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Selective $^1O_2$ quenchers, oligostilbenes, from *Vitis wilsonae*: Structural identification and biogenetic relationship

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ABSTRACT

Two previously unknown resveratrol trimers named wilsonols A–B, as well as a resveratrol tetramer named wilsonol C, were isolated from *Vitis wilsonae* Veitch, together with 12 known oligostilbenes. Their chemical structures have been elucidated by detailed analyses of 1D and 2D NMR spectroscopic data, as well as chemical evidence obtained *via* either catalysis with HRP (horseradish peroxidase) and H_2O_2 (hydrogen peroxide), acid, or UV irradiation. During the chemical processes, a biomimetic resveratrol tetramer named diviniferin B that has not been found in nature was obtained. These oligostilbenes showed potent scavenging abilities towards DPPH radicals and selective quenching effects on $^1O_2$ radicals. Furthermore, the biogenetic transformations between the 16 oligostilbenes have been elaborated chemically to provide a comprehensive mechanism of the antioxidative defense system in this plant species.

1. Introduction

In various plants in the family of the Vitaceae, oligostilbenes with complex structures are abundant compounds and play important roles, such as phytoestrogens and detoxification products of fungal metabolism of resveratrol (**5**). Furthermore, oligostilbenes may ultimately serve as lead compounds in the development of new drug entities or directly as potential medicinal agents. Consequently, considerable attention has been devoted to their studies including discovery of new oligomeric entities. In addition, it is established that **5** and oligostilbenes, as the main bioactive components of wine grapes (*Vitis vinifera*), are responsible for the unique phenomenon known as the "French Paradox" (Frankel et al., 1993, 1995; Kanner et al., 1994) in terms of attenuating the incidence of coronary heart diseases in some regions of France due to their powerful antioxidant properties.

In previous work (He et al., 2008b, 2009a, 2009b; Jiang et al., 2010), in contrast with the rather weak effects of oligostilbenes on scavenging superoxide anion ($O_2^{\cdot-}$) and hydroxyl radical (·OH), much stronger and selective quenching activities of oligostilbenes on $^1O_2$ had been implicated, which were partially associated with the positive responses of wine grapes to photooxidative stress. Under nonenzymic and enzymic conditions, the biogenetic relationship of the major constituents had been composed of some coupling reactions (He et al., 2008a) to form an integrated transformation pathway for counteracting oxidative stress in plant. LC/MS, as a rapid and sensitive tool, has been utilized to guide the fractionation of plant extracts plenty of oligostilbenes on the basis of their characteristic molecular weights and MS^n fragments. Recently, we reported the first isolation of a highly condensed resveratrol hexamer from Vitaceaeous plants, *viz.* chunganenol from *Vitis chunganensis* (He et al., 2009b).

In the present work, the study was directed to identifying more complex and active oligostilbenes from *Vitis wilsonae* Veitch to enlarge our laboratory libraries of diverse natural products for drug screening.

2. Results and discussion

Based on the indicative molecular weights of oligostilbenes and their distinguishing MS^2 fragments, the HPLC/ESI-MS^2 evaluation of 11 column chromatography (CC) fractions from the EtOAc/H_2O partition of the MeOH extract of *V. wilsonae* indicated plentiful presence of oligostilbenes in fractions 6–9. Among them, fractions 8 and 9 when processed through several rounds of CC and preparative HPLC separations gave three oligostilbenes, wilsonols A–C (**1**–**3**) as shown in Fig. 1, with other 12 known oligostilbenes (Fig. 2).

Wilsonol A (**1**), obtained as a brown amorphous powder ($[\alpha]_D^{20}$ −10.5 (c 0.423, MeOH)), gave a molecular ion peak at *m*/*z* 679.1951 ($[M-H]^-$) in the HR-ESI-MS (high-resolution electrospray ionization mass spectrometry), accounting for the elemental

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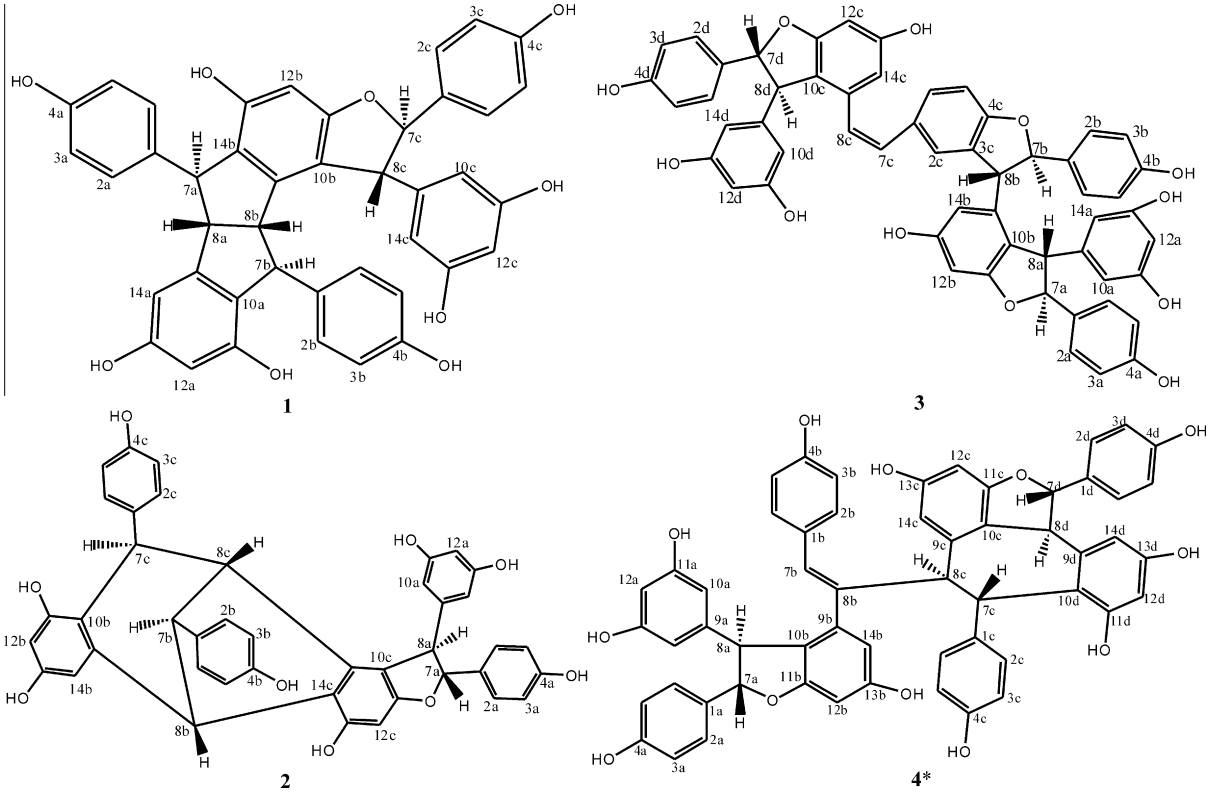

Fig. 1. Chemical structures of wilsonols A–C (**1**–**3**) isolated from *V. wilsonae* as well as a biomimetic oligostilbene diviniferin B (**4**) * from the oxidative coupling of two **7** molecules by HRP and H_2O_2.

composition $C_{42}H_{31}O_9$. Together with analysis of its ^1H NMR and ^1H–^1H COSY (correlation spectroscopy) spectra, this suggested that **1** was a resveratrol trimer containing three sets of mutually coupled aliphatic protons [δ 3.82 (1H, *d*, *J* = 6.4 Hz), 4.63 (1H, *br s*); δ 3.88 (1H, *d*, *J* = 6.4 Hz), 4.25 (1H, *br s*); δ 4.43 (1H, *br s*), 5.33 (1H, *br s*)], three sets of A_2X_2-type *ortho*-coupled aromatic protons [δ 6.70, 6.94; 6.57, 6.76; 6.80, 7.19 (each 2H, *d*, *J* = 8.5 Hz)], one set of AX_2-type *meta*-coupled aromatic protons [δ 6.17 (2H, *m*) and 6.19 (1H, *t*, *J* = 2.0 Hz)], one set of *meta*-coupled aromatic protons [δ 6.07 and 6.56 (each 1H, *br s*)], and a single aromatic proton [δ 6.32 (1H, *br s*)]. Other NMR spectroscopic analysis, especially that of the HMBC (heteronuclear multiple bond correlation) spectrum, allowed final assignments of all carbon and proton signals as shown in Table 1. The similar respective HMBC correlations of H-7a and H-8a, as well as H-7b and H-8b, in addition to the approximate chemical shifts of H-7a and H-7b as well as H-8a and H-8b, indicated the presence of pallidol (**6**) as its substructure (Ohyama et al., 1995). The ^1H–^1H coupling pattern of H-7c/H-8c characteristic of a dihydrobenzofuran ring in combination with HMBC correlations of H-7c/C-2c (6c), C-1c, C-11b, C-8c, C-10b and H-8c/C-9c, C-10c (14c), C-7c, C-1c, C-10b, C-11b established that the dihydrobenzofuran ring was coupled with one of the two resorcinol rings in **1**. Significant HMBC correlations H-12b/C-10b, C-11b, C-13b, C-14b suggested that the dihydrobenzofuran ring was connected to C-10b, consistent with that of carasiphenol C (Wang et al., 2005), which was also supported by the fact that cross-peaks H-12b/C-8b or H-8b/C-12b were not observed due to their remote distances. Furthermore, in view of biogenetic considerations, it could be deduced that **1** was generated by the oxidative coupling of one molecular unit of ε-viniferin (**7**), which is the most common dimer in Vitaceaeous plants, and one molecular unit of **5** (Fig. 3). Thus the structure depicted in Fig. 1 would be more favored than those shown in previous literature (Ohyama et al., 1995). The NOESY (nuclear overhauser effect spectroscopy) spectrum was suggestive of the relative stereochemistry for **1** as follows: NOE interactions between H-7c/H-10c (14c) and H-8c/H-2c (6c) defined the *trans*-orientation of each phenyl group connected to C-7c and C-8c; key NOE interactions between H-8b and H-8c as well as H-8b and H-2a (6a) were successfully observed, demonstrating the spatial vicinity of these protons; additionally, considering the NOE interactions between H-7a/H-14a, H-8a/H-2a (6a) and H-8b/H-2b (6b), the phenyls of the **6** moiety were situated in a *trans*-orientation to each other.

Wilsonol B (**2**) was isolated as a pale yellow amorphous powder ($([\alpha]_D^{20}$ 49.8 (c 0.292, MeOH)). Its molecular formula was established to be $C_{42}H_{32}O_9$ as confirmed by the observation of its deprotonated ion at *m*/*z* 679.1947 (calcd. 679.1974, error 3.97 ppm) in the HR-ESI-MS experiment. In the ^1H NMR and ^1H–^1H COSY spectra, the existence of three sets of mutually coupled aliphatic protons [δ 5.14 (1H, *d*, *J* = 8.2 Hz), 2.57 (1H, *d*, *J* = 8.2 Hz); δ 4.02 (1H, *br s*), 3.51 (1H, *br s*); δ 4.39 (1H, *d*, *J* = 5.5 Hz), 3.34 (1H, *d*, *J* = 5.5 Hz)], three 4-hydroxyphenyl groups [δ 6.80 (2H, *d*, *J* = 8.5 Hz), 7.00 (2H, *d*, *J* = 8.5 Hz); 6.67 (2H, *d*, *J* = 8.5 Hz), 6.77 (2H, *d*, *J* = 8.5 Hz); 5.84 (1H, *dd*, *J* = 8.5, 2.2 Hz), 6.40 (1H, *dd*, *J* = 8.5, 2.2 Hz), 6.79 (1H, *dd*, *J* = 8.5, 2.2 Hz), 7.32 (1H, *dd*, *J* = 8.5, 2.2 Hz)], one 3, 5-dihydroxyphenyl group [δ 6.02 (2H, *d*, *J* = 2.0 Hz) and 6.21 (1H, *t*, *J* = 2.0 Hz)], one *meta*-coupled dihydroxyphenyl group [δ 6.10 and 6.46 (each 1H, *d*, *J* = 2.0 Hz)], one pentasubstituted benzene ring [δ 6.06 (1H, *br s*)], and a sequence of phenolic hydroxyl groups [δ 8.29 (1H, *s*), 8.04 (2H, s), 7.99 (1H, *s*), 7.97 (1H, *s*), 7.95 (1H, *s*), 7.88 (1H, *s*), 6.55 (1H, *s*)] demonstrated that **2** was a resveratrol trimer. The unique phenomenon of four independent doublet doublets in one 4-hydroxyphenyl group was the result of the corresponding phenyl ring being fixed in one structure, identical with that of isoampelopsin F (Tanaka et al., 1998), which also caused the higher chemical shift of one aliphatic methine proton (H-8a, 2.57) as compared with the general ones (such as **1**). Table 1 shows the final assignments of all car-