Pergamon

PII: S0031-9422(97)00672-9

Phytochemistry, Vol. 47, No. 6, pp. 1157–1159, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

PHLOROGLUCINOL-MONOTERPENE ADDUCTS FROM *EUCALYPTUS GRANDIS*

INDER PAL SINGH, KAZUHIRO UMEHARA,* TATSUO ASAI,* HIDEO ETOH,*‡ MIDORI TAKASAKI† and TAKAO KONOSHIMA†

The United Graduate School of Agricultural Sciences, Gifu University (Shizuoka University), 836 Ohya, Shizuoka 422, Japan; * Department of Applied Biological Chemistry, Shizuoka University, 836 Ohya, Shizuoka 422, Japan; † Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607, Japan

(*Received in revised form* 16 *June* 1997)

Key Word Index—*Eucalyptus grandis*; Myrtaceae; leaves; euglobals-G6 and -G7; phloroglucinol-monoterpene adducts.

Abstract—Two new euglobals, G6 and G7 were isolated from the hexane fraction of the methanol extract of the leaves of *Eucalyptus grandis*. These have phloroglucinol-monoterpene structures. Both euglobals have a formyl-isovaleroyl phloroglucinol moiety fused with γ-terpinene. Their structures were elucidated on the basis of spectral evidences. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

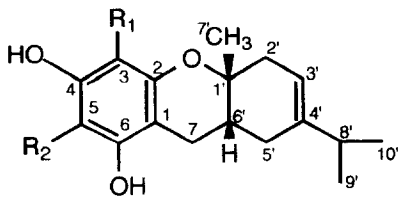
Eucalyptus is a rich source of phloroglucinol compounds which exhibit interesting biological activities. In the course of our search for bioactive compounds from natural sources, we have also reported on the presence of several bioactive compounds in *Eucalyptus* [1–4]. We have recently isolated a new phloroglucinol dimer, grandinal, from the chloroform fraction of the methanol extract of the leaves of *E. grandis* [5]. Further investigations into the hexane fraction led to the isolation of two new euglobals named euglobals G6 and G7. Euglobals G1–G5, also from the leaves of *E. grandis*, have been reported as inhibitors of Epstein Barr Virus (EBV) activation [6]. In this paper, we report on the structural elucidation of these two new compounds and their inhibitory activity against EBV activation induced by 12-*O*-tetradecanoyl phorbol-13-acetate (TPA).

RESULTS AND DISCUSSION

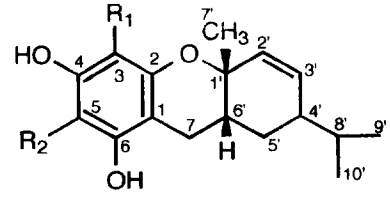
The hexane fraction of the methanol extract of air-dried leaves of *E. grandis* was fractionated as described in the Experimental section to yield euglobal G6 (**1**) and G7 (**2**) in 0.0095 and 0.011% yields, respectively. The high-resolution mass spectra of **1** and **2** revealed the molecular formula as $C_{23}H_{30}O_5$. The UV spectra of both compounds were superimposable on those of other euglobals, especially euglobal IIc (**3**) and euglobal-T1 (**4**) [7].

The high-field ^{1}H NMR spectrum of **1** showed striking similarity with that of euglobal IIc (**3**) [7]. The ^{13}C NMR and DEPT spectra showed 23 carbons and 28 hydrogens bound to carbon atoms. These NMR spectra established that **1** contained a phloroglucinol moiety (δ^C 171.6, 168.2, 161.1, 103.9, 103.5 and 100.5) containing one aldehyde group (δ^C 192.0) and one isovaleroyl group [δ^C 206.4 (*s*), 52.7 (*t*), 25.1 (*d*), 2 × 22.8 (*q*)]. The presence of a doublet carbon (δ 114.3) and a singlet carbon (δ 140.7) in the ^{13}C NMR spectrum and a one proton singlet at δ 5.23 suggested the presence of a trisubstituted double bond instead of the disubstituted double bond in **3** and **4**. The structure of the monoterpene part and its linkage to the phloroglucinol unit was elucidated by measuring ^{1}H–^{1}H COSY, HSQC and HMBC as shown in Scheme 1. HMBC correlations from H-7 to carbons at 1, 2 and 6, and from the phenolic group at δ 15.4 (6-OH) to carbons 1, 5 and 6, and from the formyl proton at δ 10.01 to carbons 3 and 4, suggested that the formyl group was located at position 3 and the isovaleroyl group at position 5. This linkage was further confirmed by differential NOE experiments. Strong through-space interactions were observed between methyl 7′ and formyl proton H-8, and between formyl proton H-8 and the phenolic proton at position 4. Also, the phenolic proton at position 6 showed strong through-space interaction with proton H-7. These interactions determined the location of isovaleroyl group at position 5 unambiguously. Other significant HMBC and ^{1}H–^{1}H COSY correlations are shown in

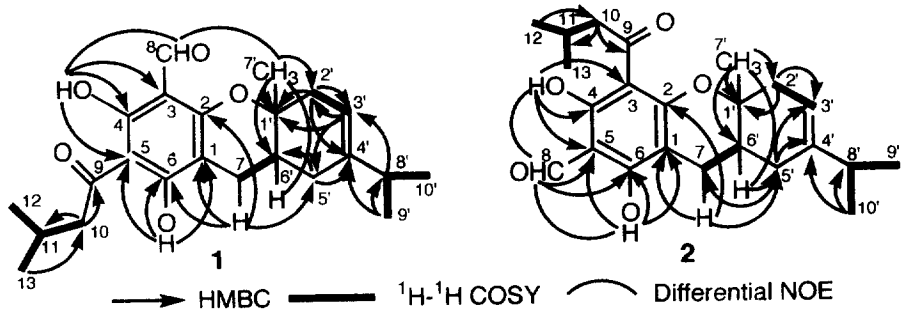
‡ Author to whom correspondence should be addressed.



1: R_1=CHO; R_2=$COCH_2CH(CH_3)_2$
2: R_1=$COCH_2CH(CH_3)_2$; R_2=CHO



3: R_1=CHO; R_2=$COCH_2CH(CH_3)_2$
4: R_1=$COCH_2CH(CH_3)_2$; R_2=CHO



Scheme 1.

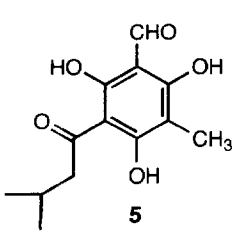
Scheme 1. Strong through-space interaction between methyl 7′ and H-6′ confirmed the *cis*-stereochemistry at these centres of euglobal-G6 as shown.

Compound **2** had the same molecular formula and similar UV spectrum as compound **1**. The high-field ^{1}H NMR showed a splitting pattern similar to euglobal T1 (**4**) [7]. The ^{13}C NMR was also similar to compound **1** but with important differences being observed only in the HMBC and differential NOE experiments. In the HMBC experiment, the phenolic proton at position 6, as well as the formyl proton H-8, were correlated with carbons 5 and 6.

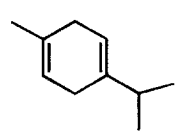
In the differential NOE experiment, the formyl proton H-8 showed strong through-space interaction with both the phenolic protons (4-OH and 6-OH). These data confirmed that the formyl group was linked to C-5 and the isovaleroyl to C-3. Interaction between methyl 7′ and H-6′ indicated a *cis*-stereochemistry at carbons 1′ and 6′.

Biogenetically, **1** and **2** may be formed by a Diels-Alder-type cyclo-addition of *o*-quinone methide derived from grandinol (**5**) [8] with γ-terpinene (**6**).

Compounds **1** and **2** were tested for inhibition of EBV early antigen activation induced by TPA. Both compounds showed *ca* 80% inhibition at 1000 mol ratio/TPA, while maintaining 70% viability of Raji cells. Activity was comparable to Euglobals IIc (**3**) and T1 (**4**), which have structures similar to **1** and **2** [6].



5



6

EXPERIMENTAL

General

Mps: uncorr. ^{1}H NMR were recorded at 500 MHz in $CDCl_3$, ^{13}C NMR at 125 MHz, using TMS as int. standard. TLC: Merck HPTLC Fertigplatten RP-18 WF_{254}s and Merck DC Fertigplatten kieselgel $60F_{254}$, the chromatograms being visualized under UV $light_{254nm}$ or by spraying with 50% EtOH in H_2SO_4. UV: hexane. IR: $CHCl_3$.

Plant material

Eucalyptus grandis was grown at the research farm of Shizuoka University.

Isolation of euglobals

The MeOH extract of air-dried leaves (1 kg) was successively extracted with hexane and CH_2Cl_2. The hexane fr. was separated into different frs by chromatography on silica gel (hexane–EtOAc). The fr. eluted with hexane–EtOAc (9:1) was subjected to recycled HPLC on ODS (MeOH–HOAc–H_2O, 100:5:3) to give compounds **1** (95 mg) and **2** (110 mg) in 0.0095 and 0.011% yields, respectively.

Euglobal-G6 (**1**).

$[\alpha]_D \pm 0°$ ($CHCl_3$ *c* 0.1). HREI-MS *m/z* 386.2092 $[M]^+$ (−0.1 mmu for $C_{23}H_{30}O_5$)]. UV λ_{max} (hexane) nm (log ε): 275 (4.33), 341 (3.25). IR ν_{max} ($CHCl_3$) cm^{-1}: 1627, 1436, 1297, 1184. ^1H NMR ($CDCl_3$, 500 MHz) δ: 15.40 (1H, *s*, 6-OH), 14.47 (1H, *s*, 4-OH), 10.01 (1H, *s*, 8-CHO), 5.23 *brt* (H-3′), 2.97 (2H, *d*, *J* = 6.7, H-10), 2.62 (1H, *dd*, *J* = 5.8 and 16.8, H-7), 2.39 (1H, *d*, *J* = 17.1, H-2′), 2.32, (1H, *dd*, *J* = 8.3 and 16.5, H-7), 2.28 (1H, *d*, *J* = 14.6 Hz, H-5′, 2.25 (1H, *septet*, *J* = 6.7 Hz, H-11, 2.19 (1H, *septet*, *J* = 7.0 Hz, H-8′), 2.13 (1H, *dd*, *J* = 4.0 and 17.7 Hz, H-2′), 2.04 (1H, *m*, H-6′), 1.96 (1H, *dd*, *J* = 3.7 and 17.7 Hz, H-5′), 1.43 (3H, *s*, H-7′), 1.00 (3H, *d*, *J* = 7.0 Hz, H-12 or 13), 0.99 (3H, *d*, *J* = 6.7 Hz, H-12 or 13), 0.98 (6H, *d*, *J* = 6.4 Hz, H-9′ and 10′). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 206.4 *s* (C-9), 192.0 *d* (C-8), 171.6 *s* (C-6), 168.2 *s* (C-4), 161.1 *s* (C-2), 140.7 *s* (C-4′), 114.3 *d* (C-3′), 103.9 *s* (C-3), 103.5 *s* (C-5), 100.5 *s* (C-1), 79.0 *s* (C-1′), 52.7 *t* (C-10), 34.5 *d* (C-8′), 34.4 *t* (C-2′), 33.3 *d* (C-6′), 30.2 *t* (C-5′), 25.1 *q* (C-7′), 25.0 *d* (C-11), 22.8 *q* (C-12 and 13), 21.5 *t* (C-7), 21.4 *q*, 21.3 *q* (C-9′ and 10′).

Euglobal-G7 (**2**).

$[\alpha]_D \pm 0°$ ($CHCl_3$ *c* 0.1). HREI-MS *m/z* 386.2101 $[M]^+$ (+0.8 mmu for $C_{23}H_{30}O_5$)]. UV λ_{max} (hexane) nm (log ε): 273 (4.49), 345 (3.62). IR ν_{max} ($CHCl_3$) cm^{-1}: 1612, 1418, 1312, 1194. ^1H NMR ($CDCl_3$, 500 MHz): δ 15.38 (1H, *s*, 4-OH), 13.22 (1H, *s*, 6-OH), 10.19 (1H, *s*, 8-CHO), 5.26 *brt* (H-3′), 2.98 (1H, *dd*, *J* = 6.4 and 14.4, H-10), 2.75 (1H, *dd*, *J* = 7.6 and 14.4, H-10), 2.67, (1H, *dd*, *J* = 5.8 and 16.8, H-7), 2.44 (1H, *d*, *J* = 17.4, H-2′), 2.36 (1H, *dd*, *J* = 7.3 and 16.8 Hz, H-7), 2.23 (1H, *dd*, H-5′), 2.22 (1H, H-2′), 2.20 (1H, H-8′), 2.17 (1H, *septet*, *J* = 7.0 Hz, H-11), 2.06 (1H, *pentet*, *J* = 6.3 Hz, H-6′), 1.94 (1H, *dd*, *J* = 17.7 and 6.4, H-5′), 1.45 (3H, *s*, H-7′), 0.99 (6H each, *d*, *J* = 7.1 Hz, H-9′ and 10′), 0.95 (3H, *d*, *J* = 7.0 Hz, H-12 or 13), 0.93 (3H, *d*, *J* = 7.0 Hz, H-12 or 13). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 206.2 *s* (C-9), 192.4 *d* (C-8), 170.1 *s* (C-4), 167.4 *s* (C-6), 162.3 *s* (C-2), 141.1 *s* (C-4′), 114.1 *d* (C-3′), 104.5 *s* (C-5). 104.2 *s* (C-3), 99.4 *s* (C-1), 79.4 *s* (C-1′), 53.3 *t* (C-10), 35.1 *t* (C-2′), 34.5 *d* (C-8′), 32.6 *d* (C-6′), 29.8 *t* (C-5′), 25.5 *d* (C-11), 25.0 *q* (C-7′), 22.9 and 22.5 *q* each (C-12 and 13), 21.4 *t* (C-7) and 21.3 *q* (C-9′ and 10′).

Biological evaluation

Inhibition of EBV-EA activation was assayed by using the method of ref. [6].

Acknowledgements—The authors thank Drs H. Tokuda and H. Nishino of the Kyoto Prefectural University of Medicine for biological evaluation of compounds.

REFERENCES

1. Satoh, H., Etoh, H., Watanabe, N., Kawagishi, H., Arai, K. and Ina, K., *Chemistry Letters*, 1992, p. 1917.
2. Singh, I. P., Takahashi, K. and Etoh, H., *Bioscience, Biotechnology, and Biochemistry*, 1996, **60**, 1522.
3. Etoh, H., Yamashita, N., Sakata, K., Ina, H. and Ina, K. *Agricultrual and Biological Chemistry*, 1990, **54**, 2443.
4 Hyodo, S., Etoh, H., Yamashita, N., Sakata, K. and Ina, K., *Bioscience, Biotechnology, and Biochemistry*, 1992, **56**, 138.
5. Singh, I. P., Etoh, H., Hayakawa, R., Takasaki, M. and Konoshima, T., *Bioscience, Biotechnology, and Biochemistry*, 1997, **67**, 921
6. Takasaki, M., Konoshima, T., Kozuka, M. and Tokuda, H., *Biological Pharmaceutical Bulletin*, 1995, **18**, 435.
7. Kokumai, M., Konoshima, T., Kozuka, M., Haruna, M. and Ito, K., *Journal of Natural Products*, 1991, **54**, 1082.
8. Crow, W. D., Osawa, T., Paton, D. M. and Willing, R. R., *Tetrahedron Letters*, 1977, p. 1073.