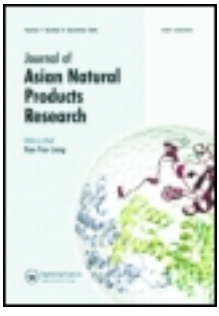

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

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Available online: 04 Oct 2006

To cite this article: Ai-Jun Hou, Yan-Ze Liu, Hui Yang, Zhong-Wen Lin & Han-Dong Sun (2000): Hydrolyzable Tannins and Related Polyphenols from Eucalyptus globulus , Journal of Asian Natural Products Research, 2:3, 205-212

To link to this article: http://dx.doi.org/10.1080/10286020008039912

JANPR, Vol. 2, pp. 205–212
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HYDROLYZABLE TANNINS AND RELATED POLYPHENOLS FROM *EUCALYPTUS GLOBULUS*

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(Received 2 August 1999; Revised 9 September 1999; In final form 22 September 1999)

Eucaglobulin (**1**), a new complex of gallotannin and monoterpene, was isolated from the leaves of *Eucalyptus globulus*. Its structure was elucidated on the basis of spectral data. Four known hydrolyzable tannins [tellimagrandin I (**2**), eucalbanin C (**3**), 2-O-digalloyl-1,3,4-tri-O-galloyl-β-D-glucose (**4**), 6-O-digalloyl-1,2,3-tri-O-galloyl-β-D-glucose (**5**)], as well as gallic acid (**6**) and (+)-catechin (**7**), were also isolated. The antibacterial effects of some of these compounds were examined.

Keywords: *Eucalyptus globulus*; Myrtaceae; Hydrolyzable tannins; Polyphenols; Antibacterial effects; Eucaglobulin

INTRODUCTION

Eucalyptus globulus Labill. (Myrtaceae) is a kind of medicinal plant widely distributed in Yunnan province of China. Its leaves, roots and fruits have been used as traditional remedies for the treatment of influenza, dysentery, enteritis, rheumatalgia and bleeding [1]. It has been well known for the

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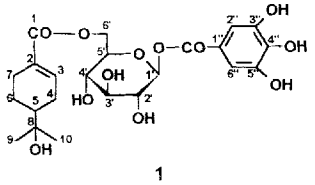
volatile terpenoid constituents of the essential oil of the leaves and fruits. In recent years, attention is focused on its unique secondary metabolites which are a number of phloroglucinol-sesquiterpene- or -monoterpene-coupled compounds named macrocarpals [2,3] and euglobals [4,5], and have been revealed to have biological activities such as HIV-RTase inhibition [2], granulation inhibition [4,5], antiviral [6] and antibacterial effects [3]. However, the tannin constituents of this species have not been studied.

In our antibacterial studies on natural products, we examined the extract of water-soluble constituents from the leaves, and found that it showed potent antibacterial activity against some bacteria. The result led to the isolation and characterization of seven hydrolyzable tannins and related polyphenols, including a novel complex of gallotannin and monoterpene, namely, eucaglobulin (**1**). Compound **1** and two main constituents of the leaves, tellimagrandin I (**2**) and eucalbanin C (**3**), exhibited antibacterial activity against several kinds of bacteria. Compounds **2**–**7** were obtained from this plant for the first time.



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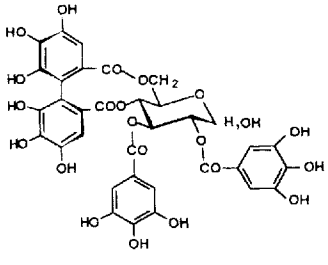

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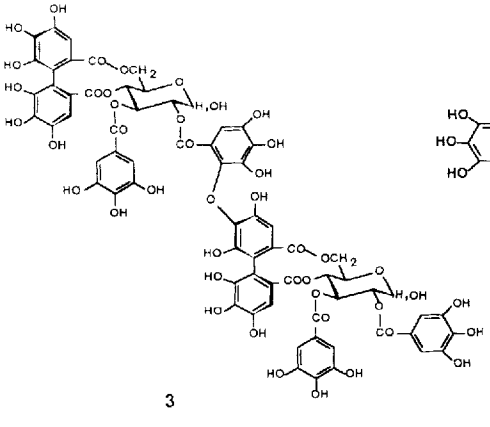

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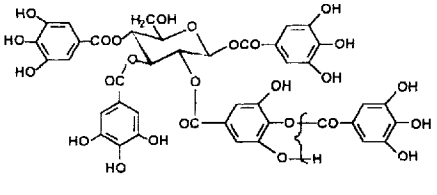

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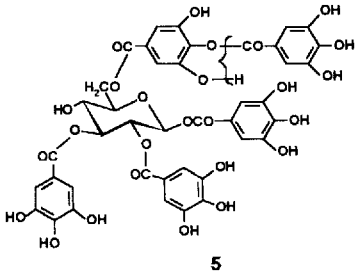

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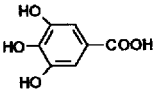

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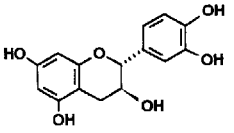

7

RESULTS AND DISCUSSION

The concentrated aqueous acetone extract of the dried leaves was fractionated by column chromatography (CC) over Diaion HP-20. The 30% and 50% methanol eluates were further purified by repeated CC over Sephadex LH-20 and MCI-gel CHP-20P to yield eucaglobulin (**1**), along with tellimagrandin I (**2**) [7,8], eucalbanin C (**3**) [9], 2-O-digalloyl-1,3,4-tri-O-galloyl-β-D-glucose (**4**), 6-O-digalloyl-1,2,3-tri-O-galloyl-β-D-glucose (**5**), gallic acid (**6**) and (+)-catechin (**7**).

Eucaglobulin (**1**), light brown amorphous powder, gave dark-blue color with $FeCl_3$, indicating the presence of galloyl group. The molecular formula $C_{23}H_{30}O_{12}$ was deduced from negative HRFABMS (found 497.1741, calcd. 497.1659) and ^{13}C NMR spectrum. The ^1H NMR spectrum exhibited one 2H-singlet at δ 7.16 ascribable to a galloyl group, one 1H-triplet at δ 6.95 due to one olefinic proton, and glucose proton signals. The configuration at C-1′ of the sugar was determined to be β by the coupling constant of the anomeric proton ($J = 7.7$ Hz). Additionally, some proton signals in the up-field region were also observed. The ^{13}C NMR and DEPT spectra of **1** confirmed the presence of the galloyl group and the β-glucose moiety. Simultaneously, they also revealed ten carbon signals owing to a monoterpene moiety with a six-membered ring, which included one carbonyl group, two unsaturated carbon atoms, two methyls, three methylene groups, one methine and one quaternary carbon. The assignment of protons and carbons was achieved on the basis of ^1H–^1H COSY and HMQC spectra. According to the results of HMBC spectrum, the position of the gallic acid ester on the glucose was determined to be at C-1′, and the monoterpene moiety was at C-6′ through an ester bond (Fig. 1). Thus, the structure of eucaglobulin was identified as **1**, which is the first example of a gallotannin possessing a monoterpene moiety (Table I).

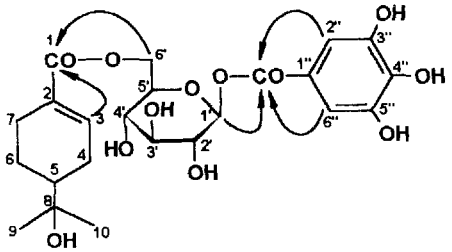

FIGURE 1 The key correlations of **1** in HMBC spectrum.

TABLE I ^{13}CNMR data of **1** (100 MHz, Me_2CO-d_6)

Carbon	δ ppm (DEPT)	*Carbon*	δ ppm (DEPT)
Monoterpene moiety		Glucose moiety	
1	167.6 (C)	1′	95.6 (CH)
2	130.8 (C)	2′	73.7 (CH)
3	141.0 (CH)	3′	77.6 (CH)
4	28.1 (CH_2)	4′	70.9 (CH)
5	45.0 (CH)	5′	75.8 (CH)
6	24.1 (CH_2)	6′	64.1 (CH_2)
7	26.0 (CH_3)	Galloyl group	
8	71.7 (C)	CO	165.7 (C)
9	27.3 (CH_3)	1″	120.8 (C)
10	26.8 (CH_3)	2″, 6″	110.2 (CH)
		3″, 5″	146.1 (C)
		4″	139.4 (C)

Besides the new compound (**1**), two ellagitannins including a dimer, a pair of gallotannins and two related phenolic compounds were also obtained. Their structures were determined as tellimagrandin I (**2**), eucalbanin C (**3**), 2-O-digalloyl-1,3,4-tri-O-galloyl-β-D-glucose (**4**), 6-O-digalloyl-1,2,3-tri-O-galloyl-β-D-glucose (**5**), gallic acid (**6**) and (+)-catechin (**7**), respectively.

The extract of water-soluble constituents from the leaves of *Eucalyptus globulus* (Eg-L) showed potent antibacterial effect against *Staphylococcus aureus*, *Staph. epidermidis*, *Candida albicans* and *Zygosaccharomyces bailii*. Compounds **1**–**3** were also evaluated for their antibacterial activity. Eucaglobulin (**1**) could inhibit *Escherichia coli* and *C. albicans*. Two main constituents, tellimagrandin I (**2**) and eucalbanin C (**3**), exhibited inhibitory effects on *E. coli*, *Staph. aureus*, *Staph. epidermidis* and *Streptococcus sanguis*. Compound **3** was also active against *Enterobacter gergoviae*. The MICs of them are listed in Table II.

TABLE II Inhibitory effects on several kinds of bacteria (MIC, ppm)

Test Substance	*E. coli* G(−)	*Staph. aureus* G(+)	*Staph. epidermidis* G(+)	*Strep. sanguis* G(+)	*E. gergoviae* G(−)	*C. albicans yeast*	*Z. bailiifungus*
Eg-L		500	34			500	500
1	500	1000	2000	1000	2000	250	
2	31	31	62	500	2000	2000	
3	31	125	62	500	500	2000	

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotation was taken on a SEPA-300 polarimeter. IR spectral data were measured on a Perkin-Elmer 577 spectrometer with KBr pellets. UV spectra were obtained on a UV 210A spectrometer. MS spectra were recorded on a VG Auto Spec-3000 spectrometer. NMR spectra were run on Bruker AM-400 and Varian VXR-500 instruments with TMS as internal standard and acetone-d_6 as solvent. Column chromatography was performed on Diaion HP-20, Sephadex LH-20 (Merck) and MCI-gel CHP-20P (Mitsubishi Chemical Industry Co. Ltd.).

Plant Material

The leaves of *E. globulus* Labill were collected in Kunming Botanical Garden in March 1997, and air-dried. The identity of plant material was verified by Prof. Zhong-Wen Lin, and a voucher specimen (970325) is deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Academia Sinica.

Extraction and Isolation

The dried and powdered leaves (3 kg) were extracted with 70% aqueous acetone and concentrated *in vacuo*. The concentrated extract was subjected to column chromatography (CC) over Diaion HP-20 with H_2O, aqueous MeOH (30% MeOH → 50%) and aqueous Me_2CO (70% Me_2CO).

A part (20 g) of the 30% MeOH elute (40 g) was subjected to CC over Sephadex LH-20 developing with aqueous MeOH. The eluate from 40% MeOH was further purified by CC over MCI-gel CHP-20P (H_2O → 10% MeOH → 15% → 20% → 30%) to yield **1** (60 mg).

A part (30 g) of the 50% MeOH elute (80 g) was chromatographed over Sephadex LH-20 developing with 60% MeOH and MeOH–H_2O–Me_2CO (8 : 1 : 1) to give five fractions. Each fraction was rechromatographed over MCI-gel CHP-20P with aqueous MeOH in stepwise gradient mode to afford **2** (100 mg), **3** (350 mg), **4** (40 mg), **5** (20 mg), **6** (5 mg), and **7** (5 mg).

Eucaglobulin (**1**): $C_{23}H_{30}O_{12}$, light brown amorphous powder; UV (MeOH) λ_{max} 218, 279.6 nm; IR (KBr) ν_{max} 3500–3200, 1695, 1680, 1600, 1440, 1340, 1320, 1250, 1070, 1027, 940, 910, 865 cm^{-1}; ^1H NMR (Me_2CO-d_6, 500 MHz) δ 7.16 (2H, s, H-2″, 6″), 6.95 (1H, t, J = 2.5 Hz, H-3), 5.66 (1H, d, J = 7.7 Hz, H-1′), 4.42 (1H, dd, J = 2.4, 11.6 Hz, H-6′a), 4.23 (1H, dd, J = 5.6, 11.6 Hz, H-6′b), 3.70 (1H, ddd, J = 2.4, 5.6, 9.7 Hz, H-5′), 3.58–3.45 (3H, m, H-2′, 3′, 4′), 2.42 (2H, br d, J = 15.6 Hz, 2 × H-7), 2.31 (2H, br d, J = 18.8 Hz, 2 × H-4), 1.99 (2H, m, 2 × H-6), 1.49 (1H, m, H-5), 1.13 (6H, s, 2 × CH_3); ^{13}C NMR data, see Table I; FABMS *m/z* 497 [M − H]$^-$ (100); negative HRFABMS *m/z* 497.1741 (calcd. for $C_{23}H_{29}O_{12}$, 497.1659).

Tellimagrandin I (**2**): $C_{34}H_{26}O_{22}$, light brown amorphous powder; ^1H NMR (Me_2CO-d_6, 400 MHz) δ 7.08, 7.07 (each s, 2H in total, galloyl), 7.01, 6.96 (each s, 2H in total, galloyl), 6.67, 6.66 (each s, 1H in total, HHDP), 6.51, 6.48 (each s, 1H in total, HHDP); glucose moiety α-anomer: δ 5.57 (d, J = 3.6 Hz, H-1), 5.16–5.08 (m, H-2, H-4), 5.89 (t, J = 10.0 Hz, H-3), 4.69 (m, H-5), 5.31–5.25 (m, H-6), 3.80 (d, J = 12.8 Hz, H-6′); β-anomer: δ 5.28 (d, J = 8.0 Hz, H-1), 5.16–5.08 (m, H-2, H-4), 5.61 (t, J = 10.0 Hz, H-3), 4.27 (m, H-5), 5.31 5.25 (m, H-6), 3.87 (d, J = 12.8 Hz, H-6′); ^{13}C NMR (Me_2CO-d_6, 100 MHz) δ 167.5, 167.4 (1C in total), 167.1, 167.0 (1C in total), 166.0, 165.7 (1C in total), 165.4, 165.0 (1C in total) (ester carbonyl); glucose moiety α-anomer: δ 90.3 (C-1), 72.2 (C-2), 70.5 (C-3), 70.2 (C-4), 66.3 (C-5), 62.8 (C-6); β-anomer: δ 95.7 (C-1), 73.3 (C-2), 72.9 (C-3), 70.2 (C-4), 71.1 (C-5), 62.8 (C-6); FABMS *m/z* 785 [M − H]$^-$ (100).

Eucalbanin C (**3**): $C_{68}H_{50}O_{44}$, light brown amorphous powder, $[\alpha]_D^{20}$ +57.2 (*c* 1.00, MeOH); UV (MeOH) λ_{max} (log ε) 217 (5.16), 270 (4.82) nm; ^1H NMR (Me_2CO-d_6, 500 MHz) δ 6.975, 6.981, 6.987 (each s, 2H in total), 7.001, 7.009, 7.017 (each s, 2H in total), 7.041, 7.047, 7.050 (each s, 2H in total) (Gal), 6.462, 6.472, 6.494, 6.513 (each s, 1H in total), 6.573, 6.577, 6.594 (each s, 1H in total), 6.625, 6.628, 6.634, 6.637 (each s, 1H in total), 6.827, 6.835, 6.855, 6.861 (each s, 1H in total), 6.953, 6.957 (each s, 1H in total) (HHDP and tergalloyl), 5.54, 5.60 (each d, J = 3.5 Hz, Glc α-anomer H-1, H-1′), 5.08, 5.10, 5.23 (each d, J = 7.0 Hz, Glc β-anomer H-1, H-1′), 5.65, 5.67, 5.69, 5.84, 5.86, 5.88 (each t, J = 10.0 Hz, Glc H-3, H-3′), 5.12 5.20 (Glc H-2, H-2′, H-4, H-4′), 5.24–5.33 (Glc H-6, H-6′), 4.65, 4.66 (each dd, J = 5.5, 10.0 Hz, Glc β-anomer H-5, H-5′), 4.25, 4.26 (each dd,

$J = 6.0, 10.0$ Hz, Glc α-anomer H-5, H-5′), 3.78, 3.83, 3.87, 3.89 (each d, $J =$ 13.0 Hz, Glc H-6, H-6′); ^{13}C NMR (Me_2CO-d_6, 125 MHz) δ 90.8, 91.2 (Glc α-anomer C-1, C-1′), 96.4, 96.6 (Glc β-anomer C-1, C-1′), 73.0, 73.7 (Glc α-anomer C-2, C-2′), 74.2, 75.0 (Glc β-anomer C-2, C-2′), 73.6, 73.7 (Glc β-anomer C-3, C-3′), 71.8, 72.0 (Glc β-anomer C-5, C-5′), 71.0, 71.1, 71.2, 71.3 (Glc α-anomer C-3, C-3′, C-4, C-4′, β-anomer C-4), 66.9, 67.0 (Glc α-anomer C-5, C-5′), 63.5, 63.9 (Glc α- and β-anomer C-6, C-6′), 166.0, 166.3 (1C in total), 166.3, 166.6 (1C in total), 166.9, 167.1, 167.8, 168.2 (each 1C), 167.9, 168.0 (1C in total), 168.3, 168.4 (1C in total) (ester carbonyl); ESIMS (50% MeOH + 0.1% $AcONH_4$) m/z 1588 $[M + NH_4]^+$ (100).

2-O-digalloyl-1,3,4-tri-O-galloyl-β-D-glucose (**4**): $C_{41}H_{32}O_{26}$, light brown amorphous powder; ^{1}H NMR (Me_2CO-d_6, 400 MHz) δ 7.18–6.96 (galloyl), 6.28 (1H, d, $J = 8.4$ Hz, Glc H-1), 5.71–5.62 (2H, m, Glc H-2, H-4), 6.02 (1H, t, $J = 9.6$ Hz, Glc H-3), 4.65 (1H, m, Glc H-5), 4.32 (1H, dd, $J = 4.8$, 12.8 Hz, Glc H-6), 4.17 (1H, dd, $J = 2.0$, 12.8 Hz, Glc H-6′); ^{13}C NMR (Me_2CO-d_6, 100 MHz) Galloyl moiety: δ 167.4–165.5 (ester carbonyl), 146.1 (C-3′, 5′), 140.1–139.3 (C-4′), 121.1–119.3 (C-1′), 110.2 (C-2′, 6′); Glucose moiety: δ 93.5 (C-1), 71.9, 71.6 (C-2), 76.0 (C-3), 69.2, 68.3 (C-4), 73.9 (C-5), 61.7 (C-6); FABMS m/z 939 $[M - H]^-$ (41), 787 $[M - H -$ galloyl$]^-$ (100).

6-O-digalloyl-1,2,3-tri-O-galloyl-β-D-glucose (**5**): $C_{41}H_{32}O_{26}$, light brown amorphous powder; ^{1}H NMR (Me_2CO-d_6, 400 MHz): δ 7.18–6.96 (galloyl), 6.12 (1H, d, $J = 8.4$ Hz, Glc H-1), 5.50–5.45 (2H, m, H-2, H-6), 5.68 (1H, t, $J = 9.6$ Hz, Glc H-3), 4.08 (1H, m, Glc H-4), 4.50 (1H, m, Glc H-5), 4.39 (1H, d, $J = 12.8$ Hz, Glc H-6′); ^{13}C NMR (Me_2CO-d_6, 100 MHz) Galloyl moiety: δ 167.4–165.5 (ester carbonyl), 146.1 (C-3′, 5′), 140.1–139.3 (C-4′), 121.1–119.3 (C-1′), 110.2 (C-2′, 6′); Glucose moiety: δ 93.4 (C-1), 71.8, 71.2 (C-2), 75.8 (C-3), 69.3, 68.3 (C-4), 73.4, 73.1 (C-5), 64.0, 63.3 (C-6); FABMS m/z 939 $[M - H]^-$ (41), 787 $[M - H -$ galloyl$]^-$ (100).

Gallic acid (**6**): $C_7H_6O_5$, colorless needles; IR (KBr) ν_{max} 3496, 3066, 2660, 1705, 1619, 1542, 1452, 1385, 1338, 1250, 1208, 956, 905, 867 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 400 MHz) δ 7.12 (2H, s, H-2, 6); ^{13}C NMR (Me_2CO-d_6, 100 MHz) δ 170.6 (COOH), 146.0 (C-3, 5), 139.4 (C-4), 121.7 (C-1), 110.4 (C-2, 6); EIMS m/z 170 $[M]^+$ (100), 153 (95), 135 (26), 125 (50), 113 (26), 79 (69).

(+)-Catechin (**7**): $C_{15}H_{14}O_6$, white amorphous powder; ^{1}H NMR (Me_2CO-d_6, 400 MHz) δ 6.94 (1H, d, $J = 2.0$ Hz, H-2′), 6.84 (1H, d, $J =$ 8.0 Hz, H-5′), 6.80 (1H, dd, $J = 2.0$, 8.0 Hz, H-6′), 6.07 (1H, d, $J = 2.4$ Hz, H-8), 5.92 (1H, d, $J = 2.4$ Hz, H-6), 4.60 (1H, d, $J = 7.6$ Hz, H-2), 4.03 (1H, m, H-3), 2.96 (1H, dd, $J = 5.2$, 16.0 Hz, H-4a), 2.59 (1H, dd, $J = 8.8$, 16.0 Hz, H-4b); ^{13}C NMR (Me_2CO-d_6, 100 MHz) δ 81.8 (C-2), 67.3 (C-3), 27.9 (C-4),

144.7 (C-5), 94.4 (C-6), 156.8 (C-7), 95.1 (C-8), 156.3 (C-9), 99.7 (C-10), 131.2 (C-1′), 114.7 (C-2′), 144.7 (C-3′), 156.0 (C-4′), 119.1 (C-5′), 114.3 (C-6′); FABMS *m/z* 289 $[M-H]^-$ (100).

Acknowledgments

We are grateful to the analytical group of Kunming Institute of Botany for recording NMR, MS, UV, IR spectra and optical rotations. Thanks also go to Prof. T. Yoshida (Okayama University, Japan) for NMR, MS measurements and structure elucidation of eucalbanin C, and P&G Company (Japan) for antibacterial assay.

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