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Novel *ent*-kaurane dimers from *Isodon rubescens* var. *rubescens*

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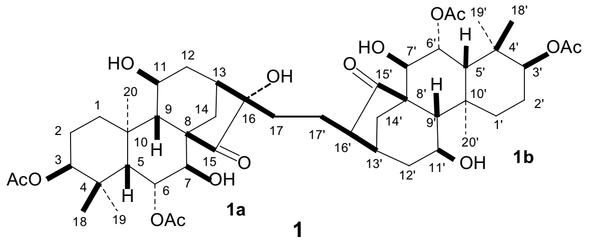
Abstract—Three novel asymmetric *ent*-kaurane dimers xindongnins M–O (**1**–**3**) were isolated from *Isodon rubescens* var. *rubescens*. Their structures were elucidated by spectroscopic methods including 2D NMR analysis. The relative stereochemistry of **1** was determined by single crystal X-ray diffraction, which also confirmed the unique linkage of a single carbon–carbon bond between the two subunits of this dimer. A biogenetic pathway was proposed for the formation of these dimers.

In our continuing phytochemical investigation of *Isodon rubescens* complex,[1a–f] we examined, with the aid of analytical and preparative HPLC, the aqueous acetone extract of the leaves of *I. rubescens* var. *rubescens* collected in Shangcheng Prefecture, Henan Province of China,[2] as a result, three novel asymmetric *ent*-kaurane dimers were isolated, together with two known compounds melissoidesin G (**4**)[3] and xingdongnin A (**5**).[4] The structures of these dimers were elucidated by the analysis of their HRMS and NMR data, especially 2D NMR spectra. These were the first examples of *ent*-kaurane dimers that possessed a rare linkage of a single carbon–carbon bond between the two structural subunits. This unique linkage was finally confirmed by single crystal X-ray analysis.



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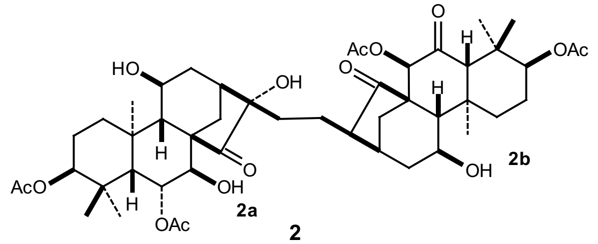

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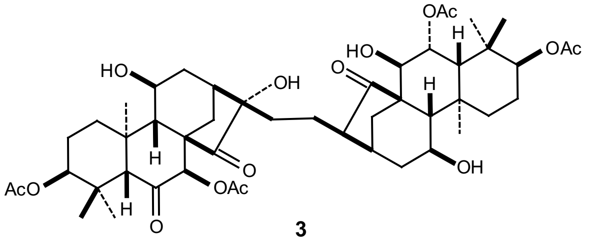

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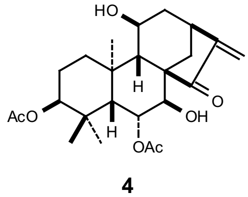

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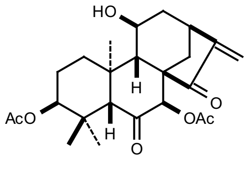

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Xindongnin M (**1**) was obtained as colorless cubes. Its molecular formula was determined to be $C_{48}H_{70}O_{15}$ by positive HRFABMS (found 887.4793, calcd 887.4811 for $C_{48}H_{71}O_{15}$).[5] Its ^{13}C NMR spectrum (Table 1) confirmed this formula, and exhibited carbon signals of two diterpene units (**1a** and **1b**) bearing four acetoxy groups in total. Each of the units was further indicated to have a kaurane skeleton by the characteristic signals of three methyl groups (C-18, 19, and 20), three methine carbons (C-5, 9, and 13), and three quaternary carbons

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Keywords: *ent*-Kaurane dimer; Xindongnins M–O; *Isodon rubescens* var. *rubescens*.

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Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data of compounds **1**–**5** in C_5D_5N (δ, J in ppm)

No	**1**		**2**		**3**		**4**	**5**
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{13}C	^{13}C
1α	1.44–1.67[a]	35.5 t	1.67[h]	35.5 t	1.61 (m)	35.0 t	35.5 t	33.5 t
1β	1.44–1.67[a]		1.47[i]		1.44 (m)			
2α	1.87–1.96[b]	22.9 t	1.54–1.64[j]	22.9 t	1.52–1.58[o]	22.6 t	23.6 t	22.6 t
2β	1.44–1.67[a]		1.54–1.64[j]		1.52–1.58[o]			
3α	4.73 (br s)	78.6 d	4.73 (br s)	78.7 d	4.66 (br s)	77.1 d	78.3 d	77.2 d
4		37.1 s		37.1 s		35.8 s	37.3 s	35.8 s
5β	2.60 (s)	42.4 d	2.60 (s)	42.4 d	3.58 (s)	55.0 d	41.9 d	54.9 d
6β	5.60 (br s)	71.9 d	5.59 (br s)	71.9 d		206.4 s	71.4 d	202.5 s
7α	4.33 (br s)	74.6 d	4.31 (br s)	74.6 d	5.55 (br s)	80.9 d	72.9 d	80.6 d
8		50.5 s		50.1 s		53.1 s	49.5 s	53.5 s
9β	2.62 (s)	60.4 d	2.60 (s)	60.4 d	2.80 (s)	59.8 d	58.6 d	59.2 d
10		38.0 s		38.2 s		44.5 s	38.1 s	44.9 s
11α	4.33 (br s)	64.4 d	4.31 (br s)	64.4 d	4.24 (br s)	63.9 d	65.7 d	64.7 d
12α	2.54[c]	32.5 t	2.50–2.54[k]	32.5 t	2.38[p]	33.7 t	41.3 t	40.7 t
12β	2.54[c]		2.50–2.54[k]		1.99[q]			
13α	2.64[d]	40.9 d	2.60 (m)	40.8 d	2.56 (m)	39.3 d	37.8 d	36.9 d
14α	2.56 (d, 12.0)	35.2 t	2.44[l]	36.6 t	2.37[p]	35.7 t		34.4 t
14β	2.18 (dd, 4.0, 12.0)		2.16[m]		2.01[q]			
15		226.5 s		225.7 s		213.1 s	213.0 s	206.7 s
16		80.6 s		80.5 s		80.6 s	149.7 s	151.1 s
17a	3.17 (m)	30.7 t	3.05 (m)	30.5 t	2.94 (m)	30.5 t	114.0 t	112.7 t
17b	2.39[e]		2.26[n]		2.63 (m)			
18	1.02 (3H, s)	28.0 q	1.00 (3H, s)	28.0 q	1.00 (3H, s)	26.8 q	27.9 q	27.0 q
19	1.01 (3H, s)	23.5 q	1.00 (3H, s)	23.5 q	1.31 (3H, s)	21.7 q	23.5 q	22.1 q
20	1.39 (3H, s)	19.6 q	1.37 (3H, s)	19.6 q	1.06 (3H, s)	18.4 q	19.4 q	18.6 q
1α′	1.44–1.67[a]	35.4 t	1.92 (m)	33.4 t	1.67–1.81[r]	34.0 t		
1β′	1.44–1.67[a]		2.26[n]		1.67–1.81[r]			
2α′	1.87–1.96[b]	22.9 t	1.54–1.64[j]	22.6 t	1.52–1.58[o]	22.3 t		
2β′	1.44–1.67[a]		1.54–1.64[j]		1.52–1.58[o]			
3α′	4.73 (br s)	78.6 d	4.65 (br s)	77.5 d	4.72 (br s)	78.3 d		
4′		37.1 s		36.1 s		36.8 s		
5β	2.57 (s)	42.4 d	3.50 (s)	55.7 d	2.55 (s)	42.1 d		
6β′	5.60 (br s)	71.9 d		206.5 s	5.51 (br s)	71.5 d		
7α′	3.93 (br s)	73.7 d	5.31 (br s)	80.6 d	3.92 (br s)	73.3 d		
8′		50.1 s		53.8 s		50.2 s		
9β′	2.43 (s)	58.4 d	2.64 (s)	58.7 d	2.41 (s)	58.0 d		
10′		38.2 s		44.4 s		37.7 s		
11α′	4.28 (br s)	64.0 d	4.20 (br s)	63.9 d	4.26 (br s)	63.7 d		
12α′	2.00 (m)	34.0 t	1.79 (m)	34.2 t	2.07 (m)	35.6 t		
12β′	2.47 (m)		1.69[h]		1.23 (m)			
13α′	2.54 (m)	33.9 d	2.44[l]	32.6 d	2.54 (m)	33.5 d		
14α′	2.58 (d, 12.0)	33.5 t	2.12[m]	34.3 t	2.59 (d, 12.0)	30.6 t		
14β′	1.24 (dd, 4.0, 12.0)		1.49[i]		2.35[s]			
15′		225.7 s		214.3 s		226.3 s		
16′	2.39[e]	57.3 s	2.30 (m)	57.1 d	2.38[s]	56.8 d		
17a′	2.97 (m)	20.8 t	2.89 (m)	20.5 t	2.90 (m)	20.5 t		
17b′	2.66[d]		2.47[l]		2.50 (m)			
18′	1.02 (s, 3H)	28.0 q	1.00 (3H, s)	27.1 q	1.00 (3H, s)	27.7 q		
19′	1.01 (s, 3H)	23.5 q	1.30 (3H, s)	22.1 q	1.01 (3H, s)	23.3 q		
20′	1.32 (s, 3H)	19.4 q	1.00 (3H, s)	18.6 q	1.31 (3H, s)	19.1 q		
Oac		170.1 s		170.1 s		170.0 s	170.6 s	169.7 s
		169.9 s		169.9 s		169.7 s	169.4 s	169.6 s
		170.1 s		169.8 s		169.6 s		
		169.9 s		169.7 s		169.5 s		
	2.10 (s, 3H)[f]	20.9 q	2.24 (3H, s)	21.3 q	2.26 (3H, s)	21.1 q	21.6 q	20.9 q
	1.84 (s, 3H)[g]	21.3 q	2.03 (3H, s)	21.1 q	2.10 (3H, s)	20.8 q	21.3 q	20.8 q
	2.10 (s, 3H)[f]	20.9 q	1.88 (3H, s)	20.9 q	1.90 (3H, s)	20.6 q		
	1.84 (s, 3H)[g]	21.3 q	1.83 (3H, s)	20.7 q	1.85 (3H, s)	20.5 q		
OH-7	6.53 (s)		6.51 (s)					
OH-11	6.46 (s)		6.41 (s)		6.49 (s)			
OH-16	7.46 (s)		7.37 (s)		7.48 (s)			
OH-7′	6.47 (s)				6.46 (s)			
OH-11′	6.27 (s)		6.32 (s)		6.24 (s)			

[a–s] Signals overlapped.

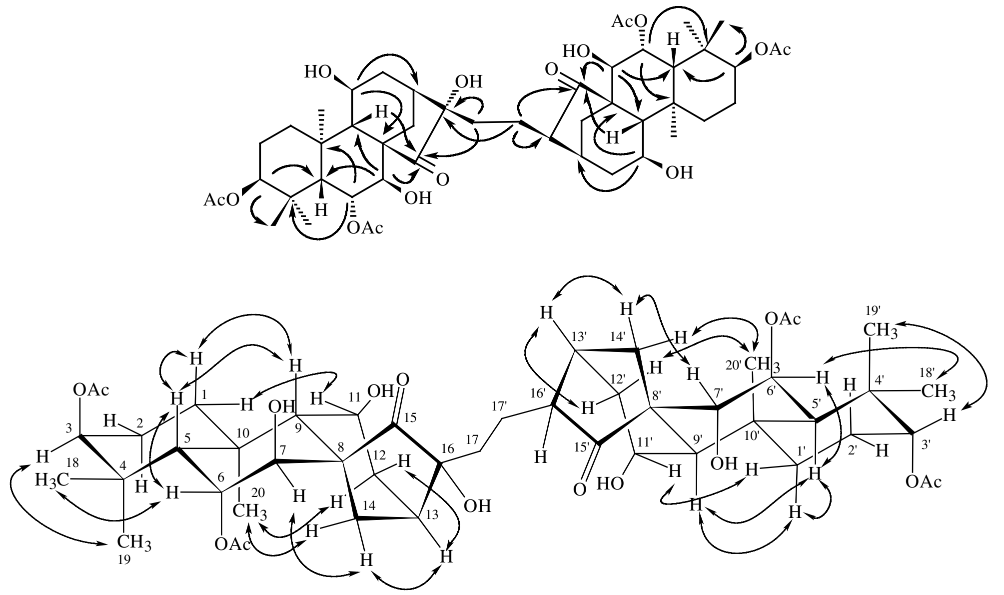


Figure 1. Selected HMBC and ROESY correlations of **1**.

(C-4, 8, and 10). Considering that all the kauranoids isolated from the genus *Isodon* possessed an *ent*-configuration, the two units (**1a** and **1b**) were presumed to also be *ent*-kauranes. A comparison of their NMR data with those of melissoidesin G (**4**, a known *ent*-kauranoid also isolated from this fraction) revealed that both **1a** and **1b** were very similar to **4** except at C-16 and C-17. The olefinic quaternary carbon (C-16) of **4** corresponded to an oxygenated quaternary carbon (δ 80.6) in **1a** and a methine carbon (δ 57.3) in **1b**, respectively, while the olefinic methylene carbon (C-17) of **4** was replaced by two methylene carbons (δ 30.7 and 20.8) in **1a** and **1b**. More importantly, the protons of these two methylene groups (δ 3.17 and 2.39; δ 2.97 and 2.66) showed ^{1}H–^{1}H COSY correlations with each other, indicating a single bond between these two carbons belonging to **1a** and **1b**. This was supported by a series of HMBC correlations between H$_2$-17 with C-13, 15, 16, and 16′ and H$_2$-17′ with C-13′, 15′, 16′, and 16 (Fig. 1).

The substituents were accordingly assigned as 3β-OAc, 6α-OAc, 7β-OH, 11β-OH, 3′β-OAc, 6′α-OAc, 7′β-OH, and 11′β-OH, respectively, by the HMBC and ROESY correlations as shown in Figure 1. The OH-16 was suggested to be in an α-orientation by the significant upfield signal of C-12 (δ 32.5) compared to that in **4**, which was caused by the γ-steric compression effect between 16β -methylene group with H-12β.[1a] Similarly, the β-orientation was deduced for 16′-methylene group. Finally, the structure of this diterpene dimer (**1**) was established as presented in Figure 1, which was confirmed by single crystal X-ray diffraction (Fig. 2).[6]

The positive HRFABMS and ^{13}C NMR data (Table 1) revealed xindongnin N (**2**) to be an analogue of **1**, a diterpene dimer having the molecular formula $C_{48}H_{68}O_{15}$.[5] It was suggested to possess a similar linkage between its two subunits, a single carbon–carbon bond on the basis of the characteristic ^{13}C NMR signals: an oxygenated quaternary carbon (δ 80.5, due to C-16), two methylene carbons (δ 30.5 and 20.5, C-17 and C-17′) and a methine carbon (δ 57.1, C-16′). In contrast to **1**, xindongnin N (**2**) was constructed from two different diterpenes units (**2a** and **2b**). **2a** Is similar to **4**, while **2b** resembled xindongnin A (**5**). The assignment of the oxygenated quaternary carbon (or the methylene carbon at δ 30.5) was the key to the structural elucidation of **2**. Detailed analysis of the COSY, HMQC, and HMBC spectra of **2** disclosed that the protons (δ 3.05 and 2.26) of the methylene group (δ 30.5, C-17) exhibited HMBCs with both the oxygenated quaternary carbon (80.5) and a carbonyl carbon (δ 225.7). And this carbonyl carbon was further indicated to be C-15 of **2a** by the HMBC correlation between the characteristic H-7 (δ 4.31) of **2a** with this carbon. Therefore, the oxygenated quaternary carbon (δ 80.5) should be assigned to C-16 of **2a**, and the structure of **2** was established to be as shown. In the same way, xindongnin O (**3**) was elucidated to be an isomer of **2**, in whose structure the oxygenated quaternary carbon (δ 80.6) was indicated to be C-16 of the unit similar to xindongnin A (**5**). According to these dimers' structures, all the NMR data of **2** and **3** were successfully assigned with the aid of their 2D NMR spectra.

The biotransformation of this kind of dimers from normal *ent*-kauranoids isolated from the genus *Isodon*, was proposed to be as shown in Scheme 1. These *ent*-kauranoids normally had the α,β-unsaturated ketone groups. The [4 + 2] cycloaddition, between the α,β-unsaturated ketone group of one diterpene unit with the olefin group of a second unit, yielded a six-membered heterocycle,[7] which linked the monomers together. The hydrolysis and rearrangement at this heterocycle then produced the title dimers. We note that this [4 + 2] cycloaddition needs rigorous conditions in organic synthesis, which are impossible in plants. It is possible

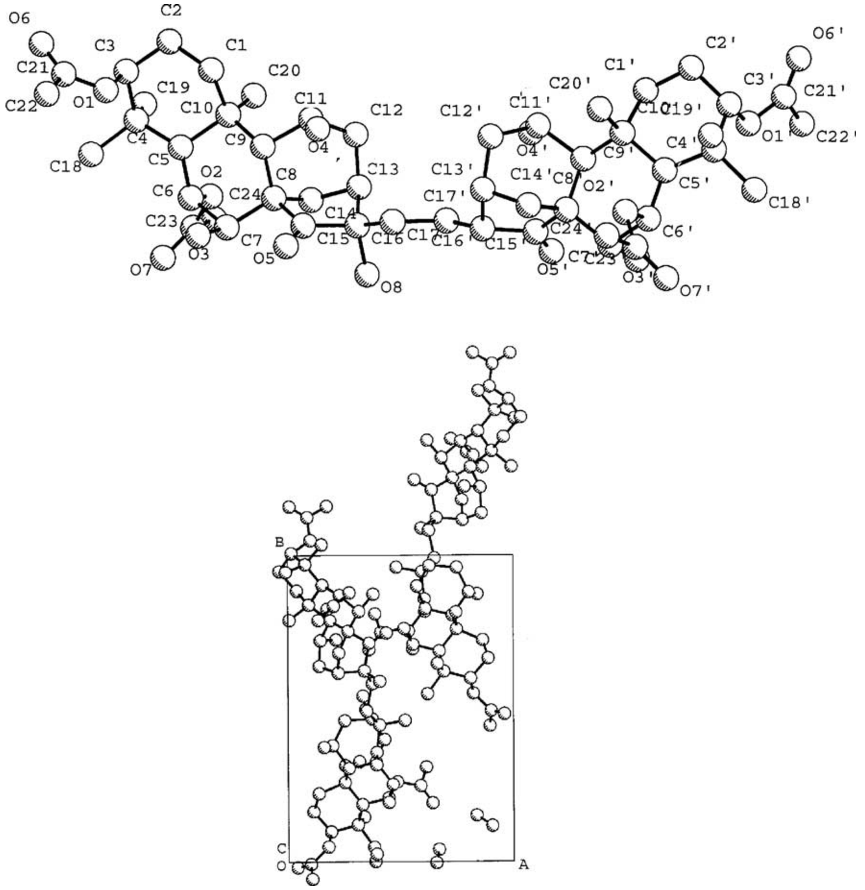
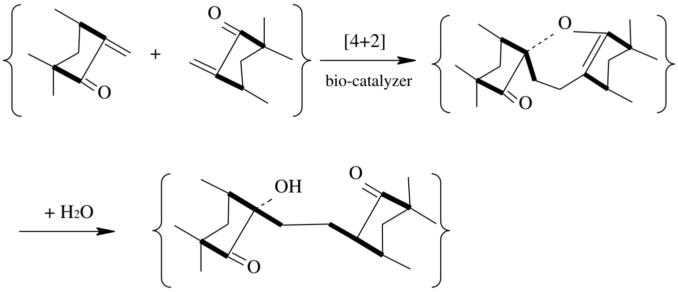


Figure 2. Crystal structure of compound **1**.



Scheme 1. Proposed biogenesis of **1**–**3**.

that in the *Isodon* plants there may be a bio-enzyme that catalyzes this reaction.

References and notes

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2. The leaves (1 kg) of *Isodon rubescens* var. *rubescens* were collected in Shangcheng Prefecture, Henan Province, PR China, in August 2002. The plant material was identified by Prof. Zhong-Wen Lin, and a voucher specimen (KIB-09-2002-Lin) was deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences.
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5. Compound **1**: colorless cubes, mp 204–206 °C; $[\alpha]_D^{22.0}$ −17.2 (MeOH, *c* 0.15); positive FABMS *m*/*z*: 887 $[M+H]^+$;

positive HRFABMS *m/z*: $[M+H]^+$ 887.4811 (calcd for $C_{48}H_{71}O_{15}$ 887.4793); IR (KBr) ν_{max}: 3444, 2939, 2879, 1731, 1433, 1375, 1241, 1033 cm^{-1}; ^1H NMR (C_5 D_5 N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz): see Table 1. Compound **2**: white amorphous powder; $[\alpha]_D^{22.0}$ −5.8 (MeOH, *c* 0.17); positive FABMS *m/z*: 885 $[M+H]^+$; positive HRFABMS *m/z*: $[M+H]^+$ 885.4633 (calcd for $C_{48}H_{69}O_{15}$ 885.4636); IR (KBr) ν_{max}: 3458, 2939, 1736, 1731, 1434, 1375, 1243, 1035 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz): see Table 1. Compound **3**: white amorphous powder; $[\alpha]_D^{22.0}$ −15.4 (MeOH, *c* 0.20) positive FABMS *m/z*: 885 $[M+H]^+$; positive HRFABMS *m/z*: $[M+H]^+$ 885.4635 (calcd for $C_{48}H_{69}O_{15}$ 885.4636); IR (KBr) ν_{max}: 3446, 2939, 1736, 1636, 1435, 1375, 1243, 1034 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz): see Table 1.

6. Crystal data for **1**. Crystals of **1**, crystallized from acetone, belong to the monoclinic space group $P2_1$. Crystal data: $C_{48}H_{70}O_{15}\cdot(CH_3OH)_2\cdot(H_2O)_{0.5}$, $M = 887.07$, $a = 12.826(1)$, $b = 16.859(1)$, $c = 12.872(1)$ Å, $\beta = 104.56(1)°$, $V = 2694.0(3)$ Å^3, $Z = 2$, $d = 1.182$ g/cm^3, Mo Kα radiation, linear absorption coefficient $\mu = 1.0$ cm^{-1}. A colorless cube of dimensions $0.20\times0.20\times0.50$ mm was used for X-ray measurements on a MAC DIP-2030K diffractometer with a graphite monochromator, maximum 2θ value of 50.0° was set. The total number of independent reflections measured was 4286, 4253 of which were considered to be observed ($|F|^2 \geqslant 3\sigma|F|^2$). The structure was solved by the direct method SHELXS-86 and expanded using difference Fourier techniques, refined by the program and method NOMCSDP[8] and full-matrix least-squares calculations. Hydrogen atoms were fixed at calculated positions. The final indices were $R_f = 0.084$, $R_w = 0.086$ ($w = 1/\sigma|F|^2$), $S = 4.542$, $(\Delta/\rho)_{max} = 0.135$, $(\Delta/\rho)_{min} = -0.520$ e/Å^3, $(\Delta/\rho)_{max} = 0.310$ e/Å^3. The crystal structure of **1** has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 226868.
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