VITISINS D AND E, NOVEL OLIGOSTILBENES FROM *VITIS COIGNETIAE* STEM BARKS

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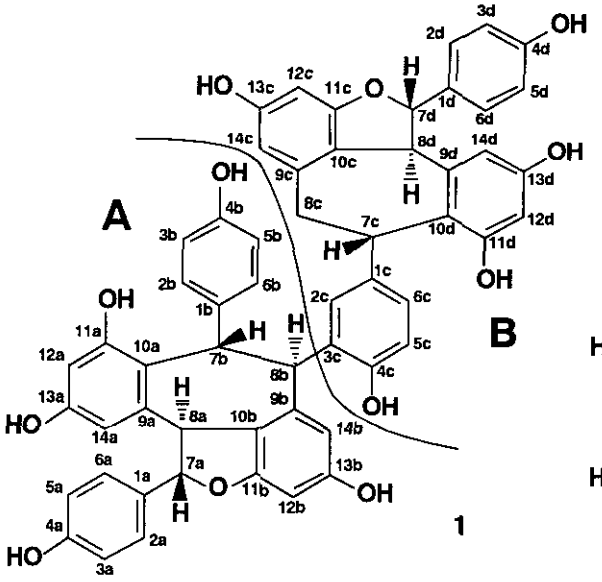
Abstract - Phytochemical investigation on oligostilbenes in a methanol extract of *Vitis coignetiae* (Japanese name: yama-budou) stem barks led to the isolation of novel oxidative resveratrol tetramer and trimer named vitisins D and E, respectively. The structures of the oligostilbenes were characterized on the basis of spectroscopic evidence.

In the course of our studies on potential hepatoprotective agents from medicinal plants, we isolated an oxidative resveratrol dimer, ε-viniferin, as a hepatoprotective constituent of the Vitaceaeous plants, *Ampelopsis brevipedunculata* var. *hancei* and *Vitis coignetiae*.[1] Our continuing phytochemical investigation on the Vitaceaeous plants revealed that they contained a variety of oligostilbenes bearing unique carbon skeletons which are biosynthesized by oxidative condensation of two - four moles of resveratrol.[2] We undertook detailed phytochemical examination of a methanol extract of *Vitis coignetiae* stem barks, leading to the isolation of a new oxidative resveratrol tetramer, vitisin D (**1**), and a new oxidative resveratrol trimer, vitisin E (**2**).

The molecular formula $C_{56}H_{42}O_{12}$ indicated for vitisin D (**1**), $[\alpha]_D$ +222.0° (*c* 0.20, MeOH), a pale brown amorphous powder, was established by a quasimolecular ion peak at *m/z* 907 $[M+H]^+$ in its FABMS and analyses of its ^1H and ^{13}C NMR spectra.[3] The molecular formula, in combination with the numbers of aliphatic and aromatic hydrogen and carbon signals, showed that vitisin D (**1**) is an oxidative resveratrol tetramer. Two-dimensional C-H COSY spectrum of vitisin D (**1**) showed the following correlations in the aliphatic carbon and hydrogen signals: δ 32.1 (δ 2.98 and 3.15), 34.8 (4.70), 39.0 (5.49), 42.2 (5.26), 47.2 (3.90), 47.6 (4.05), 87.6 (5.63) and 87.8 (5.70), indicating the presence of one methylene, five methine and two oxymethine groups. Vitisin D (**1**) was suggested to have the segment A in its molecule from its resemblance to vitisin A (**3**)[4] on the resonance positions and coupling patterns of the ^1H and ^{13}C NMR signals for three aliphatic methine, one aliphatic oxymethine, four sets of *ortho*-coupled aromatic and two sets of *meta*-coupled aromatic hydrogens and carbons. Furthermore, the presence of a 4-hydroxy-3-substituted

phenyl group in vitisin D (**1**) was indicated by similar ^{1}H and ^{13}C NMR signals for H-2c, H-5c and H-6c, and for C-1c - C-6c of vitisin D (**1**) and vitisin A (**3**). The linkage of the segment A and the phenyl group was clarified by cross peak between C-8b (δ 42.2) and H-2c (δ 5.92) in the COLOC spectrum. The ^{1}H NMR spectrum of vitisin D (**1**) showed signals for another dihydrobenzofuran moiety bearing a 4-hydroxyphenyl group (δ 3.90 and 5.63 (each 1H, d, *J*=11.0 Hz, H-8d and H-7d); 6.85 and 7.09 (each 2H, d, *J*=8.4 Hz, H-3d, 5d and H-2d, 6d)). Instead of *meta*-coupled AB_2-type aromatic hydrogen signals (H-10d, H-12d and H-14d) in vitisin A (**3**), vitisin D (**1**) exhibited *meta*-coupled hydrogen signals at δ 6.15 and 5.98 (each 1H, br s, H-12d and H-14d). In addition, olefinic hydrogen signals in vitisin A (**3**) were replaced by aliphatic methylene



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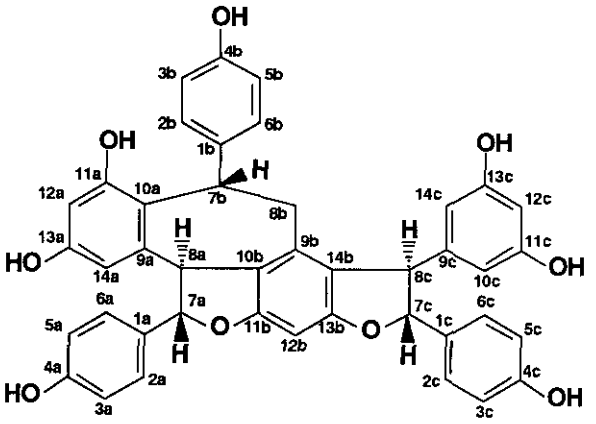

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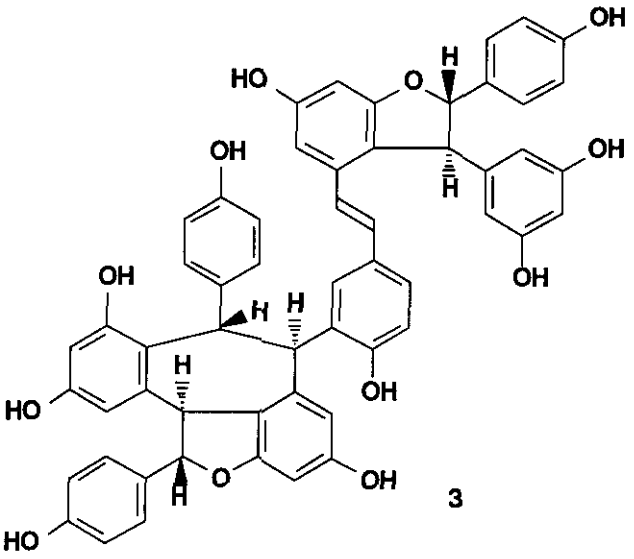

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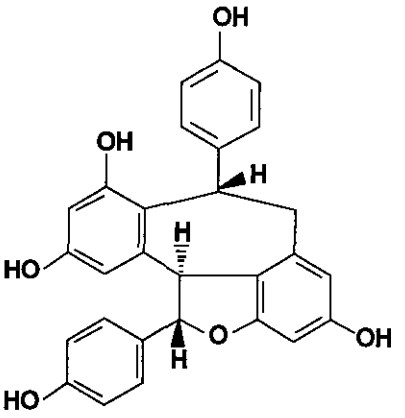

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and methine hydrogen signals at δ 2.98, 3.15 (each 1H, br d, *J*=16.8 Hz, H-8c) and 4.70 (1H, br t, *J*=4.5 Hz, H-7c) in vitisin D (**1**). These spectral data suggested that vitisin D (**1**) has the planar structure in which C-7c and C-10d in vitisin A (**3**) are connected to form a seven-membered ring, and this result was confirmed by cross peaks between C-9d (δ 141.8) - H-7c (δ 4.70) in the COLOC spectrum of vitisin D (**1**).

NOE's at H-2a(6a) and H-2b(6b) were observed by irradiation of H-8a, and irradiation of H-8b caused the same NOE's. These data indicated that H-8a, H-8b and two 4-hydroxyphenyl groups at C-7a and C-7b are spatially close. H-8d and two phenyl substituents at C-7c and C-7d were found to situate at the same directions by NOE's at H-2d(6d) and H-6c by irradiation of H-8d. Moreover, the Dreiding model study based on NOE's (H-2a(6a) - H-8d, H-2a(6a) - H-8c, H-7c - H-2c, H-7b - H-8c, and H-7b - H-2c) also clarified spatial relationship between the segments A and B.

Like ampelopsin B (**4**), the ^{1}H NMR spectrum of a novel oxidative resveratrol trimer, vitisin E (**2**), $[\alpha]_D$ +94.5° (*c* 0.20, MeOH), $C_{36}H_{32}O_9$ (FABMS *m/z*: 681 $[M+H]^+$), showed the presence of partial structures as follows: -CH-CH-O (δ 4.25 and 5.80 (each 1H, d, *J*=12.0 Hz)), -CH_2-CH- (δ 2.62 (1H, dd, *J*=3.6, 17.6 Hz), 3.39 (1H, dd, *J*=3.6, 17.6 Hz) and 5.09 (1H, t, *J*=3.6 Hz)), 3,5-dihydroxy-2-substituted phenyl group (δ 6.24 and 6.38 (each 1H, d, *J*=2.0 Hz)) and two 4-hydroxyphenyl groups (δ 6.73, 7.12 and 6.63, 6.88 (each 2H, d, *J*=8.5 Hz)). These partial structures suggested vitisin E (**2**) to be a congener of ampelopsin B (**4**). However, ^{1}H NMR signal in vitisin E (**2**) at δ 6.17 replaced *meta*-coupled doublet signal in ampelopsin B (**4**), indicating an isolated aromatic hydrogen (H-12b). Vitisin E (**2**) also showed additional hydrogen signals for a dihydrobenzofuran moiety having 4-hydroxyphenyl and 3,5-dihydroxyphenyl group (δ 4.56 and 5.41 (each 1H, d, *J*=6.0 Hz); 6.19 (2H, d, *J*=2.0 Hz) and 6.23 (1H, t, *J*=2.0 Hz); 6.88 and 7.25 (each 2H, d, *J*=8.5 Hz)). C-H long-range coupling between C-14b and H-8b and a comparative study of the ^{1}H and ^{13}C NMR signals for H-12b and C-12b of vitisin E (**2**) (δ 6.17 and 90.5) and ampelopsin B (**4**) (H-12b, H-14b: δ 6.07, 6.24; C-12b, C-14b: δ 96.2, 109.5) demonstrated that vitisin E (**2**) is a condensed compound of ampelopsin B (**4**) with resveratrol at C-13b and C-14b.

The stereochemistry of the hydrogens in two dihydrobenzofuran moieties were assigned to be *trans* from NOE's of H-8a - H-2a(6a), H-7a - H-14a, H-7c - H-10c(14c). NOE's of H-8a - H-2b(6b) and H-8b(α) - H-8c implied the relative configuration of vitisin E (**2**) as represented in the formula (**2**).

Previous studies showed that the oligostilbenes isolated from the Vitaceaeous plants have a variety of interesting biological activities such as hepatoprotective and hepatotoxic activities[1] and attaching repellent activity against the blue mussel.[6] It is worthy to investigate biological activities of vitisin D (**1**) and vitisin E (**2**).

REFERENCES AND NOTES

1. Y. Oshima, K. Namao, A. Kamijou, S. Matsuoka, M. Nakano, K. Terao, and Y. Ohizumi, *Experientia*, 1995, **51**, 63.
2. Y. Oshima, A. Kamijou, Y. Ohizumi, M. Niwa, J. Ito, K. Hisamichi, and M. Takeshita, *Tetrahedron*, 1995, **51**, 11979; and references cited herein.
3. ^{1}H NMR (300 MHz, $CDCl_3$:CD_3OD=7:3): δ 2.98 (1H, br d, *J*=16.8 Hz, H-8c), 3.15 (1H, br d, *J*=16.8 Hz, H-8c), 3.90 (1H, d, *J*=11.0 Hz, H-8d), 4.05 (1H, d, *J*=11.6 Hz, H-8a), 4.70 (1H, br t, *J*=4.5 Hz, H-7c),

5.26 (1H, d, *J*=3.7 Hz, H-8b), 5.49 (1H, d, *J*=3.7 Hz, H-7b), 5.63 (1H, d, *J*=11.0 Hz, H-7d), 5.70 (1H, d, *J*=11.6 Hz, H-7a), 5.79 (1H, br s, H-14b), 5.82 (1H, br s, H-12a), 5.89 (1H, br s, H-14a), 5.92 (1H, br s, H-2c), 5.98 (1H, br s, H-14d), 6.02 (1H, br s, H-12b), 6.07 (2H, br s, H-12c,14c), 6.15 (1H, br s, H-12d), 6.52 (1H, d, *J*=7.4 Hz, H-5c), 6.57 (1H, br d, *J*=7.4 Hz, H-6c), 6.60 (2H, d, *J*=8.4 Hz, H-3b,5b), 6.73 (2H, d, *J*=8.4 Hz, H-3a,5a), 6.85 (2H, d, *J*=8.4 Hz, H-3d,5d), 6.98 (2H, d, *J*=8.4 Hz, H-2b,6b), 7.09 (2H, d, *J*=8.4 Hz, H-2d,6d), 7.15 (2H, d, *J*=8.4 Hz, H-2a,6a).

^{13}C NMR (75 MHz, CD_3OD): δ 32.1 (C-8c), 34.8 (C-7c), 39.0 (C-7b), 42.2 (C-8b), 47.2 (C-8d), 47.6 (C-8a), 87.6 (C-7d), 87.8 (C-7a), 94.4 (C-12c), 94.8 (C-12b), 99.6 (C-12d), 100.2 (C-12a), 103.6 (C-14d), 105.1 (C-14a), 107.4 (C-14c), 109.1 (C-14b), 112.8 (C-5c), 114.0 (2C, C-3b, 5b), 114.7 (2C, C-3a, 5a), 115.0 (2C, C-3d, 5d), 118.2 (C-10c), 119.5 (C-10a), 119.6 (C-10b), 121.9 (C-10d), 123.7 (C-6c), 128.0 (2C, C-2b, 2b), 129.2 (2C, C-2a, 2a), 129.3 (2C, C-2d, 2d), 129.9 (C-2c), 130.0 (C-1d), 130.3 (C-1a), 131.3 (C-3c), 133.2 (C-1c), 134.9 (C-1b), 136.9 (C-9c), 140.1 (C-9b), 140.3 (C-9a), 141.8 (C-9d), 151.9 (C-4c), 154.2 (C-4b), 154.7 (C-13a), 155.3 (C-13d), 155.4 (C-11b), 156.2 (C-11a), 156.7 (C-11c), 157.0 (C-4a), 157.3 (C-4d), 157.4 (C-11d), 158.8 (2C, C-13b, C-13c).

4. Y. Oshima, A. Kamijou, H. Moritani, K. Namao, and Y. Ohizumi, *J. Org. Chem.*, 1993, **58**, 850.
5. ^{1}H NMR (400 MHz, acetone-d_6): δ 2.62 (1H, dd, *J*=3.6, 17.6 Hz, H-8b(β)), 3.39 (1H, dd, *J*=3.6, 17.6 Hz, H-8b(α)), 4.25 (1H, d, *J*=11.7 Hz, H-8a), 4.56 (1H, d, *J*=5.9 Hz, H-8c), 5.09 (1H, t, *J*=3.6 Hz, H-7b), 5.41 (1H, d, *J*=5.9 Hz, H-7c), 5.80 (1H, d, *J*=11.7 Hz, H-7a), 6.17 (1H, s, H-12b), 6.19 (2H, d, *J*=2.2 Hz, H-10c,14c), 6.23 (1H, t, *J*=2.2 Hz, H-12c), 6.24 (1H, d, *J*=2.2 Hz, H-14a), 6.38 (1H, d, *J*=2.2 Hz, H-12a), 6.63 (2H, d, *J*=8.4 Hz, H-3b,5b), 6.77 (2H, d, *J*=8.6 Hz, H-3a,5a), 6.78 (2H, d, *J*=8.5 Hz), 6.89 (4H, d, *J*=8.4 Hz, H-2b,6b,3c,5c), 7.12 (2H, d, *J*=8.6 Hz, H-2a,6a), 7.25 (2H, d, *J*=8.4 Hz, H-2c,6c).

   ^{13}C NMR (100 MHz, acetone-d_6): δ 31.7 (C-8b), 35.6 (C-7b), 49.4 (C-8a), 57.4 (C-8c), 88.6 (C-7a), 90.5 (C-12b), 94.4 (C-7c), 101.5 (C-12a), 102.1(C-12c or C-14a), 105.5 (C-14a or C-12c), 106.9 (2C, C-10c, C-14c), 115.6 (2C, C-3b, C-5b), 116.0 (2C, C-3a, C-5a), 116.2 (2C, C-3c, C-5c), 119.9 (C-14b), 120.9 (C-10b), 122.6 (C-10a), 128.0 (2C, C-2c, C-6c), 128.3 (2C, C-2b, C-6b), 130.0 (2C, C-2a, C-6a), 130.8 (C-1a), 134.0 (C-1c), 134.5 (C-1b or C-9b), 134.8 (C-1b or C-9b), 142.1 (C-9a), 146.9 (C-9c), 156.1 (C-4b), 156.5 (C-13a), 157.3 (C-11a), 158.3 (C-4c), 158.6 (C-4a), 159.7 (2C, C-11c, C-13c), 160.2 (C-11b or C-13b), 161.1 (C-11b or C-13b).
6. The attaching repellent activity against the blue mussel of the oligostilbenes from the Vitaceaeous plants will be reported elsewhere.

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