Tareciliosides A—G: Cycloartane Glycosides from Leaves of *Tarenna gracilipes* (Hay.) Ohwi

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From the 1-BuOH-soluble fraction of a MeOH extract of leaves of *Tarenna gracilipes*, collected in Okinawa, seven new cycloartane glycosides, named tareciliosides A—G (4—10), were isolated together with three known compounds, D-mannitol (1), (*R*)-linalool 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside (2), and mussaenoside (3). Their structures were elucidated through a combination of spectroscopic analyses.

Key words *Tarenna gracilipes*; Rubiaceae; tarecilioside; cycloartane glycoside

Rubiaceous plants are trees, shrubs, or infrequently herbs comprising about 600 genera and more than 10000 species, including some lianous forms. Many plants belonging to the Rubiaceous family have been used as drugs in traditional medicine, such as *Cinchona succirubra*, *Cephaelis ipecacuanha* and so on. The biological and chemical constituents of these plants have been isolated and identified. However, there are still numerous plants of which the biological and chemical constituents are unknown. In a search for biological and chemical constituents, we conducted a study on the plant *Tarenna gracilipes* (Hay.) Ohwi.

T. gracilipes is distributed from the Kyushu district of Japan to Taiwan, with the following botanical characters: it is an evergreen shrub with slightly pubescent sprigs, and itself-elliptical-acute leaves are opposite, glossy, usually 6—18 cm in length, and 3—8 cm in width. In summer, the white flowers are borne in corymbs at the tips of the branches. Each flower has a 5-toothed, bell-shaped calyx and a tubular, 4 or 5 petaled corolla. The petals are oval and elliptical, and longer than the tube. They twist in bud. The fruits are black and globose drupes.[1)]

Isolation work on the 1-BuOH-soluble fraction of a MeOH extract of leaves of *T. gracipes* afforded seven new cycloartane glycoside, named tareciliosides A—G (**4**—**10**), together with three known compounds, D-mannitol (**1**),[2)] (*R*)-linalool 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside (**2**),[3)] and mussaenoside (**3**).[4)] The structures of the new compounds were elucidated from spectroscopic evidence and those of known compounds were determined by comparison of the physical data with those reported.

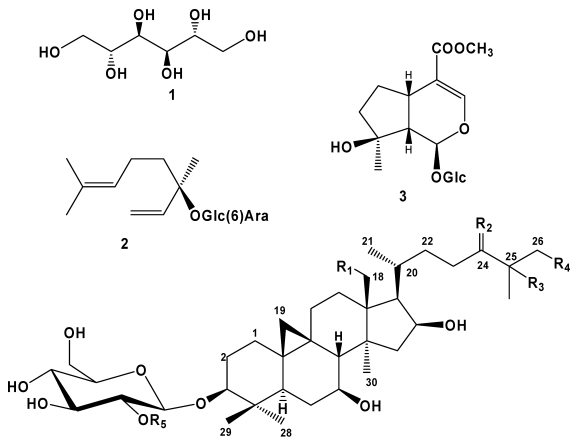
Results and Discussion

Air-dried leaves (9.90 kg) of *Tarenna gracilipes* were extracted at room temperature with methanol and then concentrated *in vacuo*. The concentrated methanol extract was extracted with *n*-hexane and then the MeOH fraction was concentrated. The concentrated MeOH layer was suspended in H_2O, and then extracted with EtOAc and 1-BuOH, successively.

The 1-BuOH-soluble extract (149 g) was subjected to a column of Diaion HP-20, using stepwise-gradient MeOH–H_2O mixtures (1 : 4, 2 : 3, 3 : 2, 4 : 1, each 6 l), and 100% MeOH (6 l) to give seven fractions. Then, fractions 2 and 5 were further subjected to silica gel column chromatography separately, using a $CHCl_3$–MeOH–H_2O solvent system with increasing polarity. The fractions eluted from these columns were combined and re-subjected to octadecylsilanized silica gel column chromatography (ODS CC), droplet counter-current chromatography (DCCC), and HPLC, successively, to afford ten compounds (**1**—**10**).

Compounds **1**, **2** and **3** were found to be known, and were identified as D-mannitol,[2)] (*R*)-linalool 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside[3)] and mussaenoside,[4)] respectively, by comparison with the data reported in the literature.

Tarecilioside A (**4**), $[\alpha]_D$ +14.5°, was isolated as an amorphous powder and its molecular formula was determined to be $C_{36}H_{62}O_{10}$ on negative-ion HR-FAB-MS. The absorption band at 3370 cm^{-1} in the IR spectrum indicated the presence of hydroxyl groups. The ^{1}H-NMR spectrum of **4** (Table 1) showed highly shielded signals characteristic of methylene



	R_1	R_2	R_3	R_4	R_5
4	H	H/β-OH	OH	H	H
5	H	H/β-OH	OH	OH	H
6	H	H/β-OH	OGlc	H	H
7	OH	H/β-OH	OXyl	H	H
8	H	H/β-OH	OXyl	H	H
9	H	O	OGlc	H	H
10	H	O	OH	H	Glc

Fig. 1. Structures

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Table 1. ^{1}H-NMR Data for Tareciliosides A—G (**4**—**10**) (400 MHz, Pyridine-d_5)

H	4	5	6	7	8	9	10
1	1.17 m	1.20 m	1.20 m	1.10 m	1.23 m	1.20 m	1.24 m
	1.50 m	1.52 m	1.50 m	1.47 m	1.58 m	1.50 m	1.53 m
2	1.85 m	1.87 m	1.86 m	1.83 m	1.86 m	1.83 m	1.86 m
	2.38 m	2.44 m	2.40 m	2.37 m	2.43 m	2.40 m	2.35 m
3	3.51 dd 12, 4	3.53 dd 12, 4	3.52 dd 12, 4	3.51 dd 12, 4	3.54 dd 12, 4	3.53 dd 11, 4	3.45 dd 12, 4
5	1.57 dd 13, 3	1.57 dd 12, 3	1.55 dd 13, 4	1.61 m	1.61 m	1.50 m	1.52 m
6	1.22 m	1.22 m	1.21 m	1.10 m	1.23 m	1.20 m	1.24 m
	2.00 m	2.03 m	2.02 m	1.98 m	2.02 m	2.02 m	2.02 m
7	3.79 ddd 10, 10, 4	3.80 ddd 9, 9, 4	3.78 ddd 10, 10, 4	3.79 br dd 10, 10	3.80 br dd 9, 9	3.80 m	3.80 m
8	2.02 d 9	2.04 d 9	2.03 d 10	2.31 d 10	2.04 d 9	2.04 d 9	2.04 d 9
11	1.21 m	1.25 m	1.22 m	1.59 m	1.24 m	1.20 m	1.22 m
	1.85 m	1.89 m	1.87 m	2.02 m	1.86 m	1.86 m	1.85 m
12	1.66 m	1.67 m	1.66 m	1.82 2H, m	1.66 m	1.66 m	1.63 m
	1.83 m	1.83 m	1.80 m	1.82 m	1.83 m	1.85 m	
15	2.31 dd 14, 5	2.32 dd 14, 5	2.29 dd 14, 5	2.72 2H m	2.32 dd 13, 4	2.32 dd 14, 4	2.32 dd 14, 5
	2.73 dd 14, 8	2.74 dd 14, 8	2.71 dd 14, 8	2.74 dd 13, 8	2.73 dd 14, 8	2.74 dd 14, 8	
16	4.75 ddd 8, 8, 5	4.76 ddd 8, 8, 5	4.71 ddd 8, 8, 5	4.70 ddd 7, 7, 5	4.74 m	4.74 m	4.76 m
17	1.80 dd 11, 8	1.82 dd 11, 8	1.77 dd 11, 8	1.95 dd 10, 8	1.80 dd 10, 7	1.76 dd 11, 8	1.75 m
18	1.46 s	1.48 s	1.44 s	3.88 d 11	1.48 s	1.44 s	1.45 s
				4.57 d 11			
19	0.22 d 4	0.22 d 4	0.22 d 4	0.21 d 4	0.23 d 4	0.22 d 4	0.20 d 4
	0.74 d 4	0.75 d 4	0.75 d 4	0.64 d 4	0.76 d 4	0.75 d 4	0.74 d 4
20	2.34 m	2.40 m	2.30 m	2.31 m	2.41 m	2.20 m	2.20 m
21	1.12 d 6	1.13 d 7	1.10 d 5	1.14 d 7	1.12 d 6	1.04 d 6	1.05 d 5
22	1.40 m	1.43 m	1.34 m	1.44 m	1.33 m	1.53 m	1.52 m
	2.48 m	2.50 m	2.46 m	2.60 m	2.50 m	2.42 m	2.40 m
23	1.72 m	1.89 m	1.68 m	1.67 m	1.66 m	3.16 ddd 18, 8, 3	3.11 ddd 18, 8, 5
	2.15 m	2.42 m	1.96 m	2.00 m	2.00 m	3.30 ddd 18, 8, 8	3.18 ddd 18, 8, 8
24	3.78 dd 10, 1	4.18 dd 10, 2	3.90 m	3.88 br d 11	3.88 br d 10		
26	1.47 s	4.08 d 10	1.49 s	1.52 s	1.51 s	1.55 s	1.51 s
		4.25 d 10					
27	1.49 s	1.61 s	1.50 s	1.51 s	1.50 s	1.58 s	1.52 s
28	1.05 s	1.07 s	1.06 s	0.98 s	1.07 s	1.07 s	1.17 s
29	1.32 s	1.34 s	1.33 s	1.31 s	1.34 s	1.34 s	1.34 s
30	1.12 s	1.12 s	1.11 s	1.20 s	1.13 s	1.12 s	1.11 s
1′	4.91 d 8	4.90 d 8	4.92 d 8	4.82 d 8	4.94[a)]	4.93 d 8	4.92 d 8
2′	4.00 dd 8, 9	4.04 dd 8, 8	4.00 dd 8, 8	4.00 dd 8, 8	4.04 dd 8, 8	4.06 dd 8, 8	4.20 dd 8, 8
3′	4.20 dd 9, 9	4.21 dd 8, 8	4.18 dd 8, 8	4.21 dd 8, 8	4.25 dd 8, 8	4.21 dd 8, 8	4.29 dd 8, 8
4′	4.16 dd 9, 9	4.20 dd 8, 8	4.14 dd 8, 8	4.18 dd 8, 8	4.20 dd 8, 8	4.20 dd 8, 8	4.25 dd 8, 8
5′	3.93 m	3.97 m	3.92 m	3.95 m	3.98 m	3.83 m	3.86 m
6′	4.35 dd 12, 5	4.40 dd 12, 5	4.36 dd, 12, 5	4.36 dd 11, 5	4.38 dd 11, 5	4.28 m	4.27 m
	4.52 dd 12, 2	4.56 dd 12, 2	4.53 dd, 12, 2	4.53 dd 11, 2	4.56 br d 11	4.42 dd 12, 2	4.52 dd 12, 2
1″			5.15 d 8	5.05 d 8	5.05 d 8	4.99 d 8	5.36 d 8
2″			3.95 dd 8, 8	3.93 dd 8, 8	3.96 dd 8, 8	4.10 dd 8, 8	4.08 dd 8, 8
3″			4.22 m	4.12 m	4.13 m	4.24 m	4.23 m
4″			4.20 m	4.13 m	4.17 m	4.22 m	4.15 m
5″			3.94 m	3.70 dd 10, 10	3.70 dd 10, 10	3.69 m	3.89 m
				4.23 dd 10, 5	4.27 dd 10, 5		
6″			4.22 m			4.38 dd 12, 5	4.21 m
			4.47 dd 12, 2			4.56 dd 12, 2	4.40 dd 12, 3

Assignments were performed by means of COSY, HSQC and HMBC experiments. s: singlet, d: doublet, m: multiplet or overlapped. *a*) Position was assigned from the HSQC spectrum, but was overlapped by the H_2O signal.

protons of a cyclopropane ring as an AX system (δ_H 0.22, 0.74, each d, J=4 Hz, H_2-19), and signals due to six tertiary methyls (δ_H 1.05, 1.12, 1.32, 1.46, 1.47, 1.49) and a secondary methyl (δ_H 1.12, d, J=6 Hz) group. The ^{13}C-NMR spectrum of tarecilioside A (**4**) exhibited signals of 36 carbons, of which 30 accounted for the aglycone moiety. The remaining signals were in good accordance with the presence of a glucose unit (Table 2). The resonances assigned to the aglycone moiety consisted of seven methyls, nine methylenes, eight methines and six quaternary carbons. The signals at δ_C 70.3, 72.2, 72.8, 80.5 and 88.6 indicated the presence of carbons each bearing a hydroxyl group. Thus, tarecilioside A (**4**) was considered to be a cycloartane-type triterpene monoglycoside.[5—10] The assignments of the proton and carbon signals, and the positions of the hydroxyl groups of **4** were established by analyses of the ^{1}H–^{1}H chemical shift correlation (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond connectivity (HMBC) spectra (Fig. 2). In the HMBC spectrum, the correlation between H_3-28 and 29 (δ_H 1.05, 1.32, respectively) and δ_C 88.6 placed one of the hydroxyl groups at the 3-position, and the position of the second hydroxyl was determined to be at the 7-position from the coupling pattern of the H-5 (δ_H 1.57, dd, J=13, 3 Hz) and H-8 (δ_H 2.02, d, J=9 Hz). The hydroxyl group at the 16-position was proved by the HMBC correlations of H-8 with C-15 (δ_C 50.7) and then H-15 (δ_H 2.31)

Table 2. ^{13}C-NMR Data for Tareciliosides A—G (**4**—**10**) (100 MHz, Pyridine-d_5)

C	4	5	6	7	8	9	10
1	31.8 t	31.8 t	31.9 t	32.3 t	31.9 t	31.8 t	31.8 t
2	29.8 t	29.8 t	29.7 t	29.8[a] t	29.8 t	29.8 t	29.8 t
3	88.6 d	88.6 d	88.6 d	88.5 d	88.6 d	88.6 d	88.7 d
4	40.9 s	41.0 s	40.9 s	41.0 s	41.0 s	41.0 s	41.0 s
5	46.4 d	46.4 d	46.4 d	47.0 d	46.4 d	46.4 d	46.4 d
6	31.9 t	32.0 t	31.8 t	32.0 t	31.8 t	32.0 t	31.9 t
7	70.3 d	70.3 d	70.3 d	70.6 d	70.3 d	70.3 d	70.3 d
8	55.3 d	55.3 d	55.1 d	55.3 d	55.2 d	55.2 d	55.1 d
9	20.3 s	20.3 s	20.3 s	20.3 s	20.3 s	20.3 s	20.2 s
10	26.9 s	26.9 s	26.9 s	27.1 s	26.9 s	26.9 s	26.9 s
11	26.8 t	26.8 t	26.8 t	26.7 t	26.8 t	26.8 t	26.8 t
12	33.2 t	33.2 t	33.1 t	30.1 t	33.2 t	33.2 t	33.2 t
13	46.1 s	46.2 s	46.1 s	47.2 s	46.2 s	46.2 s	46.2 s
14	47.1 s	47.1 s	47.0 s	51.4 s	47.1 s	47.0 s	47.1 s
15	50.7 t	50.8 t	50.6 t	52.1 t	50.8 t	50.5 t	50.3 t
16	72.2 d	72.2 d	72.1 d	72.6 d	72.1 d	71.9 d	72.1 d
17	56.8 d	56.8 d	56.9 d	55.5 d	56.9 d	56.9 d	56.8 d
18	18.8 q	18.8 q	18.7 q	65.0 t	18.9 q	18.8 q	18.7 q
19	28.6 t	28.6 t	28.5 t	29.9[a] t	28.5 t	28.6 t	28.5 t
20	31.6 d	31.6 d	31.5 d	31.6 d	31.6 d	30.5 d	30.5 d
21	18.9 q	18.9 q	18.9 q	19.1 q	18.8 q	18.4 q	18.3 q
22	35.0 t	35.0 t	35.1 t	34.4 t	35.0 t	30.9 t	30.8 t
23	29.4 t	29.1 t	29.3 t	29.1 t	29.3 t	34.4 t	34.1 t
24	80.5 d	77.9 d	79.0 d	78.9 d	79.1 d	215.5 s	218.8 s
25	72.8 s	74.8 s	80.9 s	81.0 s	81.0 s	82.9 s	76.9 s
26	26.1 q	69.3 t	21.5 q	21.4 q	21.2 q	23.8 q	27.2 q
27	25.8 q	20.0 q	24.2 q	24.0 q	24.2 q	24.7 q	27.2 q
28	15.3 q	15.4 q	15.3 q	15.4 q	15.3 q	15.4 q	15.3 q
29	25.8 q	25.9 q	25.8 q	25.8 q	25.8 q	25.8 q	25.8 q
30	20.0 q	20.2 q	20.0 q	22.7 q	20.0 q	20.0 q	20.0 q
1′	106.7 d	106.8 d	106.7 d	106.7 d	106.8 d	106.8 d	104.8 d
2′	75.7 d	75.8 d	75.7 d	75.7 d	75.8 d	75.8 d	83.3 d
3′	78.7 d	78.8 d	78.6[a] d	78.7[a] d	78.8 d	78.8[a] d	78.4 d
4′	71.8 d	71.9 d	71.8[b] d	71.9 d	71.9 d	71.8 d	71.8 d
5′	78.1 d	78.2 d	78.1 d	78.2 d	78.2 d	78.2 d	78.0 d
6′	63.0 t	63.1 t	63.0 t	63.0 t	63.1 t	63.1 t	62.9[a] t
1″			98.6 d	99.4 d	99.4 d	99.6 d	105.9 d
2″			75.3 d	75.2 d	75.2 d	75.3 d	77.0 d
3″			78.7[a] d	78.6[a] d	78.6 d	78.8[a] d	78.2 d
4″			71.8[b] d	71.1 d	71.1 d	72.1 d	71.6 d
5″			78.1 d	67.0 t	67.0 t	78.2 d	78.0 d
6″			62.8 t			62.8 t	62.8[a] t

q: CH_3, t: CH_2, d: CH, s: C. The same superscripts may be interchangeable in each column.

with C-16 (δ_C 72.2). The remaining two hydroxyl groups were placed at the 24 and 25-positions based on the HMBC correlations for H_3-26 and 27 (δ_H 1.47, 1.49, respectively) with C-24 (δ_C 80.5) and 25 (δ_C 72.8). Other HMBC correlations were also supportive of the positions of hydroxyl groups on the cycloartane skeleton (Fig. 2). The long-range correlation observed between the anomeric proton of the glucose unit (δ_H 4.91) and C-3 (δ_C 88.6) of the aglycone moiety confirmed the position of the sugar linkage. The stereochemistry of **4** was determined by analysis of the phase-sensitive rotating frame nuclear Overhauser and exchange spectroscopy (PS-ROESY) spectra, and the ^{1}H–^{1}H coupling constants (Fig. 3, Table 1). The orientation of the hydroxyl group at C-3 was concluded to be β on the basis of the coupling constant of H-3, since the H-3 (δ_H 3.51) signal was observed as a doublet of doublets due to 1,2-diaxial (J=12 Hz) and axial-equatorial coupling (J=4 Hz) with H-2ax and H-2eq, respectively. The 1,2-diaxial coupling of H-8 (δ_H 2.02, d, J=9 Hz) suggested the β configuration for the hydroxyl

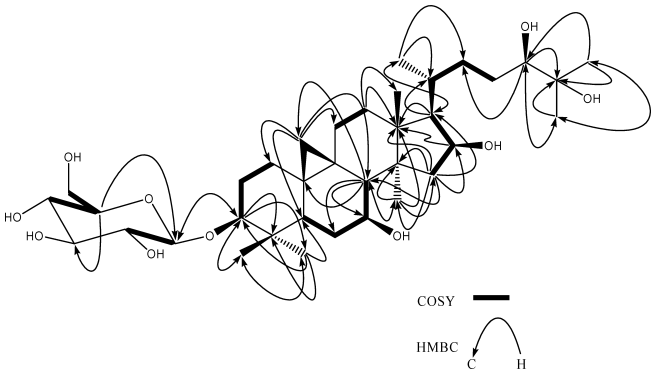

Fig. 2. HMBC and COSY Correlations of Tarecilioside A (**4**)

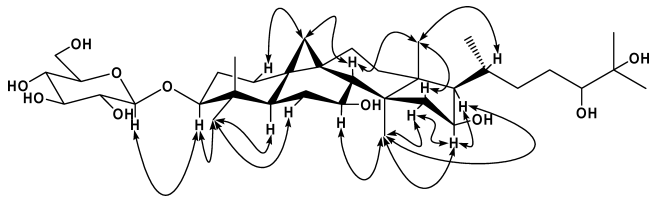

Fig. 3. Phase-Sensitive ROESY Correlations of Tarecilioside A (**4**)

group at C-7. In the case of H-16 (δ_H 4.75), the large coupling constant of H-16 with H-15ax-like (δ_H 2.73, d, J=8 Hz) and the small coupling constant of H-16 with H-15eq-like (J=5 Hz), and also the large coupling constant (J=11 Hz) with H-17 indicated the β configuration for the hydroxyl group at this position. These observations were further confirmed by the correlations observed in the PS-ROESY experiment, in which H_3-30 (δ_H 1.12) showed correlations with H-7 (δ_H 3.79) and H-16 (δ_H 4.75) (Fig. 3). The configuration of the C-24 hydroxyl group was indicated to be 24*R* on comparison with the chemical shift values of cyclounifolioside C (24*R*, C-24: δ_C 80.3)[6] and cyclocantogenin (24*S*, C-24: δ_C 77.0).[6] On acid hydrolysis of **4**, an aglycone (**4a**) and D-glucose were isolated. Additionally, the resonance for one anomeric proton (H-1′) was observed at δ_H 4.91 (d, J=8 Hz), which indicated the presence of one sugar moiety, and the mode of linkage is β and the position of the sugar linkage was confirmed to be to the hydroxy group at C-3 by the HMBC correlation between H-1′ and C-3 (δ_C 88.6). Therefore, the structure of tarecilioside A (**4**) was elucidated to be cycolartane-3β,7β,16β,24*R*,25-pentaol 3-*O*-β-D-glucopyranoside.

Tarecilioside B (**5**), $[\alpha]_D$ +10.8°, was isolated as an amorphous powder and its molecular formula was determined to be $C_{36}H_{62}O_{11}$ on negative-ion HR-FAB-MS. All the spectral data for **5** were the same as those for **4**, except for the presence of one more hydroxyl functional group than observed in **4**. In the NMR spectra of **5**, only five tertiary methyl carbons were observed, with a new primary alcohol signal [δ_C 69.2 with δ_H 4.08 (d, J=10 Hz) and 4.25 (d, J=10 Hz)]. Thus, one of the methyl groups was expected to be modified to a primary alcohol. Since the ^{13}C-NMR data for the ring portion, and C-20 and 21 of **5** were essentially the same as those for **4** (Table 2), one of the geminal methyl groups at C-25 must be oxidized to a carbinol. This was further supported by the HMBC correlation cross peak between H-24 (δ_H 4.18) and the carbinol carbon signal. The absolute configuration of the

newly formed chiral center remains to be determined. Therefore, the structure of tarecilioside B (**5**) was tentatively elucidated to be cycolartane-3β,7β,16β,24*R*,25ξ,26-hexaol 3-*O*-β-D-glucopyranoside.

Tarecilioside C (**6**), $[\alpha]_D$ +13.1°, was isolated as an amorphous powder and its molecular formula was determined to be $C_{42}H_{72}O_{15}$ on positive-ion HR-ESI-TOF-MS. From the various spectral data, **6** was also expected to be a derivative of a cycloartane glucoside and in the NMR spectra, two anomeric carbon (δ_C 106.7, 98.6) and proton (δ_H 4.92, 5.15, respectively) signals of glucoses were observed. Because the ^{13}C-NMR chemical shifts of the ring nucleus, and C-20 to 22 for **6** were not distinguishable from those of **4** and the relatively shielded chemical shift of the anomeric carbon (δ_C 98.6), a new glucoside bond was formed through the tertiary hydroxyl group at C-25. This was further confirmed by the HMBC correlation cross peak between δ_H 5.15 on C-1″ (δ_C 98.6) and C-25 (δ_C 80.9), and a significant downfield shift was observed from C-25 of **4** to that of **6** (δ_C 72.8→δ_C 80.9). On hydrolysis of **5**, only D-glucose was detected. Therefore, the structure of **6** was elucidated to be cycolartane-3β,7β,16β,24*R*,25-pentaol 3,25-di-*O*-β-D-glucopyranoside.

Tarecilioside D (**7**), $[\alpha]_D$ +17.7°, was isolated as an amorphous powder and its molecular formula was determined to be $C_{41}H_{70}O_{15}$ by positive-ion HR-ESI-TOF-MS. Tarecilioside D (**7**) was also a cycloartane diglycoside analogous to the aforementioned compounds. Glucopyranose and xylopyranose were present in the molecule, and judging from the HMBC correlation cross peaks, the former was located on the hydroxyl group at C-3 and the latter on that at C-25 (Fig. 4). The results of HR-MS and NMR experiments [δ_C 65.0 with δ_H 3.88 (d, J=11 Hz) and 4.57 (d, J=11 Hz)] indicated that tarecilioside D (**7**) possessed one more hydroxyl group on one of the methyl groups than in tareciliosiode C (**6**). The HMBC correlations of the new carbinol protons with C-13 (δ_C 47.2) and 17 (δ_C 55.5), and H-17 (δ_H 1.95) with the carbinol carbon signal supported that the C-18 methyl must be oxidized to a primary alcohol. This was further supported by a correlation peak between H_2-18 and H-20 in the PS-ROESY experiments. On acid hydrolysis of **7**, D-glucose and D-xylose were obtained. Therefore, the structure of tarecilioside D (**7**) was elucidated to be cycolartane-3β,7β,16β,18,24*R*,25-hexaol 3-*O*-β-D-glucopyranosyl 25-*O*-β-D-xylopyranoside.

Tarecilioside E (**8**), $[\alpha]_D$ +0.6°, was isolated as an amorphous powder and its molecular formula was determined to be $C_{41}H_{70}O_{14}$ on positive-ion HR-ESI-TOF-MS. The NMR data were essentially the same as those of **6**, except for the presence of xylopyranose. Thus, one of the glucopyranoses present in **6** was replaced by xylopyranose. On acid hydrolysis of **8**, D-glucose and D-xylose were obtained. The positions of sugar linkages were also the same as those in **7**, and the glucopyranose moiety was also located at the hydroxyl group at C-3 in the β-mode, since a HMBC correlation of the glucose anomeric proton (H-1′) (δ_H 4.94) with C-3 (δ_C 88.6) was observed, although H-1′ (δ_H 4.49) was overlapped by a huge water signal. Therefore, the structure of tarecilioside E (**8**) was elucidated to be cycolartane-3β,7β,16β,24*R*,25-pentaol 3-*O*-β-D-glucopyranoside, 25-*O*-β-D-xylopyranoside.

Tarecilioside F (**9**), $[\alpha]_D$ +7.1°, was isolated as an amorphous powder and its molecular formula was determined to be $C_{42}H_{70}O_{15}$ on negative-ion HR-FAB-MS. Tarecilioside F (**9**) was also a cycloartane 3,25-*O*-diglucoside analogous to **6**. A highly deshielded signal at δ_C 215.5 was expected to represent an isolated ketone functional group, and its location was determined to be at C-24 judging from the cross peaks in the HMBC and PS-ROESY spectra. Therefore, the structure of **9** was elucidated to be 24-oxo-tarecilioside C, as shown in Fig. 1.

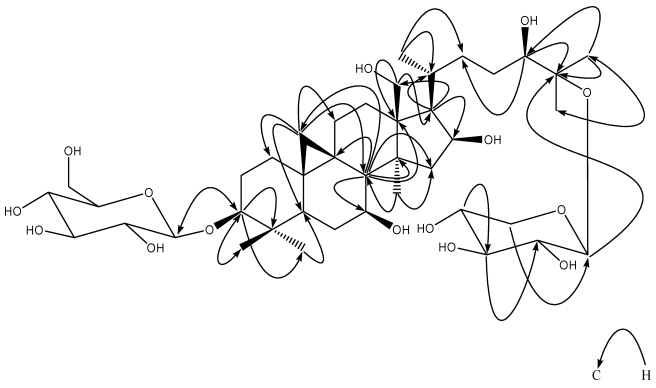

Fig. 4. HMBC Correlations of Tarecilioside D (**7**)

Tarecilioside G (**10**), $[\alpha]_D$ +15.6°, was isolated as an amorphous powder and its molecular formula was determined to be $C_{42}H_{70}O_{15}$ on positive-ion HR-ESI-TOF-MS. Tarecilioside G (**10**) was also expected to be a cycloartane diglucoside with a ketone functional group at C-24. On acid hydrolysis, only D-glucose was detected as a sugar component. However, one of the glucoses was not at the hydroxyl group at C-25, but at the 2′-position of the other glucose moiety. This was confirmed by comparison with reported ^{13}C-NMR data for Glc(2←1)Glc[11] and the HMBC experiment, in which the H-1″ proton signal (δ_H 5.36) showed a cross peak with the C-2′ carbon signal (δ_C 83.3). The glycosylation shift trend also supported the above fact that when the ^{13}C-NMR spectra of **9** and **10** were compared, the C-2′ carbon signal was shifted downfield by 7.5 ppm and the anomeric carbon was shifted upfield by 1.9 ppm. Further confirmation was provided by the acetylation experiment with acetic anhydride and pyridine, which gave a nanoacetate (**10a**). In the H–H COSY spectrum of **10a**, the H-2′ proton remained intact (δ_H 3.79, dd, J=8, 9 Hz). Therefore, the structure of tarecilioside G (**10**) was elucidated to be cycolartane-3β,7β,16β,18,25-pentaol 3-*O*-(2″-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside.

From leaves of *Tarenna gracilipes* (Rubiaceae), seven new cycloartane glycosides, named tareciliosides A—G, were isolated. Cycloartane glycosides are often found in many plant sources, for example, Leguminosae plants, *Astragalus unifoliolatus*,[6] *A. verrucosus*[12] and *A. oleifolius*.[8,13] From Rubiaceae plant, *Mussaenda pubescens*, cycloartane glycosides have also been isolated as mussaendosides, which are characteristic in having an amide bond to the terminal carboxylic acid.[14—16] However, tareciliosides do not have such amide bond, but they have a β-hydroxyl group at the 7-position. The cytotoxicity of tareciliosides was assayed by the MTT method using KB cells. But they did not show any significant activity.

It is noteworthy that from the MeOH extract, 415 g of D-mannitol was obtained. This accounted for 4% of the dried

weight of leaves. Although no medical use of *T. gracilipes* has been reported, D-mannitol has been shown to reduce the extent of ischemic injury, and to improve myocardial, renal and cerebral function.[17,18]

Experimental

General Experimental Procedures IR spectra were obtained on a Horiba Fourier Transform Infrared spectrophotometer FT-710. Optical rotation data were measured on a JASCO P-1030 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM α-400 spectrometer at 400 and 100 MHz, respectively, with tetramethylsilane (TMS) as an internal standard. HR-FAB mass spectra (negative-ion mode) and HR-ESI-TOF mass spectra (positive-ion mode) were taken on a JEOL JMS-SX 102 mass spectrometer and Applied Biosystems QSTAR XL System, respectively.

Highly-porous synthetic resin Diaion HP-20 (Φ=60 mm, L=65 cm) was purchased from Mitsubishi Chemical Co., Ltd. (Tokyo, Japan). Silica gel column chromatography (CC) was performed on silica gel 60 [(E. Merck, Darmstadt, Germany) 70—230 mesh]. Reversed-phase [octadecyl silica gel (ODS)] open CC (RPCC) was performed on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto) [Φ=50 mm, L=25 cm, linear gradient: MeOH–H_2O (1 : 9, 1.5 l)→(7 : 3, 1.5 l), 10 g fractions being collected]. The droplet counter-current chromatograph (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ=2 mm, L=40 cm), and the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O–1-PrOH (9 : 12 : 8 : 2) were used as the mobile and stationary phases, respectively. Five gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on an ODS (Inertsil; GL Science, Tokyo, Japan; Φ=6 mm, L=5 cm, flow rate 1.6 ml/min) column. Precoated silica gel 60 F_{254} TLC plates (E. Merck; 0.25 mm in thickness) were used for identification.

Plant Material Leaves of *T. gracilipes* OHWI were collected in Okinawa, Japan, in July 2002, and a voucher specimen was deposited in the Herbarium of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (02-TG-Okinawa-0705).

Extraction and Isolation Air-dried leaves of *Tarenna gracilipes* (9.90 kg) were extracted three times with MeOH (45 l×3) for one week at room temperature, and then the MeOH extract was concentrated to 6 l *in vacuo*. On evaporation of the MeOH extract, a colorless precipitate formed (**1**), which was collected by suction (415 g). A portion of the precipitates was recrystallized from EtOH to give colorless prisms. The extract was washed with *n*-hexane (6 l) and then the methanolic layer was concentrated to a viscous gum (*n*-hexane-soluble fraction: 58.8 g). The gummy residue was suspended in H_2O (6 l) and then extracted with EtOAc (6 l) to give 172 g of an EtOAc-soluble fraction. The aqueous layer was extracted with 1-BuOH (6 l) to give 149 g of a 1-BuOH-soluble fraction. The remaining water-layer was concentrated to furnish 248 g of a water-soluble fraction.

The 1-BuOH-soluble extract (149 g) was applied to highly porous synthetic resin (Diaion HP-20) CC (Φ=60 mm, L=65 cm), using a stepwise-gradient of MeOH–H_2O [(1 : 4, 6 l), (2 : 3, 6 l), (3 : 2, 6 l), (4 : 1, 6 l), and MeOH (6 l)], 500 ml factions being collected. The residue eluted with the 20% MeOH (50.7 g in fractions 2—4) eluate obtained on HP-20 CC was subjected to silica gel (1.0 kg) CC using $CHCl_3$ (6 l), $CHCl_3$–MeOH [(99 : 1, 6 l), (97 : 3, 6 l), (19 : 1, 6 l), (37 : 3, 6 l), (9 : 1, 6 l), (7 : 17, 6 l), (17 : 3, 6 l), (33 : 7, 6 l), (4 : 4, 6 l), (7 : 3, 6 l)], and $CHCl_3$–MeOH–H_2O (35 : 15 : 2, 3 l), 1 l fractions being collected. The residue (2.25 g in fractions 39—49) of the 12.5—15% MeOH in $CHCl_3$ eluate was subjected to PRCC and then the residue (79.4 mg in fractions 105—114) was purified by HPLC with 40% MeOH to afford 18.9 mg of compound **3** from the peak at 28 min (flow rate: 0.4 ml/min). The residue (228 mg in fractions 66—73) of the 25% MeOH in $CHCl_3$ eluate afforded 20.0 mg of **1**.

The residue eluted with the 60—80% MeOH (29.6 g in fractions 14—19) eluate obtained on HP-20 CC was subjected to silica gel (500 g) CC using $CHCl_3$ (3 l), $CHCl_3$–MeOH [(99 : 1, 3 l), (97 : 3, 3 l), (19 : 1, 3 l), (37 : 3, 3 l), (9 : 1, 3 l), (7 : 1, 3 l), (17 : 3, 3 l), (33 : 7, 3 l), (4 : 1, 3 l), (3 : 1, 3 l), (7 : 3, 3 l)], and $CHCl_3$–MeOH–H_2O (35 : 15 : 2, 3 l), fractions of 500 ml being collected. The residue (2.00 g in fractions 42—48) of the 15% MeOH in $CHCl_3$ eluate was subjected to PRCC. The residue (30.8 mg in fractions 211—225) was separated by DCCC to afford 9.1 mg of **9** in fractions 60—78 and 6.8 mg of compound **7** in fractions 79—88. The residue (35.9 mg in fractions 96—121) of the eluate obtained on DCCC was purified by HPLC with 57% MeOH to afford 7.20 mg of **10** from the peak at 12 min (flow rate: 0.9 ml/min). The residue (60.7 mg in fractions 122—163) of the eluate obtained on DCCC was purified by HPLC with 65% MeOH to afford 33.2 mg of **4** from the peak at 14 min.

The residue (2.50 g in fractions 49—58) of the 17.5—20% MeOH in $CHCl_3$ eluate obtained on silica gel CC was subjected to PRCC. The residue (534 mg in fractions 213—229) was separated by DCCC (105 mg in fractions 46—65) and then by HPLC with 60% MeOH to give 10.8 mg of **5** from the peak at 22 min. The residue (634 mg in fractions 230—249) was similarly separated by DCCC to give two fractions, 99.4 mg in fractions 52—61 and 261 mg in fractions 62—68. The former was finally purified by HPLC with 65% MeOH to give 37.7 mg of compound **6** from the peak at 8.1 min, and the latter gave 32.7 mg of **8** by HPLC purification with 65% MeOH from the peak at 10 min (flow rate: 1.3 ml/min).

The residue (2.51 g in fractions 59—65) of the 25% MeOH in $CHCl_3$ eluate obtained on silica gel CC was subjected to PRCC and the residue (220 mg in fractions 186—204) was purified by DCCC to give 10.9 mg of **2** in fractions 73—90.

Tarecilioside A (**4**): Amorphous powder. $[\alpha]_D^{22}$ +14.5° (c=2.48, pyridine); IR ν_{max} (film) cm^{-1}: 3370, 2935, 1459, 1379, 1252, 1161, 1076, 1036; ^{1}H-NMR (400 MHz, pyridine-d_5) and ^{13}C-NMR (100 MHz, pyridine-d_5): Tables 1 and 2, respectively; HR-FAB-MS (negative-ion mode) m/z: 653.4239 $[M-H]^-$ (Calcd for $C_{36}H_{61}O_{10}$: 653.4206).

Tarecilioside B (**5**): Amorphous powder, $[\alpha]_D^{20}$ +10.8° (c=0.827, pyridine); IR ν_{max} (film) cm^{-1}: 3367, 2938, 1442, 1379, 1256, 1163, 1076, 1034; ^{1}H-NMR (400 MHz, pyridine-d_5) and ^{13}C-NMR (100 MHz, pyridine-d_5): Tables 1 and 2, respectively; HR-FAB-MS m/z: 669.4184 $[M-H]^-$ (Calcd for $C_{36}H_{61}O_{11}$: 669.4155).

Tarecilioside C (**6**): Amorphous powder, $[\alpha]_D^{20}$ +13.1° (c=2.51, pyridine); IR ν_{max} (film) cm^{-1}: 3367, 2939, 1591, 1441, 1370, 1157, 1076, 1033; ^{1}H-NMR (400 MHz, pyridine-d_5) and ^{13}C-NMR (100 MHz, pyridine-d_5): Tables 1 and 2, respectively; HR-ESI-MS (positive-ion mode) m/z: 839.4739 $[M+Na]^+$ (Calcd for $C_{42}H_{72}O_{15}Na$: 839.4763).

Tarecilioside D (**7**): Amorphous powder, $[\alpha]_D^{20}$ +17.7° (c=2.18, pyridine); IR ν_{max} (film) cm^{-1}: 3395, 2940, 1591, 1441, 1382, 1160, 1075, 1039; ^{1}H-NMR (400 MHz, pyridine-d_5) and ^{13}C-NMR (100 MHz, pyridine-d_5): Tables 1 and 2, respectively; HR-ESI-MS (positive-ion mode) m/z: 825.4604 $[M+Na]^+$ (Calcd for $C_{41}H_{70}O_{15}Na$: 825.4606).

Tarecilioside E (**8**): Amorphous powder, $[\alpha]_D^{20}$ +0.6° (c=0.45, pyridine); IR ν_{max} (film) cm^{-1}: 3395, 2930, 1650, 1458, 1381, 1161, 1075, 1038; ^{1}H-NMR (400 MHz, pyridine-d_5) and ^{13}C-NMR (100 MHz, pyridine-d_5): Tables 1 and 2, respectively; HR-ESI-MS (positive-ion mode) m/z: 809.4648 $[M+Na]^+$ (Calcd for $C_{41}H_{70}O_{14}Na$: 809.4657).

Tarecilioside F (**9**): Amorphous powder, $[\alpha]_D^{20}$ +7.1° (c=0.693, pyridine); IR ν_{max} (film) cm^{-1}: 3368, 2937, 1705, 1461, 1381, 1163, 1076, 1033; ^{1}H-NMR (400 MHz, pyridine-d_5) and ^{13}C-NMR (100 MHz, pyridine-d_5): Tables 1 and 2, respectively; HR-FAB-MS (negative-ion mode) m/z: 813.4643 $[M-H]^-$ (Calcd for $C_{42}H_{69}O_{15}$: 813.4636).

Tarecilioside G (**10**): Amorphous powder. $[\alpha]_D^{20}$ +15.6° (c=0.43, pyridine); IR ν_{max} (film) cm^{-1}: 3376, 2935, 1702, 1458, 1370, 1165, 1075, 1034; ^{1}H-NMR (400 MHz, pyridine-d_5) and ^{13}C-NMR (100 MHz, pyridine-d_5): Tables 1 and 2, respectively; HR-ESI-MS (positive-ion mode) m/z: 837.4613 $[M+Na]^+$ (Calcd for $C_{42}H_{70}O_{15}Na$: 837.4606).

Known Compounds Isolated D-Mannitol (**1**): Colorless prisms, mp 160—160 °C (EtOH), $[\alpha]_D^{20}$ +19.6° (c=10.0, 2 ml of H_2O containing 256 mg of Borax).[2]

(*R*)-Linalool 6-*O*-α-L-Arabinopyranosyl-β-D-glucopyranoside (**2**): Amorphous powder, $[\alpha]_D^{20}$ −24.0° (c=1.00, MeOH).[3]

Mussaenoside (**3**): Amorphous powder, $[\alpha]_D^{26}$ −77.8° (c=1.26, MeOH).[4]

Acid Hydrolysis of Tarecilioside A (4) A solution of tarecilioside A (**4**) (10.0 mg) in 2 ml of 1 N HCl–dioxane (1 : 1) was heated at 100 °C for 1 h under a N_2 atmosphere. After cooling, the reaction mixture was neutralized by the addition of Amberlite IRA-96SB, and then chromatographed on silica gel (7.0 g), with elution with a linear gradient mixture of $CHCl_3$–MeOH (20 : 1, 100 ml) to (1 : 1, 100 ml), 5 ml fractions being collected. An aglycone (**4a**) and D-glucose were recovered in fractions 12—18 (2.62 mg, 38%) and 19—23 (1.74 mg, 63 %), respectively. Aglycone (**4a**), amorphous powder, $[\alpha]_D^{20}$ +23.8° (c=0.17, pyridine); ^{1}H-NMR (400 MHz, pyridine-d_5) δ: 0.29 (1H, d, J=4 Hz, H-19a), 0.76 (1H, d, J=4 Hz, H-19b), 1.05 (3H, s, H_3-28), 1.10 (3H, s, H_3-30), 1.10 (3H, d, J=6 Hz, H_3-21), 1.17 (3H, s, H_3-29), 1.44 (3H, s, H_3-26), 1.45 (3H, s, H_3-18), 1.48 (3H, s, H_3-27), 2.00 (1H, d, J=10 Hz, H-8), 2.29 (1H, dd, J=13, 4 Hz, H-15a), 2.71 (1H, dd, J=13, 8 Hz, H-15b), 3.50 (1H, dd, J=11, 4 Hz, H-3), 3.74 (1H, dd, J=10, 2 Hz, H-24), 4.71 (1H, ddd, J=8, 8, 5 Hz, H-16); ^{13}C-NMR (100 MHz, pyridine-d_5) δ: 14.7 (C-28), 18.8 (C-18), 18.9 (C-21), 20.0 (C-30), 20.3 (C-9), 25.7 (C-29), 26.0 (C-27), 26.2 (C-26), 26.9 (C-11), 27.2 (C-10), 28.9 (C-19), 29.5 (C-23), 30.0 (C-1), 31.0 (C-2), 31.5 (C-20), 32.2 (C-6), 33.2 (C-12), 35.0

(C-22), 40.8 (C-4), 46.2 (C-13), 46.4 (C-5), 47.0 (C-14), 50.6 (C-15), 55.3 (C-8), 56.9 (C-17), 70.4 (C-7), 72.2 (C-16), 72.8 (C-25), 77.9 (C-3), 80.5 (C-24); HR-ESI-MS (positive-ion mode) *m/z*: 515.3702 $[M+Na]^+$ (Calcd for $C_{30}H_{52}O_5Na$: 515.3706). D-Glucose: $[\alpha]_D^{20}$ +26.9° (*c*=0.12, 24 h after being dissolved in H_2O).

Analyses of the Sugar Moiety About 2 mg each of tareciliosides A—G (**4**—**10**) was hydrolyzed with 1 N HCl (0.1 ml) at 100 °C for 2 h. The reaction mixtures were partitioned with an equal amount of EtOAc (0.1 ml), and the water layers were analyzed with a chiral detector (JASCO OR-2090plus) on an amino column [Asahipak NH2P-50 4E, CH_3CN–H_2O (80 : 20), 1 ml/min]. Tareciliosides A (**4**), B (**5**), C (**6**), F (**9**), and G (**10**) each gave a peak for D-glucose at the retention time at 14.2 min (positive optical rotation sign), and tareciliosides D (**7**) and E (**8**) gave peaks for D-xylose and D-glucose at the retention times of 9.3 min (positive optical rotation sign) and 14.2 min (positive optical rotation sign), respectively. Peaks were identified by co-chromatography with authentic D-xylose and D-glucose.

Acetylation of Tarecilioside G Tarecilioside G (**10**) (3.4 mg) was acetylated with 50 μl each of acetic anhydride and pyridine at 25 °C for 18 h. The reagents were removed under a N_2 stream, followed by drying up *in vacuo* to give a nanoacetate (**10a**) in a quantitative yield. Nanoacetate (**10a**), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.981 (3H, s), 1.998 (3H, s), 2.002 (3H, s), 2.010 (6H, s); 2.045 (3H, s), 2.045 (3H, s), 2.055 (3H, s), 2.090 (3H, s) ($C\underline{H}_3CO\times9$), 3.67 (2H, m, H-5′, 5″), 3.79 (1H, dd, *J*=9, 8 Hz, H-2′), 4.05 and 4.08 (each 1H, each dd, each *J*=12, 2 Hz, H-6′a, H-6″a), 4.24 (2H, dd, *J*=12, 6 Hz, H-6′b, 6″b), 4.46 (1H, d, *J*=8 Hz, H-1′), 4.70 (1H, d, *J*=8 Hz, H-2″), 4.89 (1H, dd, *J*=9, 8 Hz, H-2″), 4.92 (1H, dd, *J*=9, 9 Hz, H-4′), 5.10 (1H, dd, *J*=9, 9 Hz, H-3″), 5.14 (1H, dd, *J*=9, 9 Hz, H-4″), 5.18 (1H, dd, *J*=9, 9 Hz, H-3′); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.42, 169.78, 169.98, 170.25, 170.29, 170.53, 170.57, 170.62 ($CH_3\underline{C}O\times9$); HR-ESI-MS (positive-ion mode) *m/z*: 1215.5533 $[M+Na]^+$ (Calcd for $C_{60}H_{88}O_{24}Na$: 1215.5557).

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