PROANTHOCYANIDINS FROM *RIBES NIGRUM* LEAVES ISOLATION AND STRUCTURE DETERMINATION

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KEYWORDS: *Ribes nigrum; Grossulariaceae*; black-currant leaves; proanthocyanidins; prodelphinidins.

INTRODUCTION

Black-currant leaves are traditionally used in Europe for the treatment of rheumatic disease. We have observed that the anti-inflammatory activity (in carrageenan-induced rat paw-oedema) is mainly due to proanthocyanidins and particularly to prodelphinidins [1]. Prodelphinidins are less frequently encountered in the plant kingdom than procyanidins. However, in the case of *Ribes nigrum*, the proanthocyanidin fraction contains chiefly prodelphinidins instead of procyanidins, as is also evident from the structures of the main oligomeric compounds, which are formed solely from (epi)gallocatechin units [2].

In our preceding papers [1,2] we described the occurrence in *R. nigrum* of five prodelphinidins: gallocatechin (GC) **3**, epigallocatechin (EGC) **4**, gallocatechin-(4α->8)-gallocatechin **5**, gallocatechin-(4α->8)-epigallocatechin **6** and the new trimer: gallocatechin-(4α->8)-gallocatechin-(4α->8)-gallocatechin **10**.

In continuing our studies on the constituents of black-currant leaves, we have now isolated a further five, known, compounds: catechin (C) **1**, epicatechin (EC) **2**, gallocatechin-(4α->8)-catechin **7**, gallocatechin-(4α->6)-gallocatechin **8** and catechin-(4α->8)-gallocatechin-(4α->8)-gallocatechin **9**.

EXPERIMENTAL

Extraction and isolation of the proanthocyanidins

The extraction and purification of the proanthocyanidins followed the procedure recently described [2]. The crude extract was fractionated using a medium-pressure Superformance column filled with reverse phase RP 8 and the elution was carried out with water-acetone (9:1; v/v). The purification of proanthocyanidins was effected on Sephadex LH 20, using as solvent system ethanol with increasing concentrations of methanol. The elution from the Sephadex followed the order: **1**, **2**, **3**, **4**, **7**, **5** and **6** (eluted simultaneously), **8**, **9** and **10**.

Structure determination of the proanthocyanidins

The conversion to the anthocyanidins and the identification of the lower terminal flavan-3-ol unit by treatment with 0.1 M ethanolic hydrochloric acid, were carried out as previously reported [2].

Monomers The flavan-3-ols (C **1**, EC **2**, GC **3**, and EGC **4**), were obtained and identified by comparison with authentic samples [2,3].

Dimers The main dimers GC-(4α->8)-GC **5** and GC-(4α>8)-EGC **6** have already been described [2].

GC-(4α->8)-C **7:** conversion to the anthocyanidins afforded delphinidin and cyanidin; treatment with 0.1 M HCl liberated catechin from the lower unit. $[M + H]^+$ *m/z* 595; the IR and ^{13}C NMR spectra were identical with the data given in the literature [4].

GC-(4α->6)-GC **8**: conversion to the anthocyanidins afforded delphinidin and treatment with 0.1 M HCl liberated gallocatechin from the lower unit. $[M + H]^+$ *m/z* 611; the ^1H NMR spectrum of the acetate was superimposable on that given in the literature [5]. The ^{13}C NMR and IR data, are as follows: IR $\tilde{\upsilon}_{max}^{cm-1}$ 3600-3000, 1620, 1538, 1514, 1452,1345, 1239,1204,1146,1110, 1073,1033,828,723. ^{13}C NMR: 28.6-29.3(C-4F), 38-38.8(C-4C), 67.9-68.1 (C-3F),73.5-74(C-3C),82.2(C-2F),84.1(C-2C),96.3,97.2and97.7(C-6A,C-8A, C-8D)101.6C-4aD),107.6,108.2(C-2'B,E;C-6'B,E),110.6(C-6D),131(C-1'B,E), 133.5(C-4'B,E),146.1,146.2(C-3'B,E;C-5'B,E),154.3 to 158.3(C-5A,D;C-7A,D; C-8aA,D) ppm.

Trimers The major trimer, GC-(4α->8)-GC-(4α->8)-GC **10,** has already been described [2]. A second substance **9** showed a molecular ion *m/z* 898. Conversion to the anthocyanidins gave delphinidin and cyanidin. Treatment with 0.1 M hydrochloric acid liberated gallocatechin from the lower unit.

RESULTS AND DISCUSSION

Up to now, we have isolated ten proanthocyanidins from *R. nigrum*.

*Determination of the structure of the dimers **7** and **8** and of the trimer **9**.*

Compound **7** was the first substance to be eluted after the monomers. The $[M + 1]^+$ peak *m/z* 595 suggested a dimeric structure with mixed B-ring flavan units. This was confirmed by treatment with 1.4 M hydrochloric acid, which generated cyanidin and delphinidin. Degradation by treatment with 0.1 M hydrochloric acid yielded catechin from the lower or terminal unit. Examination of the ^{13}C NMR spectrum of **7** showed signals in the aromatic region at 114.8-116 ppm, corresponding to procyanidins and at 108 ppm corresponding to C-2' and C-6' of prodelphinidins.

The chemical shifts of C-2 and C-3 indicated that the configuration is 2*R*, 3*S*, 4*S* in the two units [3,6]. These data indicated structure **7,** the same

as the GC-(4α->8)-C previously isolated from *Salix caprea* [7], *Quercus dentata* [4], *Thuya occidentalis* [8], and recently from *Cistus incanus* [5]. The IR and ^{13}C NMR spectral data were identical with those previously reported [4].

Compound **8** was eluted on Sephadex LH 20 after **5** and **6.** It has the same mol. wt (610) as the known major dimers **5** and **6** [2]. Treatment with ethanolic 0.1 M hydrochloric acid liberated gallocatechin. The IR spectrum indicated two pyrogallol rings: two distinct bands at about 1520 and 1535 cm^{-1} and a single band near 730 cm^{-1}, thus different from the spectra of procyanidin models [9]. Examination of the aromatic region of the ^{13}C NMR spectrum revealed signals at 108 ppm, corresponding to C-2' and C-6' of two pyrogallol rings (B and E), and at 146 ppm, corresponding to C-5' and C-3' of the same rings. The chemical shifts of C-2 and C-3 indicated the configuration 2*R,* 3*S,* 4*S.* Accordingly , compound **8** is gallocatechin-(4α->6)-gallocatechin, previously isolated from *Cistus incanus* [5].

The mass spectral data and the results of the hydrolysis experiments for the trimer **9** indicated a mixed B-ring oxidation pattern and suggested possible identity with a proanthocyanidin recently found in *Croton lechleri* [3]. Moreover, the ^{13}C NMR spectral data were superimposable. Hence, the compound is probably C-(4α->8)-GC-(4α->8)-GC **9.**

In summary, the antiinflammatory activity of *R. nigrum* leaves is mainly due to proanthocyanidins and particularly to prodelphinidins which are the major oligomers in the proanthocyanidin fraction.

REFERENCES

[1] M. Tits, L. Angenot, J. Damas, Y. Dierckxsens and P. Poukens, *Planta Med.* **57** (Suppl. 2), A134 (1991).

[2] M. Tits, L. Angenot, P. Poukens, R. Warin and Y. Dierckxsens, *Phytochemistry* **31**, 971-973 (1992).

[3] Y. Cai, F. Evans, M. Roberts, J. Phillipson, M. Zenk and Y. Gleba, *Phytochemistry* **30**, 2033-2040 (1991).

[4] D. Sun, H. Wong and L. Foo, *Phytochemistry* **26**, 1825-1829 (1987).

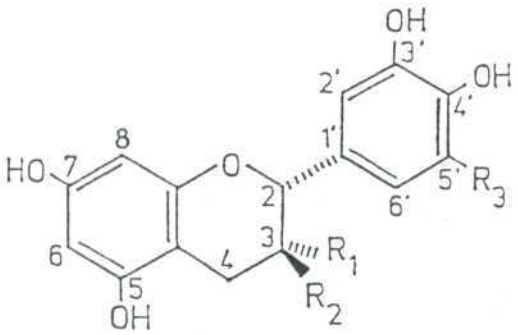
[5] F. Petereit, H. Kolodziej and A. Nahrstedt *Phytochemistry* **30**, 981-985 (1991).

[6] E. Haslam, *Phytochemistry* **16**, 1625-1640 (1977).

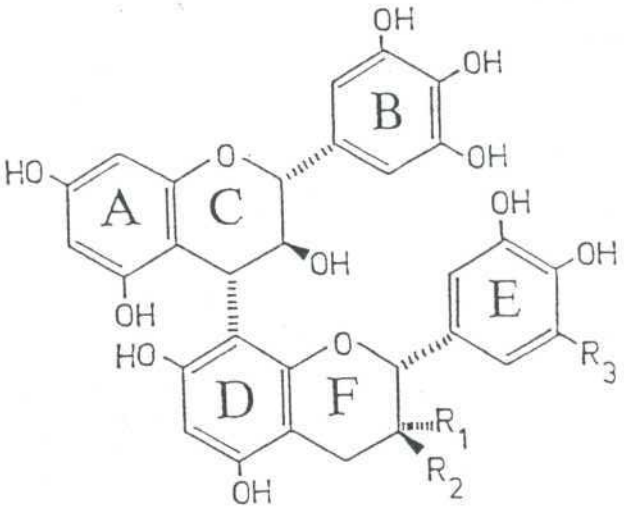
[7] L. Foo and L. Porter, *J. Chem. Soc. Perkin Trans* **I**, 1186-1190 (1978).

[8] L. Kopanski, E. Keese and G. Schnelle, *Planta Med.* **55**, 609 (1989).

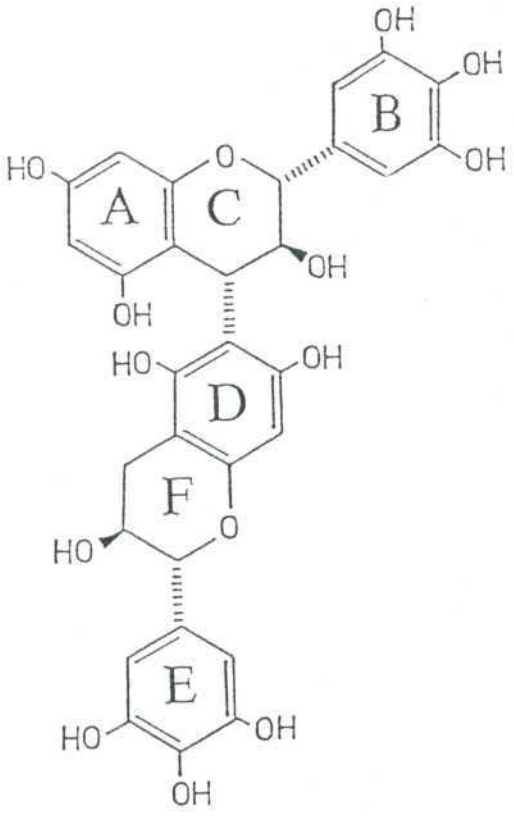
[9] L. Foo, *Phytochemistry,* **20**, 1397-1402 (1981).



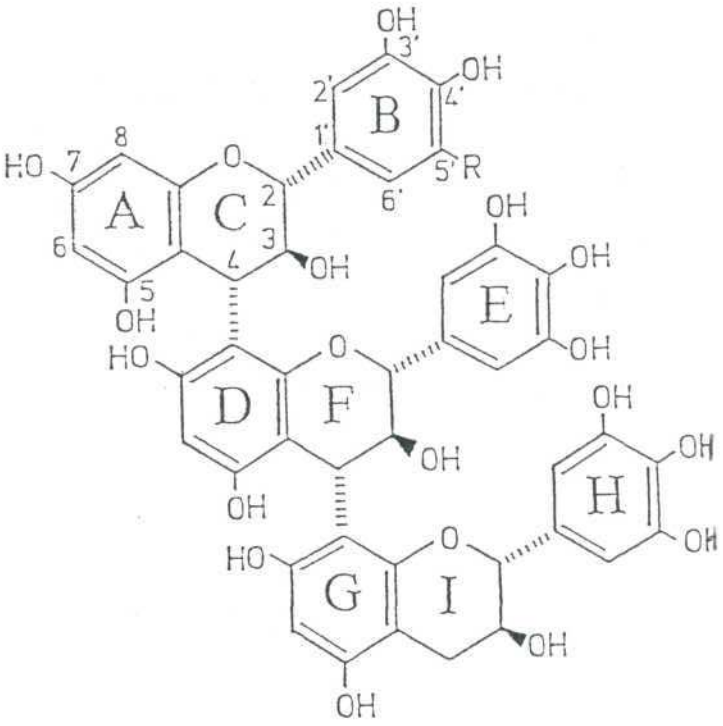
	R_1	R_2	R_3
1 C	H	OH	H
2 EC	OH	H	H
3 GC	H	OH	OH
4 EGC	OH	H	OH



	R_1	R_2	R_3
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