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Ja'afar, MK, Jamil, S, Basar, N, Bakar, MB, Sarker, SD, Flanagan, KJ and Senge, MO (2017) Lactones and Flavonoids isolated from the Leaves of *Globimetula braunii*. *Natural Product Communications*, 12 (9). pp. 1455-1458. ISSN 1934-578X

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Lactones and Flavonoids isolated from the Leaves of *Globimetula braunii*

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Received: July 5th, 2017; Accepted: July 18th, 2017

The dried powdered leaves of *Globimetula braunii* (Engler) Van Tiegh were effectively extracted by the cold extraction method. Purification of the EtOAc-soluble and MeOH-soluble extracts successfully yielded two lactones namely (*R*)-6-[(*S*)-2-hydroxy-4-(4-hydroxyphenyl)butyl]-5,6-dihydropyran-2-one (dodoneine) **1** and (1*R*,5*S*,7*S*)-[2-(4-hydroxyphenyl)ethyl]-2,6-dioxabicyclo[3.3.1]-nonan-3-one (**2**), together with five flavonoids namely quercetin **3**, (+)-catechin **4**, quercitrin **5**, rutin **6** and avicularin **7**. Their structures were established by spectroscopic means, and the absolute configuration of compound **1** was confirmed by X-ray analysis.

Keywords: *Globimetula braunii*, Lactones, Flavonoids, X-ray crystallography, Dodoneine.

Africa is endowed with rich biodiversity resources that are projected to contain up to 45,000 species of plants, out of which 5,000 species are used medicinally. The continent has a long antiquity of the use of plants and up to 80 % of the people in some Africa countries rely on orthodox medicinal plants as a source of medications [1-2]. This results from the belief that medicinal plants are more accessible, economically affordable, and more acceptable to the body, with lesser side effects than synthetic drugs [3]. There is still a dearth of updated, inclusive compilations of promising medicinal plants from the African continent. This is coupled with the unregulated devastation of the vegetation of tropical rain forest, which poses a risk to medicinal plants. There is, therefore, every need to deepen research into African medicinal flora, especially those asserted to have positive effects in severe disorders such as epilepsy. One of the African medicinal plants with scant or no scientific reports on phytochemical investigations targeted at isolating bioactive compounds is *Globimetula braunii*.

Globimetula braunii (Engler) Van Tiegh is a member of the Loranthaceae family that is represented by 75 genera and 1000 species, generally called mistletoe. It is a bushy parasitic plant found in a variety of host plants with woody stems from Ghana to Nigeria and widely dispersed across central tropical Africa. The leaves are simple, stringy and evergreen. Timbered suckers often regarded as adventitious roots connect to and infiltrate the branches of the host tree or shrub by a structure called the haustorium, through which they absorb water and nutrients from the host tree [4]. The leaves have gained relevance as being useful in folklore medicine for the management of rheumatism, headache, pains, and pulmonary problems. They are also effective against diabetes, diarrhea, hypertension, and epilepsy [4-6]. Previous studies on the anticonvulsant activity of the ethyl acetate fraction of this plant suggested that this fraction may contain psychoactive compounds and require further phytochemical investigation [7]. Thus, this study was conducted to isolate compounds from the leaves of

Globimetula braunii and here we wish to report for the first time the chemical constituents of this species.

The powdered leaves of *G. braunii* were sequentially extracted with *n*-hexane, CH₂Cl₂, and MeOH at room temperature. The MeOH crude extract was further suspended in water before partitioned with *n*-hexane, CHCl₃, EtOAc, and MeOH to yield the *n*-hexane-soluble, CHCl₃-soluble, EtOAc-soluble and MeOH-soluble extracts. Chromatographic separations using vacuum liquid chromatography (VLC) and column chromatography (CC) on silica gel of the EtOAc-soluble extract, followed by recrystallization afforded compounds **1**, **2**, **3** and **4**. Purification by VLC and CC followed by preparative thin layer chromatography (PTLC) of the MeOH-soluble extract furnished compounds **5**, **6**, and **7**. Compounds **1-7** (Figure 1) were characterized by comparison of their spectroscopic data with respective literature reports [8-15].

The first proposed structure of **1** was published in 2007 by Ouedraogo *et al.* [8]. The authors outlined the isolation and structure determination of **1** using standard spectroscopic data, sophisticated synthetic transformations and the X-ray crystallographic structure of the camphor sulfonate derivative **1a** to determine the absolute configuration (Figure 2) [8]. However, until now, no X-ray crystal structure of **1** has been published. The asymmetric unit of **1** features one complete unit of **1** accompanied by one solvent water molecule. This is noteworthy, as Ouedraogo *et al.* state that the original isolation involved the slow crystallization of an oil with one molecule of water. The overall constitution could be reliably determined as the expected (*R*)-6-[(*S*)-2-hydroxy-4-(4-hydroxyphenyl)butyl]-5,6-dihydropyran-2-one (Figure 3). As previously reported by Ouedraogo *et al.* the configurations of C6 and C2' are *R* and *S*, respectively, which, compared to the structure of **1a**, is indeed a preserved configuration [8]. The dihydropyran-2-one ring has the half-chair conformation.

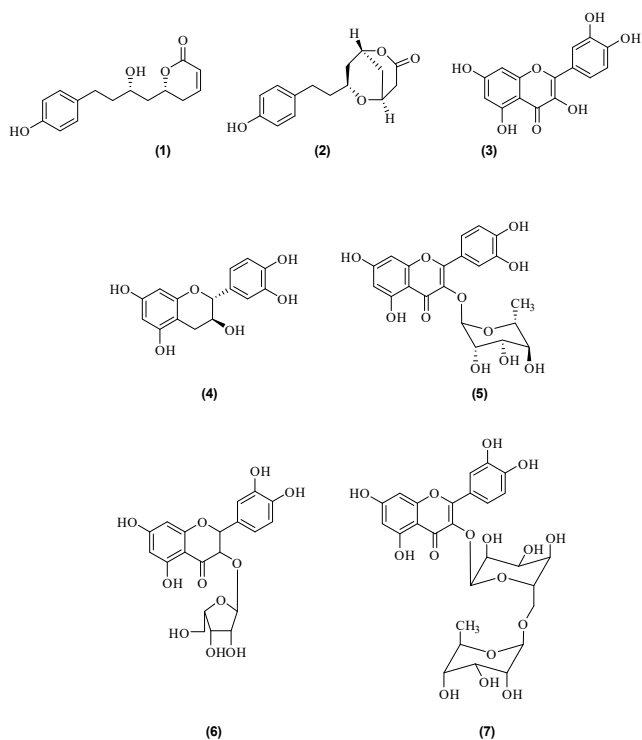


Figure 1: Structures of 1-7 isolated from *G. braunii*.

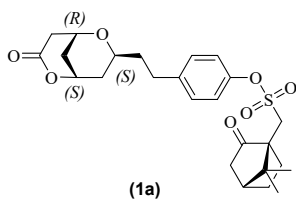


Figure 2: Camphor sulfonate 1a.

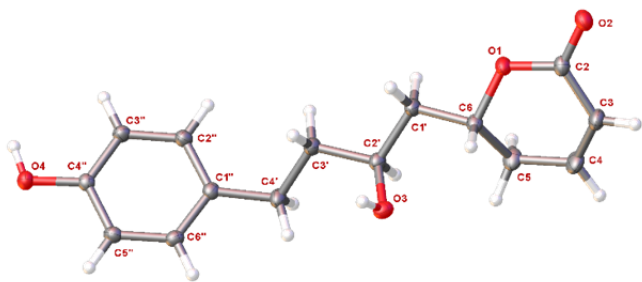


Figure 3: X-ray crystal structure of 1 (thermal displacement 50%). Solvent water molecules are omitted.

This feature is shared with other literature examples containing a dihydropyran-2-one moiety such as 8-15 (Figure 4) [16-22]. The deviations of the six-membered ring O1-C6 from its least-squares plane are quite characteristic of these compounds. The mean six-membered ring displacement of compound 1 is 0.187 Å with the C6 atom furthest away from the mean-plane at 0.314 Å. This is similar to the previously reported compounds 8-15 which have a mean six-membered ring displacement of 0.178–0.179 Å and an average C6 displacement from the mean-plane at 0.301–0.325 Å [16-22]. The rotation angle of the hydroxyphenyl moiety and dihydropyran-2-one moiety around the butyl chain of compound 1 is 129.87(6)° which, compared to compounds 8-15, falls close to the range for these types of compounds at 14.20–120.25° suggesting that high degree of rotations are possible [16-22].

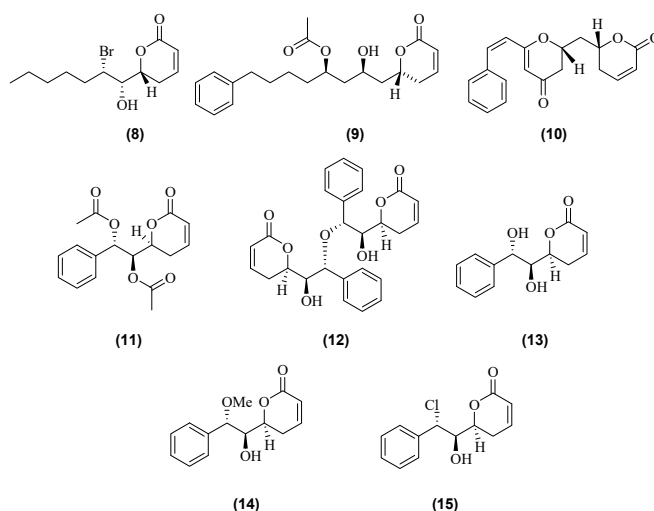


Figure 4: Examples of literature compounds containing a dihydropyran-2-one moiety.

As with many natural products implicated as potential therapeutics, the presence of hydrogen bond donors and acceptors is generally accepted as a key feature. Compound 1 features two distinct hydrogen bonding networks that are key in the molecular packing of the crystal structure. The first of these is between the phenyl hydroxy moiety (O4), the butyl hydroxy moiety (O3) and solvent water molecules (O5). As seen in Figure 5 the water molecules act as a bridge between O4 (O4-H4...O5, 2.573(1) Å) and O3 (O5-H5...O3, 2.701(1) Å) forming the hydrogen bonded network (Figure 5). The second of these networks is directly between the butyl hydroxy moiety (O3) and the ketone (O2) (O3-H3...O2, 2.771(1) Å), which forms head-to-tail overlap in the crystal packing (Figure 4). Of the aforementioned examples (8-15), these two hydrogen bonding networks are unique in their packing [16-22]. The head-to-tail overlap also removes the possibility of π -stacking within the structure inhibiting the formation of π -aggregates.

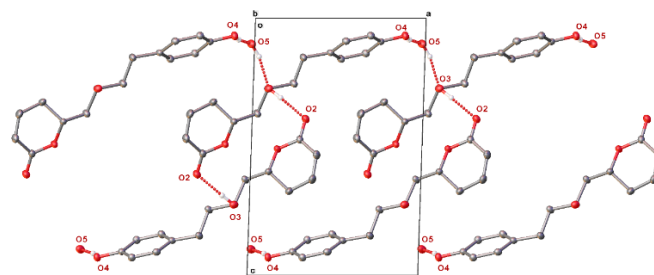


Figure 5: Molecular packing of 1. Non-hydrogen bonding hydrogen atoms are omitted for clarity. Hydrogen bonds are shown in dashed lines. Oxygen atoms implicated in hydrogen bonding are labeled. All other labels are omitted for clarity.

To the best of our knowledge, this is the first report on the isolation and structural elucidation of (*R*)-6-[(*S*)-2-hydroxy-4-(4-hydroxyphenyl)butyl]-5,6-dihydropyran-2-one (dodoneine) 1, (*1R,5S,7S*)-7-[2-(4-hydroxyphenyl)ethyl]-2,6-dioxabicyclo[3.3.1]nonan-3-one 2, quercetin 3, (+)-catechin 4, quercitrin 5, and avicularin 7 from *Globimetula braunii*. However, all the compounds have been previously reported from other Lorantheaceae plants. Compound 1 is isolated as colorless crystals with m.p. 56-58°C and $[\alpha]_D^{25} +119.3^\circ$ (*c* 0.03, acetone) (57-58°C and $[\alpha]_D^{25} +40.2^\circ$ (*c* 0.4, CHCl₃) [8]). Compound 1 has been encountered in *Tapinanthus dodoneifolius* [8] and *Pragmenanthera capitata* [9]. Compound 2 is isolated as colorless crystals with m.p. 171-172°C and $[\alpha]_D^{25}$

-46.8° (c 0.03, acetone) (170-171°C and $[\alpha]_D^{25}$ -37.5° (c 0.44, CHCl_3) [8]). Compound **2** has been isolated from *P. capitata* [9], *Globimetula dinklagei* [10], *Tapinanthus bangwensis* [23], while **3** was isolated from *P. capitata* [9] *Scurrula parasitica* [24], and *Taxillus theifer* [25]. The occurrence of **4** was also reported in *S. parasitica* [24], *T. theifer* [25], *Laranthus koi* [12] and *S. atropurpurea* [26]. Compound **5** was previously isolated from *L. koi* [12], *S. parasitica* [24], *T. theifer* [25], and *S. atropurpurea* [26]. Compound **6** has been encountered in *T. theifer* [25], *S. atropurpurea* [26] and *Tripodanthus acutifolius* [27], while **7** was previously isolated from *Psittacanthus cuneifolius* [28] and *T. kaempferi* [29-30].

Experimental

Plant material: The leaves of *Globimetula braunii* parasitizing on *Piliostigma thonningii* were collected in October 2014, from the Sheda Science and Technology complex (SHESTCO), Abuja, Nigeria. The voucher specimen for *G. braunii* (No 9016) and *P. thonningii* (No 7151) were deposited at the Herbarium section of the Biological Sciences Department, Ahmadu Bello University (ABU), Zaria, Nigeria.

Isolation procedure: Sequential extraction of the dried powdered leaves (3.3 kg) of *G. braunii* at room temperature using different polarity of organic solvents for 72 h each afforded the *n*-hexane extract (113.3 g, 3.4 %), the CH_2Cl_2 extract (71.3 g, 2.2 %) and the MeOH extract (342.0 g, 10.3 %). The MeOH extract (120 g) was further suspended in water, then successively partitioned with *n*-hexane, CHCl_3 , EtOAc and then MeOH to yield the *n*-hexane-soluble extract (5.6 g, 1.6%), the CHCl_3 -soluble extract (3.8 g, 1.1%), the EtOAc-soluble extract (19.5 g, 5.7%) and the MeOH-soluble extract (34.7 g, 10.1 %). Fractionation of the EtOAc-soluble extract (19.5 g) by silica gel VLC afforded five major fractions, GBPTE 1 - GBPTE 5. Purification of GBPTE 1 - GBPTE 3 (7.2 g) by silica gel CC gave **1** (465 mg, 2.9%) as colorless crystals, **2** (528.5 mg, 2.7%) as colorless crystals and **3** (121 mg, 0.3%) as yellow powder. Purification of GBPTE 4 (3.2 g) using silica gel CC afforded **4** (440.2 mg, 1%) as a pale brown powder. Fractionation of the MeOH-soluble extract (45 g) by silica gel VLC gave five major fractions, GBPTM 1 - GBPTM 5. Purification of GBPTM 3 - GBPTM 5 (2.7 g) by silica gel CC followed by Sephadex LH-20 CC afforded **5** (37 mg, 0.1%) as yellow solids and **6** (11.2 mg, 0.02%) as a reddish-brown powder while further purification of

several sub-fractions using PTLC gave **7** (6.8 mg, 0.01%) as a yellow powder.

Crystallographic Data of 1: Crystals were grown by dissolving the compounds in acetone and allowing for slow evaporation. Diffraction data for the compound was collected on a Bruker APEX 2 DUO CCD diffractometer using graphite-monochromated MoK_α ($\lambda = 0.71073$ Å) radiation. Crystals were mounted on a MiTeGen MicroMount and collected at 100(2) K using an Oxford Cryosystems Cobra low-temperature device using strategies described earlier [31-32]. Data were collected using omega and phi scans and corrected for Lorentz and polarization effects using the APEX software suite [33]. Using Olex2, the structure was solved with the XT structure solution program, using the intrinsic phasing solution method and refined against $|F_2|$ with XL using least squares minimization. [34-36]. Non-hydrogen atoms were refined with anisotropically thermal parameters. Hydrogen atoms were generally placed in geometrically calculated positions and refined using a riding model. All images were rendered using Olex2 [34].

Crystal Data for 1: $\text{C}_{15}\text{H}_{20}\text{O}_5$ ($M = 280.31$ g/mol): monoclinic, space group $P2_1$, $a = 9.1437(7)$ Å, $b = 6.0302(5)$ Å, $c = 13.7423(10)$ Å, $\beta = 91.9330(10)^\circ$, $V = 757.30(10)$ Å³, $Z = 2$, $T = 100.0$ K, $\mu(\text{MoK}_\alpha) = 0.092$ mm⁻¹, $D_{\text{calc}} = 1.229$ g/cm³, Flack parameter = $-0.11(0.13)$; 15738 reflections measured ($4.458^\circ \leq 2\theta \leq 63.144^\circ$), 5052 unique ($R_{\text{int}} = 0.0149$, $R_{\text{sigma}} = 0.0150$) which were used in all calculations. The final R_1 was 0.0278 ($I > 2\sigma(I)$) and wR_2 was 0.0777 (all data).

CCDC 1558020 (**1**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Acknowledgments - The authors wish to thank the Ministry of Higher Education (MOHE) for the financial support under Research University Grant (Q.J130000.2526.12H47), Science Foundation Ireland for grant support (IvP 13/IA/1894), and Faculty of Science, Universiti Teknologi Malaysia for providing the research facilities. The authors acknowledge EPSRC National Mass Spectrometry Service Centre (Department of Chemistry, University of Wales Swansea, Swansea, Wales, UK) for the MS analysis.

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