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HAEMANALYSIS: THE RELATIVE ASTRINGENCY OF PROANTHOCYANIDIN POLYMERS

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Key Word Index—Tannins; tannic acid; condensed tannins; proanthocyanidins; molecular weight; haemanalysis

Abstract—Effects of MW, stereochemistry of monomer units, and B-ring oxidation pattern on relative astringency were studied. Efficiency of protein precipitation is primarily a function of proanthocyanidin polymer (condensed tannin) size and whereas oligomeric proanthocyanidins have a relative astringency less than tannic acid, polymeric proanthocyanidins of sufficiently high average MW (M_n ~2500) are equally as efficient at precipitating haemoglobin.

INTRODUCTION

The unique feature of tannins, which are best described as complex polyphenols [1] is their ability to precipitate protein. There has been considerable interest in the mechanism by which this interaction takes place [2–4], which is usually attributed to hydrogen-bonding although non-polar interactions have also been suggested to be important [5]. Not all tannins can efficiently precipitate all soluble proteins [3; Jones, W. T., unpublished observations]. However, it does seem that haemoglobin does not display a high degree of selectivity, and is a good choice for a tannin precipitant, on the basis of measurements by Bate-Smith [6].

Bate-Smith [6] developed the technique of haemoglobin precipitation from haemolysed blood solutions by tannins into a general method of judging the 'relative astringency' (RA), or protein precipitating efficiency, of a particular tannin preparation. The RA was measured by comparison of the depth of colour, after centrifugation of the tannin-protein precipitate, of the remaining haemoglobin in the supernatant solution, after addition of a tannin, with that of a haemoglobin-tannic acid standard of similar strength. For convenience the RA comparisons were made at 50% precipitation. It was found that tannic acid was the most efficient precipitating agent and was given an RA of 1.0. On this scale chebulagic acid had an RA of 0.76, procyanidin dimers about 0.1, trimers about 0.2–0.3, and oligomers 0.4–0.5.

A deficiency of this study [6] was the fact that pure, genuinely polymeric proanthocyanidins were not available. Haemanalysis measurements on a number of proanthocyanidin polymers are reported here.

RESULTS AND DISCUSSION

Our experimental approach essentially followed that of Bate-Smith [6], but used whole blood samples kindly supplied through a local hospital. These samples were diluted to give a standard absorbance of approximately 0.2 in the final solution.

Initial experiments were carried out in distilled water as described by Bate-Smith [6], and curves of absorbance versus tannin concentration constructed. From these the end-point absorbance could be obtained, and the amount of tannin required for half-precipitation calculated. The end-point absorbance, however, was not always zero. For essentially colourless tannin preparations such as tannic acid and chinese quince the absorbance at total precipitation was approximately zero, but in some others the tannin contributed a significant absorbance at 587 nm.

Hagerman and Butler [3] in their study of comparative protein bindings with *Sorghum* proanthocyanidin showed the advantages of using buffered solutions. We therefore repeated the above measurements in pH 5.0 buffer containing sodium chloride to maintain constant ionic strength. Use of the buffer improved the consistency of the absorbance measurements and gave improved curves, three of which are shown in Fig. 1. From these plots the concentration of tannin required, in units of mg/10 ml, to precipitate half the haemoglobin could be calculated, and

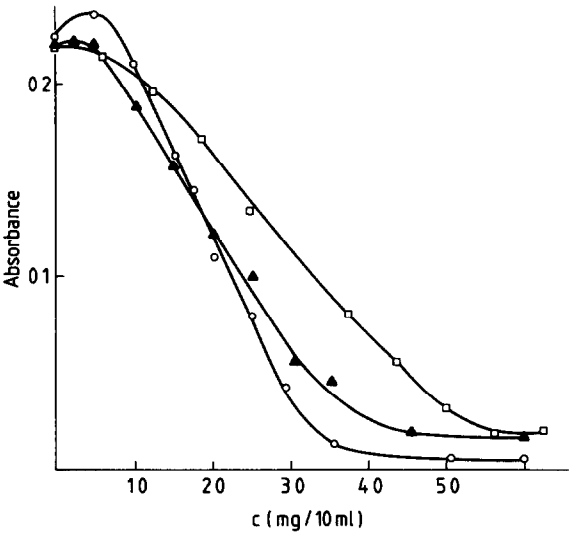

Fig. 1. Plots of residual absorbance versus tannin concentration ○, tannic acid; ▲, *Rhopalostylis* polymer; □, *Crataegus* polymer

Table 1. Relative astringency (RA) for some condensed tannins (proanthocyanidin polymers)

Polymer source	PP:PC:PD	*Cis:trans*	P_n	RA
Chaenomeles chinesis[1]	0:100:0	100:0	14.0	0.95
Aesculus × *carnea*[1]	0:100:0	100:0	11.0	0.91
Rhopalostylis sapida[1,4]	0:100.0	100:0	12.5	0.99
Strelitzia reginae[2]	25:75:0	100:0	6.6	0.91
Pinus radiata[3]	0.52:48	74:26	7.0	0.89
Pinus radiata[2]	0·20:80	90:10	9.0	1.00
Watsonia arderneι[2]	0:0:100	0:100	7.0	0.83
Crataegus oxyacantha[1]	0:100:0	100:0	4.0	0.73

Key. [1]fruit; [2]leaf; [3]phloem, [4]contains both 2*R* and 2*S* units [9]; PP = propelargonidin; PC = procyanidin; PD = prodelphinidin; *cis* and *trans* refer to the stereochemistry of the average proanthocyanidin heterocyclic rings; P_n = number average degree of polymerisation, or average chain length in terms of flavan units

the ratio of mg tannic acid/mg tannin used to obtain RA. The results for eight condensed tannins, covering a range of stereochemical and B-ring oxidation pattern differences are given in Table 1.

These results show that the predominant factor influencing RA is MW. It may be seen that provided P_n is high enough (~8–9 or 2400–2700 number average MW) the condensed tannins and tannic acid (1250 ± 60 number average MW [1]) have a very similar RA.

The lowest RA obtained was for the *Crataegus* polymer, 0.73, which has a P_n equivalent to a tetramer. As it is an all epicatechin polymer, it would perhaps be expected to approximate to the tetramer used in Bate-Smith's study [6] which had an RA of 0.35–4.0. However, the *Crataegus* polymer is polydisperse [7] and has a P_w of 13.3 as shown by low-angle laser light scattering (LALLS) measurements [7]. The polymer therefore contains a considerable proportion of chains with higher P_n values and these result in the much higher observed value of RA.

It may also be seen from Table 1 that stereochemistry and B-ring oxidation pattern have little effect. A particularly interesting example is the *Strelitzia* polymer which contains 25% of monohydroxy-B-rings (PP units) which it has been implied [4, 9] would be less efficient at protein binding than rings containing vicinal hydroxy-groups. However, it may be seen that it has a similar RA to other polymers with an equivalent P_n. It may possibly be argued that there are an insufficient proportion of PP units present to affect the binding properties. Unfortunately a polymer with a higher proportion of PP units was not at hand.

The observation that, for haemoglobin at least, both hydrolysable and condensed tannins of high average MW have equivalent protein precipitating efficiencies has interesting biological consequences. It may imply that their antibiotic activity in some situations is approximately equivalent, so that both act as broad spectrum protective agents. The other important point is that it is necessary for condensed tannins to have a proportion of genuinely polymeric chains to act with optimum efficiency, which may explain the general predominance of proanthocyanidin polymers over lower oligomers in nature.

EXPERIMENTAL

The proanthocyanidin polymers were isolated from fresh plant tissue as described previously [10]. Tannic acid was a commercial sample from Fluka.

Method for haemanalysis. Stock solutions of tannins were made up in distilled water with accurately known concns in the range 2–3 mg/ml (or higher where necessary). From these a series of dilutions were made by taking volumes ranging from 0–1.0 ml and making up the volume to exactly 1.0 ml with distilled water in a centrifuge tube. To these were added 2 ml of pH 5.0 acetate buffer containing 0.17 M NaCl [3], followed by 1.0 ml of a haemoglobin soln prepared from fresh whole blood added to distilled water and the volume adjusted to give an absorbance of *ca* 0.2 at 587 nm [6]. The contents of each tube were thoroughly mixed, and then centrifuged at 5000 rpm for 15 min. Uncomplexed haemoglobin was monitored by the residual absorbance of the uncomplexed haemoglobin at 587 nm in a 1 cm glass cell.

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