

Potent Antiviral Potamogetonyde and Potamogetonol, New Furanoid Labdane Diterpenes from *Potamogeton malaianus*

Prasat Kittakoop,^{*,†} Supakit Wanasith,[†] Patricia Watts,[†] Jarin Kramyu,[†] Morakot Tanticharoen,[†] and Yodhathai Thebtaranonth^{†,‡}

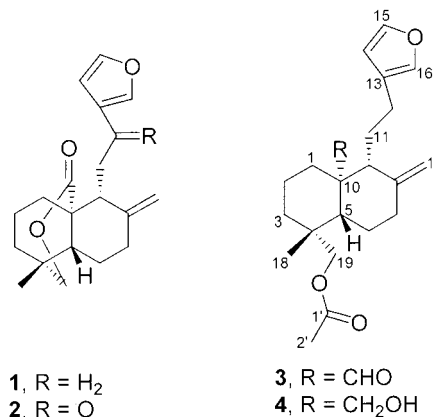
National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 73/1, Rama VI Road, Rajdhevee, Bangkok 10400, Thailand, and Department of Chemistry, Faculty of Science, Mahidol University, Rajdhevee, Bangkok 10400, Thailand

Received September 14, 2000

New furanoid labdane diterpenes, potamogetonyde (**3**) and potamogetonol (**4**), together with two known compounds, potamogetonin (**1**) and 15,16-epoxy-12-oxo-8(17),13(16),14-labdatrien-20,19-olide (**2**), were isolated from the CH_2Cl_2 extract of *Potamogeton malaianus*. The chemical structures of **1–4** were elucidated by the analyses of their spectral data, mainly by 1D and 2D NMR techniques. Potamogetonyde (**3**) and potamogetonol (**4**) exhibited potent antiviral (HSV-1) activity with respective IC_{50} values of 8 and 3 $\mu\text{g/mL}$. Compounds **1–4** possessed cytotoxicity toward insect cells (fall armyworm and mosquito larvae, IC_{50} of 11–72 $\mu\text{g/mL}$). Furanoid diterpenes **3** and **4** also exhibited cytotoxicity against the Vero cell line with respective IC_{50} 's of 31 and 28 $\mu\text{g/mL}$, while **1** and **2** were inactive at 50 $\mu\text{g/mL}$. Compounds **1–4** were inactive (at 20 $\mu\text{g/mL}$) against KB and BC cell lines and showed only weak antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra with minimum inhibitory concentrations of 50–100 $\mu\text{g/mL}$.

Covering an area of 3 km^2 in the northeast of Mahasarakham Province of Thailand, Borabue, a once fertile freshwater swamp bustling with a diversity of plants and animals now stands quiet and still, its salt-polluted water no longer able to sustain any but the most salt-tolerant forms of aquatic and terrestrial lives. The invasion of Borabue began around 1970, when salt mining was introduced to the area, and the activity increased and multiplied throughout the next decade to feed salt factories. Underground rock salt is mined by forcing down hot water to bring salt to the surface in the form of saturated salt solution. This resulted in gradual seepage of salt into the swamp, and by 1980 when the Thai government announced a ban on salt mining in the area, the Borabue swamp had been completely destroyed and transformed into a salted water reservoir. In the summer (March to April), the salt levels are up to 15–23 g/mL, but concentrations decrease to 10–13 g/mL in the rainy season (May to October). Totally different plants from those previously found in the freshwater ecology have progressively emerged; among these emerging high salt-tolerant water plants, *Potamogeton malaianus* Miq (family Potamogetonaceae) has been a uniquely dominant species to date, covering 30–40% of the reservoir area. As part of our ongoing research program on biologically active compounds from plants and microorganisms conducted at the National Center for Genetic Engineering and Biotechnology (BIOTEC),¹ we have been interested in investigating biologically active substances in *P. malaianus*. In this paper, we describe the isolation and structural elucidation of two new furanoid labdane diterpenes, namely, potamogetonyde (**3**) and potamogetonol (**4**), together with two known compounds, potamogetonin (**1**)^{2–4} and 15,16-epoxy-12-oxo-8(17),13(16),14-labdatrien-20,19-olide (**2**).⁵ The biological activities of the isolated compounds, which include antiviral and antimycobacte-

rium properties and cytotoxicity against insect cell lines (Sf9 and C6/36), BC, KB, and Vero cell lines, are also reported.



The CH_2Cl_2 extract of *P. malaianus* was sequentially purified with Sephadex LH-20 column chromatography and HPLC (C_{18} reversed-phase column), yielding potamogetonin (**1**), 15,16-epoxy-12-oxo-8(17),13(16),14-labdatrien-20,19-olide (**2**), potamogetonyde (**3**), and potamogetonol (**4**). The ^1H and ^{13}C NMR spectral data of the isolated potamogetonin (**1**) and 15,16-epoxy-12-oxo-8(17),13(16),14-labdatrien-20,19-olide (**2**) were in accordance with published values.^{2–5}

The ^1H NMR spectral data of potamogetonyde (**3**) showed similarities to that of potamogetonin (**1**), except an additional singlet methyl at δ_{H} 1.99 and an aldehyde signal at δ_{H} 9.80. The spectrum of **3** also revealed the typical characteristics of a furanoid moiety at δ_{H} 6.20 (br s), 7.15 (br s), and 7.30 (br s) and exo-methylene signals at δ_{H} 4.61 (br s) and 4.93 (br s). The ^{13}C NMR spectrum of potamogetonyde (**3**) showed 22 signals attributable to (as determined by the DEPT spectra) six methine, nine methylene, two methyl, and five quaternary carbons. The ^1H – ^1H COSY and HMQC spectral data of potamogetonyde (**3**) were employed for the assignment of protons connected to their respective carbons. The downfield shift (δ_{H} 3.72 and

* To whom correspondence should be addressed at the BIOTEC. Tel: +662-6448103. Fax: +662-6448107. E-mail: prasat@biotec.or.th.

[†] National Center for Genetic Engineering and Biotechnology.

[‡] Department of Chemistry, Mahidol University.

Table 1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR Spectral Data (CDCl_3) of Potamogetonyde (**3**) and Potamogetonol (**4**)

| C | δ_{C} , multiplicity ^a | | δ_{H} , multiplicity, J in Hz | |
|---------------------|---|----------|---|---|
| | 3 | 4 | 3 | 4 |
| 1 | 31.6, t | 33.5, t | 0.73, ddd, 3.4, 12.0, 12.0 (H-1ax) 2.47, m (H-1eq) | 0.85, ddd, 4.8, 13.5, 13.5 (H-1ax) 2.04, m (H-1eq) |
| 2 | 18.9, t | 18.8, t | 1.40, m (H-2ax) 1.47, m (H-2eq) | 1.50, m (H-2ax) 1.52, m (H-2eq) |
| 3 | 36.3, t | 36.1, t | 1.06, ddd, 4.7, 12.1, 12.1 (H-3ax) 1.68, m (H-3eq) | 1.05, ddd, 4.6, 13.2, 13.2 (H-3ax) 1.74, m (H-3eq) |
| 4 | 37.1, s | 37.2, s | | |
| 5 | 54.2, d | 55.9, d | 1.63, m | 1.40, dd, 3.2, 13.3 |
| 6 | 23.8, t | 24.4, t | 2.04, m (H-6ax) 2.13, m (H-6eq) | 1.69, m (H-6ax) 1.89, m (H-6eq) |
| 7 | 37.9, t | 38.2, t | 2.20, m (H-7ax) 2.64, ddd, 3.2, 3.2, 12.3 (H-7eq) | 2.07, m (H-7ax) 2.50, m (H-7eq) |
| 8 | 145.4, s | 149.8, s | | |
| 9 | 52.3, d | 55.5, d | 1.83, br d, 11.3 | 1.73, m |
| 10 | 53.4, s | 43.9, s | | |
| 11 | 24.1, t | 25.1, t | 1.50, m; 1.72, m | 1.67, m; 1.87, m |
| 12 | 22.5, t | 23.8, t | 2.24, m; 2.52, m | 2.23, m; 2.60, m |
| 13 | 124.4, s | 125.0, s | | |
| 14 | 110.6, d | 110.8, d | 6.20, br s | 6.27, br s |
| 15 | 142.6, d | 42.6, d | 7.30, br s | 7.36, br s |
| 16 | 138.6, d | 138.7, d | 7.15, br s | 7.21, br s |
| 17 | 107.7, t | 106.8, t | 4.61, br s; 4.93, br s | 4.81, br s; 4.95, br s |
| 18 | 26.4, q | 27.7, q | 0.96, s | 0.99, s |
| 19 | 66.2, t | 66.7, t | 3.72, d, 11.2 3.88, d, 11.2 9.80, s | 4.28, d, 11.2 3.98, d, 11.2 3.66, d, 12.2 3.74, d, 12.2 2.05, s |
| | 206.6, d | 63.8, t | | |
| OCOCH ₃ | 20.7, q | 20.9, q | 1.99, s | |
| OCHOCH ₃ | 170.7, s | 171.2, s | | |

^a Multiplicity was determined by analyses of the DEPT spectra.

3.88, AB system, $J = 11.2$ Hz) of the nonequivalent methylene protons attached to an oxygen atom (H-19) suggested the presence of an ester linkage in **3**, and the HMBC spectrum (optimized for $^nJ_{\text{HC}} = 4.0$ Hz) of potamogetonyde (**3**) clearly demonstrated the presence of the acetate linked to the oxygen at C-19 (cross-peaks observed from H-19 to C-1', C-4, and C-5, and H-2' to C-1'). The IR absorption peak at 1741 cm^{-1} also confirmed the ester functionality in **3**. The aldehyde group at C-20 in potamogetonyde (**3**) (δ_{H} 9.80, s; δ_{C} 206.6) could readily be assigned by the HMBC spectral data; correlations were evident from H-20 to C-1 and C-10, and H-9 to C-10 and C-20. The $^2J_{\text{HC}}$ of 21 Hz for the aldehyde proton (H-20) and C-10 was obtained from the HMBC spectrum of **3**. Analyses of the ^1H – ^1H COSY and HMBC spectral data of potamogetonyde (**3**) led to the complete assignment of protons and carbons in **3** (Table 1). The ^1H – ^1H COSY spectrum of potamogetonyde (**3**) showed the connectivity from H-1 to H-3, and H-5 to H-7, and also revealed that H-9 is coupled to H-11, and H-11 coupled to H-12. The HMBC spectrum of potamogetonyde (**3**) demonstrated the correlations of H-1 to C-9, H-3 to C-18, H-18 to C-4 and C-5, H-6 to C-10, H-7 to C-8 and C-17, H-9 to C-10 and C-20, H-11 to C-13, H-12 to C-13, C-14, and C-16, H-15 to C-13, and H-16 to C-13 and C-14. The ESI-TOF mass spectrum of potamogetonyde (**3**) deduced a molecular formula of **3** as $\text{C}_{22}\text{H}_{30}\text{O}_4$ [observed m/z 359.2204 ($\text{M} + \text{H}$)⁺, $\Delta -1.8$ mmu]. On the basis of these spectral data, the chemical structure of potamogetonyde (**3**) was thus conclusively elucidated.

Potamogetonyde (**3**) is a derivative of potamogetonin (**1**), which had previously been isolated from *P. ferrugineus* and chemically identified in 1976.² The chemical structure of potamogetonin (**1**) was subsequently revised in 1983–1984,^{3,4} and its absolute stereochemistry was also successfully established by the semisynthesis of **1** from stereochemically known diterpenoid analogues.³ Potamogetonyde

(**3**) exhibited a positive optical rotation similar to that of potamogetonin (**1**). It is therefore assumed that potamogetonyde (**3**) has the same absolute configuration as that of potamogetonin (**1**). The configurations of protons in potamogetonyde (**3**) were successfully assigned by analyses of the J values and ^1H – ^1H COSY and NOESY spectral data (Table 1). The NOESY spectrum of potamogetonyde (**3**) showed cross-peaks between H-1ax and H-3ax, H-1ax and H-5, H-1ax and H-9, H-5 and H-9, H-7ax and H-5, H-7ax and H-9, H-19 and H-2ax, and H-19 and H-20; this evidence supports the proposed stereochemistry of potamogetonyde (**3**).

The ^1H and ^{13}C NMR spectral data of potamogetonol (**4**) were generally similar to those of potamogetonyde (**3**), except that the aldehyde signal (δ_{H} 9.80, s) in **3** was replaced by the $-\text{CH}_2\text{OH}$ resonances (δ_{H} 3.66 and 3.74, AB system, $J = 12.2$ Hz) in **4**. The ^1H NMR spectrum of potamogetonol (**4**) showed the typical set of furanoid resonances at δ_{H} 6.27 (br s), 7.21 (br s), and 7.36 (br s), as well as exo-methylene signals at δ_{H} 4.81 (br s) and 4.95 (br s). The ^{13}C NMR spectral data of potamogetonol (**4**) exhibited 22 signals, which were classified by analyses of the DEPT spectra as five methine, 10 methylene, two methyl, and five quaternary carbons. In a fashion similar to that of potamogetonyde (**3**), the ^1H – ^1H COSY, HMQC, and HMBC spectral data were employed for the complete assignment of protons and carbons in potamogetonol (**4**) (Table 1). The ^1H – ^1H COSY spectrum of potamogetonol (**4**) readily demonstrated the connectivity from H-1 to H-3, H-5 to H-7, and H-9 to H-12. In the HMBC spectrum of potamogetonol (**4**), there were correlations from H-3 to C-4, C-5, and C-18; H-5 to C-4 and C-18; H-6 to C-4; H-7 to C-8 and C-17; H-9 to C-12; H-11 to C-13; H-12 to C-13, C-14, and C-16; H-15 to C-13; H-16 to C-13; H-17 to C-7 and C-9; H-19 to C-3, C-18, and C-1'; and H-2' to C-1'. A molecular formula of $\text{C}_{22}\text{H}_{32}\text{O}_4$ for potamogetonol (**4**) was obtained

Table 2. Biological Activities of Compounds **1–4**

| compound | cytotoxicity (IC ₅₀ , µg/mL) | | | | | antivirus (HSV-1) (IC ₅₀ , µg/mL) |
|----------|---|--------------------|------------------------|-----------------|-----------------|---|
| | Sf9 ^a | C6/36 ^b | Vero cell ^c | KB ^d | BC ^e | |
| 1 | 15 | 22 | >50 | >20 | >20 | >20 |
| 2 | 26 | 72 | >50 | >20 | >20 | >20 |
| 3 | 11 | 30 | 31 | >20 | >20 | 8 |
| 4 | 23 | 43 | 28 | >20 | >20 | 3 |

^a Ovary cells of fall armyworm (*Spodoptera frugiperda*). ^b Cells of mosquito larvae (*Aedes albopictus*). ^c Kidney fibroblast of an African green monkey. ^d Human epidermoid carcinoma of the mouth. ^e Human breast cancer cells.

from the ESI-TOF mass spectrum [observed m/z 361.2377 ($M + H$)⁺, Δ -0.2 mmu]. The IR absorption peak at 1741 cm⁻¹ indicated the presence of the ester group in **4**. On the basis of these available spectral data, the chemical structure of potamogetonol (**4**) was therefore secured.

Potamogetonol (**4**) also showed a positive optical rotation similar to that of potamogetonin (**1**); thus, it is assumed that the absolute stereochemistry of potamogetonol (**4**) was the same as that of potamogetonin (**1**). The analyses of the J values, and ¹H–¹H COSY and NOESY spectral data, assisted in the assignments of the proton's orientations in potamogetonol (**4**) (Table 1). In the NOESY spectrum of potamogetonol (**4**), there were again the NOE correlations from H-1ax to H-3ax, H-5, and H-9; H-5 to H-9; H-7ax to H-5 and H-9; and H-19 to H-2ax and H-20, confirming the proposed configuration.

Potamogetonyde (**3**) and potamogetonol (**4**) are possibly biogenetically derived from potamogetonin (**1**) by the cleavage of the lactone ring followed by acetylation. New furanoid diterpenes, potamogetonyde (**3**) and potamogetonol (**4**), exhibited potent antiviral (HSV-1) activity with IC₅₀'s of 8 and 3 µg/mL, respectively, while they showed weak cytotoxicity toward the Vero cell line (Table 2). It is worth noting that labdane diterpenes **1** and **2** were found to be inactive against HSV-1 at 20 µg/mL (Table 2). Compounds **1–4** were inactive at 20 µg/mL against BC and KB cells (Table 2). The furanoid diterpenes **1–4** exhibited weak activity against the mycobacterium *Mycobacterium tuberculosis* H37Ra (minimum inhibitory concentrations (MIC) of 100 µg/mL for **1–3** and 50 µg/mL for **4**). Diterpenoids **1–4** also possessed cytotoxicity toward insect cell lines (Sf9, fall armyworm; and C6/36, mosquito larvae) (Table 2). Potamogetonin (**1**) and potamogetonyde (**3**) were most active against the Sf9 cells (respective IC₅₀'s of 15 and 11 µg/mL for **1** and **3**) (Table 2).

Recently, there has been a report that tetranortriterpenoids isolated from the plant *Cedrela odorata* are associated with leaf rejection by the polyphagous weevil *Exophthalmus jekelianus*.⁶ In the present study, cytotoxicity toward insect cells (fall armyworm and mosquito larvae) of the isolated furanoid labdane diterpenes **1–4** implied that *P. malaianus* might produce these secondary metabolites for its defensive purposes, protecting them from insects or herbivores (mollusc and fish). This fact together with the salt tolerance ability of *P. malaianus* might be responsible for the dominance of this plant in Borabue reservoir.

To date, potamogetonin (**1**)^{2–4} and 15,16-epoxy-12-oxo-8(17),13(16),14-labdatrien-20,19-olide (**2**)⁵ have been isolated from *P. ferrugineus* and *P. nodosus*, respectively. This present work reports two additional new furanoid labdane diterpenes, potamogetonyde (**3**) and potamogetonol (**4**), in *P. malaianus*, and these labdane diterpenes are likely to be the characteristic metabolites in the plant genus *Pota-*

mogeton; as such, this chemical characteristic might be applicable for the chemotaxonomy of this plant genus.

Experimental Section

General Experimental Procedures. ¹H, ¹³C, DEPTs,⁷ ¹H–¹H COSY,⁸ NOESY,⁹ HMQC,¹⁰ and HMBC¹¹ experiments were carried out on a Bruker DRX 400 NMR spectrometer, operating at 400 MHz for proton and 100 MHz for carbon. ESI-TOF mass spectra were obtained from a Micromass LCT mass spectrometer, and the lock mass calibration was applied for the determination of the accurate mass. IR spectra and optical rotations were measured on a Perkin-Elmer 2000 spectrometer and Jasco DIP370 polarimeter, respectively. UV spectra were recorded on a Cary 1E UV–VIS spectrophotometer.

Plant Material. *P. malaianus* Miq was collected in January 2000, from Borabue reservoir, Mahasarakham Province, Thailand, and identified by Dr. Wongsatit Chuakul, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Thailand. A voucher specimen (no. PS13060031) has been deposited at the National Center for Genetic Engineering and Biotechnology, Thailand.

Extraction and Isolation. Air-dried *P. malaianus* (whole plant, 0.8 kg) was macerated in CH₂Cl₂ (4 L) for two nights. The extract was filtered and solvent evaporated, to yield 6 g of a crude extract. The CH₂Cl₂ extract was partially purified by Sephadex LH-20 column chromatography (MeOH as eluent) from which 21 fractions (100 mL each) were collected. Fractions 10–13 were combined and further purified by a preparative HPLC (reversed-phase C₁₈ column, eluted with MeCN–H₂O, 60:40), yielding potamogetonin (**1**) (95 mg), 15,16-epoxy-12-oxo-8(17),13(16),14-labdatrien-20,19-olide (**2**) (50 mg), potamogetonyde (**3**) (90 mg), and potamogetonol (**4**) (75 mg).

Bioassays. Antiviral Activity. The colorimetric method previously described by Skehan and co-workers¹² was employed for antiviral assay. Herpes simplex virus type 1 (HSV-1) was maintained in the Vero cell line (kidney fibroblast of an African green monkey), which was cultured in Eagle's minimum essential medium (MEM) with the addition of heat-inactivated fetal bovine serum (FBS) (10%) and antibiotics. The test samples were put into wells of a microtiter plate at final concentrations ranging from 20 to 50 µg/mL. The viral HSV-1 (30 PFU) was added into a 96-well plate, followed by plating of Vero cells (1 × 10⁵ cells/mL); the final volume was 200 µL. After incubation at 37 °C for 72 h, under 5% of CO₂ atmosphere, cells were fixed and stained, and optical density was measured at 510 nm. Under the screening conditions, the reference compound, Acyclovir, typically exhibited the antiviral HSV-1 with the IC₅₀ of 2–5 µg/mL.

Cytotoxicity to Insect Cell Lines. Sf9 cells (*Spodoptera frugiperda*, fall armyworm, ovary; European Collection of Animal Cell Culture (ECACC) No. 89070101) were cultured in TC100 with 10% fetal calf serum (FCS) and 1.1 g/L sodium bicarbonate, and C6/36 cells (*Aedes albopictus*, mosquito larvae; ECACC No. 89051705) were cultured in MEM, 10% FCS and 1.1 g/L Na₂CO₃. The insect cells were incubated at 28 °C. All cells were incubated for 48 h to allow attachment and initiation of growth before applying samples. The stock cells were cultured antibiotic-free and monitored for mycoplasma. Antibiotics, penicillin and streptomycin, were added to test samples. Cells were plated at an optimum concentration in a 96-well plate (cells/well) in 200 µL/well of medium. Optimum culture conditions of medium and cell seeding density for each cell line were determined: 4 × 10³ cells/well for Sf9 and 1.6 × 10⁴ cells/well for C6/36 cells. The test samples were diluted 1:100 in growth medium to give a final concentration of 200 µg/mL in 1% DMSO/medium. They were subsequently serially diluted in growth medium at a ratio of 1:2, giving concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 µg/mL. After 48 h the growth medium was aspirated from the wells and replaced with the sample at the various concentrations. The cells were exposed to the sample for 24 h. The medium with toxin was then aspirated and replaced with fresh medium, and the cultures were incubated for a further 24 h.

The cytotoxicity of the samples was quantified with a modified MTT colorimetric assay,¹³ which measures the metabolic conversion in the mitochondria of tetrazolium (blue) to formazan (yellow). Briefly, 50 μ L of 3-[4.5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) in PBS (2 mg/mL) was added to the medium in each well, and the cells were incubated for 4 h. Medium and MTT were then aspirated from the wells, and formazan was solubilized with 200 μ L of dimethyl sulfoxide (DMSO) and 25 μ L of Sorensen's glycine buffer, pH 10.5. The optical density was read with a plate reader at a wavelength of 570 nm. The average of four wells was used to determine the mean of each point. The first and last columns contained medium only and were used as the blank. The data were analyzed with the SoftMax program (Molecular Devices) to determine the dose required to inhibit 50% of the metabolic competence (IC_{50}) of the cells for each sample. Two controls were set up, one with medium and the second with DMSO (1%) in the medium, results from which showed no difference between the two controls (data not shown). Under these conditions, mitomycin exhibited an IC_{50} of 3 μ g/mL.

Antimycobacterial Test and Cytotoxicity toward BC, KB, and Vero Cell Lines. The antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA).¹⁴ Standard drugs, isoniazide (MIC of 0.040–0.090 μ g/mL) and kanamycin sulfate (MIC of 2.0–5.0 μ g/mL), were used as reference compounds for the antimycobacterial assay. The cytotoxicity assay against the BC (human breast cancer cells), KB (human epidermoid carcinoma of the mouth), and Vero cell lines was performed employing the colorimetric method;¹² the reference substance was ellipticine (IC_{50} of 0.1–0.4 μ g/mL for both BC and KB cells and 0.4–0.9 μ g/mL for Vero cell).

Potamogetonyde (3): colorless oil; $[\alpha]^{29}_D +27.4^\circ$ (*c* 1.22, $CHCl_3$); UV (MeOH) λ_{max} 201; IR (neat) ν_{max} 2939, 2858, 1741, 1644, 1511, 1460, 1378, 1239, 1040, 901, 871 cm^{-1} ; ESI-TOF MS *m/z* 359.2204 ($M + H$)⁺, calcd for $C_{22}H_{31}O_4$ 359.2222; ¹H and ¹³C NMR see Table 1.

Potamogetonol (4): colorless oil; $[\alpha]^{29}_D +15.7^\circ$ (*c* 0.76, $CHCl_3$); UV (MeOH) λ_{max} 201; IR (neat) ν_{max} 3477, 2934, 2852, 1741, 1654, 1501, 1465, 1378, 1244, 1034, 983 cm^{-1} ; ESI-TOF MS *m/z* 361.2377 ($M + H$)⁺, calcd for $C_{22}H_{33}O_4$ 361.2379; ¹H and ¹³C NMR see Table 1.

Acknowledgment. We are indebted to the Biodiversity Research and Training Program (BRT) and BIOTEC/NSTDA for financial support. Support of the antimycobacterial assay laboratory from the Thailand-Tropical Diseases Research

Program (T-2) is gratefully acknowledged. Y.T. thanks the National Science and Technology Development Agency (NSTDA) for the Senior Research Fellowship Award. We thank Ms. Prapairat Seephonkai and Mr. Suthichai Intamas for the plant specimen and for technical assistance, respectively.

Supporting Information Available: The ¹H and ¹³C NMR spectra ($CDCl_3$) and correlations observed from the NOESY spectrum of potamogetonyde (3) and potamogetonol (4). This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- (1) (a) Kittakoop, P.; Suttisri, R.; Chaichantipyuth, C.; Vethchagarun, S.; Suwanborirux, K. *J. Nat. Prod.* **1999**, *62*, 318–320. (b) Kittakoop, P.; Punya, J.; Kongsaree, P.; Lertwerawat, Y.; Jintasirikul, A.; Tanticharoen, M.; Thebtaranonth, Y. *Phytochemistry* **1999**, *52*, 453–457. (c) Ekthawatchai, S.; Isaka, M.; Kittakoop, P.; Kongsaree, P.; Sirichaiwat, C.; Tanticharoen, M.; Tarnchompoo, B.; Thebtaranonth, Y.; Yuthavong, Y. *J. Heterocycl. Chem.* **1999**, *36*, 1599–1606. (d) Isaka, M.; Punya, J.; Lertwerawat, Y.; Tanticharoen, M.; Thebtaranonth, Y. *J. Nat. Prod.* **1999**, *62*, 329–331. (e) Isaka, M.; Tanticharoen, M.; Thebtaranonth, Y. *Tetrahedron Lett.* **2000**, *41*, 1657–1660. (f) Isaka, M.; Jaturapat, A.; Kladwang, W.; Punya, J.; Lertwerawat, Y.; Tanticharoen, M.; Thebtaranonth, Y. *Planta Med.* **2000**, *66*, 473–475. (g) Yenjai, C.; Sripontan, S.; Sripajun, P.; Kittakoop, P.; Jintasirikul, A.; Tanticharoen, M.; Thebtaranonth, Y. *Planta Med.* **2000**, *66*, 277–279. (h) Boonlaksiri, C.; Oonant, W.; Kongsaree, P.; Kittakoop, P.; Tanticharoen, M.; Thebtaranonth, Y. *Phytochemistry* **2000**, *54*, 415–417.
- (2) Smith, C. R., Jr.; Madrigal, R. V.; Weisleder, D.; Mikolajczak, K. L. *J. Org. Chem.* **1976**, *41*, 1, 593–596.
- (3) Hasegawa, S.; Hirose, Y. *Chem. Lett.* **1983**, 1–4.
- (4) Bastard, J.; Duc, D. K.; Fetizon, M.; Francis, M. J.; Grant, P. K.; Weavers, R. T.; Kaneko, C.; Baddeley, G. V.; Bernassau, Jean-Marie; Burfitt, I. V.; Wovkulich, P. T.; Wenkert, E. *J. Nat. Prod.* **1984**, *47*, 592–599.
- (5) Qais, N.; Mandal, M. R.; Rashid, M. A.; Jabbar, A.; Koshino, H.; Nagasawa, K.; Nakata, T. *J. Nat. Prod.* **1998**, *61*, 156–157.
- (6) Veitch, N. C.; Wright, G. A.; Stevenson, P. C. *J. Nat. Prod.* **1999**, *62*, 1260–1263.
- (7) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323–327.
- (8) Derome, A. E.; Williamson, M. P. *J. Magn. Reson.* **1990**, *88*, 177–185.
- (9) Jeener, J.; Meier, B. H.; Bachmann, P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546–4563.
- (10) Bax, A.; Griffey, R. H.; Hawkins, B. L. *J. Magn. Reson.* **1983**, *55*, 301–315.
- (11) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093–2096.
- (12) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.
- (13) Plumb, J. A.; Milroy, R.; Kaye, S. B. *Cancer Res.* **1989**, *49*, 4435–4440.
- (14) Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *4*, 1004–1009.

NP0004404