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New pentacyclic cyclol-type naphthohydroquinone from the roots of *Pentas bussei*

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Abstract—The novel naphthohydroquinone, methyl 5,10-dihydroxy-7-methoxy-1,1,3a-trimethyl-1a,2,3,3a,10c,10d-hexahydro-1*H*-4-oxa-cyclobuta[*cd*]indeno[5,6-*a*]naphthalene-9-carboxylate **2**, was isolated from the hexane extract of dried roots of *Pentas bussei*. Its structure elucidation was done by detailed spectroscopic methods. It is the first cyclol moiety containing naphthohydroquinone to be reported, and a novel natural product from *P. bussei*.

1. Introduction

'Cyclols' belong to the wide variety of structurally complex forms shown by meroterpenoids derived from monoterpenes and phenols.[1] Cyclol units are characterized by a tricyclic terpenoid core involving a dihydropyran, a cyclopentane, and a cyclobutane ring. These units have already been found integrated into the structure of natural phenolic compounds such as cannabinoids, i.e. cannabicyclol,[2–7] isolated from *Cannabis sativa* L. (Cannabaceae), of terpenoid alkaloids, i.e. bicyclomahanimbine,[8–10] isolated from *Murraya koenigii* Spreng (Rutaceae), and more recently, of eriobrucinol **1** (and its angular regioisomers) and hydroxyeriobrucinol, a set of coumarins isolated from another rutaceous plant, *Eriostemon brucei* F. Muell.[11–13]

2. Results and discussion

Compound **2** is a minor component isolated from the hexane extract of the dried roots of *Pentas bussei* (see Section 3). It was obtained as yellowish dark crystals from hexane. EIMS showed a molecular ion peak at *m/z* 399. HREIMS provided the exact mass at *m/z* 399.1736 (calcd *m/z* 399.1808 $[M+H]^+$), and, therefore, suggesting $C_{23}H_{26}O_6$ as molecular formula which is accounting for 11 degrees of unsaturation. The IR spectrum showed absorption bands at 3409 and 1741 cm^{-1} assigned to OH functions and a carbonyl group, respectively.

The ^1H NMR and ^{13}C NMR spectra ($CDCl_3$; Table 1) of compound **2**, along with DEPT and HETCOR, showed 22 proton and twelve carbon signals in the aliphatic region corresponding to three CH_3s [δ_H 0.54 (3H, s), δ_C 19.2, CH_3-1(β); δ_H 1.36 (3H, s), δ_C 25.6, CH_3-3a; δ_H 1.49 (3H, s), δ_C 34.0, CH_3-1(α)], two CH_2s [δ_H 1.64–1.80 (2H, m), δ_C 25.3, C-2, and δ_H 1.64–1.80/2.00–2.07 (2H, m), δ_C 40.6, C-3], three CHs [δ_H 2.49 (1H, ddd, $J_{H\text{-}2(1)/H\text{-}1a}$=4.40 Hz, $J_{H\text{-}1a/H\text{-}2(2)}$=7.91 Hz, $J_{H\text{-}1a/H\text{-}10d}$=9.24 Hz), δ_C 46.7, C-1a; δ_H 2.70 (1H, dd, $J_{H\text{-}10d/H\text{-}10c}$=9.57 Hz, $J_{H\text{-}10d/H\text{-}1a}$=9.24 Hz), δ_C 41.3, C-10d; and δ_H 4.57 (1H, d, $J_{H\text{-}10c/H\text{-}10d}$=9.57 Hz), δ_C 39.0, C-10c], two OCH_3s [δ_H 3.92 (3H, s), δ_C 55.8, OCH_3-7; δ_H 3.96 (3H, s), δ_C 52.1, $COOCH_3$], and two aliphatic sp^3 quaternary carbons at δ_C 41.8 and 85.4. The aromatic region showed two sp^2 CHs and eight sp^2 quaternary carbons. In addition, an ester carbonyl carbon, and two OH groups, one of which is chelated (δ_H 12.21) were observed. HMBC analysis confirmed the proposed attribution (Table 1).

The relative stereochemistry was established by a DIFNOE and ROESY study which revealed NOE effects of 12% for H_3-1(β)/H_3-1(α), 9% for H_3-1(α)/H-1a, 5% for H-1a/H-10d, 11% for H-10d/H-10c, 4% for H-10c/H_3-1(α), and 8% for H-10d/H_3-3a (Fig. 1). These results suggested an all *cis* configuration for H-1a, CH_3-3a, H-10c, H-10d, and CH_3-1(α).

In addition, NMR data of the alicyclic system of compound **2** were fitting with those of eriobrucinol **1**.[13] The coupling systems observed therein, i.e. $J_{H\text{-}10c/H\text{-}10d}$=9.57 Hz,

Keywords: *Pentas bussei*; Rubiaceae; roots; isolation; structure elucidation; cyclol; meroterpenes; naphthohydroquinone; relative stereochemistry.
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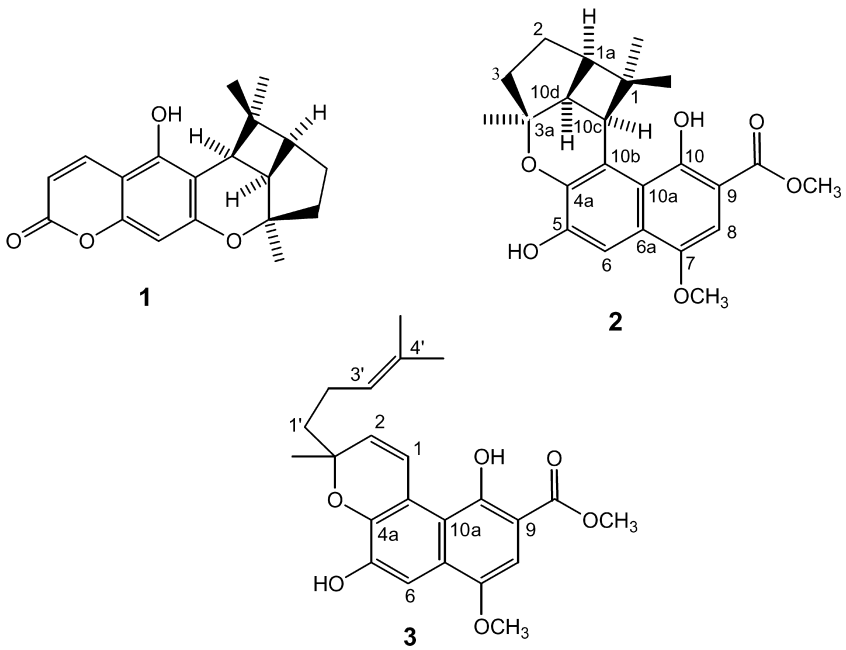

Table 1. NMR spectral data (270 and 67.5 MHz, $CDCl_3$), and observed HMBC (300 MHz for ^1H and 75 MHz for ^{13}C) correlations for compound **2**

Position	δ_H, multiplicity, (J in Hz)	δ_C	HMBC
1	–	41.8	H_3-1(α), H_3-1(β), H-10c, H-10d
1a	2.49 ddd (4.40; 7.91; 9.24)	46.7	H_2-3, H-10d, H-10c, H_3-1(α), H_3-1(β)
2	1.64–1.80 m	25.3	H_2-3, H-10d
3	1.64–1.80 m, H_2-3(1) 2.00–2.07 m, H_2-3(2)	40.6	H-10d, H-1a, H_3-3a
3a	–	85.4	H-10c, H_2-3, H_3-3a
4a	–	141.5	H-10c, OH-5, H-6
5	–	148.9	OH-5, H-6
OH-5	6.42 s	–	
6	7.60 s	102.7	OH-5
6a	–	127.8	H-8
7	–	146.8	H-8, OCH_3-7, H-6
8	6.93 s	99.5	
9	–	102.3	OH-10, H-8
10	–	157.9	OH-10, H-8
OH-10	12.21 s	–	
10a	–	119.1	OH-10, H-6, H-10c
10b	–	123.5	H-10c
10c	4.57 d (9.57)	39.0	H-1a, H-10d, H_3-1(α), H_3-1(β)
10d	2.70 dd (9.57; 9.24)	41.3	H_2-3, H_2-2, H_3-1(α), H_3-1(β)
CH_3-1 (α)	1.49 s	34.0	H_3-1(β), H-10c
CH_3-1 (β)	0.54 s	19.2	H_3-1(α), H-10c
CH_3-3a	1.36 s	25.6	H_2-3, H-10d
OCH_3-7	3.92 s	55.8	
COOCH_3-9	3.96 s	52.1	
*C*OOCH_3-9	–	172.2	COOCH_3-9, H-8

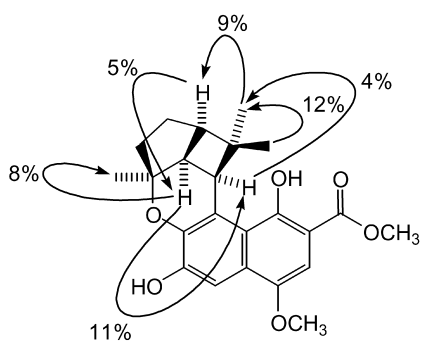

Figure 1. DIFNOE experiment for the alicyclic moiety of compound **2**.

$J_{H\text{-}10d/H\text{-}1a}$=9.24 Hz, were typical for the cyclol ring.[14,15] Highly shielded CH_3 protons such as H_3-1 resonating at δ_H 0.54 have been related to a puckered conformation of the cyclobutane ring which induces an equatorial-oriented anisotropy.[14,15] However, shielding contributions from aromatic rings have also been taken into account.[10,11,16] A Dreiding model of compound **2** showed that the CH_3-1(β) is almost lying within the shielding cone of the aromatic current, and therefore its protons are resonating at high field. This was also supported by ROESY effects observed between H_3-1(β)/H_2-3 and which are possible if the cyclobutane and

cyclopentane are puckered in such a way that CH_3-1(β) and CH_2-3 are brought closer each other, and, therefore, CH_3-1(β) is positioned over the pyranonaphthoquinone ring. Based on all the previous data, the structure of compound **2** was established as methyl 5,10-dihydroxy-7-methoxy-1,1,3a-trimethyl-1a,2,3,3a,10c,10d-hexahydro-1*H*-4-oxacyclobuta-[*cd*]indeno[5,6-*a*]naphthalene-9-carboxylate.

This compound belongs to the cyclol-type natural products mostly deriving from meroterpenes. To the best of our knowledge, no natural quinonic cyclol is yet reported in the literature. The biosynthetic pathways leading to the new naphthohydroquinone type compound could be the same as proposed in the case of natural meroterpenoid coumarins.[17] Compound **2** may derive, through a (2+2) concerted in vivo process, from the homoprenylated benzochromene **3**[18] by a light-induced mechanism. The intramolecular cycloaddition required for the formation of cyclols from appropriate prenylated compounds has been induced photochemically,[4,10,19] but also thermally,[6,10,20] and with acid catalysis.[6,10,20] Based on the fact that compound **2** could be a product of the cycloaddition of homoprenylated benzochromene **3** during the extraction and purification, the homoprenylated benzochromene **3** was subjected to exposure of daylight in acetone at room temperature for four days. However, under these conditions no trace of compound **2** was observed in the mixture and all starting material **3** was decomposed to give a more polar and very complex reaction mixture.

3. Experimental

3.1. General experimental procedures

The medium-pressure liquid chromatography (MPLC) 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^2 (Merck) and on Si gel 60 $F_{254+366}$, 20×20 cm^2, 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^2, 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 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 (300 MHz for ^{1}H NMR, 75 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 LC–MS 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^2, 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 bars. HREIMS was recorded with a Varian MAT-112S mass spectrometer.

3.2. Plant material

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

3.3. Extraction and isolation

Dried roots (613.8 g) of *P. bussei* were extracted (×3) by *n*-hexane under sonication and the combined extracts were dried under vacuum. The concentrated extract (7.52 g, 1.2252%) was chromatographed by MPLC (Medium pressure liquid chromatography) with a gradient of *n*-hexane–CH_2Cl_2 (from 90:10 to 0:100, stepwise) to afford 26 different fractions (A) monitored by TLC. Fractions A8 to A11 (1.33 g, 0.2167%) were combined and rechromatographed (MPLC) with a gradient of *n*-hexane–EtOAc (from 100:0 to 95:5, stepwise) to yield nine fractions (B). Fractions B3 to B6 (1.14 g, 0.1857%) were mixed and eluted isocratically (MPLC) with a mixture of *n*-hexane–EtOAc 2% to give three fractions (C). Fraction C2 (0.13 g, 0.0212%) was purified by preparative TLC on Si gel with *n*-hexane–Me_2CO (9:1) to afford compound **2** (22.4 mg, 0.0036%) which showed a blue band under UV light at 254 and 365 nm (R_f 0.41). The band was scraped off and compound **2** was removed from the adsorbent by extraction with Me_2CO. It was further purified by HPLC on a RP-18 column (elution with acetonitrile–water 20%).

Fractions A16 to A19 (0.55 g, 0.0896%) were eluted with *n*-hexane–EtOAc (from 70:30 to 50:50, stepwise) to yield five different fractions (B′). Recrystallization of fraction B′3 in *n*-hexane–Me_2CO (9.5:0.5) afforded stigmasterol (119 mg, 0.0019%), identified by full spectroscopic data and by comparison with data in the literature.[21,22]

3.3.1. Methyl 5,10-dihydroxy-7-methoxy-1,1,3a-trimethyl-1a,2,3,3a,10c,10d-hexahydro-1*H*-4-oxacyclobuta[*cd*]indeno[5,6-*a*]naphthalene-9-carboxylate (2). Yellow crystals (from hexane), mp 158.0–159.4°C; $[\alpha]_D^{20}$=+120.0 (*c* 1.15, $CHCl_3$); IR ν_{max} (KBr) 3409 (OH), 2953, 2860, 1741, 1660, 1626, 1517, 1449, 1365, 1272, 1238, 1153, 1027, 800 cm^{-1}; ^{1}H NMR and ^{13}C NMR (see Table 1); ESIMS *m/z* 399 $[M+H]^+$ (100), 378 (29), 367 (60), 315 (42), 289 (19), 277 (16); HREIMS *m/z* 399.1736 (calcd for $C_{23}H_{26}O_6$+H, 399.1808).

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