trans-Resveratrol-d_4, a Molecular Tracer of the Wild-Type Phytoalexin; Synthesis and Spectroscopic Properties

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Received 28 May 2008; revised 5 June 2008

Abstract: A convenient, six-step synthesis of the so far unknown *trans*-resveratrol-d_4, (*E*)-3′,4,5′-trihydroxy-2,3,5,6-tetradeuterostilbene, starting from commercially available phenol-d_6, with an overall yield of 25%, is described. The final labeled resveratrol was fully characterized by MS, IR, and ^{1}H and ^{13}C NMR spectroscopy. The isotopic distribution of the final product, determined by high resolution mass spectrometry, was as follows: d_4, 96%; d_3, 4%.

Key words: deuterated resveratrol, olefination, resveratrol, stable isotope dilution assay

Resveratrol (3,4′,5-trihydroxystilbene) is a polyphenolic phytoalexin found in more than 70 plants and many foods, including grapes, peanuts, berries, and red wine.[1] Resveratrol has recently attracted considerable attention in view of its significant biological activity, which include antioxidant and/or anti-inflammatory activity.[2] Recent data suggest that nutritional intake of resveratrol may contribute to the so-called 'French paradox', that is, the unexpectedly low incidence of coronary heart disease in the Mediterranean population, in spite of a relatively high intake of saturated fats. Resveratrol has been shown to inhibit LDL oxidation and prevent the oxidative stress in general, which results in an anti-aging effect.[2,3] Moreover, resveratrol has been classified as a phytoestrogen, due to its ability to interact to estrogenic receptors, and has been shown to exert antiproliferative and cancer-protective effects.[4]

Considering the potential beneficial effects on human health of resveratrol, the development of new, reliable, and sensitive techniques for its quantitative determination in foods appears to be of primary importance. One of the most promising methodologies for the highly sensitive quantitative determination of microcomponents in food products is currently represented by stable isotope dilution assay (SIDA).[5] Thus, resveratrol could in principle be detected and quantified in food samples by means of SIDA using, for example, a deuterated derivative such as (*E*)-3′,4,5′-trihydroxy-2,3,5,6-tetradeuterostilbene as internal standard, which, however, to our knowledge, has not been reported in the literature so far. Here we wish to present an easy and convenient synthesis for this derivative, starting from commercially available phenol-d_6, with an overall yield of 25% over six steps.

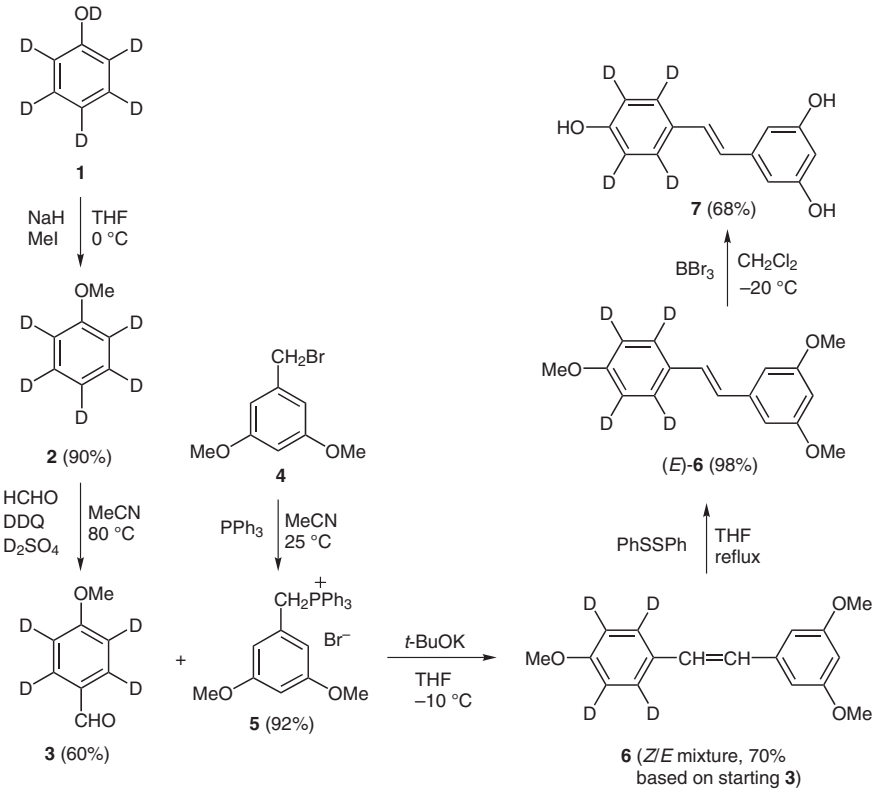
(*E*)-3′,4,5′-Trihydroxy-2,3,5,6-tetradeuterostilbene (*trans*-resveratrol-d_4, **7**) has been prepared according to the convergent synthetic strategy shown in Scheme 1.

Commercially available phenol-d_6 (**1**) was converted into 2,3,4,5,6-pentadeuteroanisole (**2**) in 90% yield by deprotonation with sodium hydride in tetrahydrofuran at 0 °C followed by quenching with iodomethane. Formylation/oxidation of **2** with formaldehyde–sulfuric acid-d_2/2,3-dichloro-5,6-dicyano-1,4-benzoquinone in acetonitrile at 80 °C led to a mixture of 3,4,5,6-tetradeutero-*o*-anisaldehyde (20% yield) and 2,4,6,7-tetradeutero-*p*-anisaldehyde (**3**, 60% yield), which could be easily separated by column chromatography. On the other hand, commercially available 3,5-dimethoxybenzyl bromide (**4**) was easily converted into (3,5-dimethoxybenzyl)triphenylphosphonium bromide (**5**) in 92% yield by the reaction with an excess of triphenylphosphine in acetonitrile at room temperature. The key step of the synthesis then consisted of the olefination reaction between **3** and **5**, to give 3′,4,5′-trimethoxy-2,3,5,6-tetradeuterostilbene (**6**) as a *E*/*Z* mixture in 70% yield based on **3**. Double bond isomerization with a catalytic amount of diphenyl disulfide in refluxing tetrahydrofuran to give the *trans*-isomer (*E*)-**6** followed by deprotection (BBr_3, CH_2Cl_2, –20 °C) eventually led to the desired deuterated resveratrol **7** in 47% yield based on deuterated aldehyde **3**. The overall yield of **7** based on starting **1** was 25% over six steps.

No H/D exchange and/or redistribution was observed in the course of the synthesis, as confirmed by the spectroscopic characterization (MS, ^{1}H NMR, ^{13}C NMR, IR) of the key intermediates **2**, **3**, and (*E*)-**6**. The final deuterated resveratrol **7** was also isotopically stable and fully characterized by MS, IR, and ^{1}H and ^{13}C NMR spectroscopy. The isotopic distribution of **7** was determined by high resolution mass spectrometry, and gave the following result: d_4, 96%; d_3, 4%. The characteristics of the new labeled resveratrol **7** are therefore suitable in view of its utilization as internal standard for the quantitative determination of naturally occurring resveratrol in food samples.

In conclusion, a convenient synthesis of a new labeled resveratrol derivative, (*E*)-3′,4,5′-trihydroxy-2,3,5,6-tetradeuterostilbene (**7**), with an overall yield of 25% over six

SYNTHESIS 2008, No. 18, pp 2953–2956
Advanced online publication: 22.08.2008
DOI: 10.1055/s-2008-1067239; Art ID: Z12408SS



Scheme 1 Synthesis of (*E*)-3′,4,5′-trihydroxy-2,3,5,6-tetradeuterostilbene (*trans*-resveratrol-d_4, **7**)

steps and an isotopic purity of 96%, has been developed. The new labeled resveratrol is isotopically stable and, therefore, suitable for its utilization as internal standard for the quantitative determination of resveratrol in food samples by means of the stable isotope dilution assay technique.

Melting points were taken on a Reichert Thermovar apparatus are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX Avance 300 spectrometer at 25 °C in $CDCl_3$ or DMSO-d_6 solns at 300 MHz and 75 MHz, respectively, with TMS as internal standard. IR spectra were taken with a Perkin-Elmer Paragon 1000 PC FT-IR spectrophotometer. MS spectra were obtained using a Shimadzu QP-2010 GC-MS apparatus at 70 eV ionization voltage. The high resolution electrospray ionization (ESI) experiments were carried out with a hybrid Q-Star *Pulsar-i* (PE SCIEX) mass spectrometer equipped with an ion spray ionization source. All samples were acquired at the optimum ion spray voltage of 4800 V by direct infusion (5 μL/min) of a soln containing the appropriate compound dissolved in MeOH (10 pmol/μL). The N_2 gas flow was set at 20 psi and the declustering and the focusing potentials were kept at 50 and 220 V relative to ground, respectively. Commercially available Renin substrate was used as calibration standard compound. The accuracy of the measurement was within 5 ppm.

Phenol-d_6 (**1**) (99% atom D), NaH (anhyd, 95%), MeI, paraformaldehyde, DDQ, D_2SO_4 (96–98 wt% in D_2O, 99.5 atom% D) 3,5-dimethoxybenzyl bromide (**4**), Ph_3P, $(PhS)_2$, and BBr_3 were commercially available (Aldrich) and were used as received.

All reactions were analyzed by TLC on silica gel 60 F_{254} and by GLC using a Shimadzu GC-2010 gas chromatograph and capillary columns with polymethylsilicone + 5% phenylsilicone as the stationary phase. Column chromatography was performed on silica gel 60 (Merck, 70–230 mesh). Evaporation refers to the removal of solvent under reduced pressure.

2,3,4,5,6-Pentadeuteroanisole (2)

The method of Kendall[6] was employed. A soln of phenol-d_6 (**1**, 1.0 g, 9.99 mmol) in anhyd THF (4 mL) was added dropwise over 1 h under N_2 to a cooled suspension (0 °C) of NaH (350.0 mg, 13.85 mmol) in anhyd THF (3 mL). The mixture was stirred at 0 °C for an additional 10 min, MeI (4.6 g, 32.4 mmol) was added rapidly. The mixture was allowed to warm up to r.t. and then refluxed for 19 h. After cooling to r.t., the mixture was quenched with H_2O (80 mL) and extracted with hexane (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), the solvent was removed by distillation at atmospheric pressure, and the residue subjected to short path distillation (bp 153–155 °C) to give **2** (1.02 g, 90%) as a colorless liquid. The spectroscopic properties of the product were in agreement with those previously reported.[7]

2,4,6,7-Tetradeutero-*p*-anisaldehyde (3)

The method of Neumann[8] was employed. A mixture of **2** (1.0 g, 8.84 mmol), paraformaldehyde (2.4 g, 79.92 mmol), D_2SO_4 (0.25 mg, ca. 2.5 mmol), and DDQ (3.61 g, 15.90 mmol) in MeCN (10 mL) was stirred under N_2 at 80 °C for 5 h. After cooling, EtOAc was added, the mixture was filtered, and the solid washed with EtOAc. The solvent used for washing the solid was added to the filtrate. The collected EtOAc phases were evaporated and the residue was purified by column chromatography (silica gel, hexane–EtOAc, 97:3) to give pure 3,4,5,6-tetradeutero-*o*-anisaldehyde (colorless oil, 245.2 mg, 20%) and 2,4,6,7-tetradeutero-*p*-anisaldehyde (**3**) (colorless oil, 746.1 mg, 60%) in this order.

IR (film): 1689, 1580, 1571, 1236 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.88 (s, 3 H, OCH_3), 9.88 (s, 1 H, CHO).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 55.6 (OCH_3), 114.0 (t, *J* = 23.8 Hz, C3, C5), 129.9 (C1), 131.7 (t, *J* = 23.8 Hz, C2, C6), 164.6 (C4), 190.9 (CHO).

GC-MS (EI, 70 eV): *m/z* (%) = 140 (73, [M^+]), 139 (100), 111 (24), 96 (24), 81 (45), 80 (18), 69 (19), 68 (20), 66 (17).

(3,5-Dimethoxybenzyl)triphenylphosphonium Bromide (4)

3,5-Dimethoxybenzyl bromide (2.0 g, 8.65 mmol) was added to a stirred soln of Ph_3P (3.0 g, 11.44 mmol) in MeCN (30 mL). The resulting mixture was stirred at r.t. for 72 h. After removal of the solvent by evaporation, the residue was solubilized in CH_2Cl_2 (30 mL). Et_2O (10 mL) was slowly added without stirring, leading to the precipitation of pure **4** (3.92 g, 92%) as colorless crystals; mp 273–274 °C. The ^{1}H NMR data of the product were in good agreement with those previously reported.[9]

IR (KBr): 1607, 1582, 1438, 1155 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.52 (s, 6 H, 2 OMe), 5.25 (d, *J* = 15.4 Hz, 2 H, CH_2P), 6.22 (t, *J* = 2.2 Hz, 2 H, H2, H6), 6.44 (q, *J* = 2.2 Hz, 1 H, H4), 7.71–7.82 (m, 12 H_{Ph}), 7.88–7.98 (m, 3 H_{Ph}).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 28.3 (d, *J* = 46.3 Hz, CH_2P), 55.0 (2 OCH_3), 100.1 (d, *J* = 3.8 Hz, $C4_{3,5\text{-MeO2C6H3}}$), 108.9 (d, *J* = 6.3 Hz, $C2_{3,5\text{-MeO2C6H3}}$, $C6_{3,5\text{-MeO2C6H3}}$), 117.8 (d, *J* = 86.3 Hz, $C1_{Ph}$), 129.9 (d, *J* = 8.8 Hz, $C1_{3,5\text{-MeO2C6H3}}$), 130.0 (d, *J* = 12.5 Hz, $C2_{Ph}$, $C6_{Ph}$), 134.0 (d, *J* = 10.0, $C3_{Ph}$, $C5_{Ph}$), 135.0 (d, *J* = 3.8 Hz, $C4_{Ph}$), 160.2 (d, *J* = 2.5 Hz, $C3_{3,5\text{-MeO2C6H3}}$, $C5_{3,5\text{-MeO2C6H3}}$).

(*E*)-3′,4,5′-Trimethoxy-2,3,5,6-tetradeuterostilbene [(*E*)-6]

The method of Tsuda[10] was employed. To a cooled (–10 °C), stirred mixture of (3,5-dimethoxybenzyl)triphenylphosphonium bromide (1.68 g, 3.41 mmol) and *t*-BuOK (381.5 mg, 3.40 mmol) in anhyd THF (50 mL) under N_2 was added **3** (400 mg, 2.85 mmol) dropwise. After additional stirring at –10 °C under N_2 for 1 h, the mixture was allowed to warm up to r.t. and then it was poured into H_2O (ca. 100 mL) and neutralized with 1 M HCl. The resulting mixture was extracted with Et_2O (3 × 30 mL) and the combined organic phases were dried (Na_2SO_4). After filtration, the solvent was evaporated and the residue purified by column chromatography (silica gel, hexane–EtOAc, 8:2), to give **6** (544 mg, 70%) as a *E*/*Z* mixture. A mixture of the latter (400 mg, 1.46 mmol) and $(PhS)_2$ (60 mg, 0.27 mmol) in anhyd THF (50 mL) was refluxed under N_2 for 2 h. After cooling, the solvent was evaporated, and the residue was purified by column chromatography (silica gel, hexane–EtOAc, 8:2), to give pure (*E*)-**6** (390 mg, 98%) as a colorless solid; mp 54–55 °C.

IR (KBr): 1591, 1456, 1204, 1151 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.83 (s, 9 H, 3 OMe), 6.36–6.42 (m, 1 H, H4′), 6.64–6.68 (m, 2 H, H2′, H6′), 6.89 (distorted d, *J* = 16.5 Hz, 1 H, HC=C*H*), 7.04 (distorted d, *J* = 16.5 Hz, 1 H, *H*C=CH).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 55.4 (2 OCH_3), 100.0 (C4′), 104.8 (C2′, C6′), 114.0 (t, *J* = 23.4 Hz, C3, C5), 126.9 (HC=*C*H), 127.5 (t, *J* = 23.4 Hz, C2, C6), 128.9 (H*C*=CH), 129.5 (C1), 140.0 (C1′), 159.6 (C4), 161.3 (C3′, C5′).

GC-MS (EI, 70 eV): *m/z* (%) = 274 (100, [M^+]), 273 (8), 259 (4), 243 (8), 228 (6), 216 (5), 200 (6), 185 (4), 173 (5), 157 (7), 156 (6), 155 (5), 145 (6), 137 (6), 119 (4).

(*E*)-3′,4,5′-Trihydroxy-2,3,5,6-tetradeuterostilbene (*trans*-Resveratrol-d_4, 7)

To a cooled (–20 °C), stirred soln of (*E*)-**6** (180.0 mg, 0.66 mmol) in anhyd CH_2Cl_2 (20 mL) under N_2 was added dropwise BBr_3 (1.30 g, 5.19 mmol). The mixture was allowed to warm up to r.t., then it was poured into ice-water, and extracted with EtOAc. The organic layer was washed with brine and dried (Na_2SO_4). After filtration, the residue was purified by column chromatography (silica gel, hexane–EtOAc, 6:4), to give pure **7** (105 mg, 68%) as a colorless solid; mp 254–256 °C.

IR (KBr): 3294, 1590, 1407, 1345, 1155 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 6.12 (s, 1 H, H4′), 6.38 (s, 2 H, H2′, H6′), 6.81 (distorted d, *J* = 16.5 Hz, 1 H, HC=C*H*), 6.93 (distorted d, *J* = 16.5 Hz, 1 H, *H*C=CH), 9.18 (s, 2 H, C3′-OH, C5′-OH), 9.53 (s, 1 H, C4-OH).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 101.7 (C4′), 104.2 (C2′, C6′), 115.0 (t, *J* = 19.0 Hz, C3, C5), 125.5 (HC=*C*H), 127.3 (t, *J* = 22.2 Hz, C2, C6), 127.7 (H*C*=CH), 127.8 (C1), 139.2 (C1′), 157.0 (C4), 158.4 (C3′, C5′).

HRMS (ES+): *m/z* [(M-d_4 + H)$^+$] calcd for $C_{14}H_9D_4O_3$: 233.1112; found: 233.1105 (96%); *m/z* [(M-d_3 + H)$^+$] calcd for $C_{14}H_{10}D_3O_3$: 232.1050; found: 232.1043 (4%).

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