

## Urea Derivatives from *Pentadiplandra brazzeana*

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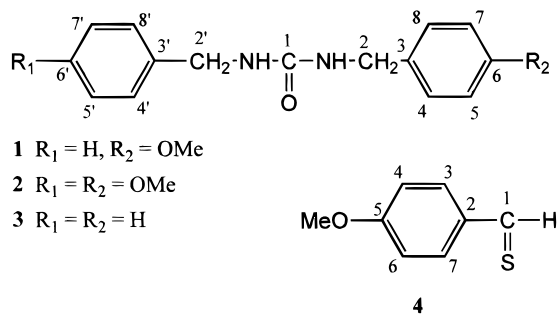
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Four urea derivatives were isolated from the roots of *Pentadiplandra brazzeana*, and their structures were elucidated by spectroscopic techniques. *N*-Benzyl-*N*-(4-methoxybenzyl)urea (**1**) is a new compound, although *N,N*-di-(4-methoxybenzyl)urea (**2**), *N,N*-dibenzylurea (**3**), and *p*-methoxythiobenzaldehyde (**4**) are reported from a natural source for the first time.

*Pentadiplandra brazzeana* Baillon (Pentadiplandraceae) is a climber with thick tuberous roots and very sweet berries, which is widespread in the savanna region of Cameroon. The root bark is a constituent of a popular ethnodiary preparation, "Nkui", which is served to mothers who have just given birth, to stimulate milk production. A root bark decoction is also a reputed folk remedy against hemorrhoids.<sup>1</sup> Children eat the sweet berries or sometimes use them to sweeten their corn porridge. Previous phytochemical studies reported the isolation of three benzylated thioureas from the root bark<sup>2</sup> and two sweet proteins, pentadin<sup>3</sup> and brazein,<sup>4</sup> as the sweet principles from the berries. As a continuation of our ongoing study of the constituents of African ethnodiary preparations,<sup>5</sup> we have reinvestigated the root extracts of *P. brazzeana*. In this paper we report the isolation and structure elucidation of four compounds from this extract, including the new urea derivative, *N*-benzyl-*N*-(4-methoxybenzyl)urea (**1**).

The CH<sub>2</sub>Cl<sub>2</sub>–MeOH (1:1) extract of the roots of *P. brazzeana* was subjected to a liquid–liquid partition using, successively, *n*-hexane, CH<sub>2</sub>Cl<sub>2</sub>, and EtOAc. Vacuum liquid column chromatography of the combined hexane and CH<sub>2</sub>Cl<sub>2</sub>-soluble fractions on Si gel followed by gel permeation chromatography of the resulting fractions through Sephadex LH-20, and finally, medium-pressure liquid chromatographic purification on Si gel, afforded the novel natural products **1**–**4**.



Compound **1**, mp 127–129 °C, was obtained as white crystals. Elemental analysis and MS data agreed with the molecular formula C<sub>16</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>. The IR spectrum exhibited strong absorptions at  $\nu_{\max}$  3300, 1615, 1615, and 1600 cm<sup>-1</sup>

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**Table 1.** <sup>1</sup>H (500 MHz) and <sup>13</sup>C (125 MHz) NMR Spectral Data and HMBC Correlations for Compound **1** in (CDCl<sub>3</sub>)

carbon	$\delta_C$	$\delta_H$	HMBC correlations (C → H)
1	158.3		H <sub>2</sub> , H <sub>2'</sub>
2	43.7	4.21 (s, 2H)	H <sub>4</sub> , H <sub>8</sub>
3	131.2		H <sub>2</sub> , H <sub>5</sub> , H <sub>7</sub>
4	128.6	7.12 (d, <i>J</i> = 8.5 Hz, 1H)	H <sub>2</sub>
5	113.9	6.79 (d, <i>J</i> = 8.5 Hz, 1H)	H <sub>4</sub>
6	158.7		Me, H <sub>4</sub> , H <sub>8</sub>
7	113.9	6.79 (d, <i>J</i> = 8.5 Hz, 1H)	H <sub>8</sub>
8	128.6	7.12 (d, <i>J</i> = 8.5 Hz, 1H)	H <sub>2</sub>
2'	44.3	4.27 (s, 2H)	H <sub>4'</sub> , H <sub>8'</sub>
3'	139.2		H <sub>2'</sub> , H <sub>5'</sub> , H <sub>7'</sub>
4'	127.3	7.21 (m, 1H)	H <sub>2'</sub> , H <sub>6'</sub>
5'	128.5	7.26 (m, 1H)	H <sub>6'</sub>
6'	127.2	7.26 (m, 1H)	H <sub>4'</sub> , H <sub>8'</sub>
7'	128.5	7.26 (m, 1H)	H <sub>6'</sub>
8'	127.3	7.21 (m, 1H)	H <sub>2'</sub>
OMe	55.2	3.76 (s, 3H)	

representing, respectively, an NH group, a carbonyl group, and a benzene ring moiety. Its UV spectrum showed absorptions at  $\lambda_{\max}$  201, 223, 275, and 282 nm, consistent with the presence of an amide group and substituted benzene rings. The <sup>13</sup>C NMR spectrum displayed signals for 16 carbons, and the HMQC spectrum showed them to be one primary, two secondary, nine tertiary, and four quaternary carbon atoms. The <sup>1</sup>H NMR spectrum revealed the presence of a *para*-disubstituted benzene ring with a typical AA'BB' spin system at  $\delta$  6.79 (d, *J* = 8.5 Hz, 2H) and 7.12 (d, *J* = 8.5 Hz, 2H) and a monosubstituted benzene ring with resonances at  $\delta$  7.21 (m, 2H) and 7.26 (m, 3H). Of the remaining protons, there were two methylene groups represented by singlets at  $\delta$  4.21 and 4.27, respectively, and one methoxy group at  $\delta$  3.76.

The above data suggest that compound **1** is a urea derivative in which the first amino residue bears a benzyl group, while the second carries a methoxybenzyl substituent. The COSY experiment and the HMBC spectrum (see connectivities in Table 1) were in agreement with the structure proposed, *N*-benzyl-*N*-(4-methoxybenzyl)urea (**1**).

Compounds **2**, **3**, and **4** were identified as *N,N*-di-(4-methoxybenzyl)urea, *N,N*-dibenzylurea, and *p*-methoxythiobenzaldehyde from their physical and spectroscopic data. These three compounds have previously been prepared by synthesis and are reported herein from nature for the first time.<sup>6–8</sup>

### Experimental Section

**General Experimental Procedures.** All melting points were recorded with a Reichert microscope and are uncorrected. The UV and the IR spectra were recorded with a Varian Cary

2290 and a Perkin–Elmer 298 spectrometer, respectively.  $^1\text{H}$  NMR (500 MHz) and  $^{13}\text{C}$  NMR (125 MHz) spectra were recorded in  $\text{CDCl}_3$  using a Bruker ARX500 spectrometer with an inverse multinuclear 5-mm probe head equipped with a shielded gradient coil. The chemical shifts ( $\delta$ ) are reported in parts per million with the solvent signals  $\delta_{\text{H}}$  7.26 and  $\delta_{\text{C}}$  77.0 as reference, while the coupling constants ( $J$ ) are given in Hertz. COSY, HMQC, and HMBC experiments were recorded with gradient enhancements using sine-shaped gradient pulses. For 2D heteronuclear correlation spectroscopy the refocusing delays were optimized for  $^1J_{\text{CH}} = 145$  Hz and  $^nJ_{\text{CH}} = 10$  Hz. MS were recorded with a JEOL SX102 spectrometer at 70 eV. Elemental analysis was performed at Lund Institute of Technology. Column chromatography was run on Merck Si gel 60 and Sephadex LH-20, while TLC were carried out on Si gel GF<sub>254</sub> precoated plates with detection accomplished by spraying with 50%  $\text{H}_2\text{SO}_4$  followed by heating at 100 °C, or by visualizing with a UV lamp at 254 nm.

**Plant Material.** The roots of *P. brazzeana* were collected from Dschang, Cameroon, in December 1995. Mr. Paul Mezili, a retired botanist of the Cameroon National Herbarium, authenticated the plant material. Voucher specimens (BUD 0307) were deposited at the Herbarium of the Botany Department of the University of Dschang.

**Extraction and Isolation.** The dried and ground roots (4 kg) were extracted overnight at room temperature by percolation with  $\text{MeOH}-\text{CH}_2\text{Cl}_2$  (1:1) (7 L), followed by pure MeOH (7 L), to yield a crude organic extract (280 g) on drying. This extract was then partitioned between *n*-hexane and 90% MeOH to give an *n*-hexane-soluble fraction (25 g) and an aqueous MeOH-soluble fraction. The aqueous MeOH fraction was adjusted to 80% MeOH and further extracted with  $\text{CH}_2\text{Cl}_2$  to yield a  $\text{CH}_2\text{Cl}_2$ -soluble fraction (62 g). The *n*-hexane and the  $\text{CH}_2\text{Cl}_2$ -soluble fractions shown by TLC to be qualitatively the same were combined and subjected to repeated column chromatography on Si gel to yield four crude compounds. Further purification of these compounds by gel permeation chromatography through a Sephadex LH-20 column (eluted with  $\text{CH}_2\text{Cl}_2$ -*n*-hexane 3:7) afforded **1** (76 mg), **2** (43 mg), **3** (23 mg), and **4** (13 mg). For compounds **2** and **4**, an additional purification by MPLC using a Baeckström Separo AB Column (i.d., 15 mm) with a continuous gradient of *n*-hexanes–EtOAc was required to obtain pure samples for analysis.

***N*-Benzyl-*N*-(4-methoxybenzyl)urea (1):** white crystals (hexane–EtOAc); mp 127–129 °C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 201 (4.45), 223 (4.05), 275 (3.16), and 282 (3.10) nm; IR (KBr)  $\nu_{\text{max}}$  3300 (NH), 1615 (C=O, urea), 1600, 1580, 1510, 1240, 1030, 800, 700  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz), see Table 1; EIMS (70 eV)  $m/z$  [ $\text{M}]^+$  270 (89), 179 (33), 149 (40), 136 (100), 121 (61), 106 (53), 91 (37), 77 (13), 51 (4); *anal.* C 71.08%, H 6.72%, N 10.32%, calcd for  $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_2$ , C 71.09%, H 6.71%, N 10.36%.

***N,N*-Di-(4-methoxybenzyl)urea (2):** white crystals (hexane–EtOAc); mp 171–173 °C (lit.<sup>6</sup> 170–172 °C); IR (KBr)  $\nu_{\text{max}}$  3325, 2920, 1615, 1580, 1510, 1250, 1175, 1030, 810  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  3.69 (6H, s, 2  $\times$  OMe), 4.17 (4H, s, H-2,-2'), 6.75 (4H, d,  $J = 8.5$  Hz, H-5,-5',-7,-7'), 7.09 (4H, d,  $J = 8.5$  Hz, H-4,-4',-8,-8');  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  158.6 (C-1,-6,-6'), 131.3 (C-3,-3'), 128.4 (C-4,-4',-8,-8'), 113.8 (C-5,-5',-7,-7'), 56.9 (C-2,-2'), 55.1 (2  $\times$  OMe); EIMS (70 eV)  $m/z$  [ $\text{M}]^+$  300 (25), 271 (17), 270 (14), 192 (4), 180 (44), 136 (100), 121 (89), 106 (15), 91 (18), 77 (14), 51 (3); *anal.* C 67.97%, H 6.72%, N 9.30%, calcd for  $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_3$ , C 67.98%, H 6.71%, N 9.33%.

***N,N*-Dibenzylurea (3):** white crystals (hexane–EtOAc); mp 168–170 °C (lit.<sup>7</sup> 166–168°); IR (KBr)  $\nu_{\text{max}}$  3320, 1620, 1570, 1420, 1250, 700  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  4.28 (4H, s, H-2,-2'), 7.21 (4H, m, H-4,-4',-8,-8'), 7.27 (6H, m, H-5,-5',-6,-6',-7,-7');  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  158.5 (C-1), 139.2 (C-3,-3'), 128.6 (C-5,-5',-7,-7'), 127.3 (C-4,-4',-8,-8'), 127.2 (C-6,-6'), 44.4 (C-2,-2'); EIMS (70 eV)  $m/z$  [ $\text{M}]^+$  240 (83), 149 (29), 136 (3), 106 (100), 91 (53), 79 (14), 77 (11), 65 (10), 51 (5); *anal.* C 74.96%, H 6.72%, N 11.60%, calcd for  $\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}$ , C 74.97%, H 6.71%, N 11.66%.

***p*-Methoxythiobenzaldehyde (4):** white crystals (hexane–EtOAc); mp 121–123 °C; IR (KBr)  $\nu_{\text{max}}$  2900, 1680, 1600, 1580, 1430, 1300, 1260, 1180, 1165, 1025, 925, 840, 770  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  3.84 (3H, s, OMe), 6.90 (2H, d,  $J = 8$  Hz, H-4,6), 8.00 (2H, d,  $J = 8$  Hz, H-3,7);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  169.5 (C-1), 163.6 (C-5), 132.1 (C-3,-7), 122.3 (C-2), 113.6 (C-4,-6), 55.4 (OMe); EIMS (70 eV)  $m/z$  [ $\text{M}]^+$  152 (98), 135 (100), 107 (9), 92 (11), 77 (13), 63 (7), 50 (3); *anal.* C 63.15%, H 5.30%, S 5.93%, calcd for  $\text{C}_8\text{H}_8\text{OS}$ , C 63.13%, H 5.03%.

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## References and Notes

- Villiers, J. F. *Flore du Cameroun*; Muséum National d'Histoire Naturelle, Laboratoire de Phanérogamie: Paris, 1973; Vol. 5, p 163.
- El Migirab, S.; Berger, Y.; Jadot, J. *Phytochemistry* **1977**, *16*, 1719–1721.
- Van der Well, H.; Larson, G.; Hladik, A.; Hladik, C. M.; Hellekant, G.; Glasser D. *Chem. Senses* **1989**, *14*, 75–79, and references cited therein.
- Ming, D.; Hellekant, G. *FEBS Lett.* **1994**, *355*, 106–108.
- Ayafor, J. F.; Tchuendem, M. H. K.; Nyasse, B.; Tillequin, F.; Anke H. *Pure Appl. Chem.* **1994**, *66*, 2327–2330.
- Atanassova, I. A.; Petrov, J. S.; Mollov, N. M. *Synth. Commun.* **1989**, *19*, 147–153.
- Leung, M.-K.; Lai, J.-L.; Lau, J.-H.; Yu, H.-h; Hsiao, H.-J. *J. Org. Chem.* **1996**, *61*, 4175–4179.
- Ming, L. G.; Toshiyuki, K.; Masakito, S.; Tadashi, N. *Chem. Express* **1993**, *8*, 53–56.

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