Pergamon

Phytochemistry, Vol. 45, No. 8, pp. 1689–1696, 1997

0031-9422/97 $17.00+0.00

PII: S0031-9422(97)00198-2

PROANTHOCYANIDINS FROM *LOTUS PEDUNCULATUS*

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(*Received in revised form* 27 *January* 1997)

Key Word Index—*Lotus pedunculatus*; Leguminosae; proanthocyanidins; dimers; polymers; phloroglucinol adducts.

Abstract—A range of flavanols and proanthocyanidin dimers were isolated and identified from *Lotus pedunculatus*. The more abundant polymeric proanthocyanidins were also isolated and their chemical constitution studied by ^{13}C NMR and by acid-catalysed degradation in the presence of excess phloroglucinol. These results showed the polymers to be highly heterogeneous, with catechin, epicatechin, gallocatechin and epigallocatechin all being constituent components of both the extenders as well as the terminating units. Epigallocatechin was the most abundant extension unit and catechin the more common terminating flavanoid. The average molecular size of the polymers was estimated by ^{13}C NMR to be *ca* eight flavanoid units and there was spectroscopic and chemical evidence to indicate that a small portion of these units were glycosylated. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Condensed tannins or proanthocyanidins (PA) are widespread in the plant kingdom and, because of their ability to interact with a range of biomolecules as well as minerals, they invariably have a significant impact on human economic activity. Their presence in foods reduces their palatability and causes processing problems, such as discolouration and haze formation. While the presence of PA has been traditionally viewed as generally undesirable, with anti-nutritional properties [1–3] associated with decreased palatability or negatively affecting digestion by immobilizing bacterial enzymes and/or forming indigestible complexes with cell wall carbohydrates [4, 5], there is now accumulating evidence to suggest that PA in small amounts are beneficial to ruminant digestion and enhance animal production. Their presence has been associated with bloat prevention, especially in cattle that have been fed a high soluble protein diet [6, 7]. Sheep fed fresh feeds containing PA, such as sainfoin, had higher nitrogen retention than those fed white clover or ryegrass which contain little or no PA [8–14]. Apparently, while digestability of protein was lower on the sainfoin diet (as evidenced by an increased nitrogen content in the faeces), this was more than compensated for by a significant reduction in urinary nitrogen loss [9–13]. Sheep fed *Lotus corniculatus* had increased flow of essential amino acids to the duodenum compared with sheep fed with alfalfa [11]. Such benefits have been reflected in better animal performance in ruminants fed PA containing forages and heifers grazing on these feeds had higher live weight gains compared with similar animals grazing on alfalfa [13]. High producing dairy cows fed *L. corniculatus*-grass haylage produced higher fat adjusted milk yields than cows fed alfalfa haylage alone [14] and sheep grazing on *L. corniculatus* had increased wool growth by *ca* 11–15% [15].

The manner in which PA impart such positive effects on ruminants is commonly believed to be attributable to their affinity for protein. The most widely accepted mechanism is the formation of complexes between PA and leaf protein in the rumen, thereby protecting dietary protein from rumen microbial degradation and concomitant loss of ammonia. These complexes dissociate under the more acidic conditions of the abomasum, thus rendering the protein available for animal digestion [13, 14]. This effect of PA on reducing dietary protein degradation by rumen microflora has now been demonstrated *in vitro* but such observations are also consistent with the theory of microbial inactivation or the inhibition of protease enzymes [16–18]. For instance, while the concentration of soluble protein and ammonia in rumen digesta was reduced in the presence of PA, the microbial biomass in the rumen was also lower [6, 11, 19].

Lotus corniculatus and *L. pedunculatus* are two PA-

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containing fodder crops which are in use in New Zealand. Both are of high nutritive value when cultivated under conditions of moderate fertility [20–22] although, when *L. pedunculatus* is raised under cold wet conditions on acidic soils, the nutritive value is significantly reduced due to elevated PA contents as a result of stress [4, 23]. The lower PA-containing *L. corniculatus* has been rated as being over 60% better than perennial ryegrass and second only to white clover for promoting lamb growth when fed *ad libitum*, in spite of the lower digestibility of this forage than that of the equivalent forages containing little or no PA [14]. However, the PA in *L. corniculatus* and *L. pedunculatus* differ substantially in their effect on ruminant digestion [11, 19]. As these differences appear not to be due to the quantity of PA in plant dry matter [24], it is therefore important to establish the nature of the chemical differences in the two PA of these forages. A detailed study of PA composition of *L. corniculatus* has already been reported [25]; the present investigation is a continuation of this work in order to provide a better understanding of the chemical structure of PA in relation to their role in ruminant nutrition.

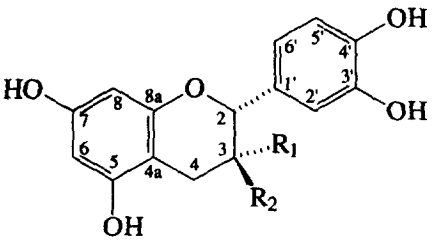
RESULTS AND DISCUSSION

The defatted aqueous acetone extract of the leaves of *L. pedunculatus* contained only low amounts of ethyl acetate extractable materials representing *ca* 1.7% of the defatted extract as compared with *ca* 4.8% in *L. corniculatus*. Examination of this fraction on cellulose 2D TLC visualized with vanillin-HCl spray revealed a highly complex mixture which, on repeated chromatography alternating between Sephadex LH20 and MCI gel CHP 20P, afforded catechin **1**, epicatechin **2**, gallocatechin **3** and epigallocatechin **4**. The presence of all these flavanols, which were regarded as representative of the terminal units of PA oligomers and polymers, in comparable relative abundance to one another, provided the first indication of a highly heterogeneous mixture of proanthocyanidins which were likely to be present in the extracts.

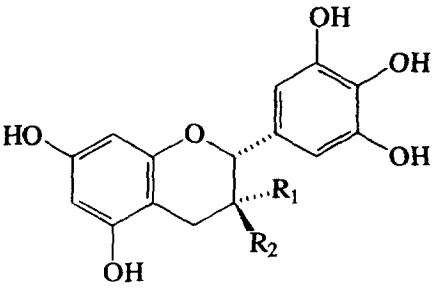
Among the procyanidin dimers were catechin ($4\alpha \rightarrow 8$)-catechin **5**, catechin ($4\alpha \rightarrow 8$) epicatechin **6**, epicatechin ($4\beta \rightarrow 8$)-catechin **7** and epicatechin ($4\beta \rightarrow 8$) epicatechin **8**, all of which were identified from their ^{13}C NMR data and confirmed by chromatographic and spectroscopic comparison with authentic materials. Another related compound **9** was also isolated and its dimeric constitution was apparent from the presence of the four clearly defined carbon signals in the heterocyclic region, with the C-2 carbon signals at δ 77.0 and 82.5 being characteristic of a 2,3-*cis* flavan unit with an appended substituent at C-4 and a 2,3-*trans* terminating unit, respectively. Compound **9** was therefore similar to **7** with a distinctive difference. An additional chemical shift observed at δ 106.8 in its ^{13}C NMR spectrum was attributable to the C-2′ and the C-6′ of a pyrogalloyl B-ring. When reacted with 5% HCl in *tert*-butanol, it yielded delphinidin, thus establishing that the upper flavan unit was an epigallocatechin moiety and that the terminating unit, by necessity, had to be catechin. Compound **9** was the more abundant proanthocyanidin dimer in *L. pedunculatus* and on the basis that the C-8 carbon was more reactive than the C-6 carbon, its interflavanoid linkage was likely to be the more commonly encountered C-4 to C-8 bond. This was subsequently confirmed by chromatographic comparison of its peracetate derivative with **11**.

The presence of a prodelphinidin dimer **10**, as evidenced by the generation of delphinidin on heating with 5% HCl in *tert*-butanol, was deduced similarly from its ^{13}C NMR spectrum. The downfield position (δ 83.5) of one of the C-2 resonances showed that the 2,3-*trans* flavanoid unit had a substituent at C-4 and, hence, that it was a gallocatechin unit. The other C-2 signal was observed at δ 79.5, which is characteristic of a flavan with a 2,3-*cis* configuration. In addition, the absence of any catechol B-ring signals and, instead, the presence of two high-field aromatic chemical shifts (δ 106.5 and 108.1), characteristic of the two sets of degenerate unsubstituted pyrogallol β-ring carbons, showed that the lower moiety was an epigallocatechin. Because of the small sample size which precluded further degradative experimentation, compound **10** was tentatively assigned as gallocatechin ($4\alpha \rightarrow 8$)-epigallocatechin from the signal multiplicities in the ^{1}H NMR spectrum and the line-broadening in some carbon chemical shifts which were suggestive of restricted rotation about the interflavanoid bond commonly associated with the C-4 to C-8 linkage where the extender unit is of the 2,3-*trans* configuration [26].

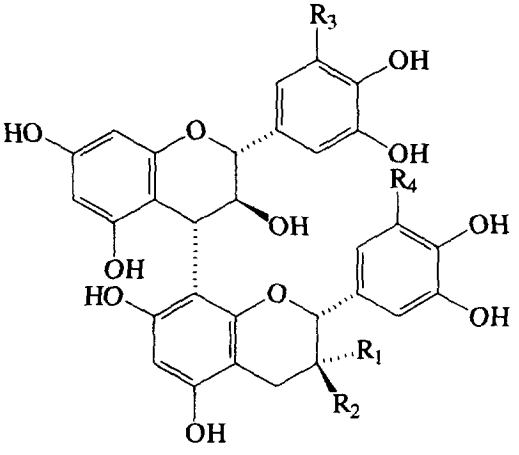
Some unresolved chromatographic fractions which resisted purification were then acetylated with acetic anhydride and pyridine, and separated by preparative TLC on silica gel. This led to the isolation and identification of epicatechin and epigallocatechin peracetates and, in addition, three other peracetates (**11**–**13**) were also isolated. The dimeric constitution of the latter three components was apparent from their ^{13}C NMR spectra showing the presence of a single interflavanoid carbon (C-4) chemical shift, an unsubstituted C-4 on the bottom or terminating flavan, together with the four heterocyclic carbon chemical shifts of the C-2 and C-3 carbons, in each case. For **11**, it was apparent that the two flavanoid units were different not only in 2,3-stereochemistry but also in the B-ring oxidation pattern. By taking advantage of the diagnostic low-field position of the unsubstituted H-4 methylene protons (δ 2.54 and 3.20) as a reference point, the location of the adjoining H-3 and H-2 protons were established through the relevant H,H couplings. The large coupling constant (J = 9.8 Hz) for H-2 showed that this lower flavanoid unit had the 2,3-*trans* stereochemistry. In addition, long-range H,C couplings (HMBC) were observed between this H-2



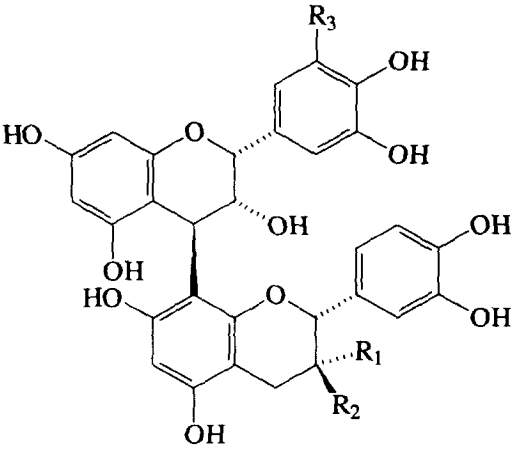
(1) $R_1 = H$, $R_2 = OH$
(2) $R_1 = OH$, $R_2 = H$



(3) $R_1 = H$, $R_2 = OH$
(4) $R_1 = OH$, $R_2 = H$

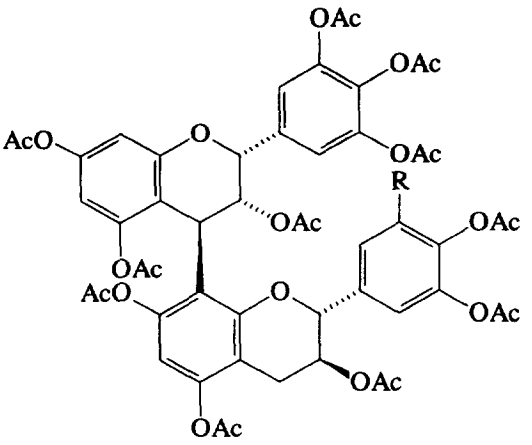


(5) R_1=H, R_2=OH, R_3=H, R_4=H
(6) R_1=OH, R_2=H, R_3=H, R_4=H
(10) R_1=OH, R_2=H, R_3=OH, R_4=OH

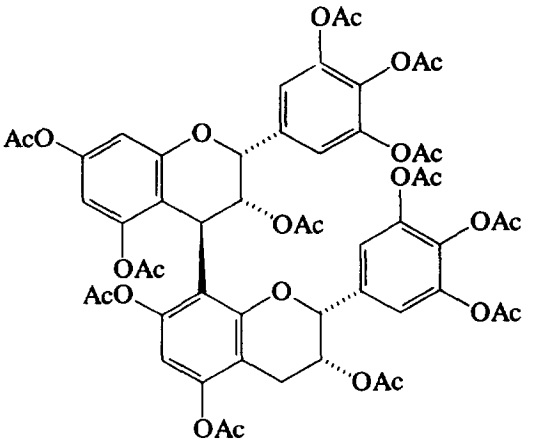


(7) R_1=H, R_2=OH, R_3=H
(8) R_1=OH, R_2=H, R_3=H
(9) R_1=OH, R_2=H, R_3=OH

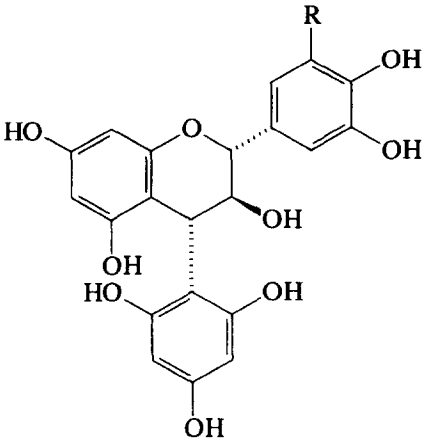
and the unsubstituted C-2′ (δ 122.3) and C-6′ (δ 125.4) of the catechol B-ring, thus establishing the lower flavan unit as catechin. The upper flavan unit had the 2,3-*cis* stereochemistry as evidenced by the small proton couplings between their respective H-2 and H-3 protons. Also, the H-2 (δ 5.42) was long range-coupled to the C-2′ and C-6′ of the pyrogallol B-ring, thus confirming the upper unit to be that of epigallocatechin. The nature of the interflavanoid linkage in **11** could also be resolved using the long-range H,C data as earlier reported for acetylated procyanidin dimers [27]. This could be more readily achieved because the acetylation of the aromatic hydroxyls caused the affected carbons to move upfield away from the C-8a carbons, thereby facilitating their chemical shift assignment from their H,C-couplings with the C-rings protons. In this manner, the upper C-8a was assigned to the signal at δ 154.0 and the corresponding carbon of the lower unit to the signal at δ 155.3. As may be observed from the H,C-correlation data (Table 1), the H-4 methine proton (δ 4.42) at the point of interflavanoid linkage correlated with both these carbons. This observation can only occur if the linkage is at C-8, and, hence, **11** was the peracetate of epigallocatechin (4$\beta \rightarrow$ 8) catechin. This assignment was further corroborated by the absence of any long-



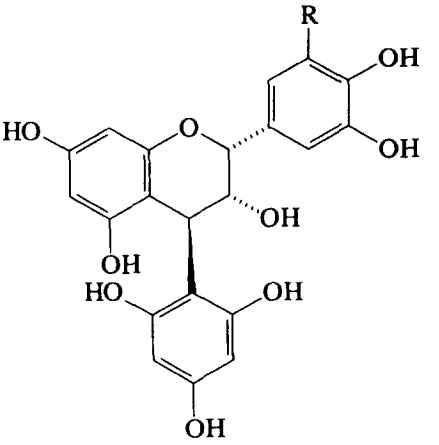
(11) R = H
(12) R = OAc



(13)



(14) R = H
(16) R = OH



(15) R = H
(17) R = OH

range H,C correlation between the A-ring proton (δ 6.68) of the lower flavan unit and the corresponding C-8a (δ 154.0).

The ^{1}H and ^{13}C NMR spectra of the peracetate **12** were similar to those of **11**, including the magnitude and H,H coupling pattern of the two heterocyclic C-ring protons. The only distinctive differences were the absences of NMR signals attributable to the catechol B-ring but, in their place, were additional peaks which were assignable to the pyrogallol B-ring. Compound **12** was therefore the acetate of epigallocatechin (4β → 8) gallocatechin. The exclusively pyrogallol B-ring type constitution of **13** was also evident from the NMR spectrum, where a strong signal observed at δ 7.26, integrating for four protons, was consistent with the presence of the two pairs of degenerate pyrogallol ring protons. This was further corroborated by observation of the two-carbon chemical shifts (δ 119.2 and 119.6) associated with these aromatic ring protons. The ^{1}H chemical shift values and coupling patterns of the heterocyclic ring protons suggested that both flavan units were of the same 2,3-*cis* stereochemistry. The spectral pattern was identical to that shown by procyanidin B2 peracetate [28]. Compound **13** was therefore the peracetate of the prodelphinidin epigallocatechin-(4β → 8) epigallocatechin.

Table 1. Inverse long-range H,C correlation (HMBC) of prodelphinidin acetate **11**

H (δ)	Correlated carbons
2.54	68.4, 113.7, 148.7, 154.0
3.20	68.4, 78.3, 113.7, 148.7, 154.0
4.32	27.2, 68.4, 122.3, 125.4, 134.5
4.42	70.7, 73.4, 111.3, 116.9, 147.9, 154.0, 155.3
5.05	78.3, 169.0
5.13	33.9, 111.3, 116.9, 169.6
5.42	70.7, 119.0, 136.0
5.99	108.6, 111.3, 149.3, 155.3
6.30	107.2, 111.3, 147.9, 149.3
6.68	113.7, 116.9, 147.9, 148.7
6.87	78.3, 125.4, 142.3
6.95	78.3, 122.3, 123.2, 142.3
7.10	125.4, 134.5, 142.3
7.16	73.4, 119.0, 134.5, 143.1

Table 2. Composition of isolated scission products from proanthocyanidin polymers of *Lotus pedunculatus* with acid in the presence of phloroglucinol

Product	Yield (mg)
Catechin	175
Epicatechin	70
Gallocatechin	70
Epigallocatechin	37
Catechin (4$\alpha \rightarrow$ 2) phloroglucinol	51
Epicatechin (4$\beta \rightarrow$ 2) phloroglucinol	215
Gallocatechin (4$\alpha \rightarrow$ 2) phloroglucinol	150
Epigallocatechin (4$\beta \rightarrow$ 2) phloroglucinol	735

The proanthocyanidin polymer which was obtained by eluting with aqueous acetone following the removal of the lower M_r oligomers from a Sephadex LH 20 column with excess 50% aqueous MeOH was shown to be a mixed polymer after acid hydrolysis, generating delphinidin and cyanidin. The ^{13}C NMR spectrum of the polymer revealed that the flavan monomers not only comprised a mixed B-ring oxidation pattern but also consisted of both 2,3-*cis* and 2,3-*trans* stereochemistries. The comparable signal intensity of these signals (δ 78.8 and 81.0) attributable to the terminal C-2 of the 2,3-*cis* and 2,3-*trans* flavan units, respectively, indicated that the polymers were terminated by such flavanoids in about the same frequency. The magnitude of the carbon peaks observed at δ 76.3 for the *cis* and δ 82.9 for the *trans* of the corresponding extender units showed these were also mixed to the extent of *ca* 1 for the *trans* to 3 for the *cis*. Further corroborative evidence on this structural variation was also available from the chemical shift of the C-4 substituted methine carbons where two signals, the more pronounced δ 36.7 peak and a minor one at δ 37.9, were clearly discernible in the spectrum. The C-4 methine of a 2,3-*cis* flavanol with a phloroglucinol substituent in this location occur upfield (δ 36.5) relative to the corresponding C-4 carbon (δ 37.9) of a 2,3-*trans* unit [29]. Hence, the observation of the peaks at δ 36.7 and 37.9, with a signal intensity ratio of *ca* 3:1 was fully consistent with this interpretation.

A relatively small signal at δ 62.6, indicated to be methylene carbons by DEPT experiments, suggested the presence of a glycoside and this was further corroborated by the presence of other sugar carbons many of which were obscured by C-ring carbons. An equivalent size carbon signal in the δ 101–102 region, which was diagnostic of the anomeric sugar carbons, suggested that a small fraction of the flavanoid units was glycosylated. This was confirmed by mild acid treatment of the polymer, which yielded glucose as a hydrolysis product. The low-field position of the anomeric carbon suggested that the sugar moieties were attached to the aliphatic 3-OH [30]. The C-3 signal (δ 65–67) of the terminal flavanoid unit was well resolved from the corresponding C-3 signal (δ 72.1) of the extender units and, thus, it was possible to estimate average chain length of the polymers to be in the order of *ca* eight flavanoid units from their respective peak intensities [31]. The sugar signals which also occurred in this general region, were expected to distort the value but, because of their relatively small contribution, the value was not expected to be affected significantly.

The nature of the B-ring hydroxylation pattern could be gauged by examination of the upfield end of the aromatic region (δ 106–120) of the ^{13}C NMR spectrum, where the unsubstituted carbons of the catechol and pyrogallol rings were located. The relatively large signal intensity observed in the δ 106–108 region was indicative of the dominant status of the pyrogallol B-ring compared with the weak signals (δ 115–119) of the catechol moiety. Thus, the polymers were predominantly of the prodelphinidin-type, with the majority of the extender units possessing the 2,3-*cis* configuration and terminated by various flavan-3-ols.

It was apparent, that while a great deal of structural information could be obtained from the ^{13}C NMR data, it was not possible to ascertain the identities of the individual units that made up the polymer chain. In order to obtain this information, which could be important in enabling a better understanding of their biological and nutritional roles for ruminants, the polymers were subjected to partial acid-catalysed cleavage in the presence of phloroglucinol. The reaction resulted in the release of the terminating units, namely catechin, epicatechin, gallocatechin and epigallocatechin, in the ratio of *ca* 5:2:2:1, respectively. The occurrence of these four flavan-3-ols in the mixture indicated the heterogeneous nature of *L. pedunculatus* proanthocyanidin polymers, with catechin being the most abundant terminating units and the remaining three being more or less evenly distributed.

The extender units were distinguished from the terminal units as phloroglucinol adducts formed by

Table 3. Composition of extender and terminal units of proanthocyanidin polymers in *Lotus corniculatus* [24] and *L. pedunculatus*

Flavan-3-ol unit	*L. corniculatus*		*L. pedunculatus*	
	Terminal (%)	Extender (%)	Terminal (%)	Extender (%)
Catechin	82	1.5	50	4.4
Epicatechin	16	67	20	18.7
Gallocatechin	0	0	20	13
Epigallocatechin	1.6	30	10	64

nucleophilic capture of the carbocations generated under the acid conditions of the reaction. Among the products were catechin (4α → 2) phloroglucinol **14**, epicatechin (4β → 2) phloroglucinol **15**, gallocatechin (4α → 2) phloroglucinol **16** and epigallocatechin-(4β → 2) phloroglucinol **17** isolated in a ratio of *ca* 1:4:3:14 respectively, showing that epigallocatechin was the dominant extender unit, a result which was in agreement with ^{13}C NMR data.

Despite their nomenclature, there are significant differences between *L. pedunculatus* and *L. corniculatus*. The former is a diploid species with 12 chromosomes, whereas the latter is a naturally occurring tetraplod (24 chromosomes) [32, 33]. This factor was also reflected by the chemical and structural differences of the proanthocyanidin polymers derived from them (Table 3).

The polymers from *L. corniculatus* are predominantly of the procyanidin-type, with epicatechin (67%) as the main extender unit and epigallocatechin (30%) making up the rest. In contrast, the polymers from *L. pedunculatus* were predominantly of the prodelphinidin type, with epigallocatechin (64%) as the major extender unit and epicatechin (18.7%) as the minor constituent unit. In addition, gallocatechin (13%) and, to a lesser extent, catechin (4.4%) also constituted the extender units of *L. pedunculatus*. In *L. corniculatus* catechin (82%) was the most prevalent terminal unit, with epicatechin essentially making up the remainder, while, in *L. pedunculatus*, the terminal units were again more heterogeneous, with all four flavan-3-ols involved in the polymeric structure. These structural differences could be expected to be expressed in their physiological and chemical properties, which, in turn, might account for the observed nutritional effects on ruminants through differences in various activities, such as protein-binding, enzyme inhibition and microbial morbidity.

EXPERIMENTAL

^{13}C NMR spectra were recorded in Me_2CO-d_6 with a small amount of D_2O for free phenols and in $CDCl_3$ for acetate derivatives. TLC were performed on Schleicher and Schuell cellulose plates and developed with solvent A (*t*-BuOH–HOAc–H_2O, 3:1:1) and solvent B (HOAc–H_2O, 3:47).

Extraction and isolation. Ground leaves of *L. pedunculatus* (cv. Grassland Maku) from *ca* 80 plants were exhaustively extracted with 70% aq. Me_2CO and the extract concd on a rotatory evaporator under red. pres. The residual extract was diluted with H_2O and washed free of lipids and waxes with CH_2Cl_2 and freeze-dried. A sample of the freeze-dried extract (80 g) was suspended in H_2O (1 l), extracted with EtOAc (5 × 200 ml) and the combined extracts dried (Na_2SO_4) and the solvent evapd to yield a solid which was dissolved in 50% aq. EtOH and fractionated on a Sephadex LH 20 column. Frs were monitored by TLC and visualized by spraying with a soln of $FeCl_3$–$K_3Fe(CN)_6$. Frs containing mixt. were further treated chromatographically alternating between MCI CHP 20 (aq. MeOH) and Sephadex LH20 (aq. EtOH), until sufficiently homogeneous samples were obtained.

Purification of proanthocyanidin polymers. The aq. fr. from the EtOAc extract was diluted with an equal vol. of MeOH and the resulting soln applied to a Sephadex LH20 column which was prepd in 50% aq. MeOH. The column was washed exhaustively with 50% aq. MeOH until the eluate was colourless and then immobilized proanthocyanidin polymers were eluted with Me_2CO–H_2O (3:2). The eluate was concd to small vol. and freeze-dried to give a light brown fluffy powder (6.7 g). ^{13}C NMR: δ 27–28, 36–38, 62–64, 66–68, 71–74, 76–77, 78–79, 80–81, 82–84, 95–98, 100–103, 106–108, 114–116, 118–120, 130–133, 143–147, 153–158.

Degradation with phloroglucinol. Purified proanthocyanidin polymers (4.5 g) were reacted with phloroglucinol (3 g) in 1% HCl in EtOH (35 ml) with continuous shaking until complete soln was effected. The soln was then concd under a stream of N_2 to *ca* 20 ml and fractionated on a Sephadex LH 20 column using EtOH as eluant. Frs were monitored by TLC and unresolved frs further fractionated by chromatographic treatment using a combination of MCI CHP 20 (MeOH–H_2O, 3:7) and Sephadex LH20 (EtOH–H_2O, 19:1 to 1:1) until sufficiently homogeneous products were obtained.

Acetylation. This was carried out by dissolving the impure materials in a minimum vol. of pyridine and adding an equal vol. of AC_2O and leaving the soln to stand at ambient conditions overnight. The reaction mixt. was evapd to dryness and the residue taken up in Me_2CO and applied to prep. silica gel TLC plates, which were developed in toluene–Me_2CO (3:1).

Catechin (**1**). Freeze-dried powder. R_f (B) 0.48. ^{13}C NMR: δ 28.8 (C-4), 68.2 (C-3), 82.5 (C-2), 95.2 (C-8), 96.2 (C-6), 100.5 (C-4a), 115.4 (C-2′), 115.8 (C-5′), 119.9 (C-6′), 131.9 (C-1′), 145.6, 145.7 (C-3′, C-4′), 157.7, 157.2, 157.6 (C-5, C-7, C-8a). ^{1}H NMR: δ 2.43–2.95 (*m*, H-4), 4.0 (*m*, H-3), 4.55 (*d*, J = 6.1 Hz, H-2), 5.88 (*bs*, H-6), 6.04 (*bs*, H-8), 6.80 (*m*, H-2′, H-5′), 6.91 (*s*, H-6′).

Epicatechin (**2**). Freeze-dried powder. R_f (B) 0.30. ^{13}C NMR: δ 29.1 (C-4), 66.9 (C-3), 79.2 (C-2), 95.7 (C-8), 96.3 (C-6), 100.7, 115.3 (C-2′), 115.6 (C-5′), 119.3 (C-6′), 132.0 (C-1′), 145.1, 146.5 (C-3′, C-4′), 156.9–158.4 (C-5, C-7, C-8a). ^{1}H NMR: δ 2.7–3.1 (*m*, H-4), 4.23 (*bs*, H-3), 4.90 (H-2), 6.98 (*bs*, H-6), 6.10 (*bs*, H-8), 6.80–6.90 (*m*, H-2′, H-5′), 7.08 (*s*, H-6′).

Gallocatechin (**3**). Freeze-dried powder. R_f (B) 0.40. ^{13}C NMR: δ 28.5 (C-4), 68.0 (C-3), 82.5 (C-2), 95.3 (C-8), 96.2 (C-6), 100.5 (C-4a), 107.4 (C-2′, C-6′), 131.1 (C-1′), 133.3 (C-4′), 146.2 (C-3′, C-5′), 156.5, 157.0, 157.4 (C-5, C-7, C-8a). ^{1}H NMR: δ 2.4–2.9 (*m*, H4), 3.98 (*m*, H-3), 4.56 (*d*, J = 6.0 Hz, H-2), 6.05 (*bs*, H-6, H-8), 6.50 (*s*, H-2′, H-6′).

Epigallocatechin (**4**). Freeze-dried powder. R_f (B) 0.25. ^{13}C NMR: δ 29.0 (C-4), 67.0 (C-3), 79.4 (C-2), 95.7 (C-8), 96.2 (C-6), 99.9 (C-4a), 106.9 (C-2′, C-6′), 131.4 (C-1′), 132.9 (C-4′), 146.1 (C-3′, C-5′), 156.8, 157.1 and 157.5 (C-5, C-7, C-8a). ^{1}H NMR: δ 2.7–3.1 (*m*, H-4), 4.22 (*bs*, H-3), 4.87 (H-2), 6.0 (*bs*, H-6), 6.10 (*bs*, H-8), 6.60 (*s*, H-2′, H-6′).

Catechin (4$\alpha \rightarrow$8) *catechin* (**5**). Freeze-dried powder. R_f (A) 0.40; R_f (B) 0.50. ^{13}C NMR: δ 28.6, 38.5, 68.8, 73.9, 81.9, 83.9, 96.4–98.1, 102.4, 107.5, 109.0, 115.9–116.9, 120.2, 121.2, 131.9, 132.5, 145.4, 145.9, 155–159; minor rotamer peaks not quoted.

Epicatechin (4$\beta \rightarrow$8) *catechin* (**7**). Freeze-dried powder. R_f (A) 0.47, R_f (B) 0.57. ^{13}C NMR: δ 29.0, 68.0, 72.0, 76.8, 82.1, 95.7, 96.1, 96.5, 101.2, 106.9, 115.2–115.9, 119.2, 120.1, 131.6, 131.8, 145–146, 154–159.

Epicatechin (4$\beta \rightarrow$8) *epicatechin* (**8**). Freeze-dried powder. R_f (A) 0.58, R_f (B) 0.62. ^{13}C NMR: 29.3, 36.9, 66.4, 72.6, 76.4, 79.3, 95.9, 96.3, 97.2, 100.5, 102.1, 107.4, 115–116, 119.2, 119.3, 131.4, 131.6, 145–146, 155–158.

Catechin (4$\alpha \rightarrow$8) *epicatechin* (**6**). Freeze-dried powder. R_f (A) 0.53, R_f (B) 0.54. ^{13}C NMR: δ 29.6, 38.9, 67.8, 73.8, 79.9, 83.9, 96.3, 97.5, 97.7, 99.5, 108.3, 114–116, 119.2, 120.3, 131–132.6, 145.6–146.5, 155.4–158.7; minor rotamer peaks not quoted.

Epigallocatechin (4$\beta \rightarrow$6) *catechin* (**9**). Freeze-dried solid. R_f (B) 0.25. ^{13}C NMR: δ 29.0, 37.1, 68.1, 72.0, 77.0, 82.5, 95.8, 96.0, 96.6, 101.3, 106.8, 107.5, 115.4, 115.7, 119.2, 120.1, 131.3, 131.8, 145.7, 145.8, 146.2, 154.7, 155.4, 157.7, 159.1, 159.3. ^{1}H NMR: δ 2.4–2.9 (*m*), 4.0 (*m*), 4.10 (*bs*), 4.50 (*d*, J = 8.2 Hz), 4.60 (*bs*) 4.90 (*m*), 6.10 (*m*), 6.51 (*m*), 6.7–7.10.

Gallocatechin (4$\alpha \rightarrow$8) *epicatechin* (**10**). Freeze-dried powder. R_f (B) 0.40. ^{13}C NMR: δ 29.2, 38.0, 66.8, 73.2, 79.5, 83.5, 96.1, 97.2, 99.1, 106.5, 108.1, 131.3, 146.0, 154.6–156.2.

Epicatechin peracetate. ^{13}C NMR: δ 20–21, 26.4, 66.8, 76.7, 108.2, 109.0, 109.7, 122.4, 123.5, 124.8, 136.1, 142.2, 149.8, (×2), 155.1, 167–171. ^{1}H NMR: δ 1.92 (*s*, Ac), 2.28–2.29 (OAc), 2.90–2.96 (*m*, H-4), 5.11 (H-3), 5.39 (*bs*, H-2), 6.57 (*d*, J = 2.2 Hz, H-8), 6.67 (*d*, J = 2.2 Hz, H-6), 7.20–7.35.

Epigallocatechin peracetate. ^{13}C NMR: δ 20.2, 20.7, 21.1 (–OAc), 26.0, 66.5 (C-3), 76.5 (C-2), 108.1 (C-8), 108.9 (C-6), 109.6 (C-4a), 119.0 (C-2′, C-6′), 134.3 (C-4′), 135.5 (C-1′), 143.3 (C-3′, C-5′), 149.7 (C-5, C-7), 154.8 (C-8a), 166.9, 167.7 (×2), 168.5, 169.0 and 170.5. ^{1}H NMR: δ 1.93, 2.27–2.29 (–OAc), 2.91 (*m*, H-4), 5.08 (*bs*, H-2), 5.38 (*m*, H-3), 6.57 (*d*, J = 2.2 Hz, H-8), 6.67 *d*, J = 2.2 Hz, H-6), 7.20 (*s*).

Epigallocatechin (4$\beta \rightarrow$8) *catechin peracetate* (**11**). ^{13}C NMR: δ 19.9–21.1, 27.2, 33.9, 68.4, 70.7, 73.4, 78.3, 107.2, 108.6, 110.7, 111.3, 113.7, 116.9, 119.0, 122.3, 123.2, 125.4, 134.5, 136.0, 141.7, 142.3, 143.1, 147.9, 148.7, 149.3, 154.0, 155.3, 166.8, 167.6, 167.8, 168.2, 168.8, 168.9, 169.6. ^{1}H NMR: δ 1.6–2.2 (OAc), 2.54, (*dd*, H-4), 3.20 (*dd*, H-4′), 4.32 (*d*, J = 9.8 Hz), 4.42 (*d*, J = 1.9 Hz), 5.05 (*m*), 5.13 (*bs*), 5.42 (*bs*), 5.99 (*d*, J = 2.2 Hz), 6.30 (*d*, J = 22 Hz). 6.68 (*s*), 6.87, 6.95, 7.10 and 7.16.

Epigallocatechin (4$\beta \rightarrow$8) *gallocatechin peracetate* (**12**). ^{13}C NMR: δ 26.5, 33.9, 68.6, 70.7, 73.4, 77.5, 107.3, 108.3, 110.8, 119.2, 119.6, 133.8, 134.6, 143.1, 143.2, 148.0, 149.3, 154.6, 154.7, 167–170. ^{1}H NMR: δ 1.6–2.35, 2.55 (*dd*), 3.20 (*dd*), 4.28 (*d*, J = 9.7 Hz), 4.43 (*d*, J = 1.9 Hz), 5.00 (*m*), 5.12 (*bs*), 5.30 (*m*), 5.45 (*bs*), 6.10 (*d*, J = 2.2 Hz), 6.29 (*d*, J = 2.2 Hz), 6.83 (*s*), 7.18 (*s*) and 7.26 (*s*).

Epigallocatechin (4$\beta \rightarrow$8) *epigallocatechin peracetate* (**13**). ^{13}C NMR: δ 26.5, 33.9, 66.6, 71.0, 73.6, 77.5, 107.2, 108.8, 110.4, 116.6, 116.7, 118.4, 119.2, 119.6, 134.0, 136.1, 143.0, 143.2, 148.0, 149.2, 154.0, 155.3, 166.8–170.6. ^{1}H NMR: δ 1.6–2.4, 2.88 (*m*), 4.43 (*m*), 4.47 (*m*), 5.11 (*m*), 5.54 (*m*), 6.07 (*d*, J = 2.2 Hz), 6.25 (*d*, J = 2.2 Hz), 6.65 (*s*), 6.90 (*s*), 7.21 (*s*), 7.26 (*s*).

Catechin (4$\alpha \rightarrow$2) *phloroglucinol* (**14**). Freeze-dried powder. R_f (A) 0.52, R_f (B) 0.61. ^{13}C NMR: δ 38.1, 73.1, 83.4, 95.8, 97.3, 100.6, 107.0, 115.2, 115.8, 120.7, 132.0, 145.4, 145.6, 157–158. ^{1}H NMR: δ 4.4–4.6 (*m*), 5.8–6.0, 6.7–7.0.

Epicatechin (4$\beta \rightarrow$2) *phloroglucinol* (**15**). Freeze-dried solid. R_f (A) 0.50, R_f (B) 0.60. ^{13}C NMR: δ 36.8, 72.4, 76.9, 95.7, 96.2, 97.1, 100.4, 106.8, 115.2, 115.6, 119.1, 132.2, 145.2, 145.4, 157.5, 157.6, 157.8, 158.3, 158.5. ^{1}H NMR: δ 3.99 (*s*, H-3), 4.54 (*s*, H-4), 5.07 (*s*, H-2), 5.94 (*s*, ArH), 6.02, 6.05 (each *d*, J = 2.3 Hz, H-6 and H-8), 6.70 (*dd*, J = 1.8, 8.18 Hz, H-6′), 6.79 (*d*, J = 8.18 Hz, H-5′), 7.0 (*d*, J = 1.8 Hz, H-2′).

Gallocatechin (4$\alpha \rightarrow$2) *phloroglucinol* (**16**). Freeze-dried powder. R_f (A) 0.40; R_f (B) 0.60. ^{13}C NMR: δ 38.1, 73.5, 84.2, 96.4, 96.3, 97.3, 100.6, 107.2, 108.1, 131.4, 133.4, 146.1, 157–158. ^{1}H NMR: δ 4.37 (*m*), 4.43 (*m*), 5.93 (*m*), 6.10 (*bs*), 6.58 (*s*).

Epigallocatechin (4$\beta \rightarrow$2) *phloroglucinol* (**17**). Freeze-dried powder. R_f (A) 0.32; R_f (B), 0.54. ^{13}C NMR: δ 36.7, 72.5, 76.9, 96.2, 96.3, 100.4, 106.7,

131.7, 132.7, 146.1, 157.6, 157.8, 158.0, 158.5, 158.6. ^{1}H NMR: δ 4.03 (*bs* H-3), 4.61 (*s*, H-4), 5.01 (*s*, H-2), 4.94, 6.04 (*bs* each, A-ring and phloroglucinol H), 6.50 (*s*, H-2′, H-6′).

Acknowledgements— The authors gratefully acknowledge the assistance of Dr Herbert Wong with the NMR data and Mr Jason Peters, AgResearch, Grasslands, for preparing crude extracts of plant material.

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