# STRUCTURES OF EUGLOBAL-G1, -G2, AND -G3 FROM *EUCALYPTUS GRANDIS*, THREE NEW INHIBITORS OF EPSTEIN- BARR VIRUS ACTIVATION[1)]

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Three new euglobals with acylphloroglucinol-monoterpene structures, named euglobal -G1 (1), -G2 (2), and -G3 (3) were isolated from the chloroform extract of the juvenile leaves of *Eucalyptus grandis* (Myrtaceae). The structures of these new compounds were determined on the basis of their spectral data. The compounds strongly inhibited the Epstein-Barr virus activation.

**KEYWORDS** euglobal-G1; euglobal-G2; euglobal-G3; *Eucalyptus grandis*; Myrtaceae; acylphloroglucinol-monoterpene structure; NMR; Epstein-Barr virus;

Our continuing research on euglobals,[2, 3b)] which have unique acylphloroglucinol-monoterpene (or -sesquiterpene) structures, led us to investigate leaves of *Eucalyptus grandis* W. Hill. We report the isolation, structure elucidation, and the inhibition of Epstein-Barr virus (EBV) activation of three new compounds, euglobal-G1 (1), -G2 (2), and -G3 (3).

Chloroform extract of the juvenile leaves of the plant was submitted to a combination of column chromatography on silica gel, low-pressure reverse-phase chromatography on Lichroprep RP-8, and HPLC with reverse-phase (ODS) and hydrophobic gel permeation columns (recycled). The yields of compounds 1, 2, and 3 from the dried leaves were 0.10%, 0.11%, and 0.009%, respectively.

Euglobal-G1 (1), colorless crystals from ethanol, mp112° C, $[\alpha]_D$ +116° ($c$ =1.0, $CHCl_3$); Euglobal-G2 (2), colorless oil, $[\alpha]_D$ + 103° ($c$ =1.0, $CHCl_3$); Euglobal-G3 (3), colorless needles, from ethanol, mp 138° C, $[\alpha]_D$ + 11° ($c$ =0.5, $CHCl_3$).

These three compounds have the same composition, $C_{23}H_{30}O_5$ (mass spectra, $M^+$: 386), and showed UV, IR, and MS data similar to those of euglobals, especially, euglobal-IIc (4) from *Eucalyptus globulus* Labill.[2a)] The UV spectra [$\lambda_{max}$ nm (ε): 277 (32000-43000), 345 (4200-5200, inflection)] were superimposable on that of grandinol (5)[4)] isolated from *E. grandis*, and showed the existence of similarly substituted phloroglucinol chromophores. The IR (3600-3300, 2950, 1620 $cm^{-1}$), $^1H$-NMR, $^{13}C$-NMR (data in Table I) for these compounds suggested a fully substituted aromatic ring bearing a formyl group, an isovaleryl group, and two hydrogen-bonded hydroxy groups. The structures for aromatic parts of 1, 2, and 3 were determined

as **A** for **1**, **B** for **2** and **3** by the measurement of $^{1}H$-$^{1}H$ COSY and COLOC of these three compounds as shown in Table I. The MS of these compounds showed a retro-Diels Alder cleavage as their major fragmentation pattern, yielding peaks at *m/z* 251 ($C_{13}H_{15}O_5$, an aromatic fragment, base peak) and *m/z* 135 ($C_{10}H_{15}$), corresponding to a monoterpene moiety fused to the aromatic moiety with an ether linkage. As shown in their formulas, euglobal-G1 (1) and -G2 (2) have the same monoterpene structure, as evidenced by $^{1}H$-$^{1}H$ COSY and $^{13}C$-$^{1}H$ COSY. The relative stereochemistry of 1 and 2 was elucidated by the NOE and NOESY data as shown in Fig. 1. In the DEPT data of euglobal-G3 (3), there were two methylene groups instead of the one methyl and methine group each in the DEPT of euglobal-G1 (1) and -G2 (2). The presence of the four-membered ring in 3 was also confirmed by $^{1}H$-$^{1}H$ COSY. In addition, the NMR data of euglobal-G3 (3) showed a partial structure (-$CH_2$-$CH_2$-CH-) in the monoterpene moiety. Accordingly, formula 3 was assigned to the structure of euglobal-G3.

Biogenetically, euglobal-G1 (1) and -G2 (2) would be derived from acylphloroglucinol precursors and α-pinene and euglobal-G3 (3) from an acylphloroglucinol and β-pinene, respectively.

As shown in Table II, these three compounds strongly inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced EBV activation as a result of screening test for inhibition of tumor promotion.[3] Further studies on the stereochemistry of euglobal-G3 and the initiation-promotion tests using ICR mice [5] of these compounds (1, 2, and 3) are now in progress.

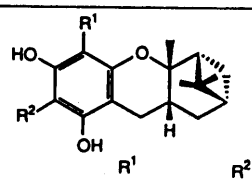
Table I. COLOC, $^{1}H$- and $^{13}C$-NMR Spectral Data for Euglobal-G1 (1), -G2 (2), and -G3 (3)

| Position | Euglobal-G1 (1) C | COLOC | H | Position | Euglobal-G2 (2) C | COLOC | H |
|---|---|---|---|---|---|---|---|
| 1 | 100.58 (C) | C(7)$H_2$, C(6)-OH | | 1 | 100.85 (C) | C(6)-OH, C(7)$H_2$, C(2')H | |
| 2 | 166.07 (C) | C(7)$H_2$ | | 2 | 164.25 (C) | C(8)-CHO, C(7)$H_2$ | |
| 3 | 104.06 (C) | C(4)-OH | | 3 | 103.75 (C) | C(8)-CHO, C(4)-OH | |
| 4 | 170.38 (C) | C(4)-OH | | 4 | 168.19 (C) | C(8)-CHO, C(4)-OH | |
| 5 | 104.69 (C) | C(8)-CHO, C(6)-OH, C(4)-OH | | 5 | 103.59 (C) | C(6)-OH, C(4)-OH | |
| 6 | 166.87 (C) | C(7)$H_2$, C(8)-CHO | | 6 | 171.29 (C) | C(6)-OH, C(7)$H_2$ | |
| 7 | 19.99 ($CH_2$) | C(3')$H_2$, C(2')H | 2.74 (dd, *J* = 15.1, 2.7 Hz)<br>2.41 (dd, *J* = 15.1, 5.9 Hz) | 7 | 19.98 ($CH_2$) | C(3')$H_2$, C(2')H | 2.67 (dd, *J* = 15.4, 2.4 Hz)<br>2.36 (dd, *J* = 15.4, 2.4 Hz) |
| 8 | 192.38 (CHO) | C(6)-OH | 10.20 (s) | 8 | 191.42 (CHO) | C(4)-OH | 9.91 (s) |
| 9 | 205.68 (C=O) | C(10)$H_2$ | | 9 | 206.12 (C=O) | C(6)-OH | |
| 10 | 52.65 ($CH_2$) | C(13)$H_3$ | 2.59 (dd, *J* = 15.3, 7.7 Hz)<br>3.01 (dd, *J* = 15.3, 6.1 Hz) | 10 | 52.73 ($CH_2$) | C(12)$H_3$, C(13)$H_3$ | 3.01-2.90 (ABX, *J* = 15.5, 6.5 Hz) |
| 11 | 24.89 (CH) | C(12)$H_3$, C(13)$H_3$<br>C(10)$H_2$ | 2.22 (m) | 11 | 24.96 (CH) | C(12)$H_3$, C(13)$H_3$<br>C(10)$H_2$ | 2.27 (nonet, *J* = 6.5 Hz) |
| 12 | 23.01 ($CH_3$) | C(13)$H_3$, C(10)$H_2$ | 0.99 (d, *J* = 6.6 Hz) | 12 | 22.82 ($CH_3$) | C(10)$H_2$ | 0.98 (d, *J* = 1.9 Hz) |
| 13 | 22.46 ($CH_3$) | C(12)$H_3$, C(10)$H_2$ | 0.94 (d, *J* = 6.6 Hz) | 13 | 22.79 ($CH_3$) | C(10)$H_2$ | 0.97 (d, *J* = 1.9 Hz) |
| 1' | 89.23 (C) | | | 1' | 87.41 (C) | | |
| 2' | 32.37 (CH) | | 2.69 (m) | 2' | 32.07 (CH) | | 2.57 (m) |
| 3' | 33.78 ($CH_2$) | | 1.32 (m)<br>2.08 - 2.19 (m) | 3' | 34.12 ($CH_2$) | | 1.25 (ddd, *J* = 11.7, 8.6, 1.7 Hz)<br>2.07-2.00 (m) |
| 4' | 40.39 (CH) | | 1.90 (m) | 4' | 40.77 (CH) | | 1.70 (m) |
| 5' | 27.64 ($CH_2$) | | 0.80 (d, *J* = 10.5 Hz)<br>2.08 - 2.19 (m) | 5' | 27.90 ($CH_2$) | | 0.77 (d, *J* = 10.6 Hz)<br>2.07-2.00 (m) |
| 6' | 55.45 (CH) | | 2.48 (t, *J* = 5.6 Hz) | 6' | 54.95 (CH) | | 2.16 (t, J = 5.6 Hz) |
| 7' | 29.17 ($CH_3$) | | 1.51 (s) | 7' | 28.71 ($CH_3$) | | 1.37 (s) |
| 8' | 40.41 (C) | | | 8' | 40.18 (C) | | |
| 9' | 28.15 ($CH_3$) | | 1.31 (s) | 9' | 28.19 ($CH_3$) | | 1.24 (s) |
| 10' | 22.75 ($CH_3$) | | 1.10 (s) | 10' | 22.66 ($CH_3$) | | 1.00 (s) |
| 4-OH | | | 15.44 (s) | 4-OH | | | 14.43 (s) |
| 6-OH | | | 13.14 (s) | 6-OH | | | 15.41 (s) |

Chemical shifts are expressed in δ (ppm) values. Spectra were recorded at 400 MHz in $CDCl_3$ (1and 3) or in $CDCl_3$-$C_6D_6$ (5:1, 2). Assignments were made by their DEPT, $^{1}H$-$^{1}H$ COSY, $^{13}C$-$^{1}H$ COSY, and NOESY experiments.

Table I. COLOC, $^1H$- and $^{13}C$-NMR Spectral Data for Euglobal-G1(1), -G2 (2), and -G3 (3) (continued)

| Position | C | Euglobal-G3 (3) COLOC | H |
|---|---|---|---|
| 1 | 101.14 (C) | C(7)$H_2$, C(7')$H_2$, C(6)-OH | |
| 2 | 161.86 (C) | C(6)-OH, C(7)-$H_2$ | |
| 3 | 104.38 (C) | C(8)-CHO, C(4)-OH | |
| 4 | 168.22 (C) | C(8)-CHO, C(4)-OH | |
| 5 | 103.36 (C) | C(4)-OH, C(6)-OH | |
| 6 | 171.24 (C) | C(7)$H_2$ | |
| 7 | 15.39 ($CH_2$) | C(7')$H_2$ | 2.55 (t, $J$ = 6.6 Hz) |
| 8 | 191.64 (CHO) | C(4)-OH | 10.00 (s) |
| 9 | 206.27 (C=O) | C(10)$H_2$, C(11)H | |
| 10 | 52.67 ($CH_2$) | C(12)$H_3$, C(13)H C(10)$H_2$ | 2.96 (d, $J$ = 6.7 Hz) |
| 11 | 25.14 (CH) | C(12)$H_3$, C(13)$H_3$ | 2.24 (m) |
| 12 | 22.77 ($CH_3$) | C(10)$H_2$, C(13)$H_3$ | 0.98 (d, $J$ = 6.7 Hz) |
| 13 | 22.76 ($CH_3$) | C(12)$H_3$, C(10)$H_2$ | 0.98 (d, $J$ = 6.7 Hz) |
| 1' | 84.91 (C) | | |
| 2' | 24.78 ($CH_2$) | | 2.05-1.62 (m) |
| 3' | 28.57 ($CH_2$) | | 2.05-1.92 (m) |
| 4' | 40.70 (CH) | | 2.05-1.92 (m) |
| 5' | 26.59 ($CH_2$) | | 2.25 (m) |
| 6' | 49.70 (CH) | | 1.60 (d, $J$ = 10.2 Hz) 2.16 (t, $J$ = 5.1 Hz) |
| 7' | 31.93 ($CH_2$) | | 2.05-1.92 (m) |
| 8' | 38.30 (C) | | |
| 9' | 27.56 ($CH_3$) | | 1.29 (s) |
| 10' | 23.25 ($CH_3$) | | 1.02 (s) |
| 4-OH | | | 14.42 (s) |
| 6-OH | | | 15.36 (s) |



1: $R^1$ = $COCH_2CH(CH_3)_2$, $R^2$ = CHO

2: $R^1$ = CHO, $R^2$ = $COCH_2CH(CH_3)_2$

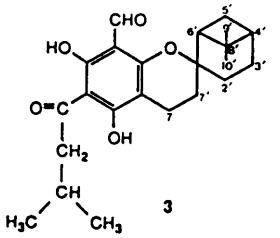


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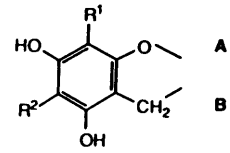
Table II. Inhibitory Effects of Euglobal-G1, -G2, and -G3 on EBV Activation

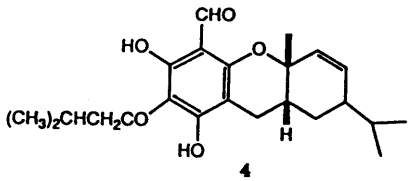
| Sample | Concentration ( Mol ratio / TPA ) 1000 | 500 | 100 | 10 |
|---|---|---|---|---|
| | % to control ( % viability of Raji cells)* | | | |
| G - 1 | 0.0 (50) | 15.6 (80) | 70.3 (80) | 100.0 (80) |
| G - 2 | 0.0 (60) | 25.4 (80) | 73.3 (80) | 100.0 (80) |
| G - 3 | 10.5 (80) | 23.6 (80) | 65.7 (80) | 100.0 (80) |

TPA (32 pMol ) 100 = positive control. *The inhibition of EBV activation was assayed by using the EBV genome-carrying human lymphoblastoid cells (Raji cells).



A : $R^1$ = $COCH_2CH(CH_3)_2$, $R^2$ = CHO

B : $R^1$ = CHO, $R^2$ = $COCH_2CH(CH_3)_2$



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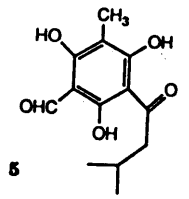

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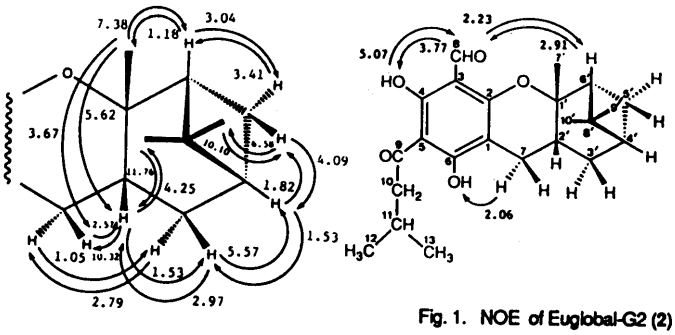

Fig. 1. NOE of Euglobal-G2 (2)

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## REFERENCES AND NOTES

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