

Condensed Tannins: A Proposed Route to 2*R*,3*R*-(2,3-*cis*)-Proanthocyanidins

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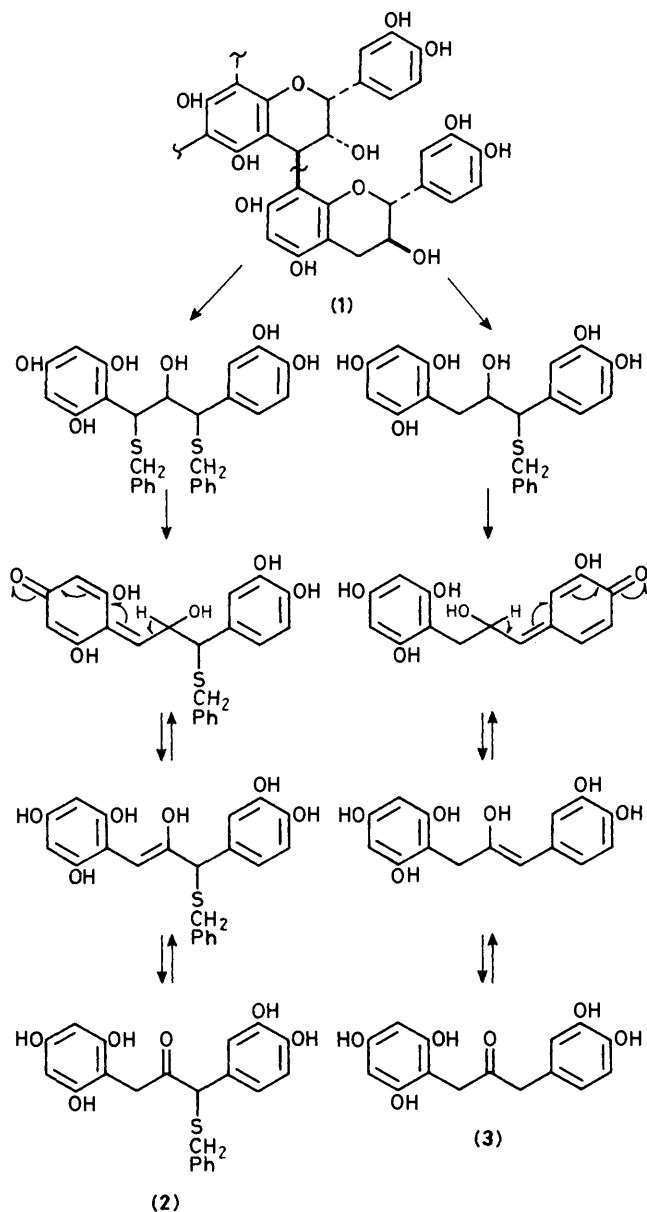
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The biogenesis of 2*R*,3*R*-(2,3-*cis*)-proanthocyanidins from 2*R*,3*R*-(2,3-*trans*)-dihydroflavonols can be accounted for by tautomerism between quinone methide and flav-3-en-3-ol intermediates.

Roux,¹ Haslam,²⁻⁴ and Stafford⁵⁻⁸ have proposed differing biogenetic schemes for the formation of proanthocyanidins (condensed tannins) in plants. All three schemes suffer from difficulties in explaining the formation of the 2*R*,3*R*-(2,3-*cis*)-proanthocyanidins that predominate in plant tannins. Stafford⁵⁻⁸ has postulated that a C-3 epimerase acts on (+)-dihydroquercetin to provide the 2*R*,3*S*-(2,3-*cis*)-dihydroflavanol that would then be reduced to the corresponding 2*R*,3*R*,4*S*- (or 4*R*-) flavan-3,4-diol. However, enzyme preparations from Douglas-fir callus do not produce any 2,3-*cis*-procyanidins.⁵⁻⁸ Haslam²⁻⁴ proposed a flav-3-en-3-ol intermediate that might be derived from an α -hydroxychalcone. A stereospecific reduction of the flav-3-en-3-ol to either 2,3-*cis*- or 2,3-*trans*-flavan-4-carbocations is then postulated to account for the formation of the proanthocyanidins, but there is debate about the presence of a carbocation. Roux¹ proposes that the α -hydroxychalcone can give either 2,3-*cis*- or 2,3-*trans*-dihydroflavonols through the familiar chalcone-flavone interconversion, but the stereochemistry of the dihydroflavonols in many plants is 2,3-*trans*, while that of the co-occurring proanthocyanidins is predominantly 2,3-*cis*, as in Douglas-fir.⁵⁻⁹

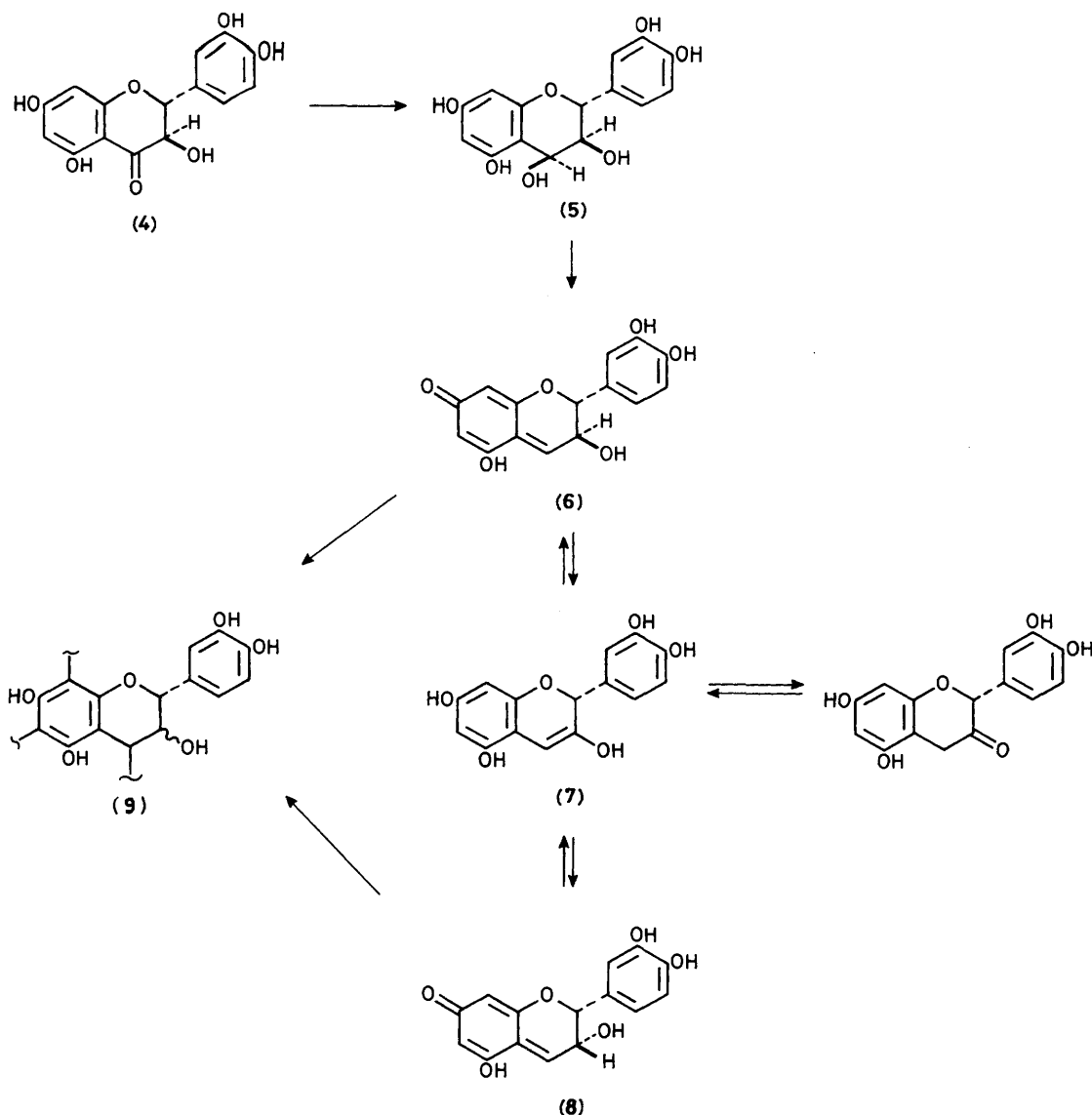
Procyanidins are readily synthesised from flavan-3,4-diols by mild base- or acid-catalysed generation of quinone methide intermediates.¹⁰⁻¹² Platt *et al.*⁴ have alluded to the tautomeric relationships of these quinone methides with flav-3-en-3-ols. Chemical evidence supporting these interconversions was obtained in recent studies of the reactions of condensed tannins in alkaline solutions.¹³ At pH 12.0 and ambient temperature, in the presence of phenylmethanethiol, procyanidins (1) are cleaved to form mono- or dibenzylsulphide derivatives from the lower and upper units, respectively. Loss of phenylmethanethiol and tautomeric rearrangement leads to the formation of two propan-2-one derivatives, (2) and (3) (Scheme 1). These ketones are analogous to Haslam's²⁻⁴ proposed flav-3-en-3-ol, which, in solution, would be in the keto form. Since these reactions are at high pH, all products are produced through cleavage of the pyran ring. However, it seems plausible that enzyme-catalysed reactions on flavan-3,4-diols could involve the formation of a quinone methide (6)¹⁰⁻¹² followed by a tautomeric rearrangement analogous to the experimentally observed reactions described in Scheme 1 to produce the flav-3-en-3-ol or its corresponding ketone without cleavage of the heterocyclic ring.

Based on these results, the following explanation is proposed for the biogenesis of either 2,3-*cis*- or 2,3-*trans*-proanthocyanidins from 2*R*,3*R*-(2,3-*trans*)-dihydroflavonols (Scheme 2). In agreement with Stafford,⁵⁻⁸ enzymatic reduction of the dihydroflavonol (4) provides the 2*R*,3*S*,4*S*-flavan-3,4-diol (5). But rather than the formation of a flavan-4-carbocation, the diol eliminates water to form a 2,3-*trans*-quinone methide (6). In agreement with Haslam,¹⁻³ it is proposed that a flav-3-en-3-ol is a central intermediate. This compound (7) is formed from the quinone methide by an enzyme-catalysed rearrangement parallel to the base-



Scheme 1. Ketones produced from the reaction of procyanidins with phenylmethanethiol and base at ambient temperature.

catalysed tautomerism observed in the reactions of the diarylpropanoids¹³ (Scheme 1). The flav-3-en-3-ol is then stereospecifically converted back into a quinone methide (6) or to (8) that will then polymerise to form the condensed tannins (9). Rearrangements of the flavan-3-ols analogous to



Scheme 2. Proposed route for biosynthesis of proanthocyanidins.

the formation of the ketone (3) (Scheme 1) could account for the formation of 2,3-*cis*-flavan-3-ols from a two step reduction of the 2,3-*trans*-dihydroflavanols.

This proposal is in full agreement with Haslam's labelling studies that show retention of the proton at C-2 and loss of a proton at C-3 of the flavan unit.²⁻⁴ By assuming different enzyme sites for the synthesis of the procyanidin and flavan-3-ol units, one can account for the differences in their hydroxylation and/or stereochemistry. Most importantly, interconversions of the quinone methides and compounds analogous to flav-3-en-3-ols have been demonstrated chemically in the formation of (2) and (3) from reactions of condensed tannins with phenylmethanethiol at alkaline pH. Our results suggest that one should look for enzymes mediating these interconversions rather than for dihydroflavanol C-3 epimerases.

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