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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 ED_{50} 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 ED_{50} values of 1.2 and 1.6 μg/mL, respectively.

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 rat[4] 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. 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

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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 epiderimoid 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

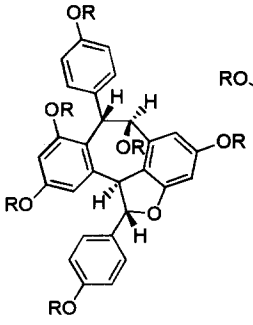
	ED_{50} (μg/mL)[a] in Cancer Cell Lines						
Compound	KB[b]	A549[b]	CAKI-1[b]	MCF-7[b]	1A9[b]	SaOS-2[b]	HCT-8[b]
1	>20 (38)[c]	>20 (7)	>20 (38)	>20 (10)	>20 (32)	>20 (8)	-[d]
10	1.2	4.8	NA[e]	4.2	7.8	>20 (41)	-
12 (1)[f]	0.6	NA	4.0	2.0	0.7	-	4.1
13 (2)	1.6	NA	8.0	7.4	3.2	-	8.0
14 (3)	3.0	NA	>10 (21)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

[a] Cytotoxicity as ED_{50} 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.

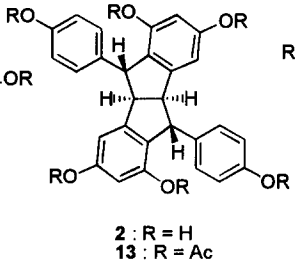
Results and Discussions. Four naturally occurring resveratrol dimers (**1–4**), five trimers (**5–9**) and two tetramers (**10** and **11**) were tested for cytotoxicity against a panel of human tumor cell lines. Hopeaphenol (**10**) showed potent cytotoxicity against KB cells with an ED_{50} value of 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.

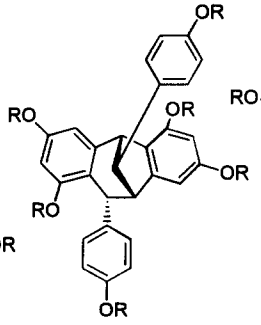
In summary, acetylation of resveratrol dimers could increase of activity and selectivity. On the other hand, certain non-acetylated oligomers such as **10**, also were cytotoxic. The increased lipophilicity of



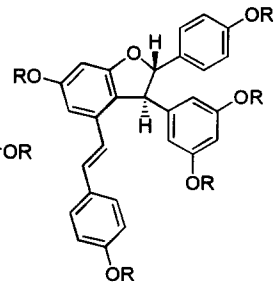
1 : R = H
12 : R = Ac



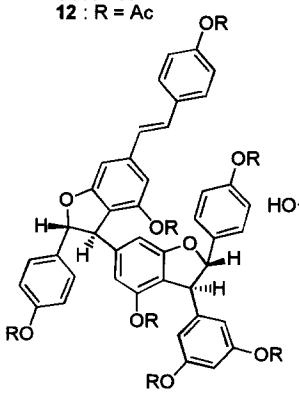
2 : R = H
13 : R = Ac



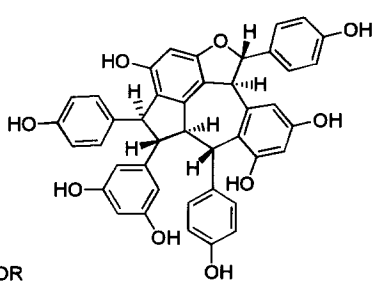
3 : R = H
14 : R = Ac



4 : R = H
15 : R = Ac



5 : R = H
16 : R = Ac



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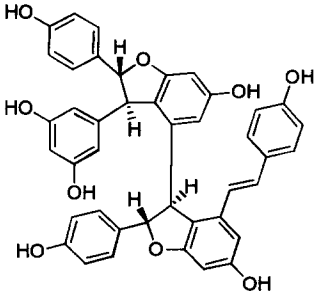

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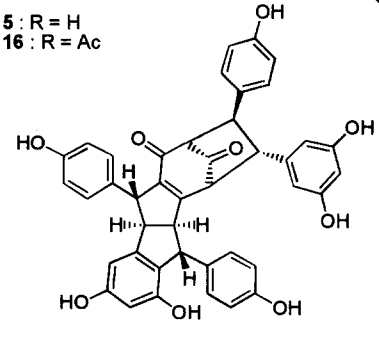

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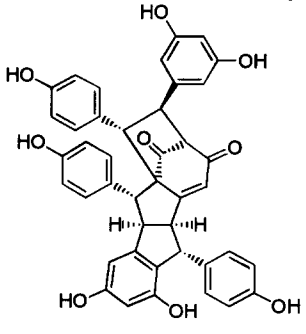

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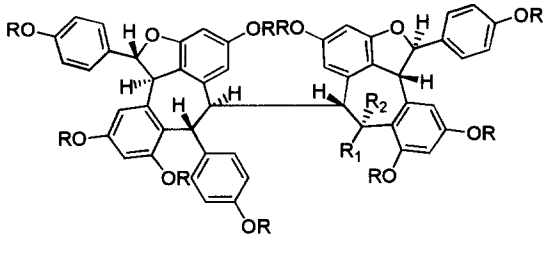
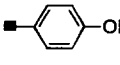
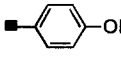
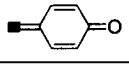


	R	R_1	R_2
10 :	H		H
17 :	Ac		H
11 :	H		

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. ^{1}H 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 E 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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