

**CHEMISTRY AND PHARMACOLOGY OF NATURAL
PRODUCTS**

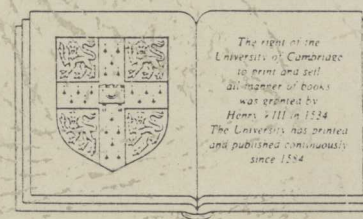
Series Editors: Professor J.D. Phillipson, *The School of Pharmacy, University of London;* Dr D.C. Ayres, *Department of Chemistry, Queen Mary College, University of London;* H. Baxter, *formerly at the Laboratory of the Government Chemist, London.*

Plant polyphenols

Vegetable tannins revisited

EDWIN HASLAM

Department of Chemistry, University of Sheffield



CAMBRIDGE UNIVERSITY PRESS

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New York New Rochelle

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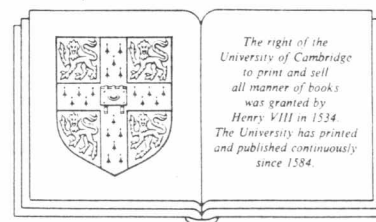
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To
Raj, colleagues, students and friends

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Preface

An author's first book is often said to be the one he should never have written. When *Chemistry of Vegetable Tannins* was published in 1966 its subject matter was doubtless considered by many not only unfashionable but also arcane and recondite, associated with the remnants of a dying trade – that of the tanning of animal hides and skins by vegetable matter to give leathers. Two decades later the chemistry and biochemistry of plant polyphenols (the author, if few others, prefers this terminology rather than the more widely used and appreciated nomenclature of vegetable tannins) may be said to have come of age scientifically. In the intervening years the subject has enjoyed a surprising renaissance and 1988 is in many ways an appropriate time to reappraise the whole field and look to future prospects. *Chemistry of Vegetable Tannins* (Academic Press, London and New York, 1966) thus provides, if nothing else, a unique bench-mark from which to judge the advances which have taken place in knowledge and understanding at this interface of botany, biochemistry and chemistry in the intervening years.

A great deal of fundamental scientific study has now unequivocally established the structures of many of the key plant polyphenols and has defined, at least in broad outline, the manner in which they are formed in plants and how they are inter-related to other important groups of natural products. This surge in knowledge of a fundamental chemical and biochemical nature has given rise in the last fifteen years to a burgeoning practical interest in the influence and role of plant polyphenols in diverse areas such as agriculture, foodstuffs and nutrition, beverages, traditional medicines and herbal remedies, chemical protection in plants, and the potential use of plant polyphenols as sources of important industrial organic chemicals.

This change in emphasis might be considered timely. Rather it illustrates the undeniable fact that the understanding of complex applied scientific problems invariably follows periods when substantial advances in fundamentals have been accomplished. The science of plant polyphenols is at just such a stage of development.

This quiet revolution has taken place moreover with the benefit of relatively modest scientific resources world-wide: sustained efforts over a long period by few groups. The perspectives developed in the present text are undeniably distinctly personal ones, the result indeed of a personal odyssey. Whilst the text draws freely on the work of many it ultimately directs principal attention to the chemical, biochemical and botanical knowledge which has been garnered concerning those plant polyphenols located in the vegetable tissues of plants – in direct lineal descent of the work of Rosenheim, Sir Robert and Lady Robinson, Dr E.C. Bate-Smith and Dr Tony Swain, pioneers of this particular facet. The text is thus a synthesis of many of the themes initiated, and ideas introduced by the author in earlier reviews, modified as necessary by the dictates of time. But what of the future? This must surely lie unerringly and increasingly in the biological aspects of the science of plant polyphenols. Structure is an absolute prerequisite to such work but certainly not an end in itself. Investigations must now focus on the properties of these substances *in vivo*.

Any insights which the author may have helped to illuminate in this area are due to a very large joint effort and the skilled, loyal and enthusiastic support over the past twenty-five years of students, colleagues and friends. Their contributions have been crucial and it is a great pleasure and important to acknowledge them. Particular mention should however be made in this context of the work of Dr E.C. Bate-Smith, and of the work of Dr Terence H. Lilley, Dr Lawrence J. Porter and the late Dr Raj K. Gupta. Dr E.C. Bate-Smith has been a constant stimulus, guide and mentor – not least through the many cryptic notes written over the past twenty years on the backs of envelopes directing the attention of a mere chemist to some new plant family or to some obscure genius. Dr Lilley, as a thermodynamicist, has taken over the past ten years the major lead opening out the whole area of polyphenol complexation to rigorous and systematic study; Dr Porter and Dr Gupta both took sabbatical periods in Sheffield in the 1970s and both made substantial and significant contributions to the then evolving story of the plant proanthocyanidins. Dr Porter has since brilliantly continued this work in New Zealand. In the preparation of this text the author also gratefully acknowledges the skilled assistance of Mrs Olga M. Bray who typed the manuscript and that of Mr Peter Silver and Mrs Susan Bowring of the Cambridge University Press and Dr David Ayres – series editor – who removed many infelicities of expression in the preparation of the text for the printer.

Polyphenols – vegetable tannins

Prologue

Leather-making is a craft and a trade of great antiquity which has, over the years, achieved a very high degree of technical development. Thus although it was possible for Henry Procter in his Cantor lectures of 1899 to describe the science of leather-making as 'still very young' the subsequent emergence of leather chemistry itself and the parallel growth of the synthetic fibres industries caused profound changes in the practices of the leather industry in the twentieth century. These contrast vividly with the skills and techniques evolved over centuries by the application of empirical methods and reasoning, and by the familiar processes of improvisation, trial and error. Tanning is the means by which an animal skin is converted to leather and in Britain, up until the dawn of the industrial revolution, most of the sole leather produced was tanned with an infusion of oak bark. A period of at least three to six months was the norm for this process of hide tannage. Today, with drum processing, similar vegetable sole tannage can now be completed within one to three days.

The rapidly increasing demands for more leather which the industrial revolution created, a century and more ago, meant that the British tanner had perforce to find new sources of vegetable tannin to supplement the indigenous oak bark. These he discovered world-wide, amongst them: myrobalans (also often written *myrabolams*, *myrabolans*, *myrabalans*; the dried fruit of an Indian tree, *Terminalia chebula*), quebracho extract (from the heartwoods of *Schinopsis lorentzii* and *S. balansae* from the Argentine), wattle or mimosa extract (from the bark of mimosa species, eg. *Acacia mollissima*, syn. *A. mearnsii*), algarobilla (the fruit bearing pods of *Caesalpinia brevifolia*), valonea (the acorn cups of the bearded oak *Quercus aegilops*), sumach (from the leaves of *Rhus* sp. grown in Mediterranean regions) and so forth. Some of the more impor-

Table 1.1. Vegetable tanning materials

| |
|--|
| <i>Barks</i> |
| Wattle (<i>Acacia</i> sp.), Mangrove (<i>Rhizophora</i> sp.), Oak (<i>Quercus</i> sp.), Spruce (<i>Picea</i> sp.), Hemlock (<i>Tsuga</i> sp.), Eucalyptus (<i>Eucalyptus</i> sp.), Avaram (<i>Cassia auriculata</i>), Babul (<i>Acacia arabica</i>), Birch (<i>Betula</i> sp.), Willow (<i>Salix caprea</i>), Pine (<i>Pinus</i> sp.), Larch (<i>Larix</i> sp.), Alder (<i>Alnus</i> sp.). |
| <i>Woods</i> |
| Quebracho (<i>Schinopsis</i> sp.), Chestnut (<i>Castanea</i> sp.), Oak (<i>Quercus</i> sp.), Cutch (<i>Acacia catechu</i>), Wandoo (<i>Eucalyptus wandoo</i>), Urunday, Tizra. |
| <i>Fruits, Fruitpods</i> |
| Myrobalans (<i>Terminalia chebula</i>), Valonea (<i>Quercus aegilops</i>), Divi-divi (<i>Caesalpinia coriaria</i>), Algarobilla (<i>Caesalpinia brevifolia</i>), Tara (<i>Caesalpinia spinosa</i>), Teripods, Sant pods. |
| <i>Leaves</i> |
| Sumach, American Sumach (<i>Rhus</i> sp.), Gambier (<i>Uncaria gambier</i>), Dhawa or Country Sumach (<i>Anogeissus latifolia</i>), Badan (<i>Bergenian crassifolia</i>). |
| <i>Roots</i> |
| Docks (<i>Rumex</i> sp.), Canaigre (<i>Rumex hymenosepalus</i>), Siberian saxifrage (<i>Saxifraga crassifolia</i>), Garouille (<i>Quercus coccifera</i>), Sea lavender. |
| <i>Plant Galls</i> |
| Oak (<i>Quercus</i> sp.), Chinese (<i>Rhus semialata</i>), Tamarisk (<i>Tamarix articulata</i>), Pistacia (<i>Pistacia</i> sp.), Aleppo (<i>Quercus infectoria</i>). |

Information obtained from Howes (1953), Haslam (1966), White (1957) and Grimshaw (1976).

tant sources of vegetable tannins which were still widely employed commercially in leather production until late into the twentieth century are listed in Table 1.1. Botanically speaking vegetable tannins occur in one form or another in a substantial part of the Plant Kingdom especially in higher plants and in particular families of dicotyledons, the most notable being perhaps the Leguminosae (eg. *Acacia* sp.), Anacardiaceae (eg. sumach, quebracho), Combretaceae (eg. myrobalan), Rhizophoraceae (eg. mangrove), Myrtaceae (eg. eucalyptus) and Polygonaceae (eg. canaigre). Many of the commercially most significant tannin materials such as wattle, mangrove, quebracho and myrobalans originate in tropical or sub-tropical climes although plants rich in tannin also occur in temperate zones. As Table 1.1 shows substantial accumulations of vegetable tannins may be found in almost any part of a plant – bark, wood, leaves, fruit, root. They also occur in seeds and increased tannin production in a plant is often associated with a particular pathological condition. The most familiar example is that of plant galls caused by insect attack. Some plant galls have an abnormally high tannin content often in excess of 60 or 70% of the dry weight. Probably the most familiar insect gall in Britain is the marble gall found on pedunculate

and sessile oak and variously known as ‘bullet gall’, ‘oak nut’ and ‘Devonshire gall’. It is plentiful particularly on young hedgerow plants and on scrub-oaks in coppices and is a hard globular object (~ 2.5 cm, diameter) growing singly or in groups. The galls arise as a result of the parasitic attack on terminal or axillary buds by the gall wasp *Andricus kollari* (*Cynips kollari*), an introduced species which reached Britain around 1830 when galls were first imported from the Middle East into Devon for dyeing cloth or ink-making. In general British oak galls have much less tannin (~ 20%) than the commercially important Aleppo gall (*Quercus infectoria*) or Chinese gall (*Rhus semialata*) which contain some three or four times this amount.

Despite the fact that by around 1950 the annual consumption of oak bark in Britain had fallen to some 1% of the total consumption of vegetable tanning materials (~ 1000 tons per annum) oak-bark tannage remains one of the very few time-tested and traditional forms of leather production still practised today – albeit on a very modest scale for the making of speciality leathers used in fashion wear and for the manufacture of orthopaedic equipment. Chestnut extract (from the wood of *Castanea sativa*) which had a similarly long and dominating influence on the leather industry in other European countries is likewise used today to a limited extent in France. These dramatic changes in the exploitation of the natural vegetable tannins for leather production are almost entirely due to the development of synthetic tannins or ‘syntans’ which have claimed considerable attention in the last fifty years. Nearly half of the classic *Text book of Tanning* written by Henry Richardson Procter and published in 1885 was devoted to vegetable tannins. A bare five pages concerned mineral tannage yet the declining patterns of utilisation of vegetable tannins within the leather industry over the past fifty years are largely due to the introduction of this class of synthetic tannins. The enormous potential of mineral tannage may be readily appreciated from the fact that for tannage to be complete collagen (the protein of animal hides and skins) must take up about half its weight of vegetable tannins whilst good leathers can be produced using basic chromium salts when the skin may absorb as little as 3% of the inorganic material.

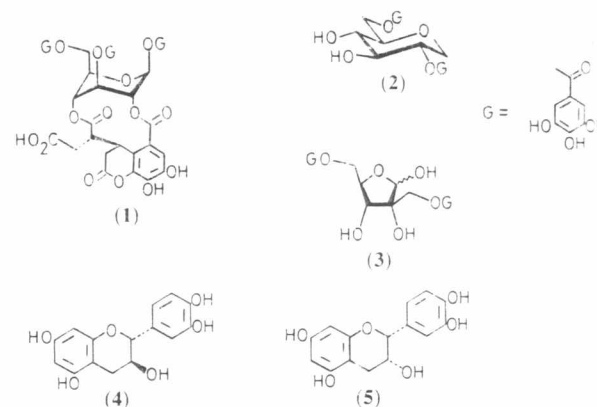
In the 21st Procter Memorial Lecture R.L. Sykes (Sykes, 1986) made a very shrewd and accurate observation:

Even now with sophisticated separation procedures the composition of commercial vegetable tannins is not completely understood although we have a working hypothesis which is adequate for most purposes. Indeed, it is probably not unfair to say that,

in commercial terms, the contribution of the physical chemists' approach . . . to vegetable tanning systems . . . has been much greater than that of the organic chemists' elucidation of the molecular structure of vegetable tannins

Some may consider the judgement harsh but it nevertheless remains true that the composition of most of the commercially important extracts used in vegetable tannage, such as wattle, chestnut, quebracho, valonea, myrobalans (Table 1.1), cannot yet be defined in sufficient detail in molecular terms either qualitatively or quantitatively.

One may speculate as to why this area of chemistry should have been so neglected and why it became so unattractive and unfashionable. A principal reason is undoubtedly that, prior to the 1960s, no industry of any significant size or importance – other than the leather industry – had any real or sustained interest in the class of compound known as vegetable tannins. Secondly and undoubtedly of equal, if not greater, importance is the nature of the chemistry involved. Emil Fischer at the turn of the century (Fischer, 1919) made some characteristically brilliant and definitive contributions to the study of the constitution of the gallo-tannins derived from Chinese galls and Aleppo galls. His work and that of Freudenberg and Karrer stimulated a great deal of interest amongst chemists (Haslam, 1966). Several important substances were isolated in a crystalline form notably chebulinic acid (**1**) from myrobalans (Freudenberg, 1919), aceritannin (**2**) from the leaves of *Acer tartaricum* native to Korea (Perkin and Uyeda, 1922), hamamelitannin (**3**) from the bark of witch hazel (*Hamamelis virginiana*) by Gruttner (Gruttner, 1898), (+)-catechin (**4**) and (–)-epicatechin (**5**) (Kostanecki and Lampe, 1906; Runge, 1821). These initial enthusiasms waned however as the great complexity of many plant extracts (often natural but frequently induced by the many and varied post-mortal processes required to derive them) was eventually realised. Crystallinity was the exception rather than the rule and studies of the chemistry of vegetable tannins had become by the 1950s one of the dark, impenetrable and inevitably neglected corners of Organic Chemistry. Its renaissance coincided with the advent in the 1950s and 60s of the armamentarium of new physical techniques and methods of isolation and structure determination. These made possible the isolation and characterisation of water soluble, amorphous natural products and they gave a new impetus to research in this area. Whilst the introduction of these techniques was partly responsible for the recent expansion in our knowledge no less significantly there has been a remarkable change in emphasis.



No longer is the interest in vegetable tannins centred solely and narrowly on their use in the leather industry but contributions have been made and information gained over a very wide field by scientists not directly concerned with leather and its manufacture. The chemistry of vegetable tannins impinges directly on both pure science and technology. For a long time that technology was simply that of leather manufacture but today these substances are recognised to be of importance in the food and beverages industry, in agriculture, in nutrition and in the re-awakening interest in herbal medicines. All these interests have helped bring about a renaissance in their chemistry. Retrospectively one can also see that the consequences of the general expansion of the opportunities for fundamental research in the 1960s and the benefits such research brings in its wake are perfectly illustrated by the vegetable tannins. The botanical interest in the vegetable tannins has provided a continuing and underlying theme to work in this area and over the past quarter of a century scientists have been able to examine in great detail not only the structure of tannins but also their position in the matrix of plant metabolism – all endeavours relatively unhindered by the pursuit of practicality and the need for direct application. This knowledge has all been gained in the 'comparative' simplicity of the situation presented by the plant *in vivo*. It provides moreover a firm basis from which one can now begin to seek to comprehend the problems which the vegetable tannins pose at the practical level in the food and allied industries, in agricultural and nutritional work and in herbal medicines and pharmacology. Not least one may now begin to try to unravel the nature, composition and properties of the complex vegetable tannin extracts used in the leather industry which are frequently derived from plant materials after numerous post-

mortal treatments such as drying, fermentation, storage and extraction. It should tell the leather technologist a great deal, although since leather-making is an art, it will surely not tell him all he wants to know! Parenthetically one should note that this brief historical episode admirably exemplifies the universal truth of Louis Pasteur's* precept and dictum:

There are no applied sciences There are only applications of Science and this is a very different matter The study of the applications of Science is very easy to anyone who is master of the theory of it.

Astringency

Knowledge is now similarly rapidly accumulating concerning the propensity of vegetable tannins to complex with macromolecules, such as proteins and polysaccharides, and with alkaloids. It will be an interesting goal for the future to rationalise this fundamental knowledge in terms of the properties of the tanners' brew and its interaction with the connective tissue of skins and hides.

It is not altogether surprising, given the intimate historical association which the oak has had with leather-making in Britain, that the word *tann* is said to be derived from the Celtic word for oak. Many of the definitions of the word *tannin* which figure currently in dictionaries and encyclopaedias derive, at least in part, from that given by Nierenstein in his book *The Natural Organic Tannins* in 1934. He described them as:

amorphous, rarely crystalline substances which are widely distributed in the Vegetable Kingdom. They are remarkable for their astringent . . . taste, and for their ability to form coloured solutions and precipitates with iron and other metals. They are also precipitated from solution by albumin, gelatin and other proteins, as well as by alkaloids. Their ability to combine with proteins is the basis of the process known as vegetable tannage.

The current definition of vegetable tannins in the Concise Oxford Dictionary (1976) reads 'any of several astringent substances got from oak-galls and various tree barks, used in preparing leather and in making ink, etc.' Other contemporary definitions mention 'Complex, amorphous, phenolic substances, used as mordants in dyeing and as medical astringents'. All refer to their vegetable origins and to their *astringency*. All foreshadow in varying degrees the other areas of scientific interest which have grown in relation to the vegetable tannins over the past 25

* Pasteur's father incidentally was a tanner.

years. Many of these, if not all, derive to a greater or lesser extent from their astringent or binding properties – their role, for example, in foodstuffs and beverages, in agriculture and nutrition and particularly as medicinal agents.

Throughout history until the beginning of the twentieth century medicine and botany came down the ages hand-in-hand and medical practice often depended largely on the use of plants. Extracts such as opium, senna and digitalis indeed still have an important place in the armoury of the modern medical practitioner. Additionally, and perhaps surprisingly, nearly half the prescriptions written annually in the USA still contain a drug of natural origin, either as the sole constituent or as one of two main ingredients. Many herbal treatments are specific in their action. One of the earliest recorded medicines consisted of an infusion of the dried shoots of a plant familiar to the ancient Chinese as 'Ma Huang'. It was a valuable remedy for coughs and as a cardiac stimulant and its active principle – ephedrine – was eventually introduced into Western medicine in 1924. Other herbal medicines are much less specific and their administration brings relief to a very wide range of conditions. Japanese and Chinese folk medicines thus frequently used plants rich in vegetable tannins (polyphenols) which are regarded as the active principles of these herbal remedies. They have been employed in the treatment of inflammation, liver injury, problems of the kidney, arteriosclerosis, blood pressure, hypertension, nervous and hormonal problems, stomach disorders, ulcers and to inhibit mucous secretions and so forth. Tannin rich plants are likewise widely used as diuretics and as antidiarrheic and hemostatic agents in both China and Japan. Typical of the very many members of this group of herbal medicines are *Geranii Herba* (Japanese name 'Gen-no-shoko' – the herb of *Geranium thunbergii*), *Psidium guajava* from S E Asia and the root of *Paeonia lactiflora* which is one of the most important Chinese crude drugs, being contained in many traditional 'Kampo' preparations. The outer skin of the root of the tree paeony ('Mudan') is used medicinally to cure disorders of the bloodstream including high blood pressure. Its usage dates back some 2000 years and demand for 'Dan pi', as its root bark is called, ensures that it is still planted by the acre in some Chinese States. Many members of this class are included in the plant family Rosaceae and it is interesting to note that in European culture today several remedies based on plants of this family persist. The astringent and stimulant character of raspberry (*Rubus idaeus*) leaf tea is thus well documented as is its use as a medicament during parturition. Similarly extracts of hawthorn (*Crataegus* sp.) find usage in Western medicine in the treatment of heart complaints.

The common characteristic of all these herbal remedies and many more is the high concentrations of polyphenols (vegetable tannins) which they contain.

Polyphenol-containing plant extracts thus have considerable efficacy in the treatment of medical disorders but against this must be set the harmful effects which these same natural compounds may also exert in mammalian systems – in particular the possible induction of gastric cancer caused by the excessive intake of herbal infusions. Many of such brews were originally recommended for the relief of specific ailments but they have often come to be regarded as tonics or beverages and are taken frequently, especially by people of low income groups. Oesophageal cancer is one of the most distressing of human afflictions and a causal relationship has been established in several communities (eg. Japan, E Turkey and N Iran) with the habitual consumption of herbal teas with a high level of tannins. The weight of circumstantial evidence of this type led one authority in this field to suggest that there is now an urgent need to explore, from the epidemiological to the molecular, the impact of tannins as plant constituents on men and animals (Morton 1978).

Polyphenols (*syn.* vegetable tannins) constitute a distinctive and unique group of higher plant metabolites. Their uniqueness lies not only in their polyphenolic character but also in their relatively large molecular size (M. wt: up to 20,000). The botanical interest, linked to the concept of astringency, has re-emerged in a new and powerful form in recent years for the vegetable tannins represent a distinctive example of the general phenomenon of *secondary* metabolism in plants. They are thought by some to constitute one of the most important groups of higher plant defensive secondary metabolites. Feeny (1975, 1976) has speculated on the possible function of polyphenols particularly in relation to the concept of plant apparency (eg. size and appearance) and the nature of a plant's chemical defences. He surmised that polyphenols (*syn.* vegetable tannins) are characteristic of the chemical defences of apparent plants and act as quantitative-dosage dependent barriers, even to insects which normally feed on leaves containing them. The relevant physiological effects of polyphenols upon herbivores derive, it is assumed, from their ability to complex with proteinaceous materials (Swain, 1977) or as succinctly stated by Bate-Smith (1973):

From the biological point of view the importance of tannins in plants lies in their effectiveness as repellants to predators, whether animal or microbial. In either case the relevant property is 'astringency' rendering the tissues unpalatable by precipitating

proteins or, by immobilizing enzymes, impeding the invasion of the host by the parasite.

Polyphenols thus have a harsh, astringent taste and produce in the mouth a feeling of constriction, dryness and roughness. These effects are thought to be due to polyphenol complexation with superficial glycoproteins within and without the epithelium, and, it is believed, they thereby render many plant tissues unacceptable as food sources to potential predators.

This text therefore seeks to embrace the various themes which surround the science of the vegetable tannins. It will thus encompass the biological as well as the strictly chemical, subjects such as classification, structure and biosynthesis; complexation with other species; the concept of astringency, its interpretation at the molecular level and its relevance to botany and agriculture, food science, nutrition and medicine.

Nomenclature and occurrence

Finally, despite Shakespeare's advice:

What's in a name? that which we call a rose

By any other name would smell as sweet.

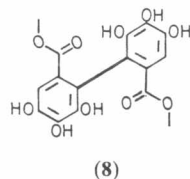
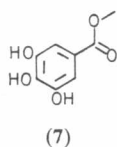
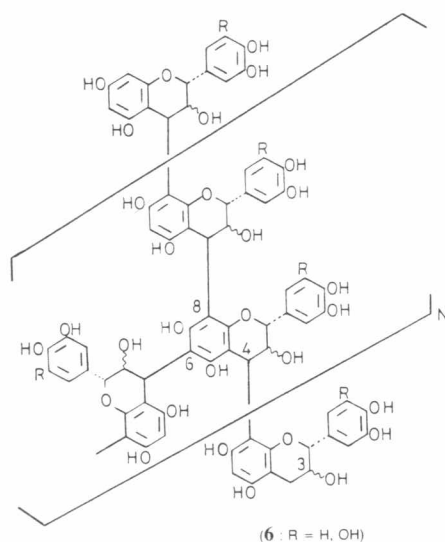
– some words on nomenclature, since the field of vegetable tannin chemistry has been bedevilled by misunderstandings, misinterpretations and changes in nomenclature which have paralleled advances in knowledge.

The word tannin has a long established and extensive usage in both the chemical and botanical literature. However a firm definition of what constitutes a vegetable tannin is not easy to give, although the reproach that everything which is isolated from plants and which gives a blue colour with ferric chloride has been described as a tannin is probably not entirely justified. In many senses the description tannin resembles the collective term alkaloid introduced by Meissner in 1818 to describe nitrogenous bases of natural occurrence but whose meaning changed repeatedly during the decades which followed and which today is not yet clearly defined.

The original implications of the word tannin clearly indicate a plant material which produces leather from hide. Probably the most acceptable definition is still that of Bate-Smith and Swain, formulated in 1962 (Bate-Smith and Swain, 1962). They adopted the earlier ideas of White (1957) and classified vegetable tannins as 'water-soluble phenolic compounds having molecular weights between 500 and 3000 and, besides giving the usual phenolic reactions, they have special properties such as the ability to precipitate alkaloids, gelatin and other proteins'. Many still prefer

the description 'tannin' which they find valuable simply because of its lack of precision. Scientifically and terminologically, plant polyphenol is to be preferred for this class of higher plant secondary metabolite if attempts are to be made to interpret their diverse properties at the molecular level.

Plant polyphenols are broadly divisible into two major groupings – the proanthocyanidins (6) and the polyesters based on gallic (7) and/or hexahydroxydiphenic acid (8) and their derivatives. As they were recorded in the earlier literature the former category corresponds with that of the condensed tannins and the latter to the hydrolysable tannins. Plant polyphenols are members of that body of natural substances, generally referred to now as secondary metabolites, which occur sporadically throughout Nature and which appear (as yet) to have no explicit role in the economy of the organism that produces them. As secondary metabolites plant polyphenols nevertheless possess several distinctive molecular characteristics which set them apart. Not only do they carry a multiplicity



of phenolic groups but their molecular weights encompass a wide range. Proanthocyanidins have been described up to 20,000 in molecular weight and esters of gallic acid and its derivatives are found with molecular weights in the region of 3000. Bate-Smith, Swain and Metcalfe (Bate-Smith and Swain 1953, Bate-Smith and Metcalfe, 1957) first drew attention to the very close similarity in the recorded systematic distribution between leucoanthocyanins (as proanthocyanidins were generally referred to at that particular time) and the diverse class of substance known in the botanical literature as 'tannins'. These authors suggested that *proanthocyanidins were most commonly responsible for the range of reactions which, up until that point, had been attributed to the presence of tannins in plants*. Subsequent detailed work in which polyphenolic metabolites have been isolated from plant materials and identified has given credence to that view. Some examples of authentic polyphenol (tannin) containing plant materials which are widely used in different areas of the World as foods, forage crops, livestock feeds, in beverages and in herbal preparations, are shown in Table 1.2. In many cases the observed properties of the plant tissue are due directly to the polyphenolic metabolites themselves. In others these phenomena are related to the presence of post-harvest transformation products of the original polyphenolic metabolites. *Black tea (Camellia sinensis)* is just such a case.

Similarly following Bate-Smith and Swain's definition of vegetable tannins (Bate-Smith and Swain, 1962) it has been customary in the intervening years, too readily perhaps, to ignore or even disregard the contribution which simple phenols (M. Wt. ~200 or less) may make to phenomena such as for example astringency. This is certainly an unwarranted assumption based on an erroneous understanding of the mechanism of phenol binding to proteins. Whilst polyphenols of the vegetable tannin class not only have the propensity to bind simultaneously to different points on the protein but also to cross-link separate protein molecules – properties which give them such a distinctive character – the capacity to bind to protein is one which derives from the chemical and physical nature of the phenolic nucleus itself. In these circumstances it is quite possible that the distinctive properties such as that of a significant astringent response may derive from plant tissues which contain (as defined above) no authentic vegetable tannins whatsoever but are nevertheless rich in simple phenolic metabolites. *Green tea*, for example, contains substantial quantities of the flavan-3-ols (9, 10, 11) and a cup of coffee is said (Challis and Bartlett, 1975) to contain some 250 mg of the ubiquitous plant phenol chlorogenic acid (12). Neither

Table 1.2. Some 'tannin' containing plants which (as leaf, fruit, bark, root, etc) have been used as foodstuffs, forage crops, livestock feeds, in beverages and in herbal preparations.

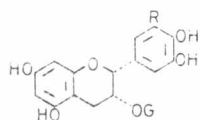
A. Proanthocyanidins

Apple (*Malus* sp.); Persimmon (*Diospyros kaki*); Grape (*Vitis vinifera*); Strawberry (*Fragaria* sp.); Blackberry, Dewberry, Raspberry (*Rubus* sp.); Plum, Cherry (*Prunus* sp.); Bilberry, Cranberry (*Vaccinium* sp.); Gooseberry, Black- and Red-Currant (*Ribes* sp.); Quince (*Cydonia* sp. *Chaenomeles chinensis*); Cocoa Bean (*Theobroma cacao*); Kola nut (*Cola acuminata*); Pear (*Pyrus* sp.); Hawthorn (*Crataegus* sp.); Rose hip (*Rosa* sp.); Chinese gooseberry (*Actinidia chinensis*); Yam (*Dioscorea alata*); Sorghum (*Sorghum* sp.); Barley (*Hordeum vulgare*); Sainfoin (*Onobrychis viciifolia*); Herbaceous legumes (*Lotus* sp., *Trifolium* sp., *Coronilla varia*, *Lespedeza cuneata*, *Lathyrus pratense*); Heather (*Calluna vulgaris*); Wattle (*Acacia* sp.); Rhubarb (*Rhei rhizoma* or *Rheum palmatum*); Myricaceae – bark (*Myrica rubra*); *Polygonum multiflorum* – root.

B. Galloyl and hexahydroxydiphenoyl esters

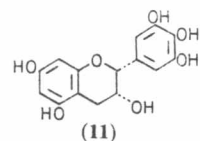
Blackberry, Dewberry, Raspberry (*Rubus* sp.); Walnut (*Juglans* sp.); Strawberry (*Fragaria* sp.); Carob pods (*Ceratonia siliqua*); Rose flower, hip (*Rosa* sp.); Pomegranate (*Punica granatum*); Acorn (*Quercus* sp.); Tea (*Camellia sinensis*); Uva-ursi (*Arctostaphylos uva ursi*); Paeony root (*Paeonia* sp.); Geranium root – geranii Herba (*Geranium* sp.); Smoke-tree (*Cotinus coggyria*); Cloves – flower buds (*Eugenia caryophyllata* or *Syzygium aromaticum*); Witch hazel (*Hamamelis* sp.); Kinmizuhiki (*Agrimonia japonica*); Ohebi-ichigo (*Potentilla kleiniana* or *Rheum palmatum*); Kibushi – leaves, fruit (*Stachyrus praecox*); Rhubarb (*Rhei rhizoma*); Casuarina (*Casuarina stricta*); Sweet gum – leaves (*Liquidaambar* sp.); Pistacio (*Pistacia vera*, *P. chinensis*); Guava (*Psidium guava*); Nupharis Rhizoma (*Nuphar japonicum*); Acacia – leaves (*Acacia miltica*); Bergenia – leaves, roots (*Bergenia crassifolia*, *B. cordifolia*, *B. purpurens*); Myricaceae – bark (*Myrica rubra*); Persimmon (*Diospyros kaki*).

(Taken from Haslam and Lilley, 1988).

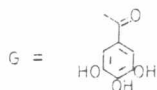


(9 : R = H)

(10 : R = OH)



(11)



(12)

green tea nor coffee contain substantial quantities of polyphenolic metabolites of the vegetable tannin class. Presumably the distinctive effects which these beverages have on the palate, derive, in part, from the presence of the relatively high concentrations of the simple phenols noted above. These and other facets of Phenol and Polyphenol Chemistry will be brought forward as they are deemed appropriate in the ensuing discussion.

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Proanthocyanidins

Introduction – leucoanthocyanins

The kind of substances that a plant contains depends upon the kind of plant that it is.

Writing in 1964 E.C. Bate-Smith (personal communication) disclaimed the originality of that statement but confidently looked forward to a phytochemistry that increasingly recognised the biological – and consequently natural – relationships between the substances that plants elaborate. 'It would be much more venturesome', he continued,

to say 'the kind of plant a plant is depends on the kind of substances that it contains' . . . The special biochemistry of a species must not only be characteristic of that species, but must inevitably be reflected in the exomorphic characters of that species. It is impossible to conceive of stable differences of morphology not being associated with equally stable physiological differences which in turn determine the processes which lead to a particular, and possibly unique, array of secondary products in the tissues of a plant.

For the time being the ball is in the chemist's court. There is so much detail to be worked out, so much work to be done to survey competently the distribution of even one kind of substance over the whole plant kingdom. At one and the same time research must be carried out in breadth and, here and there, in depth, so that the whole is not lost sight of while a part is being minutely examined.

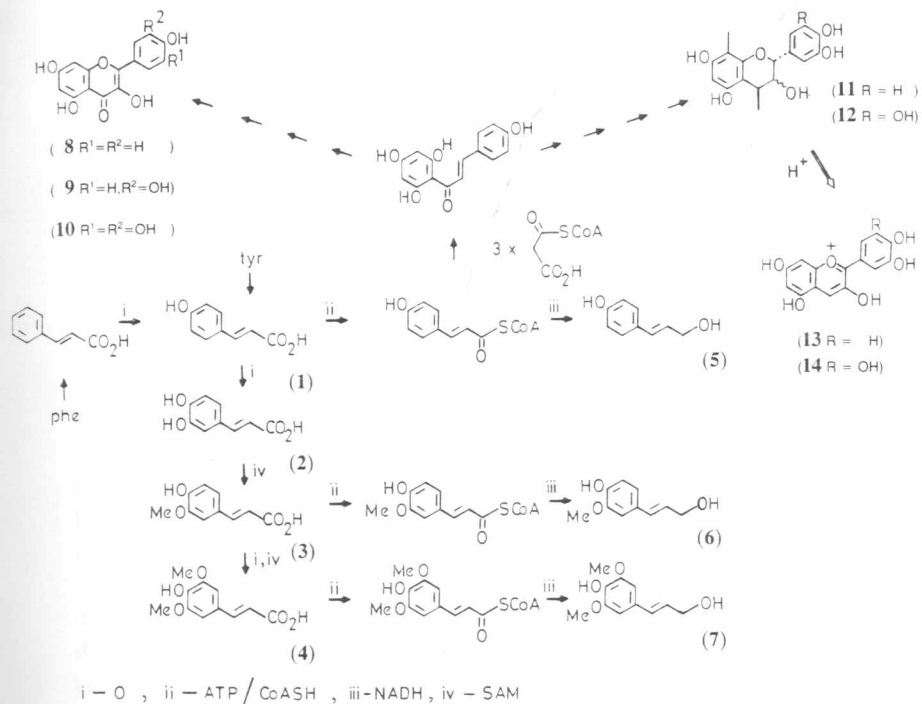
It would be unreasonable to expect that all of Bate-Smith's manifold expectations in the field of chemical taxonomy have been realised but,

over the past quarter of a century, there has been an explosion of effort and activity and a great many chemical and botanical relationships have now been described. Bate-Smith contributed in exemplary fashion to this field himself. His classical investigations of the phenolic constituents of plants and their taxonomic significance are renowned and pre-eminent in this area of research. It is obviously desirable to base systematic surveys on the examination of tissues observed at like stages of development, derived from healthy plants growing under optimal physiological conditions. Erdtmann favoured the heartwood of trees for such studies but Bate-Smith's experimental data dealt, almost exclusively, with the leaves of plants (or the nearest equivalent organs that the species possessed). Many diverse and disparate observations testify to the view that the distribution and accumulation of phenolic constituents in different tissues of a plant may vary markedly – eg. the leaves, stem, fruit and bark of *Quercus* sp.; the leaves and root of *Bergenia* sp.; the seeds, leaves and fruit-pods of members of the Leguminosae and so forth. It is nevertheless the synthetic potential of the plant as a whole which is important. However great the apparent differences between different tissues the constituents are always consistent with the chemical constitution of the plant as a whole. In this particular context therefore the synthetic capacity in the leaves is assumed to be a reflection of the synthetic capacity of the entire plant.

Bate-Smith pointed out the probability that the phenolic profile of a plant is often associated with other aspects of the chemistry and metabolism of that plant. One distinction to which he repeatedly drew attention in his work (Bate-Smith, 1953, 1956, 1958, 1959, 1962; Bate-Smith and Lerner, 1954; Bate-Smith and Metcalfe, 1957) was that between woody and non-woody plants. This distinction he suggested was sufficiently pronounced for it to be possible to speak of a typically 'woody' pattern of phenolic constituents in the leaves of certain plants. The same 'woody' phenolic constituents – apparent in a wide range of monocotyledons, gymnosperms, ferns, dicotyledons – are however not found in mosses, algae, fungi and lichens. Bate-Smith noted the structural analogies and similarities amongst these 'woody phenolics' to lignin and he postulated that they might well be concerned, in some way, with the presence of a vascular system in those plants which contain them.

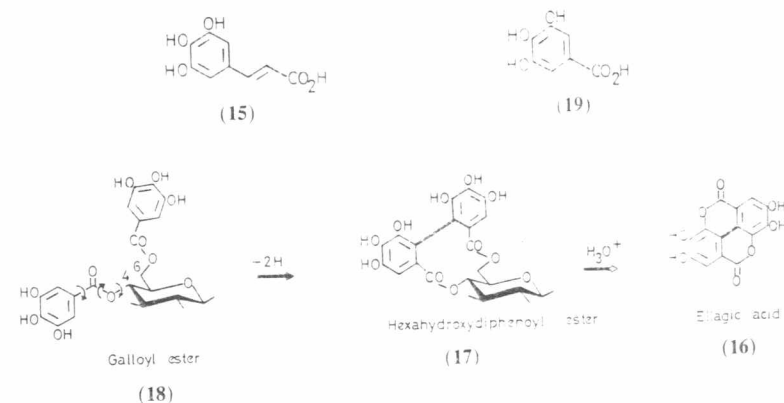
According to Bate-Smith three classes of phenolic constituent overwhelmingly predominate in the leaves of vascular plants:

- (i) leucoanthocyanins (11, 12) (*syn.* proanthocyanidins, *vide infra*) – principally those which yield cyanidin (13) and delphinidin (14) on treatment with acid,



- (ii) flavonol glycosides – principally those of kaempferol (8), quercetin (9) and myricetin (10),
- (iii) esters, amides and glycosides of various hydroxycinnamic acids – principally *p*-coumaric (1), caffeic (2), ferulic (3) and sinapic (4) acids.

The biosynthetic origin of the three classes of phenolic metabolite (i, ii, iii) is generally assumed to be intimately associated with the development of a vascular character in plants and with the development of ability to synthesise the structural polymer lignin by the diversion of L-phenylalanine (phe), and L-tyrosine (tyr) in the Gramineae, from protein synthesis. Plants use one or more of the *p*-hydroxycinnamyl alcohols (5, 6, 7) – derived by NADH reduction of the coenzyme A ester of the corresponding cinnamic acid (Zenk, 1978) – as precursors to lignin (Gross, 1978). 3,4,5-Trihydroxycinnamic acid (15) is not an intermediate in lignin biosynthesis and, except as its various *O*-methyl ethers such as sinapic acid (4), it has not been encountered in Nature. Bate-Smith found, in his taxonomic work, that hexahydroxydiphenic acid [determined as its characteristic dilactone ellagic acid (16), after acid hydrolysis] was very widely distributed in ester form (17) in plants. He suggested that it was the taxonomic equivalent of the 'missing' acid – 3,4,5-trihyd-



roxy-cinnamic acid (15). Although direct experimental evidence is still awaited there is nevertheless strong circumstantial evidence (Haslam, 1982b) to suggest that the esters (17) are derived *in vivo* by oxidative coupling of esters of gallic acid (18) and hence that gallic acid (19) is the real taxonomic equivalent of the 'missing' acid (15). Bate-Smith made several taxonomic correlations but in particular he noted the presence of leucoanthocyanins (*syn.* proanthocyanidins) and the ability of particular plant families to metabolise phenols containing the *vic*-trihydroxyaryl (pyrogallol) group. These two synthetic capabilities were accorded the characters *a* and *b* respectively and plants were broadly classified in the groups *ab*, *a₀b*, *ab₀* and *a₀b₀*. (*a₀*, *b₀* represent the situations where these synthetic capacities have been lost).

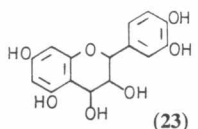
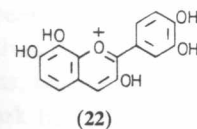
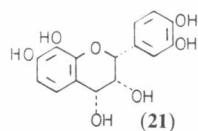
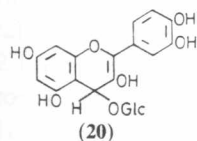
Although there can be no finality in such matters Bate-Smith considered that the evidence strongly favoured the proposition that once the ability to synthesise leucoanthocyanins (*syn.* proanthocyanidins, *a*) and the *vic*-trihydroxyl aryl (pyrogallol) group, *b*, had been lost (*a* → *a₀* and *b* → *b₀*) during evolution, these characteristics were never regained. Thus according to Bate-Smith the steps *a* → *a₀* and *b* → *b₀* were irreversible in the evolutionary sense and the trend of evolution from the earliest emergence of the dicotyledons has always been in the direction *ab* → *a₀b₀*. Since the synthesis of vegetable tannins is associated with both of the characters *a* and *b* it thus seems that the capacity to synthesise tannin is a primitive taxonomic character that tends to become lost with increasing phylogenetic specialisation. Furthermore this trend is most marked in herbaceous rather than woody plants.

During the course of these studies Bate-Smith and his colleagues also demonstrated that the presence of those substances then classified as 'leuco-anthocyanins' showed a considerable degree of correlation in their distribution with the vegetable tannins, as recorded by Metcalfe and

Chalk on the basis of histological reactions (Metcalf and Chalk, 1950). They were led indeed to speculate that 'leuco-anthocyanins' were most commonly responsible for the broad range of properties and reactions which have been attributed to the presence in plant tissues of tannins. The validity of this assumption has been presumed since that time. Leucoanthocyanins (*syn.* proanthocyanidins) are believed, on this basis, to be generally identical with the *condensed tannins* recorded in the earlier chemical and botanical literature (Haslam, 1966).

Chemically leucoanthocyanins (*syn.* proanthocyanidins) are characterised by the anthocyanidins which are formed from them when they are digested with mineral acid. With few exceptions – such as the products derived from the wood and bark of certain trees – these anthocyanidins are limited to the familiar petal pigments cyanidin (**13**) and delphinidin (**14**). Robert Boyle is credited by Pigman and his associates (Pigman *et al.*, 1953) with the first observation of this distinctive solvolytic degradation and the chemical and biochemical literature show that Staats, Laborde, Tswett and Willstätter and Nolan all made similar 'discoveries' around the turn of the century (Haslam, 1975). Rosenheim (1920) was the first to initiate studies of the chemical constitution of leucoanthocyanins during an examination of the anthocyanidin pigments of the young grape vine (*Vitis vinifera*). He put forward the hypothesis that the colourless leucoanthocyanins were glucosides of the pseudo-base of the anthocyanidins (**20**). His own observations lay dormant until the 1930s when Sir Robert and Lady Robinson in Oxford made the first systematic investigations of the distribution of leucoanthocyanins in higher plants (Robinson, R., 1936; Robinson, G.M., 1937; Robinson and Robinson, 1935, 1939). They demonstrated that leucoanthocyanins were widely found in the Plant Kingdom and that the majority yielded cyanidin (**13**) with acid and the remainder, with few exceptions, delphinidin (**14**).

Sir Robert and Lady Robinson recognised not only the generally nebulous character of much of the information at that time but they also



made tentative observations of longer term fundamental significance. On the basis of solubility differences they divided the known leucoanthocyanins into three broad classes:

- those that are insoluble in water and the usual organic solvents, or give only colloidal solutions;
- those readily soluble in water and not readily extracted from the solution by means of ethyl acetate;
- those capable of extraction from aqueous solution by ethyl acetate.

They also were mindful of the possible biosynthetic relationship between leucoanthocyanins and the anthocyanin(idin) pigments and commented:

Inevitably the transformations of these substances (leucoanthocyanins) into colouring matters of the flavylium type must occur in nature occasionally, but it does not follow that this route represents the standard mechanism . . . It is too early to attribute a predominant role to the leucoanthocyanins, but it is already safe to assume that their modification represents an auxiliary pigmentation process. It is possibly operative in autumnal reddening and in the coloration following injuries or decortication of leaves.

Following the work of Sir Robert and Lady Robinson further analytical work was carried out by Bate-Smith who examined the systematic distribution of leucoanthocyanins in plants using chromatographic techniques (Bate-Smith, 1953, 1962; Bate-Smith and Lerner, 1954). The first definitive structural work in this area was also carried out in the 1950s when King and Bottomley (1954) isolated melacacidin from Australian blackwood (*Acacia melanoxylon*) and showed its structure to be that of the tetrahydroxyflavan-3,4-diol (**21**). The compound possessed all the properties of a phloba-tannin and a leucoanthocyanin and gave with acid (10% HCl) both the flavylium salt (**22**) and a typical red-brown phlobaphen. In view of the absence of any carbohydrate molecule within the structure King and Bottomley made a revision of the nomenclature and classified the compound as a *leucoanthocyanidin*. Uncritically perhaps they also concluded that this indicated . . . 'a flavan-3,4-diol structure for this widely distributed group of natural products'.

The first real indications of the fallacy in this assumption were revealed by Forsyth (Forsyth, 1952, 1953; Forsyth and Roberts, 1958, 1960) in work with the leucoanthocyanins of the cocoa bean (*Theobroma cacao*).

Forsyth isolated the major component and showed that it was composed of two C₁₅ flavan units, one of which was (–)-epicatechin and the other a residue which yielded cyanidin (13) on treatment with acid. Forsyth formulated the leucoanthocyanin as a hemi-ketal and, although this structure has since been shown to be incorrect Forsyth's work was the first of a number of investigations which showed that the characteristic leucoanthocyanin reactions of many plant tissues were due to the presence of flavan-3-ol dimers, trimers and higher oligomers.

The need for a new systematic nomenclature was evident and Freudenberg and Weinges (1960) therefore collectively designated all the colourless substances isolated from plants which form anthocyanidins when heated with acid as *proanthocyanidins*. The name proanthocyanidin is a chemical and not a biochemical term and it does not imply any biogenetic relationship. Weinges *et al.* (1969a) subsequently reserved the term *leucoanthocyanidin* for the monomeric proanthocyanidins such as the flavan-3,4-diols and the name *condensed proanthocyanidin* for the various flavan-3-ol dimers and higher oligomers. This nomenclature is employed here except where it is deemed appropriate for illustrative reasons to use other forms of description.

Flavan-3,4-diols (leucoanthocyanidins)

(i) Occurrence and distribution

The principal leucoanthocyanidins of natural origin whose structure and stereochemistry have been fully determined are shown in Table 2.1. It may be noted that the configuration 2*S* in (–)-leucofisetinidin is antipodal at this position to the remaining members of this class and this is presumably of some biogenetic significance. Also noteworthy is the fact that all the flavan-3,4-diols listed in Table 2.1 have been isolated from the wood or bark of trees, particularly *Acacia* species, and this has prompted detailed phytochemical surveys of the flavonoids and leucoanthocyanidins of the Australian species of *Acacia* (Clark-Lewis and Dainis, 1967; Tindale and Roux, 1969). Extensive compilations of the physical, chemical and spectroscopic data related to the leucoanthocyanidins in Table 2.1 are contained in an excellent review by Weinges and his collaborators (1969a). In addition to those compounds listed in Table 2.1 several other members of the leucoanthocyanidin class of natural product have been isolated and described. These include the flavan-3,4-diol derivatives the mopanol and the peltogynols and the various leucopelargonidins, leucocyanidins and leucodelphinidins. Definitive stereochemical work has not been completed for any member of these last three

Table 2.1. Principal naturally occurring leucoanthocyanidins (flavan-3,4-diols) – occurrence, structure, stereochemistry

| Trivial names [hydroxylation] | Stereochemistry ^a | Occurrence |
|--|--|---|
| Guibourtacacidin [7,4'(OH) ₂] | 2 <i>R</i> :3 <i>S</i> :4 <i>S</i> 2 <i>R</i> :3 <i>S</i> :4 <i>R</i> 2 <i>R</i> :3 <i>R</i> :4 <i>S</i> | <i>Acacia cultriformis</i> (du Preez and Roux, 1970) <i>Guibourtia coleosperma</i> (Roux and De Bruyn, 1963) |
| (+)-Mollisacacidin, (+)-Gleditsin, Leucofisetinidin [3',4',7(OH) ₃] | 2 <i>R</i> :3 <i>S</i> :4 <i>R</i> 2 <i>R</i> :3 <i>S</i> :4 <i>S</i> 2 <i>R</i> :3 <i>R</i> :4 <i>S</i> 2 <i>R</i> :3 <i>R</i> :4 <i>R</i> 2 <i>S</i> :3 <i>R</i> :4 <i>S</i> 2 <i>S</i> :3 <i>R</i> :4 <i>R</i> 2 <i>S</i> :3 <i>S</i> :4 <i>S</i> 2 <i>R</i> :3 <i>S</i> :4 <i>S</i> 2 <i>R</i> :3 <i>S</i> :4 <i>S</i> | <i>Acacia meurnsii</i> (Keppler, 1957) <i>Gleditsia japonica</i> (Clark-Lewis and Mitsuno, 1958) <i>Acacia cultriformis</i> (du Preez and Roux, 1970) <i>Guibourtia coleosperma</i> (du Preez and Roux, 1965) <i>Guibourtia coleosperma</i> (du Preez and Roux, 1965) <i>Schinopsis lorentzii</i> (Drewes and Roux, 1964) <i>Guibourtia coleosperma</i> (du Preez and Roux, 1965) <i>Guibourtia coleosperma</i> (du Preez and Roux, 1965) <i>Neuratenia amboensis</i> (Oberhalzer <i>et al.</i> , 1980) |
| [3',4',7(OMe) ₃] [3,3',4,4',7(OMe) ₅] | | |