

PROANTHOCYANIDINS OF BARLEY AND SORGHUM; COMPOSITION AS A FUNCTION OF MATURITY OF BARLEY EARS

MICHAEL J. BRANDON, LAI YEAP FOO, LAWRENCE J. PORTER and PETER MEREDITH*

Chemistry Division; *Wheat Research Institute, Department of Scientific and Industrial Research, New Zealand

(Received 30 April 1982)

Key Word Index—*Sorghum vulgare*; *Hordeum vulgare*; Gramineae; procyanidin; prodelphinidin; oligomers; barley maturation; condensed tannins.

Abstract—*Sorghum vulgare* seeds contain a proanthocyanidin polymer consisting largely of 2,3-*cis* procyanidin units with M_n 2500. *Hordeum vulgare* ears contain low levels of proanthocyanidin oligomers containing 2–4 units, and composed largely of 2,3-*trans* procyanidin and prodelphinidin units with catechin as the terminal unit. The concentration of the oligomers in barley ears was virtually constant throughout the 33 day growth and ripening period.

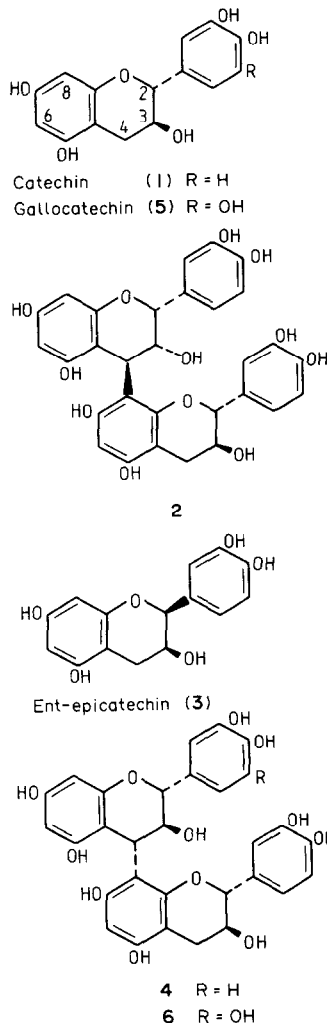
INTRODUCTION

Barley and sorghum are the only two important food grains reported to contain condensed tannins or proanthocyanidins [1]. The poor nutritional quality of some sorghum hybrids has been attributed to the presence of these compounds [1, 2], due principally to their ability to deactivate enzymes [3]. Oligomeric proanthocyanidins are considered to affect flavour and cause colloidal (chill) haze in beer [4]. Their presence, however, may not be completely undesirable; it has been suggested that they impart a certain resistance to the depredation by birds of crops [2, 5–7]. The relation between tannin levels and sorghum maturity has been studied by a number of workers [1, 8–10]. This paper reports a similar study for barley and gives details of the structures of the proanthocyanidins of both sorghum and barley.

RESULTS AND DISCUSSION

Nomenclature

A new system of proanthocyanidin nomenclature has recently been developed [11] and will be used throughout this paper. Proanthocyanidin oligomers are named in a manner analogous to the carbohydrates, in that the C-4 of the flavan unit is considered to be analogous to the C-1 anomeric sugar carbon. The configuration of the interflavanoid linkage C-4–C-8 (or C-6) is denoted by the α , β -system [12], presupposing also that the flavanoid ring system is drawn in the usual way. The basic structural units are named in terms of the familiar flavan-3-ols. The names catechin, epicatechin, galocatechin, etc. are reserved for those units with a 2*R* configuration. Those flavan-3-ol units having a 2*S* configuration are distinguished by the *enantio*-prefix [12]. Thus (+)-epicatechin (3) is now *ent*-epicatechin, and in the older system compound 2 was procyanidin B1, and compound 4 procyanidin B3.



Sorghum tannins

Proanthocyanidins of sorghum were the subject of a previous detailed study by Gupta and Haslam [1]. They isolated catechin (1) and epicatechin-(4 β →8)-catechin (2) from the ethyl acetate-soluble fraction and reported that a procyanidin (PC) polymer consisting of 2,3-*cis* PC units and a catechin (1) terminal unit was isolated in 0.8–5.0% yield.

Similarly we isolated a proanthocyanidin polymer from a high tannin variety of *Sorghum* (cv Sudax). The polymer structure was generally similar to that reported by Haslam and Gupta [1] in that the majority of proanthocyanidin units were 2,3-*cis* PC, with catechin (1) as the terminal unit. However, both ¹³C NMR and optical rotation measurements [14] showed that 20% of the units had 2,3-*trans* stereochemistry. Additionally, a small proportion of the units (8%) possessed a prodelphinidin (PD) oxidation pattern. Therefore, the structure of the sorghum tannin was somewhat more heterogeneous than implied from the earlier [1] results, and the observation that PD units are present is new, as PC units only were previously detected [1, 13].

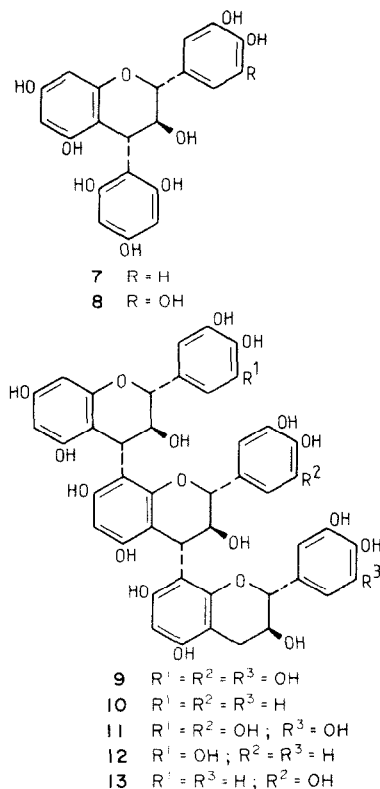
The sample of tannin isolated from Sudax had a number-average MW, \bar{M}_n of 2800 (estimated by GPC) and 2500 (estimated by ¹³C NMR [14]) and a weight average MW, \bar{M}_w of 4900 (estimated by GPC) [15]. The MW of the Sudax tannin is therefore somewhat higher than that isolated from the NK300 variety by Gupta and Haslam [1] who estimated a value of \bar{M}_n of 1700–2000 by chemical degradation.

Barley tannins

Ears of barley (cv Gwylan) were collected at regular intervals during their 33 day development and ripening period. Smaller scale preliminary examination of the lower MW polyphenols (ethyl acetate-soluble fractions) and oligomeric proanthocyanidin fractions by 2D cellulose TLC and HPLC indicated that the flavanoids in the earlier green samples, where chlorophyll was still present, possessed a similar composition. The pattern was markedly different in the mature ears. Three distinctly green and two distinctly mature samples were therefore grouped into two contrasted samples for detailed chemical study.

Monomers and dimers. Combined ethyl acetate extracts, containing the lower MW flavans, were fractionated on Sephadex LH-20 using ethanol as eluant [16]. Catechin (1) was eluted first, followed by galocatechin (5). The proportion of 1–5 was *ca* 6:1. Further elution gave a PC dimer 4 followed by a PD dimer 6.

Degradation of the PC dimer with phloroglucinol under acidic conditions [14] gave catechin-(4 α →2)-phloroglucinol (7) and catechin (1). Chromatographic comparisons with authentic material and ¹³C NMR of the dimer confirmed its structure as catechin-(4 α →8)-catechin. The authentic material had previously been isolated from *Pinus radiata* bark [17] and also synthesized by reduction of taxifolin and reaction of the borate complex with catechin in acid solution [18]. The PD dimer 6 was degraded similarly to yield catechin (1) and galocatechin-(4 α →2)-phloroglucinol (8), and the latter structure confirmed by comparison with authentic material prepared previously [14]. The



constitution of the PD dimer is therefore galocatechin-(4 α →8)-catechin (6) and this was confirmed by the ¹³C NMR spectrum. The dimer 6 is identical with the prodelphinidin previously isolated as the peracetate derivative from *Salix caprea* catkins [19].

The isolation of dimer 4 has been reported from beer [20] and from barley [21]. The dimer 6 was recently reported as a constituent of European barley [12].

The HPLC chromatograms of the ethyl acetate extracts are shown in Fig. 1. The most significant difference between the 'green' and 'mature' samples

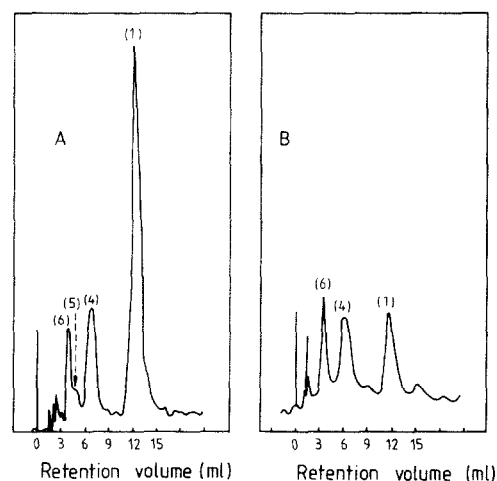


Fig. 1. HPLC chromatograms of the ethyl acetate extracts of barley. A, 'Green' sample; B, 'mature' sample. Numbers in parentheses refer to flavanoid structures in the text.

Table 1. Composition of polyflavanoids of green and mature barley

	1980 sample	1981	
		'Green'	'Mature'
(a) Ethyl acetate fractions	—	0.06	0.02
Yield (% dry wt)			
Relative yield of vanillin-reactive compounds	—	1.5	1.0
Catechin*	—	41	8
Gallocatechin (5) and prodelphinidin 6*	—	10	10
Procyanidin 4*	—	12	8
(b) Oligomer fractions			
Yield (% dry wt)	0.05	0.065	0.05
$[\alpha]_{578}^{30}$ (MeOH-H ₂ O, 1:1 v/v)	-259	-289	-306
\bar{M}_n	1600	970	1000
\bar{M}_w	3100	1100	1200
PD-PC	60:40	53:47	63:37

*In arbitrary units, based on peak heights of HPLC chromatograms monitored at 280 nm. The concentration of compounds with a 3,4,5-trihydroxyphenyl B-ring will be underestimated as they have UV maxima centred on 273 nm and a lower extinction than those with a 3,4-dihydroxyphenyl B-ring.

is the relatively high concentration of catechin (1) in the former sample. Similar observations have been reported for sorghum where there was rapid synthesis of catechin (1) in the green state, followed by a rapid decline in concentration on ripening [1]. The yield of the ethyl acetate extract in the 'green' sample was considerably higher than in the 'mature' sample (Table 1), probably accounted for by catechin (1).

Oligomeric proanthocyanidins. Samples of oligomers were extracted and purified as described previously [14] from freeze-dried barley. The concentration of the oligomeric fraction (0.002–0.06% w/w) was only *ca* 10% that of the tannins in sorghum.

The oligomers were obtained in similar yield from mature ears of Gwylan barley in two consecutive years. Although the ratio of the PD-PC units was similar in both samples, the detailed structure of the proanthocyanidins was markedly different. The 1980 season proanthocyanidins contained a significant proportion (12%) of 2,3-*cis* units (determined by ¹³C NMR [14]) and had a relatively wide MW range, with an average of 5–6 units in the oligomer chain. By contrast the sample from the 1981 season contained mainly trimers (as indicated by the similar values of \bar{M}_n and \bar{M}_w , Table 1) and was entirely 2,3-*trans* proanthocyanidin units (as indicated by ¹³C NMR [14]).

Reversed-phase HPLC revealed (Fig. 2) that the 1981 sample trimers included at least five compounds. Preparative HPLC enabled isolation of small samples of the most (a) and least (c) mobile components. The major trimer components, fraction (b), were only obtained as a mixture.

HPLC fractions were further studied by degradation with phloroglucinol and hydrochloric acid [19]. Fraction (a) yielded gallocatechin-(4 α →2)-phloroglucinol (8) and gallocatechin (5), whereas fraction (c) yielded catechin-(4 α →2)-phloroglucinol (7) and

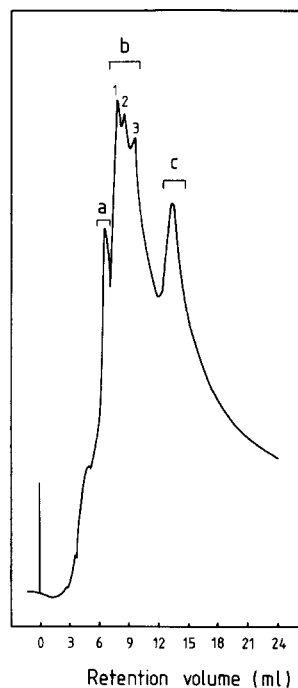


Fig. 2. HPLC chromatogram of the oligomeric fraction from 'green' barley. *a* = 9; *b*₁ = 11; *b*₂ = 12; *b*₃ = 13; *c* = 10.

catechin (1). These results indicated that the likely major component of fraction (a) was gallocatechin-(4 α →8)-gallocatechin-(4 α →8)-gallocatechin (9) and that of fraction (c) catechin-(4 α →8)-catechin-(4 α →8)-catechin (10). The latter component has been established as a component of European grown barley [21].

Degradation of fraction (b) yielded catechin (1), catechin-(4 α →2)-phloroglucinol (7) and gallocatechin-(4 α →2)-phloroglucinol (8), the latter being formed in significantly higher yield than 7. Considering that all three components of fraction (b) are likely to be trimers, and that prodelphinidins are more mobile than procyanidins on reversed-phase HPLC [Foo, L. Y., Hemingway, R. W. and Porter, L. J., unpublished results], the results lead us to suggest that these three proanthocyanidins, in order of decreasing mobility, are gallocatechin-(4 α →8)-galocatechin-(4 α →8)-catechin (11), gallocatechin-(4 α →8)-catechin-(4 α →8)-catechin (12), and catechin-(4 α →8)-gallocatechin-(4 α →8)-catechin (13).

Changes associated with maturation. The main difference between earlier and later samples is the gradual shift in the relative amount of the procyanidin trimer 10. This is present in relatively higher concentration in the earlier sample but gradually decreases in concentration to become the minor oligomeric component in mature barley. This is consistent with the somewhat higher concentration of PD units in the bulked 'mature' sample, compared with the 'green' sample (Table 1).

It is also evident that the concentration of proanthocyanidin constituents remains about constant in barley throughout the growth season and there is little change in MW. The higher concentration of ethyl acetate extractable constituents earlier in the season is largely accounted for by the variation in the absolute concentration of catechin (1). There is little evidence, therefore, in the case of barley, for the postulated increase in MW of proanthocyanidins on tissue maturation [22].

Proanthocyanidin oligomers containing propylar-gonidin units were earlier noted to be present in European strains of malting barley [2, 23]. We found no evidence of constituents containing propylar-gonidin units in our material.

EXPERIMENTAL

Analytical separations by 2D-TLC were performed on Schleicher and Schull F1400 cellulose using solvent A, *t*-BuOH-HOAc-H₂O (3:1:1) followed by solvent B, HOAc-H₂O (6:94). Spots were visualised by vanillin-HCl spray. Prep. and analytical HPLC were performed on a Spectra-Physics instrument with a C-18 reverse phase column using MeOH-HOAc-H₂O (20:1:79) as the eluting solvent operating at a flow rate of 2.0 ml/min. Components were detected using a Cecil UV variable wavelength detector set at 280 nm for analytical study and 260 nm for preparative work. NMR measurements were performed on a Varian FT-80A instrument. The number average MW's (\bar{M}_n) of the acetates were obtained by GPC on a μ Styragel 10³ Å column, THF solvent, and using flavanoid and polystyrene standards.

Barley ears (cv Gwylan) were collected from one plot at 3–5 day intervals during the harvest season from 8 January to 9 February 1981. The samples were freeze-dried, threshed and ground-up, and stored frozen until required. For preparative purposes, the samples of 8, 12 and 15 January were combined for the 'green' sample and the samples of 2 and 9 February were combined as the 'mature' sample. These two bulked samples were extracted for both the lower MW and oligomeric flavans as described previously [14, 15]. The sorghum grain (cv Sudax) was obtained from a single experimental plot. A 1 kg dry-milled sample was extracted

as for the barley samples to yield 0.5% of proanthocyanidin polymer.

Anthocyanidin estimation. A sample of proanthocyanidin material (1–5 mg) was treated with 5% HCl in *tert*-BuOH (1.0 ml) at 100° for 2 hr. The resulting anthocyanidins were separated by PC, using Forestal solvent and estimated colorimetrically.

Vanillin-HCl estimation of flavans. This was carried out on the barley samples or barley extracts by the method of Broadhurst and Jones [24].

Lower MW flavans. Combined EtOAc extracts (1.65 g) of the barley were chromatographed over Sephadex LH-20 with EtOH, the eluant being collected in 20 ml fractions and monitored by TLC on cellulose using solvent (B).

Catechin (1). Obtained from fractions 41–53 (300 mg), R_F (A) 0.65; R_F (B) 0.50.

Galocatechin (5). Obtained from fractions 54–63 (50 mg), R_F (A) 0.50; R_F (B) 0.45.

The identities of 1 and 5 were confirmed by co-chromatography on TLC and HPLC with authentic material.

Catechin-(4 α →8)-catechin (4). Obtained from fractions 75–120 (140 mg), R_F (A) 0.40; R_F (B) 0.50. ¹³C NMR (in Me₂CO-*d*₆-H₂O, 1:1 v/v): δ 28.6 (C-4'), 38.3 (C-4), 68.6 (C-3'), 73.9 (C-3), 81.9 (C-2'), 83.7 (C-2), 96.9–98.1 (C-6, C-6', C-8), 102.4 (C-4a'), 107.5 (C-4a), 109.0 (C-8'), 115.9–116.9 (C-2, C-5 of B rings), 120.2, 121.2 (C-6 of B rings), 131.9, 132.5 (C-1 of B rings), 145.4 145.9 (C-3, C-4 of B rings), 155–158.7 (C-5, C-7, C-5', C-7').

Galocatechin-(4 α →8)-catechin (6). Obtained from fractions 121–200 (170 mg), R_F (A) 0.25; R_F (B) 0.50. ¹³C NMR (in Me₂CO-*d*₆-H₂O, 1:1): δ 28.3 (C-4'), 38.3 (C-4), 68.5 (C-3'), 73.6 (C-3), 81.3 (C-2'), 83.9 (C-2), 96.3–97.6 (C-6, C-6', C-8), 101.8 (C-4a'), 107.4 (C-4a), 109 (C-8' and C-2, C-6 of the pyrogallol B ring), 115.4–116.7 (C-2, C-5 of the catechol B ring), 119.9 (C-6 of the catechol B ring), 131.7 (C-1 of both pyrogallol and catechol B rings), 145–146.4 (C-3, C-5 of the pyrogallol B ring, C-3 of the catechol B ring), 154–158 (C5, C-5', C-7, C-7').

The identities of 4 and 6 were confirmed by co-chromatography on TLC and HPLC with authentic material [17–19] and acid degradation in EtOH with phloroglucinol.

Acknowledgement—We thank Mr. S. J. McCormick, Ruakura Agricultural Research Centre, M. A. F., for the sample of sorghum.

REFERENCES

1. Gupta, R. K. and Haslam, E. (1978) *J. Chem. Soc. Perkin Trans 1*, 892.
2. Price, M. L., Stromberg, A. M. and Butler, L. G. (1979) *J. Agric. Food Chem.* **27**, 1270.
3. Goldstein, J. L. and Swain, T. (1965) *Phytochemistry* **4**, 185.
4. Jerumanis, J. (1979) *Proceedings European Brewery Convention* 309.
5. Bullard, R. W., York, J. O. and Kilburn, S. R. (1981) *J. Agric. Food Chem.* **29**, 973.
6. McMillian, W. W., Wiseman, B. R., Burns, R. E., Harris, H. B. and Greene, G. L. (1972) *Agron. J.* **64**, 821.
7. Tipton, K. W., Floyd, E. H., Marshall, J. G. and McDewitt, J. B. (1970) *Agron. J.* **62**, 211.
8. Glennie, C. W. (1981), *J. Agric. Food Chem.* **29**, 33.
9. Glennie, C. W., Kaluza, W. Z. and van Niekerk, P. J. (1981) *J. Agric. Food Chem.* **29**, 965.
10. Price, M. L., Stromberg, A. M. and Butler, L. G. (1979) *J. Agric. Food Chem.* **27**, 1270.

11. Hemingway, R. W., Foo, L. Y. and Porter, L. J. (1982) *J. Chem. Soc. Perkin Trans. 1*, 1209.
12. I. U. P. A. C. (1979) *Nomenclature of Organic Chemistry* Section F. Pergamon Press, Oxford.
13. Strumeyer, D. H. and Malin, M. J. (1975) *J. Agric. Food Chem.* **23**, 909.
14. Czochanska, Z., Foo, L. Y., Newman, R. H. and Porter, L. J. (1980) *J. Chem. Soc. Perkin Trans. 1*, 2278.
15. Foo, L. Y., Jones, W. T., Porter, L. J. and Williams, V. M. (1982) *Phytochemistry* **21**, 933.
16. Thompson, R. S., Jacques, D., Haslam, E. and Tanner, R. J. N. (1972) *J. Chem. Soc. Perkin Trans. 1*, 1387.
17. Porter, L. J. (1974) *N. Z. J. Sci.* **17**, 213.
18. Botha, J. J., Ferreira, D. and Roux, D. G. (1981) *J. Chem. Soc. Perkin Trans. 1*, 1235.
19. Foo, L. Y. and Porter, L. J. (1978) *J. Chem. Soc. Perkin Trans. 1*, 1186.
20. Eastmond, R. (1975) *J. Inst. Brew. (London)* **80**, 188.
21. Mulkay, P., Touillaux, R. and Jerumanis, J. (1981) *J. Chromatogr.* **208**, 419.
22. Goldstein, J. L. and Swain, T. (1963) *Phytochemistry* **2**, 371.
23. Silbereisen, K. and Krafczyk (1967) *Monatsschr. Brau.* **20**, 217.
24. Broadhurst, R. B. and Jones, W. T. (1978) *J. Sci. Food Agric.* **29**, 788.