Resveratrol and Two Monomethylated Stilbenes from Israeli *Rumex bucephalophorus* and Their Antioxidant Potential

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Received February 24, 2003

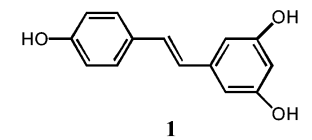
The roots of *Rumex bucephalophorus* were analyzed for resveratrol and analogues. Two stilbene-*O*-methyl derivatives were identified, in addition to resveratrol (3,5,4′-trihydroxystilbene). The stilbene-*O*-methyl derivatives were shown to be 5,4′-dihydroxy-3-methoxystilbene and 3,5-dihydroxy-4′-methoxystilbene. The antioxidant capacities of all these stilbenes were determined.

Members of the Polygonaceae are known to produce a large number of biologically active secondary metabolites, such as flavonoid glycosides,[1] anthraquinones,[2,3] tannins,[3] and other polyphenols.[4] Hydroxylated stilbenes form one of the most interesting and therapeutically important groups of plant-derived polyphenols. Among them, *trans*-resveratrol (*trans*-3,5,4′-trihydroxystilbene) and its common glycoside, piceid, are the most studied. Resveratrol has been reported to provide protection against cardiovascular diseases by having lipid-lowering activity and by inhibiting lipid peroxidation in humans.[5,6] It was found to be a potent inhibitor of tyrosine kinase (p56lck)[7] and has been widely reported to possess antifungal properties.[8] Resveratrol and piceid exert anticarcinogenic effects,[9,10] and piceid has been shown to improve blood microcirculation.[11] These effects may not only be the outcome of stilbenes' antioxidant capacity but also be due to their structural resemblance to tyrosine.[12,13]

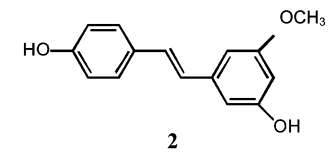
The roots and aerial parts of members of the Polygonaceae, including those of *Rumex*, have been and are still being used in ancient and current traditional herbal medicine around the world for a variety of therapeutic purposes. *Rumex* species, including *R. pictus* Forssk., *R. cyprius* Murb., *R. pulcher* L., *R. occultans sam.*, and *R. bucephalophorus* L. are native in Israel, a country with a long-documented history of traditional medicine.[14,15] Many beneficial characteristics are attributed to the use of *Rumex* roots and leaves. The roots, which have been used in medicine from ancient times, have a gentle laxative effect.[16] They are also useful against bleeding, fluxes, and some skin lesions, including bruises, burns, and swellings.[17] They are often applied as a rustic remedy for burns and scalds and as a dressing for blisters.[18] The leaves have an acid flavor and are a flavorful addition to salads.

The present work reports the isolation and identification of *trans*-resveratrol and two monomethylated stilbene derivatives from the roots of *R. bucephalophorus*. To the best of our knowledge, there has been no previous phytochemical study describing the secondary metabolites of this abundant herb genus.

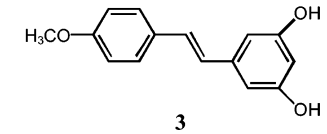
Preliminary analysis using PDA-RP-HPLC of the ethyl acetate extracts of *R. bucephalophorus* indicated that the root extract contains a few substances with absorbance spectra characteristic of *trans*-stilbenes. The three major compounds were collected for further spectral analyses and identification. Quantitation of the amounts of resveratrol and the other *trans*-stilbenes assumed that all compounds have the same response factor as *trans*-resveratrol. The level of resveratrol (compound **1**) was determined to be 165 ± 10 μg/g dry wt, and the levels of compounds **2** and **3** were 204 ± 10 and 239 ± 10 μg/g dry wt.



1
resveratrol



2
5,4'-dihydroxy-
3-methoxystilbene



3
3,5-dihydroxy-
4'-methoxystilbene

Compounds **1**, **2**, and **3** were isolated by RP-HPLC and identified by the means of UV (280 and 306 nm), GC−MS for TMS derivatives, IR, and comparison with synthetic authentic standards. Compound **3** and other stilbene-*O*-methyl derivatives were isolated previously from other plants.[19,20]

The antioxidant properties of these compounds were assessed using scavenging of the radical cation of ABTS relative to the water-soluble vitamin E analogue Trolox C (expressed as Trolox C equivalent antioxidant capacity, TEAC). TEAC values for resveratrol and the two stilbene-*O*-methyl derivatives are presented in Figure 1. Resveratrol and compound **2** showed higher antioxidant capacities than compound **3**. This is in agreement with other works that

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10.1021/np030087c CCC: $25.00 © 2003 American Chemical Society and American Society of Pharmacognosy
Published on Web 09/09/2003

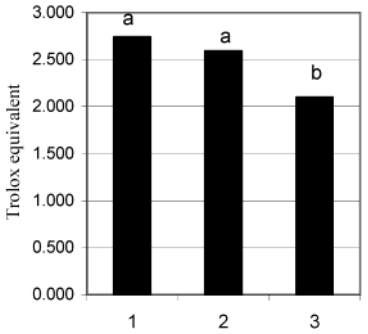

Figure 1. Trolox equivalent antioxidant capacity (TEAC) of stilbenes from the roots of *R. bucephalophorus*. Statistical comparison for all pairs was made using Tukey-Kramer HSD. Means designated by the same letter are not significantly different at $P = 0.05$.

have found that the 4′-hydroxy group of resveratrol is usually the more reactive in scavenging free radicals.[21] However, a hydroxyl group in the 4′ position is not the sole determinant of antioxidant activity, as shown by the antioxidant capacity of compound **3**.

Wide ranges of biological activities that are exhibited by plant stilbenes are thought to be due to their powerful antioxidant properties.[9,22,23] Indeed, we found that the TEAC of each molecule of resveratrol equals that of 2.74 molecules of Trolox. Even the weakest derivative studied here is more potent than Trolox. The production of these weaker antioxidants suggests that these molecules may have other roles in plants. Inhibition of pathogen enzymes may be one function, as methylated stilbenes have been shown to inhibit fungal tyrosinases.[12,13] We are currently studying the affinity of the methylated resveratrol derivatives toward tyrosine-dependent enzymes.

Experimental Section

General Experimental Procedures. HPLC-DAD was performed with a reversed-phase (RP) C18 column (25 × 4.6 mm, Goldsil, Teknokroma, Barcelona, Spain) using a linear gradient consisting of water and methanol, both acidified with 0.01% (v/v) formic acid, at a flow rate of 1 mL/min. The liner gradient after 2 min was 40% methanol and reached 55% methanol in 8 min. Stilbenoids were monitored at 306 nm. GC−MS analyses were carried out with an ion-trap system and an HT8 capillary column (25 m long, 0.22 i.d., 0.25 μm dry film) with a flow rate of 1.5 mL/min. The following temperature gradient was used: The column temperature was set to 80 °C for 1 min, raised to 250 °C at a rate of 20 °C/min, held for 1 min, raised to 290 °C at a rate of 6 °C/min, held for 2 min, and then raised to 300 °C and held for 10 min. Each analyzed sample (dried) was treated with 100 μL of BSTFA and heated to 70 °C for 15 min. The reagent was evaporated under nitrogen and the samples were dissolved in ethyl acetate.

Materials. Resveratrol, methyliodide, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, and potassium bromide were purchased from Sigma (St. Louis, MO). Sodium phosphate and sodium phosphate 7-hydrate were purchased from J. T. Baker (Phillipsburg, NJ). Anhydrous potassium carbonate and sodium chloride were purchased from BDH (Poole, UK). *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Pierce (Rockford, IL).

Synthesis. Compounds **2** and **3** were synthesized in our lab following the method of Cardona et al.[24] The synthetic products were spiked with the *Rumex* extract and chromatographed to verify their identification. The spiked samples were re-collected and analyzed by ^{1}H NMR.

TEAC. The total antioxidant capacity of resveratrol and its *O*-methyl derivatives was measured by the ABTS$^{\bullet+}$ radical cation decolorization assay involving preformed ABTS$^{\bullet+}$ radical cation.[25] Resveratrol and its *O*-methyl derivatives (100 μL) were added to 1 mL working solution of ABTS$^{\bullet+}$ and stirred continuously, and absorbance (at 734 nm) was measured after 10 min. An appropriate solvent blank was run, and absorbance of the samples was subtracted from the blank. All determinations were performed in triplicate.

Plant Material. Whole plants of *R. pictus* Forssk., *R. cyprius* Murb., *R. occultans sam*., and *R. bucephalophorus* L. were collected in April 2002 from the coastal region of Israel, 60 km south west of Jerusalem, and a voucher specimen has been deposited at the first author's research laboratory at the Institute of Biochemistry, Food Science and Nutrition of the Hebrew University of Jerusalem.

Extraction and Isolation. Roots and leaves of all species were separated, freeze-dried, and ground prior to extraction. The powders were extracted three times consecutively with acidified ethyl acetate (0.1% formic acid) at room temperature. The extract was centrifuged at 12000*g* for 15 min, and the supernatant was collected and evaporated under a nitrogen stream. The dry residues were dissolved in 40% MeOH in water and chromatographed by RP-HPLC. Fractions with a characteristic *trans*-resveratrol absorption spectrum were identified only in *R. bucephalophorus*. These fractions were collected, dried, and subjected to GC−MS and IR analyses.

Three fractions containing single pure compounds were obtained.

Compound 1: UV (MeOH) λ_{max} 280, 306; t_R 10 min and *m*/*z* 445.

Compound 2: UV (MeOH) λ_{max} 280, 306; t_R 17 min and *m*/*z* 386.

Compound 3: UV (MeOH) λ_{max} 280, 306; t_R 18 min and *m*/*z* 386.

Acknowledgment. Our thanks go to Dr. Simcha Lev-Yadun for a critical reading of the manuscript and for his comments.

Supporting Information Available: Detailed experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP030087C