# Prismatomerin, a New Iridoid from *Prismatomeris tetrandra*. Structure Elucidation, Determination of Absolute Configuration, and Cytotoxicity

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A new complex iridoid, prismatomerin (**1**), has been isolated from the leaves of *Prismatomeris tetrandra*, together with the known glucoside gaertneroside (**4**). The structures of **1** and **4** were determined by spectroscopic analysis, notably 2D NMR techniques. The (1*R*,5*S*,8*S*,9*S*,10*S*)-(−) absolute configuration of prismatomerin (**1**) was determined by comparison of the vibrational circular dichroism (VCD) spectrum calculated using density functional theory and the experimental VCD spectrum of the *O*-acetyl derivative **3**. Prismatomerin (**1**) showed remarkable antitumor activity and also interfered with mitotic spindle formation.

*Prismatomeris tetrandra* (Roxb) K. Schum belongs to the family Rubiaceae, a family of about 450 genera and 5500 species,[1] distributed mainly in tropical regions, although a small number also grow in temperate areas. *P. tetrandra* is an evergreen shrub, found in Assam, North Bengal, the Andaman Islands of India, and the hilly areas of Bangladesh. *P. tetrandra* has been used for the treatment of several ailments in traditional folk medicine: the juice of the leaves for stomachaches and poultices of the leaves for fresh wounds.[2,3] A literature survey revealed that a large number of different alkaloids, anthraquinones, anthraquinols, and iridoids with medicinal activity were isolated from the family Rubiaceae,[3,4] but only a few secondary metabolites were reported from the species *P. tetrandra*.[5] Recently, we reported the cytotoxicity of extracts of the leaves of the plant on human tumor cell lines and the isolation of some secondary metabolites.[6] We also reported on the antitumor activity of ursolic acid, also isolated from the leaves.[6] In continuation of this work, we are now reporting the isolation, structure elucidation, and bioactivity of a new representative of the rare class (only three known analogues, see Figure 1) of higher condensed iridoids, which we have named prismatomerin (**1**), and the known glucoside gaertneroside (**4**).[7] Both iridoids **1** and **4** contain an aromatic ring system as an additional feature compared to the methyl-substituted spirolactone-containing iridoids exemplified by plumericin (**5**) and isoplumericin (**6**) (the *Z*-isomer of **5**)[8−12] and the glucoside plumieride (**7**) (Figure 1).[10,11,13] The isolation of a related iridoid, oruwacin (**8**), an iridoid with a ferulate aromatic ring system, was previously reported from *Morinda lucida*, another species of Rubiaceae.[14]

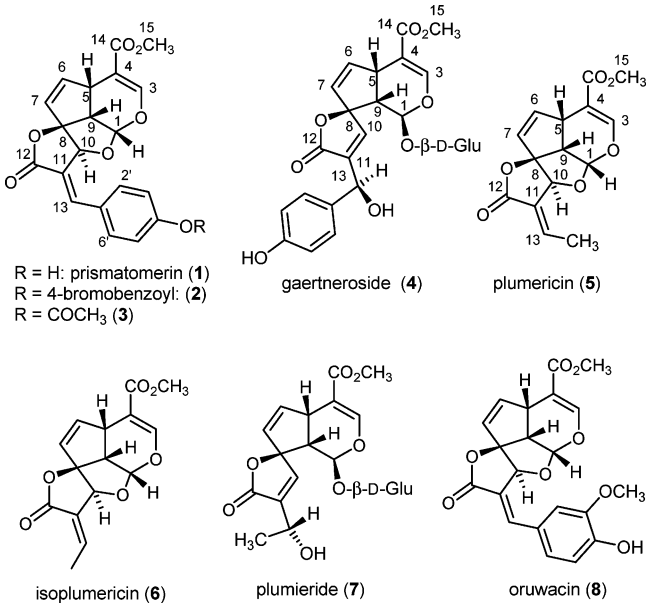


**Figure 1.** Structures of prismatomerin (**1**) and gaertneroside (**4**) and related condensed iridoids **5**−**8**.

## Results and Discussion

Dried and powdered leaves were extracted using a $CH_2Cl_2$−MeOH (1:1) solvent system. The residue obtained from this extract was suspended in $H_2O$ and partitioned with $CH_2Cl_2$. The residue of the $CH_2Cl_2$-soluble part was suspended in MeOH−$H_2O$ (9:1) and partitioned with *n*-hexane to separate the fatty and nonpolar metabolites. Repeated silica gel column chromatography of the residue of the aqueous MeOH-soluble part afforded the new iridoid prismatomerin (**1**) (70 mg) as white crystals. Chromatographic purification followed by HPLC of one of the more polar fractions of the same residue afforded the glucoside gaertneroside (**4**)[7] (15 mg) as a pale yellow solid. Preliminary biotests showed that prismatomerin (**1**) has remarkable anticancer activity; it selectively inhibited renal cancer and also showed activity against non-small-cell lung, prostate, melanoma, ovarian, and breast cancer cell lines.

The key to the assembly of the structure of **1** resided in the connectivities in the $^1$H spectrum starting from the H-1 resonance at $\delta$ 5.67. This resonance was readily identified as the signal for the acetal proton on the basis of the chemical shift of the directly bonded carbon at $\delta$ 102.4. Connectivities within the five spin systems containing the H-1 resonance were established from a COSY experiment (see Table 1). The HMBC spectrum also demonstrated some important correlations of H-1 at $\delta$ 5.67 with C-10 ($\delta$ 82.0) and of H-3 at $\delta$ 7.50 with C-1 ($\delta$ 102.4). In addition, the correlations of H-10 at $\delta$ 5.25 with C-13 ($\delta$ 144.4) and of H-13 at $\delta$ 7.81 with C-10 ($\delta$ 82.0) demonstrated the connection of the

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**Table 1.** $^1$H and $^{13}$C NMR Data of Prismatomerin (**1**) ($CDCl_3$, 500 and 125 MHz)

| no. | $\delta_H$ (mult., *J* in Hz) | $\delta_C$ | COSY | HMBC |
|---|---|---|---|---|
| 1 | 5.67 (1H, d, 5.8)[a] | 102.4 | 9-H | C-8, C-9, C-10 |
| 2 | | | | |
| 3 | 7.5 (1H, s) | 152.9 | | C-1, C-4, C-5, C-6, C-14 |
| 4 | | 109.6 | | |
| 5 | 4.08 (1H, tt, 2.0, 9.5) | 38.5 | 6-H, 7-H, 9-H | C-1, C-3, C-4, C-6, C-7, C-9 |
| 6 | 6.07 (1H, dd, 2.1, 5.4) | 141.1 | 5-H, 7-H | C-5, C-7, C-8, C-9 |
| 7 | 5.67 (1H)[a] | 126.5 | 5-H, 6-H | C-5, C-6, C-8, C-9 |
| 8 | | 104.5 | | |
| 9 | 3.56 (1H, dd, 5.8, 9.5) | 54.2 | 1-H, 5-H | C-5, C-6, C-8 |
| 10 | 5.25 (1H, bs) | 82.0 | | C-7, C-8, C-12, C-13 |
| 11 | | 120.2 | | |
| 12 | | 170.2 | | |
| 13 | 7.81 (1H, bs) | 144.4 | | C-10, C-11, C-12, C-2′/C-6′ |
| 14 | | 166.8 | | |
| 15 | 3.81 (3H, s) | 51.7 | | C-14 |
| 1′ | | 126.2 | | |
| 2′/6′ | 7.71 (2H, d, 8.6) | 133.3 | 3′-H/5′-H | C-13, C-2′/C-6′, C-3′/C-5′, C-4′ |
| 3′/5′ | 6.97 (2H, d, 8.6) | 116.3 | 2′-H/6′-H | C-1′, C-3′/C-5′, C-4′ |
| 4′ | 5.73 (1H, bs, 4′-OH) | 158.9 | | |

[a] Overlapping signals.

olefin moiety at C-11 of a tetracyclic ring system. Furthermore, the correlations of H-13 ($\delta$ 7.81) with C-2′/6′ ($\delta$ 133.3) and of H-2′/6′ ($\delta$ 7.71) with C-13 ($\delta$ 144.4) suggested the connection of the phenolic ring with the olefin moiety. The correlation of OC*H*$_3$ ($\delta$ 3.81) with C-14 ($\delta$ 166.8) proved the existence of a methyl ester group, and another correlation with C-4 ($\delta$ 109.5) confirmed that this ester moiety is connected at C-4. All of these data suggested the existence of a tetracyclic iridoid skeleton related to plumericin (**5**)[8−12] and oruwacin (**8**).[14]

The details of the relative configuration of the new iridoid **1** were then elucidated by comparison of its spectroscopic data with those of oruwacin (**8**) and plumericin (**5**), recently isolated by us from *Plumeria rubra*.[15] Although both the $^1$H and $^{13}$C NMR spectra of **1** were in close correspondence with those of **5** and **8**, some deviations could be observed due to the different residues at C-13. In the $^1$H NMR spectrum of **1**, the singlet at $\delta$ 3.92 of the aromatic methoxy group, present in the spectrum of oruwacin (**8**), was missing. Instead, the $^1$H NMR spectrum of **1** showed two symmetrical pairs of coupled two-proton resonances at $\delta$ 7.71 (H-2′ and H-6′) and at $\delta$ 6.97 (H-3′ and H-5′) (Table 1), assigned to a 1,4-disubstituted benzene ring. The DEPT 135 spectrum of **1** also showed only one resonance of a primary carbon atom at $\delta$ 51.7 for the methyl ester group at C-15. The gross structure of **1**, in comparison to **5** and **8**, was confirmed by the mass spectrum with $[M + H]^+$ at *m*/*z* 369, suggesting the molecular formula $C_{20}H_{16}O_7$, in agreement with the NMR spectra, replacing the guiacol group in **8** by a phenolic group in **1**.

Similarly, the $^1$H NMR spectrum of plumericin (**5**) was almost superposable on the coupling patterns and chemical shifts of the non-phenolic portion of **1**. In the $^1$H NMR spectrum of **5**, as expected, the two pairs of coupled 2H signals of the 1,4-disubstituted benzene ring of **1** were missing and replaced by an additional doublet at $\delta$ 2.09 ($J = 7$ Hz) caused by the methyl group at C-13, resonating at $\delta$ 15.9 in the $^{13}$C NMR spectrum. The relative configuration of plumericin (**5**) was unambiguously determined by X-ray single-crystal analysis.[15] The almost identical NMR coupling pattern and chemical shifts of all resonances apart from the phenolic portion of **1** with those of **5** proved the identical relative configuration of both iridoids. This conclusion was further supported by a 1D NOE experiment of **1**, showing considerable interactions of H-9 with the methine protons H-1 and H-5, indicating that they are cofacial. A 1D NOE experiment was also used to determine the *E*-configuration of **1**. Irradiation of the methine H-10 proton at $\delta$ 5.25 showed considerable interactions with the aromatic protons at $\delta$ 7.71 (2′- and 6′-H) and none with H-13. This is only possible with an *E*-configuration of the C-11−C-13 double bond in **1**.

In an attempt to elucidate the absolute configuration by X-ray analysis, prismatomerin (**1**) was reacted with 4-bromobenzoyl chloride to afford the 4-bromobenzoate **2** with the heavy bromine atom incorporated. However, the very fine needles of **2** were not suitable for X-ray analysis. Analysis of the NMR spectra of **2** was in total agreement with the proposed structure, especially all the 1D and 2D NMR data related to H-1 and H-7. In order to determine the absolute configuration of **1** using vibrational circular dichroism (VCD) spectroscopy, we also prepared the *O*-acetyl derivative **3** of **1** to eliminate intermolecular hydrogen bonding due to the phenolic OH group of **1**.[16] As expected, the NMR data of the *O*-acetyl derivative **3** were in good agreement with the related parts of **1** and the 4-bromobenzoate **2** (see Experimental Section).

The spectroscopic data of the second, more polar iridoid isolated from *P. tetrandra* were identical within experimental error to those of the glucoside gaertneroside (**4**), isolated from *Morinda morindoides* (Baker) Milne-Redhead (syn. *Gaertnera morindoides* Bak.).[7] The $^{13}$C NMR data were similar except for a systematic shift of all signals of ~1.4 Hz to low field of the published data. The reported specific rotation was of the same sign $\{[\alpha]^{25}_D$ +24.8 (*c* 0.75, MeOH)} but somewhat lower than that measured for our sample $\{[\alpha]^{25}_D$ +43.1 (*c* 0.75, MeOH)}.

**Absolute Configuration (AC) of 1.** The specific rotation of **1** $\{[\alpha]_D$ −136 (*c* 0.75, EtOH)} is opposite in sign to the rotations of plumericin, **5** $\{[\alpha]_D$ +179 (*c* 14.6, $CHCl_3$)[15]}, and isoplumericin, **6** $\{[\alpha]_D$ +194 (*c* 0.64, $CHCl_3$)[15]}, initially suggesting that the AC of **1** might be opposite of that of **5** and **6**. The ACs of **5** and **6** have been definitively established using VCD spectroscopy.[17] We have therefore used VCD to establish the AC of **1**. To avoid the intermolecular aggregation, commonly found in alcohols at the concentrations used in measuring VCD spectra,[16] we have used the *O*-acetyl derivative **3** of the phenolic OH group. Its VCD spectrum, measured on a 0.03 M solution in $CDCl_3$, is shown in Figure 2. Conformational analysis of **3** was carried out using DFT at the B3PW91/TZ2P level to determine the structures and populations of the conformations of **3** which are significantly populated at room temperature. The B3PW91/TZ2P VCD spectra of the 12 conformations identified were then calculated, weighted by the predicted populations, and summed, giving the conformationally averaged VCD spectrum of the 1*R*,5*S*,8*S*,9*S*,10*S* enantiomer of **3** shown in Figure 2. The calculated VCD spectrum of (1*R*,5*S*,8*S*,9*S*,10*S*)-**3** is in good agreement with the experimental spectrum (allowing for the shift to higher frequencies of the calculated spectrum, due to the neglect of anharmonicity); the assignment of the larger VCD features based on the predicted spectrum is detailed in Figure 2. Conversely, as also shown in

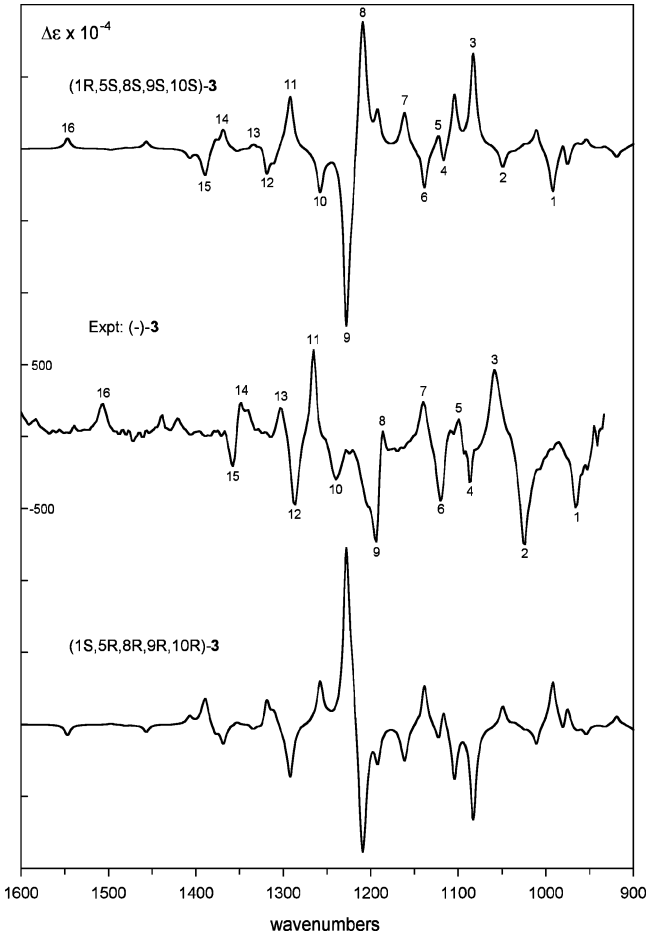


**Figure 2.** Comparison of the experimental and B3PW91/TZ2P VCD spectra of **3**. The calculated VCD spectra are obtained from the calculated vibrational frequencies and rotational strengths using Lorentzian band shapes ($\gamma = 4.0$ cm$^{-1}$).

Figure 2, the predicted VCD spectrum of (1*S*,5*R*,8*R*,9*R*,10*R*)-**3**, the enantiomer of (1*R*,5*S*,8*S*,9*S*,10*S*)-**3**, shows no agreement with the experimental spectrum. The AC of **3**, and hence the AC of **1**, is therefore unambiguously 1*R*,5*S*,8*S*,9*S*,10*S*, identical to those of **5** and **6**.

**Biological Activity. Cytotoxicity of Prismatomerin (1).** Prismatomerin (**1**) and the glucoside gaertneroside (**4**) were tested for cytotoxicity in the brine shrimp lethality assay.[18] The two compounds showed remarkable toxicity ($LD_{50}$ 72 ng/mL (195 nM) for **1** and 156 ng/mL (380 nM) for **4** at 24 h, and $LD_{50}$ below detection limit (all shrimp dead) for **1** and 92 ng/mL (224 nM) for **4** at 48 h). Prismatomerin (**1**) was then tested at NCI in an *in vitro* primary anticancer assay, a three-cell-line panel consisting of MCF7 (breast cancer), NCl-H460 (lung cancer), and SF-268 (CNS cancer). The cells showed zero growth in these tests in the presence of prismatomerin (**1**).

Prismatomerin (**1**) was then tested *in vitro* using the NCI's anticancer screen.[19] The compound was tested against these cell lines consisting of 60 human tumor cell lines, at a minimum of five concentrations at 10-fold dilutions. A 48 h continuous drug exposure was used, and a sulforhodamine B (SRB) protein assay was used to estimate cell viability and growth. The results are expressed in growth inhibition ($GI_{50}$) and cell killing activity ($LC_{50}$) values (for details see the Experimental Section and Supporting Information). The data showed that prismatomerin had high growth inhibition activity in leukemia cell lines ($GI_{50} < 10$ nM). Most of the solid tumor cell lines are less sensitive ($GI_{50} < 10$ nM to 2 $\mu$M), renal cancer cell lines being the most sensitive group.

**Table 2.** Growth Inhibition of Different Mammalian Cell Lines by Prismatomerin

| | | $IC_{50}$ [$\mu$M] | |
|---|---|---|---|
| cell line | origin | **1** | **3** |
| L-929 | murine connective tissue | 0.21 | 0.41 |
| KB-3-1 | human cervix carcinoma | 0.41 | 0.35 |
| A-549 | human lung carcinoma | 1.41 | 1.60 |
| SW-480 | human colon adenocarcinoma | 0.060 | 0.084 |

Prismatomerin showed not only a growth inhibition but also a cell killing effect in solid tumor cell lines ($LC_{50} > 100$ to 0.6 $\mu$M).

**Mode of Action of Prismatomerin (1) and the Acetate 3.** Our own assays with cultured mammalian cell lines confirmed the efficacious growth inhibitory activity of prismatomerin (**1**) and also of its *O*-acetyl derivative (**3**), which shows almost the same activity on the cell lines tested (Table 2). To further investigate the mode of action of prismatomerin, $PtK_2$ kidney cells were incubated with **1** overnight and stained for nuclei and microtubules (Figure 3). Treated cells showed a nuclear fragmentation as is known when cells are incubated with antimitotic substances such as epothilone, tubulysin, or disorazol.[20] As these compounds interfere with tubulin polymerization, we checked the cells by staining the microtubules. In these experiments, we observed no alteration in the microtubular network of nondividing cells, but we found many obviously mitotic cells showing chromosomes in a metaphase-like state that did not display a normal mitotic spindle. From these results, we assume that prismatomerin (**1**) is an antimitotic compound that interferes with spindle formation without having a direct effect on microtubules. This phenotype indicates a possibly new mode of action of an antimitotic compound and encourages more detailed investigations of the mode of action of prismatomerin and also of the related higher iridoids plumericin (**5**) and isoplumericin (**6**).

## Experimental Section

**General Experimental Procedures.** Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. The $^1$H (500 MHz) and $^{13}$C NMR (125 MHz) data are reported in ppm. Hydrogen connectivity (C, CH, $CH_2$, $CH_3$) information was obtained from DEPT-135 experiments. Proton and carbon peak assignments were based on 2D NMR analysis (COSY, HMQC, and HMBC). Column chromatography was performed on silica gel (E. Merck, 230−400 mesh), and TLC was carried out on precoated silica gel plates (PF 254). HPLC separations were performed on a Supelco Discovery RP-column (25 cm × 4.6 mm i.d.) using a Shimadzu SCL-10 AVP instrument equipped with a UV−vis detector. The samples were injected manually through a Rheodyne injector. Compound was eluted with acetonitrile−$H_2O$ (15:85) at the flow rate of 1 mL/min (retention time 11.38 min). VCD was measured using a Bomem/BioTools ChiralIR spectrometer.[17] For further general methods and instrumentation see ref 21.

**Plant Material.** Leaves and bark were collected from Chokoria, Cox's Bazar, Bangladesh, during April 2−4, 2000, and were identified by the late Professor M. Salar Khan of Bangladesh National Herbarium (BNH). A voucher specimen for this collection is maintained at BNH under the accession number DACB-29513.

**Extraction and Isolation.** The collected plant materials were separated into leaves and stems with bark, chopped into small pieces, air-dried followed by drying in an oven at 40 °C, and ground into powder. The dried leaves powder (3.0 kg) was extracted with $CH_2Cl_2$−MeOH (1:1; 6 L × 3; RT; 24 h). The extract was evaporated by a rotary vacuum evaporator and finally dried by a freeze-dryer (110 g). The dried extract (100 g) was suspended in $H_2O$, ultrasonicated, and partitioned with $CH_2Cl_2$. The residue of the $CH_2Cl_2$-soluble part was again suspended in aqueous 90% MeOH (1 L) and partitioned with *n*-hexane (1 L × 3). Repeated silica gel chromatography of the aqueous 90% MeOH extract (65 g) afforded a new iridoid, prismatomerin (**1**; 70 mg), as a white, amorphous powder, which crystallized as fine needles from 20% EtOAc in *n*-hexane. Repeated column chromatography followed by HPLC of one of the relatively polar column fractions of the same residue afforded the glucoside gaertneroside (**4**) (15 mg) as a pale yellow solid.

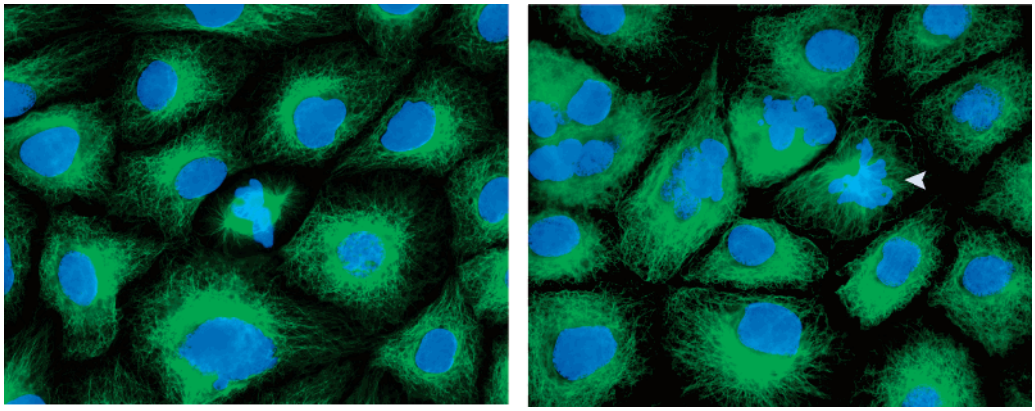

**Figure 3.** Influence of prismatomerin (**1**) on $PtK_2$ potoroo cells. Cells were stained for nuclei (blue) and microtubules (green). After incubation with **1** (0.5 $\mu$g/mL) overnight (right photo) cells show nuclear fragmentation, but no alteration in their microtubular network. The photo also shows a mitotic cell with metaphase chromosomes (arrow) but not a mitotic spindle, as displayed by the mitotic cell in the control picture (left).

**Data of prismatomerin (1):** white needles; mp 134−135 °C (dec); $R_f$ 0.45 (*n*-hexane−EtOAc, 5:1). $[\alpha]^{25}_D$ −136 (*c* 0.01, EtOH); UV ($CDCl_3$) $\lambda_{max}$ (log $\epsilon$) 317 nm (4.07); IR (KBr) $\nu_{max}$ 3512, 3123, 2962, 1732, 1696, 1644 $cm^{-1}$; ESIMS *m/z* 368 [$M^+$], 351, 337, 319, 309, 291, 275, 249, 199, 175, 163, 139, 133; FABMS *m/z* 369 [$M^+ + H$]; for $^1H$ and $^{13}C$ NMR data, see Table 1.

**Data of gaertneroside (4):** pale yellow solid; mp 150−151 °C (dec); $R_f$ 0.40 ($CH_2Cl_2$−MeOH, 20:1); $[\alpha]^{25}_D$ +43.1 (*c* 0.75, MeOH); ref 7 +24.8 (*c* 0.75 MeOH); IR (KBr) $\nu_{max}$ 3510, 3120, 2960, 2872, 1735, 1695 $cm^{-1}$; $^1H$ (500 MHz, $CD_3OD$) $\delta$ 2.93 (dd, *J* = 7.6, 4.9 Hz, 1H, 9-H), 3.23 (m, 1H, 2″-H), 3.29 (m, 1H, 5″-H), 3.41 (m, 1H, 3″-H), 3.41 (m, 1H, 4″-H), 3.72 (dd, *J* = 12.2, 4.9 Hz, 1H, 6″-H), 3.78 (s, 3H, 15-OC*H*$_3$), 3.81 (dd, *J* = 12.2, 2.2 Hz, 1H, 6″-H), 3.94 (dddd, *J* = 7.6, 4.0, 2.3 Hz, 1H, 5-H), 4.70 (d, *J* = 7.9 Hz, 1H, 1″-H), 5.18 (d, *J* = 4.9 Hz, 1H, 1-H), 5.38 (s, 1H, 13-H), 5.59 (dd, *J* = 5.6, 2.3 Hz, 1H, 7-H), 6.49 (dd, *J* = 5.6, 2.3 Hz, 1H, 6-H), 6.81 (d, *J* = 8.6 Hz, 2H, 3′-H, 5′-H), 7.32 (d, *J* = 8.6 Hz, 2H, 2′-H, 6′-H), 7.48 (s, 1H, 10-H), 7.55 (d, *J* = 1.6 Hz, 1H, 3-H); $^{13}C$ (125 MHz, $CD_3OD$) $\delta$ 38.9 (C-5), 49.4 (C-9), 50.6 (15-O*C*H$_3$), 60.8 (C-6″), 68.5 (C-13), 69.5 (C-4″), 73.1 (C-2″), 76.4 (C-3″), 76.9 (C-5″), 93.0 (C-1), 96.7 (C-8), 99.1 (C-1″), 109.5 (C-4), 114.9 (C-3′, C-5′), 128.2 (C-2′, C-6′), 128.5 (C-7), 131.9 (C-1′), 136.5 (C-11), 140.2 (C-6), 148.7 (C-10), 151.1 (C-3), 157.1 (C-4′), 167.1 (C-14), 171.0 (C-12); FABMS: *m/z* 549 [$M^+ + H$], 369, 351, 307, 289, 154, 136, 128, 107.

**4-Bromobenzoate of Prismatomerin (2).** To a stirred solution of **1** (7.9 mg, 0.021 mmol) in dry pyridine (2.0 mL) were added *p*-bromobenzoyl chloride (8.9 mg) and DMAP (20.0 mg). The reaction mixture was stirred at room temperature for about 0.5 h (TLC monitoring) and was then neutralized by addition of 1 N HCl. The mixture was extracted with $CH_2Cl_2$, washed with $H_2O$, dried over anhydrous $Na_2SO_4$, filtered, and evaporated to dryness. The resulting mixture was purified by preparative TLC followed by recrystallization with diethyl ether−*n*-hexane to afford **2** (9.0 mg, 76%) as white needles: mp 175 °C (dec); $[\alpha]^{25}_D$ −56.9 (*c* 0.42, $CHCl_3$); UV ($CDCl_3$) $\lambda_{max}$ (log $\epsilon$) 296 nm (4.47), 299 (4.47), 302 (4.49); IR (KBr) $\nu_{max}$ 2953 cm−1, 2924, 2854, 1755, 1738, 1705, 1651, 1645, 1589, 1508, 1439, 1398, 1298, 1263, 1211, 1169, 1084, 1022, 1011, 964; EIMS *m/z* 554 [$M^+ + 2$], 552 [$M$]$^+$, 368, 279, 248, 201, 182, 121, 83, 57; $^1H$ (500 MHz, $CDCl_3$) $\delta$ 3.59 (dd, *J* = 9.5, 5.8 Hz, 1H, 9-H), 3.81 (s, 3H, 15-OC*H*$_3$), 4.08 (tt, *J* = 9.5, 2.2 Hz, 1H, 5-H), 5.23 (s, 1H, 10-H), 5.66 (dd, *J* = 5.5, 2.2 Hz,1H, 7-H), 5.68 (d, *J* = 5.8 Hz, 1H, 1-H), 6.08 (dd, *J* = 5.5, 2.2 Hz, 1H, 6-H), 7.38 (d, *J* = 8.8 Hz, 2H, 3′-H, 5′-H), 7.49 (s, 1H, 3-H), 7.70[a] (d, *J* = 8.5 Hz, 2H, 2″-H, 6″-H), 7.87 (d, *J* = 8.8 Hz, 2H, 2′-H, 6′-H), 7.88 (s, 1H, 13-H), 8.09[a] (d, *J* = 8.5 Hz, 2H, 3″-H, 5″-H); $^{13}C$ (125 MHz, $CDCl_3$) $\delta$ 38.5 (C-5), 51.7 (15-O*C*H$_3$), 54.2 (C-9), 81.5 (C-10), 102.4 (C-1), 104.7 (C-8), 109.5 (C-4), 122.6 (C-3′, C-5′), 123.6 (C-11), 126.3 (C-7), 128.0 (C-1′), 129.3 (C-1″), 131.2 (C-4″), 131.7[b] (C-3″, C-5″), 132.1[b] (C-2″, C-6″), 132.3 (C-2′, C-6′), 141.4 (C-6), 143.4 (C-13), 152.8 (C-3), 153.1 (C-4′), 164.1 (−*C*OO), 166.6 (C-14), 169.4 (C-12); EIMS (70 eV, 200 °C) *m/z* (%) 552 [$M^+ + 2$] (8), 550 [$M^+$] (8), 368 (11), 279 (8), 202 (16), 200 (16), 185 (96), 183 (100), 122 (59), 121 (73), 57 (41), 45 (38). [a,b]Interchangeable assignments.

**3-*O*-Acetylprismatomerin (3):** white needles; mp 177 °C; $[\alpha]^{25}_D$ −98.3 (*c* 0.6, $CHCl_3$); further specific rotations, same concentration (578 nm: −104.6; 546 nm: −128.6; 436 nm: −336.9); UV of **3** ($CDCl_3$) $\lambda_{max}$ (log $\epsilon$) 296 nm (4.47), 299 (4.47), 302 (4.49); IR (KBr) $\nu_{max}$ 3432, 3075, 2950, 1757, 1712, 1670, 1641, 1505, 1214, 1078, 1028 $cm^{-1}$; EIMS *m/z* (%): 410 (12) [$M^+$], 368 (100), 308 (10), 279 (8), 238 (19), 201 (28), 131 (8); $^1H$ (500 MHz, $CDCl_3$) $\delta$ 2.33 (s, 3H, $COCH_3$), 3.55 (dd, *J* = 9.5, 5.8 Hz, 1H, 9-H), 3.78 (s, 3H, 15-OC*H*$_3$), 4.05 (dt, *J* = 9.5, 2.2 Hz, 1H, 5-H), 5.18 (s, 1H, 10-H), 5.62 (dd, *J* = 5.5, 2.2 Hz,1H, 7-H), 5.65 (dd, *J* = 5.8, 0.4 Hz, 1H, 1-H), 6.05 (dd, *J* = 5.5, 2.2 Hz, 1H, 6-H), 7.23 (d, *J* = 8.6 Hz, 2H, 3′-H, 5′-H), 7.46 (s, 1H, 3-H), 7.78 (d, *J* = 8.6 Hz, 2H, 2″-H, 6″-H), 7.82 (d, *J* = 1.3 Hz, 13-H); $^{13}C$ (125 MHz, $CDCl_3$) $\delta$ 38.5 (C-5), 51.7 (15-O*C*H$_3$), 54.2 (C-9), 54.2 (17-$OCH_3$), 81.5 (C-10), 102.4 (C-1), 104.7 (C-8), 109.5 (C-4), 122.6 (C-3′, C-5′), 123.4 (C-11), 126.3 (C-7), 131.0 (C-1′), 132.2 (C-2′, C-6′), 141.3 (C-6), 143.5 (C-13), 152.8 (C-3), 153.0 (C-4′), 166.6 (CO-14), 169.0 (CO-16), 169.4 (CO-12); EIMS (70 eV, 200 °C) *m/z* (%) 552 [$M^+ + 2$] (8), 550 [$M^+$] (8), 368 (11), 279 (8), 202 (16), 200 (16), 185 (96), 183 (100), 122 (59), 121 (73), 57 (41), 45 (38).

**Cell Proliferation Assay.** Cell lines were obtained from the DSMZ (L-929; KB-3-1; A-549) or ATCC (SW-480) and cultivated at 37 °C and 10% $CO_2$ in DME medium (high glucose) or McCoy's 5A medium (in the case of SW-480), both media supplemented with 10% fetal calf serum. Cell culture reagents came from Life Technologies Inc. (Gibco BRL). Growth inhibition was measured in microtiterplates. Aliquots of 120 $\mu$L of the suspended cells (50 000/mL) were added to 60 $\mu$L of serial dilutions of the test compounds. After 5 days, growth was determined using the MTT assay.[22]

**Cell Staining.** $PtK_2$ cells (ATCC CCL-56) grown on glass coverslips were fixed with cold (−20 °C) methanol−acetone (1:1) for 10 min, incubated with a primary monoclonal antibody against α-tubulin (1:500; Sigma) and then with a secondary Alexa Fluor 488 goat anti-mouse IgG antibody (1:2000; Molecular Probes), and mounted in ProLong Antifade Gold (Molecular Probes), which included DAPI to stain the nuclei.

**NCI *in Vitro* Antitumor Test Results of Prismatomerin on 60 Human Cancer Cell Lines. Growth Inhibition.** Prismatomerin showed the following 50% growth inhibition values (log $GI_{50}$ values [M]) on the following cancer cell lines of the specific cancer: leukemia [CCRF-CEM (−7.42); HL-60 (<−8.00); K-562 (<−8.00); MOLT-4 (<−8.00); RPMI-8226 (<−8.00); SR (−7.98)], non-small-cell lung cancer [A549/ATCC (−6.53); EKVX (−6.45); HOP-62 (−6.58); NCI-H226 (−6.76); NCI-H23 (−6.73); NCI-322M (−5.71); NCI-H460 (−6.62); NCI-H522 (−6.73)], colon cancer [COLO 205 (−6.27); HCT-116 (<−8.00); HCT-15, (−6.79); HT29 (−6.97); KM12 (−6.52); SW-620 (<−8.00)], CNS cancer [SF-268 (−6.58); SF-295 (−6.23); SF-539 (−6.59); SNB-19 (−6.46); SNB-75 (n.a.); U251 (−6.53)], melanoma [LOX IMVI (−7.68); MALME-3M (−6.87); M14 (−6.79); SK-MEL-2 (−6.78); SK-MEL-28 (−6.50); SK-MEL-5 (<−8.00); UACC- 257 (−6.84); UACC-62 (−6.36)], ovarian cancer [IGROV1 (−6.30); OVCAR-3 (−6.31); OVCAR-4 (−6.24); OVCAR-5 (−6.59); OVCAR-8 (−6.76); SK-OV-3 (−6.04)], renal cancer [786-0 (−7.71); A498 (−6.63); ACHN, (<−8.00); CAKI-1, (−6.90); RXF 393 (<−8.00);

SN12C (−6.76); TK-10 (−6.54); UO-31, (−6.92)], prostate cancer [PC-3 (−6.77); DU-145 (−6.94)], and breast cancer [MCF7 (−6.92); NCI/ADR-RES (−6.77); MDA-MB-231/ATCC (−6.79); HS578T (−6.59); MDA-MB-435 (−6.70); BT-549 (−6.82); T-47D (−6.42)].

**Toxicity Effect.** Prismatomerin showed the following 50% cell killing values (log $LC_{50}$ [M]) with the different cancer cell lines: leukemia [CCRF-CEM (>−4.00); HL-60 (>−4.00); K-562 (>−4.00); MOLT-4 (n.a.); RPMI-8226 (>−4.00); SR (>−4.00)], non-small-cell lung cancer [A549/ATCC (>−4.00); EKVX (−4.39); HOP-62 (−5.55); NCI-H226 (−4.64); NCI-H23 (−6.03); NCI-322M (−4.61); NCI-H460 (>−4.00); NCI-H522 (−5.87)], colon cancer [COLO 205 (>−4.00); HCT-116 (n.a.); HCT-15, (−5.42); HT29 (>−4.00); KM12 (>−4.00); SW-620 (>−4.00)], CNS cancer [SF-268 (−4.20); SF-295 (n.a.); SF-539 (>−4.00); SNB-19 (−4.57); SNB-75 (>−4.00); U251 (−4.22)], melanoma [LOX IMVI (−6.15); MALME-3M (−5.33); M14 (n.a.); SK-MEL-2 (n.a.); SK-MEL-28 (>−4.00); SK-MEL-5 (n.a.); UACC-257 (−5.00); UACC-62 (−6.24)], ovarian cancer [IGROV1 (−6.07); OVCAR-3 (−5.77); OVCAR-4 (>−4.00); OVCAR-5 (−6.09); OVCAR-8 (−5.65); SK-OV-3 (>−4.00)], renal cancer [786-0 (−6.26); A498 (−6.06); ACHN, (−5.68); CAKI-1, (−5.06); RXF 393 (−6.18); SN12C (−6.09); TK-10 (−5.40); UO-31, (−6.09)], prostate cancer [PC-3 (−5.52); DU-145 (n.a.)], and breast cancer [MCF7 (>-4.00); NCI/ADR-RES (−4.76); MDA-MB-231/ATCC (−6.07); HS578T (>−4.00); MDA-MB-435 (n.a.); BT-549 (n.a.); T-47D (−5.41)].

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**Supporting Information Available:** Dose−response curves of *in vitro* antitumor test results of prismatomerin (**1**) on 60 human tumor cell lines and 50% of cell killing ($LC_{50}$) values from the NIH investigation. This material is available free of charge via the Internet at http://pubs.acs.org.

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