Phytochemistry, 1972, Vol. 11, pp. 861 to 875. Pergamon Press. Printed in England.

PROCEEDINGS OF THE PHYTOCHEMICAL SOCIETY

A meeting of the Society was held at University College and Trinity College, Dublin on 28–30 September 1971 when the following papers were presented, under the general title:

Recent Chemistry and Biochemistry of Phenolic Compounds

REVIEWS

Role of Phenolases in the Synthesis of Plant Phenolics

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and

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ENZYMES catalysing two types of aromatic hydroxylation in plants are recognized. These involve the introduction of a phenolic hydroxyl into either a non-hydroxylated aromatic ring (e.g. phenylalanine, cinnamic acid) or *ortho* to a hydroxyl group already present (e.g. *p*-coumaric acid, tyrosine, 4-hydroxyflavonoids). The latter can be catalysed by the monophenolase (cresolase) action of phenolases, which also show catechol oxidase activity.

We have investigated a phenolase from the leaves of spinach beet (*Beta vulgaris* L. ssp. *vulgaris*)[1], which acts as a mixed function oxidase, requiring an electron donor (AH_2) as well as the substrate and molecular oxygen: $AH_2 + X.H. + O_2 \rightarrow A + X.OH + H_2O$.

The removal of a lag period in the reaction by catalytic quantities of caffeic acid or other *o*-dihydric phenols suggests that the catechol oxidase activity is part of the catalytic mechanism of hydroxylation; the reductant takes part only in reducing the *o*-quinone produced.[2] Mason[3] has proposed a mechanism in which hydroxylation requires some specific spatial relationship between protein, copper and molecular oxygen, which can be dispensed with in the direct oxidation of catechols to *o*-quinones.

With many reductants, the further oxidation of the product accompanies hydroxylation, but this secondary catechol oxidase activity was found to be suppressed when 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine was used, providing that *p*-coumaric acid was present in excess.[4] This reductant may hold the enzyme in a suitable conformation for hydroxylation and protect it from attack by *o*-quinones more effectively than ascorbate or NADH.

Distribution studies have shown the enzyme to be firmly bound to the chloroplasts of spinach beet and sugar beet.[5] Illumination of chloroplast suspensions with *p*-coumaric acid in the presence of oxygen, have produced caffeic acid without evidence of further oxidation.[6]

The lack of specificity in the hydroxylase action of phenolases[7] may bring their role into question or require control in the reactions which produce the specific phenols presented to them. Their function may be determined by relating changes in phenolase activity to those in the activity of phenylalanine ammonia-lyase and in the level and type of phenolics present in tissues. Attention is drawn to the possible action of peroxidases in hydroxylation, and to the low hydroxylase activity of many phenolase preparations with high catechol oxidase activity.

[1] P. F. T. VAUGHAN and V. S. BUTT, *Biochem. J.* **113**, 109 (1969).
[2] P. F. T. VAUGHAN and V. S. BUTT, *Biochem. J.* **119**, 89 (1970).
[3] H. S. MASON, *Nature, Lond.* **177**, 79 (1956).
[4] P. F. T. VAUGHAN and V. S. BUTT, *Biochem. J.* **111**, 32P (1969).
[5] A. M. MAYER and J. FRIEND, *Nature, Lond.* **185**, 464 (1960).
[6] D. G. BARTLETT, J. E. POULTON and V. S. BUTT, in preparation.
[7] P. F. T. VAUGHAN, V. S. BUTT, H. GRISEBACH and L. SCHILL, *Phytochem.* **8**, 1373 (1969).

Phenolases may also be important in alkaloid synthesis through their catalysis of the oxidative deamination[8,9] and decarboxylation of tyrosine and 3,4-dihydroxyphenylalanine to give C_6–C_2 aldehydes and acids, e.g. in the biogenesis of norbelladine and benzyltetrahydroisoquinolines. The conditions for the reaction with phenolases from stinging nettle (*Urtica dioica* L.) and spinach beet will be discussed; only a limited range of amino-acids appear to be decarboxylated, and these require an excess of amino-acid to catechol. Conditions for the synthesis of 4-hydroxy- and 3,4-dihydroxy-phenylacetaldehyde will be discussed.

[8] W. O. James, E. A. H. Roberts, H. Beevers and P. C. de Kock, *Biochem. J.* **43,** 626 (1948).
[9] E. M. Trautner and E. A. H. Roberts, *Austral. J. Sci.* **3B,** 356 (1950).

Neoflavanoids

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During the last decade, a number of naturally occurring compounds which fall into a new structural pattern called neoflavanoids have been discovered. The number known to-day is thirty-eight and is rapidly inrceasing. The general term *neoflavanoid* was suggested in connection with a group of natural phenolic compounds that were found in species of *Dalbergia* and *Macherium*, genera of Leguminosae-Lotoideae. However, one member of the neoflavanoid class, the 4-arylcoumarins is, to date, shown to be more prolific among genera of Guttiferae.[1] Characteristically, these are 4-phenylcoumarins with oxygen functions at the 5- and 7-positions and acyclic (alkyl or acyl) substituents at 6- and (or) 8-positions or with heterocycles (chromene or chromanone) fused to the A-ring. A lone 4-arylcoumarin exostemin[2] has been isolated from Rubiaceae.

Biogenetic studies[3] have been successfully carried out on the 4-phenylcoumarins from the Guttiferae, but only biogenetic proposals exist for the neoflavanoids of Leguminosae because of the technical difficulties of incorporation. In support of one[4] of the five pathways proposed—alkylation of phenolic or polyketide precursors with cinnamyl pyrophosphate—is the isolation of a number of cinnamyl phenols which correspond structurally to the co-occurring neoflavanoids.

The structural types now recognized as belonging to neoflavanoids are the acyclic 3,3-diarylprop-1-enes (latifolin-type) and 3-quinonyl-3-arylprop-1-enes (dalbergione-type). Two natural neoflav-3-enes have been recorded. The neoflavans brazilin (from *Caesalpinia* species) and haematoxylon (*Haematoxylon campechianum*) will also be discussed.

[1] J. Polonsky, *Bull. Soc. Chim. Fr.* 1079 (1957); R. A. Finnegan and C. Djerassi, *Tetrahedron Letters* 11 (1959); D. P. Chakraborty and B. Das, *ibid.* 5727 (1966; L. Crombie, D. Games and A. McCormick, *ibid.* 145 (1966).
[2] F. Sanchez-Viesca, E. Diaz and G. Chavez, *Ciencia Mex.* **25,** 135 (1967).
[3] G. Kuneschand and J. Polonsky, *Chem. Commun.* 317 (1967); *idem. Phytochem.* **8,** 124 (1969); *Biochemie* **53,** 431 (1971).
[4] W. D. Ollis and O. R. Gottlieb, *Chem. Commun.* 1396 (1968).

Enzymology and Regulation of Flavonoid Biosynthesis in Plants and Plant Cell Cultures

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The extensive application of labelled precursors in the *in vivo* study of the biosynthesis of flavonoids has led to a general understanding of the biogenetic interrelationships of the various classes of flavonoids. However, for the proof of the postulated biosynthetic schemes and for the study of the details of the individual reactions and their regulation it is necessary to isolate and characterize the respective enzymes. Considerable progress in this direction has been made in the last 2 or 3 yr. All but one of the eleven enzymes postulated for the biosynthesis of the flavone glycosides apiin and graveobioside B in parsley have recently been found in extracts from cell suspension cultures of this plant, in our laboratory.[1] These enzymes are: (1) phenylalanine ammonia-lyase (PAL); (2) *trans*-cinnamic acid 4-hydroxylase; (3) *p*-coumarate: CoA ligase; (4) chalcone-flavanone isomerase; (5) 'flavanone-dehydrogenase'; (6) a phenolase; (7) *S*-adenosylmethionine: luteolin 3′-*O*-methyl transferase; (8) UDP-glucose: apigenin-7-*O*-glucosyl transferase; (9) UDP-apiose:7-*O*-glucosylapigenin- 1 → 2-apiosyl transferase, and (10) UDP-apiose synthetase.

[1] K. Hahlbrock, J. Ebel, R. Ortmann, A. Sutter, E. Wellmann and H. Grisebach, *Biochim. Biophys. Acta* **244,** 7 (1971), and unpublished results.

The properties and regulation of PAL from a number of different plants have been studied extensively. Enzyme 2 is a membrane-bound enzyme of the mixed function oxygenase type which has previously been found in other plants. The enzyme catalyzing the activation of *p*-coumaric acid to the CoA-ester was partially purified from soya bean cell cultures and could be separated from the acetate activating enzyme.[2] Enzyme 4 has been detected in many plants. It can occur in the form of isoenzymes with different pH optima. Enzymes from parsley, mung bean, *Cicer arietinum*, and *Datisca cannabina* show a pronounced specificity with regard to ring A substitution (flavone numbering), whereas the substitution in the B-ring has no great influence on enzymatic activity. Chalcone glucosides cannot function as substrates. The conversion of the chalcone/flavanone to the flavone was achieved with a cell-free extract (enzyme 5), but whether this reaction is a dehydrogenation of a flavanone or an oxidation of a chalcone is not yet known.

The role of phenolases in flavonoid biosynthesis has also been explored with a phenolase from *Beta vulgaris*.[3] At present it is difficult to decide whether hydroxylation leading to 3′-hydroxyflavonoids takes place at the *p*-coumaric acid or at the flavonoid stage.

The methyltransferase (enzyme 7) has been purified 82-fold and its specificity for a number of acceptors has been determined.[4] The K_m value for luteolin and its 7-*O*-glucoside are $4{\cdot}6 \times 10^{-5}$ and $3{\cdot}1 \times 10^{-5}$ respectively, and the K_m for caffeid acid is $1{\cdot}6 \times 10^{-3}$ M. Methylation at the 3′-hydroxyl group is therefore assumed to take place at the level of the flavone or its glucoside. The two sugar transferases 8 and 9 could be completely separated on hydroxylapatite The specificity of the purified enzymes was investigated in respect to glycosyl donor and glycosyl acceptor. Glucose and apiose are transferred to apigenin in a stepwise manner to form apiin.

The levels of enzyme activities change with development of parsley seedlings.[5] In cell cultures of this plant, extractable enzyme activities increased greatly upon illumination of the cells. Two groups of enzymes can be distinguished on the basis of their responses to light. The first group comprises the three enzymes acting on substrates of the phenylpropanoid type; the second group includes all those enzymes involved exclusively in the formation of flavonoids and their glycosides. In cell cultures of *Haplopappus gracilis* dihydrokaempferol proved to be a much better precursor for cyanidin biosynthesis than the corresponding chalcone or phenylalanine.[6] A cell-free system for cyanidin biosynthesis has not yet been found. Cell cultures of mung bean (*Phaseolus aureus*) were also explored as a system for biosynthetic studies in the isoflavone series.

[2] K. HAHLBROCK and T. LINDL, unpublished results.
[3] P. F. T. VAUGHAN, V. S. BUTT, H. GRISEBACH and L. SCHILL, *Phytochem.* **8,** 1373 (1969), and unpublished results.
[4] J. EBEL, K. HAHLBROCK and H. GRISEBACH, in preparation.
[5] K. HAHLBROCK, A. SUTTER, E. WELLMANN, R. ORTMANN and H. GRISEBACH, *Phytochem.* **10,** 109 (1971).
[6] H. J. FRITSCH, K. HAHLBROCK and H. GRISEBACH, *Z. Naturforsch.* **26b,** 581 (1971).

Plant Phenolics of Pharmaceutical Interest

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THE LEADING and unifying idea in this review is Ferguson's principle, as applied to plant phenolics. Stress is laid upon the dependence of (systematic) drug action of a phenolic compound on its solubility (partition coefficient). The likelihood of reaching the site of action in adequate concentration is much higher for a compound with good rather than with low lipid solubility; and therefore, as a matter of statistics, one is more likely to detect biologically active molecules in the lipid than in the water-soluble fraction of plant extracts. Examples are given of: structure-variation within the coumarins, lipophilic coumarins (especially CNS-actions of methoxylated coumarins), the visnagins, the kawapyrones, the piperolides (from *Piper sanctum*), the lipophilic flavonoids (dependence of toxicity on distribution coefficient), the colchicine molecule.

The second section is devoted to lipophilic aromatics, which have gained some reputation in counteracting metabolic and toxic stresses. As typical examples are cited the tocopherols, the yeast benzofuran, and the silybum constituents, of which the last mentioned compounds are claimed to have 'antihepatotoxic activity'.

A section on "Phenolics as antithyroid compounds", based on the fundamental work of E. B. Astwood, summarizes briefly the papers published by E. Jeney *et al.* on the goitrogenic effects of flavonoids.

The next section "Some chemotherapeutic activities of phenolic compounds" reviews plant phenolics that are shown to have cytotoxic, anthelmintic, antifungal and antibacterial activity. Special attention is given to the observation, that the antipsoriasis activity of substances like chrysarobin is a result of their rather specific cytostatic action, in contrast to what was believed before, in that action was due to an unspecific oxidation-reduction reaction in the skin. Dhelwangin, the bactericidal component from *Pogostemon patchouli*, can be considered as the prototype of a group of $FeCl_3$-positive reacting acetogenins, which

however are not true aromatics but monocyclic 4-hydroxy-α-pyrones. More complicated members of this group are helipyrone, obtusifolin and arenarol. Finally mentioned are the antibacterial activity of the lipophilic *Calophyllum* constituents and the antifungal (especially against *Aspergillus niger*) activity of dihydrokavain.

The Plant Flavonoids: Phylogeny and Function

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TWO MAJOR changes can be discerned in the evolution of flavonoids in the vascular plants. The first concerns flavonoid class and the key oxidation at the 3-position to give flavonols, leucoanthocyanidins and anthocyanidins, the most abundant and functionally most important flavonoids in the Angiosperms. This ability, once gained, has had a dominating influence on flavonoid patterns on most plants; there is evidence, however, that, together with the ability to add a third hydroxyl to the B-ring it is lost gradually among the most highly evolved plant groups. The second concerns substitution at the 8-position, to give biflavonyls, glycosylflavones and gossypetin derivatives. The nature of the substituents appears to change as one progresses from lower to higher plants and the position of substitution can also alter with evolutionary advancement.[1,2]

The functional significance of these evolutionary sequences is not entirely clear. However, selection for flower colour has a significant effect on the types of flavonoids synthesized in the petals, not only of anthocyanins but also of yellow flavonoids and of flavone co-pigments. In the case of leaf flavonoids, the most important ecological factor is probably selection of plants by animals for food and there is increasing evidence that flavonoids can act, in appropriate circumstances, both as attractants and as repellents to feeding insects.[3,4]

Indirect evidence that leaf flavonoids have an ecological role has been the recent observation that there is a geographical component in the distribution of flavonoids in plant populations, both below and above the species level. Such studies are of value in determining the direction of migration of cosmopolitan or bipolar species, since the flavonoid types present may provide a measure or 'index' of evolutionary advancement. Examples of plant groups in which correlations between geography and flavonoid patterns include *Carex*, *Empetrum*,[5] *Rhododendron* and *Senecio*[6] and some of these cases will be discussed.

[1] J. B. HARBORNE, Evolution of flavonoids in plants. In *Recent Advances in Phytochemistry* (edited by V. C. RUNECKLES), Vol. 4, in press.
[2] J. B. HARBORNE and C. A. WILLIAMS, *Phytochem.* **10,** 367 (1971).
[3] P. FEENY, *Ecology* **51,** 565 (1970).
[4] A. G. ZIELSKE, J. N. SIMONS and R. M. SILVERSTEIN, *Phytochem.* **11,** 393 (1972).
[5] D. M. MOORE, J. B. HARBORNE and C. A. WILLIAMS, *Bot. J. Linn. Soc.* **63,** 277 (1970).
[6] C. W. GLENNIE, J. B. HARBORNE, G. D. ROWLEY and C. J. MARCHANT, *Phytochem.* **10,** 2413 (1971).

Biosynthesis and Metabolism of Phenylpropanoids

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THE BIOSYNTHESIS of aromatic C_6–C_3 amino acids and their most important metabolites, cinnamic acids and benzoic acids, is mainly already determined at the level of chorismic acid. Four major pathways will be discussed: (1) A reaction sequence via isochorismic acid to salicylic which has extensively been studied in microorganisms. (2) The involvement of an enzyme containing chorismate mutase and prephenate dehydratase leading to phenylpyruvic acid, L-phenylalanine, and cinnamic acid. (3) An enzyme complex containing chorismate mutase and prephenate dehydrogenase catalyzing the formation of *p*-hydroxyphenylpyruvic acid, the precursor of L-tyrosine and *p*-coumaric acid. (4) A pathway which appears responsible for the formation of *p*-hydroxybenzoic acid and protocatechuic acid without involvement of phenylpropanoids. Thus, the key reactions are located immediately after chorismic acid or are represented by the hydroxylation of cinnamic acid, processes which are controlled by the intracellular localization of the enzyme systems involved.

Phenylpropanoids, cinnamic acids and derivatives, are capable of being condensed with acetate units or with other C_6–C_3 units. The formation of stilbenes and of aryldihydroisocoumarins like hydrangenol was studied *in vitro*.[1] *In vivo* experiments were aimed at the elucidation of biosynthetic pathways leading to formation of the isovanillin structure of ring B of rhaponticin and phyllodulcin.[2] Most likely, in the former

[1] H. KINDL, *Z. Physiol. Chem.* **352,** 767 (1971).
[2] H. KINDL, *Naturwiss.*, in press.

case a substitution at ring B takes place when the stilbene skeleton is already built up while a cinnamic acid with a preformed isovanillin structure is the precursor of rhaponticin.

Benzoic acids can also function as starters in condensation reactions with C_2-units according to the polyacetate rule, the products being benzophenones and xanthones. In addition, benzoic acids are converted into phenols in a hydroxylation induced decarboxylation reaction.

The last section of the review deals with the conversion of phenylpropanoids into C_6–C_2 compounds. The biosynthesis of *o*-hydroxyphenylacetic acid and homogentistic acid from phenylpyruvic acid and *p*-hydroxyphenylpyruvic acid, respectively, was studied *in vivo* and *in vitro*. In certain types of plant cells an intracellular localization of the corresponding enzyme activity was possible. Direct transformation of arylalanines to the corresponding arylacetaldehyde or arylacetic acid without involvement of hydroxylation could be demonstrated utilizing cell organelles like chloroplasts[3] or glyoxysomes.[4] Finally, another reaction sequence leading to C_6–C_2 compounds must be taken into account; amino acids can be converted into arylacetaldoximes by a complex *N*-hydroxylation reaction.[3,5] These aldoximes are precursors of nitriles, cyanogenic glycosides, mustard oil glycosides, and tyrosol derivatives.

[3] H. KINDL and S. SCHIEFER, unpublished results.
[4] H. KINDL and M. RUIS, *Phytochem.* **10**, 2633 (1971).
[5] H. KINDL and S. SCHIEFER, *Phytochem.* **10**, 1795 (1971).

The Importance of Phenolics in Beverages

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PHENOLIC substances contribute to the physical and gustatory properties of all beverages derived from plants. One of the more important underlying factors determining their effects generally appears to be molecular size which influences both astringency and their ability to combine with proteins and peptides. Methods for examining the molecular size of phenolic substances are still inadequate. The use of gel filtration employing organic solvents is a partial solution to the problem but difficulties still exist in combating the effects of absorption and in obtaining reliable standard polymeric phenolic substances of known molecular weight.

The colours of red wines and tea liquors are predominantly determined by the content and nature of their phenolic substances. In addition to well defined groups of chemicals exhibiting characteristic colours (e.g. theaflavins in tea; anthocyanins in wines), complex polymers contribute very significantly to colours.

The clarity of wines and beers may be adversely effected by the appearance of non-biological hazes generally attributed to the formation of complexes between proteins or peptides and polyphenols. Polyphenols also probably influence the properties of beer foams. Cold tea liquors are rendered cloudy by the formation of insoluble complexes between caffeine and polyphenols. This has important implications for the marketing of iced tea in North America.

Taste is influenced both directly and indirectly by phenolic substances. They contribute much of the astringency and general 'mouthfeel' of tea, wines, coffee and probably cider and cocoa, and to a lesser extent, beer. Indirect contribution to the character of beverages is also made as a result of some of the reactions undergone by phenolics during the processing of the beverages. Such reactions may generate characteristic flavours and are particularly important in coffee, tea and cocoa manufacture.

The molecular size distribution of phenolic substances in beverages is determined by the nature and quantities of the largely monomeric precursors present in the original plants and the conditions to which they are subjected during processing.

Of particular significance in tea is the restricted specificity of phenolase. As a result the predominating flavan-3-ols in the green leaf tend to give rise to biflavanoid structures (bis-flavanols and the theaflavins). The formation of polymeric substances may occur by involvement of other molecular species (e.g. flavandiols) or by the action of peroxidase which has a wider specificity than the tea phenolase.

Chemistry of Biflavonoids

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THE KNOWN, characterized biflavonoids are classified according to their possible biosyntheses based on chemical analogies. The methods of structural determination appropriate to each series are briefly summarized and the limitations stressed.

The biflavonyls[1] are formed presumably by an oxidative reaction on apigenin. Chemical oxidation has led to products coupled in different modes from the natural products. The distribution of the biflavonyls is considered.

The 3,8-linked biflavonoids[2] provide the first set of biflavonoids with one unit at a lower oxidation level than a flavone. They may be thought to arise either by a phenolic oxidative phenomena or *via* nucleophilic attack on the unstable cation at C-3 of a flavanone.

The highly diverse group of 'pro-anthocyanidins' or non-hydrolysable tannins[3] are looked at, remembering that compounds are known, chemically of type (i) which fit neither title. The following subdivisions are made: (i) Substances arising by processes analogous to the acid catalysed polymerization of 4-hydroxyflavans (Weinges' group B);[4] (ii) Compounds similar to those produced by acid catalysed reactions of 3-hydroxyflavans; (iii) Compounds of the type isolated after reacting phenols with anthocyanidins (Weinges' group A);[4,5] (iv) Compounds produced by nucleophilic attack by hydroxyl groups upon C-4; (v) Theaflavin type biflavonoids;[6] (vi) Miscellaneous types.

Discussion is confined to those compounds of unambiguous structure (although other problematic substances will be mentioned).

The first group comprises compounds that illustrate a seemingly fundamental biochemical process for the production of flavonoid polymers, namely the joining of units through C-4 to C-8(6) probably by nucleophilic attack by a phenol on a stabilized benzylic carbonium ion at C-4.

The painstaking work of Freudenberg and Weinges (see ref.[4] for leading references) has provided interesting modes for the dimerization of catechol, though the importance of such reactions biochemically is open to discussion. The interactions of phenols with anthocyanidins have been well characterized and the chemically obtained compounds correspond to some isolated from natural sources, which may therefore have been produced by similar processes.

Oxidation reactions involving initially ring B of a flavonoid are well authenticated as a major route to polymeric products[1] but do not readily yield dimers. Exceptions are the theaflavins,[6] a unique class of biflavonoid. Various chemical reactions gave dimeric flavonoids belonging to classes unknown as natural products. Some of these will be mentioned.

[1] W. Baker and W. D. Ollis, in *Chemistry of Natural Phenolic Compounds*, p. 152, Pergamon Press, Oxford (1961).
[2] B. Jackson, H. D. Locksley, F. Scheinmann and W. A. Wolstenholme, *Tetrahedron Letters* 787 (1967).
[3] E. Haslam, *Chemistry of Vegetable Tannins*, p. 66, Academic Press, New York (1966).
[4] K. Weinges, W. Kaltenhäuser, H. D. Marx, E. Nader, F. Nader, J. Perner and D. Seiler, *Annalen* **711**, 184 (1968).
[5] L. Jurd and A. C. Waiss, *Tetrahedron* **21**, 1471 (1965).
[6] D. T. Coxon, A. Holmes, W. D. Ollis and V. C. Vora, *Tetrahedron Letters* 5237 (1970).

The Physiology of Flavonoid Compounds

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Flavonoid compounds have been implicated in a number of physiological phenomena in plants including, growth and movement. In the majority of cases, the effects noted appear to be correlated with the relative concentrations in the tissue of the flavonoids concerned, and with the hydroxylation patterns of their 'B' ring. Thus, any physiological activity caused by flavonoids must depend markedly on the control mechanisms involved in their biosynthesis of these and related compounds. The 'B' ring in flavonoids is formed from the shikimic acid pathway, and the products of this pathway are controlled in different ways in procaryotes, fungi, algae and higher plants. Variations in the enzymes phenylalanine ammonia lyase and the peroxidases involved in lignification are key factors in further biosynthetic transformations leading to flavonoids and must, therefore, have important physiological significance. These topics will be discussed in relation to the overall physiological rôle of flavonoid compounds.

SHORT PAPERS

Phenolic Compounds and Disease Resistance in Pyrus

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The relationship between phenolic compounds and disease resistance in plants has long been the subject of controversy. The background to this supposed relationship is critically examined and in the case of the genus *Pyrus*, the occurrence of phenolics is shown to bear no relationship to resistance to fireblight, woolly pear aphid and crown gall. The evolutionary significance of these findings will be briefly discussed.

Interactions Between Theaflavins, Flavanols and Caffeine

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WHEN a hot infusion of black tea cools down, a precipitate (tea cream) is formed which consists principally of caffeine, theaflavins and thearubigins, together with other flavonoids.[1,2] Interactions between caffeine and the polyphenols appear to be primarily responsible for this precipitation, since decaffeination inhibits cream formation.[1]

Qualitative investigations suggest that these interactions modify the mouthfeel properties of both caffeine and theaflavins.[3] To characterize these interactions in greater detail, we have investigated quantitatively the precipitation of individual theaflavins[4] and flavanols from cooled aqueous solution over a range of caffeine concentrations.

We find that; (a) precipitation from the equivalent of exact strength solutions (5% total tea solids) is minimal in the absence of caffeine, (b) Epicatechin and (−)-epigallocatechin at extract strength are completely soluble at caffeine concentrations up to 20 mg/ml, (c) In the presence of caffeine, the efficiencies of precipitation of (−)-epicatechin gallate, (−)-epigallocatechin gallate and the four principal theaflavins pass through maxima with increasing caffeine concentration.

We tentatively interpret effect (c) on the following basis. As the caffeine concentration is increased, polyphenol hydroxyl groups are increasingly hydrogen-bound to caffeine rather than to water, this accounting for the initial falling part of the solubility-caffeine concentration curve. Once a sufficient number of OH groups are so bonded, solubility reaches a minimum. If then more caffeine is added, we suggest that it interacts with caffeine already involved in H-bonded insoluble complexes by the 'stacking' mechanism described in *Chem. Commun.* pp. 524–525 (1970) so incorporating H-bond receptor sites into the complexes and increasing their solubility.

[1] E. A. H. ROBERTS, *J. Sci. Food Agric.* **14,** 700 (1963).
[2] R. F. SMITH, *J. Sci. Food Agric.* **19,** 530 (1968).
[3] D. J. MILLIN, D. J. CRISPIN and D. SWAINE, *J. Agric. Food Chem.* **17,** 717 (1969).
[4] T. BRYCE, P. D. COLLIER, I. FOWLIS and P. E. THOMAS, *Tetrahedron Letters* 2789 (1970).

The Synthesis of Isopavine Alkaloids

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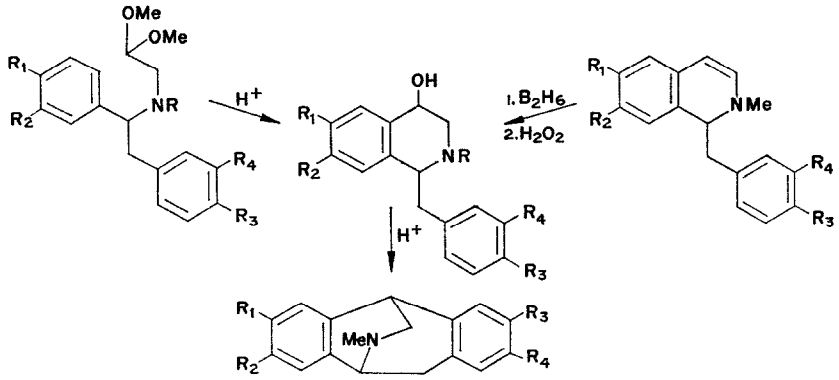
SEVERAL alkaloids are now known[1] that are based upon the 'isopavine' ring system:

	R_1	R_2	R_3	R_4
Amurensinine	O—CH_2—O		OMe	OMe
Amurensine	O—CH_2—O		OMe	OH
or	O—CH_2—O		OH	OMe
Reframidine	O—CH_2—O		O—CH_2—O	
Reframine	OMe	OMe	O—CH_2—O	
Reframoline	OH	OMe	O—CH_2—O	
or	OMe	OH	O—CH_2—O	
Thalisopavine	OME	OMe	OMe	OH

The structures have been assigned from degradative and mass spectral evidence. However, ambiguity exists over the location of the phenolic hydroxyl group in amurensine and reframoline.

In this paper the synthesis of the racemate of each alkaloid is described, based upon the double cyclization[2] of the appropriately substituted benzylamino acetal,



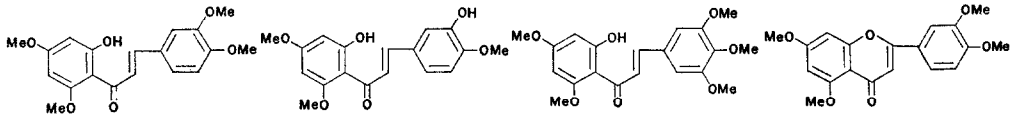
or upon the 'hydration' of a 1,2-dihydroisoquinoline, followed by cyclization.

[1] S. F. DYKE and A. C. ELLIS, *Tetrahedron* **27**, 3803 (1971), and refs therein.
[2] A. R. BATTERSBY and D. A. YEOWELL, *J. Chem. Soc.* 1988 (1958).

Some Phenolic Constituents of the Rutaceae

A. W. FRASER and J. R. LEWIS
University of Aberdeen, Scotland

A SYSTEMATIC examination of some of the Rutaceae indigenous to the Malay Peninsula, has commenced with the isolation of 4 constituents from the fruit of *Merrillia caloxylon*. Mass spectroscopic, NMR and UV measurements have shown the compounds to have the following structures:



Hitherto, the Rutaceae has yielded flavonoids containing high degrees of methylation[1,2] and these chalcones reported here follow suit. The co-occurrence of chalcones and flavones follows the well established biogenetic pathway of flavonoids.

[1] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, p. 174, Academic Press, New York (1967).
[2] T. A. GEISSMAN and D. H. G. CROUT, *Organic Chemistry of Secondary Plant Metabolism*, p. 199, Freeman Cooper (1969).

The Biosynthesis of the Coumarins of Angelica archangelica

D. E. GAMES and D. H. JAMES

Department of Chemistry, University College, Cardiff

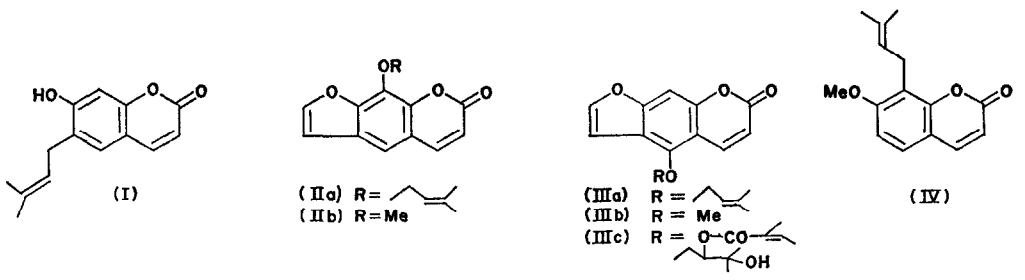
A NUMBER of suggestions[1] have been made for the biosynthetic origin of the furan ring in furanocoumarins and in most cases a coumarin with a 3-methylbut-2-enyl group *ortho* to a hydroxyl group has been postulated as an intermediate. As yet there is no evidence for such an intermediate in furanocoumarin formation though recently the involvement of such an intermediate in the biosynthesis of furanoquinoline alkaloids has been

[1] E. SPATH, *Chem. Ber.* **70A**, 83 (1937); R. D. HAWORTH, *Annual Reports*, 342 (1937); R. ANEJA, S. K. MUKERJEE and T. R. SESHADRI, *Tetrahedron* **4**, 256 (1958); W. D. OLLIS and I. O. SUTHERLAND, *The Chemistry of Natural Phenolic Compounds*, p. 74, Pergamon Press, Oxford (1961); see also refs. 3 and 4.

shown[2] and it has been established that α-hydroxyisopropyldihydrofuranocoumarins are intermediates in the formation of both linear and angular furanocoumarins.[3]

We have found in feeding experiments on the fruits of *Angelica archangelica* that [1′-^{14}C]demethylsuberosin (I) is readily incorporated into the linear furanocoumarins imperatorin (IIa) (% incorp. (i) 0·63; dilution, (d) 5·8 × 10^3), isoimperatorin (IIIa) (i, 0·045; d, 7·8 × 10^3) and bergapten (IIIb) (i, 3·8; d, 1·2 × 10^3) and degradation has established that in the case of bergapten incorporation occurs without randomization of label. These results establish the probable intermediacy of demethylsuberosin in linear furanocoumarin biosynthesis.

Other feeding experiments on the roots and fruits of *Angelica* with [1-^{14}C]sodium acetate, [1-^{14}C]phenylalanine, [3-^{14}C]cinnamic acid [1-^{14}C]*p*-coumaric acid, [2-^{14}C]umbelliferone, [2-^{14}C]daphnetin, [4-^{14}C]-mevalonate and [1-^{14}C]methionine have enabled the biosynthesis of the coumarins osthol (IV), bergapten (IIIb), xanthotoxin (IIb) and ostruthol (IIIc) in the roots, bergapten (IIIb) and xanthotoxin (IIb) in the leaves and imperatorin (IIa), isoimperatorin (IIIa) and bergapten (IIIb) in the seeds to be investigated. The results obtained will be discussed and they are generally in agreement with those obtained by most other workers.[3,4]



[2] J. F. Collins and M. F. Grundon, *Chem. Commun.* 621 (1969).

[3] W. Steck, M. El-Dakhakny and S. A. Brown, *Tetrahedron Letters* 4805 (1969); *Can. J. Biochem.* **48,** 863 (1970); W. Steck and S. A. Brown, *Can. J. Biochem.* **48,** 872 (1970).

[4] H. G. Floss and U. Mothes, *Phytochem.* **5,** 161 (1966); H. G. Floss and H. Paikert, *Phytochem.* **8,** 589 (1967); H. G. Floss, H. Guenther and L. A. Hadwiger, *Phytochem.* **8,** 585 (1969); S. A. Brown, *Phytochem.* **9,** 2471 (1970); G. Caporale, F. Dall'acqua, S. Marciani and A. Capozzi, *Z. Naturforsch.* **256,** 700 (1970).

Oxidative Browning in Mushrooms and its Economic Importance

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Horticultural Chemistry Unit, An Foras Talúntais, Kinsealy Research Centre, Co. Dublin

The Irish mushroom industry is worth about £1 million annually, £400,000 of which comes from export of fresh mushrooms to the U.K. With increased *per capita* consumption in the U.K. and the growth of the industry in Ireland it is likely that exports will treble by the middle of this decade. Fresh mushrooms have a short shelf life, mostly due to browning and water loss. A survey in 1965 showed that price losses for Irish mushrooms in the U.K. market amounted to about £275,000 over a 3-year period. This was caused largely by bad presentation and also overhandling which resulted in browning. The problem has been largely overcome by better handling; however, good methods for extending shelf life have not yet been obtained.

Browning is largely due to the action of polyphenoloxidase (PPO) on tyrosine which produces melanin pigments after a number of intermediate products have been formed. The tyrosine content of mushrooms decreases with successive flushes indicating that first flush mushrooms are very susceptible to browning. Much work has been done on the mechanism by which antioxidants, sulphite and ascorbic acid, prevent enzyme activity in mushroom extracts.

However, when these materials are applied to whole mushrooms they do not give much extension of shelf life. Prepacking in a PVC film, however, gave a 3-day extension of shelf life over unpacked mushrooms, presumably because the artificial atmosphere in the prepack slows down enzyme action.

Mushrooms for canning and drying need to be blanched to destroy enzyme action. This results in large losses of solids. Experiments on freezing mushrooms without blanching indicate that unblanched frozen mushrooms remain white, provided their temperature is kept below +10°. Above this temperature enzyme action takes place rapidly. Radiation from a ^{60}C source is claimed to give a long shelf life; however, changes in nucleic acid content of irradiated mushrooms have been reported.

Extractives from Poisonous British Plants. Alpinumisoflavone—A new Pyranoisoflavone from Laburnum alpinum

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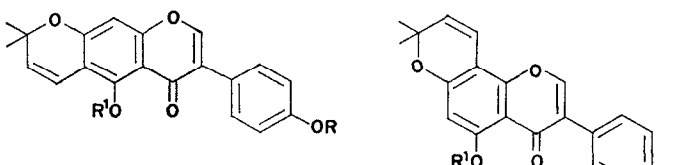
AS PART of a programme on poisonous British plants, we report structural and synthetic studies on alpinumisoflavone, which is a new pyranoisoflavone isolated from the twigs of *Laburnum alpinum* J. Presl. (Leguminosae).

Structural studies led us to formulate alpinumisoflavone as either structure Ia or IIa. A synthesis will be described which gave an isomer of the methyl ether of alpinumisoflavone either structure Ib or IIb. Since the various spectra (IR, UV, NMR and MS) failed to differentiate between the linear and angular pyranoisoflavone structures, further methods were considered based on the availability of both the linear and angular pyranoisoflavones (Ib) and (IIb).

It is known[1] that acetylation of a 5-hydroxy group can cause a diamagnetic shift in the NMR spectrum of the *peri*-proton of a pyran ring. Thus, acetylation of both synthetic and natural methyl ethers (Ib and IIb) gave acetyl derivatives (Ic and IIc) and the natural product derivative was assigned the linear structure (Ic) since the NMR spectrum shows that the pyran 4-H proton has moved up field by 0·25 ppm.

Application of the nuclear Overhauser effect provides further support for this assignment. Methylation of the hydroxy group at C-5 and double irradiation at this methyl group caused a 33% enhancement of the aromatic singlet in the synthetic compound (IId) whereas in alpinumisoflavone dimethyl ether (Id) a 10% enhancement was observed for the signal due to 4-H of the pyran ring. Thus two further aspects of NMR spectroscopy both independently support the linear pyranoisoflavone structure (Ia) for alpinumisoflavone. Alpinumisoflavone monomethyl ether (Ib) is identical with the synthetic sample[2] kindly provided by Professor T. R. Seshadri.



(I) a; R = R¹ = H
b; R = Me = R¹ = H
c; R = Me = R¹ = Ac
d; R = R¹ = Me

(II)

[1] A. ARNONE, G. CARDILLO, L. MERLINI and R. MONDELLI, *Tetrahedron Letters* 4201 (1967).
[2] A. C. JAIN, P. LAL and T. R. SESHADRI, *Tetrahedron* **1977** (1970).

Recent Work on the Chemistry of Tea Pigments

A. G. H. LEA

Brooke Bond Liebig Research Centre, Sonning, Reading

THE THEARUBIGINS of black tea give rise on paper chromatograms to streaks or areas rather than discrete spots. Fractions have been isolated by techniques which give substantially purer products (judged by freedom from monomers) than preparations previously described.[1]

An aqueous tea brew is chloroform washed, then fractionated by repeated precipitation into acetone. The precipitate is subjected to adsorption chromatography on Sephadex LH20 yielding thearubigin fraction A. The acetone soluble material is repeatedly precipitated into ether, and this precipitate is likewise purified by adsorption on LH20 to give thearubigin fraction B. Although free from monomers, A and B still comprise complex mixtures capable of further separation, e.g. by electrophoresis and gel-filtration. Together they form some 10–20% by weight of a dried aqueous tea extract. They contain approx. 0·5% N_2 which cannot be accounted for by caffeine, but do not appear to contain sugars. Simple degradative techniques show that thearubigins consist largely of polymers built up from flavanol subunits, but they also yield significant amounts of quinic and amino acids (the latter probably of protein origin) on hydrolysis, and up to 5% of gallic acid. These facts imply a role for protein and theogallin (3-galloylquinic acid[2]) in thearubigin formation, and a

[1] A. G. BROWN W. B. EYTON, H. HOLMES and W. D. OLLIS, *Phytochem.* **8,** 2333 (1969).
[2] G. V. STAGG and D. SWAINE, *Phytochem.* **10,** 1671 (1971).

method has been developed for assaying gallic and quinic acids in the same sample by GLC. The protein linkage is not clear, but there may be some relationships with humic acids[3,4] and coffee pigments, although certain sub-fractions do not contain protein.

The pigments isolated are discussed in relation to previous work,[1,5,6] particularly in connection with an analytical chromatographic method for tea brews developed in these labs.,[7] and also in relation to model experiments using enzymes and substrates from fresh green leaf.

[3] L. VUATAZ and H. BRANDENBERGER, *J. Chromatogr.* **5**, 17 (1961).
[4] S. PIERPOINT, *Biochem. J.* **112**, 609 (1969).
[5] E. A. H. ROBERTS and R. A. CARTWRIGHT, *J. Sci. Food Agric.* **8**, 72 (1957).
[6] D. J. MILLIN, D. SWAINE and L. DIX, *J. Sci. Food Agric.* **20**, 269 (1969).
[7] D. J. CRISPIN, R. H. PAYNE and D. SWAINE, *J. Chromatogr.* **37**, 118 (1968).

New Phenolic Heartwood Constituents from the Combretaceae

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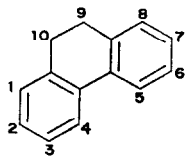
Department of Chemistry, University of Rhodesia, Salisbury

THE DARK heartwoods of several species of Combretaceae are termite and borer resistant and from these we have isolated 11 new phenanthrenes or 9,10-dihydrophenanthrenes and established their structures as:



	Substituents						
	2	3	4	6	7	9	10
I	OMe	H	OH	OMe	OH	H_2	H_2
II	OMe	H	OMe	OMe	OH	H	H
III	OMe	H	OMe	OMe	OMe	H_2	H_2
IV	OH	OMe	OMe	OH	OMe	H	H
V	OH	OMe	OMe	OH	OMe	H_2	H_2
VI	OMe	OMe	OH	OMe	OH	H	H
VII	OMe	OMe	OH	OMe	OH	H_2	H_2
VIII	OMe	OMe	OMe	OH	OH	H	H
IX	OMe	OMe	OMe	OH	OH	H_2	H_2
X	OMe	OMe	OH	OH	OH	H	H
XI	OMe	OMe	OH	OH	OH	H_2	H_2

With the exception of IV and VIII (and their dihydro-derivatives) the structures were all obtained employing the following characteristics and reactions: (a) H-5 in the phenanthrenes appears at a very characteristic low field in the NMR (τ 0·6–1·5); (b) The oxygenation pattern of the phenols was established by methylation, and identification of the products with synthetic methoxylated phenanthrenes obtained from unambiguous Pschorr syntheses. (c) Only acetoxy-groups at C-4 in the phenanthrenes showed a considerable downfield shift, compared with the 9,10-dihydrophenanthrenes, in which all acetoxy signals are the same. (d) The hydroxy-groups were placed after deuterium exchange reactions (D_2O and potassium *t*-butoxide) followed by spectroscopy.

(e) Phenanthrenes $\underset{\text{DDQ}}{\overset{H_2/PtO_2}{\rightleftharpoons}}$ Dihydrophenanthrenes.

Two phenols were isolated whose structures were both shown by the above means to be either IV or VIII. The ambiguity was resolved by the total synthesis of VIII using an iodine oxidative photo-cyclization of 3',4'-diacetoxy-3,4,5-trimethoxystilbene. Phenol II has also been synthesised by this method.

From one of the heartwoods we isolated as the major constituent 3,4-dimethoxy-4'-5-dihydroxybibenzyl (the structure of which followed from deuteriation, spectroscopy and methol ether synthesis) and which occurred together with 7 of the above phenanthrene derivatives. This unique association suggests that the phenolic bibenzyls may in fact be the precursors of the phenanthrene derivatives, arising via direct phenol oxidation. The oxygenation pattern of all the isolated compounds is in agreement with this theory. The isolated bibenzyl is unable to give a phenanthrene by direct coupling, but may of course do so via a dienone rearrangement.

The Formation of o-*Quinones in Leaf Extracts: and the Nuisance Value of Their Subsequent Reactions*

S. PIERPOINT

Rothamsted Experimental Station, Harpenden, Herts.

A BRIEF account of the *o*-quinones formed by the enzymic oxidation of plant polyphenols; their subsequent reaction with such leaf components as amino acids, auxins, enzymes and viruses; and some consequences of these reactions that emphasise the need to prevent them or be aware of them.

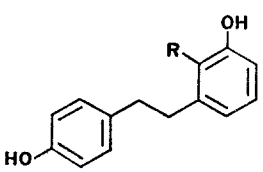
The Occurrence and Metabolism of Lunularic Acid, an Endogenous Stilbene Growth Inhibitor in Liverworts

R. J. PRYCE

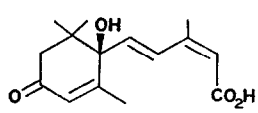
Wye College, Ashford, Kent

LUNULARIC acid (I), the dihydrostilbene carboxylic acid endogenous growth inhibitor of liverworts, has been detected in all liverworts and algae examined.[1,2] These groups of plants contain no abscisic acid (II), the ubiquitous growth inhibitor of higher plants, mosses and ferns. Higher plants, mosses and ferns contain no lunularic acid with the one known exception of *Hydrangea macrophylla*. Lunularic acid is therefore a plant growth inhibitor of some chemotaxonomic and phylogenetic significance and it is possible that in the lower green plants, liverworts and algae, it may replace the growth regulatory function of abscisic acid of higher plants.

Lunularic acid, in common with other plant stilbenes, can be biosynthesized in the liverwort *Lunularia cruciata* by a phenylpropanoid-polymalonate pathway. This was shown by successful incorporation of ^{14}C-labeled phenylalanine, acetate and hydrangenol (III) into lunularic acid by thalli of the liverwort.[2] The timecourse of metabolism of ^{14}C-lunularic acid by *L. cruciata* has been studied. At least four metabolites are produced during an 8-hr incubation period and the apparently first form of these has been identified as a new natural plant stilbene lunularin (IV). Lunularin, which is the simple decarboxylation product of lunularic acid, has been shown to be a normal and major constituent of the phenolic extract from *L. cruciata* and is also present in other liverworts. ^{14}C-labelled phenylalanine, acetate and hydrangenol are incorporated into lunularin by the liverwort but with higher specific activity dilutions than simultaneously into lunularic acid. These observations establish, for the first time, the proposed step of decarboxylation of C_{15}-stilbene-2-carboxylic acids to plant C_{14}-stilbenes in the phenylpropanoid-polymalonate biosynthetic pathway.



(I) R=CO_2H
(IV) R=H



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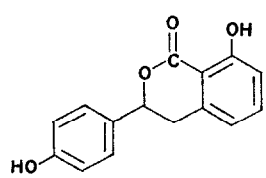

(III)

[1] R. J. PRYCE, *Planta* **97**, 354 (1971).
[2] R. J. PRYCE, *Phytochem.* **10**, 2679 (1971).

The Oxidation of a Chalcone to a Dihydroflavonol by Crystalline Horse-radish Peroxidase

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Department of Biochemistry, University of Cambridge

CRYSTALLINE horse-radish peroxidase catalysed the oxidation of 2',4,4'-trihydroxychalcone in the presence of trace amounts of H_2O_2 under aerobic conditions. One atom of oxygen was consumed for each molecule of substrate; 4',7-dihydroxyflavonol and 4',6-dihydroxyaurone were isolated from the reaction mixture. The immediate products of the reaction were probably stereoisomers of 3,4',7-trihydroxyflavanone and 4',6-dihydroxy-2-(α-hydroxybenzyl)-coumaranone, which can be readily converted non-enzymically to the flavonol and aurone respectively. The physiological significance of the reaction will be discussed in terms of a possible free radical mechanism. A close analogy may exist between flavonoid biosynthesis and lignin formation.

The Effect of Ethylene on the Biosynthesis of Phenolic Compounds in Swede Roots

M. J. C. RHODES and L. S. C. WOOLTORTON

A.R.C. Food Research Institute, Norwich

INTACT roots of Swede (*Brassica napo-brassica*) have low levels of the enzyme, phenylalanine ammonia lyase. Increases in activity of up to 50-fold can be induced by treatment of the storage root with the plant hormone, ethylene. When disks (1 cm in dia.) were cut from the root tissue and incubated in air for periods of up to 24 hr, small increases in activity of the enzyme were observed. When such disks are incubated in the presence of low concentrations of ethylene very much larger increases, 3–10-fold greater than in disks in air, were observed. The small increases in air were correlated with the stimulation of the endogenous rate of ethylene production induced by cutting the disks. The effect of ethylene in inducing increases in PAL activity in Swede roots has many features in common with other effects of ethylene on plant tissues, particularly in relation to the low levels required for stimulation and the effects of other hydrocarbons.

The fate of ^{14}C-phenylalanine fed to control and ethylene treated intact roots and tissue disks was studied and the labelling of the phenolic acid fraction and lignin oxidation products were determined. Preliminary results suggest that ethylene stimulates the synthesis of a fraction with many of the properties of lignin.

The results will be discussed in relation to the control exerted by ethylene on the activity of the enzyme PAL and on phenolic acid and lignin biosynthesis.

Properties of the Phenolase Hydroxylase System

D. A. ROBB

Department of Biochemistry, University of Strathclyde

MUSHROOM phenolase, (E.C. 1.10.3.1), oxidises monophenols and *o*-diphenols to *o*-quinones. In the presence of a quinone trapping agent, benzene sulphinate, a long lag period precedes the oxidation of monophenols and this lag is eliminated by the addition of a small amount of *o*-diphenol.[1] When radioactive 4-methylcatechol is added to *p*-cresol, benzene sulphinate and enzyme, and is subsequently recovered by chromatography on polyamide, the specific activity of the recovered diphenol is lower than that supplied; this suggests that *o*-diphenol is the primary product of monophenol oxidation. Such a conclusion is consistent with the classification of the enzyme as a hydroxylase, in which oxygen is used equally in the hydroxylation reaction and in the oxidation of an external electron donor. In a survey of reductants, the most effective in eliminating the lag associated with the oxidation of *p*-cresol is 4-methylcatechol.

Potentially, the assay of tyrosinase in the presence of benzene sulphinate provides a convenient means of following the simultaneous oxidation of monophenol and *o*-diphenol because the sulphone produced is readily determined spectrophotometrically as its anion ($E_{310\ nm}$ 9000 approx.). Thus the simultaneous measurement of oxygen utilized and the absorbance increase at 310 nm allows the amounts of monophenol and diphenol oxidized to be calculated. The precision attained in this method will be illustrated.

[1] C. A. BORDNER and J. M. NELSON, *J. Am. Chem. Soc.* **61**, 507 (1939).

A Rapidly Synthesized Tannin in Leaves of Rhus typhina

N. SCHILLING, J. A. FERGUSON and O. KANDLER

Botanisches Institüt der Universität Munich, Germany

THE LEAVES of *Rhus typhina* (Anacardiaceae) characteristically have a high tannin content. The research to be discussed has been concerned with two such tannin substances. After hydrolysis, Compound I yields glucose,

gallic acid and ellagic acid; Compound II, which shows similar characteristics is, in fact, an as yet unresolved mixture of substances.

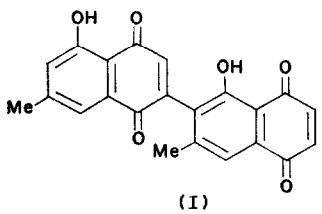
During photosynthesis in $^{14}CO_2$, both I and II are rapidly labelled. Chase experiments show that I is further metabolized, whereas II represents an end-product. A precursor-product relationship is suspected.

New Hydroxyquinones from Diospyros *Species*

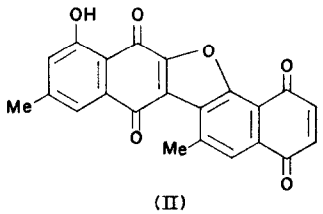
DOUGLAS SKOYLES and O. C. MUSGRAVE
University of Aberdeen, Scotland

TREES OF the genus *Diospyros* have been found in recent years to contain a wide range of hydroxylated binaphthylquinones. From *D. montana* we have isolated several new quinones (in addition to the known compounds mamegakinone, biramentacéone, diospyrin, and isodiospyrin). Three of these are related to diospyrin (I) and have the structures (II), (III) and (IV).

The novel blue pigment (V) is present in small amounts in at least eight of the *Diospyros* species which we have examined.



(I)



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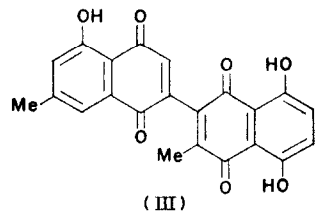

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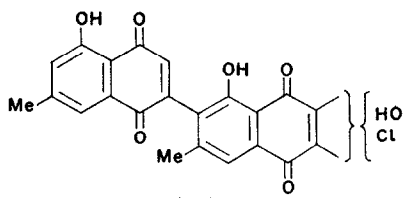

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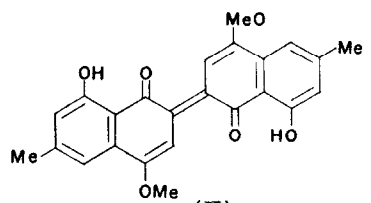

(V)

Spectroscopic Observation of Tea Quinones

C. K. WILKINS and O. KORVER
Groot Ammers, Netherlands

THE REACTION of tea flavanols with tetrachloro-*o*-benzoquinone gives products which according to their absorption spectra and some chemical properties appear to be *o*-benzoquinones. Implications regarding the mechanism of tea fermentation are discussed.

The Geography of Unusual Phenolics in Malus

A. H. WILLIAMS
Research Station, Long Ashton

THE BOTANICAL species of *Malus*, which are spread throughout the Northern Hemisphere, have in certain tissues a much wider range of unusual phenolics than was previously suspected. The pattern of their occurrence supports, in general, the presently recognized classification of *Malus* species, and accords well with their geographical distribution.

The Oxidation of Chalcone Catalysed by Peroxidase

E. WONG and J. M. WILSON
Applied Biochemistry Division, D.S.I.R., Palmerston North, New Zealand

PREVIOUS works[1,2] have shown that cell-free extracts of garbanzo and soya bean seedlings catalyse the enzymic oxidation of 2′,4,4′-trihydroxychalcone (isoliquiritigenin) to the corresponding dihydroflavonol (garbanzol), flavonol (4′,7-dihydroxyflavonol) and aurone (hispidol), the latter product being formed probably by way of the intermediate "hydrated aurone", 4′,6-dihydroxy-2-(α-hydroxybenzyl)coumaranone, which exists in diastereoisomeric forms. The enzyme responsible for these transformations in cell-free extracts of garbanzo seedlings has now been identified as peroxidase.

Studies with the purified garbanzo enzyme or with horse radish peroxidase showed the additional formation of an unstable compound OC (oxidized chalcone), as the major isolatable product of the reaction. OC has been characterized as the spirodienone isomer of 4′,7-dihydroxyflavonol, representing a new flavonoid variant. The existence of precursors of OC in these reactions has been demonstrated. Compounds representing various stereochemical modifications of the 'hydrated OC' structure have been isolated and these have been found to be precursors also of 4′,7-dihydroflavonol, the relative proportions of OC and flavonol products formed being determined by pH effects. The chemical and stereochemical relationships between these and other compounds produced have been elucidated and the existence of a pair of enantiomeric epoxides of the dehydrochalcone as the true enzymic products in these systems has been postulated. The oxidation of chalcone by peroxidase requires stoichiometric amounts of molecular oxygen and consumes catalytic quantities of hydrogen peroxide. A scheme has been devised to rationalize this novel reaction in terms of free radical mechanisms proposed for previously recorded examples of the peroxidase-oxidase reaction.

[1] E. WONG, *Biochim. Biophys. Acta* **111**, 358 (1965).
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Phytochemical Unit
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