Two New Daphniphyllum Alkaloids from *Daphniphyllum calycinum*

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Two new daphniphyllum alkaloids named 2-hydroxyyunnandaphnine D (**1**) and methyl 7-hydroxyhomodaphniphyllate (**2**), together with eight known alkaloids, daphnioldhanin D, calyciphylline F, calyciphylline B, deoxycalyciphylline B, daphnicyclidin H, macropodumine C, 9,10-epoxycalycine A, and yunnandaphnine A, were isolated from the stems and leaves of *Daphniphyllum calycinum*. Their structures and relative configurations were established on the basis of spectral evidence (including 2D-NMR) and subsequently confirmed by a single-crystal X-ray crystallographic diffraction analysis.

Introduction. – Trees of the genus *Daphniphyllum* (Daphniphyllaceae) are known to elaborate a structurally diverse group of alkaloids with unique polycyclic fused ring systems [1]. These daphniphyllum alkaloids have been attractive targets for biogenetic and synthetic studies [2]. Previous investigations on the genus *Daphniphyllum* led to the isolation of more than 60 new daphniphyllum alkaloids according to pertinent references [3–7]. To find further potentially bioactive interesting alkaloids from *D. calycinum* and to delineate their ethnomedical properties, the present study was undertaken. The investigation of the chemical constituents of the stems and leaves of *D. calycinum* collected in the Guangxi province resulted in the isolation and structural elucidation of two new daphniphyllum alkaloids.

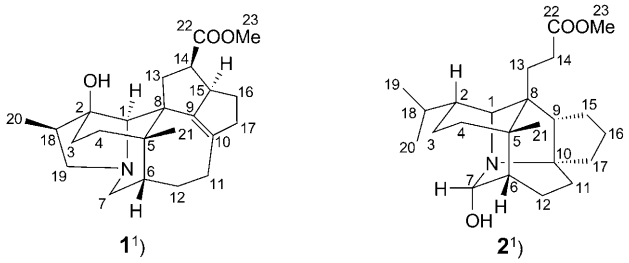
Results and Discussion. – Compound **1** was obtained as colorless crystals (MeOH). The molecular formula was determined to be $C_{23}H_{33}NO_3$ on the basis of HR-ESI-MS ($[M+H]^+$ at m/z 372.2520) with eight degrees of unsaturation. The IR spectrum of **1** exhibited absorptions for OH (3121 cm^{-1}) and C=O groups (1725 cm^{-1}). Its ^{13}C-NMR (DEPT) spectrum (*Table*) showed 23 resolved peaks corresponding to 23 C-atoms, including signals for one ester CO group, one tetrasubstituted C=C bond, three quaternary C-atoms, and five CH, nine CH_2, two Me, and one MeO group. The analysis of the ^{1}H- and ^{13}C-NMR data of **1**[1]) (*Table*) indicated that the structure of **1** is very similar to the known compound yunnandaphnine D reported in [4], except for one additional OH group (*Fig. 1*). The distinct difference between them is the following: the H–C(2) of the known compound (δ(C) 38.5 (*d*, C(2)), δ(H) 2.23 (*m*, 1 H)) is substituted by an OH group at C(2) (δ(C) 79.1 (*s*, C(2))) in **1**. In the ^{1}H,^{1}H-COSY plot of **1**, the cross-peaks H–C(6)/CH_2(7), CH_2(12), and CH_2(11), CH_2(16)/CH_2(17), and

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

Table. *^1H- and ^{13}C- NMR Data* (600 and 100 MHz, resp.) *of Compounds* **1** and **2**[1]). δ in ppm, J in Hz.

	1[a])		**2**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	2.69 (*s*)	67.1 (*d*)	2.89 (*d*, *J* = 4.7)	64.3 (*d*)
C(2) or H–C(2)		79.1 (*s*)	1.33–1.34 (*m*)	37.8 (*d*)
CH_2(3)	1.55(*dd*, *J* = 12.1, 6.3, H_a), 1.89–1.91 (*m*, H_b)	29.5 (*t*)	1.92–1.94, 1.28–1.30 (2*m*)	25.7 (*t*)
CH_2(4)	2.37(*dd*, *J* = 13.8, 6.5, H_a), 2.95(*t*, *J* = 11.3, H_b)	35.5 (*t*)	1.85–1.87, 1.45–1.48 (2*m*)	36.4 (*t*)
C(5)		36.1 (*s*)		38.0 (*s*)
H–C(6)	2.01–2.03 (*m*)	43.8 (*d*)	1.56 (*dd*, *J* = 3.2, 8.3)	47.3 (*d*)
CH_2(7) or H–C(7)	3.15 (*d*, *J* = 12.9)	57.6 (*t*)	5.11 (*d*, *J* = 3.3)	80.2 (*d*)
C(8)		47.8 (*s*)		47.2 (*s*)
C(9) or H–C(9)		145.9 (*s*)	2.38 (*t*, *J* = 5.5)	51.1 (*d*)
C(10)		134.1 (*s*)		72.1 (*s*)
CH_2(11)	2.09–2.14, 2.35–2.36 (2*m*)	26.0 (*t*)	1.70–1.73, 1.32–1.34 (2*m*)	28.6 (*t*)
CH_2(12)	1.42–1.45, 2.10–2.13 (2*m*, H)	29.3 (*t*)	1.77–1.78, 1.61–1.64 (2*m*)	17.7 (*t*)
CH_2(13)	1.43 (*dd*, *J* = 12.2, 6.4, H_a), 1.84 (*dd*, *J* = 11.7, 5.4, H_b)	38.0 (*t*)	1.82–1.84 (*m*, H_a), 1.36 (*d*, *J* = 3.4, 1 H_b)	27.3 (*t*)
H–C(14) or CH_2(14)	3.15–3.17 (*m*)	42.8 (*d*)	2.50–2.52, 2.40–2.43 (*m*)	32.3 (*t*)
H–C(15) or CH_2(15)	3.59–3.61 (*m*)	54.7 (*d*)	2.43–2.47, 1.33–1.35 (2*m*)	29.8 (*t*)
CH_2(16)	1.29–1.31, 1.80–1.83 (2*m*)	29.3 (*t*)	1.70–1.72, 1.62–1.65 (2*m*)	25.0 (*t*)
CH_2(17)	2.37 (*dd*, *J* = 13.8, 6.5, H_a), 2.67–2.70 (*m*, H_b)	43.1 (*t*)	1.73–1.75, 1.37–1.39 (2*m*)	41.3 (*t*)
H–C(18)	2.01–2.05 (*m*)	47.6 (*d*)	1.63–1.65 (*m*)	31.5 (*d*)
CH_2(19) or Me(19)	2.15–2.17, 3.30–3.32 (2*m*)	62.9 (*t*)	1.05 (*d*, *J* = 6.4)	21.2 (*q*)
Me(20)	1.04 (*d*, *J* = 7.0)	12.9 (*q*)	21.3 (*d*, *J* = 6.5)	21.3 (*q*)
Me(21)	1.29 (*s*)	24.9 (*q*)	0.92 (*s*)	26.0 (*q*)
C(22)		177.5 (*s*)		174.6 (*s*)
Me(23)	3.63 (*s*)	51.6 (*q*)	3.65 (*s*)	51.6 (*q*)

[a]) Measured in CD_3OD. [b]) Measured in $CDCl_3$.



1[1]) **2**[1])

Fig. 1. *Compounds* **1** *and* **2**, *isolated from* Daphniphyllum calycinum[1])

H–C(18)/CH_2(19) and Me(20) were observed, which indicated the following connectivities (*Fig. 2*): C(7)–C(6)–C(12)–C(11), C(16)–C(17), and C(19)–C(18)–C(20). The HMBC spectrum showed the following key correlations (*Fig. 2*): H–C(1)/C(2), C(7), C(8), C(9), and C(18), H–C(6)/C(8), CH_2(7)/C(5), CH_2(11)/C(9) and

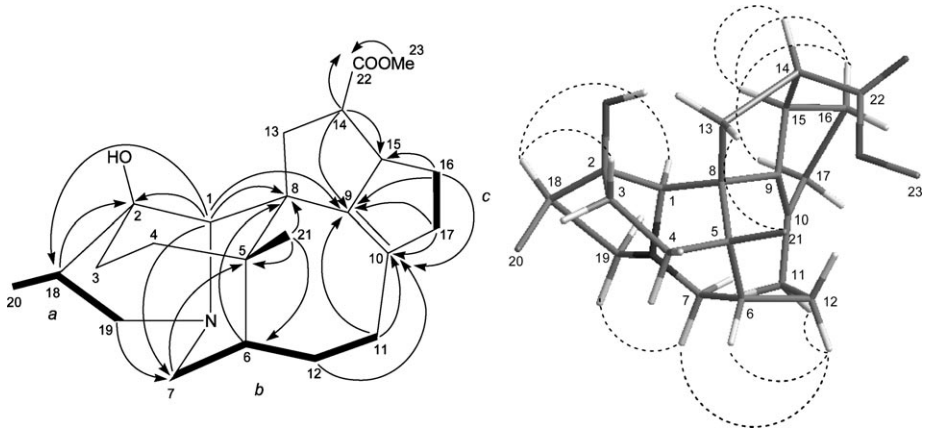


Fig. 2. *^{1}H,^{1}H-COSY* (—), *key HMBCs* (H⌒C), *selected NOESY correlations* (----), *and relative configuration of* **1** (the H-atoms of the Me groups are omitted)[1])

C(10), CH_2(12)/C(10), H–C(14)/C(9), C(15), and C(22), CH_2(16)/C(9), C(10), and C(15), CH_2(17)/C(9) and C(10), H–C(18)/C(2), CH_2(19)/C(7), Me(21)/C(6), and Me(23)/C(22), which are consistent with the proposed structure. In the light of the evidence mentioned above, the constitutional formula of **1** was established as 2-hydroxyyunnandaphnine D (*Fig. 1*), which was finally confirmed by an X-ray crystallographic diffraction analysis (*Fig. 3*). The relative configurations of all of the chiral centers of **1** were determined by the X-ray analysis, the NOESY correlations (*Fig. 2*), and comparison of chemical shifts with those of yunnandaphnine D. The same relative configuration of C(1), C(5), C(6), C(14), C(15), and C(18) in **1** as in yunnandaphnine D were deduced from the similar δ(C) and δ(H) and NOESY correlations, which were also confirmed by the X-ray analysis. The configuration of the OH group at C(2) is β-oriented, as deduced from X-ray evidence.

Compound **2** was also obtained as colorless crystals (MeOH), which exhibited a $[M+H]^+$ ion peak at *m/z* 376.28359 in the positive-ion-mode HR-ESI-MS, corresponding to the molecular formula $C_{23}H_{37}NO_3$ with six degrees of unsaturation. The IR spectrum indicated the presence of an OH (3443 cm^{-1}) and a carboxy group (1728 cm^{-1}). The ^{13}C-NMR (DEPT) spectra (*Table*) revealed 23 C-atoms due to one ester CO group, three quaternary C-atoms, and six CH, nine CH_2, three Me, and one MeO group. Comparison of the NMR data (*Table*) of **2** with the known compound methyl homodaphniphyllate isolated from *D. humile* [6], showed very similar chemical shifts values, except for one additional methoxy group, which strongly suggested an otherwise identical substitution pattern for both compounds. The distinct difference between them is the following: one H-atom at C(7) (δ(C) 47.1 (*t*); δ(H) 1.60 and 2.05 (2*m*, 2 H)) of methyl homodaphniphyllate is substituted by an OH group (δ(C) 80.2 (*d*); δ(H) 5.11 (*d*, *J* = 3.3, 1 H)) in **2**. The cross-peaks H–C(6)/H–C(7) and CH_2(12), CH_2(12)/CH_2(11), CH_2(13)/CH_2(14), CH_2(16)/CH_2(15) and CH_2(17), and H–C(18)/H–C(2), Me(19) and Me(20) were observed in the ^{1}H,^{1}H-COSY plot (*Fig. 4*). It

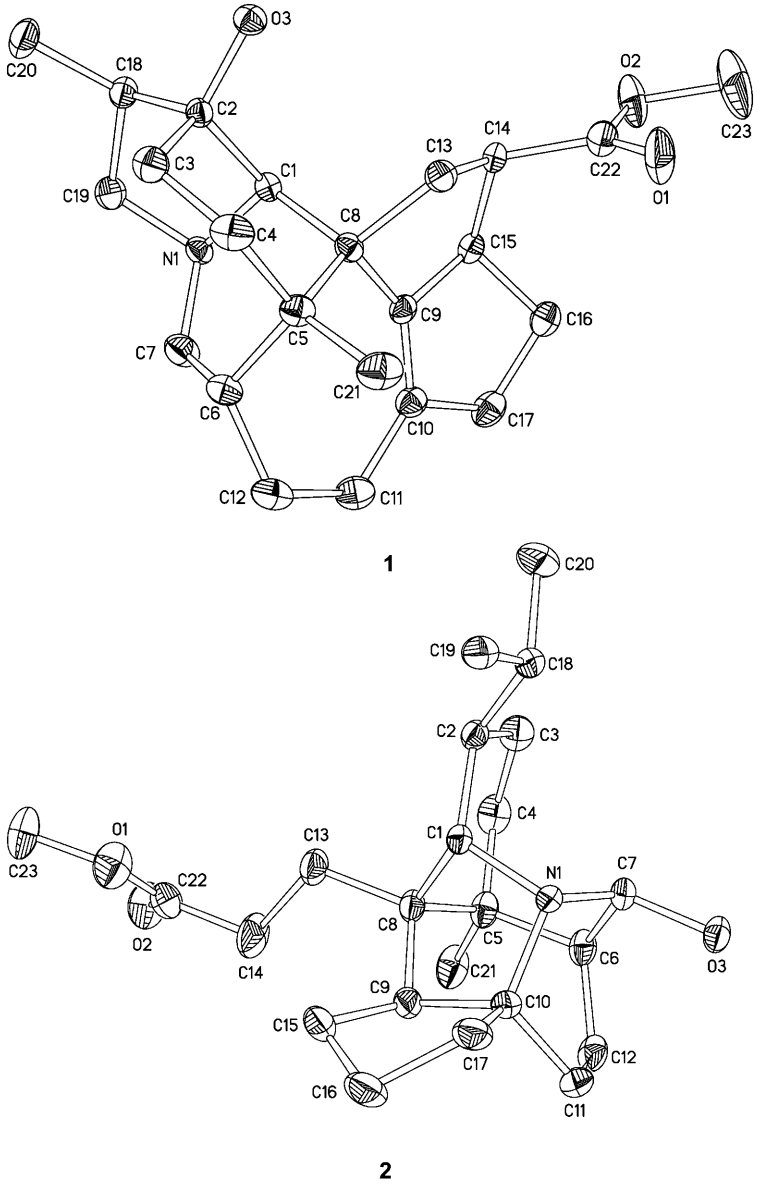


Fig. 3. *X-Ray crystal structures of* **1** *and* **2**[1])

allowed the establishment of the following connectivities: C(7)–C(6)–C(12)–C(11), C(13)–C(14), C(15)–C(16)–C(17), and C(2)–C(18)–C(19)–C(20). The HMBC spectrum showed the key correlations H–C(1)/C(2) and C(7), H–C(3)–C(1), C(2), C(4), and C(5), H–C(4)/C(2) and C(8), H–C(6)/C(4), C(5), and C(8), H–C(7)/C(10), H–C(9)–C(8) and C(15), H–C(11)/C(10), H–C(15)/C(8), H–C(17)/C(10)

and C(11), MeC(21)/C(5), C(6), C(8), and Me(23)/C(22) (*Fig. 4*). From the above considerations, the constitutional formula of **2** was established as methyl 7-hydroxy-homodaphniphyllate (*Fig. 1*). The relative configurations of all chiral centers of **2** were determined by X-ray-analysis (*Fig. 3*), NOESY correlations (*Fig. 4*), and comparison of its chemical shifts with those of methyl homodaphniphyllate.

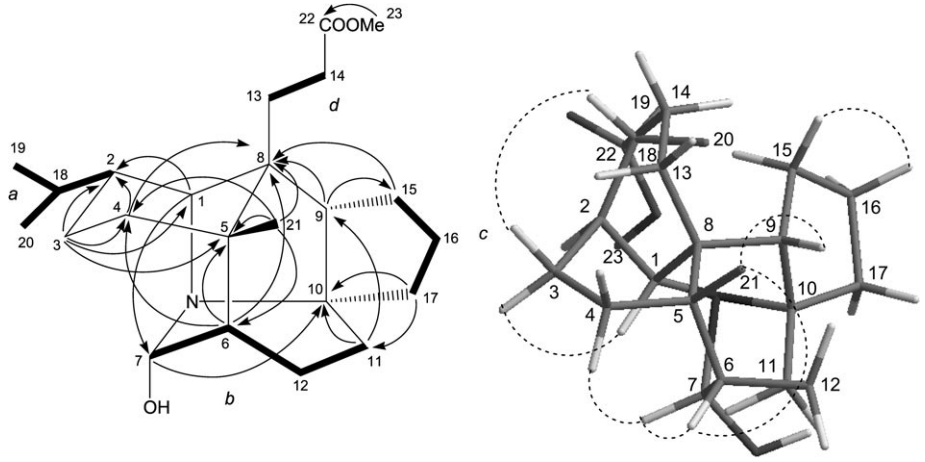


Fig. 4. *$^1H,^1H$-COSY* (—), *key HMBCs* (H⌒C), *selected NOESY correlations* (----), *and relative configuration of* **2** (the H-atoms of the Me groups are omitted)[1])

The known alkaloids daphnioldhanin D [8], calyciphylline F [9], calyciphylline B [10], deoxycalyciphylline B [11], daphnicyclidin H [12], macropodumine C [13], 9,10-epoxycalycine A [14], and yunnandaphnine A [4] were identified by comparison of their spectral data (ESI-MS, ^{1}H- and ^{13}C-NMR) with those reported in the literature.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Yantai Zhi Fu Chemical Co., Ltd.*, P. R. China), *RP-18* (12 nm, S-50 μm; *YMC Co., Ltd.*, Japan). TLC: silica gel GF_{254} plates (*Yantai Zhi Fu Chemical Co., Ltd*, P. R. China) and *Sephedax LH-20* gel (25–100 μm; *GE Healthcare Co., Ltd.*, Sweden). Optical rotations: *Perkin-Elmer-341* spectropolarimeter. UV Spectra: *Shimadzu 210-A* double-beam spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Perkin-Elmer-577* spectrometer; KBr pellets; in cm^{-1}. NMR Spectra: *Bruker-AM-600* spectrometer; δ in ppm, J in Hz; Me_4Si as internal standard, measured in $CDCl_3$ or CD_3OD. FT-MS: *Bruker-Apex-Ultra-7.0-T* spectrometer; in m/z.

Plant Material. The stems and leaves of *Daphniphyllum calycinum* were collected from Guangxi province and identified by Dr. *Hong-liang Tang*, School of Life Science, Hebei University. A voucher specimen (No. DC-200708-1) was deposited with our laboratory.

Extraction and Isolation. The powdered air-dried stems and leaves of *D. calycinum* BENTH (20 kg) were extracted with 95% EtOH three times. After evaporation, the crude extract was dissolved in 3 l of H_2O to form a suspension, and then adjusted to pH 2 with 2% HCl soln. The acidic mixture was

immediately defatted with $CHCl_3$ (6×2 l) to remove non-alkaloid components. The aq. layer was basified to pH 11 with sat. Na_2CO_3 soln. and then exhaustively exacted with $CHCl_3$ (6×2 l) to obtain the crude alkaloids (10.7 g). The crude alkaloids were subjected to CC (SiO_2, petroleum ether/$CHCl_3$ 1:0 → 0:1 and then $CHCl_3$/MeOH 1:0 → 0:1): *Fractions 1–12*. *Fr. 2* was separated by CC (SiO_2, petroleum ether/$CHCl_3$/Et_2NH 40:5:1): **2** (27.1 mg). *Fr. 3* was separated by CC (SiO_2, petroleum ether/acetone/Et_2NH 20:5:1): daphnioldhanin D (15.1 mg) and calyciphylline F (18.3 mg). *Fr. 4* was separated by CC (SiO_2, petroleum ether/acetone/Et_2NH 10:5:1): **1** (23.7 mg) and yunnandaphnine A (22.0 mg). *Fr. 5* was purified by prep. TLC (hexane/Et_2O/Et_2NH 10:5:1): 9,10-epoxycalycine A (6.4 mg). Calyciphylline B (19.5 mg) and deoxycalyciphylline B (8.7 mg) were obtained from *Fr. 6* after CC (*RP-18*, H_2O/MeOH 9:1 → 0:1; *Sephadex LH-20*, MeOH). *Fr. 9* was purified by semi-prep. HPLC (MeCN/0.2% Et_2NH 3.5:7.5 (*v*/*v*)): daphnicyclidin H (13.9 mg) and macropodumine C (21.2 mg).

2-Hydroxyyunnandaphnine D (= rel-*(3*R*,3a*S*,5a*S*,6*S*,10*R*,11*R*,12a*S*,12b*R*)-2,3,3a,5,5a,6,7,8,9,10,10a,11,12,12b-Tetradecahydro-3a-hydroxy-3,5a-dimethyl-4*H*-1,6-methanocyclopent[1,8]azuleno[4,3a-g]indole-11-carboxylic Acid Methyl Ester*; **1**). Colorless crystals (MeOH). M.p. 190–191°. $[\alpha]_D^{20} = -58.9$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3121, 2296, 2010, 1726, 1439, 1378, 1169. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 362 ($[M+H]^+$). HR-ESI-MS: 372.25195 ($[M+H]^+$, $C_{23}H_{34}NO_3^+$; calc. 372.25332).

Methyl 7-Hydroxyhomodaphniphyllate (= rel-*(3a*R*,4*S*,4a*S*,5*R*,8*S*,8a*R*,8b*S*,9*S*,10*S*)-Octahydro-9-hydroxy-8-methyl-5-(1-methylethyl)-4,8,3a-[1,2,4]butanetriylcyclopent[*b*]indole-8a(4a*H*)-propanoic Acid Methyl Ester*; **2**). Colorless crystals (MeOH). M.p. 188–189°. $[\alpha]_D^{20} = -52.7$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3443, 2861, 2013, 1868, 1843, 1728, 1437, 1379, 1265, 1147, 1052. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 365 ($[M+H]^+$). HR-ESI-MS: 376.28359 ($[M+H]^+$, $C_{23}H_{38}NO_3^+$; calc. 376.28462).

Crystal Data of **1** and **2**. Crystals of **1** and **2**, crystallized from MeOH, belong both to the orthorhombic space group *P*2(1)2(1)2(1). Crystal data of **1**: $C_{23}H_{33}NO_3$, M_r 389.52, $a = 13.4134(6)$ Å, $b = 7.6160(4)$ Å, $c = 20.4645(10)$ Å, $V = 2089.19(18)$ Å^3, $Z = 4$, $d = 1.238$ Mg/m^3; MoK_α radation, linear absorption coefficient 0.083 mm^{-1}; crystal dimensions $0.72 \times 0.14 \times 0.11$ mm; total number of independent reflections measured 10014, 6067 of which were considered to be observed ($F^2 > 3\sigma F^2$). Crystal data of **2**: $C_{23}H_{37}NO_3$, M_r 375.54, $a = 12.284(2)$ Å, $b = 16.282(3)$ Å, $c = 20.932(4)$ Å, $V = 4186.8(14)$ Å^3, $Z = 8$, $d = 1.192$ Mg/m^3; MoK_α radation, linear absorption coefficient 0.107 mm^{-1}; crystal dimensions $0.19 \times 0.03 \times 0.03$ mm; total number of independent reflections measured 23670, 10329 of which were considered to be observed ($F^2 > 3\sigma F^2$). The X-ray measurements were performed with a *Bruker-Smart-1000* diffractometer and a graphite monochromator. The structures were solved by the direct method SHELXS-97 and expanded by difference *Fourier* techniques, refined by the program and method NOMSCDP and full-matrix least-squares calculations. H-Atoms were fixed at calculated positions. The final indices were $R_f = 0.0420$ and $R_w = 0.0745$ for **1** and $R_f = 0.0547$ and $R_w = 0.0833$ for **2**. CDDC-751556 and CDDC-751557 contain the supplementary crystallographic data of **1** and **2**. These data can be obtained free of charge *via* www.ccdc.cam.ac.uk/data_request/cif.

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