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Rakanmakilactones A–F, new cytotoxic sulfur-containing norditerpene dilactones from leaves of *Podocarpus macrophyllus* var. *maki*

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Abstract—Six new S-containing norditerpene dilactones, rakanmakilactones A–F (**5**–**10**), were isolated from the leaves of *Podocarpus macrophyllus* D. Don var. *maki* Endl., along with four known norditerpene dilactones (**1**–**4**). Their structures, stereochemistry and absolute configurations were determined by spectroscopic studies (HRMS, IR, ^{1}H, ^{13}C and 2D NMR), and single-crystal X-ray analyses. Rakanmakilactones were found to have a cytotoxic effect against P388 murine leukemia cells.

1. Introduction

Podocarpus macrophyllus var. *maki* of the family Podocarpaceae is distributed from Australia to the tropical and subtropical areas of eastern Asia.[1] From the plants of the genus *Podocarpus*, a number of nor- and bisnorditerpene dilactones have been isolated,[2–4] which have various biological activities, such as antitumor,[5] insecticidal,[6] antifeedant,[7] allelopathic,[8] and fungicidal activities.[9] In our previous paper, we reported the isolation, structure elucidation, and stereochemistry, two methylsulfoxide containg norditerpene dilactones, podolactone D (**2**) and S_R-podolactone D (**3**).[10] Although sulfur-containing diterpenes are reported in fetal calf serum,[11] only very few are known in higher plants. In the present study, we conducted more thorough survey of norditerpene dilactone fractions of this plant, and separated six new norditerpene dilactones. This paper describes isolation, structural elucidation, stereochemistry, and cytotoxic activities of those new compounds (**5**–**10**).

2. Results and discussion

The $CHCl_3$-soluble portion (619.8 g) derived from a MeOH extract (8 kg) of the leaves of *P. macrophyllus* var. *maki* (46 kg), prepared as described in Section 3, was subjected to silica gel column chromatography and then Diaion HP-20 resin chromatography. The 50% MeOH and 75% MeOH elutes of the Diaion chromatography gave, when further separated and purified by silica gel column chromatography and preparative HPLC with an ODS column, compounds **1**–**4**, **5** (5.8 mg), **6** (7.3 mg), **7** (30.6 mg), **8** (43.2 mg), **9** (50.7 mg), and **10** (20.3 mg). Compounds **1**–**4** were identified as known compounds podolactone C (680 mg),[12] podolactone D (98 mg),[13] S_R-podolactone D (110 mg),[14] and Hallactone B (10.8 mg),[15] respectively, by comparing their physical and spectral data with those in literature (Fig. 1).

Compound **5** (rakanmakilactone A), colorless needles, showed a hydroxyl absorption band at 3582 cm^{-1}, and a γ-lactone carbonyl and δ-unsaturated lactone carbonyl absorption bands at 1767 cm^{-1} and 1704 cm^{-1}, respectively, in its IR spectrum. The molecular formula was assigned to $C_{20}H_{24}O_8S$ on the basis of HRESIMS, which corresponded to the molecular formula of **2** plus one oxygen or to the molecular formula of **4** minus one oxygen. The ^{1}H NMR spectrum was very similar to that of the known compound hallactone B (**4**), both having four methyls (δ 1.13, 1.29, 2.07, and 3.30, all singlets) and two pairs of doublets at δ 3.82 (1H, d, J=15.2 Hz) and δ 4.55 (1H, d, J=15.2 Hz) indicated the presence of two diastereotopic protons on a sulfur-bearing carbon (C-17), suggesting that they had the same basic structures. The major difference between their ^{1}H NMR spectra was that in **5**, there were three olefinic methine protons (δ 5.79, 5.91, 6.22) whereas in **4** there was one. The ^{13}C NMR and HMBC spectra showed the presence of two lactone carbonyl carbons, and assigned the two lactone carbonyls to at C-12 (δ 162.8) and

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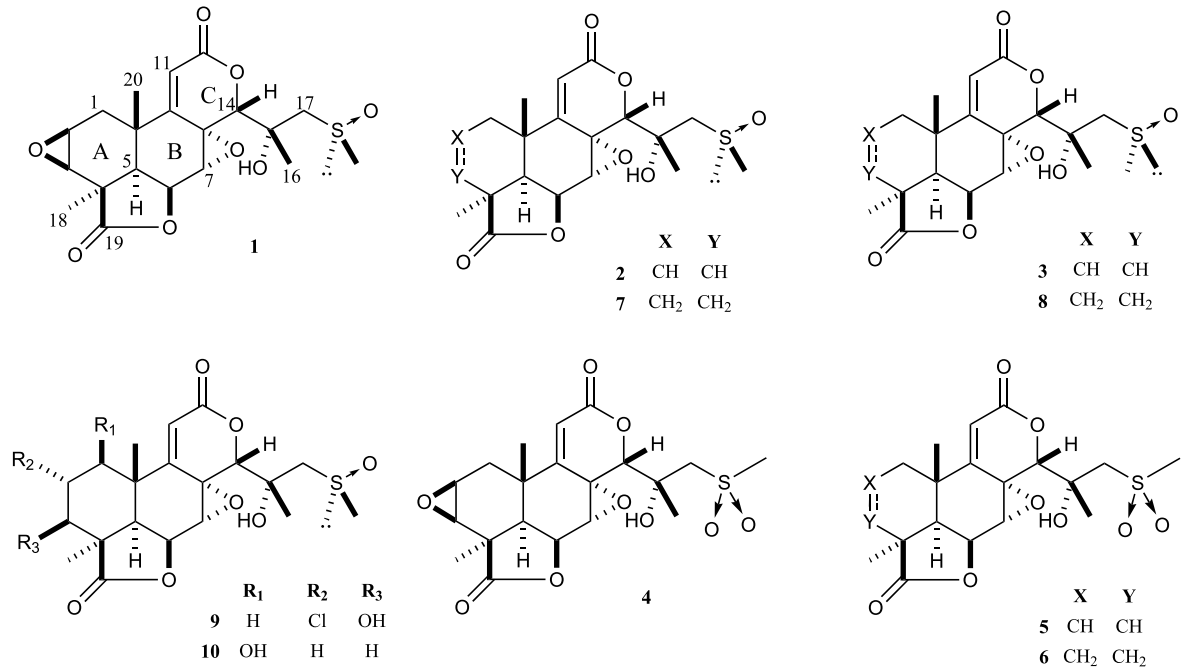


Figure 1. Structures of norditerpene dilactones.

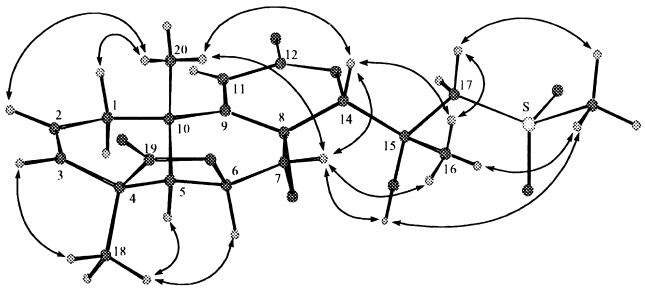


Figure 2. Selected NOESY correlations for **5**.

C-19 (δ 178.0), and assigned one of the olefinic carbon to C-11 (δ 117.6), and the other two to C-2 (δ 126.4) and C-3 (δ 128.4). The comparison of the NMR spectra of **5** with those of **2** and **4** showed that in **5** the chemical shifts of the methyl attached to sulfur in **5** (δ_C 44.4, δ_H 3.30) showed a significant downfield shift compared with the corresponding methylsulfoxide signals of **2**.[16,17] Thus, **5** was shown to the sulfur atom as sulfonyl in side chain. The stereochemistry of compound **5** was determined by the analysis of NOESY spectrum. The correlations were observed between H-7, 20-Me and H-14, between H-5 and 18-Me, H-6 and 18-Me, H-14 and 16-Me (Fig. 2). From these observations, **5** was determined to have the structure shown in Figure 1 (Table 1).

Compound **6** (rakanmakilactone B) was obtained as colorless needles. The molecular formula was assigned to $C_{20}H_{26}O_8S$ on the basis of the $[M+Na]^+$ ion that appeared at *m/z* 449.1272 in the HRESIMS. The IR spectrum showed absorbances at 3565, 1774 and 1729 cm^{-1} due to a hydroxyl and two lactone carbonyl moieties. The ^{13}C NMR spectrum showed 20 signals caused by four methyls including the one

Table 1. ^1H NMR (500 MHz) spectral data for compounds **5**–**10** in pyridine-d_5 at 300 K[a]

Position	5	6	7	8	9	10
1a	2.04 (1H, br s)	1.27–1.35 (1H)	1.24–1.34 (1H)	1.28–1.36 (1H)	1.97 (1H, d, 12.8)	4.07 (1H, dd, 6.1, 11.9)
1b	2.02 (1H, d, 5.9)	1.47–1.55 (1H)	1.46–1.56 (1H)	1.49–1.56 (1H)	2.52 (1H, dd, 4.5, 12.8)	
2a	5.79 (1H, m)	1.41–1.45 (1H, m)	1.39–1.43 (1H, m)	1.42–1.46 (1H, m)		1.84–1.92 (2H)
2b		1.47–1.55 (1H)	1.46–1.56 (1H)	1.49–1.56 (1H)	4.67 (1H, m)	
3a	5.91 (1H, dd, 1.4, 10.0)	1.27–1.35 (1H)	1.24–1.34 (1H)	1.28–1.36 (1H)	3.97 (1H, br t, 9.0)	1.50 (1H, m)
3b		2.17 (1H, m)	2.16 (1H, m)	2.17 (1H, m)		2.40 (1H, m)
5	2.06 (1H)	1.79 (1H, d, 4.5)	1.78 (1H, d, 4.5)	1.81 (1H, d, 4.5)	2.15 (1H, d, 4.1)	1.92 (1H, d, 4.6)
6	5.12 (1H, dd, 0.9, 4.9)	5.03 (1H, dd, 4.5, 1.1)	4.97 (1H, d, 4.5)	5.06 (1H, d, 4.5)	5.07 (1H, dd, 1.2, 4.1)	5.02 (1H, dd, 1.0, 4.6)
7	5.35 (1H, d, 0.9)	5.31 (1H, d, 1.1)	5.23 (1H, s)	5.28 (1H, s)	5.26 (1H, d, 1.2)	5.25 (1H, s)
11	6.22 (1H, s)	6.17 (1H, s)	6.17 (1H, s)	6.18 (1H, s)	6.45 (1H, s)	7.10 (1H, s)
14	4.88 (1H, s)	4.83 (1H, s)	4.88 (1H, s)	4.80 (1H, s)	4.93 (1H, s)	4.94 (1H, s)
16	2.07 (1H, s)	2.06 (3H, s)	1.86 (3H, s)	1.98 (3H, s)	1.86 (3H, s)	1.88 (3H, s)
17a	3.82 (1H, d, 15.2)	3.82 (1H, d, 15.2)	3.42 (1H, d, 13.7)	3.40 (1H, d, 13.7)	3.42 (1H, d, 13.7)	3.45 (1H, d, 13.7)
17b	4.55 (1H, d, 15.2)	4.56 (1H, d, 15.2)	3.79 (1H, d, 13.7)	3.93 (1H, d, 13.7)	3.79 (1H, d, 13.7)	3.81 (1H, d, 13.7)
18	1.29 (3H, s)	1.18 (3H, s)	1.16 (3H, s)	1.19 (3H, s)	1.68 (3H, s)	1.20 (3H, s)
20	1.13 (3H, s)	1.08 (3H, s)	1.09 (3H, s)	1.08 (3H, s)	1.29 (3H, s)	1.44 (3H, s)
SOMe			2.68 (3H, s)	2.71 (3H, s)	2.69 (3H, s)	2.69 (3H, s)
SO_2Me	3.30 (3H, s)	3.31 (3H, s)				

[a] Number of hydrogens, multiplicity, and *J* value in Hz are given in parentheses.

Table 2. ^{13}C NMR (125 MHz) spectral data for compounds **5**–**10** in pyridine-d_5 at 300 K

Position	5	6	7	8	9	10
1	32.5 (t)	29.6 (t)	29.6 (t)	29.5 (t)	40.7 (t)	69.1 (d)
2	126.4 (d)	17.7 (t)	17.7 (t)	17.7 (t)	61.4 (d)	29.4 (t)
3	128.4 (d)	28.6 (t)	28.6 (t)	28.6 (t)	79.2 (d)	28.2 (t)
4	44.2 (s)	41.1 (s)	41.9 (s)	42.0 (s)	47.1 (s)	42.0 (s)
5	42.8 (d)	43.2 (d)	43.4 (d)	43.3 (d)	45.0 (d)	43.8 (d)
6	72.3 (d)	72.8 (d)	72.8 (d)	72.8 (d)	72.2 (d)	72.8 (d)
7	56.0 (d)	56.1 (d)	55.9 (d)	56.0 (d)	55.9 (d)	56.1 (d)
8	58.3 (s)	58.6 (s)	58.7 (s)	58.6 (s)	58.9 (s)	59.1 (s)
9	158.3 (s)	159.4 (s)	159.8 (s)	159.5 (s)	157.7 (s)	157.8 (s)
10	35.7 (s)	36.3 (s)	36.3 (s)	36.2 (s)	38.3 (s)	41.7 (s)
11	117.6 (d)	117.1 (d)	116.9 (d)	117.0 (d)	117.3 (d)	119.9 (d)
12	162.8 (s)	163.0 (s)	163.2 (s)	163.2 (s)	163.0 (s)	163.6 (s)
14	83.7 (d)	83.8 (d)	83.2 (d)	83.8 (d)	83.2 (d)	83.2 (d)
15	72.2 (s)	72.1 (s)	73.4 (s)	72.4 (s)	73.4 (s)	73.3 (s)
16	26.7 (q)	26.7 (q)	27.7 (q)	28.2 (q)	27.6 (q)	27.8 (q)
17	59.5 (t)	59.4 (t)	61.8 (t)	63.0 (t)	61.9 (t)	62.0 (t)
18	22.5 (q)	23.9 (q)	24.0 (q)	23.9 (q)	22.2 (q)	23.7 (q)
19	178.0 (s)	180.5 (s)	180.4 (s)	180.5 (s)	176.4 (s)	180.5 (s)
20	22.7 (q)	24.4 (q)	24.4 (q)	24.4 (q)	23.5 (q)	16.9 (q)
$SOCH_3$			40.3 (q)	40.7 (q)	40.3 (q)	40.3 (q)
SO_2CH_3	44.4 (q)	44.4 (q)				

attached to a sulfonyl group (δ 44.4), four methylenes, five methines, seven quaternary carbons and two lactone carbonyls. Thus, showing that **6** was sulfur-containing norditerpene dilactone. The ^1H NMR spectrum of **6** was generally similar to that of **5**, the major difference being that **6** possessed three methylene protons, whereas **5** had only one. The $^1H-^1H$ COSY and HMBC spectra showed that the three multiplet methylene protons should be at C-1, C-2 and C-3. The NOESY spectrum of **6** showed correlations between H-7, 20-Me and H-14, between H-5, 18-Me and H-6, 18-Me and H-14, 16-ME as observed in **5**. Accordingly, **6** was determined to have the structure shown in Figure 1.

Compounds **7** (rakanmakilactone C) and **8** (rakanmakilactone D) were both obtained as colorless needles. The HRESIMS determined the molecular formulae of **7** and **8** to be $C_{20}H_{26}O_7S$ by the $[M+Na]^+$ ion peaks at *m/z* 433.1335 and *m/z* 433.1313, respectively (Calcd for $C_{20}H_{26}O_7SNa$, 433.1297). Their IR spectra had absorption peaks to be attributed to lactone carbonyl (1777 and 1779 cm^{-1}, respectively), α,β-unsaturated lactone carbonyl (1728, 1735 cm^{-1}, respectively) hydroxyl groups (3583, 3584 cm^{-1}, respectively). These ^1H NMR spectra showed the signals due to γ-lactone ring protons (H-5, H-6), four methylene protons (H-1, H-2, H-3, H-17), an olefinic proton (H-11), a carbinyl proton (H-14), three methyls (16-Me, 18-Me, 20-Me) and methylsulfoxide (side chain). The ^{13}C NMR spectra of these two compounds, together with the information from the DEPT studies, showed the presence of 20 carbons consisting of four methyls, four methylenes, five methines and seven nonprotonated carbons (Table 2). These 1D NMR data and the HMBC studies revealed that **7** and **8** were sulfur-containing norditerpene dilactones of basically the same structure with a methylsulfinyl group at C-14. Their 1D NMR data were almost identical to each other, except that the proton signal of H_b-17 of **8** was to lower than of **7** by 0.14 ppm. This is exactly the same situation that was observed between podolactone D (**2**) and S_R-podolactone D (**3**), which are the epimers at the sulfur atom; when the stereochemistry of the sulfur atom is *R*, the chemical shift of H_b-17 is known to shift to the downfield.[10,18] Accordingly, **7** and **8** were concluded to be epimers at the sulfur atom which was *S* configuration in **7** and *R* configuration in **8**. This conclusion about the structural relationship between **7** and **8** was verified, because when oxidized, they produced the same oxidation product **6** having a sulfonyl moiety on the side chain, at a yield of 85 and 84%, respectively.[19] The X-ray crystallographic analysis on single crystals of **7** and **8** finally proved that **7** had 15*R*,S_S and **8** had 15*R*,S_R configuration as shown by ORTEP representation in Figure 3 (Scheme 1).

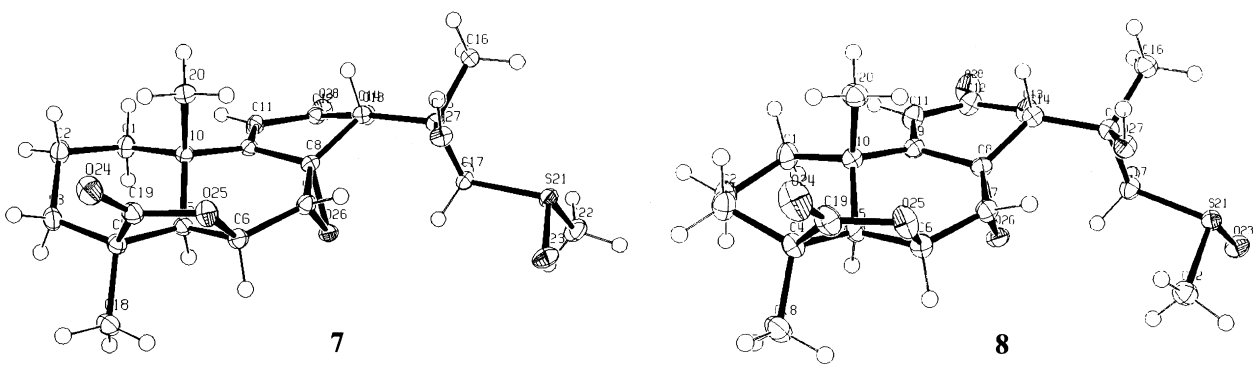
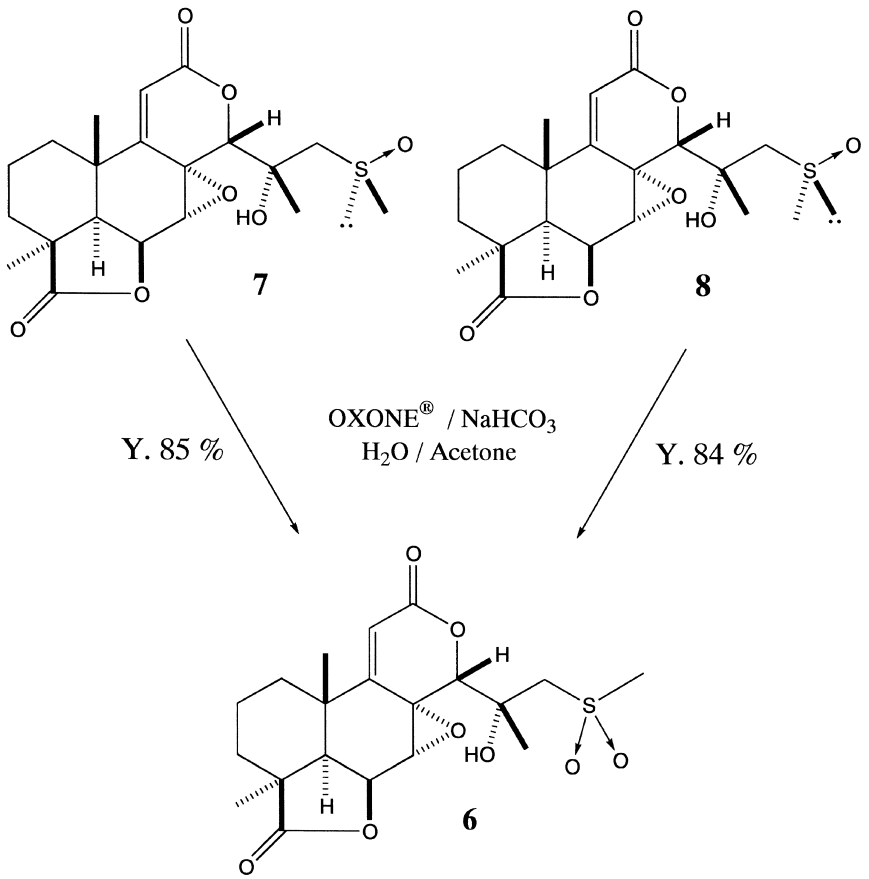


Figure 3. ORTEP representations of **7** and **8** as determined by single-crystal X-ray analysis.



Scheme 1. The oxidation of **7** and **8** to **6**.

Compound **9** (rakanmakilactone E) was isolated as colorless needles, and the IR spectrum indicated the presence of hydroxyl (3528 cm^{-1}) and lactone carbonyl groups (1767, 1725 cm^{-1}). Its elemental analysis showed the presence of a chlorine atom in the molecule (Anal. Calcd for $C_{20}H_{25}O_8ClS$, C, 52.12; H, 5.47; Cl, 7.69; S, 6.96. Found; C, 52.06; H, 5.53; Cl, 7.70; S, 7.31) and the FABMS determined its molecular formula to be $C_{20}H_{25}O_8ClS$ by the $[M+H]^+$ ion peak at *m/z* 461 with an isotope peak at *m/z* 463 (45%). The ^{13}C and ^1H NMR spectral showed the presence of a methylsulfinyl group at δ_C 40.3, δ_H 2.69 (3H, s), and three methine at δ_C 61.9, δ_H 3.42 (1H, d, 13.7) and 3.79 (1H, d, 13.7), δ_C 61.4, δ_H 4.67 (1H, m) and δ_C 79.2, δ_H 3.97 (1H, br t, 9.0) revealed that **9** was norditerpene dilactone with a chlorine atom and a hydroxy group on A ring system. Comparison of the HMBC spectra of **9** with those of the related methylsulfoxide compounds of this series (**1**, **2**, **3**, **7**, and **8**) revealed that **9** had the same basic ring structure and side chain as **1**, **2**, and **7**, and **9** was shown to be an analogue of **7** with substituents at C-2 and C-3. The $^1H-^1H$ COSY correlations observed between H-3 (δ 3.97) and hydroxyl proton (δ 7.40) and the NOESY correlations between 20-Me and H-2, and between H-3 and 18-Me and H-5, implied that **9** was concluded to be 2α-chlorine-3β-hydroxy-rakanmakilactone compound, which was verified by single-crystal X-ray analysis of **9** (Fig. 4). Known halogenated natural products are mostly from in marine organisms and fungi, and this is the first isolation of halogenated norditerpene dilactone from the family Podocarpaceae.

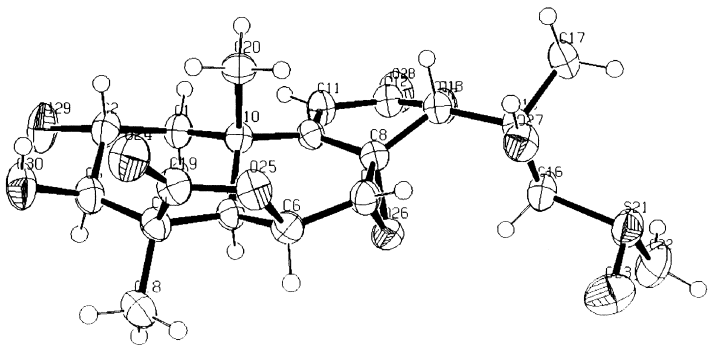


Figure 4. ORTEP representations of **9** as determined by single-crystal X-ray analysis.

Compound **10** (rakanmakilactone F) was obtained as colorless needles. The IR spectral absorptions at 3389, 1767 and 1703 cm^{-1} were assigned to a hydroxyl, a lactone carbonyl, and an α,β-unsaturated lactone carbonyl groups, respectively. Its molecular formula was determined to be $C_{20}H_{27}O_8S$ by the HRFABMS molecular ion peak at *m/z* 442.1426 $[M+H]^+$, which corresponded to those of **7** and **8** with an additional hydroxy group. The ^1H and ^{13}C NMR spectra implied that the basic structure of **10** was same as that of **7** with another hydroxyl group. The oxymethine proton signal at δ 4.07, giving an HMBC correlation with C-10 and C-20, and an NOE correlation with H-5 and Ha-3, was assigned to Ha-1. Thus, the structure of **10** was defined to have a α-hydroxyl group on H-1, as shown in Figure 5.

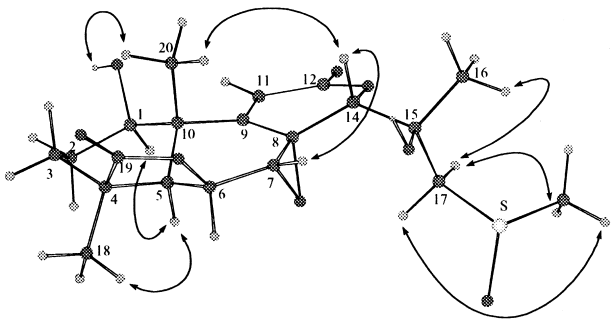


Figure 5. Selected NOESY correlations for **10**.

Many of the sulfur-containing norditerpene dilactones of this series possess a side chain with a methyl sulfinyl group (**1**, **2**, **3**, **7**, **8**, **9**, and **10**), thus producing a steric center at sulfur atom. The chemical shifts of H_a-17 and H_b-17 are summarized in Table 3. In a compound having a S_R configuration, the $\Delta_{(Hb\text{-}17\text{-}Ha\text{-}17)}$ is ca. 0.73–0.76 ppm. On the other hand, in that of compounds having S_S configuration is 0.51–0.58 ppm. This indicated the conformational difference between S_R- and S_S-type. Namely, though the intramolecular hydrogen bond in the compounds **1**, **2**, **7**, **9**, and **10** is possible to form, it may not be formed in **3** and **8** owing to 1,3-diaxial sterical effects between *S*-Me and 16-Me groups. This fact was also confirmed by comparison of single-crystal X-ray analysis data of the S_S compounds (**2**, **7**, and **9**) with those of S_R compounds (**3** and **8**).

The norditerpene dilactones have been reported to show cytotoxic activity against cultured Yoshida sarcoma cells.[5] This suggested that the biological activity of the podolactones is critically dependent on the olefinic system at 9:11, and requires the presence of a dienolide (7:8, 9:11) or the epoxy-enolide (7α:8α-9:11) moiety, and of the dilactones with less number of polar substituents (hydroxyl or ester) show a stronger activity. Compounds **1**–**3** and **5**–**10** showed a cytotoxic activity on P388 leukemia cells with IC_{50} values of 0.16, 0.23, 0.52, 0.31, 0.18, 0.29, 0.25, 5.0, and 4.3 μg/mL, respectively. The result demonstrated that the compounds having a hydroxyl group or a chlorine atom on A ring, had lower cytotoxicity as in **9** and **10**.

3. Experimental

3.1. General

Optical rotations were measured on a JASCO DIP-360 automatic digital polarimeter, IR spectra on a JASCO FT/IR 620 spectrophotometer, and Mass spectra were acquired with VG AutoSpec E and Micromass LCT (Manchester, UK) spectrometers. NMR spectra were obtained on a Brucker DRX-500 spectrometer at 300 K in C_5D_5N. The chemical shifts (δ) of proton signals are given in ppm relative to the resonances of residual C_5D_4HN at 7.21 ppm and those of carbon signals are given in ppm relative to the resonances at 135.5 ppm for C_5D_5N. Elemental analysis was carried out by using an Elemental Vario EL (Hanau, Germany) and a Mettler DL70ES elemental analyzer. Silica gel (Merck Kiesel gel 60, 70–230 μm, Kanto silica gel N 60, 63–210 μm) and Diaion® HP-20 (Mitsubishi Chemical) were used for column chromatography and precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck), RP-18 $F_{254}S$ (0.25 mm thick, Merck) plates for TLC, in which the spots were visualized by spraying of 10% H_2SO_4 solution, followed by heating. Preparative HPLC was carried out on a JASCO PU-986 equipped with a UV-970 UV detector (λ 220 nm) and Inertsil PREP-ODS column (10 μm, 20×250 mm), by using MeOH/H_2O or MeCN/H_2O at a flow rate of 10 mL/min. X-ray single-crystal analysis was taken on a Mac Science DIP diffractometer with Mo Kα radiation (λ=0.71073 Å).

3.2. 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 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).

3.2.1. Extraction and isolation. The air-dried leaves of *P. macrophyllus* var. *maki* (46 kg) were extracted with hot MeOH (180 L×3). Evaporation of the solvent yielded 8 kg of a dark green residue, which was suspended in water (8 L)

Table 3. Chemical shifts (ppm) in $CDCl_3$ for S_S (**1**, **2**, **7**, **9**, **10**) and S_R (**3**, **8**)

S_S-type				S_R-type			
Compound	H_b-17	H_a-17	Δ_{Hb-Ha}	Compound	H_b-17	H_a-17	Δ_{Hb-Ha}
2	3.53	3.02	0.51	**3**	3.63	2.90	0.73
7	3.53	3.00	0.53	**8**	3.66	2.90	0.76
1	3.51	2.99	0.52				
9	3.51	3.00	0.51				
10	3.53	3.00	0.53				

and then treated successively with hexane and $CHCl_3$ (each 8 L). The $CHCl_3$ layer was evaporated in vacuo to give a residue (619.8 g), 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 dried and the residue (200. 4 g) was subjected to Diaion HP-20 (1.4 kg) column chromatography eluting with 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) to give fractions 1–5, respectively. After removal of solvent, fraction 2 (30.6 g) was chromatographed on silica gel (600 g) eluting stepwise with a serial $CHCl_3$/MeOH mixture (20:1, 10 L; 10:1, 8 L; 5:1, 5 L; 0:1, 3 L) to give fractions I–V with the crystal of **1** (680 mg). Fraction II (9.8 g) was subjected to ODS HPLC eluting with H_2O–MeOH (73:27, v/v) and finally to preparative HPLC using H_2O–MeOH (87:13, v/v) to give **2** (98 mg), **3** (110 mg), **9** (50.7 mg) and **10** (20.3 mg). Fraction 3 gave fractions A–H when subjected to silica gel open chromatography using the same solvent system as in the case of fractions II. Fraction B was further subjected to ODS HPLC eluting with H_2O–MeCN (85:15, 76:24, v/v) to give **4** (10.8 mg), **7** (30.6 mg), and **8** (43.2 mg). Fraction H gave, when subjected to ODS HPLC using the two eluting systems H_2O–MeCN (75:25, v/v) and H_2O–MeOH (70:30, v/v), **5** (5.8 mg) and **6** (7.3 mg).

3.2.2. Rakanmakilactone A (5). Colorless needles (EtOAc–MeOH); mp 274–276 °C; $[\alpha]_D^{24}$=+13.2° (*c* 0.12, MeOH); HRESIMS *m/z* 447.1053 $[M+Na]^+$ (Calcd for $C_{20}H_{24}O_8SNa$, 447.1090); IR (film) ν_{max} 3582, 1767, 1704 cm^{-1}; ^1H, ^{13}C NMR data are given in Tables 1 and 2.

3.2.3. Rakanmakilactone B (6). Colorless needles (EtOAc–MeOH); mp 246–249 °C; $[\alpha]_D^{24}$=+13.4° (*c* 0.11, MeOH); HRESIMS *m/z* 449.1272 $[M+Na]^+$ (Calcd for $C_{20}H_{26}O_8SNa$, 449.1246); IR (film) ν_{max} 3565, 1774, 1729 cm^{-1}; ^1H, ^{13}C NMR data are given in Tables 1 and 2.

3.2.4. Rakanmakilactone C (7). Colorless needles (EtOAc–MeOH); mp 237–240 °C; $[\alpha]_D^{24}$=+61.1° (*c* 0.14, MeOH); HRESIMS *m/z* 433.1335 $[M+Na]^+$ (Calcd for $C_{20}H_{26}O_7SNa$, 433.1297); IR (film) ν_{max} 3583, 1777, 1728, 1590 cm^{-1}; ^1H, ^{13}C NMR data are given in Tables 1 and 2.

Crystal data for **7**. $C_{20}H_{26}O_7S$; orthorhombic; space group $P2_12_12_1$; unit cell dimensions *a*=7.6780(10) Å, *b*=11.0708(4) Å, *c*=22.014(6) Å; *V*=1978.93(9) $Å^3$; *Z*=4; *T*=100 K; d_{cal}=1.378 Mg m^{-3}; μ=0.203 mm^{-1}, *R*(gt)=0.0266, CCDC 216469.

3.2.5. Rakanmakilactone D (8). Colorless needles (EtOAc–MeOH); mp 253–255 °C; $[\alpha]_D^{24}$=+19.4° (*c* 0.20, MeOH); HRESIMS *m/z* 433.1313 $[M+Na]^+$ (Calcd for $C_{20}H_{26}O_7SNa$, 433.1297) IR (film) ν_{max} 3584, 1779, 1735, 1650, 1556 cm^{-1}; ^1H, ^{13}C NMR data are given in Tables 1 and 2.

Crystal data for **8**. $C_{20}H_{26}O_7S$; orthorhombic; space group $P2_12_12_1$; unit cell dimensions *a*=6.554(10) Å, *b*=13.646(4) Å, *c*=22.343(6) Å; *V*=1998.27(12) $Å^3$; *Z*=4; *T*=100 K; d_{cal}=1.364 Mg m^{-3}; μ=0.201 mm^{-1}, *R*(gt)=0.0310, CCDC 216470.

3.2.6. Oxidation of 7 and 8 to 6. Compound **7** (6.0 mg, 0.02 mmol) and sodium bicarbonate (28.0 mg, 0.33 mmol) were dissolved in a mixture of acetone (1 mL) and water (0.8 mL). To this solution was added OXONE® (51 mg) at 0 °C. After stirring at room temperature for 0.5 h, the mixture was treated with H_2O (10 mL) and the whole was extracted with $CHCl_3$ (10 mL, three times). The $CHCl_3$ solution was dried over $MgSO_4$, and evaporated in vacuo to give a residue, which was purified by ODS HPLC (H_2O–MeOH, 70:30, v/v) to afford **6** (5.3 mg, 85%). When **8** (5.1 mg, 0.01 mmol) was treated in the same way as described above, it gave **6** in 84% yield (4.5 mg).

3.2.7. Rakanmakilactone E (9). Colorless needles (EtOAc–MeOH); mp 285–287 °C $[\alpha]_D^{28}$=+11.1° (*c* 0.10, MeOH); FABMS *m/z* 461 $[M+H]^+$(100), 463 $[M+2+H]^+$(45). Anal. Calcd for $C_{20}H_{25}O_8ClS$, C, 52.12; H, 5.47; Cl, 7.69; S, 6.96. Found; C, 52.06; H, 5.53; Cl, 7.70; S, 7.31; IR (film) ν_{max} 3528, 1767, 1725 cm^{-1}; ^1H, ^{13}C NMR data are given in Tables 1 and 2.

Crystal data for **9**. $C_{20}H_{25}O_8ClS$; orthorhombic; space group $P2_12_12_1$; unit cell dimensions *a*=7.842(10) Å, *b*=12.8190(4) Å, *c*=21.9030(6) Å; *V*=2201.83(12) $Å^3$; *Z*=4; *T*=296 K; d_{cal}=1.390 Mg m^{-3}; μ=0.312 mm^{-1}, *R*(gt)=0.0352, CCDC 216471.

3.2.8. Rakanmakilactone F (10). Colorless needles (EtOAc–MeOH); mp 270–272 °C $[\alpha]_D^{28}$=+33.0° (*c* 0.12, MeOH); HRFABMS *m/z* 427.1426 $[M+H]^+$ (Calcd for $C_{20}H_{27}O_8S$, 427.1427); IR (film) ν_{max} 3389, 1767, 1703 cm^{-1}; ^1H, ^{13}C NMR data, see Tables 1 and 2.

3.3. Assay for cytotoxic activity

The cytotoxic assays were performed by using the MTT assay method. The murine P388 leukemia cells were cultured in RPMI 1640 medium (Nissui) supplemented with 5% heat-inactivated fetal bovine serum (FBS) and kanamycin (5.3 mL/L) in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The 100 μL of cell suspension was added to each well (3×10^3 cells/well) of a 96-microwell plate (Iwaki, flat bottom, treated polystyrene) and incubated for 24 h. Test compounds were dissolved in DMSO in various concentrations (100, 30, 10, 3, 1, 0.3, 0.1 μg/mL) and 10 μL of the test solution or DMSO (control) was added to each well. The plate was kept in an incubator for 48 h. After termination of cell culture by adding 20 μL MTT (5% in PBS) to each well, the plate was further incubated for 4 h. To each well was added 100 μL of 10% SDS–0.01N HCl. The plate was read on a microplate reader (MPR A4i, Tosoh) at 550 nm. A dose-response curve was plotted for each compound, and the concentrations giving 50% inhibition of the cell growth (IC_{50}) were recorded.

3.4. X-ray single crystallographic analysis

Crystallographic data for **7**, **8** and **9** reported in this paper have been deposited at the Cambridge Crystallographic Data Centre, under the reference numbers CCDC 216469, 216470, and 216471, 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).

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