New Pyranonaphthoquinone and Pyranonaphthohydroquinone from the Roots of *Pentas longiflora*

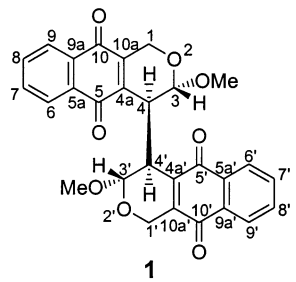
Samir El-Hady, Jacques Bukuru, Bart Kesteleyn, Luc Van Puyvelde, Tuyen Nguyen Van, and Norbert De Kimpe*

Department of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

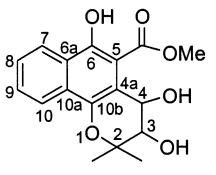
Received March 13, 2002

Several quinone type compounds were isolated from the hexane, dichloromethane, and ethyl acetate extracts of the roots of *Pentas longiflora*. The hexane extract afforded two new compounds, [(3α,3′α,4*β*,4′*β*)-3,3′]-dimethoxy-*cis*-[4,4′-bis(3,4,5,10-tetrahydro-1*H*-naphtho[2,3-*c*]pyran)]-5,5′,10,10′-tetraone (**1**) and *cis*-3,4-dihydroxy-3,4-dihydromollugin (**2**), together with six known compounds, namely, pentalongin, mollugin, *trans*-3,4-dihydroxy-3,4-dihydromollugin, methyl-2,3-epoxy-3-prenyl-1,4-naphthoquinone-2-carboxylate, tectoquinone, and 3-hydroxymollugin. From the dichloromethane extract were isolated the three known compounds 3-methoxymollugin, methyl-3-prenyl-1,4-naphthoquinone-2-carboxylate, and scopoletin, while the ethyl acetate extract afforded the known 2-methoxy-3-methylanthraquinone.

Pentas longiflora Oliver (Rubiaceae) is an important medicinal plant from Tropical East Africa.[1] In Kenya, where it is known as "Nekilango" or "Segimbe", the roots are used as a cure for tapeworm, for itchy rashes, and for pimples.[2] A decoction of the roots is mixed with milk and taken as a cure for malaria, but causes acute diarrhea and acts as a purgative.[2] In Rwanda, where the plant is called "Isagara", the traditional healers use the powder of the roots, mixed with butter, as an ointment to treat scabies and the skin disease pityriasis versicolor.[3,4] Because of the widespread use of *P. longiflora* in the traditional medicine of Central Eastern Africa, a detailed study of the constituents of the roots was undertaken. The powdered roots of *P. longiflora* were successively extracted with *n*-hexane, dichloromethane, ethyl acetate, and methanol. The different extracts were further chromatographed using several methods to provide a number of quinones. In this paper, we report on the isolation and structure elucidation of two new constituents (**1**, **2**) from the roots of *P. longiflora*.



1



2

(3α,3′α,4*β*,4′*β*)-3,3′-Dimethoxy-*cis*-[4,4′-bis(3,4,5,10-tetrahydro-1*H*-naphtho[2,3-*c*]pyran)]-5, 5′,10,10′-tetraone (**1**) was isolated from the hexane extract as yellow crystals, mp 252.8−253.5 °C (from MeOH), $[\alpha]^{21}_D$ −8.6° (*c* 0.29, $CHCl_3$). The spectral analysis displayed patterns in the ^{1}H NMR and ^{13}C NMR spectra similar to those of 3-methoxy-3,4-dihydropentalongin[5] with two exceptions. A small shift in the resonance of H_2-4 from δ_H 2.74−2.78 (2H, m, H_2-4) in 3-methoxy-3,4-dihydropentalongin[5] to δ_H 3.29 (1H, s, H-4) in **1** was observed, together with a change in intensity from two protons to one proton. The signal centered at δ_H 3.29 (1H, s, H-4) pointed to the presence of a sp^3 methine group in **1** instead of the sp^3 methylene group of 3-methoxy-3,4-dihydropentalongin.[5] The ^{13}C NMR spectrum also showed a shift in resonance of the C-4 signal from δ 27.4 in the spectrum of 3-methoxy-3,4-dihydropentalongin[5] to δ 38.7 in **1**, supporting the observed change of the carbon atom from a methylene to a methine. The elemental analysis and the mass spectrum ($[M]^+$ at *m*/*z* 486) indicated a molecular composition of $C_{28}H_{22}O_8$. The very small value of ∼0 Hz for the coupling constant ($J_{3,4}$ = 0 Hz) in the ^{1}H NMR spectrum of compound **1** indicated a pseudoequatorial position for H-4, which was confirmed by a long-range coupling (ABY) ($J_{1,4} \approx 0$ Hz), indicating that H-4 and H-1 are located in the pseudoequatorial configuration ($J_{1,4}$ to be $J^{a'a'} > J^{a'e'} \approx J^{e'a'} > J^{e'e'} < 0.5$ Hz).[6] The mass spectral fragmentation of this dimeric compound to the most abundant ion *m*/*z* 366 is explained in terms of two retro-Diels−Alder fragmentations with elimination of two units of methyl formate. Together with the previously assigned units and additional spectral data (COSY, HETCOR, IR), the structure of the isolated compound was deduced as (3α,3′α,4*β*,4′*β*)-3,3′-dimethoxy-*cis*-[4,4′-bis(3,4,5,10-tetrahydro-1*H*-naphtho[2,3]pyran)]-5,5′,10,10′-tetraone (**1**).

cis-Methyl-2,2-dimethyl-3,4,6-trihydroxy-3,4-dihydro-2*H*-naphtho[1,2-*b*]pyran-5-carboxylate (*cis*-3,4-dihydroxy-3,4-dihydromollugin) (**2**) was also isolated from the hexane extract. The ^{1}H NMR and ^{13}C NMR spectra showed nearly the same patterns as *trans*-3,4-dihydroxy-3,4-dihydromollugin.[7] The important observed difference between the ^{1}H NMR spectra of the two compounds was the coupling constant (*J*) of the two protons of the O−CH−CH−O unit at δ_H 3.85 (1H, d, *J* = 4.9 Hz, H-3) and 5.22 (1H, d, *J* = 4.9 Hz, H-4) for compound **2**, in comparison with δ_H 3.79 (1H, d, *J* = 6.4 Hz, H-3) and 5.06 (1H, d, *J* = 6.4 Hz, H-4) in the case of *trans*-3,4-dihydroxy-3,4-dihydromollugin.[7] The lower value of the coupling constant (*J*) in this case suggested the *cis* configuration of the two-coupled protons; hence *cis*-3,4-dihydroxy-3,4-dihydromollugin (**2**) was determined as the structure. The retention distance (R_f value) on a silica gel plate (Merck, silica gel 60-F_{254}) confirmed the differences between the two isomers: R_f = 0.36 for the *cis*-form,

* To whom correspondence should be addressed. Tel: +32 (0)9 264 59 51. Fax: +32 (0)9 264 62 43. E-mail: norbert.dekimpe@rug.ac.be.

10.1021/np020110e CCC: $22.00
Published on Web 08/24/2002

R_f = 0.48 for the *trans*-form. The α- or β-orientation (enantiomerism) of the two OH groups was not confirmed.

In addition, the hexane extract gave rise to the two known major compounds, pentalongin[3,8,9] and mollugin,[8,10] and four other known compounds, namely, 3-hydroxymollugin,[7] *trans*-3,4-dihydroxy-3,4-dihydromollugin,[7] methyl-2,3-epoxy-3-prenyl-1,4-naphthoquinone-2-carboxylate,[7,11] and tectoquinone.[11] Additional known compounds were isolated from the dichloromethane and ethyl acetate extracts, namely, 3-methoxymollugin,[7] methyl-3-prenyl-1,4-naphthoquinone-2-carboxylate,[12] scopoletin,[13] and 2-methoxy-3-methylanthraquinone.[14] All these compounds showed spectral data (IR, ^{1}H NMR, ^{13}C NMR, and MS) that were identical to those described in the literature.

Experimental Section

General Experimental Procedures. Melting point measurements were carried out on a Büchi melting point apparatus. Optical rotations were obtained on an AA-10 automatic polarimeter (l = 1 dm). ^{1}H and ^{13}C NMR spectra were recorded on a JEOL-JNM-EX 270 MHz FT NMR spectrometer (270 MHz for ^{1}H NMR, 67.5 MHz for ^{13}C NMR). Mass spectra were recorded at 70 eV on a Varian MAT 112 mass spectrometer using a direct inlet system. Medium-pressure liquid chromatography (MPLC) was carried out on a Büchi 687 gradient former, a Büchi 688 chromatography pump, a Büchi 684 fraction collector, a Sedex 55 light scattering detector, and Büchi borosilicate glass columns. Silica gel 60 (0.015−0.040 mm; Merck) and LiChroprep RP-18 (40−63 μm particle size; Merck) were used as column material in normal and reversed-phase separations, respectively. Centrifugal partition chromatography (CPC) was carried out using a CCC-3000 model (Pharma-Tech Research Corp., Baltimore, MD).

Plant Material. The roots of *Pentas longiflora* were collected in the Menengai Crater (Nakuru District, Kenya) at an altitude of 2400 m in July 1996. The plant was identified by G. M. Mungai and D. O. Nyakundi (National Museum of Nairobi, Kenya), and voucher herbarium species were deposited in the East African Herbarium at the National Museum in Nairobi (Kenya) (Mungai & Nyakundi, No. 464). The roots were dried in a ventilated oven at 45 °C for 3 days and powdered mechanically (Retsch GmbH, type SK1, 1100 W, 2840 u/min, DR 80B/2Q).

Extraction and Isolation. The powdered roots of *P. longiflora* (4.1 kg) were successively extracted in a percolator until exhaustion with *n*-hexane, dichloromethane, ethyl acetate, and methanol. The extracts were filtered through a filter paper and concentrated under reduced pressure at 40 °C, yielding a dark red hexane residue (44.0 g), a dark green dichloromethane residue (46.9 g), a dark red ethyl acetate residue (14.6 g), and a dark brown methanol residue (466.1 g).

Hexane Extract. The hexane extract (44 g) was adsorbed on silica gel (440 g) and submitted to MPLC (glass column, 460 × 100 mm i.d.) on silica gel. Sample application was executed with a Prep Elute dry application column (230 × 23 mm i.d.). The elution was performed using a *n*-hexane−EtOAc gradient: 5% (180 min), 10% (60 min), 25% (30 min), 50% (30 min), EtOAc 100% (30 min), and finally washed with MeOH 100% (60 min). After monitoring by TLC, 26 fractions were obtained.

Fraction 7 (*n*-hexane−EtOAc 5%) from the MPLC of the hexane extract gave mollugin (6.62 g, 0.16146% yield) as yellow crystals, mp 132.5−133.1 °C (from $CHCl_3$), lit. mp 132−134 °C.[8] Fraction 9 (*n*-hexane−EtOAc 5%) afforded pentalongin (5.87 g, 0.14317% yield) as dark red crystals, mp 160.8−161.3 °C ($CHCl_3$), lit. mp 160−161 °C.[8] Fraction 10 (5% *n*-hexane−EtOAc) was subjected to MPLC on silica gel and resulted in 12 further fractions. Fraction 5 from this separation was rechromatographed (MPLC, silica gel) two times, leading to the isolation of compound **1** (660.7 mg, 0.01612% yield), which eluted with *n*-hexane−EtOAc (99:1), while further purification of fraction 10 by CPC with *n*-hexane−EtOAc−MeOH−H_2O (15:5:5:2) as solvent system afforded 3-hydroxymollugin (160.0 mg, 0.00390% yield) as pale yellowish needles, mp 141.1−142.6 °C ($CHCl_3$), lit. mp 143−145 °C.[7] Fraction 24 (EtOAc 100%) from the hexane extract was rechromatographed on silica gel (MPLC) to afford nine different fractions. From the latter, fractions 6 and 7 were subjected to CPC with the solvent system *n*-hexane−EtOAc−MeOH−H_2O (5:6:5:2) followed by preparative TLC, using the upper layer of the previous binary system *n*-hexane−EtOAc−MeOH−H_2O (5:6:5:2) as eluent (R_f 0.36), to afford (second band) compound **2** (11.2 mg, 0.00027%), while fraction 9 was resubjected to MPLC followed by CPC using *n*-hexane−EtOAc−MeOH−H_2O (5:8:5:2) as solvent system to give *trans*-3,4-dihydroxy-3,4-dihydromollugin (6.4 mg, 0.00016% yield), mp 161−162 °C ($CHCl_3$).[7] Fraction 11 (*n*-hexane−EtOAc 5%) was further purified by CPC with *n*-hexane−EtOH−H_2O (6:5:1) as solvent system, followed by another CPC purification using *n*-hexane−EtOAc−MeOH−H_2O (9:1:5:5), affording methyl-2,3-epoxy-3-prenyl-1,4-naphthoquinone-2-carboxylate (42.5 mg, 0.00104% yield) as an oil.[7,11] On the basis of the spectral analysis, fraction 1, which eluted with CH_2Cl_2−MeOH 1%, contained tectoquinone (15.3 mg, 0.00037% yield) as the sole compound, mp 179 °C ($CHCl_3$), lit. mp 196−198 °C (toluene).[11]

Dichloromethane Extract. MPLC was conducted on the dichloromethane extract, which resulted in 10 fractions. Fraction 8 was further purified by MPLC, using a gradient of CH_2Cl_2−MeOH as solvent system to afford 15 fractions. Fraction 3 of the latter was subjected to CPC with *n*-hexane−EtOAc−MeOH−H_2O (10:6:5:2), giving rise to 3-methoxymollugin (2.6 mg, 0.00006% yield), mp 120−122 °C ($CHCl_3$), lit. mp 121−123 °C ($CHCl_3$),[7] while fractions 5 and 6 were eluted isocratically (MPLC/RP-18) with H_2O−MeOH 25% to give eight fractions, from which fraction 1 was subjected to CPC with *n*-hexane−EtOAc−MeOH−H_2O (1:1:1:1) to afford methyl-3-prenyl-1,4-naphthoquinone-2-carboxylate (3.0 mg, 0.00007% yield), mp 45.6−46.3 °C ($CHCl_3$), lit. mp 43 °C[12] and scopoletin (11.4 mg, 0.00028% yield), mp 186.9−188.3 °C, lit. mp 198−199 °C,[13] after purification by preparative TLC using MeOH−CH_2Cl_2 5% as eluent (R_f 0.62).

Ethyl Acetate Extract. MPLC was conducted on the ethyl acetate extract using RP-18 (reversed-phase) as column material, resulting in eight fractions. MPLC was again carried out on fraction 6 (H_2O−MeCN 40%) to give six further fractions, from which fraction 4, which eluted with CH_2Cl_2, afforded 2-methoxy-3-methylanthraquinone (4.5 mg, 0.00011% yield), mp 195.0−196.7 °C ($CHCl_3$), lit. mp 196−198 °C (toluene).[14]

(3α,3′α,4*β*,4′*β*)-3,3′-Dimethoxy-*cis*-[4,4′-bis(3,4,5,10-tetrahydro-1*H*-naphtho[2,3-*c*]pyran)]-5,5′,10,10′-tetraone (1): yellow crystals, mp 252.8−253.5 °C (from MeOH); $[\alpha]^{21}_D$ −8.6° (*c* 0.29, $CHCl_3$); IR (KBr) ν_{max} 1655 (C=O), 1640 (C=O), 1590 (C=C), 1330, 1295, 1175, 1120, 1060 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.29 (2 × 1H, s, H-4 and H-4′), 3.44 (2 × 3H, s, 2 × OC*H*$_3$), 4.40−4.45 (2 × 2H, m, H$_2$-1 and H$_2$-1′), 5.12 (2 × 1H, s, H-3 and H-3′), 7.72−7.76 (2 × 2H, m, H-7, H-8, H-7′, and H-8′), 8.03−8.11 (2 × 2H, m, H-6, H-9, H-6′, and H-9′); ^{13}C NMR (68 MHz, $CDCl_3$) δ 38.7 (C-4 and C-4′), 55.6 (2 × O*C*H$_3$), 56.3 (C-1 and C-1′), 99.7 (C-3 and C-3′), 126.1−126.4 (C-7, C-8, C-7′, and C-8′), 131.8 (C-5a/C-10a or C-5a′/C-10a′), 132.1 (C-5a/C-10a or C-5a′/C-10a′), 133.7 and 133.9 (C-6, C-9, C-6′, and C-9′), 140.78 (C-4a/C-9a or C-4a′/C-9a′), 140.82 (C-4a/C-9a or C-4a′/C-9a′), 183.2 and 183.3 (2 × C=O); EIMS *m*/*z* 456 [M − 30]$^+$ (3), 394 (47), 366 (100), 349 (29), 321 (13), 281 (12), 242 (25), 214 (33), 199 (27), 183 (30), 169 (14), 155 (18), 139 (14), 127 (24), 119 (14), 111 (20), 105 (33), 97 (28), 83 (28), 69 (34), 57 (54), 43 (58); *anal*. C 68.81%, H 4.32%, calcd for $C_{28}H_{22}O_8$, C 69.06%, H 4.52%.

***cis*-Methyl-2,2-dimethyl-3,4,6-trihydroxy-3,4-dihydro-2*H*-naphtho[1,2-*b*]pyran-5-carboxylate (*cis*-3,4-dihydroxy-3,4-dihydromollugin) (2):** colorless amorphous powder, mp 107.6−108 °C (from $CHCl_3$); $[\alpha]^{21}_D$ −12.9° (*c* 0.35, $CHCl_3$); IR (KBr) ν_{max} 3560−3100 (OH), 1650 (C=O), 1590 (C=C), 1440, 1240, 1150, 1100, 1040, 900, 770 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.40 (3H, s, C*H*$_3$), 1.51 (3H, s, C*H*$_3$), 3.85 (1H, d, J = 4.9 Hz, H-3), 4.06 (3H, s, OC*H*$_3$), 5.22 (1H, d, J = 4.9 Hz, H-4),

7.58−7.62 (2H, m, H-8 and H-9), 8.20−8.37 (2H, m, H-7 and H-10), 11.20 (1H, s, OH); ^{13}C NMR (68 MHz, $CDCl_3$) δ 171.2 (*C*OOCH$_3$), 155.8 (C-6), 140.8 (C-10b), 129.5 (C-9), 128.9 (C-10a), 127.0 (C-8), 125.7 (C-6a), 123.9 (C-10), 122.6 (C-7), 112.9 (C-4a), 104.6 (C-5), 77.2 (C-4), 72.3 (C-3), 52.8 (COO*C*H$_3$), 24.8 (*C*H$_3$), 22.0 (*C*H$_3$); EIMS *m*/*z* 318 $[M]^+$ (85), 301 (40), 286 (25), 257 (10), 219 (8), 215 (13), 190 (11), 145 (8), 132 (50), 119 (20), 104 (39); *anal*. C 63.78%, H 5.32%, calcd for $C_{17}H_{18}O_6$, C 64.09%, H 5.56%.

Acknowledgment. The authors are indebted to the Janssen Research Foundation (Beerse, Belgium) for financial support.

References and Notes

(1) Troupin, C. G., Ed. In *Flore du Rwanda*, S*permatophytes*; Institut National de Recherche Scientifique: Butare (Rwanda), 1985; Vol. 3, p 196.
(2) Kokwaro, J. O. *Medicinal Plants of East Africa*; East African Literature Bureau: Kampala, Nairobi, Dar es Salaam, 1976; p 190.
(3) De Kimpe, N.; Van Puyvelde, L.; Schripsema, J.; Erkelius, C.; Verpoorte R. *Magn. Reson. Chem.* **1993**, *31*, 329−330.
(4) Van Puyvelde, L.; Geysen D.; Ayobangira, F.-X.; Hakizamungu, E.; Nshimyimana, A.; Kalisa, A. *J. Ethnopharmacol.* **1985**, *13*, 209−215.
(5) El-Hady, S. Isolation and Structural Elucidation of Natural Products from *Pentas longiflora* Oliver and *Gossypioides kirkii* (Mast.) J.B. Hutch. Ph.D. Thesis, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Ghent, Belgium, 1999.
(6) Cameron, D. W.; Kingston, D. G. I.; Sheppared, N.; Todd, L. *J. Chem. Soc.* **1964**, 98−104.
(7) Itokawa, H.; Ibrahim, Z.; Qiao, Y.-F.; Takeya, K. *Chem. Pharm. Bull.* **1993**, *41*, 1869−1872.
(8) Hari, L.; De Buyck, L. F.; De Pooter, H. L. *Phytochemistry* **1991**, *30*, 1726−727.
(9) Kesteleyn, B.; De Kimpe, N.; Van Puyvelde, L. *J. Org. Chem.* **1999**, *64*, 1173−1179.
(10) Schildknecht, H.; Straub, F.; Scheidel, U. *Liebigs Ann. Chem.* **1976**, 1295−1306.
(11) Itokawa, H.; Qiao, Y.; Takeya, K. *Phytochemistry* **1991**, *30*, 637−640.
(12) Heide, L.; Leistner, E. *J. Chem. Soc., Chem. Commun.* **1981**, 334−336.
(13) Arisawa, M.; Kinghorn, A. D.; Cordell, G. A.; Farnsworth N. R. *J. Nat. Prod.* **1983**, *46*, 222−225.
(14) Sartori, G.; Bigi, F.; Tao, X.; Porta, C.; Maggi, R.; Predieri, G.; Lanfranchi, M.; Pellinghelli, M. A. *J. Org. Chem.* **1995**, *60*, 6588−6591.

NP020110E