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Eucalyptals A–C with a New Skeleton Isolated from *Eucalyptus globulus*

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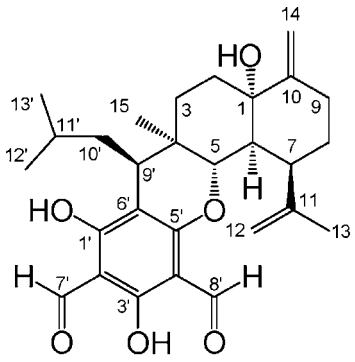
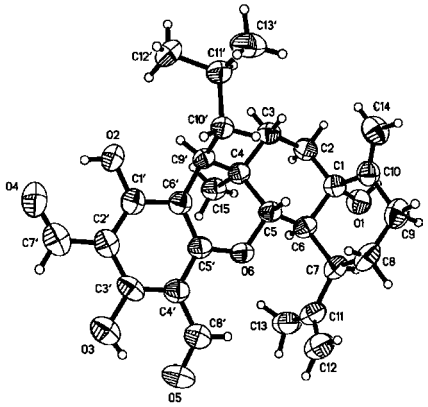
ABSTRACT



eucalyptal A (1)



Eucalyptals A–C (1–3) with a new skeleton of 3,5-diformyl-isopentyl phloroglucinol-coupled cadinane were isolated from the fruits of *Eucalyptus globulus*. Their structures were elucidated by spectroscopic analysis, and that of 1 was confirmed by single-crystal X-ray diffraction. The biosynthetic pathway of 1–3 was also postulated. Compounds 1–3 exhibited selective cytotoxicity against the HL-60 cell line.

Eucalyptus globulus Labill, a tall timber tree, grows widely in south China. Its fruits and leaves have been used as a traditional Chinese medicine to cure diseases such as flu, dysentery, eczema, and scald.[1] In the past decades, a number of unusual phloroglucinol-coupled terpenoids, named macrocarpals and euglobals, have been isolated from the genus of *Eucalyptus*,[2−8] some of which displayed a wide spectrum of significant bioactivities such as HIV-RTase inhibition,[2] granulation inhibition,[3,4] and antiviral[5] and antibacterial[6,7] effects. One of our efforts to discover the structurally diverse and biologically significant metabolites from plant resources has led to the isolation of three novel compounds (**1**−**3**) that represent a new skeleton of 3,5-diformyl-isopentyl phloroglucinol-coupled cadinane from the fruits of *E. globulus*. Herein, details of the isolation, structural elucidation, postulated biogenetic origin, and cytotoxic activities of eucalyptals A−C (**1**−**3**) are described.

The dry powder of fruits of *E. globulus* (3.0 kg) was extracted with 95% ethanol three times at ambient temperature to give the crude extract (520 g), which was dissolved

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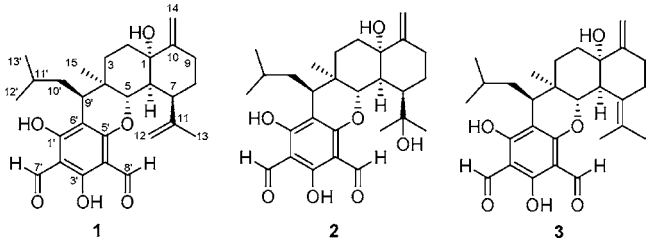
Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds **1**−**3**[a] in Pyridine-d_5

	1		**2**		**3**	
no.	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C
1		73.1		74.1		73.9
2α	2.15 (ddd, 14.2, 14.2, 4.2)	33.3	2.20 (ddd, 13.6, 13.6, 3.0)	33.7	2.19 (ddd, 14.0, 14.0, 3.1)	33.0
2β	2.45 (ddd, 14.2, 6.2, 6.2)		2.49 (br d, 13.6)		2.49 (ddd, 14.0, 3.1, 3.1)	
3α	1.35 (m)	30.5	1.41 (ddd, 13.2, 3.0, 2.7)	30.7	1.49 (ddd, 13.3, 3.1, 3.1)	30.5
3β	1.95 (m)		2.01 (m)		2.00 (ddd, 14.0,13.3, 3.1)	
4		36.5		36.8		35.9
5	4.30 (d, 11.8)	76.2	4.54 (d, 11.4)	76.2	4.28 (d, 11.6)	75.6
6	2.85 (dd, 11.8, 2.6)	46.3	2.85 (dd, 11.4,1.8)	46.4	3.57 (d, 11.6)	48.5
7	3.56 (br d, 11.9)	40.0	3.33 (br d, 12.5)	44.9		128.0
8α	2.00 (m)	24.7	2.10 (m)	22.7	2.86 (dd, 13.6, 4.2)	26.4
8β	1.75 (m)		2.00 (m)		2.24 (m)	
9α	2.94 (m)	33.1	3.00 (ddd, 13.5, 13.5, 5.6)	34.4	2.35 (m)	33.4
9β	2.38 (br d, 13.5)		2.49 (br d, 13.5)		2.99 (ddd, 13.6, 13.6, 4.7)	
10		149.0		149.4		149.6
11		150.6		71.4		127.0
12	a 4.83 (s)	108.0	1.53 (3H, s)	32.1	1.78 (3H, s)	20.9
	b 4.92 (s)					
13	1.90 (3H, s)	22.9	1.55 (3H, s)	28.5	1.86 (3H, s)	20.7
14a	5.05 (s)	110.2	5.08 (s)	110.3	5.10 (s)	109.3
14b	5.11 (s)		5.14 (s)		5.12 (s)	
15	1.04 (3H, s)	19.6	1.13 (3H, s)	20.0	1.13 (3H, s)	19.3
7′	10.32 (s)	191.9	10.31 (s)	191.9	10.32 (s)	191.9
8′	10.12 (s)	195.5	11.23 (s)	198.0	9.93 (s)	192.3
9′	2.61 (dd, 7.2, 2.8,)	38.4	2.64 (dd, 7.1, 3.0,)	38.9	2.74 (dd, 7.5, 2.9)	38.6
10′a	1.25 (m)	43.6	1.28 (m)	43.6	1.28 (m)	43.3
10′b	1.54 (m)		1.61 (m)		1.60 (m)	
11′	1.80 (m)	29.1	1.82 (m)	29.2	1.81 (m)	28.9
12′	0.99 (3H, d, 6.6)	23.9	1.01 (3H, d, 6.6)	23.9	1.01 (3H, d, 6.4)	23.9
13′	1.12 (3H, d, 6.7)	22.6	1.14 (3H, d, 6.6)	22.7	1.14 (3H, d, 6.4)	22.6
1′−6′	δ_C: 169.2 (C-1′), 104.4 (C-2′), 168.0 (C-3′), 104.5 (C-4′), 163.1 (C-5′), 107.8 (C-6′)		δ_C: 169.1 (C-1′), 104.5 (C-2′), 168.7 (C-3′), 104.8 (C-4′), 163.4 (C-5′), 108.0 (C-6′)		δ_C: 169.5 (C-1′), 104.0 (C-2′), 167.8 (C-3′), 104.4 (C-4′), 162.8 (C-5′), 107.5 (C-6′)	

[a] Recorded at 400 and 100 MHz for ^{1}H and ^{13}C, respectively.

in water (2 L) and then partitioned with petroleum ether, EtOAc, and *n*-BuOH successively. The EtOAc extract (210 g) was subjected to silica gel column chromatography eluted with petroleum ether−acetone (20:1 to 1:1, v/v) to give five major fractions 1−5. Fraction 2 was then extensively column chromatographed over silica gel, reverse-phase silica gel, and LH-20 gel to give **1** (10 mg), **2** (15 mg), and **3** (11 mg).



1 **2** **3**

Compound **1**[9] was obtained as a pale yellow crystal. The HREIMS displayed the molecular ion at *m*/*z* 468.2511 (calcd 468.2512), which is consistent with a molecular formula of $C_{28}H_{36}O_6$ with 11 degrees of unsaturation. The UV and IR spectral data closely resembled those reported for macrocarpals and euglobals that bear a 3,5-diformyl phloroglucinol subunit,[2−6] implying the presence of the same substituted chromophore. The 1D-NMR data (Table 1) and HSQC spectrum of **1** further revealed the presence of a 3,5-diformyl phloroglucinol (see Table 1), an isobutyl [δ_H 1.25 (1H, m), 1.54 (1H, m), 1.80 (1H, m), 0.99 (3H, d, J = 6.6), and 1.12 (3H, d, J = 6.7); δ_C 43.6, 29.1, 23.9, and 22.6], two tertiary methyl [δ_H 1.04 (3H, s) and 1.90 (3H, s); δ_C 19.6 and 22.9], and two terminal double bond [δ_H 5.05 and 5.11 (each 1H, s), and 4.83 and 4.92 (each 1H, s); δ_C 110.2 and 149.0, and 108.0 and 150.6] groups. The aforementioned data implied that compound **1** possessed the feature of a 3,5-diformyl-isopentyl phloroglucinol-coupled sesquiterpenoid. The 3,5-diformyl phloroglucinol group and two double bonds accounted for 8 out of the 11 double-bond equivalents. The remaining three degrees of unsaturation therefore required that compound **1** possessed three additional rings.

Three structural fragments (C-2 to C-3, C-5 to C-9, and C-9′ to C-13′) were first established by the correlations observed in the ^{1}H−^{1}H COSY spectrum (Figure 1). The connectivities of the three structural fragments, quaternary carbons, and the other functional groups were mainly

(9) **Eucalyptal A (1).** Pale yellow crystal; mp 208−209 °C; $[\alpha]^{20}_D$ −185.0 (*c* 0.140, $CHCl_3$); UV (CH_3OH) λ_{max} (log ϵ) 276 (4.74) nm; IR (KBr) ν_{max} 3425, 2950, 1633, 1442, 1384, 1305, 1193, 1157, 617 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 468 $[M]^+$ (29), 450 (9), 412 (27), 411 (100), 393 (25), 353 (13), 267 (10), 251 (6), 249 (16), 233 (13), 209 (46), 201 (40), 195 (34), 159 (17), 91 (13); HREIMS *m*/*z* 468.2511 (calcd for $C_{28}H_{36}O_6$, 468.2512).

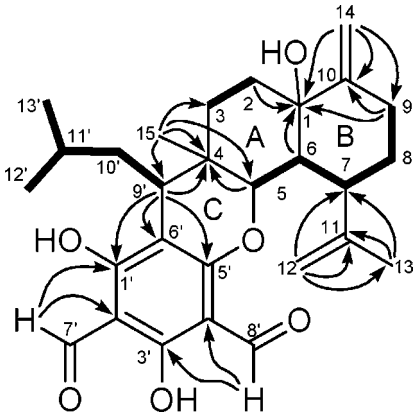


Figure 1. Key ^{1}H-^{1}H COSY (—) and HMBC (H→C) correlations of **1**.

achieved by analysis of the HMBC spectrum (Figure 1). The HMBC correlations from H_3-15 to C-3, C-4, and C-5 allowed the connection of C-3, C-5, and C-15 to the quaternary carbon C-4. The C-2 and C-6 were linked via C-1 by the correlations from H-2 and H-6 to C-1. The six-membered A-ring was thus constructed. The HMBC correlations from H_2-14 to C-1, C-9, and C-10 incorporated the exomethylene group (C-10═C-14) between C-1 and C-9 to establish the B-ring. An isopropenyl group was fixed to C-7 by the mutual HMBC correlations of H_2-12/C-7, C-11, and C-13, and H_3-13/C-7 and C-11. A cadinane sesquiterpenoid moeity in compound **1** thus emerged from the above spectral analysis. The mutual HMBC correlations of H-9′/C-1′, C-5′, and C-6′; H-7′/C-1′ and C-2′, H-8′/C-3′, and C-4′ confirmed the presence of a 3,5-diformyl-isopentyl phloroglucinol, which was coupled with cadinane sesquiterpenoid moeity via the C-4−C-9′ bond as judged by the HMBC correlations of H_3-15/C-9′ and H-9′/C-4. The only leftover uncertainty for the planar structure of **1** was the remaining one degree of unsaturation, which required the presence of an additional ring.

Observation of the ^{13}C NMR data of the reported macrocarpals and euglobals[2−8] indicated that the aromatic C-5′ bearing a hydroxyl group (as in the cases of macrocarpals) normally appeared at ca. δ_C 170 ppm, while the etherified aromatic C-5′ (as in the cases of euglobals) generally resonated at ca. δ_C 163 ppm. The relatively upfield shifted C-5′ at δ 163.1 and the heteroatom bearing C-5 resonated at δ 76.2, suggesting that an ether bridge was present between C-5′ and C-5 to form the C-ring, though no direct HMBC correlation between H-5 and C-5′ was observed. The gross structure of **1** was thus established as depicted.

The relative stereochemistry of **1** was established on the basis of the ROESY experiment (Figure 2). The strong ROESY correlations of H-6/H_3-15 and H-2α, and H_3-15/H-2α indicated that H-2α, H-6, and Me-15 adopted the axial bonds of the chair-conformational A-ring and were arbitrarily designated as the α-orientation. The ROESY correlations from H-5 to H-10′a, H-3β, and H-8β revealed that the

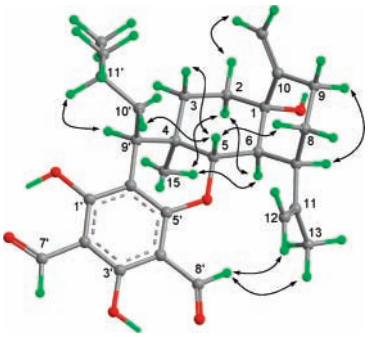


Figure 2. Selected ROESY (H↔H) correlations of **1**.

isobutyl group, H-3β, and H-8β were cofacial and β-oriented. In addition, the ROESY correlation between H-7 and H-9α placed H-7 at the α-configuration. The ROESY correlations from H-8′ to H_2-12 and H_3-13 supported the presence of the dihydropyran C-ring, which makes one of the formyl groups and the isopropenyl group approach each other in space. The above ROESY correlations also indicated the six-membered A-, B-, and C-rings of **1** were all in chair conformation, and the A-/B-rings were *cis*-fused and A-/C-rings were *trans*-fused. This conclusion was finally confirmed by the performance of a single-crystal X-ray diffraction of **1**.[10] The conformation of **1** in solution as established by ROESY spectrum is in good agreement with that in solid state as determined by X-ray study (Figure 3).

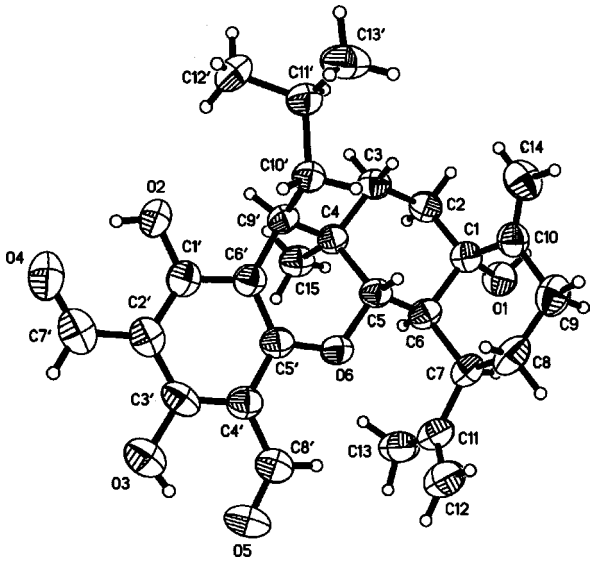


Figure 3. Single-crystal X-ray structure of **1**.

Compound **2**,[11] obtained as a yellow powder, had a molecular formula of $C_{28}H_{38}O_7$ as determined by HREIMS

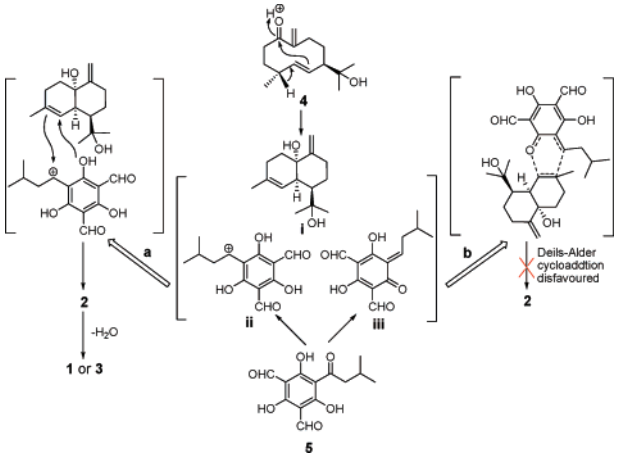
(10) Crystallographic data for eucalyptal A (**1**) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC-653049). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html.

at *m/z* 486.2602 $[M]^+$(calcd 486.2618) and its ^{13}C NMR data. The IR absorption bands at 3432 and 1627 cm^{-1} indicated the presence of hydroxyl and carbonyl functionalities. The UV absorption band at $\lambda_{max} = 276$ nm was suggestive of the presence of a 3,5-diformyl phloroglucinol chromophore.[4] The ^1H and ^{13}C NMR spectra of **2** (Table 1) bore a resemblance to those of **1**, with the notable differences being the absence of the proton and carbon resonances of an isopropenyl group and the presence of an oxygenated quaternary carbon (δ_C 71.4) and two tertiary methyls (δ_H 1.53 and 1.55; δ_C 32.1 and 28.5) that form a hydroxyisopropyl group. Further evidence came from the HMBC spectrum of **2** (Supporting Information), in which H_3-12 correlated with C-7, C-11 (δ_C 71.4) and C-13 to locate the hydroxyisopropyl group at C-7. Detailed 2D NMR analysis (HMBC, HMQC, and $^1H-^1H$ COSY) confirmed the planar structure of **2** as depicted. The relative configuration of **2** was assigned to be the same as that of **1** on the basis of 1D NMR data and the ROESY experiment in particular.

Compound **3**,[12] a yellow powder, exhibited a molecular formula of $C_{28}H_{36}O_6$ as determined by HREIMS at *m/z* 468.2518 $[M]^+$(calcd 468.2512), indicating that it was an isomer of **1**. The ^1H and ^{13}C NMR data of **3** showed high similarity to those of **1** except that the terminal Δ^{11} double bond of isopropenyl in **1** was migrated to form a tetrasubstituted exocyclic $\Delta^{7(11)}$ double bond in **3**. This was characterized by the presence of two olefinic quaternary carbons (at δ_C 128.0 and 127.0) and two allylic methyls [at δ_H 1.78 (3H, s) and 1.86 (3H, s); δ_C 20.9 and 20.7]. The structure of **3** was further confirmed by the HMBC spectrum, in which the HMBC correlations from Me-12 to C-7, C-11, and C-13 were consistent with this conclusion (Supporting Information). The stereochemistry of **3** was established to be the same as that of **1** from the ROESY spectrum.

As cadinane-type sesquiterpenoids have never been found in the genus of *Eucalyptus*, the biogenetic precursor of the key cadinane-type intermediate **i** was thus proposed to be litseagermacrane (**4**),[13] a coexisting major compound isolated in this study (Sheme 1). 4,6-Diformyl-2- isopentanoylphloroglucinol (**5**),[14] the most abundant compound in this genus, was reported to biosynthetically produce the intermediate **ii**[15] or **iii**.[16] The biogenetic pathways previously proposed for euglobals were based on the Diels−Alder cycloaddition of the intermediate **iii** (or its analogues) and the corresponging terpenoids.[8,16] If the intermediates **i** and **iii** adopted the Diels−Alder cycloaddition via route **b** (Scheme 1), it would afford

Scheme 1. Hypothetical Biogenetic Route of Compounds **1**−**3**



a stereoselective product that follows the cis rule. However, the trans-orientation of Me-15 toward H-5 in compounds **1**−**3** disfavored this route. A plausible biosynthetic pathway for compounds **1**−**3** could be therefore proposed through route **a** involving the carbocation-induced cyclization in a stepwise manner to form compound **2**, which would further transform into compound **1** or **3** by simple dehydrolyzition.

The in vitro cytotoxic activities of the eucalyptals A−C (**1**−**3**) were evaluated against HL-60 (human leukemia) and A-549 (human lung adenocarcinoma) tumor cell lines. Compounds **1**−**3** showed selective activity against HL-60 with IC_{50} values of 1.7, 6.8, and 17 μM, respectively.

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Supporting Information Available: Experimental procedures, physical and spectral data of **1**−**3**, and crystallographic data of eucalyptal A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) **Eucalyptal B (2).** Yellow powder; $[\alpha]^{20}_D$ −92.0 (*c* 0.100, $CHCl_3$); UV (CH_3OH) λ_{max} (log ϵ) 276 (4.84) nm; IR (KBr) ν_{max} 3432, 2952, 1627, 1438, 1305, 1143, 1191, 1056, 864, 609 cm^{-1}; ^1H and ^{13}C NMR see Table 1; EIMS *m/z* 486 $[M]^+$ (10), 468 (23), 450 (10), 429 (26), 411 (89), 393 (21), 251 (19), 249 (20), 209 (57), 195 (98), 159 (37), 91 (29), 84 (100), 71 (37), 57 (55), 56 (73); HREIMS *m/z* 486.2602 (calcd for $C_{28}H_{38}O_7$, 486.2618).

(12) **Eucalyptal C (3).** Yellow powder; $[\alpha]^{20}_D$ +145.0 (*c* 0.190, $CHCl_3$); UV (CH_3OH) λ_{max} (log ϵ) 278 (4.47) nm; IR (KBr) ν_{max} 3453, 2927, 1637, 1448, 1382, 1307, 1197, 846 cm^{-1}; ^1H and ^{13}C NMR see Table 1; EIMS *m/z* 468 $[M]^+$ (33), 450 (9), 411 (100), 393 (25), 345 (10), 289 (14), 251 (13), 233 (12), 209 (32), 195 (50), 185 (18), 149 (11), 109 (11), 91 (12), 69 (11), 57 (14); HREIMS *m/z* 468.2518 (calcd for $C_{28}H_{36}O_6$, 468.2512).

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