Phytochemistry, Vol. 23, No. 12, pp. 2915–2918, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

CONDENSED TANNINS: CO-OCCURRENCE OF PROCYANIDINS, PRODELPHINIDINS AND PROFISETINIDINS IN THE HEARTWOOD OF *ACACIA BAILEYANA*

LAI YEAP FOO

Chemistry Division, Department of Scientific and Industrial Research, Petone, New Zealand

(*Received* 22 *May* 1984)

Key Word Index—*Acacia baileyana* var. *purpurea*; Leguminosae; mollisacacidin; fisetinidol(4α → 8)catechin; procyanidins; prodelphinidins; profisetinidins.

Abstract—The flavonoids and condensed tannins of the heartwood of *Acacia baileyana* var. *purpurea* are described. In conformity with other *Acacia* species, the hydroxylation pattern of the flavonoids is of the resorcinol type but, in sharp contrast, the tannins are heterogeneous consisting of a mixture of the resorcinol and phloroglucinol series. Dimeric proanthocyanidins of the phloroglucinol type were absent and this exception to the general observation that they invariably co-occur with the polymers may be explained by the relative nucleophilicity of the aromatic A-rings.

INTRODUCTION

The outstanding feature of the flavonoids of the *Acacia* species is the characteristic 5-deoxy or resorcinol-type hydroxylation pattern in the A-ring [1, 2] as opposed to the common 5-oxy or phloroglucinol type in most species. The condensed tannins or proanthocyanidins are closely related to these flavonoids in the aromatic ring oxidation pattern, hence the tannins with the resorcinol type of A-ring, such as the profisetinidins and prorobinetinidins, are mostly confined to the genus *Acacia*, while the phloroglucinol analogues such as the procyanidins, prodelphinidins and propelargonidins are much more widely distributed.

Acacia baileyana var. *purpurea* is a popular garden plant cultivated especially for its attractive feathery leaves and for its flamboyant display of yellow blooms. It is a fast-growing tree and the nature of its tannins is of interest because of its close relationship to the commercially important species *A. mearnsii* [3]. This paper describes the nature of the proanthocyanidins in the heartwood of this species in relation to the simpler flavonoids that exist with them.

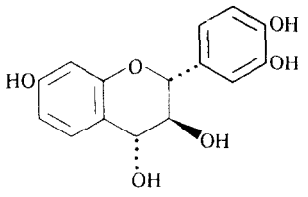
RESULTS AND DISCUSSION

Low MW polyphenols

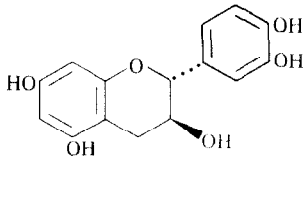
The flavonoids identified on two-dimensional paper chromatography [2] of an ether extract of the heartwood of *A. baileyana* were entirely consistent with earlier studies [1, 2] and confirmed the close association between *A. baileyana* and *A. mearnsii* [1, 3]. The resorcinol-type hydroxylation pattern of the aromatic A-ring was demonstrated by the presence of the flavonol fisetin, the dihydroflavonol fustin, the flavanone butin and the related chalcone butein together with the flavan-3,4-diol mollisacacidin (**1**). These phenolic constituents are all related chemically to one another and this, together with mollisacacidin being observed as the only constituent present in the metabolically active sapwood in *A. mearnsii*, has led to the suggestion that these phenolic precursors are all derived from the flavandiol by post-enzymatic chemical interconversion [4].

Mollisacacidin and fustin were the major constituents in the ethyl acetate extract of the heartwood and both of these were isolated by repeated chromatography over Sephadex LH20 using ethanol as the first solvent and ethanol–water (1:1) as the second. Catechin (**2**), which has the phloroglucinol or 2,4,6-trihydroxylated pattern in the A-ring, was also isolated in a low yield in the same chromatographic fraction as fustin but was separated from the latter compound by subsequent chromatography using the ethanol–water solvent. The flavanone, butin, which was chromatographically less mobile than the above three compounds, was also obtained in a small yield. All compounds were identified by their chromatographic behaviour and their identities were confirmed by ^{13}C NMR spectroscopy.

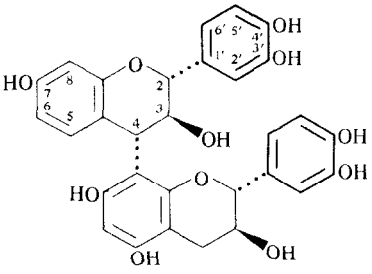
Unlike the procyanidin dimers and trimers which were separable by repeated chromatography as described above, the profisetinidins were not as amenable to this technique. The only profisetinidin dimer isolated as the free phenol was fisetinidol(4α → 8)catechin (**3**). The profisetinidin unit was readily verified by acid degradation with 3 M hydrochloric acid–*iso*-propanol (1:4) [4], which gave fisetinidin hydrochloride, identical to the anthocyanidin generated from mollisacacidin. The ^{13}C NMR chemical shifts were also consistent with the dimeric structure, the chemical shift data comparing well with those of mollisacacidin and catechin combined (see Table 1). The characteristic features in the spectrum of the dimer were the chemical shifts of the unsubstituted carbons of the resorcinol A-ring, these being at 129.7 ppm for the deoxy C-5, 109.2 ppm for C-6 and 103.4 ppm for C-8. Taken in conjunction with the acid degradation data, the simultaneous occurrence of shifts at 81.2 and 68.2 ppm corresponding to the heterocyclic C-2 and C-3 carbons, respectively, together with the one unsubstituted A-ring



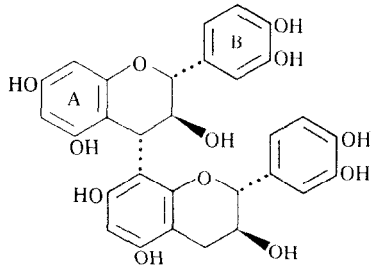
1



2



3



4

Table 1. ^{13}C NMR chemical shifts in acetone-d_6–water

Compound	C-2	C-3	C-4	C-5	C-6	C-8
Catechin	82.0	67.9	28.1	154.6	96.7	95.7
Mollisacacidin	81.9	72.2	73.9	129.7	110.1	103.0
Fisetinidol (4α → 8) catechin (**3**)	83.5	70.5	41.2	129.7	109.2	103.4
	81.2	68.2	28.0	154.6	96.3	107.9
Catechin (4α → 8) catechin (**4**)	83.2	73.5	37.9	154.6	96.4	96.4
	81.4	68.2	28.1	154.6	97.2	108.6

carbon at 96.3 ppm were characteristic of a catechin structure located in the lower or terminal position [5–7]. The position of the interflavanoid linkage on the catechin A-ring was assigned at C-8 on the basis of relative nucleophilicity, C-8 being more reactive than C-6 [8, 9]. In addition, the uncoupled singlet in the ^{1}H NMR spectrum of the acetate of **3** which was assigned to H-6 of the lower A-ring has a comparable chemical shift (δ6.70) to that of the corresponding aromatic proton of catechin (4α → 8) catechin decaacetate [6]. The ^{1}H NMR spectra of these two acetates were also very similar in the heterocyclic region with all *trans–trans* type couplings as in the catechin dimer analogue.

Another distinguishing feature in the ^{13}C NMR spectrum of the profisetinidin function was the chemical shift of C-4 at the point of linkage, this being at *ca* 41 ppm for fisetinidin (4α → 8) catechin and 38 ppm for catechin (4α → 8) catechin and in the range between 35 and 38 ppm for C-4 of procyanidins linked to another phloroglucinol A-ring [5, 7].

Heartwood polymers

The extractable heartwood polymers of *A. baileyana* consist of complex mixtures of polyphenolic components of procyanidins, prodelphinidins, profisetinidins and lignin. Separation of some of these components was achieved by taking advantage of the differences in their solubility in water and a methanol–water mixture. The water-soluble fraction consisted mostly of the phloroglucinol-type tannins, which were purified by chromatography over LH20 [7]. The IR [10] and ^{13}C NMR spectra of this polymer were entirely consistent with that of a mixed prodelphinidin–procyanidin polymer and strongly resembled the spectrum of the prodelphinidin–procyanidin polymer isolated from the phloem of *P. radiata* [7]. Degradation with phloroglucinol in the presence of acetic acid gave predominantly catechin, epicatechin (4β → 2) phloroglucinol and gallocatechin (4α → 2) phloroglucinol thus defining the terminal flavan as catechin and the monomer units as being predominantly of the epicatechin and gallocatechin stereochemical types. In addition, cyanidin and delphinidin were the only anthocyanidins produced when the polymer was treated with alcoholic hydrochloric acid.

The water-insoluble fractions obtained during the work-up were combined and dissolved in methanol. Addition of an equal volume of water to this methanolic solution resulted in a precipitate, which was filtered off through glass wool. The ^{13}C NMR spectrum showed this precipitate to be mostly lignin. The filtrate was fractionated on a column of Sephadex LH20 by eluting the

column with methanol–water (1:1). This procedure resulted in the separation of the predominantly procyanidin–prodelphinidin polymers, which were retained on the column, and the profisetinidins, which were eluted with the aqueous methanol. The retained tannin, labelled polymer B for distinction, was finally eluted with acetone–water (7:3) and degradation of polymer B with 3 M hydrochloric acid–*iso*-propanol (1:4) gave mostly cyanidin and delphinidin and a small amount of fisetinidin. The ^{13}C NMR spectrum (see Fig. 1) of polymer B was consistent with the degradation data and the ratio of prodelphinidin units to procyanidin units being lower in polymer B than in the water-soluble polymer. The presence of profisetinidin units may also be deduced from the data established from dimer **3**. The intrusion of the profisetinidin chemical shifts was the most apparent difference between the two polymers. The characteristic chemical shifts were the 41 ppm for the point of linkage at C-4 and the 129 ppm for the deoxy carbon at C-5 of the A-ring, and both of these were clearly apparent in the spectrum of polymer B together with the characteristic chemical shifts of the procyanidin and prodelphinidin moieties [7, 10]. Hence polymer B was a mixture of phloroglucinol and resorcinol types with the former type present in significantly higher levels.

The methanol–water eluants mentioned above consisted of mostly profisetinidins, which were confirmed by acid degradation to yield fisetinidin only. The ^{13}C NMR spectrum of a purified sample was consistent with that of oligomeric profisetinidins (see Fig. 1). The characteristic chemical shifts at *ca* 41.5 ppm, identified with the C-4 of the profisetinidin function, dominated that region of the spectrum as did the characteristic resorcinol A-ring shifts at 103.4, 109 and 129 ppm, which were assigned to C-8, C-6 and C-5 respectively.

The co-occurrence of procyanidins, prodelphinidins and profisetinidins in the heartwood of *A. baileyana* var. *purpurea* is in marked contrast to the reported presence of profisetinidins *only* in the heartwood of the related *A. mearnsii* [4] and it could therefore be of considerable taxonomic significance. The isolation of phloroglucinol-type tannins in the heartwood in general is also unusual, the only other reports of their detection being in the wood of the eucalypts [11].

Condensed tannins previously had been observed to be invariably accompanied by their immediate precursors, the flavanol, and the dimeric flavanoids, and these entities, being more amenable to isolation and identification than their higher analogues, had provided clues to the stereochemistry and the oxidation pattern in both the A- and B-rings of the polymers. The constituents in the heartwood of *A. baileyana* present the first outstanding exception to this rule; the accompanying catechin, mollisacacidin and the dimer fisetinidol(4α → 8)catechin would have erroneously suggested the tannins of *A. baileyana* to be exclusively of the profisetinidin polymer, very much like those present in *A. mearnsii*. The absence of the related procyanidin and prodelphinidin dimers from accompanying their polymers is a most interesting observation and may be explained on the principle of relative chemical reactivity. The generally accepted view of tannin formation has involved nucleophilic attack by the aromatic A-ring on a carbocation [12] or, as more recent evidence suggests, a quinone-methide intermediate [13, 14], either of which may be derived from a flavan-3,4-diol. In the presence of both the resorcinol- and phloroglucinol-type nucleophiles, the latter has considerably stronger nucleophilic centres [8] and hence would be preferentially involved in condensation with reactive intermediates to form higher oligomers. Catechin as well as the pro-

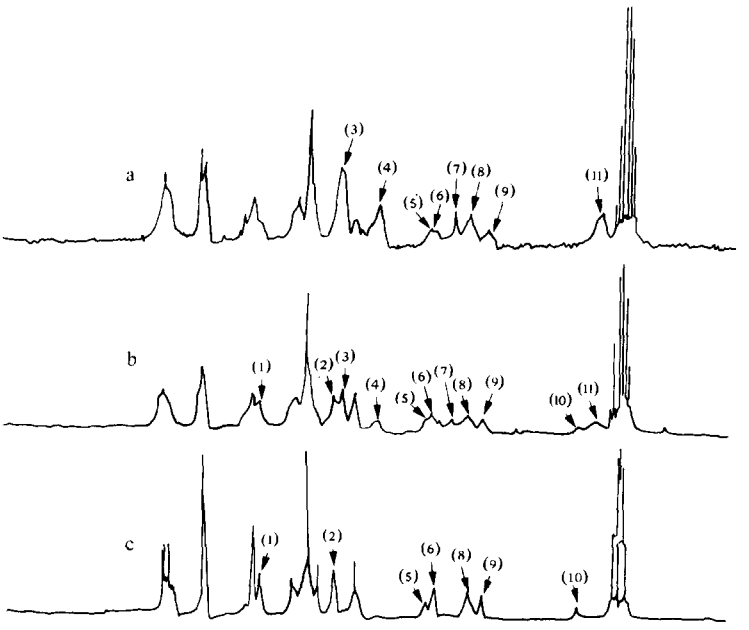


Fig. 1. ^{13}C NMR spectra of the proanthocyanidins from the heartwood of *A. baileyana* var. *purpurea*. (a) Procyanidin (PC)–prodelphinidin (PD) polymer. (b) Procyanidin (PC)–prodelphinidin (PD)–profisetinidin (PF) polymer. (c) PF polymer: (1) deoxy C-5 signal of PF units; (2) C-6 of PF units; (3) C-2′ and C-6′ of PD units; (4) C-6 and C-8 of PC and PD units; (5) C-2 of *trans* top unit; (6) C-2 of *trans* monomer unit; (7) C-2 of *cis* monomer unit; (8) C-3 of monomer units; (9) C-3 of terminal units; (10) C-4 of PF units; (11) C-4 of PC and PD units.

cyanidin and prodelphinidin dimers would therefore preferentially react with the excess mollisacacidin present in the heartwood to give rise to a mixed polymer such as polymer B.

The co-occurrence of the phloroglucinol-type tannins, the procyanidins and prodelphinidins, and the resorcinol type, the profisetinidins, raises the question of whether their biosynthesis is under the same or different enzymatic control. The occurrence of the mixed phloroglucinol–resorcinol tannins (such as polymer B) is rationalized as being non-enzymatic in origin, and the existence of discrete phloroglucinol- and resorcinol-type tannins strongly suggests that the biosynthesis of these two series of polymers is probably under separate enzymatic control.

EXPERIMENTAL

The sample of heartwood of *Acacia baileyana* var. *purpurea* was from a 5-year-old tree raised from a seedling obtained from a commercial garden centre. Specific rotations were obtained at 20° in Me_2CO. ^{13}C NMR spectra were performed in Me_2CO-d_6–H_2O (1:1) using TMS as external standard. TLC was on Schleicher and Schull cellulose using *t*-BuOH–HOAc–H_2O (3:1:1, solvent A) and HOAc–H_2O (3:47, solvent B).

Flavonoids. The low MW flavonoids from the Et_2O extract were identified by the method of Clark-Lewis and Porter [2].

Extraction procedure. The dried heartwood flakes (800 g) were left soaking in Me_2CO–H_2O (7:3) for several days and NaCl was added to the extract until two liquid phases were obtained. The upper layer was concd under red. pres. at < 40°. The residue was diluted with H_2O to twice its vol. and the resulting mixture was filtered through glass wool to remove insoluble material. The filtrate was washed with $CHCl_3$ (2 × 300 ml) and extracted with EtOAc (5 × 200 ml) to yield 8.5 g residue. The latter extraction also resulted in some precipitation, which was removed from the aq. soln by filtration through glass wool. The aq. layer was worked up as previously described [7] to yield the 'H_2O-soluble' polymer (2.1 g), $[\alpha]_{20}^{578}$ + 56.5° (*c* 0.012; Me_2CO). The polymer yielded about an equal mixture of cyanidin and delphinidin on treatment with 3 M HCl–*i*-PrOH (1:4) in a sealed tube at 100° for 1 hr. Reaction with excess phloroglucinol [14] yielded mostly catechin, epicatechin (4β → 2)phloroglucinol and gallocatechin (4α → 2)phloroglucinol.

Insoluble polymers. The insoluble materials were combined by dissolving them in MeOH. To this soln was added an equal vol. of H_2O and the resulting ppt. was immediately filtered off. This ppt. was shown to be predominantly lignin by ^{13}C NMR. The filtrate was fractionated on Sephadex LH20 by washing exhaustively with MeOH–H_2O (1:1) until the washing liquid was clear. The washings were combined and the solvents evapd to yield a residue which was purified by chromatography on Sephadex LH20 with EtOH to yield a phenolic fraction (0.3 g), $[\alpha]_{20}^{578}$ − 35.5° (*c* 0.03; Me_2CO–H_2O, 1:1). A small sample of this material when treated with 3 M HCl–*i*-PrOH (1:4) gave fisetinidin as the sole anthocyanidin.

The immobile tannins were finally eluted from the Sephadex column with Me_2CO–H_2O (7:3) to yield polymer B (2.2 g), $[\alpha]_{20}^{578}$ − 14.0° (*c* 0.02, Me_2CO–H_2O, 1:1). Degradation with 3 M HCl–*i*-PrOH (1:4) gave cyanidin, delphinidin and fisetinidin, with cyanidin as the dominant pigment. Degradation with phloroglucinol gave together with other unidentified products catechin, epicatechin (4β → 2)phloroglucinol, epigallocatechin (4β → 2)phloroglucinol and catechin (4α → 2)phloroglucinol.

The EtOAc extract was concd and the residue fractionated over Sephadex LH20 using EtOH as solvent. Fractions were collected in 20 ml tubes and monitored on cellulose TLC using solvent A. (+)-2*R*,3*S*,4*R*-3,4,7,3′,4′-Pentahydroxyflavan (mollisacacidin) was present in tubes 20–30. Rechromatography over Sephadex LH20 using EtOH–H_2O (1:1) yielded cleaner mollisacacidin, which was crystallized by leaving a sample in Me_2CO–H_2O to stand overnight; R_f 0.68 (A), 0.70 (B); mp 131–134°, $[\alpha]_D^{20}$ + 12.7° (*c* 0.08; Me_2CO). MS of sample gave a molecular ion at *m/z* 290. ^{13}C NMR δ 72.2, 73.9, 81.9, 103.0, 116.3, 116.6, 117.3, 121.3, 129.7, 145.3, 146.0, 158.0 ppm.

Catechin and fustin were present in tubes 31–56. Rechromatography over Sephadex using EtOH–H_2O (1:3) yielded catechin, which was identified by chromatographic and spectroscopic comparison with authentic material. Dihydrofisetin or fustin crystallized out from a soln of Me_2CO–H_2O by leaving the Me_2CO to evaporate off slowly overnight; R_f 0.50 (A), 0.70 (B). ^{13}C NMR: δ 73.8, 84.7, 103.7, 112.2, 112.9, 116.2, 116.7, 121.2, 129.5, 130.2, 145.5, 146.4, 164.2, 166.0, 194.5 ppm.

Butin. Further elution (tubes 58–63) gave a mixture which was rechromatographed over Sephadex LH20 using EtOH–H_2O (1:3) to give a small amount of butin, R_f 0.25 (A), 0.85 (B). ^{13}C NMR: δ 44.4, 80.3, 103.6, 111.2, 114.6, 114.9, 115.9, 119.0, 129.4, 131.8, 145.9, 146.2, 164.5, 165.5, 191.3 ppm.

Fisetinidol (4α → 8)*catechin* (**3**). Tubes 64–78 consisted mostly of a dimer, R_f 0.60 (A), 0.70 (B). ^{13}C NMR: δ 28.0, 41.2, 68.2, 70.5, 81.2, 83.5, 96.3, 101.4, 103.2, 103.4, 107.9, 109.2, 115.4, 116.2, 116.5, 116.8, 119.1, 120.8, 129.7, 131.6, 131.9, 144.7, 145.0, 145.4, 145.7, 154.2, 154.7, 155.9, 156.1, 156.3 ppm. Degradation of fisetinidol (4α → 8)catechin with 3 M HCl-*i*-PrOH (1:4) gave fisetinidin. The sample was acetylated with Ac_2O–pyridine (1:1) and the peracetate purified by prep. TLC (silica gel, C_6H_6–Me_2CO, 4:1) R_f 0.50, $[\alpha]_D^{578}$ − 72.0° (*c* 0.06; Me_2CO). ^1H NMR ($CDCl_3$): δ 1.7–2.3 (*m*), 2.9–3.2 (*m*), 4.4 (*d*, *J* = 9.3 Hz), 4.7–5.2 (*m*), 5.85 (*t*, *J* = 9.3 Hz), 6.5–7.3 (*m*).

Acknowledgements—The author thanks Dr. H. Wong for ^{13}C NMR spectra and Dr. K. Markham for the sample of *Acacia baileyana* wood.

REFERENCES

1. Tindale, M. D. and Roux, D. G. (1969) *Phytochemistry* **8**, 1713.
2. Clark-Lewis, J. W. and Porter, L. J. (1972) *Aust. J. Chem.* **25**, 1943.
3. Pettigrew, C. J. and Watson, L. (1975) *Aust. J. Botany* **23**, 833.
4. Roux, D. G. (1962) *Chem. Ind.* 278.
5. Porter, L. J., Newman, R. H., Foo, L. Y., Wong, H. and Hemingway, R. W. (1982) *J. Chem. Soc. Perkin Trans.* 1, 1217.
6. Foo, L. Y. and Porter, L. J. (1983) *J. Chem. Soc. Perkin Trans.* 1, 1535.
7. Czochanska, Z., Foo, L. Y., Newman, R. H. and Porter, L. J. (1980) *J. Chem. Soc. Perkin Trans.* 1, 2278.
8. Botha, J. J., Viviers, P. M., Ferreira, D. and Roux, D. G. (1982) *Phytochemistry* **21**, 1289.
9. Roux, D. G., Ferreira, D., Hundt, H. K. L. and Malan, E. (1975) *Appl. Polymer Symp.* No. 28, 335.
10. Foo, L. Y. (1981) *Phytochemistry* **20**, 1397.
11. Hillis, W. E. (1972) *Phytochemistry* **11**, 1207.
12. Jacques, D., Opie, C. T., Porter, L. J. and Haslam, E. (1977) *J. Chem. Soc. Perkin Trans.* 1, 1637.
13. Hemingway, R. W. and Foo, L. Y. (1983) *J. Chem. Soc. Chem. Commun.* 1035.
14. Attwood, M. R., Brown, B. R., Lisseter, S. G., Torrero, C. L., and Weaver, P. M. (1984) *J. Chem. Soc. Chem. Commun.* 177.