Polyprenylated Benzoylphloroglucinol-Type Derivatives Including Novel Cage Compounds from *Hypericum erectum*

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Hyperici erecti herba (*Hypericum erectum* Thunb.) showed a suppressive effect on generation of isovaleric acid by *Corynebacterium xerosis*. An ethyl acetate (AcOEt) soluble fraction of methanol extract of *H. erectum* showed the activity. The AcOEt fraction was separated by various successive choromatographical methods to give seven new compounds 1—7 along with some known compounds. The structures of the new compounds were elucidated to be polyprenylated benzoylphloroglucinol derivatives by means of HR-MS and NMR spectra including 2D-NMR. Some of these compounds had novel cage structures having benzoyltricyclo[3,3,1,1^{3,7}]decane and benzoyltricyclo[4,3,1,1^{3,8}]undecane skeletons arising from a polyprenylated phloroglucinol precursor by a transannular cyclization reaction. The isolated compounds were tested for suppressive activity, but they showed only weak activity.

Key words *Hypericum erectum*; Guttiferae; benzoylfuloroglucinol; cage compound; adamantane; homoadamantane

It is well known that body odors are a result of the combination of many kinds of volatile compounds. Almost of the volatile compounds related to body odor are generated from dead skin cells and extracted materials, such as sweat and sebum, through the metabolism of skin-resident microorganisms.[1—4] Vinylketones, 1-octen-3-one and *cis*-1,5-octadien-3-one, which were scented with a strong metallic odor, were generated by lipid oxidation.[5] Volatile steroids, which are one of the key compounds in body odors, are also generated through the metabolism of skin-resident microorganisms from the male hormone, testosterone.[6,7] The suppression effect of cyanogenic compounds from the kernel of *Prunus ardelmeniaca*, generating the key compound 5α-androst-16-en-3-one (androstenone) by microbial metabolism was reported.[8] Isovaleric acid is a typical malodor component that is also generated from L-leucine by a skin-resident microorganism, *Corynebacterium xerosis*. Takenaka *et al.*, reported that *C. xerosis* demonstrated strong ability to generate isovarelic acid and that the natural antimicrobial compounds from *Sophora flavescens* effectively suppressed the generation of isovaleric acid from L-leucine.[9] We found that the methanol extract of Hyperici erecti herba (*Hypericum erectum* Thunb.) had strong suppressive activity against generation of isovaleric acid from L-leucine by *C. xerosis*. From *Hypericum* genus plants, various types of biologically active compounds with an acylphloroglucinol moiety were isolated.[10—13] Some of these have interesting caged structures.[14—16] We studied isolation and structural elucidation of the constituents of *H. erectum* to give seven new polyprenylated benzoylphloroglucinol derivatives, **1**—**7**. This paper describes the isolation and structural elucidation of the novel compounds.

Results and Discussion

The MeOH extract (HYM) of *H. erectum* showed strong suppressive activity against the generation of isovarelic acid (inhibition ratio; 93.9% at 125 μg/ml). The ethyl acetate (AcOEt) soluble fraction (HYMA) and *n*-butanol (*n*-BuOH) soluble fraction (HYMB) showed the strong activity (inhibition ratio; 86.6% at 125 μg/ml and 94.2% at 125 μg/ml, respectively), but the water layer (HYMW) showed weak activity (inhibition ratio; 5.5% at 125 μg/ml). Of these active fractions, HYMA showed the presence of many constituents on TLC profiles. Therefore HYMA was purified by successive chromatographical methods such as column chromatography, HPLC and preparative TLC (PLC) as shown in Experimental to give seven new compounds, **1**—**7** along with known compounds, **8** and **9**. The structure of **8** was determined to be sampsonione A[17,18] having a novel cage structure from MS, ^{1}H-NMR, ^{13}C-NMR and 2-dimensional (2D)-NMR spectral data, and ^{1}H- and ^{13}C-NMR data of **8** were identified with the reported data of sampsonione A. Compound **9** was identified with quercetin from NMR and MS data.

The molecular formula of **1** was determined to be $C_{38}H_{48}O_4$ based on the $[M+H]^+$ ion peak at *m/z* 569.3604 ($C_{38}H_{49}O_4$) in its HR-ESI-MS (positive ion mode). The UV spectrum of **1** showed maximum absorption at 215 nm (ε 5728), 246 nm (ε 8534), indicating the presence of a conjugated system(s). The IR spectrum of **1** showed absorption bands at 2975, 2920, 1742, 1700, 1450, 1245 cm^{-1}, indicating the presence of carbonyl groups. The ^{1}H-NMR spectrum of **1** showed the presence of a phenyl group [δ_H 7.17 (2H, dd, J=7.6, 1.0 Hz), 7.27 (2H, t, J=7.9 Hz), 7.40 (1H, t, J=7.3 Hz)], nine singlet methyl groups [δ_H 1.45 (3H, s), 1.54 (3H, s), 1.58 (9H, s), 1.63 (3H, s), 1.65 (3H, s), 1.67 (3H, s), 1.74 (3H, s)], four olefinic protons [δ_H 5.01 (1H, d, J=9.3 Hz), 5.06 (1H, t, J=7.3 Hz), 5.08 (1H, t, J=7.3 Hz), 5.22 (1H, t, J=7.3 Hz)], and several methylene and methine protons as shown in Table 1. The ^{13}C-NMR spectrum of **1** showed the presence of a benzoyl group (δ_C 193.4, 135.0, 129.2×2, 128.0×2, 132.4), three carbonyl groups (δ_C 203.4, 202.5, 201.9), nine methyl groups (δ_C 16.5, 17.8, 17.9, 18.0, 23.0,

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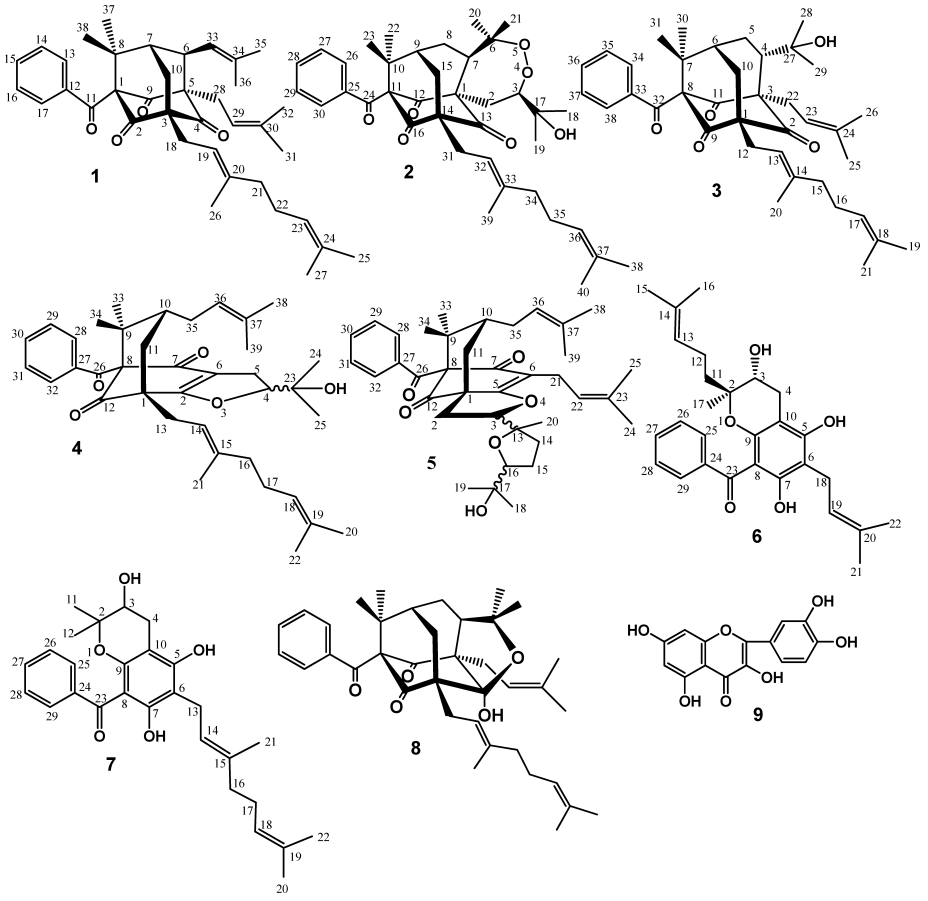

Fig. 1. Structures of Compounds Isolated from the AcOEt Soluble Fraction of *H. erectum*

23.4, 25.8, 26.1, 26.2), eight olefinic carbons (δ_C 138.7, 137.2, 134.7, 131.5, 124.2, 119.6, 118.6, 118.5), four quaternary carbons (δ_C 82.3, 72.0, 68.8, 55.0), five methylene carbons (δ_C 40.4, 40.1, 2.76, 26.8, 26.6), and two methine carbons (δ_C 51,7, 48.9). These facts indicated that **1** was a similar cage compound to **8**, having prenyl groups. The heteronuclear multiple bond correlation (HMBC) experiments of **1** showed C–H long range correlations as follows; geminal dimethyl groups at C-8 (δ_H 1.45, 3H, s, 1.54, 3H, s) to C-1, 7 and 8 (δ_C 82.3, 48.9, 55.0); H-10 (δ_H 2.49, 1H, dd, J=13.9, 2.4 Hz, 2.40, 1H, overlap) to C-2, 4, 6, 7, 8 and 18 (δ_C 202.5, 203.4, 51.7, 48.9, 55.0, 27.6); H-6 (δ_H 3.63, 1H, d, J=9.3 Hz) to C-4, 5, 8, 9, 10, 28 and 34 (δ_C 203.4, 72.0, 55.0, 201.9, 40.4, 26.6, 137.2); H-18 (δ_H 2.54, 2H, d, J=7.3 Hz) to C-2, 4, 10 and 20 (δ_C 202.5, 203.4, 40.4, 138.7); H-19 (δ_H 5.22, 1H, t, J=7.3 Hz) to C-3, 21 and 26 (δ_C 68.8, 40.1, 16.5); H-23 (δ_H 5.08, 1H, t, J=7.3 Hz) to C-21, 25 and 27 (δ_C 40.1, 25.8, 17.8); H-29 (δ_H 5.06, 1H, t, J=7.3 Hz) to C-5, 31 and 32 (δ_C 72.0, 26.2, 17.9); H-33 (δ_H 5.01, d, J=9.3 Hz) to C-5, 7, 35 and 36 (δ_C 72.0, 48.9, 26.1, 18.0); and so on as shown in Fig. 2. These data indicated that the structure of **1** was supposed as a cage compound, having an adamantane carbon skeleton with a benzoyl, a geranyl, an isopentenyl and an isobutenyl group. The HMBC data of **1** clearly showed the binding positions of the functional groups (as shown in Fig. 2). Thus, the structure of **1** was determined to be 1-benzoyl-8,8-dimethyl-3-[(*E*)-3,7-dimethyl-2,6-octadienyl]-5-(3-methyl-2-butenyl)-6-(2-methyl-1-propenyl)tricyclo[3,3,1,$1^{3,7}$]deca-2,4,9-trione having an adamantane skeleton. The relative configurations of at C-1, 3 and 7 were determined from the adamantane skeleton of **1**, but the relative configuration at C-4 was determined from the nuclear Overhauser effect spectroscopy (NOESY) experiment of **1** as shown in Fig. 3. Optical rotation ($[\alpha]_D$ −8.1°) and circular dichroism ($[\theta]_{289}$ +3204, $[\theta]_{242}$ −6032) showed that **1** was an optically active compound, but the absolute configuration of **1** was not determined. Compound **1** should be synthesized from phloroglucinol substituted with a benzoyl and some prenyl groups followed by a transannular cyclization reaction. Compound **1** was named otogirinin A. Hyperibone K[19] isolated from *H. scabrum* and pulkenetione A[20] isolated from *Clusia plukenetii* have a similar carbon skeleton (adamantane) with that of **1**, but a different substitution pattern and size of side chain moieties with those of **1**.

The molecular formula of **2** was determined to be $C_{38}H_{50}O_7$ based on the $[M+Na]^+$ ion peak at *m/z* 641.3450 in its HR-ESI-MS (positive ion mode). The UV spectrum of **2** showed maximum absorption at 214 nm (ε 6250), 243 nm (ε 8120), indicating the presence of a conjugated system. The IR spectrum of **2** showed absorption bands at 3438, 2980, 2930, 1737, 1703, 1249, 1224 cm^{-1}, indicating the presence of carbonyl groups and a hydroxyl group. The ^{1}H-NMR spectrum of **2** showed the presence of a phenyl group [δ_H 7.16 (2H, dd, J=7.3, 1.2 Hz), 7.28 (2H, t, J=8.4 Hz), 7.40 (1H, t, J=7.3 Hz)], nine singlet methyl groups [δ_H 1.06 (3H, s), 1.08 (3H, s), 1.13 (3H, s), 1.29 (3H, s), 1.42 (3H, s), 1.48 (3H, s), 1.58 (3H, s), 1.66 (3H, s), 1.69 (3H, s)], two olefinic protons [δ_H 5.06 (1H, t, J=6.0 Hz), 5.15 (1H, t, J=

Table 1. ^{1}H-NMR Data of Cage Compounds **1**—**3** ($CDCl_3$, 500 MHz)

Position	1	2	3
H-2		3.54 (1H, dd, J=14.8, 11.6 Hz)	
		1.50 (1H, overlap)	
3		4.57 (1H, dd, J=11.7, 2.9 Hz)	
4			1.93 (1H, overlap)
5			2.06 (1H, overlap)
			1.91 (1H, overlap)
6	3.63 (1H, d, J=9.3 Hz)		1.98 (1H, overlap)
7	1.57 (1H, overlap)	2.78 (1H, dd, J=10.9, 7.9 Hz)	
8		2.41 (1H, ddd, J=15.6, 11.0, 4.8 Hz)	
		1.50 (1H, overlap)	
9		2.10 (1H, overlap)	
10	2.49 (1H, dd, J=13.9, 2.4 Hz)		2.56 (1H, dd, J=13.7, 5.6 Hz)
	2.40 (1H, overlap)		1.89 (1H, d, J=13.7 Hz)
12			2.59 (2H, d, J=7.1 Hz)
13	7.17 (1H, d, J=7.6 Hz)		5.10 (1H, t, J=7.1 Hz)
14	7.27 (1H, t, J=7.6 Hz)		
15	7.40 (1H, t, J=7.6 Hz)	2.58 (1H, dd, J=14.2, 6.4 Hz)	2.00 (2H, overlap)
		1.88 (1H, d, J=14.2 Hz)	
16	7.27 (1H, t, J=7.6 Hz)		2.06 (2H, overlap)
17	7.17 (1H, d, J=7.6 Hz)		5.08 (1H, t, J=7.1 Hz)
18	2.54 (2H, d, J=7.3 Hz)	1.13 (3H, s)	
19	5.22 (1H, t, J=7.3 Hz)	1.06 (3H, s)	1.67 (3H, s)
20		1.29 (3H, s)	1.71 (3H, s)
21	2.01 (2H, t, J=6.1 Hz)	1.08 (3H, s)	1.59 (3H, s)
22	2.04 (2H, overlap)	1.42 (3H, s)	3.24 (1H, dd, J=13.9, 8.1 Hz)
			2.96 (1H, dd, J=13.9, 6.6 Hz)
23	5.08 (1H, t, J=7.3 Hz)	1.48 (3H, s)	4.42 (1H, dd, J=7.9, 6.7 Hz)
25	1.65 (3H, s)		1.52 (3H, s)
26	1.67 (3H, s)	7.16 (1H, d, J=7.3 Hz)	1.49 (3H, s)
27	1.58 (3H, s)	7.28 (1H, t, J=7.3 Hz)	
28	2.40 (2H, overlap)	7.40 (1H, t, J=7.3 Hz)	1.34 (3H, s)
29	5.06 (1H, t, J=7.3 Hz)	7.28 (1H, t, J=7.3 Hz)	1.32 (3H, s)
30		7.16 (1H, d, J=7.3 Hz)	1.45 (3H, s)
31	1.63 (3H, s)	2.62 (2H, d, J=7.3 Hz)	1.39 (3H, s)
32	1.58 (3H, s)	5.15 (1H, t, J=7.3 Hz)	
33	5.01 (1H, d, J=9.3 Hz)		
34		1.99 (2H, t, J=5.6 Hz)	7.34 (1H, d, J=7.3 Hz)
35	1.74 (3H, s)	2.03 (2H, t, J=6.0 Hz)	7.27 (1H, t, J=7.3 Hz)
36	1.58 (3H, s)	5.06 (1H, t, J=6.0 Hz)	7.37 (1H, t, J=7.3 Hz)
37	1.54 (3H, s)		7.27 (1H, t, J=7.3 Hz)
38	1.45 (3H, s)	1.66 (3H, s)	7.34 (1H, d, J=7.3 Hz)
39		1.69 (3H, s)	
40		1.58 (3H, s)	

7.3 Hz)], a methine proton bearing oxygen [δ_H 4.57 (1H, dd, J=11.7, 2.9 Hz)], and several methylene and methine protons as shown in Table 1. The ^{13}C-NMR spectrum of **2** showed the presence of a benzoyl group (δ_C 192.1, 134.8, 128.8×2, 128.0×2, 132.4), three carbonyl groups (δ_C 207.9, 205.1, 204.4), nine methyl groups (δ_C 16.5, 17.7, 17.8, 22.7, 24.9×2, 25.7, 25.9, 28.1), two oxygen-bearing quaternary carbons (δ_C 88.4, 73.0), an oxygen-bearing methine carbon (δ_C 88.9), four olefinic carbons (δ_C 138.9, 131.5, 124.1, 118.7), four quaternary aliphatic carbons (δ_C 81.8, 68.5, 66.2, 50.2), and a geranyl group, whose ^{13}C-NMR chemical shifts were similar to those of **8**. These facts indicated that **2** was a similar cage compound having a geranyl and a benzoyl group like **8**, but an isopentenyl group was modified by oxygenation. The HMBC experiments of **2** showed H–C long range correlations as follows; geminal dimethyl groups at C-17 (δ_H 1.06, 3H, s, 1.13, 3H, s) to C-3 and 17 (δ_C 88.9, 73.0); geminal dimethyl groups at C-6 (δ_H 1.08, 3H, s, 1.29, 3H, s) to C-6 and 7 (δ_C 88.4, 42.6); H-2 (δ_H 1.50, 1H, overlap, 3.54, 1H, dd, J=14.8, 11.6 Hz) to C-7, 12, 13 and 17 (δ_C 42.6, 207.9, 205.1, 73.0); H-7 (δ_H 2.78, dd, J=10.9, 7.9 Hz) to C-12 and 13 (δ_C 207.9, 205.1); H-8 (δ_H 1.50, 1H, overlap, 2.41, 1H, ddd, J=15.6, 11.0, 4.8 Hz) to C-6, 10 and 15 (δ_C 88.4, 50.2, 41.2); and so on, the same as those of **1**. These HMBC results and the molecular formula of **2** indicated that it was a cage compound having a tricyclo[4,3,1,$1^{3,8}$]undecane carbon skeleton and an *endo*-peroxide moiety as shown in Fig. 1. In the NOESY experiment, H-3 and H-7 showed NOE correlations with each other, and other NOE correlations as shown in Fig. 3 indicated a 3Hα and 7Hα configuration in relative configuration. 33-Hydroperoxyplukenetione C[21] isolated from *Clusia havetiodes* var. *stenocarpa* has the same tricyclo[4,3,1,$1^{3,8}$]undecane carbon skeleton and *endo*-peroxide moiety. The ^{13}C-NMR data of the tricyclo[4,3,1,$1^{3,8}$]undecane carbon skeleton and the *endo*-peroxide moiety ring part of both compounds showed almost the same chemical shifts. Thus, the structure of **2** was determined to be 11-benzoyl-14-([*E*]-3,7-dimethyl-2,6-octadienyl)-3-(1-hydroxy-1-methylethyl)-6,6,10,10-tetramethyl-4,5-dioxatetracyclo[7,3,3,$1^{11,14}$,$0^{1,7}$]-hexadecane-12,13,16-trione, and the rel-

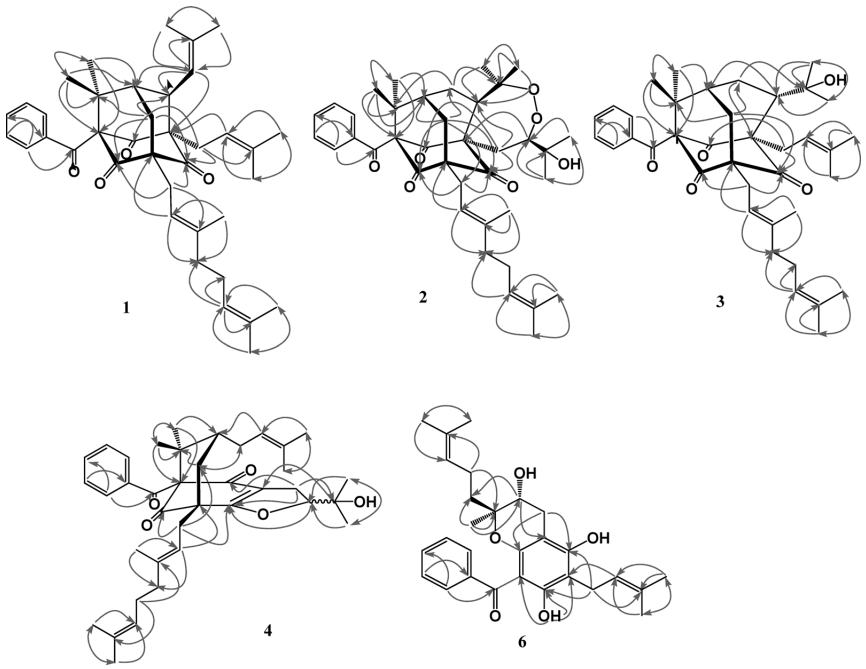

Fig. 2. Key HMBC Corelations of Compounds **1**—**4** and **6**

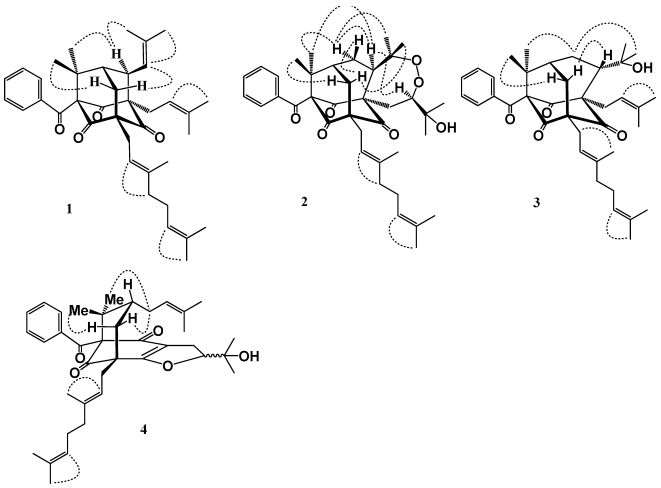

Fig. 3. Key NOE of Compounds **1**—**4**

ative stereochemistry of **2** should be the same as that of 33-hydroperoxyplukenetione C. Compound **2** was named otogirinin B.

The molecular formula of **3** was determined to be $C_{38}H_{50}O_5$ based on the $[M+Na]^+$ ion peak at *m/z* 609.3538 ($C_{38}H_{50}O_5Na$) in its HR-ESI-MS (positive ion mode). The ^{1}H-NMR spectrum of **3** showed the presence of a phenyl group [δ_H 7.27 (2H, br d, J=7.7 Hz), 7.34 (2H, t, J=7.3 Hz), 7.37 (1H, t, J=7.2 Hz)], nine singlet methyl groups [δ_H 1.32 (3H, s), 1.34 (3H, s), 1.39 (3H, s), 1.45 (3H, s), 1.49 (3H, s), 1.52 (3H, s), 1.59 (3H, s), 1.67 (3H, s), 1.71 (3H, s)], three olefinic protons [δ_H 4.42 (1H, dd, J=7.9, 6.7 Hz), 5.08 (1H, t, J=7.1 Hz), 5.10 (1H, t, J=7.1 Hz)], and several methylene and methine protons as shown in Table 1. The ^{13}C-NMR spectrum of **3** showed the presence of a benzoyl group (δ_C 195.6, 138.2, 131.2, 128.9×2, 128.0×2), three carbonyl groups (δ_C 208.7, 206.4, 205.3), nine methyl groups (δ_C 16.5, 17.8, 18.2, 23.8, 25.5, 25.8, 26.2, 26.7, 33.2), six olefinic carbons (δ_C 138.5, 136.3, 131.6, 124.2, 119.4, 119.3), an oxygen-bearing quaternary carbon (δ_C 76.7), four aliphatic quaternary carbons (δ_C 84.9, 71.5, 68.7, 50.0), and several methylene and methine carbons as shown in Table 2. These facts indicated that **3** was a similar cage compound to **2**. The HMBC experiments of **3** showed C–H long range correlations as follows; geminal dimethyl groups at C-27 (δ_H 1.32, 3H, s, 1.34, 3H, s) to C-4 and 27 (δ_C 50.8, 76.7); H-22 (δ_H 3.24, 1H, dd, J=13.9, 8.1 Hz, 2.96, 1H, dd, J=13.9, 6.6 Hz) to C-2, 4, 11 and 24 (δ_C 208.7, 50.8, 206.4, 136.3); H-10 (δ_H 2.56, 1H, dd, J=13.7, 5.6 Hz, 1.89, 1H, d, J=13.7 Hz) to C-2, 5, 7, 9 and 12 (δ_C 208.7, 30.3, 50.0, 205.3, 29.5); and so on as shown in Fig. 2. This fact indicated that the structure of **3** was a cage compound as shown in Fig. 1. The stereochemistry at C-4 was determined from an NOE experiment as shown in Fig. 3. Me-28 and 29 showed NOE correlations with H-5 and H-4 showed an NOE correlation with one of H-10, and another H-10 showed an NOE correlation with Me-31. These facts indicated that the relative configuration of the 2-hydroxyisopropyl side chain at C-4 was α. Thus, the structure of **3** was determined to be 8-benzoyl-7,7-dimethyl-1-([*E*]-3,7-dimethyl-2,6-octadienyl)-3-(3-methyl-2-butenyl)-4-(1-hydroxy-1-methylethyl)-tricyclo[4,3,1,1^{3,8}]undeca-2,9,11-trione and named otogirinin C.

The molecular formula of **4** was determined to be $C_{38}H_{50}O_5$ based on the $[M+Na]^+$ ion peak at *m/z* 609.3563 in its HR-ESI-MS (positive ion mode). The UV spectrum of **4** showed maximum absorption at 247 nm (ε 13090), and 288 nm (ε 11360), indicating the presence of a conjugated system. The IR spectrum of **4** showed absorption bands at 3450, 2975, 2930, 1725, 1700, 1625, 1405, 1222 cm^{-1}, indicating the presence of carbonyl groups and a hydroxyl group. The ^{1}H-NMR spectrum of **4** showed the presence of a phenyl group [δ_H 7.25 (2H, t, J=7.8 Hz), 7.38 (1H, t, J=7.3 Hz), 7.56 (2H, dd, J=8.3, 1.1 Hz)], nine singlet methyl groups [δ_H

Table 2. ^{1}H-NMR Data of **4**—**7** ($CDCl_3$, 500 MHz)

Position	4	5	6	7
H-2		2.54 (1H, dd, *J*=13.2, 11.0 Hz)		
		1.86 (1H, dd, *J*=13.2, 5.5 Hz)		
3		4.77 (1H, dd, *J*=10.9, 5.5 Hz)	3.70 (1H, dd *J*=7.1, 5.6 Hz)	3.59 (1H, t, *J*=5.1 Hz)
4	4.78 (1H, dd, *J*=10.5, 6.8 Hz)		2.80 (1H, dd, *J*=16.4, 5.4 Hz)	2.77 (1H, dd, *J*=16.7, 5.1 Hz)
			2.49 (1H, dd, *J*=16.4, 7.3 Hz)	2.56 (1H, dd, *J*=16.7, 5.5 Hz)
5	2.94 (1H, dd, *J*=15.1, 6.8 Hz)			
	2.87 (1H, dd, *J*=15.1, 10.6 Hz)			
10	1.55 (1H, m)	1.51 (1H, overlap)		
11	2.24 (1H, dd, *J*=14.2, 1.1 Hz)	2.33 (1H, d, *J*=14.2 Hz)	1.30 (1H, ddd, *J*=13.9, 11.5, 5.1 Hz)	0.85 (3H, s)
	2.19 (1H, dd, *J*=14.2, 6.6 Hz)	2.17 (1H, m)	1.19 (1H, ddd., *J*=13.9, 11.5, 5.4 Hz)	
12			1.55 (1H, m)	0.93 (3H, s)
			1.45 (1H, m)	
13	2.56 (2H, d, *J*=6.6 Hz)		4.84 (1H, dd, *J*=7.1, 6.8 Hz)	3.45 (2H, d, *J*=7.3 Hz)
14	5.09 (1H, br t, *J*=6.6 Hz)	1.97 (1H, overlap)		5.32 (1H, br t, *J*=6.8 Hz)
		1.74 (1H, m)		
15		1.97 (2H, overlap)	1.63 (3H, s)	
16	1.96 (2H, overlap)	3.88 (1H, t, *J*=7.3 Hz)	1.48 (3H, s)	2.11 (2H, overlap)
17	2.06 (2H, overlap)		0.90 (3H, s)	2.15 (2H, overlap)
18	5.05 (1H, br t, *J*=6.6 Hz)	1.16 (3H, s)	3.43 (2H, d, *J*=7.3 Hz)	5.06 (1H, br t, *J*=6.8 Hz)
19		1.25 (3H, s)	5.31 (1H, t, *J*=7.3 Hz)	
20	1.65 (3H, s)	1.25 (3H, s)		1.69 (3H, s)
21	1.71 (3H, s)	3.09 (1H, dd, *J*=14.2, 7.5 Hz)	1.80 (3H, s)	1.84 (3H, s)
		2.97 (1H, dd, *J*=14.2, 7.5 Hz)		
22	1.57 (3H, s)	5.03 (1H, t, *J*=7.5 Hz)	1.85 (3H, s)	1.61 (3H, s)
24	1.22 (3H, s)	1.59 (3H, s)		
25	1.31 (3H, s)	1.59 (3H, s)	7.48 (1H, d, *J*=7.6 Hz)	7.44 (1H, d, *J*=7.5 Hz)
26			7.35 (1H, t, *J*=7.6 Hz)	7.36 (1H, t, *J*=7.5 Hz)
27			7.43 (1H, t, *J*=7.6 Hz)	7.43 (1H, t, *J*=7.5 Hz)
28	7.56 (1H, d, *J*=7.8 Hz)	7.45 (1H, d, *J*=7.6 Hz)	7.35 (1H, t, *J*=7.6 Hz)	7.36 (1H, t, *J*=7.5 Hz)
29	7.25 (2H, t, *J*=7.8 Hz)	7.20 (1H, t, *J*=7.6 Hz)	7.48 (1H, d, *J*=7.6 Hz)	7.44 (1H, d, *J*=7.5 Hz)
30	7.38 (1H, t, *J*=7.8 Hz)	7.37 (1H, t, *J*=7.6 Hz)		
31	7.25 (2H, t, *J*=7.8 Hz)	7.20 (1H, t, *J*=7.6 Hz)		
32	7.56 (1H, d, *J*=7.8 Hz)	7.45 (1H, d, *J*=7.6 Hz)		
33	1.49 (3H, s)	1.40 (3H, s)		
34	1.35 (3H, s)	1.49 (3H, s)		
35	2.28 (1H, m)	2.17 (2H, m)		
	1.95 (1H, m)			
36	4.96 (1H, br t, *J*=6.6 Hz)	4.89 (1H, t, *J*=7.2 Hz)		
38	1.70 (3H, s)	1.70 (3H, s)		
39	1.58 (3H, s)	1.54 (3H, s)		
OH at C-7			12.5 (1H, s)	12.6 (1H, s)

1.22 (3H, s), 1.31 (3H, s), 1.35 (3H, s), 1.49 (3H, s), 1.57 (3H, s), 1.58 (3H, s), 1.65 (3H, s), 1.70 (3H, s), 1.71 (3H, s)], three olefinic protons [δ_H 4.96 (1H, br t, *J*=6.6 Hz), 5.05 (1H, br t, *J*=6.6 Hz), 5.09 (1H, br t, *J*=6.6 Hz)], an oxygen-bearing methine proton [δ_H 4.78 (1H, dd, *J*=10.5, 6.8 Hz), and several methylene and methine protons, as shown in Table 1. The ^{13}C-NMR spectrum of **4** showed the presence of a benzoyl group (δ_C 193.4, 136.8, 132.2, 128.2×2, 128.1×2), two carbonyl groups (δ_C 207.1, 188.2), an oxygen-bearing olefinic carbon (δ_C 176.5), eight olefinic carbons (δ_C 176.5, 138.7, 132.5, 131.9, 124.9, 123.8, 119.4, 118.8), nine methyl groups (δ_C 16.9, 17.7, 18.0, 22.7, 23.5, 25.7, 25.9, 26.0, 26.8), three aliphatic quadrant carbons (δ_C 77.8, 53.6, 49.3), oxygen-bearing quaternary and methine carbons (δ_C 71.9, 93.0), six methilene carbons (δ_C 40.4, 38.9, 30.0, 29.5, 27.8, 26.5) and a methine carbon (δ_C 48.4). These facts indicated that **4** was a different type of compound from the cage compounds, **1**, **2** and **3**. The phloroglucinol part of **4** take an intramolecular enol-ether form. The HMBC experiments showed H–C long range correlations as follows; geminal dimethyl groups at C-23 (δ_H 1.22, 3H, s, 1.31, 3H,s) to C-4 and 23 (δ_C 93.0, 71.9); H-4 (δ_H 4.78, 1H, dd, *J*=10.5, 6.8 Hz) to C-2, 6, 23, 24 and 25 (δ_C 176.5, 118.8, 71.9, 23.5, 26.0); H-5 (δ_H 2.94, 1H, dd, *J*=15.1, 6.8 Hz, 2.87, 1H, dd, *J*=15.1, 10.6 Hz) to C-2, 7 and 23 (δ_C 176.5, 188.2, 71.9); H-11 (δ_H 2.24, 1H, dd, *J*=14.2, 1.1 Hz, 2.19, 1H, dd, *J*=14.2, 6.6 Hz) to C-2, 9, 12 and 35 (δ_C 176.5, 49.3, 207.1, 30.0); geminal dimethyl groups at C-9 (δ_H 1.35, 3H, s, 1.49, 3H, s) to C-8, 9 and 10 (δ_C 77.8, 49.3, 48.4); and so on as shown in Fig. 3. These results showed that **4** had the same 4-oxo-tricyclo[6,3,1,$0^{1,5}$]dodecane skeleton as sampsonione M,[18] while substitution pattern of side chain groups of **4** was different with that of sampsonione M. Thus, the planer structure of **4** was determined as shown in Fig. 1. The relative stereochemistry at C-10 was determined based on NOE correlations in the NOESY spectrum. H-35 showed NOE correlations with Me-33 and H-11eq, and Me-34 showed a correlation with H-11ax. These results indicated that the stereochemistry of the isopentenyl moiety was an axial configuration, but the configuration at C-4 could not be determined. Thus, the structure of **4** was determined to be 8-benzoyl-9,9-dimethyl-1-([*E*]-3,7-dimethyl-2,6-octadienyl)-10-(3-methyl-

2-butenyl)-4-(1-hydroxyl-1-methylethyl)-3-oxatricyclo [6,3,1,$0^{2,6}$]dodeca-2(6)-ene-7,12-dione and named otogirinin D.

The molecular formula of **5** was determined to be $C_{38}H_{50}O_6$ based on the $[M+H]^+$ ion peak at *m/z* 603.3658 in its HR-ESI-MS (positive ion mode). The ^{1}H-NMR spectrum of **5** showed the presence of a phenyl group [δ_H 7.45 (2H, d, J=7.7 Hz), 7.37 (1H, t, J=7.5 Hz), 7.20 (2H, br d, J=7.6 Hz)], nine singlet methyl groups [δ_H 1.16 (3H, s), 1.25 (6H, s), 1.40 (3H, s), 1.49 (3H, s), 1.54 (3H, s), 1.59 (6H, s), 1.70 (3H, s)], two olefinic protons [δ_H 5.03 (1H, t, J=7.5 Hz), 4.89 (1H, t, J=7.2 Hz)], two oxygen-bearing methine groups [δ_H 4.77 (1H, dd, J=10.9, 5.5 Hz), 3.88 (1H, t, J=7.3 Hz)], and several methylene and methine groups, as shown in Table 1. The ^{13}C-NMR spectrum of **5** showed the presence of a benzoyl group (δ_C 193.5, 137.0, 131.9, 128.1×2, 127.9×2), two carbonyl groups (δ_C 205.7, 193.8), nine methyl groups (δ_C 27.4, 27.0, 25.9, 25.8, 24.5, 22.9, 22.3, 17.8, 17.7), two oxygen-bearing quaternary carbons (δ_C 83.8, 71.2), six olefinic carbons (δ_C 172.4, 133.0, 132.9, 124.4, 119.4, 115.7), six methylene carbons (δ_C 36.6, 33.1, 32.4, 29.0, 26.5, 22.4), and three quaternary methine carbons (δ_C 77.3, 58.5, 49.3). These facts indicated that **5** had a similar ring structure to that of **4**. The HMBC experiments showed C–H long correlations as follows; geminal dimethyl at C-17 (δ_H 1.16, 3H, s, 1.25, 3H, s) to C-16 and 17 (δ_C 85.3, 71.2); H-3 (δ_H 4.77, 1H, dd, J=10.9, 5.5 Hz) to C-1, 2, 5, 13, 14, and 20 (δ_C 58.5, 32.4, 172.4, 83.8, 33.1, 22.9); H-16 (δ_H 3.88, 1H, t, J=7.3 Hz) to C-14, 15, 17, 18 and, 19 (δ_C 33.1, 26.5, 71.2, 24.5, 27.4); H-2 (δ_H 2.54, 1H, dd, J=13.2, 11.0 Hz, 1.86, 1H, dd, J=13.2, 5.5 Hz) to C-1, 3, 5, 11, 12 and 13 (δ_C 58.5, 88.5, 172.4, 36.6, 205.7, 83.8); H-21 (δ_H 3.09, 1H, dd, J=14.2, 7.5 Hz, 2.97, 1H, dd, J=14.2, 7.5 Hz) to C-5, 6, 7, 22 and 23 (δ_C 172.4, 115.7, 193.8, 119.4, 133.0); and so on as shown in Fig. 2. These results indicated that **5** had the same 4-oxo-tricyclo[6,3,1,$0^{1,5}$]dodeca-5-ene skeleton as sampsonione K.[18] Thus, the structure of **5** was determined to be 8-benzoyl-6,10-di-(3-methyl-2-butenyl)-3-[5-(1-hydroxy-1-methylethyl)-2-methyltetrahydrofuran-2-yl]-4-oxatricyclo[6,3,1,$0^{1,5}$]dodecane-7,12-dione. The relative configurations at C-1, 8 and 10 were determined from the similarity of stereochemistry with **4**. The stereochemistry at C-4 and the absolute stereochemistry could not be determined. Compound **5** was named otogirinin E and was an isomer of sampsonione K in the position of a benzoyl and a geranyl group.

The molecular formula of **6** was determined to be $C_{28}H_{34}O_5$ based on the $[M+Na]^+$ ion peak at *m/z* 473.2265 in its HR-ESI-MS (positive ion mode). The ^{1}H-NMR spectrum of **6** showed the presence of a phenyl group [δ_H 7.48 (2H, dd, J=7.6, 1.2 Hz), 7.43 (1H, t, J=6.8 Hz), 7.35 (2H, t, J=7.6 Hz)], five singlet methyl groups [δ_H 0.90 (3H, s), 1.48 (3H, s), 1.63 (3H, s), 1.80 (3H, s), 1.85 (3H, s)], two olefinic protons [δ_H 5.31 (1H, t, J=7.3 Hz), 4.84 (1H, dd, J=7.1, 6.8 Hz)], an intramolecular hydrogenbonded hydroxyl proton [δ_H 12.5 (1H, s)], an oxygen-bearing methine group [δ_H 3.70 (1H, dd, J=7.1, 5.6 Hz)], and four methylene groups [δ_H 3.43 (2H, d, J=7.3 Hz), 2.80 (1H, dd, J=16.4, 5.4 Hz), 2.49 (1H, dd, J=16.4, 7.3 Hz), 1.55 (1H, m), 1.45 (1H, m), 1.30 (1H, ddd, J=13.9, 11.5, 5.1 Hz), 1.19 (1H, ddd, J=13.9, 11.5, 5.4 Hz). The ^{13}C-NMR spectrum of **6** showed the presence of a benzoyl group (δ_C 203.0, 142.9, 130.4, 127.7×2, 127.5×2), trisubstituted phloroglucinol moiety (δ_C 161.3, 160.3, 153.5, 105.4, 105.3, 98.5), five methyl groups (δ_C 25.9, 25.7, 18.0, 17.7, 17.5), an oxygen-bearing methine carbon (δ_C 67.3), an oxygen-bearing quaternary carbon (δ_C 79.7), four olefinic carbons (δ_C 136.4, 131.9, 124.0, 121.3), and four methylene carbons (δ_C 37.3, 25.5, 21.8, 21.2). These facts indicated that **6** was a benzoyl benzopyran derivative with prenyl side chains. The HMBC experiments of **6** showed C–H long correlations as follow; a methyl at C-2 (δ_H 0.90, 3H, s) to C-2, 3 and 11 (δ_C 79.7, 67.3, 37.3); H-3 (δ_H 3.70, 1H, dd, J=7.1, 5.6 Hz) to C-2, 4, 10, 11 and 17 (δ_C 79.7, 25.5, 98.5, 37.3, 17.5); H-4 (δ_H 2.80, 1H, dd, J=16.4, 5.4 Hz, 2.49, 1H, dd, J=16.4, 7.3 Hz) to C-2, 5 and 9 (δ_C 79.7, 160.3, 153.5); OH at C-7 (δ_H 12.5, 1H, s) to C-6, 7 and 8 (δ_C 105.4, 161.3, 105.3); H-18 (δ_H 3.43, 2H, d, J=7.3 Hz) to C-5, 7 and 20 (δ_C 160.3, 161.3, 136.4); and so on as shown in Fig. 2. These data indicated that the plane structure of **6** was as shown in Fig. 1. The relative configurations at C-2 and 3 were determined from an NOE experiment. Hα at C-4 showed a correlation with the methyl group at C-2, and H at C-3 showed correlations with Hβ at C-4 and H-11. These results showed that the relative configuration between the methyl group at C-2 and OH at C-3 was *cis*. Thus, the structure of **6** was determined to be 8-benzoyl-3,5,7-trihydroxy-6-(3-methyl-2-butenyl)-2-(4-methyl-3-pentenyl)-2-methylchromane without an absolute configuration. Compound **6** was named otogirinin F.

The molecular formula of **7** was determined to be $C_{28}H_{34}O_5$ based on the $[M+Na]^+$ ion peak at *m/z* 473.2276 in its HR-ESI-MS (positive ion mode). The ^{1}H-NMR spectrum of **7** showed the presence of a phenyl group [δ_H 7.44 (2H, d, J=8.3 Hz), 7.43 (1H, t, J=7.3 Hz), 7.36 (2H, t, J=7.5 Hz)], five singlet methyl groups [δ_H 1.84 (3H, s), 1.69 (3H, s), 1.61 (9H, s), 0.93 (3H, s), 0.85 (3H, s)], two olefinic protons [δ_H 5.32 (1H, br t, J=6.8 Hz), 5.06 (1H, br t, J=6.8 Hz)], an oxygen-bearing methine group [δ_H 3.59 (1H, t, J=5.1 Hz), four methine groups [δ_H 2.77 (1H, dd, J=16.7, 5.1 Hz), 2.56 (1H, d, J=16.7, 5.5 Hz), 3.45 (2H, d, J=7.3 Hz), 2.15 (2H, overlap), 2.11 (2H, overlap)], and an intramolecular hydrogen-bonding OH [δ_H 12.60 (1H, s)]. The ^{13}C-NMR spectrum of **7** showed the presence of a benzoyl group (δ_C 200.3, 143.0 130.1, 127.5×2, 127.3×2), three oxygen-bearing aromatic carbons (δ_C 161.4, 160.8, 153.5), three aromatic carbons neighboring the phenolic hydroxyl group on both sides (δ_C 105.3, 105.1, 98.0), four olefine carbons (δ_C 140.1, 132.3, 123.6, 121.8), five methyl groups (δ_C 25.7, 24.0, 21.0, 17.8, 16.2), an oxygen-bearing quaternary carbon (δ_C 77.5), and an oxygen-bearing methine carbon (δ_C 68.7). The ^{1}H- and ^{13}C-NMR data of **7** showed similar signals to those of **6**. The HMBC experiments of **7** showed C–H long range correlations as follows; geminal dimethyl at C-2 (δ_H 0.93, 3H, s, 0.85, 3H, s) to C-2 and 3 (δ_C 77.5, 68.7); H-3 (δ_H 3.59, 1H, t, J=5.1 Hz) to 2, 4, 10, 11 and 12 (δ_C 77.5, 25.7, 98.0, 21.0, 24.0); H-4 (δ_H, 2.77, 1H, dd, J=16.7, 5.1 Hz, 2.56, 1H, dd, J=16.7, 5.5 Hz) to C-2, 3, 5, 9 and 10 (δ_C 77.5, 68.7, 160.8, 153.5, 98.0); H-13 (δ_H 3.45, 2H, d, J=7.3 Hz) to C-5, 6, 7, 14 and 15 (δ_C 160.8, 105.1, 161.4, 121.8, 140.1); Me-21 (δ_H 1.84, 3H, s) to C-14 and 16 (δ_C 121.8, 39.8); H-18 (δ_H 5.06, 1H, br t, J=6.8 Hz) to C-16, Me-20 and Me-22 (δ_C 39.8, 25.7, 17.8); and so on as shown

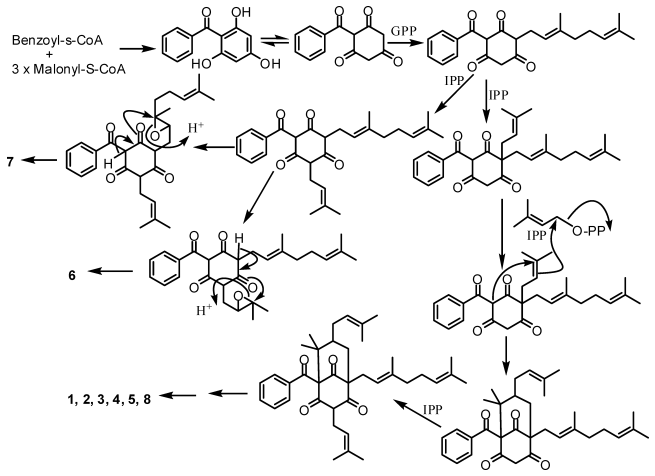

Fig. 4. Hypothetical Biosynthetic Pathway of Compounds **1**—**8** from Benzoylphloroglucinol

in Fig. 2. From these data, the structure of **7** was determined to be 8-benzoyl-2,2-dimethyl-6-([*E*]-3,7-dimethyl-2,6-octadienyl)-3,5,7-trihydroxychromane. The absolute configuration of **7** was not determined. Compound **7** was named otogirinin G. Compound **6** was derived from the precursor through cyclization at a geranyl part, but compound **7** should be derived from the precursor through cyclization at an isopentenyl part.

Compounds **1**—**8** should be biosynthesized from benzoylfuloroglucinol with some prenyl moieties, which was derived from a benzoyl-*S*-CoA and three malonyl-*S*-CoA, and followed by prenylation. The prenylated common intermediates were transformed by three dimensional transannular cyclization at the prenyl parts to give several different type compounds as shown in Fig. 4. We isolated interesting polyprenylated benzoylphloroglucinol derivatives and some of them had novel cage structures. Compound **1** has tricycle[3,3,1,$1^{3,7}$]decane carbon skeleton (adamantane) and **2**, **3** and **8** had a tricycle[4,3,1,$1^{3,8}$]undecane carbon skeleton (homoadamantane).

AcOEt-soluble and *n*-BuOH-soluble fractions of the MeOH extract of *H. erectum* showed strong suppressive activity (100% inhibithion ratio at 125 μg/ml in both fractions) against isovaleric acid generation from L-leucine by *C. xerosis*, but the isolated compound from the AcOEt soluble fraction showed only weak activity (77% inhibition ratio at 125 μg/ml in the case of **6**). We found that some of the purified compounds decomposed after isolation. Thus, we assumed that strong active constituents were unstable under isolation conditions, and decomposed during the extraction and isolation processes.

Table 3. ^{13}C-NMR Data of **1**—**7** ($CDCl_3$, 125 MHz)

Position	1	2	3	4	5	6	7
C-1	82.3	66.2	68.7	53.6	58.5		
2	202.5	31.1	208.7	176.5	32.4	79.7	77.5
3	68.8	88.9	71.5		88.5	67.3	68.7
4	203.4		50.8	93.0		25.5	25.7
5	72.0		30.3	27.8	172.4	160.3	160.8
6	51.7	88.4	44.4	118.8	115.7	105.4	105.1
7	48.9	42.6	50.0	188.2	193.8	161.3	161.4
8	55.0	31.4	84.9	77.8	77.3	105.3	105.3
9	201.9	44.5	205.3	49.3	49.3	153.5	153.5
10	40.4	50.2	42.0	48.4	47.8	98.5	98.0
11	193.4	81.8	206.4	38.9	36.6	37.3	21.0
12	135.0	207.9	29.5	207.1	205.7	21.2	24.0
13	129.2	205.1	119.4	29.5	83.8	124.0	21.7
14	128.0	68.5	138.5	119.4	33.1	131.9	121.8
15	132.4	41.2	40.3	138.7	26.5	25.7	140.1
16	128.0	204.4	26.9	40.4	85.3	17.7	39.8
17	129.2	73.0	124.2	26.5	71.2	17.5	26.2
18	27.6	24.9	131.6	123.8	24.5	21.8	123.6
19	118.6	25.9	25.8	131.9	27.4	121.3	132.3
20	138.7	28.1	16.5	25.7	22.9	136.4	25.7
21	40.1	17.8	17.8	16.9	22.4	25.9	16.2
22	26.8	22.7	34.4	17.7	119.4	18.0	17.8
23	124.2	24.9	119.3	71.9	133.0	203.0	200.3
24	131.5	192.1	136.3	23.5	25.8	142.9	143.0
25	25.8	134.8	26.2	26.0	17.8	127.5	127.3
26	16.5	128.8	18.2	193.4	193.5	127.7	127.5
27	17.8	128.0	76.7	136.8	137.0	130.4	130.1
28	26.6	132.4	26.7	128.2	128.1	127.7	127.5
29	118.5	128.0	33.2	128.1	127.9	127.5	127.3
30	134.7	128.8	23.8	132.2	131.9		
31	26.2	29.6	25.5	128.1	127.9		
32	17.9	118.7	195.6	128.2	128.1		
33	119.6	138.9	138.2	22.7	27.0		
34	137.2	40.0	128.9	26.8	22.3		
35	26.1	26.7	128.0	30.0	29.0		
36	18.0	124.1	131.2	124.9	124.4		
37	23.0	131.5	128.0	132.5	132.9		
38	23.4	25.7	128.9	25.9	25.9		
39		16.5		18.0	17.7		
40		17.7					

Experimental

Optical rotations were recorded on a JASCO P-1010 polarimeter at 25 °C. The UV spectrum was observed by an U-2001 spectrophotometer (Hitachi). The IR spectrum was observed by a FT-IR spectroscopy (Perkin-Elmer). One-dimensional (1D) and two-dimensional (2D) ^{1}H- and ^{13}C-NMR spectra were measured on a Unity INOVA 500 spectrometer (Varian) and Bruker (600 MHz) (^{1}H-NMR; 500 and 600 MHz, ^{13}C-NMR; 125 and 150 MHz). HR-ESI-TOF-MS spectra were obtained on a Micromass Q-TOF micro mass spectrometer (Waters Corp., Milford, U.S.A.). Preparative and analytical HPLC were performed on a reverse phase column (Mightysil RP-28 and 8, Kanto Chemical Co., Inc.) with CH_3CN–H_2O solvent systems. Silica gel 60N (100—210 μm, Kanto Chemical Co., Inc.) was used for column chromatography. Analytical and preparative thin layer chromatography (PLC) were carried out on pre-coated Kieselgel 60 F_{254} (0.25 mm thickness plate for analysis and 0.5 mm thickness plate for preparation, respectively, Merck) and spots were visualized by spraying the plates with 50% H_2SO_4, followed by heating.

Plant Material Dry aerial parts of *Hypericum erectum* were purchased from Matsuura Yakugyou Co., Ltd. on May, 2005. Part of the sample was deposited in the laboratory of natural product chemistry of the Prefectural University of Hiroshima.

Extraction and Isolation The chipped aerial part of *Hypericum erectum* (1 kg) was extracted with methanol (MeOH) under reflux to give the MeOH extract (108 g). The MeOH extract was partitioned between ethyl acetate (AcOEt) and water to give an AcOEt-soluble fraction (HYMA, 52 g). The water layer was extracted with *n*-butanol (*n*-BuOH) to give *n*-BuOH-soluble (HYMB, 21 g) and water soluble (HYMW, 33 g) fractions. Of these fractions, HYMA and HYMB showed strong suppressive activity (100% inhibition at 125 μg/ml) against generation of isovaleric acid by *C. xerosis*. HYMA showed the presence of various compounds on its TLC profile. Thus, we isolated the constituents from HYMA.

HYMA (51 g) was choromatographed on a silica gel column using a gradient-*n*-hexane–AcOEt solvent system to give many fractions, which were compiled into 16 fractions (HYMA-1—HYMA-16) according to TLC profiles. These fractions were examined for inhibition activity on isovaleric acid generation, and HYMA-2, HYMA-3, HYMA-4, and HYMA-10 showed strong activity (100% inhibition at 125 μg/ml). Of these fractions, HYMA-3 showed the highest fraction-yield (11.3 g); thus, HYMA-3 was further purified by silica gel column chromatograohy, PLC and HPLC, successively, to give compound **1** (192 mg), **2** (67 mg), **3** (8 mg) and **4** (27 mg). HYMA-2 (2 g) was also purified by PLC and HPLC to give compound **2** (43 mg).

HYMA-10 (2.0 g) was purified by PLC and HPLC to give compounds **5** (6 mg), **6** (16 mg) and **7** (20 mg).

Otogirinin A (**1**): Colorless amorphous solid. HR-ESI-MS *m/z:* 569.3604 $[M+H]^+$ (Calcd for $C_{38}H_{49}O_4$; 569.3631). $[\alpha]_D$ −8.1° (c=0.08, MeOH). CD; $[\theta]_{289}$ +3204, $[\theta]_{242}$ −6032 (MeOH). UV λ_{max} nm (ε); 215 (5728), 246 (8534) (MeOH). IR ν_{max} cm^{-1}; 2975, 2920, 1742, 1700, 1450, 1245 (KBr). ^{1}H-NMR was shown in Table 1 and ^{13}C-NMR in Table 3.

Otogirinin B (**2**): Colorless amorphous solid. HR-ESI-MS *m/z*: 641.3450 $[M+Na]^+$ (Calcd for $C_{38}H_{50}O_7Na$; 641.3454). $[\alpha]_D$ +12.0° (c=0.14, MeOH). CD; $[\theta]_{253}$ +1033, $[\theta]_{220}$ +1395 (MeOH). UV λ_{max} nm (ε); 214 (6250), 243 (8120), 281sh (1360) (MeOH). IR ν_{max} cm^{-1}; 3438, 2980, 2930, 1737, 1703, 1249, 1224 (KBr). ^{1}H-NMR is shown in Table 1 and ^{13}C-NMR in Table 3.

Otogirinin C (**3**): Colorless amorphous solid. HR-ESI-MS *m/z*: 609.3538 $[M+Na]^+$ (Calcd for $C_{38}H_{50}O_5Na$; 609.3556). ^{1}H-NMR is shown in Table 1 and ^{13}C-NMR in Table 3.

Otogirinin D (**4**): Colorless amorphous solid. HR-ESI-MS *m/z*: 609.3563 $[M+Na]^+$ (Calcd for $C_{38}H_{50}O_5Na$; 609.3556). 587.3779 $[M+H]^+$ (Calcd for $C_{38}H_{51}O_5$; 587.3737). $[\alpha]_D$ +160.0° (c=0.03, MeOH). UV λ_{max} nm (ε); 247 (13090), 288 (11360) (MeOH). IR ν_{max} cm^{-1}; 3450, 2975, 2930, 1725, 1700, 1625, 1405, 1222 (KBr). ^{1}H-NMR is shown in Table 2 and ^{13}C-NMR in Table 3.

Otogirinin E (**5**): Colorless amorphous solid. HR-ESI-MS *m/z*: 603.3658 $[M+H]^+$ (Calcd for $C_{38}H_{51}O_6$; 603.3686). ^{1}H-NMR is shown in Table 2 and ^{13}C-NMR in Table 3.

Otogirinin F (**6**): Colorless amorphous solid. HR-ESI-MS *m/z*: 473.2265 $[M+Na]^+$ (Calcd for $C_{28}H_{34}O_5Na$; 473.2304). ^{1}H-NMR is shown in Table 2 and ^{13}C-NMR in Table 3.

Otogirinin G (**7**): Colorless amorphous solid. HR-ESI-MS *m/z*: 473.2276 $[M+H]^+$ (Calcd for $C_{28}H_{34}O_5Na$; 473.2304). ^{1}H-NMR is shown in Table 2 and ^{13}C-NMR in Table 3.

Evaluation of Suppressive Activity of Isovaleric Acid Germination by *Corynebacterium xerosis*. Bacteria, Media, and Chemicals *C. xerosis* was subdivided from the American Type Culture Collection (ATCC) and the ATCC number was 7094. We used tryptone soya broth (TSB) in the case of *C. xerosis*, to which we added 0.1% yeast extract and 0.1% Tween 80; these media were purchased from Becton, Dickinson and Company (Sparks, MD, U.S.A.). Other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Biotransformation Assays We conducted the biotransformation assays for isovaleric acid generation according to Takenaka *et al.*'s method[9] with slight modifications. We evaluated the inhibitory effect of fractions or isolated constituents from *Hypericum erectum* on isovaleric acid generation.

Before the assay, *C. xerosis* was pre-cultured, aerobically with shaking, in 1000 ml conical flasks at 37 °C in 500 ml of medium. The pre-culturing was continued until an optical density (A_{655}) of 1.3 was reached and 200 ml of them was then harvested by centrifugation (2500 rpm, 10 min). The precipitate was rinsed with an adequate amount of saline solution and centrifuged again under the same conditions. The rinsed precipitate was resuspended in 100 ml of 0.05 M phosphate buffer (pH 6.0) and used as the bacterial dispersion.

We mixed 2.5 ml of 0.05 M phosphate buffer (pH 6.0), including 1.0% L-leucine and 2.5 ml of the bacterial dispersion and each sample was added to the mixture. The mixture was incubated aerobically, with shaking, at 37 °C for 6 h. A reaction mixture with ethanol instead of with a sample was defined as the control. After incubation, aliquots of the mixture (2.5 μl) were separated for viable cell counts.

The residual mixture was filtered through a sterile 0.22-micron filter. We added 0.5 ml of 10% sulfuric acid and 3 ml of ethyl acetate into 2 ml of the filtrate to extract lipophilic substances. Extraction was performed by vortex-mixing and centrifugal separation (2500 rpm, 5 min). The organic extract was analyzed by GC and the inhibitory effect was calculated.

Measurement of Viable Cell Counts Aliquots of an assay medium (2.5 μl) were diluted one hundred times with TSB in a 96-well plate, and a ten-fold dilution was repeated eight times. After dilution, the 96-well plates were incubated aerobically at 37 °C for 2 d. After incubation, the optical density at A_{595} was measured with a micro plate reader (Spectra Image, Tecan, Austria). We estimated viable cell counts from the dilution rate, which exhibited turbidity.

GC Analysis GC analysis was conducted using a GC353B (GL Sciences, Inc., Tokyo, Japan) with a CP-FFAP CB column, 25 m long with an i.d. of 0.32 mm (Varian, Inc., CA, U.S.A.). The GC column was maintained at a temperature of 150 °C for 2 min. The column was subsequently programmed to be heated from an initial temperature of 150 to 220 °C at 10 °C/min, and this was maintained at 220 °C for 2 min. Injector and detector temperatures were both 250 °C. The carrier gas for the column was helium, and the sample volume for injection was 5 μl.

Isovaleric acid generation was determined by comparing peak areas of the sample and blank. The generated isovaleric acid was quantified using a standard curve for authentic isovaleric acid.

Statistical Analysis Any significant differences in the mean values between groups were determined by using an analysis of variance (ANOVA) and Fisher's protected least significant difference (PLSD).

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