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SHORT COMMUNICATION

DETERMINATION OF THE DEGREE OF POLYMERIZATION OF LEUCOANTHOCYANIDINS

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Abstract—From studying a series of hawthorn condensed tannins, it has been found that the colorimetric method for determining the degree of polymerization of flavans based on the transformation of leucoanthocyanidins into anthocyanidins (V/LA coefficient) gives much less reliable results than molecular sieving and the colorimetric method based on the use of Folin–Denis reagent (V/FD coefficient).

INTRODUCTION

LEUCOANTHOCYANIDINS (flavan-3,4-diols) occur in plants as monomers, dimers and polymers. Dimeric and polymeric molecules can be comprised exclusively of flavan-3,4-diol units or they can contain, additionally, flavan-3-ol units such as (+)catechin and (−)epicatechin. Their ability to yield anthocyanidins upon heating with mineral acids is a common property. This transformation is widely used for the quantitative determination of leucoanthocyanidins and in 1963 Goldstein and Swain[1] used it, together with some other colour reactions specific for phenols, as a means of determining the degree of polymerization of high-molecular-weight flavans.

Although condensed tannins have been extensively studied, no method has been elaborated for determining their absolute molecular weights. The colorimetric methods described by Goldstein and Swain[1, 2] allow merely the determination of their degree of polymerization. In the course of studies on the macromolecular leucoanthocyanidins of hawthorn,[3–6] the results of using these colorimetric techniques have now been compared with those obtained by the molecular sieving.

RESULTS

Six fractions, differing in their degree of polymerization, were obtained by separating the leucoanthocyanidins of the leaves of hawthorn on Sephadex LH 20 gel columns with methanol–water as eluent.[7] The order of the elution of the individual fractions from the column allows approximate classification of the compounds according to the size of the molecule.

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The relative differences in molecular weight were then determined using the method based on the colorimetric estimation of free phenolic groups (reaction with Folin–Denis reagent) and on the estimation of unsubstituted phloroglucinol units in the flavans (reaction with the vanillin reagent; V/FD coefficient).[1,2] Since all but one ((−)epicatechin) of the flavan fractions studied gave some colour with acid, the method based on the determination of the anthocyanidins formed and simultaneous determination of unsubstituted phloroglucinol system (V/LA coefficient)[1] was also applied. The results of these experiments are presented in the Table.

TABLE 1. ORDER OF ELUTION FROM THE SEPHADEX LH 20 GEL, V/FD AND V/LA COEFFICIENTS FOR THE HAWTHORN FLAVAN FRACTIONS

Flavan fractions in order of increasing molecular weight (inverse order of elution from the Sephadex LH 20 gel)	V/FD	V/LA
(−) Epicatechin	0·90	—
Dimeric leucocyanidin[5]	0·87	11·50
Oligomeric leucoanthocyanidin I	0·36	0·97
Oligomeric leucoanthocyanidin II	0·47	1·60
Polymeric leucoanthocyanidin	0·10	2·80
Copolymer of leucoanthocyanidin and (−) epicatechin	0·01	16·60
(−) Epicatechin polymer	0·05	10·80

The data presented in the table indicate satisfactory correlation between the order of elution from Sephadex LH 20, representing increasing molecular weight, and the decreasing values of the V/FD coefficient. Values deviating from the expected were obtained for the oligomeric leucoanthocyanidins and for the fraction of leucoanthocyanidin–catechin copolymer but these deviations are probably caused by the adsorption phenomena accompanying molecular sieving.

On the other hand, the values of the V/LA coefficient, which according to Goldstein and Swain should also decrease with increasing polymerization, show no correlation with respect to the data of the first two columns of the table. Unexpectedly high are the values of the V/LA coefficient obtained for the polymeric fractions and in particular for the fraction of (−)epicatechin polymers. This fraction contains no detectable amounts of leucoanthocyanidins, in spite of the fact that with the Swain–Hillis reagent it gives products absorbing at 540 nm.

DISCUSSION

The results presented indicate that two of the three methods of determining polymerization in flavans give comparable results. These results confirm the usefulness of the method proposed by Goldstein and Swain[1] for this purpose (V/FD coefficient). They also show that gel-filtration, which until now has been applied in flavan chemistry only for separation purposes,[8–11] can be successfully applied to the estimation of molecular weight.

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On the other hand, the results obtained show that the transformation of leucoanthocyanidins into flavylium salts cannot be used for this purpose. This transformation includes a number of reactions such as liberation of the flavan unit from a dimer or polymer, oxidation of the flavandiol heterocyclic ring and dehydration. These reactions are accompanied by some secondary processes, leading to the formation of high-molecular insoluble products called phlobaphenes. Thus, the yield of the transformation depends basically on the structure of the leucoanthocyanidin and varies from a few per cent for complex leucoanthocyanidins, to 30 per cent for the dimeric leucocyanidin of the leaves of hawthorn[3] and to 40 per cent for leucofisetinidin.[12]

Goldstein and Swain assumed that yield of the transformation of leucoanthocyanidins into anthocyanidins does not change significantly above a certain degree of polymerization. This assumption may be correct for compounds which differ only in their degree of polymerization, as e.g. the leucofizetinidins.[13] However, the mixtures of high-molecular leucoanthocyanidins occurring in plant material contain, as a rule, compounds which differ in molecular weight, structure of sub-units and type of bonds between them. These differences substantially affect yields of the reactions leading to formation of the flavylium salts. Besides, it has been found that although catechin polymers, upon heating with acids, do not transform into anthocyanidins in chromatographically detectable amounts, they are converted under these conditions into soluble coloured products which interfere with the determinations.

It seems that the V/LA coefficient represents a parameter characteristic for particular compound or fraction and it can be useful for identification of a compound or determination of the degree of purity of a preparation. However, the value of this coefficient cannot be translated into information concerning the molecular weight of a compound studied.

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