Phytochemistry, Vol. 24, No. 3, pp. 567–569, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

ISOLATION OF THREE NATURALLY OCCURRING O-β-GLUCOPYRANOSIDES OF PROCYANIDIN POLYMERS

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(*Received* 29 *May* 1984)

Key Word Index—*Cydonia oblonga*; Rosaceae; ripe fruit; polymers; condensed tannins; *O*-β-glucopyranosides.

Abstract—Three *O*-β-glucosides of procyanidin polymers (condensed tannins) have been isolated from the ripe fruits of *Cydonia oblonga* (quince) and the barks of *Pinus brutia* and *Picea abies*.

Proanthocyanidins and the co-occurring polyhydroxyflavan-3-ols generally occur as free phenols in nature [1, 2], or with moderate frequency as 3-*O*-gallates and rarely as 7-*O*- [3] or 3,5-di-*O*- [1] gallates. There are now a number of examples known where flavan-3-ols are glycosylated. These include catechin 7-*O*-arabinoside from *Polypodium vulgare* [4, 5], catechin 7-*O*-β-xylopyranoside from the twig bark of *Ulmus americana* [6, 7], the 7-*O*-β-D-glucoside of epicatechin 3'-*O*-methyl ether from *Symplocos uniflora* [8], catechin 5-*O*-β-D-glucopyranoside from Chinese medicinal rhubarb and *ent*-catechin-7-*O*-β-D-glucopyranoside from *Rhaphiolepis umbellata* bark [9].

Although there have been a number of claims of isolation of glycosides of 'leucoanthocyanidins' of various types [10–13], none is supported by satisfactory proofs of structure. This communication reports the isolation of three procyanidin polymers containing *O*-β-D-glucopyranoside functions.

During a survey of the condensed tannins of edible fruits [14], it was noted that the ^{13}C NMR spectrum of the procyanidin polymers isolated from unripe and ripe quince fruits differed significantly. Whereas the polymer isolated from unripe fruit possessed the typical 20 MHz ^{13}C NMR spectrum associated with a structure consisting entirely of epicatechin-4 procyanidin units and an epicatechin terminal unit [15, 16], the polymer isolated from ripe fruits was observed to contain some extra signals. The same samples run at higher field (62.9 MHz) yielded spectra in which these signals were clearly resolved and which occurred between δ60 and 105, Fig. 1. The spectrum of the ripe quince polymers (Fig. 1b) shows that the signals are much sharper than normally associated with the bulk polymer signals, which are broadened due to chemical shift anisotropy [16].

The chemical shifts of the extra signals suggested that they were in positions expected for a hexose sugar derivative. This was confirmed by acid hydrolysis of the ripe quince polymer which gave glucose as the only product. Comparison of the observed chemical shifts with those of suitable model compounds (Table 1) showed that they were typical of a pyranoglucoside β-glycosidically linked to an aliphatic hydroxyl group, the position of C-1 being diagnostic. There is only one position on the polymer units consistent with this observation, the 3-hydroxyl of the heterocyclic ring (see structure **1**).

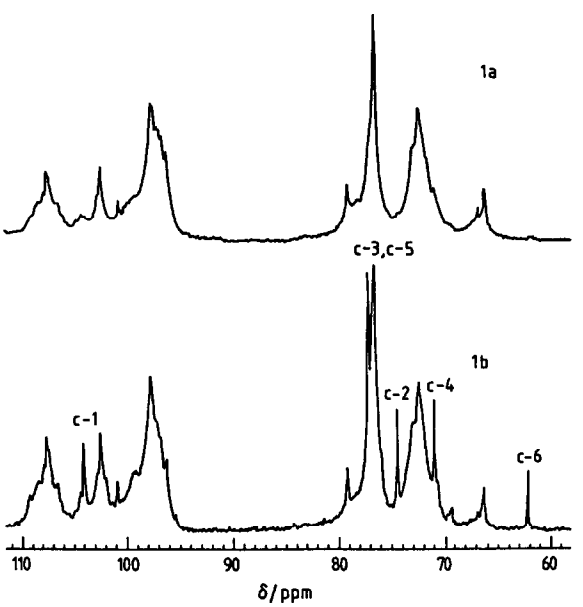


Fig. 1. ^{13}C NMR spectrum of the δ60–110 region of the procyanidin polymer from quince fruit. (a) Unripe. (b) Ripe.

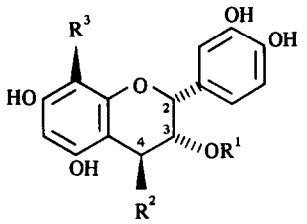
Reference to the ^{13}C NMR spectra of disaccharides [17] and diglycosides [18] shows that substitution of glucose linked through C-1 induces a downfield shift of δ9–10 on the methine carbon carrying the substituted hydroxy group, compared with the shift before glucosylation. In contrast, the shift on an aromatic oxygen is near zero. The ^{13}C NMR chemical shifts of C-3 of the epicatechin-4 procyanidin monomer units (**1**) are at δ72.4 [16] and the epicatechin chain-terminating groups (**2**) are at δ66.2 [16]. If either are glucosylated, signals would also be expected at δ81–82 and δ75–76, respectively. As no signals are observed at all in the δ81–82 region (see Fig. 1b) then it is likely that the glucosylpyranose function must reside exclusively on the epicatechin terminal unit in this polymer (**3**), the δ75–76 signal being masked by the large C-2 epicatechin signal at δ76.6. This interpretation is also

Table 1. Observed ^{13}C NMR chemical shifts for β-glucoside residues in tannins and model systems*

Carbon No.	Laminiaribiose [17]	Quince tannin	Phenolic linked†	Spruce tannin	*P. brutia* tannin
C-1	104.0	103.8	101.5	101.5	~102
C-2	74.6	74.3	73.0	74.1	74.5
C-3	76.7	77.1	76.3	77.0	76.8
C-4	70.7	70.9	69.4	70.7	70.8
C-5	76.7	77 1	76.7	77.0	76.8
C-6	~62	62.2	60.4	61.9	62.1

*Run at 62.9 MHz (quince) or 20 MHz (others), chemical shifts in δ-values.

†Average of glucose values for catechin 5-*O*-glucoside and *ent*-catechin 7-*O*-glucoside from Ref. [9]. Small differences are expected in the δ-values as the spectra in Ref. [9] were run in DMSO-d_6 + heavy water.



1 R^1 = H, R^2 = R^3 = epicatechin
2 R^1 = R^2 = H, R^3 = epicatechin
3 R^1 = Glc, R^2 = H, R^3 = epicatechin

supported by the fact that the glucose signals are very narrow, implying a homogeneous chemical environment, which would not be the case if the sugar resided on the epicatechin-4 units.

In contrast, the glucosylpyranose moieties must be attached to phenolic hydroxyl functions in the *Pinus brutia* and *Picea abies* tannins. This is shown by the chemical shift of C-1 being at δ101–102, characteristic of a sugar attached to an oxygen on an aromatic ring [18]. Further support for this view came from the fact that the sugar signals were much broader in the bark tannins than those observed for quince, which implies that they are attached to a more diverse range of chemical environments.

The number of sugar residues attached to the flavanoid groups on the bark tannins were also much greater than for the quince tannin. Relative signal intensities in the ^{13}C NMR spectra implied at least one sugar residue per flavanoid unit for these tannins. This was also supported by estimates of flavanoid unit concentrations based as the vanillin–hydrochloric acid method [15]. Typical $E^{1\%}_{500}$ values for procyanidin polymers similar to these are *ca* 260 [15], whereas the measured values for the *Pinus brutia* and *Picea abies* tannins were 164 and 183, respectively. These imply that the proportion of flavanoid units on a weight basis was 63% and 70%, respectively, for the two polymers. The MWs of the glucosyl and flavanoid units are 162 and 288, respectively, from which it may be calculated that the ratio of glucosyl to flavanoid units is 1:0.96 for the *P. brutia* and 1:1.27 for the spruce polymer, respectively—or close to one sugar unit per flavanoid unit.

Attempts to elucidate the points of glucosylation on the flavanoid units by mild acid hydrolysis in the presence of phloroglucinol or phenylmethanethiol [15] failed because of the similar lability of the glucosidic and interflavanoid bonds.

The current study shows that not only are flavan-3-ols, but also proanthocyanidin polymers, glycosylated. The current observations also support the view that the occurrence of glycosylation in these natural products may simply be associated with a co-incidental high concentration of UDP-glucose and transferase enzyme in the plant tissue. Thus, the glucosylated procyanidin only appears in quince fruits on ripening when the general sugar level has risen, and, at least in the case of spruce bark, the procyanidins are associated with high concentrations of stilbene glucosides [19, 20]. These observations may also possibly explain the inextractability which has been commonly observed for certain tannins [21]. While these may in part be explained by high M_r or binding of tannin to cell wall polysaccharides and protein, it is also possible that condensed tannins are glycosidically bound to cell wall hemicelluloses, in a similar way to that suggested for lignin [22].

EXPERIMENTAL

^{13}C NMR spectra were recorded at 20 or 62.9 MHz in $[^2H_6]Me_2CO$–H_2O (1:1) at 30° using an external TMS reference with correction for solvent magnetic susceptibility. The procyanidin polymers were extracted and purified as described previously [15]. Analysis of the tannins by the vanillin–HCl method was also performed as described previously [15].

Sugar analyses were performed by hydrolysis of the tannins in 1 M CF_3COOH under reflux for 4 hr. The soln was evaporated to dryness and the sugars converted to their TMS ethers; and the derivatized material analysed on a 2 m × 4 mm i.d. packed column with SE-52 liquid phase, programmed at 100–250°, 8°/min.

Conversion of the polymer to anthocyanidins and analysis as described previously [15] showed that all proanthocyanidins produced only cyanidin.

The tannins were subjected to acidic cleavage in HCl in the presence of phloroglucinol or HOAc in the presence of phenylmethanethiol and the progress of the reactions were monitored by 2D-TLC on cellulose using TBA and 6% HOAc as solvents [15]. The pattern of products was consistent with the quince tannin being composed of epicatechin-4 procyanidin and epicatechin terminal units, whereas the products from *P. brutia* or *P. abies* were consistent with polymers being composed of epicatechin-4 (the major unit in both cases) and catechin-4 procyanidin units, together with catechin terminal units. No other vanillin reactive products were detected in significant concentration.

The polymers had the following $[\alpha]_{578}$ values, all measured at *c* 0.2–0.5 and in MeOH–H_2O (1:1). Quince tannin (unripe), +131°; quince tannin (ripe), +125°; *Pinus brutia* tannin, −19.1°; spruce tannin, +38.0°.

Acknowledgements—To Dr. S. R. Johns and Mr. R. I. Willing of the Division of Applied Organic Chemistry, CSIRO, Melbourne, for the high field NMR spectrum of the quince polymers. To Dr. C. Ayla, Institut für Holzchemie und Chemische Technologie des Holzes, Hamburg-Reinbek, West Germany for

the sample of *Pinus brutia* bark and to Dr. R. Marutzky, Wilhelm-Klauditz Institut, Braunschweig, West Germany for the sample of spruce bark.

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