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NEW CYTOTOXIC NORDITERPENE DILACTONES FROM LEAVES OF *PODOCARPUS MACROPHYLLUS* VAR. *MAKI*

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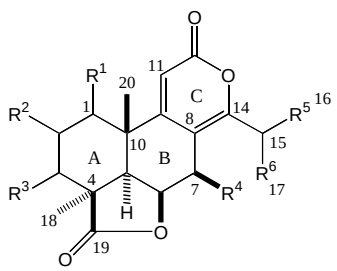
Abstract – Four new nor- and bisnorditerpene dilactones, rakanmakilactones G (**1**), H-J (**3**-**5**), one new norditerpene dilactone apioside (**2**), and one new totarane diterpene (**6**) were isolated from a MeOH extract of the leaves of *Podocarpus macrophyllus* D. Don var. *maki* Endl. of the family Podocarpaceae. The structures of **1**-**6** were established by spectroscopic studies, including 1D and 2D NMR spectral analysis, and single-crystal X-Ray crystallographic analysis. Those rakanmakilactones from this plant were found to have a cytotoxic effect on P388 murine leukemia cells.

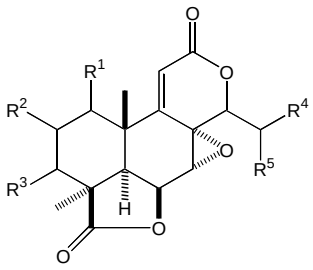
INTRODUCTION

More than 50 bioactive nor- and bisnorditerpene dilactones and several totarane diterpenoids have been isolated from plants of the genus *Podocarpus* of the family Podocarpaceae,[1-5] and the diterpene dilactones are classified into the following three major subgroups A-C, according to the structures of the B/C ring systems.[6] In subgroup A, the B/C ring is α-pyrone [8(14),9(11)-dienolide], in subgroup B,

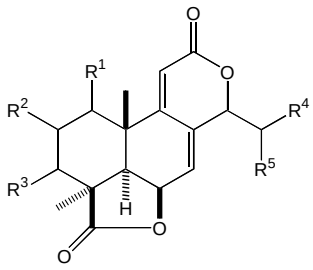
Subgroup A
α-pyrone type



Subgroup B
7α:8α-epoxy-9:11-enolide type



Subgroup C
7:8, 9:11-dienolide type



7α,8α-epoxy-9(11)-enolide, and in subgroup C, 7(8),9(11)-dienolide. In our previous paper,[7,8] we reported the isolation and characterization of ten sulfur-containing subgroup B norditerpene dilactones from a $CHCl_3$ soluble fraction of the leaves of *Podocarpus macrophyllus* D. Don var. *maki* Endl., all

showing a cytotoxic activity on P388 murine leukemia cells. By further investigation on this plant, six new diterpenes, i.e. two subgroup A diterpene dilactones [rakanmakilactone G (**1**), rakanmakilactone G-7β-*O*-β-D-apiofuranoside (**2**)], three subgroup B diterpene dilactones (rakanmakilactones H-J, **3**-**5**), and one totarane diterpene (4β-carboxy-17-hydroxy-19-nor-totarol, **6**), were isolated along with three known compounds, nagilactone B (subgroup A, **7**),[9] inumakilactone A (subgroup B, **8**),[9-11] and 4β-carboxy-19-nor-totarol (**9**).[12,13] In the present paper, we report the isolation, structural elucidation and cytotoxic activities of these newly isolated compounds.

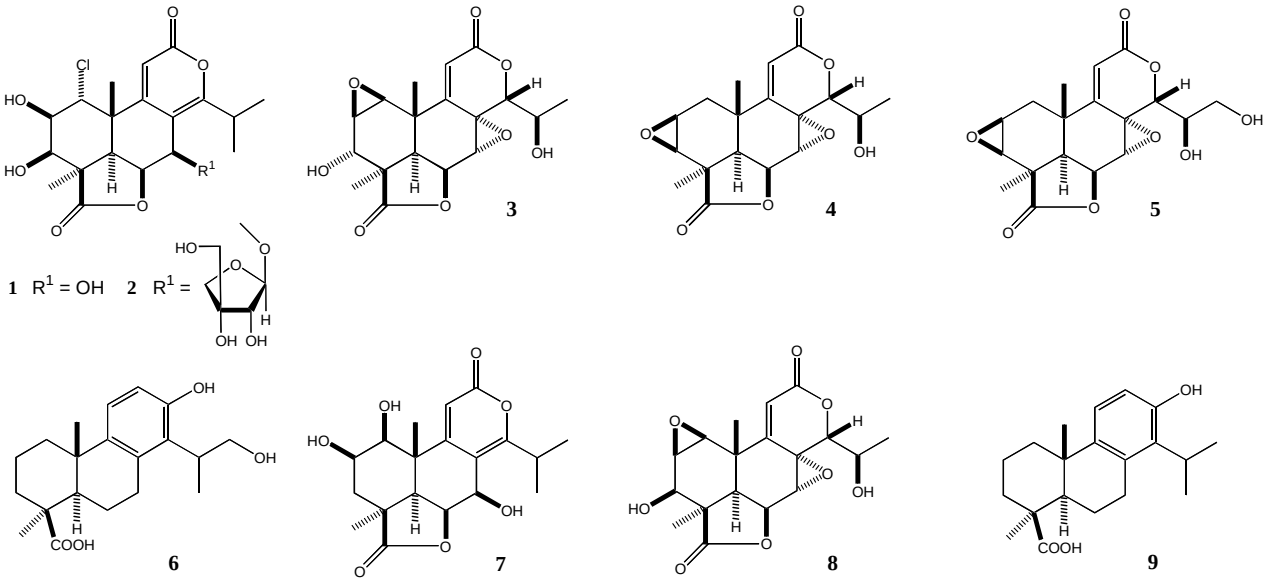


Figure 1.

RESULTS AND DISCUSSION

By repeated silica gel open column chromatography and preparative reversed phase HPLC as described in the EXPERIMENTAL, the MeOH extract (8 kg) of the leaves of *P. macrophyllus* var. *maki* (46 kg), gave compounds (**1**) (rakanmakilactone G, 20.3 mg), (**2**) (rakanmakilactone G-7β-*O*-β-D-apiofuranoside, 103.6 mg), (**3**) (rakanmakilactone H, 1.3 mg), (**4**) (rakanmakilactone I, 17.5 mg), **(5**) (rakanmakilactone J, 10.8 mg), and (**6**) (4β-carboxy-17-hydroxy-19-nor-totarol, 30.8 mg), along with known compounds (**7**) (nagilactone B, 30.2 mg), (**8**) (inumakilactone A, 8.7 mg), and (**9**) (4β-carboxy-19-nor-totarol, 50.2 mg). Identification of those known compounds (**7**-**9**) was made by comparing their physical and spectral data with those in literature (Figure 1).[9-13]

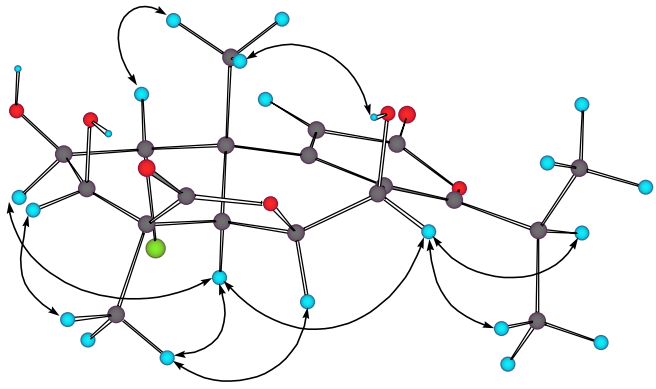


Figure 2. Selected NOE Correlations for **1**.

Compound (**1**) (rakanmakilactone G), obtained as colorless needles, had the molecular formula

$C_{19}H_{23}O_7Cl$ as determined by its HRFABMS (*m/z* 399.1209 $[M+H]^+$). FABMS of **1** also suggested the presence of a chlorine atom by the $[M+H]^+$ ion peak at *m/z* 399 (100 %) and chlorine isotope (^{37}Cl) peak at *m/z* 401(42 %). The characteristic IR spectral absorptions of subgroup A diterpene dilactones were observed at 1781 cm^{-1} (γ-lactone group), 1679, 1618, and 1537 cm^{-1} (α-pyrone group). The profiles of the NMR spectra were generally quite similar to those of nagilactone B (**7**), a 7β-hydroxylated subgroup A diterpene dilactone isolated also from this plant, implying that **1** and **7** had the same basic structure. The ^{13}C NMR spectrum of **1** displayed signals assignable to four methyls, eight methines, two quaternary carbons, and five sp^2 carbons. Two of the methyl signals at δ_C 19.8 and δ_C 20.9, correlating with the signals at δ_H 1.37 (3H, d, J = 6.8 Hz) and δ_H 1.24 (3H, d, J = 6.8 Hz), respectively, in the HMQC

Table 1. ^1H NMR (500 MHz) Spectral Data for (**1-9**) in pyridine-d_5 at 300 K.

position	**1**[a]	**7**[a]	**2**[a]	**3**[a]	**8**[a]
1a	5.14 (1H, d, 2.8)	4.33 (1H, d, 7.1)	5.10 (1H, d, 2.8)	3.68 (1H, d, 4.6)	3.68 (1H, d, 4.2)
1b					
2a	4.81 (1H, br d, 2.8)	4.29 (1H, m)	4.77 (1H, br d, 2.8)	3.58 (1H, dd, 2.8, 4.6)	3.56 (1H, dd, 4.2, 5.0)
2b					
3a	4.43 (1H, br s)	2.11 (1H, dd, 3.8, 13.4)	4.38 (1H, dd, 5.2, 10.9)		4.69 (1H, t, 5.0)
3b		2.74 (1H, t, 13.4)		4.73 (1H, dd, 2.8, 5.6)	
5	2.74 (1H, d, 4.9)	1.92 (1H, d, 6.8)	2.67 (1H, d, 4.7)	2.37 (1H, d, 5.0)	2.18 (1H, d, 5.1)
6	5.42 (1H, dd, 4.9, 8.9)	5.18 (1H, dd, 6.8, 8.4)	5.50 (1H, dd, 4.7, 9.2)	5.29 (1H, dd, 0.8, 5.0)	5.15 (1H, d, 5.1)
7	5.73 (1H, dd, 1.7, 8.9)	5.65 (1H, d, 8.4)	5.68 (1H, dd, 9.2)	5.17 (1H, d, 0.8)	5.18 (1H, s)
11	6.56 (1H, s)	7.02 (1H, s)	6.53 (1H, s)	6.87 (1H, s)	6.84 (1H, s)
14				4.78 (1H, d, 8.6)	4.79 (1H, d, 8.7)
15	3.54 (1H, sept, 6.8)	3.49 (1H, sept, 6.8)	3.41 (1H, sept, 6.8)	4.39 (1H, m)	4.39 (1H, m)
16	1.37 (3H, d, 6.8)	1.32 (3H, d, 6.8)	1.33 (1H, d, 6.8)	1.60 (3H, d, 5.7)	1.61 (3H, d, 5.9)
17	1.24 (3H, d, 6.8)	1.26 (3H, d, 6.8)	1.17 (1H, d, 6.8)		
18	1.76 (3H, s)	1.47 (3H, s)	1.68 (3H, s)	1.60 (3H, s)	1.42 (3H, s)
20	2.42 (3H, s)	2.00 (3H, s)	2.20 (3H, s)	1.10 (3H, s)	1.60 (3H, s)
OH	5.23 (1H, br s)		5.19 (1H, d, 10.9)	7.03 (1H, d, 5.7)	7.00 (1H, d, 5.9)
	7.97 (1H, br s)		6.33 (1H, br s)	8.27 (1H, d, 5.6)	7.53 (1H, d, 5.0)
	8.29 (1H, br s)		6.65 (1H, br t 5.6)		
			7.25 (1H, br d, 5.4)		
			8.29 (1H, br d, 5.7)		
1'			5.87 (1H, d, 2.0)		
2'			4.81 (1H, br d, 3.2)		
4'a			4.94 (1H, d, 9.3)		
b			4.46 (1H, d, 9.3)		
5'a			4.29 (1H, dd, 5.5, 11.4)		
b			4.24 (1H, dd, 5.5, 11.4)		

position	**4**[a]	**5**[a]	**6**[a]	**9**[a]
1a	1.75 (1H, d, 14.6)	1.73 (1H, d, 14.6)	1.44-1.48 (1H)	1.41-1.44 (1H)
1b	2.23 (1H, dd, 14.6, 1.8)	2.18 (1H, dd, 1.6, 14.6)	2.35-2.42 (1H)	2.35-2.42 (1H)
2a	3.39 (1H, m)	3.37 (1H, m)	1.66 (1H, m)	1.65 (1H, m)
2b			2.35-2.42 (1H)	2.35-2.42 (1H)
3a	3.29 (1H, d, 3.7)	3.28 (1H, d, 3.7)	1.14 (1H, ddd, 13.3, 13.3, 3.9)	1.13 (1H, ddd, 12.8, 12.4, 2.1)
3b			2.50-2.57 (1H)	2.52-2.56 (1H)
5	1.85 (1H, d, 4.6)	1.84 (1H, d, 4.5)	1.63 (1H, m)	1.63 (1H, m)
6a	5.20 (1H, d, 4.6)	5.23 (1H, s)	2.50-2.57 (1H)	2.52-2.56 (1H)
6b			2.35-2.42 (1H)	2.35-2.42 (1H)
7a	5.09 (1H, s)	5.22 (1H, d, 3.7)	2.83 (1H, m)	2.82 (1H, m)
7b			3.30 (1H, dd, 16.6, 4.8)	3.14 (1H, dd, 16.7, 3.4)
11	6.22 (1H, s)	6.17 (1H, s)	7.24 (1H, d, 8.6)	7.17 (1H, d, 8.5)
12			7.09 (1H, d, 8.6)	7.04 (1H, d, 8.5)
14	4.65 (1H, d, 8.7)	5.16 (1H, d, 9.0)		
15	4.34 (1H, m)	4.36 (1H, m)	3.73 (1H, br s)	3.51 (1H, br s)
16a	1.57 (3H, d, 6.2)	4.35 (1H, m)	1.80 (3H, d, 7.1)	1.64 (3H, d, 6.8)
16b		4.27 (1H, m)		
17			4.44 (2H, m)	1.72 (3H, d, 6.8)
18	1.43 (3H, s)	1.43 (3H, s)	1.48 (3H, s)	1.50 (3H, s)
20	1.43 (3H, s)	1.32 (3H, s)	1.12 (3H, s)	1.46 (3H, s)
OH	6.96 (1H, br d, 2.5)	6.72 (1H, br s)		
		7.23 (1H, d, 6.7)		

[a] Number of hydrogens, mulriplicity, and J values in Hz are given in parentheses.

spectrum, were assigned to the isopropyl group at the side chain. The other two methyl carbon signals at δ_C 23.0 and δ_C 25.1 correlating with the singlet methyl signals at δ_H 1.76 and δ_H 2.42, respectively, were assigned to C-18 and C-20 methyls, respectively. The major structural difference between **1** and **7**, suggested by the ^{1}H and ^{13}C NMR spectral data, was in the C-1/C-2/C-3 subunit. In **1**, two secondary hydroxyl groups were attached to C-2 and C-3, as demonstrated by its ^{1}H-^{1}H COSY and HMBC spectra. The chemical shift of H-1 of **1** was in the lower field than the corresponding signal of **7** by 0.5 ppm, demonstrating that the chlorine atom was located at C-1. The NOESY spectrum clearly indicated that the hydrogens at positions 5, 6, and 7 were *syn*, relative to each other, and the correlations observed between H-1/20-Me, H-2/H-5, H-3/H-5, H-3/18-Me, and 7-OH/20-Me suggested that the chlorine was of α-configuration, and the two hydroxy groups at C-2 and C-3 were of β-configuration. Accordingly, **1** was shown to have the structure given in Figure 1.

Compound (**2**) (rakanmakilactone G-7β-*O*-β-D-apiofuranoside) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{24}H_{31}O_{11}Cl$ by the $[M+H]^+$ ion peak at 531.1630 (*Calcd* for $C_{24}H_{32}O_{11}Cl$, 531.1633) in the HRESIMS. As in the case of **1**, ESIMS of **2** suggested the presence of a chlorine atom by the $[M+H]^+$ ion peak at *m/z* 531 (100 %) and chlorine isotope (^{37}Cl) peak at *m/z* 533 (20 %). ESIMS also suggested that **2** was a glycoside by the fragment ion peak $[MH-C_5H_{10}O_5]^+$ at *m/z* 381. The IR spectrum showed the absorptions of hydroxyl (3251 m^{-1}), γ-lactone (1768 m^{-1}), and α-pyrone groups (1695, 1625, and 1548 cm^{-1}) to show that it was a subgroup A diterpene dilactone. Its ^{1}H NMR spectrum showing the signals due to four methyls, and the ^{13}C NMR spectrum containing signals assignable to two lactone carbonyl carbons, and four olefinic carbons, also showed that **2** was a subgroup A norditerpene dilactone. The ^{1}H and ^{13}C NMR spectra of **2** were almost identical to those of **1**, except that **2** had the signals caused by the sugar moiety and that the chemical shifts of 20-Me (δ_H 2.20) in **2** showed an upfield shift compared with the corresponding methyl signal of **1** (δ_H 2.42). The NOESY correlations observed between H-1/20-Me, H-3/H-5, H-3/18-Me, and H-6/18-Me indicated that the chlorine was of α-configuration, and the hydroxy group at C-3 of β-configuration as in **1**, implying that **2** was a glycoside of **1**. Its ^{13}C NMR spectrum and the HMBC correlations between H-1'/C-7 demonstrated that the pentose unit (δ_C 111.3, 78.2, 80.8, 76.4, and 65.3) was connected to the C-7 oxygen atom. The sugar component was identified as D-apiose by acid hydrolysis of **2** followed by the HPLC analysis of the hydrolysate using an aminopropyl-bonded silica gel column column and an optical rotation detector. The ^{13}C NMR shifts of the anomeric carbon of the apiosyl at δ 111.3 indicated that the glycoside linkage was β.[14] The chemical shifts of ^{1}H NMR of the hydrolysed aglycone was exactly the same as those of **1**. Accordingly, **2** was determined to be rakanmakilactone G-glycoside with *O*-β-D-apiose moiety at C-7 of β-configuration as shown in Figure 1.

Compound (**3**) (rakanmakilactone H) was determined to have the molecular formula $C_{18}H_{20}O_8$ on the basis of the data derived from the ^{13}C NMR (18 carbons) and ^{1}H NMR (20 protons) spectra and the HRESIMS spectra (*m*/*z* 365.1252 $[M+H]^+$). The IR spectrum indicated the presence of γ-lactone carbonyl (1757 cm^{-1}), δ-lactone carbonyl (1693 cm^{-1}) and hydroxy groups (3583 cm^{-1}). The molecular formulae of **3** and **8** were the same and their carbon and proton NMR spectra were very similar to each other, implying that they had the same basic structure. The major difference between **3** and **8**, as demonstrated by the NMR spectral data, involved the stereochemistry at C-3 on the A ring. The methyl signal at δ_H 1.10 (20-Me) was correlated with the signal at δ_H 4.73 in the NOESY spectrum. So the signal at δ_H 4.73 was determined to be of β-H on C-3, and thus the hydroxyl group at C-3 to be α. The single crystal X-Ray crystallographic analysis confirmed that **3** had an α-hydroxyl group at C-3 and *R* configuration at C-15 (Figure 3). Thus, rakanmakilactone H (**3**) was shown to be 3-epiinumakilactone A (Figure 1).

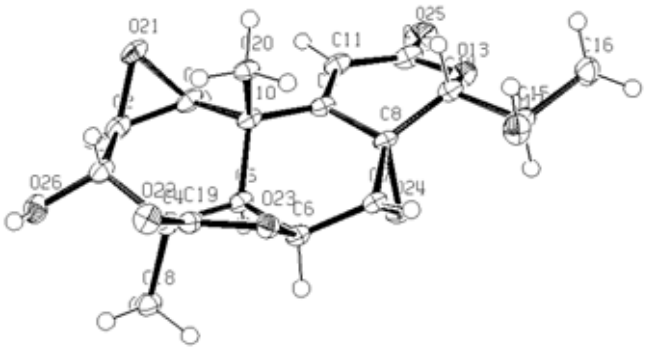


Figure 3. ORTEP representation of **3** as determined by single-crystal X-Ray

Table 2. ^{13}C NMR (125 MHz) Spectral Data for **1 - 9** in Pyridine-d_5 at 300K.

position	**1**	**7**	**2**	**3**	**8**	**4**	**5**	**6**	**9**
1	65.3	71.5	65.1	55.1	55.9	30.5	30.5	40.8	40.9
2	73.1	65.4	73.0	58.0	51.1	51.9	51.9	20.8	20.9
3	71.6	35.1	71.4	68.1	68.3	52.9	52.8	38.2	38.3
4	45.8	42.8	45.3	49.6	49.0	43.2	43.1	43.9	44.1
5	46.8	46.9	46.7	47.0	45.5	43.1	43.2	52.4	52.6
6	74.4	75.1	73.8	73.2	71.8	73.4	73.4	22.1	22.3
7	60.9	60.0	67.2	56.0	56.1	55.7	55.9	30.8	30.7
8	112.0	111.7	110.1	57.2	57.2	57.3	57.6	135.2	134.4
9	162.1	166.5	161.7	157.1	158.2	158.9	159.0	140.5	140.4
10	42.2	42.4	41.9	37.7	37.9	35.7	35.7	38.8	39.0
11	107.0	107.8	107.3	119.8	119.3	117.2	117.2	125.1	124.5
12	162.2	162.6	161.8	163.1	163.3	163.2	163.1	115.8	115.4
13								154.9	155.0
14	170.8	169.7	171.7	82.8	82.9	82.9	78.8	129.0	131.6
15	29.7	29.5	30.1	63.7	63.8	63.7	68.8	36.6	28.2
16	19.8	20.7	19.9	21.0	21.1	21.0	63.2	15.7	20.7
17	20.9	20.2	20.6					66.3	20.9
18	23.0	23.9	22.5	17.4	25.3	21.1	21.1	29.0	29.1
19	179.2	181.7	179.1	181.2	176.7	177.2	177.2	180.0	180.1
20	25.1	18.6	25.0	21.4	20.8	25.6	25.4	23.8	23.9
1'			111.3						
2'			78.2						
3'			80.8						
4'			76.4						
5'			65.3						

Compound (**4**) (rakanmakilactone I), obtained as colorless needles, had the molecular formula $C_{18}H_{20}O_7$ as determined by the high resolution ESIMS quasimolecular ion peak at *m/z* 349.1263 $[M+H]^+$. It was also a subgroup B diterpene, showing the IR absorptions of γ-lactone carbonyl and δ-lactone carbonyl at 1769 and 1693 cm^{-1}, respectively. The ^1H NMR spectrum showed γ-lactone ring protons on the A/B ring system (H-5 at δ_H 1.85 and H-6 at δ 5.20), and an olefinic proton (H-11 at δ_H 6.22) as in **3**. The multiplet proton signal at δ_H 4.34 (H-15) was assigned to the carbinyl hydrogen, which coupled to a doublet methyl signal (δ_H 1.57, d, *J* = 6.2) and a hydroxy proton (δ_H 6.96, br d, *J* = 2.5) as demonstrated in its ^1H-^1H COSY and HMBC spectra. These facts revealed that **4** had the same hydroxyl group at its side chain as in **3** and **8**. These spectra also demonstrated that **4** had a methylene carbon at C-1 (δ_C 30.5) and an epoxide group formed between C-2 (δ_C 51.9) and C-3 (δ_C 52.9). The NOESY correlation between H-2/H-5, H-3/H-5, and H-3/Me-18 suggested that these protons and the methyl group were all of α-configuration, and accordingly, the epoxide group between C-2 and C-3 was β-oriented. Correlations between 20-Me/H-7, 20-Me/H-14, and H-7/H-14 meant that the epoxide group of B-ring and the side chain at C-14 were both of α-configuration. The stereochemistry at C-15 was established to be *R* by X-Ray crystallography (Figure 4).

Compound (**5**) (rakanmakilactone J), obtained as colorless needles, was shown to have the molecular formula $C_{18}H_{20}O_8$ by HRESIMS. Analysis of the ^1H and ^{13}C NMR spectra of **5** and the comparison of these data with those of **4** implied that they had the same skeleton structure with an epoxide group between C-2 and C-3. Moreover, in the NOESY spectrum the methyl signal at δ_H 1.32 (20-Me) correlated with the signals at H-11, H-14, and H-7, and the other methyl signal at δ_H 1.43 (18-Me) correlated with the H-2, H-3 and H-5, thus revealing that the epoxide group of 2:3 was β-oriented, and that the side chain was α-oriented as in **4**. The difference between **4** and **5** was shown to be in the side chain structure, **5** having an additional hydroxy group at C-16. In the ^1H, ^{13}C, and DEPT spectra of **5** were signals

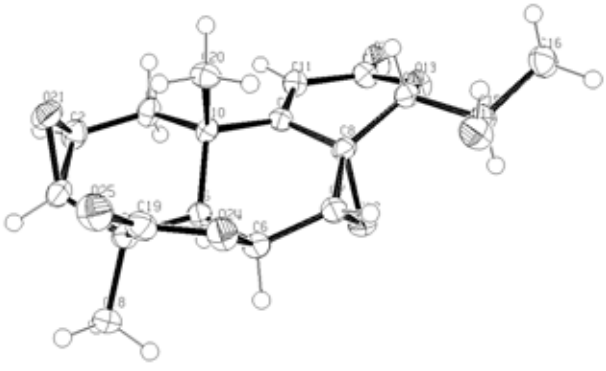


Figure 4. ORTEP representation of **4** as determined by single-crystal X-Ray

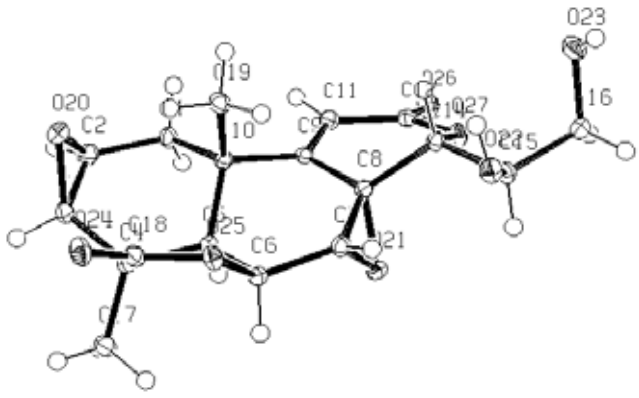


Figure 5. ORTEP representation of **5** as determined by single-crystal X-Ray

assignable to a methylene residue at δ_C 63.2 and δ_H 4.35 (1H, m) and 4.27 (1H, m), instead of the C-16 methyl signals in **4**. The ^{1}H-^{1}H COSY spectra showed that the hydroxy proton signals at δ_H 6.72 (1H, br s) and δ_H 7.23 (1H, d, J = 6.7 Hz) were coupled to H-16 and H-15, respectively, indicating that **5** had a primary hydroxyl group at C-16 and a secondary hydroxyl group at C-15. The configuration at C-15 was determined to be *R* by single-crystal X-Ray analysis (Figure 5).

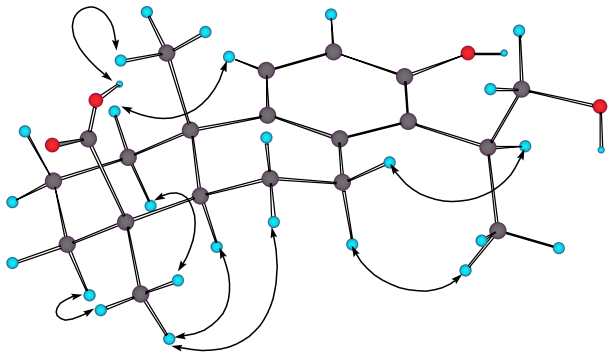


Figure 6. Selected NOE Correlations for **6**.

Compound (**6**) (4β-carboxy-17-hydroxy-19-nor-totarol) having the molecular formula $C_{20}H_{28}O_4$ as determined by HRFABMS (*m/z* 333.2041, $[M+H]^+$) exhibited the IR absorptions due to a hydroxyl group (3375 cm^{-1}), an aromatic ring (1589 and 1556 cm^{-1}), and a carbonyl group (1693 cm^{-1}), implying that it was a totarane diterpene. Its ^{13}C NMR spectrum was very similar to that of **9**, implying that they had the same basic structure, and showing the presence of three rings in the structure, one ring being aromatic, and one having a carboxyl group on it (δ 180. 0). The ^{1}H NMR spectrum of **6** showed signals of five methylenes, and a pair of aromatic protons (δ 7.24 and 7.09, each 1H, d, J = 8.6 Hz) as in that of **9** (δ 7.17 and 7.04, each 1H, d, J = 8.5 Hz). The difference was observed in the side chain. **6** did not give the NMR signals assignable to the isopropyl. Instead, it gave a signal of a carbon resonating at δ_C 66.3, with H-17 proton signals at a lower field (δ 4.44, 2H), showing that one of the methyl groups at C-15 in **9** was an oxygen-bearing methylene group in **6**. The combined analysis of the ^{1}H-^{1}H COSY, HMQC, and HMBC experiments showed that **6** had a totarane skeleton with a primary hydroxyl group at C-17.

Table 3. Cytotoxicity of several diterpenes against P-388 leukemia cells

Compound[a]	IC_{50} (μg/mL)
1	>100
2	>100
3	-
4	0.18
5	0.55
6	>100
7	1.9
8	0.23
9	18

[a] **3** could not be tested because of the small amount isolated.

All those diterpenes isolated in the present study, i.e., subgroup A dilactones (**1**, **2** and **7**), subgroup B dilactones (**4**, **5**, and **8**), and totarane diterpenes (**6** and **9**) were tested for their cytotoxic activities on P388 leukemia cells and the results are given in Tab. 3. Hayashi *et al*. suggested that the conjugated systems of C-ring in norditerpene dilactones should be the essential structural part to provide the molecule with a cytotoxic activity, since fully saturated compound was totally inactive, and that the dilactones with fewer polar substituents (hydroxyl or ester) had a stronger activity.[15] Our result supported their suggestion. The most potent of the present diterpenes, subgroup B dilactones (**4**, **5**, and **8**), gave the IC_{50} values of 0.18, 0.55, and 0.23 μg/mL, respectively. As regards subgroup A

dilactones, the chlorinated dilactones (**1**, **2**), were inactive, and **7** had only a moderate activity.

In the present study, from leaves of *Podocarpus macrophyllus* var. *maki*, we isolated six new compounds and determined their structures. Of them, **1** and **2** were shown to contain a chlorine atom in the molecule. A number of halogenated natural products have been obtained from marine organisms and fungi.[16] Occurrence of plant-origin chlorinated compounds are, however, very rare. From the family Podocarpaceae, only one chlorinated norditerpene has been reported.[8] **2** is a glycoside and is the first example of norditerpene dilactone glycoside whose sugar component is apiose. The glycoside is also unique in that the sugar is linked to C-7, and not to a side chain- or ring A as in other cases.[17-19] The results of the cytotoxic activity assay of these new compounds may add further data for the studies of the activity-structure relationship of nor-, bisnorditerpene dilactones.

EXPERIMENTAL

General Method

Melting points were determined on a Yanaco MP-3 apparatus and are recorded uncorrected. IR spectra were recorded on a JASCO FT/IR 620 spectrophotometer, and the optical rotations on a JASCO DIP-360 automatic digital polarimeter. The NMR spectra were recorded in C_5D_5N on a Brucker DRX-500 spectrometer at 300K. The chemical shifts (δ) of proton signals are given in ppm relative to the proton resonance of the residual C_5D_4HN at 7.21 ppm with the *J* values given in Hz, and those of carbon signals in ppm relative to the resonance at 135.5 ppm due to C_5D_5N. Mass spectra were recorded on a VG AutoSpec E and Micromass LCT (Manchester, UK) spectrometers. Preparative HPLC was carried out on a JASCO PU-986 equipped with a UV-970 UV detector (λ 220 nm) and a Inertsil PREP-ODS column (10 μm, 20 × 250 mm), by using a MeOH/H_2O or a MeCN/H_2O solvent system at a flow rate of 10 mL/min. The HPLC analysis of apiose was carried out using an optical rotation detector, SHODEX OR-2 (Showadenko Co. Ltd.). X-Ray single-crystal analysis was made on a Mac Science DIP diffractometer with Mo Kα radiation (λ = 0.71073 Å).

Plant Material

The leaves of *Podocarpus macrophyllus* D. Don var. *maki* Endl. were collected in Chiba, Japan, in October 2000. The botanical identification was made by one of the authors, K. Takeya, Professor of Plant Chemistry of Tokyo University of Pharmacy and Life Science. A voucher specimen has been deposited in the herbarium of Tokyo University of Pharmacy and Life Science. (00JCP09)

Extraction and Isolation

The air-dried leaves of *P. macrophyllus* (46 kg) were extracted with MeOH (180 L × 3) at 55°C for 12 h. The MeOH extract was concentrated to give a residue (8 kg), which was suspended in water and treated successively with hexane, $CHCl_3$, and *n*-BuOH to give a hexane-, a $CHCl_3$-, a *n*-BuOH soluble fractions.

The $CHCl_3$ soluble fraction gave, on removal of the solvent, 619.8 g of a residue, which was placed on a silica gel column (9.5 × 35 cm) and eluted sequentially with $CHCl_3$ (15 L), $CHCl_3$/MeOH (20:1, v/v, 25 L), $CHCl_3$/MeOH (7:3, v/v, 20 L) and MeOH (10 L). The $CHCl_3$/MeOH (20:1, v/v) eluate was concentrated, and the residue (200. 4 g) was subjected to Diaion HP-20 (1.4 kg) column chromatography using H_2O (10 L), H_2O/MeOH (1:1, v/v, 25 L), H_2O/MeOH (1:3, v/v, 25 L), MeOH (15 L) and acetone (5 L). The H_2O/MeOH (1:1, v/v) fraction (30.6 g) was chromatographed by using silica gel (600 g), and stepwise elution with $CHCl_3$/MeOH (20:1, 10 L, 10:1, 8 L, 5:1, 5 L, v/v) and finally with MeOH (3 L) to give fractions I-V. By ODS HPLC with H_2O-MeOH (75:15, v/v) and subsequent ODS HPLC using H_2O-MeOH (85:15, v/v), fraction I (9.0 g) gave compound (**4**) (17.5 mg). Fraction II (9.8 g) gave **3** (1.3 mg) by analogous ODS HPLC with H_2O-MeOH (73:27, v/v), and by further elution of the ODS HPLC column using H_2O-MeOH (87:13, v/v), it gave **1** (20.3 mg), **5** (10.8 mg), **7** (30.2 mg), and **8** (8.7 mg). The H_2O/MeOH (1:3, v/v) eluate of Diaion HP-20 column chromatography was also subjected to silica gel open chromatography using the same solvent system as for the H_2O/MeOH (1:1, v/v) eluate to give fractions A-H. By ODS HPLC eluting with H_2O-MeOH (50:50, v/v), fraction B gave **6** (30.8 mg), and **9** (50.2 mg). The n-BuOH soluble fraction was concentrated under reduced pressure, and the residue (1746.8 g) was subjected to a Diaion HP-20 column chromatography, eluting sequentially with H_2O (67 L), H_2O/MeOH (1:1, v/v, 74 L), H_2O/MeOH (1:3, v/v, 51.8 L), and MeOH (11.1 L). The H_2O/MeOH (1:1, v/v) fraction (658.6 g) was subjected to ODS MPLC using H_2O-MeOH (80:20, v/v) to give two fractions (1 and 2). Fraction 1 was subjected to preparative HPLC using H_2O-MeCN (88:12, v/v) to give **2** (103.6 mg).

X-Ray Single Crystallographic Analysis.

For X-Ray analysis, samples were recrystallized from EtOAc-MeOH. Crystallographic data for **3**, **4** and **5** reported in this paper have been deposited at the Cambridge Crystallographic Data Centre, under the reference numbers CCDC 220830, 220831, and 220832, respectively. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk)

Rakanmakilactone G (1) Colorless needles (H_2O-MeOH); mp 234-236 °C; $[\alpha]^{28}_D$ +146.0° (*c* 0.16, MeOH); HRFABMS *m/z* 399.1209 $[M+H]^+$ (*Calcd* for $C_{19}H_{24}O_7Cl$, 399.1211); IR (film) ν_{max} 3290, 1781, 1679, 1618, 1537cm^{-1}; ^1H and ^{13}C NMR spectral data given in Tables 1 and 2, respectively.

Rakanmakilactone G-7β-*O*-β-D-apiofuranoside (2) Colorless amorphous powder (H_2O-MeCN); mp 215-218 °C; $[\alpha]^{28}_D$ +62.5° (*c* 0.12, MeOH); HRFABMS *m/z* 531.1630 $[M+H]^+$ (*Calcd* for $C_{24}H_{32}O_{11}Cl$, 531.1633); IR (film) ν_{max} 3251, 1768, 1695, 1625, 1548 cm^{-1}; ^1H and ^{13}C NMR spectral data given in Tables 1 and 2, respectively.

Acid Hydrolysis of 2. A solution of **2** (4.8 mg) in 0.1 M HCl (1 mL) was heated at 100 °C for 0.5 h under an Ar atmosphere. After cooling, H_2O (5 mL) was added to the mixture, and the aqueous solution was extracted with $CHCl_3$ (3 × 5 mL). The $CHCl_3$ extract was washed with brine, dried over Na_2SO_4, and evaporated to give an aglycone fraction (1.8 mg). The aglycone was identified as **1** by the ^1H and ^{13}C NMR spectral analysis. The H_2O layer was passed through a short Amberlite IRA-400 column and evaporated to dryness to give a sugar fraction (1.2 mg). The sugar fraction was dissolved in MeOH/H_2O (2:8) and after passing through a Sep-Pak C_{18} cartridge, it was analyzed by HPLC using MeCN/H_2O (85:15). The sugar component was identified as D-apiose by the HPLC retention time, t_R 6.97 min (D-apiose^{20} t_R 7.01 min) and the sign (positive) of optical rotations.

Rakanmakilactone H (3) Colorless needles (H_2O-MeOH); mp 245-246 °C; $[\alpha]^{24}_D$ +15.8° (*c* 0.11, MeOH); HRESIMS *m/z* 365.1252 $[M+H]^+$ (*Calcd* for $C_{18}H_{21}O_8$, 365.1236); IR (film) ν_{max} 3583, 1757, 1693 cm^{-1}; ^1H and ^{13}C NMR data given in Tables 1 and 2, respectively.

*Crystal data for **3***. $C_{18}H_{20}O_8 \cdot CH_2O$ (EtOAc-MeOH); orthorhombic; space group $P2_12_12_1$; unit cell dimensions *a* = 6.3000 (2) Å, *b* = 12.8070 (12) Å, *c* = 21.885 (2) Å; *V* = 1765.8 (2) Å^3; *Z* = 4; *T* = 293 K; d_{cal} = 1.483 Mg m^{-3}; μ = 0.119 mm^{-1}, R(gt) = 0.0360.

Rakanmakilactone I (4) Colorless needles (H_2O-MeOH); mp 296-297 °C; $[\alpha]^{24}_D$ +11.6° (*c* 0.16, MeOH); HRESIMS *m/z* 349.1263 $[M+H]^+$ (*Calcd* for $C_{18}H_{21}O_7$, 349.1287); IR (film) ν_{max} 3407, 1769, 1693 cm^{-1}; ^1H and ^{13}C NMR spectral data given in Tables 1 and 2, respectively.

*Crystal data for **4***. $C_{18}H_{20}O_7$ (EtOAc-MeOH); orthorhombic; space group $P2_12_12_1$; unit cell dimensions *a* = 8.107 (3) Å, *b* = 10.979 (2) Å, *c* = 18.286 (6) Å; *V* = 1627.6 (8) Å^3; *Z* = 4; *T* = 100 K; d_{cal} = 1.422 Mg m^{-3}; μ = 0.110 mm^{-1}, R(gt) = 0.0455.

Rakanmakilactone J (5) Colorless needles (H_2O-MeOH); mp 292-293 °C; $[\alpha]^{24}_D$ +29.5° (*c* 0.15, MeOH); HRESIMS *m/z* 387.1067 $[M+Na]^+$ (*Calcd* for $C_{18}H_{20}O_8Na$, 387.1056); IR (film) ν_{max} 3584, 1769, 1694 cm^{-1}; ^1H and ^{13}C NMR spectral data given in Tables 1 and 2, respectively.

*Crystal data for **5***. $C_{18}H_{20}O_8 \cdot CH_4O$ (EtOAc-MeOH); monoclinic; space group $P2_1$; unit cell dimensions *a* = 6.4510 (5) Å, *b* = 12.769 (2) Å, *c* = 10.952 (2) Å; β = 103.410 (7)°; *V* = 877.6 (2) Å^3; *Z* = 2; *T* = 100 K; d_{cal} = 1.500 Mg m^{-3}; μ = 0.120 mm^{-1}, R(gt) = 0.0345.

4β-Carboxy-17-hydroxy-19-nor-totarol (6) Colorless amorphous powder (H_2O-MeOH); mp 109-110 °C; $[\alpha]^{24}_D$ +75.9° (*c* 0.12, MeOH); HRFABMS *m/z* 333.2041 $[M+H]^+$ (*Calcd* for $C_{20}H_{29}O_4$, 333.2066); IR (film) ν_{max} 3375, 1693, 1589, 1556 cm^{-1}; ^1H and ^{13}C NMR spectral data given in Tables 1 and 2, respectively.

Assay of Cytotoxic Activity.

Samples were assayed for their cytotoxic activity on P388 leukemia cells by the MTT method described previously.[8]

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