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Three new iridolactone derivatives from the whole plant of *Brillantaisia owariensis* P. Beauv

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Abstract

Owariensisone B-D, three new lactone iridoids, identified as lactone of α -(3-acetoxymethyl-2-hydromethylcyclopent-3-en-yl) ethanoic acid (**1**), lactone of α -(4- β -acetoxy-3- β -acetoxymethyl-2-hydromethyl-3-hydroxycyclopentyl) ethanoic acid (**2**) and lactone of α -(4- β -acetoxy-3- α -acetoxymethyl-2-hydromethyl-3-hydroxycyclopentyl) ethanoic acid (**3**) were isolated from the *n*-hexane extract of the whole plant of *Brillantaisia owariensis*. Their structures were established by interpretation of their spectral data, mainly HR-TOFESIMS, 1D-NMR (^1H , ^{13}C) and 2D-NMR (^1H - ^1H COSY, HSQC, HMBC and ROESY) and by comparison with the literature data.

Keywords: *Brillantaisia owariensis*, Acanthaceae, Lactone iridoids, Owariensisone.

Introduction

The genus *Brillantaisia* belongs to the family Acanthaceae and is found growing throughout tropical Africa and Madagascar and occupies a prominent position in traditional medicine (Ngbolua et al., 2013). Eleven species are known in Cameroon, and among these, *Brillantaisia owariensis* P. Beauv (Synonym *Brillantaisia patula* T. Anderson), a large erect shrub with a purple-blue flowers (Heine, 1963). It is found growing in Nigeria, Togo, West Cameroon and across Uganda and Angola. The leaves are used for the treatment of anaemia (Ngbolua et al., 2013), rheumatism, menstrual pain, stomach ache and for their antiplasmodial and analgesic potentials (Asai et al., 2012; Makambila-Koubemba et al., 2011; Mbatchi et al., 2006). Previous study indicated that the alcoholic extract has antibacterial and antioxidant activities (Aluko et al., 2014; Faparusi et al., 2012,) but the phytochemical investigation were limited. Our previous contribution reported the isolation of one new lactone iridoid in the methanol extract with flavonoid and glycosides (Foning Tebou et al., 2016). In an extension of our studies, the *n*-hexane soluble part of the methanol extract of the whole plant of *B. owariensis* was examined in greater detail and three novel lactone iridoids (**1-3**) were isolated in minor concentration.

2. Results and discussion

Purification of the *n*-hexane soluble fraction of the crude MeOH extract afforded three new compounds, owariensisone B, C and D (**1-3**) (Fig. 1).

Compound **1** was obtained as yellowish gum. Its molecular formula was determined as $\text{C}_{11}\text{H}_{14}\text{O}_5$ on the basis of its HR-TOFESIMS spectrum, exhibiting a pseudo-molecular ion

peak at m/z 249.0734 $[M+Na]^+$ (calcd. for $C_{11}H_{14}O_5Na$. 249.0739) with five degrees of unsaturation. Its 1H -NMR spectrum (Table 1) shows one olefinic proton at δ_H 5.83 (H-7, brqt, $J = 1.6$ Hz), two pairs of *gem*-coupled methylene protons at δ_H 2.60 and 2.65 (H-6 α and H-6 β), and at δ_H 2.80 and 2.83 (H-4 α and H-4 β , each d, $J = 14.6$ Hz), two pairs of oxymethylene protons at δ_H 4.59 and 4.72 (H-10 α and H-10 β , each d, $J = 13.2$ Hz) and δ_H 4.26 and 4.58 (H-1 α and H-1 β), and one methine proton at δ_H 2.99 (H-9, dd, $J = 6.5, 4.9$ Hz) attributed to the boschnialactone type skeleton (Sakan et al., 1967; Sisido et al., 1968; Callant et al., 1983; Tanaka et al., 1993; Hilgraf et al., 2012). The signal observed at δ_H 2.09 (3H, H-2', s) indicated the presence of an acetyl group in this compound. This was supported by the ^{13}C -NMR spectrum exhibiting eleven carbon signals including two ester carbonyls at δ_C 174.4 (C-3) and 172.4 (C-1'), an acetoxymethyl carbon at δ_C 20.7 (C-2'), two ethylenic carbons at δ_C 136.7 (C-8) and 131.1 (C-7), four methylene carbons at δ_C 68.4 (C-1), 62.5 (C-10), 48.9 (C-6) and 44.4 (C-4), one methine carbon at δ_C 55.4 (C-9) and one quaternary carbon at δ_C 78.9 (C-5) bearing an hydroxy group. These NMR data are very closed to owariensisone except for a supplementary acetyl group in C-10 position (Foning Tebou et al., 2016). The $^1J_{C-H}$ correlation in the HSQC spectrum allowed us to attribute to each carbon the corresponding proton. Thus, the two methylene protons at δ_H 2.60 and 2.65 (H-6) were linked to carbon C-6 and those at δ_H 2.80 and 2.83 (H-4) were correlated to carbone C-4, and the two pairs oxymethylene protons group at δ_H 4.26 and 4.58 (H-1) and 4.59 and 4.72 (H-10) were linked to carbon C-1 and C-10, respectively. In the COSY spectrum, protons at δ_H 2.60 and 2.65 (H-6) were correlated with proton at δ_H 5.83 (H-7). Mains correlations were also observed between the protons at δ_H 4.26 and 4.58 (H-1) and proton at δ_H 2.99 (H-9).

The two *gem*-methylene systems H-1 and H-4 were allocated to the δ -lactone group by the HMBC cross peaks with the carbonyl carbon at δ_C 174.4 thus confirming its C-3 position. From the HMBC spectrum, correlations observed between the methylene protons H-10 and carbons C-7, C-8 and C-9 suggested that this oxymethylene was located at C-8. The carbonyl of the methyl ester at 172.4 (C-1') was assigned to C-10 by long-range correlation between protons at δ_H 2.09 (H₃-2') and carbon at δ_C 62.5 (C-10).

The β -orientation of hydrogen at C-9 and hydroxyl group at C-5 was supported by the biosynthetic pathway to iridomyrmercin, isoiridomyrmercin and owariensisone, respectively (Lunn, 1961; Foning Tebou et al., 2016), as in usual iridoids. This is confirmed by the coupling constants of H-9 from 6.5 and 4.9 Hz with both protons H-1, characteristic of *pseudo*-axial-axial and *pseudo*-axial-equatorial system as in owariensisone. On the basis of afore mentioned information, the structure of compound **1** was elucidated as 10-*O*-acetyl

owariensisone or lactone of α -(3-acetoxymethyl-2-hydroxymethylcyclopent-3-en-yl) ethanoic acid named owariensisone **B** (Fig. 1).

Compound **2** was obtained as yellowish gum. Its molecular formula was determined as $C_{13}H_{18}O_8$ on the basis of its pseudo-molecular ion peak at m/z 325.0905 $[M+Na]^+$ (calcd. for $C_{13}H_{18}O_8Na$ 325.0899) in the HR-TOFESIMS spectrum, with five degrees of unsaturation. The 1H -NMR spectrum (Table 1) shows signals for two acetyl groups [δ_H 2.09 (3H, H-2', s), 2.07 (3H, H-2'', s); δ_C 172.5 (C-1'), 171.8 (C-1''), 20.7 (C-2'), 21.0 (C-2'')] and signals of iridoid lactone: two pairs of methylene protons at δ_H 2.00 and 2.43 (H-6 α , dd, J = 14.9, 1.8 Hz and H-6 β , dd, J = 14.9, 5.0 Hz) and δ_H 2.73 and 2.85 (H-4 α , dd, J = 14.8, 0.8 Hz and H-4 β , d, J = 14.8 Hz), two pairs of oxymethylene protons at δ_H 4.49 and 4.53 (H-1 α , dd, J = 11.9, 7.7 Hz and H-1 β , dd, J = 11.9, 10.0 Hz) and δ_H 4.12 and 4.38 (H-10 α , d, J = 11.6 Hz and H-10 β , d, J = 11.6 Hz), one methine proton at δ_H 2.57 (H-9, dd, J = 10.0, 7.7 Hz) and one oxymethine proton at δ_H 5.07 (H-7, dd, J = 5.0, 1.8 Hz). This was supported by the ^{13}C -NMR spectrum exhibiting signals of an ester carbonyl at δ_C 174.6 (C-3), four methylene carbons at δ_C 66.6 (C-1), 67.4 (C-10), 45.3 (C-6) and 44.9 (C-4), two methine carbons at δ_C 80.9 (C-7) and 51.9 (C-9) and two quaternary carbons at δ_C 82.1 (C-8) and 80.1 (C-5) bearing each an hydroxy group (Table 1). Their attributions were assigned by analysis of COSY and $^1J_{C-H}$ HSQC spectra. In the COSY spectrum, proton at δ_H 5.07 (H-7) was correlated with protons at δ_H 2.00 and 2.43 (H-6). Another correlation was also observed between the protons at δ_H 4.49 and 4.53 (H-1) and proton at δ_H 2.57 (H-9). As compared to compound **1**, the ethylenic group in $\Delta_{7,8}$ was replaced by two oxycarbonates at C-7 and C-8 as in patriscabrol (Kouna et al., 1994). Analysis of HMBC spectrum showed correlations between the protons at δ_H 4.12 and 4.38 (H-10) and carbons at δ_C 82.1 (C-8), 80.9 (C-7) and 67.4 (C-9) suggesting that this oxymethylene was located at C-8 as in **1**. The carbonyl of the methyl ester at 172.5 (C-1') was assigned to C-10 by long-range correlation with protons H-10, while the one at 171.8 (C-1'') was linked to C-7 by the long-range correlation with proton at δ_H 5.07 (H-7). The position of the lactone at δ_C 174.6 was determined as in **1**. The β -orientation of H-9 was deduced from the coupling constant of 10.0 and 7.7 Hz with protons H-1 α -axial and H-1 β -equatorial, respectively. The α -equatorial orientation of H-7 was deduced from the coupling constant of 5.0 and 1.8 Hz with H-6 α -axial and H-6 β -equatorial, respectively as observed for similar protons in jioglutoside (Morota et al., 1989) and patriscabrol (Kouna et al., 1994). Thus, the hydroxyl at C-7 is β -oriented. As observed in jioglutoside the δ -lactone ring has a boat conformation (1B4) (Morota et al., 1989), as observed by the ROESY correlations between H-1 and H-4 α -axial. Correlations observed in the ROESY spectrum between H-9 and H-10 β , indicated a β -

orientation for C-10 and an α -orientation of hydroxyl at C-8 position (Fig. 2). On the basis of afore mentioned information, the structure of compound **2** was elucidated as lactone of α -(4- β -acetoxy-3- β -acetoxymethyl-2-hydroxymethyl-3-hydroxycyclopentyl) ethanoic acid named owariensisone **C** (Fig.1).

Compound **3** was obtained as yellowish gum. It has the same molecular formula $C_{13}H_{18}O_8$ as compound **2**, and differs from the latter only by the orientation of C-8 oxymethylene. Its 1H -NMR spectrum (Table 1) was so closed to that of **2** and its ^{13}C -NMR spectrum is almost superimposable on that of **2**, except for the signal of oxymethylene C-10 δ_C 65.0 (Δ -2.4 ppm) and methine C-9 δ_C 54.0 (Δ +2.1 ppm). From the analysis of HMBC spectrum, the oxymethylene, the carbonyl of the methyl ester and the position of the lactone have been solved as in **2**. The absence of correlation between H-10 and H-9 in the ROESY spectrum indicated the α orientation of C-10 (Fig.2). On the basis of afore mentioned information, the structure of compound **3** was elucidated as lactone of α -(4- β -acetoxy-3- α -acetoxymethyl-2-hydroxymethyl-3-hydroxycyclopentyl) ethanoic acid named owariensisone **D** (Fig.1).

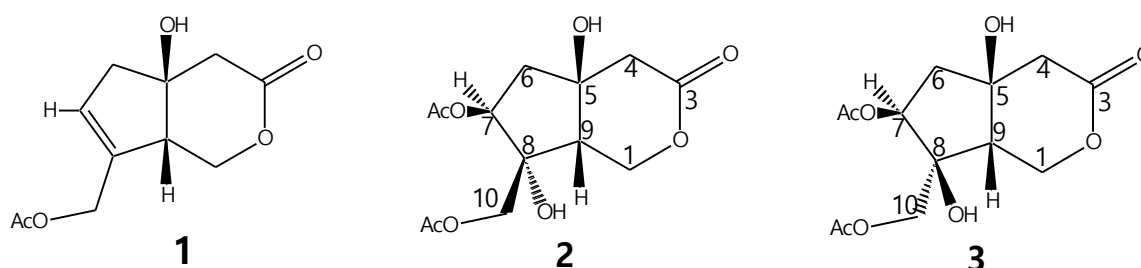


Fig.1. Structures of compounds **1-3** isolated from the *n*-hexane soluble extract of *Brillantaisia owariensis*.

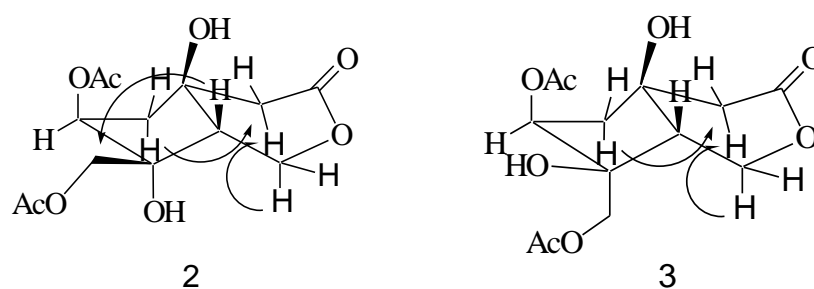


Fig.2. Noesy effects in compounds **2-3**

Table 1: ¹H-NMR (600 MHz) and ¹³C-NMR (150 MHz) data of compounds **1-3** in CD₃OD.

N°	1		2		3	
	¹³ C	¹ H (<i>J</i> in Hz)	¹³ C	¹ H (<i>J</i> in Hz)	¹³ C	¹ H (<i>J</i> in Hz)
1	68.4	4.26 (1H, dd, 11.9, 6.5) 4.58 (1H, dd, 11.9, 4.9)	66.6	4.49 (1H, dd, 11.9, 7.7) 4.53 (1H, dd, 11.9, 10.0)	66.9	4.21 (1H, t, 11.2) 4.57 (1H, dd, 11.6, 6.2)
3	174.4		174.6		174.5	
4	44.4	2.80 (1H, d, 14.6) 2.83 (1H, d, 14.6)	44.9	2.73 (1H, dd, 14.8, 0.8) 2.85 (1H, d, 14.8)	44.7	2.57 (1H, d, 15.1) 2.78 (1H, d, 15.1)
5	78.9		80.1		80.4	
6	48.9	2.60 (1H, dq, 18.1, 1.0) 2.65 (1H, dm, 18.1)	45.3	2.00 (1H, dd, 14.9, 1.8) 2.43 (1H, dd, 14.9, 5.0)	46.1	1.91 (1H, dd, 14.5, 1.6) 2.46 (1H, dd, 14.5, 5.0)
7	131.1	5.83 (1H, brqt, 1.6)	80.9	5.07 (1H, dd, 5.0, 1.8)	80.8	5.17 (1H, dt, 5.0, 1.6)
8	136.7		82.1		82.1	
9	55.4	2.99 (1H, dd, 6.5, 4.9)	51.9	2.57 (1H, dd, 10.0, 7.7)	54.0	2.45 (1H, dd, 11.1, 6.2)
10	62.5	4.59 (1H, d, 13.2) 4.72 (1H, d, 13.2)	67.4	4.12 (1H, d, 11.6) 4.38 (1H, d, 11.6)	65.0	3.91 (1H, d, 11.6) 4.18 (1H, d, 11.6)
11	172.4		172.5		172.4	
12	20.7	2.09 (3H, s)	20.7	2.09 (3H, s)	20.7	2.09 (3H, s)
13			171.8		171.2	
14			21.0	2.07 (3H, s)	21.0	2.07 (3H, s)

3. Experimental

3.1. General

IR spectra were recorded with a Shimadzu FT-IR-8400S (Shimadzu, France) spectrophotometer. ¹H and ¹³C-NMR spectra were recorded on a Bruker Avance III 600 spectrometer equipped with a cryoprobe (¹H at 600 MHz and ¹³C at 150 MHz). 2D NMR experiments were recorded by means of standard Bruker microprograms (Xwin-NMR version 2.1 software TopSpin 3.2). Chemical shifts (δ) are reported in parts per million (ppm) using the residual solvent signals as secondary reference relatively to TMS ($\delta = 0$), while the coupling constants (*J* values) are given in Hertz (Hz). TOF-ESIMS and HR-TOFESIMS spectra were recorded using a Micromass Q-TOF micro instrument (Manchester, UK) equipped with an electrospray source. The samples were introduced by direct infusion in a solution of MeOH at a rate of 5 μ L min⁻¹. The optical rotations were measured on a Bellingham & Stanley ADP 220 polarimeter (Bellingham + Stanley Ltd., United Kingdom). Column chromatography was run on Merck silica gel 60 (70-230 mesh) and gel permeation on Sephadex LH-20 while TLC was carried out on silica gel GF₂₅₄ pre-coated plates with detection accomplished by spraying with 50% H₂SO₄ followed by heating at 100°C, or by visual inspection under UV lamp at 254 and 365 nm.

3.1. Plant material

The whole plant of *B. owariensis* was collected at Tsinkop, Menoua Division, West Region of Cameroon, in October 2013. Authentication was done by Mr Victor Nana, a botanist of the

Cameroon National Herbarium, Yaoundé, where the voucher specimen (N° 34376/HNC) has been deposited.

3.2.Extraction and isolation

The air-dried and plant material (4 Kg) was powdered and extracted at room temperature with methanol (3 x 15L, 72h). The solvent was evaporated under reduced pressure, leaving an extract (110 g). Part of this extract (100 g) was extracted with *n*-hexane yielding **30** g fraction after evaporation to dryness. Part of *n*-hexane-soluble fraction (**25** g) was subjected to silica gel column chromatography using Hex-EtOAc (9:1 to 1:0) gradient graduated elution. Many fractions of 250 mL each were collected and combined on the basis of their TLC profiles to give 3 fractions: A, B and C. Fraction B (5.1 g) was purified on silica gel column chromatography using an isochratic eluent system Hex-EtOAc (1:1). Fractions of 10 mL were collected and combined on the basis of TLC profiles to give 3 sub-fractions B₁, B₂ and B₃. Sub-fraction B₂ (102 mg) was subjected to silica gel column chromatography using Hex-EtOAc (3:2) as eluent to give compound **1** (8 mg) and a mixture of **2** and **3**. This mixture was combined to sub-fractions B₁ and B₃ and purified by chromatography on silica gel column chromatography to yield compounds **3** (12 mg) and **2** (9mg).

New compounds

Owariensisone B: Yellowish gum; ¹H and ¹³C-NMR data, see Table 1; $[\alpha]_D^{21} -5.2$ (MeOH *c* 0.06); IR (NaCl) ν_{\max} (cm⁻¹) 3350-3300, 1070, 1040 (OH), 1643 (C=O), 1605, 1580, 1520 (C=C aromatic), 1650 (C-O); HR-TOFESIMS (positive ion mode) *m/z*: 249.0734 [M+Na]⁺ (calcd. for C₁₁H₁₄O₅Na 249.0739).

Owariensisone C: Yellowish gum; ¹H and ¹³C-NMR data, see Table 1; $[\alpha]_D^{21} -20$ (MeOH *c* 0.05); IR (NaCl) ν_{\max} (cm⁻¹) 3350-3300, 1070, 1040 (OH), 1643 (C=O), 1605, 1580, 1520 (C=C aromatic), 1650 (C-O); HR-TOFESIMS (positive ion mode) *m/z*: 325.0905 [M+Na]⁺ (calcd. for C₁₃H₁₈O₈Na 325.0899).

Owariensisone D: Yellowish gum; ¹H and ¹³C-NMR data, see Table 1; $[\alpha]_D^{21} -87.5$ (MeOH *c* 0.02); IR (NaCl) ν_{\max} (cm⁻¹) 3350-3300, 1070, 1040 (OH), 1643 (C=O), 1605, 1580, 1520 (C=C aromatic), 1650 (C-O); HR-TOFESIMS (positive ion mode) *m/z*: 325.0892 [M+Na]⁺ (calcd. for C₁₃H₁₈O₈Na 325.0899).

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