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PROANTHOCYANIDINS FROM THE ROOTS OF *FRAGARIA VESCA*

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Key Word Index—*Fragaria vesca*; Rosaceae; proanthocyanidins; procyanidins.

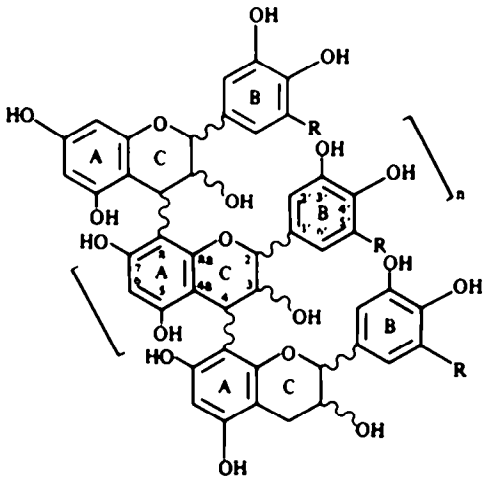
Abstract—Water-soluble proanthocyanidins obtained by fermentation of a tannin extract from *Fragaria vesca* were shown, mainly by ^{13}C NMR and HPLC, to consist of procyanidins B_1, B_2 and B_5 together with catechins.

INTRODUCTION

The rhizomes of *Fragaria vesca* L. (Rosaceae) are rich in proanthocyanidin polymers of undefined molecular weight. Determination of their structure involves assay of their constituent proanthocyanidins. Depolymerization is not possible by chemical means, but can be performed by fermentation [1]. We report here the determination of the structure of water-soluble proanthocyanidins obtained in this way.

RESULTS AND DISCUSSION

According to the biogenetic mechanisms proposed by Haslam [2], most proanthocyanidins are derived from the condensation of catechin or gallocatechin units via a 4-C/8-C bond. Hence the overall formula of the water-soluble proanthocyanidins obtained by fermentation is **1**. The catechin units may be (+)-catechin or (−)-epicatechin. The gallocatechin units may be (+)-gallocatechin or (−)-epigallocatechin. (+)-Catechin and (+)-gallocatechin both have a *trans*-configuration, while their epimers have a *cis*-configuration. Thus, the structure of the proanthocyanidins (**1**) depends on three main variables [3]: the relative proportions of procyanidin and prodelphinidin units, i.e. the PC:PD ratio; the stereochemistry of the heterocyclic nucleus of the monomer units, i.e. the *cis–trans* ratio; and the degree of polymerization.



proanthocyanidin **1**

R = H: catechin or procyanidin moiety
R = OH: gallocatechin or prodelphinidin moiety

The leucoanthocyanin reaction releases a cyanidin unit from proanthocyanidin polymer **1**; PC:PD = 100:0. In addition, the UV spectrum is symmetrical with respect to the maximum at 280 nm, which is characteristic of a homogeneous procyanidin polymer [3]. However, neither the leucoanthocyanin reaction nor UV spectrophotometry can prove the presence of prodelphinidins, which differ from procyanidins only by an extra hydroxyl group on the B-ring. This difference is reflected in the IR spectrum between 1600 and 1500 cm^{-1} and between 780 and 730 cm^{-1}. The absorption bands characteristic of procyanidins are at 1520 and 780 cm^{-1} and those of prodelphinidins are at 1535, 1520 and 730 cm^{-1} [4]. The spectrum of proanthocyanidin polymer **1** shows bands only at 1520 and 780 cm^{-1}, characteristic of a homogeneous procyanidin polymer; PC:PD = 100:0.

IR spectrophotometry can also be used to determine the *cis–trans* ratio. The units with the *cis*-configuration have a characteristic absorption band at about 800 cm^{-1}. According to Foo [4], when this band has about the same intensity as that of the procyanidins at 780 cm^{-1} the polymer contains over 70% of units with the *cis*-configuration. This is the case for proanthocyanidin polymer **1**, thus the *cis–trans* ratio is 7:3. This value agrees with that obtained by polarimetry.

Czochanska *et al.* [3] discovered that the specific rotation $[\alpha]_D^{20}$ of a proanthocyanidin polymer is linked to the molar fraction X of the units of the *cis*-configuration by a simple linear relation: $[\alpha]_D^{20}$(polymer) = $17X - 320(1 - X)$. Polymer **1** has a specific rotation of +54.2°. It must therefore contain about 76% of units with the *cis*-configuration. The results obtained by IR spectrophotometry and polarimetry were confirmed by ^{13}C NMR spectroscopy.

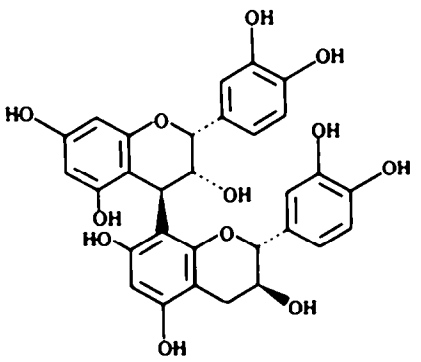
We compared the spectrum of polymer **1** with those of homogeneous polymers of procyanidin and prodelphi-

nidin studied by Czochanska *et al.* [3]. All the signals due to the three A, B and C nuclei were assigned. Those close to 146 ppm are due, according to Czochanska, to the quaternary 3- and 4-carbons of the procyanidin units and the 3- and 5-carbons of the prodelphinidin units. The spectrum studied showed a single signal at 146.1 ppm, attributable therefore solely to the procyanidin units; PC:PD = 100:0. The signals at 84 and 77 ppm correspond, respectively, to the two carbons of the units of *trans*- and *cis*-configuration. The *cis–trans* ratio is obtained by integration of the two peaks. The proportion of units of the *cis*-configuration in polymer **1** is 75.5%; *cis*:*trans* = 75.5:24.5. The degree of polymerization, i.e. the number of monomer units, can be obtained by integration of the signals at 72–73 ppm and 67–68 ppm. These chemical shifts correspond to the 3-carbon of the monomer units and that of the end unit.

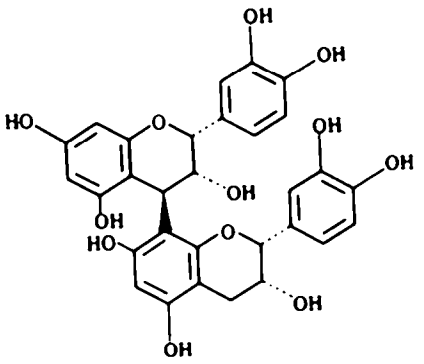
However, for polymer **1** the end unit signal is too weak to be measured accurately. Accordingly, a different method was sought to determine the degree of polymerization. Chromatography on Sephadex LH20 of the acetylated procyanidins allowed variously polymerized fractions to be isolated. Thus, ethanol elutes monomers, dimers and trimers; methanol elutes tetramers; and 70% aqueous acetone elutes more highly polymerized fractions [5]. Acetylated procyanidins (**1**) were eluted entirely with ethanol. They must therefore be at most trimers: $n \leqslant 3$.

Qualitative analysis was carried out by two-dimensional chromatography on cellulose plates based on the method proposed by Haslam for the separation of procyanidins on paper [2]. Five spots were obtained, four of which were identified. Their R_f values were compared with those of controls run under the same conditions. They were monomers (+)-catechin and (−)-epicatechin and the dimers procyanidin B_1 (**2**) and procyanidin B_2 (**3**).

High-performance liquid chromatography of procyanidin polymer **1** was performed under conditions similar to those used by Vande Casteele *et al.* [6]. As shown in Table 1, this method allowed a third dimer to be identified, procyanidin B_5 (**4**), and the concentration of each constituent to be calculated. The monomers and the three dimers B_1, B_2 and B_5 accounted, respectively, for 27.9 and 58% of the sample analysed.



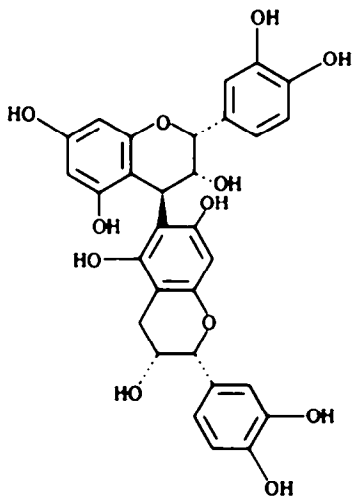
procyanidin B_1 **2**



procyanidin B_2 **3**

Table 1. HPLC analysis of *Fragaria* proanthocyanidins

Procyanidins	*RR*, (min)	Concentration (%)
Unknown	4.75	12.5
(+)-Catechin	7.15	26
(−)-Epicatechin	13.55	1.9
Procyanidin B_2	15.80	15.7
Procyanidin B_5	16.50	3.9
Procyanidin B_1	17.10	38.4



procyanidin B_5 **4**

EXPERIMENTAL

Isolation of proanthocyanidins. Tannin extract was depolymerized by fermentation as described previously [7]. 100 mg water-soluble proanthocyanidins obtained thereby was dissolved in 2 ml MeOH–H_2O (1:1) and applied to a column of Sephadex LH20. Purified proanthocyanidins were eluted as a discrete, visible band with 150 ml EtOH. This solvent was removed *in vacuo* at 40°.

PC:PD ratio and determination of stereochemistry of the monomer unit. Determination of leucoanthocyanin was per-

formed by the method of Bate-Smith [8]: 5 mg proanthocyanidins dissolved in 1 ml H_2O was heated with 10 ml *n*-BuOH HCl (19:1) for 40 min at 90–95°. The anthocyanidin formed was measured at 550 nm. Optical rotations and UV spectra were recorded in H_2O: $[\alpha]_D^{20}$ + 54.2° (H_2O; *c* 0.5) and $\lambda_{max}^{H_2O}$ nm: 280.

IR (KBr) for **1**: ν_{max} cm^{-1}: 3700–3100 (OH), 1600 (>C=C<), 1520, 1200–1100 (C–O), 800, 780. ^{13}C NMR spectra for **1** were recorded at 60 MHz in DMSO. The chemical shifts are given in ppm: δ 38 (C-4), 71.8 (C-3), 76.9 (C-2 *cis*), 84.9 (C-2 *trans*), 97.8 (C-6), 103 (C-4a), 105.9 (C-8), 115.6 (C-2'), 117.2 (C-5'), 119.2 (C-6'), 134.6 (C-1'), 146.1 (C-3' and C-4'), 156.8 (C-8a), 157.1 (C-7), 157.4 (C-5).

Evaluation of degree of polymerization. Acetylation was carried out with Ac_2O–pyridine at 25° for 12 hr. Removal of reagents gave a residue which was chromatographed on Sephadex LH20 using EtOH, MeOH and Me_2CO–H_2O (7:3) successively as eluting solvents.

2D-TLC of proanthocyanidins was carried out on cellulose (Merck, 0.25 mm) using the solvent systems (A) HOAc–H_2O (3:47) and (B) *n*-BuOH HOAc H_2O (14:1:5). Procyanidin B_1: R_f (A) 0.37, R_f (B) 0.16 (green); procyanidin B_2: R_f (A) 0.45, R_f (B) 0.26 (blue); (−)-epicatechin: R_f (A) 0.42, R_f (B) 0.55 (violet); (+)-catechin: R_f (A) 0.46, R_f (B) 0.67 (violet).

HPLC was conducted with a two-pump Model 510, solvent programmer 680, U6K injector and UV detector Model 490 (Waters). A Delsi Icap 50 integrator calculator was used for all computations. Columns: prepacked analytical column Lichrosorb RP-18 10 μ (250 × 4 mm) (Merck) and short precolumn of μ-Bondapak C_{18} (Waters). Gradient: two solvents were used: (C) HCO_2H H_2O (1:19) and (D) MeOH. The elution profile was: 0–2 min, 7% D in C (isocratic); 2–8 min, 7–15% D in C (linear gradient); 8–25 min, 15–75% D in C (linear gradient); 25–27 min, 75–80% D in C (linear gradient); 27–29 min, 80% D in C (isocratic). Flow rate 2.5 ml/min, temp. 20°, column pressure 150 kg/cm^2, UV detection 280 nm. RR_ts: (+)-catechin 7.15 min; (−)-epicatechin 13.55 min; procyanidin B_1 17.10 min; procyanidin B_2 15.80 min and procyanidin B_5 16.50 min.

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REFERENCES

1. Vennat, B. (1985) Th. Doct. Etat ès Sci. Pharm., Clermont-Ferrand.
2. Haslam, E. (1977) *Phytochemistry* **16**, 1625.
3. Czochanska, Z., Foo, L. Y., Newman, R. H. and Porter, L. J. (1980) *J. Chem. Soc. Perkin Trans* 1, 2278.
4. Foo, L. Y. (1981) *Phytochemistry* **20**, 1397.
5. Foo, L. Y. (1982) *Phytochemistry* **21**, 1741.
6. Vande Casteele, K., Geiger, H., De Loose, R. and Van Sumere, C. F. (1983) *J. Chromatogr.* **259**, 291.
7. Pourrat, A., Vennat, B. and Pourrat, H. (1983) *Ann. Pharm. Fr.* **41**, 597.
8. Bate-Smith, E. C. (1973) *Phytochemistry* **12**, 907.