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 I was established by spectroscopic methods.

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

As a part of our continuing chemical studies on euglobals that have unique acylphloroglucinol-mono-terpene (or -sesquiterpene) structures and biological studies on the potential anti-tumor-promoting activities of natural products, we have investigated *Eucalyptus incrassata* Labill. (Myrtaceae). In the previous paper, we reported the isolation and structural elucidation of twelve euglobals from *E. globulus*, three euglobals from *E. grandis*; and two euglobals from *E. tereticornis*; 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).

According to the analysis by LC/atmospheric pressure ionization (API-MS), 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 (I), 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* and also by their HPLC and TLC behavior, IR and 1H-NMR spectra.

Compound 1 has the same composition, C_{28}H_{38}O_{5} (M^+, 545), as compounds 2 and 3, and exhibited UV, IR and MS spectral data similar to those of reported euglobals that have sesquiterpene structures. In addition, the 13C-NMR spectrum and the distortionless enhancement by polarization transfer (DEPT) experiments on I 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 (I) was achieved using 2D-NMR spectra and difference nuclear Overhauser effect (NOE) experiments as follows. The 1H-13C long range correlation spectroscopy (COSY) of I 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'-H2) 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 methylene protons at δ 0.74 (14'-Me), together with the methylene protons at δ 1.76 (3'-H2). 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 1H-13C correlations are indicated by arrows in Fig. 1. From these results on I and by reference to chemical shift values of 2 and 3, 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 (I) could be assigned by 1H-1H COSY, DEPT experiments, 1H-13C COSY and 1H-13C long range COSY spectra, as shown in Table I.

In addition, difference NOE experiments (in CDCl3)
Fig. 1a. Part of the $^1$H-$^{13}$C Long Range COSY Spectrum of I (in CDCl$_3$)
Fig. 1b. Correlation ($^{13}$C $^1$H) in the $^1$H-$^{13}$C Long Range COSY Spectrum of I

on I 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'z-H). Irradiation of the signal of 3'z-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 $\alpha$-side of the cyclopetanone ring in compound I 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'z-H). Some other significant difference NOE results are indicated by arrows in Fig. 2. From these difference NOE results of I and from a study of Dreiding models, the structure and relative configuration of euglobal-In-I were assigned as I, exclusive of the absolute configuration.

Of these three compounds, I and 3 exhibited remarkable inhibitory effects on EBV-EA activation (more than 80% inhibition at a $1 \times 10^{-3}$ mol ratio of compound/TPA and more than 40% inhibition at a $5 \times 10^{-2}$ mol ratio of compound/TPA) and 2 exhibited significant inhibitory effects on EBV-EA activation (100% and more than 70% inhibition at $1 \times 10^{-3}$ and $5 \times 10^{-2}$ mol ratios of compound/TPA, respectively). 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
mass spectrometer. Preparative HPLC was carried out on a Japan Analytical Industry LC-09 with a reversed-phase [JAI-EL-ODS, S-343-15 (20 x 250 mm)] column using CH$_2$CN (5.0 ml/min) as eluent. Pre-coated silica gel plates (Kieselgel 60 F254, 0.25 mm, Merck) were used for analytical TLC and tigucalbs were detected under UV light (365 nm) and by spraying with 10% H$_2$SO$_4$ 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 x 150 mm, solvent: MeOH/ACOH-H$_2$O (100:5:3), flow rate: 1 ml/min] with a UV (280 nm) detector.  

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$_6$H$_6$ followed by C$_6$H$_6$-CHCl$_3$ (1:1) to yield a crude euglobal fraction (907 mg). The fraction was recrystomographed 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, [α]$_D$ = −32.3° (c = 0.8, CHCl$_3$), UV$_{max}$ (nm): 277 (24900), 343 (3300). IR (CHCl$_3$ cm$^{-1}$): 3500, 2950, 1625, 1420, 1330, 1180. EI-MS m/z: 454 (M$^+$, C$_{22}$H$_{16}$O$_7$), 411 (M$^+$ − C$_3$H$_7$), 397, 251 (M$^+$ − C$_{19}$H$_{12}$), 203, 195, 163 (base). HR-MS: Calcd for C$_{24}$H$_{16}$O$_7$: 454.2717. Found: 454.2744. 1H- and 13C-NMR: Given in Table I.

Compound 2: Colourless needles, mp 169–171 °C (from CHCl$_3$), [α]$_D$ +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 13C-NMR spectra).  

Compound 3: Colourless prisms, mp 184–185 °C (from CHCl$_3$), [α]$_D$ = −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 13C-NMR spectra).  

References and Notes


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).