

# Reviews

## Natural Polyphenols (Vegetable Tannins) as Drugs: Possible Modes of Action

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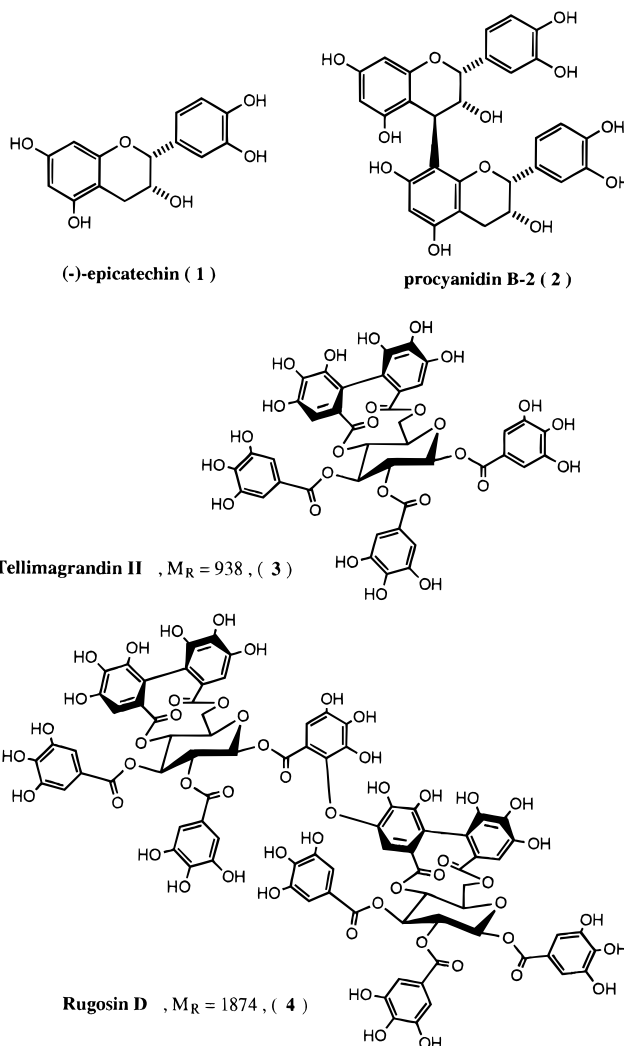
### Introduction

Natural products from plants and microorganisms traditionally have provided the pharmaceutical industry with one of its most important sources of "lead" compounds in the search for new drugs and medicines. Over the past two decades, researchers have also turned to many of the traditional folk medicines—invariably a "cocktail" of natural products—to uncover the scientific basis of their remedial effects, endeavors which have their roots as much in a desire to improve the efficacy as to enhance the ethics of modern medical practice. In this context, particular attention has been given by various groups in the Far East, Europe, and America to those traditional herbal medicines rich in polyphenols (vegetable tannins). Some typical examples of such medicines and the polyphenols which they contain<sup>1,2</sup> are shown in Table 1.

The ripe fruits of hawthorn (*Crataegus* sp.) provide one of the best tonic remedies for the heart and circulatory system. They act in a normalizing way upon the heart, depending on the need, stimulating or depressing its activity.<sup>3,4</sup> Hawthorn is a rich source of the flavan-3-ol (–)-epicatechin and proanthocyanidins related to (–)-epicatechin (1), e.g., epicatechin-(4β→8)-epicatechin [procyanidin B<sub>2</sub> (2)].

Meadowsweet is likewise one of the best digestive remedies available. It acts to protect and soothe the mucous membranes of the digestive tract, reducing excess acidity and easing nausea. It is used in the treatment of heartburn, hyperactivity, gastritis, and peptic ulceration and relieves the pain of rheumatism. Its gentle astringency is very useful in the treatment of diarrhea in children. Two of its principal polyphenolic constituents<sup>3,4</sup> are tellimagrandin II (3) and rugosin D (4); the latter polyphenol is formally derived by loss of two hydrogen atoms from two molecules of the "monomer" tellimagrandin II.

Very recently, and related generally to this area of research, there has arisen a considerable interest in the possibility that the impact of several diseases which afflict mankind may be ameliorated, if not prevented, by the simple expedient of improving the dietary intake of nutrients with antioxidant properties. Their role has been described with varying degrees of certitude<sup>5</sup> as follows: *important*, vitamin E; *thought to be important*, vitamin C; *probably important*, β-carotene and carotenoids; *possibly important*, plant phenolics (such as tannins and flavonoids). Thus, although proof of a cause and effect relationship does not exist, substantial interest has recently been engendered by the epidemiological evidence which points to a reduced risk of certain degenerative diseases by the consumption of beverages



containing polyphenols,<sup>6</sup> in particular green tea and red wines, both rich sources of polyphenols based on the flavan-3-ol carbon-oxygen skeleton.

**Vegetable Tannins: Polyphenols.** The word tannin has a long and well-established usage in the scientific literature which relates specifically to the application of plant extracts in the manufacture of leather. In this context, it is an important etymological legacy. Probably the most acceptable definition of vegetable tannins is still that of Bate-Smith and Swain,<sup>7</sup> formulated in 1962. They adopted the earlier ideas of White<sup>8</sup> and classified these higher plant metabolites as

water-soluble phenolic compounds having molecular-weights between 500 and 3,000 and, besides giving the usual phenolic reactions, they have special properties such as the ability to precipitate alkaloids, gelatin and other proteins.

**Table 1.** Some Medicinal Plants Containing Polyphenolic Metabolites

1. Tree Peony ( <i>Paeonia lactiflora</i> )	outer skin of the root; used to cure disorders of the bloodstream, including high blood pressure. Principal polyphenolic metabolites: gallotannins
2. Bearberry ( <i>Arctostaphylos uva-ursi</i> )	dried leaves; infusions have a soothing astringent effect and have value as a diuretic in kidney disorders and ailments of the bladder and urinary tract. Principal polyphenolic metabolites: gallotannins, arbutin, galloyl esters of arbutin
3. Agrimony ( <i>Agrimonia</i> sp.)	roots and dried aerial parts of the plant; used as an astringent on the digestive system, as a diuretic, and as a haemostatic agent. Principal polyphenolic metabolites: ellagitannins
4. Geranii Herba ( <i>Geranium maculatum</i> , <i>G. thunbergii</i> )	dried rhizome and leaves; used as an astringent and antihemorrhagic and antiinflammatory agent. Principal polyphenolic metabolites: ellagitannins
5. Meadowsweet ( <i>Filipendula ulmaria</i> )	aerial parts of the plant, leaves and flowers used as an infusion; employed as a mild astringent, antirheumatic antiinflammatory agent, and as a diuretic. Principal polyphenolic metabolites: ellagitannins
6. Raspberry ( <i>Rubus idaeus</i> )	leaves and fruit; mild astringent used in disorders of the digestive system, raspberry leaf tea traditionally used during pregnancy. Principal polyphenolic metabolites: ellagitannins
7. Hawthorn ( <i>Crataegus</i> sp.)	leaves and berries; used as astringent for digestive system, diuretic, cardiac tonic in treatment of high blood pressure. Principal polyphenolic metabolites: proanthocyanidins

In the 1960's the advent of new methods of isolation and analysis gradually revolutionized the depth of scientific understanding and appreciation of this field; natural polyphenols became available as single pure entities, and this has made possible recent extensive studies of the relationship of structure to biological and pharmacological activity.

It is now possible to describe in broad terms the nature of plant polyphenols. They are secondary metabolites widely distributed in various sectors of the higher plant kingdom.<sup>1,2</sup> They are distinguished by the following general features:

(a) *Water Solubility.* Although when pure some plant polyphenols may be difficultly soluble in water, in the natural state polyphenol–polyphenol interactions usually ensure some minimal solubility in aqueous media.

(b) *Molecular Weights.* Natural polyphenols encompass a substantial molecular weight range from 500 to 3000–4000. Suggestions that polyphenolic metabolites occur which retain the ability to act as tannins but possess molecular weights up to 20 000 must be doubtful in view of the solubility proviso.

(c) *Structure and Polyphenolic Character.* Polyphenols, per 1000 relative molecular mass, possess some 12–16 phenolic groups and 5–7 aromatic rings.

(d) *Intermolecular Complexation.* In addition to giving the usual phenolic reactions, they have the ability to precipitate some alkaloids, gelatin, and other proteins from solution. These complexation reactions are of intrinsic scientific interest as studies in molecular recognition and as the basis of possible biological function.

(e) *Structural Characteristics.* Plant polyphenols are based upon two broad structural themes:-

**(1) Galloyl and Hexahydroxydiphenoyl Esters and Their Derivatives.** These metabolites are almost invariably found as multiple esters with D-glucose,<sup>1,2</sup> and a great many can be envisaged as being derived from the key biosynthetic intermediate  $\beta$ -1,2,3,4,6-pentagalloyl-D-glucose, e.g., tellimagrandin II (**3**) and rugosin D (**4**). Derivatives of hexahydroxydiphenic acid are assumed to be formed by oxidative coupling of vicinal galloyl ester groups in a galloyl D-glucose ester, Figure 1.

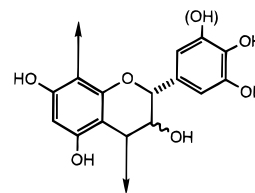
Gallic acid is most frequently encountered in plants in ester forms. These may be classified into several broad categories: (i) simple esters, (ii) depside metabo-

lites (*syn* gallotannins), (iii) hexahydroxydiphenoyl and dehydrohexahydroxydiphenoyl esters (*syn* ellagitannins), and (iv) “dimers” and “higher oligomers” formed by oxidative coupling of “monomers”, principally those of class iii above. Thus, rugosin D (**4**), *vide supra*, may be envisaged as being derived by C–O oxidative coupling of two molecules of tellimagrandin II (**3**).

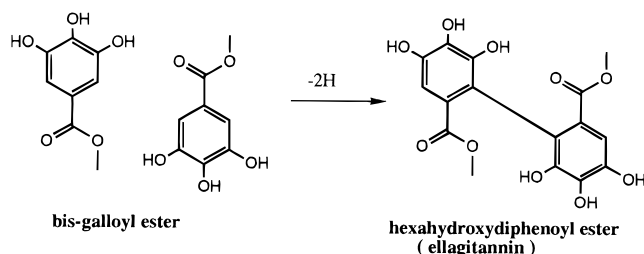
Gallic acid metabolites often accumulate in substantial quantities in plant tissues. The apotheosis of this characteristic is the storage (up to 70% of the dry weight) of complex polyphenols of the gallotannin class in Chinese galls (*Rhus semialata*). Similarly, the vegetative tissues of the green tea flush (*Camellia sinensis*) may contain up to 25–30% of phenolic flavan-3-ols, prominent amongst which are (–)-epigallocatechin and (–)-epicatechin and their 3-gallate esters.

Gallic acid metabolites are not universally distributed in higher plants. They occur within clearly defined taxonomic limits in both woody and herbaceous dicotyledons. Ellagitannins are widely distributed in the lower Hamamelidae, Dilleniidae, and Rosidae (the HDR-complex) and have been used as prominent chemotaxonomic markers. It has been suggested that the low degree of diversification in gallate-dominated taxa may be as a result of the electron scavenging properties of these metabolites which, in turn, inhibits oxidation, the most important reaction in the synthesis of secondary metabolites.

**(2) Condensed Proanthocyanidins.** The fundamental structural unit in this groups is the phenolic flavan-3-ol (“catechin”) nucleus.<sup>1</sup> Condensed proanthocyanidins exist as oligomers (soluble), containing two to five or six *catechin* units, and polymers (insoluble). The flavan-3-ol units are linked principally through the 4 and the 8 positions (**5**). In most plant tissues, the

flavan-3-ol oligomeric unit (**5**)

polymers are of greatest quantitative significance, but there is also usually found a range of soluble molecular



**Figure 1.** Biogenesis of hexahydroxydiphenyl esters (ellagitannins).

**Table 2.** Some Physiological and Pharmacological Actions of Polyphenols

i	bacteriocidal action
ii	molluscicidal action
iii	anthelmintic action
iv	antihepatotoxic action
v	stimulation of Phagocytic cell iodination
vi	inhibition of human immunodeficiency viral replication (HIV)
vii	inhibition of human simplex virus (HSV)
viii	inhibition of glucosyl transferases of <i>Streptococcus mutans</i> (dental caries)
ix	inhibition of ascorbate autooxidation (green tea)
x	inhibition of lipoxygenase dependent peroxidation; "French Paradox"
xi	host-mediated antitumor activity: cytotoxic effects, inhibition of tumor promotion, inhibition of Ornithine decarboxylase (ODC) response
xii	inhibition of xanthine oxidase and monoamine oxidase

species—monomers, dimers, trimers, *etc.* These polymers are, metaphorically speaking, the base of the "metabolic iceberg". In the tissues of some plants such as ferns and fruit such as the persimmon (*Diospyros kaki*) there is an overwhelming preponderance of these polymeric forms. They are also of frequent occurrence in plant gums and exudates.

**Biological and Pharmacological Activity.** Notable studies in this area have been made by several groups worldwide.<sup>9–19</sup> *In vitro* testing has identified a wide range of potentially significant biological activities which are exhibited by natural polyphenols, and a selection of these is shown in Table 2. Although these studies have revealed important differences in pharmacological activity between individual polyphenols and between classes of different polyphenols, overall they suggest some selectivity rather than high specificity toward particular biological targets. Thus, Okuda and his collaborators<sup>14</sup> demonstrated significant inhibition of both the cytopathic effect of HIV and the expression of HIV antigen in human lymphotropic virus type I (HTLV-1)-positive MT-4 cells by several hydrolyzable tannins but not by the condensed proanthocyanidins tested. The anti-HIV activity of the various galloyl and hexahydroxydiphenyl esters increased with increasing molecular size, suggesting some link to this quantity and/or their polyanionic character *in situ*. It should also be pointed out that, as yet, it is not clear how or if these complex polyphenolic substrates are absorbed from the gut, and this lack of precise knowledge of the fate of these compounds in the human body remains a major weakness in this area.

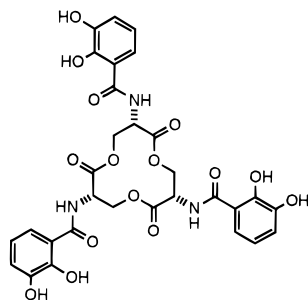
Nevertheless, it is also clear that polyphenols have a number of physical and chemical properties—associated principally with the possession of a concatenation of phenolic nuclei within the molecule—in common. These properties, moreover, probably underly, *at least in part*,

their physiological and pharmacological actions. It is therefore suggested that polyphenols probably exert certain of their roles in the medical treatment of diseased states by virtue of three distinctive *general* characteristics which they all possess to a greater or lesser degree and which derive in essence from the properties of the simple phenolic nucleus itself, namely, (i) **their complexation with metal ions (iron, manganese, vanadium, copper, aluminum, calcium, *etc.*)**, (ii) **their antioxidant and radical scavenging activities**, and (iii) **their ability to complex with other molecules including macromolecules such as proteins and polysaccharides.**

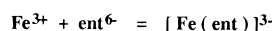
### Metal Ion Complexation

Pliny first described the blue-black color produced when an aqueous infusion of oak galls is treated with iron salts, and its use in the analysis of mineral waters and in the manufacture of inks was noted as early as the sixteenth century. Thereafter, the reproach that "anything that gives a blue-black colour with iron salts has been termed a tannin" has often seemed well merited. Nevertheless, this property of molecules, such as natural polyphenols, with catechol and pyrogallol nuclei of forming strong complexes with metal ions such as iron, vanadium, manganese, aluminum, calcium, *etc.* is not only a distinctive one but also an important one. In view of the importance of these metals to living systems, it is logical to presume that species which form strong complexes with them, such as polyphenols, may well modify their biological activities. Insofar as the transition metals (vanadium, iron, manganese, copper, and cobalt) themselves are concerned, they have properties which clearly distinguish them from other metals found in living systems (*e.g.*, sodium, potassium, and magnesium), namely,<sup>20</sup> (i) they are good Lewis acids, can act as  $\pi$ -electron donors, and are red-ox active, (ii) biological systems have evolved in such a way that, while much of the particular element may remain free, a considerable fraction is bound up with particular organic ligands, *e.g.*, Fe—haem, and (iii) functional properties, such as red-ox potentials, are very sensitive to ligand coordination.

The case of iron may, at this juncture, be taken as representative of these various metals. Iron is common to all life and is the most abundant transition metal found in the biosphere; its involvement in biological systems is, however, complicated. It is involved not only in red-ox catalysis but has numerous other functions, *e.g.*, storage and transport of oxygen, electron transfer, hydroxylation reactions, utilization of hydrogen peroxide, and superoxide dismutation.<sup>21</sup> Because iron in its most common form is not readily available (the solubility of ferric hydroxide is  $10^{-38}$ ), many microorganisms produce siderophores—low molecular mass chelating agents that bind and solubilize iron. The one which binds iron the most strongly under physiological conditions is the siderophore enterochelin (*ent*, **6**), formed by *Escherichia coli*. The siderophore employs the three dihydroxybenzoyl rings to give a charged octahedral triscatecholate  $\Delta$ -*cis* complex. The formation constant with ferric iron and the macrocycle of  $10^{49} \text{ M}^{-1}$  is the highest reported for a siderophore.<sup>22</sup> The mechanism of capture and binding of the transition metal ion is clearly very similar to that deployed by natural polyphenols in their complexation of such ions.



enterochelin (enterobactin), (6)



The average adult male contains ~4.5 g of iron, absorbs some 1 mg per day from the diet, and excretes about the same quantity when in iron balance. Plasma iron turnover accounts for some 35 mg per day, and slight disturbances of iron metabolism readily leads to iron overload or iron deficiency. About two-thirds of body iron is located in hemoglobin, with lesser amounts in myoglobin, various enzymes, the transport protein transferrin, and a small transit pool of iron chelates (nature uncertain). Otherwise, iron that is not required is stored in ferritin which consists of a protein shell surrounding an iron core which holds up to 4500 iron ions per molecule of protein.

In humans, iron is of particular medical concern because of its involvement in various red-ox reactions, its effect on infectious organisms, and the diseases of iron overload and iron deficiency. Antimicrobial activity through iron depletion is, for example, well documented. Thus, infection of humans by *Escherichia coli* is inhibited by the iron-chelating lactoferrin present in human milk but restored by augmentation with additional iron. Lactoferrin is also released in septic infections by degranulation of blood circulating leucocytes. There seems, *a priori*, therefore very good reason to think that natural polyphenols have the potential (should they possess the ability to penetrate to particular sites in the human body), to modulate physiological reactions involving iron and other transition metals. Experimental developments in this area are eagerly awaited.

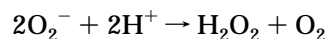
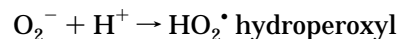
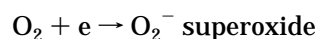
### Antioxidant Activity

Increasing attention is being given to the role of free radicals and other oxidants in the mechanism of action of many toxins and, in recent years, their involvement in the pathophysiology of major chronic diseases. Thus, reactive oxygen species have been implicated in various human diseases including the processes of aging, cancer, multiple sclerosis, Parkinson's disease, autoimmune disease, senile dementia, inflammation and arthritis, and atherosclerosis. Many chronic diseases are also exacerbated by imbalances or perturbations in fatty acid and lipid metabolism. It is thought, for example, that excess dietary fatty acids are conducive to atherosclerosis and coronary arterial disease and uncontrolled lipid oxidation (enzymic or nonenzymic) is associated with arthritis, cancer, and atherogenesis. While there is little doubt that cellular prooxidant states are implicated in many of the diseases noted above, it is not yet clear that they are the causative agents. Ames has argued that a deficiency of micronutrients that protect

against oxidative damage to DNA is a major contributor to human cancer; Halliwell,<sup>21</sup> on the other hand, has stated that increased oxidant formation is usually a *consequence* of disease.

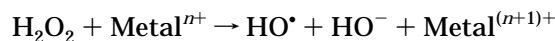
In cellular prooxidant states the intracellular concentration of activated forms of oxygen (reactive oxygen species) is increased, presumably because cells either overproduce these reactive substances or are deficient in their ability to destroy them.<sup>23</sup> The reactivity of oxygen can be enhanced in a number of ways. One-electron reduction of oxygen produces the superperoxide radical  $\text{O}_2^-$ . This species is formed in almost all aerobic cells, a major source being the "leakage" of electrons onto oxygen from various components of the cellular electron transport chain (e.g., mitochondria). Any reaction undergone by  $\text{O}_2^-$  in aqueous media will be in competition with its spontaneous dismutation to hydrogen peroxide. Superperoxide dismutase enzymes, which are specific for  $\text{O}_2^-$ , have evolved a surface arrangement of charge which accelerates the decomposition of  $\text{O}_2^-$  to hydrogen peroxide. Protonation of  $\text{O}_2^-$  produces the hydroperoxyl radical  $\text{HO}_2^\bullet$ , which is less polar than  $\text{O}_2^-$  and somewhat more reactive.

#### *superperoxide: generation and dismutation*



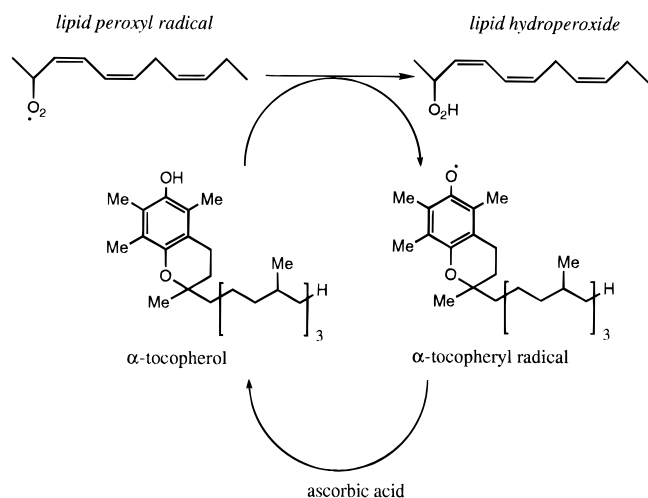
The moderate reactivity of both  $\text{O}_2^-$  and hydrogen peroxide in aqueous media makes it unlikely that the oxidative damage done to cells can be directly attributable to these species. In general, it is thought more probable that the damage incurred arises due to their conversion to more reactive radical species—notably hydroperoxyl  $\text{HO}_2^\bullet$  and hydroxyl  $\text{HO}^\bullet$ . However the less reactive  $\text{O}_2^-$  and hydrogen peroxide may well be more damaging in the wider sphere since they can diffuse away from their site of generation and induce the formation of  $\text{HO}^\bullet$  at remote cellular locations. *In vivo* most of the hydroxyl radicals generated come from the metal ion (e.g.,  $\text{Fe}^{3+}$ )-catalyzed breakdown of hydrogen peroxide.

#### *hydroxyl radical generation*



The highly reactive hydroxyl radical, once formed, can then initiate the process of lipid peroxidation by abstraction of a hydrogen atom from an unsaturated aliphatic lipid side chain, eventually giving rise by an autocatalytic chain reaction to a lipid hydroperoxide.

Oxidative stress represents a disturbance of the prooxidant/oxidant balance of the body toward the former state and may arise from environmental or other external sources or by the endogenous production of free radicals accompanying diseased states. Cells and tissues normally possess antioxidant defense mechanisms to ensure the removal of reactive oxygen species—those that are controlled endogenously (e.g., superoxide dismutase) and those [e.g., antioxidants such as  $\alpha$ -tocopherol (vitamin E), ascorbic acid (vitamin C), and  $\beta$ -carotene] that are provided by dietary and other



**Figure 2.** Lipid peroxidation–chain reaction blocking action of  $\alpha$ -tocopherol.

**Table 3.** Rate Constants ( $\text{M}^{-1} \text{s}^{-1}$ ,  $\times 10^5$ ) for the Reaction of Some Strongly Oxidizing Radicals with Various Flavonoids and Natural Phenols<sup>a</sup>

compd	$\text{O}_2^-$	$\text{RCO}_2^\bullet$	$\text{HO}^\bullet$
gallic acid (9)		4.5	
propyl gallate (8)		170	
ascorbic acid (13)		1300	
fisetin	0.13	4100	
kaempferol	0.024		46 000
quercetin	0.47	390	43 000
eriodictyol (12)			31 000
hesperitin (10)	0.059		58 000
(+)-catechin (14)	0.18	61	66 000
(-)-epicatechin (1)		73	64 000

<sup>a</sup> Table compiled from data derived from various sources.<sup>24–26</sup> Rates of radical reaction are directly comparable for each reactive oxygen species but are not directly comparable between reactive oxygen species.

means. Vitamins E and C are known and important antioxidants. Vitamin E is the general name given to a group of lipid-soluble compounds of which  $\alpha$ -tocopherol is the most familiar. It is found in lipoproteins and membranes and acts to block the chain reaction of lipid peroxidation by scavenging the intermediate peroxy radicals which are generated, Figure 2. The highly sterically hindered  $\alpha$ -tocopheryl radical is much less reactive in attacking other fatty acid side chains and may be converted back to the parent phenol by ascorbic acid, thus breaking the chain reaction. The water-soluble vitamin C (ascorbic acid) itself has many physiological roles; antioxidant activity (such as recycling of vitamin E in membranes and lipoproteins) is only one of them. Paradoxically, however, it should also be noted that, *in vitro*, vitamin C is also capable of prooxidant activity. It has long been known, for example, that the combination of ascorbate and ferrous ions generates hydroxyl radicals which can induce lipid peroxidation.

It is in this context of cellular prooxidant states and lipid peroxidation that intense speculation has very recently been generated in relation to the possible role which simple plant phenols and polyphenols, either as components of herbal medicinal extracts or as part of the regular diet, may have in the treatment/amelioration/prevention of the various diseases noted earlier which are associated with the presence of prooxidant states. According to Halliwell,<sup>5</sup> plant phenols and

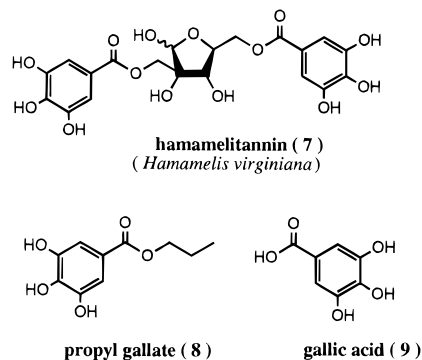
**Table 4.** Radical Scavenging Activities of Hamameli Tannin, Gallic Acid, and Propyl Gallate

compd	superperoxide anion, $\text{O}_2^-$	hydroxyl radical, $\text{HO}^\bullet$	singlet oxygen
hamamelitannin (7)	1.31	5.46	45.5
gallic acid (9)	1.01	78.0	69.8
propyl gallate (8)	1.41	86.5	66.7
ascorbic acid (13)	23.3	18.8	120.4

<sup>a</sup>  $\text{IC}_{50}$  values ( $\mu\text{M}$ ) corresponding to 50% inactivation of active oxygen species, values determined by ESR spin trapping. Data from ref 27.

polyphenols are “possibly important as antioxidants”, and it is clear that the present speculation is best now replaced by the search for hard scientific evidence in this area.

Plant phenols and polyphenols are known to inhibit lipid peroxidation and lipoxygenases *in vitro*, and information has been accumulated over the past few years demonstrating their ability to scavenge radicals such as hydroxyl, superoxide, and peroxy, which are known to be important in cellular prooxidant states. Some idea of the relative reactivity of these reactive oxygen species toward phenolic substrates may also be gleaned from these observations. Typical data showing the radical scavenging efficiency of various phenols are shown in Table 3 for simple phenols and flavonoids<sup>24–26</sup> and in Table 4 for the natural bis-galloyl ester hamamelitannin<sup>27</sup> (7).



Studies to date indicate that, despite its name, the superperoxide ion is fairly innocuous. The propensity of superperoxide ion to remove protons from substrates accounts for its reactivity with acidic reductants and their overall oxidation. Electrochemical studies with catechol derivatives, ascorbic acid, and  $\alpha$ -tocopherol provide clear evidence that these substrates are not oxidized in protic media by direct one-electron transfer to superperoxide ion.<sup>28,29</sup> The primary step involves abstraction of a proton from the substrate by superperoxide to give the substrate anion and the disproportionation products of superperoxide (oxygen and hydrogen peroxide). The substrate anion is then oxidized by oxygen in a multistep process.

Evidence thus exists which supports the contention that the beneficial action of natural polyphenols in traditional medicines toward certain diseases may well derive from their ability to scavenge reactive oxygen species in cellular prooxidant states. However, while the propensity of natural plant phenols and polyphenols to act *in vitro* with reactive oxygen species in the manner predicted for natural antioxidants is clear, it should nevertheless also be pointed out that they may

additionally (like ascorbate) in some circumstances show prooxidant characteristics.<sup>26</sup> Thus, it has been demonstrated that the food antioxidant propyl gallate and plant phenols can in fact also react with ferrous ions in the presence of hydrogen peroxide to produce reactive oxygen species which can subsequently damage other biological molecules.

**Green Tea and the "French Paradox".** The present intense interest in the possibility that many major human degenerative diseases may involve, in their etiology, free radical processes and that antioxidant nutrients may be capable of delaying or even preventing these processes has origins which extend back to at least the 1920's and the discovery of vitamin E. Much of our present knowledge comes from epidemiological studies and indicates that incidence of some forms of cancer and cardiovascular disease appears to be lower in populations with a larger than average intake of antioxidant nutrients such as vitamins C and E and various carotenoids.<sup>5</sup> Extended dietary surveys in the U.S. have revealed that the calculated dietary intake of essential antioxidants is inversely related to the risk of coronary heart disease and certain forms of cancer.<sup>30</sup> Plasma concentrations of dietary-derived antioxidants have revealed inverse correlations with the incidence of these diseases, although with a different rank order of antioxidants:

*decreased cancer risk:*

$\beta$ -carotene > vitamin C > vitamin E

*decreased coronary heart disease risk:*

vitamin E >  $\beta$ -carotene > vitamin C

It has been suggested that the following levels of intake (mg. per day) in the diet are likely to provide blood levels of the nutrients consonant with a low risk of degenerative disease:<sup>31,32</sup> vitamin E (40–60 mg/day), vitamin C (150 mg/day), and  $\beta$ -carotene (9–12 mg/day). In itself, this is a thought-provoking observation given, for example, that in the United Kingdom the estimated levels of dietary intake of these substances is generally low. Thus, for  $\beta$ -carotene it is, in the 16–24 age group, ~1.6–1.9 mg/day and, in the 50–64 age group, ~2.4–2.8 mg/day.

It is against this background, and the observations of Szent-Gyorgi<sup>33–35</sup> in the 1930's, that the current excitement concerning the consumption of polyphenol-rich green teas and red wines should be viewed. Following his discovery of vitamin C, Szent-Gyorgi and his colleagues reported a number of findings that other substances in fruit juices probably have a synergistic effect on the actions of vitamin C. The general thrust of their observations is evident from these extracts from their papers:

Variou chemical and clinical observations have led to the assumption that ascorbic acid is accompanied in the cell by a substance of similar importance and related activity. In the absence of both substances, the symptoms of the lack of ascorbic acid (**scurvy**) prevail and conceal symptoms of the deficiency of the second substance.

These results suggest that this great group of vegetable dyes, the flavons or flavonols, also play an important rôle in animal life, and that the dyes are of a vitamin nature.

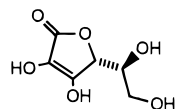
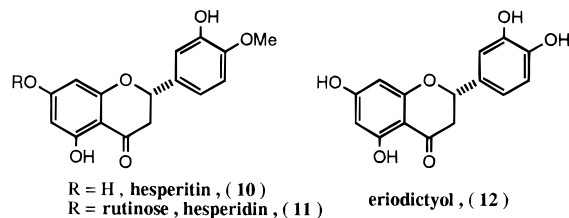
These results suggest that experimental scurvy, as commonly known, is a deficiency disease caused by the combined lack of vitamin C and P.

The therapeutic effects observed after the administration of 'citrin' in man in septic conditions, also accompanied by polyarthritis and endocarditis, suggest that the age old beneficial effect of fruit juice is partly due to its vitamin P content.

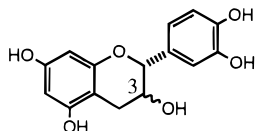
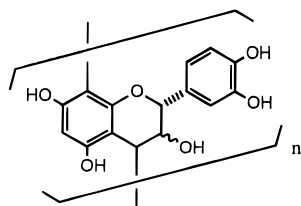
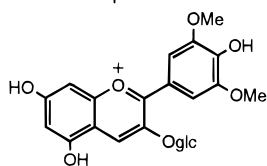
In the event, although the story of vitamin P continued in Hungary and France well into the postwar years, substantive proof of the existence of this additional vitamin P was never forthcoming. However it seems reasonable to assume that flavonoid compounds, such as hesperitin (**10**), hesperidin (**11**), and eriodictyol (**12**), in the fruit juices were largely responsible for the observations, acting as antioxidants themselves or to enhance and protect the actions of vitamin C.

Contemporaneously, interest has revived in this work following observations in two other areas. Epidemiological evidence seems to indicate that consumption of polyphenol rich common items in the diet is associated with an increase in plasma antioxidant potential, and this might therefore have an important role to play in the modulation of exposure to cellular oxidative stress.<sup>10,11,36–38</sup> Examination of WHO data shows marked differences in mortality from coronary heart disease among various countries—especially between French and U.S. and U.K. populations. Subjects who have similar intakes of saturated fatty acids, similar risk factors, and comparable plasma cholesterol levels show a much lower incidence of death from coronary heart disease in France. This has been attributed to regular consumption of red wines—to the alcohol and/or polyphenolic contents of the red wine. Thus, a full-bodied young red wine contains up to 4.0 g/L of phenolics—principally flavan-3-ols (**1**, **14**), oligomeric procyanidins (**15**), and anthocyanin (**16**) pigments,<sup>39</sup> and attention has been directed, principally, but not exclusively, to these particular constituents to rationalize the epidemiological data. However, it should be very clearly pointed out as a word of caution, if not scepticism, that not all workers in this field share this enthusiasm for the view that the phenolic constituents of red wines may act as antioxidants for low-density lipoprotein (LDL) and so exert an antiatherogenic effect.<sup>40–43</sup> The title of one such paper<sup>42</sup> perceptively encapsulates this present uncertainty: "*Take two glasses of wine and see me in the morning*". More detailed biological observations are required to substantiate the epidemiological evidence at this stage.

Epidemiological evidence, principally from Japan and China, likewise strongly suggests that the habitual consumption of green tea as a beverage may protect both against cancer and the development of coronary heart disease. Attention has been similarly focused, with the presumption that polyphenols may ameliorate conditions of oxidative stress, upon the major polyphenolic component of the green tea flush (*Camellia sinensis*), namely (–)-epigallocatechin 3-*O*-gallate. Although the amounts may vary, dependent upon cultural and climatic conditions, flavanols usually constitute up to 20–30% of the dry matter of the fresh green tea leaf; of these, (–)-epigallocatechin (~2.5%), (–)-epigallocatechin 3-*O*-gallate (**17**; ~10.5%), and (–)-epicatechin 3-*O*-gallate (**18**; ~2.75%) overwhelmingly predominate. At this stage, the position in regard to this problem is very similar to that described for red wines—experimental evidence in relation to the fate, after ingestion, of these phenolic compounds in humans is still awaited. Until

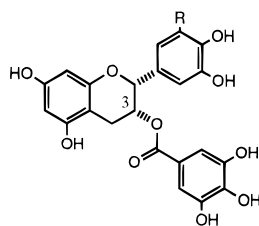


ascorbic acid, vitamin C, (13)

3*S*, (+)-catechin (14)  
3*R*, (-)-epicatechin (15)oligomeric procyanidins  
(16)

malvidin-3-glucoside (17)

such time, the validity of the claims made for these dietary polyphenols should be treated with similar reserve.



R = OH; (-)-epigallocatechin-3-O-gallate (18)

R = H; (-)-epicatechin-3-O-gallate (19)

### Polyphenol Complexation

Although the uses of polyphenols as medicinal agents may be summarized under several broad headings, *e.g.*, Tables 1 and 2, many of their actions appear to devolve on their ability to complex with proteins and polysaccharides. They thus aid the healing of wounds, burns, and inflammations. In doing so, they act to produce an impervious layer [polyphenol-protein and/or polysaccharide complex] under which the natural healing processes can occur. Similar complexation processes probably take place internally; gut secretions are hindered, thus protecting the underlying mucosa from toxins and other irritants in the bowels. **This propensity to bind to proteins also presumably accounts for the fact that polyphenols inhibit virtually every enzyme that are tested with *in vitro*.**<sup>1,44</sup>

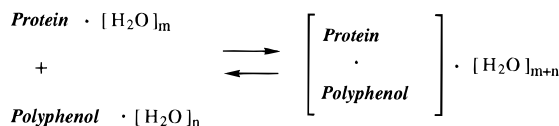
Assessment of the medical significance of polyphenol inhibition of a particular enzyme, determined *in vitro*, is therefore dependent on the, as yet, unanswered questions relating to the absorption and penetration of ingested polyphenols to the desired site(s) of action *in vivo*. These problems do not arise where the enzymes are extracellular. Thus, the mutans groups of streptococci, *Streptococcus mutans* and *Streptococcus sobrinus*, are principal cariogenic organisms, and their major ecological niche is the tooth surface and dental plaque. The mutans streptococci synthesize water-insoluble glucan from sucrose by the action of glycosyl transferases, resulting in a firm attachment of the mutans streptococci to the tooth surface. This leads eventually to the formation of dental plaque and the development of dental caries. Recently, several polyphenols present in green tea extracts were demonstrated to inhibit glucan synthesis from sucrose by the glycosyl transferases of *Streptococcus sobrinus*,<sup>45</sup> and administration of polyphenolic extracts of Oolong (semifermented) tea led to a highly significant reduction in dental caries in experimental animals.<sup>46</sup> It was suggested that such extracts (like betel nuts) may well be very useful for controlling dental caries in humans, presumably *via* their inhibition of the glycosyl transferases.

In an early study of plant viral infection, Cadman<sup>47</sup> suggested that polyphenolic extracts of the leaf of raspberry (*Rubus idaeus*) probably act on most viruses by clumping the virus particles together into complexes which are largely uninfected. In later work, others have similarly deduced that viral inactivation, *in vitro*, is directly attributable to preferential binding of the polyphenol to the protein coat of the virus.<sup>48</sup> In a systematic study of the antiviral activity of a very wide range of natural products, Vlietinck and his colleagues<sup>49</sup> concluded that polyphenols act principally by binding to the virus and/or the protein of the host cell membrane and thus arrest absorption of the virus. In consequence, polyphenols are probably only viricidal in nature.

Whatever finally emerges as the *in vivo* role of polyphenols in relation to their action of enzymes, viruses, and other proteinaceous materials, Tables 1 and 2, the mechanism of interaction of polyphenols and proteins has been a subject of detailed scrutiny over the past two decades. Present knowledge will doubtless form the fundamental basis for the future analysis of *in vivo* physiological and pharmacological studies. An outline of the principal features of the phenomenon of protein-polyphenol interactions is therefore given below.

The complexation of polyphenols with proteins is a specific example of the phenomenon of molecular recognition, and some of the general features of this form of association are delineated in Table 5.

**Hydrophobic Effects.** Albert Szent-Gyorgi once cryptically remarked, "Biology has forgotten water, or never discovered it". The presence of water is intuitively accepted and invariably tacitly ignored, but the role of water and water solubility is one of the key factors in the phenomenon of polyphenol complexation. The degree of order of the solvent externally is generally proportional to its proximity to hydrophilic groups on the molecular surfaces of the substrates where water molecules may be anchored by hydrogen bonding to acceptor and donor functionalities (*e.g.*, hydroxyl, car-



**Figure 3.** Polyphenol–protein association: structural reorganization of water molecules.

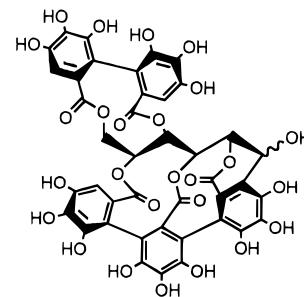
**Table 5.** Facets of Polyphenol–Protein Interactions

- (i) Complexation is essentially a surface phenomenon, maximized at or near the isoelectric point of the protein. Interactions are dynamic and time dependent; conformational flexibility in both the polyphenol and the protein are important complimentary factors leading to strong interactions. The mechanism of association may be likened to “fitting a hand into a glove”. Unless secondary chemical/biochemical reactions take place complexations of this type are generally, but not invariably, reversible.
- (ii) proteins which are small and compact with a tightly folded secondary and tertiary structure have a poor affinity for polyphenols. Conversely, proline-rich proteins (e.g., gelatin, salivary proteins) which have an open, random coil type of conformation have a high affinity for polyphenolic substrates.
- (iii) Polyphenols, *via* their aromatic nuclei and phenolic groups, act as multidentate ligands on the protein surface. Molecular size of the polyphenol is therefore important, and in the galloyl-D-glucose series the efficacy of binding increases as the number of galloyl groups increases: tri < tetra < penta.
- (iv) The principal driving forces toward association are “hydrophobic effects”, and these are enhanced by “hydrogen bonding” of the phenolic groups to points on the protein, in particular, the carbonyl groups of tertiary (prolyl) peptides.

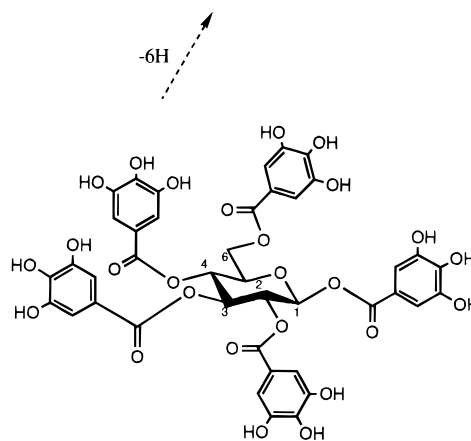
bonyl, amide, *etc.*). During the association processes, the reorganization of the various solvation shells provides an important driving force for the interactions to take place Figure 3.

Hydrophobic forces are difficult to define since they arise from the presence in the complexing species of groups or regions which have a general *dislike* for water. These groups or regions shy away from water and seek to coalesce. Although van der Waals forces may make a substantive contribution to the stability of the resultant complex, the main contribution comes from an entropic effect. In the vicinity of the surface of groups and regions in molecules which have a dislike for water there is performed a layer of partially ordered water molecules, likened by some to “icebergs”. The anarchic distaste of water for such orderly regimentation drives these groups and regions (in different molecules) to associate and congregate together, if at all possible. In doing so, the overall surface areas exposed to water are reduced and parts of the highly structured “icebergs” are thus returned to the random bulk state of water. It is believed that polyphenols contain several groups or regions—aromatic rings, the carbon–hydrogen skeleton of sugars, *etc.*, which provide a multiplicity of sites of a potentially hydrophobic nature to participate in such interactions.

A corollary of these observations is that where a polyphenol molecule is very water soluble, *i.e.* is “perfectly happy” in an aqueous medium, then there will be very little driving force toward complexation with proteins. The importance of water solubility can be readily seen by reference to  $\beta$ -1,2,3,4,6-penta-*O*-galloyl-D-glucose (**20**) and two highly condensed unique open-chain derivatives castalagin/vescalagin (**19**). The latter molecules are formally six hydrogens less than their



vescalagin / castalagin, ( 19 )



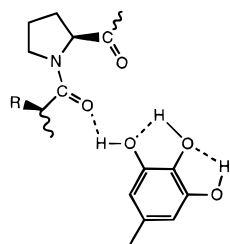
$\beta$ -1,2,3,4,6-pentagalloyl-D-glucose, ( 20 )

presumed biosynthetic precursor  $\beta$ -1,2,3,4,6-penta-*O*-glucose; all three molecules possess five aromatic nuclei and 15 phenolic hydroxyl groups. Castalagin and vescalagin are very nicely crystalline and highly soluble in water, *not* extracted therefrom by ethyl acetate, and show  $K$  [octanol/water] = 0.1. In contrast,  $\beta$ -1,2,3,4,6-penta-*O*-galloyl-D-glucose has a very limited solubility in water, from which it is readily extracted by ethyl acetate and shows a value of  $K$  [octanol/water] = 32. The strength of association  $\beta$ -1,2,3,4,6-penta-*O*-galloyl-D-glucose (**20**) with protein is several orders of magnitude greater than both vescalagin and castalagin (**19**), whose affinity for proteins is very poor.

Similarly, the assay of polyphenol/protein affinity usually takes place in laboratory conditions which rarely mirror the complexities of the situation *in vivo* where the interaction is probably taking place amidst a “cocktail” of other substrates and inorganic salts which may well alter the fundamental characteristics of the polyphenol. Thus, for example, the solubility of  $\beta$ -1,2,3,4,6-penta-*O*-galloyl-D-glucose (**20**) in glass distilled water at 20 °C can be enhanced from 1.0 to 40 mM by the addition of hexanoic acid/sodium hexanoate (1:1; pH 4.7), thereby reducing its affinity for protein. Likewise, the presence of caffeine in solutions of  $\beta$ -1,2,3,4,6-penta-*O*-galloyl-D-glucose (**20**) similarly reduces the extent of association with proteins by providing an alternative substrate with a strong affinity for the polyphenol.

**Hydrogen Bonding.** Hydrogen bonding is the second effect which dominates polyphenol complexation, and it is thought to be of importance because of its directionality and the overall strength derived from a multiplicity of hydrogen bonds radiating from the





**Figure 4.** Cooperatively reinforced hydrogen bonding of a phenolic group with the tertiary carbonyl group of a prolyl peptide.

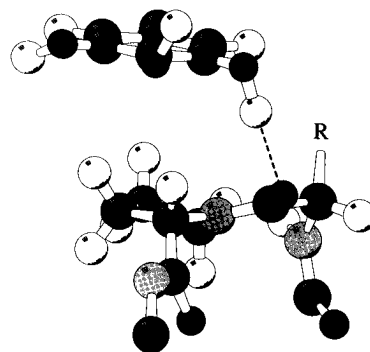
polyphenolic substrate. Molecular recognition *via* the depolymerization of hydrogen bonds in an aqueous medium is a manifestation of the eventual compromise between solute–solute interactions (*i.e.*, complexation) and the forces of solvation. Thus, potential hydrogen-bonding groups in a substrate are usually also water-solubilizing groups (*e.g.*, the phenolic hydroxyl groups in a polyphenol, the carbonyl group of a peptide) and, thus, themselves readily participate in the water matrix. Therefore, as a precondition hydrogen bonds formed by both substrates to water must first be broken; the energetics of interaction thus depends on the stability of the hydrogen bonds both *made* and *broken*. Interactions of this type may also be marginally favorable since there may be a net gain in entropy due to the release of substrate-bound water to the bulk medium upon complexation.

There is now good evidence that the carbonyl function in tertiary amides (such as prolyl peptides and caffeine) is a much more effective hydrogen bond acceptor than that in primary and secondary amides.<sup>50–52</sup> The reason for this improved binding has been suggested to be the poorer solvation of the tertiary amide function. A ligand hydrogen bonding to the prolyl group therefore requires the breaking of fewer hydrogen bonds in comparison to, say, the secondary amide group. Additionally, the methylene substituents of the tertiary amide nitrogen “donate” electrons into the peptide bond, causing it to be electron rich. The carbonyl group has thus an enhanced hydrogen bond acceptor capability. Polyfunctional hydrogen bonding networks deployed and formed cooperatively between the phenolic hydroxyl groups of the polyphenol (proton donors) and the carbonyl groups of prolyl peptides in the protein structure (proton acceptors), Figure 4, therefore contribute significantly as a second phase in the association process. In order that the possibilities for hydrogen bonding are maximised it is imperative that both substrates are conformationally mobile, hence, the time-dependent and dynamic nature of this second stage of association.

**Proline-Rich Peptides and Proteins.** Polyphenols bind most strongly to extended proteins with a high proline content.<sup>53</sup> Amongst the proteins which fall typically into this category are the following:

(i) **Salivary proline-rich proteins** [acidic (~30%), basic (~25%), and glycosylated (~17%)] are major components are parotid and submandibular saliva in humans and other mammals. Proline accounts for 25–42 residue %; in addition, there are high contents of glutamic acid (glutamine) and glycine. Together, these amino acids account for 70–88% of the amino acids in the protein.

(ii) **Caseins** are a major groups of phosphoproteins synthesized and stored during mammalian lactation.



**Figure 5.** Model of the proposed interaction between a galloyl ester group and a prolyl residue with its preceding peptide bond.

They constitute some 80% of the proteins of bovine milk. The principal caseins are  $\alpha_{s1}$ ,  $\alpha_{s2}$ ,  $\beta$ , and  $\kappa$  and contain 4.8–16.8 proline residue %.

(iii) **Collagen (gelatin)** is probably the most widely abundant protein in the animal kingdom. Its properties are remarkably diverse, and the molecule contains some 24 proline/hydroxyproline residue %.

In a comprehensive study, using high-resolution NMR, the interaction of the polyphenol  $\beta$ -1,2,3,4,6-penta-*O*-galloyl-D-glucose (**20**) with two peptides (19 and 22 amino acids in length) that are typical of the repeat unit of a salivary protein from mice has been examined.<sup>54,55</sup> This study showed that the main binding sites on the peptides are the proline residues themselves together with the preceding amide bond and the associated amino acid. The interaction has been interpreted as being principally a hydrophobically driven association between a galloyl ring and the open, flat and rigid hydrophobic surface of the pyrrolidine ring face containing the  $C_{\alpha}$  proton. Hydrogen bonding of the tertiary amide carbonyl group of the proline residue with a phenolic hydroxyl group of the polyphenol is presumed to be a secondary interaction helping to stabilise the complex, Figure 5.

Thus, proline-rich proteins (*e.g.*, gelatin, salivary proteins) which have an open, random coil type of conformation have a high affinity for polyphenols not only as a result of their extended structures but also by virtue of the presence of the proline residues themselves which, in a figurative sense, provide “sticky patches” on the protein for the phenolic nuclei of the polyphenolic substrate.

Parenthetically, the interaction of plant polyphenols with the proline-rich protein collagen in skins which forms the basis for the production of leather will, in all probability, turn out to be a special case, arising from the nature of the supramolecular assembly of collagen molecules in the collagen fibrils and fibers. The individual triple-helical collagen molecules may be regarded as stiff rodlike structures whose staggered form of organization in the collagen fiber gives rise to a liquid crystal-like supramolecular assembly containing “holes” or “gap zones”. It is these latter regions which it is thought constitute the principal sites at which polyphenol molecules may interact and embed themselves within the collagen structure (E. Haslam, unpublished observations).

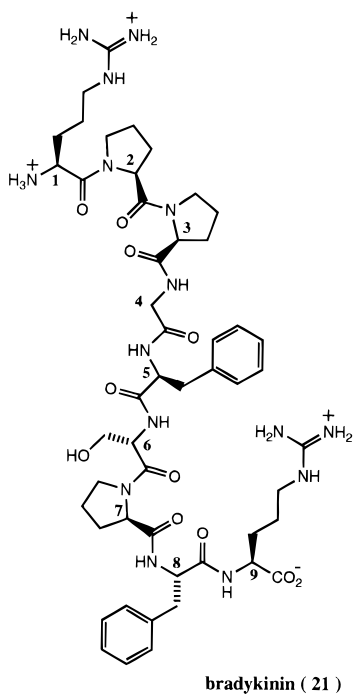
This affinity of natural polyphenols for proline-rich proteins also extends to very simple peptides which are proline-rich and/or hydrophobic in character. In this

**Table 6.** Bradykinin: Association with Polyphenols and Precipitation by Polyphenols

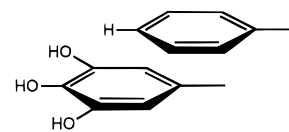
polyphenol	$K_a^a/M^{-1}$	threshold concn <sup>b</sup>
$\beta$ -1,2,3,4,6-Penta-O-galloyl-D-glucose ( <b>20</b> )	33.7	<0.2 mM
$\beta$ -1,3,4,6-Tetra-O-galloyl-D-glucose	20.5	<0.5 mM
$\beta$ -1,3,6-Tri-O-galloyl-D-glucose	4.4	<0.75 mM
procyanidin B-2 ( <b>2</b> )	8.2	~1.0 mM

<sup>a</sup> Association constants ( $K_a$ ) determined by titration of aliquots of polyphenol into a solution of bradykinin in D<sub>2</sub>O/DMSO-*d*<sub>6</sub> (4:1) at 295 K, pH 5.0, and measurement of chemical shift changes of the protons of bradykinin. The maximum chemical shift change upon saturation with the polyphenol  $\Delta\delta(\text{max})$  was calculated by a curve fitting procedure and the association constant calculated as previously described.<sup>54</sup> <sup>b</sup> The threshold precipitation values for polyphenols were those observed for the initiation of precipitation of bradykinin from a 2.0 mM aqueous solution at 295 K.

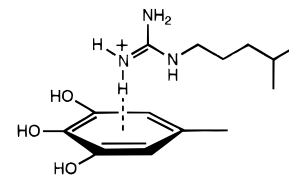
context, the association of polyphenols with bioactive peptides such as angiotensin I and II and bradykinin is of particular interest. All three peptides are, for example, readily precipitated from aqueous media by polyphenols (Table 6). Bradykinin (**21**,  $M_r = \sim 1060$ ) is



a nonapeptide (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and is released from its plasma protein precursor(s) by certain snake venoms or enzymes with trypsin-like activity. Its physiological and pharmacological activities are manifold. They include stimulation of smooth muscle, inhibition of neurotransmission in the spinal cord, the release of catecholamines in the adrenal medulla, induction of acute arterial hypotension, powerful vasodilation, increased capillary permeation, leucocyte migration and accumulation, and the initiation of pain. Bradykinin may be a mediator of conditions ranging from functional vasodilation to acute inflammation in the human body. It is well recognized that the conformation of a hormone can be related to its biological activity. In aqueous media, NMR and CD studies suggest that bradykinin is in rapid equilibrium among many conformers and does not show any persistent structural features such as  $\beta$ -turns or internal hydrogen bonds.<sup>56,57</sup> However, in DMSO the peptide

Phenylalanine residues -  $\pi$ - $\pi$  stacking

Arginyl residues - hydrogen bonding

**Figure 6.** Additional modes of complexation of polyphenols with the proline-rich peptide bradykinin.

probably assumes a more rigid conformation with  $\beta$ -bends at both the C- and N-termini, stabilized by electrostatic interactions between the two arginine side chains which are juxtaposed as a consequence of the folding pattern.<sup>58</sup>

Bradykinin is proline rich and with two phenyl residues is hydrophobic in character; its strong association with and precipitation by natural polyphenols is therefore not unexpected. Studies of the complexation of bradykinin with polyphenols have therefore been conducted (Md. Lokman Kahn, E. Haslam, T. H. Lilley, and M. P. Williamson, unpublished observations) in D<sub>2</sub>O-DMSO-*d*<sub>6</sub> (20%) using the high-resolution NMR techniques previously described.<sup>54</sup> Association constants were determined (Table 6), and these show very similar trends to those predicted from earlier work (Table 5, iii).

No evidence was obtained to show that the peptide underwent significant changes from a random coil conformation upon complexation with the polyphenols. Although the most significant proton chemical shift changes in the bradykinin were associated with each of the three proline residues and the two phenylalanine groups, suggesting that these amino acid side chains were participating preferentially in the complexation with polyphenol, it is thought to be improbable that there is a specific mode of binding between the polyphenol and peptide substrates. Rather, the driving force is visualized as the relatively unselective association of the aromatic nuclei of the polyphenol with hydrophobic groups [Pro, Figure 5; Phe ( $\pi$ - $\pi$  interactions), Figure 6] on the nonapeptide followed by secondary hydrogen bonding reinforcing this initial complexation.

The role of the side chain of the amino acid arginine (arg) in these complexations remains unclear. However, analogues of bradykinin, which lack either of the terminal arginine groups at positions 1 and 9, were significantly inferior in their capacity to bind the polyphenol  $\beta$ -1,2,3,4,6-penta-O-galloyl-D-glucose (**20**), Table 7, suggesting that these residues may also have a specific role to play in the binding of polyphenolic substrates. It is therefore interesting to note that as a result of earlier crystallographic studies of proteins Perutz and Levitt<sup>59</sup> embarked upon an investigation to discover if the energy of conventional electrostatic interactions between amino and benzene groups is strong enough to speak of a hydrogen bond. Simple energy calculations showed that there was a significant

**Table 7.** Association of  $\beta$ -1,2,3,4,6-Penta-*O*-galloyl-D-glucose (20) with derivatives of Bradykinin

peptide deriv	$K_a^a/M^{-1}$
<i>desarginyll(1)bradykinin</i> : Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	20
<i>desarginyll(9)bradykinin</i> : Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe	20.4
<i>peptide(1-5)</i> : Arg-Pro-Pro-Gly-Phe	~13.0

<sup>a</sup> See footnote a of Table 6 for information concerning association constants.

interaction between a hydrogen bond donor (like  $-\text{NH}-$  and  $-\text{NHC}(\text{NH})\text{NH}_2$ ) and the center of a benzene ring, which acts as a hydrogen bond acceptor. Hunter and his colleagues<sup>60</sup> have similarly shown that the  $\pi$ -electrons of an aromatic ring may, in certain circumstances, represent adequate hydrogen bond acceptors. In the case of an "electron rich" phenolic nucleus this would doubtless possess an enhanced hydrogen bond acceptor capability, and therefore, such interactions may be of some importance at a secondary stage in polyphenol/bradykinin and polyphenol/salivary protein interactions.

Molecular models show that the unstructured random coil of a penta/hexapeptide is sufficient to bridge two adjacent galloyl ester groups in a substrate such as  $\beta$ -1,2,3,4,6-penta-*O*-galloyl-D-glucose (20). This minimum structural feature for bidentate binding is borne out by the relatively strong complexation of the bradykinin analogue—peptide 1–5—with  $\beta$ -1,2,3,4,6-penta-*O*-galloyl-D-glucose, Table 7.

Clearly, it would be now of considerable interest to ascertain if and how polyphenols may modify the *in vivo* physiological actions of bradykinin.

## Epilogue

Insofar as the possible modes of action of natural polyphenols as drugs and medicines are concerned, there is clear evidence that they have the potential to act in the three general areas specified earlier (*i.e.*, transition metal ion complexation, as antioxidants in cellular prooxidant states, and by association with proteins and peptides). What is urgently required at this juncture is evidence concerning their absorption, penetration, and metabolism in the human body. Until such time the evidence for their remedial effects remains based upon epidemiological evidence rather than scientific observation.

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