New Phenanthrene Derivatives from *Maxillaria densa*[1]

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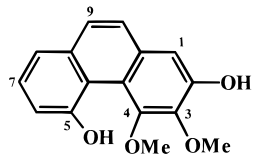
Two new phenanthrene derivatives, 2,5-dihydroxy-3,4-dimethoxyphenanthrene (**1**) and 9,10-dihydro-2,5-dihydroxy-3,4-dimethoxyphenanthrene (**2**), were isolated from an extract prepared from the whole plant of the orchid *Maxillaria densa* with spasmolytic activity. In addition, four known compounds, namely 2,7-dihydroxy-3,4-dimethoxyphenanthrene, 9,10-dihydro-2,7-dihydroxy-3,4-dimethoxyphenanthrene (**3**), 2,5-dihydroxy-3,4,9-trimethoxyphe-nanthrene, and 2,7-dihydroxy-3,4,9-trimethoxyphenanthrene, were obtained. The structures of the isolated compounds were elucidated by spectroscopic methods. In the case of phenanthrene derivatives **1** and **3**, the structures were unambiguously assigned by X-ray analysis.

As part of our effort to discover natural products with potential use as spasmolytic agents, we have reported that some phenanthrenes and stilbenoids from *Scaphyglottis livida* (Lindley) Schltr. (Orchidaceae) possess significant smooth-muscle relaxing properties.[2] Furthermore, pharmacological and radioimmunoassay evidence indicated that their spasmolytic activity was mediated by the system nitric oxide/cGMP.[2] Subsequently, we have screened several Mexican orchid extracts for their ability to relax the spontaneous rat ileum contraction and accordingly have selected *Maxillaria densa* Lindley for fractionation. The present report describes the isolation and structure elucidation of several phenanthrene derivatives, including the new natural products **1** and **2**, from a pharmacologically active extract of this epiphytic orchid.[3]

After the initial observation of the significant inhibition of the spontaneous rat ileum contractions induced by a $CHCl_3$−MeOH (1:1) extract (IC_{50}, 0.62 ± 0.13 μg/mL) prepared from the whole plant of *M. densa,* large-scale extraction and fractionation was undertaken. Altogether six phenenthrene derivatives were isolated and characterized from this bioactive extract, comprised by 2,5-dihydroxy-3,4-dimethoxyphenanthrene (**1**), 9,10-dihydro-2,5-dihydroxy-3,4-dimethoxyphenanthrene (**2**), 2,7-dihydroxy-3,4-dimethoxyphenanthrene,[4] 9,10-dihydro-2,7-dihydroxy-3,4-dimethoxyphenanthrene (**3**),[5] 2,5-dihydroxy-3,4,9-trimethoxyphenanthrene,[6] and 2,7-dihydroxy-3,4,9-trimethoxyphenanthrene.[7] Compounds **1** and **2** are new natural products. The structures of the known compounds were ascertained by comparison of their physical and spectroscopic properties with those reported in the literature. In the case of compounds **1** and **3**, the structures were unambiguously assigned by X-ray analysis.

Compound **1** was obtained as yellow crystals. EIMS and ^{13}C NMR spectroscopy established its molecular formula as $C_{16}H_{14}O_4$. The IR spectrum contained bands for hydroxyl groups (3528 and 3180 cm^{-1}) and the UV spectrum showed absorptions typical for phenanthrene derivatives.[4−12] The NMR spectra of compound (**1**) (Table 1) were similar to those of other 2,3,4,5-tetrasubstituted phenanthrenes.[6,10] As in fimbriol B,[6] the ^1H NMR spectrum of **1** exhibited two doublets (J = 9.0 Hz) at δ_H 7.53 and 7.39 attributable to H-9 and H-10, respectively, as diagnostic signals. An ABX system [δ_H 7.47 (dd, J = 8.0 and 7.5 Hz, H-7), 7.38 (dd, J = 7.8 and 1.5 Hz, H-8) and 7.25 (dd, J = 7.8 and 1.5 Hz, H-6)] and a singlet for an isolated benzene proton at δ_H 7.22 (s, H-1) were also observed. In addition, resonances for two hydroxyl (δ_H 10.32 and 6.41) and two methoxyl (δ_H 4.10 and 3.79) groups were observed. The ^{13}C NMR data and HMQC correlations supported the above assignments. A detailed analysis of the NOESY (Table 1) and HMBC spectra confirmed the position of the methoxyl and hydroxyl groups on the phenanthrene skeleton. A subsequent study by X-ray crystallography confirmed the proposed structure (Figure 1). Compound **1** crystallized with two chemically identical but crystallographic different molecules. The methoxyl groups are oriented in opposite directions in order to minimize steric repulsion. The most striking feature of this compound resides in the notable deviation from planarity of the phenanthrene moiety (angle between the least-squares planes of the lateral benzene rings: molecule A = 17.0°; molecule B = 18.7°). The phenanthrene backbone seems to be stabilized by an intramolecular hydrogen bond between the hydroxyl [O-4 (O-24)] and the methoxyl groups [O-3 (O-23)] on both molecules, although with different strengths judging by the H···O distance (Table 4). In the crystal, the molecules are arranged into tetramers by forming hydrogen bonds, alternating molecules A and B in a cyclic fashion (Figure 2).



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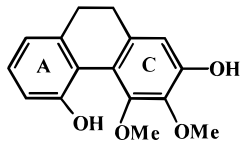

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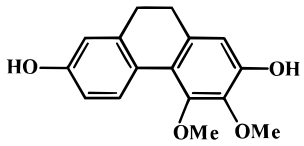

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Compound **2** had the composition $C_{16}H_{16}O_4$, as determined by MS and ^{13}C NMR, differing from **1** by two mass units. This observation as well as the UV[7] and NMR data (Table 1) suggested that **2** was the 9,10-dihydro derivative

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Table 1. ^{1}H (500 MHz, $CDCl_3$, *J* (Hz)) and ^{13}C NMR (125 MHz, $CDCl_3$) Data of Compounds **1** and **2**[a]

	1			**2**		
position	^{1}H	^{13}C	NOESY[b]	^{1}H	^{13}C	NOESY[b]
1	7.22 (s)	109.9	H-10	6.77 (s)	111.6	CH_2-10
2		147.1			148.9	
3		140.7			138.6	
4		148.1			148.1	
4a		116.7			118.8	
4b		117.9			120.1	
5		153.7			153.6	
6	7.25 (dd, 7.8, 1.5)	116.2	H-7, OH-5	6.96 (dd, 8.3, 1.3)	118.1	H-7, OH-5
7	7.47 (dd, 8.0, 7.5)	127.3	H-6, H-8	7.15 (dd, 7.2, 7.2)	128.2	H-8, H-6
8	7.38 (dd, 7.8, 1.5)	120.6	H-7	6.85 (dd, 7.3, 1.3)	119.1	CH_2-9, H-7
8a		134.2			140.5	
9	7.53 (d, 9.0)	128.1	H-10			
10	7.39 (d, 9.0)	125.9	H-9, H-1			
CH_2-9				2.71 (m)	31.1	H-8, CH_2-10
CH_2-10				2.64 (m)	30.7	H-1, CH_2-9
10a		130.9			137.4	
OH-2	6.41 (brs)		OMe-3	5.65 (brs)		
OH-5	10.32 (s)		OMe-4, H-6	8.51 (brs)		H-6, OMe-4
OMe-3	3.79 (s)	62.0	OMe-4, OH-2	3.99 (s)	61.7	OMe-4
OMe-4	4.10 (s)	62.5	OMe-3, OH-5	3.75 (s)	62.1	OH-5, OMe-3

[a] Assigned by HMQC, HMBC, and DEPT spectra. [b] Key ^{1}H−^{1}H correlations.

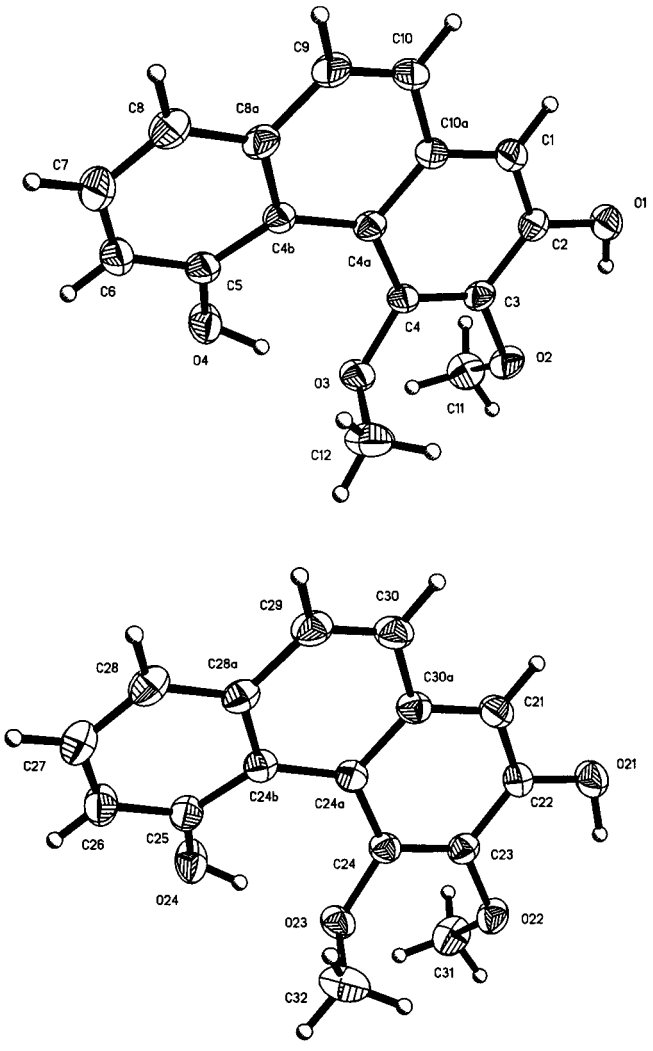

Figure 1. ORTEP diagrams for compound **1** (30% probability ellipsoids) showing two crystallographically independent molecules with atom numbering scheme.

of compound **1**. The most obvious differences between the NMR spectra of the two compounds resulted from the presence of two methylene signals in **1** [δ_H 2.71 (2H, m, H-9) and 2.64 (2H, m, H-10) in the ^{1}H NMR spectrum; δ_C 31.1 (C-9) and 30.7 (C-10) in the ^{13}C NMR spectrum], instead of the aromatic resonances attributed to H-9/C-9 and H-10/C-10 in **2**. In addition, the chemical shift values for the aromatic protons and carbons of rings A and C in **2** (Table 1) were shifted diamagnetically in comparison to those in **1**. As in the case of compound **1**, the position of the substituents in the dihydrophenanthrene core was deduced from the NOESY (Table 1) and HMBC data. Thus, the cross-peaks between H-1 (δ_H 6.77)/CH_2-10 (δ_H 2.64), and H-1 (δ_H 6.77)/OH-2 (δ_H 5.65) in the NOESY spectrum allowed the placement of one hydroxyl group at C-2. On the other hand, the correlations CH_2-9 (δ_H 2.71)/H-8 (δ_H 6.85), H-8 /H-7 (δ_H 7.15), H-7 /H-6 (δ_H 6.96), H-6/OH-5 (δ_H 8.51), OH-5/OMe-4 (δ_H 3.75), and OMe-4/OMe-3 (δ_H 3.99) indicated that the second hydroxyl and the methoxyl groups were located at C-5, C-3, and C-4, respectively. Furthermore, the HMBC correlations C-5/H-6, C-5/H-7, C-6/OH-5, C-1/OH-2, C-2/H-1, C-3/H-1, C-3/OMe-3, and C-4/OMe-4 confirmed the allocations for these functional groups.

The structure of compound **3** was also assigned unequivocally as 9,10-dihydro-2,7-dihydroxy-3,4-dimethoxyphenanthrene by X-ray crystallography. The molecular structure of **3** is illustrated in Figure 3. This dihydro compound deviates considerably from planarity (angle between the least-squares planes of the lateral benzene rings: 25.6°), mainly due to the twisted-boat conformation of the central six-member ring (Cremer and Pople parameters:[13] Q = 0.491 Å, θ = 110.3°, ϕ = 29.31°). In the crystal, helical ribbons are formed by hydrogen bonds between the hydroxyl group attached to C-2 and the methoxyl group at C-3 of the symmetrically related molecule, extended and inter-linked by interaction between the hydroxyl groups at C-7 (Figure 3b).

The spasmolytic activity and the mode of action of the isolated compounds will be described elsewhere.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns apparatus and are uncorrected. IR spectra were obtained using KBr disks on a Perkin-Elmer 599 B spectrophotometer. UV spectra were registered on a Shimadzu 160 UV apparatus in MeOH solution. NMR spectra including COSY spectra, NOESY, HMBC and HMQC experiments were recorded on a Varian UNITY PLUS 500

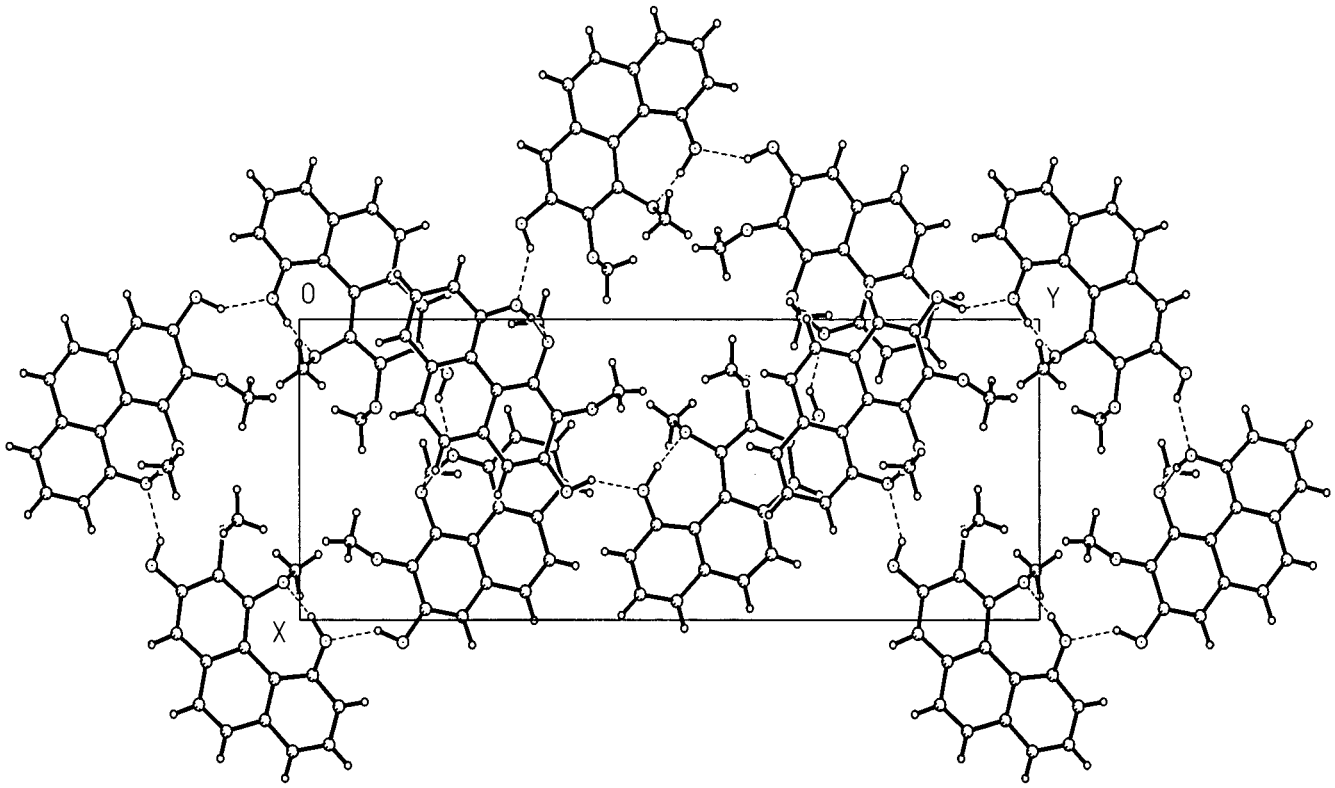

Figure 2. Unit cell viewed down the *c* axis for compound **1**.

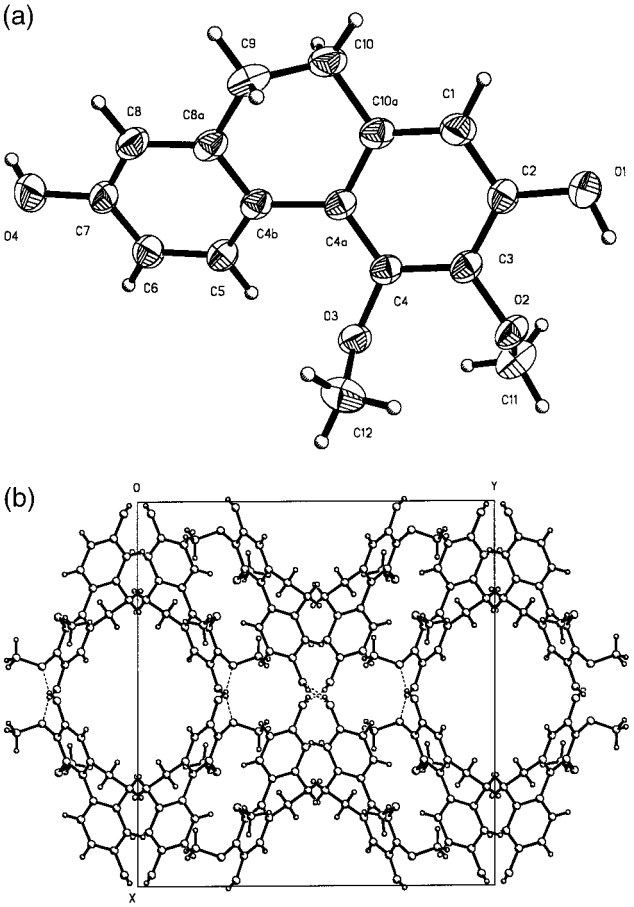

Figure 3. (a) ORTEP diagram for compound **3** (30% probability ellipsoids) showing the crystallographic atom numbering scheme. (b) Unit cell viewed down the *c* axis.

spectrometer in $CDCl_3$ or DMSOd_6 either at 500 MHz (^{1}H) and 125 MHz (^{13}C), using tetramethylsilane (TMS) as an internal standard. EIMS were performed using a JEOL JMS-AX505 HA spectrometer, at an ionization energy of 70 eV. HPLC was carried out with a Waters HPLC instrument equipped with Waters UV photodiode array detector (900) set at 230 nm, using a silica gel column (19 mm id X 300 mm) at a flow rate of 6.7 mL min^{-1}. Control of the equipment, data acquisition, processing and management of chromatographic information were performed by the Millenium 2000 software program (Waters). Column chromatography: silica gel 60 (Merck, 30−70 mesh). TLC: silica gel 60 F_{254} (Merck).

Plant Material. Whole plants of *M. densa* Lindley (Orchidaceae) were collected in July 1996 from Ejido Ruíz Cortínez, Catemaco, State of Veracruz, México. A voucher specimen (Carmona 96-1) is preserved at the Instituto de Ecología Herbarium (XAL), Xalapa, Veracruz.

Extraction and Isolation. The air-dried plant material (2.2 kg) was ground and extracted exhaustively by maceration at room temperature with a mixture of MeOH−$CHCl_3$ (1:1). After filtration, the extract was concentrated in vacuo to yield 161.2 g of residue. The concentrated extract was fractionated by column chromatography on silica gel (775 g), eluting with *n*-hexane, followed by a gradient of hexane/EtOAc (10:0→0:10) and finally with EtOAc/MeOH (10:0→5:5). Altogether, 215 fractions (600 mL each) were collected and pooled based on their TLC profiles to yield seven major fractions (FI to FVII). According to a pharmacological evaluation,[2] fraction VI was the most active, and induced 80% inhibition of the spontaneous contractions of the rat ileum, when tested at the IC_{50} of the original extract (0.62 μg/mL).

Fraction FVI (17.31 g) was further chromatographed on a Si gel column (759 g) and eluted with a gradient of hexane/EtOAc (10:0→0:10). This procedure led to the isolation of compounds **1** (205.2 mg), 2,7-dihydroxy-3,4-dimethoxyphenanthrene (150 mg), **3** (1.27 g), 2,5-dihydroxy-3,4,9-trimethoxyphenanthrene (1.55 g), 2,7-dihydroxy-3,4,9-trimethoxy-phenanthrene (1.85 g), and a mixture of compounds **1** and **2** (45 mg). Compounds **1** and **3** as well as the mixture of **1** and **2** were obtained from the fractions eluted with hexane−AcOEt 7:3. The remaining compounds were isolated from the fractions eluted with hexane−AcOEt 6:4. The mixture was resolved by

HPLC (the eluants were 94% hexane, 3% *i*-PrOH, and 3% MeOH) to yield additional amounts of **1** (15 mg) and **2** (25 mg).

2,5-Dihydroxy-3,4-dimethoxyphenanthrene (1). Yellow crystals; mp 118−119 °C; UV (MeOH) λ_{max} (log ϵ) 258 (4.78), 280 (4.36), 300 (4.19), 310 (4.19), 330 sh (3.60) nm; IR (KBr) ν_{max} 3528, 3180, 1622, 1603, 1564, 1526, 1467, 1431, 1355, 1272, 1095, 997, 938, 862 cm^{-1}; ^1H and ^{13}C NMR (Table 1); EIMS *m*/*z* 270 $[M]^+$ (100), 255 (28), 237 (9), 227 (31), 212 (48), 184 (9), 155 (15), 139 (13), 128 (10).

9,10-Dihydro-2,5-dihydroxy-3,4-dimethoxyphenanthrene (2). Yellow powder; mp 123−124 °C; UV (MeOH) λ_{max} (log ϵ) 272 (3.45), 280 (3.35), 296 sh (3.24), 304 (3.45) nm; IR (KBr) ν_{max} 3388, 2942, 1614, 1581, 1486, 1456, 1345, 1298, 1244, 1224, 1059, 999, 927, 823 cm^{-1}; ^1H and ^{13}C NMR (Table 1); EIMS *m*/*z* 272 $[M]^+$ (100), 257 (32), 239 (11), 225 (38), 212 (8), 197 (22), 183 (3), 169 (12), 153 (3), 141 (4), 115 (2).

The identification of the known compounds obtained in this investigation was accomplished by comparison of their spectral data (UV, MS, ^1H and ^{13}C NMR) with those previously described for 2,7-dihydroxy-3,4-dimethoxyphenanthrene,[4] 9,10-dihydro-2,7-dihydroxy-3,4-dimethoxyphenanthrene (**3**),[5] 2,5-dihydroxy-3,4,9-trimethoxyphenanthrene,[6] and 2,7-dihydroxy-3,4,9-trimethoxyphenanthrene.[7]

X-ray Crystallographic Analysis of Compounds 1 and 3. The molecular structures of compounds **1** and **3** were analyzed by X-ray diffraction crystallography following very similar procedures. The crystal data for the two samples and details of the experimental results are shown in Table 2.[14] For each sample, crystals were mounted, in air, on glass fibers. Accurate cell parameters were determined by refinement from the setting of 25 reflections and diffraction intensities measured at 293 K using an $\omega-\theta$ scan method on a Siemens P4/PC diffractometer equipped with graphite-monochromated radiation. The intensities of three standard reflections, recorded every 100 collected reflections, showed no changes. All data sets were corrected for Lorentz−polarization effects but no absorption corrections were applied.

The structure of each compound was determined by direct methods (SIR92)[15] and refined by full-matrix least-squares methods using SHELXTL97.[16] Hydrogen atoms attached to carbon atoms were set to ride on the parent C atoms, and for those bonded to O atoms their positional parameters were refined, on both cases an isotropic temperature factor 1.2 times the U*eq* of the parent atom was used. The non-hydrogen atoms were refined with anisotropic thermal parameters.

Pharmacological Testing. The smooth muscle relaxant effect of the extract and fractions was demonstrated using the isolated rat ileum test.[2] Papaverine (IC_{50} ($(1.55 \pm 0.12) \times 10^{-6}$ M) was used as positive control.

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Supporting Information Available: Tables of crystal data and experimental crystallographic details, of atomic coordinates and equivalent isotropic displacement parameters, and hydrogen bond schemes for compounds **1** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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