# Structures of Euglobals-G1, -G2, -G3, -G4, and -G5 from *Eucalyptus grandis* [1)]

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**Five new euglobals with acylphloroglucinol-monoterpene structures ($C_{23}H_{30}O_5$), named euglobals-G1 (1), -G2 (2), -G3 (3), -G4 (4), and -G5 (5) were isolated from the chloroform extract of the juvenile leaves of *Eucalyptus grandis* W. HILL (Myrtaceae). The structures and relative stereochemistries of 1—5 have been elucidated on the basis of their two-dimensional (2D)-NMR spectra and difference in nuclear Overhauser effects (NOE) experiments.**

**Keywords** *Eucalyptus grandis*; Myrtaceae; euglobal; phloroglucinol-monoterpene; two-dimensional-NMR

As a part of our continuing chemical studies on euglobals[2)] which have unique acylphloroglucinol-monoterpene (or -sesquiterpene) structures, and our biological studies on the potential anti-tumor-promoting activities of natural products,[3)] we have investigated euglobals from the leaves of *Eucalyptus grandis* W. HILL (Myrtaceae).

In the previous papers, we reported the isolation and structural elucidation of 12 euglobals from *E. globulus*,[2)] two euglobals from *E. tereticornis*,[4)] three euglobals from *E. incrassata*,[5)] and four euglobals from *E. blakelyi*.[6)] In addition the inhibitory effects of some euglobals and related compounds on Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) were reported.[7)] On the other hand, according to the analysis by LC-atmospheric pressure ionization (API-MS),[8)] it was confirmed that more than five euglobals having a monoterpene skeleton were present in the juvenile and adult leaves of *E. grandis*.

In this paper, we describe the details of the isolation and structural elucidation of new euglobals (**1**—**5**) from the juvenile leaves of *E. grandis*.[1)]

The chloroform extract of the air-dried juvenile leaves of this plant was subjected to column chromatography on silica gel to give the crude euglobal fraction. This crude fraction was further purified by means of HPLC to provide five new euglobals, named euglobal-G1 (**1**, 0.097% from the dried leaves), -G2 (**2**, 0.114%), -G3 (**3**, 0.009%), -G4 (**4**, 0.003%) and -G5 (**5**, 0.001%). Compounds (**1**—**5**) have the common composition $C_{23}H_{30}O_5$ (MS, [$M^+$] 386), suggesting that they have phloroglucinol-monoterpene structures.

**Euglobal-G1 (1) and -G2 (2)** Euglobal-G1 (**1**) and -G2 (**2**) showed UV, IR and MS data similar to those of reported euglobal, euglobal-IIc (**6**), from *E. globulus*[2)] and *E. tereticornis*.[4)] In addition, the $^{13}$C-NMR spectra and the distortionless enhancement by polarization transfer (DEPT) experiments on both **1** and **2**, respectively, disclosed the presence of nine quarternary carbons which included one carbonyl and six phenyl carbons, one aldehyde carbon, four methine carbons, four methylene carbons, and five methyl carbons. The structural elucidations of **1** and **2** were carried out using two-dimensional (2D)-NMR spectra and nuclear Overhauser effect (NOE) experiments as follows. The correlation spectroscopy *via* long-range coupling (COLOC) experiments of **1** and **2** were performed in order to confirm the connectivities of the monoterpene moiety and sub-

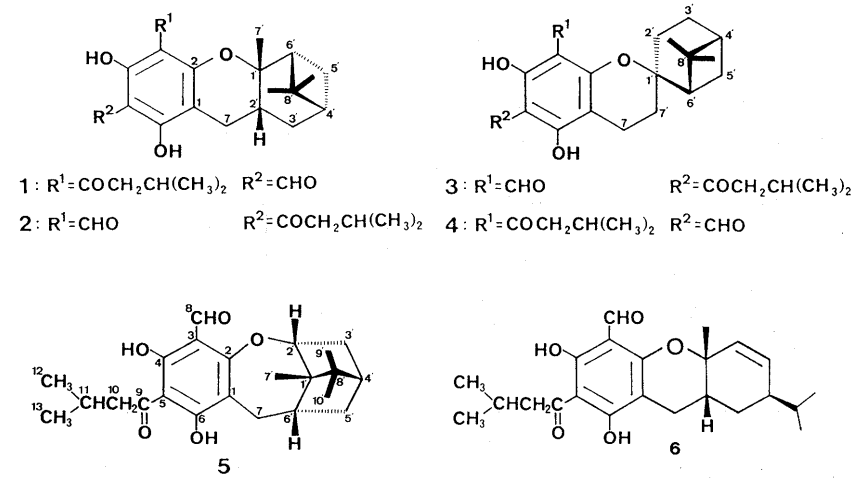



Chart 1

TABLE I. $^1H$- and $^{13}C$- Chemical Shift Values ($\delta$) of Euglobal-G1 (**1**) and -G2 (**2**)

| Carbon No. | Euglobal-G1 (**1**) | | Euglobal-G2 (**2**)[a] | |
|---|---|---|---|---|
| | $^{13}C$ | $^1H$ | $^{13}C$ | $^1H$ |
| 1 (=C–)[b] | 100.58 | | 100.85 | |
| 2 (=C–) | 166.07 | | 164.25 | |
| 3 (=C–) | 104.06 | | 103.75 | |
| 4 (=C–) | 170.38 | | 168.19 | |
| 5 (=C–) | 104.69 | | 103.59 | |
| 6 (=C–) | 166.87 | | 171.29 | |
| 7 (>$CH_2$) | 19.99 | $\alpha$ 2.74 (1H, dd, $J$=15.1, 2.7 Hz)<br>$\beta$ 2.41 (1H, dd, $J$=15.1, 5.9 Hz) | 19.98 | $\alpha$ 2.67 (1H, dd, $J$=15.4, 2.4 Hz)<br>$\beta$ 2.36 (1H, dd, $J$=15.4, 6.1 Hz) |
| 8 (–CHO) | 192.38 | 10.20 (1H, s) | 191.42 | 9.91 (1H, s) |
| 9 (>C=O) | 205.68 | | 206.12 | |
| 10 (>$CH_2$) | 52.65 | 2.59 (1H, dd, $J$=15.3, 7.7 Hz)<br>3.01 (1H, dd, $J$=15.3, 6.1 Hz) | 52.73 | 2.90—3.01 (2H, ABX, $J$=15.5, 6.5 Hz) |
| 11 (>CH–) | 24.89 | 2.22 (1H, m) | 24.96 | 2.27 (1H, sept, $J$=6.5 Hz) |
| 12 (–$CH_3$) | 23.01 | 0.99 (3H, d, $J$=6.6 Hz) | 22.82 | 0.98 (3H, d, $J$=6.5 Hz) |
| 13 (–$CH_3$) | 22.46 | 0.94 (3H, d, $J$=6.6 Hz) | 22.79 | 0.97 (3H, d, $J$=6.5 Hz) |
| 1′ (>C<) | 89.23 | | 87.41 | |
| 2′ (>CH–) | 32.37 | 2.69 (1H, m) | 32.07 | 2.57 (1H, m) |
| 3′ (>$CH_2$) | 33.78 | $\alpha$ 1.32 (1H, ddd, $J$=10.2, 8.2, 2.0 Hz)<br>$\beta$ 2.14 (1H, m) | 34.12 | $\alpha$ 1.25 (1H, ddd, $J$=11.7, 8.6, 1.7 Hz)<br>$\beta$ 2.04 (1H, m) |
| 4′ (>CH–) | 40.39 | 1.90 (1H, m) | 40.77 | 1.80 (1H, m) |
| 5′ (>$CH_2$) | 27.64 | $\alpha$ 0.80 (1H, d, $J$=10.5 Hz)<br>$\beta$ 2.14 (1H, m) | 27.90 | $\alpha$ 0.77 (1H, d, $J$=10.6 Hz)<br>$\beta$ 2.04 (1H, m) |
| 6′ (>CH–) | 55.45 | 2.48 (1H, t, $J$=5.6 Hz) | 54.95 | 2.16 (1H, t, $J$=5.6 Hz) |
| 7′ (–$CH_3$) | 29.17 | 1.51 (3H, s) | 28.71 | 1.37 (3H, s) |
| 8′ (>C<) | 40.41 | | 40.18 | |
| 9′ (–$CH_3$) | 28.15 | 1.31 (3H, s) | 28.19 | 1.24 (3H, s) |
| 10′ (–$CH_3$) | 22.75 | 1.10 (3H, s) | 22.66 | 1.00 (3H, s) |
| 4-OH | | 15.44 (1H, s) | | 14.43 (1H, s) |
| 6-OH | | 13.14 (1H, s) | | 15.41 (1H, s) |

a) Spectra were recorded at 400 MHz in $CDCl_3$ (**1**) or in $CDCl_3$–$C_6D_6$ (5:1, **2**). b) Multiplicities were determined by DEPT and $^1H$–$^{13}C$ COSY spectra.

stituent groups on the phloroglucinol part, as shown in Fig. 1a and 1b. In the COLOC experiment [in $CDCl_3$–$C_6D_6$ (5:1)], **2** was shown to have $^1H$–$^{13}C$ correlations between one of the methylene protons (7-$H_2$) at $\delta$ 2.67 and carbons at $\delta$ 164.25 (C-2), 171.29 (C-6) and 87.41 (C-1′), between one of the methylene protons (5′-$H_2$) at $\delta$ 0.77 and carbons at $\delta$ 87.41 (C-1′) and 40.18 (C-8′), and also between two methylene protons (7-$H_2$, 5′-$H_2$) and the carbon at $\delta$ 34.12 (C-3′). Further, correlations were observed between two methyl protons at $\delta$ 1.24 (9′-$H_3$) and 1.00 (10′-$H_3$) and carbons at $\delta$ 40.77 (C-4′) and 54.95 (C-6′), between the methyl protons at $\delta$ 1.37 (7′-$H_3$) and carbons at $\delta$ 32.07 (C-2′) and C-6′, and between the methine proton at $\delta$ 2.57 (2′-H) and the carbon at $\delta$ 28.71 (C-7′).

In the phloroglucinol moiety, the aldehyde proton at $\delta$ 9.91 (8-CHO) was correlated with carbons at $\delta$ 164.25 (C-2), 103.75 (C-3) and 168.19 (C-4). The hydroxyl proton at $\delta$ 15.41 (6-OH) was correlated with carbons at $\delta$ 100.85 (C-1), 103.59 (C-5), 171.29 (C-6) and 206.12 (C-9). Some other long-range $^1H$–$^{13}C$ correlations are indicated by arrows in Fig. 1b. From these results, the positions of an aldehyde and an isovaleroyl groups of **2** were concluded to be at C-3 and C-5, respectively. All proton and carbon signals of euglobal-G2 (**2**) could be assigned by $^1H$–$^1H$ COSY, DEPT experiments, $^1H$–$^{13}C$ COSY and COLOC spectra, as shown in Table I.[9] Furthermore, an incredible natural abundance double quantum transfer experiment (INADEQUATE) (in $CDCl_3$) of **2** was performed in order to confirm the above connectivities. Compound **2** was shown to have $^{13}C$–$^{13}C$ correlations between a phenyl carbon (C-3) and aldehyde carbon (C-8), between a phenyl carbon (C-5) and carbonyl carbon (C-9), and also between a phenyl carbon (C-1) and methylene carbon (C-7). In addition, correlations were observed between a quarternary carbon (C-8′) and methine carbons (C-6′ and C-4′). All $^{13}C$–$^{13}C$ correlations were observed as shown in Fig. 2. Therefore, the plane structure of **2** was supported by the INADEQUATE.

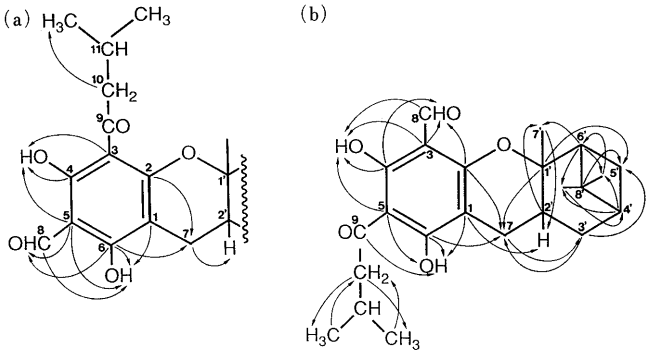
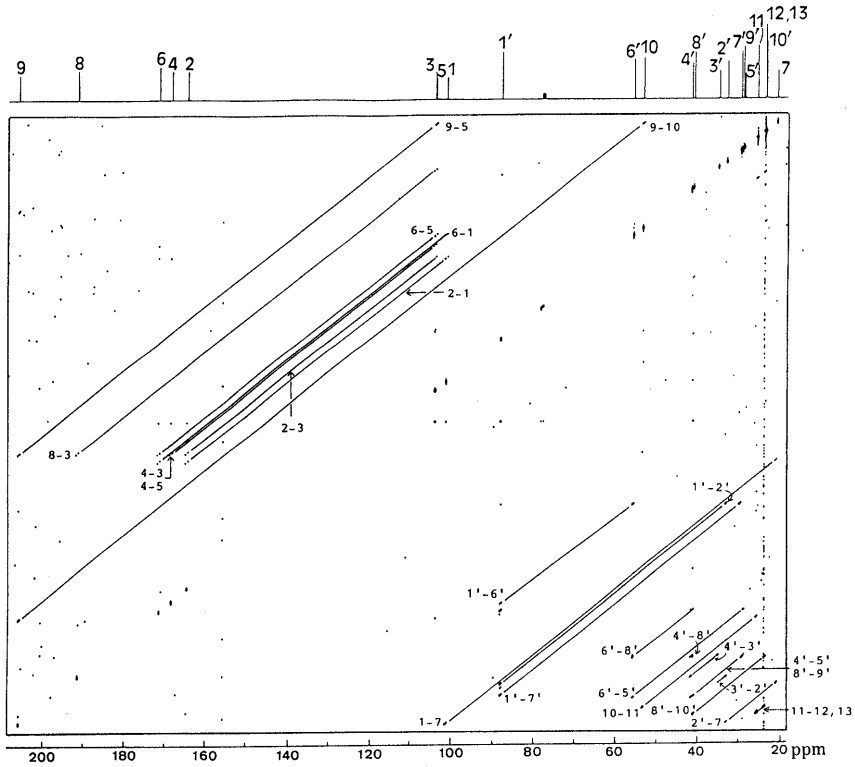



Fig. 1. Correlation ($^{13}C$ ⌒ $^1H$) in the COLOC Spectrum of Phloroglucinol Moiety of **1** (a) and in the COLOC Spectrum of **2** (b)

On the other hand, in the COLOC of compound **1**, $^1H$–$^{13}C$ correlations were observed between the hydroxyl proton at $\delta$ 15.44 (4-OH) and carbons at $\delta$ 104.06 (C-3),



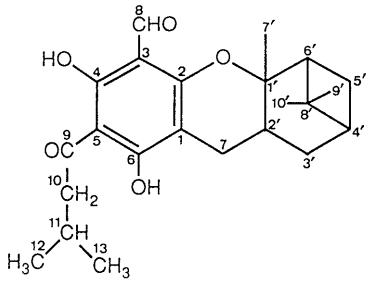




Fig. 2. $^{13}C$–$^{13}C$ Correlation in the INADEQUATE Spectrum of **2**

170.38 (C-4) and 104.69 (C-5), between the aldehyde proton at $\delta$ 10.20 (8-CHO) and carbons at C-5 and 166.87 (C-6), and also between the hydroxyl proton at $\delta$ 13.14 (6-OH) and carbons at $\delta$ 100.58 (C-1), C-5, C-6 and 192.38 (C-8).

From these results the positions of an aldehyde and an isovaleroyl group were concluded to be at C-5 and C-3, respectively, as shown in Fig. 1a. In the COLOC of **1**, the $^{1}H$–$^{13}C$ long-range correlations of the monoterpene moiety were very similar to those of **2**. Therefore, the plane structure of **1** was also supported by the INADEQUATE.

Next, the relative stereostructures of euglobals-G1 (**1**) and -G2 (**2**) were deduced on the basis of the following NOE experiments. In the NOE experiments of **2**, irradiation of the signal of the methine proton (2′-H) enhanced the signal intensities of 7$\beta$-H, 3′$\beta$-H and 10′-methyl protons. Irradiation of the signal of 7′-methyl protons enhanced the signal intensities of 7$\beta$-H and 2′-H. From these NOEs, it was concluded that the dihydropyrane/cyclohexane ring fusion should be a *cis* configuration. Some other significant NOE results are indicated by arrows in Fig. 3. From these NOE results of **2**, and in consideration of the Dreiding models, the relative stereostructure of euglobal-G2 was assigned as **2**, exclusive of the absolute configuration.

Further, the NOEs of the monoterpene moiety of **1** were also closely similar to those of **2**. Therefore, the relative stereostructure of euglobal-G1 is assigned as **1**, exclusive of the absolute configuration.

**Euglobals-G3 (3) and -G4 (4)** Euglobal-G3 (**3**) and -G4 (**4**) have the same composition, $C_{23}H_{30}O_5$ (MS, $[M]^+$ 386), as compounds **1** and **2**, and showed UV, IR and MS data similar to those of reported euglobals that have a

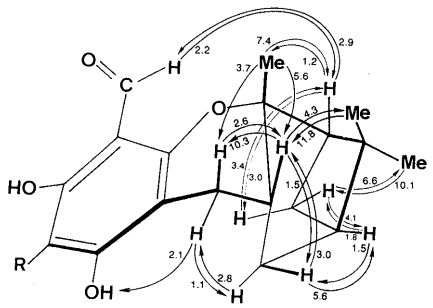



Fig. 3. Significant Enhancement of Signal Intensity (%) by NOE Experiments of **2**

monoterpene skeleton.

In addition, the $^{13}C$-NMR spectra and DEPT experiments of **3** and **4**, respectively, clarified the presence of nine quarternary carbons which included one carbonyl and six phenyl carbons, one aldehyde carbon, three methine carbons, six methylene carbons, and four methyl carbons. In compounds **3** and **4**, there were two methylene carbons instead of one methyl and methine carbons in compounds **1** and **2**. The structural elucidations of **3** and **4** were carried out using 2D-NMR spectra and NOE experiments as follows. The presence of the four-membered ring in **3** and **4** was also confirmed by $^{1}H$–$^{1}H$ COSY. The $^{1}H$–$^{13}C$ long-range COSY of **3** and **4** were measured in order to confirm the connectivities of the monoterpene moiety and substituent groups on the phloroglucinol part. In the $^{1}H$–$^{13}C$ long-range COSY [in $CDCl_3$–$C_6D_6$ (1 : 1)] of **3**, the methylene protons at $\delta$ 2.55 (7-$H_2$) were correlated with phenyl carbons at $\delta$ 161.86 (C-2) and 171.24 (C-6). The quaternary carbon at $\delta$ 84.91

TABLE II. $^1H$- and $^{13}C$- Chemical Shift Values ($\delta$) of Euglobal-G3 (**3**) and -G4 (**4**)

| Carbon No. | Euglobal-G3 (**3**)[a] | | Euglobal-G4 (**4**) | |
|---|---|---|---|---|
| | $^{13}C$ | $^1H$ | $^{13}C$ | $^1H$ |
| 1 (=C–)[b] | 101.14 | | 100.35 | |
| 2 (=C–) | 161.86 | | 163.32 | |
| 3 (=C–) | 104.38 | | 104.73 | |
| 4 (=C–) | 168.22 | | 166.85 | |
| 5 (=C–) | 103.36 | | 104.26 | |
| 6 (=C–) | 171.24 | | 169.94 | |
| 7 (>$CH_2$) | 15.39 | 2.55 (2H, t, $J$=6.6 Hz) | 15.18 | 2.57 (2H, t, $J$=6.8 Hz) |
| 8 (–CHO) | 191.64 | 9.99 (1H, s) | 192.43 | 10.18 (1H, s) |
| 9 (>C=O) | 206.27 | | 205.69 | |
| 10 (>$CH_2$) | 52.67 | 2.95 (2H, d, $J$=6.7 Hz) | 53.11 | 2.95 (2H, d, $J$=7.0 Hz) |
| 11 (>CH–) | 25.14 | 2.27 (1H, sept, $J$=6.7 Hz) | 24.66 | 2.24 (1H, m) |
| 12 (–$CH_3$) | 22.77 | 0.96 (3H, d, $J$=6.7 Hz) | 22.77 | 0.97 (3H, d, $J$=6.6 Hz) |
| 13 (–$CH_3$) | 22.76 | 0.96 (3H, d, $J$=6.7 Hz) | 22.68 | 0.95 (3H, d, $J$=6.6 Hz) |
| 1′ (>C<) | 84.91 | | 86.67 | |
| 2′ (>$CH_2$) | 24.73 | $\beta$ 1.71 (1H, m) | 24.83 | 2.02 (2H, m) |
| | | $\alpha$ 1.87 (1H, m) | | |
| 3′ (>$CH_2$) | 28.57 | 1.75 (2H, m) | 28.67 | 2.02 (2H, m) |
| 4′ (>CH–) | 40.70 | 1.85 (1H, m) | 40.46 | 2.02 (1H, m) |
| 5′ (>$CH_2$) | 26.59 | $\alpha$ 1.49 (1H, d, $J$=10.2 Hz) | 27.32 | $\alpha$ 1.63 (1H, d, $J$=10.1 Hz) |
| | | $\beta$ 2.12 (1H, dt, $J$=10.2, 5.1 Hz) | | $\beta$ 2.32 (1H, m) |
| 6′ (>CH–) | 49.70 | 2.02 (1H, t, like, $J$=5.1 Hz) | 49.42 | 2.19 (1H, t like, $J$=5.3 Hz) |
| 7′ (>$CH_2$) | 31.93 | $\beta$ 1.65 (1H, m) | 31.32 | $\beta$ 1.89 (1H, m) |
| | | $\alpha$ 1.79 (1H, m) | | $\alpha$ 2.00 (1H, m) |
| 8′ (>C<) | 38.30 | | 38.28 | |
| 9′ (–$CH_3$) | 27.56 | 1.17 (3H, s) | 19.37 | 1.30 (3H, s) |
| 10′ (–$CH_3$) | 23.25 | 0.83 (3H, s) | 23.38 | 1.03 (3H, s) |
| 4-OH | | 14.59 (1H, s) | | 15.37 (1H, s) |
| 6-OH | | 15.53 (1H, s) | | 13.18 (1H, s) |

a) Spectra were recorded at 400 MHz in $CDCl_3$–$C_6D_6$ (1 : 1, **3**) or $CDCl_3$ (**4**). b) Multiplicities were determined by DEPT and $^1H$–$^{13}C$ COSY experiments.

(C-1′) was correlated with methylene protons at $\delta$ 2.55 (7-$H_2$) and $\delta$ 1.49 and 2.12 (5′-$H_2$). The methylene carbon at $\delta$ 31.93 (C-7′) was correlated with methylene protons at $\delta$ 1.71 and 1.87 (2′-$H_2$). The methine carbons at $\delta$ 40.70 (C-4′) and 49.70 (C-6′) were correlated with two methyl protons (9′-$H_3$ and 10′-$H_3$). The long-range $^1H$–$^{13}C$ correlations of the phloroglucinol moiety of **3** were observed to be almost similar to **2**. Some other significant long-range $^1H$–$^{13}C$ correlations are indicated by arrows in Fig. 4. From these results, the plane structure of the monoterpene moiety of **3** was clarified and the positions of aldehyde and isovaleroyl groups of **3** were concluded to be at C-3 and C-5, respectively, the same as compound **2**. All proton and carbon signals of euglobal-G3 (**3**) could be assigned by $^1H$–$^1H$ COSY, DEPT experiments, $^1H$–$^{13}C$ COSY and $^1H$–$^{13}C$ long-range COSY, as shown in Table II.[9]

On the other hand, in the $^1H$–$^{13}C$ long-range COSY of compound **4**, correlations were observed between the hydroxyl proton (4-OH) and carbons at C-3, C-4 and C-5, between the aldehyde proton (8-CHO) and carbons at C-5 and C-6, and also between the hydroxyl proton (6-OH) and carbons at C-1, C-5, C-6 and C-8. Therefore, the positions of aldehyde and isovaleroyl groups of **4** were concluded to be at C-5 and C-8, respectively, the same as compound **1**. The $^1H$–$^{13}C$ long-range correlations of the monoterpene moiety of **4** were very similar to those of **3**.

Next, the NOE experiments were performed in order to confirm the relative stereostructures of **3** and **4**. In the NOE experiments of **3**, irradiation of the signal of one of

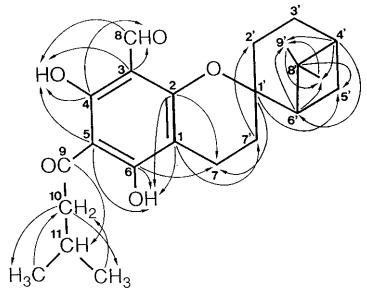



Fig. 4. Correlation ($^{13}C$ ⌒ $^1H$) in the $^1H$–$^{13}C$ Long-Range COSY Spectrum of **3**

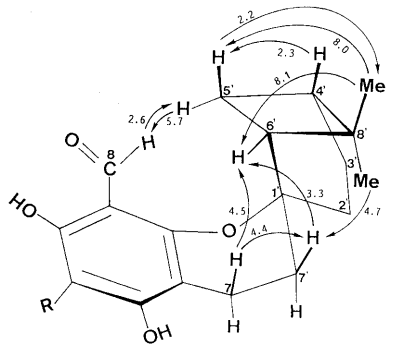



Fig. 5. Significant Enhancement of Signal Intensity (%) by NOE Experiments of **3**

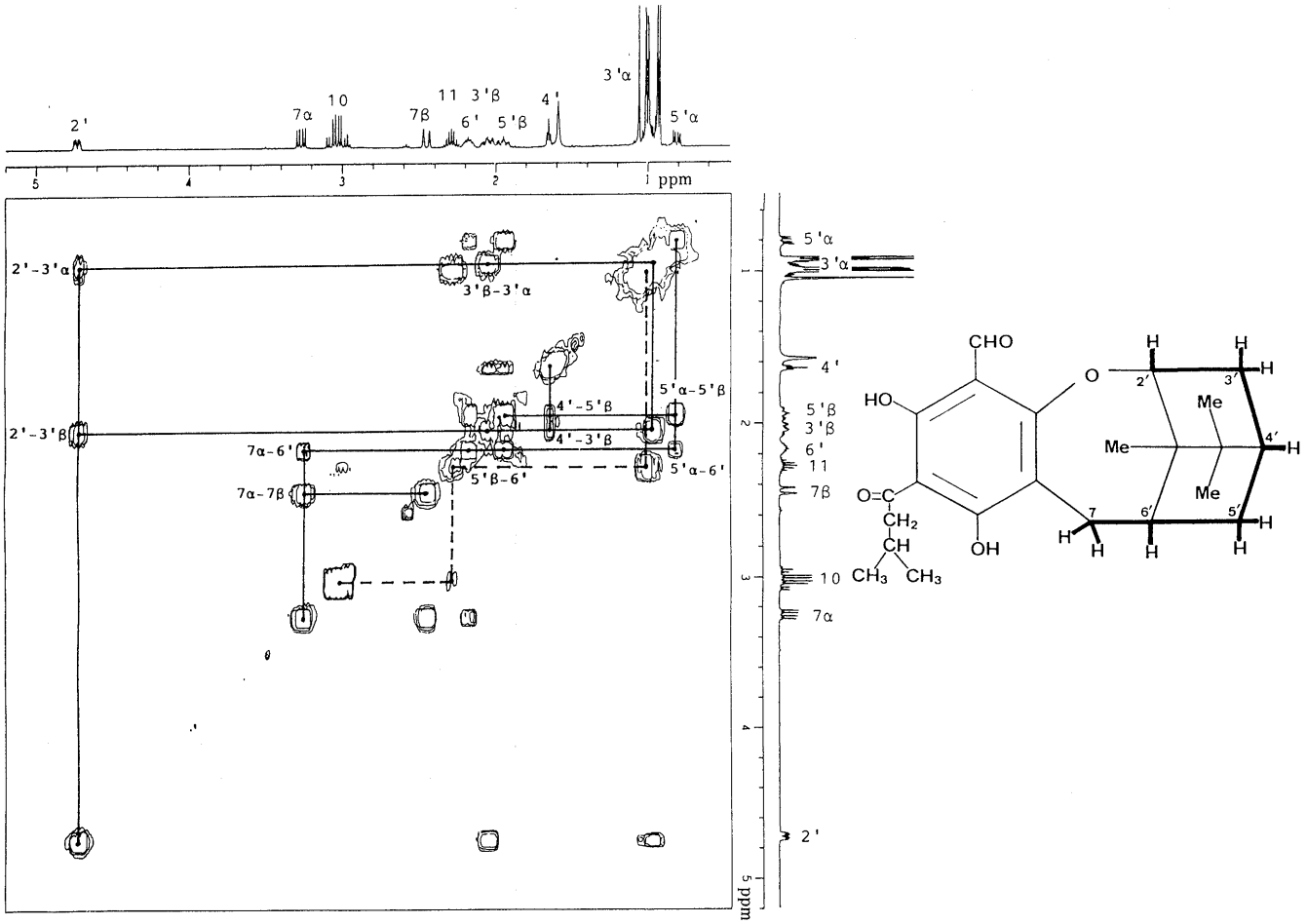



Fig. 6. Correlation ($^1$H–$^1$H) in the $^1$H–$^1$H COSY Spectrum of **5**

the methylene protons at $\delta$ 1.65 (7′$\beta$-H) enhanced the signal intensity of the methine proton at $\delta$ 2.02 (6′-H). The NOEs were observed between one of the methylene protons at $\delta$ 1.49 (5′$\alpha$-H) and the aldehyde proton at $\delta$ 9.99 (8-CHO). From these NOEs, it was concluded that a 7′$\beta$-H and C1′–C6′ bond were located on the $\beta$-side of the dihydropyrane ring on euglobal-G3 (**3**), as shown in Fig. 5.

On the other hand, in the NOE experiments of **4**, irradiation of the signal of one of the methylene protons at $\delta$ 1.89 (7′-$\beta$H) enhanced the signal intensity of the methine proton at $\delta$ 2.19 (6′-H). NOEs were also observed between one of the methylene protons at $\delta$ 1.63 (5′$\alpha$-H) and the methylene protons at $\delta$ 2.95 (10-$H_2$). From these results, it was concluded that a 7′$\beta$-H and C1′–C6′ bond were located on the $\beta$-side of the dihydropyrane ring on euglobal-G4 (**4**), the same as in the case of euglobal-G3 (**3**).

Consequently, the relative stereostructures of euglobals-G3 and -G4 were assigned as **3** and **4**, exclusive of the absolute configurations.

**Euglobal-G5 (5)** Euglobal-G5 (**5**) also has the same composition, $C_{23}H_{30}O_5$ (MS, $[M]^+$ 386), as compounds **1**—**4**, and showed UV, IR and MS data similar to those of reported euglobals having a monoterpene skeleton. In addition, $^{13}$C-NMR spectra and DEPT experiments of **5** disclosed the presence of nine quarternary carbons which included one carbonyl and six phenyl carbons, four methine carbons, one aldehyde carbon, four methylene carbons and five methyl carbons, similarly to **1** and **2**. The structural elucidation of **5** was carried out using 2D-NMR spectra and difference NOE experiments as follows. The plane structure of euglobal-G5 (**5**) was constructed on the basis of $^1$H–$^1$H COSY and $^1$H–$^{13}$C long-range COSY spectra. Thus, the $^1$H–$^1$H COSY and $^1$H–$^{13}$C COSY of **5** indicated the presence of a partial structure from C-2′ to C-7, as shown in Fig. 6. In the $^1$H–$^{13}$C long-range COSY of **5**, one of the methylene protons (7-$H_2$) at $\delta$ 3.26 was correlated with a phenyl carbon at $\delta$ 164.95 (C-2), quaternary carbon at $\delta$ 51.70 (C-1′) and methylene carbon at $\delta$ 31.59 (C-5′). The methyl protons at $\delta$ 1.05 (7′-$H_3$) were correlated with methine carbons at $\delta$ 88.48 (C-2′) and 37.04 (C-6′). Further, correlations were observed between the methine proton at $\delta$ 1.64 (4′-H) and carbons at C-2′ and C-6′, between two methyl protons at $\delta$ 0.93 and 0.92 (9′-$H_3$ and 10′-$H_3$) and two methyl carbons at $\delta$ 18.59 and 19.37 (C-10′ and C-9′), respectively, and also between one of the methylene protons (3′-$H_2$) at $\delta$ 1.00 and a carbon at C-1′. In the phloroglucinol moiety, the aldehyde proton at $\delta$ 9.97 (8-CHO) was correlated with carbons at $\delta$ 108.48 (C-3) and 167.44 (C-4). The hydroxyl proton at $\delta$ 15.39 (6-OH) was correlated with carbons at $\delta$ 113.37 (C-1), 105.78 (C-5) and 172.13 (C-6). Some other long-range $^1$H–$^{13}$C correlations are indicated by arrows in Fig. 7. From these results, the positions of aldehyde and isovaleroyl groups of **5** were concluded to be at C-3

and C-5, respectively. All proton and carbon signals of euglobal-G5 (**5**) could be assigned by DEPT experiments and 2D-NMR spectra, as shown in Table III.

Next, the relative stereostructure of euglobal-G5 (**5**) was deduced on the basis of the difference in NOE. NOEs were observed between the methyl protons at $\delta$ 0.93 (9′-$H_3$) and 4′-H, 6′-H and 5′$\beta$-H. NOEs were also observed between the methyl protons at $\delta$ 0.92 (10′-$H_3$) and 2′-H, 4′-H and 3′$\beta$-H. Furthermore, NOEs were observed between the methyl protons at $\delta$ 1.05 (7′-$H_3$) and 2′-H, 6′-H and 7$\beta$-H. NOEs were again observed between 6′-H and 7$\alpha$-H and 5′$\beta$-H. From these NOEs, it was concluded that the bicyclo[2.2.1]heptane ring was located on the $\alpha$-side of the seven-membered ring. The chemical shift values of 5′$\alpha$-H ($\delta$ 0.81) and 3′$\alpha$-H ($\delta$ 1.00) which, were observed at a relatively higher field, supported that these protons were located on the aromatic ring. Some other significant NOEs are indicated by arrows in Fig. 8.

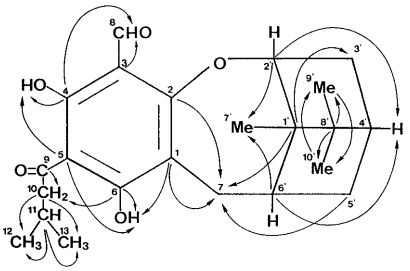


Fig. 7. Correlation ($^{13}$C⌒$^{1}$H) in the $^{1}$H–$^{13}$C Long-Range COSY Spectrum of **5**

TABLE III. $^{1}$H- and $^{13}$C- Chemical Shift Values ($\delta$) of Euglobal-G5 (**5**) in $CDCl_3$

| Carbon No. | $^{13}$C | $^{1}$H |
|---|---|---|
| 1 (=C–)[a] | 113.37 | |
| 2 (=C–) | 164.95 | |
| 3 (=C–) | 108.48 | |
| 4 (=C–) | 167.44 | |
| 5 (=C–) | 105.78 | |
| 6 (=C–) | 172.13 | |
| 7 (>$CH_2$) | 21.43 | $\beta$ 2.44 (1H, dd, $J$=15.4, 1.4 Hz) |
| | | $\alpha$ 3.26 (1H, dd, $J$=15.4, 6.8 Hz) |
| 8 (–CHO) | 193.17 | 9.97 (1H, s) |
| 9 (>C=O) | 206.92 | |
| 10 (>$CH_2$) | 53.03 | 2.99 (1H, dd, $J$=15.9, 6.7 Hz) |
| | | 3.06 (1H, dd, $J$=15.9, 6.6 Hz) |
| 11 (>CH–) | 24.89 | 2.28 (1H, sept, $J$=6.7 Hz) |
| 12 (–$CH_3$) | 22.82 | 1.00 (3H, d, $J$=6.7 Hz) |
| 13 (–$CH_3$) | 22.75 | 0.99 (3H, d, $J$=6.7 Hz) |
| 1′ (>C<) | 51.70 | |
| 2′ (>CH–) | 88.48 | 4.73 (1H, ddd, $J$=11.0, 4.6, 2.6 Hz) |
| 3′ (>$CH_2$) | 31.51 | $\alpha$ 1.00 (1H, m) |
| | | $\beta$ 2.07 (1H, m) |
| 4′ (>CH–) | 43.15 | 1.64 (1H, t, $J$=4.4 Hz) |
| 5′ (>$CH_2$) | 31.59 | $\alpha$ 0.81 (1H, dd, $J$=12.6, 4.9 Hz) |
| | | $\beta$ 1.96 (1H, m) |
| 6′ (>CH–) | 37.04 | 2.17 (1H, m) |
| 7′ (–$CH_3$) | 12.26 | 1.05 (3H, s) |
| 8′ (>C<) | 49.94 | |
| 9′ (–$CH_3$) | 19.37 | 0.93 (3H, s) |
| 10′ (–$CH_3$) | 18.59 | 0.92 (3H, s) |
| 4-OH | | 14.27 (1H, s) |
| 6-OH | | 15.39 (1H, s) |

a) Multiplicities were determined by DEPT and $^{1}$H–$^{13}$C COSY spectra.

From these difference NOE experiments of **5**, and from the consideration of Dreiding models, the relative stereostructure of euglobal-G5 was assigned as **5**, exclusive of the absolute configuration.

In conclusion, we have isolated five new euglobals with acylphloroglucinol-monoterpene structures, named euglobals-G1 (**1**), -G2 (**2**), -G3 (**3**), -G4 (**4**), and -G5 (**5**) from the juvenile leaves of *Eucalyptus grandis*.

We will now consider the biogenesis of these euglobals. These compounds were thought to be derived biogenetically from a isovaleroylphloroglucinol dialdehyde (**7**) and a monoterpene by a Diels–Alder type condensation involving the double bond of monoterpene and one of the aldehydic carbonyls in **7**. In the case of euglobals-G1 (**1**) and -G2 (**2**), the phloroglucinol precursor (**7**) would be condensed with the 2,3-double bond of $\alpha$-pinene (**8**). In the case of euglobals-G3 (**3**) and -G4 (**4**), **7** would be

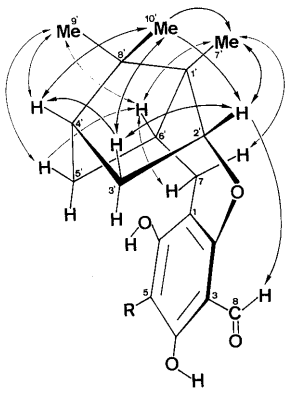


Fig. 8. Significant Enhancement of Signal Intensity by Difference NOE Experiments of **5**

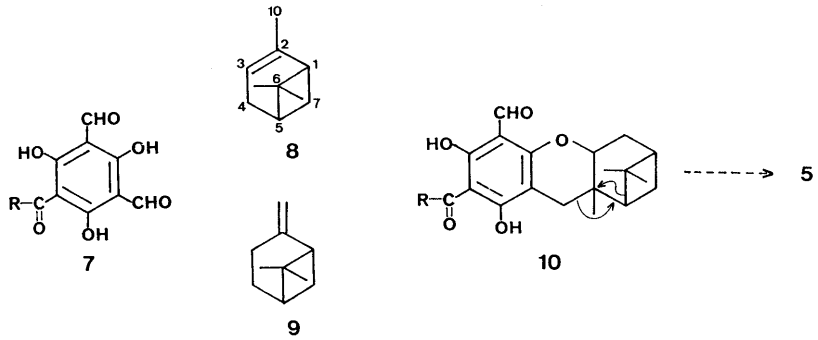


Chart 2

condensed with the 2,10-double bond of β-pinene (**9**). Further, euglobal-G5 (**5**) would presumably be derived from **7** and **8**, followed by bond migrations of compound **10**, as shown in Chart 2.

These compounds (**1**—**5**) strongly inhibited EBV-EA activations induced by TPA, which was used as the primary screening test of their potential anti-tumor-promoting activities.[10] Therefore, these *in vitro* results suggest that these euglobals might be valuable anti-tumor-promoters.

## Experimental

Melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were measured on a Shimadzu IR-480 spectrometer in $CHCl_3$, and UV spectra were obtained on a Shimadzu 210-A spectrophotometer in 95% EtOH. $^1$H- and $^{13}$C-NMR spectra were recorded on a Varian XL-300 spectrometer in $CDCl_3$ using TMS as an internal standard. 2D-NMR and NOE spectra were recorded on JEOL JNM GX-400 and Bruker AM 400 spectrometers. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. MS and HRMS were taken under electron impact (EI) conditions using a Hitachi M-80 mass spectrometer at 20 eV having a direct inlet system. 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, and SD-8 with a hydrophobic gel permeation column [JAIGEL-1H (20 × 600 mm) -2H (20 × 600 mm)] using $CHCl_3$ (3.0 ml/min) as an eluent. Silica gel (Merk, Kieselgel 60, 70—230 mesh) was using for column chromatography. Precoated silica gel plates (Merk, Kieselgel 60 $F_{254}$, 0.25 mm) were used for analytical TLC, and euglobals were detected under a UV lamp (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-100 LC-API, L-6200) using a reversed-phase column [YMC-ODS, A-302 (4.6 × 150 mm), solvent: MeOH–AcOH–$H_2O$ (100 : 5 : 3), flow rate: 1 ml/min] with a UV (280 nm) detector.[11]

**Plant Material** The juvenile leaves of *E. grandis* were collected in Nagoya in January 1988. A voucher specimen was deposited at the Herbarium of Kyoto Pharmaceutical University.

**Extraction and Isolation** The air dried juvenile leaves (903 g) of *E. grandis* were extracted with $CHCl_3$ at room temperature, and the solvent was evaporated *in vacuo* to give dark green tar (113.16 g, 12.53% from the dried leaves). The $CHCl_3$ extract (36.25 g) was chromatographed on silica gel with $C_6H_6$ followed with $C_6H_6$–$CHCl_3$ (1 : 1) to yield a crude euglobal fraction (4.21 g). The fraction (2.75 g) was rechromatographed on ODS by preparative HPLC to yield four fractions (A—D), and each fraction was purified by recycle preparative HPLC with ODS and a hydrophobic gel permeation column.

From fraction A, euglobal-G1 (**1**, 182.9 mg, 0.097%) was isolated and euglobals-G2 (**2**, 215.4 mg, 0.114%) and -G3 (**3**, 17.0 mg, 0.009%) were isolated from the fractions C and D, respectively. Fraction B was purified to afford euglobals-G4 (**4**, 5.3 mg, 0.003%) and -G5 (**5**, 2.5 mg, 0.001%).

**Euglobal-G1 (1)** Colorless needles, mp 112—114 °C (from EtOH), $[\alpha]_D^{23}$ +116.3° ($c$ = 1.0, $CHCl_3$). UV $\lambda_{max}$ nm (ε): 275 (31500), 345 (5200). IR cm$^{-1}$: 3600—3300, 2950, 1630, 1610, 1429, 1315, 1190. MS *m/z*: 386 ($M^+$), 251 ($M^+ - C_{10}H_{15}$, base), 195 (*m/z* 251 − $C_4H_8$), 135 ($M^+ - C_{13}H_{14}O_5$), 93. HR-MS: Calcd for $C_{23}H_{30}O_5$: 386.2093. Found: 386.2102. $^1$H- and $^{13}$C-NMR: Given in Table I.

**Euglobal-G2 (2)** Colorless oil, $[\alpha]_D^{23}$ +102.8° ($c$ = 1.0, $CHCl_3$). UV $\lambda_{max}$ nm (ε): 277.5 (39700), 341 (4200). IR cm$^{-1}$: 3600—3300, 2950, 1620, 1425, 1300, 1185. MS *m/z*: 386 ($M^+$), 251, 195, 135, 93 (*m/z* 135 − $C_3H_7$, base). HR-MS: Calcd for $C_{23}H_{30}O_5$: 386.2093. Found: 386.2091. $^1$H- and $^{13}$C-NMR [$CDCl_3$–$C_6D_6$ (5 : 1)]: Given in Table I. $^1$H-NMR ($CDCl_3$) δ: 2.72 (1H, dd, $J$ = 15.4, 2.4 Hz, 7α-H), 2.45 (1H, dd, $J$ = 15.4, 6.1 Hz, 7β-H), 9.93 (1H, s, 8-CHO), 2.93—3.04 (2H, ABX, $J$ = 15.5, 6.5 Hz, 10-$H_2$), 2.26 (1H, sept, $J$ = 6.6 Hz, 11-H), 0.99 (3H, d, $J$ = 6.5 Hz, 12-$H_3$), 0.98 (3H, d, $J$ = 6.5 Hz, 13-$H_3$), 2.69 (1H, m, 2′-H), 1.32 (1H, ddd, $J$ = 11.7, 8.6, 1.7 Hz, 3′α-H), 2.14 (1H, m, 3′β-H), 1.88 (1H, m, 4′-H), 0.82 (1H, d, $J$ = 10.6 Hz, 5′α-H), 2.14 (1H, m, 5′β-H), 2.25 (1H, t, $J$ = 5.8 Hz, 6′-H), 1.46, 1.30, 1.10 (each 3H, s, 7′-, 9′-, 10′-$H_3$), 14.35 (1H, s, 4-OH), 15.34 (1H, s, 6-OH)

**Euglobal-G3 (3)** Colorless needles, mp 136—138 °C (from EtOH), $[\alpha]_D^{23}$ +10.7° ($c$ = 0.5, $CHCl_3$). UV $\lambda_{max}$ nm (ε): 277 (43400), 339 (4400). IR cm$^{-1}$: 3600—3300, 2950, 1620, 1430, 1295, 1185. MS *m/z*: 386 ($M^+$), 251 (base), 148, 93. HR-MS: Calcd for $C_{23}H_{30}O_5$: 386.2093. Found: 386.2096. $^1$H- and $^{13}$C-NMR [$CDCl_3$–$C_6D_6$ (1 : 1)]: Given in Table II. $^1$H-NMR ($CDCl_3$) δ: 2.55 (2H, t, $J$ = 6.6 Hz, 7-$H_2$), 10.00 (1H, s, 8-CHO), 2.96 (2H, d, $J$ = 6.7 Hz, 10-$H_2$), 2.24 (1H, m, 11-H), 0.98 (6H, d, $J$ = 6.7 Hz, 12,13-$H_3$), 1.84 (2H, m, 2′-$H_2$), 1.99 (3H, m, 3′-$H_2$, 4′-H), 1.60 (1H, d, $J$ = 10.2 Hz, 5′α-H), 2.25 (1H, m, 5′β-H), 2.16 (1H, t, $J$ = 5.1 Hz, 6′-H), 1.99 (2H, m, 7′-$H_2$), 1.29, 1.02 (each 3H, s, 9′-, 10′-$H_3$), 14.42 (1H, s, 4-OH), 15.36 (1H, s, 6-OH).

**Euglobal-G4 (4)** Colorless oil, $[\alpha]_D^{28}$ +14.1° ($c$ = 1.12, $CHCl_3$). UV $\lambda_{max}$ nm (ε): 275 (34500), 345 (5100). IR cm$^{-1}$: 3600—3300, 2950, 1630, 1610, 1415, 1310, 1190. MS *m/z*: 386 ($M^+$), 343, 251 (base), 195, 148, 93. HR-MS: Calcd for $C_{23}H_{30}O_5$: 386.2093. Found: 386.2096. $^1$H- and $^{13}$C-NMR: Given in Table II.

**Euglobal-G5 (5)** Colorless oil, $[\alpha]_D^{28}$ +213.8° ($c$ = 0.46, $CHCl_3$). UV $\lambda_{max}$ nm (ε): 277 (41500), 339 (4300). IR cm$^{-1}$: 3600—3300, 2950, 1620, 1425, 1290, 1160. MS *m/z*: 386 ($M^+$), 329, 251 (base), 195, 193,135, 108, 93. HR-MS: Calcd for $C_{23}H_{30}O_5$: 386.2093. Found: 386.2100. $^1$H- and $^{13}$C-NMR: Given in Table III.

**Acknowledgement** We are grateful to Higashiyama Zoological and Botanical Gardens, City of Nagoya, for providing the plant material.

## References and Notes

1) Structures of euglobals-G1, -G2 and -G3 were reported as a preliminary communication. M. Takasaki, T. Konoshima, T. Shingu, H. Tokuda, H. Nishino, A. Iwashima, M. Kozuka, *Chem. Pharm. Bull.*, **38**, 1444 (1990).
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8) M. Takasaki, T. Konoshima, M. Kozuka, K. Hashimoto, Abstracts of Papers, The 144th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, March 1994, II, p. 235.
9) The chemical shift values of $^1$H-NMR in $CDCl_3$ of **2** and **3** are reported in the experimental section, and those of the monoterpene moiety of **2** and **3** are very similar to those of **1** and **4**, respectively.
10) The results of the primary screening test of euglobals-G1—G5 and the results of anti-tumor-promoting activities on two-stage carcinogenesis test *in vivo* will be reported elsewhere.
11) LC/API-MS were measured under the following conditions. (Nebrizer temp.: 250 °C, Desolvation temp.: 399 °C and drift voltage: 50 V).