Euglobal-IIIa, a novel acylphloroglucinol-sesquiterpene derivative from *Eucalyptus robusta*: absolute structure and cytotoxicity

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Abstract: Euglobal-IIIa (1), a novel acylphloroglucinol-sesquiterpene derivative, and a known analogue, have been isolated from leaves of *Eucalyptus robusta*. The structures was elucidated by extensive spectroscopic data and by comparison with data reported in literature, while the absolute configuration of 1 was determined by the X-ray diffraction analysis. Compound 1 exhibited comparable cytotoxicity with that of cisplatin against five human cancer cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW480 with IC₅₀ values of 15.7, 15.5, 17.6, 14.3, and 21.8 μM, respectively.

Keywords: *Eucalyptus robusta*, acylphloroglucinol-sesquiterpene, absolute structure, cytotoxicity

Introduction
Euglobals are a group of caryophyllene-based monoterpene (or sesquiterpenoid) derivatives which have been found to be abundant in the genus *Eucalyptus*.¹ Pharmacological investigations of them showed antitumor, antimicrobial, and granulation inhibiting activities.² As part of our efforts to search for significant antitumor agents, a novel acylphloroglucinol-sesquiterpene derivative, named euglobal-IIIa (1), and a known analogure, were isolated from leaves of *E. robusta* Smith, a tree up to 20 meters distributed in Yunnan and Sichuan province, China. The structure of 1 was established on the basis of extensive spectroscopic methods and the absolute configuration was determined by the single crystal X-ray diffraction analysis, while the known compound was identified as sideroxylonal B (2) by comparison with data reported in literature.³ The cytotoxicity of two compounds against five human cancer cell lines was evaluated.

Results and Discussion

An acetone extract of *E. Robusta* was partitioned between H₂O and EtOAc. The isolation of the EtOAc lay afforded a new caryophyllene-based terpenoid, named as euglobal-IIIa (1), along with an analogue, sideroxylonal B (2).

Euglobal-IIIa (1), colorless crystals, was found to possess a molecular formula of C₃₅H₄₆O₈ as assigned by HREIMS at m/z 472.2828 [M]⁺ (calcd. 472.2825 [M]⁺), implying nine degrees of unsaturation. The UV spectrum showed the existence of a phenyl group based on the maximum absorption bands at 282 and 232 nm, while the FT-IR spectrum exhibited absorption bands for carbonyl groups (1629 cm⁻¹) and hydroxy groups (3556 and 3441 cm⁻¹).

The ¹H NMR spectrum displayed two downfield singlets at δ₁ 10.15 (1H, s) and 9.98 (1H, s) ascribable for two aldehyde groups, an olefinic signal at δ₇ 5.29 (1H, dd, J = 6.4, 3.2 Hz), six methyl signals (including four singlets at δ₁ 0.71, 1.07, 1.24, and 1.69 and two doublets at δ₁ 0.71 and 0.91) (Table 1). The ¹³C NMR spectrum displayed 28 carbon resonances which could be assigned as nine quaternary carbons, seven methines, six methylenes, and six methyls (Table 1). These information suggested that compound 1 possessed three rings. Of the carbon resonances, six quaternary signals at δ₁ 105.7, 169.0, 103.8, 167.8, 104.6, and 163.9, together with two aldehyde carbons at δC 191.8 (d) and 191.9 (d) established an acylphloroglucinol moiety (ring A, Figure 1).⁴ Preliminary analysis of ¹H–¹H COSY spectrum readily established an isopentane group which was connected to C-1 as revealed by the key HMBC correlation of δ₁ 2.68 (1H, m, H-7) with δC 105.7 (C-1), 163.6 (s, C-2), and 169.0 (s, C-6) (Figure 1).

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Further analyses of $^1$H–$^1$H COSY spectrum established another two partial structures a and b, which were linked to form the ring C according to HMBC correlations including $\delta_H$ 2.09 (1H, m, H-1') with $\delta_C$ 85.1 (s, C-10') and 36.2 (d, C-7) and $\delta_H$ 2.03 (1H, m, H-3'a) and 2.28 (1H, m, H-3'b) with $\delta_C$ 134.4 (s, C-4'), and $\delta_C$ 1.80 (2H, m, H-9') with $\delta_C$ 85.1 (s, C-10') (Figure 1). In addition, a methyl signal at $\delta_H$ 1.69 (3H, s, H-14') showing HMBC correlations with $\delta_C$ 134.4 (s, C-4') and 41.5 (t, C-3') suggested the methyl placed at C-4', while the correlation of $\delta_H$ 1.07 (1H, m, H-9') (Figure 1). Since compound 1 exhibited the same characteristic UV absorption maxima at 254 and 328 nm, and IR absorption at 3445 and 1676 cm$^{-1}$ in DMSO-d$_6$, compound 1 was active to HL-60, SMMC-7721, A-549, MCF-7, and SW480, compound 2 was inactive to all the tested strains (IC$_{50}$ > 40 μM) (Table 2). The results showed that compound 1 displayed comparable cytotoxicity with that of cisplatin against SMMC-7721, A-549, MCF-7, and SW480, but compound 2 was inactive to all the tested strains (IC$_{50}$ > 40 μM) (Table 2).

![Figure 1](image.png)

**Figure 1.** Key 2D NMR correlations of 1 with the X-ray structure showing absolute configuration.

Table 1. $^1$H and $^1$C NMR data for 1 (CDCl$_3$, $\delta$ in ppm and $\delta$ in Hz).$^{a}$

| No. | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
|-----|------------|------------|------------|------------|
| 1   | 105.7 (s)  | 2'         | 1.47 m     | 32.5 (t)   |
| 2   | 163.9 (s)  | 3'         | 2.03 m     | 41.5 (t)   |
| 3   | 104.6 (s)  | 4'         |            |            |
| 4   | 167.8 (s)  | 5'         | 5.29 dd (6.4, 3.2) | 123.9 (d) |
| 5   | 103.8 (s)  | 6'         | 2.25 m     | 25.1 (t)   |
| 6   | 169.9 (s)  | 7'         | 1.87 m     | 44.7 (d)   |
| 7   | 2.68 m     | 36.2 (d)   | 8'         | 2.03 m     |
| 8   | 10.15 s    | 191.9 (d)  | 9'         | 1.80 m     |
| 9   | 9.98 s     | 191.8 (d)  | 10'        | 85.1 (s)   |
| 10  | 1.53 m     | 37.9 (t)   | 11'        | 73.5 (s)   |
| 11  | 1.54 m     | 25.2 (d)   | 12'        | 1.24 s     |
| 12  | 0.71 d (6.1) | 23.8 (q)  | 13'        | 1.28 s     |
| 13  | 0.91 d (6.1) | 24.3 (q)  | 14'        | 1.07 s     |
| 14  | 2.09 m     | 39.8 (d)   | 15'        | 1.69 s     |

*Assignments may be reversed.

Table 2. Cytotoxicity of 1 and 2 (IC$_{50}$, μM).

|             | HL-60 | SMMC-7721 | A-549 | MCF-7 | SW480 |
|-------------|-------|-----------|-------|-------|-------|
| Cisplatin   | 15.7  | 15.5      | 17.6  | 14.3  | 21.8  |
| 1           | > 40  | > 40      | > 40  | > 40  | > 40  |
| Compound 2  | > 40  | > 40      | > 40  | > 40  | > 40  |

*Positive control.

**Experimental Section**

**General Experimental Procedures.** Melting points were determined on an X-4 micro melting point apparatus. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained with a Tensor 27 spectrophotometer with KBr pellets. 1D and 2D spectra were run on a Bruker DRX-500 spectrometer with TMS as an internal standard. Chemical shifts ($\delta$) were expressed in ppm with reference to the solvent signals. Mass spectra were recorded on a Waters AutoSpec Primier P776 instrument or an API QSTAR Pulsar i spectrometer. X-ray diffraction was performed on a Bruker SMART APEX-II diffractometer using graphite-monochromated Cu Kα radiation. Column chromatography (CC) was performed using silica gel (200–300 mesh and H, Qingdao Marine Chemical Co. Ltd., Qingdao, People’s Republic of China). Fractions were monitored by TLC (GF254, Qingdao Marine Chemical Co. Ltd., Qingdao, People’s Republic of China). Plant Material. The leaves of E. robusta were obtained from Kunming Botanical Garden, Kunming, China, and identified by Prof. Xiao Chen. A specimen (No. 2009716E) has been deposited at Kunming Institute of Botany, Chinese Academy of Sciences.

**Extraction and Isolation.** An air-dried sample (5 kg) was extracted in acetone at room temperature, and a crude extract was obtained after three times, which was partitioned between H$_2$O and EtOAc. The EtOAc lay was separated by CC over silica gel (100–200 mesh, Qingdao Marine Chemical Ltd., China) eluted with petroleum ether : acetone in a gradient of 0:1.
Euglobal-IIIa (1): colorless crystals (MeOH); mp 182–184 °C; [α]D
20 + 142.9 (c = 0.05, CHCl3); UV (CHCl3) λmax (log ε): 282 (3.93), 232 (3.37), 207 (3.42), 202 (3.42), 192 (3.41); IR (KBr) νmax: 3556, 3441, 2951, 1629, 1442, 1313, 1177, 860 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; EIMS m/z 472 [M⁺]; HREIMS m/z 472.2828 [M⁺] (calcd for C₃₀H₂₈O₁₀ 472.2825).

Crystal data for euglobal-IIIa (1): C₃₀H₂₈O₁₀ M = 472.60; orthorhombic, space group P2₁2₁2₁; a = 8.11740(10) Å, b = 10.9874 (2) Å, c = 29.3316 (5) Å, α = 90.00, β = 90.00, γ = 90.00, V = 2616.06 (7) Å³, Z = 4, d = 1.2000 g/cm³, crystal dimensions 0.45 × 0.25 × 0.15 mm was used for measurement on a Bruker SMART APEX II diffractometer using graphite-monochromated Cu Kα radiation. The total number of reflections measured was 9380, of which 4335, were observed, J > 2σ(J). Final indices: R₁ = 0.0599, wR₂ = 0.1719. Crystallographic data for the structure of I have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 809489). Copies of the data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk

Cytotoxicity Assay. Five human cancer cell lines, breast cancer MCF-7, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, colon cancer SW-480, and lung cancer A-549 cells, were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO₂ at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates. Briefly, 100 μL adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with initial density of 1 x 10⁵ cells/mL. Each tumor cell line was exposed to the test compound dissolved in DMSO at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μM in triplicates for 48 h, with cisplatin (Sigma, USA) as a positive control. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC₅₀ values were calculated by Reed and Muench's method.⁶

Electronic Supplementary Material

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s13659-011-0021-9 and is accessible for authorized users.

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References

[1] (a) Sawada, T.; Kozuka, M.; Komiya, T.; Amano, T.; Goto, M. Chem. Pharm. Bull. 1980, 28, 2546–2548. (b) Amano, T.; Komiyama, T.; Hori, M.; Goto, M.; Kozuka, M.; Sawada, T. J. Chromatogr. 1981, 208, 347–355. (c) Kokumai, M.; Konoshima, T.; Kozuka, M.; Haruna, M.; Ito, K. J. Nat. Prod. 1991, 54, 1082–1086. (d) Takasaki, M.; Konoshima, T.; Kozuka, M.; Haruna, M.; Ito, K.; Crow, W. D.; Paton, D. M. Chem. Pharm. Bull. 1994, 42, 2113–2116. (e) Cheng, Q.; Snyder, J. K. J. Org. Chem. 1988, 53, 4562–4567. (f) Takasaki, M.; Konoshima, T.; Kozuka, M.; Haruna, M.; Ito, K.; Yoshida, S. Chem. Pharm. Bull. 1994, 42, 2177–2179.

[2] (a) Takasaki, M.; Konoshima, T.; Fujitani, K.; Yoshida, S.; Nishimura, H.; Tokuda, H.; Nishino, H.; Iwashima, A.; Kozuka, M. Chem. Pharm. Bull. 1990, 38, 2737–2739. (b) Takasaki, M.; Konoshima, T.; Kozuka, M.; Tokuda, H. Biol. Pharm. Bull. 1995, 18, 435–438. (c) Takasaki, M.; Konoshima, T.; Etoh, H.; Pal Singh, I.; Tokuda, H.; Nishino, H. Cancer Lett. 2000, 155, 61–65. (d) Bharate, S. B.; Bhutani, K. K.; Khan, S. I.; Tekwani, B. L.; Jacob, M. R.; Khan, I. A.; Singh, I. P. Bioorg. Med. Chem. 2006, 14, 1750–1760. (e) Kozuka, M.; Sawada, T.; Mizuta, E.; Kasahara, F.; Amano, T.; Komiya, T.; Goto, M. Chem. Pharm. Bull. 1982, 30, 1964–1973.

[3] Satoh, H.; Etoh, H.; Watanabe, N.; Kawagishi, H.; Arai, K.; Ina, K. Chem. Lett. 1992, 21, 1917–1920.

[4] Fu, H. Z.; Luo, Y. M.; Li, C. J.; Yang, J. Z.; Zhang, D. M. Org. Lett. 2010, 12, 656–659.

[5] Mosmann, T. J. Immunol. Methods 1983, 65, 55–63.

[6] Reed, L. J.; Muench, H. Am. J. Hyg. 1938, 27, 493–497.