Four Euglobals from *Eucalyptus blakelyi*

Midori Takasaki,*[,a] Takao Konoshima,[a] Mutsuo Kozuka,[a] Mitsumasa Haruna,[b] Kazuo Ito,[b] and Shigeo Yoshida[c]

Kyoto Pharmaceutical University,[a] Misasagi, Yamashina-ku, Kyoto 607, Japan, Faculty of Pharmacy, Meijo University,[b] Yagoto, Tempaku-ku, Nagoya 468, Japan, and The Institute of Physical and Chemical Research,[c] Hirosawa, Wako 351, Japan. Received April 26, 1994; accepted June 20, 1994

From the juvenile leaves of *Eucalyptus blakelyi*, a new euglobal, euglobal-Bl-1 (1), having a phloroglucinol-monoterpene structure, has been isolated along with the known euglobals-Ib (2), -Ic (3) and -IIa (4). The structure and stereochemistry of euglobal-Bl-1 (1) and the stereochemistry of euglobal-Ib (2) were established by spectroscopic evidence.

Keywords *Eucalyptus blakelyi*; euglobal-Bl-1; phloroglucinol-monoterpene; Myrtaceae; eublobal-Ib; eublobal-Ic

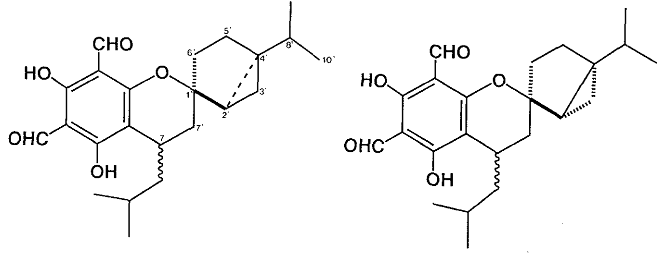
As a part of our continuing chemical studies on euglobals[1)] that have unique acylphloroglucinol-monoterpene (or -sesquiterpene) structures and biological studies on the potential anti-tumor-promoting activities (cancer chemopreventive activities) of natural products,[2)] we have investigated the constituents of the juvenile leaves of *Eucalyptus blakelyi* Maiden (Myrtaceae). We previously reported the isolation and structural elucidation of twelve euglobals from *E. globulus*,[3)] three from *E. grandis*,[4)] two from *E. tereticornis*[5)] and three from *E. incrassata*,[1)] and the inhibitory effects of these euglobals and related compounds on Epstein–Barr virus early antigen (EBV-EA) activation as a primary indicator for anti-tumor-promoting activity.[6)]

Analysis by LC/atmospheric pressure ionization (API)-MS[7)] confirmed that more than four euglobals having monoterpene structure existed in the juvenile leaves of *E. blakelyi*.

In this paper, we report the isolation and structural elucidation of a new euglobal, euglobal-Bl-1 (**1**), and three known euglobals (**2**, **3**, and **4**) from the juvenile leaves of *E. blakelyi*. Compounds **3** and **4** were identified with authentic samples of euglobal-Ic and -IIa, respectively, obtained from *E. globulus* by their HPLC and TLC behavior, and IR and ^{1}H-NMR spectra.[3)] Their structure and stereochemistry were reconfirmed on the basis of 2D-NMR spectra and difference nuclear Overhauser effect (NOE) experiments.

Compound **1** has the same composition, $C_{23}H_{30}O_5$ (MS [M^+] 386), as compounds **2**, **3** and **4**, and showed UV, IR and MS data similar to those of reported euglobals that have monoterpene skeleton, especially euglobal-Ib (**2**). The structural elucidation of euglobal-Bl-1 and the determination of stereochemistry of euglobal-Ib were carried out using 2D-NMR spectra and difference NOE experiments as follows.[8)] The ^{1}H–^{13}C long range correlation spectroscopy (COSY) of euglobal-Bl-1 was measured in order to confirm the connectivities of the partial structure and substituent groups. As shown in Fig. 1, the methylene protons at δ 2.13 and 1.90 (7′-H_2) are correlated with the carbons at δ 31.91 (C-6′) and 105.91 (C-1). The methylene protons at δ 0.85 (3′-H_2) and δ 1.73 (5′-H_2) are correlated with the carbon at δ 88.42 (C-1′). The methylene protons at δ 1.73 (5′-H_2) are correlated with the carbon at δ 11.65 (C-3′). One of the methylene protons (10-H_2) at δ 2.04 is correlated with the carbon at δ 36.41 (C-7′). Some other significant long-range ^{1}H–^{13}C correlations are indicated by arrows in Fig. 1. From these results, the positions of two aldehydes, isobutyl and isopropyl groups of euglobal-Bl-1 (**1**) were concluded to be at C-3, C-5, C-7 and C-4′, respectively, as the same as euglobals-Ib (**2**), -Ic (**3**) and -IIa (**4**). All proton and carbon signals of **1** and **2** could be assigned as shown in Table I on the basis of their distortionless enchancement by polarization transfer (DEPT), ^{1}H–^{13}C COSY, and ^{1}H–^{13}C long range COSY spectra.

The relative stereochemistries of **1** and **2** were determined and those of **3** and **4** were reconfirmed by difference NOE experiments. On compound **1**, the NOEs were observed between 7′β-H (δ 1.90) and 2′-H (δ 1.31), one of the methylene protons (10-H_2) at δ 2.04 and 2′-H, and another of these (10-H_2) at δ 1.28 and 7′β-H, re-



7β-isobutyl : euglobal-Bl-1 (**1**)
7α-isobutyl : euglobal-Ib (**2**)

7β-isobutyl : euglobal-Ic (**3**)
7α-isobutyl : euglobal-IIa (**4**)

Chart 1

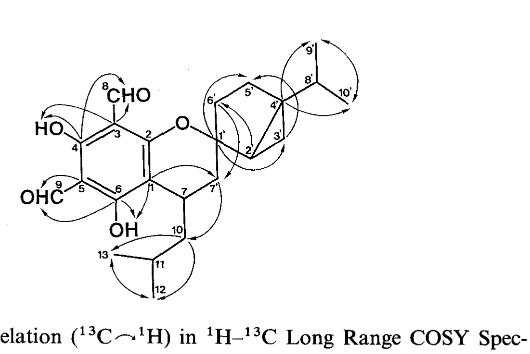


Fig. 1. Correlation (^{13}C⌒^{1}H) in ^{1}H–^{13}C Long Range COSY Spectrum of **1**

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Table I. ^1H and ^{13}C Chemical Shift Values of Compounds **1** and **2** (in $CDCl_3$)

	Euglobal-Bl-1 (**1**)			Euglobal-Ib (**2**)	
	^{13}C	^1H		^{13}C	^1H
1	105.91		1	106.33	
2	164.92		2	165.47	
3	104.64		3	104.67	
4	167.93	13.43 (4-OH)	4	167.91	13.41 (4-OH)
5	104.04		5	104.17	
6	169.69	13.47 (6-OH)	6	169.77	13.52 (6-OH)
7	25.92	2.97 (1H, m)	7	25.96	3.09 (1H, m)
8[a]	192.39	10.05	8[a]	192.41	10.03
9[a]	191.78	10.14	9[a]	191.84	10.16
10	43.14	2.04 (1H, ddd, $J=13.4$, 10.3, 3.2 Hz)	10	43.05	2.15 (1H, ddd, $J=13.7$, 10.4, 3.5 Hz)
		1.28 (1H, m)			1.15 (1H, ddd, $J=13.7$, 10.4, 4.0 Hz)
11	25.59	1.77 (1H, m)	11	25.46	1.75 (1H, m)
12[b]	24.04	0.93 (3H, d, $J=6.6$ Hz)	12[b]	24.08	0.90 (3H, d, $J=6.5$ Hz)
13[b]	21.04	0.99 (3H, d, $J=6.6$ Hz)	13[b]	20.85	0.98 (3H, d, $J=6.5$ Hz)
1′	88.42		1′	88.59	
2′	30.66	1.31 (1H, dd, $J=8.0$, 3.1 Hz)	2′	28.15	1.21 (1H, dd, $J=8.0$, 3.7 Hz)
3′	11.65	α0.85 (1H, dd, $J=5.2$, 3.1 Hz)	3′	12.34	β0.76 (1H, dd, $J=5.2$, 3.7 Hz)
		β0.51 (1H, dd, $J=8.0$, 5.2 Hz)			α0.38 (1H, dd, $J=8.0$, 5.2 Hz)
4′	33.64		4′	34.37	
5′	25.29	1.73 (2H, m)	5′	24.20	
6′	31.91	1.36 (1H, m), 1.70 (1H, m)	6′	33.91	1.70—1.80 (4H, m)[d]
7′	36.41	α2.13 (1H, dd, $J=14.3$, 7.6 Hz)	7′	38.04	α1.71 (1H, dd, $J=14.0$, 9.6 Hz)
		β1.90 (1H, dd, $J=14.3$, 7.6 Hz)			β2.23 (1H, dd, $J=14.0$, 7.6 Hz)
8′	32.35	1.44 (1H, m)	8′	32.60	1.30 (1H, q, $J=7.0$ Hz)
9′[c]	19.64	0.97 (3H, d, $J=6.8$ Hz)	9′[c]	19.57	0.94 (3H, d, $J=6.8$ Hz)
10′[c]	19.54	0.90 (3H, d, $J=6.8$ Hz)	10′[c]	19.50	0.93 (3H, d, $J=6.8$ Hz)

a—c) Assignments are interchangeable among or signals with the same superscript in any one spectrum. *d*) These signals are overlapping other signals.

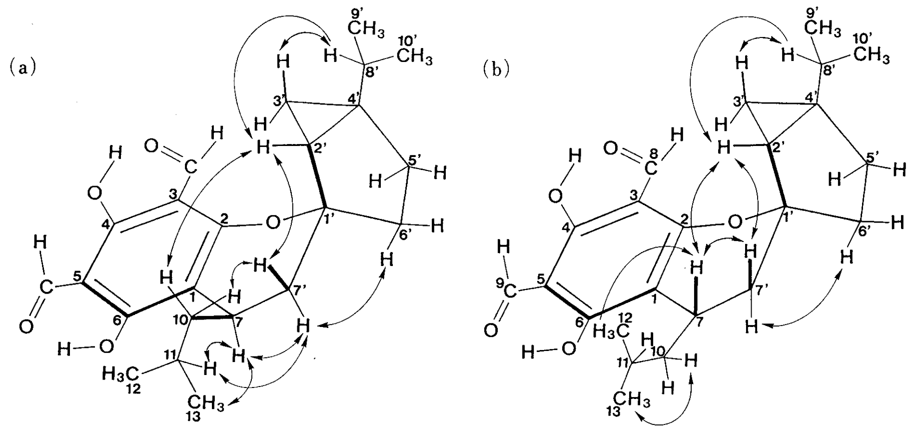

Fig. 2. Significant Enhancement of Signal Intensity by NOE Experiments of **1**(a) and **2**(b)

spectively. The NOEs were also observed between 7′α-H (δ 2.13) and 7-H (δ 2.97), 11-H (δ 1.77) and 7-H, the methyl protons at δ 0.99 (12 or 13-H_3) and 7-H, and between 7′α-H and 11-H, respectively. From these results, it was concluded that the 7′β-H, C1′-C2′ bond and isobutyl moiety (C7-C10 bond) were located at the β-side of the dihydropyrane ring on euglobal-Bl-1 (**1**) as shown in Fig. 2a.

In the difference NOE experiments of euglobal-Ib (**2**), on the other hand, the NOEs were observed between 2′-H (δ 1.21) and 7′β-H (δ 2.23), 2′-H and 7-H (δ 3.09), and 7′β-H and 7-H, respectively. Therefore, it was concluded that 7-H, one of the methylene protons at δ 2.23 and C1′-C2′ bond were located at the β-side of the dihydropyrane ring on euglobal-Ib (**2**) as shown in Fig. 2b. On compound **4**, the NOEs were observed between 3′-H and 7′β-H and between 2′-H and 7-H. Some other significant NOEs are indicated by arrows in Fig. 3. In the case of **3**, the NOE between 3′-H and 7′β-H was also observed as on compound **4**. Furthermore, from the facts that our compounds (**1**—**4**) have small $[\alpha]_D$ values and each of the euglobals (**3**, **4**) had been obtained as a racemate from *E. globulus*,[1] each compound (**1**—**4**) in this report is also presumably in each racemic mixture. From these difference NOE results of **1**—**4**, and from consideration of the Dreiding models, the structures and relative configurations

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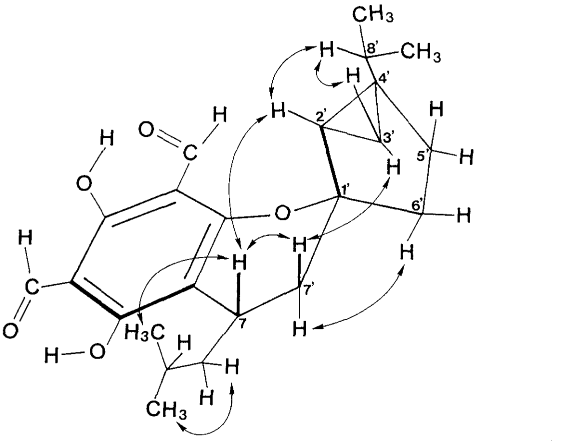

Fig. 3. Significant Enhancement of Signal Intensity by NOE Experiments of **4**

of euglobals-Bl-1 and -Ib were assigned as **1** and **2** as each racemate.

In these four compounds, **1** and **2** showed strong inhibitory effects on EBV-EA activation (more than 70—80% inhibition at 1×10^3 mol ratio of compound/TPA and about 50% inhibition at 1×10^2 mol ratio), and **3** and **4** exhibited remarkable inhibitory effects on EBV-EA activation (more than 80% inhibition at 1×10^3 mol ratio of compound/TPA and 20—30% inhibition at 1×10^2 mol ratio).

Experimental

General Experimental Procedure 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 27°C. MS were determined on a Hitachi M-80 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 an eluent. Pre-coated Si gel plates (Kieselgel 60 F254, 0.25 mm, Merck) were used for analytical TLC, euglobals were detected under UV lamp (365 nm) and sprayed 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 UV (280 nm) detector.[9]

Plant Material The juvenile leaves of *E. blakelyi* were collected in Australia in 1990, and a voucher specimen was deposit at the Herbarium of Kyoto Pharmaceutical University.

Extraction and Isolation The air-dried juvenile leaves (270 g) of *E. blakelyi* were extracted with $CHCl_3$ at room temperature, and the $CHCl_3$ solution was evaporated *in vacuo* to afford a dark residue (15.44 g). The residue was applied to column chromatography on Si gel with C_6H_6 followed by C_6H_6–$CHCl_3$ (1:1) to yield a crude euglobal fraction (1.15 g). The fraction was rechromatographed on ODS by preparative HPLC to yield three fractions (A—C), and each fraction was purified by recycle preparative HPLC. From fraction A, euglobal-Bl-1 (**1**, 30.8 mg) was isolated together with euglobal-Ib (**2**, 23.1 mg). From fraction B, euglobal-Ic (**3**, 47.0 mg) was isolated, and euglobal-IIa (**4**, 17.9 mg) was isolated from fraction C.

Euglobal-Bl-1 (**1**): Colorless prisms, mp 98—100 °C (from $CHCl_3$), $[\alpha]_D$ +11.3° (c=0.985, $CHCl_3$). UV λ_{max} nm (ε): 277 (36000), 340 (4300), IR ($CHCl_3$) cm^{-1}: 3450, 2950, 1620, 1435, 1300, 1175. EI-MS *m/z*: 386 (M^+, $C_{23}H_{30}O_5$), 251 ($M^+ - C_{10}H_{15}$, base), 195 ($C_9H_7O_5$). *Anal.* Calcd for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.27; H, 8.12. ^{1}H- and ^{13}C-NMR: Given in Table I.

Compound (**2**): Colorless needles, mp 118—120 °C (from $CHCl_3$), $[\alpha]_D$ +2.27 (c=0.65, $CHCl_3$), was directly identified by comparison with an authentic sample of euglobal-Ib (HPLC behavior and IR, ^{1}H- and ^{13}C-NMR spectra).[3] ^{1}H- and ^{13}C-NMR: Given in Table I.

Compound (**3**): Colorless prisms, mp 105—107 °C (from $CHCl_3$), $[\alpha]_D$ +1.98° (c=0.60, $CHCl_3$), was directly identified by comparison with an authentic sample of euglobal-Ic (HPLC behavior and IR, ^{1}H- and ^{13}C-NMR spectra).[3]

Compound (**4**): Colorless prisms, mp 115—117 °C (from $CHCl_3$), $[\alpha]_D$ +26.7° (c=0.71, $CHCl_3$), was directly identified by comparison with an authentic sample of euglobal-IIa (HPLC behavior and IR, ^{1}H- and ^{13}C-NMR spectra).[3]

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8) In the previous paper,[3] the relative stereochemistry at C-7 of euglobal-Ib (**2**) had not been confirmed.
9) LC/API-MS were measured under the following conditions: Nebrizer temp.: 250°C, desolvation temp.: 399 °C and drift voltage: 50 V.