Cytotoxic Falcarinol Oxylipins from *Dendropanax arboreus*

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The crude organic extract of *Dendropanax arboreus* was selected as a candidate for bioassay-guided fractionation on the basis of its relatively selective cytotoxicity to a subset of cell lines within the National Cancer Institute's disease-oriented in vitro tumor-screening panel. The major compound responsible for the in vitro cytotoxicity was falcarinol (**1**). Several other known compounds were isolated and found to be cytotoxic, including dehydrofalcarinol (**2**), a diynene (**3**), falcarindiol (**4**), and dehydrofalcarindiol (**5**). In addition, two novel polyacetylenes, dendroarboreols A (**6**) and B (**7**), were isolated and characterized by standard and inverse-detected NMR methods. Compounds were selected from this series for absolute stereochemical determination using the modified Mosher method and preliminary in vivo evaluation using a LOX melanoma mouse xenograft model.

The family Araliaceae is notable as a rich source of C_{17} polyacetylenes,[2,3] but only two reports have appeared of this type of compound in *Dendropanax*. Two antifungal C_{18} homologues of falcarinol (**1**) were described some two decades ago from *Dendropanax trifidus*,[4] while 16,17-didehydrofalcarindiol (**5**) was very recently reported from Costa Rican specimens of *Dendropanax arboreus*.[5] Although a diverse selection of polyacetylenes has been isolated from this family, biological evaluations of these compounds, with the possible exception of falcarinol, have been largely preliminary in nature. Falcarinol, synonymous with "panaxynol",[2,6] has been variously reported as a skin sensitizer and irritant,[7,8] toxic trace constituent of carrot,[9−12] probable phytoalexin,[13] and thromboxane inhibitor.[14] Panaxynol reportedly is a common constituent of many plant parts[2,3] but is especially well known from roots of *Panax ginseng*.[2,15−17] To our knowledge, ginseng was not used as an antitumor herbal in traditional Asiatic medicine; however, a recent study has claimed a correlation between ginseng consumption and decreased cancer risk.[18] Falcarinol reportedly showed selective in vitro cytotoxicity against L-1210[16] and MK-1, B-16, and L-929 cancer cell lines compared to normal cell cultures.[17] Preliminary studies on the antitumor mechanism(s) of falcarinol have been undertaken.[16]

We present here chemical and biological data for falcarinol (**1**) and six related compounds (**2**−**7**), which were isolated through bioassay-guided fractionation of leaf extracts of *D. arboreus* (L.) Decne. & Planch. (Araliaceae). The extract was initially selected for study based upon its relatively pronounced in vitro cytotoxicity to a subset of cell lines contained within the National Cancer Institute's (NCI) screening panel. Complete NMR assignments are presented for novel natural products **6** and **7**, as well as for compound **2** (16,17-didehydrofalcarinol) and **5** (16,17-didehydrofalcarindiol), for which the literature assignments were either incomplete or in error.[20] Because of perceived stereochemical problems in this series,[15,21,22] we produced MTPA (Mosher) esters of compounds **1**, **3**, and **4** for absolute stereochemical determination. Sufficient amounts of compounds **1**, **2**, and **5** were isolated and subjected to preliminary in vivo antitumor evaluations.[23]

Results and Discussion

Solvent−solvent partitioning concentrated the cytotoxicity in the CCl_4- and $CHCl_3$-soluble portions of the crude organic extract. Following Sephadex LH-20 size-exclusion and cyano-bonded-phase chromatography of the CCl_4 solubles, the major cytotoxic constituent of this fraction was identified as falcarinol (**1**) on the basis of its spectroscopic features.[6,9−11,24−26] The absolute stereochemistry was tentatively assigned as 3*S* based on optical rotation measurements and comparison to literature values.[6,25,27] A trailing peak from the preparative HPLC of falcarinol was identified as 16,17-didehydrofalcarinol (**2**),[25] mainly by its NMR spectra. As some of the literature NMR assignments appeared incomplete or inadvertently transposed,[20] we provide corrected data, including firm assignments by HMBC for the acetylene carbons (see Table 1). The absolute stereochemistry of **2** was assigned as 3*S* by comparison of its optical rotation to that of falcarinol.

The requirement for substantial amounts of falcarinol and related derivatives for preliminary in vivo xenograft testing necessitated our processing all of the CCl_4 solubles, as well as the comparably cytotoxic $CHCl_3$-soluble extracts. The $CHCl_3$ fraction was subjected to Sephadex LH-20 gel permeation chromatography, vacuum−liquid chromatography on aminopropyl-bonded phase, and preparative HPLC on aminopropyl-bonded phase[28] to give a series of diyne diols. The first fraction to elute in the HPLC analysis was the diynene **3**; optical rotation measurements indicated that **3** was probably the enantiomer of a compound reported previously from *Panax ginseng*[29] and the family Umbelliferae.[30−32] We have confirmed, via HMBC experiments, the ^{13}C-NMR assignments tentatively assigned earlier.[29,32] Ahn and Kim[28] had reported that their isolate was cytotoxic.

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Table 1. ^{1}H- and ^{13}C-NMR Assignments for Compounds **2**, **5**, **6**, and **7**[a]

	dehydrofalcarinol (**2**)[b]				dehydrofalcarindiol (**5**)[c]				dendroarboreol A (**6**)[b]				dendroarboreol B (**7**)[b]			
C no.	δ^{13}C	δ^1H	m	*J* (Hz)	δ^{13}C	δ^1H	m	*J* (Hz)	δ^{13}C	δ^1H	m	*J* (Hz)	δ^{13}C	δ^1H	m	*J* (Hz)
1	117.0	5.24	dt	10.4, 1.3	117.4	5.24	dt	10.2, 1.0	9.3	0.99	t	7.3	117.3	5.25	dt	10.2, 1.3
		5.44	dt	17.4, 1.3		5.45	dt	17.1, 1.0						5.46	dt	17.1, 1.3
2	136.1	5.92	ddd	17.4, 10.4, 5.4	135.8	5.92	ddd	17.1, 10.2, 1.0	30.6	1.72	m		135.9	5.94	ddd	17.1, 10.2, 5.4
3	63.5	4.91	ddd	6.8, 5.4, 1.3	63.5	4.92	m		64.1	4.36	t	6.6	63.7	4.96	m	
4	74.3				78.3				80.7				80.4			
5	71.2				70.3				68.9[f]				70.9[i]			
6	64.0				68.7				68.9[f]				73.6[i]			
7	80.1				79.8				79.1				77.5			
8	17.6	3.01	br d	6.5	58.6	5.18	br dd	8.3, 5.4	58.6	5.18	br d	8.3	108.1	5.75	br d	15.4
9	122.0	5.36	dtt	10.8, 6.5, 1.5	127.8	5.50	ddt	10.8, 8.3, 1.0	127.8	5.50	ddt	10.8, 8.3, 1.5	149.9	6.31	dd	15.4, 6.1
10	132.9	5.49	dtt	10.8, 7.3, 1.5	134.6	5.59	ddt	10.8, 1.0, 7.4	134.5	5.60	ddt	10.8, 7.3, 1.5	72.0	4.18	ddt	6.1, 1.2, 6.8
11	27.1	2.00	m		27.6	2.09	ddt	7.4, 1.0, 7.0	27.6	2.09	dq	1.5, 7.3	36.8	1.52	q	6.8
12	28.7[d]	1.35	m		28.7[e]	1.37	m		29.1	1.37	m		25.0	1.31	m	
13	28.6[d]	1.29	m		28.6[e]	1.30	m		28.7[g]	1.31	m		28.9[h]	1.31	m	
14	29.0[d]	1.35	m		29.1[e]	1.37	m		28.6[g]	1.37	m		28.7[h]	1.37	m	
15	33.7	2.03	m		33.7	2.04	br q	6.8	33.7	2.03	br dt	6.8, 7.3	33.6	2.02	q	7.0
16	139.0	5.79	ddt	17.1, 10.8, 6.8	139.0	5.79	ddt	16.9, 10.3, 6.8	139.0	5.79	ddt	17.1, 10.3, 6.8	138.9	5.78	ddt	14.7, 10.3, 7.0
17	114.2	4.93	dq	10.8, 1.5	114.4	4.92	dq	10.3, 1.5	114.3	4.92	ddd	10.3, 2.5, 1.5	114.4	4.91	br d	10.3
		4.99	dq	17.1, 1.5		4.97	dq	16.9, 1.5		4.98	ddd	17.1, 3.5, 1.5		4.98	br d	14.7
3-OH		1.87	d	6.8		1.92	d	6.8								
8-OH						1.83	d	5.4								

[a] Spectra obtained on a Varian VXR 500 MHz instrument with $CDCl_3$ as solvent and internal reference. [b] Assignments are based on COSY, HMQC, and HMBC experiments. [c] Assignments are based on coupling constant analysis and comparison to **2** and falcarindiol **4**.[36,37] [d–g] Assignments are interchangeable within each letter. [h] Two discrete signals were observed at δ 68.93 and 68.86. [i] Assignments are based on comparison to diynene **3**.

Table 2. Stereochemical Analysis of Compounds **1**, **3**, and **4** with (*R*)- and (*S*)-MTPA (Mosher) Derivatives[a]

MTPA esters of compd no.	$\Delta\delta = \delta_{S\text{-ester}} - \delta_{R\text{-ester}}$ (Hz)								carbinol confign		
	H-1$_E$	H-1$_Z$	H-2	H-8	H-9	H-10	H-11	H-12	C-3	C-8	C-10
falcarinol (**1**)	−29	−41	−50	+6	+4	+3	+2	~0	S		
diynene **3**	−30	−40	−50	+3	+5	*b*	~0	*c*	S		?
falcarindiol (**4**)	−27	−37	−44	*b*	−43	−18	−11	−6	S	S	

[a] Referenced to the $CDCl_3$ solvent. $\Delta\delta$ measured in Hz at 500 MHz field strength. [b] Resonances of protons directly attached to esterified center are not subject to analysis. [c] Resonances not discernible in overlapped multiplets.

The penultimate peak of the preparative aminopropyl HPLC was identified as falcarindiol (**4**), long known as a constituent of many umbelliferous plants.[19,21,26,30−35] The optical rotation of our falcarindiol was comparable to that reported for (3*R*,8*S*)-falcarindiol.[21,36] However, it was difficult to reconcile production of a 3*R* metabolite in a plant containing such an abundance of (3*S*)-falcarinol. This uncertainty and the lack of firm stereochemical assignments for **3** prompted us to produce MTPA (Mosher) esters of these natural products for absolute stereochemical determination using the modified Mosher method.[37,38] Both the *S* and *R* esters of **1**, **3**, and **4** were produced from the (*R*)- and (*S*)-MTPA acid chlorides, respectively. After chromatographic cleanup, ^{1}H−^{1}H COSY experiments were used to assign the ^{1}H-NMR spectra of each derivative. The difference (due to anisotropic effects) in chemical shifts between each set of like protons was tabulated using the equation $\Delta\delta = \delta_S - \delta_R$ (see Table 2).

Because dextrorotatory falcarinol (**1**) was deduced to be 3*S* by optical rotation,[25,27] it served as a useful model compound for this NMR study, particularly because CD studies had provided ambiguous results.[15,22] Indeed, the results of the Mosher analysis (Table 2) established the absolute stereochemistry of our falcarinol to be 3*S*.[22] These results, therefore, substantiated the assignment of 3*R* stereochemistry to levorotatory falcarinol.[27] The anisotropic effect in the MTPA esters was detectable even beyond the C4−C7 diacetylene “spacer arm”, as evident from the small, but measurable $\Delta\delta$ values for H-8 to H-11. It was also apparent that these derivatives prefer a solution conformation in which H_Z of the terminal olefin is closer than H_E to the esterified center.

Though the 3*S* stereochemistry of diynene **3** was apparent from the virtually identical $\Delta\delta$ values for the H-1 and H-2 protons in MTPA esters of both **1** and **3**, the stereochemistry at C-10 could not be defined by this method. Although $\Delta\delta$ values for H-8 and H-9 of MTPA esters of **3** appear to indicate that C-10 is *S*, the magnitudes of these $\Delta\delta$ values are very small and comparable to those observed in **1**. Moreover, no appreciable $\Delta\delta$ could be measured at H-11 and H-12.

In falcarindiol (**4**), as in its congeners from *D. arboreus*, the stereochemistry at C-3 is clearly *S*. The magnitudes of $\Delta\delta$ at the 1- and 2-positions have been somewhat diminished relative to those in the MTPA esters of falcarinol (**1**). We surmise that this is due to the anisotropic effects of the additional MTPA ester at C-8. The C-8 MTPA esters showed negative $\Delta\delta$ values, which decreased in absolute value from H-9 through H-12, thus dictating 8*S* stereochemistry for falcarindiol (**4**). Had we relied merely on optical rotations for stereochemical determination, we would have made an erroneous assignment at C-3. Curiously, the optical rotations reported for (3*R*,8*S*)-falcarindiol[21,36] are, within experimental error, equivalent to the values we report for our isolate of this compound (see Experimental Section). A possible explanation is that C-3 in falcarindiol makes a much smaller contribution to the optical activity (on the order of falcarinol) than does the C-8 center, so that the optical rotations of both 8*S* diastereomers of falcarindiol would nearly coincide. However, all other reports of falcarindiol appear to show the same

relative stereochemistry as our isolate by ^{1}H- and ^{13}C-NMR analysis. Unfortunately, Lemmich did not report detailed NMR data for comparison.[36]

The most polar peak from the preparative HPLC was identified on the basis of its spectral properties (see Table 1 and Experimental Section) as 16,17-didehydrofalcarindiol (**5**). A literature search revealed that **5** was first found in extracts of *Artemesia borealis* (Asteraceae)[39] and more recently from *D. arboreus* from Costa Rica.[5] It was presumed that the stereochemistry was 3*S*,8*S*, as in falcarindiol.

The second peak eluting from the preparative HPLC appeared to contain a mixture of novel polyacetylenes by ^{1}H NMR. Subsequent C_{18} HPLC yielded an oil that analyzed for $C_{17}H_{24}O_2$ by HRMS. Its UV spectrum was similar to those of compounds **1**, **2**, and **4**, indicative of a conjugated diyne. This was confirmed by ^{13}C NMR, which showed four nonprotonated (by HMQC) acetylene resonances as well as a terminal olefin (δ 139.0, 114.3). Another olefin (δ 134.5, 127.8) accounted for the last unsaturation inherent in the molecular formula. ^{1}H-NMR analyses indicated that the other terminus consisted of a spin system that began with a methyl triplet (δ 0.99). A $^{1}H-^{1}H$ COSY experiment showed that this terminus was coupled to a methylene (δ 1.72) that was further coupled to a hydroxyl-bearing methine (δ 4.36, t), marking the end of the spin system. The COSY spectrum also showed a separate spin system that began at the allylic hydroxy-bearing methine (δ 5.18), as in compounds **4** and **5**. This methine was coupled to a cis (J = 10.8 Hz) olefin resonance (δ 5.50), which, in turn, was coupled to its vicinal partner (5.60). A correlation was observed between this latter signal and an allylic methylene (δ 2.09). Beyond this, the spin system extended to the overlapped methylene envelope (δ 1.31–1.37) and could not be traced further with certainty. In the spin system associated with the terminal olefin, the proton at δ 5.79 was cis-coupled to a proton at δ 4.92, trans-coupled to one at δ 4.98, and vicinally coupled to an allylic methylene at δ 2.03. This spin system also correlated to the overlapped methylene envelope and could not be traced further.

After the ^{13}C resonances had been assigned by HMQC (see Table 1), partial structures were linked using an HMBC experiment. Key correlations included those observed between the carbinol proton at δ 4.36 and the acetylene carbons at δ 80.7 and 68.9. A $^{3}J_{CH}$ between the δ 1.72 methylene and the 80.7 resonance firmly placed the carbinol at 64.1 vicinal to the 80.7 acetylene. On the opposite side of the diyne, we observed a correlation between the δ 5.18 carbinol proton and an acetylene at δ 79.1, as well as a $^{3}J_{CH}$ between the same proton and one of the two interior alkyne carbons near δ 68.9. A $^{3}J_{CH}$ between the olefin at δ 5.50 and the δ 79.1 acetylene carbon firmly placed this center on the opposite side of the diacetylene from the 80.7 resonance and dictated placement of the 58.6 carbinol vicinal to the 79.1 signal. On the basis of the above data and comparison to compounds **4** and **5**, compound **6** must be 1,2-dihydro-16,17-didehydrofalcarindiol, to which we have assigned the trivial name dendroarboreol A.

Another novel trace metabolite, dendroarboreol B (**7**), was isolated following amino-bonded phase HPLC of fractions from the C_{18} HPLC procedure, which yielded dendroarboreol A (**6**). All data (see Table 1 and Experimental Section) were consistent with assignment of compound **7** as the 16,17-didehydro analogue of diynene **3**. The paucity of both **6** and **7** precluded the possibility of defining their absolute configurations.

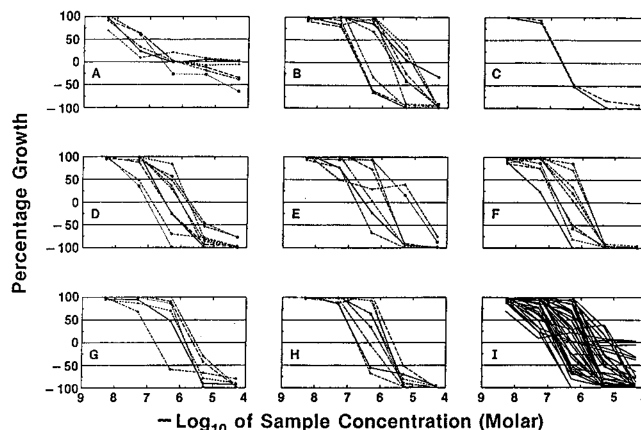
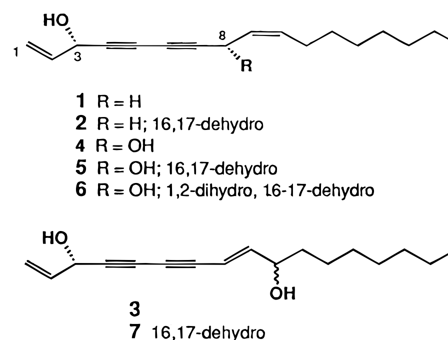


Figure 1. Dose–response curves for falcarinol (**1**) in the in vitro human disease-oriented tumor cell line screening panel: (A) leukemia, (B) non-small cell lung, (C) small cell lung, (D) colon, (E) CNS (brain), (F) melanoma, (G) ovarian, (H) renal, (I) composite of all cell lines.

Table 3. Comparison of *in Vitro* Cytotoxicity Potencies[a]

compound	GI_{50}[b]	TGI[b]	LC_{50}[b]
1[c]	2.2×10^{-7}	7.6×10^{-7}	3.8×10^{-6}
2	4.0×10^{-7}	1.3×10^{-6}	4.8×10^{-6}
3	4.4×10^{-7}	1.4×10^{-6}	4.7×10^{-6}
4	6.6×10^{-7}	2.2×10^{-6}	7.6×10^{-6}
5	2.1×10^{-6}	3.0×10^{-6}	1.0×10^{-5}
6[d]	4.6×10^{-6}	1.4×10^{-5}	2.5×10^{-5}
7[d]	5.0×10^{-7}	1.9×10^{-6}	7.6×10^{-6}

[a] In NCI 60-cell-line human tumor screening panel; data reported (molarity) are overall panel averages of quadruplicate tests, unless otherwise noted. [b] GI_{50}—drug-treated cells' net growth is 50% of untreated controls; TGI—no net growth of drug treated cells; LC_{50}—50% net cell death in drug-treated cells vs. starting levels. [c] Values based on triplicate tests. [d] Values based on duplicate tests.



The in vitro differential cytotoxicity profile of falcarinol is depicted in Figure 1 as a compilation of dose-response curves.[40] The patterns of differential cytotoxicity for compounds **2**–**5** and **7** were virtually superimposable, although subtle differences in potency were observed through the series. Table 3 provides a comparison of mean GI_{50}, TGI, and LC_{50} values for **1**–**7**. Inclusion of the 16,17-olefin led to a slight reduction in potency in all three cases (**1** → **2**, **4** → **5**, **3** → **7**). The even lower potency observed for dendroarboreol A (**6**) may be due to saturation of the $\Delta^{1,2}$ olefin.

Because of its relatively high degree of in vitro sensitivity to this series of compounds, the LOX mela-

noma cell line was selected for use in in vivo experimental therapeutic studies. This particular melanoma line grows very aggressively in athymic nude mice, making possible a range of experimental models from relatively simple ones through advanced metastatic models.[23] To provide maximal sensitivity for preliminary in vivo evaluations, tumor cells were implanted in the peritoneal cavity of mice, and test compounds, formulated in sesame oil, were injected intraperitoneally. Treatment was administered on a daily times five schedule. Results of testing are summarized in Table 4.

The initial testing of **1** indicated a high degree of toxicity. All animals treated at doses of 3.51 mg/kg or greater died acutely. However, as indicated in Table 4, some evidence of therapeutic activity was observed at the lowest doses tested. In a subsequent test, with dosing up to the maximum tolerated dose (MTD) indicated from the first experiment, a modest therapeutic effect was again observed. Testing of **5** failed to indicate any evidence of therapeutic activity, even at doses twofold higher than the MTD for **1**. The initial test of **2** produced the strongest therapeutic effect observed with this series of compounds. As illustrated in Figure 2, 40% long-term survivors were observed at a dose of 2.0 mg/kg. In another experiment, a direct comparison of **2** with **1** was performed. As indicated in Table 4, **2** did produce a better therapeutic effect in this comparison, but the extent of activity was less dramatic than that observed in the initial experiment.

Thus, we have demonstrated some potential for in vivo antitumor activity with this series of compounds. Compound **2** appears to have the greatest activity in the LOX melanoma model employed. However, considering the overall very modest amount of activity that could be demonstrated even in this minimal therapeutic challenge, the ip−ip model, and the relative instability of the enynols, we have not pursued further in vivo investigations of these compounds.

Table 4. Falcarinol In Vivo Experiments[a]

falcarinol (**1**) dose (mg/kg)	number of mice with tumors	median day of death	percent increase in lifespan[b]
Expt 1			
control	19/20	21	
225.0	0/10	2	
112.5	0/10	3	
56.25	0/10	3	
28.12	0/10	4	
14.06	0/10	4.5	
7.03	0/10	5.5	
3.51[c]	0/10	6.5	
1.76	10/10	22	5
0.88	10/10	22	5
Expt 2			
control	19/20	20	
3.0	10/10	20	
2.0	10/10	19.5	
1.33	10/10	20	
0.88	10/10	18	
0.59	10/10	22.5	13
0.39	10/10	19	
0.17	10/10	21	5
Expt 3			
control	20/20	15	
3.0	10/10	17	13[e]
2.0	10/10	16	7
1.33	10/10	15	
0.88	10/10	16	7
0.59	10/10	16	7
dehydrofalcarindiol (**5**) expt 1			
control	20/20	15	
6.0	10/10	15	
3.0	10/10	14.5	
2.0	10/10	14	
1.33	10/10	15.5	3
0.88	10/10	14	
0.59	9/9	15	
0.39	10/10	14.5	
0.26	10/10	15	
dehydrofalcarinol (**2**) expt 1[f]			
control	20/20	15	
3.0	8/10	22	47[e]
2.0	6/10	19	27[e]
1.33	9/10	18	20
0.88	10/10	15	
0.59	9/10	18	20[e]
0.39	10/10	15	
0.26	10/10	18	20
0.17	10/10	15	
expt 2[d]			
control	20/20	15	
3.0	10/10	18	20[e]
2.0	10/10	16	7
1.33	10/10	15	
0.88	10/10	17	13
0.59	10/10	15	

[a] Human LOX melanoma cells were inoculated intraperitoneally on day 0 in female athymic nude mice. Mice were given 5 daily treatments ip days 1 through 5. Vehicle control mice were treated with sesame oil. Experiments were continued until all animals succumbed to tumor or 120 days after tumor cell inoculation. [b] Percent increase in lifespan was calculated using median day of death parameters for control (C) and test (T) groups according to the following formula: $(T-C)/C \times 100$. [c] All falcarinol doses at and above 3.51 produced 100% acute (<7-day) lethality. [d] Falcarinol Experiment 3 and dehydrofalcarinol Experiment 2 were conducted concurrently as a head-to-head comparison using a common control group. [e] Statistically significant difference ($p < 0.05$) from vehicle control group based on a modified Wilcoxon test. [f] See Figure 2 for details of this experiment.

Experimental Section

Collection, Extraction, and Chromatography. Leaves of *D. arboreus* (488 g dry wt) were collected from a 7−8-m tree in Municipio Utuado, Puerto Rico, in September 1987, at an elevation of 800 m. Voucher specimens have been deposited in the herbaria of the New York Botanical Garden and the Smithsonian Institution. The leaves were air dried in the field, stored at −20 °C for three days, ground to a fine powder, and extracted overnight at room temperature in CH_2Cl_2−MeOH (1:1), followed by MeOH. Solvents were evaporated in vacuo to yield 33 g of crude organic extract, of which 22 g were sequentially partitioned among hexane, CCl_4, $CHCl_3$, and increasingly polar mixtures of MeOH−H_2O.

The CCl_4 solubles (5.7 g) were applied to a Sephadex LH-20 column (5 × 96 cm, monitored @ 232 nm) and eluted with hexane−CH_2Cl_2−MeOH (2:5:1) to yield a UV-active fraction (1.85 g). This fraction was further purified by vacuum and flash cyano-bonded-phase columns (40 μm) eluted with increasingly polar mixtures of MeOtBu−heptane. Final preparative separation was accomplished by HPLC (Rainin-Dynamax-cyano, 8 μm, 4.1 × 25 cm, 50 mL/min, monitored @ 258 nm); elution with hexane−iPrOH (49:1) yielded 500 mg falcarinol (**1**) and a tailing peak. The tailing peak was chromatographed further on Diol (7 μm, 1 × 25 cm, hexane−

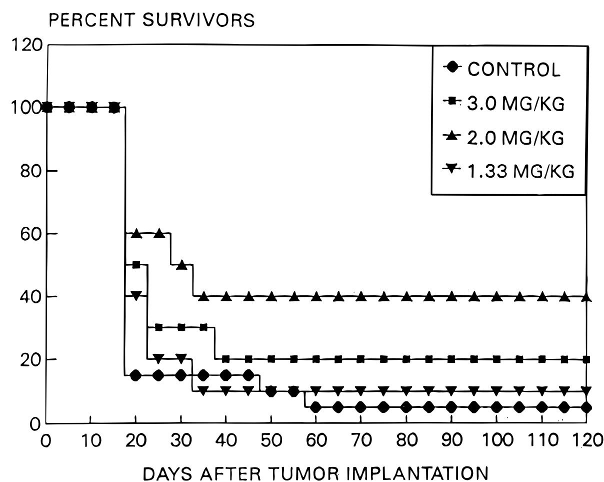


Figure 2. Human LOX melanoma cells (1×10^7 cells) were inoculated intraperitoneally on day 0 in female athymic nude mice. Mice were given five daily treatments with dehydrofalcarinol (**2**) ip on days 1−5. Vehicle control mice were treated with sesame oil. The experiment was terminated 120 days after tumor cell inoculation. Surviving treated animals were observed to be tumor-free at necropsy. The surviving control animal showed evidence of ascites. Data illustrated are for the top three doses used in dehydrofalcarinol Experiment 1 (Table 4). Doses of 3.0 and 2.0 mg/kg produced survival curves significantly different from the vehicle controls ($p < 0.05$, modified Wilcoxon test).

EtOAc, 9:1) and C_{18} (8 μm, 1 × 25 cm, MeOH−H_2O, 4:1) HPLC columns to yield 30 mg 16,17-didehydrofalcarinol (**2**).

The $CHCl_3$ solubles (2.3 g) were applied to the same Sephadex LH-20 column described above (monitored at 285 nm) and eluted with the same solvents to yield 400 mg of a strongly UV-active mixture. This fraction was subjected to vacuum chromatography on amino-bonded phase. Fractions eluting from 20 to 50% iPrOH−hexane (170 mg) were pooled and separated by HPLC (Rainin Dynamax-amino, 8 μm, 2.1 × 25 cm, diode array detection) with heptane−iPrOH (9:1) to give four major peaks. The first major peak to elute displayed a strong UV absorbance at 285 nm and was further purified using C_{18} and cyano HPLC to yield 21 mg of the known diynene **3**. The penultimate peak lacked the 285 nm absorption but was detected at 205 nm. C_{18}-bonded phase HPLC yielded 26 mg of a pure compound, identical in planar structure to falcarindiol (**4**). The most polar major peak (43 mg) was 16,17-didehydrofalcarindiol (**5**). The second major peak (7.4 mg) from the preparative amino-bonded-phase HPLC described above was still a mixture. Purification on a C_{18} HPLC column yielded 1.8 mg of the previously undescribed dendroarboreol A (**6**). Earlier eluting C-18 HPLC fractions were pooled for a final HPLC separation on amino-bonded phase, yielding 1.8 mg of the novel metabolite dendroarboreol B (**7**).

Characterization of (3*S*)-Falcarinol (1). A complete spectral data set (UV, IR, NMR, MS) corresponded fully with literature values,[6,9−11,24−26] with the exception of optical rotation: $[\alpha]^{25}$ (λ, nm) +29° (589), +30° (578), +35° (546), 66° (436), +110° (365) (*c* 0.57, $CHCl_3$); $[\alpha]^{24}_D$ +7° (*c* 1.0, Et_2O). Literature values for (3*R*)-falcarinol[6,25,27] were levorotatory at various wavelengths in these same solvents.

(3*S*)-16,17-didehydrofalcarinol (2): colorless oil; $[\alpha]^{26}$ (λ, nm) +10° (589), +11° (578), 13° (546), +25° (436), +47° (365) (*c* 0.94, Et_2O); lit., $[\alpha]^{23}_{578}$ −5.0° (*c* 13.9, Et_2O);[25] HREIMS *m/z* 242.1695 $[M]^+$,$C_{17}H_{22}O$, 2.5 mmu dev); other spectroscopic data in accord with literature values[20,25] except for ^1H and ^{13}C NMR (see Table 1).

(3*S*)-diynene (3): colorless oil; $[\alpha]^{24}$ (λ, nm) +37° (589), 38° (578), +44° (546), +78° (436), +140° (365) (*c* 0.3, $CHCl_3$), lit. $[\alpha]_D$ −38° (*c* 0.7, $CHCl_3$);[29] other relevant spectrochemical data in agreement with literature values.[28−32]

(3*S*,8*S*)-Falcarindiol (4): colorless oil; $[\alpha]^{26}$ (λ, nm) +300° (589), +320° (578), +360° (546), +670° (436) (*c* 0.14, Et_2O); lit. (3*R*,8*S*), $[\alpha]^{20}$ (λ, nm) +284° (589), +621° (436) (*c* 1, Et_2O);[21,35] other spectral features consistent with literature data.[18,26,33−34]

(3*S*,8*S*)-16,17-Didehydrofalcarindiol (5): colorless oil; $[\alpha]^{26}$ (λ, nm) +310° (589), +320° (578), 370° (546), 690° (436) (*c* 0.38, Et_2O); UV λ_{max} (Et_2O) 218, 234, 247, 261 nm (ϵ 1700, 980, 930, 549); EIMS (probe, 70 eV) *m/z* (rel int) 258 ($[M]^+$, 0.02), 239 (1), 197 (3), 183 (5), 171 (10), 157 (27), 141 (25), 129 (81), 115 (66), 105 (39), 91 (100), 77 (85); HREIMS *m/z* 258.1593 ($[M]^+$,$C_{17}H_{22}O_2$, 2.7 mmu dev); for ^1H and ^{13}C NMR, see Table 1.

Dendroarboreol A (1,2-dihydro-16,17-didehydrofalcarindiol) (6): colorless oil; $[\alpha]^{24}$ (λ, nm) +250° (589), 260° (578), +300° (546), +560° (436) (*c* 0.2, $CHCl_3$); UV λ_{max} (EtOH) 220, 234, 245, 259 nm (ϵ 1800, 1200, 1100, 750); EIMS (probe) 70 eV *m/z* (rel int) 260 ($[M]^+$, 0.15), 227 (6), 213 (6), 199 (6), 185 (9), 171 (11), 159 (36), 145 (28), 131 (64), 117 (47), 105 (31), 91 (100), 77 (58); HREIMS *m/z* 260.1773 ($[M]^+$, $C_{17}H_{24}O_2$, 0.4 mmu dev). For ^1H and ^{13}C NMR, see Table 1.

Dendroarboreol B (7): colorless oil; $[\alpha]^{24}$ (λ, nm) +34° (589), 35° (578), +42° (546), +81° (436), +150° (365) (*c* 0.2, $CHCl_3$); UV λ_{max} (EtOH) 214, 242, 255, 270, 286 nm (ϵ 33 000, 6500, 13 000, 18 000, 14 000); EIMS (probe 70 eV *m/z* (rel int) 258 ($[M]^+$, 1), 197 (3), 183 (3), 169 (4), 161 (17), 143 (11), 133 (19), 125 (38), 115 (100), 107 (34), 103 (33), 91 (35), 77 (71); HREIMS *m/z* 258.1606 ($[M]^+$, $C_{17}H_{22}O_2$, 1.4 mmu dev); for ^1H and ^{13}C NMR see Table 1.

MTPA (Mosher) Esters of Compounds 1, 3, and 4. The best yields (ca. 75%) were obtained by dissolving 5 mg of natural product in 0.5 mL dry pyridine. To these solutions were added a granule of catalytic DMAP and a 4× molar excess of α-methoxy-α-(trifluoromethyl)-phenylacetic acid chloride (MTPA-Cl). Reactions were stirred under N_2 overnight at room temperature and then quenched by removal of solvent *in vacuo*. Reaction products were applied in minimal CH_2Cl_2 to a plug of cyano-bonded phase, which was eluted under vacuum with hexane. The desired nonpolar product always eluted in the first 50-mL fraction. The product could be further purified when necessary on cyano-bonded-phase HPLC (hexane−iPrOH, 99:1). Both the (*S*)-ester [from (*R*)-MTPA-Cl] and (*R*)-ester [from (*S*)-MTPA-Cl] were produced for each of the three natural products. The six derivatives were characterized by 500 MHz ^1H NMR according to the formula $\Delta\delta = \delta_S - \delta_R$. Results are summarized in Table 2.

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References and Notes

(1) Current address: Nutraceutical Corporation, Ogden, Utah 84403.
(2) Hansen, L.; Boll, P. M. *Phytochemistry* **1986**, *25*, 285−293.
(3) Bohlmann, F.; Burkhardt, T.; Zdero, C. *Naturally Occurring Acetylenes*; Academic Press: London, 1973; p 547.
(4) Kawazu, K.; Noguchi, H.; Fujishita, K.; Iwasa, J.; Egawa, H. *Tetrahedron Lett.* **1973**, *14*, 3131−3132.
(5) Setzer, W. N.; Green, T. J.; Whitaker, K. W.; Moriarity, D. M.; Yancey, C. A.; Lawton, R. O.; Bates, R. B. *Planta Med.* **1995**, *61*, 470−471.
(6) Terada, A.; Tanoue, Y.; Kishimoto, D. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 2977−2980.
(7) Hansen, L.; Boll, P. M. *Phytochemistry* **1986**, *25*, 529−530.
(8) Gafner, F.; Epstein, W.; Reynolds, G.; Rodriguez, E. *Contact Dermatitis* **1988**, *19*, 125−128.
(9) Crosby, D. G.; Aharonson, H. *Tetrahedron* **1967**, *23*, 465−472.
(10) Bentley, R. K.; Thaller, V. *J. Chem. Soc., Chem. Commun.* **1967**, 439−440.
(11) Yates, S. G.; England, R. E. *J. Agric. Food Chem.* **1982**, *30*, 317−320.
(12) Lund, E. D.; White, J. M. *J. Sci. Food Agric.* **1990**, *51*, 507−516.
(13) Batista, U. G.; Higgins, V. J. *Can. J. Bot.* **1991**, *69*, 822−830.
(14) Kuo, S.-C.; Teng, C.-M.; Lee, J.-C.; Ko, F.-N.; Chen, S.-C.; Wu, T.-S. *Planta Med.* **1990**, *56*, 164−167.
(15) Shim, S. C.; Koh, H. Y.; Chang, S. *Tetrahedron Lett.* **1985**, *26*, 5775−5776.
(16) Kim, Y. S.; Jin, S. H.; Kim, S. I.; Hahn, D. R. *Arch. Pharm. Res.* **1989**, *12*, 207−213.
(17) Matsunaga, H.; Katano, M.; Yamamoto, H.; Fujito, H.; Mori, M.; Takata, K. *Chem. Pharm. Bull.* **1990**, *38*, 3480−3482.
(18) Yun, T. K.; Choi, S. Y. *Int. J. Epidemiology* **1990**, *19*, 871−876.
(19) Cunsolo, F.; Ruberto, G.; Amico, V.; Piattelli, M. *J. Nat. Prod.* **1993**, *56*, 1598−1600.
(20) Harada, R.; Iwasaki, M. *Phytochemistry* **1982**, *21*, 2009−2011.
(21) Lemmich, E. *Phytochemistry* **1981**, *20*, 1419−1420.
(22) Bernart, M. W.; Hallock, Y. F.; Cardellina, J. H., II; Boyd, M. R. *Tetrahedron Lett.* **1994**, *35*, 993−994.
(23) Shoemaker, R. H.; Dykes, D.; Plowman, J.; Mayo, J. G.; Fodstad, O.; Boyd, M. R. *Cancer Res.* **1991**, *51*, 2837−2841.
(24) Gafner, F.; Reynolds, G. W.; Rodriguez, E. *Phytochemistry* **1989**, *28*, 1256−1257.
(25) Bohlmann, F.; Niedballa, U.; Rode, K.-M. *Chem. Ber.* **1966**, *99*, 3552−3562.
(26) Tanaka, S.; Ikeshiro, Y.; Tabata, M.; Konoshima, M. *Arzneim. Forsch.* **1977**, *27*, 2039−2041.
(27) Larsen, P. K.; Nielsen, B. E.; Lemmich, J. *Acta Chem. Scand.* **1969**, *23*, 2552−2554.
(28) Ahn, B.-Z.; Kim, S. I. *Planta Med.* **1988**, *54*, 183.
(29) Hirakura, K.; Morita, M.; Nakajima, K.; Ikeya, Y.; Mitsuhashi, H. *Phytochemistry* **1992**, *31*, 899−903.
(30) Schulte, K. E.; Wulfhorst, G. *Arch. Pharm.* **1977**, *310*, 285−292.
(31) Schulte, K. E.; Potter, B. *Arch. Pharm.* **1977**, *310*, 945−956.
(32) Baba, K.; Tabata, Y.; Kozawa, M.; Kimura, Y.; Arichi, S. *Shoyakugaku Zasshi* **1987**, *41*, 189−194; *Chem. Abstr.* **1988**, *108*, 143050a.
(33) Villegas, M.; Vargas, D.; Msonthi, J. D.; Marston, A.; Hostettmann, K. *Planta Med.* **1988**, *54*, 36−37.
(34) Nitz, S.; Spraul, M. H.; Drawert, F. *J. Agric. Food Chem.* **1990**, *38*, 1445−1447.
(35) Kern, J. R.; Cardellina, J. H., II *J. Nat. Prod.* **1982**, *45*, 774−776.
(36) Lemmich, E. *Phytochemistry* **1979**, *18*, 1195−1197.
(37) Ohtani, I.; Kusukmi, T.; Kashman, Y.; Kakisawa, H. *J. Org. Chem.* **1991**, *56*, 1296−1298.
(38) Rieser, M. J.; Hui, Y.; Rupprecht, J. K.; Kozlowski, J. F.; Wood, K. V.; McLaughlin, J. L.; Hanson, P. R.; Zhuang, Z.; Hoye, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 10 203−10 213.
(39) Wang, Y.; Toyota, M.; Krause, F.; Hamburger, M.; Hostettmann, K. *Phytochemistry* **1990**, *29*, 3101−3105.
(40) Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91−109.

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