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FLAVAN AND PROCYANIDIN GLYCOSIDES FROM THE BARK OF BLACKJACK OAK

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Key Word Index—*Quercus marilandica*; Fagaceae; blackjack oak; bark; flavan-3-ols; procyanidins; glucoside; rutinoside; condensed tannin.

Abstract—The bark of blackjack oak contains (+)-catechin, (−)-epicatechin, (+)-3-*O*-[β-D-glucopyranosyl]-catechin, catechin-(4α→8)-catechin, epicatechin-(4β→8)-catechin as well as the novel 3-*O*-[β-D-glucopyranosyl]-catechin-(4α→8)-catechin and 3-*O*-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-catechin, as major components. The latter rutinoside of catechin is especially interesting because of the exceptionally small $J_{2,3}$ C-ring coupling. The glucoside of catechin-(4α→8)-catechin is only the second proanthocyanidin found in nature in which a 3-*O*-glycoside is present in the upper 'extender unit' of a dimer.

INTRODUCTION

Blackjack oak (*Quercus marilandica* Muenchh.) grows throughout the southeastern United States. Because of its small size and comparatively poor wood quality, it is not classified as a commercial tree species. A sampling of blackjack oak collected from across the southern United States suggested a typical tree would be 39 years of age, 6 inches in diameter, and only 33 feet in height [1]. Interest in the chemistry of blackjack oak has derived from the fact that this tree grows profusely in northeast Texas and northwest Louisiana where large-scale lignite mining was proposed. Whereas markets are readily available for pine and other commercial hardwoods, uses for large quantities of blackjack oak are not evident. This tree also is a problem in management of industrial pine plantations because it finds little commercial use when it must be cut during site preparation for southern pine planting. Commonly, the small, low-quality oaks are piled into windrows and burned.

Both the bark and heartwood (usually dark brown-purple) contain large amounts of phenolic extractives, and there has been speculation as to the possible value of the wood and bark as a source of chemicals. Some data on the 'total extractive content' (alcohol–benzene followed by ethanol extraction) indicated about 16.0 and 6.9% extractives in the bark and whole stemwood, respectively [2], and there are 'tannin' content estimates ranging from 7.7 to about 9.2% in bark [3]. No detailed analyses of the structure of the polyphenols in blackjack oak bark extracts were found; thus we undertook this investigation.

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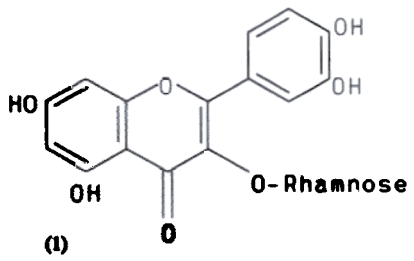
RESULTS AND DISCUSSION

Acetone–water extraction of the finely ground bark at ambient temperature gave a red-brown extract that, after partition between ethyl acetate and water, gave 1.05% ethyl acetate solubles and 8.76% water solubles. Cellulose TLC of the ethyl acetate-soluble extract suggested large amounts of catechin and several oligomeric procyanidins. The water-soluble fraction contained several compounds that gave a red coloration with vanillin–HCl at comparatively high R_f values in the 6% acetic acid dimension.

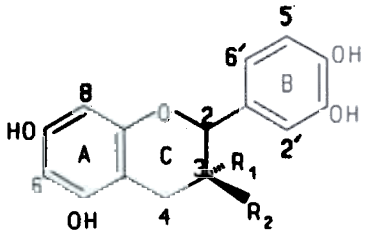
Separation of the ethyl acetate-soluble fraction on LH-20 Sephadex by elution with ethanol, combining fractions, and separating again with methanol–water mixtures afforded (+)-catechin (2), that, after acetylation with acetic anhydride in pyridine, gave an amorphous solid from preparative TLC that showed $[\alpha]_D$ +30.4°, ($CHCl_3$; *c* 1.0), (lit. +39.7°) [4], establishing the absolute stereochemistry as 2*R*,3*S*. Only small amounts of epicatechin (3) were observed (by cellulose TLC and ^{13}C NMR) as impurities in fractions containing (2), so it was not isolated.

The dominant dimeric procyanidin isolated from the ethyl acetate-soluble material was the common catechin-(4α→8)-catechin (procyanidin B-3) (4). Its identity was established by comparison of chromatographic properties and the ^{13}C NMR spectral data (Table 1) with the authentic compound synthesized by reaction of (+)-catechin with dihydroquercetin in the presence of sodium borohydride (Steynberg and Hemingway, unpublished results). A second dimeric procyanidin isolated from the ethyl acetate-soluble extract was the common epicatechin-(4β→8)-catechin (procyanidin B-1) (5). Chro-

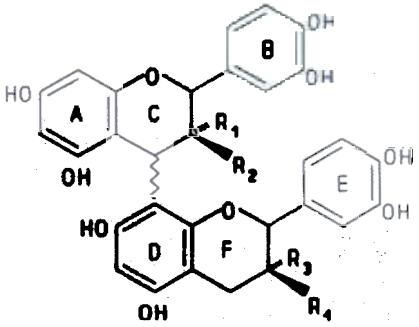
Oak Bark Flavan Glycosides



(1)



(2) R_1=H, R_2=OH
(3) R_1=OH, R_2=H
(6) R_1=H, R_2=O-β-D-glucose
(7) R_1=H, R_2=O-β-D-glucosyl-O-α-L-rhamnose



(4) R_1,R_2=H; R_3,R_4=OH, ⌇ = ····
(5) R_1,R_4=OH; R_2,R_3=H, ⌇ = —
(8) R_1,R_3=H, R_4=OH; R_2=O-β-D-glucose, ⌇ = ····
(9) R_1,R_3=H, R_2,R_4=OH, ⌇ = —
(10) R_1,R_4=H, R_2,R_3=OH, ⌇ = —

matographic properties and ^{13}C NMR spectrum were identical with 5 isolated previously from southern pine bark [5].

The comparatively large proportions of procyanidin derivatives in the methanol–water eluted fraction suggested the presence of flavan-glycosides. Therefore, this bark extract was much more like *Quercus miyagii* [6] than either *Quercus falcata* [7] (southern red oak) where large amounts of the yellow pigment quercetin-3-*O*-rhamnoside (1) were found or *Quercus dentata* [8] that was characterized by both prodelphinidins and their gallate esters. The wide variation in structures found in these *Quercus* species is particularly interesting.

The water-soluble extract was separated on LH-20 Sephadex by first eluting with methanol–water (1:1) to recover a carbohydrate-rich fraction (also containing flavan- and procyanidin-glycosides) and then acetone–water (1:1) to remove absorbed high molecular weight procyanidins. Repeated LH-20 Sephadex column chromatography of the methanol–water eluted fraction with different methanol/water ratios afforded three flavan-glycosides. One of these compounds has ^{13}C NMR spectral data (Table 1) essentially identical to those reported for 3-*O*-[β-D-glucopyranosyl]-catechin (6) [6]. This compound was first synthesized by Weinges and Seiler [9] and later isolated from *Quercus miyagii* by Nishioka and co-workers [6]. Negative ion FAB-mass spectrometry gave a strong $[M-H]^-$ peak at *m/z* 409, as expected for this compound, so it was not investigated further.

The ^{13}C NMR spectral data (Table 1) and high resolution FAB-mass spectrometry [*m/z* 599.1946, consistent for $C_{27}H_{35}O_{15}$] of a second flavan-glycoside (7) isolated from this fraction, indicated both glucose and rhamnose together with catechin in the molecule. The ^{1}H NMR spectral data (Table 2) showed that this compound was a rutinoside [α-L-rhamnopyranosyl-(1→*O*→6)-β-D-glucopyranosyl] derivative of catechin (7). The essential features of the structure of 7 were established through 45° COSY and NOE experiments following procedures used by Steynberg and co-workers [10], who proved the structure of a stilbene-rutinoside (*E*)-3,4′-dimethoxy-5-*O*-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]stilbene in a similar manner.

An α-L-rhamnopyranosyl unit was indicated by the anomeric H-1 at δ4.73, with J = 2.0 Hz and the methyl at δ1.20 with J = 6.0 Hz. These assignments were supported by the 45° COSY spectrum that showed correlation between the rhamnose methyl and the double doublets at δ3.68, with J = 3.8 and 10.0 Hz and at δ3.63, with J = 6.0 and 10.0 Hz for the rhamnose H-3 and H-5, respectively. An α-linkage between the C-1 of the rhamnosyl and C-6 of the glucosyl unit was established by irradiation of the anomeric rhamnose proton at δ4.73 that resulted in enhancement of the H-4 of the rhamnose at δ3.38 and conspicuous absence of NOE to the rhamnose H-3 and H-5 as well as the strong NOE of the H-6 of glucose at δ3.54.

Substitution of (+)-catechin with a glycopyranosyl unit was indicated by the anomeric H-1 at δ4.30, with J = 8.0 Hz and by H-4 at δ3.25, with J = 9.0 Hz. The 45° COSY spectrum also showed that the H-2, H-3, H-4 and H-5 (Table 2) of the glucosyl unit were all in the narrow range of δ3.1 to 3.4, and these, as in earlier work [10], were elucidated from a 2D J-resolved experiment. An *O*-β-linkage of the glucosyl unit to the C-3 of the catechin heterocyclic ring was established from a NOE experiment that showed association of the anomeric glucose proton at δ4.30 with the catechin C-ring H-2 and H-3 protons at δ4.17 and 4.90 as well as to the H-3 and H-5 of the glucose at δ3.32 and 3.39 (i.e. diaxial), respectively. The CD spectrum showed molecular ellipticity maxima as a weak $\theta = 1.863E+0.3$ at 282 nm, a weak $\theta = +6.939E+0.2$ at 253.5 nm, and a strong $\theta = -5.264E+0.3$ at 230 nm indicating the expected 2*R*,3*S* absolute stereochemistry.

The ^{13}C NMR spectral data (Table 1) were assigned through the assistance of a C–H HETCOR spectrum. In

Table Selected ^{13}C NMR assignments* for flavan- and procyanidin-glycosides

Flavan Sugar	C-6 C-1	C-8 C-2	C-2 C-3	C-3 C-4	C-4 C-5	C-1' C-6	C-2'	C-6'
(2) (+)-Catechin[A]	96.2	95.4	82.3	68.4	28.0	132.1	115.1	119.8
(4) Catechin-(4α→8)-	97.1	96.1	83.5	73.2	38.3	132.8	115.3	120.1
catechin[A]	97.1	108.1	82.1	68.4	28.2	131.7	115.3	120.9
(5) Epicatechin-(4α→8)-	97.0	95.8	76.8	73.0	37.1	132.8	115.5	119.8
catechin[A]	96.5	108.0	82.5	68.5	28.2	132.2	115.5	120.3
(6) 3-(*O*-β-D-glucosyl)-	104.0	75.1	77.5	71.6	77.8	62.8		
catechin[M]	—	—	80.2	76.2	26.0	—	115.5	120.1
(7) 3-*O*-[α-L-Rhamnosyl-1-	101.3	71.1	71.6	73.1	68.9	17.9		
6-*O*-β-D-glucosyl]-	103.5	74.2	76.3	70.7	75.8	67.5		
catechin[A]	94.9	96.0	79.0	75.8	25.8	131.3	114.3	120.1
(8) 3-*O*-β-D-Glucosyl-	103.2	75.4	77.1	71.0	77.1	62.5		
catechin-(4α→8)-	97.3	96.8	82.4	81.1	37.3	131.7	115.4	120.7
catechin[M]	96.2	109.0	82.4	68.8	28.7	132.1	116.0	119.8

*^{13}C NMR spectral data (δ A = d_6-acetone/D_2O 30.3; M = d_4-MeOH/D_2O 49.9). Carbons not assigned are indicated by (—).

Table 2. Selected ^{1}H NMR assignments for flavan- and procyanidin-glycosides

	Unit	H-1	H-2	H-3	H-4	H-5	H-6
7	Catechin						
	δ		4.90	4.17	2.70 (2H)		
	J		6.0	6.0	6.0		
	Glucose						
	δ	4.30	3.07	3.32	3.25	3.39	3.54, 3.94
	J	8.0	8.0, 8.5	8.5, 8.5	8.5, 9.0	*br m*	6.0, 9.0
	Rhamnose						
	δ	4.73	3.89	3.68	3.38	3.63	1.20
	J	2.0	2.0, 3.8	3.8, 10.0	10.0, 10.0	6.0, 10.0	6.0
8	Catechin						
	δ		4.21	4.48	4.37		5.81
	J		10.0	7.0, 10.0	7.0		2.0
	Glucose						
	δ	3.33	2.73	2.83	2.99	2.59	3.49, 3.33
	J	7.5	7.5, 9.0	9.0, 9.0	9.0, 9.0	*br m*	3.0, 12.0; 4.0, 12.0
	Catechin						
	δ		4.51	3.68	2.62; 2.40		6.08
			7.0	*br m*	5.5, 16.0		
					8.0, 16.0		

^{1}H NMR spectral data (7 in d_6-acetone/D_2O; 8 in d_4-MeOH/D_2O).

d_6-acetone–D_2O, the rhamnose C-6 methyl is at δ17.9 and the C-4 of the catechin C-ring is at δ25.8. In addition, the carbon resonances at δ96.1 and 94.9 ppm were assigned to the C-8 and C-6 A-ring carbons through correlation with the *meta*-coupled (J = 2.5 Hz) protons at δ5.85 and 5.97, thus showing that C-glycosylation did not occur on the A-ring.

The assignments in which the C–H HETCOR experiment was more important include C-2 and C-3 of the heterocyclic C-ring of the catechin unit and the two sugar units. The C-2 of the C-ring appears at δ79.0 as evidence for correlation of this signal with the proton doublet at δ4.90 (J = 6.0 Hz). Substitution at C-3 is evident from the downfield shift (Δδ = 7.4 ppm) of the C-3 signal to δ75.8 that was correlated with the proton multiplet at δ4.17 assigned to the proton at C-3 of the C-ring.

Carbon signals assigned to glucose included those at: δ67.5 for C-6; δ75.8 correlated with a broad multiplet at δ3.39 for C-5; δ70.7 correlated with a triplet (J = 9.0 Hz) at δ3.25 for C-4; δ76.3 correlated with a triplet (J = 9.0 Hz) at δ3.32 for C-3; δ74.2 correlated with a double doublet at δ3.07 (J = 8.0 and 9.0 Hz) for C-2; and the signal at δ103.5 correlated with the doublet at 4.30 (J = 8.0 Hz) for C-1. Assignment of the carbon resonances for rhamnose were similarly based on the C–H HETCOR experiment including those at: δ68.9 correlated with the double doublet

($J = 6.0$ and 10.0 Hz) at $\delta 3.63$ assigned to C-5; $\delta 73.1$ to C-4 (no clear correlation); $\delta 71.6$ to the double doublet ($J = 3.8$ and 10.0 Hz) at $\delta 3.68$ to C-3; and $\delta 71.1$ to C-2. The carbon signal at $\delta 101.3$ was clearly correlated with the proton at $\delta 4.73$ with $J = 2.0$ Hz and therefore assigned to the rhamnose C-1.

This compound is especially interesting from a conformational viewpoint. The comparatively small $J_{2,3}$ coupling observed for the catechin unit (6.0 Hz) suggested that this compound exists in solution with a high proportion of A-conformation [11], possibly because of the lower steric energy achieved by having the catechol B-ring and the bulky rutinosyl substituent diaxial as was observed previously in penta-*O*-acetyl-(+)-catechin [12]. At the same time, a NOE experiment showed weak but significant enhancement of the H-2, H-5 and H-6 of the catechol B-ring as well as the H-2 of the rhamnose by irradiation of the glucose H-6 signal that was buried in the DOH signal, implying equatorial orientations of the B-ring and the rutinosyl substituent. These results might be explained by either a distorted C-ring conformation stabilized by hydrogen bonding or to A/E flipping with a time averaged high proportion of A conformer in the C-ring [13].

To examine that possibility, the conformations of **2**, **6** and **7** were studied using the MMX forcefield in PC Model. Optimization of **7** with hydrogen bonding considered showed that a large number of differing conformations (A, E, as well as intermediate distorted C-ring conformations) gave similar (± 2 kcal mol^{-1}) total steric energy. Because no conformation appears to be highly favoured, the conclusion reached from this work suggests that the molecule is flexible and can adopt a wide range of conformations (including A and E conformers) that, when averaged, result in the 6 Hz $J_{2,3}$ coupling constant. Because of the unusually small coupling constant, molecular modelling work continues on this compound.

The ^{13}C NMR spectrum of the third glycoside (**8**) suggested that it is a glucoside of catechin-(4$\alpha \rightarrow$8)-catechin (Table 1). The high-resolution FAB-mass spectrum showed a *m/z* of 741.2056 consistent with $C_{36}H_{37}O_{17}$ required for a monoglucoside of a biscatechin dimer. The ^{1}H NMR spectrum was consistent with such a structure but showed the presence of two rotameric forms in a relative proportion of 59:41 when recorded in d_6-acetone–D_2O. Interestingly, and fortunately, **8** existed in predominantly one rotameric form when dissolved in d_4-methanol (Table 2). The ^{1}H NMR spectrum (Table 2) was assigned through the assistance of a 45° COSY spectrum recorded in d_4-methanol. This spectrum showed correlation of the C-ring H-2 doublet ($\delta 4.21$ $J_{2,3} = 10.0$ Hz) and H-4 doublet ($\delta 4.37$ $J_{3,4} = 7.0$ Hz) with the H-3 double doublet at $\delta 4.48$. The F-ring H-2 doublet is superimposed on the latter signal as is shown by correlation with the multiplet at $\delta 3.68$, that in turn is correlated with H-4$_{ax}$ and H-4$_{eq}$ double doublets at $\delta 2.42$ and 2.62.

The 45° COSY and C–H HETCOR spectra (with ^{13}C NMR assignments as shown in Table 1) also support the assignment of the A-ring C-6 and C-8 *meta*-coupled protons at $\delta 5.66$ and 5.77 ($J = 2.5$ Hz) as well as ^{13}C assignments of $\delta 97.3$ and 96.8, respectively, with the H-6 (D) proton appearing as a singlet at $\delta 5.94$ and the carbon resonance at $\delta 96.2$ (Table 2). The C–H HETCOR experiment permitted unequivocal assignment of the C-3 of glucose at $\delta 77.1$, the C-3 of the upper chain extender unit (C-ring) at $\delta 81.1$ and the C-2 of the chain extender and terminal unit (F-ring) superimposed at $\delta 82.4$. In addition, assignments for the protons of the B- and E-rings were evident from correlations between the double doublets of H-6 (B) and H-6 (E) at $\delta 6.33$ and 6.15 and the carbon resonances at $\delta 120.7$ and 119.8 with the *ortho*-coupled H-5 (B) and H-5 (E) signals at $\delta 6.56$ and 6.52 and carbon resonances almost superimposed at 116.0, respectively. The assignments of the H-2 (B) and H-2 (E) resonances then follow at $\delta 6.63$ and 6.49 with carbon resonances at $\delta 116.5$ and 115.4, respectively.

Assignment of the *O*-β-linkage of the glucosyl unit to the C-3 of the upper unit was made through NOE experiments. Irradiation of the glucose H-1 showed strong NOE with the glucose H-3 and H-5 at $\delta 2.83$ and 2.59 [proving a glucopyranosyl unit], and 3-*O*-β substitution of the upper chain extender unit (C-ring) was evident from the strong NOE between the glucose H-1 and the C-ring H-3 and H-4 at $\delta 4.48$, $J = 7.0$ and 10.0 Hz and 4.37, $J = 7.0$ Hz, respectively. Nishioka and co-workers isolated two rhamnosides of catechin-(4$\alpha \rightarrow$8)-catechin, one substituted at the C-3 of the C-ring and the other at the C-3 of the F-ring from *Quercus miyagii* [6]. Analogous glucosides have also been reported as natural products by Nishioka's group [14].

Compound **8** was methylated and acetylated to provide a derivative that would allow assignment of the location of the interflavanoid bond. A NOE experiment showed that irradiation of the H-6 (D) singlet at $\delta 6.14$ resulted in the enhancement of two methoxyl signals at $\delta 3.77$ and 3.88, thus confirming a (4$\rightarrow$8) interflavanoid bond.

A CD spectrum showed ellipticity maxima $\theta(-5.948E + 0.3)$ at 289 nm, a broad but very weak (+) shoulder between 279 and 251 nm, and a very strong negative Cotton effect ($-4.826E + 0.4$) at 226 to about 228 nm indicating a 4α (C-ring) orientation of the DEF flavanyl moiety. When taken in conjunction with the ^{1}H NMR coupling constants of the C-ring protons, the latter Cotton effect then establishes the 2*R*,3*S*,4*R* absolute stereochemistry of this heterocyclic ring.

An interesting feature of **8** is that this compound has a (4$\alpha \rightarrow$8) interflavanoid bond, in contrast to the corresponding rhamnoside [15], **8** shows only one rotational isomer in spectra recorded in d_4-methanol, and the C-ring conformation in **8** must be distorted toward a C-2 sofa conformation to account for the coupling constants of $J_{2,3} = 10.0$; $J_{3,4} = 7.0$ Hz. When the spectra are recorded in d_6-acetone-D_2O two rotamers in approximate proportions of 59:41 are observed for **8**. In addition, a NOE experiment in which irradiation of the glucose H-1 at $\delta 3.33$ resulted in enhancement of both the C-ring H-3 and H-4 at $\delta 4.48$ and 4.36, a result that would be expected from a C-2 sofa conformation. Procyanidins with 2,3-

trans-3,4-*cis* stereochemistry are rarely found, but Kolodziej [16] and Delcour [17] have described catechin-(4β→8)-catechin (**9**) and catechin-(4β→8)-epicatechin (**10**) as acetate and methyl ether acetate derivatives where coupling constants were $J_{2,3}=9.6$; $J_{3,4}=6.5$ and $J_{2,3}=9.5$; $J_{3,4}=5.5$ Hz, respectively. The 2,3-*trans*-3,4-*trans* stereochemistry of **8** is of interest in comparison to coupling constants observed in **9** and **10**. It is clear that an unequivocal assignment of the stereochemistry of these compounds cannot be made on the basis of heterocyclic ring coupling constants.

Previous work on the conformation of penta-*O*-acetyl-(+)-catechin and the discussion on the conformation of **7** above show that bulky substituents at C-3 force these compounds to assume an 'A-conformation' (a reverse half-chair heterocyclic ring conformation in which both the pyrocatechol B-ring and the C-3 substituents are axial) [11, 12]. If the chain extender precursor had a glucosyl group at C-3, the carbocation (or quinone methide) intermediate could assume an A-conformation making the 4β substitution more favourable to a nucleophile. However, if glucosylation occurred after formation of the procyanidin dimer, one would expect to find the (4α→8) linked isomer as observed in isolation of **4** and **8** from this extract.

In conclusion, blackjack oak bark contains substantial amounts of procyanidins that might find uses similar to those proposed for other conifer bark tannins such as southern pine. No gallic acid or hydrolysable tannins were isolated. The conformation of **7** is particularly interesting because it serves as a good example of the effect of hydrogen bonding forcing a distorted conformation of the heterocyclic C-ring. The 2,3-*trans*-3,4-*trans* stereochemistry of **8** is important with respect to its possible biosynthesis. Despite the predominant polymeric procyanidin structure, the comparatively high proportion of carbohydrate and especially the presence of flavan- and procyanidin-glycosides may limit the usefulness of these bark extracts in wood adhesive applications.

EXPERIMENTAL

The whole bark was removed from a composite sample of blackjack oak stems with a draw knife and air-dried in the dark. The dried bark was ground in a Wiley mill and extracted with Me_2CO–H_2O (1:1), for several days in aluminum foil covered jars. The Me_2CO was removed on a rotary evaporator and the aqueous suspension was extracted repeatedly (four to five times) with an equal volume of EtOAc. The EtOAc-soluble and H_2O-soluble fractions were freeze-dried.

Cellulose TLC was on Baker-flex plates cut to 6.5 × 6.5 cm and developed with t-BuOH–HOAc–H_2O (TBA) (3:1:1) in the first dimension followed by 6% HOAc (6-AA) in the second dimension. Most plates were visualized by spraying with vanillin–HCl, but some were sprayed with either ferric chloride/potassium ferricyanide or diazotized nitroaniline. Column chromatography was performed using both large 2.5 × 80.0 cm (for initial separations) and 1.0 × 50.0 cm columns packed with LH-20 Sephadex pre-swollen in the appropriate solvent. EtOH and MeOH–H_2O mixtures of various proportions were used as eluting solvents. Typically, every third fraction was spotted on a cellulose TLC plate developed with 6-AA to monitor the elution of the compounds from the columns.

^{13}C NMR spectra were recorded in d_4-CD_3OD or d_6-Me_2CO with D_2O added in amounts needed to keep the compound soluble. For the flavan-glycosides, both ^{1}H and ^{13}C NMR experiments also were made using a 300 MHz spectrometer and to perform a number of 2D NMR experiments (COSY, HETCOR, *J*-resolved) to assign spectra. Mass spectral determinations were made at the Midwest Center for Mass Spectrometry with partial support by the National Science Foundation, Biology Division (Grant No. DIR9017262). CD spectra were recorded in MeOH scanning the range of 200–360 nm and calculating molecular ellipticity θ as M_rs were known from MS and NMR data.

(+)-*Catechin* (**2**). R_f 0.60 and 0.50 on cellulose TLC in TBA and 6-AA, respectively. After acetylation with acetic anhydride and isolation on prep. silica gel TLC (sol. = C_6H_6–Me_2CO, 9:1, 2X) obtained an amorphous solid $[\alpha]_D$ + 30.4° ($CHCl_3$; *c* 1.0). ^{13}C NMR: Table 1.

(−)-*Epicatechin* (**3**). R_f 0.55 and 0.45 on cellulose TLC in TBA and 6-AA, respectively, was isolated only in enriched form with (+)-catechin impurities.

Catechin-(4α→8)-*catechin* (**4**). R_f 0.55 and 0.41 on cellulose TLC in TBA and 6-AA, respectively. ^{13}C NMR: Table 1.

Epicatechin-(4β→8)-*catechin* (**5**). R_f 0.47 and 0.58 on cellulose TLC in TBA and 6-AA, respectively. ^{13}C NMR: Table 1.

3-O-[β-D-*glucopyranosyl*]-*Catechin* (**6**). R_f 0.50 and 0.59 on cellulose TLC in TBA and 6-AA, respectively. ^{13}C NMR: Table 1. Negative ion FAB-MS $[M-H]^-$ m/z = 409.

3-O-[α-L-*rhamnopyranosyl*-(1→6)-β-D-*glucopyranosyl*]-*Catechin* (**7**). A light tan amorphous solid, R_f 0.51 and 0.76 on cellulose TLC in TBA and 6-AA, respectively. ^{13}C NMR: Table 1; ^{1}H NMR: Table 2. Positive ion FAB-MS $[M+H]=599$, high resolution FAB-MS m/z = 599.1946 consistent for $C_{27}H_{35}O_{15}$. CD (2.20 mg/25 ml MeOH): 214 nm, $\theta=-2.995E+02$; 230 nm, $\theta=-5.264E+0.3$; 248 nm, $\theta=-1.478E+0.2$; 253.5 nm, $\theta=+6.939E+0.2$; 265.5 nm, $\theta=+5.682E\rightarrow0.1$; 282.0 nm, $\theta=-1.863E+0.3$.

3-O-[β-D-*glucopyranosyl*]-*Catechin*-(4α→8)-*catechin* (**8**). A light tan amorphous solid, R_f 0.47 and 0.62 on cellulose TLC in TBA and 6-AA, respectively. ^{13}C NMR: Table 1; ^{1}H NMR: Table 2. FAB-MS $[M+H]=741.2$, high resolution FAB-MS m/z = 741.2056 consistent with $C_{36}H_{37}O_{17}$. CD (1.93 mg/25 ml MeOH): 217.5 nm, $\theta=-1.208E+0.3$; 226.0 nm, $\theta=-4.826E+0.4$; 228.0 nm, $\theta=4.769E+0.4$; 251.0 nm, $\theta=+1.617E+0.2$; 279.0 nm, $\theta=+1.418E+0.2$; 289 nm $\theta=-5.948E+0.3$.

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