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BRIEF COMMUNICATIONS

STILBENES FROM STEMS OF PRIMORSKY GRAPE VARIETY ALPHA AND THEIR ANTIOXIDANT ACTIVITY

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Grapevine waste (stems, runners) is a vineyard waste generated during grape harvesting that is usually discarded without further use, although it contains significant concentrations of stilbenes, which are useful for human health [1]. Vegetable stilbenes are polyphenolic phytoalexins with antifungal properties that are synthesized by plants of the family Vitaceae to protect them from various types of phytopathogens [2]. Much research has shown the efficacy of these compounds to treat cardiovascular and autoimmune diseases [3], aging [4], and neurodegenerative pathologies [5]. They possess antioxidant, antimicrobial, anti-inflammatory, and anticancer activity and are widely used in the food industry. These effects of stilbenes are mainly due to their antioxidant activity because of free-radical destruction [6].

The most adaptable grape variety of those previously imported to Primorsky Krai was the Alpha variety, which demonstrated high frost tolerance and was resistant to various fungal diseases. An industrial viticulture based on this grape variety began in the region. The Alpha variety is an interspecies hybrid produced by natural hybridization of the two varieties *Vitis labrusca* L. and *V. riparia* Michx., the physical origin of which was the state of Minnesota (USA) [7]. Each grape variety is known to be characterized by its own polyphenol composition [8]. Therefore, the goal of the present work was to isolate and establish the structures of the main oligomeric stilbenes from stems of the Alpha variety collected in southern Primorsky Krai of the Russian Federation and to study their antioxidant properties. An herbarium specimen of the Alpha variety (voucher No. 267600) was located in the bioresource collection of FSR of Agricultural Biotechnology of the Far East named after A. K. Chaiki.

The dry EtOH extract (13 g) of dried stems of the Alpha variety was chromatographed over a column packed with polyamide 6DF (Sigma-Aldrich, USA) using $CHCl_3$–EtOH with a gradient of increasing EtOH content from 0 to 100%. The resulting stilbene fractions were analyzed by HPLC and HPLC-UV-MS [9] and successively chromatographed over a column (10 × 0.8 cm) of silica gel (Sigma-Aldrich, USA) using $CHCl_3$ with a gradient of EtOH content increasing to 100% and then over a column (15 × 0.5 cm) packed with Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden) using MeOH.

Stilbene fractions were additionally purified over YMC-GEL ODS-A (Supelco, USA) using H_2O with a gradient of increasing EtOH content from 20 to 100% to give five oligomeric stilbenes **1**-**5** in yields of 15, 12, 12, 7, and 13 mg, respectively. ^1H and ^{13}C NMR spectra of **1**–**5** were recorded in Me_2CO-d_6 at 30°C on Avance III DRX-700 and Avance DRX-500 instruments (Bruker, Karlsruhe, Germany). High-resolution mass spectra using electrospray ionization (ESI-HR-MS) of pure compounds were determined on an LCMS-IT-FOR liquid-chromatograph–mass-spectrometer (Shimadzu, Japan) equipped with an LC-20A HPLC, an SPD-M20A diode-array detector, and a TOR mass spectrometer with an ion trap. Constituents were separated over a Discovery HS C-18 column (150 × 2.1 mm, 3 μm) thermostatted at 36°C according to a previously published method [9].

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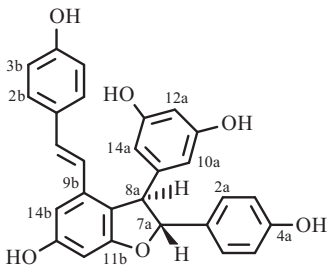

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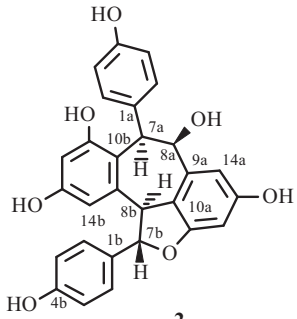

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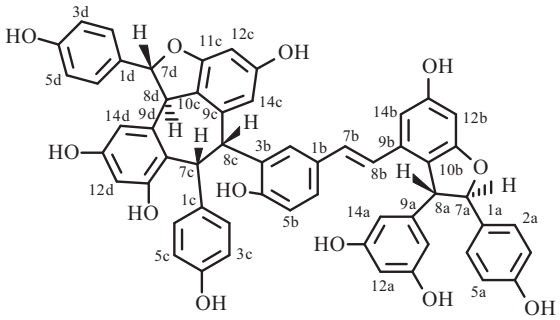
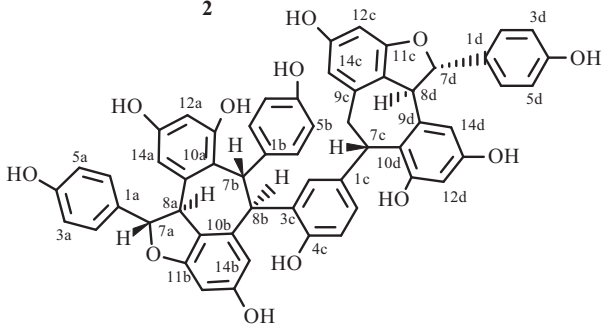


3, 5

3: 7*β*-8*β*-*trans*; **5**: 7*β*-8*β*-*cis*



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The structures of **1**–**5** were determined by spectral methods (^{1}H and ^{13}C NMR) as *trans*-*ε*-viniferin (**1**), (+)-ampelopsin A (**2**), (+)-vitisin A (**3**), (+)-vitisin D (**4**), and (+)-*cis*-vitisin A (**5**).

Compounds **1**–**5** were isolated for the first time from Alpha variety.

***trans*-*ε*-Viniferin (1)**, $C_{28}H_{22}O_6$, yellow amorphous compound, $[\alpha]_D^{20}$–5° (*c* 0.1 MeOH); lit. data: –38.6° (*c* 0.12, MeOH) [10]. UV (MeOH, λ_{max}, nm): 280, 320. Mass spectrum *m/z* 453.1312 $[M-H]^-$ (calcd for $C_{28}H_{21}O_6$, 453.1338). ^{1}H NMR (δ, ppm, J/Hz): 4.48 (1H, d, J = 5.5, H-8a), 5.42 (1H, d, J = 5.5, H-7a), 6.24 (3H, s, H-10a, 12a, 14a), 6.33 (1H, d, J = 1.8, H-12b), 6.71 (1H, d, J = 16.5, H-8b), 6.72 (1H, d, J = 1.8, H-14b), 6.73 (2H, dd, J = 8.7, 2.0, H-3b, 5b), 6.83 (2H, dd, J = 8.5, 2.0, H-3a, 5a), 6.90 (1H, d, J = 16.5, H-7b), 7.18 (2H, dd, J = 8.7, 2.0, H-2b, 6b), 7.20 (2H, dd, J = 8.5, 2.0, H-2a, 6a). ^{13}C NMR (δ, ppm): 57.1 (C-8a), 93.4 (C-7a), 96.9 (C-12b), 102.1 (C-12a), 104.2 (C-14b), 106.9 (C-10a, 14a), 116.1 (C-3a, 5a), 116.3 (C-3b, 5b), 119.8 (C-10b), 123.8 (C-8b), 127.9 (C-2a, 6a), 128.9 (C-2b, 6b), 129.9 (C-1b), 130.1 (C-7b), 133.9 (C-1a), 136.4 (C-9b), 147.5 (C-9a), 158.2 (C-4a, 4b), 159.6 (C-11a, 13a), 159.7 (C-13b), 162.5 (C-11b) [11].

(+)-Ampelopsin A (2), $C_{28}H_{22}O_7$, white amorphous compound, $[\alpha]_D^{20}$ +181° (*c* 0.1 MeOH); lit. data: –191.96° (*c* 0.96 MeOH) [12]. UV (MeOH, λ_{max}, nm): 281. Mass spectrum *m/z* 469.1265 $[M-H]^-$ (cacld for $C_{28}H_{21}O_7$, 469.1287). ^{1}H NMR (δ, ppm, J/Hz): 4.15 (1H, d, J = 11.3, H-8b), 5.39 (1H, d, J = 4.6, H-8a), 5.44 (1H, d, J = 4.6, H-7a), 5.75 (1H, d, J = 11.3, H-7b), 6.14 (1H, d, J = 1.8, H-12a), 6.20 (1H, d, J = 2.0, H-14b), 6.42 (1H, d, J = 2.0, H-12b), 6.60 (1H, d, J = 1.8, H-14a), 6.62 (2H, dd, J = 8.6, 2.0, H-3a, 5a), 6.76 (2H, dd, J = 8.6, 1.8, H-3b, 5b), 6.90 (2H, dd, J = 8.6, 2.0, H-2a, 6a), 7.10 (2H, dd, J = 8.6, 1.8, H-2b, 6b). ^{13}C NMR (δ, ppm): 43.9 (C-7a), 49.6 (C-8b), 70.8 (C-8a), 88.5 (C-7b), 97.2 (C-12a), 101.5 (C-12b), 105.5 (C-14b), 110.5 (C-14a), 115.5 (C-3a, 5a), 116.0 (C-3b, 5b), 118.4 (C-10b), 118.9 (C-10a), 128.7 (C-2a, 6a), 130.0 (C-2b, 6b), 131.0 (C-1b), 132.7 (C-1a), 140.1 (C-9a), 140.1 (C-9a), 143.2 (C-9b), 156.0 (C-4a), 157.3 (C-13b), 158.5 (C-4b), 158.9 (C-11b), 159.0 (C-13a), 160.2 (C-11a) [11–13].

(+)-Vitisin A (3), $C_{56}H_{42}O_{12}$, white amorphous compound, $[\alpha]_D^{20}$ +158° (*c* 0.1 MeOH); lit. data: +195.1° (*c* 1.1, MeOH) [14]. UV (MeOH, λ_{max}, nm): 280, 320. Mass spectrum *m/z* 905.2581 $[M-H]^-$ (calcd for $C_{56}H_{41}O_{12}$, 905.2598). ^{1}H NMR (δ, ppm, J/Hz): 4.24 (1H, d, J = 11.2, H-8d), 4.40 (1H, d, J = 5.2, H-8a), 5.34 (1H, d, J = 5.2, H-7a), 5.37 (1H, d, J = 3.6, H-7c), 5.50 (1H, d, J = 3.6, H-8c), 5.88 (1H, d, J = 11.2, H-7d), 6.06 (3H, m, H-12c, 12d, 14d), 6.08 (1H, d, J = 2.0, H-2b), 6.16 (2H, d, J = 2.2, H-10a, 14a), 6.21 (1H, t, J =2.2, H-12a), 6.25 (1H, d, J = 2.0, H-12b), 6.27 (1H, br.s, H-14c), 6.38 (2H, br.s, H-7b, 8b), 6.53 (1H, d, J = 2.0, H-14b), 6.65 (2H, dd, J = 8.5, 2.0, H-3c, 5c), 6.70 (1H, d, J = 8.0, H-5b), 6.77 (2H, dd, J = 8.5, 2.0, H-3d, 5d), 6.83 (2H, dd, J = 8.5, 1.9, H-3a, 5a), 6.86 (1H, dd, J = 8.0, 2.0, H-6b), 7.04 (2H, dd, J = 8.5, 2.0, H-2c, 6c), 7.14 (2H, dd, J = 8.5, 2.0, H-2d, 6d), 7.20 (2H, dd, J = 8.5, 1.9, H-2a, 6a). ^{13}C NMR (δ, ppm): 40.7 (C-7c), 41.3 (C-8c), 49.5 (C-8d), 57.1 (C-8a), 88.5 (C-7d), 93.9 (C-7a), 96.1 (C-12d), 96.6 (C-12b), 100.9 (C-12c), 102.1 (C-12a), 104.7 (C-14b), 105.0 (C-14c), 106.9 (C-10a, 14a), 110.1 (C-14d), 115.4 (C-5b), 115.5 (C-3c, 5c), 116.0 (C-3d, 5d), 116.2 (C-3a, 5a), 118.9 (C-10b), 120.1 (C-10c), 120.4 (C-10d), 122.8 (C-8b), 123.7 (C-6b), 127.9 (C-2a, 6a), 128.8 (C-2c, 6c), 129.0 (C-1b), 130.1 (C-2d. 6d), 131.1 (C-7b), 131.2 (C-1d), 132.5 (C-2b, 3b), 134.6 (C-1a), 135.5 (C-1c), 136.6 (C-9b), 141.3 (C-9d), 142.3 (C-9c), 147.2 (C-9a), 155.1 (C-4b), 155.9 (C-4c), 157.9 (C-13d), 158.2 (C-4a), 158.5 (C-4d), 158.8 (C-13c), 159.6 (C-13b), 159.8 (C-11a, 13a), 160.0 (C-11d), 160.4 (C-11c), 162.6 (C-11b) [11, 14].

TABLE 1. Antioxidant Activity of Stilbenes **1**–**5**

Compound	DPPH* IC_{50} μg/mL	ABTS* IC_{50} μg/mL	ABTS* (trolox equivalents)
Dry extract of stems	78.0 ± 1.4	11.74 ± 0.31	0.28
1	28.6 ± 0.50	7.47 ± 0.24	0.44
2	337.5 ± 12.7	14.29 ± 0.12	0.23
3	53.5 ± 2.3	10.2 ± 0.24	0.30
4	56.8 ± 0.60	9.32 ± 0.52	0.36
5	18.8 ± 0.3	4.69 ± 0.32	0.71
6	28.0 ± 0.3	6.96**	0.48
Trolox	8.0	3.32 ± 0.05	1.00

*All experiments conducted in triplicate, Student criterion and SigmaPlot 14 (Systat Software Inc., USA) used to determine statistical significance; **literature data for **6** [17].

(+)-Vitisin D (4), $C_{56}H_{42}O_{12}$, white amorphous compound, $[\alpha]_D^{20}$ +210° (*c* 0.1 MeOH); lit. data: +222.0° (*c* 0.20, MeOH) [15]. UV (MeOH, λ_{max}, nm): 281. Mass spectrum *m/z* 905.2571 $[M - H]^-$ (calcd for $C_{56}H_{41}O_{12}$, 905.2598). ^{1}H NMR (δ, ppm, J/Hz): 2.94 (1H, d, J = 16.7, H-8c), 3.13 (1H, d, J = 16.7, H-8c), 4.11 (2H, m, H-8a, H-8d), 4.79 (1H, br.s, H-7c), 5.36 (1H, br.s, H-8b), 5.60 (1H, br.s, H-7b), 5.64 (1H, d, J = 11.2, H-7d), 5.80 (1H, d, J = 11.7, H-7a), 5.86 (1H, br.s, H-14b), 5.97 (1H, br.s, H-12a), 6.00 (1H, br.s, H-12b), 6.05 (1H, br.s, H-14a), 6.10 (3H, br.s, H-12c, 14c, 14d), 6.14 (1H, br.s, H-2c), 6.28 (1H, br.s, H-12d), 6.45 (1H, br.s, H-6c), 6.53 (1H, br.s, H-5c), 6.62 (2H, dd, J = 8.1, 2.0, H-3b, 5b), 6.75 (2H, dd, J = 8.5, 2.0, H-3a, 5a), 6.87 (2H, dd, J = 8.5, 2.0, H-3d, 5d), 6.98 (2H, dd, J = 8.1, 2.0, H-2b, 6b), 7.17 (4H, dd, J = 8.5, 2.0, H-2a, 6a, 2d, 6d). ^{13}C NMR (δ, ppm): 33.8 (C-8c), 36.0 (C-7c), 40.3 (C-7b), 43.1 (C-8b), 49.1 (C-8a), 49.5 (C-8d), 88.3 (C-7d), 88.5 (C-7a), 95.5 (C-12b), 96.1 (C-12c), 101.1 (C-12d), 101.4 (C-12a), 105.1 (C-14d), 106.4 (C-14a), 108.7 (C-14c), 109.8 (C-14b), 113.9 (C-5c), 115.3 (C-3b, 5b), 115.8 (C-3a, 5a), 116.1 (C-3d, 5d), 117.1 (C-10c), 119.1 (C-10a), 120.0 (C-10b), 122.2 (C-10d), 124.7 (C-6c), 129.0 (C-2b, 6b), 130.3 (C-2a, 6a, 2d, 6d), 130.5 (C-1d), 131.5 (C-2c), 131.6 (C-1a), 131.8 (C-3c), 134.3 (C-1c), 135.7 (C-1b), 137.8 (C-9c), 141.0 (C-9b), 141.6 (C-9a), 142.8 (C-9d), 152.9 (C-4c), 155.5 (C-4b), 156.4 (C-13a, 13d), 156.8 (C-11c), 157.2 (C-11a), 158.2 (C-4a, 4d), 158.3 (C-11d, 13b, 13c), 160.1 (C-11b) [15].

(+)-*cis*-Vitisin A (5), $C_{56}H_{42}O_{12}$, yellow amorphous compound, $[\alpha]_D^{20}$ +444° (*c* 0.1 MeOH); lit. data: +184° (*c* 0.5 CH_3OH) [14]. UV (MeOH, λ_{max}, nm): 282. HR-ESI-MS *m/z* 905.2532 $[M - H]^-$ (calcd for $C_{56}H_{41}O_{12}$, 905.2598). ^{1}H NMR (δ, ppm, J/Hz): 4.10 (1H, d, J = 5.4, H-8a), 4.31 (1H, d, J = 11.4, H-8d), 5.38 (1H, d, J = 5.4, H-7a), 5.40 (1H, br.s, H-8c), 5.50 (1H, br.s, H-7c), 5.85 (2H, br.s, H-7b, 8b), 5.95 (1H, d, J = 11.4, H-7d), 6.05 (1H, br.s, H-12d), 6.11 (1H, dd, J = 8.1, 1.8, H-6b), 6.13 (2H, br.s, H-12c, 14c), 6.15 (2H, br.s, H-10a, 14a), 6.24 (1H, br.s, H-12a), 6.27 (1H, br.s, H-12b), 6.36 (1H, br.s, H-14d), 6.53 (1H, br.s, H-14b), 6.69 (2H, dd, J = 8.5, 2.0, H-3c, 5c), 6.70 (1H, dd, J = 8.1, 1.8, H-4b), 6.73 (1H, d, J = 8.1, H-5b), 6.79 (2H, dd, J = 8.5, 2.0, H-3d, 5d), 6.84 (2H, dd, J = 8.5, 2.0, H-3a, 5a), 6.92 (2H, dd, J = 8.5, 2.0, H-2a, 6a), 7.09 (2H, dd, J = 8.5, 2.0, H-2c, 6c), 7.17 (2H, dd, J = 8.5, 2.0, H-2d, 6d) [14].

Compound **6** was identified in stems of Alpha variety using HPLC-UV-HR-MS as *trans*-resveratrol by direct comparison with a sample isolated earlier from wood of *Maackia amurensis* [16]. Thus, the main constituents of stems of the Primorsky grape Alpha variety were two dimeric stilbenes (**1** and **2**) and three tetrameric ones (**3**–**5**) with oligomeric stilbenes **1**–**3** and **5** contained primarily in organs of the plant *V. vinifera* [11], while the tetramer of resveratrol (+)-vitisin D (**4**) was isolated earlier from *V. coignetiae* [14]. Table 1 presents data for the antioxidant activity of stilbenes **1**–**6** (DPPH and ABTS acceptor effects).

Antiradical activity was determined from the reaction with the free radical diphenylpicrylhydrazyl (DPPH) using a modified method [18]. An EtOH solution (3 mL) of DPPH (10^{-4} M) was treated with solutions of the stilbenes in the concentration range 1–90 μg/mL. The solutions were stored in a dark place. Absorption at 517 nm was measured after 20 min on a Shimadzu UV 1240 spectrophotometer.

The percent inhibition was calculated using the formula

$$\text{Inhibition (\%)} = 100 - (A_{sample} \times 100/A_{control}),$$

where A_{sample} is the optical density of the sample solution at 517 nm and $A_{control}$, the optical density of DPPH solution (10^{-4} M).

The IC_{50} value designates the compound concentration required to trap 50% of DPPH radicals in 20 min. Its values were found from curves for the dependence of the percent inhibition on concentration. Antiradical activity was determined

from the reaction with ABTS radical [2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] using the literature method [19, 20]. Trolox was used as a standard. Results were expressed as IC_{50} values (μg/mL) and in Trolox equivalents (ratio of IC_{50} of Trolox to IC_{50} of the sample).

All isolated stilbenes possessed antioxidant properties. They rapidly absorbed DPPH radicals and ABTS cation-radicals with activities from 0.23 to 0.71 Trolox eq. (+)-*cis*-Vitisin A (**5**), *trans*-ε-viniferin (**1**), and *trans*-resveratrol (**6**) characteristically had the greatest parameters. Tetrameric stilbenes **3** and **4** had average antioxidant parameters, while dimeric stilbene (+)-ampelopsin A (**2**) was the weakest antioxidant. The dry extract of grape stems showed exceedingly high activity for absorption of DPPH and ABTS radicals that was comparable to that of the pure stilbenes (Table 1). This suggested that the grape stems contained more highly active polyphenolic antioxidants. Further research may unleash the full potential of this plant as a natural source of antioxidants with possible use in health. The results could have practical value for using grapes (Alpha variety) in the agriculture, food, and pharmaceutical industries.

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