

## PROANTHOCYANIDINS FROM *RIBES NIGRUM* LEAVES TLC ANALYSIS

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### INTRODUCTION

The ten proanthocyanidins isolated from *R. nigrum* (black-currant) can be subdivided into three groups, according to the increasing degree of polymerization:

- **four monomers** including catechin **1**, epicatechin **2**, galocatechin **3**, epigallocatechin **4**;
- **four dimers** comprising two major prodelphinidins: galocatechin-(4 $\alpha$ ->8)-galocatechin **5** and galocatechin-(4 $\alpha$ ->8) epigallocatechin **6** and two minor compounds, galocatechin-(4 $\alpha$ ->8)-catechin **7** and galocatechin-(4 $\alpha$ ->6)-galocatechin **8**;
- **two trimers** including the new prodelphinidin galocatechin-(4 $\alpha$ ->8)-galocatechin-(4 $\alpha$ ->8)-galocatechin **10** and the mixed proanthocyanidin, catechin-(4 $\alpha$ ->8)-galocatechin-(4 $\alpha$ ->8) galocatechin **9**.

Here we report on the identification and TLC-fingerprint of these ten proanthocyanidins. The simultaneous examination of their R<sub>f</sub> values and colorations with the vanillin-HCl reagent gives structural and stereochemical informations which is of use in the quality control of medicinal products based on *R. nigrum* leaves.

### EXPERIMENTAL

Each proanthocyanidin solution was prepared by dissolving 1 mg of substance in 1 ml methanol. The solutions (10 $\mu$ l) were spotted on HPTLC silicagel 6OF254 plates (10 x 20 cm) Merck® as 5 mm. bands. Plates were developed in the ascending mode in a saturated chamber (15 min) at room temperature, using the upper phase of ethyl acetate-water-formic acid-acetic acid (70:20:3:2;v/v/v/v) as mobile phase [1]. Following development, the plates were dried and sprayed with a 1 % vanillin solution in methanol-hydrochloric

acid (8:2:v/v). The colours of the bands were observed 5 min and 24 hours after spraying. The R<sub>f</sub> values were as follows: **1**(0.89), **2**(0.875), **3**(0.80), **4**(0.78), **5**(0.41), **6**(0.42), **7**(0.51), **8**(0.6), **9**(0.21), **10**(0.15).

## RESULTS AND DISCUSSION

Examination of the plates 5 min. after spraying with vanillin-HCl reagent showed that flavan-3-ols (monomers and oligomers) afforded a red colour, which allows the proanthocyanidins and flavonoids present in the leaves of *R. nigrum* to be distinguished; however, the next day, re-examination of the plate allowed some of the stereoisomers to be differentiated.

Prodelphinidins comprising gallocatechin unit(s) only (**3,5,8,10**) changed to violet at room temperature, while those containing epigallocatechin unit(s) (**4,6**) became browner.

Evaluation of the R<sub>f</sub> values revealed a characteristic TLC finger-print for the *R. nigrum* proanthocyanidins.

### Four points may be noted:

I. R<sub>f</sub> values decrease with increasing degree of polymerization (R<sub>f</sub> 0.75-0.9 for monomers; 0.4-0.6 for dimers and 0.15-0.3 for trimers). This order is the same on silica gel with dibutyl ether-diethyl ether-isobutanol-acetic acid (5:2:2:1:v/v/v/v) [2]; nevertheless, this solvent system does not provide a good separation between the dimers and trimers. On cellulose, the R<sub>f</sub> values do not depend on the average molecular weight.

II. Procyanidins are eluted before prodelphinidins of equivalent constitution.

III. Dimers with a (4 $\alpha$ ->8) inter-flavan linkage are eluted after their (4 $\alpha$ ->6) isomers; this elution order is reverse of that described earlier on Sephadex LH 20.

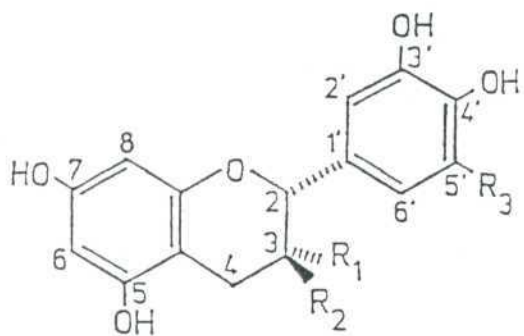
IV. R<sub>f</sub> values of C-3 diastereoisomers are too close to allow quantitative densitometric determination of each constituent.

The HPTLC method, however, can be used for the quantitative determination of some of the major oligomeric prodelphinidins. These observations corroborate and complete the previous studies on the chromatographic behaviour of flavan-3-ols [3].

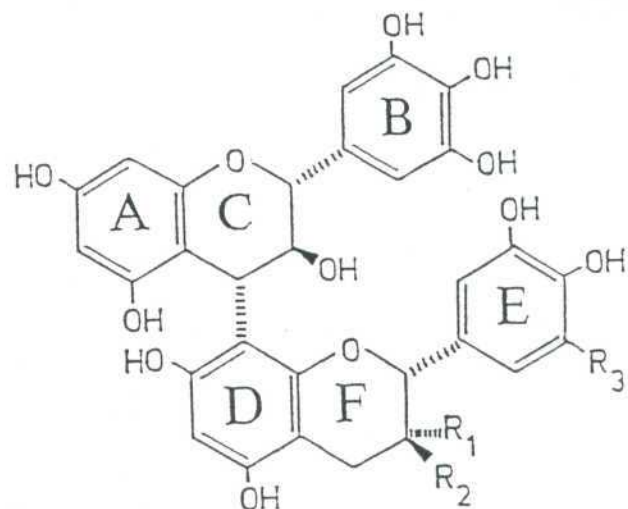
In summary, the main oligomeric prodelphinidins of *R. nigrum* leaves can be identified by HPTLC; this method could also be utilized as a proanthocyanidin screen for determining their occurrence in other plants and for quantification of the major dimers. We are currently investigating the development of an appropriate densitometric method.

## REFERENCES

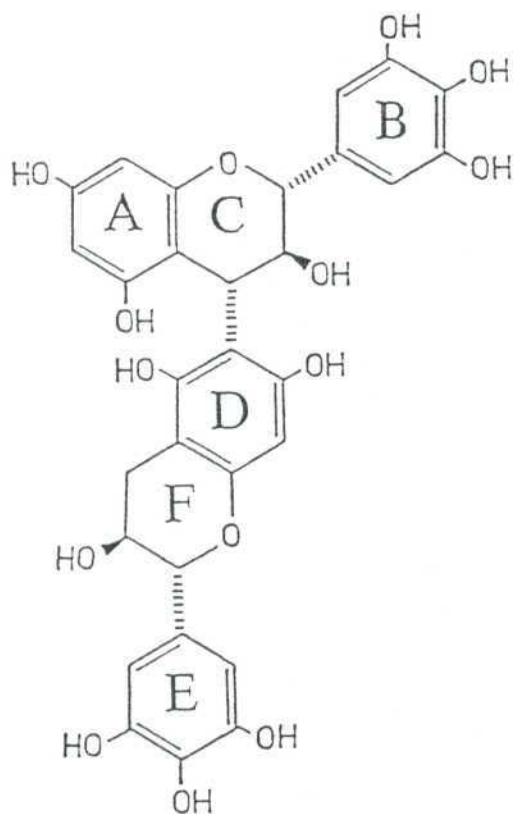
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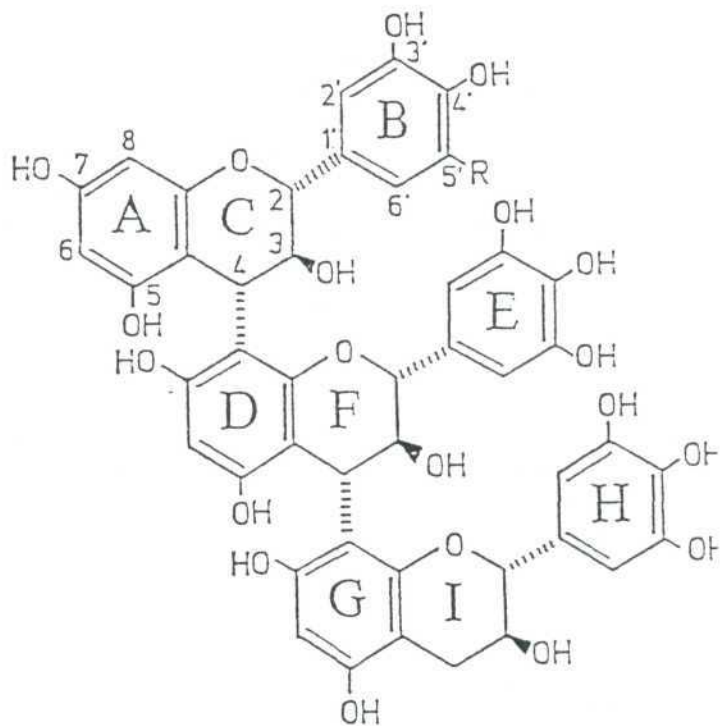
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
1 C	H	OH	H
2 EC	OH	H	H
3 GC	H	OH	OH
4 EGC	OH	H	OH



	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
5 GC-(4 α->8)GC	H	OH	OH
6 GC-(4α->8)EGC	OH	H	OH
7 GC-(4α->8)C	H	OH	H



8 GC-(4α->6)GC



9 C-(4 α->8)-GC-(4α->8)-GC	R = H
10 GC-(4α->8)-GC-(4α->8)-GC	R = OH