of Phyllanthus flexuosus

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11β-HYDROXY-D: A-FRIEDOOLEAN-1-EN-3-ONE FROM THE STEM BARK OF *PHYLLANTHUS FLEXUOSUS*

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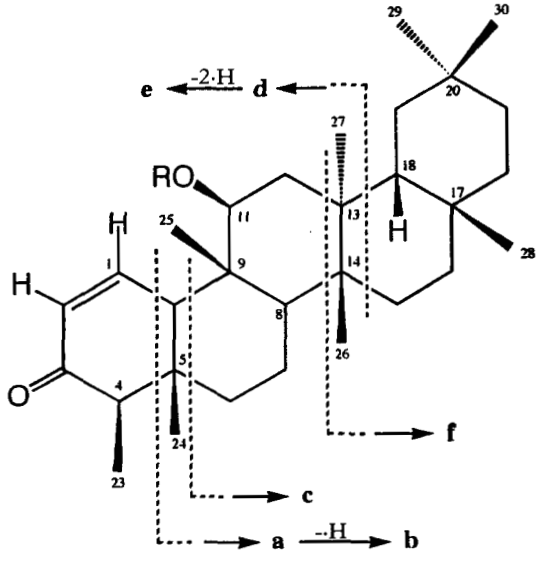
ABSTRACT.—A new pentacyclic triterpene ketol was isolated from the stem bark of *Phyllanthus flexuosus* and the structure was established on the basis of spectral data interpretation and single-crystal X-ray analysis as 11β-hydroxy-D: A-friedoolean-1-en-3-one bearing a stretched conformation of the *cis*-fused D and E rings.

Previously, we have reported that extracts of the leaves and the stem bark of *Phyllanthus flexuosus* (Sieb. et. Zucc.) Muell.-Arg. (Euphorbiaceae) contain *ent*-3β-hydroxykaur-16-ene and 14 triterpenes including ocotillol-II, oleana-9(11),12-dien-3β-ol, oleana-11,13(18)-dien-3β-ol, olean-12-en-3β,15α-diol, olean-12-en-3β,24-diol, oleana-11,13(18)-dien-3β,24-diol, olean-12-en-3β,15α,24-triol, lup-20(29)-en-3β,15α-diol (1–3) and trichadenic acid B (4), together with bergenin (1) and several novel tannins (5). Further investigation of the CH_2Cl_2 extract of the stem bark has led to the isolation of an unidentified triterpene ketol [**1**]. This paper describes the characterization of **1**.

RESULTS AND DISCUSSION

Compound **1** was assigned a molecular formula of $C_{30}H_{48}O_2$ from the hreims. It gave a positive purple color with the Liebermann-Burchard reagent and showed the presence of a hydroxyl group (ν max 3475 and 1073 cm^{-1}) and an α,β-unsaturated cyclohexenone ring (λ max 234 and 259 nm; ν max 1665 cm^{-1}) in its uv and ir spectra. The ^1H- and ^{13}C-nmr spectra (Table 1) exhibited signals for seven quaternary methyl groups, one secondary methyl group [δ_H 1.00 (3H, d); δ_C 6.75 (q)], an axially oriented secondary carbinolic methine proton [δ_H 3.73 (1H, dd); δ_C 77.43 (d)], two protons on a cis-



1 R=H
2 R=Ac

TABLE 1. ^{1}H- (300 MHz) and ^{13}C-Nmr (74.5 MHz) Chemical Shifts of Compounds **1** and **2** in $CDCl_3$ Solution (TMS=0).[a]

Position	Compound			
	1		**2**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	8.08 dd (10.8, 2.0)	129.49 (d)	7.22 dd (10.8, 2.0)	130.20 (d)
2	6.02 dd (10.8, 3.1)	153.43 (d)	5.97 dd (10.8, 3.1)	150.47 (d)
3		201.65 (s)		201.17 (s)
4		57.80 (d)		57.91 (d)
5		44.15 (s)		44.58 (s)
6		39.91 (t)		39.76 (t)
7		17.26 (t)		16.92 (t)
8		51.54 (d)		51.79 (d)
9		42.83 (s)		41.42 (s)
10		37.38 (d)		31.81 (d)
11	3.73 dd (11.3, 4.8)	77.43 (d)	4.94 dd (11.3, 4.8)	80.98 (d)
12		42.23 (t)		37.06 (t)
13		38.27 (s)		38.30 (s)
14		41.31 (s)		41.22 (s)
15		32.23 (t)		32.32 (t)
16		35.38 (t)		35.74 (t)
17		30.01 (s)		29.99 (s)
18		42.54 (d)		42.37 (d)
19		35.52 (t)		35.52 (t)
20		28.16 (s)		26.13 (s)
21		32.63 (t)		32.51 (t)
22		39.28 (t)		39.34 (t)
23	1.00 d (6.5)	6.75 (d)	0.99 d (6.5)	6.82 (d)
24	0.82 s	14.02 (q)	0.81 s	14.10 (q)
25	1.00 s	15.10 (q)	1.06 s	15.76 (q)
26	1.02 s	19.86 (q)	1.11 s	19.17 (q)
27	1.10 s	19.62 (q)	1.17 s	22.01 (q)
28	1.19 s	32.09 (q)	1.19 s	32.03 (q)
29	1.01 s	31.64 (q)	0.99 s	31.35 (q)
30	0.96 s	35.06 (q)	0.95 s	35.18 (q)
OCOMe	—	—	2.09 s	20.23 (q)
OCOMe	—	—	—	170.40 (s)

[a]Assignments were made by 2D ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY nmr experiments.

disubstituted double bond in a conjugated enone system combined with an allylic methine group [δ_H 6.02 (1H, dd, J=10.8 and 3.1 Hz) and 8.08 (1H, dd, J=10.8 and 2.0 Hz); δ_C 129.43 (d) and 153.43 (d)]. Acetylation of **1** in the usual manner afforded a monoacetate [**2**], in which the ^{1}H-nmr signal of the carbinolic methine group was shifted to low-field at δ 4.94 (1H, dd). Conversely, two proton signals of the conjugated enone grouping in **2** showed considerable diamagnetic shifts at δ 5.97 (1H, dd) and 7.22 (1H, dd) by the introduction of the acetoxy group, suggesting the hydroxy group of **1** to be spatially close to the double bond. In the eims spectrum, **1** showed fragment ion peaks, corresponding to the fragmentation of a D:A-friedoolean-1-en-3-one (6) bearing a hydroxyl group on the B or C ring, at m/z 357 $[C_{25}H_{41}O]^+$ (ion **a**), 356.3068 $[C_{25}H_{40}O]^+$ (ion **b**), 316.2747 $[C_{22}H_{36}O]^+$ (ion **c**), 289 $[C_{19}H_{29}O_2]^+$ (ion **d**), 287 $[C_{19}H_{27}O_2]^+$ (ion **e**), and 205.1954 $[C_{15}H_{25}]^+$ (ion **f**). Furthermore, comprehensive analyses of the above ^{1}H-nmr and eims spectral data indicated that the equatorial secondary hydroxy group in **1** may be located at the 11β-position in the D:A-friedoolean-1-en-3-one skeleton.

The 2D ^{1}H-^{1}H COSY, 2D ^{1}H-^{13}C COSY and long-range 2D ^{1}H-^{13}C COSY nmr analyses supported the above assumption. In the long-range 2D ^{1}H-^{13}C COSY nmr spectrum of **1**, the signal of Me-25 exhibited correlations with those of C-8, C-9, C-10, and C-11. Figure 1 summarizes the observed ^2J and ^3J H-C correlations. Accordingly, the structure of compound **1** must be 11β-hydroxy-D:A-friedoolean-1-en-3-one.

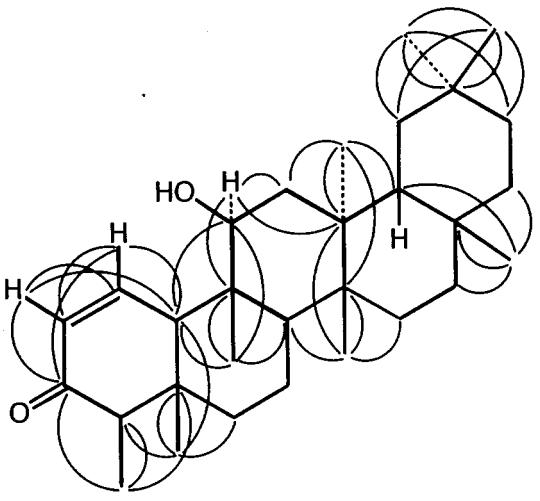


FIGURE 1. Long-range ^{1}H-^{13}C COSY interactions of **1**.

The complete structure involving stereochemistry was established by single-crystal X-ray analysis. The crystal structure of **1** was solved by direct methods. The non-hydrogen atom fractional coordinates and thermal parameters are listed in Table 2. Figure 2 shows the ORTEP diagram of **1** together with the atomic numbering scheme. In this structure, the A ring has a twisted-chair conformation due to the presence of the Δ^1-en-3-one chromophore, while the *cis*-fused D and E rings adopt a deformed boat form and a boat form, respectively, characterizing the stretched conformation in spite of the absence of any other substituents on the C, D, and E rings (7). As shown by the stereoview of the crystal packing in Figure 3, the structures of compound **1** are stabilized by intermolecular O(2)-H...O(1) hydrogen bonds [O(2)...O(1)=2.80(1) Å] and by van der Waals contacts among the neighboring molecules.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were measured on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were taken in $CHCl_3$ using a Jasco DIP-140 polarimeter. Uv spectra were run in EtOH employing a Hitachi 150-20 spectrophotometer. Ir spectra were recorded as KBr disks with a Perkin-Elmer 1720X Ft-ir spectrometer. ^{1}H- and ^{13}C-nmr spectra were obtained in $CDCl_3$ on a Varian XL-300 instrument at 300 MHz and 74.5 MHz, respectively, using TMS as internal standard. Eims were recorded at 70 eV (probe) on a Hitachi M-80 double-focusing mass spectrometer equipped with a M-003 data processor. Si gel 60 (Merck, 70–230 mesh) and alumina 90 (Merck, 70–230 mesh) were used for cc. Si gel HF_{254} (Merck, 0.25 mm thick) and PF_{254} (Merck, 1 mm thick) were employed for tlc and prep. tlc, respectively.

PLANT MATERIAL AND EXTRACTION AND ISOLATION.—The extraction and isolation of lup-20(29)-en-3β,15α-diol, olean-12-en-3β,15α-diol and ocotillol-II from the CH_2Cl_2 extract of the stem bark of *P. flexuosus* (3.70 kg), collected in August 1990, in Mt. Hiei, Shiga Prefecture, Japan, has been previously reported (3). Purification by Si gel cc of the extract with $CHCl_3$ as eluent successively afforded sitosterol, mp 132–136°, *m/z* 414 $[M]^+$, 2.584 g, a gummy residue [**A**] (3.359 g) and a triterpene mixture [**B**] (3.70 g), from fraction nos. 65–75, 76–90, and 91–136 (each fraction, 500 ml). Among these products, the

TABLE 2. Non-Hydrogen Atom Fractional Coordinates and Thermal Parameters of Compound **1**, with Estimated Standard Deviation in Parentheses.

Atom	x	y	z	U_{eq}
C(1)	0.4604(6)	0.0778(4)	0.8404(6)	0.060(4)
C(2)	0.4758(7)	−0.0029(5)	0.8219(8)	0.072(5)
C(3)	0.4355(6)	−0.0659(4)	0.9013(7)	0.054(4)
C(4)	0.3967(5)	−0.0360(3)	1.0162(5)	0.045(3)
C(5)	0.3391(4)	0.0471(3)	0.9964(5)	0.035(3)
C(6)	0.3001(5)	0.0785(4)	1.1140(6)	0.046(3)
C(7)	0.2637(5)	0.1683(4)	1.1060(6)	0.042(3)
C(8)	0.3439(4)	0.2275(3)	1.0655(4)	0.031(3)
C(9)	0.3776(4)	0.2045(3)	0.9388(5)	0.035(3)
C(10)	0.4134(4)	0.1117(3)	0.9488(5)	0.034(3)
C(11)	0.4661(5)	0.2616(4)	0.9127(6)	0.048(3)
C(12)	0.4447(5)	0.3533(4)	0.9348(6)	0.043(3)
C(13)	0.4141(4)	0.3742(3)	1.0597(5)	0.034(3)
C(14)	0.3235(4)	0.3216(3)	1.0901(5)	0.036(3)
C(15)	0.3012(6)	0.3348(4)	1.2213(7)	0.054(4)
C(16)	0.3351(7)	0.4187(5)	1.2748(6)	0.064(4)
C(17)	0.3431(5)	0.4939(4)	1.1909(6)	0.049(3)
C(18)	0.3891(4)	0.4695(4)	1.0694(5)	0.038(3)
C(19)	0.4757(5)	0.5260(4)	1.0404(6)	0.048(3)
C(20)	0.4585(5)	0.6200(4)	1.0549(6)	0.048(3)
C(21)	0.4209(6)	0.6378(4)	1.1800(7)	0.059(4)
C(22)	0.4078(6)	0.5587(4)	1.2510(6)	0.055(4)
C(23)	0.3431(8)	−0.1047(4)	1.0818(7)	0.068(4)
C(24)	0.2557(5)	0.0279(4)	0.9144(7)	0.053(4)
C(25)	0.3043(6)	0.2138(4)	0.8412(6)	0.056(4)
C(26)	0.2347(5)	0.3498(4)	1.0196(7)	0.050(4)
C(27)	0.5001(5)	0.3539(4)	1.1402(7)	0.048(3)
C(28)	0.2427(6)	0.5332(5)	1.175(1)	0.074(5)
C(29)	0.3888(6)	0.6517(4)	0.9611(7)	0.061(4)
C(30)	0.5563(6)	0.6647(5)	1.0341(9)	0.070(5)
O(1)	0.4315(7)	−0.1380(3)	0.8708(6)	0.104(5)
O(2)	0.4990(6)	0.2498(3)	0.7969(5)	0.093(4)

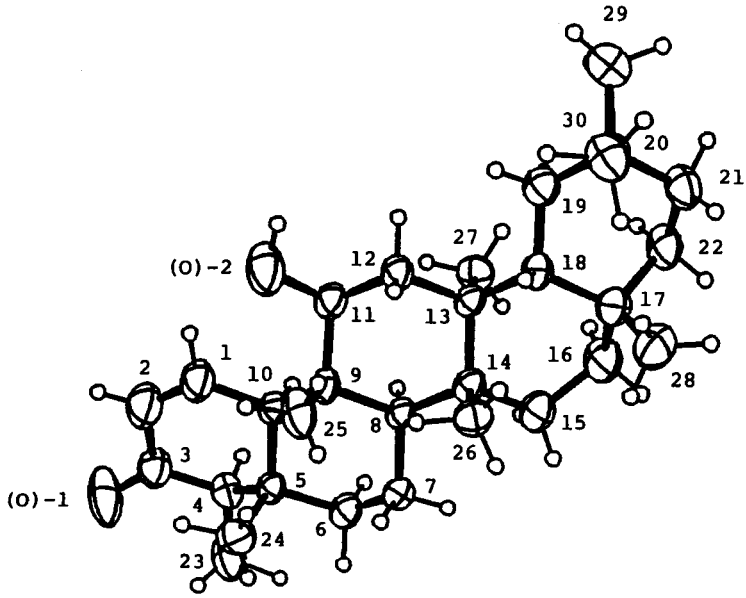


FIGURE 2. ORTEP diagram of compound **1**.

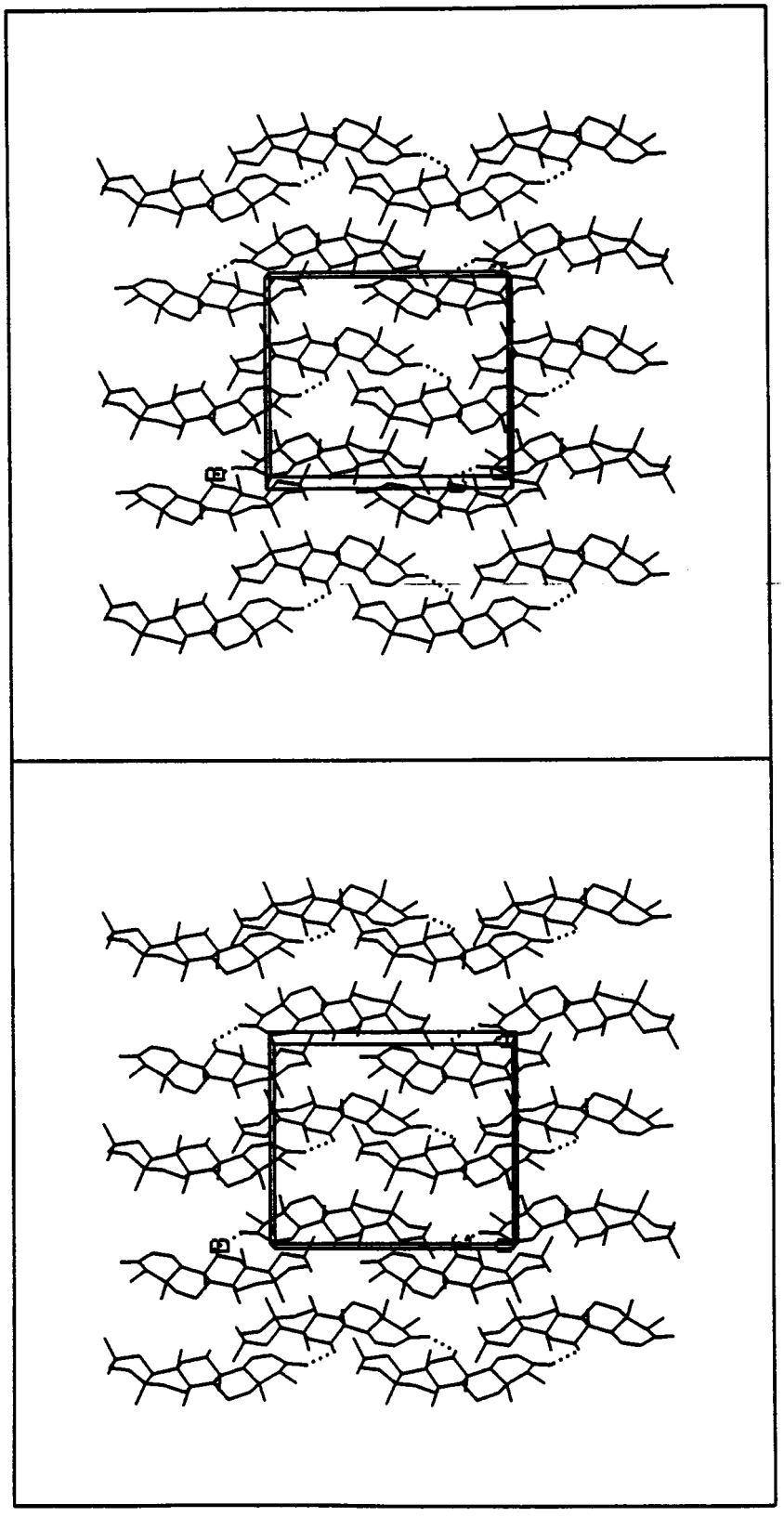


FIGURE 3. Stereoview of the crystal packing of compound **1**, viewed from the *c*-axis. Possible intermolecular hydrogen bonds are shown in dotted lines.

mixture B has already been reported to contain three triterpenoids described above (3). Repeated cc of residue **A** on alumina (150 g) furnished a crude crystalline solid (41 mg) from the fractions eluted with C_6H_6 (fraction nos. 7–18; each fraction, 100 ml), which was purified by prep. tlc (plate: 20×20 cm, 1 mm thick; solvent: C_6H_6-$CHCl_3$-EtOAc, 2:2:1) to give compound **1** (22 mg). Successive elution of the column with C_6H_6-$CHCl_3$ (10:1–5:1) afforded olean-12-en-3β,15α-diol (1.654 g), identical in all respects (mmp, $[\alpha]_D$, co-tlc, ir, ^1H- and ^{13}C-nmr, and eims) with an authentic sample mentioned above.

Compound **1**.—Prisms, mp 282.5–285° (MeOH/$CHCl_3$), $[\alpha]^{23}D$ −2.6° (*c*=0.24, $CHCl_3$), uv λ max (EtOH) (ε) 234 (4100) and 259 nm (6300) (conjugated cyclohexenone ring); ir ν max 3645 (OH), 2920, 2820, 1665 (C=C-C=O), 1466, 1450, 1383, 1375, 1210, 1195, 1173, 1115, 1073, 1060, 1018, 980, 840, 814, 780, and 740 cm^{-1}; hreims $[M]^+$ *m/z* 440.3659 ($C_{30}H_{48}O_2$ requires 440.3658); eims *m/z* $[M]^+$ 440 (16), $[M-Me]^+$ 425 (5), $[M-H_2O]^+$ 422 (8), $[M-Me-H_2O]^+$ 407 (5), (ion **a**) 357 (7), (ion **b**) 356 (27), (ion **c**) 316 (23), (ion **d**) 289 (2), (ion **e**) 287 (2), (ion **f**) 205 (100), and 191 (20).

Acetylation of **1**.—Compound **1** (13 mg) was added into an equivolume mixture of pyridine and Ac_2O (each 1 ml) and the mixture was kept at room temperature overnight. The usual workup afforded a solid, which was purified by prep. tlc (plate: 20×20 cm, 1 mm thick; solvent, $CHCl_3$) to give the corresponding keto-acetate [**2**], 13 mg, mp 207–210.5° (MeOH/$CHCl_3$), $[\alpha]^{23}D$ +1.4° (*c*=0.16, $CHCl_3$); uv λ max (EtOH) (ε) 232 (5800) and 258 nm (9600) (conjugated cyclohexenone ring); ir ν max 3060 (C=C), 2930, 2820, 1720 (OAc), 1665 (C=C-C=O), 1465, 1450, 1382, 1367, 1235 (OAc), 1210, 1180, 1073, 1015, 990, 840, 808, 774, and 738 cm^{-1}; ^1H and ^{13}C nmr, see Table 1; eims *m/z* $[M-HOAc]^+$ 422 (42), $[M-Me-HOAc]^+$ 407 (12), (ion **b**) 358 (4), [ion **a**−HOAc]$^+$ 339 (5), [ion **b**−CH_2CO] 316 (2), 273 (13), 259 (6), 218 (14), (ion **f**) 205 (100), and 191 (26).

CRYSTAL DATA OF COMPOUND **1**.—A single crystal of **1** was obtained by recrystallization from a mixed solvent of MeOH and $CHCl_3$. $C_{30}H_{48}O_2$, mol wt=440.711, orthorhombic, space group $P2_12_12_1$, *a*=13.883 (2) Å, *b*=16.040 (2) Å, *c*=11.454 (1) Å: α=β=γ=90.0°: *V*=2550.5 (5) Å^3, D_c=1.148 g cm^{-3}, *Z*=4. The intensities up to 2θ=130° were measured on a Rigaku automatic four circle diffractometer with graphite-monochromated Cu-Kα radiation. A total of 2137 reflections with $F_o > 2\sigma F_o$ was used for the structure analysis by direct methods. The non-hydrogen atoms were refined anisotropically by the block-diagonal least-squares method on a Micro VAX II computer at the Computer Center of Osaka University of Pharmaceutical Sciences. The geometrically ideal positions of H-atoms were calculated in the final refinement with isotropic thermal parameters; their electron densities were ascertained on a difference Fourier map. The structure was finally refined to *R*=0.0609 (R_w=0.0743). The atomic scattering factors and anomalous scattering corrections were taken from International Tables for X-ray Crystallography (8). The final non-hydrogen atom fractional coordinates and thermal parameters are listed in Table 2.[1] All calculations were performed using the TEXAN (9) crystallographic software package of Molecular Structure Corporation.

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[1]Atomic coordinates, thermal parameters, bond lengths and bond angles, and torsion angles for this structure have been deposited with the Cambridge Crystallographic Data Centre and may be obtained on request from Dr. Olga Kennard, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CBZ 1EZ, UK.