SHORT REPORT

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A New Phloroglucinol Derivative Isolated from *Hypericum afrum*, a Plant Endemic to Algeria

Farida Larit [1,2], Francisco León[1], Narayan D. Chaurasiya[3], Babu L. Tekwani[3], Samira Benyahia[4], Samir Benayache[5] and Stephen J.Cutler[1*]

[1]*Department of BioMolecular Sciences, Division of Medicinal Chemistry, The University of Mississippi, University, MS 38677, United States*
[2]*Faculté des Sciences Exactes, Département de Chimie, Université Constantine 1, 25000 Constantine, Algeria*
[3]*National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, United States*
[4]*Laboratoire de Synthèse Organique, Modélisation et Optimisation des Procèdes (LOMOP), Université Badji Mokhtar, Faculté des Sciences, Département de Chimie, 23000 Annaba, Algeria*
[5] *Unité de Recherche Valorisation des Ressources Naturelles, Molécules Bioactives et Analyse Physico-Chimique et Biologique (VARENBIOMOL), Université des Frères Mentouri, Constantine, Algeria*

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Abstract: A new phloroglucinol derivative, identified as 3-benzoyl-3-hydroxy-5-(3-methylbut-2-en-1-yl)cyclopentane-1,2,4-trione (**1**), together with eight previously reported compounds, quercetin, myricitrin, hypericin, biapeginin, pseudohypericin, myricetin, 1,3,5,6-tetrahydroxyxanthone, and β-sitosterol were isolated from the chloroform, ethyl acetate and butanol extracts of the aerial part of *Hypericum afrum* (Lam.). Their structures were elucidated by spectroscopic analyses, including 1D-, 2D-NMR and HRESIMS. The EtOAc extract showed moderate MAO-A inhibition with an IC_{50} value of 3.35 µg/mL. Bioassay-guided fractionation of the EtOAc extract resulted in the isolation of quercetin as the active component exhibiting MAO-A inhibitory activity with an IC_{50} value of 1.25 µM.

Keywords: *Hypericum*; phloroglucinol derivatives; MAO-A and MAO-B; spectroscopic analyses.

1. Plant Source

The genus *Hypericum* (Hypericaceae) comprises more than 480 species with worldwide distribution except in the Antarctica. It is found in different habitats including a variety of temperate, subtropical and tropical (high altitudes) regions, and isn't observed in places with extreme aridity and salinity [1]. The popular interest in *Hypericum* species have been based on their pharmacological properties and their use in traditional medicines around the world. In fact, *H. perforatum*, commonly

* Corresponding author: E Mail: cutler@olemiss.edu

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known as St. John's wort, is used as poultice, decoction or infusion for sedative and tonic functions and more commonly to treat mild to moderate depression [1, 2]. The extracts of *H. perforatum* are available as dietary supplements in the United States and as a botanical medicine in Europe. It is one of the top best-selling botanicals for more than a decade in the US, with $ 5.6 million in 2013 sales [3] and € 70 million in 2004 sales in Germany (latest data available) [1]. Pharmacological use of the *H. perforatum* and its economic impact prompted the phytochemical study of different plants belonging to the same genus. The predominant secondary metabolites isolated from this genus are phenolic compounds including hypericin, pseudohypericin, hyperfirin, hyperforin, quercetin and derivatives, chlorogenic acid and other flavonoids and phenolic acid, as well as, phloroglucinol and its derivatives [4].

The plant *Hypericum afrum* (Lam.) was collected in the El Kala region (El Tarf, Northeastern Algeria) in July of 2011 and identified by Belouahem-Abed Djamila from Institut National de recherche forestière. Station de recherche d'El Kala (El Tarf). Algeria. A voucher specimen (UM-10012014) has been deposited in the culture collection of the Department of BioMolecular Sciences, University of Mississippi. *H. afrum* is an endemic species growing in the wetlands in north-eastern Algeria. This plant grows in different forms existing as a shrub or herbaceous plant depending on its biological adaptation to the dampness of the environment [5]. The phytochemical study of *H. afrum* yielded a new phloroglucinol derivative **1**. Additionally, the bioassay-guided fractionation of the EtOAc extract resulted in the isolation and identification of the flavonoid quercetin, possessing selective inhibition of the human MAO-A enzyme.

2. Previous Studies

No phytochemical study has been reported

3. Present Study

Air-dried aerial parts (1000 g) of *H. afrum* were macerated at room temperature with EtOH–H_2O (80:20, v/v) for 24 h, three times. After filtration, the filtrate was concentrated and dissolved in H_2O (800 mL). The resulting solution was extracted successively with $CHCl_3$, EtOAc and n-butanol. The organic phases were dried with Na_2SO4, filtered and concentrated in vacuum at room temperature to obtain the following extracts: chloroform (1 g), EtOAc (7.9 g), and n-butanol (15.92 g).

The chloroform extract was subjected to silica gel column chromatography (230–400 mesh) using a step-gradient system hexane/$CHCl_3$ and then with increasing percentages of MeOH to afford ten fractions (FC1–FC10) obtained by combining the eluates on the basis of TLC analysis. FC4 (50 mg, hexane/$CHCl_3$ 7:3) yielded β-sitosterol (14 mg) through crystallization with MeOH. FC6 (350 mg, $CHCl_3$ 100%) was subjected to SPE RP-18 column chromatography (CC), using MeOH/H_2O elution to give six subfractions SFC4-1 to SFC4-6. Fraction SFC4-1 (200mg) was subjected to Sorbadex 20-LH column chromatography using CH_2Cl_2/MeOH (1:1, v/v) elution and yielded compound **1** (50 mg). The EtOAc extract was chromatographed on a silica gel column (CH_2Cl_2/MeOH, gradient elution in a high polarity) to yield 10 fractions FE1 to FE10 according to their TLC behavior. From fraction FE3 (423 mg, CH_2Cl_2/MeOH 3%) quercetin (10 mg) was obtained as a yellow precipitate, the liquid supernatant was chromatographed on Sorbadex 20-LH eluted with CH_2Cl_2/MeOH (1:1), yielding six subfractions, subfraction four was subsequently purified by Sorbadex 20-LH eluted with CH_2Cl_2/MeOH (1:1) to yield hypericin (2 mg). The fraction FE4 (115 mg, CH_2Cl_2/MeOH 2%) was rechromatographed on Sorbadex 20-LH eluted with CH_2Cl_2/MeOH (1:1) to furnish biapigenin (15.6 mg). Fraction FE6 (125 mg, CH_2Cl_2/MeOH 5%) was chromatographed by Sorbadex 20-LH eluted with CH_2Cl_2/MeOH (1:1) and finally purified by preparative TLC developed with $CHCl_3$/MeOH (10:1) to afford pseudohypericin (2 mg) and myricetin (7.3 mg) and 1,3,5,6-tetrahydroxyxanthone (5 mg). The fraction FE7 (1 g, CH_2Cl_2/MeOH 10%) yield myricitrin (15 mg) as a brownish powder precipitate.

*3-Benzoyl-3-hydroxy-5-(3-methylbut-2-en-1-yl)cyclopentane-1,2,4-trione (**1**)*: Yellow amorphous powder; mp 120-121 ºC; $[\alpha]^{25}_{D}$ + 21 º (c 0.5, MeOH); UV (MeOH) λ_{max} (log ε): 339 (4,82); IR (KBr) ν_{max}: 3430, 1741, 1625, 1448, 1416, 1221, 770 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.04 ppm (3H, s, CH_3-6), 1.54 ppm (3H, s, CH_3-4′), 1.56 ppm (3H, s, CH_3-5′), 2.28-2.40 (2H, m, CH_2-1′), 4.93 (1H, t , *J* = 7,7 Hz, CH-2′), 7.29 (2H, t, *J* = 7,6 Hz, CH-3″ and CH-5″), 7.42 (1H, t, *J* = 7,4 Hz, CH-4″), 7.55 (2H, d, *J* = 7,5 Hz, CH-2″ and CH-6″); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 213.9 (C, C-4), 200.3 (C, C-1), 194.7 (C, C-α), 181.5 (C, C-2), 139.0 (C, C-1″), 135.2 (C, C-3′), 132.1 (C, C-4″), 129.0 (CH, C-2″ and C-6″), 127.7 (CH, C-3″ and C-5″), 121.9 (C, C-3), 118.6 (CH, C-2′), 50.6 (C, C-5), 34.2 (CH_2, C-1′), 25.8 (CH_3, C-4′), 19.0 (CH_3, C-6), 17.6 (CH_3, C-5′); negative HRESIMS: *m/z* 313.1077 [M-H]$^-$ (calcd for $C_{18}H_{17}O_5$, 313.1070).

Compound **1** was isolated as a yellow powder and its molecular formula was established as $C_{18}H_{18}O_5$ by negative HRESIMS (*m/z* 313.1077 [M-H]$^-$). The IR spectra showed the absorptions for OH (3430cm^{-1}), and carbonyl (1747 cm^{-1} and 1625 cm^{-1}) groups. The ^{1}H NMR spectrum showed characteristic signals for three methyl groups at δ_H 1.56 ppm (3H, s), 1.54 ppm (3H, s), and 1.04 ppm (3H, s) the first two methyl corresponding to a gem dimethyl group attached to a double bond; along with the signals at δ_H 2.40-2.28 ppm (2H, m) characteristic of aliphatic protons; and vinylic proton at δ_H 4.93 ppm (1H, t, *J* = 7,7 Hz) suggested the presence of a prenyl group in the molecule, which was confirmed by COSY and HMBC (Figure 1) experiments. Also in the ^{1}H NMR spectrum three signals at δ_H 7.55 ppm (2H, d, *J* = 7,5 Hz), 7.42 ppm (1H, t, *J* = 7,4 Hz) and 7.29 ppm (1H, t, *J* = 7,6 Hz) indicated a monosubstituted phenyl group. The ^{13}C NMR and DEPT spectra of 1 disclosed 18 carbons, which were indicative of four ketone carbonyl carbons at δ_C 213.9, 200.3, 194.7 and 181.5 ppm; additional four quaternary carbons, six methine, three methyl and one methylene carbon. HMBC correlations (Figure 1) permit joining to the remaining methyl and determining the connections among the rest of the structural fragments. Thus, the structure for **1** has been established and proposed to be 3-benzoyl-3-hydroxy-5-(3-methylbut-2-en-1-yl)cyclopentane-1,2,4-trione. The stereochemistry of the two stereogenic centers have not been successfully determined. The known compounds were identified by comparison of their spectra and physical data with the available literature [6-8].

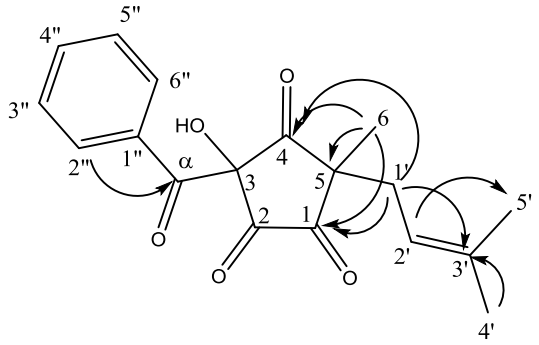

Figure 1. Selected ^{1}H -^{13}C HMBC correlations of compound **1**.

Interestingly, the presences of compounds with two or three keto cyclopentane moiety like compound 1 only have been reported from *Humulus lupulus* as phloroglucinol derivatives [9]. In the same case, the high content of benzoylphloroglucinols in *Hypericum* genus [4, 10], suggested a benzoylphloroglucinol as precursor for compound **1**.

Human MAO-A and MAO-B inhibition assay: The extracts, fractions and constituents of *H. afrum*, were evaluated using MAO-A and-B enzymatic assays as previously reported [11]. The ethyl acetate extract showed moderate inhibition for MAO-A and B, following a bioassay-guided fractionation strategy. The compound responsible for that activity was identified as quercetin (Table 1). Quercetin has previously been identified as MAO-A inhibitor with comparable values in this report [12].

Table 1. Inhibition of recombinant human Monoamine Amine Oxidase-A and B by crude extract, fractions, and pure constituents of *Hypericum afrum*.

Samples	**MAO-A IC_{50}** (μg/mL)	**SD**	**MAO-B IC_{50}** (μg/mL)	**SD**
EtOAc extract[#]	3.35	0.035	13.500	0.7071
Fraction FE3[#]	2.17	0.01	4.12	0.59
Compound[*] **1**	>100	--	71.12	4.24
Quercetin[*]	1.25	0.050	16.50	0.50
Phenelzine[*a]	0.268	0.0257	0.1430	0.025
Clorgyline[*b]	0.0076	0.0005	----	-----
Deprenyl[*c]	----	----	0.050	0.0122

[a] Positive control for both MAO enzyme; [b] Positive control selective for MAO-A; [c] selective for MAO-B. [*]Shows inhibition = μM and [#] shows inhibition = (μg/mL)

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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