# $S_R$-Podolactone D, a New Sulfoxide-Containing Norditerpene Dilactone from *Podocarpus macrophyllus* var. *maki*

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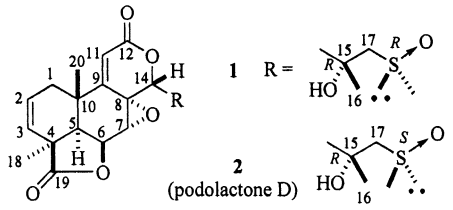
*Received July 25, 2002*

$S_R$-Podolactone D (**1**), a new norditerpene dilactone having a methylsulfoxide moiety, was isolated from the leaves of *Podocarpus macrophyllus* D. Don var. *maki* Endl. along with known podolactone D (**2**, $S_S$-podolactone D). The structures and absolute configurations of compounds **1** and **2** were elucidated by spectral methods (HREIMS, IR, $^1$H, $^{13}$C, and 2D NMR) and finally confirmed by single-crystal X-ray analyses. The cytotoxic effects of compounds **1** and **2** on P388 murine leukemia cells were also examined.

A number of podolactones, norditerpene dilactones, have been isolated from plants of the genus *Podocarpus* (family Podocarpaceae)[1] and filamentous fungi.[2,3] Because of their antitumor,[4−6] insecticidal,[7] antifeedant,[8] allelopathic,[9] and fungicidal activities,[10] these compounds are of considerable pharmaceutical interest. In continuation of our studies on antitumor agents from higher plants, a MeOH extract of the leaves of *Podocarpus macrophyllus* var. *maki* yielded a new compound, epipodolactone D (**1**), and the known podolactone D (**2**).[11] Both compounds are norditerpene dilactones with a methylsulfoxide moiety. In this paper, we describe their isolation, structural elucidation, and cytotoxic activities.




The leaves of *Podocarpus macrophyllus* var. *maki* were extracted with hot MeOH, and the crude MeOH extract was partitioned between $H_2O$ and hexane. The aqueous layer was extracted with $CHCl_3$. The $CHCl_3$ extract, showing a moderate cytotoxicity ($IC_{50}$ 16 $\mu$g/mL) on the P388 cell line, was subjected to silica gel column chromatography and then Diaion HP-20 column chromatography. Further purification by reversed-phase HPLC gave compounds **1** and **2** (See Experimental Section).

Compound **2** was obtained as colorless needles, whose spectral data including HREIMS, IR, and $^1$H and $^{13}$C NMR were identical with those of podolactone D,[11] originally isolated from *Podocarpus neriifolius* by Galbraith et al.[11] Key NOESY correlations are shown in Figure 1. However, the stereochemistry at C-15 and that of the sulfur atom were not established, and the compound possessed a 1,2-double bond in the A ring system. Arora et al. reported that the compound possessed a 2,3-double bond rather than a 1,2-double bond, on the basis of the X-ray analysis[5] of a related compound, podolide.[12] Our present 2D NMR (HMQC, HMBC, and NOESY) spectral data showed that both compounds should possess a 2,3-double bond in the A ring system, and a single-crystal X-ray diffraction analysis of **2** determined that the stereochemistry at C-15 and that of the sulfur atom were 15*R* and $S_S$, respectively. Thus, podolactone D (**2**) was determined to have the structure shown by the ORTEP representation in Figure 2.

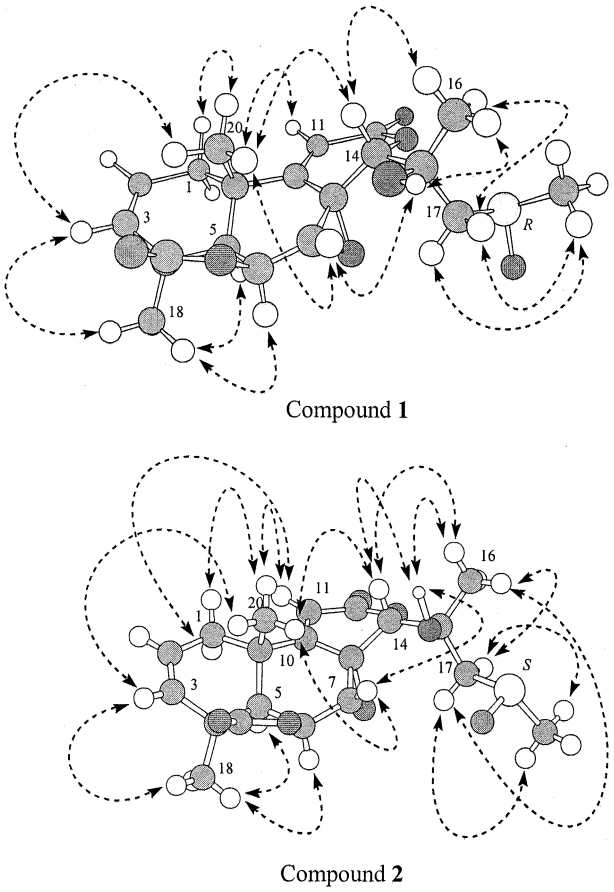



**Figure 1.** Key NOESY correlations observed in compounds **1** and **2**.

Compound **1** was obtained as colorless needles. The HREIMS of both **1** and **2** was appropriate for molecular formula $C_{20}H_{24}O_7S$. The IR spectra suggested lactone carbonyl (1774 cm$^{-1}$), $\alpha,\beta$-unsaturated lactone carbonyl (1727 cm$^{-1}$), hydroxyl (3583 cm$^{-1}$), and sulfoxide (1024 cm$^{-1}$) groups. The $^1$H and $^{13}$C NMR spectra of **1** were very similar to those of **2**,[11] except that the proton signals of Me-17 and -17b of compound **1** appeared at lower field than those of **2** in the $^1$H NMR spectrum, and the carbon signals for C-14, C-15, C-16, and C-17 were slightly different from those for the corresponding carbons in compound **2** (see

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10.1021/np020334x CCC: $25.00 
Published on Web 01/01/2003

**Table 1.** $^1$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) Spectral Data for Compounds **1** and **2** in Pyridine-$d_5$ at 300 K

| | rakanmakilactone A (**1**) | | | podolactone D (**2**) | | |
|---|---|---|---|---|---|---|
| position | $\delta_C$ | $\delta_H$[a] | HMBC ($^1$H) | $\delta_C$ | $\delta_H$[a] | HMBC ($^1$H) |
| 1a | 32.5 | 2.04 (1H, br s) | 2, 3, 5, 20-Me | 32.3 | 2.01 (1H, br t) | 2, 3, 5, 20-Me |
| 1b | | 2.02 (1H, d, 6.0) | | | 1.99 (1H, dd, 6.0, 1.5) | |
| 2 | 126.4 | 5.78 (1H, m) | 1a, 1b | 126.2 | 5.76 (1H, ddd, 9.8, 6.0, 2.3) | 1a, 1b, 5 |
| 3 | 128.4 | 5.90 (1H, br d, 9.8) | 1a, 1b, 18-Me | 128.2 | 5.87 (1H, dd, 9.8, 1.5) | 1a, 1b, 18-Me |
| 4 | 44.2 | | 2, 3, 5, 18-Me | 44.0 | | 3, 5, 18-Me |
| 5 | 42.9 | 2.08 (1H, d, 4.9) | 1a, 1b, 3, 6, 7, 11, 18-Me, 20-Me | 42.7 | 2.04 (1H, d, 5.0) | 1a, 1b, 3, 6, 7, 1,, 18-Me, 20-Me |
| 6 | 72.3 | 5.14 (1H, d, 4.9) | 7 | 72.1 | 5.03 (1H, dd, 4.9, 1.0) | 7, 16-Me |
| 7 | 55.9 | 5.34 (1H, s) | 6 | 55.6 | 5.26 (1H, d, 1.0) | 6 |
| 8 | 58.2 | | 6, 7, 11, 14 | 58 | | 6, 7, 11, 14 |
| 9 | 158.4 | | 5, 11, 20-Me | 158.4 | | 1a, 20-Me |
| 10 | 35.6 | | 1a, 1b, 2, 5, 6, 11, 20-Me | 158.4 | | 1a, 1b, 5, 6, 11, 20-Me |
| 11 | 117.5 | 6.23 (1H, s) | 7 | 117.2 | 6.20 (1H, s) | |
| 12 | 163.1 | | 11 | 162.9 | | 11 |
| 14 | 83.7 | 4.85 | 6, 16-Me, 17a, 17b, OH | 82.9 | 4.91 (1H, s) | 6, 16-Me |
| 15 | 72.5 | | 7, 14, 16-Me, 17a, 17b, OH | 73.2 | | 14, 16-Me, 17a, 17b, SOMe |
| 16 | 28.2 | 1.99 (3H, s) | 14, 17a, 17b | 27.5 | 1.86 (3H, s) | 14, 17a, 17b |
| 17a | 63.1 | 3.42 (1H, d, 13.7) | 14, 16-Me | 61.7 | 3.42 (1H, d, 13.7) | 14, 16-Me, SOMe |
| 17b | | 3.93 (1H, d, 13.7) | SOMe, OH | | 3.77 (1H, d, 13.7) | |
| 18 | 22.5 | 1.29 (3H, s) | 3,5 | 22.3 | 1.26 (3H, s) | 5 |
| 19 | 178.0 | | 5, 18-Me | 177.8 | | 5, 18-Me |
| 20 | 22.7 | 1.12 (3H, s) | 1a, 1b, 5, 11 | 22.5 | 1.13 (3H, s) | 1a, 1b, 5 |
| SOMe | 40.7 | 2.70 (3H, s) | 17a, 17b | 440.1 | 2.66 (3H, s) | 17a, 17b |
| OH | | 8.16 (1H, s) | | | 7.56 (1H, s) | |

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

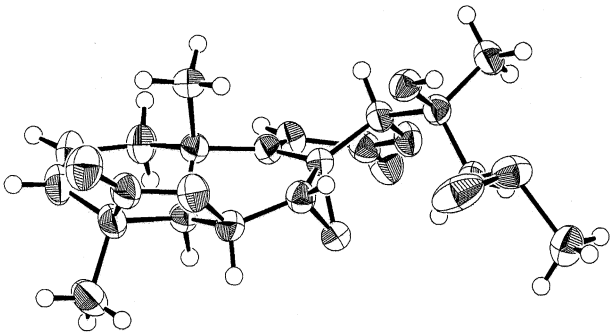

**Figure 2.** ORTEP representation of compound **2** ($S_S$-podolactone D) as determined by single-crystal X-ray analysis.

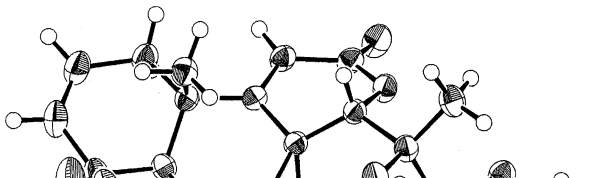

**Figure 3.** ORTEP representation of compound **1** ($S_R$-podolactone D) as determined by single-crystal X-ray analysis.

Table 1). In the NOESY experiment, NOEs were observed between Me-16 and 17a and 17b in **2**, whereas only between Me-16 and 17a in **1**. These facts revealed that **1** and **2** are epimers having opposite configurations at the sulfoxide group. Compound **1** crystallized in an infrequently observed space group *P*1, with an asymmetric unit (Figure 3). The absolute structure of **1** was determined to be $S_R$-podolactone D, as shown (Figure 3).

Compounds **1** and **2** showed moderate cytotoxic activity on P388 murine leukemia cells with $IC_{50}$ values of 0.52 and 0.23 $\mu$g/mL, respectively, indicating that the sulfur atom stereochemistry slightly affected the cytotoxic activity.

In summary, known **2** and its epimer at the sulfur atom, **1**, were isolated from a MeOH extract of the leaves of *Podocarpus macrophyllus* var. *maki*. The structural studies determined that both compounds possess a 2,3-double bond system and that the stereochemistry at C-15 and that of sulfur atom in **1** were 15*R* and $S_R$, respectively, whereas those of **2** were 15*R* and $S_S$, respectively.

## Experimental Section

**General Experimental Procedures.** Optical rotations were determined on a JASCO DIP-360 digital polarimeter, and IR spectra were recorded on a JASCO FT/IR 620 spectrophotometer. NMR spectra were obtained on a Bruker DRX-500 spectrometer at 300 K. The chemical shifts ($\delta$) are reported in ppm relative to the residual $C_5D_4HN$ resonance at 7.19 ppm for $^1$H NMR and to the $C_5D_5N$ resonance at 135.3 ppm for $^{13}$C NMR. Mass spectra were obtained with a VG AutoSpec E spectrometer. Preparative HPLC was carried out on a JASCO PU-986 equipped with a UV-970 UV detector ($\lambda$ 220 nm) and a Inertsil PREP-ODS column, (10 $\mu$m, 20 × 250 mm), by using MeCN−$H_2O$ (15:85, v/v) 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 ($\lambda$ = 0.71073 Å).[13−16]

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

**Extraction and Isolation.** The air-dried leaves (3.5 kg) were extracted with hot MeOH (16 L × 3 times). The solvent was removed to give a crude MeOH extract (600 g), which was partitioned between hexane (2 L) and $H_2O$ (2 L). The aqueous layer was extracted with $CHCl_3$ (2 L × 3). The $CHCl_3$ extract (6 L) was evaporated in vacuo to give a residue (40 g), which was applied to a silica gel column (Merck Kieselgel 60, 1.2 kg, 70−230 mesh) and eluted with $CHCl_3$ containing an increasing amount of MeOH. The $CHCl_3$−MeOH (20:1, v/v) fraction (3.2 g) was subjected to ion-exchange resin (DIAION HP-20, 50 g) column chromatography using a $H_2O$−MeOH gradient system. The fraction eluted with $H_2O$−MeOH (75:25, v/v) was concen-

trated in vacuo to give a residue (0.17 g), which was then repeatedly chromatographed on ODS HPLC using both eluting systems MeOH−$H_2O$ (35:65, v/v) and MeCN−$H_2O$ (85:15, v/v), to give compounds **1** (11.9 mg, $t_R$ 146 min) and **2** (8.4 mg, $t_R$ 154 min).

***S_R*-Podolactone D (1):** colorless needles (EtOAc−hexane); mp 201−203 °C; $[\alpha]_D$ +22.8° (*c* 0.18, MeOH); IR (film) $\nu_{max}$ 3583, 1774, 1727, 1454, 1024 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, Table 1; HREIMS *m*/*z* 408.1255 (calcd for $C_{20}H_{24}O_7S$, 408.1243).

**Podolactone D (2):** colorless needles (EtOAc−hexane); mp 200−202 °C (lit.[11] mp 261−266 °C); $[\alpha]_D$ +60.0° (*c* 0.12, MeOH); IR (film) $\nu_{max}$ 3584, 1769, 1721, 1007 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, Table 1; HREIMS *m*/*z* 408.1236 (calcd for $C_{20}H_{24}O_7S$, 408.1243).

**X-ray Crystallographic Studies.[17] Crystal data for *S_R*-podolactone D (1):** $C_{20}H_{24}O_7S$; fw 408.469; colorless needles, triclinic, space group *P*1, unit cell dimensions $a = 6.2490(8)$ Å, $b = 7.135(2)$ Å, $c = 11.525(2)$ Å, $V = 479.3(2)$ $Å^3$, $Z = 1$; $d_{calc} = 1.415$ Mg $m^{-3}$; $\mu$ (M*o* K$\alpha$, $\lambda = 0.71073$ Å) = 0.21 $mm^{-1}$.

**Crystal data for podolactone D (2):** $C_{20}H_{24}O_7S$; fw 408.469; colorless needles, orthorhombic, space group $P2_12_12_1$, unit cell dimensions $a = 7.6030(5)$ Å, $b = 10.468(2)$ Å, $c = 24.782(3)$ Å, $V = 1972.4(4)$ $Å^3$, $Z = 4$; $d_{calc} = 1.376$ Mg $m^{-3}$; $\mu$ (M*o* K$\alpha$, $\lambda = 0.71073$ Å) = 0.20 $mm^{-1}$.

**Supporting Information Available:** X-ray crystallographic data for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Crystallographic data for **1** and **2** reported in this paper have been deposited at the Cambridge Crystallographic Data Centre, under the reference numbers CCDC-189959 and -189958. 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).

NP020334X