Macaflavanones A−G, Prenylated Flavanones from the Leaves of *Macaranga tanarius*

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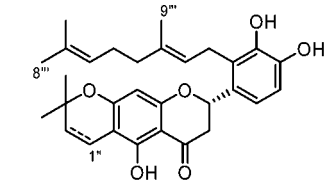
Phytochemical investigation of leaves of *Macaranga tanarius* resulted in the isolation of seven new prenylated flavanones, macaflavanones A−G (**1**−**7**), along with two known compounds, nymphaeol C (**9**) and the diterpene kolavenol. The structures of the new compounds were elucidated by means of a combination of spectroscopic methods and chemical conversion. The absolute structure of tanariflavanone B (**8**), isolated from the title plant, was also resolved. The cytotoxic activities of isolated flavanones were assayed using two cell lines, with macaflavanone G (**7**) being the most active compound in each case.

Macaranga tanarius (L.) Benth. Müll.-Arg. (Euphorbiaceae) is an evergreen tree of about 4 to 10 m in height distributed widely in tropical zones of Asia, such as in southern Japan, the Okinawa Islands, mainland China, and the Malay Peninsula.[1] This species is called the "parasol leaf" tree due to its huge leaves.[2] It is also known as the "pioneer plant", exhibits a symbiotic relationship with ants, and grows initially in fairly sterile land, which becomes fertile. The isolation of diterpenoids,[3,4] flavanoids,[5,6] megastigmane glucoside gallates,[7] and hydrolyzable tannins[8] has been reported from this species. Some biological activities have also been reported, such as allelopathic,[5] antiulcer,[6] inhibitory activities of cycloxygenase by the crude extract from *M. tanarius*,[6] and radical-scavenging activity for its flavanoids[6] and for megastigmane glucoside gallate.[7] This paper deals with the isolation of seven new prenylated flavanones (**1**−**7**) from *M. tanarius*.[2]

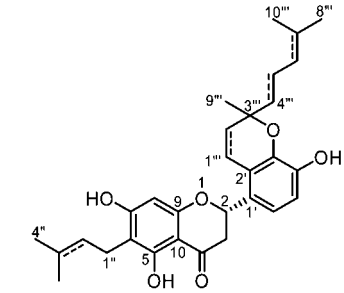
Results and Discussion

Air-dried leaves of *M. tanarius* were extracted three times with MeOH, and the resultant concentrated extract was partitioned with solvents of increasing polarity. The EtOAc-soluble fraction was separated by column chromatography and high-performance liquid chromatography, leading to the purification of nine prenylflavanones (**1**−**9**) and a diterpene. The structures of the new compounds (**1**−**7**) were elucidated on the basis of spectroscopic and chemical evidence. The known compounds tanariflavanone B (**8**),[5] nymphaeol C (**9**),[9] and kolavenol[10,11] were identified by comparison of spectroscopic data with those reported in the literature.

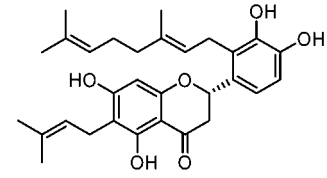
Macaflavanone A (**1**), $[\alpha]_D$ +27.6, was isolated as an amorphous powder, and its elemental composition was determined to be $C_{30}H_{36}O_6$ by positive-ion HRTOFESIMS. The IR spectrum showed that compound **1** contains hydroxyl (3435 cm^{-1}) and conjugated ketone (1642 cm^{-1}) functional groups. Altogether, 30 carbon signals were observed in the ^{13}C NMR spectrum, and these were assigned to two singlet methyls and three singlet methyls on double bonds, as well as 17 sp^2 carbon signals, which were ascribable to tetrasubstituted and pentasubstituted aromatic rings, two trisubstituted double bonds, and one carbonyl group, six methylenes, an oxymethine, and an oxygenated quaternary carbon atom. The highly deshielded signal at δ_H 12.4 in the ^1H NMR spectrum together with the above NMR data implied that **1** is a flavonoid with prenyl side chains. A closely related compound, tanariflavanone A, previously isolated from the same plant, possesses a five-carbon unit on the A-ring and a 10-carbon unit on the B-ring.[5] The HMBC (Figure 2) correlations from the dimethyl groups on the double bond to the aromatic B-ring [i.e., the final correlations from δ_H 3.46 (H_2-1‴) to δ_C 128.6 (C-1′) and 142.5 (C-3′)] confirmed that the 10-carbon unit is connected to the C-2 position of the B-ring, and those from the dimethyl groups on an aliphatic carbon [i.e., δ_H 2.62 on δ_C 15.7 (C-1″) to δ_C 161.5 (C-5) and 162.9 (C-7)] also confirmed that the five-carbon unit is connected to the C-6 position of the A-ring. Judging from the degree of unsaturation and the presence of the oxygenated quaternary carbon atom, the five-carbon unit in **1** must



1 macaflavanone A
2 $\Delta^{1''}$ macaflavanone B



3 $\Delta^{2''}$ $\Delta^{1'''}$ $\Delta^{6'''}$ 3‴*S* macaflavanone C
3a 3‴*S* hexahydromacaflavanone C
8 $\Delta^{2''}$ $\Delta^{1'''}$ $\Delta^{6'''}$ 3‴*R* tanariflavanone B
8a 3‴*R* hexahydrotanariflavnone B
4 $\Delta^{2''}$ $\Delta^{4'''}$ $\Delta^{6'''}$ 3‴*R* macaflavanone D
5 $\Delta^{2''}$ $\Delta^{4'''}$ $\Delta^{6'''}$ 3‴*S* macaflavanone E
6 $\Delta^{2''}$ $\Delta^{6'''}$ 3‴*S* macaflavanone F
7 $\Delta^{2''}$ $\Delta^{6'''}$ 3‴*R* macaflavanone G



9 nymphaeol C

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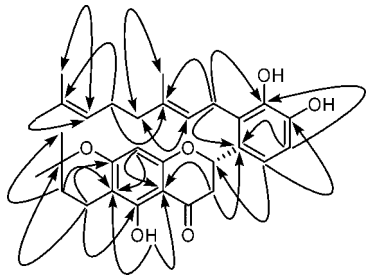

Figure 1. HMBC correlations of **1**.

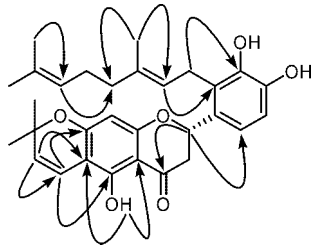

Figure 2. Diagnostic HMBC correlations of **2**.

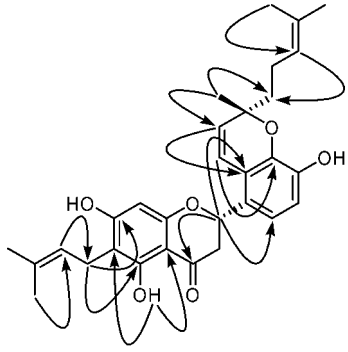

Figure 3. Diagnostic HMBC correlations of **3**.

form a ring similar to that in tanariflavanone A.[5] Therefore, the planar structure of macaflavanone A (**1**) was elucidated as a dehydroxy derivative of tanariflavanone A, as shown. The absolute stereochemistry at C-2 was established as *S* from the negative Cotton effect ($\Delta\varepsilon$ −5.7) at 294 nm and the positive value ($\Delta\varepsilon$ +1.1) at 333 nm in the CD spectrum.[12]

Macaflavanone B (**2**), $[\alpha]_D$ +24.1, was isolated as an amorphous powder, and its elemental composition was determined to be $C_{30}H_{34}O_6$, which is two mass units less than **1**. The ^{1}H and ^{13}C NMR spectra were essentially the same as those of **1**, except for the signals of the five-carbon unit. Two methylene signals were replaced by a disubstituted *cis* double bond [δ_C 115.3 with δ_H 6.62 (d, J = 10 Hz) and 126.2 with δ_H 5.49 (d, J = 10 Hz)]. Therefore, the structure of macaflavanone B (**2**) was elucidated as 1″,2″-dehydromacaflavanone A, as indicated. The HMBC correlations shown in Figure 2 supported the structure proposed for **2**.

Macaflavanone C (**3**), $[\alpha]_D$ +50.6, and tanariflavanone B (**8**), $[\alpha]_D$ +19.6, were each isolated as an amorphous powder and gave the same molecular formula of $C_{30}H_{34}O_6$, as determined by HRTOFESIMS. Although the ^{1}H and ^{13}C NMR spectra of **3** were indistinguishable from those of tanariflavanone B, which was also isolated from the same plant in the current work (**8**) and by Tseng et al. ($[\alpha]_D$ +28.2), they showed different retention times by HPLC [ODS (H_2O−MeOH, 3:17), 34 and 38 min, respectively].[5] The HMBC correlations shown in Figure 3 supported the structure proposed for **3**. Tseng et al. reported partial CD spectroscopic data [a negative Cotton effect at 287 nm (θ = −16 570) and a positive Cotton effect at 340 nm (θ = +12 390)], which confirmed the stereochemistry of the C-2 position of tanariflavanone B (**8**) as *S*. Macaflavanone C (**3**) had a negative Cotton effect at 294 nm ($\Delta\varepsilon$ −4.1) and a positive Cotton effect at 335 nm ($\Delta\varepsilon$ +1.3), while tanariflavanone B (**8**), isolated in this experiment, had a negative Cotton effect at 290 nm ($\Delta\varepsilon$ −2.8) and a positive Cotton effect at 336 nm ($\Delta\varepsilon$ +1.0), and so these compounds were also assigned the *S* stereochemistry at the C-2 positions. Thus, the difference in retention times, which means that they are different compounds, must have arisen from the remaining chiral center at C-3‴. The B-ring and C-1‴, -2‴, -3‴, and the oxygen atom at the C-3′ position of **3** and **8** form a chromene ring, whose CD data were compared with those by Nozoe et al.[13] and Kikuchi et al.[14] The distinct positive Cotton effect at 261 nm ($\Delta\varepsilon$ +5.0) enabled the assignment of the absolute stereochemistry at the C-3‴ position of macaflavanone C (**3**) as *S*, and the *R* configuration was assigned to the C-3‴ position of tanariflavanone B (**8**) based on the negative Cotton effect at 275 nm ($\Delta\varepsilon$ −1.1). Although Tseng et al. did not assign the absolute configuration at the C-3‴ position of tanariflavanone B (**8**) in their paper, due to the resemblance of optical rotation values, it was also deduced to have the 3‴*R* configuration.

Macaflavanone D (**4**), $[\alpha]_D$ −9.5, and macaflavanone E (**5**), $[\alpha]_D$ +75.6, were also each isolated as as an amorphous powder, and both molecular formulas were determined to be $C_{30}H_{34}O_6$, which is the same as those of macaflavanone C (**3**) and tanraiflavanone B (**8**). The ^{1}H and ^{13}C NMR spectra of **4** and **5** were also indistinguishable, similar to those of **3** and **8**. The NMR signals of the A- and B-rings of **4** and **5** were the same as those of **3** and **8**. However, a *cis* double bond, which was evident for **3** and **8**, was replaced by a *trans* double bond [δ_H 5.59 (d, J = 15 Hz) and 6.34 (dd, J = 15, 11 Hz)], which was then conjugated with another double bond. The only possible site of the *trans* double bond was between C-4‴ and 5‴. Therefore, macaflavanone D (**4**) and macaflavanone E (**5**) are positional isomers at the 1‴−2‴ double bond in macaflavanone C (**3**) and tanariflavanone B (**8**), as shown. The absolute configuration at the C-2 positions was assigned as *S* based on the CD spectrum, but **4** and **5** had no chromene ring on the B-ring. The stereochemistry at the C-3‴ position will be discussed later.

Macaflavanone F (**6**), $[\alpha]_D$ +25.0, and macaflavanone G (**7**), $[\alpha]_D$ +75.9, were also each isolated as an amorphous powder, and their molecular formulas were determined to be $C_{30}H_{36}O_6$, which is two mass units more than in the case of macaflavanone C (**3**) and tanariflavanone B (**8**), and macaflavanone D (**4**) and macaflavanone E (**5**). The NMR signals of the A- and B-rings of **6** and **7** were also essentially the same as those of **3**, **4**, **5**, and **8**, and one of the double bonds in the 10-carbon side chain must be reduced to a single bond. Since four methyl groups were still on the double bonds, judging from their chemical shifts (δ_H 1.81 and 1.75, and δ_H 1.60 and 1.68), terminal double bonds were retained in **6** and **7**, and thus the double bond at 1‴ in **3** and **8** or that at 4‴ in **4** and **5** was expected to be reduced, as shown.

To establish the absolute stereochemistry at the 3‴-positions of macaflavanones D−G (**4**−**7**), macaflavanone C (**3**) and tanariflavanone B (**8**) were catalytically hydrogenated over PtO_2 and H_2 to give the corresponding hexahydro compounds (**3a** and **8a**, respectively). The structure of the reduced product (**3a**) was confirmed by one- and two-dimensional NMR spectroscopy (Figure 4), and other physical data also supported the structure. The reduced products could be separated by HPLC by ODS with a solvent system of H_2O−MeOH (3:17) at a flow rate of 1.0 mL/min, with **3a** (2*S*,3‴*S*) being eluted at 72 min and **8a** (2*S*,3‴*R*) at 86 min. Macaflavanones D−G (**4**−**7**) were similarly hydrogenated over PtO_2 to give the corresponding hexahydro (**4a** and **5a**) and tetrahydro (**6a** and **7a**) derivatives. HPLC analysis under the same conditions revealed that **4a** and **6a** are the same compound as **3a**, and **5a** and **7a** are the same compound as **8a**. Thus, the absolute configurations at the 3‴-position were established to be *R* for **4** (β-9‴-Me) and *S* for **6** (β-9‴-Me), and *S* for **5** (α-9‴-Me) and *R* for **7** (α-9‴-

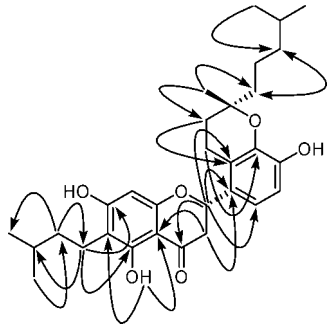

Figure 4. Diagnostic HMBC correlations of **3a**.

Me). The 9‴-β-Me stereoisomers of **3** (34 min), **3a**, **4** (27.5 min), and **6** (39 min) were eluted faster than the respective 9‴-α-Me stereoisomers, **8** (38 min), **8a**, **5** (32 min), and **7** (45 min), under the same conditions.

The cytotoxic activities of the isolated compounds were assayed against the KB and A549 cell lines by means of the 3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide (MTT) method. The isolated compounds did not show any selectivity between these cell lines. Macaflavanone G (**7**) showed IC_{50} values of 12.3 ± 3.0 and 13.4 ± 2.1 μM for KB and A549 cells, respectively. This was the most potent compound against both of these cell lines.

Experimental Section

General Experimental Procedures. Optical rotations and CD spectra were measured on a JASCO P-1030 polarimeter and a JASCO J-720 spectropolarimeter, respectively. IR and UV spectra were measured on Horiba FT-710 and JASCO V-520 UV/vis spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were taken on a JEOL JNM α-400 spectrometer at 400 and 100 MHz, respectively, with tetramethylsilane as an internal standard. Positive-ion HRTOFESIMS was performed with an Applied Biosystem QSTAR XL system ESI (Nano Spray)-TOFMS.

A highly porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel open CC and reversed-phase [octadecyl silica gel (ODS)] CC were performed on silica gel 60 (Merck, Darmstadt, Germany) and Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) [Φ = 50 mm, L = 25 cm, linear gradient: MeOH−H_2O, fractions of 10 g being collected], respectively. HPLC was performed on an ODS column (Inertsil; GL Science, Tokyo, Japan; Φ = 6 mm, L = 25 cm), and the eluate was monitored with a UV detector at 254 nm and/or a refractive index monitor.

Plant Material. Leaves of *Macaranga tanarius* were collected in Okinawa, Japan, in June 2003, and the plant was identified by one of the authors (T.S.). A voucher specimen has been deposited in the Herbarium of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (03-MT-Okinawa-0630).

Extraction and Isolation. The air-dried leaves of *M. tanarius* (12.1 kg) were extracted three times with MeOH (45 L) for a week at room temperature. The MeOH extract was concentrated to 3 L, and then 150 mL of H_2O was added to make a 95% aqueous solution. This solution was defatted with 3.0 L of *n*-hexane, and then the methanolic layer was concentrated to a viscous gum. The gummy residue was suspended in 3.0 L of H_2O and then extracted with 3.0 L each of EtOAc and 1-BuOH successively, to afford, in turn, 801 and 374 g of EtOAc-soluble and 1-BuOH-soluble extracts. The *n*-hexane layer and the remaining H_2O layer were concentrated to furnish 70.0 and 499 g of *n*-hexane- and H_2O-soluble fractions, respectively.

A portion (437 g) of the EtOAc-soluble extract was subjected to silica gel (500 g) CC with elution by *n*-hexane−EtOAc [(20:1, 6 L), (5:1, 6 L), (4:1, 6 L), (3:1, 6 L), (2:1, 6 L), and (1:1, 6 L)], EtOAc (6 L), and MeOH (6 L), with 1 L fractions being collected. The residue (145 g) in fractions 8−16 was then subjected to silica gel (2.0 kg) CC, eluting with toluene (6 L), toluene−Me_2CO [(100:1, 6 L), (70:1, 3 L), (50:1, 3 L), (40:1, 3 L), (30:1, 3 L), (10:1, 3 L), and (5:1, 3 L)], Me_2CO (3 L), and MeOH (8 L), and 1 L fractions were collected. The residue (102 g) in fractions 19−28, obtained on the second silica gel CC, was subjected to Diaion HP-20 (2 L) CC by elution with MeOH−H_2O (3:1, 9 L), MeOH (9 L), MeOH−$CHCl_3$ (1:1, 9 L), and $CHCl_3$ (9 L), with again l L fractions being collected. The residue (68.7 g) in fractions 10−18 was separated by ODS CC with MeOH−H_2O (1:1, 2 L → 1:0, 2 L). Next, the residue (2.45 g) in fractions 247−276 was purified by HPLC with MeOH−H_2O−CH_2Cl_2 (9:1:0.02) to give four fractions, namely, 366 mg from the peaks between 31−37 min, 474 mg from the peaks between 37−46 min, 72.1 mg from the peaks between 53−58 min, and 22.3 mg from those between 73−78 min. Final HPLC purification (MeOH−H_2O, 83:7) was performed for the first residue to afford 7.3 mg of **4**, 6.3 mg of **5**, and 26.2 mg of **3** from the peaks at 48, 55, and 58 min, respectively. The second residue gave 4.9 mg of **2** on HPLC separation (MeCN−H_2O, 13:7) from the peak at 86 min. Compounds **1** (6.1 mg) and **2** (15.3 mg) were isolated from the third residue by HPLC (MeOH−H_2O, 4:1), from the peaks at 54 and 57 min. From the fourth residue, 3.3 mg of kolavenol was obtained by HPLC (MeOH−H_2O, 87:13) from the peak at 35 min. The residue (21.8 g) in fractions 19−36 obtained by Diaion HP-20 CC was separated by two ODS CC separations with MeOH−H_2O (1:1, 1 L → 1:0, 1 L) and then MeOH (1 L) to $CHCl_3$ (1 L), to yield two fractions, 1.29 g in fractions 1−120 and 1.54 g in fractions 122−133. The first fraction was subjected to Sephadex LH-20 CC (Φ = 20 mm, L = 120 cm) with $CHCl_3$−MeOH (1:1), with fractions of 13 g being collected, and then the residue (390 mg) in fractions 42−55 was separated by HPLC (MeOH−H_2O, 17:3) to give a mixture (57.8 mg) of **8** and **6** from the peak between 44 and 47 min and 21.0 mg of **7** from the peak at 52 min. The mixture was separated by repeated HPLC (MeOH−2-propanol−H_2O, 3:3:4) to afford 18.7 mg of **6** from the peak at 199 min and 11.9 mg of **8** from the peak at 211 min. Further amounts of **1** (28.9 mg) and **2** (18.0 mg) were isolated from the second fraction by Sephadex LH-20 CC (239 mg in fractions 127−146) and then by HPLC (MeOH−H_2O, 4:1) from the peaks at 78 and 82 min, respectively.

An aliquot (2.05 g) of the residue in fractions 29−35 obtained on the second silica gel CC was separated by ODS CC with a linear gradient of MeOH in H_2O (1:9, 1 L → 1:0, 1 L), followed by HPLC purification of the residue (1.31 g) in fractions 141−151 with MeOH−H_2O−CH_2Cl_2 (9:1:0.02) to give 38.9 mg of **9** from the peak at 28 min.

Known Compounds Isolated. Tanariflavanone B (8): yellow, amorphous powder; $[\alpha]_D^{23}$ +19.6 (*c* 0.79, $CHCl_3$); CD (*c* 2.79 × 10^{-5} M, MeOH) Δε (nm) −3.5 (214), +8.0 (238), −1.1 (275), −2.8 (290), +1.0 (336).[5]

Nymphaeol C (9): yellow, amorphous powder; $[\alpha]_D^{24}$ +25.0 (*c* 0.20, $CHCl_3$); CD (*c* 2.79 × 10^{-5} M, MeOH) Δε (nm) +3.9 (227), −2.1 (293), +0.71 (339).[11]

Kolavenol: colorless oil; $[\alpha]_D^{20}$ −24.1 (*c* 0.21, $CHCl_3$).[9,10]

Macaflavanone A (1): off-white, amorphous powder; $[\alpha]_D^{30}$ +27.6 (*c* 0.41, $CHCl_3$); IR ν_{max} (film) 3435, 2923, 1642, 1578, 1444, 1287, 1156, 1117, 1094 cm^{-1}; UV (log ε) λ_{max} (MeOH) 226 (4.21), 294 (4.22), 335 sh (3.50) nm; CD (*c* 2.46 × 10^{-5} M, MeOH) Δε (nm) +18.7 (222), +1.7 (263 sh), −5.7 (294), +1.1 (333); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.4 (1H, s, OH-5), 6.98 (1H, d, J = 8 Hz, H-6′), 6.83 (1H, d, J = 8 Hz, H-5′), 5.91 (1H, s, H-8), 5.48 (1H, dd, J = 13, 3 Hz, H-2), 5.19 (1H, br t, J = 7 Hz, H-2‴), 5.03 (1H, brt, J = 7 Hz, H-6″), 3.46 (2H, d, J = 7 Hz, H_2-1‴), 3.11 (1H, dd, J = 17, 13 Hz, H-3ax), 2.74 (1H, dd, J = 17, 3 Hz, H-3eq), 2.62 (2H, t, J = 7 Hz, H_2-1″), 2.10 (2H, m, H_2-5‴), 2.07 (2H, m, H_2-4‴), 1.79 (2H, t, J = 7 Hz, H_2-2″), 1.78 (3H, s, CH_3-9‴), 1.67 (3H, s, CH_3-8‴), 1.59 (3H, s, CH_3-10‴), 1.33 (6H, s, CH_3-4″ and 5″); ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HRTOFESIMS (positive-ion mode) *m*/*z* 493.2564 $[M + H]^+$ (calcd for $C_{30}H_{37}O_6$, 493.2584).

Macaflavanone B (2): off-white, amorphous powder; $[\alpha]_D^{30}$ +24.1 (*c* 1.02, $CHCl_3$); IR ν_{max} (film) 3436, 2925, 1644, 1632, 1568, 1451, 1287, 1153, 1115, 1088 cm^{-1}; UV (log ε) λ_{max} (MeOH) 235 (4.13), 272 (4.33), 292 (4.12), 356 (3.42) nm; CD (*c* 6.24 × 10^{-5} M, MeOH) Δε (nm) +0.81 (214tr), +2.9 (235), +4.0 (262), −0.86 (297), +0.22 (345); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.3 (1H, s, OH-5), 6.97 (1H, d, J = 8 Hz, H-6′), 6.83 (1H, d, J = 8 Hz, H-5′), 6.62 (1H, d, J = 10 Hz, H-1″), 5.93 (1H, s, H-8), 5.51 (1H, dd, J = 13, 3 Hz, H-2), 5.49 (1H, d, J = 10 Hz, H-2″), 5.18 (1H, td, J = 7, 1 Hz, H-2‴), 5.03 (1H, br t, J = 7 Hz, H-6‴), 3.46 (2H, d, J = 7 Hz, H_2-1‴), 3.11 (1H, dd, J = 17, 13 Hz, H-3ax), 2.74 (1H, dd, J = 17, 3 Hz, H-3eq), 2.10 (2H, m, H_2-5‴), 2.07 (2H, m, H_2-4‴), 1.78 (3H, d, J = 1 Hz, H_3-9‴), 1.67 (3H, s, H_3-8‴), 1.59 (3H, s, H_3-10‴), 1.43 (6H, s, CH_3-4″ and 5″);

Table 1. ^{13}C NMR Spectroscopic Data for Macaflavanones A−G (**1**−**7**) and Tanariflavanone B (**8**) (100 MHz in $CDCl_3$)

carbon	1	2	3	4	5	6	7	8
2	76.3	76.5	76.0	76.1	75.7	75.8	75.9	76.2
3	42.6	42.5	42.6	42.2	42.0	42.1	42.0	42.6
4	196.3	196.4	196.3	196.5	196.7	196.6	196.6	196.3
5	161.5	158.5	161.1	161.3	161.3	161.4	161.3	161.2
6	102.3	103.1	107.3	107.0	107.0	107.1	107.0	107.2
7	162.9	162.5	163.8	163.7	163.7	163.7	163.7	163.8
8	96.2	96.3	95.5	95.5	95.5	95.4	95.4	95.6
9	160.6	162.1	161.4	161.3	161.4	161.4	161.3	161.4
10	102.2	102.9	103.0	103.0	103.0	103.0	103.0	103.0
1′	128.6	128.3	124.9	127.3	127.2	127.3	127.2	125.0
2′	126.4	126.4	119.0	119.9	120.0	119.6	119.7	119.0
3′	142.5	142.6	139.8	141.1	141.1	140.9	141.0	139.8
4′	144.9	144.9	145.2	145.1	145.7	146.0	146.0	145.2
5′	113.0	113.1	114.6	112.1	112.1	112.0	111.9	114.6
6′	119.2	119.1	118.8	118.0	117.9	117.7	117.8	118.8
1″	15.7	115.3	21.2	21.2	21.2	21.2	21.1	21.2
2″	32.0	126.2	121.5	121.5	121.5	121.5	121.5	121.5
3″	77.2	78.3	135.2	135.5	135.3	135.3	135.4	135.5
4″	26.8	28.5	25.8	25.8	25.8	25.8	25.8	25.8
5″	26.7	28.4	17.9	17.9	17.8	17.9	17.8	17.9
1‴	25.6	25.5	118.9	19.6	19.4	19.1	19.1	118.8
2‴	121.3	121.3	130.9	32.2	32.2	31.0	31.0	130.9
3‴	139.3	139.1	79.1	77.2	77.2	76.9	76.9	79.1
4‴	39.6	39.6	40.8	132.7	132.6	39.3	39.3	40.8
5‴	26.3	26.3	22.8	125.8	125.8	22.4	22.4	22.8
6‴	123.8	123.7	123.8	124.1	124.1	124.0	123.9	123.8
7‴	132.3	132.2	132.1	136.5	136.5	131.9	131.9	132.1
8‴	25.7	25.7	25.6	26.0	26.0	25.6	25.6	25.7
9‴	16.3	16.3	26.1	26.9	27.1	23.9	23.9	26.2
10‴	17.7	17.7	17.6	18.4	18.3	17.6	17.6	17.7

^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HRTOFESIMS (positive-ion mode) *m/z* 491.2434 $[M + H]^+$ (calcd for $C_{30}H_{35}O_6$, 491.2428).

Macaflavanone C (3): off-white, amorphous powder; $[\alpha]_D^{30}$ +50.6 (*c* 1.75, $CHCl_3$); IR ν_{max} (film) 3389, 2918, 1636, 1601, 1455, 1306, 1153, 1077 cm^{-1}; UV (log ε) λ_{max} (MeOH) 235 (4.26), 274 sh (4.07), 291 (4.17), 333 (3.61) nm; CD (*c* 5.34 × 10^{-5} M, MeOH) $\Delta\varepsilon$ (nm) −4.9 (211), +15.2 (233), +5.0 (261), −4.1 (294), +1.3 (335); ^1H NMR (400 MHz, $CDCl_3$) δ 12.3 (1H, s, OH-5), 6.89 (1H. d. *J* = 8 Hz, H-6′), 6.83 (1H, d, *J* = 8 Hz, H-5′), 6.60 (1H, d, *J* = 10 Hz, H-1‴), 5.98 (1H, s, H-8), 5.67 (1H, d, *J* = 10 Hz, H-2‴), 5.49 (1H, dd, *J* = 13, 3 Hz, H-2), 5.25 (1H, t, *J* = 7 Hz, H-2″), 5.09 (1H, t, *J* = 7 Hz, H-6‴), 3.34 (2H, d, *J* = 7 Hz, H_2-1″), 3.12 (1H, dd, *J* = 17, 13 Hz, H-3ax), 2.74 (1H, dd, *J* = 17, 3 Hz, H-3eq), 2.10 (2H, m, H_2-5‴), 1.81 (3H, s, H_3-5″), 1.76 (2H, m, H_2-4‴), 1.74 (3H, s, H_3-4″), 1.67 (3H, s, H_3-8‴), 1.57 (3H, s, H_2-10‴), 1.42 (3H, s, H_3-9‴); ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HRTOFESIMS (positive-ion mode) *m/z* 491.2421 $[M + H]^+$ (calcd for $C_{30}H_{35}O_6$, 491.2428).

Macaflavanone D (4): off-white, amorphous powder; $[\alpha]_D^{30}$ −9.5 (*c* 0.49, $CHCl_3$); IR ν_{max} (film) 3434, 2922, 1634, 1492, 1451, 1304, 1153, 1079 cm^{-1}; UV (log ε) λ_{max} (MeOH) 237 (4.38), 257 sh (4.02), 289 (4.16), 334 sh (3.54) nm; CD (*c* 2.86 × 10^{-5} M, MeOH) $\Delta\varepsilon$ (nm) +21.7 (216), −6.8 (239), +0.39 (262), −5.4 (293), +0.86 (323); ^1H NMR (400 MHz, $CDCl_3$) δ 12.4 (1H, s, OH-5), 6.94 (1H, d, *J* = 8 Hz, H-6′), 6.84 (1H, d, *J* = 8 Hz, H-5′), 6.34 (1H, dd, *J* = 15, 11 Hz, H-5‴), 5.95 (1H, s, H-8), 5.78 (1H, d, *J* = 11 Hz, H-6‴), 5.59 (1H, d, *J* = 15 Hz, H-4‴), 5.42 (dd, *J* = 13, 3 Hz, H-2), 5.25 (1H, t, *J* = 7 Hz, H-2″), 3.35 (2H, d, *J* = 7 Hz, H_2-1″), 3.12 (1H, dd, *J* = 17, 13 H-3ax), 2.77 (1H, m, H-1‴a), 2.74 (1H, dd, *J* = 17, 3 Hz, H-3eq), 2.73 (1H, m, H-1‴b), 1.95 (2H, m, H_2-2‴), 1.81 (3H, s, H_3-5″), 1.75 (6H, s, H_3-4″ and 8‴), 1.69 (3H, s, H_3-10‴), 1.48 (3H, s, H_3-9‴); ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HRESITOFMS (positive-ion mode) *m/z* 491.2424 $[M + H]^+$ (calcd for $C_{30}H_{35}O_6$, 491.2428).

Macaflavanone E (5): off-white, amorphous powder; $[\alpha]_D^{30}$ +75.6 (*c* 0.42, $CHCl_3$); IR ν_{max} (film) 3396, 2920, 1632, 1602, 1492, 1303, 1452, 1153, 1079 cm^{-1}; UV (log ε) λ_{max} (MeOH) 237 (4.38), 275 sh (4.01), 292 (4.15), 338 sh (3.51) nm; CD (*c* 2.47 × 10^{-5} M, MeOH) $\Delta\varepsilon$ (nm) −13.3 (207), +19.1 (232), +1.5 (268 sh), −2.3 (295), +1.3 (331); ^1H NMR (400 MHz, $CDCl_3$) δ 12.4 (1H, s, OH-5), 6.95 (1H, d, *J* = 8 Hz, H-6′), 6.85 (1H, d, *J* = 8 Hz, H-5′), 5.97 (1H, s, H-8), 6.31 (1H, dd, *J* = 15, 11 Hz, H-5‴), 5.77 (1H, d, *J* = 11 Hz, H-6‴), 5.56 (1H, d, *J* = 15 Hz, H-4‴), 5.43 (1H, dd, *J* = 13, 3 Hz, H-2), 5.25 (1H, t, *J* = 7 Hz, H-2″), 3.32 (2H, d, *J* = 7 Hz, H_2-1″), 3.12 (1H, dd, *J* = 17, 13 Hz, H-3ax), 2.85 (1H, m, H-1‴a), 2.70 (1H, dd, *J* = 17, 3 Hz. H-3eq), 2.61 (1H, m, H-1‴b), 1.96 (2H, m, H_2-2‴), 1.81 (3H, s, H_3-5″), 1.75 (6H, s, H_3-4″ and 8‴), 1.67 (3H, s, H_3-10‴), 1.50 (3H, s, H_3-9‴); ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HRTOFESIMS (positive-ion mode) *m/z* 491.2423 $[M + H]^+$ (calcd for $C_{30}H_{35}O_6$, 491.2428).

Macaflavanone F (6): off-white, amorphous powder; $[\alpha]_D^{25}$ +25.0 (*c* 1.25, $CHCl_3$); IR ν_{max} (film) 3367, 2927, 1637, 1603, 1493, 1451, 1307, 1155, 1082, 1024 cm^{-1}; UV (log ε) λ_{max} (MeOH) 212 (4.46), 230 sh (4.32), 290 (4.18), 335 sh (3.54) nm; CD (*c* 3.74 × 10^{-5} M, MeOH) $\Delta\varepsilon$ (nm) +9.2 (226), −3.1 (292), +1.3 (335); ^1H NMR (400 MHz, $CDCl_3$) δ 12.4 (1H, s, OH-5), 6.94 (1H, d, *J* = 8 Hz, H-6′), 6.83 (1H, d, *J* = 8 Hz. H-5′), 5.97 (1H, s, H-8), 5.45 (1H, dd, *J* = 13, 3 Hz, H-2), 5.25 (1H, t, *J* = 7 Hz, H-2″), 5.11 (1H, t, *J* = 7 Hz, H-6‴), 3.35 (2H, d, *J* = 7 Hz, H_2-1″), 3.13 (1H, dd, *J* = 17, 13 Hz, H-3ax), 2.87 (1H, m, H-1‴a), 2.73 (1H, dd, *J* = 17, 3 Hz, H-3eq), 2.68 (1H, m, H-1‴b), 2.10 (2H, m, H_2-5‴), 1.88 (2H, m, H_2-2‴), 1.81 (3H, s, H_3-5″), 1.75 (3H, s, H_3-4″), 1.68 (2H, m, H_2-4‴), 1.68 (3H, s, H_3-8‴), 1.60 (3H, s, H_3-10‴), 1.34 (3H, s, H_3-9‴); ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HRTOFESIMS (positive-ion mode) *m/z* 515.2424 $[M + Na]^+$ (calcd for $C_{30}H_{36}O_6Na$, 515.2404).

Macaflavanone G (7): off-white, amorphous powder; $[\alpha]_D^{30}$ +75.9 (*c* 1.40, $CHCl_3$); IR ν_{max} (film) 3367, 2925, 1634, 1601, 1492, 1449, 1306, 1152, 1079 cm^{-1}; UV (log ε) λ_{max} (MeOH) 220 (4.11), 234 (4.15), 289 (4.11), 335 sh (3.63) nm; CD (*c* 2.13 × 10^{-5} M, MeOH) $\Delta\varepsilon$ (nm) +25.2 (218), −2.3 (293), +2.0 (330); ^1H NMR (400 MHz, $CDCl_3$) δ 12.4 (1H, s, 5-OH), 6.93 (1H, d, *J* = 8 Hz, H-6′), 6.82 (1H, d, *J* = 8 Hz, H-5′), 5.97 (1H, s, H-8), 5.44 (1H, dd, *J* = 13.3 Hz, H-2), 5.25 (1H, t, *J* = 7 Hz, H-2″), 5.11 (1H, t, *J* = 7 Hz, H-6‴), 3.35 (2H, d, *J* = 7 Hz, H_2-1″), 3.15 (1H, dd, *J* = 17, 13 Hz, H-3ax), 2.86 (1H, m, H-1‴a), 2.74 (1H, dd, *J* = 17, 3 Hz, H-3eq), 2.70 (1H, m, H-1‴b), 2.11 (2H, m, H_2-5‴), 1.85 (2H, m, H_2-2‴), 1.81 (3H, s, H_3-5″), 1.75 (3H, s, H_3-4″), 1.69 (3H, s, H_3-8‴), 1.66 (2H, m, H_2-4‴), 1.60 (3H, s, H_3-10‴), 1.34 (3H, s, H_3-9‴); ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HRTOFESIMS (positive-ion mode) *m/z* 515.2416 $[M + Na]^+$ (calcd for $C_{30}H_{36}O_6Na$, 515.2404).

Catalytic Hydrogenation of Macaflavanones C−G (3−7) and Tanariflavanone B (8). Macaflavanone C (**3**) (10.0 mg) and tanariflavanone B (**8**) (10.0 mg) in 1 mL of MeOH were hydrogenated with PtO_2 (1.0 mg) and H_2 for 1 h, and then the catalyst was removed by filtration to give hexahydromacaflavanone C (**3a**) (3.2 mg) and hexahydrotanariflavanone B (**8a**) (4.4 mg), respectively. With the same method, macaflavanones D (**4**) (2.1 mg) and E (**5**) (2.2 mg) gave hexahydromacaflavanones D (**4a** = **3a**) (1.2 mg) and E (**5a** = **8a**) (1.6 mg), respectively. Macaflavanones F (**6**) (2.1 mg) and G (**7**) (1.7 mg) gave tetrahydromacaflavanones F (**6a** = **3a**) (0.9 mg) and G (**7a** = **8a**) (1.3 mg), respectively.

Hexahydromacaflavanone C (3a): amorphous powder; $[\alpha]_D^{24}$ +14.0 (*c* 0.17, CH_3OH); IR ν_{max} (film) 3388, 2954, 1637, 1602, 1452, 1301, 1157 cm^{-1}; UV (log ε) λ_{max} (MeOH) 210 (4.41), 230 sh (4.23), 290 (4.05) nm; CD (*c* 3.40 × 10^{-5} M, MeOH) $\Delta\varepsilon$ (nm) +14.6 (218), +1.4 (260), −3.0 (292), +1.1 (333); ^1H NMR (400 MHz, $CDCl_3$) δ 12.3 (1H, s, OH-5), 6.95 (1H, d, *J* = 8 Hz, H-6′), 6.84 (1H, d, *J* = 8 Hz, H-5′), 5.94 (1H, s, H-8), 5.68 (1H, s, OH-4′), 5.46 (1H, dd, *J* = 13, 3 Hz, H-2), 3.14 (1H, dd, *J* = 17, 13 Hz, H-3ax), 2.85 (1H, ddd, *J* = 17, 8, 6 Hz, H-1‴a), 2.72 (1H, dd, *J* = 17, 3 Hz, H-3eq), 2.68 (1H, ddd, *J* = 17, 6, 6 Hz, H-1‴b), 2.56 (2H, td, *J* = 7, 3 Hz, H_2-1″), 1.92 (1H, ddd, *J* = 14, 8, 6 Hz, H-2‴a), 1.82 (1H, dt, *J* = 14, 6 Hz, H-2‴b), 1.62 (2H, m, H_2-4‴), 1.60 (1H, m, H-3″), 1.57 (1H, m, H-7‴), 1.39 (4H, m, H_2-2″ and 5‴), 1.31 (3H, s, H_3-9‴), 1.19 (2H, m, H_2-6‴), 0.96 (6H, d, *J* = 7 Hz, H_3-4″ and 5″), 0.88 (6H, d, *J* = 7 Hz, H_3-8‴ and 10‴); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.6 (C-4), 162.1 (C-7), 161.9 (C-5), 161.0 (C-9), 141.0 (C-3′), 146.0 (C-4′), 127.4 (C-1′), 119.6 (C-2′), 117.7 (C-6′), 112.0 (C-5′), 109.4 (C-6), 103.1 (C-10), 94.9 (C-8), 77.2 (C-3‴), 75.9 (C-2), 42.3 (C-3), 39.8 (C-4‴), 39.3 (C-6‴), 38.0 (C-2″), 30.9 (C-2‴), 28.2 (C-3″), 27.9 (C-7‴), 24.0 (C-9‴), 22.58 (C-4″ and 5″), 22.56 (C-8‴ and 10‴), 21.4 (C-5‴), 19.8 (C-1″), 19.1 (C-1‴); HRTOFESIMS (positive-ion mode) *m/z* 497.2902 $[M + H]^+$ (calcd for $C_{30}H_{41}O_6$, 497.2897).

Hexahydrotanariflavanone B (8a): amorphous powder; $[\alpha]_D^{24}$ +64.6 (*c* 0.323, CH_3OH); IR ν_{max} (film) 3374, 2953, 1637, 1601, 1451, 1301, 1157 cm^{-1}; UV (log ε) λ_{max} (MeOH) 209 (4.40), 230 sh (4.25), 290 (4.13) nm; CD (*c* 3.26 × 10^{-5} M, MeOH) $\Delta\varepsilon$ (nm) +20.0 (218),

+1.5 (260), −3.0 (293), +1.3 (332); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.3 (1H, s, OH-5), 6.94 (1H, d, J = 8 Hz, H-6′), 6.84 (1H, d, J = 8 Hz, H-5′), 5.94 (1H, s, H-8), 5.68 (1H, s, OH-4′), 5.45 (1H, dd, J = 13, 3 Hz, H-2), 3.15 (1H, dd, J = 17, 13 Hz, H-3ax), 2.85 (1H, ddd, J = 17, 8, 6 Hz, H-1‴a), 2.73 (1H, dd, J = 17, 3 Hz, H-3 eq), 2.69 (1H, ddd, J = 17, 6, 6 Hz, H-1‴b), 2.56 (2H, td, J = 7, 3 Hz, H_2-1″), 1.90 (1H, ddd, J = 14, 8, 6 Hz, H-2‴a), 1.84 (1H, dt, J = 14, 6 Hz, H-2‴b), 1.61 (3H, m, H-3″ and H_2-4‴), 1.55 (1H, m, H-7‴), 1.41 (2H, m, H_2-2″), 1.39 (2H, m, H_2-5‴), 1.33 (3H, s, H_3-9‴), 1.19 (2H, m, H_2-6‴), 0.96 (6H, d, J = 7 Hz, H_3-4″ and 5″), 0.88 (6H, d, J = 7 Hz, H_3-8‴ and 10‴); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.6 (C-4), 162.2 (C-7), 161.8 (C-5), 161.0 (C-9), 146.0 (C-4′), 141.0 (C-3′), 127.4 (C-1′), 119.7 (C-2′), 117.7 (C-6′), 112.0 (C-5′), 109.5 (C-6), 103.1 (C-10), 94.9 (C-8), 77.2 (C-3‴), 75.9 (C-2), 42.2 (C-3), 39.7 (C-4‴), 39.3 (C-6‴), 38.0 (C-2″), 30.8 (C-2‴), 28.2 (C-3″), 27.9 (C-7‴), 24.1 (C-9‴), 22.57 (C-4″ and 5″), 22.55 (C-8‴ and 10‴), 21.4 (C-5‴), 19.8 (C-1″), 19.1 (C-1‴); HRTOFESIMS (positive-ion mode) m/z 497.2903 $[M + H]^+$ (calcd for $C_{30}H_{41}O_6$, 497.2897).

Hexahydromacaflavanone D (4a): HRESITOFMS (positive-ion mode) m/z 497.2902 $[M + H]^+$ (calcd for $C_{30}H_{41}O_6$, 497.2897).

Hexahydromacaflavanone E (5a): HRESITOFMS (positive-ion mode) m/z 497.2903 $[M + H]^+$ (calcd for $C_{30}H_{41}O_6$, 497.2897).

Tetraahydromacaflavanone F (6a): HRESITOFMS (positive-ion mode) m/z 497.2905 $[M + H]^+$ (calcd for $C_{30}H_{41}O_6$, 491.2897).

Tetrahydromacaflavanone G (7a): HRESITOFMS (positive-ion mode) m/z 497.2884 $[M + H]^+$ (calcd for $C_{30}H_{41}O_6$, 497.2897).

Biological Assays. The cytotoxicities of the isolated compounds toward KB cells and A 549 cells were determined by means of a MTT assay. KB cells were inoculated at a density of 3×10^3 cells/well in 90 μL of Dulbecco's modified Eagle medium containing 10% fetal bovine serum, supplemented with amphotericin B (Sigma Co., Ltd.) (0.5 μg/ml) and kanamycin sulfate (Meiji Confectionery Co., Ltd.) (100 μg/mL) on 96-well plates and then incubated at 37 °C under 5% CO_2. After 24 h, 10 μL of a sample solution and etoposide in 10% DMSO was added, followed by further incubation for 72 h. The medium was removed, and 100 μL of MTT (0.5 mg/mL) in Dulbecco's modified Eagle medium was added. After 1.5 h, the medium was removed, 100 μL of DMSO was added to lyse the cells, and then the absorbance of MTT formazan in each well was measured with a microplate reader at L_1 = 540 nm and L_2 = 620 nm. Activity was calculated as follows:

% inhibition = $[1 - \text{sample } (Abs_{L1} - Abs_{L2})/\text{control}(Abs_{L1} - Abs_{L2})] \times 100$.

The results are expressed as the means with SD for triplicate experiments. Etoposide was used as a positive control, and IC_{50} values of 1.25 ± 0.47 and 8.37 ± 1.0 μM were found for KB and A549 cells, respectively.

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References and Notes

(1) Hatusima, S. *Flora of Ryukyus, Added and Corrected*; The Biological Society of Okinawa: Okinawa, Japan, 1975; p 373.
(2) Eck, G.; Fiala, B.; Linsenmair, K. E. *J. Chem. Ecol.* **2001**, *27*, 1979–1996.
(3) Hui, W.-H.; Ng, K.-K.; Fukamiya, N.; Koreeda, M.; Nakanishi, K. *Phytochemistry* **1971**, *10*, 1617–1620.
(4) Hui, W.-H.; Li, M.-M.; Ng, K.-K. *Phytochemistry* **1975**, *14*, 816–817.
(5) Tseng, M.-H.; Chou, C.-H.; Chen, Y.-M.; Kuo, Y.-H. *J. Nat. Prod.* **2001**, *64*, 827–828.
(6) Phommart, S.; Sutthivaiyakit, P.; Chimnoi, N.; Ruchirawat, S.; Sutthivaiyakit, S. *J. Nat. Prod.* **2005**, *68*, 927–930.
(7) Matsunami, K.; Takamori, I.; Shinzato, T.; Aramoto, M.; Kondo, K.; Otsuka, H.; Takeda, Y. *Chem. Pharm. Bull.* **2006**, *54*, 1403–1407.
(8) Lin, J.-H.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull* **1990**, *38*, 1218–1223.
(9) Yakushijin, K.; Shibayama, K.; Murata, H.; Furukawa, H. *Heterocycles* **1980**, *14*, 397–402.
(10) Misra, R.; Pandey, R. C.; Dev, S. *Tetrahedron Lett.* **1964**, *5*, 3751–3759.
(11) Monti, H.; Tiliacos, N.; Faure, R. *Phytochemistry* **1999**, *51*, 1013–1015.
(12) Gaffield, W. *Tetrahedron* **1970**, *26*, 4093–4108.
(13) Nozoe, S.; Hirai, K.; Snatzke, F.; Snatzke, G. *Tetrahedron* **1974**, *30*, 2773–2776.
(14) Kikuchi, T.; Mori, Y.; Yokoi, T.; Nakazawa, S.; Kuroda, H.; Masada, Y.; Kitamura, K.; Kuriyama, K. *Chem. Pharm. Bull.* **1983**, *31*, 106–113.

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