Phytochemistry, Vol. 27, No. 10, pp. 3277–3280, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00

POTENTILLANIN, A BIFLAVANOID AND A PROCYANIDIN GLYCOSIDE FROM *POTENTILLA VISCOSA**

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(*Received* 26 *February* 1988)

Key Word Index—*Potentilla viscosa*; Rosaceae; potentillanin; bisflavanoid; flavan-3-ol glycoside; procyanidin glycoside; proanthocyanidin.

Abstract—An investigation of the root of *Potentilla viscosa* has led to the isolation and characterization of a novel 6′,8-linked bisflavanoid, potentillanin and a procyanidin B-3 3′-*O*-glucoside. Furthermore, the occurrence of (+)-catechin and its 3-*O*-glucoside, procyanidins B-3 and C-2 and afzelechin-(4α→8)-catechin was demonstrated.

INTRODUCTION

In continuing our chemical investigation of tannins in Rosaceous plants, we have isolated (+)-catechin glycoside (**2**), procyanidin B-3 glycoside (**5**) and potentillanin (**7**), together with (+)-catechin and dimeric and trimeric proanthocyanidins consisting entirely of 2,3-*trans* flavan-3-ol units, from the root of *Potentilla viscosa*, a herb growing in the northern areas of China. The isolation of potentillanin (**7**) is particularly significant, as the 6′,8-binding of two flavan-3-ols is unique although 4,8-or 4,6-linked bisflavanoids (proanthocyanidins) occur very commonly in the plant kingdom. The occurrence of flavan-3-ol and procyanidin glycosides as major metabolites is of chemotaxonomic importance, since in contrast to ubiquitous flavonoid glycosides such compounds are rarely found in Nature.

RESULTS AND DISCUSSION

The aqueous acetone extract of the roots of *Potentilla viscosa* was initially chromatographed on Sephadex LH-20 with water containing increasing proportions of methanol [2] to afford three fractions. The first fraction containing a mixture of phenolic glycosides and dimeric and trimeric proanthocyanidins was separated by repeated Sephadex LH-20 (EtOH, 80% MeOH), MCI-gel (H_2O–MeOH) and Fuji-gel (H_2O–MeOH) chromatographies to furnish compounds **2** and **4**–**7**. The ^{1}H NMR spectra of **4** and **6** showed extremely complicated signal patterns, indicating the occurrence of intramolecular steric interactions [3, 4]. However, relatively large coupling constants of each aliphatic signal suggested the presence of flavan-3-ol units with 2,3-*trans* configuration. In addition, from the R_f values on TLC, **4** and **6** were considered to be dimeric and trimeric procyanidins, respectively. Final structural assignments of these compounds to procyanidins B-3 [3] and C-2 [5] were made by comparing their ^{1}H NMR data with those of authentic samples.

The ^{1}H and ^{13}C NMR spectra of the major compound (**2**) closely resembled those of (+)-catechin (**1**), except for the presence of additional signals in the aliphatic regions. The appearance of an anomeric proton signal at δ 4.32 (*d*, $J=7$ Hz), as well as six aliphatic carbon signals the chemical shifts being similar to those of methyl β-D-glucoside, suggested that **2** is a glucoside of (+)-catechin. Furthermore, the fairly lowfield shift (δ 77.3) of the C-3 signals, as compared with that (δ 68.0) of **1**, clearly indicated the location of the glucose moiety at the C-3 position. The identity of **2** with (+)-catechin 3-*O*-β-D-glucopyranoside was confirmed by comparison of the $[\alpha]_D$ and ^{1}H and ^{13}C NMR spectra with those of a sample [6] recently isolated from *Quercus miyagii*.

Compound **5** gave, on treatment with HCl, a red colour characteristic of proanthocyanidins. The ^{1}H and ^{13}C NMR spectra were closely correlated with those of **4**, showing similar complex signal patterns, but differed clearly in the observation of sugar signals. Degradation of **5** with acid in the presence of benzylmercaptan [5] afforded (+)-catechin 3-*O*-glucoside (**2**) and (+)-catechin 4-benzylthioether, the latter being characterized by its conversion with Raney nichel into (+)-catechin. Since it is evident that the former compound is derived from the lower part, the location of the glucose moiety could be established to be at the lower catechin unit. In addition, close similarities of aliphatic signal patterns (δ4.0–5.0) in the ^{1}H NMR spectra of **5** and **4** concluded the mode and the point of the linkage of the two units to be 4α-8. Thus, **5** was characterized as catechin-(4α → 8)-catechin 3-*O*-β-D-glucopyranoside. An attempt to cleave the sugar linkage with acids or enzymes was unsuccessful, but the presence of the glucose moiety can be readily deduced from the ^{1}H NMR spectrum which characteristically shows a pair of anomeric doublets at δ 5.05 and 5.14 (1H in total, each

*Part 70 in the series 'Tannins and Related Compounds'. For Part 69 see ref. [1].

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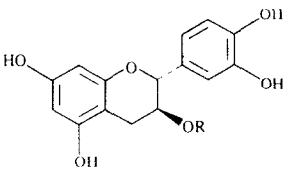
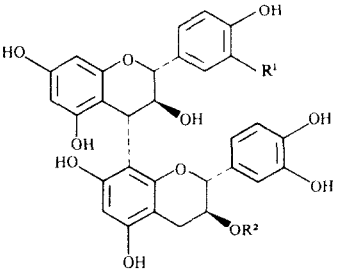
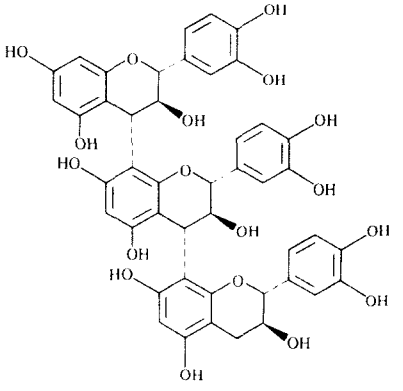


R
1 H
2 Glc



	R^1	R^2
3	H	H
4	OH	H
5	OH	Glc



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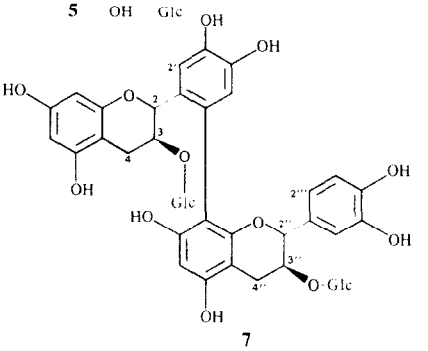


7

6 Hz), despite the duplicated signal patterns in the regions of δ 2.5–4.5 and 5.7–7.1

Compound **7** [negative FABMS *m/z*: 901 $(M-H)^-$] did not form an anthocyan pigment on treatment with acid. The ^{13}C NMR spectrum suggested **7** to consist of 2 mol of **2**, showing a similar but duplicated signal pattern. The major chemical shift differences in the ^{13}C NMR spectra were the lowfield shifts of C-8 (or C-6)(δ 107,8) and C-6′ (δ 124.2) signals which appeared as singlets in the off-resonance spectrum, suggesting that the two catechin units are linked through a carbon–carbon linkage at these positions. In the ^1H NMR spectrum, the appearance of each pair of signals due to H-2 (δ 5.58, 5.01), H-3 (δ 4.25, 4.21) and H-4 (δ 2.95, 2.86 and 2.72, 2.24) further supported the presence of two flavan-3-ol units, while the observation of one A-ring singlet at δ6.33 and two B-ring singlets at δ 6.65 and 6.69 clearly indicated the point of the interflavanoid linkage to be at the 6′-8 (or 6′-6) position. However, the coupling constant (almost 0 Hz) of the H-2 signal at δ 5.58 was inconsistent with the 2,3-*trans* configuration. Examination of the Dreiding model suggested that in the case of the normal half-chair conformation of the 'upper' C-ring, there is a strong steric interaction between the sugar moiety in the upper unit and the lower A-ring, and the upper C-ring therefore prefers the half-boat conformation. Furthermore, in order to elucidate the stereochemistry, the NOE correlation spectrum was measured, and the results are summarized in Fig. 1. The correlation between the H-2 and the sugar anomeric proton signal clearly indicated that glucose is located at the C-3 position and also that the two flavan-3-ol units are of (+)-catechin (2,3-*trans*) -type. In addition, the observation of NOE between the signals of H-3 (upper unit) and H-2′ (lower unit) confirmed the 6′-8 linkage of the two flavan units.

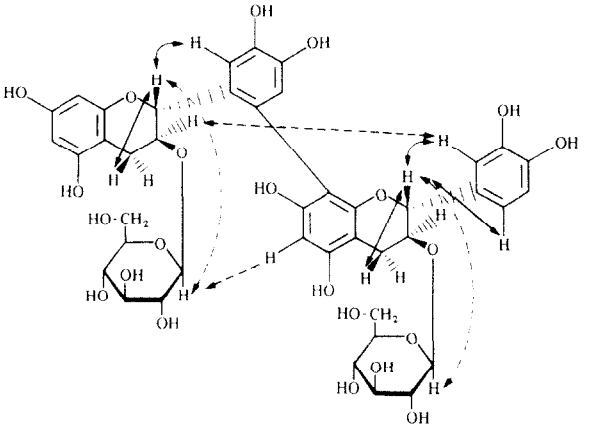
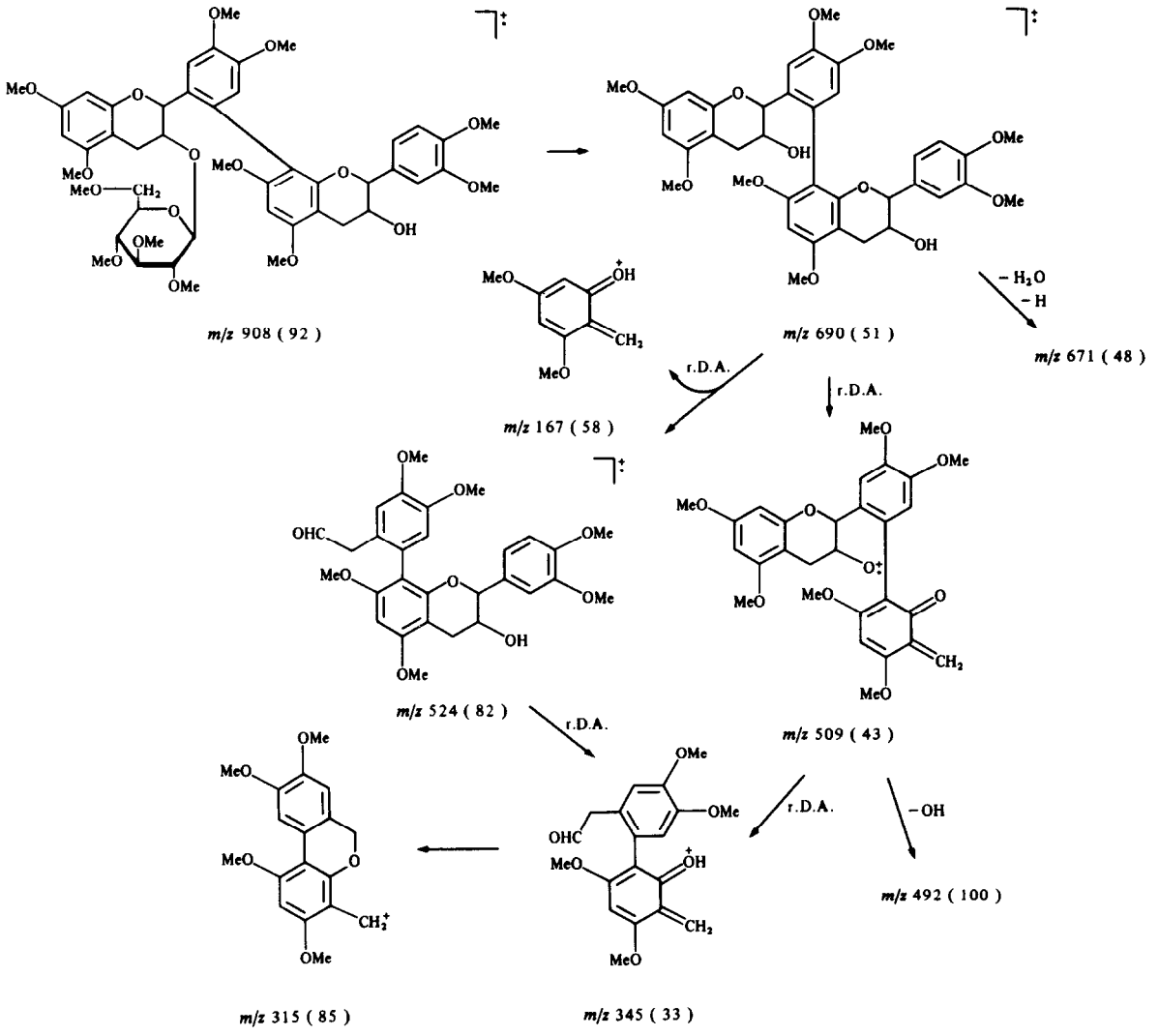


Fig. 1. Observed NOEs and long-range couplings for potentillanin (7) (←——→: NOE; ⟷: long-range coupling).

Further structural support was obtained by EIMS analysis of the partial hydrolysate (**7a**) of the permethyl ether. The molecular ion peak and highly characteristic peaks were identified and rationalized as shown in Scheme 1. Among these, the observation of prominent peaks at *m/z* 345 and 315, originated by retro-Diels–Alder-type fissions of the two C-rings, indicated that the two flavan units are bonded at the B-and A-rings through a carbon-carbon linkage.

Based on these findings, potentillanin has structure **7**. It should be noted that the glucose moiety in the upper unit resisted hydrolysis with acids and enzymes.

The second fraction consisted of (+)-catechin (**1**) and a proanthycyanidin dimer (**3**), which were separated by



Scheme 1. Diagnostic fragment ions (relative intensities in parentheses) in the EIMS of **7a**.

Sephadex LH-20 chromatography in ethanol. The ^{1}H NMR spectrum of **3** was quite similar to that of **4**, except for the aromatic resonances arising from the B-ring. The observation of a doublet at δ 7.25 (J = 8 Hz), although the other signals were complicated by conformational isomerism, suggested the presence of one afzelechin-type unit in the molecule. Finally, the structure (**3**) was confirmed by comparison of its $[\alpha]_D$ and ^{1}H NMR data with those of an authentic samples [7]. The final fraction was found to consist of proanthocyanidin polymers, and was not examined further.

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR spectra were recorded at 100 (in part 270) and 25.05 MHz, respectively, with TMS as ref. Negative FABMS were measured at 2 kV (ion source accelerating voltage) with glycerol as matrix. TLC was conducted on silica gel with a solvent system of C_6H_6–HCO_2Et–HCO_2H (1:7:1), and spots visualized by a $FeCl_3$ spray and the anisaldehyde–H_2SO_4 reagent.

Plant material. Potentialla viscosa was collected near Tumen City, Yan Bian Autonomous District, Jilin Province, China, and was identified by Prof. Deng Ming-lu and Prof. Gao Shi-xian, Changchun Traditional Chinese Medicine College. A voucher specimen is deposited at the Herbarium, Faculty of Pharmaceutical Sciences, Kyushu University.

Extraction and isolation. Air-dried, ground material (7.46 kg) of *P. viscosa* was extracted × 3 with 60% aq. Me_2CO at room temp. and the concd extract, after removal of ppt. by filtration, was subjected to Sephadex LH-20 CC. Elution with H_2O containing increasing amounts of MeOH yielded three fractions. The first fraction was repeatedly CC on Sephadex LH-20 (EtOH and 80% aq. MeOH), MCI-gel (H_2O–MeOH) and Fuji-gel ODS G3 (H_2O–MeOH) to give compounds **2** (4.77 g), **4** (7 mg), **5** (141 mg), **6** (70 mg) and **7**(390 mg). The second fraction was subjected to Sephadex LH-20 CC with EtOH to afford compounds **1** (2.8 g) and **3** (100 mg).

Potentillanin (7). An off-white amorphous powder, $[\alpha]_D^{18}$ −80° [Me_2CO–H_2O (1:1); c 1.0]. Negative FABMS m/z (rel. int.): 901 $[M-H]^-$ (17.2), 739 $[M-glc]^-$(2.7), 721 (2.8). ^{1}H NMR (270 MHz, Me_2CO-d_6 + D_2O): δ 2.24 (1H, *dd*, J = 4, 17 Hz, H-4), 2.72

(1H, *d*, $J = 17$ Hz, H-4), 2.86 (1H, *dd*, $J = 16$, 6 Hz, H-4″), 2.95 (1H, *dd*, $J = 16$, 4 Hz, H-4″), 3.0–4.0 (glc-H), 3.79 (1H, *d*, $J = 8$ Hz, glc. H-1), 4.21 (1H, *m*, H-3), 4.25 (1H, *m*, H-3″), 4.38 (1H, *d*, $J = 8$ Hz, glc. H-1), 5.01 (1H, *d*, $J = 5$ Hz, H-2″), 5.58 (1H, *s*, H-2), 5.99 (1H, *d*, $J = 2$ Hz, H-6), 6.02 (1H, *d*, $J = 2$ Hz, H-8), 6.33 (1H, *s*, H-6″), 6.65 (1H, *s*, H-5′), 6.69 (1H, *s*, H-2′), 6.72 (1H, *dd*, $J = 7$, 2 Hz, H-6″), 6.82 (1H, *d*, $J = 7$ Hz, H-5‴), 6.85 (1H, *d*, $J = 2$ Hz, H-2‴). ^{13}C NMR (Me_2CO–$d_6 + D_2O$): δ 22.3 (*t*, C-4), 25.7 (*t*, C-4″), 61.4, 62.3 (*t*, glc. C-6), 70.3, 71.0 (*d*, glc C-4), 73.7, 74.0 (*d*, glc C-2), 74.5, 75.2 (*d*, C-3), 76.5, 76.9 (x2), 77.2 (x2) (*d*, C-2 and glc C-3, 5), 79.5 (*d*, C-2″), 94.8 (*d*, C-8), 96.1 (*d*, C-6), 96.4 (*d*, C-6″), 99.0, 100.7 (*s*, C-4a, 4a″), 102.7, 103.0 (*d*, glc C-1), 107.8 (*s*, C-8″), 113.1, 114.7, 116.3, 120.5 (*s*, C-2′, 5′, 2″, 5″), 119.6 (*s*, C-6‴), 124.2 (*s*, C-6′), 130.9 (*s*, C-2′), 131.9 (*s*, C-2‴), 144.2, 144.6, 145.4, 145.6 (*s*, C-3′, 4′, 3‴, 5‴), 153.9, 154.0, 155.7, 155.9, 157.0, 157.3 (*s*, C-5, 7, 8a, 5″, 7″, 8a″). (Found: C, 52.52; H, 5.50. $C_{42}H_{46}O_{22}{\cdot}3H_2O$ requires: C, 52.53; H, 5.46%.)

Enzymatic hydrolysis of **7**. A soln of **7** (50 mg) in Ac buffer (pH 4.5) was incubated with snail acetone powder (prepared from *Helix pomatia*, Sigma) at 40° for 4 days. The solvent was evapd off under red. pres., and the residue treated with MeOH. The MeOH-soluble portion was CC on MCI-gel with H_2O containing increasing proportions of MeOH to give the mono-desgalloyl derivative (10 mg), an off-white amorphous powder, $[\alpha]_D^{17}$ -61.6° (MeOH; *c* 0.5). ^{1}H NMR (Me_2CO–$d_6 + D_2O$): δ 4.12 (1H, *m*, H-3), 4.62 (1H, *d*, $J = 8$ Hz, H-2″), 5.46 (1H, *br s*, H-2), 5.92 (1H, *d*, $J = 2$ Hz, H-6), 6.00 (1H, *d*, $J = 2$ Hz, H-8), 6.25 (1H, *s*, H-6″), 6.53 (1H, *s*, H-5′), 6.56 (1H, *d*, $J = 8$ Hz, H-5‴), 6.64 (1H, *br d*, $J = 8$ Hz, H-6‴), 6.68 (1H, *s*, H-2′), 6.74 (1H, *br s*, H-2‴).

Permethylation of **7**, *followed by methanolysis*. A mixture of **7** (48 mg), $(Me)_2SO_4$ (0.6 ml) and K_2CO_3 (1.0 g) in dry Me_2CO (8 ml) was heated under reflux for 8 hr. After filtration of ppt., the reaction mixture was CC on silica gel eluting successsively with C_6H_6, C_6H_6–Me_2CO (4:1) and the lower layer of $CHCl_3$–MeOH–H_2O (7:3:1). The $CHCl_3$–MeOH–H_2O eluate was concd to dryness, and the residue was treated with MeI (0.5 ml) and freshly prepared Ag_2O (0.2 g) in DMF(0.5 ml). After stirring overnight at room temp., the inorganic salts were removed by filtration, and the filtrate concd under red. pres. The residue was heated under reflux with 1.5 M methanolic HCl for 24 hr. After cooling, the reaction mixture was neutralized with Amberlite IRA 410 (OH^- form) resins, and subjected to CC on silica gel. Elution with C_6H_6–Me_2CO (1:1) afforded the methanolysate (**7a**) (2 mg), a white amorphous powder, $[\alpha]_D^{17}$-48.0° ($CHCl_3$; *c* 0.24). EIMS:Scheme 1. ^{1}H NMR ($CDCl_3$): δ 3.30, 3.35, 3.48, 3.56, 3.57, 3.68, 3.69, 3.76, 3.84, 3.87, 3.88, 3.93, (each 3H, s, OMe x 12), 4.63 (1H, *d*, $J = 8$ Hz, H-2″), 5.38 (1H, *br s*, H-2), 5.99 (1H, *d*, $J = 2$ Hz, H-6), 6.18 (1H, *d*, $J = 2$ Hz, H-8), 6.56 (1H, *s*, H-5′), 6.70 (1H, *d*, $J = 8$ Hz, H-5‴), 6.74 (1H, *br d*, $J = 8$ Hz, H-6‴), 6.83 (1H, *s*, H-2′), 6.84 (1H, *br s*, H-2‴).

Acid hydrolysis of **7**. A soln of **7** (3 mg) in 0.5 MH_2SO_4 was heated on a water bath for 3 hr. The reaction mixture was neutralized with $BaCO_3$ and the products were analysed by cellulose TLC. Only the spot [R_f 0.42 (*n*-BuOH–pyridine–H_2O (6:4:3); R_f 0.20 (the upper layer of *n*-BuOH–AcOH–H_2O (4:1:5)] of glucose was detected by aniline–hydrogen-phthalate spray.

Procyanidin B-3 3′-O-β-D-*glucopyranoside* (**5**). A brown amorphous powder, $[\alpha]_D^{18}$-166.5° [Me_2CO–H_2O (1:1); *c* 1.0]. The ^{1}H NMR spectrum was complicated by conformational isomerism. (Found: C, 51.85; H, 5.23. $C_{36}H_{36}O_{17}{\cdot}5H_2O$ requires: C, 52.05; H, 5.52.)

Thiol degradation of **5**. A mixture of **5** (50 mg), benzylmercaptan (2 ml) and AcOH (2 ml) in EtOH was refluxed for 10 hr. The reaction mixture was concd under red. pres. to give an oily residue, which was CC on Sephadex LH-20. Elution with EtOH separated products which were further CC on Sephadex LH-20. Elution with H_2O–MeOH (2:3) yielded (+)-catechin 3-*O*-β-D-glucopyranoside (**2**). Successive elution with H_2O–MeOH (1:4) afforded a mixture of benzylthioethers, which was treated at room temp. with Raney Ni in EtOH–AcOH (9:1) for 1 hr. After removal of the catalyst by filtration, the filtrate was subjected to CC on Sephadex LH-20 in H_2O–MeOH (2:3) to furnish (+)-catechin (**1**) (2 mg).

Acid hydrolysis of **5**. A soln of **5** (3 mg) in 0.5 M H_2SO_4 (5 drops) was heated on a water bath for 1 hr. The reaction mixture was treated in the same way as described above to yield glucose.

(+)-*Catechin* 3-*O*-β-D-*glucopyranoside* (2). Colourless needles (H_2O), mp 227–228°, $[\alpha]_D^{18}$-13.3° [Me_2CO–H_2O (1:1); *c* 1.0]. ^{1}H NMR (Me_2CO–$d_6 + D_2O$): δ 2.73 (2H, *d*-like, $J = 5$ Hz, H-4), 4.32 (1H, *d*, $J = 7$ Hz, glc. H-1), 4.92 (1H, *d*, $J = 6$ Hz, H-2), 5.89 (1H, *d*, $J = 2$ Hz, H-6), 6.00 (1H, *d*, $J = 2$ Hz, H-8), 6.64 (1H, *dd*, $J = 2$, 8 Hz, H-6′), 6.76 (1H, *d*, $J = 8$ Hz, H-5′), 6.84 (1H, *d*, $J = 2$ Hz, H-2′). ^{13}C NMR ($Me_2CO + D_2O$): δ 28.4 (C-4), 62.5 (glc C-6), 71.1 (glc C-4), 74.6 (glc C-2), 75.6 (C-3), 77.1 (glc C-5), 77.3 (glc C-3), 79.3 (C-2), 95.3 (C-8), 96.4 (C-6), 100.0 (C-4a), 103.5 (glc C-1), 114.6 (C-2′), 116.0 (C-5′), 119.1 (C-6′), 131.7 (C-1′), 145.6 145.7 (C-3′,4′), 156.0 (C-8a), 156.9 (C-5), 157.4 (C-7).

(+)-*Catechin* (**1**). Colourless needles (H_2O), mp 172–174°, $[\alpha]_D^{18}$ + 13.1° [Me_2CO–H_2O (1:1); *c* 1.1]. ^{1}H NMR (Me_2CO + D_2O): δ 2.50 (1H, *dd*, $J = 8$, 16 Hz, H-4), 2.92 (1H, *dd*, $J = 6$, 16 Hz, H-4), 3.98 (1H, *m*, H-3), 4.54 (1H, *d*, $J = 8$ Hz, H-2), 5.88 (1H, *d*, $J = 2$ Hz, H-6), 6.04 (1H, *d*, $J = 2$ Hz, H-8, 6.72 (1H, *dd*, $J = 2$, 8 Hz, H-6′), 6.84 (1H, *d*, $J = 8$ Hz, H-5′), 6.90 (1H, *d*, $J = 2$ Hz, H-2′).

Procyanidin B-3 (**4**). A brown amorphous powder, $[\alpha]_D^{18}$ − 158.0° [Me_2CO–H_2O (1:1); *c* 0.5]. The ^{1}H NMR showed a complex signal pattern due to the occurrence of conformational isomerism.

Procyanidin C-2 (**6**). A brown amorphous powder, $[\alpha]_D^{18}$ − 247.4° [Me_2CO–H_2O (1:1); *c* 0.9]. The ^{1}H NMR spectrum was complicated by conformational isomerism.

Afzelechin-(4α-8)-*catechin* (**3**). An off-white amorphous powder, $[\alpha]_D^{18}$ − 197.8° [Me_2CO–H_2O (1:1); *c* 1.0].

Acknowledgements–We are grateful to Mr H.-y. Zhang and Mr Y.-h. Di (the Academy of Traditional Chinese Medicine and Material Medica of Jilin Province) for collection of plant material, and to Mr Y. Kashiwada (Kyushu Unitersity) for performing part of this work. We are also indebted to Mr Y. Tanaka, Miss K. Soeda and Mr R. Isobe (Kyushu University) for measurements of ^{13}C NMR, ^{1}H NMR and MS, respectively, One of us (B.Z.) wishes to thank the Government of the Peoples Republic of China and the Academy of Traditional Chinese Medicine and Material Medica of Jilin Province for the award of a scholarship.

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