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# PROANTHOCYANIDINS: GROSS CHEMICAL STRUCTURES BY INFRARED SPECTRA

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**Key Word Index**—Proanthocyanidin polymers; procyanidin; prodelphinidin units; *cis–trans* configurations; infrared data; structures.

**Abstract**—IR studies of four proanthocyanidin dimers and all four 4-(2′,4′,6′-trihydroxyphenyl) derivatives of the flavan monomer units of proanthocyanidins revealed differences in certain vibrational frequencies which can be related to the structure of polymeric proanthocyanidins. The structural data derived from IR studies are fully consistent with the data obtained by other methods.

## INTRODUCTION

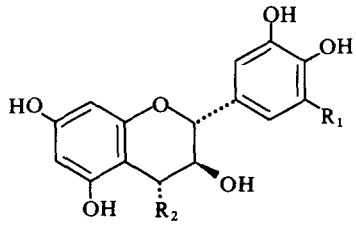
Although condensed tannins or proanthocyanidins have long been known, only recently have significant advances been made in the elucidation of their structures [1, 7]. In the majority of polymeric proanthocyanidins, the structural units are based on (+)-catechin (**1**), (−)-epicatechin (**2**), (+)-gallocatechin (**3**) and (−)-epigallocatechin (**4**). A complete chemical definition of the polymer would entail identifying and estimating these monomer units. The employment of IR spectroscopy towards this end is discussed in the light of what has been established in previous studies [8].

The choice of synthetic proanthocyanidins **5–8** rather than the unsubstituted flavans **1–4** as models for polymeric proanthocyanidins (**13**) was made mainly because their IR spectra are more akin to the spectra of the natural proanthocyanidins.

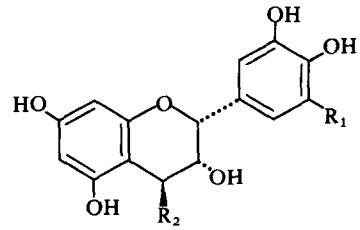
## RESULTS AND DISCUSSION

### *B-ring hydroxylation pattern*

*The* 1540–1520 $cm^{-1}$ *region.* The gallocatechins differ from the catechins in having an extra hydroxyl group in the B-ring and this distinction is seen in the 1540–1520 $cm^{-1}$ region, commonly attributed to the skeletal stretching modes of the aromatic ring. The spectra of the gallocatechin derivatives or the prodelphinidin compounds **7**, **8** and **11** exhibit two distinct



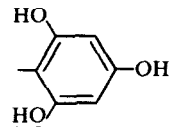
**1** $R_1 = R_2 = H$



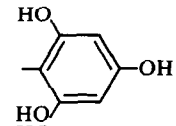
**2** $R_1 = R_2 = H$

**3** $R_1 = OH, R_2 = H$

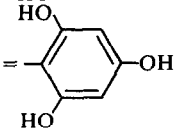
**4** $R_1 = OH, R_2 = H$



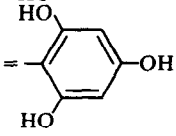
**5** $R_1 = H, R_2 =$



**6** $R_1 = H, R_2 =$



**7** $R_1 = OH, R_2 =$



**8** $R_1 = OH, R_2 =$

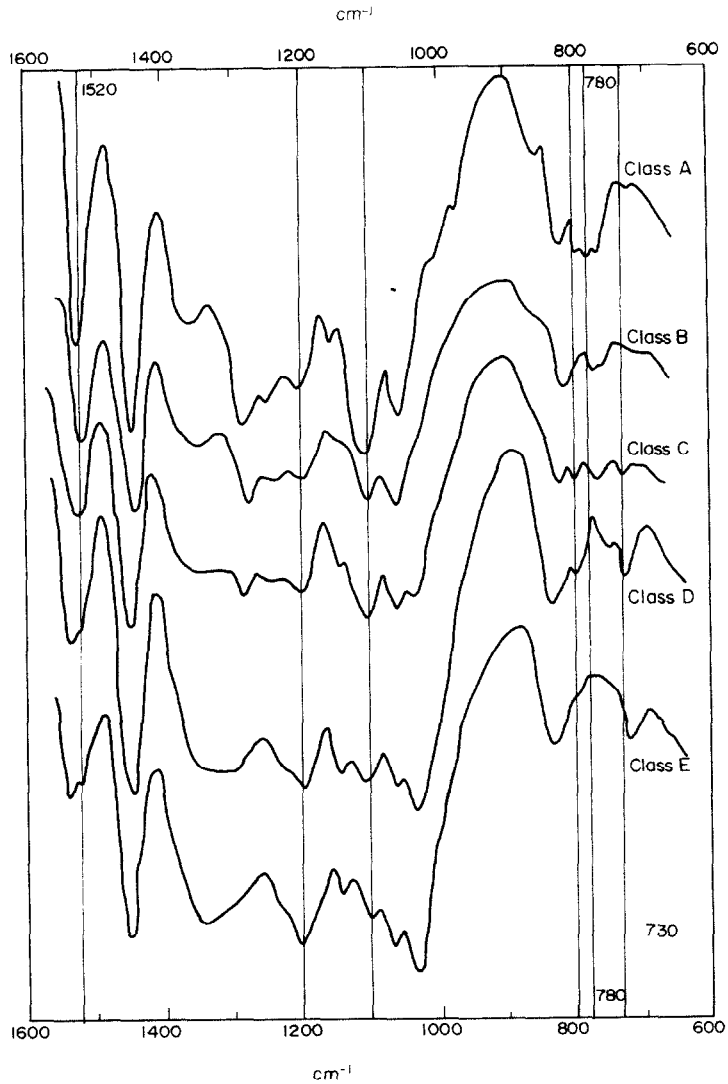
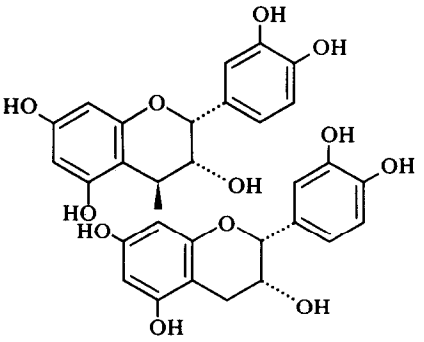



Fig. 1. IR spectra of proanthocyanidin polymers. Class A of *Cydonia oblonga*, class B of *Pinus radiata* (middle bark), class C of *Pinus radiata* (phloem), class D of *Ribes sanguineum* (fruit) and class E of *Ribes sanguineum* (leaf).

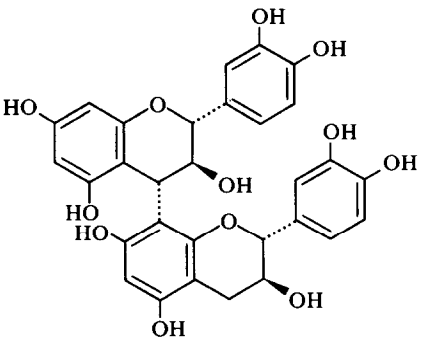
peaks at about 1520 and 1535 $cm^{-1}$, while only a single band at about 1520 $cm^{-1}$ was observed in the spectra of the catechin or the procyanidin models **5**, **6**, **9** and **10**.

The IR spectra of polymeric proanthocyanidins of known structures indicate that these distinguishing vibrational characteristics are equally applicable to the polymers. The predominantly prodelphinidin polymers [8] isolated from *Ribes nigra*, *R. sanguineum* and *Lotus pedunculatus* exhibit the characteristic doublet in their IR spectra (Fig. 1, classes D and E) while the predominantly procyanidin polymers, such as those isolated from *Vaccinium oxycoccos* and *Cydonia oblonga*, had only a single band in their spectra (see Fig. 1, classes A and B). Observations made on the spectra of 26 polymeric proanthocyanidins of known structures revealed that the characteristic double peak at about 1520 $cm^{-1}$ is only apparent in the spectra of proanthocyanidins containing a minimum of about 60 per cent of the prodelphinidin units. The spectra of polymers containing fewer prodelphinidin units, such as that from *Pinus radiata* phloem (which contains 48% prodelphinidin units) [8], show just one peak in this region (Fig. 1, class C). This is consistent with the observation that only a single band is present in the spectrum of the mixed dimer **12** (Fig. 2). However, the IR spectra of such mixed proanthocyanidins exhibit some degree of broadening in the band at about 1520 $cm^{-1}$.

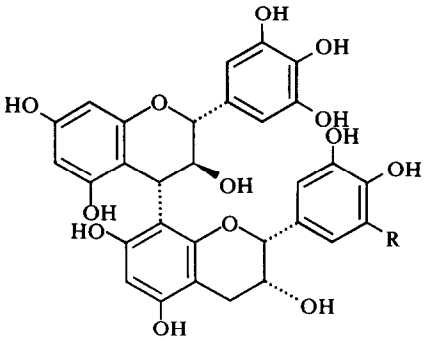
*The* 780–730 $cm^{-1}$ *region.* The B-ring hydroxylation pattern is also reflected in the 780–730 $cm^{-1}$ region where the out-of-plane deformations of the hydrogen atoms of the aromatic rings absorb. Since the deformation frequency is known to be determined by the position rather than the nature of the substituents [10], absorption bands in this region provide a means of distinguishing between procyanidin and prodelphinidin polymers. In the spectra of the procyanidin models **5**, **6**, **9** and **10** the more pronounced bands in this region occur at 780–770 $cm^{-1}$ while the spectra of the prodelphinidin models **7**, **8** and **11** show pronounced bands at a lower wave number near 730 $cm^{-1}$ (see Figs. 2 and 3). More importantly, in the spectrum of the mixed dimer where the upper unit is



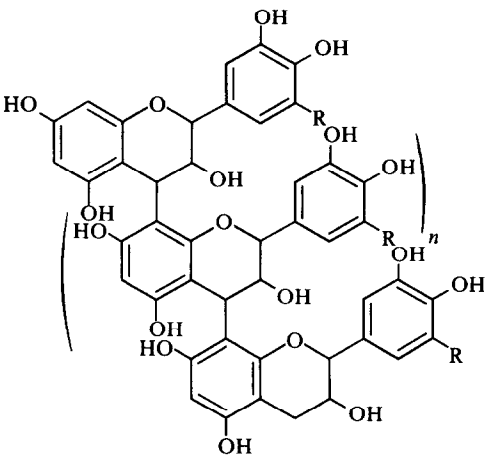
**9**



**10**



**11** R = OH
**12** R = H



**13**, where R = H or OH and $n$ = 1, 2, 3, ...

gallocatechin and the lower unit is epicatechin, the absorption bands at around 775 and 730 $cm^{-1}$ are of about the same order of intensity (Fig. 2, **12**). Although these bands are relatively weak, they nonetheless provide a convenient method of estimating the relative proportions of the catechin and the gallocatechin types in a polymer.

*The configuration of the flavan units*

Both the catechin and the gallocatechin units have in common the *trans* configuration at C-2 and C-3 in the heterocyclic ring while their epimers have the *cis* configuration. The absorption in the 800–795 $cm^{-1}$ region shows the nature of such configurations. In the spectra of models **6**, **8** and **9** where all the flavan units are of the *cis* configuration, there is an absorption band in the 800–795 $cm^{-1}$ region (see Figs. 2 and 3). In contrast no absorption is evident in the spectra of the *trans* compounds **5**, **7**, and **10** (see Figs. 2 and 3). In the spectra of the dimers **11** and **12** (Fig. 2) where both configurations are present, the 795 $cm^{-1}$ band is still distinct although considerably weaker. It is therefore possible, with some experience, to employ the spectrum to estimate the relative proportion of these two configurations in a polymer. The presence of neighbouring bands, particularly the procyanidin bands between 780 and 770 $cm^{-1}$, tends to obscure further this already weak band, but from observations made from the spectra of the 26 polymeric proanthocyanidins of known structures, it is possible to make the following generalization: where the 795–800 $cm^{-1}$ band is only observed as a shoulder, it may be assumed that the *cis*-monomer units in a polymer are less than about 30% (Fig. 1, class E); where the band is distinctive, although relatively weak, the polymers are made up of flavan units with approximately equal proportions of *cis*- and *trans*-type configurations (Fig. 1, class B); when the 795–800 $cm^{-1}$ band is about the same intensity as the procyanidin or prodelphinidin bands at 770 and 730 $cm^{-1}$ respectively (see Fig. 1 classes A, C and D) the flavans with the *cis* configuration form the bulk of the polymer.

The above discussion has been restricted to two regions between 1540 and 1530 $cm^{-1}$ and more particularly between 800 and 700 $cm^{-1}$ mainly because the differences in the vibrational frequencies in these regions are simply related to the structures of proanthocyanidins. The absorption patterns, particularly between 1300 and 1000 $cm^{-1}$, also show considerable differences and the transition from a wholly procyanidin polymer to the predominantly prodelphinidin type is very apparent in Fig. 1. The broad band at about 1350 $cm^{-1}$ and absorptions at 1200 and 1035 $cm^{-1}$ increase in intensity

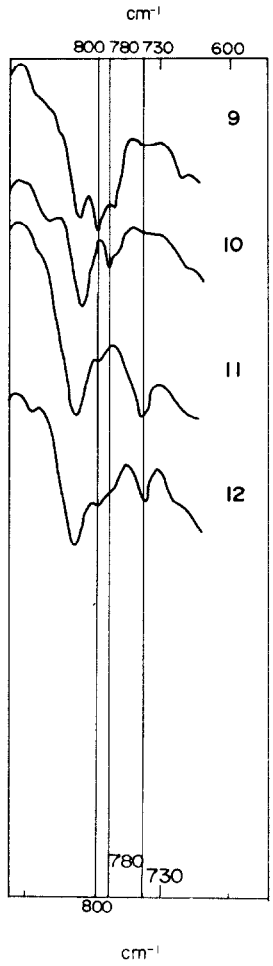



Fig. 2. IR spectra of proanthocyanidin dimers. **9**, Epicatechin-4,8-epicatechin; **10**, catechin-4,8-catechin; **11**, gallocatechin-4,8-epigallocatechin; **12**, gallocatechin-4,8-epicatechin.

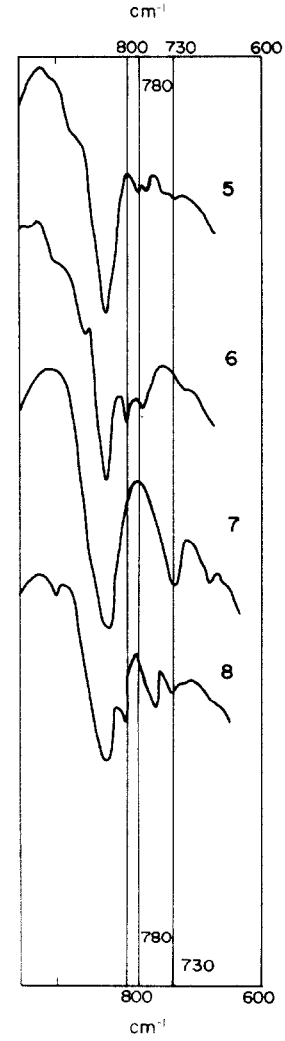



Fig. 3. IR spectra of 4-(2′,4′,6′-trihydroxyphenyl) derivatives of flavan-3-ols. **5**, 4-(2′,4′,6′-trihydroxyphenyl) catechin; **6**, 4-(2′,4′,6′-trihydroxyphenyl) epicatechin; **7**, 4 -(2′,4′,6′-trihydroxyphenyl) gallocatechin; **8**, 4-(2′,4′,6′-trihydroxyphenyl) epigallocatechin.

relative to the common 1430 $cm^{-1}$ band with increasing proportion of prodelphinidin units while the reverse is true for the 1100 $cm^{-1}$ band. Although these absorptions are more intense than the bands in the 800–700 $cm^{-1}$ region, their relative intensities are more difficult to estimate. They nevertheless provide valuable confirmation of the findings from other regions of the spectrum.

The 26 proanthocyanidin polymers identified in previous studies [8] may be conveniently grouped solely from their IR spectra into five classes A–E (see Fig. 1). Each class has the following features:

Class A: Polymer mainly of the procyanidin type with the monomers having the *cis* configuration (i.e. the monomer units being mainly of the epicatechin type only).

Class B: Polymer mainly of the procyanidin type with monomers having the *cis* and *trans* configurations (i.e. the monomer units being of about equal mixture of the catechin and epicatechin type).

Class C: Polymer of the mixed procyanidin and prodelphinidin type with the monomers having both the *cis* and *trans* configurations (i.e. the monomer units being mainly of epicatechin, epigallocatechin with significant proportion of catechin and gallocatechin types).

Class D: Polymer mainly of the prodelphinidin type with the monomers having the *cis* configuration (i.e. the monomer units being mainly of the epigallocatechin type).

Class E: Polymer mainly of the prodelphinidin type with the monomers having mainly the *trans* configuration (i.e. the monomer units being mainly of the gallocatechin type).

Table 1. Comparison of proanthocyanidin data derived by IR method with those obtained by other established methods [8, 9]

| Class type derived from IR spectra | Proanthocyanidin source | Data from other methods | |
|---|---|---|---|
| | | PC:PD* | *cis:trans* |
| A | *Vaccinium corymbosum* (unripe fruit) | 100:0 | 95:5 |
| (where PC > 70, PD < 30; | *Vaccinium oxycoccos* (unripe fruit) | 78:22 | 87:13 |
| *cis* > 70, *trans* < 30)† | *Betula alba* (catkins) | 84:16 | 82:18 |
| | *Aesculus carnea* (unripe fruit) | 100:0 | 93:7 |
| | *Chaenomeles sinensis* (unripe fruit) | 100:0 | 94:6 |
| | *Lotus corniculatus* (root) | 80:20 | 87:13 |
| | *Cotoneaster serotina* (unripe fruit) | 93:7 | 90:10 |
| | *Salix fragaria* (leaf) | 95:5 | 87:13 |
| | *Cydonia oblonga* (unripe fruit) | 100:0 | 95:5 |
| | *Aesculus hippocastanum* (unripe fruit) | 100:0 | 97:3 |
| | *Astinidia chinensis* (leaf) | 88:12 | 97:3 |
| | *Photinia glabrescens* var. *rubra* × *P. serrulata* | 100:0 | 97:3 |
| B | *Pinus radiata* (middle bark) | 90:10 | 41:59 |
| (where PC > 70, PD < 30; | *Salix caprea* (catkins) | 78:22 | 38:62 |
| *cis* ≃ *trans*) | | | |
| C | *Ribes grossularia* (unripe fruit) | 63:37 | 77:23 |
| (where PC ~ PD; | *Pinus radiata* (phloem) | 52:48 | 74:26 |
| *cis* > 70, *trans* < 30) | *Ribes nigra* (unripe fruit) | 40:60 | 74:26 |
| | *Grevillea robusta* (leaf) | 39:61 | 72:28 |
| D | *Ribes sanguineum* (fruit) | 23:77 | 72:28 |
| (where PC < 30, PD >70; | *Onobrychis viciifolia* (leaf) | 23:77 | 87:13 |
| *cis* > 70, *trans* < 30) | *Lotus pedunculatus* (leaf) | 20:80 | 80:20 |
| | *Ribes rubrum* (leaf) | 10:90 | 92:8 |
| | *Grevillea rosmarinifolia* (leaf) | 18:82 | 90:10 |
| | *Lotus pendunculatus* (root) | 23:77 | 73:27 |
| E | *Ribes nigra* (leaf) | 6:94 | 14:86 |
| (where PC < 30, PD >70; | *Ribes sanguineum* (leaf) | 10:90 | 12:88 |
| *cis* < 30, *trans* > 70) | | | |

* Procyanidin units, PD = prodelphinidin units.

† These are arbitrary figures. The value 70% or more is equated to being predominant while 30% or less is regarded as minor.

There are, however, slight variations between the spectra of some of the members of each class mainly because of smaller differences in the proportion of monomer units and perhaps also some unknown structural factors. The five classes satisfactorily account for all the proanthocyanidins isolated so far, but it is obvious that some polymers may fit into more than one of these classes. There are also obviously other classes possible yet to be described. The validity of using IR data to determine the gross chemical structure of polymeric proanthocyanidins is illustrated in Table 1. The structural features derived from the IR spectra of the polymers compare most favourably with structural data established by $^{13}C$ NMR and chemical degradation methods [8, 9].

The 4-(2′,4′,6′-trihydroxyphenyl) derivatives of the flavans and the proanthocyanidin dimers have in common a relatively intense band at about 1150 $cm^{-1}$ while the polymeric proanthocyanidins, in contrast, show insignificant absorption in this region. The significance of this difference is open to speculation, but aside from this absorption there is a remarkable similarity of the spectrum of the procyanidin dimer **9** with that of the procyanidin polymer (Fig. 1, class A) and of the prodelphinidin dimer **11** with the polymeric prodelphinidin isolated from the leaves of *Ribes sanguineum* (Fig. 1, class E). This observation is another direct confirmation of the exclusiveness of the poly-flavan-3-ol nature of polymeric proanthocyanidins.

## EXPERIMENTAL

The IR spectra were obtained with KBr pellets (1.5–2.0 mg sample in a 13 mm pellet) on a Perkin–Elmer Model 580 Infrared Spectrophotometer.

The identification of the proanthocyanidin dimers and the synthesis of the 4-(2′,4′,6′-trihydroxyphenyl) derivatives were carried out as previously described [11–13]. The purity of these compounds was checked by TLC on cellulose using 6% HOAc

and *n*-BuOH–HOAc–$H_2O$ (3:1:1) as solvents. The isolation and structural characterization of the proanthocyanidin polymers by $^{13}C$ NMR and chemical degradation methods were those described previously [8, 9].

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