Plant "Polyphenolic" Small Molecules Can Induce a Calorie Restriction-Mimetic Life-Span Extension by Activating Sirtuins: Will "Polyphenols" Someday Be Used as Chemotherapeutic Drugs in Western Medicine?

Stéphane Quideau*[a]

Plant phenols and polyphenols have long been regarded as a pool of natural products with promising biological activities for the development of therapeutic substances. Certain herbs, spices and plant extracts, rich in phenolic compounds, have been used for thousands of years in traditional Eastern medicines. The literature abounds in reports, in particular by Japanese scientists,[1–3] on the identification of polyphenols as active principles of these alternative medications. The regular intake of fruits and vegetables is today highly recommended in the Western diet, mainly because the polyphenols they contain are thought to play important roles in long-term health and reducing the risk of chronic and degenerative diseases.[4, 5] Moreover, this increasing recognition of the benefits brought by plant phenols and polyphenols to human health has sparked a new appraisal of various plant-derived food and beverages, such as olive oil, chocolate, apple and citrus juices, coffee, tea and wine. Their high content in phenolic substances has recently fuelled numerous investigations that, again, unveiled the therapeutic significance of these natural products, and yet the potential of polyphenol-based drugs has so far remained untapped in Western conventional medicinal approaches.

The reasons for this relative disapproval of polyphenols by the pharmaceutical industry are unclear. It is true that plant polyphenols have long been considered as structurally undefined and water-soluble oligomers only capable of complexing alkaloids and proteins (i.e., tanning), and chelating metallic ions in unspecific manners. Hence, standard extraction protocols of plant secondary metabolites usually involve a step to ensure the complete removal of all polyphenolic compounds in order to avoid "false-positive" results in screening against a given biomolecular target.[6]

But what exactly is a polyphenol? The Bate–Smith–Haslam definition has somewhat broadened out over the years.[7, 8] The term "polyphenol" (*syn* vegetable tannins) refers to water-soluble phenolic compounds having molecular masses between 500 and 3000–4000 Da, possessing 12–16 phenolic groups and 5–7 aromatic rings per 1000 relative molecular mass, and expressing special properties such as the ability to precipitate proteins and alkaloids. Today, many simpler plant phenols are referred to as "polyphenols", which now basically means any structure that features at least one di- or trihydroxyphenyl unit. Thus, plant "polyphenols" encompass several classes of structurally and biosynthetically diverse natural products that comprise small- to medium-sized molecules ($\mu M <$ ca. 1500 Da) and oligomeric to polymeric structures. Polyhydroxystilbenes, such as resveratrol (**6**, vide infra), and flavonoids, which include flavones, isoflavones, flavanols, flavanones, chalcones, aurones and anthocyanins, are examples of "polyphenolic" small molecules. Some of these simple plant phenols, such as the flavanols, can oligomerise through condensation and/or oxidation reactions to furnish polyphenolic proanthocyanidins (*syn* condensed tannins) and complex theaflavins.[4, 7] The metabolism of gallic acid (3,4,5-trihydroxybenzoic acid) leads to a myriad of polyphenolic ester derivatives of polyols, mainly glucose, that can be regrouped into two major subclasses, the gallotannins and the ellagitannins (*syn* hydrolysable tannins).[8, 9] Red-brown algae also produce polyphenolic oligomers, called phlorotannins, that are derived from dehydrogenative coupling of phloroglucinol (1,3,5-trihydroxybenzene).[7] Furthermore, general phenylpropanoid metabolism furnishes various derivatives of hydroxycinnamic acids that express some polyphenolic characters, among which are the caffeic (3,4-dihydroxycinnamic) acid-derived chlorogenic acids (*syn* caffetannins) and rosmarinic acids (*syn* labiataetannins).[1, 3, 4] In fact, any type of natural polyol that is acylated by some polyhydroxyphenylcarbonyl unit, among the most common are the caffeoyl, galloyl and its dimeric hexahydroxybiphenoyl units, is today classified under one or other of the "polyphenol" subclasses, as long as it expresses some polyphenolic tannin-like characteristics.

This entanglement of structure types is far from providing a clear picture of the plant polyphenol family. The inclusion of many simple plant phenols in this family

[a] *Prof. Dr. S. Quideau*
Institut Européen de Chimie et Biologie
2 rue Robert Escarpit
33607 Pessac Cedex (France)
and Centre de Recherche en Chimie Moléculaire
Laboratoire de Chimie des Substances Végétales
Université Bordeaux 1
351 cours de la Libération
33405 Talence Cedex (France)
Fax: (+33) 5-40-00-64-39
E-mail: s.quideau@iecb.u-bordeaux.fr

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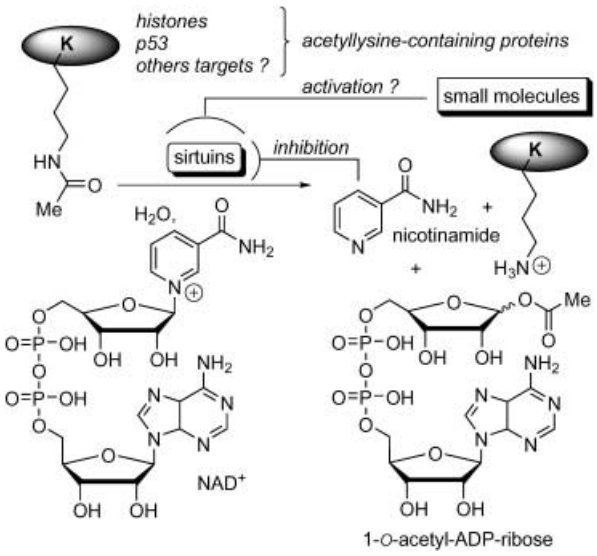
can be attributed to an important property that all phenols share to some extent, that is, their ability to scavenge oxidatively generated free radicals. This antioxidant activity is frequently cited as the main event responsible for the reduction of age-related diseases, such as neurodegeneration, carcinogenesis and cardiovascular diseases, including atherosclerosis.[5, 10] Besides this general mode of action based on the chemical reactivity inherent in the phenol function, plant phenols and polyphenols can also physically and specifically interact with biomolecules, including therapeutically significant enzymes.[1–3, 10]

More evidence of such biomechanistic implications of plant phenols in disease control has recently been provided by Howitz et al.[11] These researchers are investigating the positive health effects of calorie restriction, which is considered as a mild stress that promotes defence responses in a broad range of organisms, from yeasts to mammals. They earlier reported that the pace of ageing in *Saccharomyces cerevisiae* is governed by *PNC1* (pyrasinamidase/nicotinamidase 1), a calorie-restriction responsive gene that depletes nicotinamide, a physiological inhibitor of the silent information regulator 2 (Sir2) protein (Figure 1).[12]

This enzyme belongs to a large family of evolutionarily conserved nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylases called sirtuins. In yeasts, the Sir2-mediated deacetylation of acetylated lysine residues, such as those found in the *N* termini of histones,[13, 14] promotes the cleavage of the *N*-glycosidic bond between nicotinamide and ADP-ribose in NAD^+ (Scheme 1).[15] The released nicotinamide acts as a strong noncompetitive inhibitor of Sir2, thereby negatively regulating the activity of this enzyme.[16] Furthermore, this inhibition of Sir2 by nicotinamide increases the recombination of ribosomal DNA (rDNA) repeats, which can cause the accumulation of extrachromosomal circular rDNA up to levels that are toxic for old cells, hence causing aging. In *S. cerevisiae*, a single extra copy of the *SIR2* gene is sufficient to counteract the adverse effect of nicotinamide while extending lifespan by 40%.[16, 17]



Scheme 1. *Sirtuin-mediated deacetylation of acetylated lysine residues, such as those found in the N termini of histones and in the active form of the p53 tumour suppressor, produces 1-O-acetyl-ADP-ribose and nicotinamide, which in turn negatively regulates sirtuins.*

Finding small molecules capable of modulating the activity of sirtuins thus appeared as a valuable idea for understanding the function of these silencing enzymes better (Scheme 1). It is in a library of plant "polyphenolic" small molecules comprising stilbenes, chalcones, flavones, isoflavones, flavanones, flavanols and anthocyanidins that Howitz et al.[11] found several activators of sirtuins. The first sirtuin they investigated was SIRT1 (sirtuin-1), the closest human homologue of the yeast Sir2 enzyme that also promotes cell survival by negatively regulating the p53 tumour suppressor[18] by deacetylation of its lysine residue 382 (K382). A fluorescent assay was performed, by using the acetylated lysine-containing synthetic epitope $RHKK_{382}(Ac)$ of p53, to evaluate the modulation of SIRT1 activity. The flavones quercetin (**1**) and fisetin (**2**), the stilbenes piceatannol (**4**) and resveratrol (**6**), and the chalcone butein (**5**) stimulated SIRT1 from about five- to thirteenfold (Scheme 2).

A total of 15 simple plant phenols were found to activate SIRT1 by more than twofold. A majority of these sirtuin-activating compounds, referred to as STACs, display a *meta*-positioning of their phenolic hydroxyl groups in their A ring *trans*-oriented to a *para*-hydroxylated B ring around the ethylene unit of the stilbene, chalcone or flavone skeleton. When the A

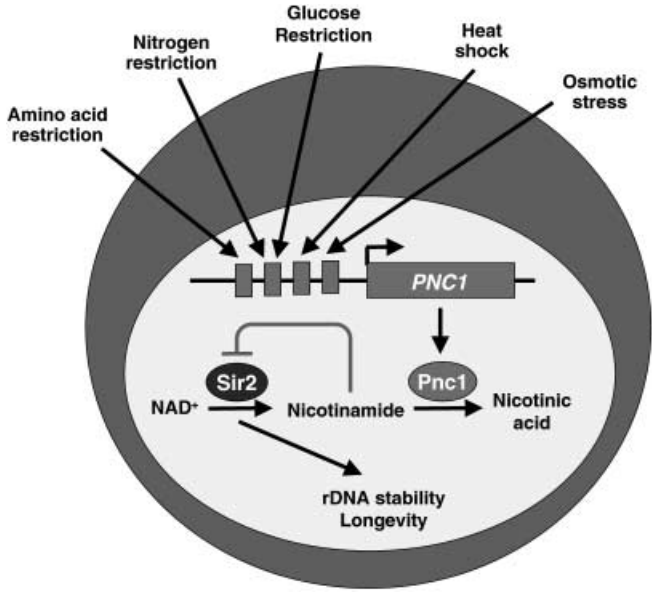
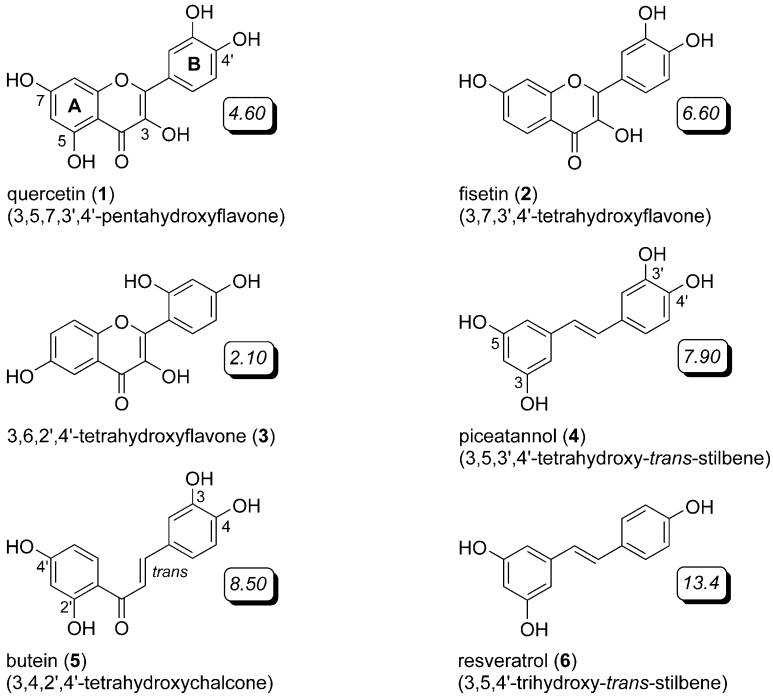


Figure 1. *Calorie restriction and other stimuli, such as heat and osmotic stresses, extend the life span in the budding yeast S. cerevisiae by increasing the expression of PNC1, which encodes an enzyme that converts nicotinamide into nicotinic acid. The resulting depletion of nicotinamide is sufficient to activate the NAD^+-dependent* Sir2 *histone deacetylase, which is thought to increase longevity by stabilizing rDNA. Reproduced with permission from D. Sinclair.*[11]



Scheme 2. *Plant phenolic sirtuin-activating compounds (STACs). The framed figures are the ratios of the rate of deacetylation of the p53 – 382 RHKK(Ac) synthetic epitope by SIRT1 in the presence of NAD^+ and the plant phenol to the control rate in the absence of the plant phenol.*

ring does not feature a *meta*-positioning of its phenolic hydroxyl groups, then a catecholic B ring appears necessary to maintain significant activity. For example, 3,6,2′,4′-tetrahydroxyflavone (**3**) is a rather poor activator of SIRT1, but fisetin (**2**) (3,7,3′,4′-tetrahydroxyflavone) activates it sixfold (Scheme 2). The screened catechins (i.e., flavan-3-ols) were all poor activators, or even inhibitors, of SIRT1. This lack of activity might be due to the relative conformational flexibility of their C ring, which does not have rigidifying sp^2 centres at the 1-, 2-, 3-, or 4-locus. Thus, the possibility of coplanarity between the hydroxylated A and B rings appears to be essential for activity. Of particular note is the fact that the most potent STAC, resveratrol (**6**; 3,5,4′-trihydroxy-*trans*-stilbene), is found in grapes and red wine, and it is already known for its putative mitigation of age-related diseases,[5, 10] including cancer by inhibiting cyclooxygenase (COX)[19] and cardiovascular disease by stimulating the oestrogen receptor.[20]

Evaluation of the effects of the identified STACs in vivo was first carried out with the Sir2-expressing yeast *S. cerevisiae*. Butein (**5**), fisetin (**2**) and resveratrol (**6**) increased its average lifespan by 31%, 55% and 70%, respectively. Since **6** had no effect on the lifespan of a *sir2* null mutant, whereas it did increase that of a *pnc1* null mutant (Figure 1), it seems that the extension of longevity is due to a direct stimulation of Sir2. Furthermore, **6** was found to reduce the frequency of rDNA recombination in a Sir2-dependent manner, even in the presence of nicotinamide. Howitz et al.[11] then turned back to SIRT1 with the aim of assessing if STACs could stimulate it in vivo. Using an antibody that specifically recognizes the acetylated form of p53-K382 (Ac-K382), they found that human embryonic kidney (HEK) and U2OS osteosarcoma cells treated with 0.5 μM resveratrol (**6**) showed a marked decrease (up to 75%) in their level of Ac-K382 and a concomitant increase in their survival under DNA-damaging conditions, such as ionizing and ultraviolet radiation. Although higher concentrations of **6** (> 50 μM) had the opposite effect, it remains clear that this phenolic stilbene has the capability of promoting cell survival in vivo by activating SIRT1.

This capability of SIRT1 to deactivate the p53 tumour-suppressing enzyme under DNA-damaging conditions might appear somewhat puzzling, for one of the consequences of silencing p53 activity will be, on the one hand, to diminish the chances of a cell to become senescent or apoptotic,[18] hence supporting the contention that **6** can, in some systems, act as a tumour promoter.[20] On the other hand, delaying apoptosis can give cells more time to repair damage and to prevent unnecessary cell death under various stress conditions. The extent of deacetylation of p53 by SIRT1 is controlled by several factors, such as cellular concentrations of its cofactor NAD^+ and of its reaction product and negative regulator, nicotinamide, which could conceivably be generated from the action of SIRT1 on other acetylated targets. One possible explanation of this apparent SIRT1/p53 dichotomy would be that cells are capable of subtly exploiting the interplay between these two enzymes to tip the scales towards either tumour suppression or life-span extension,[21] depending on the type and level of stress they are subjected to. Calorie restriction is an example of a low-intensity stress that is known to promote the survival and longevity of diverse sirtuin-expressing eukaryotes. In plants, phenolic secondary metabolites are often generated in response to various attacks and environmental stresses. Certain plant phenols, such as the stilbenes, chalcones and flavones screened by Howitz et al.,[11] might very well participate in the plant defence mechanism by activating sirtuins. Howitz et al.[11] had another enlightening thought, that is, that fungi and animals living in a symbiotic relationship with plants could stimulate their own sirtuins using plant-derived phenolic activators. Structural studies are now needed to understand how a simple plant phenol like resveratrol (**6**) can activate sirtuins, while counteracting the effect of nicotinamide. The work reported by Howitz et al.[11] constitutes an important new testimony to the biological significance of plant phenols, not only as mere antioxidants, but also as specific modulators of protein functions. This work opens up a novel avenue of investigation towards a better understanding of age-related diseases by using plant phenols as chemical probes.

While we are waiting for the development of "longevity drugs", we should learn from fungi, and indeed recommend the intake of phenol-rich plant-derived food. And yes! we can add a few glasses of wine to our diet! In 1934, Désiré Cordier (1858 – 1940), founder of the famous Cordier wine trading house, organized the first "longevity festival" in Saint-Julien-Beychevelle (Médoc, France), after having noted that life expectancy in this wine-producing area near Bordeaux was 45% higher than the national average. All the centenarians from the Médoc came to celebrate wine as the "elixir of life"...

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