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Condensed vegetable tannins: Biodiversity in structure and biological activities

Tess De Bruyne*, Luc Pieters, Hendrik Deelstra, Arnold Vlietinck

Department of Pharmaceutical Sciences, University of Antwerp (UIA), Universiteitsplein 1, B-2610 Antwerp, Belgium

Abstract

Proanthocyanidins, as an important class of secondary plant metabolites, are in many cases the active principles of the medicinal plants from which they are isolated. The structural complexity and conformational properties of the lower molecular weight oligomers have been investigated thoroughly, while the chemistry of the polymers still remains a difficult topic. Shikimate-derived phenolics like flavonoids and tannins are widely distributed in plant kingdom and are thus not of interest as classificatory tool; oxidation levels however are indicative in the attribution of evolutionary status among phyla and within each phylum. The main biological and pharmacological effects reported for condensed tannins can be classified into antibacterial and antiviral activities, enzyme inhibition, anti-oxidative effects, anti-mutagenic and antitumoral properties, next to some more specific interactions e.g. with vascular and cardial systems and inflammation processes. Their anticipated interaction with biological systems originates in principle directly from the physical and chemical properties of the polyphenolic skeleton, although prominent individual differences have been observed.

Keywords: Condensed tannins; Proanthocyanidins; Structural variability; Biological diversity; Biological activities

1. Introduction

The group of vegetable tannins or plant polyphenols is composed of two classes, the "hydrolysable" and the "condensed" tannins. The first group encompasses polyesters of gallic and hexahydroxydiphenic acid (gallotannins and ellagitannins, respectively), whereas the latter groups oligomers and polymers composed of flavan-3-ol nuclei.

* Corresponding author. Tel./Fax: + 32 3 820 27 09.

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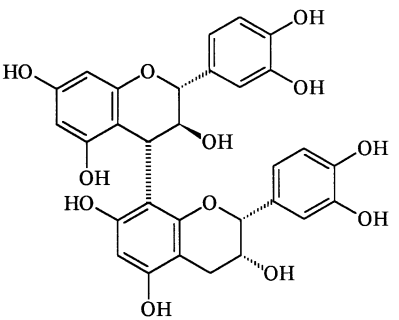
These condensed tannins are now commonly referred to as proanthocyanidins or, more broadly, as polyflavanoids, in which also the structurally related oxidatively coupled flavanoids are included. In an additional class of polyphenols, named the "complex" tannins, a flavan-3-ol unit is connected to a gallo- or ellagitannin through a carbon–carbon linkage. All these tannins are derived from the shikimate/chorismate pathway.

2. Chemosystematics of plant polyphenols

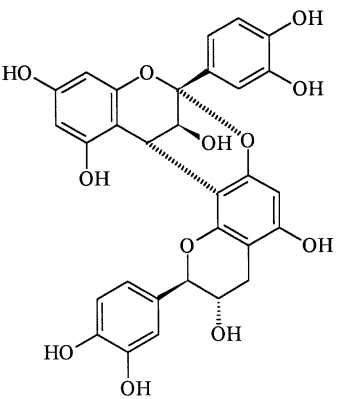
Whereas proanthocyanidins are widely distributed in higher plants, particularly in conifers, hydrolyzable tannins are of limited distribution in nature. Gallic acid metabolites occur within clearly defined taxonomic limits in woody and herbaceous dicotyledons; and the ellagitannins are found in the lower Hamamelidae, Dilleniidae and Rosidae and have been used as important chemotaxonomic markers thereof. Gottlieb (1992) developed the redox theory as an explanation for the occurrence of secondary metabolites by the observance of evolutionary directions. Shikimate-derived polyphenols follow analogous evolutionary development in all lineages of terrestrial plants. He observed two evolutionary chemical channels. One pathway explains chemical disparity among phyla. From Bryophyta onwards, expansion of the shikimate pathway through a reductive sequence leads first to cinnamyl alcohols, precursors of lignans and lignins from Pteridophyta onwards and then to propenylphenols and allylphenols, precursors of neolignanes from gymnosperms onwards. Flavonoids however, as condensation products of p-coumaric acid with three malonate units, preceded lignoids in this evolutionary history. From this point on, flavonoids and lignoids developed independently. Bryophytes contain p-coumaric acid and caffeic acid derivatives, as well as flavones. An analogous pattern is still seen in Psilophytatae and Lycopodiatae. In the Equisitales, flavonols start to predominate over flavones. In ferns, flavonols are accompanied by the related proanthocyanidins. The production of the dihydro-analogues seems to be an advanced feature in evolution; and from now on, diversifications in numerous oxidation patterns occur in parallel. In angiosperms, deoxygenation of position 5 and rarely of position 7 is now encountered. Further development of angiosperms was then accompanied by evolutionary canalisation, so that flavonoid variation is most prominent in the primitive angiosperms. Gradually dihydroflavonol, flavonol, and proanthocyanidin synthesis is downregulated until flavones become important again in the most advanced orders. This evolutionary pathway is accompanied by a trend towards replacement of the phenolics by acetate-derived, mostly aliphatic compounds during the advanced stages of evolutionary processes in plants. The other chemical evolution channel, justifying chemical diversity within each phylum, involves progressive increase in oxidation level within each metabolic class. An increase in oxidation level enhances intra- and intermolecular reactivity and promotes therefore diversification of the metabolic pool. Moreover, the introduction of a new class of polyphenols often represses the expression of a previous dominant one. Shikimate-derived phenolics are thus not of interest as classificatory tool, but are useful in the attribution of evolutionary status of a taxon.

3. Structural complexity within the proanthocyanidin group (Hemingway, 1989; Ferreira and Bekker, 1996)

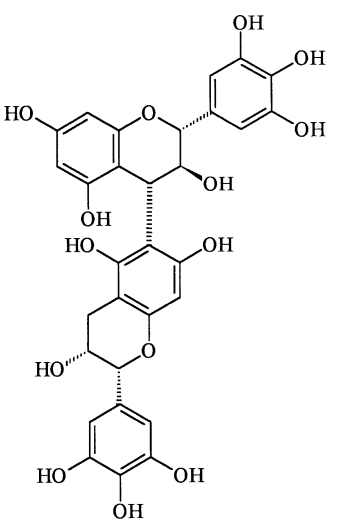
Proanthocyanidins are built by coupling at C-4 of an electrophilic flavanyl unit, generated from a flavan-4-ol or a flavan-3,4,-diol, to a nucleophilic flavanyl unit, often a flavan-3-ol. Structural variability is primarily seen in well-defined variation in hydroxylation pattern, stereochemistry at the three chiral centres, the location and type of interflavan linkage and the structure of the terminal unit. Furthermore, derivatisations as O-methylation, C- and O- glycosylation and O-galloylation are frequently reported and structural complexity is most prominently present in the



catechin- (4α→8)- epicatechin (procyanidin B 4)



ent-epicatechin- (2α→7,4α→8)- catechin (pavettanin A 2)



gallocatechin- (4α→6)- epigallocatechin

Fig. 1. Some representatives of condensed vegetable tannins.

rearrangement products of proanthocyanidins. Variation in hydroxylation pattern classifies the proanthocyanidins into several subgroups: propelargonidins (3,4′,5,7-OH), procyanidins (3,3′,4′,5,7-OH), prodelphinidins (3,3′,4′,5,5′,7-OH), proguibourtinidins (3,4′,7-OH), profisetinidins (3,3′,4′,7-OH), prorobinetinidins (3,3′,4′,5′,7-OH), proteracacidins (4′,7,8-OH; only synthetical), promelacacidins (3′,4′,7,8-OH), proapigeninidins (4′,5,7-OH) and proluteolinidins (3′,4′,5,7-OH). Of these subgroups, procyanidins are the most common one. They are present in barks of woody plants, whereas the prodelphinidins are major constituents of the leaves of conifers. The hydroxylation pattern in ring A, having either a phloroglucinol or a resorcinol substitution, has important implications on the type of interflavan linkages observed. Most proanthocyanidins are linked between C-4 of the "preceding" unit and C-6 or C-8 of the next flavan A-ring. In procyanidins and prodelphinidins, $4 \rightarrow 8$ linkages are stereochemically favoured, but do not occur exclusively. Usually, both $4 \rightarrow 8$ and $4 \rightarrow 6$ linkages are present in a ratio of 3:1. In 5-deoxy proanthocyanidins however, $4 \rightarrow 6$ linkages are predominant. Furthermore, in this 5-deoxy series, the interflavan bond is remarkably stable under a variety of conditions, including those conventionally used for the controlled cleavage of the interflavan bond. (Ferreira and Bekker, 1996) Both within the propelargonidins and procyanidins numerous representatives with an additional ($2\beta \rightarrow O \rightarrow 7$) interflavan ether linkage and categorised as A-class proanthocyanidins have been isolated. Those compounds are characterised by a high degree of conformational stability in contrast to the B-type proanthocyanidins linked via only one bond, and therefore lack the dynamic rotational isomerism for which B-type proanthocyanidins are reknown.

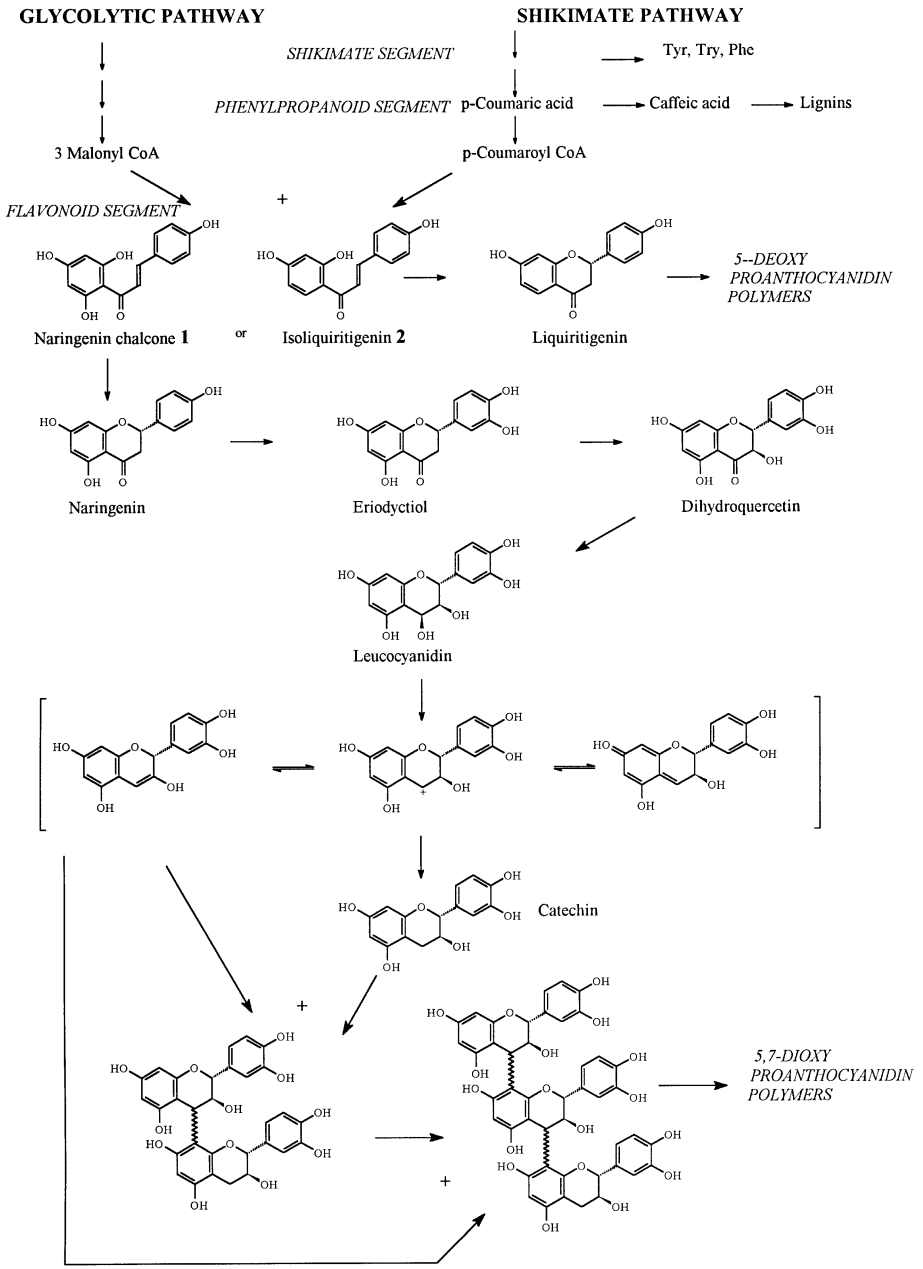
Stereochemistry at the three chiral centres of the heterocyclic ring is an additional complexicity enhancing factor. Most proanthocyanidins have a 2R absolute configuration; exceptions being found in monocotyledons and in selected dicotyledonous families such as *Rhus*, *Uncaria*, *Polygonum*, *Rapheolepsis* and *Schinopsis* (2S-configuration is denoted by the prefix *ent*). The 5-deoxy compounds, predominantly restricted to the Leguminosae and Anacardiaceae, have generally a 2,3-*trans* stereochemistry; while plants outside these taxa contain 2,3-*cis* procyanidins and prodelphinidins as major tannin compounds (2,3 *cis*/*trans* ratio between 90:10 and 50:50). At C-4, stereochemistry is determined by the hydroxylation pattern of the A-ring. Within the 5-deoxy class, 2,3-*trans*-3,4-*trans* and 2,3-*trans*-3,4-*cis* isomers are found in similar ratios; in the procyanidins and prodelphinidins on the other hand, 3,4-*cis* isomers are rather rare. Here, interflavonoid linking occurs thus under strong stereoselective control. The majority of proanthocyanidins has flavan-3-ols as chain terminating units, although within the profisetinidins also flavonols and dihydroflavanols have been described. (+)-Catechin is the most popular terminating unit among the flavan-3ols, throughout the profisetinidins, prodelphinidins and procyanidins; homopolymers of (−)-epicatechin are rather seldomly encountered in certain isolated species. This lead to the hypothesis of a separate metabolic pool for chain extender and terminal units. (Hemingway, 1989) A more elaborate discussion on the structure of proanthocyanidins, covering conformation, derivatives and polymer features is found in the papers by Hemingway (1989), by Steynberg et al. (1992) and by Ferreira and Bekker (1996).

4. Biosynthesis

Phenolic plant compounds, encompassing all aromatic molecules from the simple aromatic amino acids to the condensed tannins, are products of the "plant aromatic pathway", which consists of three main sections: the shikimate segment that produces the aromatic amino acids phenylalanine, tyrosine and tryptophan, the phenylpropanoid segment that produces the cinnamic acid derivatives that are precursors of flavonoids and lignans and the flavonoid route that produces the diverse flavonoid compounds. Indications exist that those sections may function as one membrane associated metabolic unit, localised in the cytoplasm. It has since been demonstrated that e.g. chalcon synthase is situated on the endoplasmic reticulum. (Hrazdine, 1992) The continuation of this biosynthetic route to the 5,7 dioxy-proanthocyanidins starts with the condensation of three molecules malonyl-CoA and one molecule p-coumaryl-CoA to naringenin chalcone, catalysed by chalcone synthase. Chalcone isomerase subsequently regulates the cyclisation to naringenin, which serves as the precursor for the flavan-3,4-diols, the flavan-3-ols and ultimately for the procyanidins and prodelphinidins. For the biosynthesis of the 5-deoxy proanthocyanidins, a route in which they are derived from liquiritigenin via isoliquiritigenin, has been proposed. This metabolic pathway is explained by a concerted action of chalcon synthase with a NADPH-dependent reductase. (Lewis and Yamamoto, 1989) The main route to the 2,3-*trans* procyanidins proceeds through the following sequence: naringenin → eriodyctiol → dihydroquercetin → leucocyanidin → catechin. The oligomeric forms are synthesised by the sequential addition of a quinone-methide intermediate derived from the flavan-3,4-diols to a flavan-3-ol or to a pre-existing chain. (Scheme 1) The enzymes involved are arranged in multienzyme complexes associated with the endoplasmic reticulum, in which intermediates could be transferred immediately from one enzymatic site to the other. (Stafford, 1989) The enzymology of the 2,3-*cis* pathway remains hypothetical. A pathway proposing a selective α-hydroxylation at C-3 of a flavanone to produce the 2,3-*cis* isomer in contrast to a β-hydroxylation to produce the more common 2,3-*trans* isomer is brought forward, next to other approaches involving either quinone–methide intermediates, or a C-3 epimerase. (Stafford, 1989; Hergert, 1989) Unambiguous information on the biosynthetic mechanisms involved for proanthocyanidins with various hydroxylation patterns, *ent*-isomers and various coupling products remains still unavailable.

5. Biological significance of proanthocyanidins

Many medicinal plants used for a range of ailments and disorders contain vegetable tannins as their active principles. In vitro assays revealed a variety of significant biological activities, as summarised by Haslam in 1996. Although differences in pharmacological activity are observed between individual polyphenols or between different classes of vegetable tannins, most of these variabilities can be approached as some selectivity rather than specificity towards a specific target system. Their anticipated interaction with biological systems originates primarily from their



Scheme 1. Biosynthetic route to flavan-3-ols and proanthocyanidins.

characteristical ability to form complexes, both with metal ions and with macromolecules such as proteins and polysaccharides, and from their anti-oxidative and radical scavenging properties. All those interactions at the basis of their physiological and pharmacological interactions, are in principle directly derived from the physical and chemical properties of the polyphenolic skeleton. However, in a recent publication on the inhibition of radioligand binding to a panel of 16 receptors by plant polyphenols, Zhu et al. (1997) concluded that "some phenolic compounds including tannins did show specific activities at the receptor level, which could not be explained solely in terms of protein binding". The most susceptible receptors to phenolic binding were β-adrenergic, 5-HT1 and opiate receptors, while some of the compounds tested showed selectivity for a single or for two receptors. These results suggest that although vegetable tannins all have intrinsically the same chemical tools for interference with biological systems, the effective manifestation of a physiological effect is strongly dependent on the chemical surroundings and conformation of polyphenol and target. In a comparative investigation we therefore evaluated the effects of procyanidin dimers as antibacterial, antiviral (HSV, HIV), radical scavenging and complement modulating agents. (De Bruyne et al., 1996; in press) An overview of other reports on biological activities of proanthocyanidins is summarised hereafter.

6. Antimicrobial and antiviral properties

The antimicrobial effects of tannins have been widely recognised. In 1986, Kakiuchi et al. screened several traditional Chinese medicines for antibacterial activity against *Streptococcus mutans*, a primary cariogenic bacterium. Gallotannin-rich extracts inhibited the adherence of *S. mutans* to smooth surfaces. This conclusion was formulated in a paper by Hada et al. (1989) that showed considerable anti-glucosyltransferase (anti-GTF) action for gallotannins and procyanidins. Hattori et al. denoted in 1990 a potent anti-GTF activity for theaflavins and for galloylated flavan-3-ols. In 1993 then, Nakahara and coworkers demonstrated that oolong tea polyphenols strongly inhibited some GTF-types of *S. mutans*, while catechins did not inhibit GTF significantly. Since oolong tea is semifermented, which results in polymerisation, the conformational changes of such a polymerisation should be critically important for the demonstrated effect. Recently, Sakanaka et al. (1996) reported on the inhibition of green tea polyphenols on the growth and cellular adherence of the oral bacterium *Porphyromonas gingivalis*, responsible for the majority of adult periodontitis cases.

Baldé et al. (1988, 1990a and b, 1991) demonstrated antibacterial activities of *Pavetta owariensis* extracts and pure doubly linked proanthocyanidins against *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Neisseria gonorrhoea* at $10\,mg\,ml^{-1}$ concentrations. Antibacterial potency increased with the number of flavanol unities for the doubly linked proanthocyanidins obtained by bio-assay guided isolation.

Sakagami et al. (1992) screened 86 tannin-related compounds for the stimulation of monocyte iodination and of interleukin-1 production. Since it had been reported that fixation of iodide in PMN and monocytes ingesting tuberculosis bacilli was parallel to

the cellular peroxidase content (Simmons and Karnovsky, 1973), it suggests a correlation with bactericidal activity. Interleukin-1 enhances host resistance against bacterial infection (Ozaki et al., 1987). Galloylated condensed tannins showed the most potent activity in these assays, which could account for their antibacterial properties. Antiviral activity is due to the binding of tannin molecules to the protein coat of the virus or to the host cell membrane. Virus adsorption and consequently virus penetration is thus arrested. In some cases however, the binding causes only minor changes on the viral surface, still allowing penetration, but preventing the uncoating of the virus. Takechi et al. (1985) investigated the relationship between antiherpetic activity and structure of tannins. The activities of hydrolysable tannins were dependent on the number of galloyl or hexahydroxydiphenoyl groups, while those of condensed tannins increased with the degree of condensation. The more active tannins, however, were the more cytotoxic.

In 1989, Fukuchi et al. showed the antiviral activities on *Herpes simplex* virus (HSV-1,HSV-2)-infected African green monkey kidney cells and on human adenocarcinoma cells of several plant extracts, hydrolysable tannins and galloylated condensed tannins. They demonstrated by means of radiolabelled virus particles that the anti-HSV effect was due to inhibition of virus adsorption.

Baldé et al. (1988, 1989, 1990a and b, 1991) tested extracts of *Pavetta owariensis* bark and purified doubly linked dimers, trimers and tetramers for their in vitro antiviral activities. Antiviral properties were most prominent against *Herpes simplex* and *Coxsackie* viruses, whereas a weak activity against *Semliki forest*, *VSV* and *poliomyelitis* viruses could be observed at nontoxic concentrations. No activity was shown against the measles virus.

In 1992, Nakashima et al. noticed significant inhibition for the cytopathic effects of human immunodeficiency virus (HIV) and for the expression of HIV-antigen in human lymphotropic virus type I (HTLV-1)-positive MT-4 cells by several hydrolysable tannins. Here, too, anti-HIV activity was at least partly mediated by adsorption–inhibition, although complete inhibition of HIV-binding did not occur, even at elevated concentrations ($12.5\ g\ ml^{-1}$).

Ferrea et al. reported (1993a–d) on the in vitro antiviral activities of alkaline autooxidized catechinic acid (AOCA) against HSV-1 and HIV-1 viruses. Catechin and condensed "catechinic" tannins were seen to produce AOCA via rearrangement to catechinic acid and subsequent autooxidation. They also demonstrated (1993e) antiviral properties against HSV-1 and HSV-2 viruses for a *Combretum micrantum* extract, prepared 7 days before the assay. The activity was absent in freshly prepared extracts. They could therefore conclude that inactive precursors were transformed into the active compound, which had similar spectroscopic and antiviral properties as AOCA. AOCA, especially the polymers with M_r between 10,000 and 30,000, were seen to inhibit the viruses even after viral penetration, an activity comparable to that of the oxidized polymerisate of caffeic acid (KOP).

In 1994, Ubillas et al. isolated a condensed tannin with an average chain length of seven units, composed of both procyanidin and prodelphinidin-type B-ring moieties, from the latex of *Croton lechleri* (Sangre de Drago). The proanthocyanidin oligomer exhibited antiviral activities against respiratory syncytial virus (RSV), influenza A

virus (FLU-A) and parainfluenza virus (PIV) comparable to ribavirin. Additionally, inhibition of herpes viruses types 1 and 2 (HSV-1, HSV-2) and hepatitis A and B viruses was observed. The antiviral mechanism was said to be derived from its binding to components of the viral envelope, resulting in inhibition of viral attachment and penetration of the plasma membrane.

Inhibitors of reverse transcriptase (RTase) could be effective in diseases caused by retroviruses (e.g. AIDS and adult T-cell leukemia). Kakiuchi et al. (1985 and 1991) denoted ellagitannins and also several condensed tannins as potent inhibitors of RTase. Galloylation, the extent of oligomerisation, the difference in interflavan-linkage and the stereochemistry of the 3-hydroxyl function influence strongly the inhibitory capacity. The inhibitory effect of these tannins is due to the prevention of nucleic acid–enzyme complex formation (Vlietinck et al., 1998).

In 1992 however, Okuda's group (Nakashima et al.) reported on the inhibition of the HIV cytopathic effect and the HIV antigen expression in HTLV-I-positive MT-4 cells by hydrolysable tannins, while claiming that condensed tannins showed only negligible activity.

7. Enzyme-inhibiting properties

The general protein-complexing effects of polyphenols can cause enzyme inhibition. In certain specific cases however, other inhibition mechanisms were observed. Wagner et al. (1991) studied the inhibiting properties of several medicinal plant extracts on angiotensin I-converting enzyme (ACE). The antihypertensive and diuretic activities of these herbal drugs were due to proanthocyanidins and γ-glutamyl peptides. The screening of various pure phenolic compounds (flavanoids and proanthocyanidins) then suggested chelation of the zinc-atom in the active centre of the enzyme as a probable reaction mechanism. In the group of proanthocyanidins, higher oligomers and $(4 \rightarrow 6)$ linked dimers were found to be more active than their $(4 \rightarrow 8)$ counterparts.

Ellagitannins and complex tannins were found to be more effective inhibitors of protein kinase C than gallotannins and condensed tannins by Kashiwada et al. (1992).The selectivity was shown by the lack of inhibition versus c-AMP-dependent protein kinase, while the phorbol displacement assay pointed to interaction with the regulatory site of the enzyme.

Hatano et al. (1990) investigated the inhibitory effects of both hydrolysable and condensed tannins on xanthine oxidase (XOD) activity. The number of phenolic groups, the location of acyl-groups in hydrolysable tannins and the galloylation and degree of polymerisation in proanthocyanidins affected the strength of inhibition remarkably. Absence of correlation between the inhibitory effect and the binding activity to haemoglobin for several tannins proved that the inhibition was not due to the non-specific binding to proteins. Since xanthine oxidase is used in a test system to evaluate the superoxide-anion radical scavenging ability of polyphenols, their O_2-scavenging (1989) and the XOD-inhibiting properties were compared. They concluded that the order of strength of inhibition of xanthine oxidase (XOD) among the

tannins was considerably different from that of the inhibitory effects on the O_2°-generation from the hypoxanthine–XOD system, and that therefore the inhibition of O_2-generation is caused by their radical scavenging effect, rather than by the XOD-inhibition, which was confirmed in a recent publication by Cos et al. (1998) for (−)-epigallocatechin.

8. Mutagenicity, antimutagenicity and antitumoral activity

Since procyanidins are consumed in considerable doses every day (± 1 g polyphenols/day) through the intake of tea, wine, beer and several vegetables and fruits, the careful examination of possible mutagenic properties is very important. In the past several epidemiological studies have attributed the relatively high incidence of oesophageal cancer in certain areas to the consumption of proanthocyanidins from red wines, teas and medicinal herbs. Yu et al. (1987) screened procyanidins with different degrees of polymerisation in the *Salmonella* mutagenesis assay system. They were found to be non-mutagenic; the observed mutagenicity of one compound was found to be due to the presence of the mutagen rutin as impurity. Rutin is indeed hydrolysed by the intestinal microflora to quercetin, which is known to be mutagenic in the *Salmonella* assay (Brown et al., 1983).

On the other hand, Gichner et al. (1987) noticed antimutagenic effects of caffeic, gallic and chlorogenic acids towards mutagenicity induced by N-nitroso compounds in the Ames *Salmonella* assay.

Miyamoto et al. (1987) investigated the antitumour activities of 63 tannins and polyphenols. He concluded that the active compounds against sarcoma-180 tumours in mice could be found in the group of ellagitannins, while condensed tannins showed negligible activity. Since he observed that the antitumour effect of agrimoniin (1988) could be partly due to its induction of interleukin-1β secretion, additional to the increase of natural killer cell activity and stimulation of macrophages (Murayama et al., 1992), he was prompted to investigate the correlation between antitumour activity of hydrolysable tannins and interleukin-1 production of macrophages. Nevertheless, no clear correlations could be observed (Miyamoto et al., 1993), suggesting that this activity is both due to their particular structure and to general complexating properties.

Kashiwada et al. (1992) evaluated the selective cytotoxicity of 57 tannins and related compounds against several human tumour cell lines. For gallotannins, quinic acid cores seem to be essential for cytotoxicity. Within the ellagitannin group, C-glycosylation, a 4,6 (S)-hexahydroxydiphenic acid (HHDP) moiety and the position of the HHDP group are also important characteristics. Condensed tannins need a galloyl group at C-3 for increased cytotoxicity. Additional flavan-units or ether linkages (A-type proanthocyanidins) also promote cytotoxic action. In a recent study, the inhibitory effect of (−)-epigallocatechin gallate (EGCG) on the promotional stage of radiation-induced mouse oncogenic transformation was reported. (Komatsu, 1997) EGCG and green tea extract also showed interesting effects in the prevention of gastrointestinal carcinogenesis. (Yamane et al., 1995, 1996) Green tea catechins were

also tested and proved effective growth inhibitors on MCF-7 breast carcinoma, HT-29 colon carcinoma, A-427 lung carcinoma and UACC-375 melanoma (Valcic et al., 1996).

9. Anti-oxidative properties

Recently, considerable interest has been evoked on the impact of the pro-oxidant/anti-oxidant status on the prevention or amelioration of several degenerative diseases. Cellular pro-oxidant states, with an increased concentration of reactive oxygen species and free radicals, are involved in the pathophysiology of major chronic diseases like ageing, cancer, inflammation, atherosclerosis, multiple sclerosis, etc. Restoration of the oxidative balance by removal of those reactive oxygen species is controlled endogenously (e.g. by superoxide dismutase) or by the administration of anti-oxidants. In the dietary intake of anti-oxidants, plant polyphenols seem to play an important role, next to the vitamins E and C and the carotenoids.

Procyanidins B1 and B3 were evaluated as anti-oxidants for linoleic acid in aqueous systems by Ariga et al. (1988). They showed stronger anti-oxidant activity than ascorbic acid and α-tocopherol. From a study of their radical scavenging properties towards radicals induced from an aqueous dispersion of methyl linoleate, it was seen that the dimeric procyanidins can trap eight peroxyl radicals (cf. ascorbic acid traps one radical; α-tocopherol traps two radicals). The higher the degree of polymerisation, the more radicals are scavenged per molecule (Ariga et al., 1990).

Uchida et al. (1987) reported the radical scavenging action of galloylated condensed tannins for 1,1-diphenyl-2-picrylhydrazyl (DPPH)-radical, as well as for superoxyde anions and for $\cdot OH$ and $\cdot OOH$. All mentioned radicals were scavenged dose-dependently. The authors also decided that the ability for radical scavenging was proportional to the degree of polymerisation, which was contradicted by Ricardo da Silva et al. (1991), since with higher oligomerisation, also the number of galloyl groups increased in the investigated compounds.

Ricardo da Silva et al. (1991) tested various procyanidins for their scavenging activities for superoxyde radical $O_2^{-\cdot}$ and hydroxyl radical $\cdot OH$ in aqueous models. Galloylation, preferably in 3′-position, increases the scavenging ability for both $O_2^{-\cdot}$ and $OH^{\cdot}$. $O_2^{-\cdot}$-scavenging was more prominent for (4 → 8)-coupled dimers than for (4 → 6)-linked procyanidins; a difference not seen for $OH^{\cdot}$-scavenging activity.

Hatano et al. (1989) investigated the scavenging effects on the $O_2^{-\cdot}$ radical generated in the hypoxanthine–xanthine oxidase system by electron spin resonance. They also observed a stronger scavenging effect for galloylated (ortho-trihydroxyl structure) hydrolysable and condensed tannins, and for higher polymers. The prominent influence of ortho-trihydroxyl groups was probably due to the stability of the phenoxy radicals formed.

By means of the molecular orbital method (MNDO), Zhao et al. (1992) calculated that for catechin the more active hydroxyls are those on the benzene ring of the chromane. Therefore, those hydroxyls will be the first attacked upon reaction with oxygen free radicals. In 1996, Nanjo et al. published their investigations on the

structure–activity relationships of the scavenging effects of tea catechins on 1,1-diphenyl-2-picrylhydrazyl radical. (−)-Epigallocatechin gallate proved a better inhibitor of the formation of 8-oxodeoxyguanosine and 3-nitrotyrosine than ascorbate or glutathione; generation of these reaction products are considered as initiating steps in atherosclerosis. (Fiala et al., 1996)

10. Various biological properties

10.1. Anti-inflammatory effects

In 1991, Tits et al. showed an important anti-inflammatory activity in the carrageenan rat paw edema model for prodelphinidins, isolated from *Ribes nigrum.*

10.2. Vascular and cardial effects

Besides inhibition of the angiotensin-I converting enzyme (ACE) by procyanidins as mentioned before (cf. enzyme inhibiting properties), other vascular effects were reported. Calixto et al. (1986) denoted that tannic acid affects calcium availability for the contraction of smooth and cardiac muscles. Indeed, by complexating Ca^{2+}, tannic acid shows a hypotensive effect. Nevertheless, also the involvement of histamine release inhibition should be taken into account as suggested by the partial antagonism of the hypotensive response upon pretreatment with antihistaminica.

Chang et al. described in 1989 the inhibition of platelet aggregation in human and rat plasma by two trimeric procyanidins and one dimer thioether. This could be explained by the inhibition of platelet thromboxane biosynthesis from arachidonic acid by cyclooxygenase, observed for the same products. The most active compound, the dimer thioether, also inhibited 12-lipoxygenase and thus 12-HETE formation.

More recently, Pattichis et al. (1993) showed that the "phenolic flavanoid fraction" of red wine, but not that of white wine, released platelet 5-hydroxytryptamine in vitro. This could be the mechanism by which red wines can induce migrainous headaches. Maffei Facinó et al. (1996) reported on the protective effect of a high-molecular weight procyanidin fraction from *Vitis vinifera* in myocardial ischemia and reperfusion injury. This effect is probably the consequence of the radical scavenging properties of the procyanidin fraction, and of their chelating effect on Fe^{2+} and Cu^{2+}, the catalysts of the free radical cascade in vascular and cardiac tissue.

10.3. Anti-ulcer activity

Vennat et al. reported in 1989 on the anti-ulcer properties of procyanidins, isolated from *Fragaria vesca.* Moreover, they formed procyanidin–cimetidine complexes in which the procyanidins increased the water solubility of cimetidine and blocked its cyanamide function, thus preventing the formation of genotoxic nitrosamines in gastric medium.

A possible explanation was brought forward by Murakami et al. (1992), who observed that catechins, gallocatechins and their gallic esters inhibited gastric H^+,K^+-ATPase. Inhibition leads to the reduction of gastric acid secretion. The inhibitory activity was proportional to the number of hydroxyl groups of the tested compounds.

10.4. Pulmonary inflammation

The observation that inhalation of cotton mill dust caused acute pulmonary inflammation, prompted several research groups to isolate the responsible compounds. Condensed tannins alter the functionality of alveolar macrophages, as seen by Kreofsky et al. (1992). Tannins inhibit the ability of those cells to produce reactive oxygen intermediates and promote arachidonic acid release.

10.5. Anti-diarrhoeal activity

Galvez et al. reported in 1993 on the anti-diarrhoeal activity of a *Sclerocarya birrea* proanthocyanidin against magnesium sulphate, castor oil and arachidonic acid experimentally induced diarrhoea in mice. For prostaglandin E_2 induced diarrhoea, the proanthocyanidin was only active at higher dose (150 mg kg^{-1}). They postulated that the antidiarrhoeal activity of the proanthocyanidin is related to an inhibition of intestinal motility and that its action is concentrated at the level of the effector cells.

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