An Investigation of Diterpenes from the Leaves of *Rabdosia trichocarpa* and Their Antibacterial Activity against Oral Microorganisms

Kenji OSAWA,*[,a] Hideyuki YASUDA,[a] Takashi MARUYAMA,[a] Hiroshi MORITA,[b] Koichi TAKEYA,[b] Hideji ITOKAWA,[b] and Katsuji OKUDA[c]

Department of Basic Research, Lotte Central Laboratory Co., Ltd.,[a] Numakage 3-1-1, Urawa, Saitama 336, Japan, Department of Pharmacognosy, Tokyo College of Pharmacy,[b] Horinouchi 1432-1, Hachioji, Tokyo 192-03, Japan, and Department of Microbiology, Tokyo Dental College,[c] Masago 1-2-2, Mihama-ku, Chiba 261, Japan.
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The ethanolic extract of *Rabdosia trichocarpa* leaves showed antibacterial activity against cariogenic mutans streptococci and periodontopathic *Porphyromonas gingivalis*. Chromatographic separation and purification of the extract afforded ten diterpenes, including one novel *ent*-kauren type compound named trichoranin. Some of these compounds possess potent antibacterial activity against these oral micro-organisms, indicating that these diterpenes may be useful natural substances for the maintenance of oral health.

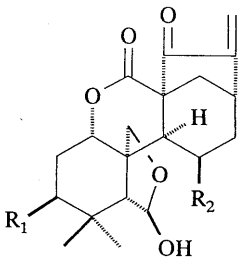
Keywords *Rabdosia trichocarpa*; Labiatae; trichoranin; antibacterial activity; diterpen; oral micro-organism

Dental caries and periodontal disease are the major oral infectious diseases. It has been shown that members of the mutans streptococci group, mainly *Streptococcus mutans* itself and *Streptococcus sobrinus*, are important human cariogenic bacteria which synthesize insoluble glucan from sucrose, adhere to tooth surfaces, and produce acids.[1] *Porphyromonas gingivalis* is a gram-negative, anaerobic rod which contains lipopolysaccharides,[2] exhibits proteolytic enzymatic activities[3] and other periodontopathic factors. These microorganisms are common in human periodontal lesions and are major pathogens for adult periodontitis. They have been implicated as etiological agents of dental caries and periodontal disease. To prevent such diseases, it is important to control the growth of these bacteria in the oral cavity by using compounds with antibacterial properties.[4] In a previous paper,[5] we reported that several isoflavanones from *Swartzia polyphylla* showed potent antibacterial activity against cariogenic bacteria. In the course of our antibacterial studies using natural products, we observed that an ethanolic extract prepared from the dried leaves of *Rabdosia trichocarpa* exhibited potent antibacterial activity against cariogenic and periodontopathic bacteria. Plants of the genus *Rabdosia* are known to contain many kinds of diterpenes. Several reports have examined their chemistry and biological activities as antimutagenic,[6] antitumor[7] and antibacterial[8] agents. In the present study, fractionation of the active extract of *R. trichocarpa*, followed by bioassay, led to the isolation and characterization of ten diterpenes with antibacterial effects against oral micro-organisms.

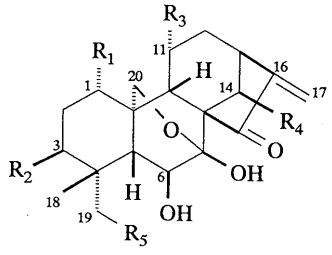
Results and Discussion

Ethanol-soluble material was extracted from the dried leaves of *Rabdosia trichocarpa* (Labiatae). The extract showed appreciable antibacterial activity against *S. mutans* Ingbritt and *P. gingivalis* ATCC 33277 using the paper disk assay method (inhibitory zones were formed at 50 μg/disk against both micro-organisms). Following partition with *n*-hexane, ether, and ethyl acetate, the antibacterial activity was found to be concentrated in the ether-soluble fraction. This fraction was subjected to silica-gel column chromatography to yield several sub-fractions. From the biologically-active sub-fractions, ten diterpenes (**1**—**10**) were isolated by repeated HPLC separation and recrystallization. Among these ten, nine of the compounds were identified as enmein (**1**),[9] nodosin (**2**),[10] oridonin (**3**),[11] effusanin A (**4**),[11] effusanin B (**5**),[12] longikaurin A (**6**),[13] longikaurin B (**7**),[12] longikaurin D (**8**),[14] and lasiokaurin (**9**).[11] We carried out a structural investigation of the novel tenth diterpene and identified it as trichoranin (**10**).

Trichoranin (**10**) was obtained as colorless crystals, mp 218—220 °C. The high resolution mass spectrum of **10** supported the molecular formula of $C_{22}H_{30}O_7$ (M^+ 406.2002, requires 406.1992). Its infrared (IR) absorption indicated the presence of hydroxyl(3430 cm^{-1}) and carbonyl (1691, 1722, 1745 cm^{-1}) groups. Trichoranin has a five membered ketone conjugated with an *exo*-methylene group (UV: 239 nm (log ε, 3.97); IR: 1745, 1691 cm^{-1}, $^1H\delta$: 5.29, 5.94 (each 1H, s); $^{13}C\delta$: 116.40 (t), 153.67 (s) (*exo*-methylene), 210.15 (s) (ketone)). Comparison of the



1: R_1=OH, R_2=H
2: R_1=H, R_2=OH



3: R_1=R_4=OH, R_2=R_3=R_5=H
4: R_1=OH, R_2=R_3=R_4=R_5=H
5: R_1=OAc, R_2=R_3=R_4=R_5=H
6: R_1=R_2=R_3=R_5=H, R_4=OH
7: R_1=R_2=R_3=H, R_4=OH, R_5=OAc
8: R_1=R_2=R_4=H, R_3=OH, R_5=OAc
9: R_1=OAc, R_2=R_3=R_5=H, R_4=OH
10: R_1=R_3=R_4=H, R_2=OH, R_5=OAc

Fig. 1

spectral data described above with those of congeneric diterpenes **3**—**10** suggested the basic skeleton of **10** was *ent*-7β,20-epoxy-kaur-16-en-15-one-7α-ol, containing two secondary hydroxyl groups (^{1}Hδ: 7.06 (1H, d, J=10.1 Hz), 6.16 (1H, br s); ^{13}Cδ: 73.53 (d), 69.15 (d)) and one acetoxyl group (^{1}Hδ: 1.92 (3H, s); ^{13}Cδ: 20.55 (q), 170.70 (s)). By comparing these analytical data of trichoranin with those of longikaurin D (**8**) (see Experimental), it was clear that these compounds were isomers, differing only in the location of their hydroxyl groups. In the ^{13}C-NMR data of trichoranin, C-2 (25.70, t) and C-4 (42.29, s) signals were shifted down field from that of longikaurin D, indicating that one secondary hydroxyl group was located at the C-3 position. This suggestion was confirmed by the fact that the carbon signal bearing a hydroxyl group at δ 69.15 (d) showed a cross-peak with the *tert*-methyl protons in the heteronuclear multiple-bond correlation (HMBC) spectrum.[15] The stereochemistry of the hydroxyl group at C-3 was assigned to be in the β-orientation from the coupling pattern of the proton signal of H-3 at δ 3.73 (1H, t, J=2.9 Hz) in methanol-d_4. Another secondary hydroxyl group was attached to the C-6, whose location was established from the ^{1}H-NMR chemical shift and coupling pattern of H-6 (δ 4.46 (dd, J=6.4, 10.1 Hz)); this was also supported by nuclear Overhauser effect (NOE) effects between H-6 and H-5, H-18, H-19. Catalytic hydrogenation of **10** gave a dihydrocompound which had a β-oriented methyl group (^{1}Hδ: 1.01 (3H, d, J=6.9 Hz))[16] and exhibited a negative Cotton effect at 299.6 nm, which indicated that the absolute configuration of the D ring was α.[17] Based on these data, trichoranin was assigned the structure **10** as shown in Fig. 1. NOE experiments also supported this structure. The ^{1}H- and ^{13}C-NMR assignments of trichoranin are listed in Table I.

The antibacterial activities of these diterpenes isolated from *R. trichocarpa* against cariogenic and periodontopathic bacteria were estimated using the paper disk method and the results are summarized in Table II. As can be seen, these compounds had no appreciable activity against *Actinomyces viscosus* ATCC 15987. As far as the other organisms were concerned, all the tested *Rabdosia* diterpenes exhibited some inhibitory effect, except for longikaurin D (**8**), but the degree of this effect varied considerably. Nodosin, oridonin, longikaurin B, and lasiokaurin had relatively strong antibacterial activities against cariogenic bacteria. Among the *ent*-kauren type diterpenes (**3**—**10**), oridonin, longikaurin B and lasiokaurin have a hydroxyl group at the C-14 position. The results suggest that this structural feature might be related to their highly antibacterial properties. Among the periodontopathic bacteria, no significant differences in inhibitory activity were observed among these compounds, except for longikaurin D. Kubo *et al.* have reported that the structural requirement for activity in the group of *Rabdosia*

Table I. ^{13}C and ^{1}H Spectral Assignment of Trichoranin (**10**) in Pyridine-d_6

C	^{13}C	DEPT	^{1}H (J)
1	22.64	t	1.05, m
2	25.70	t	1.85, m
			1.76, m
3	69.15	d	4.13, overlap (3.73, t (2.9))[a]
4	42.29	s	
5	55.93	d	2.43, d (6.4)
6	73.53	d	4.46, dd (6.4, 10.1)
7	96.04	s	
8	60.09	s	
9	49.93	d	1.69, m
10	36.17	s	
11	16.60	t	1.27, m
12	29.50	t	2.10, m
			1.59, dd (8.4, 12.3)
13	34.90	d	2.88, dd (4.1, 9.5)
14	26.52	t	2.44, dd (4.5, 12.3)
			2.26, d (12.3)
15	210.15	s	
16	153.67	s	
17	116.40	t	5.94, s
			5.27, s
18	32.33	q	1.70, s
19	67.81	t	4.66, d (11.4)
			4.61, d (11.4)
20	66.68	t	4.12, d (10.2)
			4.04, d (10.2)
18 OCOC$\underline{H}_3$	20.55	q	1.92, s
18 O$\underline{C}$OCH$_3$	170.70	s	
7 OH			8.82, br s
6 OH			7.06, d (10.1)
3 OH			6.16, br s

Chemical shift values are in ppm. The coupling constants (J values) in parentheses are in Hz. *a*) The value in methanol-d_4.

Table II. Antibacterial Activity of Diterpenes from *Rabdosia trichocarpa* against Cariogenic and Periodontopathic Bacteria

Samples	Cariogenic bacteria				Periodontopathic bacteria			
	S.m[a] Ingbritt	*S.m*[a] LA7	*S.s*[b] 6715	*S.s*[b] B13	*P.g*[c] FDC 381	*P.g*[c] 33277	*A.v*[d] 15987	*F.n*[e] 25586
Enmein (**1**)	+	++	++	++	++	+++	−	++
Nodosin (**2**)	++	+++	++	++	+++	+++	+	+++
Oridonin (**3**)	++	++	+++	+++	++	+++	+	++
Effusanin A (**4**)	++	+	++	++	++	++	−	++
Longikaurin B (**7**)	+++	++	+++	+++	+++	+++	+	++
Longikaurin D (**8**)	−	−	−	−	+	+	−	+
Lasiokaurin (**9**)	++	+++	+++	+++	++	++	−	++
Trichoranin (**10**)	+	+	+	+	++	++	−	++
Dihydrooridonin	−	−	−	−	−	−	−	−

Antibacterial activity was determined by paper disk assay. Inhibitory zone was formed; +++, at 12.5 μg/disk; ++, at 25 μg/disk; +, at 50 μg/disk; − no inhibitory zone formed at 50 μg/disk. *a*) *Streptococcus mutans*, *b*) *Streptococcus sobrinus*, *c*) *Porphyromonas gingivalis*, *d*) *Actinomyces viscosus*, *e*) *Fusobacterium nucleatum*.

TABLE III. MIC of Diterpenes from *Rabdosia trichocarpa* against *S. mutans*, *S. sobrinus* and *P. gingivalis* (μg/ml)

Samples	*S. mutans* Ingbritt	*S. sobrinus* 6715	*P. gingivalis* 33277
Enmein (**1**)	200 (50)	ND	12.5 (3.13)
Nodosin (**2**)	100 (25)	50 (25)	12.5 (1.56)
Oridonin (**3**)	100 (25)	50 (25)	6.25 (1.56)
Effisanin A (**4**)	100 (25)	50 (50)	12.5 (3.13)
Longikaurin B (**7**)	50 (25)	50 (50)	6.25 (1.56)
Longikaurin D (**8**)	>200 (>200)	>200 (100)	12.5 (3.13)
Lasiokaurin (**9**)	100 (50)	ND	12.5 (3.13)
Trichoranin (**10**)	200 (100)	100 (50)	12.5 (6.25)
Thymol	100 (100)	200 (100)	100 (50)

( ): the final dilute concentration possessing apparent antibacterial effect (the absorbance is less than half the value of the control at 660 nm). ND: not done.

diterpenes requires an exocyclic methylene conjugated with a ketone; this active portion is highly reactive towards sulfhydryl groups essential for biological function.[8] All the compounds isolated in this study have this structural moiety in the D ring. We prepared dihydrooridonin by hydrogenation of oridonin with Pd–C, according to a procedure described earlier,[18] and investigated its antibacterial activity. The strong antibacterial action against cariogenic and periodontopathic bacteria disappeared completely following this slight structural modification of oridonin.

We further examined the growth inhibitory effects of these diterpenes with respect to *S. mutans* Ingbritt, *Streptococcus sobrinus* 6715 and *P. gingivalis* ATCC 33277 (Table III) by the broth dilution method. As shown in Table III, these diterpenes,except for longikaurin D, inhibit *S. mutans* and *S. sobrinus* at concentrations of 50—200 μg/ml. The antibacterial activities of *Rabdosia* diterpenes against *S. mutans* and *S. sobrinus* were slightly greater than that of thymol, which is known as a wide-spectrum antibacterial agent. These compounds possessed potent antibacterial activity against *P. gingivalis* ATCC 33277. Oridonin and longikaurin B were the most active compounds and bacterial growth was completely inhibited at 6.25 μg/ml. These two compounds and nodosin showed appreciable inhibitory activity, even at 1.56 μg/ml. The inhibitory activity of these diterpenes on *P. gingivalis* as greater than that of thymol (MIC, 100 μg/ml). *Rabdosia* diterpenes are known to exhibit highly specific antibacterial activity against gram-positive bacteria, such as *Bacillus subtilis*[8]; however, their inhibitory effects on gram-negative bacteria, such as *P. gingivalis*, have not been reported. In this study, we found that the *Rabdosia* diterpenes exhibited antimicrobial effect against not only gram-positive bacteria, but also gram-negative periodontopathic bacteria. However the antibacterial activity of these diterpenes was not as powerful as that exhibited by antibiotics such as tetracycline–HCl (bacterial growth was completely prevented at 0.78 μg/ml (*S. mutans* Ingbritt) and 0.39 μg/ml (*P. gingivalis* ATCC 33277) under the same conditions). Continuous use of antibiotics solely for the prevention of oral disease should be avoided because of the possibility resistant bacteria may appear. Our findings suggest that these diterpenes may be among the most effective agents for preventing the growth of both cariogenic and periodontopathic bacteria from natural sources.

Experimental

All melting points were recorded on a Yanagimoto MP-3 micro melting point apparatus and were uncorrected. Spectral data were obtained using the following instruments: optical rotation on a JASCO DIP-4, circular dichroism (CD) on a JASCO J-700, IR on a Perkin-Elmer 1750, UV on a Shimadzu UV-160, NMR on a Bruker AM 400 and MS on a Hitachi M-80. High-performance liquid chromatography (HPLC) was carried out on a Shimadzu LC-8A system with a Senshu pak silica-5251-S column for normal phase separation and a Senshu pak ODS-5251-SH column for reversed phase separation.

Chemicals The standard antibacterial agent, thymol, was purchased from Kokusan Chemical Co., and tetracycline–HCl was purchased from Sigma Chemical Co., U.S.A.

Test Bacterial Strains and Culture Media The four cariogenic bacteria, *S. mutans* Ingbritt, *S. mutans* LA7, *S. sobrinus* B13, *S. sobrinus* 6715, and the four periodontopathic bacteria, *P. gingivalis* ATCC 33277, *P. gingivalis* FDC 381, *A. viscosus* ATCC 15987, *Fusobacterium nucleatum* ATCC 25586, were tested. *S. mutans*, *S. sobrinus* and *A. viscosus* were cultured on Brain heart infusion agar (BHI, Difco) at 37 °C. *P. gingivalis* and *F. nucleatum* were cultured on blood agar medium which consisted of Trypticase soy agar (BBL) supplemented with 5 μg/ml hemin, 0.5 μg/ml menadione and 10% defibrinated horse blood at 37 °C in an anaerobic chamber with an atmosphere of 10% carbon dioxide, 10% hydrogen, and 80% nitrogen.

Assay of Antibacterial Activity Paper Disk Method: Each precultured organism was inoculated onto a BHI agar plate (*S. mutans*, *S. sobrinus* and *A. viscosus*) or blood agar medium plate (*P. gingivalis* and *F. nucleatum*). The paper disk (8 mm in diameter, Advantec) which contained the test substance was placed on the seeded medium. The BHI agar plates were incubated at 37 °C for 24 h and the blood agar medium plates were incubated at 37 °C for 48 h in the anaerobic atmosphere. The diameter of the inhibitory zone was then measured.

Measurement of Growth Inhibitory Effect: Two-fold serial dilutions of the test substances were prepared with ethanol and sterilized by passing through a filter (0.22 μm, Millipore). Then 100 μl of a given dilution of the test substance was mixed with 4.85 ml of liquid BHI broth (*S. mutans* and *S. sobrinus*) or Trypticase soy broth containing 5 μg/ml hemin and 0.5 μg/ml menadione (*P. gingivalis*) in glass tubes (15 mm in diameter). A control culture medium was prepared by adding 100 μl of ethanol. An aliquot of 50 μl of the bacterial suspension, adjusted to an optical density (OD) of 0.8 (550 nm), was inoculated into media containing test substances. The culture test tubes were incubated at 37 °C in an aerobic (*S. mutans* Ingbritt and *S. sobrinus* 6715) or anaerobic (*P. gingivalis*) atmosphere. Bacterial growth was monitored by measuring the increase in absorbance (OD at 660 nm) using a digital colorimeter (ICM Co.,). The minimum inhibitory concentration (MIC) of each test substance was taken as that required to produce on absorbance less than 0.5 of the control tube for each bacterium.

Extraction and Isolation Ethanol soluble material was extracted from the dried leaves of *Rabdosia trichocarpa* (1.9 kg), purchased from Uchida Wakanyaku Co. (Japan), at room temperature for 10 d. A total of 60.21 g of ethanol-soluble material was obtained. The extract was fractionated into *n*-hexane, ether, ethyl acetate and water soluble fractions, respectively. The active fraction (ether soluble fraction) was subjected to silica-gel column chromatography. The column was eluted with dichloromethane–methanol (100 : 0—0 : 100, v/v) and separated into nine fractions (A-I) including active fractions B, C, and F. Fraction B was separated by silica-gel HPLC using dichloromethane–ethyl acetate–methanol (94 : 6 : 0.6 v/v) as mobile phase and on ODS HPLC with methanol–water (55 : 45—100 : 0, v/v) as mobile phase to afford effusanin B (**5**). Fraction C was separated on ODS HPLC with methanol–water (50 : 50—100 : 0, v/v) as mobile phase, and each fraction was further purified by on ODS HPLC with acetonitrile–water (30 : 70—50 : 50, v/v) as mobile phase to give enmein (**1**), nodosin (**2**), effusanin A (**4**), longikaurin A (**6**), longikaurin B (**7**), longikaurin D (**8**), lasiokaurin (**9**), and trichoranin (**10**). Fraction F, purified using silica-gel HPLC with dichloromethane–methanol (93 : 7, v/v) as mobile phase, afforded oridonin (**3**).

Enmein (1) Colorless crystals, mp >300 °C, $[\alpha]_D$ −172.0° (c=0.043, EtOH). IR (KBr): 3453, 2947, 1749, 1703, 1351, 1258, 1072 cm^{-1}. UV

λ_{max}^{EtOH} nm (log ε): 231.8 (3.89). MS *m/z* (%): 363 ($M^+ + 1$, 8), 344 (10), 183 (100), 165 (46), 91 (52). ^{1}H-NMR (pyridine-d_5) δ: 5.93, 5.25 (each 1H, s, H-17), 5.90 (1H, s, H-6), 5.42 (1H, dd, J=6.0, 11.6 Hz, H-1), 4.56, 4.38 (each 1H, d, J=8.9 Hz, H-20), 3.81 (1H, d, J=2.9 Hz, H-3), 2.85 (1H, dd, J=4.2, 8.9 Hz, H-13), 1.33 (3H, s, H-18), 1.04 (3H, s, H-19).

Nodosin (2) Colorless crystals, mp 283—288 °C, $[\alpha]_D$ −200.0° (c=0.017, EtOH). IR (KBr): 3525, 2949, 1745, 1704, 1335, 1236, 1044 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 230.8 (3.85). MS *m/z* (%): 363 ($M^+ + 1$, 5), 344 (15), 167 (44), 150 (45), 91 (100). ^{1}H-NMR (pyridine-d_5) δ: 5.95, 5.30 (each 1H, d, J=0.8 Hz, H-17), 5.80 (1H, overlap, H-1), 5.80 (1H, s, H-6), 5.10 (1H, d, J=4.0 Hz, H-11), 4.55, 4.32 (each 1H, d, J=8.9 Hz, H-20), 3.14 (1H, dd, J=4.4, 8.9 Hz, H-13), 1.01 (3H, s, H-19), 0.98 (3H, s, H-18).

Oridonin (3) Colorless crystals, mp 250—252 °C, $[\alpha]_D$ −54.6° (c=0.097, EtOH). IR (KBr): 3382, 3317, 1710, 1646, 1457, 1272, 1065, 944 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 237.0 (3.95). MS *m/z* (%): 364 (M^+, 52), 318 (16), 204 (18), 177 (24), 133 (32), 105 (60), 85 (100). ^{1}H-NMR (pyridine-d_5) δ: 6.26, 5.49 (each 1H, s, H-17), 5.31 (1H, s, H-14), 4.76, 4.38 (each 1H, d, J=10.1 Hz, H-20), 4.24 (1H, d, J=6.8 Hz, H-6), 3.62 (1H, dd, J=6.0, 11.0 Hz, H-1), 3.19 (1H, d, J=9.1 Hz, H-13), 1.28 (3H, s, H-19), 1.04 (3H, s, H-18).

Effusanin A (4) Colorless crystals, mp 262—265 °C, $[\alpha]_D$ −76.0° (c=0.050, EtOH). IR (KBr): 3252, 2944, 1705, 1640, 1424, 1272, 1168 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 239.2 (3.94), 205.6 (3.65). MS *m/z* (%): 348 (M^+, 97), 269 (30), 162 (30), 105 (70), 79 (100). ^{1}H-NMR (pyridine-d_5) δ: 5.98, 5.29 (each 1H, s, H-17), 4.76, 4.36 (each 1H, d, J=10.0 Hz, H-20), 4.26 (1H, dd, J=6.5, 10.6 Hz, H-6), 3.68 (1H, t, J=9.1 Hz, H-1), 2.93 (1H, d, J=9.1 Hz, H-13), 1.29 (3H, s, H-19), 1.13 (3H, s, H-18).

Effusanin B (5) Colorless crystals, mp 258—260 °C, $[\alpha]_D$ −66.7° (c=0.027, EtOH). IR (KBr): 3250, 2946, 1724, 1646, 1495, 1370, 1253 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 240.0 (3.90), 204.0 (3.60). MS *m/z* (%): 390 (M^+, 76), 269 (32), 228 (25), 149 (34), 105 (58), 85 (100). ^{1}H-NMR (pyridine-d_5) δ: 5.96, 5.27 (each 1H, s, H-17), 4.84 (1H, dd, J=5.4, 11.4 Hz, H-1), 4.47, 4.27 (each 1H, dd, J=1.4, 10.1 Hz, H-20), 4.22 (1H, dd, J=6.3, 10.6 Hz, H-6), 2.91 (1H, dd, J=4.0, 9.5 Hz, H-13), 2.06 (3H, s, –OCOCH$_3$), 1.23 (3H, s, H-19), 1.08 (3H, s, H-18).

Longikaurin A (6) Colorless crystals, mp 222—224 °C, $[\alpha]_D$ −86.6° (c=0.147, EtOH). IR (KBr): 3380, 2933, 1711, 1645, 1452, 1241, 1059 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 235.6 (3.78), 205.0 (3.68). MS *m/z* (%): 348 (M^+, 48), 151 (83), 131 (42), 105 (100). ^{1}H-NMR (pyridine-d_5) δ: 6.27, 5.50 (each 1H, s, H-17), 5.13 (1H, s, H-14), 4.19 (1H, dd, J=6.3, 10.0 Hz, H-6), 4.15, 3.93 (each 1H, d, J=9.9 Hz, H-20), 3.17 (1H, d, J=9.5 Hz, H-13), 1.20 (3H, s, H-19), 1.05 (3H, s, H-18).

Longikaurin B (7) Colorless crystals, mp 232—235 °C, $[\alpha]_D$ −84.6° (c=0.052, EtOH). IR (KBr): 3179, 2929, 1735, 1710, 1639, 1241, 1175, 1063 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 235.4 (3.88). MS *m/z* (%): 406 (M^+, 48), 346 (88), 149 (52), 105 (71), 79 (100). ^{1}H-NMR (pyridine-d_5) δ: 6.27, 5.51 (each 1H, s, H-17), 5.10 (1H, s, H-14), 4.67, 4.42 (each 1H, d, J=11.1, H-19), 4.36 (1H, dd, J=6.6, 10.4 Hz, H-6), 4.11, 4.01 (each 1H, d, J=10.2 Hz, H-20), 3.16 (1H, d, J=9.4 Hz, H-13), 1.97 (3H, s, –OCOCH$_3$), 1.39 (3H, s, H-18).

Longikaurin D (8) Colorless needles, mp 266—270 °C, $[\alpha]_D$ −112.5° (c=0.013, EtOH). IR (KBr): 3533, 3287, 2929, 1722, 1706, 1645, 1251 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 238.4 (3.82). MS *m/z* (%): 406 (M^+, 10), 346 (42), 297 (20), 209 (35), 150 (100). ^{1}H-NMR (pyridine-d_5) δ: 5.99, 5.32 (each 1H, s, H-17), 5.28, 4.22 (each 1H, d, J=9.0 Hz, H-20), 4.73, 4.49 (each 1H, d, J=11.0 Hz, H-19), 4.46 (1H, overlap, H-11), 4.45 (1H, overlap, H-6), 3.15 (1H, dd, J=4.2, 9.4 Hz, H-13), 1.96 (3H, s, –OCOCH$_3$), 1.46 (3H, s, H-18). ^{13}C-NMR (pyridine-d_5) δ: 210.9 (s, C-15), 170.7 (s, –O<u>C</u>OCH$_3$), 154.2 (s, C-16), 115.6 (t, C-17), 96.4 (s, C-7), 74.2 (d, C-6), 69.5 (t, C-20), 67.0 (t, C-19), 65.4 (d, C-11), 61.7 (t, C-5), 59.9 (s, C-8), 54.5 (d, C-9), 41.3 (t, C-12), 37.6 (s, C-10), 37.5 (s, C-4), 36.3 (t, C-1), 34.8 (d, C-13), 30.7 (t, C-3), 28.3 (q, C-18), 27.3 (t, C-14), 20.7 (q, –OCO<u>C</u>H$_3$), 18.6 (t, C-2).

Lasiokaurin (9) Colorless needles, mp 223—226 °C, $[\alpha]_D$ −68.0° (c=0.050, EtOH). IR (KBr): 3445, 3206, 2953, 1726, 1646, 1371, 1251 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 238.0 (3.88), 203.2 (3.61). MS *m/z* (%): 406 (M^+, 80), 300 (43), 150 (54), 105 (100). ^{1}H-NMR (pyridine-d_5) δ: 6.25, 5.48 (each 1H, s, H-17), 5.24 (1H, s, H-14), 4.81 (1H, dd, J=5.4, 11.3 Hz, H-1), 4.51, 4.32 (each 1H, d, J=10.2, H-20), 4.22 (1H, dd, J=6.6, 10.4 Hz, H-6), 3.18 (1H, d, J=9.8 Hz, H-13), 2.03 (3H, s, –OCOCH$_3$), 1.19 (3H, s, H-19), 1.09 (3H, s, H-18), 1.01 (3H, d, J=6.9 Hz, H-17).

Trichoranin (10) Colorless crystals, mp 218—220 °C, $[\alpha]_D$ −106.7° (c=0.060, EtOH). IR (KBr): 3430, 2929, 1745, 1722, 1691, 1406, 1276, 1023, 801 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 238.6 (3.97). MS *m/z* (%): 406 (M^+, 16), 346 (100), 245 (30), 199 (24), 105 (44).

Hydrogenation of Oridonin and Trichoranin Hydrogenation was performed according to the method of Kubo *et al.*[17] Oridonin (100 mg) and trichoranin (10 mg) were hydrogenated with 10% Pd–C in dioxane until 1 eq of H_2 had been consumed to afford dihydrooridonin (77.1 mg) and dihydrotrichoranin (9.0 mg), respectively. Dihydrooridonin: colorless crystals, mp 235—237 °C. IR (KBr): 3295, 2936, 1713, 1455, 1177, 1084 cm^{-1}. MS *m/z* (%): 366 (M^+, 10), 320 (68), 221 (32), 189 (30), 85 (100). ^{1}H-NMR (pyridine-d_5) δ: 5.38 (1H, s, H-14), 4.73, 4.39 (each 1H, d, J=10.2 Hz, H-20), 4.17 (1H, dd, J=6.0, 10.5 Hz, H-6), 3.64 (1H, d, J=9.1 Hz, H-1), 3.35 (1H, t, J=7.1 Hz, H-13), 1.25 (3H, s, H-19), 1.16 (3H, d, J=7.1 Hz, H-17), 1.13 (3H, s, H-18). Dihydrotrichoranin: colorless amorphous solid. IR (KBr): 3379, 2936, 1716, 1458, 1241, 1031 cm^{-1}. MS *m/z* (%): 408 (M^+, 5), 183 (45), 152 (44), 105 (100). CD(EtOH) [θ] (nm): −66140 (299.6). ^{1}H-NMR (pyridine-d_5) δ: 4.69, 4.65 (each 1H, d, J=11.4 Hz, H-19), 4.43 (1H, dd, J=5.8, 10.9 Hz, H-6), 4.17 (1H, s, H-3), 4.10, 4.03 (each 1H, d, J=10.2 Hz, H-20), 1.93 (3H, s, –OCOCH$_3$), 1.01 (3H, d, J=6.9 Hz, H-18).

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