Cytotoxic Bisnor- and Norditerpene Dilactones Having 7α,8α-Epoxy-9,11-enolide Substructure from *Podocarpus macrophyllus* D. Don

Kimihiko Sato,[a] Yasunori Inaba,[a] Hyun-Sun Park,[b] Toshiyuki Akiyama,[b] Tetsuo Koyama,[b] Haruhiko Fukaya,[a] Yutaka Aoyagi,[a] and Koichi Takeya*,[a]

[a] *School of Pharmacy, Tokyo University of Pharmacy & Life Science; 1432–1 Horinouchi, Hachioji, Tokyo 192–0392, Japan: and* [b] *The Kochi Prefectural Makino Botanical Garden; 4200–6 Godaisan, Kochi, Kochi 781–8125, Japan.*
Received January 5, 2009; accepted April 4, 2009; published online April 17, 2009

Fourteen new bisnor- and norditerpene dilactones, makilactones E—R, having a 7α:8α-epoxy-9,11-enolide substructure, were isolated from a methanolic extract of the root and the bark of *Podocarpus macrophyllus* D. Don (Podocarpaceae) with thirteen known bisnor- and norditerpenoids, and the structures of those new bisnor- and norditerpenoids were determined on the basis of their spectroscopic studies. Of the thirteen known ones isolated, the structures of two, *i.e.*, podolactone B and inumakilactone B, were revised on the basis of X-ray crystallographic analysis and spectroscopic analysis. Many of the compounds of this type isolated in this study showed potent cytotoxic activities against P388 murine leukemia cells.

Key words *Podocarpus macrophyllus*; norditerpene dilactone; cytotoxic activity; 7α,8α-epoxy-8,11-enolide substructure

Podocarpus macrophyllus D. Don (Podocarpaceae) (Japanese name: *Inumaki*) is a dioecious evergreen tree distributed in the subtropical areas of south eastern China, Taiwan, and Japan. From this plant, flavonoids, bisflavonoids, norditerpenoids, and ecdysons have been obtained.[1—4] In our recent study, several bisnor- and norditerpenoids having potent cytotoxic activities against P388 murine leukemia cells were isolated and their structures were determined.[5—7] In the present study, from the root and bark of this plant, we isolated fourteen new bisnor- and norditerpenoides, makilactones E—R, along with thirteen known ones. This paper describes the isolation and structural elucidation of those new bisnor- and norditerpenoides, revision of the structures of two of the thirteen known ones isolated and their cytotoxic activities.

Results and Discussion

Dried root of *Podocarpus macrophyllus* D. Don (22.66 kg) was extracted with MeOH (45 l×3) at room temperature. The combined MeOH extract was concentrated and the residue (614 g) was subjected to Diaion® HP-20 resin column chromatography (10 cm×40 cm) eluting sequentially with H_2O/MeOH (1 : 0, 1 : 1, 1 : 4, 0 : 1) and acetone (each 5 l). By silica gel column chromatography ($CHCl_3$–MeOH) and subsequent repeated reversed-phase HPLC (MeCN–H_2O, MeOH–H_2O), the fraction eluted with H_2O/MeOH (1 : 1) (71.6 g) gave ten new bisnor- and norditerpenoids, makilactones E [(**1**) 1.8 mg], F [(**2**) 13.0 mg], G [(**3**) 95.0 mg], J [(**6**) 9.6 mg], K [(**7**) 13.0 mg], L [(**8**) 6.0 mg], M [(**9**) 2.0 mg], O [(**11**) 6.0 mg], P [(**12**) 1.2 mg] and Q [(**13**) 64.0 mg] and four known bisnor- and norditerpenoides, podolactone B[9] [(**15**) 23.0 mg], rakanmakilactone H[7] [(**20**) 12.0 mg], inumakilactone A[7] [(**21**) 3.5 mg] and inumakilactone A 15-*O*-β-D-glucoside[2] [(**23**) 6.8 g]. By analogous chromatographic separation, the fraction eluted with H_2O/MeOH (1 : 4) (82.4 g) gave makilactones H [(**4**) 3.3 mg], I [(**5**) 11.0 mg], N [(**10**) 3.9 mg] and R [(**14**) 15.6 mg] along with known ones, inumakilactone B[10] [(**16**) 24.0 mg], 3-deoxy-2α-hydroxynagilactone E[11] [(**18**) 62.0 mg], nagilactone E[11] [(**19**) 46.0 mg], salignone M[12] [(**22**) 9.4 mg], podolactone D[5] [(**24**) 6.5 mg] and rakanmakilactone C[6] [(**27**) 4.0 mg].

By the procedures described above, a MeOH extract of the bark of this plant gave three known norditerpenoids, nagilactone G[8] [(**17**) 7.0 mg], rakanmakilactone D[6] [(**25**) 11.8 mg] and sellowin B[13] [(**26**) 1.3 mg].

Makilactone E (**1**) was isolated as colorless needles, mp 283 °C. The molecular formula was determined to be $C_{18}H_{21}O_8Cl$ from the $[M+Na]^+$ ion peak at *m*/*z* 423.0815 in HR-electrospray ionization (ESI)-MS. IR absorption bands for hydroxyl and, γ-lactone and δ-lactone carbonyl groups were observed at 3437 cm^{-1}, 1765 cm^{-1} and 1715 cm^{-1}, respectively. As seen in Table 2, the ^{13}C-NMR spectrum of **1** was very similar to that of nagilactone G (**17**), a known norditerpenoid also isolated from the bark of this plant, excepting for the signals due to the C-14 side chain and C-1, C-2, and C-3 of ring A substructures. Two of the three methyl carbon signals at δ_C 22.3 and δ_C 26.1 correlating with the singlet proton signals at δ_H 1.64 and 2.00, respectively, in the heteronuclear multiple quantum correlation (HMQC) spectrum, were assigned to C-18 and C-20, respectively. The other methyl carbon signal (δ_C 21.0) correlating with the doublet methyl signal at δ_H 1.57 was assigned to C-17 of the side chain in the HMQC spectrum. The proton signal of Me-20 (δ_H 2.00) correlated with C-1 (δ_C 63.6) and that of Me-18 (δ_H 1.64) with C-3 (δ_C 71.4) in heteronuclear multiple bond connectivity (HMBC). In the nuclear Overhauser effect spectroscopy (NOESY) spectrum, the proton signal of Me-20 (δ_H 2.00) correlated with H-7 (δ_H 5.18). These observations showed that when C-14 side chain is removed, **1** had the same carbon skeleton structure as **17**[8] (hereafter, this basic carbon skeleton structure is referred to as "desisopropylnagilactone G unit"). The ^{13}C-NMR spectrum showed that none of C-1, C-2 and C-3 was methylene carbon. In the correlation spectroscopy (COSY) spectrum, the proton signals H-2 (δ_H 4.69) and H-3 (δ_H 4.35) correlated with the hydroxyl group protons OH-2 (δ_H 8.30) and OH-3 (δ_H 5.48), respectively, and the proton signal at δ_H 4.39 assigned to H-15 correlated with a hydroxyl group at δ_H 7.01. In the NOESY spectrum, the proton signal H-1 (δ_H 4.97) correlated with Me-20 (δ_H 2.00), and those of H-2 (δ_H 4.69) and H-3 (δ_H 4.35) both

* To whom correspondence should be addressed. e-mail: takeyak@toyaku.ac.jp

Table 1. ^{1}H-NMR Data of New (**1**—**14**) and Known (**15**—**17**) Bisnor- and Norditerpenoids

Position	Nagilactone G (**17**)[a]	Makilactone E (**1**)[a]	Makilactone F (**2**)[b]	Makilactone G (**3**)[b]	Makilactone H (**4**)[b]	Makilactone I (**5**)[b]
1	1.49 (1H, m)	4.97 (1H, d, 3.0)	4.03 (1H, dd, 8.6, 2.8)	4.68 (1H, br m)	4.07 (1H, br m)	4.06 (1H, m, overlapped)
	1.26 (1H, m)					
2	1.51 (1H, m)	4.69 (1H, br m)	4.32 (1H, m, overlapped)	4.64 (1H, br m)	1.89 (1H, m, overlapped)	1.89 (1H, m)
	1.40 (1H, m)				1.89 (1H, m, overlapped)	1.86 (1H, m)
3	2.15 (1H, ddd, 14.3, 6.1, 6.1)	4.35 (1H, m)	4.02 (1H, m, overlapped)	4.43 (1H, m)	1.50 (1H, m, overlapped)	1.50 (1H, m)
	1.31 (1H, dd, 14.3, 6.6)				2.40 (1H, ddd, 14.7, 6.2, 6.2)	2.41 (1H, ddd, 14.8, 6.4, 6.4)
5	1.79 (1H, d, 4.6)	2.75 (1H, d, 4.2)	2.19 (1H, d, 4.2)	2.85 (1H, d, 4.1)	1.94 (1H, d, 4.5)	1.92 (1H, d, 4.6)
6	5.09 (1H, br d)	5.34 (1H, dd, 4.2, 1.2)	5.27 (1H, dd, 4.2, 0.9)	5.32 (1H, d, 4.1)	5.15 (1H, d, 4.5)	5.13 (1H, d, 4.6)
7	4.22 (1H, s)	5.18 (1H, d, 1.3)	5.13 (1H, d, 0.9)	5.19 (1H, s)	5.10 (1H, s)	4.39 (1H, s)
11	6.14 (1H, s)	6.62 (1H, s)	7.23 (1H, s)	6.77 (1H, s)	7.10 (1H, s)	7.03 (1H, s)
14	4.56 (1H, d, 3.4)	4.75 (1H, d, 8.5)	4.72 (1H, d, 8.7)	4.76 (1H, d, 8.8)	4.70 (1H, d, 8.8)	4.90 (1H, d, 6.0)
15	1.96 (1H, m)	4.39 (1H, m)	4.37 (1H, m)	4.39 (1H, m)	4.35 (1H, br m)	2.27 (1H, m)
16	1.16 (3H, d, 6.8)					1.32 (3H, d, 6.8)
17	1.01 (3H, d, 6.8)	1.57 (3H, d, 6.2)	1.58 (3H, d, 6.0)	1.58 (3H, d, 6.0)	1.57 (3H, d, 6.3)	4.08 (1H, br m)
						4.28 (1H, br d)
18	1.15 (3H, s)	1.64 (3H, s)	1.68 (3H, s)	1.62 (3H, s)	1.19 (3H, s)	1.20 (3H, s)
19						
20	1.07 (3H, s)	2.00 (3H, s)	1.62 (3H, s)	1.94 (3H, s)	1.47 (3H, s)	1.44 (3H, s)
SOMe						
OH-1			7.09 (1H, br d)	7.28 (1H, br d)	6.74 (1H, br d)	6.73 (1H, br d)
OH-2		8.30 (1H, br d)	7.58 (1H, br s)	7.38 (1H, br d)		
OH-3		5.48 (1H, d, 8.7)	6.61 (1H, br d)	5.52 (1H, d, 10.1)		
OH-15		7.01 (1H, d, 5.8)	6.95 (1H, d, 5.5)	6.97 (1H, d, 5.5)	6.94 (1H, br d)	
OH-17						6.14 (1H, br s)

a) ^{1}H (600 MHz) NMR data measured in pyridine-d_5. *b*) ^{1}H (500 MHz) NMR data measured in pyridine-d_5.

Table 1. (Continued)

Position	Makilactone J (**6**)[b]	Makilactone K (**7**)[b]	Makilactone L (**8**)[b]	Makilactone M (**9**)[b]	Makilactone N (**10**)[b]	Makilactone O (**11**)[b]
1	4.10 (1H, m)	4.10 (1H, br d)	3.64 (1H, d, 4.6)	3.62 (1H, d, 4.2)	1.43 (1H, m)	3.67 (1H, d, 4.2)
					1.69 (1H, m)	
2	2.36 (1H, ddd, 12.0, 12.0, 12.0)	2.34 (1H, ddd,12.2, 12.2, 12.2)	3.54 (1H, dd, 4.6, 2.8)	3.51 (1H, dd, 5.5, 4.2)	1.92 (1H, m)	3.55 (1H, dd, 5.5, 4.2)
	2.60 (1H, ddd, 12.0, 4.6, 4.6)	2.59 (1H, m)			2.07 (1H, m)	
3	3.99 (1H, m)	3.98 (1H, br s)	4.70 (1H, dd, 5.5, 2.8)	4.65 (1H, dd, 5.5, 5.1)	3.82 (1H, m)	4.67 (1H, dd, 5.5, 5.5)
5	1.98 (1H, d, 4.1)	1.96 (1H, d, 4.0)	2.31 (1H, d, 5.1)	2.14 (1H, d, 5.5)	1.89 (1H, d, 4.2)	2.15 (1H, d, 5.5)
6	5.21 (1H, dd, 4.1, 1.4)	5.17 (1H, d, 4.0)	5.20 (1H, d, 5.1)	5.13 (1H, d, 5.5)	5.07 (1H, d, 4.2)	5.08 (1H, d, 5.5)
7	4.42 (1H, s)	5.24 (1H, s)	5.32 (1H, s)	5.28 (1H, s)	5.28 (1H, s)	5.40 (1H, s)
11	7.13 (1H, s)	7.13 (1H, s)	6.81 (1H, s)	6.75 (1H, s)	6.20 (1H, s)	6.84 (1H, s)
14	4.93 (1H, d, 6.0)	5.12 (1H, s)	5.12 (1H, s)	5.24 (1H, d, 9.2)	4.80 (1H, s)	4.91 (1H, s)
15	2.29 (1H, m)			4.37 (1H, m, overlapped)		
16	1.33 (3H, d, 6.9)	1.84 (3H, s)	1.85 (3H, s)		1.98 (3H, s)	1.99 (3H, s)
17	4.09 (1H, m)	4.28 (1H, d, 11.4)	4.28 (1H, dd, 11.5, 6.0)	4.27 (1H, br d)	3.40 (1H, d, 13.8)	3.43 (1H, d, 13.8)
	4.27 (1H, ddd, 10.6, 4.6, 4.6)	4.51 (1H, d, 11.4)	4.52 (1H, dd, 11.5, 6.0)	4.36 (1H, br d)	3.93 (1H, d, 13.8)	3.95 (1H, d, 13.8)
18	1.60 (3H, s)	1.58 (3H, s)	1.57 (3H, s)	1.38 (3H, s)	1.54 (3H, s)	1.40 (3H, s)
19						
20	1.56 (3H, s)	1.63 (3H, s)	1.10 (3H, s)	1.45 (3H, s)	1.28 (3H, s)	1.50 (3H, s)
SOMe					2.70 (3H, s)	2.70 (3H, s)
OH-1	6.92 (1H, d, 5.0)	6.90 (1H, br s)				
OH-2						
OH-3	6.32 (1H, d, 8.7)	6.25 (1H, br s)	8.25 (1H, d, 5.5)	7.48 (1H, d, 5.1)	6.16 (1H, br d)	7.56 (1H, br d)
OH-15		7.18 (1H, br s)	7.26 (1H, s)	6.68 (1H, br s)	8.16 (1H, s)	8.17 (1H, s)
OH-17	6.15 (1H, dd, 5.1, 5.1)	6.35 (1H, br s)	6.38 (1H, dd, 6.0, 6.0)	7.17 (1H, br s)		

a) ^{1}H (600 MHz) NMR data measured in pyridine-d_5. *b*) ^{1}H (500 MHz) NMR data measured in pyridine-d_5.

Table 1. (Continued)

Position	Makilactone P (**12**)[b]	Makilactone Q (**13**)[b]	Makilactone R (**14**)[b]	Podolactone B (**15**)[b]	Inumakilactone B (**16**)[b]
1	3.67 (1H, d, 4.6)	4.07 (1H, br dd)	1.74 (1H, d, 14.5) 2.19 (1H, dd, 14.5, 2.0)	3.65 (1H, d, 3.7)	3.64 (1H, d, 4.1)
2	3.56 (1H, dd, 4.6, 2.8)	1.50 (1H, ddd, 14.7, 8.8, 6.1) 2.39 (1H, ddd, 14.7, 6.1, 6.1)	3.38 (1H, m, overlapped)	3.53 (1H, dd, 5.1, 3.7)	3.55 (1H, d, 6.0, 4.1)
3	4.68 (1H, m)	1.87 (1H, m) 1.88 (1H, m)	3.28 (1H, d, 3.7)	4.66 (1H, dd, 5.1, 5.1)	4.68 (1H, dd, 6.0, 5.0)
5	2.34 (1H, d, 5.1)	1.93 (1H, d, 4.6)	1.82 (1H, d, 4.6)	2.12 (1H, d, 5.1)	2.18 (1H, d, 5.1)
6	5.21 (1H, d, 5.1)	5.09 (1H, 4.6, 1.1)	5.12 (1H, dd, 4.6, 1.1)	5.06 (1H, d, 5.1)	5.14 (1H, dd, 5.1, 1.0)
7	5.41 (1H, s)	5.34 (1H, d, 1.1)	5.30 (1H, d, 1.1)	5.32 (1H, s)	3.97 (1H, d, 1.0)
11	6.87 (1H, s)	7.08 (1H, s)	6.21 (1H, s)	6.78 (1H, s)	6.84 (1H, s)
14	4.90 (1H, s)	4.86 (1H, s)	4.78 (1H, s)	5.13 (1H, d, 0.9)	5.41 (1H, d, 7.8)
15					5.99 (1H, ddd, 17.0, 10.6, 7.8)
16	2.00 (3H, s)	1.99 (3H, s)	1.97 (3H, s)	1.85 (3H, s)	
17	3.43 (1H, d, 13.8)	3.43 (1H, d, 13.7)	3.39 (1H, d, 13.8, overlapped)	4.28 (1H, dd, 11.5, 6.0)	5.39 (1H, d, 10.6)
	3.94 (1H, d, 13.8)	3.94 (1H, d, 13.7)	3.89 (1H, d, 13.8)	4.53 (1H, dd, 11.5, 6.0)	5.55 (1H, d, 17.0)
18	1.59 (3H, s)	1.20 (3H, s)	1.43 (3H, s)	1.37 (3H, s)	1.40 (3H, s)
19					
20	1.00 (3H, s)	1.40 (3H, s)	1.35 (3H, s)	1.60 (3H, s)	1.53 (3H, s)
SOMe	2.70 (3H, s)	2.69 (3H, s)	2.67 (3H, s)		
OH-1		6.75 (1H, d, 4.7)			
OH-2					
OH-3	8.27 (1H, br d)			7.50 (1H, d, 5.1)	7.57 (1H, br d)
OH-15	8.21 (1H, br s)	8.15 (1H, s)	8.15 (1H, s)	7.23 (1H, s)	
OH-17				6.34 (1H, dd, 6.0, 6.0)	

a) ^{1}H (600 MHz) NMR data measured in pyridine-d_5. *b*) ^{1}H (500 MHz) NMR data measured in pyridine-d_5.

Table 2. ^{13}C-NMR Data of of New (**1**—**14**) and Known (**15**—**17**) Bisnor- and Norditerpenoids

Position	Nagilactone G (**17**)[a]	Makilactone E (**1**)[a]	Makilactone F (**2**)[b]	Makilactone G (**3**)[b]	Makilactone H (**4**)[b]	Makilactone I (**5**)[b]	Makilactone J (**6**)[b]	Makilactone K (**7**)[b]	Makilactone L (**8**)[b]
1	29.5 (t)	63.6 (d)	74.0 (d)	73.0 (d)	69.1 (d)	69.1 (d)	68.9 (d)	68.9 (d)	55.0 (d)
2	17.7 (t)	73.1 (d)	75.5 (d)	74.2 (d)	29.5 (t)	29.5 (t)	39.4 (t)	39.4 (t)	58.0 (d)
3	28.6 (t)	71.4 (d)	78.6 (d)	72.9 (d)	28.2 (t)	28.2 (t)	72.2 (d)	72.2 (d)	68.0 (d)
4	42.1 (s)	44.6 (s)	45.9 (s)	45.1 (s)	42.1 (s)	42.1 (s)	45.7 (s)	45.6 (s)	49.6 (s)
5	43.5 (d)	40.6 (d)	44.6 (d)	40.8 (d)	44.0 (d)	44.1 (d)	45.0 (d)	44.8 (d)	46.9 (d)
6	72.8 (d)	72.6 (d)	72.9 (d)	73.3 (d)	73.0 (d)	72.8 (d)	72.8 (d)	73.0 (d)	73.3 (d)
7	54.2 (d)	55.6 (d)	56.2 (d)	56.0 (d)	56.1 (d)	55.2 (d)	55.3 (d)	56.2 (d)	56.0 (d)
8	58.6 (s)	57.6 (s)	58.5 (s)	57.9 (s)	57.9 (s)	59.0 (s)	59.6 (s)	60.1 (s)	58.4 (s)
9	159.2 (s)	155.5 (s)	156.9 (s)	157.7 (s)	157.9 (s)	157.8 (s)	157.1 (s)	157.5 (s)	157.4 (s)
10	36.1 (s)	42.6 (s)	42.3 (s)	42.0 (s)	41.6 (s)	41.6 (s)	42.5 (s)	42.7 (s)	37.8 (s)
11	117.4 (d)	119.5 (d)	120.8 (d)	118.6 (d)	120.2 (d)	120.0 (d)	120.2 (d)	119.8 (d)	119.2 (d)
12	163.9 (s)	163.3 (s)	163.8 (s)	163.9 (s)	163.9 (s)	164.3 (s)	164.3 (s)	164.3 (s)	163.6 (s)
14	82.8 (d)	83.0 (d)	83.0 (d)	83.1 (d)	82.9 (d)	82.4 (d)	82.3 (d)	84.3 (d)	84.2 (d)
15	26.9 (d)	63.8 (d)	63.6 (d)	63.8 (d)	63.6 (d)	34.5 (d)	34.5 (d)	75.0 (s)	75.2 (s)
16	16.6 (q)					16.1 (q)	16.0 (q)	24.3 (q)	24.5 (q)
17	21.3 (q)	21.0 (q)	21.0 (q)	21.1 (q)	21.0 (q)	62.5 (t)	62.5 (t)	65.7 (t)	65.6 (t)
18	23.9 (q)	22.3 (q)	22.4 (q)	22.6 (q)	23.7 (q)	23.7 (q)	22.0 (q)	21.9 (q)	17.4 (q)
19	180.6 (s)	178.1 (s)	177.5 (s)	178.7 (s)	180.6 (s)	180.5 (s)	177.7 (s)	177.8 (s)	181.2 (s)
20	24.9 (q)	26.1 (q)	17.4 (q)	24.2 (q)	17.0 (q)	17.2 (q)	16.4 (q)	16.3 (q)	21.4 (q)
SOMe									

a) ^{13}C (150 MHz) NMR data measured in pyridine-d_5. *b*) ^{13}C (125 MHz) NMR data measured in pyridine-d_5.

Table 2. (Continued)

Position	Makilactone M (**9**)[b]	Makilactone N (**10**)[b]	Makilactone O (**11**)[b]	Makilactone P (**12**)[b]	Makilactone Q (**13**)[b]	Makilactone R (**14**)[b]	Podolactone B (**15**)[b]	Inumakilactone B (**16**)[b]
1	55.9 (d)	29.4 (t)	55.9 (d)	55.1 (d)	69.1 (d)	30.6 (t)	55.9 (d)	55.9 (d)
2	51.0 (d)	28.6 (t)	51.1 (d)	58.0 (d)	29.5 (t)	51.9 (d)	51.0 (d)	51.0 (d)
3	68.3 (d)	72.9 (d)	68.2 (d)	68.0 (d)	28.3 (t)	52.8 (d)	68.3 (d)	68.3 (d)
4	49.0 (s)	45.6 (s)	49.0 (s)	49.6 (s)	42.1 (s)	43.2 (s)	49.0 (s)	48.9 (s)
5	45.5 (d)	45.0 (d)	45.3 (d)	46.8 (d)	43.8 (d)	42.9 (d)	45.4 (d)	45.7 (d)
6	71.8 (d)	72.6 (d)	71.7 (d)	73.2 (d)	72.9 (d)	73.4 (d)	71.9 (d)	71.4 (d)
7	56.3 (d)	56.1 (d)	56.3 (d)	56.2 (d)	56.3 (d)	55.9 (d)	56.1 (d)	53.9 (d)
8	57.4 (s)	59.1 (s)	58.3 (s)	58.3 (s)	59.2 (s)	58.5 (s)	58.4 (s)	57.1 (s)
9	158.4 (s)	159.4 (s)	158.0 (s)	156.9 (s)	157.6 (s)	158.6 (s)	158.5 (s)	157.2 (s)
10	37.9 (s)	36.8 (s)	37.9 (s)	37.8 (s)	41.7 (s)	35.8 (s)	37.9 (s)	37.9 (s)
11	119.1 (d)	116.7 (d)	119.1 (d)	119.6 (d)	120.0 (d)	117.1 (d)	118.6 (d)	119.9 (d)
12	163.2 (s)	163.2 (s)	163.1 (s)	162.9 (s)	163.7 (s)	163.0 (s)	163.7 (s)	162.9 (s)
14	78.9 (d)	83.9 (d)	83.8 (d)	83.8 (d)	83.9 (d)	83.9 (d)	84.3 (d)	81.1 (d)
15	68.9 (s)	72.4 (s)	72.5 (s)	72.6 (s)	72.4 (s)	72.4 (s)	75.2 (s)	129.2 (d)
16		28.2 (q)	28.3 (q)	28.3 (q)	28.2 (q)	28.2 (q)	24.5 (q)	
17	63.3 (t)	63.1 (t)	63.1 (t)	63.1 (t)	63.2 (t)	63.1 (t)	65.5 (t)	123.6 (t)
18	25.3 (q)	22.0 (q)	25.3 (q)	17.4 (q)	23.8 (q)	21.1 (q)	25.3 (q)	25.2 (q)
19	176.7 (s)	177.9 (s)	176.6 (s)	181.1 (s)	180.5 (s)	177.1 (s)	176.7 (s)	176.5 (s)
20	20.5 (q)	23.9 (q)	20.8 (q)	21.4 (q)	17.0 (q)	25.6 (q)	20.7 (q)	21.2 (q)
SOMe		40.7 (q)	40.8 (q)	40.7 (q)	40.8 (q)	Makilactone R (**15**)[b]		

a) ^{13}C (150 MHz) NMR data measured in pyridine-d_5. *b*) ^{13}C (125 MHz) NMR data measured in pyridine-d_5.

with H-5 (δ_H 2.75), suggesting that Cl-1 was α-oriented, and the two hydroxyls OH-2 and OH-3 were β-oriented. The results of the single crystal X-ray crystallographic analysis confirmed that the chlorine at C-1 was α-oriented, OH-2 and OH-3 were β-oriented and the configuration at C-15 was *R*. Thus, the structure of **1** was determined to be as shown in Fig. 1. The ORTEP is given in Fig. 2.

Makilactone F (**2**) was isolated as colorless needles, mp 289 °C. The $[M+Na]^+$ ion peak at *m/z* 405.1127 in HR-ESI-MS determined the molecular formula to be $C_{18}H_{22}O_9$. IR absorption bands for hydroxyl and, γ-lactone and δ-lactone carbonyl groups were observed at 3340 cm^{-1}, 1766 cm^{-1} and 1694 cm^{-1}, respectively. The ^{13}C-NMR spectrum of **2** was quite similar to that of **1** excepting for the signal due to C-1, implying that **2** also had the desisopropylnagilactone G unit as **1**. The proton signal at δ_H 4.37 assigned to H-15 was correlated with the hydroxyl proton at δ_H 6.95 in the COSY spectrum, implying that the C-14 side chain structure was also the same as that in **1**. In the COSY spectrum, the protons H-1 (δ_H 4.03), H-2 (δ_H 4.32) and H-3 (δ_H 4.02) correlated with the hydroxyl protons at δ_H 7.09, δ_H 7.58 and δ_H 6.61, respectively, to show that those hydroxyls were at C-1, C-2 and C-3, respectively. In the NOESY spectrum, H-2 (δ_H 4.32) correlated with Me-20 (δ_H 1.62), H-1 (δ_H 4.03) correlated with H-5 (δ_H 2.18), and H-3 (δ_H 3.99) correlated with Me-18 (δ_H 1.68), suggesting that OH-1, OH-2 and OH-3 were β-, α-, and β-oriented, respectively. The single crystal X-ray crystallographic analysis confirmed that the hydroxyl groups OH-1 and OH-3 were β-oriented, OH-2 was α-oriented, and the configuration at C-15 was *R*. Accordingly, the structure of **2** was determined to be as shown in Fig. 1.

Makilactone G (**3**) was isolated as colorless needles, mp 276 °C. The molecular formula was determined to be $C_{18}H_{22}O_9$ from the $[M+Na]^+$ ion peak at *m/z* 405.1127 in HR-ESI-MS. IR absorption bands for hydroxyl and, γ-lactone and δ-lactone carbonyl groups were observed at 3308 cm^{-1}, 1762 cm^{-1} and 1691 cm^{-1}, respectively. Its molecular formula was the same as that of **2**, and its NMR profiles were quite similar to those of **2**. Accordingly, **3** was considered to be a diastereomer of **2**. As in the case of **2**, the protons H-1 (δ_H 4.68), H-2 (δ_H 4.64) and H-3 (δ_H 4.43) correlated with the protons of OH-1 (δ_H 7.28), OH-2 (δ_H 7.38) and OH-3 (δ_H 5.52), respectively. In the NOESY spectrum, H-1 (δ_H 4.68) correlated with Me-20 (δ_H 1.94), and the protons H-2 (δ_H 4.64) and H-3 (δ_H 4.43) correlated with H-5 (δ_H 2.85), suggesting that H-1 was β-oriented and H-2 and H-3 were α-oriented. The single crystal X-ray crystallographic analysis confirmed that the hydroxyl group at C-1 was α-oriented, that the hydroxyl groups at C-2 and C-3 were both β-oriented, and that the configuration at C-15 was *R*. Accordingly, the structure of **3** was determined to be as shown in Fig. 1.

Makilactone H (**4**) was isolated as colorless needles, mp 272 °C. The molecular formula was determined to be $C_{18}H_{22}O_7$ from the $[M+Na]^+$ ion peak at *m/z* 373.1246 in HR-ESI-MS. IR absorption bands for hydroxyl and, γ-lactone and δ-lactone carbonyl groups were observed at 3465 cm^{-1}, 1761 cm^{-1} and 1692 cm^{-1}, respectively. The ^{13}C-NMR spectrum of **4** showed that it had a quite similar structure to that of **3** and that C-1 was oxygen-linked, which implied that **4** also had the desisopropylnagilactone G unit. The proton signal of H-15 (δ_H 4.35) correlated with the hydroxyl proton signal at δ_H 6.94 in the COSY spectrum to show that **4** had the same C-14 side chain structure as **3**. The proton signal H-1 (δ_H 4.07) correlated with the hydroxyl proton at δ_H 6.74 in the COSY spectrum. In the NOESY spectrum, Me-20 (δ_H 1.47) correlated with OH-1 (δ_H 6.74), suggesting that OH-1 was β-oriented. The single crystal X-ray crystallographic analysis confirmed that the hydroxyl group at C-1 was β-oriented and that the configuration at C-15 was *R*. Accordingly, the structure of **4** was determined to be as shown in Fig. 1.

Makilactone I (**5**) was isolated as colorless needles, mp 296 °C. The molecular formula was determined to be

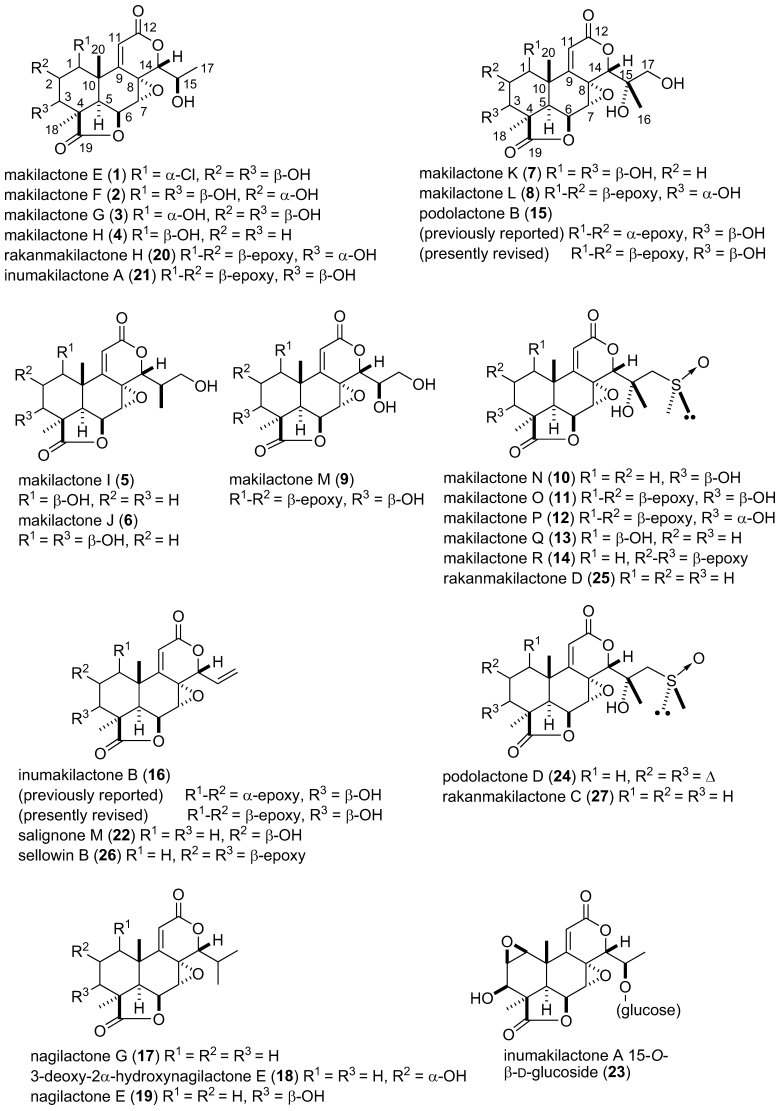


Fig. 1. New Bisnor- and Norditerpene Dilactones (**1**—**14**) and Known Ones (**15**—**27**) Isolated from the Root and Bark of *Podocarpus macrophyllus* D. DON

$C_{19}H_{24}O_7$ from the $[M+Na]^+$ ion peak at *m/z* 387.1407 in HR-ESI-MS. IR absorption bands for hydroxyl and, γ-lactone and δ-lactone carbonyl groups were observed at 3418 cm^{-1}, 1752 cm^{-1} and 1706 cm^{-1}, respectively. The ^{13}C-NMR spectrum of **5** was quite similar to that of **4**, excepting for the signals due to C-16 and C-17, implying that also **5** had the desisopropylnagilactone G unit as **4**, with a different side chain at C-14. In the HMQC spectrum, the methyl carbon signal at δ_C 16.1 correlated with the doublet methyl proton signal at δ_H 1.32, and was assigned to C-16 of the side chain. The H_2-17 methylene protons (δ_H 4.08 and δ_H 4.28) correlated with the proton of OH-17 (δ_H 6.14) in the COSY spectrum, showing that C-17 was a hydroxyl methylene group, and that the C-14 side chain of **5** was 1-methyl-2-hydroxyethyl group. The ^{13}C-NMR spectrum implied that C-1 was linked to oxygen. The proton H-1 (δ_H 4.06), correlating with C-20 (δ_C 17.2) in the HMBC, correlated with the hydroxyl proton at δ_H 6.73 in the COSY spectrum, implying that the hydroxyl group was at C-1. In the NOESY spectrum, the proton H-1 (δ_H 4.06) correlated with H-5 (δ_H 1.92), suggesting that H-1 was α-oriented. The single crystal X-ray crystallographic analysis confirmed that hydroxyl group at C-1 was β-oriented and the configuration at C-15 was *R*. Accordingly, the structure of **5** was determined to be as shown in Fig. 1. The ORTEP is given in Fig. 2.

Makilactone J (**6**) was isolated as colorless needles, mp 284 °C. The molecular formula was determined to be $C_{19}H_{24}O_8$ from the $[M+Na]^+$ ion peak at *m/z* 403.1352 in HR-ESI-MS. IR absorption bands for hydroxyl and, γ-lactone and δ-lactone carbonyl groups were observed at 3515 cm^{-1}, 1766 cm^{-1} and 1716 cm^{-1}, respectively. The ^{13}C-NMR spectrum was quite similar to that of **5**, implying that **6** had the desisopropylnagilactone G unit, too. The spectrum also implied that C-1 and C-3 were linked to oxygen. The methylene proton signals of H_2-17 (δ_H 4.09 and δ_H 4.27) correlated with the hydroxyl proton at δ_H 6.15 in the COSY spectrum, which revealed that C-17 was hydroxymethylene and that the C-14 side chain structure of **6** was also the same as that in **5**. In the COSY spectrum, H-1 (δ_H 4.10) and H-3 (δ_H 3.99) correlated with the hydroxyl group protons at δ_H 6.92 and δ_H 6.32, respectively. In the NOESY spectrum, the proton signal of OH-1 (δ_H 6.92) correlated with Me-20 (δ_H

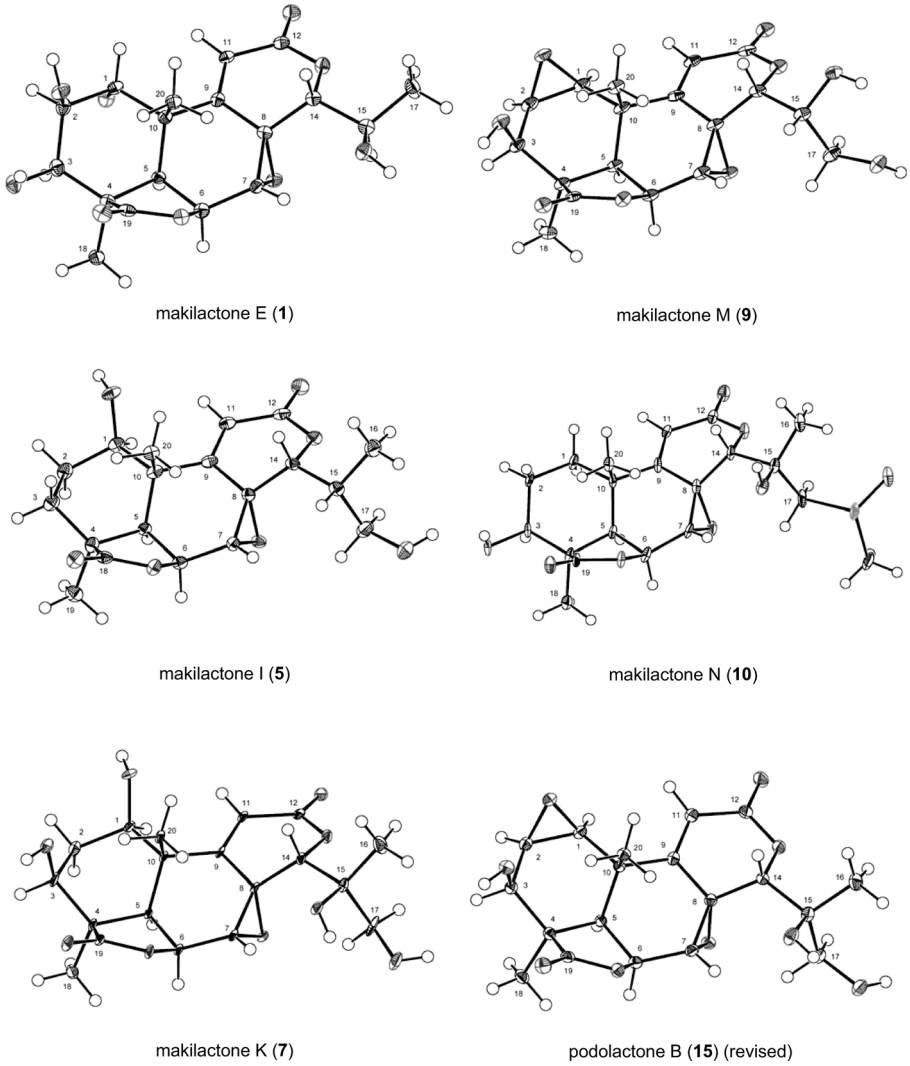


Fig. 2. ORTEP Representation for Compounds **1**, **5**, **7**, **9**, **10**, and Podolactone B (**15**) Revised

1.56), and H-3 (δ_H 3.99) correlated with H-5 (δ_H 1.98), suggesting that OH-1 and OH-3 were both β-oriented. The single crystal X-ray crystallographic analysis confirmed that the hydroxyl groups at C-1 and at C-3 were both β-oriented and that the configuration at C-15 was *R*. Accordingly, the structure of **6** was determined to be as shown in Fig. 1.

Makilactone K (**7**) was isolated as colorless needles, mp 268 °C. The molecular formula was determined to be $C_{19}H_{24}O_9$ from the $[M+Na]^+$ ion peak at *m/z* 419.1291 in HR-ESI-MS. IR absorption bands for hydroxyl and, γ-lactone group and δ-lactone carbonyl groups were observed at 3449 cm^{-1}, 1769 cm^{-1} and 1703 cm^{-1}, respectively. Its ^{13}C-NMR spectrum showed that **7** also had the desisopropylnagilactone G unit as **6**, with C-1 and C-3 connected to oxygen. In the HMQC spectrum, one of the three methyl carbon signals (δ_C 24.3) correlated with the singlet methyl proton signal at δ_H 1.84 which was assigned to C-16 of the side chain. Further, C-15 (δ_C 75.0) correlated with OH-15 (δ_H 7.18) in the HMBC spectrum. In the COSY spectrum the methylene proton signals H_2-17 (δ_H 4.28 and δ_H 4.51) correlated with OH-17 (δ_H 6.35) to show that C-17 was a hydroxymethyl group. Those observations revealed that C-15 was an asymmetric carbon connected to one methyl, one hydroxyl and one hydroxymethyl, which represented the C-14 side chain of **7**. The COSY spectrum showed that H-1 (δ_H 4.10) and H-3 (δ_H 3.98) correlated with the hydroxyl protons of OH-1 (δ_H 6.90) and OH-3 (δ_H 6.25), respectively. In the NOESY spectrum, both H-1 (δ_H 4.10) and H-3 (δ_H 3.98) correlated with H-5 (δ_H 1.96), suggesting that both OH-1 and OH-3 were of β-orientation. The single crystal X-ray crystallographic analysis confirmed that the hydroxyl groups at C-1 and C-3 were β-oriented and that the configuration at C-15 was *S*. Accordingly, the structure of **7** was determined to be as shown in Fig. 1.

Makilactone L (**8**) was isolated as colorless needles, mp 288 °C. The molecular formula was determined to be $C_{19}H_{22}O_9$ from the $[M+Na]^+$ ion peak at *m/z* 417.1160 in HR-ESI-MS. IR absorption bands for hydroxyl and, γ-lactone and δ-lactone carbonyl groups were observed at 3436 cm^{-1}, 1782 cm^{-1} and 1707 cm^{-1}, respectively. The carbon NMR spectrum and the 1D NMR profiles revealed that also **8** had the desisopropylnagilactone G unit with the C-14 side chain of the same structure as that in **7**, and that C-1, C-2 and C-3 were all linked to oxygen. In the COSY spectrum, the only hydroxyl group (δ_H 8.25) correlated with H-3 (δ_H 4.70), implying the presence of an epoxy group between C-1

and C-2. In the NOESY spectrum, the proton of OH-3 (δ_H 8.25) correlated with Me-18 (δ_H 1.57), and the proton of H-3 (δ_H 4.70) correlated with Me-20 (δ_H 1.10) to suggest that OH-3 was α-oriented. No correlation was noted between H-1 and Me-20, suggesting that H-1 was not β-oriented, and accordingly the 1 : 2-epoxy was β-oriented. The single crystal X-ray crystallographic analysis confirmed that OH-3 was α-oriented, 1 : 2-epoxy was β-oriented, and the configuration at C-15 was *S*. Accordingly, the structure of **8** was determined to be as shown in Fig. 1.

Makilactone M (**9**) was isolated as colorless needles, mp 293 °C. The molecular formula was determined to be $C_{18}H_{20}O_9$ from the $[M+Na]^+$ ion peak at *m/z* 403.0996 in HR-ESI-MS. IR absorption bands for hydroxyl and, γ-lactone and δ-lactone carbonyl groups were observed at 3543 cm^{-1}, 1761 cm^{-1} and 1707 cm^{-1}, respectively. The ^{13}C-NMR spectrum of **9** showed that it also had the desisopropylnagilactone G unit, that C-1, C-2 and C-3 were all linked to oxygen and that the C-14 side chain structure of **9** was similar to that of **8**, with C-16 missing in **9**. In the COSY spectrum, the methylene protons of H_2-17 (δ_H 4.27 and δ_H 4.36) correlated with the hydroxyl group proton at δ_H 7.17, implying that C-17 was a hydroxymethyl group, and H-15 (δ_H 4.37) correlated with another hydroxyl group proton (δ_H 6.68), implying that C-15 had a hydroxy group. Accordingly, the C-14 side chain of **9** was shown to be 1,2-dihydroxyethyl group. H-3 (δ_H 4.65) correlated with a hydroxyl group proton at δ_H 7.48 in the COSY, implying that the hydroxyl was at C-3 and accordingly the epoxy group was between C-1 and C-2. The proton of OH-3 (δ_H 7.48) correlated also with Me-20 (δ_H 1.45), and H-2 (δ_H 3.51) correlated with H-5 (δ_H 2.14) in the NOESY spectrum, suggesting that OH-3 was β-oriented and the 1 : 2-epoxy group was β-oriented. The single crystal X-ray crystallographic analysis confirmed that OH-3 was β-oriented, the 1 : 2-epoxy group β-oriented and that the configuration at C-15 was *R*. Accordingly, the structure of **9** was determined as shown in Fig. 1. The ORTEP is given in Fig. 2.

Makilactone N (**10**) was isolated as colorless needles, mp 273 °C. The molecular formula was determined to be $C_{20}H_{26}O_8S$ by HR-ESI-MS molecular ion peak at *m/z* 449.1224 $[M+Na]^+$. IR absorption bands for hydroxyl and, γ-lactone and δ-lactone carbonyl groups were observed at 3392 cm^{-1}, 1773 cm^{-1} and 1724 cm^{-1}, respectively. The ^{1}H- and ^{13}C-NMR spectra implied that **10** also had the desisopropylnagilactone G unit, that C-3 was linked to oxygen, and that the sulfoxide-containing C-14 side chain structure was the same as that in podolactone D (**24**)[5] or in rakanmakilactone D (**25**),[6] also isolated in the present study. The proton signal H-3 (δ_H 3.82) correlated with both H-5 (δ_H 1.89) and Me-18 (δ_H 1.54) in the NOESY spectrum, suggesting that OH-3 was β-oriented. The single crystal X-ray crystallographic analysis confirmed that the hydroxyl group at C-3 was β-oriented, that the configurations at C-15 and *S* of the sulfoxide were both *R*. Accordingly, the structure of **10** was determined to be as shown in Fig. 1. The ORTEP is given in Fig. 2.

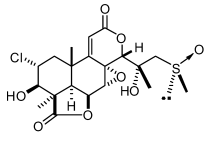
Makilactone O (**11**) was isolated as colorless needles, mp 292 °C. The molecular formula was determined to be $C_{20}H_{24}O_9S$ by HR-ESI-MS molecular ion peak at *m/z* 463.1024 $[M+Na]^+$. IR absorption bands for hydroxyl and, γ-lactone and δ-lactone carbonyl groups were observed at 3366 cm^{-1}, 1773 cm^{-1} and 1697 cm^{-1}, respectively. ^{13}C-NMR showed that **11** also had the desisopropylnagilactone G unit, that the sulfoxide-containing C-14 side chain structure was the same as that in **10**, and that C-1, C-2 and C-3 were all linked to oxygen. The proton signal of H-3 (δ_H 4.67) correlated with both H-5 (δ_H 2.15) and Me-18 (δ_H 1.40) in the NOESY spectrum, suggesting that H-3 was α-oriented. No correlation was observed between the protons H-1 and H-2 and the proton Me-20 to show that neither H-1 nor H-2 was β-oriented. The single crystal X-ray crystallographic analysis confirmed that OH-3 at C-3 was β-oriented, that the 1 : 2-epoxy group was β-oriented and that the configurations at C-15 and *S* of the sulfoxide were both *R*. Accordingly, the structure of **11** was determined to be as shown in Fig. 1.

As regards the stereochemistry of the sulfur in the sulfoxide group of the type as seen in **10**, we found that the difference between the chemical shifts of the two C-17 methylene protons in the ^{1}H-NMR was related to the stereochemistry of the sulfoxide group of this type.[6] Table 3 lists the chemical shift values of the two H_2-17 methylene protons and the dif-

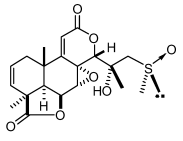
Table 3. Chemical Shifts of C-17 Methylene Protons (ppm) in Pyridine-d_5 for Norditerpenes with Sulfoxide-Containing C-14 Side Chains of S_S-15*R* (**24**, **27**, **28**, **30**, **33**) and of S_R-15*R* (**10**—**14**, **25**, **33**) Configurations[a]

	S_S-15*R* type				S_R-15*R* type		
Compounds	H-17b	H-17a	Δ_{Hb-Ha}	Compounds	H-17b	H-17a	Δ_{Hb-Ha}
Podolactone D (**24**)[b]	3.77	3.42	0.35	S_R-Podolactone D (**33**)[b]	3.93	3.42	0.51
Rakanmakilactone C (**27**)[c]	3.79	3.42	0.37	Rakanmakilactone D (**25**)[c]	3.93	3.40	0.53
Rakanmakilactone F (**28**)[c]	3.81	3.45	0.36	Makilactone N (**10**)	3.93	3.40	0.53
Podolactone C (**30**)[c]	3.77	3.41	0.36	Makilactone O (**11**)	3.95	3.43	0.52
Rakanmakilactone E (**32**)[c]	3.79	3.42	0.37	Makilactone P (**12**)	3.94	3.43	0.51
				Makilactone Q (**13**)	3.94	3.43	0.51
				Makilactone R (**14**)	3.89	3.39	0.50

a) ^{1}H (500 MHz) NMR data measured in pyridine-d_5. *b*) Ref. 5, *c*) Ref . 6.



rakanmakilactone E (**32**)



S_R-podolactone D (**33**)

ferences between them in the new makilactones N—R (**10**—**14**) and known norditerpenoids having a sulfoxide-containing C-14 side chain, podolactone D (**24**) and rakanmakilactones C (**27**) and D (**25**) isolated in the present study, and four reference norditerpenoids having a C-14 side chain of this type whose sulfur configuration is properly defined, rakanmakilactones E (**32**), and F (**28**), podolactone C (**30**) and S_R-podolactone D (**33**). In **10**, **11**, **24**, **25**, **27**, **32**, and **33**, the configurations of sulfur in the sulfoxide group and C-15 have been verified directly by the single crystal X-ray crystallographic analysis. In **28** and **30**, it has been defined indirectly. Table 3 shows that in compounds of S_S-15*R* type, the $\delta_{Hb}-\delta_{Ha}$ value is in the range of 0.35—0.37 ppm, whereas in those of S_R-15*R* type, the $\delta_{Hb}-\delta_{Ha}$ value is in the range of 0.50—0.53 ppm. Some compounds of this series may not produce good crystals to allow direct determination of the configuration by X-ray crystallography. In such cases, this rule may be used to define the configuration at sulfur. This system was actually applied in the case of **12**, **13**, and **14** of the present compounds, as seen in Table 3.

Makilactone P (**12**) was isolated as colorless needles, mp 271 °C. The molecular formula was determined to be $C_{20}H_{24}O_9S$ by HR-ESI-MS molecular ion peak at *m/z* 463.1020 $[M+Na]^+$. IR absorption bands for hydroxyl and, γ-lactone and δ-lactone carbonyl groups were observed at 3362 cm^{-1}, 1768 cm^{-1} and 1719 cm^{-1}, respectively. The molecular formula and the ^{13}C- and ^1H-NMR profiles of **12** were almost the same as those of **11**, suggesting that **12** was a diastereomer of **11**. In the NOESY spectrum, H-3 (δ_H 4.68) correlated with Me-20 (δ_H 1.00), and both H-1 (δ_H 3.67) and H-2 (δ_H 3.56) correlated with H-5 (δ_H 2.34), implying that OH-3 was α-oriented and the 1 : 2-epoxy group was β-oriented. The stereochemistry at C-15 and at the sulfur of the sulfoxide were both decided to be *R* on the basis of the difference between δ_{Hb} (3.94 ppm) and δ_{Ha} (3.43 ppm), which was 0.51 ppm (Table 3). Accordingly, the structure of **12** was determined to be an epimer of **11**, as shown in Fig. 1.

Makilactone Q (**13**) was isolated as colorless needles, mp 293 °C. The molecular formula was determined to be $C_{20}H_{26}O_8S$ from the $[M+Na]^+$ ion peak at *m/z* 449.1205 in HR-ESI-MS. IR absorption bands for hydroxyl and, γ-lactone group and δ-lactone carbonyl groups were observed at 3308 cm^{-1}, 1760 cm^{-1} and 1698 cm^{-1}, respectively. The ^{13}C-NMR spectrum showed that **13** had the desisopropylnagilactone G unit with the C-14 side chain of the same structure as in **12** and with C-1 linked to oxygen. H-1 (δ_H 4.07) correlated with a hydroxyl group (δ_H 6.75) in the COSY spectrum to show that the hydroxyl group was OH-1. In the NOESY spectrum, H-1 (δ_H 4.07) correlated with H-5 (δ_H 1.93), and OH-1 (δ_H 6.75) correlated with Me-20 (δ_H 1.40) to show that OH-1 was β-oriented. The difference between the chemical shifts of the two C-17 methylene protons, δ_{Hb} (3.94 ppm)$-$ δ_{Ha} (3.43 ppm) was 0.51 ppm. Following the rule described above (Table 3), the stereochemistry of the sulfoxide and C-15 in the side chain of **13** were both concluded to be *R*. By oxidation, **13** and rakanmakilactone F (**28**),[6] a known norditerpene of defined structure, gave an identical oxidation product **29** in satisfactory yields, as shown in Chart 1. Consequently, the structure of **13** was determined to be as shown in Fig. 1. with C-15 of *R* configuration. **13** was shown to be an epimer of rakanmakilactone F (**28**) at the sulfur atom configuration.

Makilactone R (**14**) was isolated as colorless needles, mp 292 °C. The molecular formula was determined to be $C_{20}H_{24}O_8S$ from the $[M+Na]^+$ ion peak at *m/z* 447.1073 in HR-ESI-MS. IR absorption bands for hydroxyl and, γ-lactone and δ-lactone carbonyl groups were observed at 3371 cm^{-1}, 1775 cm^{-1} and 1725 cm^{-1}, respectively. The ^{13}C-NMR spectrum showed that **14** also had the desisopropylnagilactone G unit with the same C-14 side chain structure as in **13**. It also showed the presence of oxygen-linked C-2 and C-3, which formed 2 : 3-epoxy. The proton signal of H-2 (δ_H 3.38) correlated with H-5 (δ_H 1.82) in the NOESY spectrum, suggesting that the epoxy was β-oriented. The difference between the two proton signals of H_2-17, δ_{Hb} (3.89 ppm)$-\delta_{Ha}$ (3.39 ppm), was 0.50 ppm, to imply that the both stereochemistry of C-15 and the sulfoxide group in **14** were *R*. Oxidation of **14** and of podolactone C (**30**),[5] a known norditerpene of defined structure, gave an identical oxidation product **31** in satisfactory yields. Consequently, the structure of **14** was determined to be as shown in Fig. 1 with C-15 of *R* configuration. **14** was shown to be an epimer of **30** at the sulfur atom in the side chain.

Podolactone B (**15**) was isolated as colorless needles, mp 261 °C. The molecular formula was determined to be $C_{19}H_{22}O_9$ from the $[M+Na]^+$ ion peak at *m/z* 417.1185 in

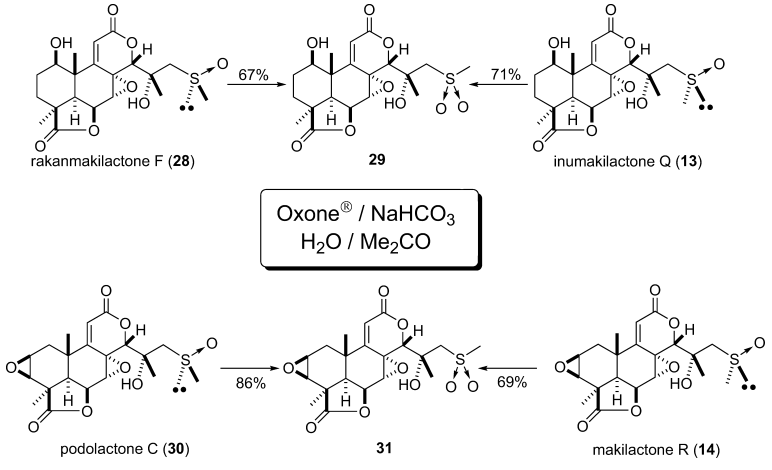


Chart 1. Oxidation of **13**, **14**, **28**, and **30** with Oxone®/Me_2CO Oxidation System

HR-ESI-MS. Its IR absorption bands for hydroxyl and, γ-lactone and δ-lactone carbonyl groups observed at 3395 cm^{-1}, 1771 cm^{-1} and 1723 cm^{-1}, respectively. Its molecular formula and ^{1}H- and ^{13}C-NMR profiles were exactly the same as those reported previously for podolactone B.[9] So, the presently isolated **15** was identified as podolactone B. The structure reported for podolactone B is shown in Fig. 1. In the present study, detailed 2D NMR studies demonstrated, however, that H-1 (δ_H 3.65) and H-2 (δ_H 3.53) correlated with H-5 (δ_H 2.12) in the NOESY spectrum, implying that the 1 : 2-epoxy was β-oriented, not α-oriented as previously reported. The single crystal X-ray crystallographic analysis confirmed that OH-3 was β-oriented, the 1 : 2-epoxy group was β-oriented, and the configuration at C-15 was *S*. Accordingly, the structure of **15** was now revised to be as shown in Fig. 1. The ORTEP is given in Fig. 2.

Inumakilactone B (**16**) was isolated as colorless needles, mp 288 °C. The molecular formula was determined to be $C_{18}H_{18}O_7$ from the $[M+H]^+$ ion peak at *m/z* 347.1120 in HR-ESI-MS. IR absorption bands for hydroxyl and, γ-lactone and δ-lactone carbonyl groups were noted at 3518 cm^{-1}, 1755 cm^{-1} and 1700 cm^{-1}, respectively. The mp, molecular formula, IR data, and the profiles of the ^{1}H- and ^{13}C-NMR spectra of **16** were exactly the same as those reported for inumakilactone B.[10] So, **16** was identified as inumakilactone B. The structure previously given to inumakilactone B is shown in Fig. 1. Detailed 2D NMR studies, however, showed that the signals H-1 (δ_H 3.64) and H-2 (δ_H 3.55) correlated with H-5 (δ_H 2.18), and that of OH-3 (δ_H 7.57) correlated with H-20 (δ_H 1.53) in the NOESY spectrum (Fig. 3). The fact implied that H-1 and H-2 were α-oriented and OH-3 was β-oriented, and accordingly, the 1 : 2-epoxy group was β-oriented, and not α-oriented as reported. The structure of inumakilactone B was thus revised to be as shown in Fig. 1.

All these bisnor- and norditerpene dilactones isolated in the present study [**1**—**14**: new bisnor- and norditerpenoids; **15** and **16**: known ones whose structures were now revised: **17**—**27**: known ones, two synthetic analogues **29** and **31** and camptothecin (**32**) as a positive control, were tested for their cytotoxic activities against P388 murine leukemia cells. The results are given in Table 4. Excepting **1**—**3**, and **23**, all the bisnor- and norditerpene dilactones showed cytotoxic activities. Among them, nagilactone G (**17**) showed almost the same cytotoxic activity as that of camptothecin (**32**).

In summary, fourteen new bisnor- and norditerpene dilactones, makilactones E—R (**1**—**14**), having a 7α : 8α-epoxy-9,11-enolide substructure, were isolated from methanolic extracts of the root and the bark of *Podocarpus macrophyllus* D. Don (Podocarpaceae) with thirteen known bisnor- and norditerpene dilactones. The structures of the new bisnor- and norditerpenoids were determined on the basis of their spectroscopic studies. Further, the structures of two known bisnor- and norditerpenes, *i.e.*, podolactone B (**15**) and inumakilactone B (**16**), were revised on the basis of X-ray crystallographic analysis and spectroscopic analysis. In the present study, 27 different bisnor- and norditerpene dilactones

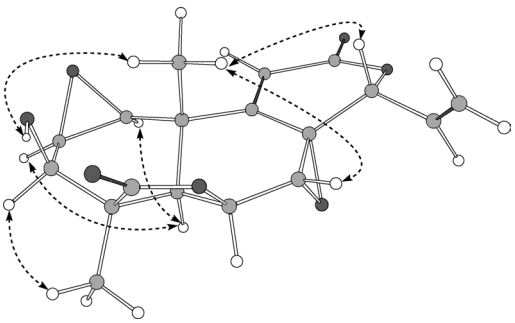


Fig. 3. Selected NOE Correlations for Inumakilactone B (**16**) (Revised)

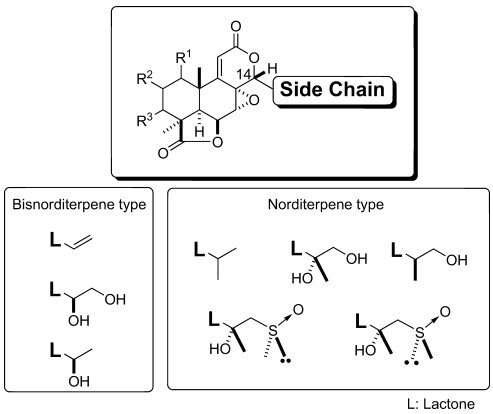


Fig. 4. Structure Diversity in the C-14 Side Chains of Presently Isolated Bisnor- and Norditerpene Dilactones

Table 4. Cytotoxic Activities of the Presently Isolated Norditerpenoids and Camptothecin (**32**, Positive Control) against P388 Murine Leukemia Cells (IC_{50} μg/ml)

Compounds	IC_{50} (μg/ml)	Compounds	IC_{50} (μg/ml)
Makilactone E (**1**)	>100	Inumakilactone B (**16**)	0.22
Makilactone F (**2**)	20	Nagilactone G (**17**)	0.08
Makilactone G (**3**)	50	3-Deoxy-2α-hydroxynagilactone E (**18**)	0.20
Makilactone H (**4**)	0.69	Nagilactone E (**19**)	0.25
Makilactone I (**5**)	0.75	Rakanmakilactone H (**20**)	0.55
Makilactone J (**6**)	9.0	Inumakilactone A (**21**)	0.35
Makilactone K (**7**)	7.0	Salignone M (**22**)	0.20
Makilactone L (**8**)	2.0	Inumakilactone-β-D-glucoside (**23**)	>100
Makilactone M (**9**)	5.8	Podolactone D (**24**)	1.5
Makilactone N (**10**)	0.80	Rakanmakilactone D (**25**)	1.6
Makilactone O (**11**)	0.50	Sellowin B (**26**)	0.60
Makilactone P (**12**)	6.0	Rakanmakilactone C (**27**)	1.1
Makilactone Q (**13**)	6.5	**29**	0.44
Makilactone R (**14**)	2.3	**31**	4.4
Podolactone B (**15**)	1.0	Camptothecin (**32**)	0.045

were isolated from one plant material. Those 27 compounds, however, had the same carbon ring system, and were different apart from the ring A substituents, only in the C-14 side chain structure. Those various C-14 side chains may be grouped as in Fig. 4. The fact may suggest that the side chain is more liable to various biological enzymatic effects in the plant. Except for **1**—**3** and **23**, all the present bisnor- and norditerpene dilactones showed cytotoxic activity.

Experimental

General Experimental Procedures Optical rotations were measured on a JASCO DIP-360 automatic digital polarimeter, IR spectra on a JASCO FT/IR 620 spectrophotometer, and Mass spectra on VG AutoSpec E and Micromass LCT (Manchester, U.K.) spectrometers. NMR spectra were obtained on a Bruker DRX-500 and AV-600 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 7.19 ppm and those of carbon signals are given in ppm relative to the resonances at 135.5 ppm for C_5D_5N. Silica gel (Merck Kiesel gel 60, 70—230 μm, Kanto silica gel N 60, 63—210 μm) and Diaion® HP-20 (Mitsubishi Chemical Co., Ltd.) 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, on 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-octadecyl silica (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$K\alpha$ radiation (λ=0.71073 Å).

Plant Material The root and the bark of *Podocarpus macrophyllus* D. Don were collected in Kochi, Japan, in November 2004. The botanical identification was made by K. Takeya, Professor of Plant Chemistry of Tokyo University of Pharmacy and Life Science. A voucher specimen (08JCP18) has been deposited in the herbarium of Tokyo University of Pharmacy and Life Science.

Extraction and Isolation a) From Root: The air dried root of *Podocarpus macrophyllus* D. Don (22.66 kg) was extracted with MeOH (45 l×3, 1 d each time) at room temperature. The combined MeOH extract was concentrated and the residue (614 g) was subjected to Diaion HP-20® resin column chromatography (10 cm×40 cm) eluting sequentially with H_2O/MeOH (1 : 0, 1 : 1, 1 : 4, 0 : 1) and acetone (each 5 l). The fraction eluted with H_2O/MeOH (1 : 1) was concentrated to give a residue, F (71.6 g), which was subjected to silica gel chromatography eluting with $CHCl_3$/MeOH (1 : 0, 19 : 1, 9 : 1, 2 : 1, 0 : 1) to give five fractions. The fraction eluted with $CHCl_3$/MeOH (2 : 1), F-4 (20.0 g), was recrystallized from MeOH to give inumakilactone A-15-*O*-β-D-glucoside[11] [(**23**) 6.8 g]. The fraction eluted with $CHCl_3$/MeOH (19 : 1), F2 (8.9 g), was subjected to ODS-HPLC eluting with H_2O/MeCN (93.5 : 6.5) to give eleven fractions. The 4th fraction, F2-4, gave inumakilactone A[7] [(**21**) 3.5 mg], and the 8th fraction F2-8 gave rakanmakilactone H [7] [(**20**) 12 mg]. The 11th fraction, F2-8, gave, when subjected to ODS-HPLC eluting with H_2O/MeCN (85 : 15), thirteen fractions, of which the 12th fraction, F2-11-12 gave makilactone E [(**1**) 1.8 mg]. Another silica gel chromatography fraction, eluted with $CHCl_3$/MeOH (9 : 1), F3 (7.7 g), was subjected to ODS-HPLC eluting with H_2O/MeCN (88 : 12) to give seven fractions, F3-1—F3-7. The fractions F3-2 (459 mg) and F3-6 (388 mg) gave, when recrystalized from MeOH, podolactone B[9] [(**15**) 23 mg] and makilactone Q [(**13**) 64 mg], respectively. The mother liquid of F3-2 was further treated with ODS-HPLC with H_2O/MeCN (88 : 12) to give thirteen fractions, F3-2-1—F3-2-13, the 6th fraction of which, F3-2-6, gave makilactone K [(**7**) 13.0 mg]. When treated with HPLC eluting with $CHCl_3$/MeOH (5 : 1), the 10th fraction, F3-2-10, gave three fractions, the 2nd fraction of which, F3-2-10-2, gave, when treated by ODS-HPLC eluting with H_2O/MeCN (85 : 15), makilactone O [(**11**) 6.0 mg] and makilactone M [(**9**) 2.0 mg]. F3-3 gave, by ODS-HPLC eluting with H_2O/MeCN (93 : 7), eight fractions, of which the 5th fraction F3-3-5 (92 mg) was recrystallized from MeOH to give makilactone F [(**2**) 13 mg], whereas the 6th fraction F3-3-6 (33 mg) gave by ODS-HPLC eluting with H_2O–MeCN (95 : 5) makilactone L [(**8**) 6 mg]. By ODS-HPLC eluting with H_2O–MeCN (93.5 : 6.5), F3-3-7 (139 mg) gave six fractions. The 4th fraction, F3-3-7-4, was makilactone G [(**3**), 95 mg], and the 5th fraction, F3-3-7-5 was makilactone P [(**12**) 1.2 mg]. F3-3-8 (161 mg) was subjected to ODS-HPLC eluting with H_2O/MeCN (90 : 10) to give four fractions. The 2nd fraction F3-3-8-2 (88 mg) gave, when recrystallized from MeOH, a crystalline substance (25 mg), which, when treated with ODS-HPLC eluting with H_2O/MeCN (91 : 9), gave makilactone J [(**6**) 9.6 mg]. The fraction eluted with H_2O/MeOH (1 : 4) in Diaion HP-20® column chromatography was concentrated to give a residue, E (82.4 g), which was subjected to silica gel chromatography eluting with $CHCl_3$/MeOH (1 : 0, 19 : 1, 9 : 1, 2 : 1, 0 : 1) to give five fractions, E1—E5. The 2nd fraction E2 (12.6 g) was further subjected to silica gel chromatography eluting with $CHCl_3$/MeOH (30 : 1) to give fourteen fractions, E2-1—E2-14. The 2nd fraction, E2-2 (3.8 g), was recrystallized from MeOH to give a crystalline substance (106 mg), which, when treated with ODS-HPLC eluting with H_2O/MeCN (65 : 35) gave inumakilactone B[10] [(**16**) 24 mg] and nagilactone E[11] [(**19**) 46.0 mg]. The 3rd fraction, E2-3, gave, when treated with ODS-HPLC eluting with H_2O–MeCN (60 : 40), salignone M[12] [(**22**) 9.4 mg], podolactone D[5] [(**24**) 6.5 mg], rakanmakilactone C[6] [(**27**) 4.0 mg] and 3-deoxy-2α-hydroxynagilactone E[11] [(**18**) 62.0 mg]. The 6th fraction E2-6 (378 mg) was recrystallized from MeOH to give a crystalline mass (27.0 mg) and mother liquid (350 mg). The crystalline mass gave, when subjected to ODS-HPLC eluting with H_2O/MeOH (72 : 28), makilactone R [(**14**) 15.6 mg] and makilactone I [(**5**) 11.0 mg], whereas the mother liquid gave, by ODS-HPLC eluting with H_2O/MeOH (75 : 25), makilactone E [(**1**) 3.0 mg], and makilactone H [(**4**) 3.3 mg]. The 7th fraction E2-7 was recrystallized from MeOH to give makilactone E [(**1**) 65 mg]. The 8th fraction E2-8 gave, by ODS-HPLC eluted with H_2O/MeOH (72 : 28), makilactone N [(**11**) 3.9 mg].

b) From Bark: Air-dried bark of *Podocarpus macrophyllus* D. Don (3.2 kg) was extracted with hot MeOH (3×45 l). The combined MeOH extract was concentrated and the residue (284 g) was subjected to Diaion HP-20® resin column chromatography (10 cm×40 cm) eluting with H_2O/MeOH (1 : 0, 1 : 1, 1 : 4, 0 : 1) and acetone (each 5 l). The fraction eluted with 80% MeOH was concentrated to give a residue RE (10.4 g), which was subjected to silica gel column chromatography eluting with $CHCl_3$/MeOH (19 : 1) to give two fractions RE1 (635 mg) and RE2 (896 mg). By ODS-HPLC eluting with H_2O/MeCN (60 : 40), RE1 gave sellowin B[13] [(**26**) 1.3 mg]. By ODS-HPLC eluting with H_2O/MeOH (70 : 30) and then with H_2O/MeCN (45 : 55), RE2 gave rakanmakilactone D[6] [(**25**) 11.8 mg]. The fraction eluting with 100% MeOH in HP-20® resin column chromatography was concentrated to give a residue RF (18.4 g) and, which was subjected to silica gel column chromatography eluting with $CHCl_3$ and then $CHCl_3$/MeOH (19 : 1) to give sixteen fractions. The 2nd fraction RF2 (164 mg) was subjected to silica gel column chromatography eluting with hexane/AcOEt (8 : 1) to give four fractions. The 2nd fraction RF2-2 (18 mg) gave, when treated with ODS-HPLC eluting with H_2O/MeOH (62 : 38) nagilactone G[8] [(**17**) 7.0 mg].

Makilactone E (**1**): Colorless needles, mp 283 °C (H_2O–MeOH). $[\alpha]_D^{24}$ +53.2° (*c*=0.08, MeOH); IR (film) 3437 (OH), 1765 (C=O), 1715 (C=O) cm^{-1}; HR-ESI-MS *m/z*: 423.0815 [Calcd for $C_{18}H_{21}O_8ClNa$ 423.0823 $(M+Na)^+$]. ^1H- and ^{13}C-NMR, see Tables 1 and 2.

Makilactone F (**2**): Colorless needles, mp 289 °C (H_2O–MeOH). $[\alpha]_D^{24}$ −90.0° (*c*=0.04, MeOH); IR (film) 3340 (OH), 1766 (C=O), 1694 (C=O) cm^{-1}; HR-ESI-MS *m/z*: 405.1127 [Calcd for $C_{18}H_{22}O_9Na$ 405.1162 $(M+Na)^+$]. ^1H- and ^{13}C-NMR, see Tables 1 and 2.

Makilactone G (**3**): Colorless needles, mp 276 °C (H_2O–MeOH). $[\alpha]_D^{24}$ +11.9° (*c*=0.06, MeOH); IR (film) 3308 (OH), 1762 (C=O), 1691 (C=O) cm^{-1}; HR-ESI-MS *m/z*: 405.1127 [Calcd for $C_{18}H_{22}O_9Na$, 405.1162 $(M+Na)^+$]. ^1H- and ^{13}C-NMR, see Tables 1 and 2.

Makilactone H (**4**): Colorless needles, mp 272 °C (H_2O–MeOH). $[\alpha]_D^{24}$ +11.1° (*c*=0.10, MeOH); IR (film) 3465 (OH), 1761 (C=O), 1692 (C=O) cm^{-1}; HR-ESI-MS *m/z*: 373.1246 [Calcd for $C_{18}H_{22}O_7Na$, 373.1263 $(M+Na)^+$]. ^1H- and ^{13}C-NMR, see Tables 1 and 2.

Makilactone I (**5**): Colorless needles, mp 296 °C (H_2O–MeOH). $[\alpha]_D^{24}$ −13.2° (*c*=0.16, MeOH); IR (film) 3418 (OH), 1752 (C=O), 1706 (C=O) cm^{-1}; HR-ESI-MS *m/z*: 387.1407 [Calcd for $C_{19}H_{24}O_7Na$, 387.1420 $(M+Na)^+$]. ^1H- and ^{13}C-NMR, see Tables 1 and 2.

Makilactone J (**6**): Colorless needles, mp 284 °C (H_2O–MeOH). $[\alpha]_D^{24}$ +33.9° (*c*=0.05, MeOH); IR (film) 3515 (OH), 1766 (C=O), 1716 (C=O) cm^{-1}; HR-ESI-MS *m/z*: 403.1352 [Calcd for $C_{19}H_{24}O_8Na$, 403.1369 $(M+Na)^+$]. ^1H- and ^{13}C-NMR, see Tables 1 and 2.

Makilactone K (**7**): Colorless needles, mp 268 °C (H_2O–MeOH). $[\alpha]_D^{24}$ −17.0° (*c*=0.13, MeOH); IR (film) 3449 (OH), 1769 (C=O), 1703 (C=O) cm^{-1}; HR-ESI-MS *m/z*: 419.1291 [Calcd for $C_{19}H_{24}O_9Na$, 419.1318 $(M+Na)^+$]. ^1H- and ^{13}C-NMR, see Tables 1 and 2.

Makilactone L (**8**): Colorless needles, mp 288 °C (H_2O–MeOH). $[\alpha]_D^{24}$ +75.1°(*c*=0.15, MeOH); IR (film) 3436 (OH), 1782 (C=O), 1707 (C=O) cm^{-1}; HR-ESI-MS *m/z*: 417.1160 [Calcd for $C_{19}H_{22}O_9Na$, 417.1162 $(M+Na)^+$]. ^1H- and ^{13}C-NMR, see Tables 1 and 2.

Makilactone M (**9**): Colorless needles, mp 293 °C (H_2O–MeOH). $[\alpha]_D^{24}$

+58.3° (c=0.05, MeOH); IR (film) 3543 (OH), 1761 (C=O), 1707 (C=O) cm^{-1}; HR-ESI-MS m/z: 403.0996 [Calcd for $C_{18}H_{20}O_9Na$, 403.1005 $(M+Na)^+$]. ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Makilactone N (**10**): Colorless needles, mp 273 °C (H_2O–MeOH). $[\alpha]_D^{24}$ +76.4° (c=0.15, MeOH); IR (film) 3392 (OH), 1773 (C=O), 1724 (C=O) cm^{-1}; HR-ESI-MS m/z: 449.1224 [Calcd for $C_{20}H_{26}O_8SNa$, 449.1211 $(M+Na)^+$]. ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Makilactone O (**11**): Colorless needles, mp 292 °C (H_2O–MeOH). $[\alpha]_D^{24}$ +14.3° (c=0.15, MeOH); IR (film) 3366 (OH), 1773 (C=O), 1697 (C=O) cm^{-1}; HR-ESI-MS m/z: 463.1024 [Calcd for $C_{20}H_{24}O_9SNa$, 463.1039 $(M+Na)^+$]. ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Makilactone P (**12**): Colorless needles, mp 271 °C (H_2O–MeOH). $[\alpha]_D^{24}$ +48.3° (c=0.15, MeOH); IR (film) 3362 (OH), 1768 (C=O), 1719 (C=O) cm^{-1}; HR-ESI-MS m/z: 463.1020 [Calcd for $C_{20}H_{24}O_9SNa$, 463.1039 $(M+Na)^+$]. ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Makilactone Q (**13**): Colorless needles, mp 293 °C (H_2O–MeOH). $[\alpha]_D^{24}$ −11.9° (c=0.10, MeOH); IR (film) 3308 (OH), 1760 (C=O), 1698 (C=O) cm^{-1}; HR-ESI-MS m/z: 449.1205 [Calcd for $C_{20}H_{26}O_8SNa$, 449.1246 $(M+Na)^+$]. ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Makilctone R (**14**): Colorless needles, mp 292 °C(H_2O–MeOH). $[\alpha]_D^{24}$ −12.4° (c=0.08, MeOH); IR (film) 3371 (OH), 1775 (C=O), 1725 (C=O) cm^{-1}; HR-ESI-MS m/z: 447.1073 [Calcd for $C_{20}H_{24}O_8SNa$, 447.1090 $(M+Na)^+$]. ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Podolactone B (**15**): Colorless needles (H_2O–MeOH), mp 261 °C $[\alpha]_D^{24}$ +12.1° (c 0.10, MeOH); IR (film) 3395 (OH), 1771 (C=O), 1723 (C=O) cm^{-1}; HR-ESI-MS m/z: 417.1185 [Calcd for $C_{19}H_{22}O_9Na$, 417.1162 $(M+Na)^+$]. ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Inumakilactone B (**16**): Colorless needles, mp 288 °C (H_2O–MeOH). $[\alpha]_D^{24}$ +8.9° (c=0.30, MeOH); IR (film) 3518 (OH), 1755 (C=O), 1700 (C=O) cm^{-1}; HR-ESI-MS m/z: 347.1120 [Calcd for $C_{18}H_{19}O_7$, 347.1131 $(M+H)^+$]. ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Oxidation of 13 Oxone® (49.3 mg) was added to a solution of compound **13** (4.9 mg, 0.01 mmol) and $NaHCO_3$ (28.8 mg, 0.34 mmol) in a mixture of Me_2CO (1 ml) and H_2O (0.8 ml) at 0 °C. The mixture was stirred at rt for 0.5 h. After addition of H_2O (10 ml), the mixture was extracted with $CHCl_3$ (10 ml×3). The organic layer was dried over $MgSO_4$, filtered, and evaporated *in vacuo* to give a solid mass, which was purified by ODS-HPLC (30% MeOH) to afford **29** (3.48 mg, 71%) as colorless amorphous solid, mp 260—263 °C; $[\alpha]_D^{24}$ +14.6° (c=0.044, MeOH). HR-ESI-MS m/z: 443.1372 [Calcd for $C_{20}H_{27}O_9S$, 443.1372 $(M+H)^+$]. ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 7.06 (1H, s), 5.34(1H, d, 0.9), 5.06 (1H, d, 4.6), 4.88 (1H, s), 4.55 (1H, d, 15.1), 4.06 (1H, m), 3.80 (1H, d, 15.1), 3.30 (3H, s), 2.39 (1H, m), 2.06 (3H, s), 1.91 (1H, d, 4.6), 1.88 (1H, m), 1.50 (1H, m), 1.40 (3H, s), 1.20 (3H, s). ^{13}C-NMR (pyridine-d_5, 100 MHz) δ: 180.5, 163.4, 157.5, 120.1, 83.9, 72.9, 72.1, 69.0, 59.5, 59.1, 56.4, 44.4, 43.7, 42.0, 41.7, 29.5, 28.2, 26.7, 23.7, 17.0.

Oxidation of 28 From **28** (2.4 mg, 0.005 mmol), **29** was prepared according to the procedure described above. Yield: 67%. $[\alpha]_D^{24}$ +12.6° (c=0.044, MeOH).

Oxidation of 14 From **14** (5.1 mg, 0.01 mmol), **31** was prepared according to the procedure described above. Yield: 69%. Colorless amorphous solid, mp 248—248 °C; $[\alpha]_D^{24}$ +32.1° (c=0.057, MeOH). HR-ESI-MS m/z: 441.1214 [Calcd for $C_{20}H_{25}O_9S$, 441.1219 $(M+H)^+$]. ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 6.20 (1H, s), 5.31 (1H, s), 5.10 (1H, d, 5.1), 4.80 (1H, s), 4.51 (1H, 15.2), 3.77 (1H, d, 15.2), 3.39 (1H, d, 3.7), 3.28 (1H, d, 3.7), 3.27 (3H, s), 2.19 (1H, 13.0), 2.04 (3H, s), 1.81 (1H, d, 5.1), 1.73 (1H, d, 13.0), 1.43 (3H, s), 1.35 (3H, s). ^{13}C-NMR (pyridine-d_5, 100 MHz) δ: 177.1, 162.7, 158.5, 117.1, 83.9, 73.3, 72.1, 59.4, 58.4, 55.9, 52.8, 51.9, 44.4, 43.1, 42.8, 35.8, 30.6, 26.7, 25.6, 21.1.

Oxidation of 30 From **30** (5.1 mg, 0.01 mmol), **31** was prepared according to the procedure described above. Yield: 86%. $[\alpha]_D^{24}$ +32.0° (c=0.057, MeOH).

X-Ray Crystallographic Studies Crystal Data for Makilactone E (**1**): $C_{18}H_{21}ClO_8$; FW 400.80; orthorhombic, space group $P2_12_12_1$, unit cell dimensions a=8.026(5) Å, b=9.671(6) Å, c=21.698(14) Å, V=1684.2(19) Å^3, Z=4; d_{calc}=1.581 Mg m^{-3}; μ (Mo$K\alpha$, λ=0.71073 Å)=0.275 mm^{-1}.[14]

Crystal Data for Makilactone F (**2**): $C_{19}H_{26}O_{10}$; FW 414.40; monoclinic, space group $P2_1$, unit cell dimensions a=9.629(3) Å, b=7.731(2) Å, c=12.605(4) Å, V=917.8(5) Å^3, Z=2; d_{calc}=1.499 Mg m^{-3}; μ (Mo$K\alpha$, λ=0.71073)=0.122 mm^{-1}.[15]

Crystal Data for Makilactone G (**3**): $C_{19}H_{24}O_8$; FW 380.38; space group $C2$, unit cell dimensions a=18.142(4) Å, b=8.2019(19) Å, c=12.666(3) Å, V=1743.6(7) Å^3, Z=4; d_{calc}=1.449 Mg m^{-3}; μ (Mo$K\alpha$, λ=0.71073)=0.113 mm^{-1}.[16]

Crystal Data for Makilactone H (**4**): $C_{18}H_{22}O_7$; FW 350.36; orthorhombic, space group $P2_12_12_1$, unit cell dimensions a=7.8064(8) Å, b=11.7079(11) Å, c=17.5929(17) Å, V=1607.9(3) Å^3, Z=4; d_{calc}=1.447 Mg m^{-3}; μ (Mo$K\alpha$, λ=0.71073)=0.112 mm^{-1}.[17]

Crystal Data for Makilactone I (**5**): $C_{19}H_{26}O_8$; FW 82.40; orthorhombic, space group $P2_12_12_1$, unit cell dimensions a=7.745(5) Å, b=12.253(7) Å, c=18.630(11) Å, V=1768.1(18) Å^3, Z=4; d_{calc}=1.437 Mg m^{-3}; μ (Mo$K\alpha$, λ=0.71073)=0.112 mm^{-1}.[18]

Crystal Data for Makilactone J (**6**): $C_{19}H_{24}O_8$; FW 380.38; orthorhombic, space group $P2_12_12_1$, unit cell dimensions a=7.923(2) Å, b=11.700(3) Å, c=18.240(5) Å, V=1690.8(8) Å^3, Z=4; d_{calc}=1.494 Mg m^{-3}; μ (Mo$K\alpha$, λ=0.71073)=0.117 mm^{-1}.[19]

Crystal Data for Makilactone K (**7**): $C_{19}H_{24}O_9$; FW 396.38; orthorhombic, space group $P2_12_12_1$, unit cell dimensions a=8.000(2) Å, b=11.969(3) Å, c=17.770(5) Å, V=1701.4(8) Å^3, Z=4; d_{calc}=1.547 Mg m^{-3}; μ (Mo$K\alpha$, λ=0.71073)=0.124 mm^{-1}.[20]

Crystal Data for Makilactone L (**8**): $C_{20}H_{26}O_{10}$; FW 426.41; orthorhombic, space group $P2_12_12_1$, unit cell demensions a=6.286(6) Å, b=12.910(13) Å, c=22.70(2) Å, V=1842(3) Å^3, Z=4; d_{calc}=1.537 Mg m^{-3}; μ (Mo$K\alpha$, λ=0.71073)=0.124 mm^{-1}.[21]

Crystal Data for Makilactone M (**9**): $C_{18}H_{20}O_9$; FW 380.34; orthorhombic, space group $P2_12_12_1$, unit cell dimensions a=7.817(5) Å, b=9.657(6) Å, c=20.536(13) Å, V=1550.3(17) Å^3, Z=4; d_{calc}=1.630 Mg m^{-3}; μ (Mo$K\alpha$, λ=0.71073)=0.132 mm^{-1}.[22]

Crystal Data for Makilactone N (**10**): $C_{20}H_{26}O_9S$; FW 442.47; orthorhombic, space group $P2_12_12_1$, unit cell dimensions a=6.130(2) Å, b=13.032(5) Å, c=25.276(9) Å, V=2019.2(12) Å^3, Z=4; d_{calc}=1.455 Mg m^{-3}; μ (Mo$K\alpha$, λ=0.71073)=0.212 mm^{-1}.[23]

Crystal Data for Makilactone O (**11**): $C_{20}H_{24}O_9S$; FW 440.45; orthorhombic, space group $P2_12_12_1$, unit cell dimensions a=5.9157(17) Å, b=12.339(4) Å, c=27.082(8) Å, V=1976.8(10) Å^3, Z=4; d_{calc}=1.480 Mg m^{-3}; μ (Mo$K\alpha$, λ=0.71073)=0.216 mm^{-1}.[24]

Crystal Data for Podolactone B (**15**): $C_{20}H_{26}O_{10}$; FW 426.41; monoclinic, space group $P2_1$, unit cell dimensions a=6.8110(5) Å b=6.7080(5) Å, c=21.0675(15) Å, V=952.58(12) Å^3, Z=2; d_{calc}=1.487 Mg m^{-3}; μ (Mo$K\alpha$, λ=0.71073)=0.120 mm^{-1}.[25]

Assay for Cytotoxic Activity The cytotoxic assay was performed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (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. A portion of 100 μl of the cell suspension was added to each well (3×10^3 cells/well) of a 96 microwell plate (Iwaki, flat bottomed, treated polystyrene) and the plate was 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 the 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 SDS 10% solution in 0.01 N 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.

Acknowledgements This work was supported by a Grant-in-aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. The authors are grateful to Dr. Motoko Watanabe for help with writing the manuscript.

References and Notes

1) Saeki I., Sumitomo M., Kondo T., *Holzforschung*, **24**, 83—86 (1970).
2) Hayashi T., Kakisawa H., Ito S., Chen Y. P., Hsu, H.-Y., *Tetrahedron Lett.*, **1972**, 3385—3388 (1972).
3) Kodama M., Kabuto C., Sunagawa M., Ito S., *Tetrahedron Lett.*, **1977**, 2909—2910 (1977).
4) Omoto T., Yoshida O., *Chem. Pharm. Bull.*, **28**, 1894—1899 (1980).
5) Park H.-S., Takahashi Y., Fukaya H., Aoyagi Y., Takeya K., *J. Nat. Prod.*, **66**, 282—284 (2003).
6) Park H.-S., Yoda N., Fukaya H., Aoyagi Y., Takeya K., *Tetrahedron*, **60**, 171—177 (2004).
7) Park H.-S., Kai N., Fukaya H., Aoyagi Y., Takeya K., *Heterocycles*, **63**, 347—357 (2004).
8) Amaro-Luis J. M., Dignora C. U., *J. Nat. Prod.*, **51**, 1249—1250 (1988).

9) Galbraith M. N., Horn D. H. S., *Chem. Commun.*, **1970**, 170—171 (1970).
10) Ito S., Sunagawa M., Kodama M., Honma H., *Chem. Commun.*, **1971**, 91—93 (1971).
11) Kubo I., Ying B.-P., *Phytochemistry*, **30**, 1967—1969 (1991).
12) Matlin S. A., Prazeres M. A., Bittner M., Silva M., *Phytochemistry*, **23**, 2863—2866 (1984).
13) Arora S. K., Bates R. B., Chou P. C. C., *J. Org. Chem.*, **41**, 2458—2461 (1976).
14) Crystallographic data for compound **1** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 712026. Copies of the data can be obtained, free of charge, on application to the Director, CCDC (e-mail: deposit@ccdc.cam.ac.uk).
15) Crystallographic data for compound **2** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 712027. Copies of the data can be obtained, free of charge, on application to the Director, CCDC (e-mail: deposit@ccdc.cam.ac.uk).
16) Crystallographic data for compound **3** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 712028. Copies of the data can be obtained, free of charge, on application to the Director, CCDC (e-mail: deposit@ccdc.cam.ac.uk).
17) Crystallographic data for compound **4** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 712029. Copies of the data can be obtained, free of charge, on application to the Director, CCDC (e-mail: deposit@ccdc.cam.ac.uk).
18) Crystallographic data for compound **5** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 712030. Copies of the data can be obtained, free of charge, on application to the Director, CCDC (e-mail: deposit@ccdc.cam.ac.uk).
19) Crystallographic data for compound **6** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 712031. Copies of the data can be obtained, free of charge, on application to the Director, CCDC (e-mail: deposit@ccdc.cam.ac.uk).
20) Crystallographic data for compound **7** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 712032. Copies of the data can be obtained, free of charge, on application to the Director, CCDC (e-mail: deposit@ccdc.cam.ac.uk).
21) Crystallographic data for compound **8** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 713233. Copies of the data can be obtained, free of charge, on application to the Director, CCDC (e-mail: deposit@ccdc.cam.ac.uk).
22) Crystallographic data for compound **9** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 712034. Copies of the data can be obtained, free of charge, on application to the Director, CCDC (e-mail: deposit@ccdc.cam.ac.uk).
23) Crystallographic data for compound **10** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 712035. Copies of the data can be obtained, free of charge, on application to the Director, CCDC (e-mail: deposit@ccdc.cam.ac.uk).
24) Crystallographic data for compound **11** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 712036. Copies of the data can be obtained, free of charge, on application to the Director, CCDC (e-mail: deposit@ccdc.cam.ac.uk).
25) Crystallographic data for compound **15** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 712033. Copies of the data can be obtained, free of charge, on application to the Director, CCDC (e-mail: deposit@ccdc.cam.ac.uk).