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Two novel 3,4-*seco*-trinorlanostane triterpenoids isolated from *Ganoderma fornicatum*

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Abstract—Two novel 3,4-*seco*-25,26,27-trinorlanostane triterpeniod compounds, fornicatins A and B (**1** and **2**) have been isolated from the fruiting body of *Ganoderma fornicatum*. The structural elucidation of **1** and **2** were accomplished by extensive NMR analysis. The relative stereochemistry of **2** was established by single crystal X-ray crystallography, which also confirmed the novel carbon skeleton of the new triterpenoid. An ether linkage of C-4 with C-7 in **1** is unprecedented in natural triterpenoids. Both compounds were tested for their inhibitory effects on rabbit platelet aggregation induced by PAF, ADP, or AA.

The genus *Ganoderma* (Ganodermataceae) has been used as folk medicine since ancient time, and it has been included in the most highly rated herb category in terms of multiple benefit and absence of side effects.[1] *Ganoderma lucidum* (lingzhi) is such an example which, lately, has gained popularity worldwide as a health-supplement, especially in the Pacific regions, like Japan, China, Korea, Taiwan, Thailand, Malaysia, Vietnam, Indonesia, and the United States. Researchers in China and Japan found that *Ganoderma* contained ganoderic acids, which have been claimed not only to lower blood pressure and cholesterol, and inhibit platelet aggregation,[2–4] but also to have antitumor and antimetastatic activities.[5] Ganoderic acids are the only known source of a group of triterpenes, which have a molecular structure similar to steroid hormones. So far, more than 110 triterpenes have been reported to be isolated from the genus *Ganoderma*.[6–14] In fact, ganoderic acids contents are used in Japan to determine lingzhi's (*G. lucidum*) quality and authenticity. Our recent investigation on the bioactive triterpenoid compounds of *Ganoderma fornicatum* led to the isolation of two novel trinorterpenoids, fornicatins A and B (**1** and **2**), which represented a novel carbon skeleton of 3,4-*seco*-25,26,27-trinorlanostane triterpenoid. The structures of both compounds were identified by extensive NMR spectroscopic means including[1] ^1H–^1H COSY, HMQC, HMBC, and ROESY techniques. The stereochemistry of **2** was also confirmed by X-ray analysis. Both compounds have been evaluated for their in vitro inhibitory activity against platelet aggregation induced by PAF, ADP, or AA. In this paper, we wish to report the isolation and structural elucidation of compounds **1** and **2**.

The fruiting body of *G. fornicatum* (R.) Pat. (1.5 kg) purchased from Honghe Prefecture of Yunnan Province, People's Republic of China, were powdered and macerated in 70% aqueous Me_2CO. The extract was filtered, concentrated in vacuo to a suitable volume, and then partitioned between water and EtOAc. Compounds **1** and **2** were isolated from the EtOAc extract.

Since the NMR spectra of most triterpenoids reported from the genus *Ganoderma* were measured in C_5D_5N, this routine solvent was also selected for our NMR experiments on the outset. However, later we found that both **1** and **2** were unstable in C_5D_5N, which complicated 2D NMR data collection. Switching to CD_3OD as the NMR solvent solved the problem.

Compound **1** gave a quasi-molecular ion peak at m/z 475 $[M-H]^-$ in its negative FABMS spectrum, and was assigned a molecular formula of $C_{27}H_{40}O_7$, which was confirmed by HRESIMS (calcd 475.2696) and NMR spectral data (Tables 1 and 2).

Keywords: *Ganoderma fornicatum*; Ganodermataceae; Trinorlanostane; Fornicatins A and B; NMR; X-ray.
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Table 1. The ^{1}H NMR data of compounds **1**–**2** (500 MHz, in ppm)

No	**1**		**2**	
	(C_5D_5N)	(CD_3OD)	(C_5D_5N)	(CD_3OD)
1	3.04–3.08 (1H, m)	2.36–2.40 (1H, overlap)	3.24–3.28 (1H, m)	2.52–2.56 (1H, overlap)
	2.21–2.25 (1H, overlap)	1.83–1.80 (1H, overlap)	2.29–2.24 (1H, overlap)	1.48–1.52 (1H, overlap)
2	2.57–2.61 (1H, overlap)	2.35–2.39 (1H, overlap)	2.63–2.67 (1H, m)	1.68–1.72 (1H, overlap)
	2.21–2.25 (1H, overlap)	2.19–2.23 (1H, m)	2.22–2.26 (1H, overlap)	2.19–2.22 (1H, overlap)
5α	2.54–2.57 (1H, overlap)	2.17–2.19 (1H, overlap)	2.48–2.52 (1H, overlap)	2.25–2.28 (1H, overlap)
6	2.16–2.21 (2H, overlap)	2.12–2.17 (2H, overlap)	2.27–2.31 (2H, overlap)	1.93–1.97 (2H, overlap)
7α	4.24 (1H, d, $J = 3.8$ Hz)	4.29 (1H, br s)	4.70 (1H, t, $J = 8.0$ Hz)	4.47 (1H, t, $J = 8.2$ Hz)
12	2.62–2.69 (2H, overlap)	2.64 (1H, d, $J = 18.2$ Hz)	2.70 (1H, d, $J = 16.4$ Hz)	2.86 (1H, d, $J = 16.4$ Hz)
		2.43 (1H, d, $J = 18.2$ Hz)	2.83 (1H, d, $J = 16.4$ Hz)	2.52 (1H, d, $J = 16.4$ Hz)
15	1.98–2.02 (1H, overlap)	1.92–1.96 (1H, m)	2.97–3.03 (1H, m)	2.52–2.56 (1H, overlap)
	1.34–1.39 (1H, overlap)	1.45–1.48 (1H, overlap)	1.65–1.70 (1H, overlap)	1.70–1.74 (1H, overlap)
16	1.96–2.00 (1H, overlap)	2.08–2.12 (1H, m)	1.95–2.01 (1H, m)	1.97–2.03 (1H, m)
	1.39–1.44 (1H, overlap)	1.48–1.51 (1H, overlap)	1.47–1.52 (1H, overlap)	1.43–1.47 (1H, overlap)
17α	1.65–1.68 (1H, overlap)	1.76–1.80 (1H, overlap)	1.62–1.67 (1H, overlap)	1.71–1.75 (1H, overlap)
18	1.03 (3H, s)	1.01 (3H, s)	1.04 (3H, s)	0.94 (3H, s)
19	1.68 (3H, s)	1.38 (3H, s)	1.48 (3H, s)	1.22 (3H, s)
20	1.46–1.50 (1H, overlap)	1.51–1.54 (1H, overlap)	1.50–1.54 (1H, overlap)	1.47–1.51 (1H, overlap)
21	0.84 (3H, d, $J = 5.6$ Hz)	0.92 (3H, d, $J = 6.5$ Hz)	0.89 (3H, d, $J = 5.7$ Hz)	0.91 (3H, d, $J = 5.7$ Hz)
22	2.44–2.48 (1H, m)	1.84–1.87 (1H, overlap)	2.46–2.50 (1H, overlap)	1.83–1.89 (1H, m)
	2.02–2.06 (1H, overlap)	1.30–1.36 (1H, m)	2.04–2.09 (1H, m)	1.30–1.35 (1H, m)
23	2.55–2.60 (1H, overlap)	2.30–2.36 (1H, m)	2.59–2.65 (1H, m)	2.33–2.39 (1H, m)
	1.43–1.48 (1H, overlap)	2.22–2.28 (1H, m)	1.44–1.48 (1H, overlap)	2.25–2.29 (1H, overlap)
28	3.80 (1H, d, $J = 10.7$ Hz)	3.39 (1H, d, $J = 11.0$ Hz)	5.04 (1H, s)	4.99 (1H, s)
	3.62 (1H, d, $J = 10.7$ Hz)	3.15 (1H, d, $J = 11.0$ Hz)	4.97 (1H, s)	4.75 (1H, s)
29	1.72 (3H, s)	1.39 (3H, s)	1.83 (3H, s)	1.79 (3H, s)
30	0.90 (3H, s)	1.06 (3H, s)	1.24 (3H, s)	1.25 (3H, s)

Obviously discernible in the ^{1}H NMR spectrum (Table 1, in C_5D_5N) were four sharp methyl singlets at δ_H 0.90 (3H, s), 1.03 (3H, s), 1.68 (3H, s), and 1.72 (3H, s), one methyl doublet at δ_H 0.84 (3H, d, $J = 5.6$ Hz), and a pair of AB doublets at δ_H 3.80 (1H, d, $J = 10.7$ Hz) and 3.62 (1H, d, $J = 10.7$ Hz). The ^{13}C NMR and DEPT (Table 2, in C_5D_5N) spectra of **1** exhibited four tertiary methyls and one secondary methyl, nine methylenes (including an oxymethylene), four methines (including an oxymethine), four quaternary carbons (including an oxygen-bearing one), two carboxylic carbonyl groups, and an α,β-unsaturated ketone group [δ_C 161.41 (s), 135.44 (s), 199.78 (s)], which was further supported by the λ_{max} absorption at 263.2 (4.6) nm in the UV spectrum.[7,15] Considering the fact that the structures of triterpenoids isolated thus far from the genus *Ganoderma* were similar to those of steroids,[8] compound **1** was tentatively presumed to have the basic skeleton of a 25,26,27-trinorlanosta-24-oic acid triterpenoid with an α,β-unsaturated ketone moiety. Furthermore, comparison of the NMR data of compound **1** with those of a known compound, methyl lucidenate H (**3**), displayed that the two compounds were structurally very similar in rings B, C, D, and side chain at C-17, except for one more carbonyl group at C-15 in compound **3**.[8] It could be deduced that compound **1** also contained a conjugated system at C-8, 9, and 11, an oxygenation at C-7, and an oxymethylene attached to C-4, which was verified by the HMBC spectrum (Fig. 1). By detailed comparison of the ^{13}C NMR data of two compounds, we found that an oxymethine (C-3) and a quaternary carbon (C-4) of **3** were replaced by a carboxylic group and an oxygen-occurring quaternary carbon, respectively, in **1**. These differences could be explained to be the result of oxidative cleavage between C-3 and C-4 of **1**, which was confirmed by the noticeable correlations of the carboxyl carbonyl carbon

Table 2. The ^{13}C NMR (DEPT) data of compounds **1**–**2** (125 MHz, in ppm)

No	**1**		**2**	
	(C_5D_5N)	(CD_3OD)	(C_5D_5N)	(CD_3OD)
1	38.0 (t)	38.1 (t)	32.7 (t)	33.0 (t)
2	29.6 (t)	29.9 (t)	30.8 (t)	30.6 (t)
3	176.4 (s)	177.9 (s)	176.5 (s)	178.0 (s)
4	87.3 (s)	87.9 (s)	146.5 (s)	147.1 (s)
5	48. (d)	48.7 (d)	45.0 (d)	45.8 (d)
6	32.1 (t)	32.6 (t)	35.5 (t)	35.4 (t)
7	72.9 (d)	74.1 (d)	67.9 (d)	69.0 (d)
8	161.4 (s)	163.7 (s)	165.3 (s)	166.8 (s)
9	135.4 (s)	136.4 (s)	137.4 (s)	138.6 (s)
10	41.6 (s)	42.2 (s)	41.3 (s)	41.9 (s)
11	199.8 (s)	202.4 (s)	200.4 (s)	202.9 (s)
12	50.9 (t)	51.6 (t)	52.1 (t)	52.6 (t)
13	45.3 (s)	46.2 (s)	47.3 (s)	48.2 (s)
14	50.9 (s)	52.0 (s)	53.4 (s)	54.2 (s)
15	30.1 (t)	30.2 (t)	32.4 (t)	32.8 (t)
16	27.2 (t)	27.8 (t)	27.8 (t)	28.3 (t)
17	50.2 (d)	51.1 (d)	50.1 (d)	50.9 (d)
18	18.0 (q)	18.7 (q)	17.7 (q)	17.8 (q)
19	25.5 (q)	25.6 (q)	22.4 (q)	22.5 (q)
20	36.2 (d)	37.1 (d)	36.2 (d)	37.1 (d)
21	18.1 (q)	18.3 (q)	18.5 (q)	18.7 (q)
22	31.9 (t)	32.1 (t)	31.9 (t)	32.3 (t)
23	31.6 (t)	32.0 (t)	31.9 (t)	32.0 (t)
24	176.4 (s)	177.9 (s)	176.5 (s)	177.7 (s)
28	71.3 (t)	71.3 (t)	115.6 (t)	115.9 (t)
29	25.0 (q)	24.9 (q)	23.4 (q)	23.5 (q)
30	24.5 (q)	24.8 (q)	27.6 (q)	27.8 (q)

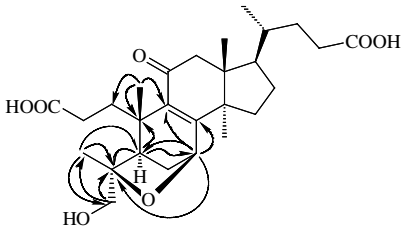


Figure 1. Key HMBC correlations of **1**.

at δ_C 176.42 (s) with H-1 at δ_H 3.06 (1H, m), and of the oxygenated quaternary carbon at δ_C 87.30 (s) with the two oxymethylene protons at δ_H 3.80 and 3.62 (each 1H, d, $J = 10.7$ Hz) and a methyl at δ_H 1.72 (3H, s) in the HMBC spectrum of **1**. Meanwhile, the outstanding HMBC crosspeak of the C-4 signal with the oxymethine proton at δ_H 4.24 (1H, d, $J = 3.8$ Hz) due to H-7, undoubtedly demonstrated that there was an oxygen-bridge between C-4 and C-7, which also accounted for the largely down-field shifted chemical signal of C-4 at δ_C 87.30. Such an ether linkage is unprecedented in natural triterpenoids.

The stereochemistry of the oxy-group at C-7 was established to be β-oriented by NOE correlation of H-7 with CH_3-30α in the ROESY spectrum (Fig. 2). The interactions of the methyl at C-4 with CH_3-18β, along with the correlation of H-5α with the oxymethylene proton [δ_H 3.62 (1H, d, $J = 10.7$ Hz)] in the ROESY spectrum of **1**, finally revealed the configurations of the methyl and the oxymethylene at C-4 to have β- and α-orientations, respectively, which also indicated that the methyl-28 was oxygenated to the oxymethylene. Thus, compound **1** was characterized as 4,7β-epoxy-28-hydroxy-11-oxo-3,4-*seco*-25,26,27-trinorlanosta-8-en-3,24-dioic acid, named fornicatin A. An attempt of growing single crystals of **1** did not succeed, and X-ray diffraction analysis of **1** could not be realized.

Compound **2** was obtained as colorless crystals and possessed a molecular formula $C_{27}H_{40}O_6$ as determined by HRESIMS (found 459.2733, calcd 459.2747), which revealed that the molecular formula of **2** comprised one oxygen less than that of **1**. Comparison of the ^{1}H NMR spectrum of **2** with that of **1** indicated that two compounds bore close similarities, except that the two doublets at δ_H 3.80 and 3.62 (each 1H, d, $J = 10.7$ Hz) arising from the oxymethylene (C-28) in **1** were replaced by two singlets at 5.04 and 4.97 (each 1H, s), typical of an *exo*-methylene, in **2**. All the above data suggested that **2** contained a double bond existing between C-4 and C-28. The presence of two carbon signals at δ_C 146.53 (s) and 115.61 (t) in the ^{13}C NMR spectrum of **2** gave support to this proposition. The assignment of all the carbons and protons of **2** was finally achieved on the basis of 2D NMR analysis, and the structure of **2** was elucidated to be 7β-hydroxy-11-oxo-3,4-*seco*-25,26,27-trinorlanosta-4(28),8-dien-3,24-dioic acid, named fornicatin B.

Fortunately, compound **2** was obtained as colorless columnar crystals after several recrystallizations. The analysis of the single crystal X-ray diffraction[16] (Fig. 3) of **2** confirmed the structure of **2** as proposed, featuring the new skeleton of 3,4-*seco*-25,26,27-trinorlanosta-3,24-dioic acid triterpenoid.

The possibility of compounds **1** and **2** being an artifact produced during the separation could be excluded since the extraction and isolation processes did not involve the use of temperatures above 60 °C or of acid and alkali. Compounds **1** and **2** are the first naturally occurring 25,26,27-trinorlanostane triterpenoids with the cleavage of the bond between C-3 and C-4 in ring A, and are structurally unique among the triterpenoids previously found in the *Ganoderma* fungi. Furthermore, both compounds are so far the most hydrophilic ganoderic acids, which have ever been discovered from the genus *Ganoderma*, because of the occurrence of an additional carboxylic group in their molecules.

According to the reports that some of those ganoderic acids could inhibit platelet aggregation,[2–4] compounds **1** and **2** were evaluated for their in vitro inhibitory activity against rabbit platelet aggregation induced by PAF (platelet activating factor), ADP (adenosine diphosphate), or AA (arachidonic acid) using the same bioassay methods as previously described.[19] Ginkgolide B (BN52021) and acetylsalicylic acid (ASA) were used as positive control, and 2% PEG (polyethylene glycol) was used as contrast. Compounds **1** and **2** both exhibited modest inhibitory activity on PAF-induced rabbit platelet aggregation. Compound **1** also displayed weak

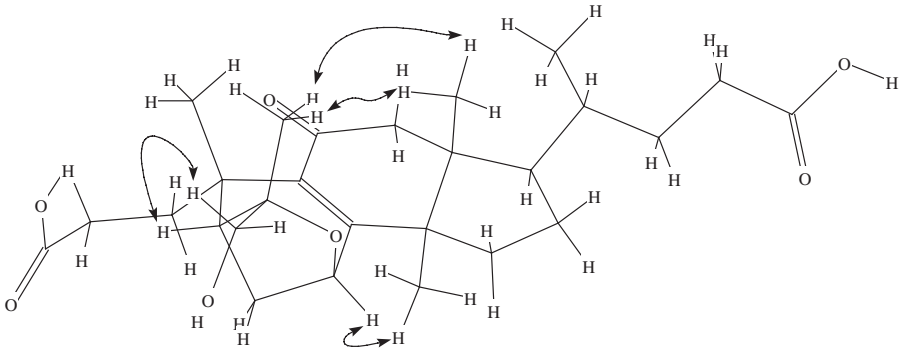


Figure 2. Key NOESY correlations of **1**.

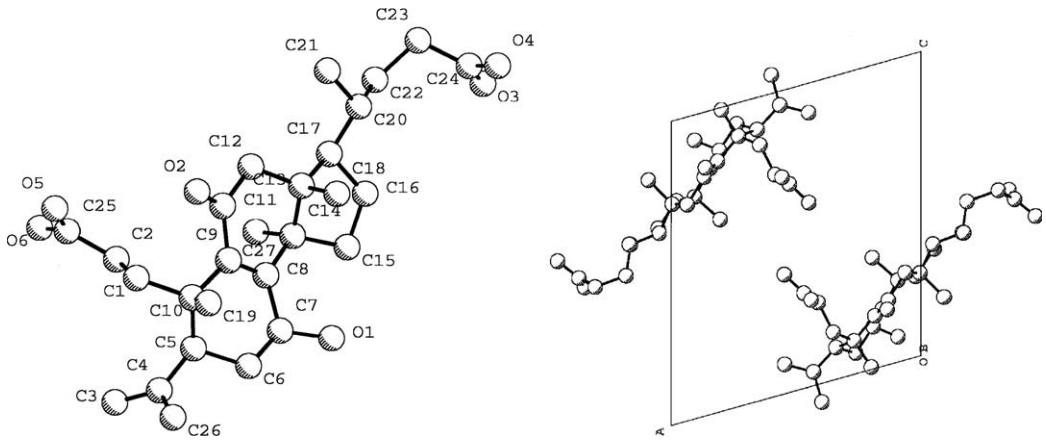


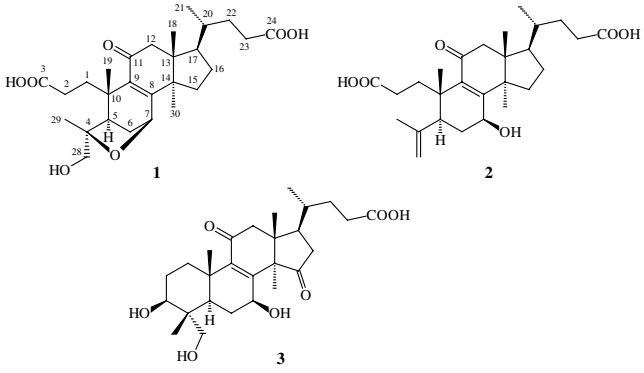
Figure 3. X-ray crystal structure of **2**.

Table 3. Percentage inhibition of compounds **1**–**2** on the aggregation of rabbit platelets induced by PAF, AA, ADP ($x \pm s$, $n = 3$)

Compound (240 μmol)	Aggregation % (Inhibition %)		
	PAF (4.5 nmol)	ADP (5 μmol)	AA (240 μmol)
2%PEG	62.5 ± 4.4	56.5 ± 6.5	62.2 ± 6.6
1	41.2 ± 1.0 (33.9 ± 5.3)*	45.0 ± 7.0 (20.6 ± 3.6)	65.2 ± 7.6 (−15.4 ± 4.7)
2	44.3 ± 1.7 (28.8 ± 6.8)*	58.3 ± 7.9 (−3.0 ± 2.4)	65.4 ± 5.4 (−16.2 ± 8.0)
BN52021	(80.2 ± 4.4)		
ASA			(86.0 ± 1.8)

* $P < 0.01$, as compared with control (*t*-test).

inhibition against platelet aggregation induced by ADP. No other inhibitory effects were observed (Table 3).



Acknowledgements

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15. Compound **1**: white powder. $[\alpha]_D^{21}$ +66.7 (*c* 0.2, MeOH); UV (MeOH) λ_{max}: 263.2 (4.6) nm; IR (KBr) ν_{max}: 3427, 2962, 1727, 1716, 1683, 1652, 1558, 1539, 1386, 1290, 1185, 1092, 1039, 981, 798, 679, 612, 580 cm^{-1}; ^1H and ^{13}C NMR, data see Tables 1 and 2; negative FABMS *m/z* 475 $[M-H]^-$ (100), 461 (13), 445 (14), 443 (13), 401 (35); HRESIMS *m/z* found 475.2689 $[M-H]^-$ calcd 475.2696, $C_{27}H_{39}O_7$; EIMS *m/z* 445 (8), 440 (8), 427 (6), 399 (7), 385 (31), 370 (61), 355 (10), 337 (6), 329 (9), 319 (7), 295 (6), 277 (7), 269 (8), 251 (8), 243 (13), 227 (72), 213 (31), 201 (50), 189 (63), 174 (38), 159 (44), 145 (39), 133 (30), 129 (30), 95 (30), 81 (25), 69 (34), 55 (100). Compound **2**: colorless crystal. $[\alpha]_D^{21}$ +260.0

(*c* 0.2, MeOH); UV (MeOH) λ_{max}: 256.6 (4.4) nm; IR (KBr) ν_{max}: 3536, 3439, 2971, 2937, 1703, 1651, 1456, 1419, 1378, 1290, 1211, 1182, 1045, 951, 891, 611, 459 cm^{-1}; ^1H and ^{13}C NMR, data see Tables 1 and 2; Negative FABMS *m/z* 459 $[M-H]^-$ (76), 443 (100), 429 (5), 415 (6), 399 (10), 387 (17), 369 (10), 355 (3); HRESIMS *m/z* found 459.2733, calcd 459.2747, $C_{27}H_{39}O_6$; EIMS *m/z* 440 (21), 368 (60), 353 (20), 315 (18), 225 (34), 213 (35), 199 (50), 187 (44), 171 (40), 159 (40), 145 (37), 131 (34), 91 (45), 81 (31), 69 (40), 57 (57), 55 (100).

16. A crystal of dimensions $0.05\times0.10\times0.30$ mm was used for X-ray measurements on a MAC DIP-2030 K diffractometer with a graphite monochromator, with maximum 2θ value of 50.0°. The total number of independent reflections measured was 2792, of which 2172 were considered to be observed ($|F|^2 \geqslant 8\sigma|F|^2$). Crystal data: $C_{27}H_{40}O_6$, $M = 460.61$, monoclinic system, space group: $P2_1$, $a = 12.050$ (1), $b = 7.910$ (1), $c = 13.971$ (1) Å, $\beta = 105.49$ (1)°, $V = 1283.3$ (1) $Å^3$, $Z = 2$, $d = 1.192$ g cm^{-3}, Mo Kα radiation, linear absorption coefficient $\mu = 1.0\,cm^{-1}$. The structure was solved by the direct method SHELX-86[17] and expanded using difference Fourier techniques, refined by the program and method NOMCSDP[18] and full-matrix least-squares calculations. Hydrogen atoms were fixed at calculated positions. The final indices were $R_f = 0.059$, $R_w = 0.061$ ($w = 1/\sigma|F|^2$). Crystallographic data for the structure has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 226882. Copies of the data can be obtained, free of charge, on application to the CCDC via www.ccdc.cam.ac.uk/conts/retrieving.html (or 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44(0)223 336033, e-mail: deposit@ccdc.cam.ac.uk).
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