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EUCALYPTONE FROM *EUCALYPTUS GLOBULUS*

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Key Word Index—*Eucalyptus globulus*; Myrtaceae; eucalyptone; antibacterial activity; cariogenic bacteria; glucosyltransferase.

Abstract—A new cariostatic compound named eucalyptone was isolated from the leaves of *Eucalyptus globulus*. The structure of this compound was elucidated by spectroscopic methods.

INTRODUCTION

Plants of the genus *Eucalyptus* contain many kinds of phloroglucinol derivatives, such as macrocarpals and euglobals, and some of these compounds possess interesting biological activities [1–3]. In our continuing studies [4, 5] on cariostatic compounds from natural sources, an isopentyl phloroglucinol–sesquiterpene coupled compound, named eucalyptone (**1**), was isolated from the leaves of *Eucalyptus globulus*. This compound has a unique sesquiterpene moiety with both a five-membered ring and a cyclopropane ring system. This paper deals with the structural analysis and cariostatic activities of this compound.

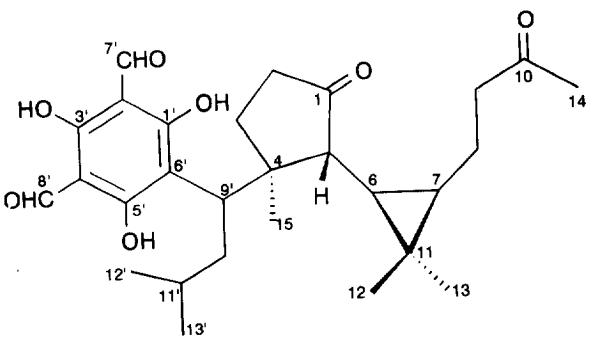

Fig. 1. Molecular structure of **1**.

RESULTS AND DISCUSSION

The 50% EtOH-soluble material from the dried leaves of *E. globulus* showed appreciable antibacterial activity against cariogenic bacteria and also inhibited the enzyme glucosyltransferase (GTase) [6]. An active principal was found in the EtOAc-soluble fraction. This fraction was subjected to silica gel column chromatography followed by silica gel and ODS HPLC to afford compound **1**, which we have named eucalyptone, along with other macrocarpals.

Eucalyptone (**1**) was obtained as colourless powder. The high-resolution FAB mass spectrum supported the molecular formula of $C_{28}H_{39}O_7$ and the UV and IR data were similar to those of macrocarpals [1–3]. The ^{1}H and ^{13}CNMR spectra established that **1** contained a phloroglucinol moiety [δC 172.4(*s*) × 2, 173.2(*s*), 107.1(*s*), 107.0(*s*) and 105.6(*s*)] containing two aldehyde groups [δH 10.48 and 10.50 (each 1H, *s*), δC 191.8 and 191.9 (each *d*)], and a methine moiety [δH 3.51 (1H, *dd*, J = 11.8, 4.0 Hz), δC 39.7(*d*)] bearing an isobutyl side chine [δC 36.3(*t*), 27.3(*d*), 21.6 and 24.4 (each *q*)]. They also showed the presence of one carbonyl group [δC 221.3(*s*)], one acetyl group [δH 2.07 (3H, *s*), δC 208.3(*s*), 29.6(*q*)], two methine groups forming a cyclopropane ring [δH 0.61 (1H, *dd*, J = 7.0, 2.0) and 0.61 (1H, *d*, J = 7.0), δC 27.3 and 26.5 (each *d*)], the *tert* methyl, four methylene and one methine group, and two quaternary carbons in a sesquiterpene moiety. The carbon linkage and carbonyl and acetyl connected sites for the sesquiterpene part of **1** were determined by means of 2D-NMR techniques, including ^{1}H-^{1}H COSY, HMQC and HMBC spectra at 500 MHz (Table 1). As can be seen from formula **1**, eucalyptone (**1**) has a unique, sesquiterpene moiety containing both a five-membered ring and a cyclopropane ring system. The relative configurations at C-4, 5, 6 and 9 were elucidated as shown in Fig. 1 by NOE correlations in the NOESYPH spectrum.

Eucalyptone (**1**) has antibacterial activity against cariogenic bacteria (MIC: 12.5 μg ml^{-1} against *Streptococcus mutans* Ingbritt, *Streptococcus sobrinus* 6715 and *S. sobrinus* B13; 6.25 μg ml^{-1} against *S. mutans* LA7) and an inhibitory effect on adherent water-insoluble glucan synthesis by GTase (97.6 and 44.0% inhibition at concentration of 100 and 10 μg ml^{-1}) prepared from the supernatant of *S. sobrinus* 6715 [6]. These data indicate that **1** might be a promising natural substance for the development of a new cariostatic drug.

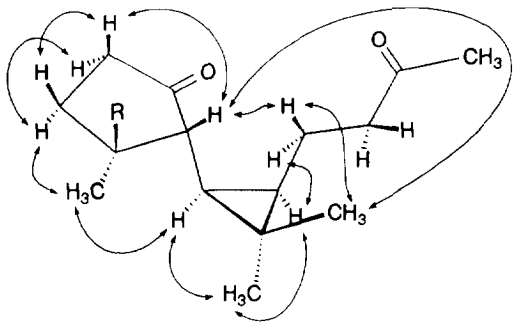

Fig. 2. NOE relationships of **1** in pyridine-d_5; R = isopentyl phloroglucinol.

Table 1. ^{13}C and ^{1}H spectral data, and HMBC correlations of **1** in pyridine-d_5

C	^{13}C (DEPT)	HMBC	^{1}H (J)
1	221.3 (*s*)	H-2, 3, 5	
2	35.8 (*t*)		α2.37 *ddd* (10.4, 10.4, 3.5)
			β2.65 *m*
3	32.4 (*t*)	H-15	α1.75 *dd* (10.4, 3.5)
			β2.17 *ddd* (10.4, 10.4, 2.3)
4	47.6 (*s*)	H-9′, 2, 15	
5	20.7 (*t*)	H-15	2.50 *d* (7.0)
6	27.3 (*d*)	H-5, 12, 13	0.61 *dd* (7.0, 2.0)
7	26.5 (*d*)	H-5, 12, 13	0.61 *d* (7.0)
8	44.4 (*t*)		2.30 *m*
			1.78 *dd* (9.3, 7.0)
9	54.8 (*d*)		2.54 *dd* (9.3, 7.0)
			2.68 *m*
10	208.3 (*s*)	H-9, 14	
11	18.2 (*s*)	H-12, 13	
12	16.0 (*q*)	H-13	1.13 *s*
13	28.9 (*q*)	H-6, 7, 12	1.11 *s*
14	29.6 (*q*)		2.07 *s*
15	21.6 (*q*)	H-9′	1.37 *s*
1′	172.4 (*s*)	H-7′	
2′	107.1 (*s*)	H-7′	
3′	173.2 (*s*)	H-7′, 8′	
4′	107.0 (*s*)	H-8′	
5′	172.4 (*s*)	H-8′	
6′	105.6 (*s*)	H-9′, 10′	
7′	191.8 (*d*)		10.48 *s*
8′	191.9 (*d*)		10.50 *s*
9′	39.7 (*d*)	H-5, 3, 15	3.51 *dd* (11.8, 4.0)
10′	36.3 (*t*)	H-9′, 12′, 13′	2.71 *dd* (13.4, 4.0)
			1.40 *dd* (13.4, 4.1)
11′	27.3 (*d*)	H-12′, 13′	1.55 *m*
12′	21.6 (*q*)	H-13′	0.89 *d* (6.2)
13′	24.4 (*q*)	H-12′	0.92 *d* (6.2)

Chemical shift values are in ppm. The coupling constants (J values) in parentheses are in Hz.

EXPERIMENTAL

General. HPLC: Senshu Pak silica-5251-S for normal phase and Senshu Pak ODS-5251-SS for reversed phase.

Plant material. The dried leaves of *E. globulus* used in this experiment were purchased from Charis Seijiyo, Tokyo in 1994.

Assay of antibacterial activity. Aliquots (100 μl) of two-fold serial dilutions of the test substance were placed into the wells of a flat-bottomed 96-well plate (Costar). Subsequently, 100 μl of two-fold concentrated Brain Heart Infusion broth (Difco) containing $1–2 \times 10^5$ c.f.u. ml^{-1} test organisms were inoculated into each well. The assay mixtures were incubated at 37° for 24 hr, and the minimum inhibitory concentration (MIC) recorded as the lowest concentration of test substance to inhibit growth.

Assay of GTase inhibitory activity. See previous paper [6].

Extraction and isolation. The dried leaves (1 kg) of *E. globulus* were extracted with 5 l 50% EtOH, to give 233 g of conct. extract. The extract (100 g) was fractionated into EtOAc (29 g), *n*-BuOH (31 g) and water (43 g)-soluble fractions. The EtOAc-soluble fraction was subjected to silica gel CC with CH_2Cl_2–MeOH (20:1–2:1 stepwise elution) and separated into 10 fractions. The biologically active fraction (896 mg) was separated by silica gel HPLC with CH_2Cl_2–MeOH (17:3) and ODS HPLC with MeOH–H_2O (9:1) to afford **1** (27.4 mg) along with other macrocarpals.

Eucalyptone (**1**). Powder, $[\alpha]_D$ + 44.6° (EtOH; c 0.19); IR ν_{max}^{KBr} cm^{-1}: 3411, 2955, 1711, 1625; UV λ_{max}^{EtOH} (log ε): 274 nm (4.15); HI FAB-MS (pos.) *m/z* 487 (calc. for $C_{28}H_{39}O_7$ 487.2696; found: 487.2685).

REFERENCES

1. Nishizawa, N., Emura, M., Yasuda, Y., Ogawa, K. and Hamanaka, N. (1992) *Tetrahedron Letters* **33**, 2983.
2. Kozuka, M., Sawada, T., Kasahara, F., Mizuta, E., Amano, T., Komiya, T. and Goto, M. (1982) *Chem. Pharm. Bull.* **30**, 1952.
3. Murata, M., Yamakoshi, Y., Homma, S., Arai, K. and Nakamura, Y. (1992) *Biosci. Biotech. Biochem.* **56**, 2062.
4. Osawa, K., Yasuda, H., Maruyama, T., Morita, H., Takeya, K. and Itokawa, H. (1992) *Chem. Pharm. Bull.* **40**, 2970.
5. Osawa, K., Yasuda, H., Maruyama, T., Morita, H., Takeya, K. and Itokawa, H. (1994) *Chem. Pharm. Bull.* **42**, 922.
6. Onogi, A., Osawa, K., Yasuda, H., Sakai, A., Morita, H. and Itokawa H. (1993) *Shoyakugaku Zasshi* **47**, 423.