

Isolation and Characterization of Novel Stilbene Derivatives from Riesling Wine

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Besides the already known stilbenes *trans*-resveratrol as well as isomeric piceids, seven novel stilbene derivatives have been isolated from a commercial Riesling wine. The newly identified compounds included the monostilbene 2,4,6-trihydroxyphenanthrene-2-*O*-glucoside, as well as two isomeric resveratrol-2-*C*-glucosides. In addition, four dimeric stilbenes, i.e., *cis*- and *trans*- ϵ -viniferin diglucoside as well as pallidol glucoside and pallidol diglucoside, have also been obtained for the first time from Riesling wine.

Keywords: Antioxidants; white wine; Riesling; resveratrol-2,4,6-trihydroxyphenanthrene 2-*O*-glucoside; resveratrol 2-*C*-glucosides; ϵ -viniferin diglucosides; pallidol glucoside; pallidol diglucoside.

INTRODUCTION

Hydroxylated stilbenes are phytoalexins that are produced by plants in response to fungal infections (Langcake and Pryce, 1977; Jeandet et al., 1995a,b, 1997). Resveratrol (stilbene-3,5,4'-triol) is a wine constituent that has attracted an enormous research interest in recent years due to its antioxidant activity. Resveratrol is known to occur in wine in free and glycosidically bound form. Free *trans*- and *cis*-resveratrols **1a,b** (cf. Figure 2) are present in a concentration range of 0.2–13 mg/L in red wines and 0.1–0.8 mg/L in white wines, respectively. For the bound forms of resveratrol, the so-called piceids **2a,b** (cf. Figure 2) concentration are reported to be in a range of 0.3–9 mg/L in red and 0.1–2.2 mg/L in white wines (Lamuela-Raventós et al., 1995; Goldberg et al., 1996a,b; Sato et al., 1997; Dietrich et al., 1999). Ribeiro de Lima et al. (1999) even determined piceids in Portuguese red wines in concentrations up to 68 mg/L. The antioxidant activity of resveratrol has been linked to a reduced mortality from coronary heart disease (CHD) (Frankel et al., 1993). Resveratrol also inhibits human platelet aggregation in vitro (Orsini et al., 1997) and modulates eicosanoid synthesis toward a pattern likely to be protective against CHD (Kimura et al., 1985; Pace-Asciak et al., 1995). More recently, Gehm et al. (1997) have reported estrogenic properties of resveratrol that may also contribute to the reported cardioprotective effects of wine consumption.

Many recent papers have described methods to assay the content in wine (Lamuela-Raventós et al., 1995; Goldberg et al., 1995) as well as in grape juice (Romero-Pérez et al., 1999) of resveratrol isomers **1a,b** and their corresponding glucosides **2a,b**. Also, the influence of different wine making techniques on resveratrol concentration in the finished wine has been studied by different research groups (Mattivi et al., 1995; Pezet and Cuenat, 1996; Castellari et al., 1998). Despite this effort,

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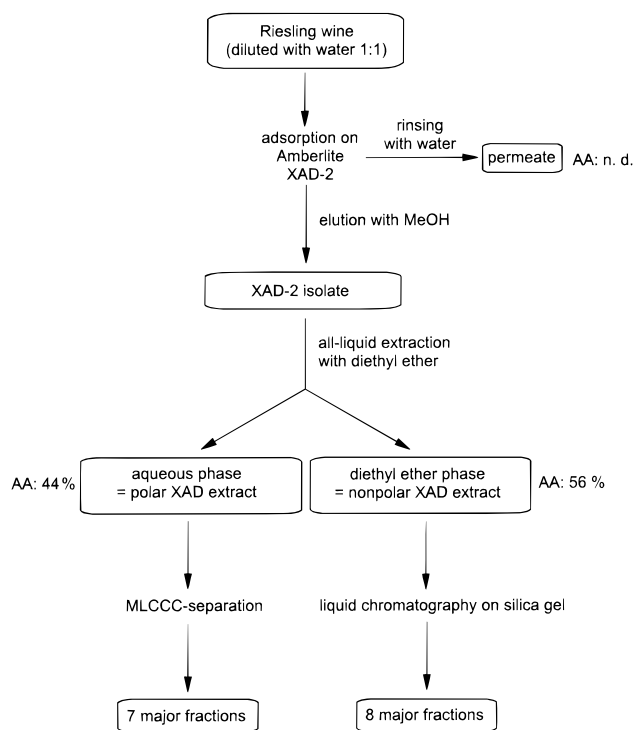


Figure 1. Protocol for the isolation of antioxidant compounds from Riesling wine. AA = antioxidant activity, n.d. = not detectable.

little is known about the occurrence of additional stilbene derivatives in wine. Lamikanra et al. (1996) tentatively identified mono-, di-, and tetrahydroxystilbenes in red wines using gas chromatography–mass spectrometry. Moreover, the occurrence of 3,5,3',4'-tetrahydroxystilbene 3-*O*- β -glucoside (*trans*-astringin) in white and red wines has been reported by Ribeiro de Lima et al. (1999). In the course of our studies on antioxidants in white wine, seven new stilbene derivatives have been isolated. Structure elucidation and antioxidant testing of these new wine constituents will be reported here.

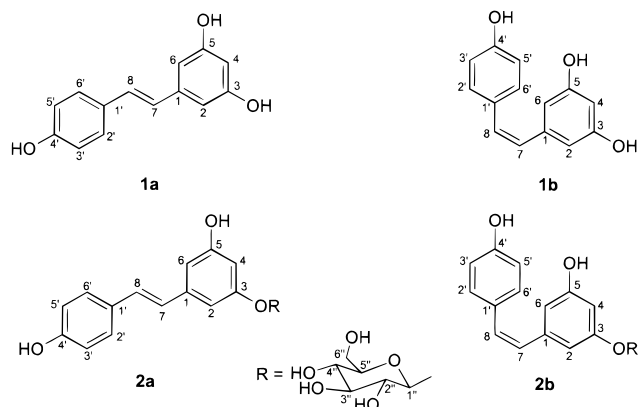


Figure 2. Known resveratrol derivatives isolated from Riesling wine: **1a**, *trans*-resveratrol; **2a**, *trans*-piceid; **2b**, *cis*-piceid.

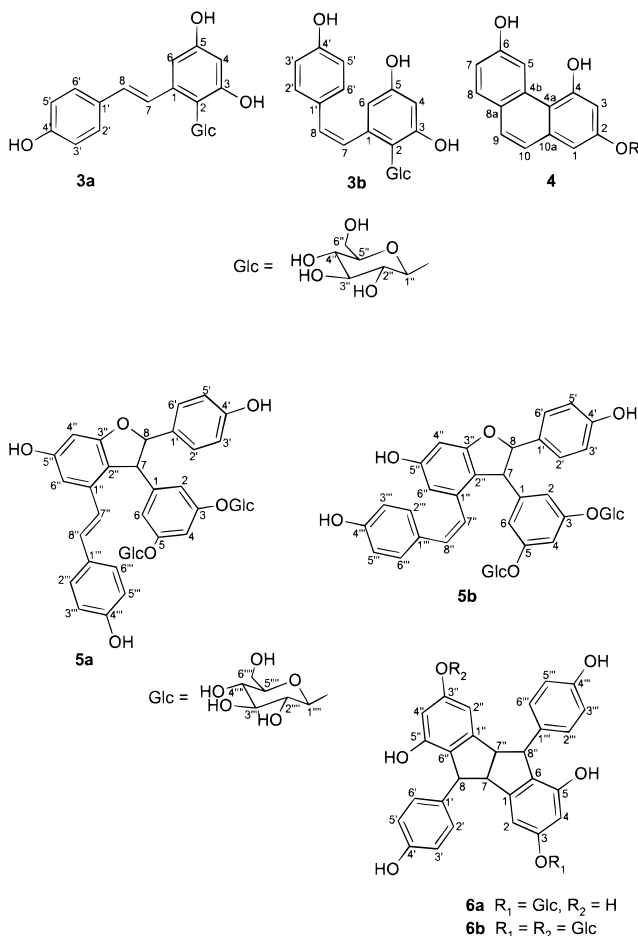


Figure 3. Resveratrol derivatives newly isolated from Riesling wine: **3a,b**, resveratrol 2-*O*-glucosides; **4**, 2,4,6-trihydroxyphenanthrene 2-*O*-glucoside; **5a,b**, ϵ -viniferin diglucosides; **6a**, pallidol 3-*O*-glucoside; **6b**, pallidol 3,3'-diglucoside.

MATERIALS AND METHODS

All commercial chemicals used were of analytical grade quality. Solvents were redistilled before use or purchased in HPLC-grade quality.

Isolation and Identification of Stilbene Derivatives. A commercial Riesling wine (100 L, QbA quality, Rheinpfalz, 1992 vintage) was worked up as shown in Figure 1. Each step of the workup was checked by measuring the antioxidant activity of the obtained fractions using the β -carotene bleaching method (Baderschneider et al., 1999). Initially, the wine was diluted with water (1:1) and passed through a column of Amberlite XAD-2 resin (Günata et al., 1985). The column was

then rinsed with water. The permeate consisted mainly of sugars and organic acids and did not reveal any antioxidant activity. The XAD isolate obtained after exhaustive elution of the column with methanol was concentrated under reduced pressure and subsequently partitioned into polar and nonpolar fractions by all-liquid extraction using diethyl ether as solvent. Since both fractions showed almost equal antioxidant activity each isolate was further fractionated.

The polar XAD extract (aqueous phase of the all-liquid extraction) was first separated by multilayer coil countercurrent chromatography (Multilayer Coil Separator-Extractor P.C. Inc., Potomac, MD) using the preparative device (85 m \times 2.6 mm i.d. PTFE tubing; solvent system: $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ 7:13:8; flow rate 1.5 mL/min). TLC monitoring of the collected fractions allowed to group them into 7 combined fractions. The most polar fractions 1 and 2 that showed highest antioxidant activity were further fractionated by analytical MLCCC (160 m \times 1.6 mm i.d. PTFE tubing, solvent system: $\text{EtOAc}/\text{BuOH}/\text{H}_2\text{O}$ 3:2:5; flow rate 1.0 mL/min). The subfractions were purified by gel chromatography on Sephadex LH20 (solvent: MeOH). Final purification by preparative HPLC (Eurospher 100 RP-18 column, 5 μm , 250 \times 16 mm, Knauer Säulentechnik, Berlin, eluent: gradients of 2% acetic acid/MeOH) yielded 8 mg of compound **2a**, 0.5 mg of compound **2b**, 8 mg of compound **3a**, 6 mg of compound **3b**, 3.6 mg of compound **4**, 3.2 mg of compound **5a**, 1.2 mg of compound **5b**, 3.5 mg of compound **6a** and 2.0 mg of compound **6b**. Spectral data of the isolated compounds are given below: **2a**: DCI-MS: pseudo molecular ion at m/z 408 $[\text{M}(390) + \text{NH}_4]^+$. ^1H and ^{13}C NMR spectral data were identical with the data published by Waffo-Tégue et al. (1996) for *trans*-piceid. **2b**: DCI-MS: pseudo molecular ion at m/z 408 $[\text{M}(390) + \text{NH}_4]^+$. ^1H NMR spectral data were in good agreement with data obtained by Mattivi et al. (1995) for *cis*-piceid. **3a/3b**: DCI-MS: pseudo molecular ion at m/z 408 $[\text{M}(390) + \text{NH}_4]^+$. ^1H and ^{13}C NMR spectral data are shown in Tables 1 and 2. **4**: DCI-MS: pseudo molecular ion at m/z 406 $[\text{M}(388) + \text{NH}_4]^+$. ^1H and ^{13}C NMR spectral data are shown in Tables 1 and 2. **5a/5b**: ESI-MS (negative mode): pseudo molecular ion at m/z 777 $[\text{M}(778) - \text{H}]^-$; MS/MS of m/z 777 at m/z 615 $[\text{M} - \text{H}^+ - \text{anhydroglucose}]^-$. ^1H NMR spectral data of both compounds are shown in Table 3; ^{13}C NMR spectral data of compound **5a** are gathered in Table 4. **6a**: DCI-MS: pseudo molecular ion at m/z 634 $[\text{M}(616) + \text{NH}_4]^+$. ^1H and ^{13}C NMR spectral data are shown in Tables 3 and 4. **6b**: ESI-MS (negative mode): pseudo molecular ion at m/z 777 $[\text{M}(778) - \text{H}]^-$; MS/MS of m/z 777 at m/z 615 $[\text{M} - \text{H}^+ - \text{anhydroglucose}]^-$. ^1H NMR spectral data are shown in Table 3.

Compound **1a** was isolated from the Et_2O fraction of the XAD extract. After concentration of the Et_2O phase the isolate was fractionated by conventional liquid chromatography on silica gel. Fractions obtained by gradient elution using different mixtures of solvents of increasing polarity (pentane, diethyl ether, ethyl acetate, methanol, acidified methanol) were monitored by TLC and finally grouped into 8 major fractions. Fraction 4 being highest in antioxidant activity was further fractionated by gel chromatography on Sephadex LH20 using methanol as eluent. Subsequent purification of subfraction 3 by preparative HPLC (conditions as mentioned above) yielded 12 mg of pure compound **1a**. ESI-MS (negative mode): pseudo molecular ion at m/z 227 $[\text{M}(228 - \text{H})]^-$. ^1H and ^{13}C NMR data are in good agreement with the literature data (Mattivi et al., 1995).

Nuclear Magnetic Resonance (NMR) Spectroscopy. ^1H and ^{13}C NMR spectral data were recorded on Fourier transform Bruker AM 360, AC 250, and AMX 300 spectrometers with TMS as internal reference standard.

Mass Spectrometry. DCI-MS (Desorption Chemical Ionization Mass Spectrometry) was carried out with a Finnigan TSQ 70 mass spectrometer at 70 eV using ammonia as reactant gas. ESI-MS/MS data (electrospray ionization ion trap multiple mass spectrometry) was obtained using a Bruker Esquire-LC-MS/MS system with electrospray ionization in the negative mode.

Table 1. ¹H NMR Spectral Data of Isolated Monomeric Resveratrol Derivatives (CD₃OD, 360 MHz, Coupling Constants in Hertz, δ Relative to TMS)

| 3a | | | | 3b | | | 4 | | | |
|-------|------|---------|-----------|------|---------|-------|-------|------|--------|---------|
| atom | δ | signal | J | δ | signal | J | atom | δ | signal | J |
| H4 | 6.24 | 1H, d | 2 | 6.22 | 1H, d | 2.5 | H1 | 6.99 | 1H, d | 2.5 |
| H6 | 6.55 | 1H, d | 2 | 6.14 | 1H, d | 2.5 | H3 | 6.86 | 1H, d | 2.5 |
| H7 | 6.76 | 4H, m | | 6.44 | 1H, d | 12 | H5 | 8.99 | 1H, d | 2.5 |
| H8 | 6.76 | 4H, m | | 6.60 | 1H, d | 12 | H7 | 7.02 | 1H, dd | 8.5/2.5 |
| H2' | 7.36 | 2H, m | | 7.04 | 2H, m | | H8 | 7.65 | 1H, d | 8.5 |
| H3' | 6.76 | 4H, m | | 6.56 | 2H, m | | H9 | 7.52 | 1H, d | 8.5 |
| H5' | 6.76 | 4H, m | | 6.56 | 2H, m | | H10 | 7.32 | 1H, d | 8.5 |
| H6' | 7.36 | 2H, m | | 7.04 | 2H, m | | | | | |
| H1'' | ~4.8 | a | | 4.64 | 1H, d | 9.5 | H1'' | 5.26 | 1H, d | 8.5 |
| H2'' | 3.87 | 1H, t | 9 | 3.92 | 1H, dd | 9.5/9 | H2'' | 3.90 | 1H, t | 8.5 |
| H3'' | 3.52 | 1H, t | 9 | 3.39 | 1H, t | 9 | H3'' | 3.57 | m | |
| H4'' | 3.46 | 1H, t | 9 | 3.49 | 1H, t | 9 | H4'' | 3.57 | m | |
| H5'' | 3.39 | 1H, ddd | 9/4.5/2.5 | 3.18 | 1H, ddd | 9/4/3 | H5'' | 3.57 | m | |
| H6''a | 3.77 | 1H, dd | 12/4.5 | 3.71 | 1H, d | 4 | H6''a | 3.77 | 1H, dd | 12/5 |
| H6''b | 3.88 | 1H, dd | 12/2.5 | 3.72 | 1H, d | 3 | H6''b | 3.95 | 1H, dd | 12/1.5 |

^a Overlapped by solvent signal.

Table 2. ¹³C NMR Spectral Data of Isolated Monomeric Resveratrol Derivatives (CD₃OD, 62.9 MHz, δ Relative to TMS)

| 3a | | 3b | | 4 | |
|------|-------|------|-------|------|-------|
| atom | δ | atom | δ | atom | δ |
| C1 | 141.8 | C1 | 142.3 | C1 | 107.3 |
| C2 | 115.8 | C2 | 114.9 | C2 | 158.9 |
| C3 | 158.5 | C3 | 158.8 | C3 | 103.4 |
| C4 | 103.5 | C4 | 103.7 | C4 | 157.3 |
| C5 | 158.8 | C4a | 159.0 | C4a | 115.5 |
| C6 | 106.2 | C4b | 109.2 | C4b | 133.1 |
| C7 | 126.4 | C5 | 128.6 | C5 | 113.4 |
| C8 | 130.9 | C6 | 131.0 | C6 | 156.7 |
| C1' | 130.9 | C7 | 129.7 | C7 | 115.9 |
| C2' | 129.0 | C8 | 131.8 | C8 | 130.5 |
| C3' | 116.4 | C8a | 115.7 | C8a | 127.3 |
| C4' | 158.5 | C9 | 157.7 | C9 | 128.9 |
| C5' | 116.4 | C10 | 115.7 | C10 | 124.6 |
| C6' | 129.0 | C10a | 131.8 | C10a | 137.6 |
| C1'' | 80.0 | C1'' | 80.0 | C1'' | 102.5 |
| C2'' | 74.4 | C2'' | 73.8 | C2'' | 74.8 |
| C3'' | 80.0 | C3'' | 80.0 | C3'' | 78.3 |
| C4'' | 71.6 | C4'' | 71.3 | C4'' | 71.2 |
| C5'' | 82.2 | C5'' | 82.2 | C5'' | 78.6 |
| C6'' | 62.7 | C6'' | 62.3 | C6'' | 62.5 |

Determination of Antioxidant Activity. The antioxidant activity of fractions obtained during the workup was monitored using the β-carotene bleaching test as described previously (Baderschneider et al., 1999). Antioxidant capacity of the pure compounds was determined as Trolox equivalents using the TAS measurement (Miller et al., 1993). The previously published procedure (Baderschneider et al., 1999) was slightly modified. Metmyoglobin was prepared by oxidation of commercial myoglobin by potassium ferricyanide and purified prior to use on a Sephadex G-15-120 column. The concentration of metmyoglobin was determined spectrophotometrically and the solution diluted to 25 μM. A solution (1 mM) of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) in phosphate buffer (pH 7.4) was prepared together with an aqueous solution of hydrogen peroxide (0.5 mM). For calibration of the measurement, Trolox standards (0.05, 0.1, 0.15 and 0.2 mM, respectively) were prepared. Samples were dissolved to final concentrations which were expected to reveal antioxidant activities of about 1 Trolox equivalent (mmol Trolox/mmol compound). Assays were performed at room temperature. One hundred microliters of H₂O for the blank, standard solutions, or sample solutions was added to semimicrocuvettes containing 800 μL phosphate buffer, 600 μL of the ABTS solution and 200 μL of the metmyoglobin solution, respectively. After mixing, the initial absorbance at 734 nm was read (A₁). To start the reaction 300 μL of the hydrogen peroxide solution were added

and exactly after 6 min the final absorbance was read (A₂).

$$\Delta A = A_2 - A_1$$

[ΔA(blank) - ΔA] was calculated for the standards as well as for the samples. A Trolox calibration curve was obtained by plotting the concentration of the standard solutions versus [ΔA(blank) - ΔA]. For the samples, Trolox equivalents (mmol Trolox/mmol compound) were calculated by dividing the Trolox concentration obtained from the calibration curve by the sample concentration.

RESULTS AND DISCUSSION

In a preceding communication (Baderschneider et al., 1999), the antioxidant activity of Riesling wine has been evaluated using four different testing methods (LDL oxidation, TAS measurement, β-carotene bleaching, Pryor's screening test). Importantly, the antioxidant activity was found to be almost equally distributed between a fraction that contained less polar, diethyl ether (Et₂O) soluble compounds and the XAD-2 fraction, in which polar wine constituents, such as glycosides and flavonoids, were enriched. In an effort to isolate the white wine constituents responsible for the antioxidant activity, each of the isolates has been further fractionated: the Et₂O-isolate by conventional liquid chromatography on silica gel and the XAD-2 isolate by the support-free technique of multilayer coil counter-current chromatography (MLCCC). So far, 93 compounds that belong to different classes of wine constituents (i.e., stilbenes, flavonoids, and cinnamates) were obtained in pure form. Due to the known importance of stilbenes as antioxidants in wine (Frankel et al., 1993; Goldberg et al., 1996b), members of this class of secondary plant metabolites will be described in the present paper.

Seven stilbene derivatives were newly isolated and characterized by spectroscopic methods. Their structures were mainly deduced from one- and two-dimensional NMR spectral data. Assignments of proton and carbon resonances were made on the basis of ¹H-¹H COSY, heteronuclear HMQC, and HMBC 2D chemical shift correlations.

Identification of C-Glucosides of Resveratrol (3a,b). On the basis of their NMR spectral data, compounds **3a** and **3b** were characterized as *trans*- and *cis*-3,5,4'-trihydroxystilbene 2-C-glucosides (cf. Figure 3). To the best of our knowledge, these C-glucosides have

Table 3. ¹H NMR Spectral Data of Isolated Resveratrol Dimers (CD₃OD, 360 and 300 MHz, Respectively, Coupling Constants in Hertz, δ Relative to TMS)

| atom | 5a | | | 5b | | | 6a | | | 6b | | |
|-------------|----------|----------|----------|----------|--------|----------|----------|--------|----------|----------|----------|----------|
| | δ | signal | <i>J</i> | δ | signal | <i>J</i> | δ | signal | <i>J</i> | δ | signal | <i>J</i> |
| H2 | 6.61 | 1H, d | 1.5 | 6.55 | 1H, d | 2 | 6.87 | 1H, d | 2 | 6.87 | 1H, d | 2 |
| H4 | 6.78 | 1H, t | 1.5 | 6.74 | 1H, s | | 6.39 | 1H, d | 2 | 6.39 | 1H, d | 2 |
| H6 | 6.60 | 1H, d | 1.5 | 6.57 | 1H, d | 2 | | | | | | |
| H7 | 4.52 | 1H, d | 6 | 3.97 | 1H, d | 6 | 3.77 | 1H, d | 7 | 3.78 | 1H, brs | |
| H8 | 5.34 | 1H, d | 6 | 5.22 | 1H, d | 6 | 4.50 | 1H, s | | 4.53 | 1H, brs | |
| H2''/H6' | 7.15 | 2H, m | | 6.95 | 2H, m | | 6.93 | 2H, m | | 6.93 | 2H, m | |
| H3''/H5' | 6.77 | 2H, m | | 6.72 | 2H, m | | 6.66 | 2H, m | | 6.65 | 2H, m | |
| H2'' | | | | | | | 6.51 | 1H, d | 1.5 | 6.87 | 1H, d | 2 |
| H4'' | 6.27 | 1H, d | 2 | 6.38 | 1H, s | | 6.10 | 1H, d | 1.5 | 6.39 | 1H, d | 2 |
| H6'' | 6.65 | 1H, d | 2 | 6.39 | 1H, s | | | | | | | |
| H7'' | 6.53 | 1H, d | 16 | 6.06 | 1H, d | 12 | 3.73 | 1H, d | 7 | 3.78 | 1H, brs | |
| H8'' | 6.84 | 1H, d | 16 | 6.24 | 1H, d | 12 | 4.49 | 1H, s | | 4.53 | 1H, brs | |
| H2'''/H6''' | 7.06 | m | | 6.91 | 2H, m | | 6.91 | 2H, m | | 6.93 | 2H, m | |
| H3'''/H5''' | 6.67 | m | | 6.60 | 2H, m | | 6.65 | 2H, m | | 6.65 | 2H, m | |
| H1'''' | ~4.8 | <i>a</i> | | 4.81 | 2H, d | 7.5 | 4.90 | 1H, d | 7.5 | ~4.8 | <i>a</i> | |
| H2'''' | 3.40 | m | | 3.40 | m | | 3.44 | m | | 3.40 | m | |
| H3'''' | 3.40 | m | | 3.40 | m | | 3.44 | m | | 3.40 | m | |
| H4'''' | 3.40 | m | | 3.40 | m | | 3.44 | m | | 3.40 | m | |
| H5'''' | 3.40 | m | | 3.40 | m | | 3.44 | m | | 3.40 | m | |
| H6''''a | 3.66 | 2H, dd | 12.5/4.5 | 3.68 | 2H, dd | 12.5/4.5 | 3.70 | 1H, dd | 12.5/5.5 | 3.70 | 2H, dd | 12.5/5.5 |
| H6''''b | 3.82 | 2H, brd | 12.5 | 3.80 | 1H, dd | 12.5/2 | 3.93 | 1H, dd | 12.5/2 | 3.93 | 2H, dd | 12.5/2 |
| | | | | 3.86 | 1H, dd | 12.5/2 | | | | | | |

^a Overlapped by solvent signal.

Table 4. ¹³C NMR Spectral Data of *trans*- ϵ -Viniferindiglucoside (5a) and Pallidol Glucoside (6a) (CD₃OD, 62.9 MHz, δ Relative to TMS)

| 5a | | 6a | | 5a | | 6a | |
|------|----------|----------|--------|----------|----------|------|----------|
| atom | δ | δ | atom | δ | δ | atom | δ |
| C1 | 147.5 | 150.7 | C1'' | 137.0 | 150.8 | | |
| C2 | 111.6 | 105.0 | C2'' | 119.3 | 103.4 | | |
| C3 | 160.5 | 160.2 | C3'' | 163.0 | 159.4 | | |
| C4 | 104.4 | 104.1 | C4'' | 97.0 | 102.7 | | |
| C5 | 160.5 | 155.5 | C5'' | 160.0 | 155.6 | | |
| C6 | 111.6 | 126.8 | C6'' | 104.5 | 123.6 | | |
| C7 | 57.7 | 61.1 | C7'' | 123.5 | 60.7 | | |
| C8 | 94.7 | 54.7 | C8'' | 130.2 | 54.6 | | |
| C1' | 133.8 | 138.2 | C1''' | 130.7 | 138.0 | | |
| C2' | 128.3 | 129.2 | C2''' | 128.9 | 129.2 | | |
| C3' | 116.4 | 116.0 | C3''' | 116.6 | 116.0 | | |
| C4' | 158.6 | 156.4 | C4''' | 158.5 | 156.3 | | |
| C5' | 116.4 | 116.0 | C5''' | 116.6 | 116.0 | | |
| C6' | 128.3 | 129.2 | C6''' | 128.9 | 129.2 | | |
| | | | C1'''' | 102.4 | 102.6 | | |
| | | | C2'''' | 74.8 | 75.0 | | |
| | | | C3'''' | 77.9 | 77.1 | | |
| | | | C4'''' | 71.3 | 71.5 | | |
| | | | C5'''' | 78.0 | 78.3 | | |
| | | | C6'''' | 62.4 | 62.7 | | |

been detected for the first time in wine. The elaborated structures for **3a,b** were confirmed by DCI-MS measurements, which indicated a molecular mass of 390 amu. While the mass spectra showed the same molecular weight for *C*-glucosides **3a,b** and isomeric piceids, respectively, the proton NMR spectra revealed the lack of one aromatic proton, thus leading to a tetra- instead of a trifold substituted benzene unit. Additionally the coupling constant of 9.5 Hz for the anomeric proton and the downfield shift by 0.5 ppm of H-2 of the glucose unit (cf. Table 1) indicated the presence of a *C*-glucosidic linkage. The ¹³C NMR (Table 2) proved the presence of a *C*-glucoside since the characteristic signal for the anomeric *C*-atom around 100 ppm was lacking. On the basis of a heteronuclear correlation experiment the chemical shift for C-1 of the glucose moiety was assigned to be 80 ppm.

Identification of 2,4,6-Trihydroxyphenanthrene 2-O-Glucoside (4). This phenanthrene glucoside was obtained from MLCCC fraction 2 after careful purification using a series of separation steps. As to the hypothetical formation of **4** from *cis*-piceid **2b**, photochemical reactions as well as enzymatic activities can be considered as being likely. The phenanthrene derivative **4** is to the best of our knowledge reported here for the first time as natural product.

Identification of Dimeric Stilbene Derivatives (5a,b and 6a,b). Dimeric stilbenes have been isolated from grapevine leaves after infection with *Botrytis cinerea* (Langcake and Pryce, 1977; Jeandet et al., 1997). Up to now, a presence of these substances in wine has not been reported. Dimeric stilbenes have been demonstrated to be formed by oxidative processes from the parent compound resveratrol. In experiments with horseradish peroxidase, a 40% conversion of resveratrol into viniferin was reported (Langcake and Pryce, 1977). Two compounds newly detected in Riesling wine are identical with those isolated from grapevine leaves by Langcake and Pryce (1977), except that the wine constituents are attached to a β -D-glucose unit. NMR and mass spectral data are in good agreement with published data. The position of the glucosidic linkage was deduced from the observed downfield shift by 4.8 ppm for C-2 and C-6 and 2.5 ppm for C-4, respectively (cf. Table 4). The resonances for H-2, H-4, and H-6 were downfield shifted by 0.4–0.5 ppm (cf. Table 3). Two further dimeric stilbene derivatives were identified as pallidol 3-*O*-glucoside **6a** and pallidol-3,3''-*O*-diglucoside **6b**, respectively. The aglycon pallidol was for the first time isolated from *Cissus pallida*, a member of the Vitaceae (Khan et al., 1986). Our NMR spectral data are in good agreement with the data published by Khan et al. (1986). Substitution of the hydroxyl group at C-3 by a glucose moiety caused a slight downfield shift of H-2 and H-3, respectively. For compound **6b**, ESI-MS revealed a molecular weight of 778 amu. The MS/MS fragmentation pattern of the pseudo-molecular ion of *m/z* 777 showed the consecutive loss of two glucose

Table 5. Antioxidant Potential Expressed as Trolox Equivalents of Newly Isolated Resveratrol Derivatives in Comparison to Resveratrol and *trans*-Piceid

| compd | Trolox equivalents (mmol Trolox/mmol) |
|--|---------------------------------------|
| <i>trans</i> -resveratrol (1a) | 2.6 |
| <i>trans</i> -resveratrol-3- <i>O</i> -glucoside (2a) | 1.5 |
| <i>trans</i> -resveratrol-2- <i>C</i> -glucoside (3a) | 2.6 |
| <i>trans</i> - ϵ -viniferindiglucoside (5a) | 4.3 |
| pallidol glucoside (6a) | 4.5 |

units as well of the loss of two hydroxyphenyl moieties (m/z 615, 521, 453, and 359). This indicated the presence of a pallidol diglucoside. The fact that in the NMR spectra only half of the number of the expected resonance signals were observed led us to conclude that the pallidol moiety was symmetrically substituted by two glucose units. Hence, the structure **6b** could be assigned.

Antioxidant Activity of the Newly Identified Stilbenes. The relation between moderate wine consumption and reduced risk for coronary heart disease has been the subject of many publications. Initially, the compound many studies focused on was the stilbene derivative resveratrol, which is present in wine in free and glycosidically bound form. In more recent years, it became obvious that the entire polyphenol fraction has to be taken into consideration (Dietrich et al., 1999). Thus, the antioxidative activity of the newly identified stilbenes has been evaluated. The data obtained are shown in Table 5. With an antioxidative activity of 2.6–4.5 Trolox equivalents, compounds **3–6** are on a molar basis equal or even more potent as the well-known resveratrols. Further studies using Riesling wines from various geographic regions and vintages will be necessary to determine the contribution of the novel stilbene derivatives to the overall antioxidant activity of wine.

SUMMARY

It is evident that the class of wine stilbenes comprises more compounds as was previously known. In the white wine under investigation, mono-, di-, and tetrahydroxystilbenes that have been tentatively identified by Lamikanra et al. (1996) in red wine could not be detected. Instead, seven novel stilbene derivatives were isolated and characterized for the first time in wine. Apart from the clarification of the absolute configuration of compounds **5a,b** and **6a,b**, a complete set of NMR spectral data is presented for the new wine constituents which will enable their identification in additional natural sources.

LITERATURE CITED

- Baderschneider, B.; Luthria, D.; Waterhouse A. L.; Winterhalter, P. Antioxidants in white wine (cv. Riesling): I. Comparison of different testing methods for antioxidant activity. *Vitis* **1999**, *38*, 127–131.
- Castellari, M.; Spinabelli, U.; Riponi, C.; Amati, A. Influence of some technological practices on the quantity of resveratrol in wine. *Z. Lebensm. Unters. Forsch. A* **1998**, *206*, 151–155.
- Dietrich, H.; Pour Nikfardjam, M.; Patz, C.-D. Importance and occurrence of resveratrol in red and white wines. In *Deutsches Weinbau-Jahrbuch*; Waldkircher Verlag: Waldkirch, Germany, 1999; pp 221–230.
- Frankel, E. N.; Waterhouse, A. L.; Kinsella, J. E. Inhibition of human LDL oxidation by resveratrol. *Lancet* **1993**, *341*, 1103–1104.
- Gehm, B. D.; McAndrews, J. M.; Chien, P.-Y.; Jameson, J. L. Resveratrol, a polyphenolic compound found in grapes and wine is an agonist for the estrogen receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14138–14143.
- Goldberg, D. M.; Karumanchiri, A.; Ng, E.; Yan, J.; Diamandis, E. P.; Soleas, G. J. Direct gas chromatographic-mass spectrometric method to assay *cis*-resveratrol in wines: preliminary survey of its concentration in commercial wines. *J. Agric. Food Chem.* **1995**, *43*, 1245–1250.
- Goldberg, D. M.; Tsang, E.; Karumanchiri, A.; Diamandis, E. P.; Soleas, G.; Ng, E. Method to assay the concentration of phenolic constituents of biological interest in wines. *Anal. Chem.* **1996a**, *68*, 1688–1694.
- Goldberg, D. M.; Ng, E.; Karumanchiri, A.; Diamandis, E. P.; Soleas, G. J. Resveratrol glucosides are important components of commercial wines. *Am. J. Enol. Vitic.* **1996b**, *47*, 415–420.
- Günata, Y. Z.; Bayonove, C. L.; Baumes, R. L.; Cordonnier, R. E. The aroma of grapes. I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *J. Chromatogr.* **1985**, *331*, 83–90.
- Jeandet, P.; Bessis, R.; Sbaghi, M.; Meunier, P.; Trollat, P. Production of phytoalexin resveratrol by grapes as a response to *Botrytis* attack under natural conditions. *J. Phytopathol.* **1995a**, *143*, 135–139.
- Jeandet, P.; Bessis, R.; Sbaghi, M.; Meunier, P.; Trollat, P. Resveratrol content of wines of different ages: Relationship with fungal disease pressure in the vineyard. *Am. J. Enol. Vitic.* **1995b**, *46*, 1–4.
- Jeandet, P.; Breuil, A. C.; Adrian, M.; Weston, L. A.; Debord, S.; Meunier, P.; Maume, G.; Bessis, R. HPLC analysis of grapevine phytoalexins coupling photodiode array detection and fluorimetry. *Anal. Chem.* **1997**, *69*, 5172–5177.
- Khan, M. A.; Nabi, S. G.; Prakash, S.; Zaman, A. Pallidol, a resveratrol dimer from *Cissus pallida*. *Phytochemistry* **1986**, *25*, 1945–1948.
- Kimura, Y.; Okuda, H.; Arichi, S. Effects of stilbenes on arachidonate metabolism in leucocytes. *Biochim. Biophys. Acta* **1985**, *834*, 275–278.
- Lamikanra, O.; Grimm, C. C.; Rodin, J. B.; Inyang, D. Hydroxylated stilbenes in selected American wines. *J. Agric. Food Chem.* **1996**, *44*, 1111–1115.
- Lamuela-Raventós, R. M.; Romero-Pérez, A. I.; Waterhouse, A. L.; de la Torre-Boronat, M. C. Direct HPLC analysis of *cis*- and *trans*-resveratrol and piceid isomers in Spanish red *Vitis vinifera* wines. *J. Agric. Food Chem.* **1995**, *43*, 281–283.
- Langcake, P.; Pryce, R. J. A new class of phytoalexins from grapevines. *Experientia* **1977**, *33*, 151–152.
- Mattivi, F.; Reniero, F.; Korhammer, S. Characterization and evolution in red wine vinification of resveratrol monomers. *J. Agric. Food Chem.* **1995**, *43*, 1820–1823.
- Miller, N. J.; Rice-Evans, C. A.; Davies, M. J.; Gopiathan, V.; Milner, A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin. Sci.* **1993**, *84*, 407–412.
- Orsini, F.; Pelizzoni, F.; Verotta, L.; Aburjai, T. Isolation, synthesis, and antiplatelet aggregation activity of resveratrol-3-*O*- β -D-glucopyranoside and related compounds. *J. Nat. Prod.* **1997**, *60*, 1082–1087.
- Pace-Asciak, C. R.; Hahn, S.; Diamandis, E. P.; Soleas, G.; Goldberg, D. M. The red wine phenolics *trans*-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: Implication for protection against coronary heart disease. *Clin. Chim. Acta* **1995**, *235*, 207–219.
- Pezet, R.; Cuenat, Ph. Resveratrol in Wine: Extraction from skin during fermentation and post-fermentation standing of must from Gamay grapes. *Am. J. Enol. Vitic.* **1996**, *47*, 287–298.
- Ribeiro de Lima, M. T.; Waffo-Tégou, P.; Teissedre, P. L.; Pujolas, A.; Vercauteren, J.; Cabanis, J. C.; Mérillon, J. M. Determination of stilbenes (*trans*-astringin, *cis*- and *trans*-

- piceid, and *cis*- and *trans*-resveratrol) in Portuguese wines. *J. Agric. Food Chem.* **1999**, *47*, 2666–2670.
- Romero-Pérez, A. J.; Ibern-Gómez, M.; Lamuela-Raventós, R. M.; de la Torre-Boronat, M. C. Piceid, the major resveratrol derivative in grape juices. *J. Agric. Food Chem.* **1999**, *47*, 1533–1536.
- Sato, M.; Suzuki, Y.; Okuda, T.; Yokotsuka, K. Content of resveratrol, piceid, and their isomers in commercially available wines made from grapes cultivated in Japan. *Biosci. Biotech. Biochem.* **1997**, *61*, 1800–1805.
- Waffo-Tégue, P.; Decendit, A.; Vercautern, J.; Deffieux, G.; Mérillon, J.-M. *Trans*-resveratrol 3-O- β -glucoside (piceid) in cell suspension cultures of *Vitis vinifera*. *Phytochemistry* **1996**, *42*, 1591–1593.

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