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Prenylated chromones and coumarins from the leaves of *Billburttia capensoides* Sales & Hedge (Apiaceae)

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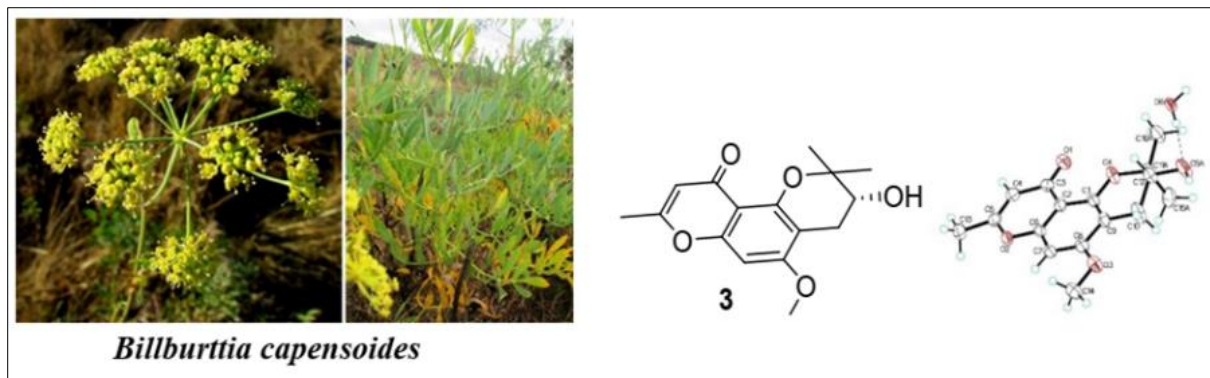
Abstract

Billburttia Sales & Hedge is a new genus endemic to Madagascar, which belongs to the Apiaceae family and comprises of two species, namely *Billburttia capensoides* and *B. vaginoides*. The present work was undertaken in a view to explore secondary metabolites from the leaves of *B. capensoides*, contributing to have more knowledge on the chemical profile of this species. Liquid-liquid partition followed by chromatographic separation of its ethanolic extract led to the isolation of two new prenylated chromones, 2'*R*-hydroxy-7-*O*-methylallopeucenin and the known coumarins imperatorin and xanthotoxin. Their chemical structures were established on the basis of spectroscopic methods including nuclear magnetic resonance (NMR) and high resolution mass spectrometry, and by comparison with the reported data in the literature. The structure of the compound 3 was confirmed by single-crystal X-ray diffraction. This is the first report on the nonvolatile constituents of *B. capensoides*. The presence of imperatorin and xanthotoxin supported that the new endemic genus *Billburttia* belongs to the Apiaceae family. All these data may induce further research on this species in order to find new chemical entities with biological and chemotaxonomic interests.

Keywords: *Billburttia capensoides*; Apiaceae; Chemical constituents; Prenylated chromones; Coumarins

Graphical abstract

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1. Introduction

Billburttia (Apiaceae) is a new endemic genus of Madagascar and comprises only two species, namely *B. capensoides* Sales & Hedge and *B. vaginoides* S. & H [1]. These two species were initially placed in the genus *Peucedanum* (Apiaceae) before being transferred into *Billburttia* [2]. The present report is focusing on *B. capensoides* which is a shrub of 2m in height, encountered in the central part of Madagascar and locally known under the vernacular name Volotaratsitsina. The plant is traditionally used for the treatment of jaundice, convulsion and common cold [3]. The composition of the essential oils from the two *Billburttia* species has been previously reported with *p*-mentha-1,3,8-triene, terpinolene and dill apiole as the major components [4].

As a continuation of our study on medicinal plants endemic to Madagascar in a view to searching for new secondary metabolites and biologically active natural substances, we carried out a phytochemical investigation of the leaves of *B. capensoides*. Fractionation of the ethanol extract led to the isolation of three prenylated chromones (1-3) and the two coumarins (4 and 5). The isolation and structural identification of the two new chromone derivatives 1 and 2 as well as the X-ray analysis of 3 are described herein.

2. Material and methods

2.1. Plant material

The leaves of *B. capensoides* were collected in the region of Itasy, district of Arivonimamo, Commune of Manalalondo in the central part of Madagascar in September 2015. A voucher specimen (No. ST1494) is preserved in the Herbarium of the National Center of Applied Pharmaceutical Research, Antananarivo, Madagascar.

2.2. Methods

2.2.1. General experimental procedures

The NMR spectra were recorded on a Bruker Avance-400 operating at 400 MHz for ^1H and 100 MHz for ^{13}C . Preparative HPLC was performed using Shimadzu LC-20AB pumps coupled with a semi-preparative Purospher STAR C_{18} column (5 μm , 250 x 10 mm), a Shimadzu variable wavelength SPD-20A detector and a CBM-20A/20A lite Prominence system controller. Silica gel 60 (35 – 70 μm , Fluka) was used for normal-phase flash column chromatography and silica gel 60 RP-18 (40 – 60 μm , EMD) for reversed-phase flash column chromatography. TLC analysis was carried out on Si gel 60 F_{254} (Merck) or reversed-phase 60 RP-18 F_{254}S (EMD). All solvents were distilled before use.

2.2.2. Extraction and Isolation

The dried leaves of *B. capensoides* (440 g) were ground into powder and extracted by maceration with EtOH (1.5 L) for 48 hours. After filtration and evaporation under reduced pressure of the solvent, the resulting ethanol extract (39.9 g) was taken in MeOH (250 mL) and defatted with *n*-hexane (3 x 200 mL). The methanol layer was evaporated to dryness and the residue was partitioned successively between chloroform and water, ethyl acetate and water, and *n*-butanol and water. Evaporation under reduced pressure of all the solvents used during this preliminary fractionation yielded 4.1 g of hexane-soluble, 10.1 g of chloroform-soluble, 2.6 g of ethyl acetate-soluble, 5.9 g of *n*-butanol-soluble (5.9 g) and 14 g of water-soluble fractions.

Part of the chloroform-soluble fraction (4 g) was applied to a silica gel flash column chromatography eluted with hexane/ ethyl acetate (9:1 to 0:1) then with ethyl acetate/MeOH (19:1 to 4:1) to give nine fractions (A – I). Imperatorin (4, 10.9 mg) crystallized from fraction B. Fraction C (1.2 g) was subjected to ODS flash chromatography eluted with MeOH/H₂O (1:4 to 9:1) to afford xanthotoxin (5, 10.5 mg) and more imperatorin (4, 14.6 mg). Fraction H (1.1 g) was further separated on flash ODS column eluted with MeOH/H₂O (1:4 to 4:1) to afford five sub-fractions: H-1 to H-5. Compound 2 (12.4 mg) crystallized from sub-fraction H-3. Sub-fraction H-4 was purified on ODS HPLC eluting with MeOH/H₂O (11:9) to furnish 1 (5.4 mg, $t_R = 27.7$ min) and 3 (1.8 mg, $t_R = 47.3$ min).

Compound 1 white solid; $[\alpha]^{23}_D + 13.8$ (c 1.2 methanol); IR (NaCl) ν_{max} 3418 (OH), 2925 (aromatic methines), 1660 (conjugated carbonyl), 1586 (conjugated C=C) cm^{-1} . 1H NMR (CDCl₃, 400 MHz) δ 6.44 (1H, s, H-8), 6.09 (1H, s, H-3), 3.93 (3H, s, OMe-7), 3.59 (1H, ddd, $J = 8.4$ Hz, H-2'), 2.98 (1H, dd, $J = 14, 2.4$ Hz, H-1'a), 2.80 (dd, $J = 14, 10.4$, H-1'b), 2.39 (3H, s, Me-2), 1.32 (3H, s, Me-4'), 1.31 (3H, s, Me-5'); ^{13}C NMR (CDCl₃, 100 MHz) δ 182.4 (C-4), 166.7 (C-2), 163.1 (C-7), 158.9 (C-5), 156.9 (C-8a), 110.4 (C-6), 108.9 (C-3), 105.2 (C-4a), 89.9 (C-8), 78.9 (C-2'), 72.9 (C-3'), 56.1 (OMe-7), 25.9 (C-1'), 25.0 (C-4'), 23.7 (C-5'), 20.5 (Me-2). $[M+Na]^+ m/z$ 331.1160 (calculated for C₁₆H₂₀O₆Na⁺ 331.1152).

Compound 2 colorless crystal; $[\alpha]^{23}_D + 13.8$ (c 1.2 methanol); IR (NaCl) ν_{max} 3418 (OH), 2925 (aromatic methines), 1660 (conjugated carbonyl), 1586 (conjugated C=C) cm^{-1} . 1H NMR (CDCl₃, 400 MHz) δ 6.76 (1H, s, H-3), 6.51 (1H, s, H-5), 3.95 (3H, s, OMe-7), 3.88 (1H, t, $J = 5.2$ Hz, H-2'), 2.91 (1H, dd, $J = 17.6, 5.4$ Hz, H-1'a), 2.72 (1H, dd, $J = 17.6, 5.2$ Hz, H-1'b), 2.41 (3H, brd, $J = 0.8$ Hz, Me-2), 1.46 (3H, s, Me-4'), 1.41 (3H, s, Me-5'); ^{13}C NMR (CDCl₃, 100 MHz) δ 177.7 (C-4), 166.0 (C-2), 163.0 (C-6), 159.1 (C-8a), 153.7 (C-8), 109.7 (C-3), 106.1 (C-4a), 106.1 (C-7), 91.0 (C-5), 78.6 (C-3') 68.2 (C-2'), 56.1 (OMe-7), 26.1 (C-1'), 24.5 (C-4'), 21.7 (C-5'), 20.2 (Me-9). $[M+Na]^+ m/z$ 313.1046 (calculated for C₁₆H₁₈O₅Na⁺ 313.1046).

Compound 3 Colorless crystal; $[\alpha]^{23}_D + 42$ (c 0.1 methanol); IR (NaCl) ν_{max} 3418 (OH), 2925 (aromatic methines), 1660 (conjugated carbonyl), 1586 (conjugated C=C) cm^{-1} . UV (MeOH) λ_{max} (log ϵ) 202 (4.45), 230 sh (4.2), 250 sh (4.02), 257 (4.02), 290 (3.7). 1H NMR (CDCl₃, 400 MHz) δ 6.43 (1H, s, H-3), 6.19 (1H, s, H-8), 3.92 (3H, s, OMe-7), 3.86 (1H, t, $J = 5.2$, H-2'), 2.87 (1H, dd, $J = 17.6, 5.2$ Hz, H-1'a), 2.69 (1H, dd, $J = 17.6, 5.2$ Hz, H-1'b), 2.31 (3H, s, Me-2), 1.46 (3H, s, Me-4'), 1.42 (3H, s, Me-5'); ^{13}C NMR (CDCl₃, 100 MHz) δ 178.7 (C-4), 165.0 (C-2), 160.1 (C-8a), 163.3 (C-7), 154.9 (C-5), 106.8 (C-4a), 112.7 (C-3), 106.8 (C-6), 92.4 (C-8), 79.5 (C-3'), 69.8 (C-2'), 57.3 (OMe-7), 27.6 (C-1'), 25.9 (C-4'), 23.1 (C-5'), 21.3 (Me-9). Crystal data of **3**: C₁₆H₁₈O₅, M = 294.58, monoclinic crystal system, crystal size 0.258 × 0.165 × 0.116 mm³, space group *I*2, $a = 6.63491(13)$ Å, $b = 15.1675(2)$ Å, $c = 14.2847(2)$ Å, $\alpha = 90^\circ$, $\beta = 94.61^\circ$, $\gamma = 90^\circ$, $V = 1432.88(4)$ Å³, reflections collected 10289, parameters 251. The CIF file for **3** has been deposited in the Cambridge Crystallographic Data Centre [deposition number: CCDC 1957061].

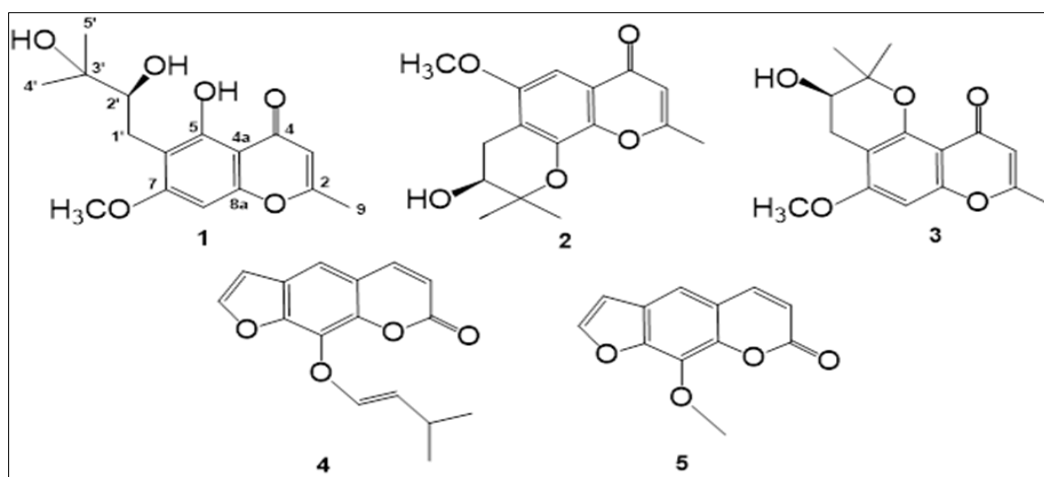


Figure 1 Structures of compounds 1-5 isolated from *Billburttia capensisoides*

3. Results and discussion

The ethanol extract of the leaves from *B. capensisoides* was liquid-liquid partitioned to give hexane-, chloroform-, butanol- and water-soluble fractions. Fractionation of the chloroform-soluble part by a combination of chromatographic techniques, including normal phase silica gel and reversed phase silica gel (ODS C₁₈) open columns, as well as reversed phase ODS-HPLC led to the isolation of two new compounds 1 and 2, 2'*R*-hydroxy-7-*O*-methylallopeucenin (3) and the

two known coumarins imperatorin (4) and xanthotoxin (5) (Fig. 1). The structures of the known compounds 4 and 5 were identified by spectroscopic analyses including 1D and 2D NMR experiments and by comparison with data reported in the literature [5,6,7,8].

Compound 1 was obtained as a white solid and its molecular formula was deduced to be $C_{16}H_{20}O_6$ (requires seven degrees of unsaturation) by the observation of a sodiated molecular ion peak at m/z 331.1160 (calcd for $C_{16}H_{20}O_6Na^+$ 331.1152) in HRESIMS analysis. The IR spectrum showed the presence of conjugated carbonyl stretching at 1660 cm^{-1} , aromatic and δ olefinic absorption band at 2926 cm^{-1} and hydroxyl group at 3410 cm^{-1} . The profile of its UV spectrum is very similar to those of previously described chromone [9]. The 1H NMR spectrum showed signals consistent with the presence of a C-5 chelated hydroxyl group (δ 13.11, s), one isolated aromatic proton (δ 6.44, s, H-8), one methyl (δ 2.39, s) attached to a double bond, one olefinic proton (δ 6.09, s, H-3) and one methoxy group (δ 3.93, s). The ^{13}C NMR spectrum of 1 disclosed 16 carbon resonances consistent with a ketone carbonyl (δ 182.4, C-4), four oxygenated sp^2 hybridized carbons (δ 166.7, C-2; δ 163.1, C-7; δ 158.9, C-5; δ 156.9, C-8a), two substituted sp^2 hybridized carbons (δ 110.4, C-6; δ 105.2, C-4a), two olefinic methines (δ 89.9, C-8; δ 108.9, C-3) and signals ascribable to a 2,3-dihydroxy-3-methylbutyl unit as confirmed by HSQC and HMBC experiments. The basic skeleton of 1 was achieved by the interpretation of the HMBC spectrum starting from the olefinic proton H-3 and aromatic one H-8. The proton H-3 (δ 6.09, s) correlated with the carbons at δ 20.5 (CH₃-2), 105.2 (C-4a), 166.7 (C-2), and the carbonyl carbon at δ 182.4 (C-4). Similarly, the proton H-8 (δ 6.44, s) correlated with the carbons at δ 105.2 (C-4a), 110.4 (C-6), 156.9 (C-8a) and 163.1 (C-7). In addition, strong 4J coupling was observed between the proton H-8 and the carbonyl carbon C-4, and between the proton H-3 and the aromatic carbon C-5 (δ 158.9). From these NMR spectroscopic findings and by comparison with literature data [10,11], a 2-methylchromone skeleton was established for 1. The attachment of the 2,3-dihydroxy-3-methylbutyl unit at C-6 was evidenced by the HMBC cross-peaks between the methylene protons at δ 2.80 and 2.98 (H-1') and the three aromatic carbons at δ 110.4 (C-6), 158.9 (C-5), and 163.1 (C-7) (Fig. 2). The closest analogs are (2'S)-perforatin C (6) and its enantiomer (2'R)-perforatin C (7), which have been reported from *Harrisonia perforata* (Rutaceae) [12] (Fig. 3). The NMR spectroscopic data of 1 are very similar to those of 6/7, except for the chemical shifts arising from the aromatic rings. Since C-2' is the only chiral center in these molecules, the (S)-configuration was tentatively assigned to 1 based on the positive value of its optical rotation as that of 6. Accordingly, the structure of 1 was proposed to be (2'S)-5-hydroxy-7-methoxy-2-methyl-6-(2,3-dihydroxy-3-methylbutyl)chromone.

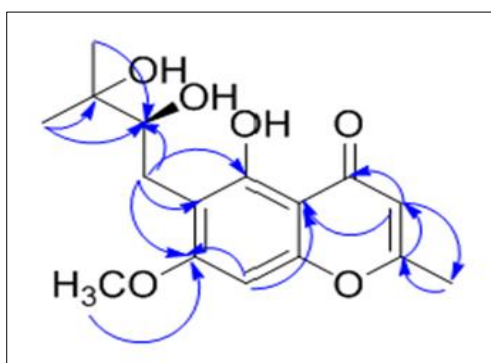


Figure 2 Key HMBC correlations observed in 1

Compound 2 was obtained as a white solid with a molecular formula of $C_{16}H_{18}O_5$ as determined by the presence of a sodiated molecular ion peak at m/z 313.1046 [$M+Na$]⁺ (calculated for $C_{16}H_{18}O_5Na^+$ 313.1046) in the HRESIMS spectrum. The IR spectrum showed the presence of hydroxyl and carbonyl groups. Comparison of the 1H and ^{13}C NMR spectra of 2 with those of 1 revealed that it also possessed a 2-methylchromone skeleton, but without hydrogen-bonded hydroxyl group. This was supported by the correlations observed in the HMBC spectrum between the olefinic methine proton (H-3, δ 6.76, s) and the methyl (δ 20.2), aromatic carbon (C-4a, δ 106.1) and carbonyl C-4 carbon (δ 177.7), and by the 3J long-range correlations between the aromatic proton H-5 (δ 6.51, s) and C-7 (δ 106.1), C-8a (δ 159.1) and C-4 (δ 177.7) (Fig. 4). Moreover, the HMBC spectrum displayed correlations from the germinal methyl groups (δ 1.41, s; 1.46, s) to C-2' (δ 68.2) and C-3' (δ 78.6), and from the methylene protons H-1' (δ 2.72, dd, $J = 17.6, 5.2$ Hz and δ 2.91, dd, $J = 17.6, 5.4$ Hz) to the oxygenated aromatic carbons C-6 (δ 163.0) and C-8 (δ 153.7). These data suggest that carbons C-3' and C-8 are linked by an ether function and the five carbons C-1', C-2', C-3', C-7 and C-8 form a 3',3'-dimethyl-dihydropyran ring. Complete assignment of the 1H and ^{13}C signals of compound 2 was achieved by the interpretation of correlations observed in the HSQC and HMBC spectra. Two closely related compounds, (2'S)-2'-hydroxy-7-O-methylallopecenin (8) and (2'S)-5-O-methylhamaudol (9), have been previously reported from *Diplolophium buchananii* of the Apiaceae family [13] (Fig. 3). The major difference in the gross chemical structures of the three

compounds 2, 8 and 9 is the position of the dihydropyran ring on the 2-methylchromene skeleton. Regarding specifically the optical rotations of 8 and 9, the strong negative value for 8 becomes slightly positive for 9, while conserving the (2'*S*)-configuration in both of them. Accordingly, the (2'*S*)-stereochemistry was tentatively ascribed to 2 based on the fairly positive value of its optical rotation. In light of the above results, the structure of 2 was established as depicted in Fig. 1.

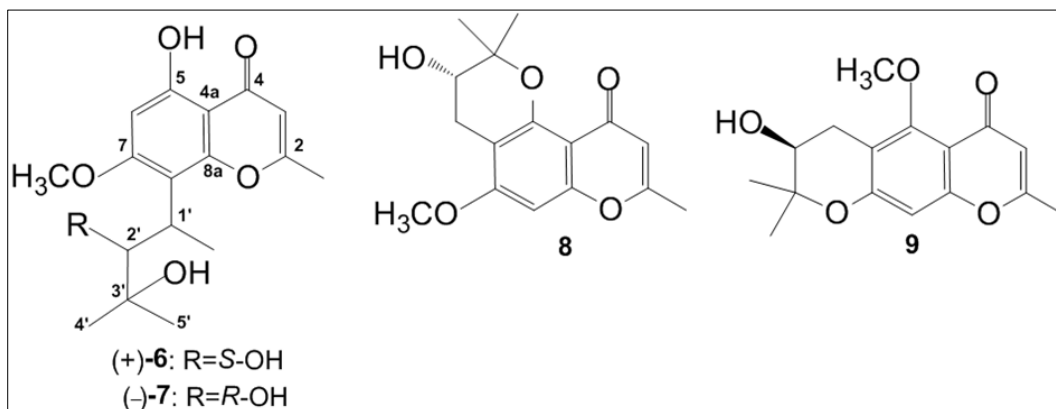


Figure 3 Structures of compounds 6-9

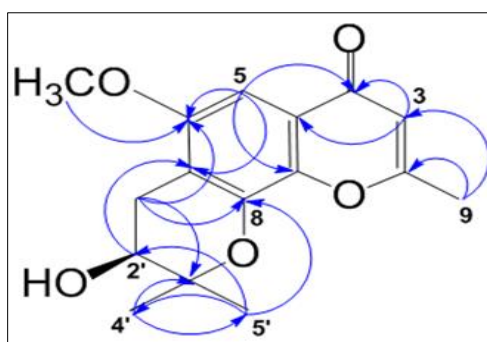


Figure 4 Key HMBC correlations observed in 2

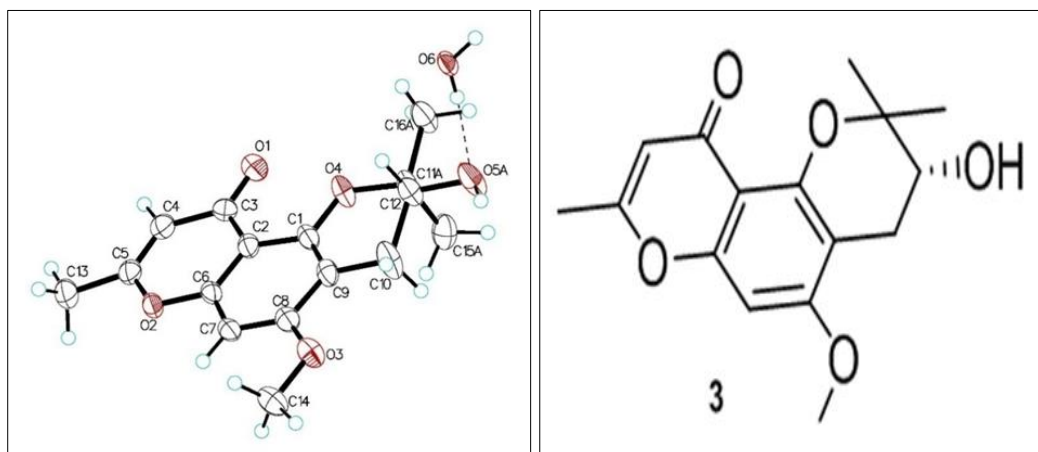


Figure 5 Anisotropic displacement ellipsoid drawing (50% probability) of 3

Compound **3** was obtained as a colorless crystal. Its NMR spectroscopic data were superimposable with those of (2'*S*)-2'-hydroxy-7-*O*-methylallopeucenin (**8**) [13]. To confirm the proposed structure of **3**, including the absolute configuration at C-2', a crystal structure was obtained via single-crystal X-ray diffraction (Fig. 5). As shown in the X-ray crystallographic data, the structure of **3** was enantiomeric to 2'*S*-hydroxy-7-*O*-methylallopeucenin (**8**). The relative strong positive value of the optical rotation of **3** is in agreement with this result. Thus the structure of **3** was determined to be 2'*R*-hydroxy-7-*O*-methylallopeucenin (or 2'*R*-hydroxy-3',3',2-trimethyl-7-methoxy-3,4-dihydropyrano[2,3-*f*]10*H*-chromen-4-one).

Prenylated chromones have been reported to exhibit anti-HIV, antiproliferative and anti-inflammatory activities [14,15]. Coumarins have been considered as one of the major biochemical markers of the Apiaceae family.

4. Conclusion

The present study suggests that *B. capensoides* is a new source of prenylated chromones, as exemplified by the two novel compounds **1** and **2**. Moreover, the isolation of the prenylated chromone **3** was described herein for the first time although it was established to be the (2'*R*)-enantiomer of the reported 2'*S*-hydroxy-7-*O*-methylallopeucenin by X-ray crystallographic analysis. The presence of imperatorin (**4**) and xanthotoxin (**5**) in *B. capensoides* supported that the new endemic genus *Billburttia* belongs to the Apiaceae family. All the above data may induce further research on this species in order to find new chemical entities with biological and chemotaxonomic interests.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No competing financial interests to declare.

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