Euglobal T1, a New Euglobal from Eucalyptus tereticornis

Midori Kokumai, Takao Konoshima, Mutsuo Kozuka, Mitsumasa Haruna, and Kazuo Ito

J. Nat. Prod., **1991**, 54 (4), 1082-1086• DOI: 10.1021/np50076a025 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 4, pp. 1082-1086, Jul-Aug 1991

EUGLOBAL T1, A NEW EUGLOBAL FROM *EUCALYPTUS TERETICORNIS*

MIDORI KOKUMAI,* TAKAO KONOSHIMA, MUTSUO KOZUKA,

Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607, Japan

MITSUMASA HARUNA, and KAZUO ITO

Faculty of Pharmacy, Meijo University, Yagoto, Tempaku-ku, Nagoya 468, Japan

ABSTRACT.—From the juvenile leaves of *Eucalyptus tereticornis*, a new euglobal having a phlorophene-monoterpene structure, euglobal T1 [**1**], has been isolated along with the known euglobal IIc [**2**]. The structure and stereochemistry of **1** were established by spectroscopic evidence.

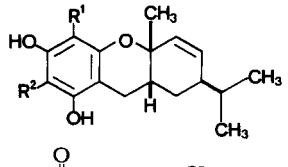
As a part of our continuing chemical studies on euglobals (1) that have unique acylphloroglucinol-monoterpene (or -sesquiterpene) structures and biological studies on the potential anti-tumor-promoting activities of natural products (2–5), we have investigated *Eucalyptus tereticornis* Sm. (Myrtaceae). In the previous paper, we reported the isolation and structure elucidation of twelve euglobals from *Eucalyptus globulus* (1,6) and three euglobals from *Eucalyptis grandis* (7), and the inhibitory effects of these euglobals and related compounds on Epstein-Barr virus (EBV) activation (7,8).

In this paper, we report the isolation and structure elucidation of a new euglobal, euglobal T1 [**1**], and a known compound, euglobal IIc [**2**], from the juvenile leaves of *E. tereticornis*. Euglobal T1 [**1**] showed weak inhibition, similar to that of euglobal IIc [**2**] (8), on Epstein-Barr virus activation.

Compound **2** was identified with an authentic sample of euglobal IIc obtained from *E. globulus* (6) by the hplc and tlc behavior, and ir and ^{1}H-nmr spectra.

Compound **1** has the same composition, $C_{23}H_{30}O_5$ (ms [M]$^+$ 386), as compound **2** and showed uv, ir, and ms data similar to those of reported euglobals that have monoterpene structures, especially euglobal IIc (6). The structure elucidation of euglobal T1 and the reconfirmation of the stereochemistry of euglobal IIc were carried out using 2D nmr spectra and difference nOe experiments as follows.

The ^{1}H-^{13}C long range COSY of euglobal T1 [**1**] was measured in order to confirm the connectivities of the partial structure and substituent groups. As shown in Figure 1, the methylene protons at δ 2.69 and 2.40 (H_2-7) are correlated with the carbons at δ 79.19 (C-1′), 99.64 (C-1), 162.05 (C-2), and 167.04 (C-6), and the proton at δ 13.20 (6-OH) is correlated with the carbons at δ 99.64 (C-1), 104.42 (C-5), and 167.04 (C-6). The aldehyde proton at δ 10.18 (8-CHO) is correlated with the carbons at δ 104.42 (C-5) and 167.04 (C-6), and the proton at δ 15.40 (4-OH) is correlated with the carbons at δ 103.97 (C-3) and 169.94 (C-4). Some other significant long-range ^{1}H-^{13}C correlations are indicated by arrows in Figure 1. From these results, the positions of the



1 R^1=-C(=O)-CH_2-CH<(CH_3, CH_3), R^2=-C(=O)-H

2 R^1=-C(=O)-H, R^2=-C(=O)-CH_2-CH<(CH_3, CH_3)

aldehyde and the isovaleryl groups of euglobal T1 were concluded to be at C-5 and C-3, respectively.

The ^{1}H-^{13}C long range COSY spectrum of euglobal IIc showed the correlations of the aldehyde proton at δ 10.03 (8-CHO) with the carbons at δ 103.73 (C-3) and 168.09 (C-4), the proton at δ 14.46 (4-OH) with the carbons at δ 103.73 (C-3) and 168.09 (C-4), and the proton at δ 15.39 (6-OH) with the carbons at δ 103.41 (C-5), 100.51 (C-1), and 171.36 (C-6). Some other significant long range correlations are indicated in Figure 2, supporting the reported result that the positions of the aldehyde

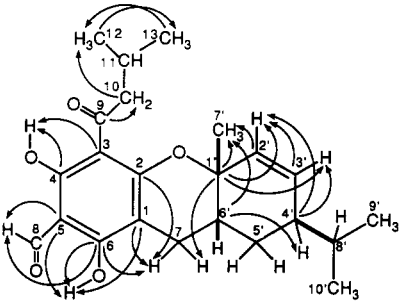


FIGURE 1. Correlation ($^{13}C \curvearrowright {}^{1}H$) in ^{1}H-^{13}C long-range COSY spectrum of euglobal T1 [**1**].

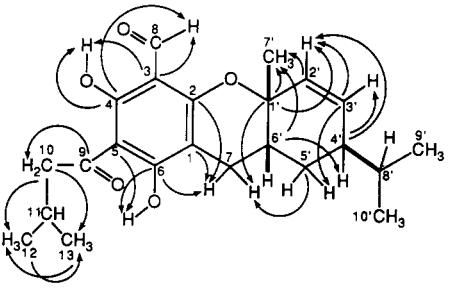


FIGURE 2. Correlation ($^{13}C \curvearrowright {}^{1}H$) in ^{1}H-^{13}C long-range COSY spectrum of euglobal IIc [**2**].

and the isovaleryl groups on euglobal IIc were at C-3 and C-5, respectively. All proton and carbon signals of euglobal IIc and T1 could be assigned by ^{1}H-^{1}H COSY, DEPT experiment, ^{1}H-^{13}C COSY, and ^{1}H-^{13}C long range COSY spectra, as shown in Table 1.

The relative stereochemistry of euglobal IIc had been deduced from the values of ^{13}C chemical shifts of C-1′ and C-2′ and from consideration of Dreiding models (3). In this paper, the difference nOe experiments were performed to support the reported results on the structure of euglobal IIc [**2**] and to confirm the relative stereochemistry of euglobal T1 [**1**]. In the difference nOe spectra of **2**, irradiation at δ 1.49 (H_3-7′) enhanced the signal intensities of the olefinic proton (H-2′ at δ 5.69), methine proton (H-6′ at δ 2.07), aldehyde proton (8-CHO at δ 10.03), and H-7β (at δ 2.65). Irradiation at the methine proton (H-6′ at δ 2.07) enhanced the signal intensities of the methyl protons (H_3-7′ at δ 1.49) and one of the methylene protons at δ 2.65 (H-7β), and irradiation at one of the methylene protons at δ 2.65 (H-7β) enhanced the intensities of methine proton at δ 2.07 (H-6′) and the methyl protons at δ 1.49 (H_3-7′). Some other significant difference nOe results are indicated by arrows in Figure 4. From these results, the relative stereochemistry of euglobal IIc [**2**] was reconfirmed as shown in Figure 4.

In the difference nOe spectra of **1**, irradiation at δ 1.54 (H_3-7′) enhanced the signal intensities of the olefinic proton (H-2′, at δ 5.73), methine proton (H-6′, at δ 2.08) and one of the methylene protons (H-7β, at δ 2.69). Irradiation at the methine proton (H-4′, at δ 2.14), enhanced the signal intensities of the olefinic proton (H-3′, at δ 5.78), one of the methylene protons (H-7α, at δ 2.40) and the methylene protons (H_2-5′, at δ 1.75), and irradiation at the methyl protons (H_3-9′, -10′, at δ 0.96 and 0.93) enhanced the signal intensities of the olefinic proton (H-3′, at δ 5.78) and the methine proton (H-4′, at δ 2.14) (Figure 3). From these difference nOe results of **1** and from the consideration of Dreiding models, the structure and relative configuration of euglobal T1 were assigned as **1** exclusive of the absolute configuration.

TABLE 1. ^{13}C and ^{1}H Chemical Shift Values of Euglobals T1 [**1**] and IIc [**2**].[a]

Position	Compound **2**		Position	Compound **1**	
	^{13}C	^{1}H		^{13}C	^{1}H
1	100.51		1	99.64	
2	160.83		2	162.05	
3	103.73		3	103.97	
4	168.09		4	169.94	
5	103.41		5	104.42	
6	171.36		6	167.04	
7	20.41	2.65 (1H, dd, J = 16.6, 6.3 Hz) 2.38 (1H, dd, J = 16.6, 7.8 Hz)	7	20.10	2.69 (1H, dd, J = 16.7, 6.3 Hz) 2.40 (1H, dd, J = 16.7, 7.8 Hz)
8	191.83	10.03 (1H, s)	8	192.33	10.18 (1H, s)
9	206.32		9	205.93	
10	52.68	2.96 (2H, d, J = 6.8 Hz)	10	53.14	2.96 (1H, dd, J = 14.8, 6.8 Hz) 2.85 (1H, dd, J = 14.8, 7.1 Hz)
11	25.07	2.25 (1H, m)	11	25.41	2.20 (1H, m)
12	22.77	0.98 (6H, d, J = 6.6 Hz)	12	22.73	0.98 (3H, d, J = 6.7 Hz)
13			13	22.65	0.97 (3H, d, J = 6.6 Hz)
1′	78.45		1′	79.19	
2′	130.61	5.69 (1H, br d, J = 10.1 Hz)	2′	130.42	5.71 (1H, br d, J = 10.2 Hz)
3′	133.12	5.74 (1H, dd, J = 10.1, 2.9 Hz)	3′	133.40	5.78 (1H, dd, J = 10.2, 2.9 Hz)
4′	38.75	2.13 (1H, m)	4′	38.70	2.14 (1H, m)
5′	27.59	1.75, 1.69 (each 1H, m)	5′	27.34	1.75, 1.70 (each 1H, m)
6′	33.21	2.07 (1H, m)	6′	32.66	2.08 (1H, m)
7′	27.49	1.49 (3H, s)	7′	27.61	1.54 (3H, s)
8′	31.80	1.72 (1H, m)	8′	31.75	1.71 (1H, m)
9′	20.04	0.95 (3H, d, J = 6.8 Hz)	9′	20.01	0.96 (3H, d, J = 6.8 Hz)
10′	19.81	0.93 (3H, d, J = 6.7 Hz)	10′	19.79	0.93 (3H, d, J = 6.7 Hz)
6-OH		15.39 (1H, s)	6-OH		13.20 (1H, s)
4-OH		14.46 (1H, s)	4-OH		15.40 (1H, s)

[a]Ppm (δ) relative to TMS. ^{13}C chemical shifts were assigned on the basis of DEPT and ^{1}H-^{13}C COSY experiments, and ^{1}H chemical shifts were assigned on the basis of ^{1}H-^{1}H and ^{1}H-^{13}C COSY experiments.

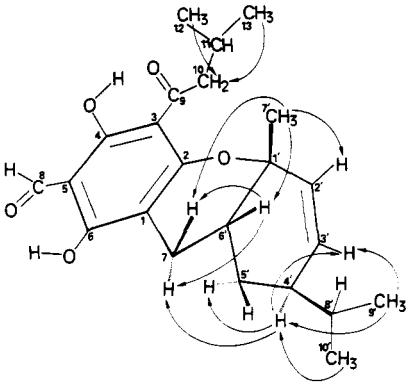


FIGURE 3. Significant enhancement of signal intensity by difference nOe experiments of euglobal T1 [**1**].

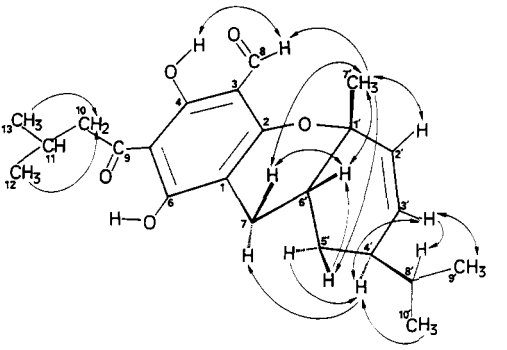


FIGURE 4. Significant enhancement of signal intensity by difference nOe experiments of euglobal IIc [**2**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were obtained on a Shimazu 210-A spectrophotometer in 95% EtOH, and ir spectra were measured on a Shimadzu IR-408 spectrometer. ^{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 difference nOe spectra were recorded on a JEOL JNM GX-400 spectrometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter at 27°. Mass spectra were determined on a Hitachi M-80 mass spectrometer. 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 MeCN (5.0 ml/min) as an eluent. Pre-coated Si gel plates (Kieselgel 60 F254, 0.25 mm, Merck) were used for analytical tlc, and euglobals were detected by spraying with 10% H_2SO_4 solution containing anisaldehyde followed by heating.

PLANT MATERIAL.—The juvenile leaves of *E. tereticornis* were collected in Nagoya, Japan, on 10 January 1988. A voucher specimen was deposited at the Herbarium of Kyoto Pharmaceutical University.

EXTRACTION AND ISOLATION.—The air-dried juvenile leaves (50 g) of *E. tereticornis* were extracted with $CHCl_3$ at room temperature, and the $CHCl_3$ extract was evaporated in vacuo to give a dark brown tar (5.76 g). The residue was chromatographed on Si gel eluting with C_6H_6 followed with C_6H_6-$CHCl_3$ (1:1) to yield a crude euglobal fraction (331.9 mg). The fraction was rechromatographed on ODS by hplc to yield a new euglobal (85.4 mg) for which the name euglobal T1 [**1**] was proposed. Euglobal IIc [**2**] (91.2 mg) was also isolated by the reversed-phase hplc.

EUGLOBAL T1.—Colorless oil: $[\alpha]_D$ −143.7° (c = 0.39, $CHCl_3$); ir ($CHCl_3$) 3600–3400, 2950, 1615 cm^{-1}; uv (95% EtOH) λ max (ε) 278 (21,600), 343 (5600); eims *m/z* $[M]^+$ 386, 251, 195, 148, 93; hrms found 386.2063, calcd for $C_{23}H_{30}O_5$, 386.2090.

EUGLOBAL IIc.—Euglobal IIc was identified by comparison with ir, ^{1}H- and ^{13}C-nmr spectra, and chromatographic data (hplc and tlc) of an authentic sample.

ACKNOWLEDGMENTS

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

LITERATURE CITED

1. M. Kozuka, T. Sawada, E. Mizuta, F. Kasahara, T. Amano, T. Komiya, and M. Goto, *Chem. Pharm. Bull.*, **30**, 1964 (1982), and references cited therein.
2. T. Konoshima, E. Okamoto, M. Kozuka, H. Nishino, H. Tokuda, and M. Tanabe, *J. Nat. Prod.*, **51**, 1266 (1988).
3. T. Konoshima, M. Takasaki, M. Kozuka, A. Inada, T. Nakanishi, H. Tokuda, and T. Matsumoto, *Shoyakugaku Zasshi*, **43**, 135 (1989).
4. B. Diallo, M. Vanhaelen, R. Vanhaelen, T. Konoshima, M. Kozuka, and H. Tokuda, *J. Nat. Prod.*, **52**, 879 (1989).

5. H. Tokuda, T. Konoshima, M. Kozuka, and T. Kimura, *Oncology*, **48**, 77 (1991).
6. M. Kozuka, T. Sawada, F. Kasahara, E. Mizuta, T. Amano, T. Komiya, and M. Goto, *Chem. Pharm. Bull.*, **30**, 1952 (1982).
7. M. Takasaki, T. Konoshima, T. Shingu, H. Tokuda, H. Nishino, A. Iwashima, and M. Kozuka, *Chem. Pharm. Bull.*, **38**, 1444 (1990).
8. M. Takasaki, T. Konoshima, K. Fujitani, S. Yoshida, H. Nishimura, H. Tokuda, H. Nishino, A. Iwashima, and M. Kozuka, *Chem. Pharm. Bull.*, **38**, 2737 (1990).

Received 4 February 1991