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POLYPHENOLS FROM *Vitis amurensis* STEMS

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The search for sources of natural antioxidants among Far-East plants has uncovered promising species, including Amur grape (*Vitis amurensis* Rupr.).

The chemical composition of Amur grape roots has been investigated in sufficient detail. They contain a dimer and trimer of resveratrol, amurensin A and amurensin B, respectively [1], a dimer amurensin H [2], trimers amurensins C and D, pentamers amurensins E and F [3], and tetramers amurensins I-M [4].

Stems of Amur grape have not been investigated. Stems of other grape species contain the monomeric stilbenes resveratrol and piceatannol in addition to various oligomers of resveratrol such as piceatannol, resveratrol, and pallidol from *V. vinifera* [5]; tetramers vitisins A and D from *V. coignetiae* [6]; and resveratrol, dimers (-)-ε-viniferin, ampelopsin A, betulifols A and B, trimer ampelopsin D, and tetramers vitisin A, hopeaphenol, and heyneanol from *V. betulifolia* [7]. (-)-ε-Viniferin, ampelopsins A and C, and heyneanol have been identified in *V. heyneana* [8] and (+)-ε-viniferin, gnetin, vitisin A, hopeaphenol, and flexuosol A in *V. flexuosa* [9].

Stems of *V. amurensis* were collected in August 2002 in Khasan region of Primor′ye territory. The ethanol extract of stems (980 g) purified of bark produced an alcohol extract (36 g). A part of the dry alcohol extract (9.0 g) was dissolved in the minimal amount of alcohol, diluted with water, and re-extracted successively with hexane (1.60 g), benzene (0.26 g), and ethyl acetate (3.94 g). The ethyl acetate extract was investigated.

Preparative column chromatography used glass columns with KSK silica gel and the solvent systems C_6H_6:$(CH_3)_2CO$ and $CHCl_3$:$(CH_3)_2CO$ with a gradient of increasing acetone. The pure compounds were further purified over Sephadex LH-20 using $CHCl_3$:C_2H_5OH with detection on a Uvicord SLKB.

Six compounds (**1**-**6**) were isolated from the ethyl acetate extract. Preliminary analysis by UV, IR, and NMR spectroscopy indicated that **1** and **2** were resveratrol and piceatannol, respectively; **3**-**6**, dimers of resveratrol ε-viniferin, pallidol, ampelopsin A, and isoampelopsin F.

UV spectra were recorded on a Cary-Varian 219 in MeOH; IR spectra, on a Specord M 82; mass spectra, in an LKB-9000 S mass spectrometer with direct sample introduction into the ion source at 18 and 70 eV potential. PMR spectra were obtained at 300 and 500 MHz (δ, ppm, 0 = TMS, acetone-d_6). Optical rotation was determined on a Perkin—Elmer 131 spectropolarimeter.

Compound 3. The PMR spectra of **3** agreed well with those for (+)-ε-viniferin [1]. The optical rotation angle for our sample was negative.

Compound 4. The PMR spectrum of **4** showed that the molecule was symmetric and contained two 1,4-disubstituted and two 1,2,3,5-tetrasubstituted benzene rings in addition to four aliphatic methine protons at δ 3.75 (2H, br.s, H-8a, 8b), 3.95 (1H, d, J = 6.0 Hz, H-7a), 5.32 (1H, d, J = 5.8 Hz, H-7b). This together with other properties agreed well with data for the resveratrol dimer pallidol [10].

Compound 5. The PMR spectrum of **5** showed that the molecule contained two 1,4-disubstituted and two 1,2,3,5-tetrasubstituted benzene rings, an aliphatic hydroxyl appearing as a broad singlet (δ 3.77), and two pairs of mutually coupled methine protons (δ 4.15/5.75 and 5.41/5.45). The SSCC of the methine protons of a dihydrofuran ring and methine protons H-7a and H-8 agreed with these same ones for (-)-ampelopsin A [11]. However, the chemical shifts for H-7b were markedly different. The NMR spectra of **5** and (+)-ampelopsin A differed slightly [1]. H—H COSY, CH COSY, and HMBC experiments enabled **5** to be identified as (+)-ampelopsin A.

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Compound 6. The substitution patterns of the benzene rings in the PMR and the chemical shifts and SSCC of methine protons δ 3.36 (H-8a), 3.65 (H-7b), 4.14 (H-8b), and 4.2 (H-7a) were close to those of (-)-isoampelopsin F [12]. However, the C-8a proton appeared as a triplet with J = 4.5 Hz instead of a broad doublet and the optical rotation angle had the opposite sign. A comparison of the PMR and ^{13}C NMR spectral data of **6** and (+)-isoampelopsin F [13] showed slight differences in the chemical shifts and SSCC for the methine protons. H—H COSY, CH COSY, and HMBC experiments enabled **6** to be identified as (+)-isoampelopsin F.

(-)-ε-Viniferin (**3**, 45.0 mg). Cream powder, $C_{28}H_{22}O_6$, $[\alpha]_D$ -50° (*c* 1.0, C_2H_5OH). EIMS (*m*/*z*): 454 [M]. UV spectrum (λ_{max}, MeOH, nm): 285, 321 (sh). IR spectrum (ν_{max}, KBr, cm^{-1}): 3360, 1600, 1510. PMR spectrum (acetone-d_6, δ, ppm, J/Hz): 4.47 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 = 2.2, H-12b), 6.71 (1H, d, J = 16.4, H-7b), 6.72 (1H, d, J = 2.2, H-14b), 6.74 (2H, d, J = 8.2, H-3b, 5b), 6.84 (2H, J = 8.2, H-3a, 5a), 6.91 (1H, d, J = 16.4, H-8b), 7.19 (2H, d, J = 8.2, H-2b, 6b), 7.21 (2H, d, J = 8.2, H-2a, 6a), 8.18 (2H, 2 × OH), 8.34 (1H, OH), 8.37 (1H, OH), 8.42 (1H, OH).

(-)-Pallidol (**4**, 8.0 mg). Brown powder, $C_{28}H_{22}O_6$, $[\alpha]_D$ -40° (*c* 0.5, C_2H_5OH). EIMS (*m*/*z*): 454 [M]. UV spectrum (λ_{max}, MeOH, nm): 285. IR spectrum (ν_{max}, KBr, cm^{-1}): 3348, 1603, 1510. PMR spectrum (acetone-d_6, δ, ppm, J/Hz): 3.75 (2H, br.s, H-8a, 8b), 3.95 (1H, d, J = 6.0, H-7a), 5.20 (1H, d, J = 5.8, H-7b), 6.05 (2H, d, J = 2.4, H-12a, 12b), 6.18 (2H, t, J = 2.1, H-14a, 14b), 6.79 (4H, d, J = 8.5, H-3a, 5a, 3b, 5b), 7.05 (4H, d, J = 8.5, H-2a, 6a, 2b, 6b), 8.05 (4H, s, 4 × OH), 8.37 (2H, s, 2 × OH). ^{13}C NMR spectrum (acetone-d_6, δ, ppm): 54.2 (2C, d, C-7a, 7b), 60.6 (2C, d, C-8a, 8b), 102.7 (2C, d, C-12a, 12b), 103.5 (2C, d, C-14a, 14b), 115.9 (4C, d, C-3a, 5a, 3b, 5b), 123.4 (2C, d, C-10a, 10b), 129.2 (2C, d, C-2b, 6b), 129.5 (2C, s, C-2a, 6a), 137.9 (2C, d, C-1a, 1b), 150.4 (2C, d, C-9a, 9b), 155.4 (2C, d, C-11a, 11b), 156.5 (2C, d, C-4a, 4b), 159.5 (2C, d, C-13a, 13b).

(+)-Ampelopsin A (**5**, 9.0 mg). Cream powder, $C_{28}H_{22}O_7$, $[\alpha]_D$ +50° (*c* 1.0, C_2H_5OH). EIMS (*m*/*z*): 470 [M]. UV spectrum (λ_{max}, MeOH, nm): 285. IR spectrum (ν_{max}, KBr, cm^{-1}): 3355, 1610, 1515, 1446. PMR spectrum (acetone-d_6, δ, ppm, J/Hz): 4.15 (1H, d, J = 11.3, H-8b), 5.41 (1H, d, J = 4.8, H-8a), 5.45 (1H, d, J = 4.8, H-7a), 5.75 (1H, d, J = 11.3, H-7b), 6.14 (1H, d, J = 2.1, H-12a), 6.23 (1H, d, J = 1.8, H-12b), 6.42 (1H, d, J = 2.3, H-14b), 6.60 (1H, d, J = 2.1, H-14a), 6.62 (2H, d, J = 8.6, H-3a, 5a), 6.77 (2H, d, J = 8.6, H-3b, 5b), 6.88 (2H, d, J = 8.6, H-2a, 6a), 7.11 (2H, d, J = 8.6, H-2b, 6b), 3.77 (1H, br.s, OH-8a), 8.09, 8.13, 8.25, 8.33, 8.47 (5H, br.s, 5 × OH). ^{13}C NMR spectrum (acetone-d_6, δ, ppm): 43.7 (1C, s, C-7a), 49.4 (1C, s, C-8b), 71.0 (1C, s, C-8a), 88.4 (1C, s, C-7b), 96.9 (1C, s, C-12a), 101.4 (1C, s, C-12b), 105.3 (1C, s, C-14b), 110.3 (1C, s, C-14a), 115.3 (2C, s, C-3b, 5b), 115.8 (2C, d, C-3a, 5a), 118.1 (1C, s, C-10b), 118.7 (1C, s, C-10a), 128.5 (2C, d, C-2b, 6b), 129.8 (2C, d, C-2a, 6a), 130.7 (1C, s, C-1b), 132.5 (1C, s, C-1a), 140.3 (1C, s, C-9a), 142.9 (1C, s, C-9b), 155.9 (1C, s, C-4a), 157.2 (1C, s, C-11b), 158.1 (1C, s, C-4b), 155.0 (1C, s, C-13b), 159.0 (1C, s, C-13a), 160.0 (1C, s, C-11a).

(+)-Isoampelopsin F (**6**, 13.0 mg). Light brown powder, $C_{28}H_{22}O_6$, $[\alpha]_D$ +120° (c 1.0, C_2H_5OH). EIMS (m/z): 454 [M]. UV spectrum (λ_{max}, MeOH, nm): 280. IR spectrum (ν_{max}, KBr, cm^{-1}): 3310, 1614, 1510, 1454. PMR spectrum (acetone-d_6, δ, ppm, J/Hz): 3.36 (1H, t, J = 4.5, H-8a), 3.65 (1H, s, H-7b), 4.14 (1H, s, H-8b), 4.20 (1H, d, J = 6.0, H-7a), 6.07 (1H, d, J = 2.2, H-12a), 6.16 (1H, d, J = 2.4, H-12b), 6.45 (1H, d, J = 2.4, H-14b), 6.52 (1H, d, J = 2.0, H-14a), 6.57 (2H, d, J = 8.6, H-3b, 5b), 6.76 (2H, d, J = 8.6, H-3a, 5a), 6.79 (2H, d, J = 8.6, H-2b, 6b), 7.10 (2H, d, J = 8.6, H-2a, 6a), 7.30 (1H, s, 11b-OH), 7.78 (1H, s, 11a-OH), 7.86 (1H, s, 13a-OH), 7.92 (1H, s, 13b-OH), 7.93 (1H, s, 4b-OH), 7.99 (1H, s, 4a-OH). ^{13}C NMR spectrum (acetone-d_6, δ, ppm): 47.0 (s, C-7a), 49.5 (1C, s, C-8b), 50.0 (1C, s, C-7b), 58.0 (1C, s, C-8a), 101.7 (2C, d, C-12a, 12b), 104.0 (1C, s, C-14a), 105.5 (1C, s, C-14b), 113.2 (1C, s, C-10b), 115.3 (2C, d, C-3b, 5b), 115.4 (2C, d, C-3a, 5a), 127.7 (1C, s, C-10a), 129.0 (2C, d, C-2b, 6b), 129.7 (2C, d, C-2a, 6a), 135.3 (1C, s, C-1b), 138.2 (1C, s, C-1a), 147.1 (1C, s, C-9a), 147.4 (1C, s, C-9b), 156.0 (2C, d, C-4a, 4b), 157.6 (1C, s, C-11b).

Resveratrol (**1**, 35.0 mg) and piceatannol (**2**, 28.0 mg) were identified by comparing their physicochemical properties and NMR spectra with authentic samples.

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REFERENCES

1. K.-Sh. Huang and M. Lin, *J. Asian Nat. Prod. Res.*, **2**, 21 (1999).
2. K.-Sh. Huang, M. Lin, and Y. H. Wang, *Chin. Chem. Lett.*, **10**, 817 (1999).
3. K.-Sh. Huang, M. Lin, L. N. Yu, and M. N. Kong, *Tetrahedron*, **56**, 1321 (2000).
4. K.-Sh. Huang, M. Lin, and G.-F. Cheng, *Phytochemistry*, **58**, 357 (2001).
5. J.-C. Delaunay, C. Costagnino, C. Cheze, and J. Vercauteren, *J. Chromatogr. A*, **964**, 123 (2002).
6. Y. Oshima, A. Kamijou, H. Moritani, K. Namao, and Y. Ohizumi, *J. Org. Chem.*, **58**, 850 (1993).
7. W. Li, B. Li, and Y. Chen, *Phytochemistry*, **49**, 1393 (1998).
8. W.-W. Li, L.-Sh. Ding, B.-G. Li, and Y.-Z. Chen, *Phytochemistry*, **42**, 1163 (1996).
9. W.-W. Li, B.-G. Li, and Y.-Z. Chen, *J. Nat. Prod.*, **61**, 646 (1998).
10. M. Oshima, T. Tanaka, M. Iinuma, and K. Gobo, *Chem. Pharm. Bull.*, **42**, 2117 (1994).
11. T. Tanaka, T. Ito, Y. Ido, T.-K. Son, K. Nakaya, M. Iinuma, M. Ohyama, and V. Chelladurai, *Phytochemistry*, **53**, 1015 (2000).
12. T. Tanaka, M. Oshima, K. Morimoto, F. Asai, and M. Iinuma, *Phytochemistry*, **48**, 1241 (1998).
13. H.-F. Luo, L.-P. Zang, and C.-Q. Hu, *Tetrahedron*, **57**, 4849 (2001).