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Macrocarpals H, I, and J from the Leaves of *Eucalyptus globulus*

Kenji Osawa* and Hideyuki Yasuda

Department of Basic Research, Lotte Central Laboratory Co., Ltd., Numakage 3-1-1, Urawa, 336 Saitama, Japan

Hiroshi Morita, Koichi Takeya, and Hideji Itokawa

Department of Pharmacognosy, School of Pharmacy, Tokyo University of Pharmacy and Life Science, Horinouchi 1432-1, Hachioji, 192-03 Tokyo, Japan

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A 50% EtOH extract of *Eucalyptus globulus* leaves yielded eight phloroglucinol−sesquiterpene-coupled constituents, including three novel compounds named macrocarpals H, I, and J. Some of these compounds possessed antibacterial activity against oral pathogenic microorganisms with MIC values ranging from 0.20 μg/mL to 6.25 μg/mL. Inhibition of glucosyltransferase activity by these compounds was also noted.

Dental caries and periodontal disorders are major infectious diseases. It is now apparent that Gram-positive bacteria such as the mutans streptococci group, mainly *Streptococcus mutans* and *Streptococcus sobrinus*, are important human cariogenic bacteria that synthesize insoluble glucans from sucrose by secreting glucosyltransferase (GTase), adhere to tooth surfaces, and produce acids.[1,2] Many workers also consider that the causative microorganisms of periodontal diseases are specific anaerobic Gram-negative bacteria,[3] including *Porphyromonas gingivalis*, which possesses lipopolysaccharides,[4] proteolytic enzymes,[3] and other periodontopathic factors.[5] To prevent such dental diseases, it may be important to control the growth of these bacteria in the oral cavity with the use of compounds having antibacterial properties.[6,7] In previous papers, we have reported that several isoflavanones from *Swartzia polyphylla*,[8] and some diterpenes from *Rabdosia trichocarpa*[9,10] showed potent antibacterial activity against cariogenic and periodontopathic bacteria. In the course of our antibacterial studies using natural products, we observed that a 50% EtOH extract prepared from dried leaves of *Eucalyptus globulus* Labill. (Myrtaceae) exhibited potent antibacterial activity against cariogenic and periodontopathic bacteria. This extract also inhibited adherent H_2O-insoluble glucan synthesis by extracellular GTase prepared from *S. sobrinus* 6715.[11] Plants of the genus *Eucalyptus* have been shown to produce a number of phloroglucinol−sesquiterpene- or −monoterpene-coupled compounds, namely, the macrocarpals[12,13] and euglobals.[14,15] Several reports have revealed biological activities such as HIV-RTase inhibition,[12] granulation inhibition,[14,15] and antiviral[16] and antibacterial[13] effects. In the present study, fractionation of an active extract of *E. globulus*, followed by bioassay, led to the isolation and characterization of eight phloroglucinol−sesquiterpene-coupled compounds.

A 50% EtOH-soluble material was extracted from the dried leaves of *E. globulus*. The extract showed appreciable antibacterial activity against *S. mutans* Ingbritt and *P. gingivalis* ATCC 33277 using the broth dilution method (MICs were 12.5 and 6.25 μg/mL, respectively), and an inhibitory effect of GTase (44.9% inhibition at 10 μg/mL). An aqueous solution of the 50% EtOH extract was partitioned successively with EtOAc

* To whom correspondence should be addressed. Phone: 048-837-0162. FAX: 048-863-1511.

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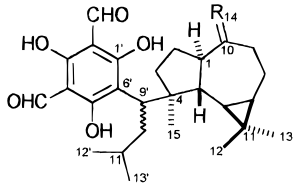
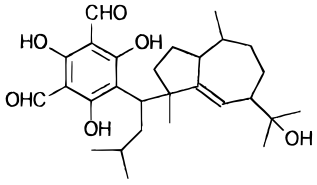
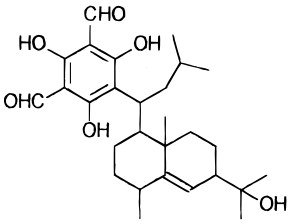
and *n*-BuOH. The biological activity was found to be concentrated in the EtOAc-soluble fraction. This fraction was subjected to Si gel column chromatography to yield several subfractions. From the biologically active subfractions, eight phloroglucinol−sesquiterpene-coupled compounds (**1**−**4**, **6**−**9**) were isolated by repeated HPLC separation. Among these eight compounds, five were identified as the known macrocarpals A (**1**), B (**2**), C (**3**), and D (**4**)[12] and eucalyptone (**6**).[17] We have carried out a structural investigation of the three novel compounds isolated, namely, macrocarpals H (**7**), I (**8**), and J (**9**).

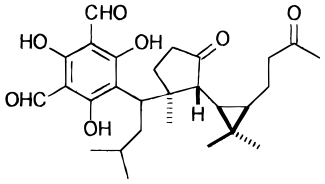




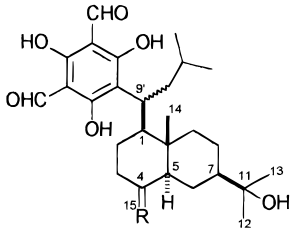
1 R=α-OH, β–Me; 9'β-isobutyl
2 R=α-OH, β–Me; 9'α-isobutyl
3 R=CH_2; 9'β-isobutyl

4





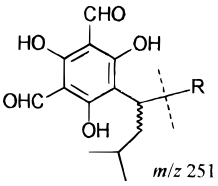
5 **6**



7 R=CH_2
8 R=α-OH, β-Me
9 R=α-OH, β-Me

Results and Discussion

Compound **7** was obtained as a pale yellow powder, and its HREIMS was consistent with a molecular formula of $C_{28}H_{40}O_6$. The IR and UV data of **7** were closely comparable to those of macrocarpals A−E (**1**−**5**). From an inspection of its ^{1}H- and ^{13}C-NMR spectra, **7** was found to possess an isobutyl side chain [^{1}H NMR: δ 2.28, 1.29, 1.53, and two doublet methyls at δ 1.12 and 0.93 (J = 6.4)], a phloroglucinol unit [^{13}C NMR: δ 171.1 (s) × 2, 169.0 (s), 107.2 (s), 106.6 (s), and 113.8 (s)] bearing two aldehydes (^{1}H NMR: δ 10.51 and 10.54), and a methine carbon (^{1}H NMR: δ 3.73) bearing an isobutyl side chain. These NMR data and the fragment ion peak of EIMS at *m*/*z* 251 suggested **7** as having the common structural unit of macrocarpals A−E (**1**−**5**) as shown in Figure 1. The NMR data of **7** were closely related to those of macrocarpal E (**5**).[12] Since these compounds had the same molecular formula, compounds **5** and **7** were assigned as isomers having a eudesmane-type sesquiterpene skeleton. Based on ^{1}H−^{1}H COSY, HMQC, and HMBC experiments, the ^{1}H- and ^{13}C-NMR spectral assignments for **7** are summarized in Tables 1 and 2. The HMBC experiments showed that the exomethylene protons at δ 4.86 and 4.65 were correlated with the C-3 and C-5 signals, which indicated that the exomethylene functionality was present at the $\Delta^{4(15)}$ position. The HMBC spectrum also showed that two methyl protons assigned to H-12 and H-13 (δ 1.35 and 1.36) were correlated with an oxygen-bearing methine carbon (δ 71.4) and C-7, which indicated that the hydroxyl group was located at the C-11 position. Other HMBC correlations as shown in Table 2 also supported the proposed structure of macrocarpal H (**7**). The relative stereochemistry of **7** was elucidated as in Figure 2 from a phase-sensitive NOESY NMR experiment.



m/*z* 251

Figure 1. Partial structure of the macrocarpals.

Macrocarpals I (**8**) and J (**9**) were obtained as pale yellow powders. The common molecular formula for **8** and **9**, $C_{28}H_{42}O_7$, was derived by HREIMS. The IR and UV data of these two compounds were closely related to those of compound **7**, which indicated that they also have a similarly substituted phloroglucinol moiety. The ^{1}H- and ^{13}C-NMR data of **8** and **9** also suggested that these compounds possess an isobutyl side chain and a phloroglucinol moiety bearing two aldehydes as in **7**. The fragment ion peak from the EIMS at *m*/*z* 251 suggested the same common structural unit of the macrocarpals as shown in Figure 1. In addition, these two compounds exhibited the same fragmentation pattern in the EIMS and showed similarities in their ^{1}H- and ^{13}C-NMR spectral data as shown in Tables 1 and 2. The ^{13}C-NMR spectra of **8** and **9** showed the presence of four methyls, five methylenes, three methine groups, and three quaternary carbons, which included two carbons bearing a hydroxyl group in the sesquiterpene moiety. ^{1}H−^{1}H COSY, HMQC, and HMBC NMR experiments on these two compounds were carried out to confirm the carbon linkage and connectivity of the hydroxyl groups. These 2D-NMR methods revealed that the two compounds had the eudesmane-type sesquiterpene skeleton. Comparison of their spectral data with those of **7** suggested the substitution of a methyl signal at δ 1.28 (**8**) or 1.41 (**9**) in place of the exomethylene signals at δ 4.86 and 4.65 in **7**. The HMBC NMR spectra of **8** and **9** showed that the two compounds had almost the same correlation patterns between their proton and carbon signals, and indicated that these substances are stereochemical isomers. The HMBC spectra of these two compounds showed that the H-15 methyl at δ 1.28 in **8** and at δ 1.41 in **9**, and the H-2, H-3, and H-5 signals were correlated with an oxygen-bearing methine carbon at δ 71.0 (**8**) or δ 71.1 (**9**), and the two methyl protons assigned to H-12 and H-13 were correlated with another oxygen-bearing methine carbon at δ 71.6 (**8**) or δ 71.6 (**9**) and C-7, respectively. These correlations indicated that the two hydroxyl groups of **8** and **9** were in the same positions at C-4 and C-11 in the sesquiterpene moiety. From the NOE results of **8** and **9**, the relative configurations at C-1, C-4, C-5, C-7,

Table 1. ^{1}H-NMR Data of Compounds **7**−**9** in Pyridine-d_5[a]

protons(s)	compound **7**	**8**	**9**
1	2.03, m	1.75, br d, J = 11.5 Hz	1.90 br d, J = 12.5 Hz
2	α 2.50, d, J = 13.0 Hz	α 2.09, dd, J = 11.5, 2.8 Hz	α 2.50, br d, J = 12.5 Hz
	β 1.72, ddd, J = 13.0 Hz	β 1.46, m	β 1.70, ddd, J = 12.5, 12.5, 3.0 Hz
3	α 2.21, ddd, J = 13.0, 12.5, 4.9 Hz	α 1.90, dd, J = 12.0, 3.8 Hz	α 1.96, ddd, J = 12.5, 12.5, 3.0 Hz
	β 2.50, d, J = 12.5 Hz	β 2.05, ddd, J = 12.0, 2.8, 2.8 Hz	β 2.19, ddd, J = 12.5, 3.0, 3.0 Hz
5	1.90, br d, J = 9.8 Hz	1.72, dd, J = 11.3, 2.5 Hz	1.63, d, J = 12.0 Hz
6	α 2.00, m	α 2.73, d, J = 11.5 Hz	α 2.71 br d, J = 9.9 Hz
	β 1.52, m	β 1.52, m	β 1.50, m
7	1.48, m	1.63, m	1.55, m
8	α 1.80, br d, J = 7.7 Hz	α 1.95, br d, J = 12.0 Hz	α 1.79, br d, J = 10.0 Hz
	β 1.50, m	β 1.60, m	β 1.52, m
9	α 1.23, dd, J = 13.0, 4.5 Hz	α 1.36, dd, J = 12.3, 4.3 Hz	α 1.15, m
	β 2.56, d, J = 13.0 Hz	β 2.37, ddd, J = 12.3, 2.5, 2.5 Hz	β 2.51, d, J = 12.3 Hz
12	1.35, s	1.41, s	1.37, s
13	1.36, s	1.43, s	1.38, s
14	0.97, s	1.22, s	1.19, s
15	4.86, s	1.28, s	1.41, s
	4.65, s		
7′	10.51, s	10.53, s	10.48 s
8′	10.54, s	10.54, s	10.53, s
9′	3.73, ddd, J = 13.0, 2.4, 2.4 Hz	3.89, ddd, J = 11.6, 4.0, 4.0 Hz	3.77, ddd, J = 12.8, 3.3, 3.3 Hz
10′	2.28, ddd, J = 13.0, 13.0, 2.4 Hz	2.71, dd, J = 11.6, 11.6 Hz	2.35, ddd, J = 12.8, 12.8, 2.4 Hz
	1.29, m	1.55, m	1.43, m
11′	1.53, m	1.57, m	1.57, m
12′	0.93, d, J = 6.4 Hz	1.05, D, J = 5.8 Hz	1.16, d, J = 6.6 Hz
13′	1.12, d, J = 6.4 Hz	0.95, d, J = 5.8 Hz	0.96, d, J = 6.6 Hz

[a] Chemical shift values are in ppm.

Table 2. ^{13}C-NMR Data and HMBC Correlations of Compounds **7**−**9** in Pyridine-d_5[a]

carbon	7		8		9	
	δ_C	HMBC correlation	δ_C	HMBC correlation	δ_C	HMBC correlation
1	53.8 d	H-3, 14, 9′	54.8 d	H-3, 14, 9′	54.3 d	H-3, 14, 9′
2	27.6 t	H-1	25.2 t	H-3	24.0 t	
3	37.9 t	H-15	45.3 t	H-1, 15	45.0 t	H-15
4	152.1 s	H-2, 3, 5	71.0 s	H-2, 3, 5, 15	71.1 s	H-2, 3, 5, 15
5	51.8 d	H-1, 3, 9, 14, 15	57.1 d	H-9, 3, 14, 15	57.3 d	H-3, 9, 14, 15
6	25.8 t	H-5, 8	22.6 t	H-5, 8	22.4 t	H-5
7	49.7 d	H-6, 9, 12, 13	50.4 d	H-9, 12, 13	50.5 d	H-9, 6, 12, 13
8	23.4 t	H-6	23.6 t	H-6, 7, 9	23.5 t	H-6, 7, 9
9	38.0 t	H-14	43.4 t	H-14	41.6 t	H-14
10	41.5 s	H-1, 2, 5, 14, 9′	39.9 s	H-5, 6, 8, 14	39.9 s	H-2, 5, 6, 14
11	71.4 s	H-12, 13	71.6 s	H-12, 13	71.6 s	H-12, 13
12	27.3 q	H-7, 13	27.7 q	H-13	27.5 q	H-13
13	28.2 q	H-7, 12	28.0 q	H-12	28.2 q	H-12
14	12.2 q	H-1, 5	15.9 q	H-1, 5	14.9 q	H-1, 5
15	105.1 t	H-3, 5	23.4 q	H-3, 5	23.4 q	H-3, 5
1′	171.1 s	H-7′, 9′	171.9 s	H-7′, 9′	171.4 s	H-7′, 9′
2′	107.2 s	H-7′	106.9 s	H-7′	107.4 s	H-7′
3′	171.1 s		171.7 s		171.4 s	
4′	106.6 s	H-8′	106.8 s	H-8′	106.7 s	H-8′
5′	169.0 s	H-8′, 9′	170.4 s	H-8′	169.2 s	H-8′
6′	113.8 s	H-9′	108.9 s	H-9′	113.7 s	H-9′
7′	192.2 d		192.0 d		192.1 d	
8′	192.2 d		192.2 d		192.1 d	
9′	30.3 d	H-1, 10′	31.6 d	H-10′	29.8 d	H-10′
10′	39.4 t	H-9′, 12′, 13′	44.5 t	H-9′, 12′, 13′	39.6 t	H-9′, 12′, 13′
11′	26.9 d	H-12′, 13′	27.4 d	H-12′, 13′	27.0 d	H-12′, 13′
12′	22.3 q	H-10′, 13′	21.9 q	H-10′, 13′	22.3 q	H-10′, 13′
13′	24.9 q	H-12′	24.5 q	H-12′	25.0 q	H-12′

[a] Chemical shift values are in ppm.

and C-10 were elucidated as shown in Figure 3, which showed the presence of the same sesquiterpene moiety in these two compounds. In the ^{13}C-NMR spectra of **8** and **9**, there were no significant differences in the chemical shift values of sesquiterpene moiety except for the isobutyl side chain at C-6′, C-9′, and C-10′. These spectroscopic data indicated that structures **8** and **9** had opposite stereochemistry at the C-9′ position. Further studies on the relative configuration at C-9′ of these compounds are in progress.

The antibacterial activities of compounds **1**−**9** were determined using the broth dilution method, and the MIC against oral pathogenic microorganisms are summarized in Table 3. As can be seen from Table 3, all the phloroglucinol derivatives inhibit cariogenic and periodontopathic bacteria at concentrations of < 100 μg/mL and < 25 μg/mL, respectively, and their antibacterial properties were greater than that of thymol, which is used as a wide-spectrum oral antibacterial agent.[18] Macrocarpals A (**1**), B (**2**), C (**3**), D (**4**), and H (**7**) had

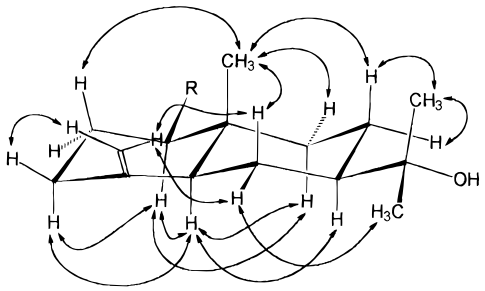
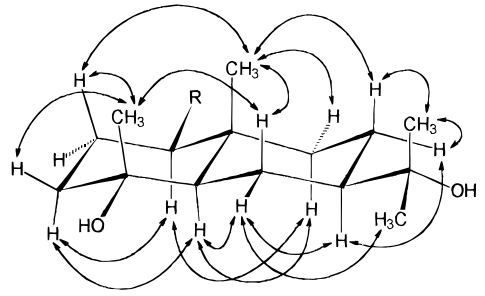


Figure 2. NOE relationships of macrocarpal H (**7**) (R = isopentyl phloroglucinol moiety).



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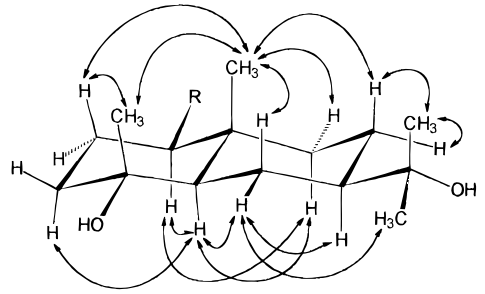

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Figure 3. NOE relationships of macrocarpals I (**8**) and J (**9**) (R = isopentyl phloroglucinol moiety).

relatively strong antibacterial activity against cariogenic and periodontopathic bacteria. These compounds inhibited the growth of all test bacteria except for *Fusobacterium nucleatum* 25586 at concentrations between 0.20 and 3.13 μg/mL. Macrocarpals I (**8**) and J (**9**) did not significantly inhibit these bacteria, in spite of having an isopentyl phloroglucinol unit, and there was no difference in MIC values between these two compounds. These results suggest that the antibacterial potency of these phloroglucinol−sesquiterpene-coupled compounds might be regulated by the structure of the sesquiterpene moiety. Macrocarpals I (**8**) and J (**9**) have two hydroxyl groups in the sesquiterpene moiety, a factor that might also be related to low antibacterial properties. Macrocarpals A (**1**), B (**2**), and C (**3**) are known to exhibit highly specific antibacterial activity against Gram-positive bacteria including *Staphylococcus aureus* and *Bacillus subtilis*;[10] however, their inhibitory effects on Gram-negative bacteria, such as periodontopathic bacteria, have not been reported.

In the present study, we have found that the phloroglucinol−sesquiterpene derivatives from *E. globulus* exhibited antibacterial effects against not only Gram-positive bacteria, but also Gram-negative periodontopathic bacteria. The inhibitory effects of these phloroglucinol derivatives for the adherent H_2O-insoluble glucan synthesis by GTase are summarized in Table 4. All of these compounds strongly inhibited the GTase activity at a concentration of 100 μg/mL, and macrocarpals A (**1**), B (**2**), C (**3**), D (**4**), and H (**7**) exhibited more than 60% inhibition even at 10 μg/mL. These inhibitory effects were greater than that of (−)-epigallocatechin gallate, which is known as a GTase inhibitor.[19] The present findings indicate that these phloroglucinol derivatives isolated from *E. globulus* may be useful natural substances for the maintenance of oral health.

Experimental Section

General Experimental Procedures. Optical rotations were measured on Jasco DIP-4 polarimeter. Spectral data were obtained using following spectrometers; IR on a Perkin-Elmer 1750, UV on a Shimadzu UV-160, ^{1}H and ^{13}C NMR on a Bruker AM 400 and a Bruker AM 500, respectively, and MS on a VG Auto Spec. HPLC was carried out on a Shimadzu LC-8A system with a Senshupak silica-5251-S column for normal-phase separations and a Senshupak ODS-5251-SH column for reversed-phase separations.

Plant Material. The dried leaves of *E. globulus* used in this investigation were purchased from Charis Seijyo Co., Tokyo, in 1994. A voucher specimen has been deposited in the herbarium of Tokyo University of Pharmacy and Life Science.

Chemicals. Thymol was purchased from Kokusan Chemical Co., Tokyo, and (−)-epigallocatechin gallate was purchased from Kurita Industry Co., Tokyo.

Extraction and Isolation. The dried leaves (1 kg) of *E. globulus* were extracted with 5 L of 50% EtOH at 80 °C for 3 h. The extract was filtered and concentrated *in vacuo* to yield 190 g of dark brownish extract. The concentrated extract (100 g) was fractionated into

Table 3. Antibacterial Activity of Compounds **1**−**9** from *E. globulus* against Cariogenic and Periodontopathic Bacteria

	MIC[a] (μg/mL)									
	cariogenic bacteria (Gram-positive)					periodontopathic bacteria (Gram-negative)				
compound	A	B	C	D	E	F	G	H	I	J
1	0.78	0.78	1.56	1.56	0.39	0.39	0.78	0.39	0.78	6.25
2	3.13	1.56	3.13	3.13	0.39	0.78	3.13	1.56	1.56	3.13
3	0.39	0.39	1.56	1.56	0.20	0.20	1.56	0.39	0.39	0.78
4	1.56	0.78	3.13	0.78	0.39	0.39	1.56	0.39	1.56	6.25
6	12.5	12.5	25	25	3.13	1.56	3.13	0.78	3.13	6.25
7	3.13	1.56	3.13	3.13	0.39	0.78	3.13	0.78	1.56	6.25
8	50	25	100	100	12.5	6.25	12.5	12.5	12.5	25
9	50	25	100	100	12.5	6.25	12.5	12.5	12.5	25
thymol	400	400	200	400	200	200	200	200	100	50

[a] Bacterial species: A, *S. mutans* Ingbritt; B, *S. mutans* LA7; C, *S. sobrinus* 6715; D, *S. sobrinus* B13; E, *A. viscosus* 15987; F, *P. gingivalis* 33277; G, *P. melaninogenica* 25845; H, *P. intermedia* 25611; I, *C. ochracea* 33596; J, *F. nucleatum* 25586.

Table 4. Inhibition of Adherent Water-Insoluble Glucan Synthesis by Compounds **1**−**9**

	% inhibition (μg/mL)[a]		
compound	100	10	1
1	97.1	75.4	9.7
2	97.1	67.6	10.1
3	98.9	92.6	18.9
4	99.0	78.2	17.3
6	97.6	44.0	4.4
7	97.6	64.0	2.9
8	86.9	26.3	0
9	77.1	29.7	6.9
EGCg[b]	26.5	10.5	3.0

[a] % Inhibition was expressed as follows: % inhibition = (control OD_{550} − test OD_{550})/control OD_{550} × 100. [b] (−)-Epigallocatechin gallate.

EtOAc-, *n*-BuOH-, and H_2O-soluble fractions, sequentially. The EtOAc-soluble fraction (29.4 g) was subjected to Si gel column chromatography with CH_2Cl_2−MeOH mixtures (1:0 to 2:1 stepwise elution) as eluents, to give 10 fractions. A biologically active fraction was further separated by Si gel HPLC with CH_2Cl_2−MeOH (17:3 to 49:1), and each fraction was further purified by ODS HPLC with MeOH−H_2O (9:1−1:0) and CH_3CN−H_2O (9:1−1:0) to give macrocarpals A (**1**) (109 mg), B (**2**) (130 mg), C (**3**) (229 mg), D (**4**) (18.2 mg), eucalyptone (**6**) (27.4 mg), and three new compounds named macrocarpals H (**7**) (23.0 mg), I (**8**) (23.0 mg), and J (**9**) (28.4 mg).

Macrocarpal H (7): pale yellow powder; $[\alpha]_D$ −39.2° (*c* 0.020, EtOH); UV (EtOH) λ max (log ϵ) 275 (4.20) nm; IR (KBr) ν max 3441, 2953, 1642, 1444, 1382, 1312, 1181 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; HREIMS *m/z* $[M]^+$ 472.2815 (calcd for $C_{28}H_{40}O_6$, 472.2825); EIMS *m/z* $[M]^+$ 472 (20), 454 (65), 414 (20), 251 (98), 195 (100).

Macrocarpal I (8): pale yellow powder; $[\alpha]_D$ −49.1° (*c* 0.028, EtOH); UV (EtOH) λ max (log ϵ) 274 (4.32) nm; IR (KBr) ν max 3429, 2931, 1623, 1459, 1383, 1311, 1184 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; HREIMS *m/z* $[M - H_2O]^+$ 472.2809 (calcd for $C_{28}H_{40}O_6$, 472.2825); EIMS *m/z* $[M - H_2O]^+$ 472 (8), 454 (30), 251 (97), 195 (100).

Macrocarpal J (9): pale yellow powder; $[\alpha]_D$ −62.5° (*c* 0.048, EtOH); UV (EtOH) λ max (log ϵ) 275 (4.19) nm; IR (KBr) ν max 3429, 2953, 1623, 1459, 1383, 1313, 1179, 1020 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; HREIMS *m/z* $[M - H_2O]^+$ 472.2802 (calcd for $C_{28}H_{40}O_6$, 472.2825); EIMS *m/z* $[M - H_2O]^+$ 472 (8), 454 (33), 251 (98), 195 (100).

Antibacterial Assay. Broth dilution method: aliquots (100 μL) of twofold serial dilutions of each test substance containing 2% EtOH were placed into wells of a flat-bottomed 96-well plate (Costar). A control was prepared by adding 100 μL of 2% EtOH. Subsequently, 100 μL of 2-fold-concentrated medium [brain heart infusion (BHI) broth for *S. mutans*, *S. sobrinus*, and *Actinomyces viscosus*; Tripticase soy broth containing 5 μg/mL hemin and 0.5 μg/mL menadione for *P. gingivalis*, *Prevotella melaninogenica*, *Prevotella intermedia*, and *Capnocytophaga ochracea*; BHI broth containing 5 μg/mL hemin and 0.5 μg/mL menadione for *F. nucleatum*], containing 1−2 × 10^5 colony-forming units/mL of test organisms, were inoculated into each well. These were incubated at 37 °C for 24 h under aerobic conditions for *S. mutans*, *S. sobrinus*, and *A. viscosus* and for 48 h under anaerobic conditions (10% carbon dioxide, 10% hydrogen, and 80% nitrogen) for *P. gingivalis*, *P. melaninogenica*, *P. intermedia*, *C. ochracea*, and *F. nucleatum*. Bacterial growth was monitored by measuring the increase in absorbance at OD_{550nm} using a microplate reader (Corona Electric Co.). The minimum inhibitory concentration (MIC) was recorded as the lowest concentration of test substance to inhibit growth.

Preparation of Crude GTase. *S. sobrinus* 6715 was grown in BHI broth at 37 °C for 24 h. The supernatant obtained by centrifugation (12 000 × g, 20 min) of the culture was brought to 60% saturation with $(NH_4)_2SO_4$. The precipitate formed was collected, dissolved in 10 mM potassium phosphate buffer (pH 6.5), and dialyzed against the same buffer.

Assay of GTase Inhibitory Activity. The inhibition test of GTase activity was carried out according to a previous study.[11,20] A mixture (1.0 mL) containing crude GTase, 1% sucrose, 2% MeOH, and test compound in 50 mM potassium phosphate buffer (pH 6.5) was incubated at 37 °C for 16 h at an angle of 30° to the horizontal. The glucan produced that adhered to the glass surface was dispersed by sonic oscillation in 6.0 mL H_2O. The amount of adhered insoluble glucan was measured by turbidimetry at 550 nm. The percentage of glucan synthesis was calculated in comparison with turbidity in the absence of the test compound as a control (total glucan = 0.3−0.4 at OD_{550nm}).

References and Notes

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