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Soluble polyphenols: Synthesis and bioavailability of 3,4′,5-tri(α-D-glucose-3-*O*-succinyl) resveratrol

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Dedicated to Professor Gianfranco Scorrano on the occasion of his 70th birthday

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ABSTRACT

We report the development of a chemical modification method of general applicability to polyphenols, which increases solubility to influence absorption. Glucosyl groups were added to the resveratrol kernel via a succinate linker, yielding 3,4′,5-tri-(α-D-glucose-3-*O*-succinyl) resveratrol. The construct was only slowly hydrolyzed in acid and at pH 6.8, but it was destroyed by blood esterases in less than 1 h. In rats its administration resulted in a blood concentration versus time curve shifted to longer times in comparison to resveratrol, a useful modulation of pharmacokinetics. The area-under-curve parameter and the metabolite mix were similar to those of resveratrol. The method may be advantageously employed to solubilize other polyphenols and to make them more palatable.

Optimization of bioavailability is fundamental for the full realization of the biomedical potential of nutraceuticals such as plant polyphenols. The research efforts of dozens of groups worldwide have proven that many polyphenols possess potentially very useful biochemical properties,[1] offering promise for the fight against aging, cancer, cardiovascular diseases and chronic inflammation. The promise however is only partially fulfilled: polyphenols are poorly absorbed and are present in the circulatory streams at very low levels, mostly as conjugates produced by phase II metabolism in the intestinal enterocytes and in the liver. Solubility is recognized as a key factor for bioaccessibility and thus bioavailability.[2] The problems and approaches to the improvement of the bioavailability of antioxidants and polyphenols via formulations and modifications have been reviewed.[3]

The generally low solubility of polyphenol aglycones can be attributed largely to a tendency to form aggregates via hydrophobic interaction of the aromatic systems and hydrogen-bond formation by the hydroxyls. In nature polyphenols occur mainly as glycosylated derivatives. The current paradigm describing the absorption of these compounds in the intestine is that they are first deglycosylated at the intestinal wall, and then enter the cell by diffusion or via transport by carriers.[4] The presence of glycosyl groups may nonetheless improve bioavailability by influencing phenomena taking place upstream of entry into enterocytes. A well-studied case is that of quercetin and its 3-O-glycosides.[5] Kinetics and extent of absorption by rats depend strongly not only on the presence, but also on the identity of the sugar group, with uptake being most efficient for the most soluble derivatives. Following nature's lead, as a proof of principle we linked a prototypical glycosyl group, glucose, to a model polyphenol, resveratrol (**1**), via succinic acid.

Synthetic procedures[6] were optimized using 4,4′-dihydroxybiphenyl as a model compound. The final optimized protocol was then successfully applied to resveratrol (Scheme 1). In the first step of the synthesis of a succinyl linker is attached to the 3-hydroxyl group of a glucose molecule used in a protected form (diacetone-D-glucose, DAG) to avoid reactions of the other hydroxyls, yielding diacetone-α-D-glucose-3-*O*-succinyl ester (**2**). Per-esterification of 4,4′-dihydroxybiphenyl or **1** to give 4,4′-di(diacetone-α-D-glucose-3-*O*-succinyl)-biphenyl or 3,4′,5-tri(diacetone-α-D-glucose-3-*O*-succinyl)-resveratrol (**3**), respectively, was then performed by activating the free carboxylic group of **2** with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), using

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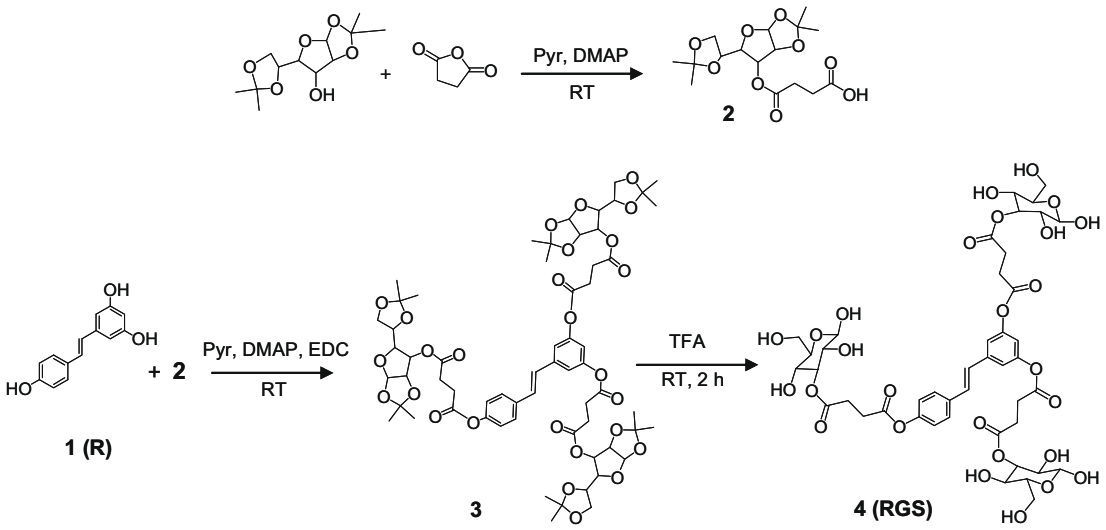

Scheme 1. Synthesis of 3,4′,5-tri(α-D-glucose-3-*O*-succinyl)-resveratrol (RGS, **4**).

4-*N*,*N*-dimethylamino pyridine (DMAP) as catalyst. The final step consisted in the deprotection of the glucose hydroxyl groups with trifluoroacetic acid (TFA), affording 4,4′-di(α-D-glucose-3-*O*-succinyl)-biphenyl or 3,4′,5-tri(α-D-glucose-3-*O*-succinyl)-resveratrol (**4**; RGS), respectively. The solubility of **4** in water at room temperature is at least 90 mg/mL (89 mM). The major resveratrol phase II metabolites were also synthesised according to published procedures[7] as described in the Supplementary data (materials and methods). They were used as standards to identify metabolites.

RGS (**4**) underwent slow hydrolysis in 1 N HCl (Fig. 1). After 6 h at 37 °C, 80% of the starting material was still present as such, with the main product of hydrolysis being the di-substituted derivatives. At pH 6.8 in phosphate-buffered saline (PBS) disappearance was faster. The process consisted in the loss of one entire glucose-*O*-succinyl moiety at a time. Loss of the first one was nearly complete after 6 h, as showed by LC–MS analysis.[8] Formation of resveratrol was observed after 28 h. At no time were detectable amounts of products arising from the loss of glucose molecules formed.

In pharmacokinetics experiments (see Supplementary data for the experimental procedures) individual variability is generally high. We therefore performed our experiments utilizing the same set of rats for the administration of both resveratrol and the new derivative **4**. The identity of the circulating species was confirmed by LC–MS analysis using standards and by enzymatic treatment (Fig 2). In the case of resveratrol, blood analyses revealed relatively high amounts of resveratrol sulfate- and glucuronide-conjugates. In agreement with the literature reports,[9] C_{max} for the sum of all resveratrol-derived species (9.4 ± 8.5 μM) was reached at about 60 min after administration (Fig. 3A). The concentration versus time curves of all metabolites followed the same pattern. Intact resveratrol appeared only at low levels (C_{max}: 1.2 ± 1.2 μM), and the corresponding peak earlier, at about 10 min. Administration of **4** resulted in a delayed absorption, with the maximum concentration of total species in blood reached after approximately 4 h (Fig. 3B). The overall C_{max} was 5.4 ± 4.0 μM. The species present in the bloodstream were also in this case resveratrol (at low levels) and mostly its metabolites, in proportions similar to those observed after administration of resveratrol itself (Fig. 4). When values of the area under concentration–time curves (AUC) are compared, no significant differences can be noted depending on whether resveratrol or **4** was administered. Similar results were obtained administering **4** as a water, rather than a DMSO, solution (not shown; $N = 3$).

Compound **4** is water-soluble and its stability versus chemical hydrolysis is satisfactory. In particular, it is nearly stable in an acidic environment mimicking that of the stomach, so that it can be supposed to survive the gastric stage with only very limited destruction. Interestingly, hydrolysis of the succinate–resveratrol bond is much faster than that of the succinate–glucose bond, so that **4** is destroyed via successive losses of whole glucosylsuccinyl groups. Administration of this solubilized form of resveratrol re-

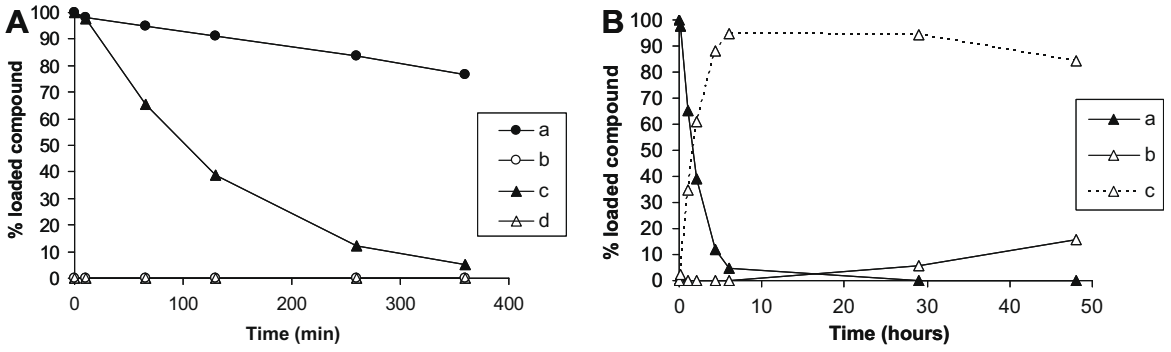

Figure 1. Stability of **4** in media mimicking gastric and intestinal environment (37 °C). (A) Over 6 h: (a) **4** and (b) **1** in 1 N HCl; (c) **4** and (d) **1** in PBS, pH 6.8. (B) Over 48 h: (a) **4**, (b) **1**, (c) mono- and di-(α-D-glucose-3-*O*-succinyl)-resveratrol in PBS, pH 6.8. Data are expressed as % of the initial molar amount used.

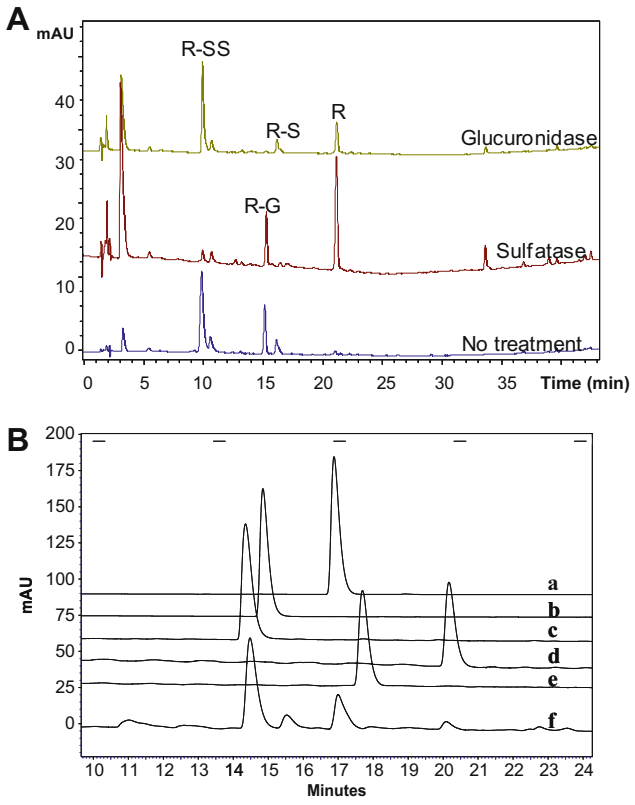

Figure 2. Identification of resveratrol metabolites. (A) HPLC–UV chromatograms (320 nm) of a blood sample withdrawn 4 h after intragastric administration of **4**. The chromatograms were obtained from aliquots of the same sample: without any enzymatic treatment, and after treatment with sulfatase or with glucuronidase, as indicated. R-S: resveratrol sulfate; R-G: resveratrol glucuronide; R: resveratrol. (B) Comparison between synthetic resveratrol conjugates and in vivo metabolites. HPLC chromatograms recorded at 320 nm of: (a) R-3-glucuronide; (b) R-4′-glucuronide; (c) R-3,4′-disulfate; (d) R-3-sulfate; (e) R-4′-sulfate; (f) rat blood sample collected 30 min after administration of 0.22 mmol/kg resveratrol.

sults in delayed absorption in comparison to the aglycone, without a significant difference in total absorption. Thus, administration of a proper mix of aglycone and derivative is expected to produce a prompt as well as long-lasting increase in circulating and body levels of the polyphenol and of its metabolites. The delay may be tentatively attributed to the time needed for complete hydrolysis of **4** to resveratrol in the intestinal tract, with the subsequent absorption of the resveratrol thus formed.

While polyphenols generally occur in nature as glycosylated derivatives, in many cases these products are not commercially available. Furthermore, monoglycosylation in some cases does not make the molecule soluble. For example rutin (quercetin-3-*O*-rutinoside, an abundant glycoside of quercetin) only dissolves at about 1 g in 8 L of water,[10] that is, about 2×10^{-4} M (2.3 g/L at pH 9 and decreasing with pH[11]). Quercitrin and genistin, common glucosides of quercetin and genistein, respectively, have very low solubility in cold water.[10] The procedures described above provide a straightforward approach to soluble polyphenol prodrugs. Linkage to more than one sugar residue is expected to lead to products with higher water solubility. Bioavailability may be improved by optimizing the choice of the glycosyl component or by linkage to a polymeric soluble molecule. New synthetic polyphenols are beginning to be explored as drugs and they are certain to be affected by the same (or worse) bioavailability problems as the natural compounds. Modulating solubility may have an impact and it would facilitate administration.

Modification of OHs may have to do not only with bioavailability, but also with complex formation with salivary proteins in the mouth. Sensations of bitterness and astringency are often associated with these compounds and in particular with their polymers,[12] but they would not be expected to be induced by derivatives with 'capped' hydroxyls. The other components forming RGS, succinic acid and glucose, are molecules already abundant and ubiquitous in the body, and thus certainly safe or beneficial as nutrients. Therefore, this type of molecules may find relevant technological applications in the food industry, such as, in the formulation of fortifying ingredients and supplements.

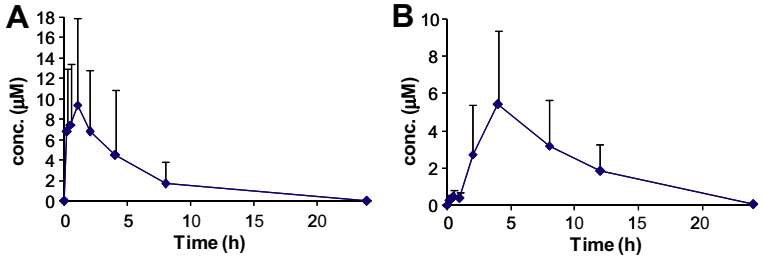

Figure 3. Pharmacokinetics of (A) **1** and (B) **4**. Mean values of total circulating species derived from the respective parent compounds ($N = 3$).

A

	AUC (μM * h)	
Circulating specie	Resveratrol	RGS
R	1,1 ± 1,1	0,2 ± 0,2
R-S	2,4 ± 1,7	3,5 ± 2,6
R-G	35,5 ± 36,9	21,2 ± 15,8
R-SS	14,4 ± 20,0	27,0 ± 18,7
Total	53,4 ± 57,7	51,8 ± 37,2

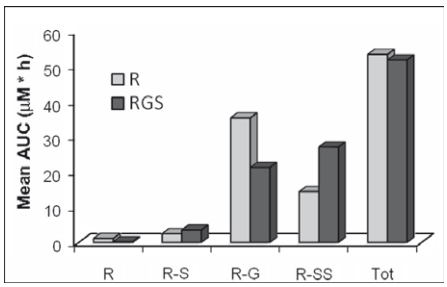

Figure 4. Mean AUC values for resveratrol and its main metabolites. The determinations were performed using three rats, each of which received both resveratrol and **4** with a 2-week interval. R: resveratrol; R-S: resveratrol sulfate; R-G: resveratrol glucuronide; R-SS: resveratrol disulfate. (A) Error notations are standard deviations. (B) Column plots of the data tabulated in A (error notations omitted).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.114.

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6. Synthesis and characterization of compounds.
Diacetone-α-D-glucose-3-O-succinyl ester (**2**). Synthesis was performed modifying a published procedure.[13] Briefly, DMAP (120 mg, 0.98 mmol, 0.26 equiv) and succinic anhydride (1.6 g, 16 mmol, 4.2 equiv) were added to a solution of DAG (1 g, 3.8 mmol) in dry pyridine (15 mL). After stirring for 20 h at room temperature, the mixture was diluted in $CHCl_3$ (30 mL) and washed with 0.5 N HCl (6 × 50 mL). The organic layer was dried over $MgSO_4$ and filtered. The solvent was finally evaporated under reduced pressure to afford 1.08 g of the desired product (78%). ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 1.47,1.36,1.27 (s, 12H, CH_3); 2.64 (m, 4H, CH_2); 3.99 (m, 2H, CH-4, CH-5); 4.17 (m, 2H, CH-6); 4.45 (m, 1H, CH-2); 5.22 (d, 1H, CH-3); 5.82 (d, 1H, CH-1);10.7 (br, 1H, OH).
4,4′-Di(diacetone-α-D-glucose-3-O-succinyl)-biphenyl. DMAP (120 mg, 0.98 mmol, 1.3 equiv), EDC (0.58 g, 3 mmol, 4 equiv) and 4,4′-diidroxybiphenyl (**2**; 140 mg, 0.75 mmol) were added to a solution of **3** (1.08 g, 3 mmol, 4 equiv) in dry pyridine (15 mL). After stirring for 24 h at room temperature, the mixture was diluted in $CHCl_3$ (30 mL) and washed with 0.5 N HCl (6 × 50 mL) and then with 5% $NaHCO_3$ (3 × 50 mL). The organic layer was dried over $MgSO_4$ and filtered. The solvent was finally evaporated under reduced pressure, to afford 560 mg of the desired product (86%). ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 1.44, 1.33, 1.23 (s, 24H, CH_3); 2.72–2.84 (m, 8H, CH_2); 3.98 (m, 4H, CH-4, CH-5); 4.16 (m, 4H, CH-6); 4.45 (m, 2H, CH-2); 5.22 (m, 2H, CH-3); 5.79 (d, 2H, CH-1); 7.09 (m, 4H, H-3, H-5, H-3′, H-5′, J_{3-2} = 8.1 Hz); 7.47 (m, 4H, H-2, H-6, H-2′, H-6′, J_{2-3} = 8.1 Hz). MS-ESI (CH_3CN): *m*/*z* 893, $[M+Na]^+$.
4,4′-Di(α-D-glucose-3-O-succinyl)-biphenyl. 4,4′-Di(diacetone-α-D-glucose-3-*O*-succinyl)-biphenyl (150 mg, 0.17 mmol) was dissolved in 12 M TFA (3 mL). After stirring for 1.5 h at room temperature, the product was precipitated with diethyl ether (10 mL) three times, and the solvent decanted after each precipitation. The white solid was dried under vacuum and then dissolved in 3 mL water to hydrolyze trifluoroacetic esters at glucose hydroxyls. The solution was finally lyophilized to afford 98 mg of the desired product (81%). ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 2.73–2.8 (m, 8H, CH_2); 3.07–5.05 (m, CH_2 D-glucose); 7.21 (d, 4H, H-3, H-5, H-3′, H-5′, J_{3-2} = 8.1 Hz); 7.69 (d, 4H, H-2, H-6, H-2′, H-6′, J_{2-3} = 8.1 Hz). MS-ESI (CH_3CN): *m*/*z* 733, $[M+Na]^+$.
3,4′,5-Tri(diacetone-α-D-glucose-3-O-succinyl)-resveratrol (**3**). DMAP (120 mg, 0.98 mmol, 1.8 equiv), EDC (0.58 g, 3 mmol, 5.7 equiv) and **1** (120 mg, 0.53 mmol) were added to a solution of **3** (990 mg, 2.75 mmol, 5.2 equiv) in dry pyridine (15 mL). After stirring for 24 h at room temperature, the mixture was diluted in $CHCl_3$ (30 mL) and washed with 0.5 N HCl (6 × 50 mL). The organic layer was dried over $MgSO_4$ and filtered. The solvent was finally evaporated under reduced pressure and the residue was purified by flash chromatography using ethylacetate/hexane 5:3 as eluent to afford 475 mg of the desired product (74%). ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 1.49, 1.39, 1.28 (s, 36H, CH_3); 2.77–2.9 (m, 12H, CH_2); 4.04–4.08 (m, 6H, CH-4,CH-5); 4.21 (m, 6H, CH-6); 4.5 (m, 3H, CH-2); 5.29 (m, 3H, CH-3); 5.85 (d, 3H, CH-1); 6.85 (t, 1H, H-4, *J* = 2 Hz); 6.92–6.99 (d, 1H, H-7, *J* = 16 Hz); 7.07–7.11 (d, 2H, H-5′, H-3′, *J* = 8.6 Hz); 7.10 (d, 2H, H-2, H-6, *J* = 2 Hz);7.49 (s, 2H, H-2′, H-6′, *J* = 8.6 Hz). ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.39,1.27,1.19,1.18 (s, 36H, CH_3); 2.7–2.85 (m, 12H, CH_2); 3.8–3.9 (m, 6H, CH-4, CH-5); 4.15 (m, 6H, CH-6); 4.49 (m, 3H, CH-2); 5.04 (m, 3H, CH-3); 5.86 (d, 3H, CH-1); 6.83 (t, 1H, H-4, *J* = 2 Hz); 7.12 (d, 2H, H-3′, H-5′, *J* = 8.6 Hz); 7.25 (d, 2H, H-2, H-6, *J* = 2 Hz); 7.28 (d, 1H, H-7, *J* = 16 Hz);7.59 (d, 2H, H-2′, H-6′, *J* = 8.7 Hz). MS-ESI (CH_3CN): *m*/*z* 1277, $[M+Na]^+$.
3,4′,5-Tri(α-D-glucose-3-O-succinyl)-resveratrol (**4**). Compound **3** (150 mg, 0.12 mmol) was dissolved in 12 M TFA (3 mL). After stirring for 1.5 h at room temperature, the product was precipitated with diethyl ether (10 mL) three times, and the solvent decanted after each precipitation. The white solid was dried under nitrogen and then dissolved in 3 mL water to hydrolyze trifluoroacetic esters at glucose hydroxyls. The solution was finally lyophilized to afford 121 mg of the desired product (98%). ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 2.74–2.83 (d, 12H, CH_2); 3.07–5.06 (m, CH_2 D-glucose); 6.88 (s, 1H, H-4); 7.16 (d, 2H, H-3′, H-5′, *J* = 7.9 Hz); 7.28 (s, 2H, H-2, H-6); 7.31–7.36 (d, 1H, H-7, *J* = 16 Hz);7.64–7.67 (d, 2H, H-2′, H-6′, *J* = 8.1 Hz). MS-ESI (CH_3CN): *m*/*z* 1037, $[M+Na]^+$.
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