A Benzochromene from the Roots of *Pentas bussei*

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A new homoprenylated benzochromene, methyl-5,10-dihydroxy-7-methoxy-3-methyl-3-[4-methyl-3-pentenyl]-3*H*-benzo[*f*]chromene-9-carboxylate (**1**), was isolated from the roots of *Pentas bussei*, collected from Kenya. The structure of this new compound was determined by spectroscopic data interpretation.

Recently, a growing interest has been accorded to the phytochemical study of the genus *Pentas* (Rubiaceae). For example, *Pentas longiflora* Oliv. has been found to be an important source of quinones.[1,2] Examples include pentalongin, which possesses algicidal activity,[3] as well as the new tetracyclic quinone, isagarin,[4,5] and mollugin.[2] We report here on the isolation and the structural elucidation of a new naphthohydroquinone of the benzochromene type, methyl-5,10-dihydroxy-7-methoxy-3-methyl-3-[4-methyl-3-pentenyl]-3*H*-benzo[*f*]chromene-9-carboxylate (**1**), from *Pentas bussei* K. Krause. No previous phytochemical study is reported in the literature on this plant, which is a woody herb or shrub, about 0.5−4 m high, sometimes scrambling.[6] The plant was collected in Kenya, where it is called "Mdobe" or "Mudobe" in a local dialect (Digo) and where a decoction of the roots is taken as a remedy against gonorrhea, syphilis, and dysentery.[7]

The hexane extract of the powdered roots of the plant was subjected to a three-step purification procedure involving medium-pressure liquid chromatography (MPLC) on Si gel, preparative TLC, and HPLC (see Experimental Section), to afford compound **1** as a yellow solid. HRMS provided the exact mass at *m*/*z* 399.1735 (calcd *m*/*z* 399.1808 $[M + H]^+$), suggesting $C_{23}H_{26}O_6$ as the molecular formula, with an unsaturation index of 11. The ^{1}H NMR, ^{13}C NMR, and HMBC NMR spectra (Table 1) revealed the presence of a 4-methyl-3-pentenyl side chain from the observation of two methyl groups [δ_H 1.57 (3H, s), δ_C 17.62, C-5′ and δ_H 1.66 (3H, s), δ_C 25.56, C-6′], two methylene groups [δ_H 1.76−1.86 (2H, m), δ_C 40.33, C-1′ and δ_H 2.08−2.22 (2H, m), δ_C 22.78, C-2′], and one methine and a quaternary olefinic carbon forming a carbon−carbon double bond with the respective NMR spectral data δ_H 5.04−5.14 (1H, br t, J = 7.1 Hz), δ_C 123.82, C-3′, and δ_C 131.96, C-4′. The data were similar to those found in the literature for this alkenyl chain in other compounds.[8−11] In addition, an oxygenated quaternary carbon was shown from the ^{13}C NMR spectrum at δ_C 78.95 (C-3). A *cis* vicinally coupled two-proton olefinic system (as an AX coupling system) was observed in the ^{1}H NMR spectrum with the protons resonating at δ_{H-2} 5.66 and δ_{H-1} 8.02 (1H each, d, J = 10.4 Hz). The ^{13}C NMR and HETCOR spectra showed that their corresponding carbons resonated at δ_C 123.37 (C-1) and δ_C 127.81 (C-2). A further methyl group [δ_H 1.47 (3H, s); δ_C 25.61, C-7′] and an ester carbonyl carbon (δ_C 171.98) were also identified.

Table 1. NMR Spectral Data (67.5 and 270 MHz, $CDCl_3$) and Observed HMBC (500 MHz for ^{1}H and 125 MHz for ^{13}C) Correlations

position	δ_C	δ_H mult. (J in Hz)	HMBC
1	123.37	8.02 d (10.4)	H-2
2	127.81	5.66 d (10.4)	H-1′, H-7′
3	78.95		H-1, H-2, H-1′, H-2′, H-7′
4a	140.97		H-1, OH-5, H-6
5	147.30		OH-5, H-6
OH-5		6.05 s	
6	105.33	7.60 s	OH-5
6a	127.18		H-8
7	146.96		H-6, H-8, OC*H*$_3$-7
8	99.59	6.92 s	
9	103.53		H-8, OH-10
10	157.60		H-8, OH-10
OH-10		12.26 s	
10a	115.98		H-1, H-6, OH-10
10b	117.35		H-1, H-2
1′	40.33	1.76−1.86 m	H-2, H-2′, H-3′, H-7′
2′	22.78	2.08−2.22 m	H-1′, H-3′
3′	123.82	5.04−5.14 br t (7.1)	H-1′, H-2′, H-5′, H-6′
4′	131.96		H-2′, H-5′, H-6′
5′	17.62	1.57 s	H-3′, H-6′
6′	25.56	1.66 s	H-3′, H-5′
7′	25.61	1.47 s	H-1′, H-2
OC*H*$_3$-7	55.78	3.91 s	
COOC*H*$_3$-9	52.15	3.97 s	
*C*OOCH$_3$-9	171.98		H-8, COOC*H*$_3$-9

The long-range HMBC spectrum showed that H-7′, H-1, and H-2, together with H-1′ and H-2′, all gave a strong coupling with the oxygenated quaternary carbon C-3. This suggested that the side chain must be located at C-3. This was also supported by the observation of a fragment at *m*/*z* 315 ($M^+ - 83$) in the ESIMS spectrum corresponding to $[M - (CH_2)_2CH{=}C(CH_3)_2]^+$, a loss of the side chain by an ether α-cleavage. This type of cleavage has been observed in similar compounds.[8] The stereochemistry at C-3 was not determined because of expected problems with Mosher derivatization of the multiple OH functionalities in the molecule. Due to the limited amount of natural product available and due to the expected lack of regiocontrol for the derivatization of the OH functions, efforts to determine the stereochemistry at C-3 were not performed. The HMBC correlations showed a strong coupling between H-2 and the aromatic quaternary carbon C-10b (δ_C 117.35) and between

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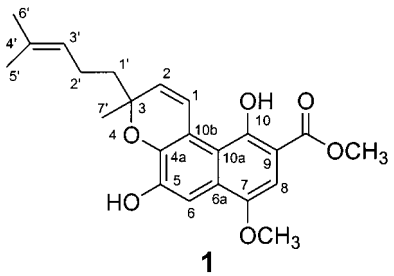
H-1 with the aromatic carbon C-4a (δ_C 140.97), which was assigned as the oxygenated aromatic carbon C-4a involved in the pyran ring.

In addition, a combination of ^{1}H NMR, ^{13}C NMR, DEPT, and HETCOR spectra revealed the presence of two aromatic CH units, respectively [δ_H 6.92 (1H, s), δ_C 99.59, C-8 and δ_H 7.60 (1H, s), δ_C 105.33, C-6], along with two methoxy groups [δ_H 3.91 (3H, s), δ_C 55.78, O*CH*$_3$-7 and δ_H 3.97 (3H, s), δ_C 52.15, COO*CH*$_3$-9]. The two remaining signals were deduced as being two hydroxy protons substituted at aromatic rings [δ_H 6.05 (1H, s), OH-5 and δ_H 12.26 (1H, s), OH-10]. The presence of a methoxycarbonyl group (COOCH$_3$-9) was established first, from the ^{13}C NMR spectrum, which revealed an ester-like carbonyl carbon at δ_C 171.98 and, second, by the HMBC spectrum, which showed a strong long-range coupling between the methyl protons (COOC*H*$_3$-9) and the carbonyl carbon (*C*OOCH$_3$-9). The locations of this methoxycarbonyl, the two hydroxy groups, and the methoxy group on the aromatic rings were established by the HMBC experiment. The significant deshielding of the OH-10 proton was taken as an indication that this hydroxy group is located in an *ortho* position with respect to the methoxycarbonyl group. Long-range coupling of this proton with the two quaternary aromatic carbons δ_C 103.53 (C-9) and δ_C 115.98 (C-10a) confirmed this proposed arrangement. The hydroxyl proton was strongly coupled to a carbon at δ_C 157.60, which is unequivocally carbon C-10 (^2J long-range coupling) on which the hydroxy group is directly substituted. In addition, long-range coupling of H-8 (δ_H 6.92) with the carbonyl carbon, C-9, and C-7 gave further evidence for the substitution pattern of H-8. The location of OH-5 was confirmed by a strong coupling of its proton with C-6 and with the oxygenated quaternary carbon C-4a (δ_C 140.97). The location of C-6 was confirmed by long-range coupling of the proton H-6 with C-4a, C-5, C-7, and C-10a.

In conclusion, on the basis of the analysis of all the spectroscopic data obtained, the structure of compound **1** was established as methyl-5,10-dihydroxy-7-methoxy-3-methyl-3-[4-methyl-3-pentenyl]-3*H*-benzo[*f*]chromene-9-carboxylate (**1**). This is the first time that a compound possessing this type of skeleton has been isolated from a plant source. A related but less functionalized tetracyclic skeleton, 2*H*-naphtho[1,2-*f*][1]benzopyran, was described previously in a compound isolated from *Bauhinia rufescens* Lam.[12] However, the latter compound has a completely different oxygenation and substitution pattern. In other words, a novel heterocyclic system was found in the tricyclic natural compound **1**.



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Experimental Section

General Experimental Procedures. The MPLC (medium-pressure liquid chromatography) system was composed of a Büchi 687 Gradient former, a Büchi 688 chromatography pump (maximum pressure: 40 bar), a Büchi 684 fraction collector, a Sedex 55 light scattering detector, and Büchi Borosilicat glass columns. Column chromatography was conducted on Si gel 60 (0.015−0.040 mm, Merck). Analytical TLC and preparative TLC were performed on Si gel plates 60 $F_{254+366}$, 20 × 20 cm (Merck), and on Si gel 60 $F_{254+366}$, 20 × 20 cm, 2 mm (Merck), respectively. The system for preparative HPLC was composed of two HPLC pumps 422 (maximum pressure: 200 bar), a Kromasil C_{18} (25 × 2 cm, i.d., 5 μm) column, a UV HPLC detector 430, and a Retriever II fraction collector. Detection was carried out at 254 nm.

The melting point measurement was carried out with a Büchi melting point apparatus. The optical rotation was obtained on an AA-10 automatic polarimeter (l = 1 dm). The UV spectrum was recorded on a Varian Cary 50 probe spectrophotometer, and the IR spectrum was obtained using a Perkin-Elmer 1310 infrared spectrometer. 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). The HMBC NMR data were obtained with a Bruker Avance DRX-500 spectrometer (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR). For all NMR experiments, $CDCl_3$ and TMS were used as the solvent and as the internal standard, respectively.

ESIMS was performed using the LCMS technique with mass spectra recorded on a Waters ZMD spectrometer coupled to an LC system using a Waters Alliance 2690 separation module with 996 PDA detector, a Waters Xterra MS C_{18} (50 × 4.6 mm, i.d., 2.5 μm) column with a gradient of 10 mM $HCOONH_4$ (0.1% HCOOH)−acetonitrile (0.1% HCOOH) from 85:15 to 100:0 (%) for 5 min, and a flow rate of 1.2 mL min^{-1}. Maximum pressure: 300 bar. HREIMS was recorded with a Varian MAT-112S mass spectrometer.

Plant Material. Roots of *Pentas bussei* K. Krause were collected at Shimba Hills, Kenya, in May 1999. The plant was identified by S.G.M and F.P.M. A voucher herbarium specimen (LVP-PB) was deposited at the herbarium of the Department of Botany of Ghent University.

Extraction and Isolation. The ground, dried roots of the plant (613.8 g) were extracted exhaustively (×3) by *n*-hexane under sonication at room temperature. The combined extracts were evaporated under a vacuum. The concentrated extract (7.52 g, 1.23%) was chromatographed by MPLC with a gradient of hexane−CH_2Cl_2 (from 90:10 to 100:0, stepwise) as eluent to afford 26 different fractions (A) monitored by TLC. Fractions A8−A11 (1.33 g, 0.22%) were combined and rechromatographed (MPLC) with a gradient of hexane−EtOAc (from 100:0 to 95:5, stepwise) as eluent to afford nine fractions (B). Fractions B3 to B6 (1.14 g, 0.19%) were mixed and eluted isocratically (MPLC) with a mixture of 2% ethyl acetate in hexane to afford three further fractions (C). Fraction C3 (0.68 g, 0.11%) was purified by preparative TLC on Si gel with hexane−Me_2CO (9:1) to afford compound **1** (purity about 90%), which showed yellow and blue bands under UV light at 254 and 365 nm, respectively (R_f 0.32). These bands were scraped off and compound **1** was removed from the adsorbent by extraction with Me_2CO. It was further purified by HPLC on a RP-18 column (elution with 12.5% of water in acetonitrile) to obtain pure compound **1** (350.6 mg, 0.06%).

Methyl-5,10-dihydroxy-7-methoxy-3-methyl-3-[4-methyl-3-pentenyl]-3*H*-benzo[*f*]chromene-9-carboxylate (1): yellow solid (from hexane); mp 89.9−92.1 °C; $[\alpha]^{21}_D$ +34.8° (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 252 (4.63), 255 (4.57), 272 (4.62), 283 (4.62) nm; IR ν_{max} (KBr) 3455 (OH), 2960, 2919, 2848, 1632, 1593, 1455, 1359, 1273 cm^{-1}; ^{1}H NMR and ^{13}C NMR (see Table 1); ESIMS *m*/*z* 399 [M + H]$^+$ (73), 367 (100), 355 (12), 315 (74), 283 (13), 279 (19), 257 (12); HREIMS *m*/*z* 399.1735 (calcd for $C_{23}H_{26}O_6$ + H, 399.1808).

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