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New formylated phloroglucinol compounds from *Eucalyptus globulus* foliage

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ABSTRACT

Two new acylphloroglucinols were isolated from the leaves of *Eucalyptus globulus* Labill and identified as macrocarpals P (**1**) and Q (**2**). Structural elucidations were carried out using conventional 1D and 2D NMR and mass spectrometry together with complementary techniques (UV and IR). Macrocarpal Q was a diastereoisomer of macrocarpal E (**3**), configuration of which was not precised. Simultaneous isolation of macrocarpals E and Q allowed to determine the configurations of both compounds. The diformylphloroglucinol (**4**) was also isolated as well as already known compounds grandinol, macrocarpals D, I, L, N, O and am-1.

1. Introduction

Eucalyptus trees constitute a rich source of formylated phloroglucinol compounds (FPCs) including simple and complex acylphloroglucinol-terpenes as the euglobals, macrocarpals and sideroxylonals (Eschler et al., 2000). Their presence was only demonstrated in the Eucalyptus until recently where they were isolated in an Australian sawfly feeding on Eucalyptus plants (Yin et al., 2013). FPCs are endowed with several interesting biological effects (Ghisalberti, 1996): euglobals possess anti-viral (Takasaki et al., 1990), anti-HIV (Wichtl and Anton, 2003) and anti-tumoral properties (Takasaki et al., 2000). Sideroxylonals are strong inhibitors of the human plasminogen activation (Neve et al., 1999) and antibacterial compounds (Satoh et al., 1992). Macrocarpals have antibacterial properties against dermatophytes cariogenic and periodonthopathic bacteria (Osawa et al., 1996) and seem to stimulate the synthesis of ceramides in *corneum stratum* (Ishikawa et al., 2012) (Fig. 1).

Our interest in the macrocarpals stemmed from the observation that macrocarpals A, B, C, D, E and M were inhibitors of the uptake of catecholamines (serotonin, dopamine and norepinephrine) and consequently, were of potential interest for central nervous system therapies (Fiorini-Puybaret et al., 2008; Fiorini-Puybaret and Joulia, 2009). Sixteen macrocarpals have been described in the literature and known as macrocarpals A-O and -am-1 also named eucalyptone (Shibuya et al., 2001; Singh et al., 1999; Osawa et al., 1996; Singh and Etoh, 1995; Osawa et al., 1995; Nishizawa et al., 1992). All but macrocarpal E are of defined stereochemistry. During our investigations, we made use of supercritical CO_2 extraction of Eucalyptus leaves and obtained a 5.2% yield of an FPCs enriched extract with 22% of macrocarpals, which allowed to isolate and characterize new and minor FPCs.

2. Results and discussion

Dried and powdered leaves of *Eucalyptus globulus* were extracted by supercritical CO_2 with ethanol as cosolvant. The crude extract was purified by liquid-liquid extraction under basic and acid conditions. The purified extract was fractionated by

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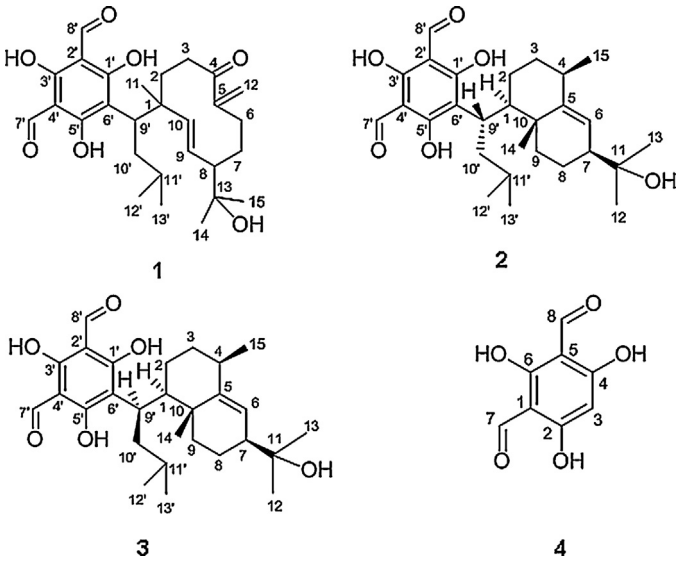

Fig. 1. Chemical structures of compounds **1**–**4**.

preparative RP-HPLC into 49 fractions. The final stages of purification for each compound were semi-preparative RP-HPLC. Fractions 13, 27, 24 and 4 respectively contained compounds **1** (1.8 mg), **2** (1.5 mg), **3** (2 mg) and **4** (6.6 mg). Grandinol (4.5 mg), macrocarpals I (2.2 mg), N (6.3 mg), eucalyptone (29 mg), O (12.2 mg) and D (23.4 mg) were respectively obtained from fractions 12, 10, 15, 16, 26 and 29. All these compounds were identified by UV and IR spectroscopy, 1D and 2D NMR experiments (^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMBC, HSQC, ROESY) and high-resolution mass spectrometry.

Compound **1** was purified as a white powder from fraction 13. According to its high-resolution mass spectrum (HRToFMS: *m*/*z* 486.2628 [M−H]$^-$), its molecular formula was deduced to be $C_{28}H_{38}O_7$. Its UV spectrum contained three absorption maxima (UV (EtOH) λ_{max} (log ε): 218 (3.28), 275 (3.32), 391 (2.81) nm) and resembled those of other macrocarpals, suggesting the presence of the same substituted phloroglucinol chromophore. IR spectrum was similar to that of macrocarpal N and eucalyptone suggesting that these compounds and **1** possessed identical chemical functions. Comparison of the spectral data of **1** with those of eucalyptone and macrocarpal N, which were the unique Eucalyptus compounds with this molecular formula, demonstrated that **1** was a new macrocarpal. These compounds were proven to be different by retention times measurements (**1**: 19.63 min, eucalyptone: 23.73 min and macrocarpal N: 23.09 min). The ^{13}C NMR spectra of compound **1** (Table 1) showed the presence of 28 carbons including 10 quaternary, 7 tertiary, 6 secondary and 5 primary carbons, with signals corresponding to a phloroglucinol moiety (δ_C 106.3, 110.0, 170.0 and 171.0), bearing two aldehyde groups (δ_C 193.1 and 193.2), a ketone function (δ_C 207.8), a quaternary carbon atom substituted by an hydroxyl group (δ_C 74.0) and four ethylenic carbons (δ_C 154.2, 129.7, 140.2 and 120.8). The ^{1}H NMR spectrum confirmed the presence of two formyl groups (δ_H 10.08 et 10.1), four ethylenic functions (δ_H 5.60, 5.49 and 5.26 (2H)) and five methyl groups (δ_H 0.79, 0.81, 0.84, 0.90 and 1.15). COSY, HSQC and HMBC experiments confirmed the connectivities (Fig. 2). The equivalence of H_9 and H_{10} was raised by adding C_6D_6 to the $CDCl_3$ solution and their coupling constant was measured at 17 Hz, suggesting a *trans* relationship. NMR did not allow to go further as far as absolute and relative configurations of the three asymmetric centers were concerned. An ECD spectrum was measured for **1** (see Supplementary material) in the hope of being able to assign the configuration of the benzylic carbon atom (as

Table 1
^{13}C and ^{1}H NMR data of macrocarpal P (**1**) and macrocarpal Q (**2**) (500 MHz, in CD_3OD, δ in ppm).

Position	**1**		Position	**2**	
	δ_C	δ_H (*J* in Hz)		δ_C	δ_H (*J* in Hz)
1	46.6 (s)	–	1	53.2 (d)	1.58, m
2	41.0 (t)	*a 1.90*, m	2	21.6 (t)	*a* 1.77, qd, (13; 3.4)
		b 2.13, t (13.1)			*b* 2.09, dq, (13; 3.4)
3	34.9 (t)	*a* 2.04, m	3	35.1 (t)	1.57, m
		b 2.93, t (13.1)			1.64, m
4	207.9 (s)	–	4	41.0 (d)	2.44, m
5	154.2 (s)	–	5	152.3 (s)	–
6	34.1 (t)	*a* 1.88, m	6	122.9 (d)	5.42, d (3.4)
		b 2.77, d (13.1)			
7	24.8 (t)	1.77, m	7	46.6 (d)	1.97, m
8	52.2 (d)	1.80, m	8	20.9 (t)	1.5, m
					1.58, m
9	129.7 (d)	5.26, m	9	36.7 (t)	1.38, ddd (12.8; 12; 3.4)
					1.66, m
10	140.2 (d)	5.26, m	10	40.7 (s)	–
11	19 (q)	1.15 (s)	11	74.3 (s)	–
12	120.8 (t)	5.49 (s)	12/13	27.2 (q)	1.04, s
		5.6 (s)		27.5 (q)	1.06, s
13	74 (s)	–	14	23.1 (q)	1.15,s
14/15	24.1 (q)	0.81, s	15	22.5 (q)	1.17, d (7.5)
	29 (q)	0.90, s			
1′	170 or 171 (s)	–	1′/5′	168.4 (s)	–
2′	106.3 (s)	–	2′/4′	105.9/106.5 (s)	–
3′	169.6 (s)	–	3′	170.5 (s)	–
4′	106.3 (s)	–	6′	116.1 (s)	–
5′	170 or 171 (s)	–	7′/8′	193.1 (d)	10.11, s
6′	110 (s)	–	9′	31.5 (d)	3.41, dt (12.5; 3.6)
7′/8′	193.1 (d)	10.08, s	10′	40.2 (t)	*a* 1.22, m
	193.2 (d)	10.1, s			*b* 2, m
9′	43 (d)	3.33, m	11′	27.8 (d)	1.14, m
10′	36.8 (t)	*a* 1.17, m	12′/13′	22.4 (q)	0.94, d (6.4)
		b 2.29, t (11.2)		25.0 (q)	0.80, d (6.4)
11′	27.9 (d)	1.17, m			
12′/13′	21.7 (q)	0.79, d (6.1)			
	24.7 (q)	0.84, d, (6.1)			

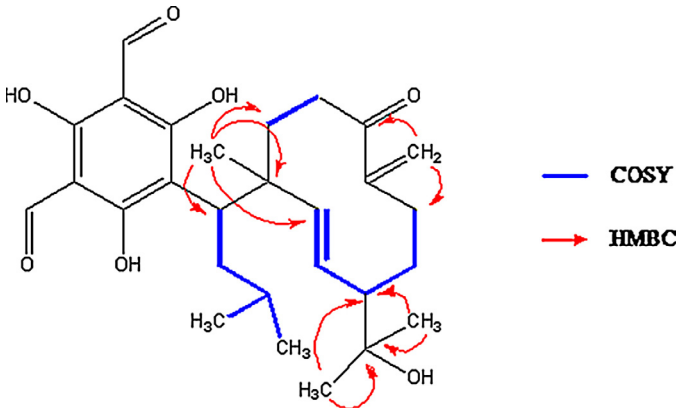

Fig. 2. Key ^{1}H–^{1}H COSY and HMBC correlations of **1**.