

ANTITUMOR AGENTS 200.¹ CYTOTOXICITY OF NATURALLY OCCURRING RESVERATROL OLIGOMERS AND THEIR ACETATE DERIVATIVES

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Abstract: Eleven resveratrol oligomers and six acetylated derivatives were evaluated for in vitro cytotoxicity against a panel of human tumor cell lines. The acetate of (-)-ampelopsin A (**12**) showed potent and selective cytotoxic activity with ED₅₀ values of 0.6, 0.7 and 2.0 µg/mL against KB, 1A9 and MCF-7 cells, respectively. Hopeaphenol (**10**) and pallidol hexaacetate (**13**) also showed significant cytotoxicity against KB cells with ED₅₀ values of 1.2 and 1.6 µg/mL, respectively. © 1999 Elsevier Science Ltd. All rights reserved.

Resveratrol and its oligomers are widely distributed in the families Vitaceae and Dipterocarpaceae.² Monomeric resveratrol was investigated recently as a chemopreventive agent,³ and displayed tumor growth inhibition in the rat⁴ and induction of p53-dependent apoptosis.⁵ Nevertheless, a systematic study has not been reported to date on antitumor activity of resveratrol oligomers.

In our previous studies, resveratrol oligomers were isolated from *Sophora* (Leguminosae),^{6–11} *Parthenocissus*, *Cyphostemma* (Vitaceae),^{12,13} and *Hopea* (Dipterocarpaceae)¹⁴ species, and their structures elucidated. Now, we have screened 11 compounds against several tumor cell lines: (-)-ampelopsin A (**1**), pallidol (**2**), isoampelopsin F (**3**), ε-viniferin (**4**) as dimers; gnetin E (**5**), davidiol A (**6**), miyabenol C (**7**), leachianol A (**8**), leachianol B (**9**) as trimers; hopeaphenol (**10**), stenophyllol A (**11**) as tetramers. Herein we describe the results for these compounds and the acetates of **1–5** and **10**.

Materials. Compound **1** was isolated from the bark of *Hopea parviflora*, **2** and **3** from the bark of *Parthenocissus tricuspidata*, and **5** from the root of *Cyphostemma bainesii*. Compounds **4–7** (except **5**), **8–9**, and **10–11** were obtained from the root of *Sophora davidii*, *S. leachiana* and *S. stenophylla*, respectively. The isolation procedure and spectroscopic data of all compounds were described in the previous papers.^{6–14}

The acetates (**12–17**)¹⁵ were prepared by the following general method.¹⁵ Each oligomer (3 to 5 mg) was dissolved in pyridine (1 mL), and acetic anhydride (0.5 mL) was added. After 12 h, the mixture was

poured into cold water, then partitioned with ethyl acetate. The ethyl acetate layer was concentrated in vacuo, and purified by preparative TLC (benzene:acetone, 5:1). Yields were 60–70%.

Cytotoxicity Assays. The in vitro cytotoxicity assay was carried out according to procedures described in Rubinstein et al.¹⁶ The human tumor cell line panel constituted of epidermoid carcinoma of the nasopharynx (KB), lung carcinoma (A549), ileocecal carcinoma (HCT-8), melanoma (SK-MEL-2), and renal (CAKI-1), breast (MCF-7), and ovarian (1A9) cancers. The results are shown in Table 1.

Table 1. In Vitro Cytotoxicity of Resveratrol Oligomers and Their Acetate Derivatives

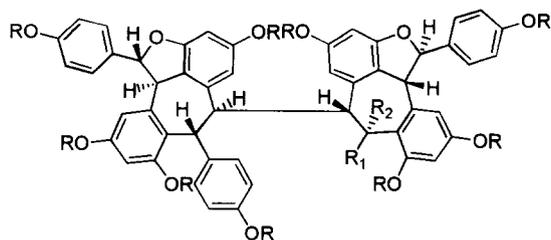
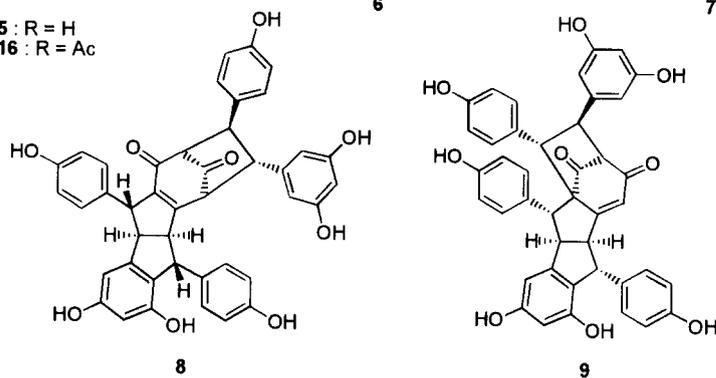
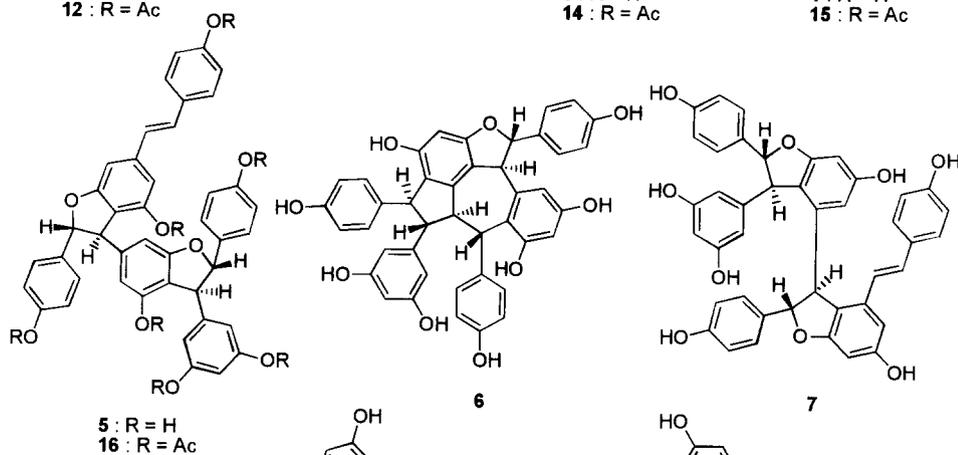
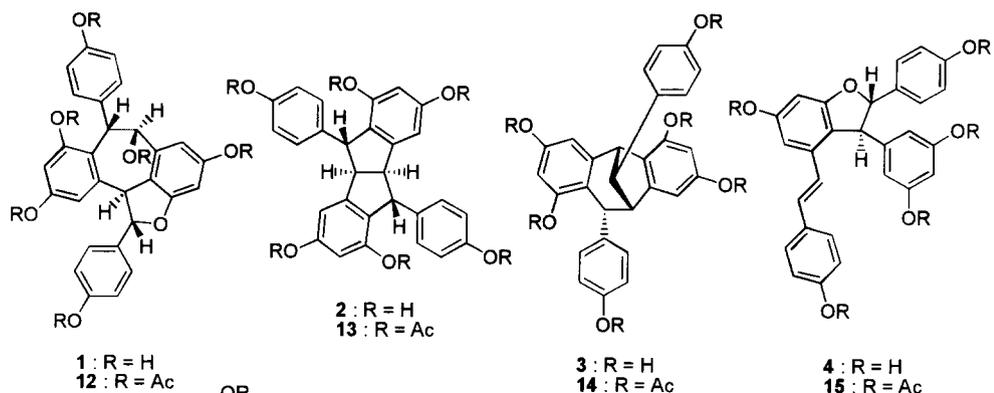
Compound	ED ₅₀ (μg/mL) ^a in Cancer Cell Lines						
	KB ^b	A549 ^b	CAKI-1 ^b	MCF-7 ^b	1A9 ^b	SaOS-2 ^b	HCT-8 ^b
1	>20 (38) ^c	>20 (7)	>20 (38)	>20 (10)	>20 (32)	>20 (8)	- ^d
10	1.2	4.8	NA ^e	4.2	7.8	>20 (41)	-
12 (1)^f	0.6	NA	4.0	2.0	0.7	-	4.1
13 (2)	1.6	NA	8.0	7.4	3.2	-	8.0
14 (3)	3.0	NA	>10 (21)	>10 (43)	6.0	-	>10 (36)
15 (4)	7.5	20.0	NA	4.0	5.8	7.5	-

^a Cytotoxicity as ED₅₀ for each cell line, the concentration of compounds that causes a 50% reduction in absorbance at 562 nm relative to untreated cells using the SRB assay. ^b Human epidermoid carcinoma of the nasopharynx (KB), human lung carcinoma (A549), human renal cancer (CAKI-1), human breast cancer (MCF-7), human ovarian cancer (1A9), human osteosarcoma (SaOS-2) and human ileocecal carcinoma (HCT-8). ^c Inhibition was less than 50% at the highest concentration tested. The percent inhibition observed in such cases is given in the parentheses. ^d - = not tested. ^e NA = not active at the highest concentration tested. ^f The number of the corresponding oligomer is given in the parentheses.

Results and Discussions. Four naturally occurring resveratrol dimers (**1–4**), five trimers (**5–9**) and two tetramers (**10** and **11**) were tested for cytotoxicity against a panel of human tumor cell lines. Hopeaphenol (**10**) showed potent cytotoxicity against KB cells with an ED₅₀ value of 1.2 μg/mL, although stenophyllol A (**11**), which is an oxidative derivative of **10**, was not effective. This result suggested that 4-hydroxyphenyl moieties on the seven-membered rings of hopeaphenol could enhance cytotoxicity.

Furthermore, the acetate derivatives (**12–17**) also were evaluated as shown in Table 1. In general, the acetates (**12–15**) showed significantly increased cytotoxic activity compared with their corresponding resveratrol dimers. Compound **12** was the most potent of the acetylated compounds. It was quite active against rapidly replicating cell lines (KB and 1A9; 0.6 and 0.7 μg/mL) and had significant activity against MCF-7 (2.0 μg/mL), while it was inactive against A549 and SK-MEL-2 (melanoma cells; data not shown), indicating that **12** possesses significant tumor-type selectivity. Other compounds (**13–15**) showed similar pattern of activity indicating a common mechanism of action. However, the acetates of the trimer and tetramer (**16** and **17**) displayed no cytotoxicity in this study.

In summary, acetylation of resveratrol dimers could increase of activity and selectivity. On the other hand, certain non-acetylated oligomers such as **10**, also were cytotoxic. The increased lipophilicity of



	R	R ₁	R ₂
10 :	H		H
17 :	Ac		H
11 :	H		

the acetate might play a role in transport and cellular penetration and uptake. We will continue our cytotoxic evaluation of additional resveratrol oligomers and their derivatives to extend the structure-activity relationship studies of this compound class.

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References and Notes

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15. ^1H NMR (300 MHz, CDCl_3): (-)-*Ampelopsin A* hexaacetate (**1a**): δ 2.03 (3H, s), 2.27 (6H, s), 2.29 (9H, s), 4.24 (1H, d, $J = 10$ Hz), 5.26 (1H, d, $J = 5$ Hz), 5.97 (1H, d, $J = 10$ Hz), 6.56 (1H, d, $J = 2$ Hz), 6.68 (1H, d, $J = 5$ Hz), 6.76 (1H, d, $J = 2$ Hz), 6.79 (1H, br s), 7.05 (2H, d, $J = 8$ Hz), 7.08 (2H, d, $J = 8$ Hz), 7.22 (2H, d, $J = 8$ Hz); *Pallidol* hexaacetate (**2a**): δ 1.69 (6H, s), 2.28 (6H, s), 2.30 (6H, s), 4.17 (2H, t like m), 4.44 (2H, t like m), 6.76 (2H, d, $J = 2$ Hz), 6.88 (2H, d, $J = 2$ Hz), 7.05 (4H, d, $J = 9$ Hz), 7.16 (2H, d, $J = 9$ Hz); *Isoampelopsin F* hexaacetate (**3a**): δ 1.56, 2.14, 2.25, 2.26, 2.30, 2.36 (3H each, s), 3.58 (1H, d, $J = 6$ Hz), 3.89 (1H, s), 4.18 (1H, s), 4.70 (1H, d, $J = 6$ Hz), 5.74 (1H, d, $J = 2$ Hz), 5.80 (1H, dd, $J = 8, 2$ Hz), 6.68 (1H, dd, $J = 8, 2$ Hz), 6.75 (1H, d, $J = 2$ Hz), 6.78 (1H, d, $J = 2$ Hz), 6.92 (2H, d, $J = 8$ Hz), 7.10 (2H, d, $J = 8$ Hz), 7.40 (1H, dd, $J = 8, 2$ Hz); *ε-Viniferin* pentaacetate (**4a**): δ 2.27 (9H, s), 2.30, 2.34 (3H each, s), 4.60 (1H, d, $J = 7$ Hz), 5.60 (1H, d, $J = 7$ Hz), 6.53 (1H, d, $J = 15$ Hz), 6.65 (1H, d, $J = 2$ Hz), 6.85 (2H, d, $J = 2$ Hz), 6.92 (1H, d, $J = 15$ Hz), 6.98 (2H, d, $J = 9$ Hz), 7.10 (2H, d, $J = 9$ Hz), 7.18 (2H, d, $J = 9$ Hz), 7.34 (2H, d, $J = 9$ Hz); *Gnetin F* heptaacetate (**5a**): δ 1.77 (3H, s), 1.92 (3H, d, $J = 5$ Hz), 2.27 (6H, s), 2.31 (9H, s), 4.49 (2H, m), 5.53 (1H, dd, $J = 9, 2$ Hz), 5.66 (1H, dd, $J = 8, 2$ Hz), 6.47 (1H, d, $J = 3$ Hz), 6.68 (1H, br s), 6.76 (2H, d, $J = 2$ Hz), 6.80 (1H, d, $J = 2$ Hz), 6.88 (1H, d, $J = 2$ Hz), 7.02 (1H, d, $J = 3$ Hz), 7.11 (10H, m), 7.31 (2H, dd, $J = 8, 2$ Hz), 7.40 (2H, dd, $J = 8, 2$ Hz), 7.52 (2H, d, $J = 8$ Hz); *Hopeaphenol* decaacetate (**10a**): δ 1.89, 2.13, 2.26, 2.29, 2.30 (6H each, s), 4.07 (2H, br s), 4.19 (2H, d, $J = 10$ Hz), 4.94 (2H, d, $J = 2$ Hz), 5.12 (2H, br s), 6.00 (2H, d, $J = 10$ Hz), 6.13 (2H, d, $J = 2$ Hz), 6.88 (4H, d, $J = 8$ Hz), 6.92 (2H, br s), 7.07 (4H, d, $J = 8$ Hz), 7.08 (4H, d, $J = 8$ Hz), 7.13 (2H, d, $J = 2$ Hz), 7.23 (4H, d, $J = 8$ Hz).
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