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When quinones meet amino acids: chemical, physical and biological consequences

Review Article

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Summary. Quinones and amino acids are usually compartmentally separated in living systems, however there are several junctions in which they meet, react and influence. It occurs mainly in wounded, cut or crushed plant material during harvest, ensiling or disintegrating cells. Diffusing polyphenols are oxidized by polyphenol oxidases (PPOs) to quinonic compounds, which associate reversibly or irreversibly with amino acids and proteins. The reaction takes place with the free nucleophilic functional groups such as sulfhydryl, amine, amide, indole and imidazole substituents. It results in imine formation, in 1,4-Michael addition via nitrogen or sulphur and in Strecker degradation forming aldehydes. The formation and activity of quinone–amino acids conjugates influences the colour, taste, and aroma of foods. Physical and physiological phenomena such as browning of foods, discoloration of plants during processing, alteration of solubility and digestibility, formation of humic substances, germicidal activity, cytotoxicity and more occur when quinones from disintegrating cells meet amino acids. The mechanisms of toxicity and the pathways by which PCBs may be activated and act as a cancer initiator include oxidation to the corresponding quinones and reaction with amino acids or peptides. Sclerotization of insect cuticle is a biochemical process involving also the reaction between quinones and amino acid derivatives.

Keywords: Quinones – Amino acids – Polyphenols – Polyphenyloxidase – Browning of foods – Catechol amino acid adducts

1. Introduction

Astrobiologists at the Astrochemistry lab at NASA Ames demonstrated recently that amino acids and quinones are two of the compounds generated upon ultraviolet light irradiation of deep-space-like ''ices'', simulating conditions that are commonplace in interstellar space (Dworkin et al., 2002). Both group of molecules are of importance in current living organisms and play essential roles in the metabolism of life on Earth and maybe on other planets.

The quinones and the amino acids are usually compartmentally separated and do not cooperate in living systems. They act in parallel and not with each other. However there are several junctions in which they meet, react and influence. It occurs mainly in wounded, cut or crushed leaves during harvest, ensiling or disintegrating cells. Various diffusing phenols are than oxidized by polyphenol oxidases (PPO) and the resulting quinonic compounds associate reversibly or irreversibly with the amino acids. Many physical and physiological phenomena, such as enzymic browning of foods, discoloration of various plants during processing, alteration of solubility, changes in digestibility and in phatogenic viruses infectivity, formation of humic substances, allergic contact dermatitis and cutaneous inflammation, eye cataract formation, germicidal activity, cytotoxicity and more, were found to be connected to the formation and activity of quinone–amino acids conjugates.

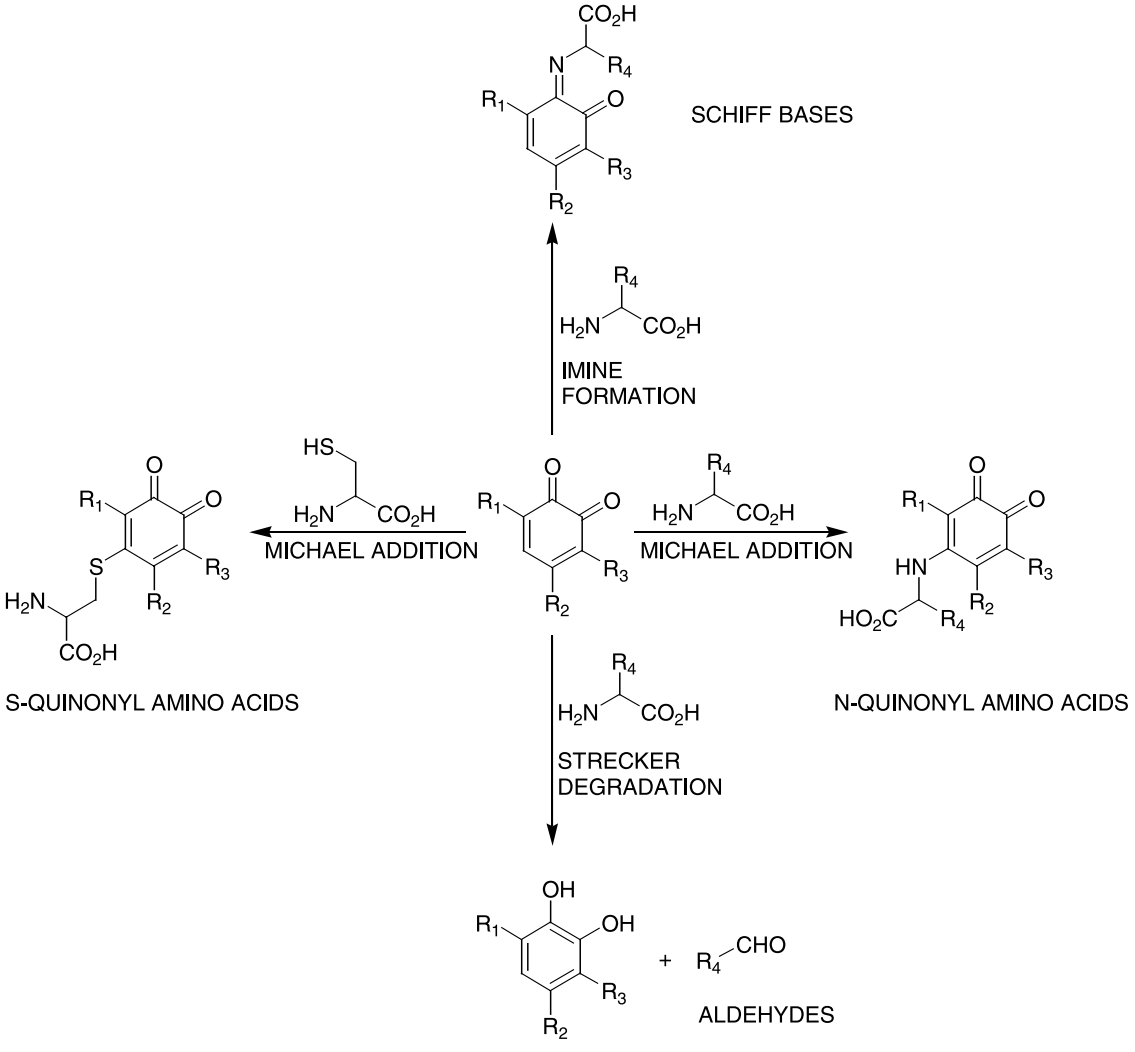
Moreover, the colour, flavour and aroma of various natural products as tobacco, coffee, cocoa, tea, wine and others are affected by similar reactions of quinones and amino acids. The toxicity of PCB's to animals and in exposed human populations is the result of the oxidation of its hydroquinonic metabolites to quinones. It was found that PCB–quinone–amino acids adducts play an important role in the in vivo rat liver cancer initiation. Sclerotization of insect cuticle is an extremely important biochemical process in which internal chemical interactions between structural proteins, catechols and oxidative enzymes form catechol–amino acids adducts which are responsible for the physical properties of the mature exoskeleton.

This review tries to enlighten some of these activities and phenomena that occur when quinones meet amino acids.

2. PPO and enzymic browning of food

Processing conditions of various natural products has a profound influence on the colour, aroma and flavour of the end products. The mechanism of enzymic browning of foods, which in most cases affects adversely the quality of food and its control, was lately reviewed (Pilizota and Subaric, 1998). An important factor was found to be the polyphenol oxidation reaction between free amino acids, peptides or proteins and quinonic compounds. The later compounds are formed in wounded, cut or crushed leaves during harvest, ensiling or disintegrating cells. Polyphenol oxidases (monooxygenase, PPO) are ubiquitous copper metalloenzymes of angiosperms, which catalyze the oxidation of the colourless phenols to coloured quinones at the expense of O_2. More specifically these enzymes catalyze the o-hydroxylation of a monophenol followed by its oxidation to the o-diquinone (cresolase activity) or the oxidation of an o-dihydroxyphenol to the o-diquinone (catecholase activity). PPO is present in many organs and tissues but is released to the cytosol only upon wounding, senescence or deterioration of the organelle.

The quinonoid reaction products formed by PPO are highly reactive electrophilic molecules, which undergo a series of non-enzymatic secondary reactions to yield brown polymeric pigments. The o-quinones react with themselves or act to covalently modify and crosslink a variety of cellular nucleophiles. They react with functional groups of proteins or amino acids such as sulfhydryl, amine, amide, indole and imidazole substituents. Primary products are Schiff bases, N-quinonyl derivatives, S-quinonyl

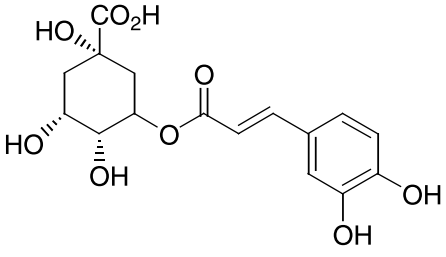


Scheme 1. Possible primary, non-enzymatic reactions that may occur when oxidized polyphenols meet amino acids

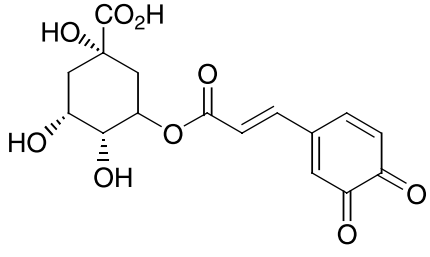
derivatives and simple aldehydes evolving from the Strecker degradation (Yaylayan, 2003) (see Scheme 1). The oxidative degradation at high temperatures, of α-amino acids by 1,2-dicarbonyls and o-quinones is known as the Strecker degradation in food science (Schönberg and Moubacher, 1952). The importance of the Strecker degradation is based on its ability to produce structurally related aldehydes (with one carbon atom less than the α-amino acids, 'Strecker aldehydes') and 2-aminocarbonyl compounds, both critical intermediates in the generation of aromas in processed foods. The formation of quinone adducts (usually brown or black colored) is the reason of pigmentation of various living tissues both in the plant and mammal kingdom. It usually represents the primary detrimental effect of PPO in post harvest physiology and food processing and is the primary reason for the interest in PPOs in food technology.

Chlorogenic acid is the major polyphenol in foods derived from plants and is a good substrate for PPO. Chlorogenic acid quinone (CQA-Q, see Fig. 1), which is the oxidative product of chlorogenic acid by PPO, is an important intermediate compound in enzymic browning. CQA-Q was lately prepared, and its properties and the relationship with browning were examined (Murata et al., 2002). It was shown that in addition to the reaction of CQA-Q with amino acids, H_2O_2 might also play an important role in the formation of the brown colour by enzymic browning.

In order to prevent the deteriorating effect of enzymic browning, various inhibitors are applied during processing and storage of foods. The inhibition of enzymic browning generally proceeds *via* the following three mechanisms: (1) direct inhibition of PPO, (2) non-enzymic reduction of the o-quinones to derivatives of o-diphenols and (3) chemical modification or removal of phenolic substrates of PPO. Various sulfite or ascorbate additives used in the food, wine and beverage industry are the most efficient multifunctional agents in control of enzymic browning.



CHLOROGENIC ACID



CHLOROGENIC ACID QUINONE

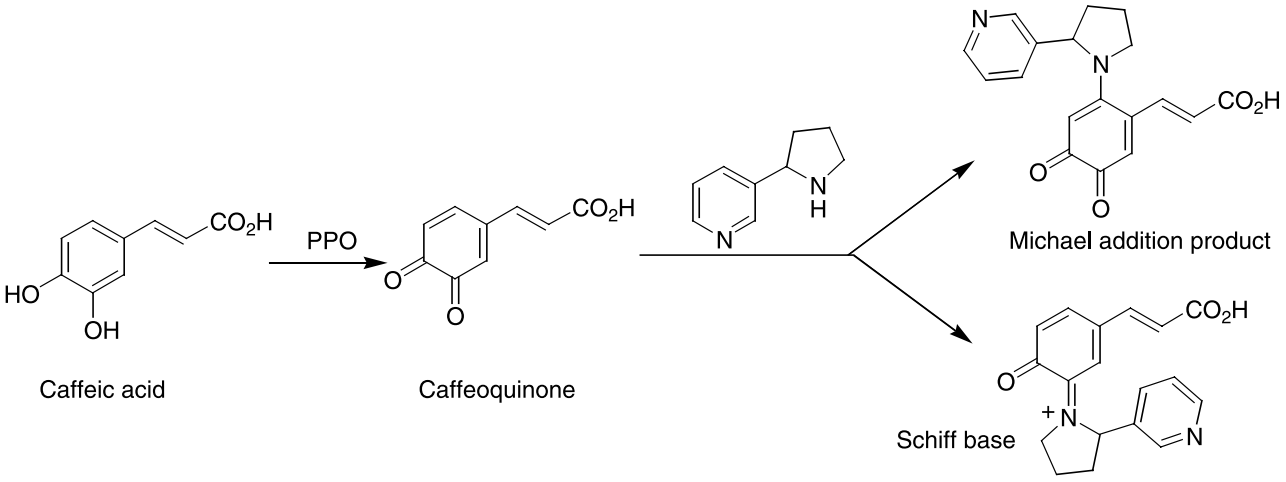
Fig. 1. Chlorogenic acid and its quinone

The amino acid cysteine can act as an inhibitor of enzymatic browning. Thus, the effect of cysteine and cysteine-quinone addition compound on apple PPO activity was investigated by HPLC and polarography, using 4-methylcatechol chlorogenic acid (CG), and (−)-epicatechin (EC) as substrate (Richard-Forget, 1992). Cysteine at a higher concentration prevented colour development by trapping o-quinones as colorless addition compounds while at low amounts the o-quinones formed in excess co-oxidized the cystein-quinone conjugates, leading to a phenol regeneration with a deep colour formation.

3. Colour, flavour and aroma of tobacco

The colour of dried tobacco leaves depends on the drying time and conditions. Artificial drying, yielded lemon yellow, sun drying gave orange-yellow, and shadow drying gave brownish red colors (Yunoshev, 1957, 1958). Increase in drying time causes accumulation of orange, brown and red colours. The changes from yellow to darker colors were caused by the oxidation of polyphenolic substances. In an aqueous extract of tobacco, the content of the non-oxidized polyphenolic substances decreased with the increase of the drying time. The chlorogenic and caffeic acid content in the red-brownish tobacco was 4–7 times smaller, than in the lemon yellow one. Accumulation of quinonoid substances increased the oxidative deamination process (Strecker degradation) of amino acids. During post-harvest processing of tobacco, proteins are hydrolysed, decreasing the content of bound amino acids from 19 to 12 dry-wt.% of the protein fractions. In addition to their reaction with the quinones, the released amino acids react also with sugars and aldehydes forming melanoidins and other products contributing to the desirable colour, flavour and aroma of processed tobacco (Devdariani and Labartkava, 1986).

Direct proof of the involvement of PPO in the oxidation process was obtained. Thus, PPO preparations from green tobacco leaves oxidized chlorogenic and caffeic acids to o-quinones, which reacted in vitro with benzenesulfinic acid to form the appropriate sulfones (Chenikov et al., 1975). The o-quinones react also with nornicotine to



Scheme 2. Oxidation of caffeic acid and formation of a Schiff base and Michael addition product

produce a red color absorbing at 570 nm (Weeks et al., 1993, 1995). Asparagine, alanine, glutamic acid and also nicotine, myosmine and pyrrolidine, failed to react with a mixture of chlorogenic acid and PPO. Similar red color was obtained in aqueous-ethanolic extracts of tobacco grade FR and cherry red tobacco (CR) but a brown color from orange tobacco grade F. Analysis showed that CR and FR grade tobaccos contained higher levels of nornicotine than F grade tobacco. Model chemical reactions of nornicotine with o- and p-quinones indicated both Schiff base formation and Michael-type addition (Scheme 2).

4. Colour, flavour and taste of tea

Tea is grown in about 30 countries and consumed worldwide. It is the most widely consumed beverage excepting water. Tea is manufactured in three basic forms: green tea, which is prepared in such a way as to preclude the oxidation of green leaf polyphenols; black tea, in which production oxidation is promoted so that most of these substances are oxidized, and oolong tea which is a partially oxidized product (Graham, 1992). Factors affecting tea yield and tea quality include geography, climate, leaf size, and processing methods. Black tea accounts for almost 80% of the world's tea production and is the most important source of polyphenol in the world. However, little is known about the chemistry of black tea polyphenols due to their complexity. Fresh tea leaves are unusually rich in the flavan-3-ol group of polyphenols known as catechins which may constitute up to 30% of the dry leaf weight. Other polyphenols include theaflavins and thearubigins and their glycosides, and depsides such as chlorogenic acid, coumarylquinic acid and one unique to tea, theogallin (3-galloylquinic acid). In addition to the normal complement of plant cell enzymes, tea leaves contains an active PPO which catalyzes the aerobic oxidation of the catechins when the leaf cell structure is disrupted during black tea manufacture. The catechin quinones formed during oxidation, initiate the formation of many of the hundreds of volatile compounds found in the black tea aroma fraction.

Tea has been particularly associated with decreased risk of various proliferative diseases such as cancer and atherosclerosis in humans (Demeule et al., 2002). Various studies have provided evidence that the polyphenols are the strongest biologically active agents in green tea. Recent observations have raised the possibility that green tea catechins, in addition to their antioxidative properties, also affect the molecular mechanisms involved in angiogenesis, extracellular matrix degradation, regulation of cell death and multidrug resistance.

It is evident that the value of a tea depends on the aroma of its infusion, which varies with the age of the leaves, the development of the plant and the methods employed for the preparation of the leaves (Protopopescu, 1937). There are great differences in the quality of the products even during the different harvests in one year. Buds and first leaves give the best tea. Proper rolling, fermentation and roasting produce important modifications in compounds and enhance the value. The classical fermentation treatment of tea is an enzymic oxidation process in which the polyphenol constituents of tea such as (−)-epigallocatechin and its gallate are oxidized to the corresponding o-quinones. The various quinones produced by the enzymatic oxidations (catechin quinone,

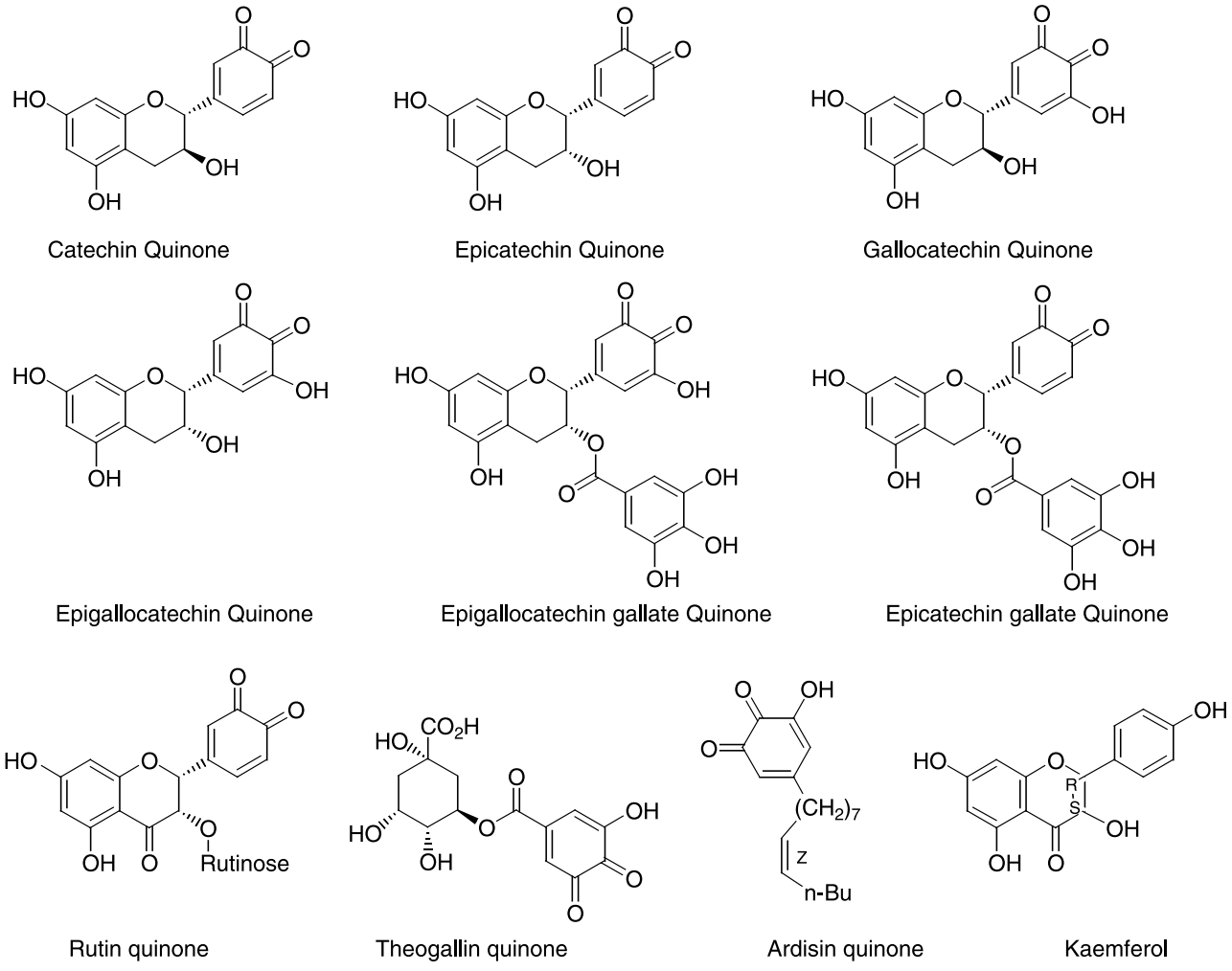


Fig. 2. Polyphenyl quinones in tea

epicatechin quinone, gallocatechin quinine etc.) (Fig. 2) undergo condensation reactions, which result in a series of compounds, including bisflavanols, theaflavins, epitheaflavic acids, and thearubigens (Fig. 3). These compounds together with essential oils and some amino acids impart the characteristic taste, flavour and color properties of black tea (Chang, 1975). The same quinones can be obtained by chemical oxidation of the polyphenols with tetrachloro-o-benzoquinone (Korver et al., 1973). This chemical reaction produces new absorptions at 474 nm or at 372 nm, which can be used for spectroscopic identification.

The more advanced heat treatment proved superior to the older fermentation processes. In the heat treatment those compounds, which give tea its flavor and bouquet (e.g. tannins and quinones) are not destroyed or rendered insoluble as in the fermentation process (Bokuchava, 1968). Moreover, the vitamin P content in heat-treated leaves remains at 50–70 mg/g, which satisfy the daily human requirement. Flavour-active substances originate from: (1) formation of terpenes by various enzymic reactions, (2) formation of hexanal, 2-hexenal, and 3-hexenol by enzymic oxidation of tea glycolipids, (3) Strecker degradation of amino acids by quinones, (4) transformation of carotenoids by oxidative cleavage and cyclization reactions, (5) secondary transformations of the above types of substances (Lai et al., 1987).

In several varieties, used for tea preparation, other phenolic compounds are the dominant oxidizable compounds. The leaves of *A. compressa* (AC) have been used in folk medicine to treat various liver disorders including liver cancer. The total polyphenol content, antioxidant capacity and quinone reductase activity of *A. compressa* tea were characterized and determined in comparison to mate (MT) and green (GT) teas. The major polyphenols in AC were not catechins but gallic acid, epicatechin gallate, ardisin

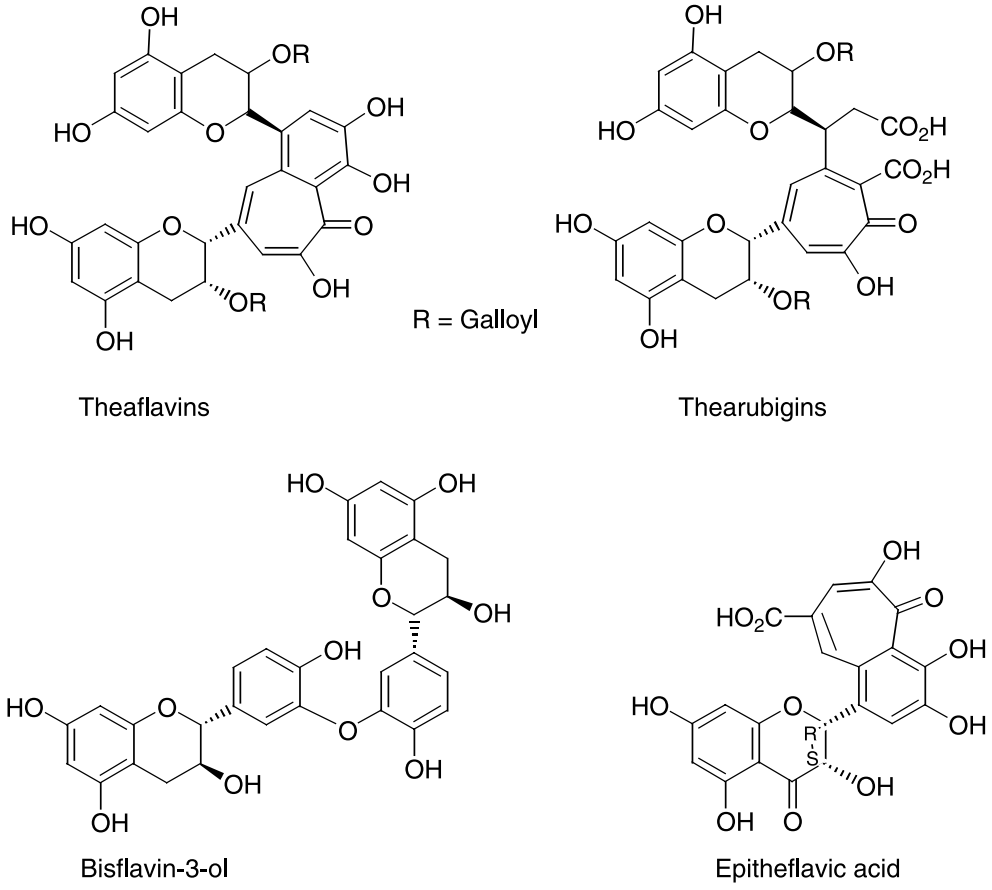


Fig. 3. General structure of theaflavins, thearubigins, bisflavanols and epitheflavic acid

and kaempferol (Fig. 2). The total polyphenol value of AC was significantly lower than GT and MT and the antioxidant capacity correlated with total polyphenol values (Chandra and Gonzalez de Mejia, 2004).

Experimentation with amino acids began already in 1954 (Bokuchava et al., 1954). Addition of different amino acids to hot aqueous solutions of tea tannin, led to the development of various odors. Phenylalanine produced a rose-like odor, glutamic acid or alanine gave a "flower-like" odor, tryptophan and tyrosine yielded an unpleasant odor, norleucine gave a spicy odor, threonine gave a wine-like odor, and cystine produces no odor at all. As no aroma was developed when an aldehyde-binding reagent is added (e.g. dimedone), it was deduced that aldehydes, formed *via* oxidation of the amino acids by the quinones, are the odor-bearing substances. A later report (Mayuranathan and Gopalan, 1966) states that no reaction and no aroma formation was observed between solutions of black tea or extracted tea quinones with aspartic acid or phenylalanine in brews at 70°–100°. However, a short discussion contradicting this conclusion was later published as a letter to the editor (Sanderson, 1966), stating that tea quinones do cause oxidative deamination of amino acids during tea fermentation. A bioelectrode, consisting of an oxygen electrode and a biocatalytic membrane containing L-amino acid oxidase, was used to measure amino acid concentrations in tea infusions (Horie et al., 1993). The values determined by this method were highly correlated with those determined by conventional colorimetric methods. Since high-quality teas show high total amino acid values, this enzyme electrode can be used for the evaluation of the quality of tea.

In vitro model fermentation experiments using purified catechins were very useful in elucidation the structure of some novel oxidation products of theaflavins in black tea pigments. (Tanaka and Kouno, 2003). Production and accumulation of catechin dimer quinones (Fig. 4) during tea fermentation were chemically confirmed by trapping it as phenazine derivatives (Tanaka et al., 2002). Treatment of fermented tea leaves with o-phenylenediamine yielded phenazine derivatives of the epigallocatechin dimer and its galloyl esters, in which two flavan units were linked at

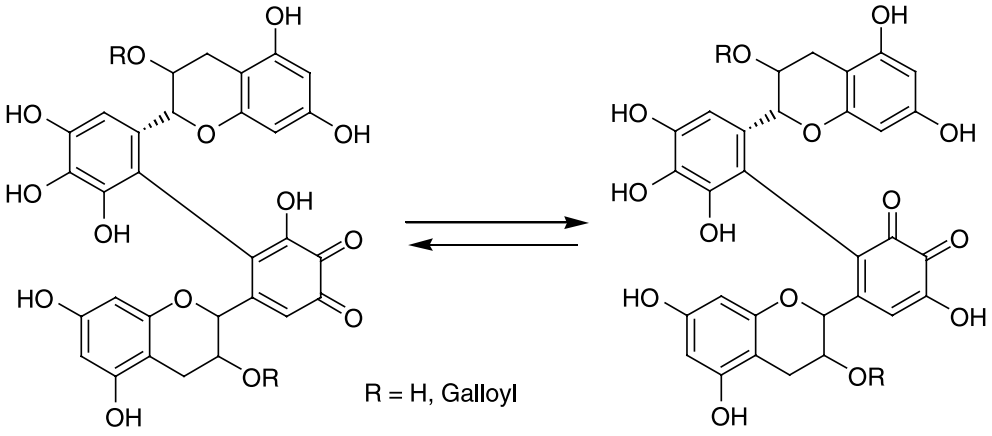


Fig. 4. Catechin dimer quinones

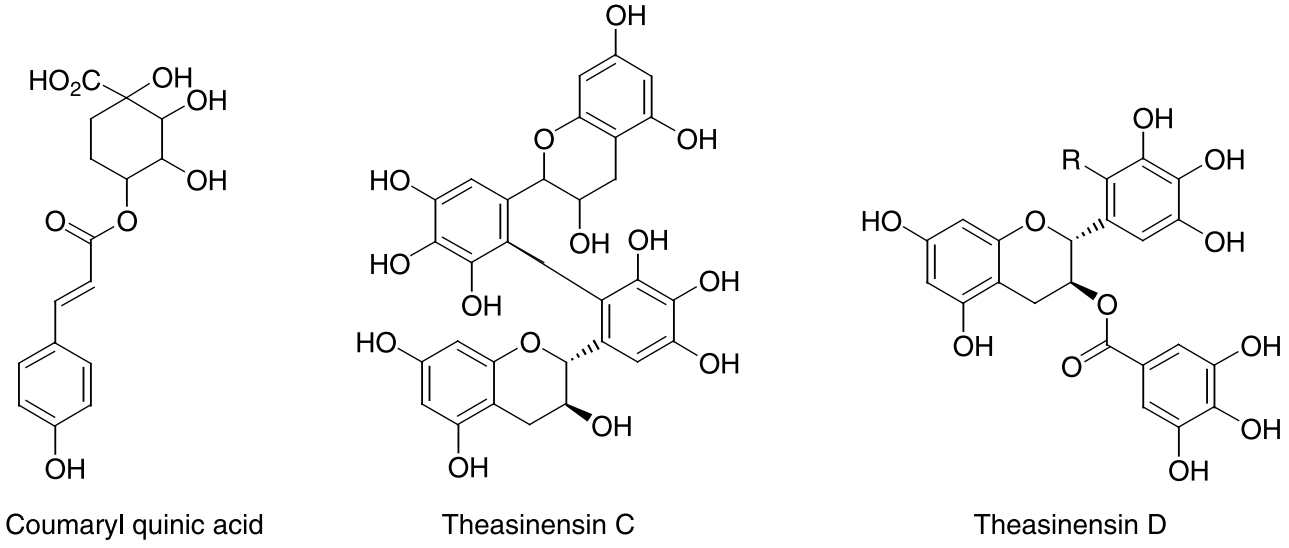


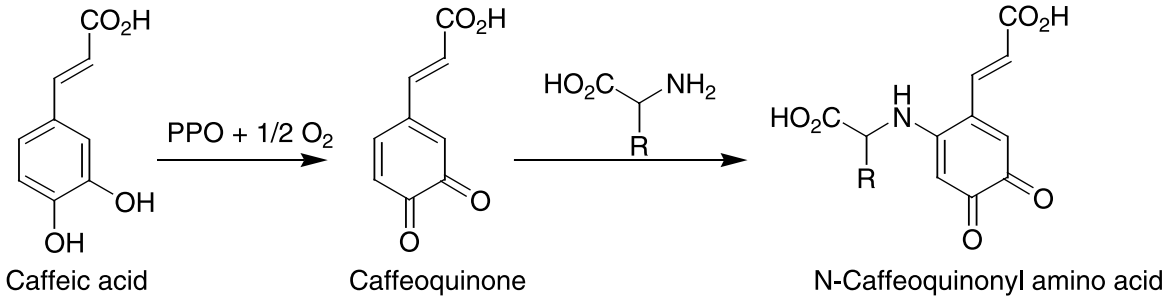
Fig. 5. Coumaryl quinic acid and the Theasinensins

the B-rings through a C–C bond. Phenazine derivatives of monomeric quinones of epigallocatechin were not isolated. When fermented tea leaves were heated, the quinone dimers were converted to theasinensins (Fig. 5), which are constituents of black tea, suggesting that the asinensins are generated by reduction of the quinone dimers during the heating and drying steps in black tea manufacture.

5. Colour, flavour and taste of cocoa and coffee

During cocoa fermentation, the phenolic compounds originally compacted into vacuoles of specific cells diffuse through the cotyledon. The phenols are oxidized and the resulting quinonic compounds associate reversibly with proteins by hydrogen bonds or, irreversibly, by condensation with the nucleophilic groups of amino acids, peptides, proteins and polysaccharides. The total phenols in cocoa is reduced during fermentation to 30% of the initial value and the (−)-epicatechin (Fig. 2), principal substrate of cocoa PPO, is reduced by 90%, with a proportional increase in catechin content. The PPO activity in ungerminated beans, are correlated to oxygen uptake. Thus, the addition of some amino acids or peptides (e.g. L-proline or glycylglycine) after the stage of quinone formation, exert a strong influence on oxygen uptake (Purr et al., 1960, 1964).

Development of the typical intense flavour of cocoa requires a controlled high-temperature roasting step. Cocoa



Scheme 3. 1,4-Addition reaction of caffeoquinone with amino acids

roasting is usually carried out at temperatures between 110 and 200 °C for approximately 45 minutes. The wonderful chocolate flavour arises from a balanced combination of 400–500 compounds such as aldehydes, ethers, thiazoles, phenols, sulfur compounds, pyrazines, ketones, alcohols, furans and more, which forms during roasting, from the flavour precursors produced by fermentation. Roasting induces the Maillard-type reaction, which starts from a condensation of carbonyl group of sugar and amino group of amino acid and amino residue of peptide or protein, and develops to a complex reaction network and produces variety of volatiles, color compounds and polymers, which are collectively referred to as Maillard reaction products. The first stage of the Maillard reaction consists of the reaction between the carbonyl group of the sugar with the amino group of the amino acid, to form a Schiff's base, which then rearranges to an amino ketose, also called the Amadori product. The intermediate stage involves the degradation of the Amadori product, which gives a variety of reactive compounds. These compounds react further in the final stages of the Maillard reaction, which is condensation to give high molecular weight polymers known as melanoidins. Roasting also brings about the oxidation of polyphenolic compounds to quinones, which also react with amino acids and peptides. Indeed, the process reduces the amino acid levels by *50%*. Aerobic fermentation of cocoa seeds results in release of polyphenols, peptides and amino acids. In the presence of oxygen the polyphenols react with the amino acids (quinone tanning reaction) thus reducing the amount of amino N compounds in the hydrolysate (Biehl, 1967). Insolubilization of cacao proteins during processing or fermentation is also attributed to quinone tanning of the proteins by the quinones and the reaction with free amino groups (Biehl, 1963).

As in the case of tea, the colour intensities of extracts of different coffee brands is a result of different activities of PPO. The enzyme oxidizes the chlorogenic acid to an o-quinone, which in turn react with amino acids (Scheme 3) giving rise to the different colour intensities (Melo and Amorim, 1975). The green colour development in coffee beans was studied using model systems. It was found that caffeic acid or its ester is oxidized by PPO to caffeoquinone which reacts with glycine methyl ester to produce a green colour with maximum absorbance at 690 nm. Reaction of the quinone with free amino acids (e.g. glutamic or aspartic), peptides or proteins, form a blue pigment which in the presence of excess caffeoquinone, becomes a green pigment (Guyot et al., 1988). The coloured product detracts from the appearance and can impact the taste of the coffee.

6. Colour and flavour of wines

Polyphenols in wine predominantly arise from the grapes and are primarily flavonoids including chlorogenic, isochlorogenic, caffeic, shikimic, and p-coumaric acids (Fig. 6). They are crucial components in wines because of their basic importance to the colour and flavour. Flavonoids (catechines and proanthocyanidins) are also responsible for discolouration, turbidity and flavour changes in wines. There is a big difference between oxidized and non-oxidized wine. Chemical and enzymatic oxidation of phenolic compounds lead to the formation of highly reactive quinones, which interact with amino acids and peptides to reduce sugars and alcohol and give rise to chromophores and volatile substances. Interaction of polyphenols and amino acids in wines, under various oxygen conditions yielded both aminophenols and aminoquinones (Nilov et al., 1973). Both enzymic and non-enzymic oxidation studies with wine phenols showed that ascorbic acid, but not glutathione, acts as a direct reductant for the PPO-generated o-quinones. These quinones react with nucleophiles such as glutathione and phloroglucinol to produce adducts with regenerated hydroquinone structure and lowered oxidation-reduction potential compared to the original phenols (Singleton and Cilliers, 1995).

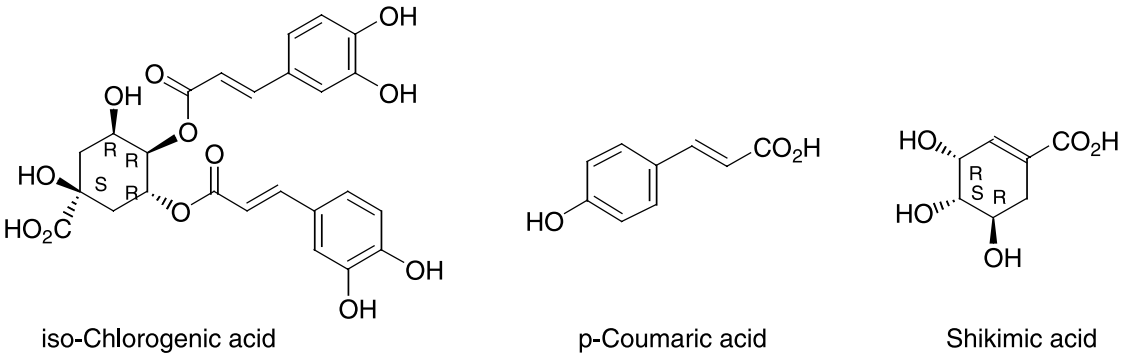


Fig. 6. Some polyphenols from wine

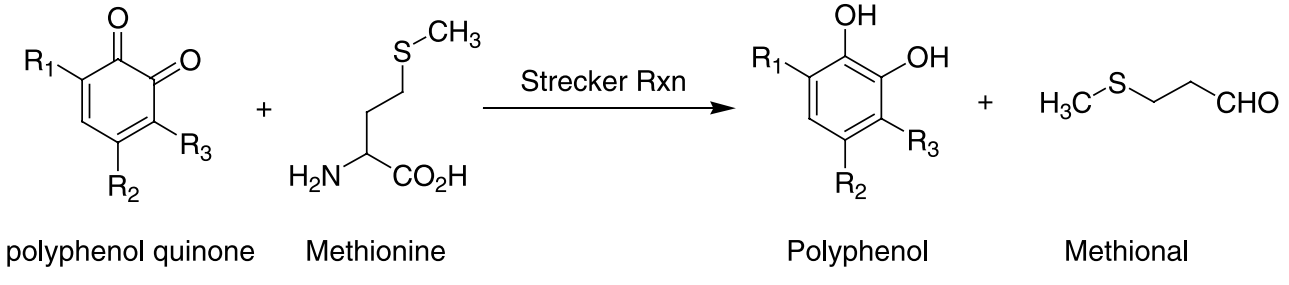
Although glutathione in high proportion prevents browning, it stimulates oxygen uptake in the reaction with caffeic acid. Trapping of primary quinones by glutathione competes with reactions leading to must discoloration but maintains high levels of oxidizable compounds especially flavan-3-ols, which serve as browning precursors in wine (Cheynier et al., 1995).

The quinones also convert ascorbic acid to dehydroascorbic acid and deaminate some amino acids. This results in loss of freshness, yellowing or browning of the wine and turbidity. In wine production, must and young wines are protected from oxidation by air and H_2SO_3 is held at 10–25 mg/l (Hennig and Burkhardt, 1957).

In champagne production by continuous method when oxygen was present in excess (up to 4.4 mg/l), polyphenols were oxidized to quinones and these oxidized amino acids. This led to an enrichment of surface-active materials, giving better led to an enrichment of surface-active materials, giving better taste, bouquet, sparkle and foam (Rodopulo, 1961).

Methional was found to be an important odorant. Thus, in oxidized wines methional can reach more than 200 Odor Units, whereas in non-oxidized samples, it was not possible to detect methional. It was proposed that a Strecker degradation of methionine is the most likely pathway for forming the unpleasant odorant methional from methionine and not the direct peroxidation of methionol. Oxidation of wine ortho-diphenols, form the dicarbonyl compounds ortho-quinones, which induce the Strecker reaction of amino acids (Escudero et al., 2000).

Consequently, the removal of polyphenols is a common practice in winemaking to prevent discolouration and/or to obtain stabilization against oxidation, especially in white and rose wines. Wine technology tends towards adoption of systems for extraction of the must, which limit the solubilization of oxidizable substances and toward the use of fining (gelatin and casein), adsorbants (active carbon) and polyvinylpolypyrrolidone. The use of these additives in wine production is aimed at reducing the level of polyphenols and compounds that catalyze their transformation, particularly enzymes and heavy metals. In order to limit the amount of treatment with chemical-physical agents, it has been suggested that enzymes active against polyphenols (oxidases, hydrolases and transferases) be used in the prefermentative phase. The aim of this is to set off a process of enzymatic oxidation of polyphenols in the must under controlled conditions, so as to destabilize them and accelerate the process of polymerization and flocculation. The possibility of eliminating or achieving reactivity loss of the polyphenols responsible for wine instability



Scheme 4. Formation of methional by the Strecker reaction

has been tested on the bench and pilot scale using tannase, phenolase and laccase. The laccase was more effective than the other enzymes tested and produces a wine with a more stable colour. The wines treated with laccase had different and better organoleptic madeirization-resistant features. This enzymatic treatment is highly effective, preferable, or practically identical to traditional processing, especially when using must without the addition of sulfur dioxide. (Winetech, project title IWBT 1/12).

7. Discoloration of leeks, cauliflower, potato and other plants

Wounded, cut or crushed plants during harvest, ensiling or disintegrating cells all are characterized by discoloration. In most cases, the discoloration results from polyphenols oxidation, which is followed by the reaction of the quinones with amino acids. Here, we will describe several interesting examples.

a) The edible parts of **leeks** (*Allium porrum*) were analyzed for the possible causes of discoloration during processing. The red pigment formed is water soluble and unstable in neutral or alkaline media. The absorption spectrum peaked at 520 nm and the minimum was at 460 nm. Three phenols were isolated from the etheric extract. Evidently after cell rupture, enzymic oxidation of the phenols yielded the quinones, which then reacted with amino acids to yield the pink-red pigment (Korner and Berk, 1967).
b) The non-enzymatic browning of **cauliflower** homogenate was evaluated during storage at temperatures ranging between 40–80 °C. The browning followed 1st order kinetics at higher temperatures and zero order at lower temperatures. Results of model experiments showed that the discoloration is due to the reaction of free amino acids and other amine derivatives with quinones produced by oxidation of natural polyphenols. Red primary condensation products were transformed into brown pigments by subsequent secondary reactions (Pokorny et al., 1975).
c) Black spot related pigments were partially purified from bruised tubers of **potato** cultivars. Chemical characterization showed that these pigments consist of protein and a relatively small amount of covalently bound constituents. Chlorogenic acid may take part in the black spot formation, but is not essential for the discoloration. It was suggested that the pigments are products of unregulated reactions between nucleophilic amino acid residues in proteins and quinones. The quinones are presumably derived from endogenous substrates of PPO in disintegrated cells (Stevens and Davelaar, 1996).
d) The peroxidase- and PPO-catalyzed oxidations of (+)-catechin yield several products showing different degrees of polymerization, which are apparently responsible for the pigment decay and the associated browning reaction that occurs in processed **strawberry fruits** and their derived foods. It was suggested that brown polymer formation, in strawberries is mainly due to PPO, although peroxidase also plays an important role. It is apparently auto-regulated by product (dehydrodicatechin A) inhibition (Lopez-Serrano and Barcelo, 2002).
e) Oxidant air pollutants, induces discoloration on foliage of susceptible plants. In one study, plants of *Phaseolus vulgaris* humlis L. with nearly fully expended leaves were treated wit ozone and examined for presence of induced pigments (Howell and Kremer, 1973). The pigments formed were polymers with molecular weight range of 10,000 to 26,000. It was postulated that the damage to the membrane permitted phenols and phenolase to react and to produce quinones. The quinones thus formed, polymerise with amino acids and proteins. This oxidative process by air pollutants reduces nutritional values in leafy food and forage crops.
f) PPO is also involved in the browning reaction of **red clover** leaves, when cut or crushed and exposed to air. PPO starts the browning process by oxidizing endogenous phenols to quinones, which contain electrophilic sites. These sites react with nucleophilic sites of other compounds such as amino acids or proteins. In a series of experiments it was shown that PPO activity of leaf tissue extracts, is reflected in the extent of quinones (or phenols) binding to amino acids or proteins (Lee et al., 2004).
g) The foliage and fruit of the **tomato** plant *L. esculentum* contains PPO and peroxidases (POD) that are compartmentally separated from o-dihydroxyphenolic substrates in situ. However, when leaf tissues are damaged by insect feeding, the enzyme and phenolic substrates come in contact, resulting in the rapid oxidation of phenolics to o-quinones. When the tomato fruitworm *Heliothis zea* or the beet armyworm *Spodoptera exigua* feed on tomato foliage, substantial amounts of the ingested chlorogenic acid is oxidized in the insect gut, by PPO, to chlorogenoquinone (Fig. 1). The digestive enzymes of the fruitworm have the potential to further activate foliar oxidase activity in the gut. Chlorogenoquinone is a highly reactive electrophilic molecule that readily binds covalently to nucleophilic groups of

amino acids and proteins. In particular, the –SH and $–NH_2$ groups of amino acids are susceptible to binding or alkylation. In experiments with tomato foliage (Felton et al., 1989), the relative growth rate of the fruitworm was negatively correlated with PPO activity. As the tomato plant matures, foliar PPO activity may increase nearly 10-fold, whereas the growth rate of the fruitworm is severely depressed. In tomato fruit, the levels of PPO are highest in small immature fruit but are essentially negligible in mature fruit. The growth rate of larvae on fruit was also negatively correlated with PPO activity, with the fastest larval growth rate occurring when larvae fed on mature fruit. The reduction in larval growth is proposed to result from the alkylation of amino acids/protein by o-quinones, and the subsequent reduction in the nutritive quality of foliage. This alkylation reduces the digestibility of dietary protein and the bioavailability of amino acids. This mechanism of digestibility reduction may be extrapolatable to other plant-insect systems because of the ubiquitous co-occurrence of PPO and phenolic substrates among vascular plant species.

The infectivity of the nuclear polyhedrosis virus, HzSNPV, to *Heliothis zea* was significantly reduced when viral occlusion bodies were exposed to the plant phenolic chlorogenic acid in the presence of PPO (Felton and Duffey, 1990). Chlorogenic acid is rapidly oxidized to the o-quinone, chlorogenoquinone by foliar PPO of the tomato plant, *Lycopersicon esculentum*, when foliage is damaged during feeding by larval *H. zea.* Chlorogenoquinone, a powerful oxidizing agent, covalently binds to the occlusion bodies of HzSNPV and significantly reduces their digestibility and solubility under alkaline conditions. This binding is proposed to interfere with the infection process by impairing the release of infective virions in the midgut.

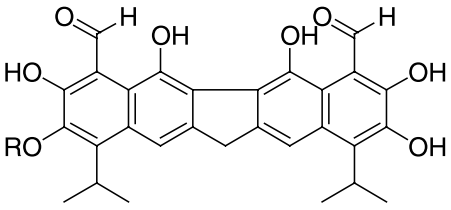
The same authors later discussed the importance of phenolics and phenolic oxidizing enzymes in determining protein quality to insect herbivores. They showed that treatment of selected dietary proteins (i.e. casein, soy protein, gluten, zein, and tomato foliar protein) with chlorogenic acid and PPO significantly reduced protein quality to *S. exigua* larvae, as measured by larval growth rate (Felton et al., 1992). The reduction in growth was negatively correlatable with the content of lysine, histidine, cysteine, and methionine for each protein. These amino acids contain nucleophilic centers (e.g. –SH, $–NH_2$) that are susceptible to alkylation by quinones formed from the enzymic oxidation of chlorogenic acid. Treatment of the proteins with chlorogenic acid and PPO caused significant losses in alkylatable amino acids (i.e. cysteine, histidine, methionine, and lysine). The amount of chlorogenic acid bound to each protein was significantly increased by PPO activity and was dependent upon the content of the alkylatable amino acids.

Effects of orthoquinone alkylation of amino acids on the toxicity of an important microbial insecticide, *B. thuringiensis kurstaki* (BTk), to larval *H. zea* were also studied (Ludlum et al., 1991). BTk Incubated with these phytochemicals and fed to larvae was more toxic than untreated BTk. Digestibility experiments suggest that alkylation enhanced the solubilization and/or proteolysis of crystal proteins in vivo, altering digestibility and pathogenic viruses infectivity.

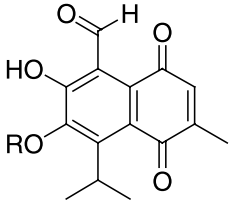
h) Quinones are found in all parts of the **kok-saghyz** plant except the resting dormant seeds and the latex. The seeds lack quinones owing to absence of PPO. The concentration of quinones rises as the seeds sprout and is maximum in about 6-day plants in sunlight The highest levels of PPO are found in leaf areas, followed in order by root dermal layers, latex, and root matter. The quinones are not found if trituration is performed in ascorbic acid solutions since the quinones act as H-transfer agents between ascorbic acid and atmospheric oxygen. The kok-saghyz quinones deaminate amino acids like glycine and alanine, yielding glyoxalic and pyruvic acids. In these reactions no external H-consumption is observed. If trituration is done in presence of $HONH_2$ the colour formation is more intense, indicating the presence of phenols capable of giving such colour tests, and also capable of being oxidized to the quinones (Kolesnikov, 1952).

i) The Akagare disease (red disease) of **rice plants** is characterized by reddish brown spots on leaf blades accompanied by growth retardation, and often occurs in poorly drained muck lowland and sand soils. The disease is attributable to potassium deficiency causing unusual metabolic conditions in plants. Thus, terminal oxidase is converted from cytochrome c oxidase to some other oxidase system, and the Embden-Meyerhof system in glycolysis is changed to the Warburg-Dickens system, due to increase in oxidative functions. Polyphenols produced in these processes are oxidized to quinones, which react with amino acids to form brownish spots, characteristic symptoms of the disease (Takahashi, 1970).

j) In cultivated **cottons**, pigment glands in achlorophyllous plant parts contained predominantly gossypol and



Gossypol (R = H) and
Methyl gossypol (R = Me)



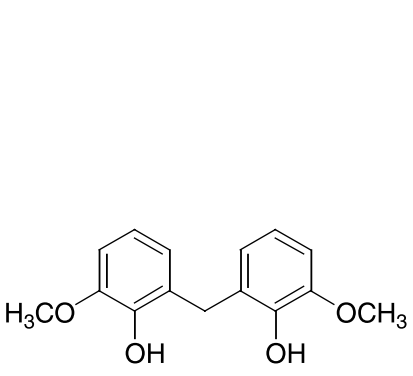
Hemigossypolone (R = H) and
Methyl hemigossopolone (R = Me)

Fig. 7. Gossipols and gossipolones

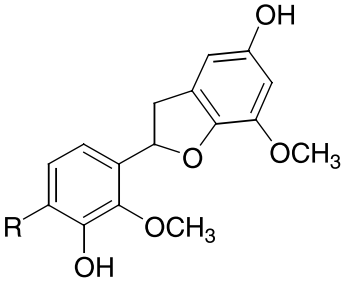
its methyl and di-methyl ethers. Another red pigment was found in the glands of young green tissues and identified as the sesquiterpenoid quinone hemigossypolone (Fig. 7). This special quinone is probably obtained during oxidation of gossypol. It was suggested that the red coloration of the envelope cells surrounding the gland sac, results from imine formation between hemigossypolone and amino acids (Bell et al., 1978).

8. Formation of humic substances

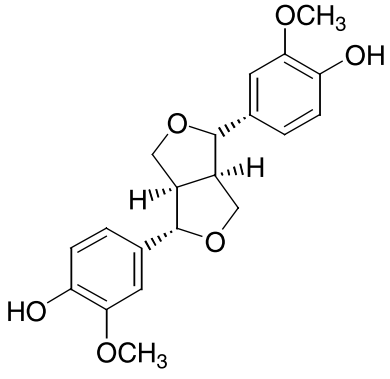
The formation of humic substances (recalcitrant soil organic matter) is one of the least understood aspects of humus chemistry and one of the most intriguing. This process involves the gradual incorporation of carbon from dead plants and microbes into stable organic matter called humus, which is responsible for the dark color in many soils. Studies on this subject are of long-standing (Swaby and Ladd, 1962) and continued research is justified on theoretical and practical grounds. Several pathways, including degradation and condensation probably occur during the decay of plants and formation of humic substances. Flaig's concept about humus formation (Flaig, 1971, 1997; Schulten and Schnitzer, 1997) is widely accepted. Lignin, freed of its linkage with cellulose during decomposition of plant residues, is subjected to oxidative splitting with the formation of primary structural unit derivatives e.g. phenylpropane units of lignin aryl ethers, diaryl ethers, biphenyls, phenylcoumarane and pinoresinol (Fig. 8). The side-chains of the lignin-building units are oxidized, demethylation occurs, and the resulting polyphenols are converted to ortho- or para-quinones by PPO enzymes. The quinones arising from the lignin, react with amino acids and peptides released by soil microbes and also with other N-containing compounds to form complex durable, dark-colored humic polymers. Study on the factors affecting the fate of amino acids in soils and various sediments during diagenesis, proved that the amino acids are indeed incorporated into humic-like substances through reactions with quinones (Stevenson, 1974). Model humic acids were synthesized by reacting quinones, formed from phenols, with amino acids and compared with natural humic acids (Swaby and Ladd, 1963). In a later synthetic project, quinonyl amino acid were prepared in which the amino acids are directly attached to the quinone nucleus (Fig. 9) and their hydrolytic mode was studied (Cranwell and Haworth, 1971). It



Diarylmethane



Phenyl coumarane



(+)-Pinoresinol

Fig. 8. Some primary structural unit derivatives formed upon splitting of lignin

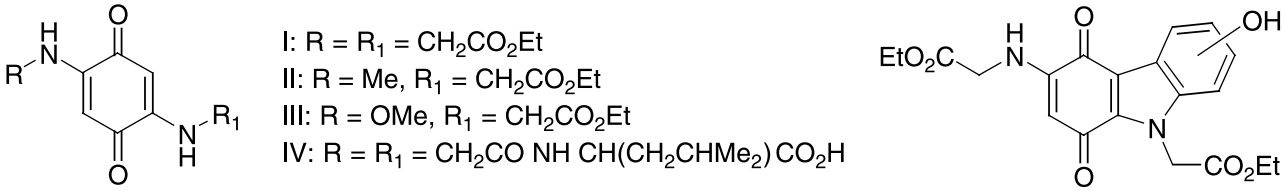
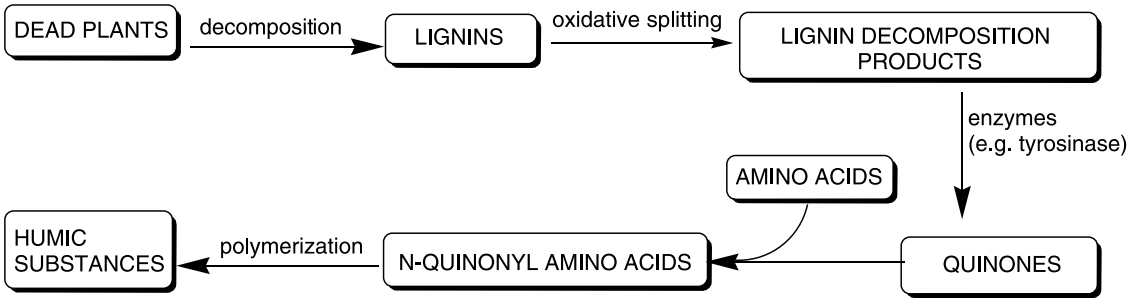


Fig. 9. Various N-quinonyl amino acids



Scheme 5. Proposed mechanism of humic acid formation

was found that in 6N HCl, the dducts behaved as vinylogous amides but the release of the amino acids was very low. It was the first proof that compounds of this type contribute to the stability of soil organic nitrogenous compounds. A proposed method for synthesis of humic substances of agricultural interest involves inserting phenolic or quinonyl groups in polymers containing benzene rings and subsequently subjecting the polymers to oxidative conditions in which ammonia or amino acids are inserted (Carpena et al., 1984). Novolak resins were prepared by nitrosation of phenol-formaldehyde polymer and oxidized with $K_2S_2O_8$ in presence of amino acids to yield humic acids. Hydroquinone-formaldehyde polymer and its quinone oxidation products were also used in synthetic humic acid formation.

Lately it was shown that the humification process is mediated by the enzyme tyrosinase, followed by non-enzymic polymerzation of the resulting quinones with amino acids to form humic polymers (Amonette et al., 2003). Tyrosinase increases the reaction rate between oxygen and humus precursors, such as phenols and hydroxybenzoic acids, to form quinones. As humic polymers are degrad by microbes less easily than the precursor molecules, they survive to diffuse into small pores in soil aggregates where they are stabilized for decades, if not centuries. It was found that an alkaline, porous material called "fly ash" (a by-product of coal combustion) speeds up the normal humification process by promoting the reaction of the quinones with the amino acids and providing small pores to protect humic polymers (Amonette et al., 2004; Kim et al., 2005).

9. PCBs quinones–amino acid adducts

Polychlorinated biphenyls (PCBs) were commercially manufactured and used as industrial chemicals in diverse applications. They are highly persistent and contaminate our environment. Their persistence is due to a general resistance towards chemical and biochemical degradation, which resulted in worldwide environmental contamination. They cause various toxic effects in animals and in exposed human populations. In 1996 it was suggested that PCBs undergo cytochrome P-450-catalyzed hydroxylations to form chlorinated dihydroxybiphenyl metabolites (Amaro et al., 1996). In order to study the reactivity of PCB-derived quinones, selected chlorophenyl 1,2- and 1,4-benzoquinones were prepared (Fig. 10) and their rate of reactivity toward the amino acids: glycine, L-arginine, L-histidine- and L-lysine was determined under pseudo-first-order conditions at pH 7.4.

The rate constants ranged from 0.45 up to $0.75\,\mathrm{min}^{-1}\,\mathrm{M}^{-1}$, but higher rates were obtained under conditions of higher pH. In contrast, the reaction of the chloroquinones with sulfur nucleophiles, e.g. glutathione or N-acetyl-L-cysteine was instantaneous. The mechanisms of toxicity and the pathways by which PCBs may be activated and act as an in vivo rat liver cancer initiator were, later, thoroughly studied. Using lower halogenated

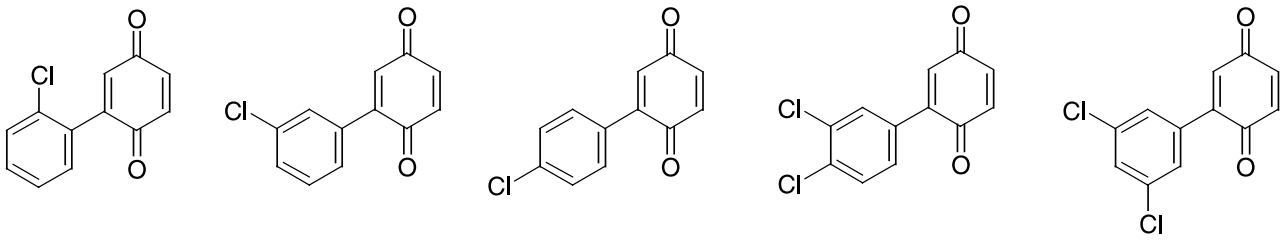


Fig. 10. Several PCBs quinones studied with amino acids

PCBs, it was found that PCB–quinone–amino acids adducts play an important role in the metabolism. PCBs are metabolized by cytochromes P-450 to mono- and dihydroxylated compounds (catechols and hydroquinones). Dihydroxy-PCBs can potentially be oxidized enzymatically by various peroxidases (including lactoperoxidase), prostaglandin synthase and cytochrome P-450, to the corresponding quinones. PCB–quinones are strong electrophiles that can react with both sulfur and nitrogen nucleophiles. It reacts with amino acids, individual DNA bases (nucleotides) and DNA to form stable adducts in vitro (Robertson et al., 2000). PCB quinones react also with glutathione (GSH) and produce superoxide and other ROS both in vitro and in HL-60 cells, and oxidative DNA damage in the form of DNA strand breaks in vitro. The resulting hydroquinone-glutathione addition product can undergo a second and third cycle of oxidation and GSH addition, with the formation of di- and tri-GSH–PCB adducts. The oxidation of PCB hydroquinone metabolites to quinones in cells followed by the binding of quinones to GSH and to protein sulfhydryl groups and the resulting oxidative stress may be important aspects of the toxicity of these compounds in animals (Srinivasan et al., 2001, 2002).

10. Germicidal activity, cytotoxicity and cutaneous inflamation

The mechanism of the germicidal action of quinones as compared with that of phenols was already studied 90 years ago (Cooper, 1913). It was observed that quinone reacts with egg albumin, casein, proteines of horse serum, gelatin and Witte peptone to form ''intensely coloured compounds''. As the addition of formalin to the reaction mixture completely inhibited the colour reaction, the conclusion was that the amino groups or imino groups of the proteins condense with the ketonic group of the quinone. Later, a relation was found between the activity of quinones with proteins and their bactericidal activity (Morgan and Cooper, 1924). It was also claimed that the germicidal power of the quinones is due to their chemical reactivity towards simple cell constituents, such as some of the amino acids (Cooper and Haines, 1928). In 1935 it was shown that certain bacteria give a red colouring reaction with quinones. Amino acids, amines, and other nucleophiles gave the same reaction (Fujita and Kodama, 1935). Later studies revealed that when a suspension of dysentery bacteria was added to a quinone solution, a cherry-red colour was obtained in a few minutes. Of a number of strains of dysentery bacteria examined, some were positive and others negative to this reaction. *Shigella flexneri* and *Shigella sonnei* were also positive to this test. The bacterial quinone reaction was influenced by minute amounts of alkali or acid in short periods (Yoneyama, 1957). Phenanthrenquinone (Fig. 11) inhibits the growth of *Staphylococcus aureus* in broth at a concentration of 1:200,000. Other benzoquinones and naphthoquinones showed similar bacteriostatic activity. A parallelism seems to exist between the dehydrogenating power of the quinone on amino acids and their bacteriostatic action. This suggest a possible interaction between the quinones and the amino

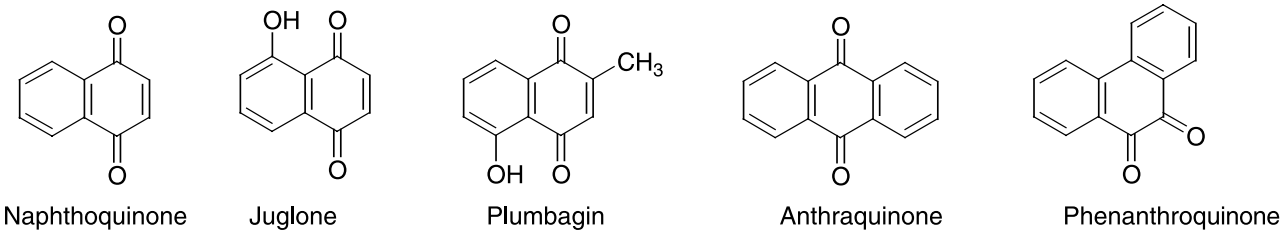


Fig. 11. Several cytotoxic quinones

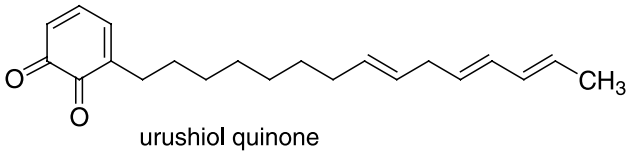


Fig. 12. Urushiol ortho-quinone

acids (Marini-Bettolo and Del Pianto, 1947). The decreasing toxicity of p-benzoquinone to yeast with time was studied and found to be due to the combination of the quinone with amino acids (Oka, 1962).

Herbal preparations derived from black walnut have been used as hair dyes and skin colorants in addition to being applied topically for the treatment of acne, inflammatory diseases, ringworm, and fungal, bacterial, or viral infections. Juglone (5-hydroxy-1,4-naphthoquinone) and plumbagin (5-hydroxy-3-methyl-1,4-naphthoquinone) (Fig. 10) are yellow pigments found in black walnut (*Juglans regia*). The cytotoxicity of these quinones to HaCaT keratinocytes was studied. Exposure to juglone or plumbagin (1–20 mM) resulted in a concentration-dependent decrease in cell viability. The cytotoxicity of these quinones is due to two different mechanisms, namely, redox cycling and reaction with glutathione (GSH). While most of the activity is attributed to the redox cycling mechanism, which forms free radicals and peroxides, the cytotoxicity of juglone may also involve nucleophilic addition to GSH (Inbaraj and Chignell, 2004).

The ability of small molecules such as urushiol present as a wax on the poison ivy leaf surface, to cause allergic contact dermatitis (rhus dermatitis) has fascinated immunologists for decades. Current dogma suggests that these epicutaneously applied catechol-containing molecules serve as haptens to conjugate with larger proteins via reactive o-quinone intermediates (Fig. 12). These complexes are then recognized as foreign antigens by the immune system and elicit a hypersensitivity reaction (Griffiths and Nickoloff, 1989).

Polyphenols are capable of inducing cutaneous inflammation reaction when in contact with the skin. The mechanism of allergy probably involves oxidation of phenols and polyphenols to quinones. These quinones are Michael acceptors susceptible to attack by nucleophiles present in amino acids such as lysine (amino group), cysteine (thiol group), and histidine (imidazole group) (Benezra, 1990).

11. Cuticle sclerotization and tanning

Sclerotization of insect cuticle is an extremely important biochemical process for the successful survival of most insects. Cuticle sclerotization or tanning occurs during each stage of insect development to harden and stabilize the newly secreted exoskeleton. The structural polymers protein and chitin make up the bulk of the cuticle, and chemical interactions between these biopolymers with quinonoid tanning agents are largely responsible for the physical properties of the mature exoskeleton. Already 50 years ago (Hackman and Todd, 1953) it was established that the first step in the hardening of the cockroach exocuticle is the reaction of a protein or an amino acid with a polyphenol derivative (e.g. 3,4-dihydroxybenzoic acid) secreted by the right colleterial gland, in the presence of an oxidase. The actual hardening involves an oxidation of the dihydroxy acids to an ortho-quinone followed by condensation with the NH_2 (possibly also SH) groups of the protein. The attachment of the NH_2 to the quinone nucleus, occures at position 4 and (or) 5. Subsequently indole may be formed followed by oxidative polymerization or reoxidation to the quinone followed by complex condensation reactions. Several deaminated and undeaminated derivatives of o-dihydroxybenzenes were found in the abdominal cuticle of adult desert locusts both before and after it has naturally hardened (Malek, 1961). They are probably derived from phenolic substance formed in the blood by enzymatic oxidation of tyrosine. The cuticle is incapable of hydroxylating the naturally occurring aromatic amino acids.

Later it was shown that when insects harden or sclerotize their exoskeletons, quinones of N-acylated catecholamines, such as N-acetyldopamine (NADA) or N-β-

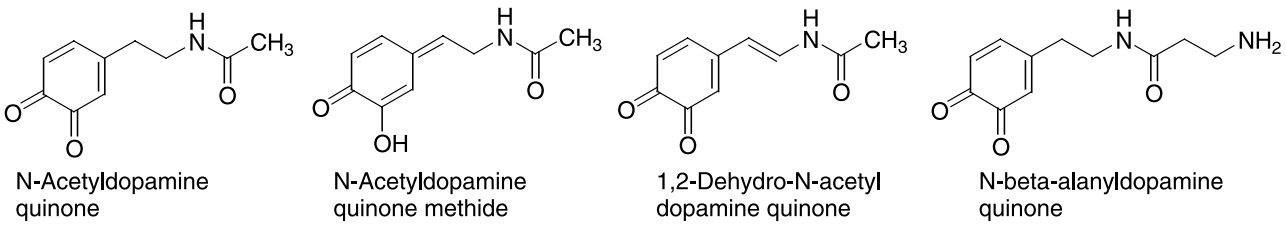


Fig. 13. Quinones and quinine methides participating in cuticle sclerotization

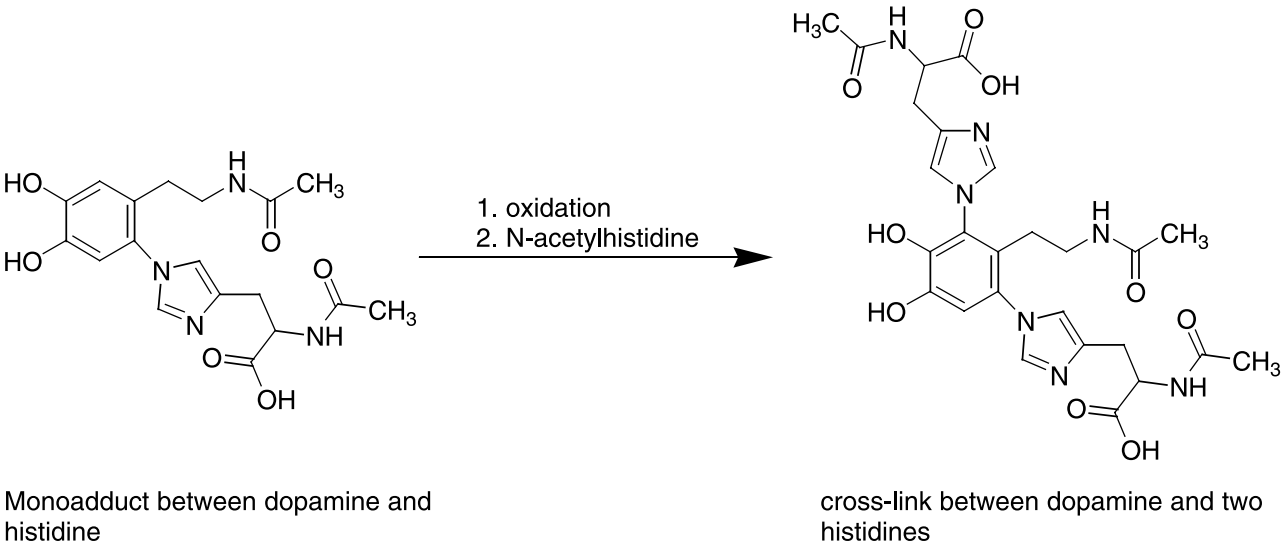


Fig. 14. Mono-adduct and cross-links between imidazole nitrogens derived from histidine and N-acetyldopamine

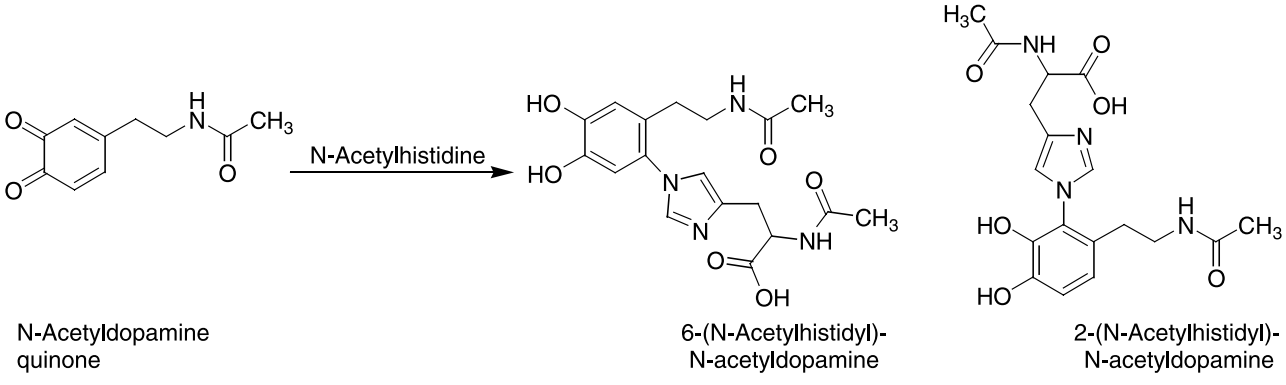
alanyldopamine (NBAD) undergo nucleophilic addition reactions with amino acids such as histidine in cuticular proteins.

The bulk of the studies carried out with N-acetyldopamine have led to the development of three different modes of sclerotization mechanisms:

1) Quinone tanning involving quinones as sclerotizing agents.
2) Quinone methide sclerotization using quinone methide as the sclerotizing agents.
3) α,β-Sclerotization also commonly known as β-sclerotizing which uses 1,2-dehydro-N-acetyldopamine and its derivatives (Fig. 14).

Discovery of the two new enzymes: quinone isomerase which generates N-acetyldopamine quinone methide from N-acetyldopamine quinone and quinone methide isomerase that produces 1,2-dehydro-N-acetyldopamine from N-acetyldopamine quinone methide, led to the unification of the above three mechanisms (Rickets and Sugumaran, 1994).

Laccase is hypothesized to play an important role in insect cuticle sclerotization by oxidizing catechols in the cuticle to their corresponding quinones, which then catalyze protein cross-linking reactions. Laccase is an enzyme with p-diphenol oxidase activity that is a member of a group of proteins collectively known as multicopper, or blue copper oxidases. The current hypothesis for cuticle sclerotization involves the formation of adducts and cross-links between nucleophilic imidazole nitrogens of histidyl residues in the proteins and electrophilic ring or side-chain carbons of ortho-quinones and para-quinone methides derived from the catechols, N-acetyldopamine, N-beta-alanyldopamine, and 3,4-dihydroxyphenylethanol (Kramer et al., 2001).



Scheme 6. Products from the reaction of N-acetyldopamine with N-acetylhistidine

In order to identify the products of the reaction that occurs during cuticle sclerotization, the reaction between N-acetyldopamine quinone and N-acetyl histidine (a protein model nucleophile) have been investigated (Xu et al., 1996). Two major products: 6-[N-(N-acetylhistidyl)]-N-acetyldopamine and 2-[N-(N-acetylhystidyl)]-N-acetyldopamine, were obtained (Scheme 6). The researchers demonstrate that both side chain and aromatic ring carbons of N-β-alanyldopamine are modified in crosslinking reactions with histidine residues in cuticular proteins during insect cuticle sclerotization (Xu et al., 1996a).

The marine polychaete *P. californica* (Sabellariidae) lives within a tube that it constructs by cementing together material such as sand and shells. Most of the carbon and nitrogen in the cement is proteinic, similar to the silk protein sericin, which is the sticky outer covering of silk fibers, but other organic materials are also present. It is suggested that the amino acid DOPA (3,4-dihydroxyphenylalanine), which is present as 2.6% of the total residues, acts to stabilize the material through quinone tanning (Jensen and Morse, 1988).

12. More effects of quinone–amino acids adducts

a. Cataractogenic activity

Cataracts in rabbit eyes are caused by daily feeding of 1 g/kg naphthalene. The cataracts were in the form of striations radiating from the periphery towards the centre of the lens, occasional brown coloration in the lens, yellow colour and blue fluorescence of the aqueous and vitreous humors, degeneration of the retina, and crystals in the vitreous. It was suggested that naphthlenediol and its glucuronide are involved in the metabolism of the naphthalene (Van Heyningen and Pirie, 1968). These compounds are very unstable in neutral solutions and are rapidly auto-oxidized to the highly reactive quinone. The presence of the quinone was sufficient to account for all the deleterious effects of naphthalene feeding on the eye, since it reacts at low concentration with proteins, co-enzymes and amino acids.

The cataractogenic activity of p-quinones was studied. Human lens proteins remained soluble, while approximately 40% of rabbit lens proteins precipitated following treatment with 1,4-benzoquinone. Apparently, the quinone oxidizes the lens protein SH groups to SS bonds and forms 2,5-disubstituted products with the ε-NH_2 group of lysine and with the imidazole group of histidine in the protein. This was indicated by amino acid reactions with benzoquinone and by the observed loss of lysine and histidine in the quinone-treated human proteins. All the reactions were apparently intramolecular, since no increases in the molecular wight of the lens proteins or their subunits were observed. It seems that any cataractogenic action of p-quinones may be due to insolubilization of lens proteins (Ikemoto and Augusteyn, 1976).

b. Pigments in hair and feathers

Pheomelanins from chicken feathers are structurally different from eumelanins. A degradation study (Minale et al., 1967) suggested that pheomelanins are derived from coupling reactions between cysteine and quinones, produced upon dopa oxidation. Further studies on pigments in Saddle feathers from New Hampshire chickens and human red hair, point to the presence of sulfur in phaeomelanins and related compounds. This and other data suggested that these pigments are formed by a reaction of the thiol groups of amino acids with quinones produced by the oxidation of dopa. Red and violet pigments as such are not present in feathers or hair but are artifacts that arise from yellow-orange pigments during the extraction procedures (Prota and Nicolaus, 1967).

Studies on tanned skin or leather showed much less lysine and hydroxylysine as compared to their concentration in untanned skin or leather. It is suggested that these two amino acids, having a free ε-amino groups, react with quinones. The amino acid-quinone addition product, hydrolyse very slowly compared to normal peptidic compounds and that accounts for their lower concentration in the hydrolysate (Enders et al., 1965).

13. Conclusions and possible future research

In this review we tried to enlighten some of the activities and phenomena that occur when quinones meet amino acids in living systems. In these systems the quinones (or polyphenols) and the amino acids are usually compartmentally separated and do not cooperate. However there are several junctions where they do meet do react with each other and influence. It occurs mainly in wounded, cut or crushed leaves during harvest, ensiling or in disintegrating cells. Diffusing polyphenols are than oxidized by polyphenol oxidases (PPOs) and the resulting quinonic compounds associate reversibly or irreversibly with amino acids or with peptides. While most polyphenols and amino acids are colourless, the quinone–amino acids conjugates are coloured, thus, enzymic browning of foods is a sign of disintegrated or infected cells. Physical and physiological phenomena are connected to the formation and activity of

such coloured conjugates. Several examples are: the colour, flavour and aroma of various natural products (e.g. tobacco, coffee, cocoa, tea, wine) are affected by reactions of quinones and amino acids; the toxicity of PCB's to humans and animals is the result of its oxidation to quinones and formation of PCB–quinone–amino acids adducts; sclerotization of insect cuticle, include enzymatic catechol–amino acids adducts formation, responsible for the physical properties of the exoskeleton.

This field is wide and its various aspects have been extensively studied. However many questions are still to be answered and advanced research is needed on the following and other subjects: a) isolation and characterization of naturally N- and S-quinonyl amino acids and quinonyl peptides. b) Development of anti-browning reagents, by inhibiting either the enzymatic oxidation of poly-phenols or the non-enzymatic reaction between the quinones and the amino acids. c) Research on the mechanism of activity between the naturally occurring quinones and the various amino acids and peptides, mode of PCBs activity, sclerotization mechanisms and more. d) Developing sophisticated analytical and spectroscopic methods for the detection and quantization of the quinones–amino acids adducts. e) Studies on the biological activity, inhibitory influence and medicinal feasibility of the various adducts.

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