Euglobal-In-1, a New Euglobal from *Eucalyptus incrassata*

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From the juvenile leaves of *Eucalyptus incrassata*, a new euglobal having an acylphloroglucinol-sesquiterpene structure, euglobal-In-1 (1), has been isolated along with the known euglobal-III (2) and -V (3). The structure and stereochemistry of 1 were established by spectroscopic methods.

Keywords *Eucalyptus incrassata*; euglobal-In-1; phloroglucinol-sesquiterpene; Myrtaceae; euglobal-III; euglobal-V

As a part of our cotinuing chemical studies on euglobals[1] that have unique acylphloroglucinol-monoterpene (or -sesquiterpene) structures and biological studies on the potential anti-tumor-promoting activities of natural products,[2] we have investigated *Eucalyptus incrassata* LABILL (Myrtaceae). In the previous paper, we reported the isolation and structural elucidation of twelve euglobals from *E. globulus*,[1] three euglobals from *E. grandis*[3] and two euglobals from *E. tereticornis*[4]; we also studied the inhibitory effects of these euglobals and related compounds on Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[5]

According to the analysis by LC/atmospheric pressure ionization (API)-MS,[6] it was confirmed that more than five euglobals having sesquiterpene skeletons were existent in the juvenile leaves of *E. incrassata*.

In this paper, we report the isolation and structural elucidation of a new euglobal, euglobal-In-1 (**1**), and two known euglobals (**2**, **3**) from the juvenile leaves of *E. incrassata*. Compounds **2** and **3** were identified by comparison with authentic samples of euglobal-III and -V, respectively, obtained from *E. globulus*[1] and also by their HPLC and TLC behavior, IR and ^{1}H-NMR spectra.

Compound **1** has the same composition, $C_{28}H_{38}O_5$ (M^+, 454), as compounds **2** and **3**, and exhibited UV, IR and MS spectral data similar to those of reported euglobals that have sesquiterpene structures.[1] In addition, the ^{13}C-NMR spectrum and the distortionless enhancement by polarization transfer (DEPT) experiments on **1** showed the presence of two aldehyde carbons (δ 191.86, 191.88), two olefinic carbons (δ 123.31, 143.56), two quarternary carbons (δ 45.38, 53.93), five methine carbons (δ 25.86, 31.78, 35.08, 36.71, 96.03), five methylene (δ 21.85, 25.98, 34.08, 39.08, 41.09) and six methyl carbons (δ 14.58, 21.42, 21.82, 21.87, 24.46, 28.38) together with six phenyl carbons. The structural elucidation of euglobal-In-1 (**1**) was achieved using 2D-NMR spectra and difference nuclear Overhauser effect (NOE) experiments as follows. The ^{1}H–^{13}C long range correlation spectroscopy (COSY) of **1** was measured in order to confirm the connectivities of the sesquiterpene moiety and substituent groups on the phloroglucinol part as shown in Fig. 1. The methine proton at δ 2.89 (7-H) is correlated with the carbons at δ 45.38 (C-4′), 96.03 (C-5′), 108.52 (C-1) and δ 164.56 (C-2). The methylene protons at 1.51 and 1.76 (3′-H_2) are correlated with the carbons at δ 96.03 (C-5′) and 53.93 (C-1′). The methine proton at δ 4.39 (5′-H) is correlated with the carbons at δ 28.38 (C-15′), 34.99 (C-2′), 39.08 (C-3′) and 164.56 (C-2). In addition, the methyl protons at δ 1.36 (15′-Me) are correlated with the carbons at δ 36.71 (C-7), 39.08 (C-3′) and 96.03 (C-5′). The quarternary carbon at δ 53.93 (C-1′) is correlated with the olefinic proton at δ 5.20 (6′-H) and the methyl protons at δ 0.74 (14′-Me), together with the methylene protons at δ 1.76 (3′-H_2). The phenyl carbons at δ 168.17 (C-4) and 168.46 (C-6) are correlated with the aldehyde protons at δ 10.06 (8-CHO) and 10.19 (9-CHO), respectively. Some other significant long-range ^{1}H–^{13}C correlations are indicated by arrows in Fig. 1. From these results on **1** and by reference to chemical shift values of **2** and **3**,[7] the positions of two aldehydes and the isobutyl and isopropyl groups of euglobal-In-1 were concluded to be located at C-3, C-5, C-7 and C-7′, respectively. All proton and carbon signals of euglobal-In-1 (**1**) could be assigned by ^{1}H–^{1}H COSY, DEPT experiments, ^{1}H–^{13}C COSY and ^{1}H–^{13}C long range COSY spectra, as shown in Table I.

In addition, difference NOE experiments (in $CDCl_3$)

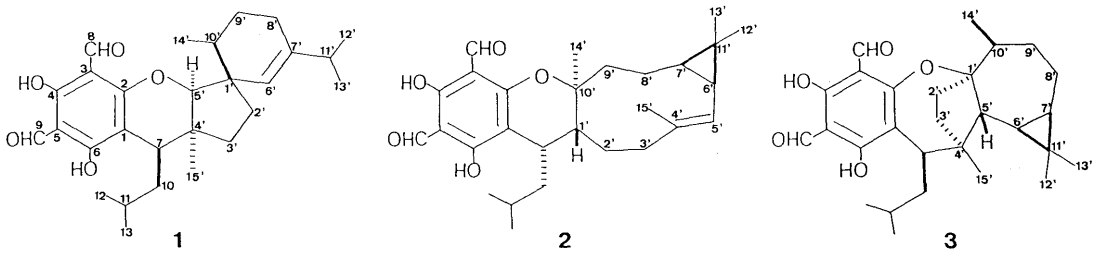

Chart 1

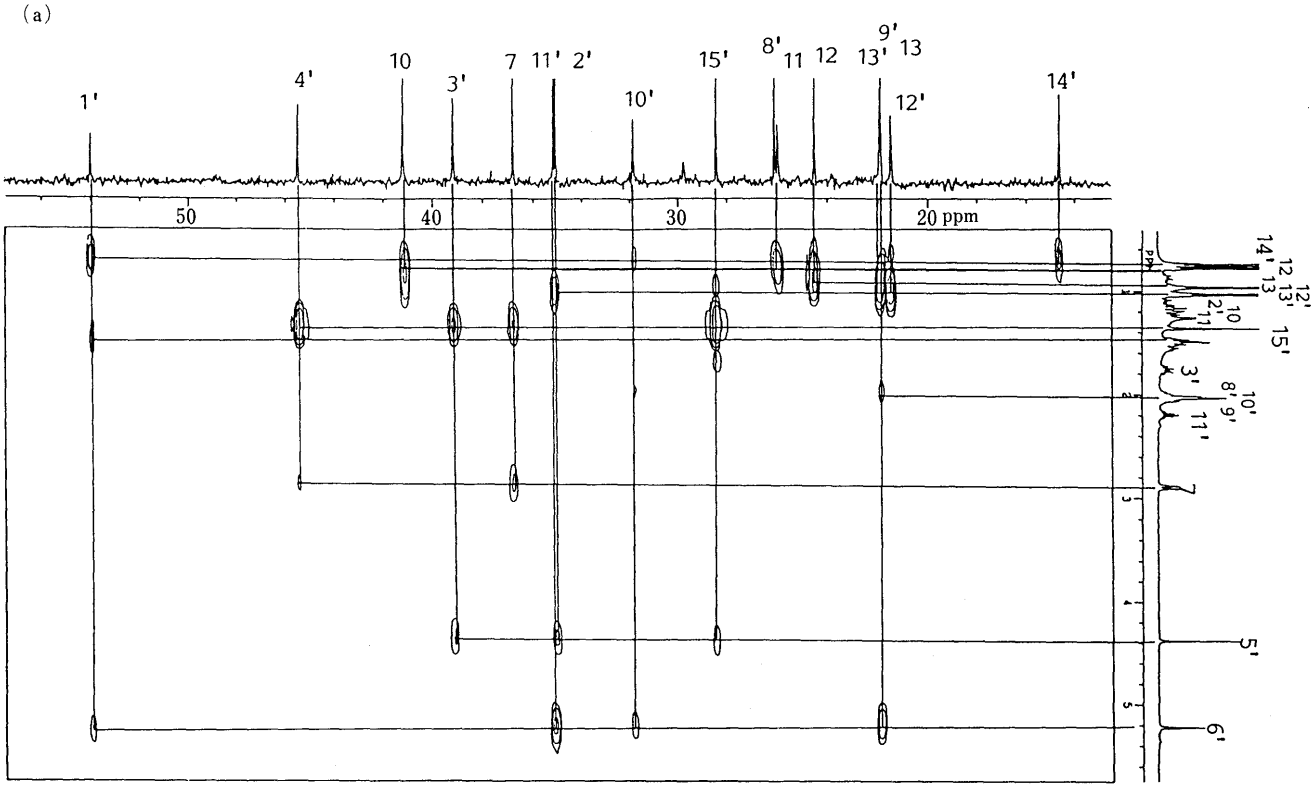

Fig. 1a. Part of the ^{1}H–^{13}C Long Range COSY Spectrum of **1** (in $CDCl_3$)

Fig. 1b. Correlation ($^{13}C \curvearrowright {}^{1}H$) in the ^{1}H–^{13}C Long Range COSY Spectrum of **1**

on **1** were performed in order to confirm the relative stereochemistry. Irradiation of the signal of the methine proton (5′-H) enhanced the signal intensities of the 15′-methyl protons, 6′-H and 10′-H. Irradiation of the signal of the 15′-methyl protons enhanced the signal intensities of 7-H, 5′-H, 6′-H and one of the methylene protons (3′α-H). Irradiation of the signal of 3′α-H enhanced the signal intensities of 5′-H, 15′-methyl protons and olefinic proton (6′-H). From these NOEs, it was concluded that the 5′-H, 15′-Me and C1′–C6′ bond were located on the α-side of the cyclopentane ring in compound **1** as shown in Fig. 2. Irradiation of the signal of the methine proton (10′-H) enhanced the signal intensities of the 5′-H, aldehyde proton (8-CHO) and one of the methylene protons (8′α-H). Some other significant difference NOE results are indicated by arrows in Fig. 2. From these difference NOE results of **1** and from a study of Dreiding models, the structure and relative configuration of euglobal-In-1 were assigned as **1**, exclusive of the absolute configuration.

Of these three compounds, **1** and **3** exhibited remarkable inhibitory effects on EBV-EA activation (more than 80% inhibition at a 1×10^3 mol ratio of compound/TPA and more than 40% inhibition at a 5×10^2 mol ratio of compound/TPA) and **2** exhibited significant inhibitory effects on EBV-EA activation (100% and more than 70% inhibition at 1×10^3 and 5×10^2 mol ratios of compound/TPA, respectively).[2] These results suggest that compound **2** would be valuable as an anti-tumor-promoter in carcinogenesis and two-stage carcinogenesis testing *in vivo* of **2** is now in progress.

Experimental

General Experimental Procedures UV spectra were obtained on a Shimadzu 210-A spectrophotometer in 95% EtOH, and IR spectra were measured on a Shimadzu IR-408 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian XL-300 spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. 2D-NMR and difference NOE spectra were recorded on a JEOL JNM GX-400 spectrometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter at 28 °C. MS were determined on a Hitachi M-80

TABLE I. ^1H- and ^{13}C-Chemical Shift Values of Compounds **1** and **3** (in $CDCl_3$)

	Euglobal-In-1 (**1**)			Euglobal-V (**3**)	
	^{13}C	^1H		^{13}C	^1H
1	108.52		1	116.77	
2	164.56		2	167.69	
3	104.56		3	108.84	
4	168.17	13.33 (4-OH)	4	167.75	13.56 (4-OH)
5	103.93		5	104.58	
6	168.46	13.12 (6-OH)	6	169.96	13.38 (6-OH)
7	36.71	2.89 (1H, dd, J=3.3, 11.5 Hz)	7	42.89	3.27 (1H, m)
8	191.88	10.06	8	193.46	10.24
9	191.86	10.19	9	192.11	10.21
10	41.09	1.17 (1H, m), 1.46 (1H, m)	10	43.20	1.13—1.18 (2H, m)
11	25.86	1.24 (1H, m),	11	25.73	1.18—1.24 (1H, m)
12	24.46	0.77 (3H, d, J=6.3 Hz)	12[a]	21.52	0.94 (3H, d, J=5.7 Hz)
13	21.82	0.96 (3H, d, J=6.3 Hz)	13[a]	24.63	0.75 (3H, d, J=5.7 Hz)
1′	53.93		1′	105.32	
2′	34.99	1.13 (1H, m), 1.47 (1H, m)	2′	36.03	1.68—1.75 (2H, m)
3′	39.08	β1.51 (1H, m), α1.76 (1H, m)	3′	37.55	1.55—1.68 (2H, m)
4′	45.38		4′	47.08	
5′	96.03	4.39 (1H, s)	5′	49.14	2.13 (1H, d, J=12.0 Hz)
6′	123.31	5.20 (1H, br s)	6′	26.27	0.33 (1H, dd, J=8.8, 12.0 Hz)
7′	143.56		7′	23.73	0.66 (1H, ddd, J=5.6, 8.8, 12.0 Hz)
8′	25.98	α1.56 (1H, m), β2.00 (1H, m)	8′	20.23	α1.82—1.92 (1H, m), β1.30—1.49 (1H, m)
9′	21.85	2.00 (2H, m)	9′	29.79	1.78—1.85 (2H, m)
10′	31.78	2.00 (1H, m)	10′	40.18	2.47 (1H, m)
11′	35.08	2.19 (1H, spt, J=6.9 Hz)	11′	19.48	
12′	21.42[a]	1.03 (6H, d, J=6.9 Hz)	12′[b]	28.66	1.08 (3H, s)
13′	21.87[a]		13′[b]	15.78	1.09 (3H, s)
14′	14.58	0.74 (3H, d, J=7.9 Hz)	14′	17.31	1.00 (3H, d, J=6.9 Hz)
15′	28.38	1.36 (3H, s)	15′	24.00	1.20 (3H, s)

a, b) Assignments with the same superscripts in each column may be interchangeable.

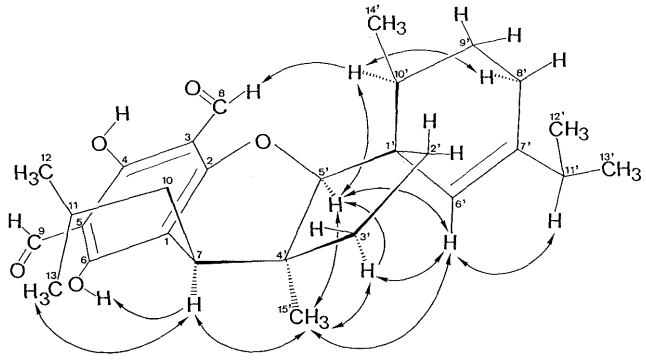

Fig. 2. Significant Enhancement of Signal Intensity by NOE Experiments of **1**

mass spectrometer. Preparative HPLC was carried out on a Japan Analytical Industry LC-09 with a reversed-phase [JAIGEL-ODS, S-343-15 (20 × 250 mm)] column using CH_3CN (5.0 ml/min) as eluent. Pre-coated silica gel plates (Kieselgel 60 F254, 0.25 mm, Merck) were used for analytical TLC and euglobals were detected under UV light (365 nm) and by spraying with 10% H_2SO_4 solution containing anisaldehyde, followed by heating. LC/API-MS was measured on a Hitachi LC/MS system (M-1000 LC-API, L-6200) using a reversed-phase column [4.6 × 150 mm, solvent: MeOH–AcOH–H_2O (100 : 5 : 3), flow rate: 1 ml/min] with a UV (280 nm) detector.[8]

Plant Material The juvenile leaves of *E. incrassata* were collected in Australia in May 1991. A voucher specimen was deposited at the Herbarium of Kyoto Pharmaceutical University.

Extraction and Isolation The air-dried juvenile leaves (64 g) of *E. incrassata* were extracted with $CHCl_3$ at room temperature, and the $CHCl_3$ extract was evaporated *in vacuo* to give a dark green tar (6.49 g). The residue was chromatographed on silica gel with C_6H_6 followed by C_6H_6–$CHCl_3$ (1 : 1) to yield a crude euglobal fraction (907 mg). The fraction was rechromatographed on ODS using preparative HPLC to give five fractions (A—E), and each fraction was purified by recycle preparative HPLC. From fraction A, euglobal-III (**2**, 45.6 mg) was isolated and a new euglobal (euglobal-In-1, **1**, 24.8 mg) was isolated together with euglobal-V (**3**, 103.8 mg) from fraction D.

Euglobal-In-1 (**1**): Colourless oil, $[\alpha]_D$ −32.3° (c=0.8, $CHCl_3$). UV λ_{max} nm (ε): 277 (24600), 343 (3300). IR ($CHCl_3$) cm^{-1}: 3500, 2950, 1625, 1440, 1300, 1180. EI-MS *m/z*: 454 (M^+, $C_{28}H_{38}O_5$), 411 ($M^+-C_3H_7$), 397, 251 ($M^+-C_{15}H_{23}$), 203, 195, 163 (base). HR-MS: Calcd for $C_{28}H_{38}O_5$: 454.2717. Found: 454.2744. ^1H- and ^{13}C-NMR: Given in Table I.

Compound **2**: Colourless needles, mp 169—171 °C (from $CHCl_3$), $[\alpha]_D$ +190.9° (c=0.45, $CHCl_3$), was directly identified by comparison with an authentic sample of euglobal-III (HPLC behavior and IR, ^1H- and ^{13}C-NMR spectra).[1]

Compound **3**: Colourless prisms, mp 184—185 °C (from $CHCl_3$), $[\alpha]_D$ −294.7° (c=1.0, $CHCl_3$), was directly identified by comparison with an authentic sample of euglobal-V (HPLC behavior and IR, ^1H- and ^{13}C-NMR spectra).[1] ^1H- and ^{13}C-NMR: Given in Table I.[7]

References and Notes

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7) Although the ^{1}H- and ^{13}C-NMR signal assignments of euglobal-V (**3**) had been reported in ref. 1, their signals could be newly assigned on the bases of 2D-NMR spectra of **3**, and some signal assignments were revised as shown in Table I in this report.
8) LC/API-MS were measured under following conditions. (Nebrizer temp.: 250 °C, Desolvation temp.: 399 °C and drift voltage: 50 V).