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REVIEW

α-HYDROXYCHALCONES AS INTERMEDIATES IN FLAVONOID BIOGENESIS: THE SIGNIFICANCE OF RECENT CHEMICAL ANALOGIES*

DAVID G. ROUX and DANEEL FERREIRA

Department of Chemistry, University of the Orange Free State, Bloemfontein, South Africa

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Key Word Index—α-Hydroxychalcones; distribution; flavonoid biogenesis; 2-Hydroxy-2-benzylcoumaranones; 2,3-*cis*- and 2,3-*trans*-flavanoids.

Abstract—α-Hydroxychalcones are shown to be prominent metabolites amongst the flavonoids of certain heartwoods. Their existence mainly in the keto-form confers properties which are presumably responsible for their previous oversight. Indications of the wider distribution of α-hydroxychalcones raises interesting speculation regarding their hitherto unconsidered role in the biogenesis of 3-hydroxyflavonoids. Cyclization involving the β-position of the *trans*-enolic isomer of α-hydroxychalcones to form both 2,3-*cis*- and 2,3-*trans*-dihydroflavonols, infers for the first time biosynthetic paths which lead by way of successive reductions to 2,3-*cis*- and 2,3-*trans*-diastereoisomers of flavan-3,4-diols and flavan-3-ols, and hence by condensation via carbonium ion or quinone-methide intermediates to polyflavonoid tannins. Alternative cyclization to the α-position, or more likely to the equivalent carbonyl of the keto-form, represents the only feasible mode of biogenesis of 2-hydroxy-2-benzylcoumaranones. Newly-established associations of the complete range of peltogynoid analogues in heartwoods, often with α-hydroxychalcones or their peltogynoid equivalent, permits similar conjecture.

INTRODUCTION

WHILE the source of the C_{15}-skeleton of flavonoid compounds is beyond dispute,[1,2] the sequence of changes which result in the formation of a relatively diverse group of compounds, based on variations in the oxidation level of the C_3-portion of the molecule, remains ill-defined.[3] Continuing uncertainty regarding the immediate biogenetic sequence which leads to 3-hydroxyflavonoids may conceivably be attributed to the operation of more than one mechanism amongst the diversity of plant species, or to temporary oversight of key intermediates.

The purported central role of the chalcone-flavanone pair has gained general acceptance[3–7] due to specific observations which are not compelling in terms of the extent of precursor incorporation or of their general applicability. Furthermore, introduction of the

* Based on a paper read at the 22nd Meeting of the South African Chemical Institute, Pretoria in July, 1972.

[1] HARBORNE, J. B. (1967) *Comparative Biochemistry of Flavanoids*, p. 251, Academic Press, London.

[2] PACHECO, H. (1969) *Bull. Soc. France Physiol. Vegetale* **15**, 3; GRISEBACH, H. and BARZ, W. (1969) *Naturwissenchaften* **56**, 538.

[3] PELTER, A., BRADSHAW, J. and WARREN, R. F. (1971) *Phytochemistry* **10**, 835.

[4] GRISEBACH, H. and OLLIS, W. D. (1961) *Experientia* **17**, 4.

[5] GEISSMAN, T. A. (1967) in *Biogenesis of Natural Compounds* (BERNFELD, P., ed.), 2nd edn, p. 743, Pergamon Press, Oxford.

[6] BU'LOCK, J. D. (1965) *The Biosynthesis of Natural Products*, p. 86, McGraw-Hill, New York.

[7] BIRCH, A. J. (1963) in *Chemical Plant Taxonomy* (SWAIN, T. ed.), p. 148, Academic Press, New York.

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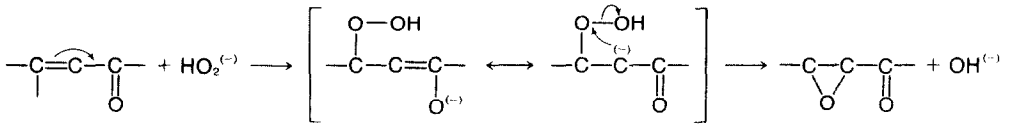
3-hydroxyl group, leading to the highly important production of generically-related flavononols, flavan-3,4-diols, flavan-3-ols and condensed tannins, implies either epoxidation of the suggested chalcone prior to cyclization, as postulated for the AFO-reaction; oxidation of flavanones at the enolic position followed by hydration to dihydroflavonols (flavanonols);[6-7] or phenolic oxidation of 4-hydroxychalcones, 4′-hydroxydihydro-flavonols or -flavones.[3]

These proposals are regarded as tentative considering that natural chalcone epoxides are as yet unknown, α,β-unsaturated ketones reacting either very slowly or not at all with peracids;[8] that although synthetic 2′-OR chalcone epoxides are known,[9] their epoxidation with H_2O_2 requires strongly alkaline conditions (AFO-reaction) and represents a Michael-type mechanism involving attack by $HO_2^{(-)}$[8,10] (Scheme 1), and that mechanisms proposed for the oxidation of flavanones are similarly doubtfully feasible *in vitro* (e.g. direct oxidation to give a flavanone C_3-cation followed by reaction with $^{(-)}OH$,[7] or enolization of the flavanone followed by attack by the equivalent of $^{(+)}OH$[6]). More likely, in view of the existence of many 3,8-linked biflavonoids, is an attack by water or by an hydroxyl radical at the α-position of a chalcone radical,[3] giving the equivalent of an α-hydroxychalcone. An obvious criticism, however, is that the formation of those flavonoids which are devoid of B-ring (4′- or 2′-) hydroxylation is excluded on the basis of this mechanism (see later).



SCHEME 1. FORMATION OF CHALCONE EPOXIDES.

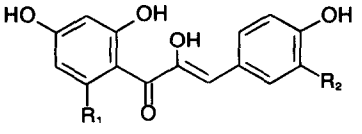
Unconsidered hitherto is the role of a new group of natural α-hydroxychalcones in the formation of reduced flavonoid ring systems. These new compounds probably originate more conventionally from *p*-hydroxyphenylpyruvic acid and malonate (or acetate) units.

DISTRIBUTION OF α-HYDROXYCHALCONES AND RELATED COMPOUNDS

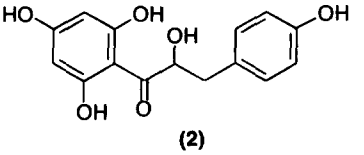
Our recent isolation of the first of the above new groups of natural flavonoids, α,2′,3,4,4′-pentahydroxychalcone (**1**. R_1=H, R_2=OH), from the heartwood of the gum copal (*Trachylobium verrucosum*: Leguminosae; Caesalpinioideae)[11] and latterly from *Peltogyne pubescens* and *P. venosa*,[12] was followed by almost immediate recognition of two others, α,2′,4,4′,6′-pentahydroxychalcone (**1**. R_1=OH, R_2=H)[13] and α,2′,3,4,4′,6′-hexahydroxychalcone (**1**. R_1=R_2=OH)[14] from species of the same genus (*Berchemia zeyheri* and *B. discolor* respectively: Sapindaceae), but unrelated to the above. All are present as prominent

[8] MARCH, J. (1968) *Advanced Organic Chemistry, Reactions, Mechanism and Structure*, p. 620. McGraw-Hill, New York.
[9] LITKEI, G. and BOGNÁR, R. (1973) *Acta Chim. Hung.* **77**, 93.
[10] BUNTON, C. A. and MINKOFF, G. J. (1949) *J. Chem. Soc.* 665.
[11] VAN DER MERWE, J. P., FERREIRA, D., BRANDT, E. V. and ROUX, D. G. (1972) *J. Chem. Soc. Chem. Commun.* 521.
[12] MALAN, E. and ROUX, D. G. (1974) *Phytochemistry*. In press.
[13] VOLSTEEDT, F. DU R., RALL, G. J. H. and ROUX, D. G. (1973) *Tetrahedron Letters* 1001.
[14] VOLSTEEDT, F. DU R., RALL, G. J. H., FERREIRA, D. and ROUX, D. G., (unpublished work on *Berchemia discolor*).

components (5–15%) relative to the total flavonoid content of the respective heartwoods. They bear the same relationship to α,2',4,4',6'-pentahydroxydihydrochalcone (**2**), isolated recently from the leaves of *Podocarpus nubigena* (Podocarpaceae)[15] as does conventional chalcones to the rarer dihydrochalcones.[16]

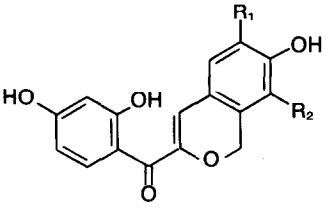


(1)



(2)

The peltogynoid equivalent of α-hydroxychalcones (α-alkoxychalcones) corresponding to mopanol (**3**. R_1=H, R_2=OH) was recently found in *Goniorrhachis marginata* (Caesalpinioideae) by Gottlieb and de Sousa,[17] and also its isomer corresponding to peltogynol (**3**. R_1=OH, R_2=H) was isolated by us[18] from *Acacia carnei* (Leguminosae; Mimosoideae) (*cf.* ref. [19]).



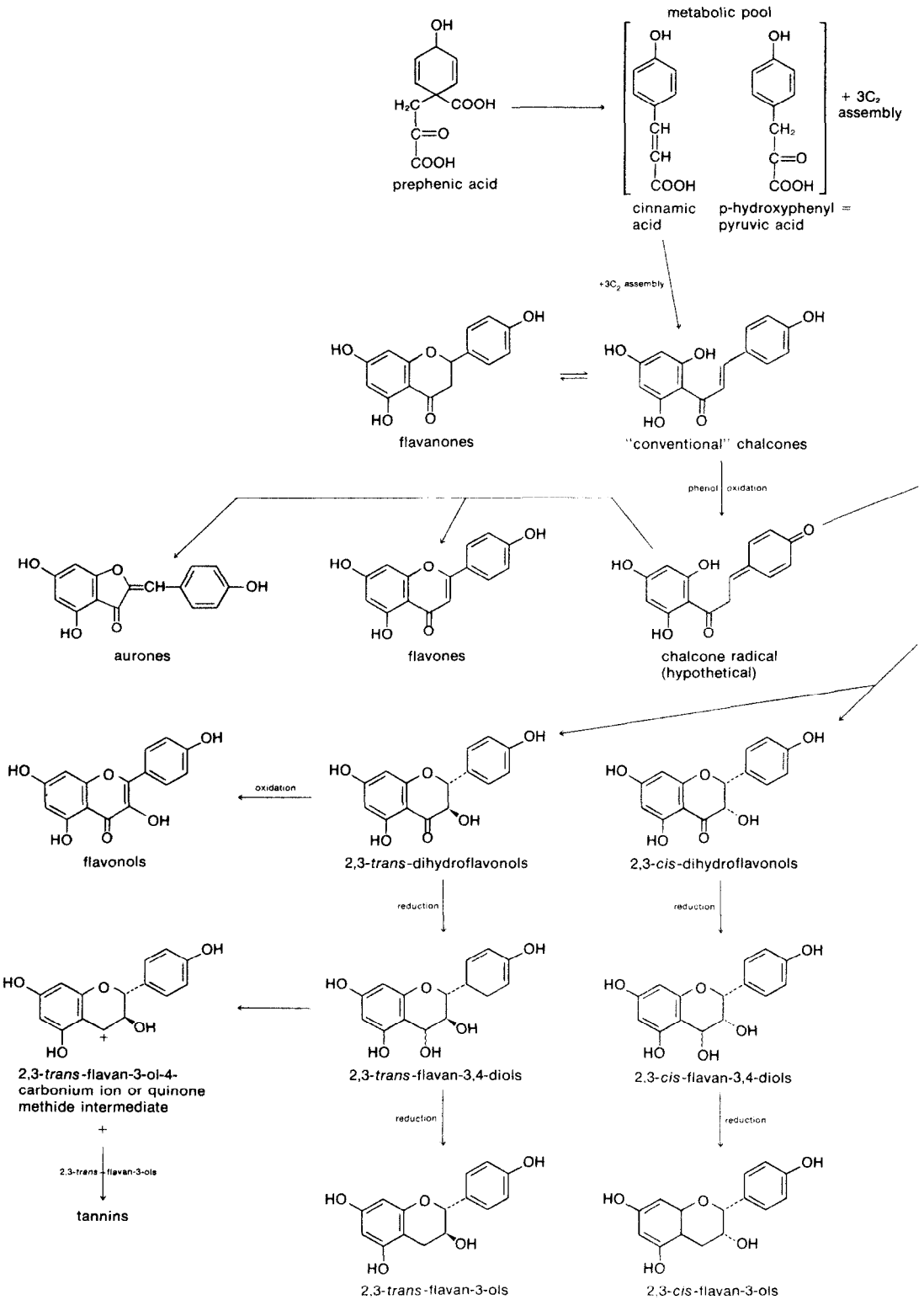
(3)

Such random distribution located within a short lapse of time and without screening of plant material, is taken to be indicative of a wider distribution of α-hydroxychalcones or their equivalent.

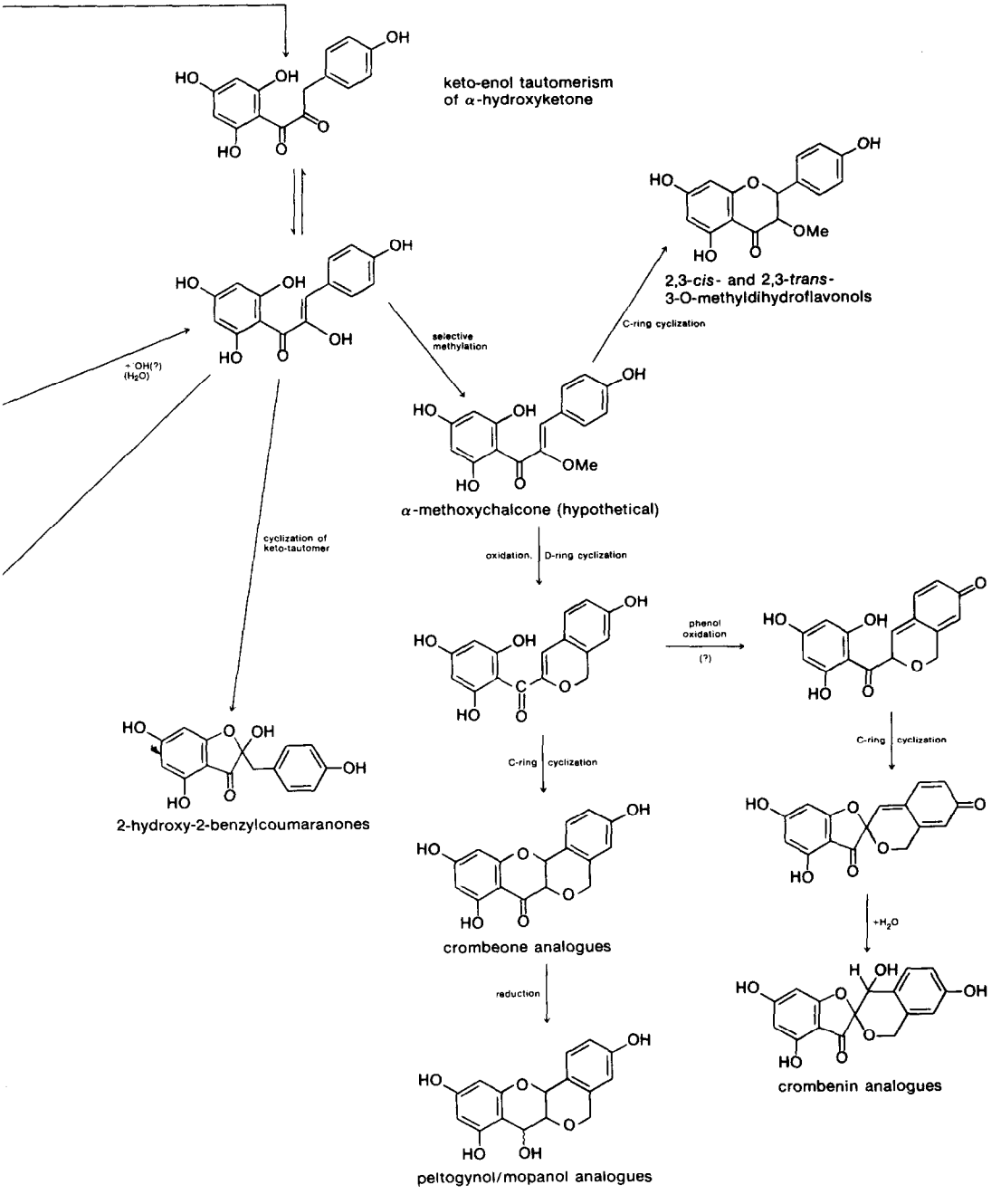
PROPERTIES OF α-HYDROXYCHALCONES AND THEIR PREVIOUS OVERSIGHT

Previous oversight of α-hydroxychalcones was possibly due to a combination of their colourless nature;[11,13] their complete overlap with 2-hydroxy-2-benzylcoumaranone analogues on paper chromatograms;[11,13] their partial conversion into the 2-hydroxy-2-benzylcoumaranone derivative during methylation with dimethyl sulphate–potassium carbonate in dry acetone;[13] and their unusual mobility in water (R_f 0·60 in 2% HOAc) during paper chromatography.[11–14] The first- and last-mentioned properties contrast with the bright yellow and complete immobility(R_f 0) of conventional chalcones in the same system. The masking of α-hydroxychalcones by 2-hydroxy-2-benzylcoumaranones closely parallels Oyamada's[20] classic discovery of the concealment of colourless and usually predominant dihydroflavonols by associated yellow flavonols.

[15] BHAKUNI, D., BITTNER, M., SILVA, M. and SAMMES, P. G. (1973) *Phytochemistry* **12**, 2777.
[16] WILLIAMS, A. H. in *Comparative Phytochemistry* (SWAIN, T. ed.), p. 297, Academic Press, London.
[17] GOTTLIEB, O. R. and RÉGO DE SOUSA, J. (1972) *Phytochemistry* **11**, 2841.
[18] BRANDT, E. V., FERREIRA, D. and ROUX, D. G., (unpublished work on *Acacia carnei*, *A. crombei* and *A. peuce*).
[19] TINDALE, M. D. and ROUX, D. G. (1969) *Phytochemistry* **8**, 1713; (1974) *ibid.* **13**, 829.
[20] OYAMADA, T. (1934) *J. Chem. Soc. Japan* **55**, 1256; (1939) *Annalen* **538**, 44.



SCHEME 2. THE POSSIBLE ROLE OF



α-HYDROXYCHALCONES IN FLAVONOID BIOSYNTHESIS.

The strong adsorption of conventional chalcones on cellulose in aqueous systems is related to their planarity,[21] and conversely the mobility of α-hydroxychalcones and hence their non-planarity must be attributed to the keto-form and to keto-enol tautomerism (see Scheme 2). Substitution reactions, namely acetylation and methylation shift the equilibrium to those enolic forms, both *cis* and *trans*,[13] in which the compounds are identified.[11, 14] By contrast the properties of achromation and mobility of the free phenol suggests that the keto-form (a phenylbenzyl-α-diketone) predominates in nature.

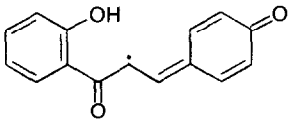
The mobility of α-hydroxychalcones on cellulose substrates in aqueous medium indicates the possibility of their translocation from the points of biogenesis in plant tissue, a property which cannot be shared by "conventional" chalcones.

α-HYDROXYCHALCONES IN FLAVONOID, TANNIN AND PELTOGYNOÏD BIOGENESIS

Dihydroflavonols, flavan-3,4-diols, flavan-3-ols and flavonols

Chalcones have long been implicated as compounds which possibly occupy a central role in flavonoid biogenesis.[1-7] However, the ease with which the new group of α-hydroxychalcones may be accommodated into a biogenetic scheme for most flavonoid and also peltogynoid analogues, suggests that the newly-discovered variations present useful alternatives, at least in some instances.

For example, cyclization of the enolic form of α-hydroxychalcones could account for the biogenesis of both 2,3-*cis*- and 2,3-*trans*-dihydroflavonols (Scheme 2). The association of natural 3-*O*-methyldihydroflavonols with the corresponding α-hydroxychalcone and the chemical analogy for this conversion were demonstrated recently.[11,22] Thus cyclization of α-methoxychalcones with sodium acetate/ethanol/water through β-addition (Michael-type of reaction) gives a 1:2 ratio of 2,3-*cis*- and 2,3-*trans*-dihydroflavonols. This represents the exact proportion in which they occur in the heartwood of *Trachylobium verrucosum*.[11,22] The ease of this conversion under relatively neutral conditions (pH 8·4) contrasts with the strong alkali required presumably for both anion formation at the 2′-hydroxyl and subsequent base-catalysed cleavage of the epoxide group in the AFO-synthesis of 3-hydroxyflavanones from conventional chalcones. For the same sequences Pelter *et al.*[3] suggest an alternative scheme, as yet unsubstantiated by chemical analogy, which is based on the tautomeric form (**4**) of a radical (**5**) formed by phenol oxidation of a conventional 4-hydroxychalcone. Attack by water or an hydroxyl radical on this chalcone radical or its cyclized form introduces the 3-hydroxyl group into the C_{15}-flavonoid skeleton.



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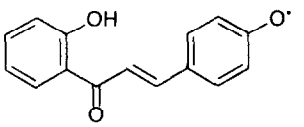


(5)

Subsequent reductions of these dihydroflavonols lead feasibly to 2,3-*cis*- and 2,3-*trans*-flavan-3,4-diols and eventually to corresponding flavan-3-ols. These classes of compounds, as is well known, form flavonols and anthocyanidins by oxidative and elimination reactions respectively. Cyclization of the *trans*-enolic form of α-hydroxychalcones is, therefore,

[21] ROUX, D. G., MAIHS, E. A. and PAULUS, E. (1961) *J. Chromat.* **5**, 9.
[22] FERREIRA, D., VAN DER MERWE, J. P. and ROUX, D. G., *J. Chem. Soc. Perkin I* (1974) In press.

of the utmost importance considering its relative ease, and the provision of the first chemical analogy for the natural origin of 2,3-*cis*- and 2,3-*trans*-diastereoisomers of dihydroflavonols, leucoanthocyanidins and catechins.

Evidence of the relative ease of conversions amongst flavonoids (reduced ring systems) is the apparent equilibrium (+)-flavan-3,4-diols ⇌ (+)-dihydroflavonols ⇌ (−)-flavanones formed as side-reaction to the substitution (S_N1) reaction of (+)-flavan-3,4-diols at C_4 with mercaptoacetic (thioglycollic) acid.[23–25] The basis of this oxidative-reductive conversion with retention of configuration at C_2 and C_3 (where applicable) is not fully understood at present.

The initial steps in these *in vitro* sequences are consistent with our observations on the composition of heartwood components of many species of the Mimosoideae, Caesalpinioideae and Anacardiaceae, where dihydroflavonols and flavan-3,4-diols (or their peltogynoid equivalents) occupy positions of great prominence, while flavanones and chalcones represent minor components.[19]

2-Hydroxy-2-benzylcoumaranones and aurones

The alternative method of cyclization to the α-position of the enolic form of α-hydroxychalcones (addition in the anti-Michael sense), or more likely to the corresponding carbonyl group of the keto isomer, readily leads to 2-hydroxy-2-benzylcoumaranones which possess 5-membered heterocyclic rings of exceptional stability. Such cyclization requires acid conditions for the enolic ether form,[11,22] while those for the keto-form are as yet unestablished.

The prominence of related α-hydroxychalcones and 2-hydroxy-2-benzylcoumaranones in *B. zeyheri* and *B. discolor*, and also their association in *T. verrucosum* (where the benzylcoumaranone represents a minor component) appears to substantiate the above.

Further conversions of 2-hydroxy-2-benzylcoumaranones to aurones require drastic conditions (conc. H_2SO_4 *in vitro*)[26] and accordingly the biogenesis of aurones may proceed via (α-hydroxybenzyl) coumaranones as postulated by Wong[27] or more likely via the phenol oxidation mechanism proposed by Pelter *et al.*[3] on the basis of chemical analogy. They postulate the same tautomeric form (**4**) of the radical derived from a conventional 4-hydroxychalcone as intermediate.

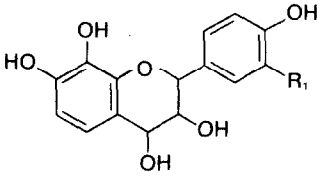
Condensed tannins

Flavan-3-ol-4-carbonium ions originating most likely from protonation of flavan-3,4-diols by organic acids, or the corresponding quinone methides derived from the same source, by loss of the elements of water, will form condensed tannins by repetitive condensation with a second flavonoid unit, provided that a sufficiently strong nucleophilic site is available on the latter.[28,29] These are usually furnished by A-rings of phloroglucinol-type catechins, or in rare instances by resorcinol-type catechins and flavan-3,4-diols (where phloroglucinol-type catechins are absent), or by phloroglucinol-type flavan-3,4-diols (where these presumably predominate initially).[29] Notable is the presence of monomeric

[23] Du Preez, I. C., Ferreira, D. and Roux, D. G. (1971) *J. Chem. Soc.* (*C*) 336.
[24] Du Preez, I. C., Fourie, T. G. and Roux, D. G. (1971) *Chem. Commun.* 333.
[25] Fourie, T. G., Du Preez, I. C. and Roux, D. G. (1972) *Phytochemistry* **11**, 1763.
[26] King, H. G. C. and White, T. (1961) *J. Chem. Soc.* 3539.
[27] Wong, E. (1967) *Phytochemistry* **6**, 1227.
[28] Ferreira, D., Hundt, H. K. L. and Roux, D. G. (1971) *Chem. Commun.* 1257.
[29] Roux, D. G. (1972) *Phytochemistry* **11**, 1219.

flavan-3,4-diols and phloroglucinol catechins in most heartwoods and barks where tannin-formation predominates.

Where pyrogallol-type A-rings are present in flavan-3,4-diols, as in the melacacidins (**6.** R_1=OH)[19,25] or teracacidins (**6.** R_1=H)[19,30,31] natural condensed tannins are absent since potential 4-carbonium ions should be less stable (poorer delocalization on the A-rings) or quinone methide intermediates less readily formed, and neither A- or B-rings provide strong nucleophilic centres.



(6)

The above observations lead to the conclusion that the extent and position of phenolic substitution on the A-rings of flavan-3,4-diols and flavan-3-ols determine the likelihood and ease of condensed tannin formation.

These, however, contrast to some extent with conclusions by Haslam *et al.*[32] drawn from their correlation of condensed tannin structure (based exclusively on phloroglucinol-type flavonoids) with associated (+)-catechin and (−)-epicatechin content in 29 plant species. Here quinone methides resulting from oxidation of the catechins are suggested as intermediates in tannin formation, but the exceptional reactivity and hence the possible contribution of phloroglucinol-type flavan-3,4-diol analogues appears to have been overlooked.

Mopanols, peltogynols and their analogues

Methylation of the α-hydroxyl of the new class of α-hydroxychalcones, followed by oxidative cyclization of the resultant α-methoxyl (enol ether) group with the benzene B-ring (D-ring formation)[33,34] could result in chalcones which are analogues of mopanol and peltogynol. Examples of the latter have recently been isolated by Gottlieb and de Sousa[17] from *Goniorrhachis marginata* (the mopanol analogue) and by us[18,19] from *Acacia carnei* (peltogynol analogue) as indicated before. These may undergo further cyclization (C-ring formation) to mopanones and peltogynones,[35,17] and final reduction to mopanols and peltogynols (Scheme 2). The planarity of both chalcone intermediates would favour the proposed cyclizations. An α-hydroxychalcone (**1.** R_1=H, R_2=OH) is in fact associated with both (+)-mopanol and (+)-peltogynol and their 4-epimers in *T. verrucosum*,[11] *P. pubescens* and *P. venosa*.[12] The α-OH group has acidic character[11–14] as it methylates with ease with diazomethane, and numerous analogies exist for the methylation of acidic (phenolic) hydroxyls *in vivo*.

Phenol oxidation of peltogynoid chalcones followed by cyclization would yield quinone methide intermediates, and eventually 4′-hydroxyisochroman-3′-spiro-2-coumaran-3-ones

[30] DREWES, S. E. and ROUX, D. G. (1966) *Biochem. J.* **98**, 493.
[31] MALAN, E. and ROUX, D. G., (unpublished work on *Acacia galpinii*).
[32] THOMPSON, R. S., JACQUES, D., HASLAM, E. and TANNER, R. J. N. (1972) *J. Chem. Soc. Perkin I*, 1387; HASLAM, E. (1969) *J. Chem. Soc. (C)*, 1824.
[33] WAISS, A. C. and CORSE, J. (1965) *J. Am. Chem. Soc.* **87**, 2068.
[34] WAISS, A. C., LUDIN, R., LEE, R. E. and CORSE, J. (1967) *J. Am. Chem. Soc.* **89**, 6213.
[35] BRANDT, E. V., FERREIRA, D. and ROUX, D. G. (1971) *Chem. Commun.* 116.

after reaction with the elements of water. This represents the only feasible sequence leading to crombenin,[36] as yet the only natural compound of this class (Scheme 2).

The α-methoxychalcone which represents the hypothetical primary precursor of the mopanol–peltogynol analogues may also undergo initial C-ring cyclization (β-addition) to form 2,3-*cis*- and 2,3-*trans*-3-*O*-methyldihydroflavonols. These exist in *T. verrucosum*,[11] *A. carnei*,[18] *A. peuce*,[18] *P. pubescens*[12] and *P. venosa*[12] in association with mopanols and peltogynols[11,12] or peltogynols only[18] in all instances.

All of the above suggestions are supported by simple synthesis, apart from the initial step of D-ring formation from α-methoxychalcones and the phenol oxidation of peltogynoid chalcones as above. The feasibility of D-ring formation (oxidative, photolytic) to give both mopanol and peltogynol analogues has, however, been demonstrated by Waiss *et al.*[34] for 3-methoxyflavones. Recent parallels for the incorporation of an "extra" carbon atom are to be found in the enzymic cyclization of 2′-methoxyisoflavones to rotenoids[37] and similar formation of 3-benzylchroman-4-ones.[38]

CONVENTIONAL CHALCONES IN FLAVONOID BIOSYNTHESIS

Biosynthetic studies (see refs [1–6]) have emphasized the significance of cinnamic acids and chalcones as flavonoid precursors. The oxidation level of the C_3-portion of these molecules requires that epoxidation[4,5] or phenol oxidation[3] of chalcones should represent the intermediate step in an alternative method of biosynthesis of dihydroflavonols (see Introduction).

Evidence as regards the role of chalcones is well established, but for the fact that the suggested epoxide-induced biosynthesis[4,5] of dihydroflavonols would not present simultaneous opportunity for the formation of the now common 2-hydroxy-2-benzylcoumaranones, but would provide (α-hydroxybenzyl)coumaranones via the alternative method of cyclization.[27] In this connection it is interesting to note that the three α-hydroxychalcones (**1**)[11,13,14] are all associated with 2-hydroxy-2-benzylcoumaranones.

Similarly the phenol oxidation mechanism proposed by Pelter *et al.*[3] for the 3-hydroxylation of flavonoids, requires the presence of 4-(or 2-)hydroxyl function in "conventional" chalcones,[3] and is thus apparently not applicable to the biosynthesis of dihydroflavonols such as pinobanksin, strobobanksin and alpinone which lack B-ring hydroxylation. Notable too is that among the 3,8-biflavonoids whose natural existence lends support to the suggestion of phenol oxidation of chalcones,[3] the mechanism appears to be strictly limited to 4-hydroxychalcones i.e. yielding structures which possess 4′-hydroxyl function in the B-ring of the "upper" unit only.

POSSIBLE BIOGENETIC STEPS LEADING TO α-HYDROXYCHALCONES

Consideration of the structure of the α-diketone tautomer (see scheme) suggests that *p*-hydroxyphenylpyruvic acid, of which the ester significantly exists as the keto and two *cis*-

[36] BRANDT, E. V., FERREIRA, D. and ROUX, D. G. (1972) *J. Chem. Soc. Chem. Commun.* 392.
[37] CROMBIE, L., FREEMAN, P. W. and WHITING, D. A. (1973) *J. Chem. Soc. Perkin I* 1285.
[38] DEWICK, P. M. (1973) *J. Chem. Soc. Chem. Commun.* 438.
[39] GAULT, H. and WEICK, R. (1920) *Compt. Rend.* **170**, 1392; *ibid.* **171**, 395; (1922) *Bull. Soc. Chim. France* **31**, 867, 993.

trans enol isomerides[39] (see α-hydroxychalcones[13]) as the most likely precursor of α-hydroxychalcones from the metabolic pool. Phenylpyruvic acid was previously considered as key intermediate by Underhill *et al.*[40] and was also cited by Wong.[41]

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