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Total Synthesis as a Resource in Drug Discovery: The First In Vivo Evaluation of Panaxytriol and Its Derivatives

Heedong Yun,[†] Ting-Chao Chou,[‡] Huajin Dong,[‡] Yuan Tian,[§] Yue-ming Li,[§] and Samuel J. Danishefsky*[,†,⊥]

Department of Chemistry, Columbia University, 3000 Broadway, New York, New York, 10027 and Laboratory for Bioorganic Chemistry, Preclinical Pharmacology Core Facility, and Laboratory of Biochemistry and Pharmacology, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021

s-danishefsky@ski.mskcc.org

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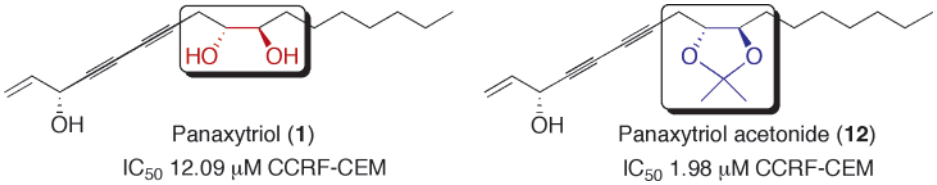
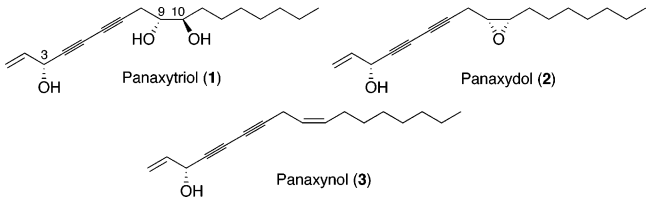


We have conducted key preliminary studies into the in vitro and in vivo cytotoxicity of panaxytriol. Through total synthesis, we prepared and evaluated several synthetic panaxytriol analogues, each of which exhibited enhanced cytotoxicity relative to the natural product. Consequently, we have begun to chart the first in vitro SAR map for the compound, which suggests that the C_3 hydroxyl functionality is not critical for biological activity and that, in fact, engagement of the C_9-C_{10} diol as an acetonide actually leads to notably enhanced cytotoxicity. Furthermore, through in vivo investigations, we demonstrated that panaxytriol and panaxytriol acetonide (**12**) moderately suppress tumor growth with little or no toxicity. Finally, preliminary in vitro evaluation of panaxytriol indicates that it possesses neurotrophic activity.

Introduction

Red ginseng, the steamed and dried root of *Panax ginseng* C. A. Meyer, is widely used as folk medicine throughout Asia. Numerous therapeutic benefits have been ascribed to this popular dietary supplement, which is commonly used for its purported analeptic, erythropoietic, and cytotoxic properties.[1] The latter attribute, in particular, has been corroborated through a number of in vitro and in vivo biological studies of the red ginseng extract. In addition, several components of red ginseng have been isolated and evaluated for their antitumor properties, including panaxytriol (**1**),[2,3] panaxydol (**2**),[4] and panaxynol (**3**).[5] Of these, we were particularly interested in panaxytriol (**1**), which demonstrates in vitro inhibitory activity against a range of tumor cells, including human gastric carcinoma (MK-1),[6] mouse lymphoma (P388D1),[7] and human breast carcinoma (Breast M25-SF).[8] In addition, in one in vivo investigation, panaxytriol reportedly suppressed the growth of B16 melanoma cells that had been transplanted into mice.[9]



[†] Columbia University.
[‡] Preclinical Pharmacology Core Facility, Sloan-Kettering Institute for Cancer Research.
[§] Laboratory of Biochemistry and Pharmacology, Sloan-Kettering Institute for Cancer Research.
[⊥] Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research.

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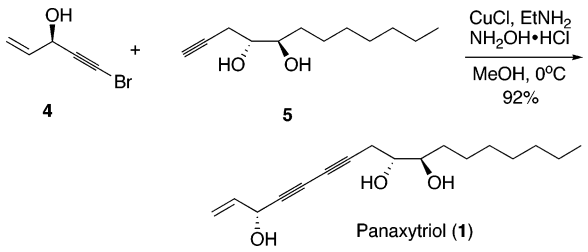
In launching our program toward the total synthesis and biological evaluation of panaxytriol, we were mindful of the unique attributes of this cytotoxic natural product. It is, in fact, very rare to find a documented instance of a tumor retardant isolated from a food product. Thus, given the widespread availability of the source, red ginseng, as well as the lack of toxicity that would be expected from a food component, even a very moderate cytotoxic effect would be considered to be particularly noteworthy in the context of panaxytriol.

To date, a number of syntheses have been reported for panaxytriol.[10] We recently disclosed our own total synthesis, which we hoped would allow access to significant amounts of the natural product for extensive biological investigations.[10i] We report, herein, the development of a slightly modified route, which has allowed us to prepare multigram quantities of synthetic panaxytriol. The synthetic material was evaluated for its cytotoxic properties in a number of in vitro and in vivo contexts. Using our streamlined panaxytriol synthesis as a guide, we prepared several synthetic analogues, whose activities were compared to the natural product. With these results, we have begun to chart an SAR map for panaxytriol.

Finally, we recently became aware of a disclosure from the laboratory of Kurimoto et. al. on the in vitro neurotrophic activity of the organic extracts of red ginseng.[11] The authors did not attempt to identify the neurotrophically active components of the red ginseng extract; however, with ample synthetic panaxytriol on hand, we sought to determine whether this compound was a contributor to the observed activity. This line of investigation is in keeping with a broad program in our laboratory that is devoted to the total synthesis and biological evaluation of nonpeptidyl small molecule neurotrophic factors. We report the results of this preliminary investigation herein.

Modified Synthesis of Panaxytriol. As previously reported,[10i] the defining transformation of our synthesis of panaxytriol (**1**) is the Cadiot−Chodkiewicz coupling[12] of alkynyl bromide **4** and alkyne **5**, which constitutes the final step of the synthesis. Despite the overall convergence and efficiency of the original route, we found that the preparation of intermediate **5** was not particularly amenable to reaction scale-up. Our first task would be to modify our synthesis of **5** to allow for the facile preparation of multigram quantities of panaxytriol.

The previously described synthesis of alkyne **5** commenced with Sharpless asymmetric dihydroxylation[13] of



the corresponding TBS ether of **6** to afford **7** in approximately 99% ee. Protection of the resultant diol as an acetonide, followed by cleavage of the TBS ether, and subsequent bromination provided intermediate **8**. The latter was subjected to acid-induced deprotection followed by epoxide formation to afford **9**, which was then alkylated with Li/acetylide to give rise to coupling partner **5**. Unfortunately, the conversion of **7** to **8** was found to be quite sluggish when the reaction was performed on a large scale (∼20 mmol). We have developed an improved route to **5** that converges on intermediate **9** from the original synthesis. Thus, Sharpless asymmetric dihydroxylation of **6**, followed by TBDPS protection of the primary alcohol, furnished intermediate **10**. Following acetonide protection of the diol, the TBDPS group was removed and the resultant primary alcohol was converted to an iodide to afford **11**. The latter was then deprotected and epoxidized to provide intermediate **9** in good overall yield from **6**. The epoxide was converted to the terminal alkyne (**5**), which was coupled with alkynyl bromide **4** according to the previously disclosed method, to provide panaxytriol (**1**) in 51% overall yield from **6**. This modified route has been used to prepare up to 10 g of synthetic panaxytriol.

Preparation of Synthetic Analogues of Panaxytriol. We next prepared several synthetic derivatives of panaxytriol, in the hopes of constructing an SAR profile with respect to antitumor activity. Thus, analogues were prepared in which the C_9-C_{10} glycol was protected as an acetonide (**12**), the C_3 alcohol was oxidized to a ketone (**13**), and the C_3 of the acetonide derivative was oxidized to a ketone (**14**).

Biological Investigations: Antitumor Activity. The in vitro cytotoxicities of panaxytriol (**1**) and its derivatives (**12**, **13**, and **14**) were evaluated against multidrug resistance (MDR)-sensitive (CCRF-CEM) and MDR-resistant (CCRF-CEM/VBL) cell lines (Table 1). We were interested to find that each of the panaxytriol derivatives (**12**, **13**, and **14**) exhibited a stronger inhibitory effect than did panaxytriol itself. On the basis of these results, it would appear that the C_3 alcohol function is not required for biological activity (cf. **1** versus **13**). The acetonide derivative, **12**, the most active analogue, is roughly 6-fold more potent than panaxytriol. Importantly, each of the four compounds examined retained its potency against the CCRF-CEM/VBL cell line, which displays a multidrug resistance phenotype. These results provide strong evidence that panaxytriol and its derivatives are not substrates for the P-glycoprotein and thus would not be vulnerable to multidrug resistance.

It is of note that the presence of the acetonide functionality appears to confer an increase in cytotoxicity, as evidenced by the fact that both acetonide derivatives (**12**

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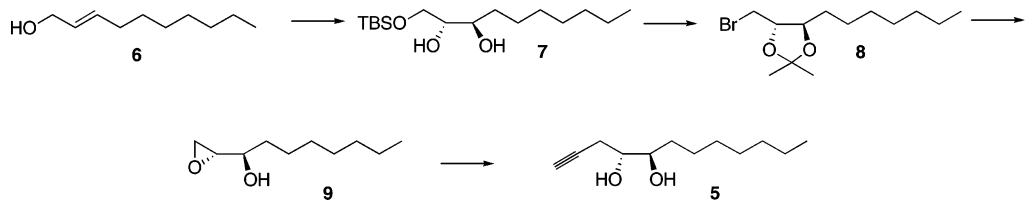
SCHEME 1



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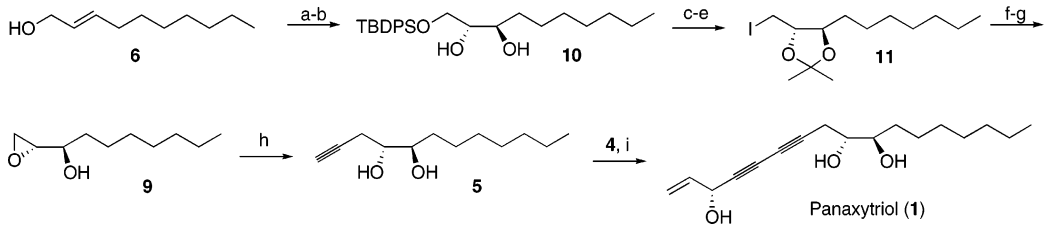


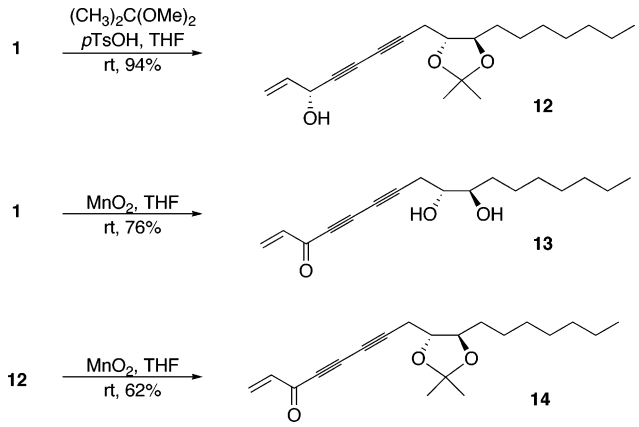
[a] Reaction conditions: (a) AD-mix-β, $CH_3SO_2NH_2$, $^tBuOH/H_2O$ = 1:1, 0 °C, 1 day, 85%, >99% ee; (b) TBDPSCl, TEA, DMAP, CH_2Cl_2, 90%; (c) $(CH_3)_2C(OCH_3)_2$, *p*-TsOH, CH_2Cl_2, 99%; (d) TBAF, THF, 0 °C to room temperature, 3 h, 100%; (e) PPh_3, I_2, Imidazole, CH_2Cl_2, 94%; (f) 1 N HCl, 3 days; (g) K_2CO_3, 91% for two steps; (h) Li/acetylide EDA complex, HMPA, THF, 0 °C to room temperature, 85%; (i) CuCl, $EtNH_2$, $NH_2OH{\cdot}HCl$, MeOH, 0 °C, 92%.

TABLE 1. In Vitro Cytotoxicities against Tumor Cell Lines[a]

	IC_{50} (μM)	
compound	CCRF-CEM	CCRF-CEM/VBL[b]
1	12.09 ± 1.57	14.56 ± 6.92 (1.20x)
12	1.98 ± 0.20	2.41 ± 0.05 (1.22x)
13	4.49 ± 0.41	6.29 ± 1.41 (1.40x)
14	3.23 ± 0.78	3.84 ± 0.08 (1.19x)

[a] XXT assay following 72-hr inhibition. CCRF-CEM is a human T-cell acute lymphoblastic leukemia cell line. CCRF-CEM/VBL overexpresses P-glycoprotein and displays an MDR phenotype to MDR-associated oncolytics. [b] Resistance to vinblastine was 400-fold as indicated by IC_{50} increases (0.0020 ± 0.0007 μM in CCRF-CEM compared to 0.80 ± 0.10 μM in CCRF-CEM/VBL cell line).

and **14**) exhibited greater in vitro activity than do their diol counterparts (**1** and **13**). Hypothesizing that incor-



poration of the acetonide moiety may alter the conformational properties of the compound in solution, we set out to evaluate the conformations of panaxytriol (**1**) and the panaxytriol acetonide (**12**) through NMR spectroscopy and computational techniques. An extensive 2D NMR study was conducted, and selected nOe effects are shown in Figure 1. Our computational analysis, using a quantum mechanics-based gNMR program,[14] projected the

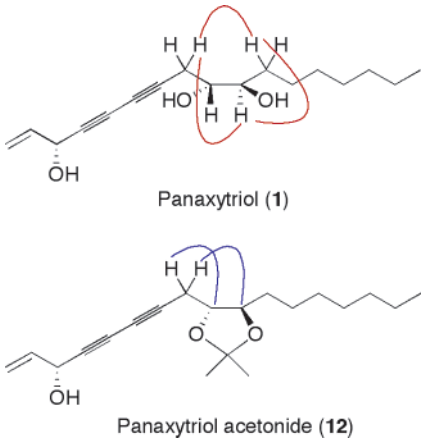


FIGURE 1. nOe effects of **1** and **12**.

TABLE 2. Coupling Constants of 1 and 12

1	*J*(Hz)	**12**	*J*(Hz)
H8a/8b	17.09	H8a/8b	17.47
H8a/9	5.97	H8a/9	5.32
H8b/9	5.61	H8b/9	5.64
H9/10	4.01	H9/10	7.87
H10/11a	6.51	H10/11a	3.18
H10/11b	5.83	H10/11b	8.64
H11a/11b	13.71	H11a/11b	13.71

characteristic coupling constants for these two compounds as shown in Table 2. On the basis of observed 2D nOe effects and gNMR simulation, Newman projections along the C_9-C_{10} bond were obtained for **1** and **12**. As shown in Figure 2, panaxytriol (**1**) has a bent

(14) Proton spectra were simulated by using the gNMR program (Cherwell Scientific Ltd., now Adept Scientific). The procedure is as follows: initial guesses for chemical shifts and proton−proton couplings were used to generate a theoretical multiplet band shape. Transitions were assigned to observed peaks, and then using least-squares methods, the theoretical band shape was fit to the experimental one. Then, transitions were reassigned, and if needed, the procedure was repeated until a best fit was obtained. As a final step, all proton multiplets were compared with theoretical spectra generated by the best-fit values.

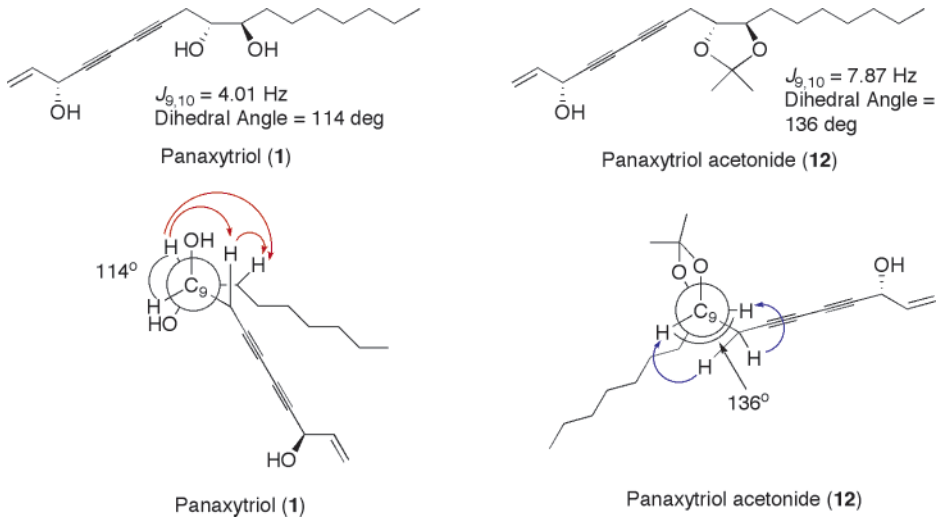


FIGURE 2. Newman projections of **1** and **12** along the C9−C10 bonds. Arrow indicates strong nOe effect.

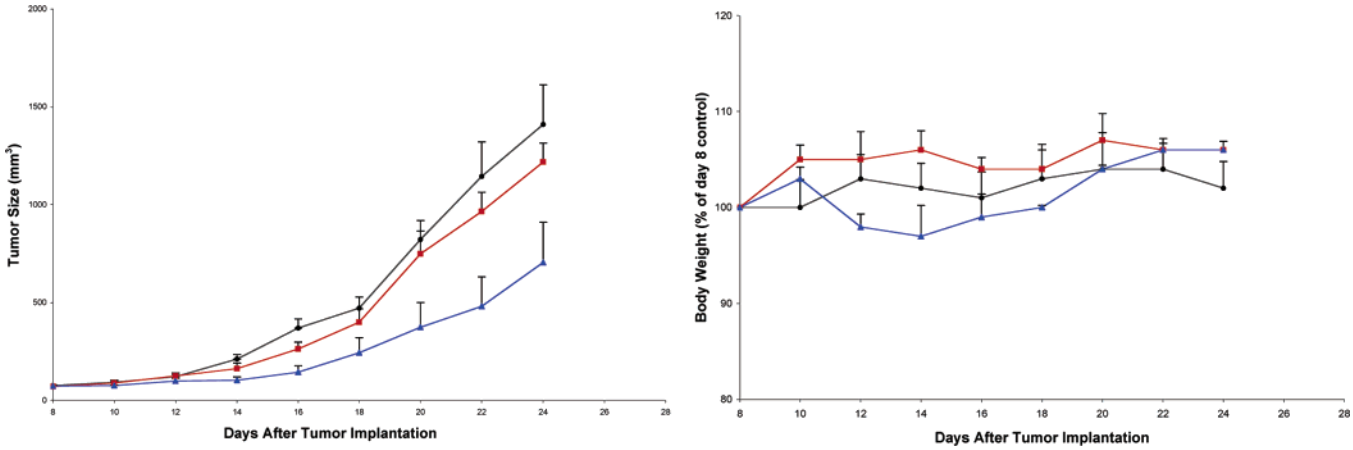


FIGURE 3. Therapeutic effect of panaxytriol (**1**) in nude mice bearing MX-1 xenograft (iv injection). Black (●) Control (n = 4). Red (■) **1** 30 mg/kg Q2Dx3, 50 mg/kg Q2Dx3, 75 mg/kg Q2Dx3. Blue (▲) **1** 50 mg/kg Q2Dx3, 75 mg/kg Q2Dx3, 100 mg/kg Q2Dx2. Body weight is the difference between total weight and tumor weight.

structure, while the acetonide (**12**) has a fairly linear structure in solution. These findings suggest that the notable increase in biological activity of panaxytriol acetonide (**12**) relative to that of panaxytriol (**1**) may reflect the impact of the conformation of the C_9-C_{10} region.

We next sought to investigate the in vivo efficacy of panaxytriol (**1**) and panaxytriol acetonide (**12**). Thus, nude mice bearing human mammary carcinoma xenograft MX-1 were treated with panaxytriol (**1**) or panaxytriol acetonide (**12**) at various dosages through the slow iv infusion protocol developed in our laboratories.[15] Mice treated with 30 mg/kg of panaxytriol exhibited some suppression of tumor growth, but no appreciable reduction in tumor mass was observed (Figure 3). At elevated dosage levels, improved inhibitory effects were observed. The panaxytriol acetonide (**12**) demonstrated enhanced in vivo potency, inhibiting tumor growth at levels as low as 10 mg/kg (Figure 4). Once again, elevated dosages led to enhanced tumor growth suppression, although treatment with compound **12** did not lead to a shrinkage in the tumor mass. Notably, even at the highest dosage levels (100 mg/kg), no body weight decrease was observed upon treatment with either compound **1** or **12**. We emphasize that these biological findings are particularly noteworthy, not because of any remarkable level of cytotoxic activity, but rather because appreciable levels of in vivo tumor growth suppression have been observed in a commonly used dietary supplement. Consequently, the antitumor activity achieved with panaxytriol is apparently not accompanied by any toxic side effects, even at high dosage levels.

Biological Investigations: Neurotrophic Activity. We sought to determine whether panaxytriol might be a contributor to the neurotrophic activity observed in the organic extracts of red ginseng. Thus, rat pheochromocytoma cells (PC12) were treated with 50 ng/mL NGF and with 60 μM panaxytriol for 96 h. The cells were then compared to a similarly prepared control, lacking only panaxytriol. As shown in Figure 5, although neurite growth was observed in the absence of panaxytriol, the sample treated with 60 μM panaxytriol demonstrated significantly enhanced neurite outgrowth. This finding confirms that panaxytriol is a neurotrophically active

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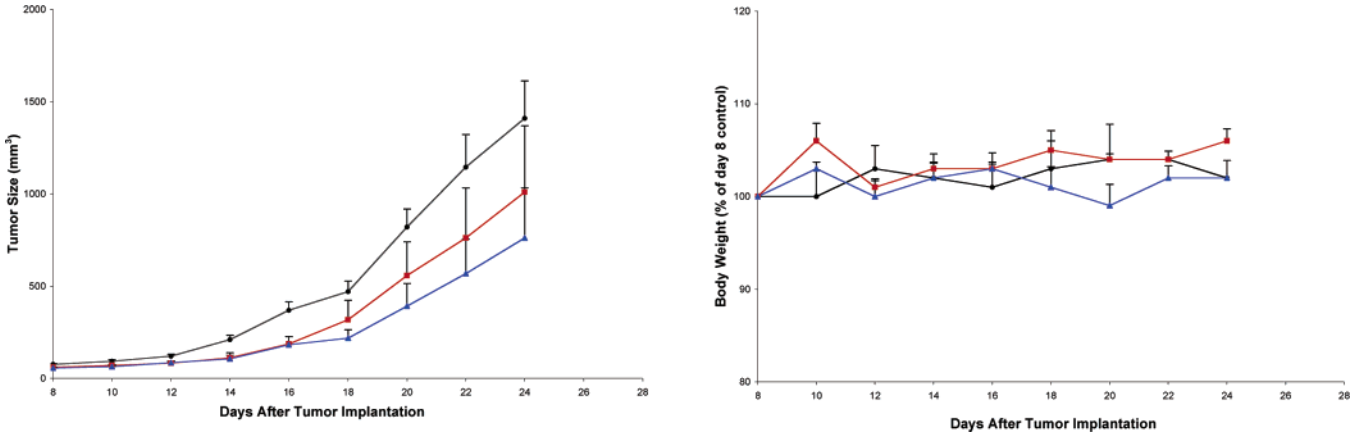

FIGURE 4. Therapeutic effect of panaxytriol acetonide (**12**) in nude mice bearing MX-1 xenograft (iv injection). Black (●) Control ($n = 4$). Red (■) **12** 10 mg/kg Q2Dx3, 30 mg/kg Q2Dx3, 50 mg/kg Q2Dx2. Blue (▲) **12** 20 mg/kg Q2Dx3, 50 mg/kg Q2Dx3, 100 mg/kg Q2Dx2. Body weight is the difference between total weight and tumor weight.

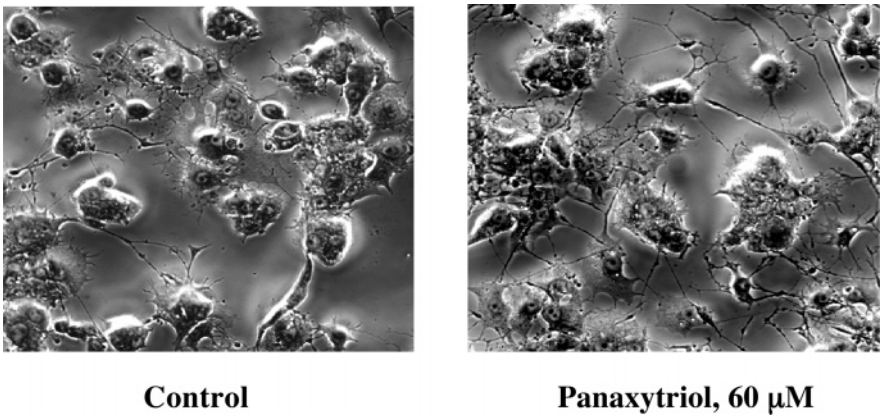

FIGURE 5. Images of neurons.

compound that contributes to the neurotrophic activity observed for red ginseng.

In summary, we have conducted key preliminary studies into the in vitro and in vivo cytotoxicity of panaxytriol. Through total synthesis, we prepared and evaluated several synthetic panaxytriol analogues, each of which exhibited enhanced cytotoxicity relative to the natural product. Consequently, we have begun to chart the first SAR map for the compound, which suggests that the C_3 hydroxyl functionality is not critical for biological activity and that, in fact, engagement of the C_9-C_{10} diol as an acetonide actually leads to notably enhanced cytotoxicity. On the basis of the results of in vitro studies, we have obtained strong evidence that panaxytriol and its derivatives are not substrates for the P-glycoprotein and, therefore, are not vulnerable to the onset of multidrug resistance. Furthermore, through in vivo investigations, we demonstrated that panaxytriol (**1**) and panaxytriol acetonide (**12**) moderately suppress tumor growth with little or no toxicity. Finally, preliminary in vitro evaluation of panaxytriol indicates that it possesses neurotrophic activity.

Obviously the research described above fosters a fresh new range of questions: (i) Is there any relationship between the manifestation of neurotrophic activity and the anticancer activity exhibited by panaxytriol (and red ginseng)? (ii) At the molecular level, what are the biological targets for each of the activities? (iii) Is there cooperativity between the various components of red ginseng contributing to its overall activity profile? While these questions remain presently unanswered, the research described above already touches on potentially important possibilities that invite more detailed exploration. The style of chemotherapeutically based oncology involves the use of very powerful cytotoxic agents. These, of course, raise significant issues of selectivity of attack on cancer cells and normal cells (i.e., therapeutic index). Also, potent anticancer drugs are inactivated through the phenomenon of MDR. The research described herein, by implication, envisions an alternate direction. Perhaps there may well be room for relatively weak antitumor agents, provided that the toxicity risk factor is dramatically reduced. In this view, a key determinant would be the chronic nature of treatment that such agents might well require.

Given the results described above, it may well be appropriate to look for and investigate compounds of this genre that offer mild antiproliferative properties in the absence of toxicity. Studies along these lines as well as studies dedicated to the discovery of the molecular targets of panaxytriol, its analogues, and, more broadly, red ginseng, are planned.

Experimental Section

Compound 12. To a THF solution of compound **1** (0.61 g, 2.191 mmol) were added $Me_2C(OMe)_2$ (3 mL, 21.91 mmol) and

p-TsOH (42 mg, 0.2191 mmol) at room temperature. After overnight, the reaction mixture was quenched with saturated $NaHCO_3$. After aqueous workup, it was purified by column chromatography (hexane/ethyl acetate 15:1 to 7:1) to give compound **12** (0.6567 g, 94%) as a colorless oil: R_f 0.19 (hexane/ethyl acetate = 8:1); $[\alpha]^{25.7}_D$ +5.0 (*c* 0.47, acetone); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.95 (ddd, 1H, J = 17.0, 10.1, 5.3 Hz), 5.46 (d, 1H, J = 17.0 Hz), 5.25 (d, 1H, J = 10.1 Hz), 4.91 (d, 1H, J = 5.3 Hz), 3.80 (dt, 1H, J = 7.7, 4.2 Hz), 3.72 (dt, 1H, J = 7.9, 5.3 Hz), 2.60 (m, 2H), 1.2−1.7 (m, 12H), 1.37 (s, 6H), 0.89 (t, 3H, J = 6.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.3, 117.6, 109.1, 80.9, 78.6, 77.2, 75.0, 71.5, 66.9, 64.0, 33.5, 32.4, 30.3, 29.8, 28.0, 27.7, 26.6, 24.2, 23.3, 14.8; IR (neat) ν 3434.8, 2927.4, 2856.3, 2256.2, 1716.7, 1458.2, 1377.4, 1242.1, 1220.6, 1066.2, 985.9, 930.6 cm^{-1}; HRMS calcd for [M − CH_3 − H] 303.1960; found 303.1946.

Compound 13. To a THF solution of compound **1** (6 mg, 0.02155 mmol) was added MnO_2 (22 mg, 0.251 mmol) at room temperature. After overnight, the reaction mixture was filtered through a short column of Celite and the solvent was removed. The concentrated reaction mixture was purified by column chromatography (hexane/ethyl acetate 4:1 to 2:1) to give compound **13** (4.5 mg, 76%) as a colorless oil: R_f 0.19 (hexane/ethyl acetate = 3:1); $[\alpha]^{20.7}_D$ +14.4 (*c* 0.44, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.55 (d, 1H, J = 17.3 Hz), 6.41 (dd, 1H, J = 17.3, 10.0 Hz), 6.22 (d, 1H, J = 10.0 Hz), 3.72 (m, 1H), 3.61 (m, 1H), 2.68 (d, 2H, J = 6.2 Hz), 1.2−1.6 (m, 12H), 0.88 (t, 3H, J = 6.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.1, 138.1, 134.8, 86.5, 77.6, 73.4, 72.3, 71.2, 66.1, 34.0, 32.3, 29.9, 29.6, 25.9, 25.7, 23.0, 14.9; IR (neat) ν 3300.3, 2945.4, 2850.4, 2231.9, 2150.6, 1650.8, 1607.1, 1463.4, 1400.9, 1257.2, 1163.5, 1132.3, 1094.8, 1026.0, 976.1, 938.6, 788.6 cm^{-1}; HRMS calcd for [M + H] 277.1804; found 277.1808.

Compound 14. To a THF solution of compound **12** (29.9 mg, 0.09389 mmol) was added MnO_2 (81.6 mg, 0.9389 mmol) at room temperature. After overnight, the reaction mixture was filtered through a short column of Celite and the solvent was removed. The concentrated reaction mixture was purified by column chromatography (hexane/ethyl acetate 4:1 to 2:1) to give compound **14** (18.4 mg, 62%) as a colorless oil: R_f 0.48 (hexane/ethyl acetate = 8:1); $[\alpha]^{22.6}_D$ +8.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.57 (d, 1H, J = 17.3 Hz), 6.41 (dd, 1H, J = 17.4, 10.0 Hz), 6.22 (d, 1H, J = 10.0 Hz), 3.77 (m, 2H), 2.69 (m, 2H), 1.59 (m, 2H), 1.41 (s, 6H), 1.26−1.40 (m, 10H), 0.88 (t, 3H, J = 6.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.1, 138.2, 134.7, 109.3, 85.6, 80.7, 78.2, 71.2, 66.2, 33.2, 32.2, 30.0, 29.5, 27.8, 27.4, 26.3, 24.2, 23.0, 14.5; IR (neat) ν 2985.9, 2929.1, 2857.5, 2236.0, 2153.0, 1734.0, 1717.0, 1645.5, 1616.4, 1456.5, 1379.2, 1290.0, 1243.0, 1163.6, 1070.0, 980.0, 789.3 cm^{-1}; HRMS calcd for [M + H] 317.2117; found 317.2123.

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Note Added after ASAP Publication. In the Figure 4 caption Q2Dx2 incorrectly read Q2Dx3 in the version published ASAP November 12, 2005; the corrected version was published ASAP November 14, 2005.

Supporting Information Available: Characterization data for compounds **1** and **12**−**14**. ^{1}H NMR and ^{13}C NMR spectra for **1**, **5**, **12**−**14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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