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Polyphenols from peanut skins and their free radical-scavenging effects

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Abstract

Separation of the water-soluble fraction of peanut skins led to the isolation of five proanthocyanidins. Based on the spectroscopic investigation and partial acid catalyzed degradation, their structures were determined to be epicatechin-(2β→*O*→7, 4β→6)-[epicatechin-(4β→8)]-catechin (1), epicatechin-(2β→O→7, 4β→8) epicatechin-(4β→8)-catechin-(4α→8)-epicatechin (2), and procyanidins B2 (**3**), B3 (**4**) and B4 (**5**). The absolute configuration of the new compounds was determined from their circular dichroism curves and the ^{1}H NMR spectra of analysis of flavan-3-ols formed by thiolytic degradation of **1** and **2** in the presence of a chiral dirhodium complex (dirhodium tetra-(*R*)–(trifluoromethyl) phenyl acetate).

Keywords: *Arachis hypogaea*; Leguminosae; Peanut skin; Proanthocyanidins

1. Introduction

Peanut skins are used to treat chronic haemorrhage and bronchitis in Chinese traditional medicine (Jiansu Xin Medical College, 1977). Six A-type proanthocyanidins (Lou et al., 1999) and flavonoids (Lou et al., 2001) have been isolated from the water-soluble phenolic fraction of skin of the mature seed of peanut, *Arachis hypogaea*. In this study, five oligomeric proanthocyanidins were isolated from the water-soluble fraction of peanut skins. Among them, two were identified as new polyphenols based on their spectral data, their chemical conversion to monomeric flavan-3-ol and their ^{1}H NMR spectra in the presence of a chiral dirhodium complex (dirhodium tetra-(*R*)–(trifluoromethyl)phenyl acetate, $Rh_2(DTPA)_4$).

2. Results and discussion

Peanut skins were extracted with boiling water and the extract was then fractionated by adsorption on HP-20 resin (Lou et al., 1999). The fraction obtained by elution with 70% (v/v) aqueous ethanol was then subjected to chromatography using Toyopearl HW-40 and eluted with aqueous ethanol and acetone. Fractions 11 and 12, obtained by eluting with 50% (v/v) acetone in water, from Toyopearl HW 40 contained oligomeric proanthocyanidins as detected by a characteristic orange coloration with anisaldehyde-sulfuric acid reagent (Morimoto et al., 1987). When subjected to further separation on Sephadex LH-20 and preparative HPLC, compounds **1**–**5** were obtained.

Compounds **3** and **4** were identified as procyanidins B2 and B3 by direct comparison of their R_f values obtained by TLC and R_t values obtained by HPLC with those of authentic samples, and **5** was identified as procyanidin B4 by direct comparison of its NMR

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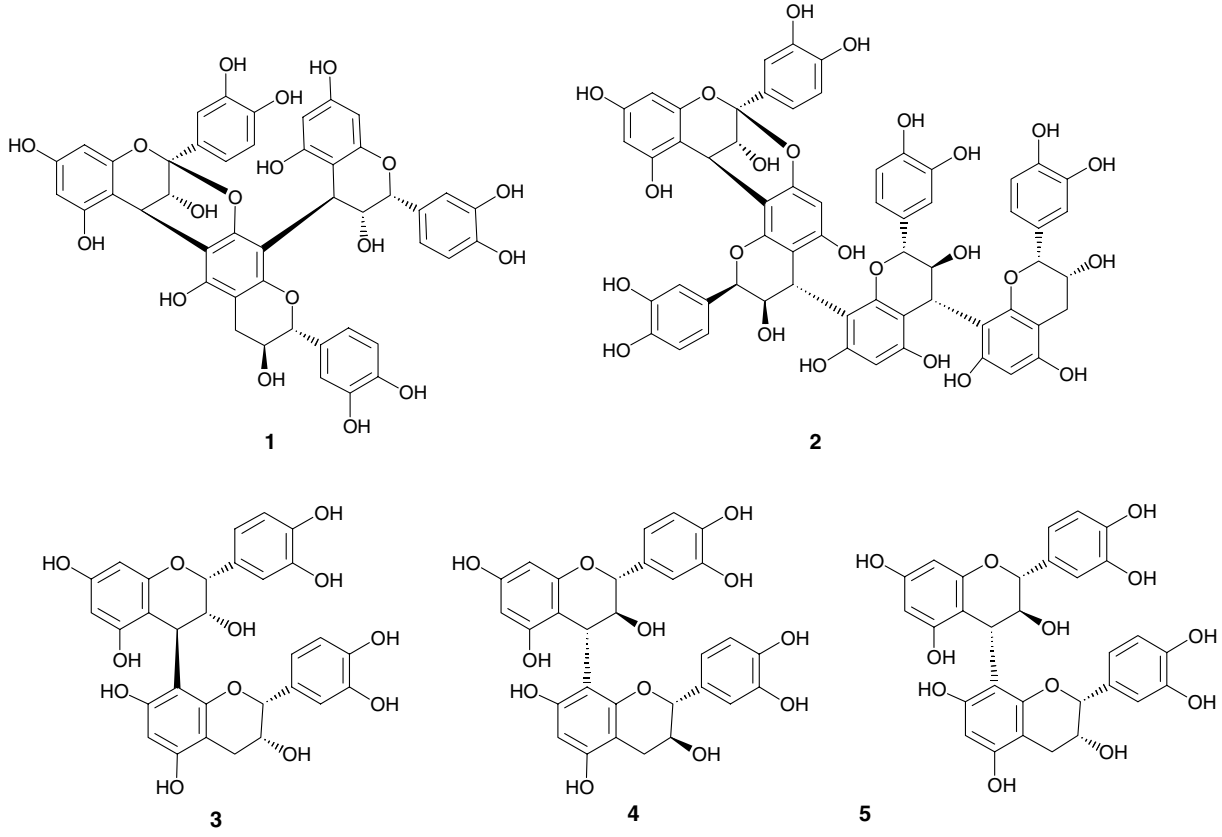
spectral data with that of the known compound (Hatano and Hemingway, 1997) (Scheme 1).

Compound **1** was obtained as an off-white amorphous powder. Its molecular composition was determined to be $C_{45}H_{36}O_{18}\cdot 2H_2O$ based on the results of elemental analysis and the presence of molecular ions at *m/z* 865 ($M^+ + 1$) in FAB-MS, which revealed that **1** is a trimeric flavan-3-ol. The presence of three flavanyl units was also indicated by ^{13}C resonances at δ 68.0 (C-3), 30.8 (C-4), 82.4 (C-2′), 68.77 (C-3′), 26.8 (C-4′), 78.0 (C-2″), 74.5 (C-3″), and 37.7 (C-4″), arising from flavanyl heterocyclic rings (rings C, I and F) and one ketal carbon at δ 101.8 (C-2) as assigned by an HMBC experiment. Proof of the existence of an A-type proanthocyanidin unit was obtained in the ^1H NMR spectrum, which showed an isolated AB coupling system at δ 4.05 (*d*, $J = 3.8$ Hz, H-3) and 4.34 (*d*, $J = 3.8$ Hz, H-4) (C ring), while one ketal carbon was seen at δ 101.8 (C-2) in the ^{13}C NMR spectrum (Jacques et al., 1974). The two flavan-3-ol units of this A-type entity in **1** were deduced to be linked through C_4 (C ring) and C'_6 (D ring) based on the absence of NOE between H-4 and the protons on the E ring or H-2′ (F ring) in NOESY (Bilia et al., 1996). Two sets of *meta*-coupled protons at δ 6.02 (1H, *d*, $J = 2.0$ Hz) and 6.09 (1H, *d*, $J = 2.0$ Hz), and δ 6.06 (1H, *d*, $J = 1.8$ Hz) and 5.63 (1H, *d*, $J = 1.8$ Hz), were assigned to the protons of the A and G rings in ^1H-^1H COSY, and this confirmed that another flavan-3-ol unit is linked to the A-type proanthocyanidin entity through C''_4 (I ring) and C'_8 (D ring). The long-range correlations of H-4″ (I ring) at δ 4.83 with C'_8 at δ 111.7, C'_7 at δ 151.7, C'_9 at δ 152.8 (D ring) and H-2′ (F ring) at δ 5.07 (1H, *d*, $J = 4.3$ Hz) with C'_9 in the HMBC also confirmed this $C''_4 \rightarrow C'_8$ linkage (Fig. 1).

The terminal flavanol unit was determined to be 2,3-*cis* (I ring) from the singlet at δ 5.34 (1H, s, 2″-H) and the corresponding carbon at δ 78.0 (C-2″), while the middle unit (the doubly-substituted terminal unit) was deduced to have a 2,3-*trans* configuration (F ring) from the proton signal at δ 5.07 (1H, br. *d*, $J = 7.5$ Hz, 2′-H) in ^1H NMR and δ 82.4 (C-2′) in ^{13}C NMR. Circular dichroism measurement (Fig. 2) reflected the β orientation of the flavanyl substitutes, i.e., an R configuration, at both the C-4 (C ring) and C-4″ (I ring) positions for a high-amplitude positive Cotton effect at a diagnostic wavelength of 240nm ($\Delta\varepsilon_{240} + 26.18$) (Barrett et al., 1979; Botha et al., 1981). Treatment of **1** with benzylthiol/acetic acid in ethanol yielded **6**, which was identified to be epicatechin-(2β→*O*→7, 4β→6)-catechin by direct comparison of its ^1H NMR spectrum with that previously reported (Lou et al., 1999), confirming the absolute configuration of the A-type entity. The thioether



Scheme 1.

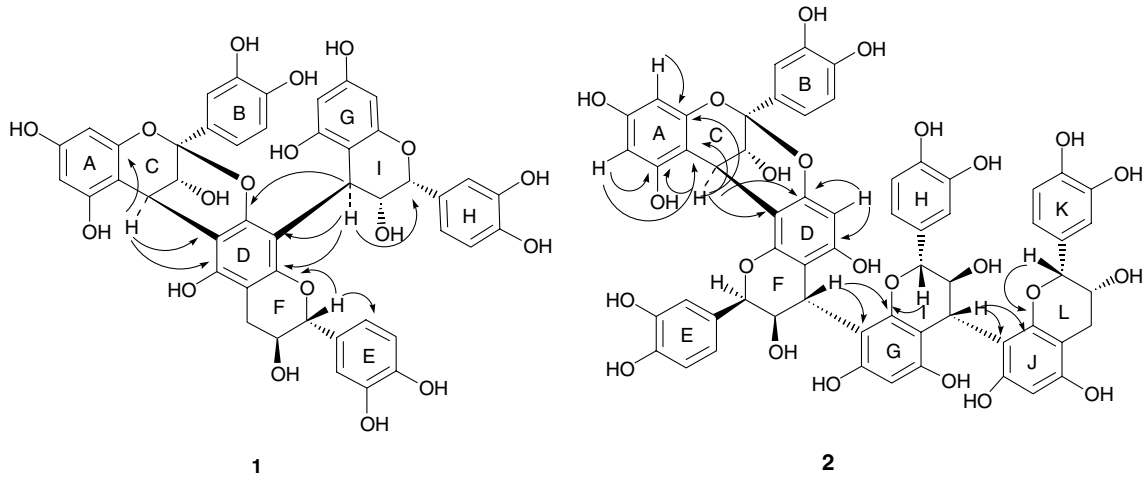


Fig. 1. Long-range correlations between H and C as observed in HMBC.

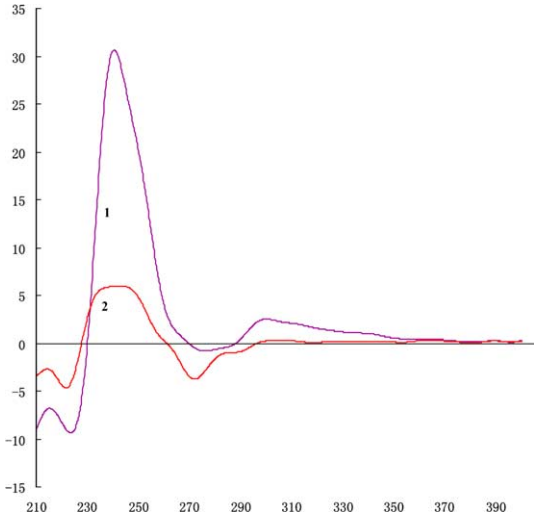


Fig. 2. CD measurement of **1** and **2**.

(**7**), derived from the lower terminal unit in the thiolytic degradation of **1**, was desulfurized with Raney nickel (Morimoto et al., 1987) to give **8**. Compound **8** was then methylated with CH_2N_2 to form **9**, the structure of which was finally confirmed to be tetra-*O*-methyl ether of epicatechin by measurement of its ^{1}H NMR in the presence of $Rh_2(DTPA)_4$). In the presence of $Rh_2(DTPA)_4$), the proton signals of the A- and C-rings all shifted to a lower field while the protons of the B-ring shifted up-field, possibly because the chiral reagent was closer to the A- and C-rings in the solution. These diastereomeric dispersions due to complexation in the ^{1}H NMR spectrum allowed us to identify epicatechin and *ent*-epicatechin subunits, as well as their enantiomeric ratio, as illustrated in Fig. 3.

Consequently, the structure of **1** was determined to be epicatechin-(2β→*O*→7,4β→6)-[epicatechin-(4β→8)]-catechin according to the nomenclature for A-type oligoflavanoids (Kolodziej et al., 1993). This is a branched A-type proanthocyanidin with C_4–C_6 and C_4–C_8 linkages to the same A ring of a flavanyl unit (Foo and Hemingway, 1984) (see Schemes 2 and 3).

The FAB-MS of **2** indicated an $[M+l]^+$ ion at *m/z* 1153, consistent with a flavan-3-ol tetramer. Although the occurrence of conformational isomers of **2** makes the ^{1}H NMR spectrum even more complex, only one conformer (which account for more than 90%) appeared to be the major constituent responsible for the possible hindrance of its structure, as revealed in the 600 MHz ^{1}H NMR spectrum (recorded in CD_3OD), and this made it possible to assign its NMR spectroscopic data, the ^{13}C NMR spectrum of **2**, the presence of four flavanyl units was also indicated by C-3 signals at δ 67.0, 67.2, 70.8, and 74.9, and C-4 signals at δ 29.0 (*d*), 38.0 (*d*), 38.5 (*d*) and 23.7 (*t*). The presence of an A-type proanthocyanidin in the tetrameric chain was evident from the characteristic ketal carbon resonance at δ 100.3 as determined by HMBC and the typical AB coupling protons at δ 3.59 (1H, *d*, *J*=3.5 Hz, H-3) and 4.03 (1H, *d*, *J*=3.5 Hz, H-4) (A ring) in the ^{1}H–^{1}H COSY. This A-type proanthocyanidin unit was deduced to be at the top of the structure based on the occurrence of *meta* coupling aromatic protons in the A-ring at δ 5.96 (1H, *d*, 1.8 Hz, H-6) and 5.84 (1H, *d*, *J*=1.8 Hz, H-8) which correlated with the same C-7 (δ 156.6) (A ring) as well as the correlations between H-6 and C-5 (154.4), and H-4 and C-5 in HMBC (Fig. 1). The NOE effects between H-4 and H-2′ (δ 5.6, 1H, s) (F ring) revealed that the interflavanoid linkage of this A-type proanthocyanidin is through C_2-O-C_7' and C_4–C_8' (Bilia et al., 1996).

The terminal flavan-3-ol had a 2,3-*cis* configuration (L ring) as suggested by the coupling constants of the aliphatic proton at δ 4.92 (1H, *s*, H-2‴). A carbon resonance at δ 80.2 also confirmed this 2,3-*cis* configuration. The remaining flavan-3-ol unit with a 2,3-*trans* configuration

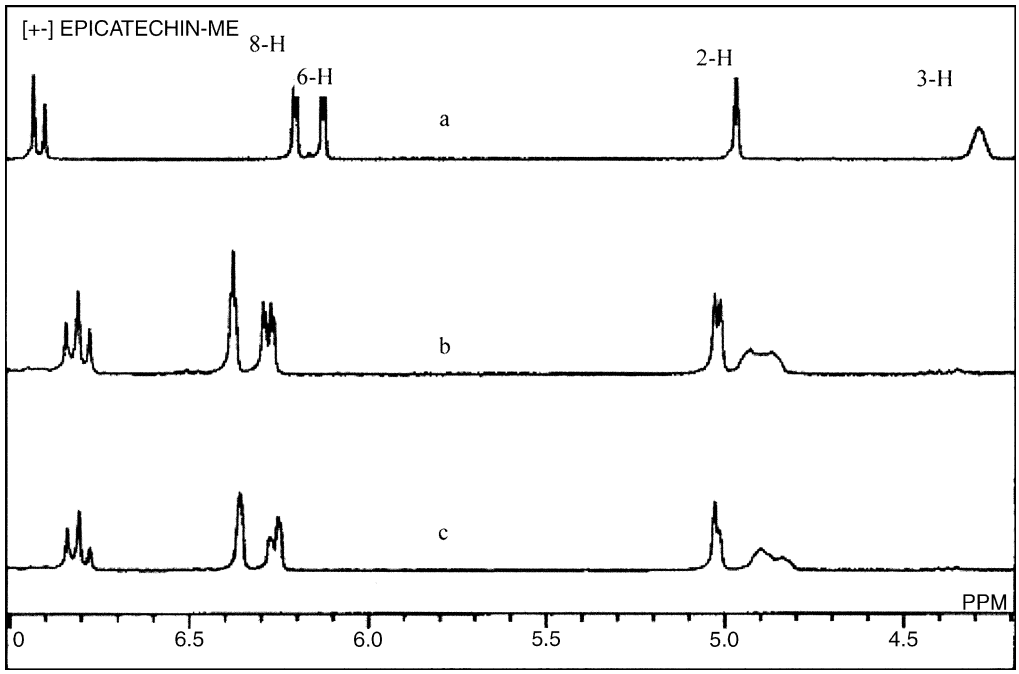
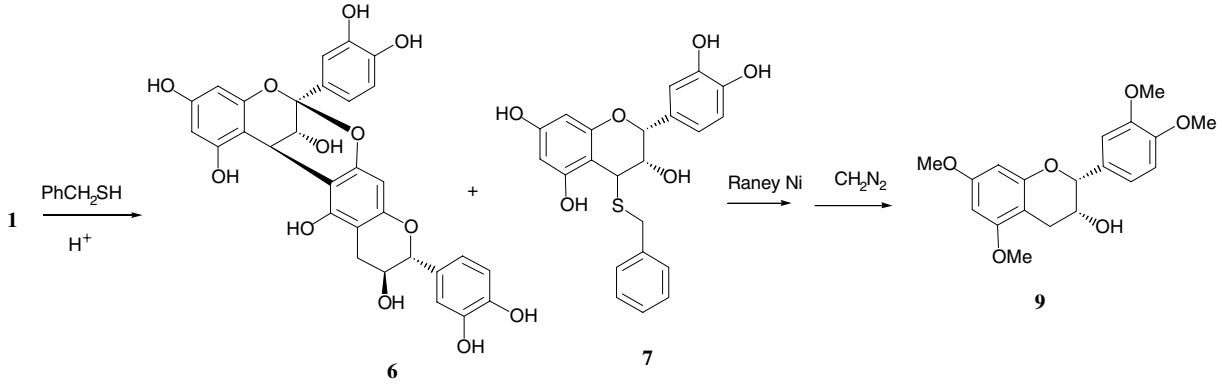


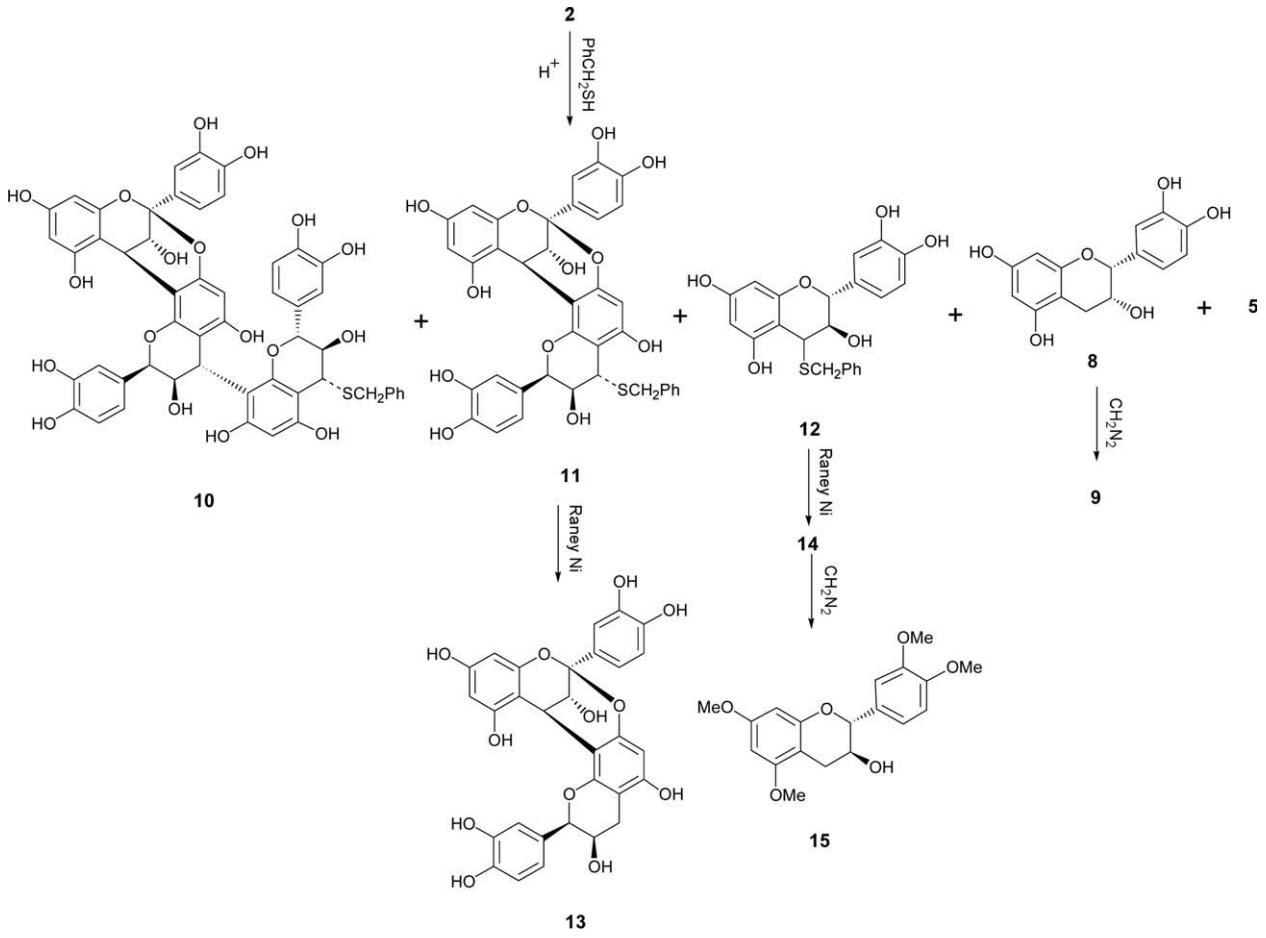
Fig. 3. Enantioselectivity of tetramethyl ether of epicatechin by ^{1}H NMR spectra recorded with $CDCL_3$ as solvent (Hameed et al., 1998). (a) 1:1 mixture of **9** and the tetra-*O*-methyl ether of *ent*-epicatechin (**9a**) without $Rh_2(DTPA)_4$); (b) 1:1 mixture of **9** and **9a** in the presence of $Rh_2(DTPA)_4$); (c) 2:1 mixture of **9** and **9a** in the presence of $Rh_2(DTPA)_4$). The signals below 7.0 ppm overlapped with that of the chiral reagent, while no significant chemical shift change was observed for the signals of methoxyl groups above 4.3 ppm.



Scheme 2. Thiolytic degradation of **1**.

was determined based on the chemical shifts of the protons at δ 3.93 (1H, brd, J=6.6 Hz, H-2″), a unique up-field chemical shift due to steric interactions with linked flavanyl units, 4.41 (1H, *d*, J=6.6 Hz, H-3″) and 4.43 (1H, br.*s*, H-4″) (I ring) in ^{1}H NMR, and the carbon resonance at δ 84.0 (C-2″) in ^{13}C NMR. The interflavanyl linkages were determined to be C'_4—C''_8 and C''_4—C'''_8, respectively, based on the presence of long-range correlations of H-4′ with C''_8 (δ 109.7) and C''_9 (δ 151.6), H-2″ with C''_9, as well as H-4″ with C'''_8 (δ 108.7) and C'''_9 (δ 154.4), and H-2‴ with C'''_9, respectively.

The thiolytic degradation of **2** led to the formation of **5** and **8**, which were identified to be procyanidin B4 and epicatechin, respectively, by direct comparison, as well as **10**, **11** and **12**. The desulfurization of **11** yielded **13**, which was identified to be proanthocyanidin A-2, as previously reported (Lou et al., 1999). Compound **12**, when desulfurized and subjected to methylation with CH_2N_2, was converted to **15**. The structure of **15** was unambiguously identified to be the 3′,4′,5,7-tetramethyl ether of catechin by measurement of its ^{1}H NMR spectra in the presence of $Rh_2(DTPA)_4$ (Fig. 4). Moreover, the CD spectrum of **2** revealed two positive Cotton effects at 243 nm ($\Delta\varepsilon$ 5.22) and 236 nm ($\Delta\varepsilon$ 5.39), indicating an β-orientation of the 4′-flavanyl substituent (Botha et al., 1981). Accordingly, the structure of 2 was determined to be epicatechin-(2β→*O*→7, 4β→8)-epicatechin-(4β→8)-catechin-(4α→8)-epicatechin.



Scheme 3. Thiolytic degradation of **2**.

Oligomeric proanthocyanidins have been shown to have various biologically significant effects (Lou et al., 2000). Similar A-type proanthocyanidins in cranberry prevent the adherence of P-fimbriated *Escherichia coli* isolates from the urinary tract to cellular surfaces and have been used as an effective therapeutic agent in the treatment and prevention of urinary tract infections (Foo et al., 2000). In this paper, the scavenging activities of compounds **1**–**5** toward the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical were determined (Table 3). The IC_{50} values of **1**–**5** were about 1.0 μM. These results showed that the proanthocyanidins in peanut skins have free radical-scavenging effects, which could protect the seed fatty residue from oxidation.

3. Experimental

3.1. General experimental procedures

Melting points were determined on a Yanaco micro-melting point apparatus and are uncorrected. UV and IR. spectra were taken using a Shimadzu UV 240 spectrophotometer and a Nicolet Nexus FT/IR-470 spectrophotometer, respectively. ^{1}H NMR and ^{13}C NMR were measured using a Brucker Avance 600 NMR spectrometer (600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR). Chemical shifts are given in δ (ppm), based on TMS. FAB-MS were measured with a JEOL JM-HX110 mass spectrometer. CD was recorded with a JASCO J-15 spectro-polarimeter. Preparative HPLC was performed using a Waters 600-996 on a Prepak Cartridge 25×100 ODS column (Waters) with a mixture of methanol and water as a mobile phase and detection at 280 nm. The flow rate of the mobile phase was 3.5 ml/min. TLC was performed on precoated aluminum sheets (Rp-18 F_{254}, 0.2 mm, Merck) with CH_3OH–H_2O (5:5). Column chromatography was carried out using Diaion HP 20 (Nippon Rensui Co.), Sephadex LH-20 (Pharmacia Biotech, Sweden), Toyopearl HW40 (fine grade from Tosoh, Japan), MCI CH 20P (Mitsubishi Chemical Co.), and silica gel 300 (Qingdao Chemical Co., Ltd.). $Rh_2(DTPA)_4$ was generously provided by Prof. Helmut Duddeck of Hannover University, Germany.

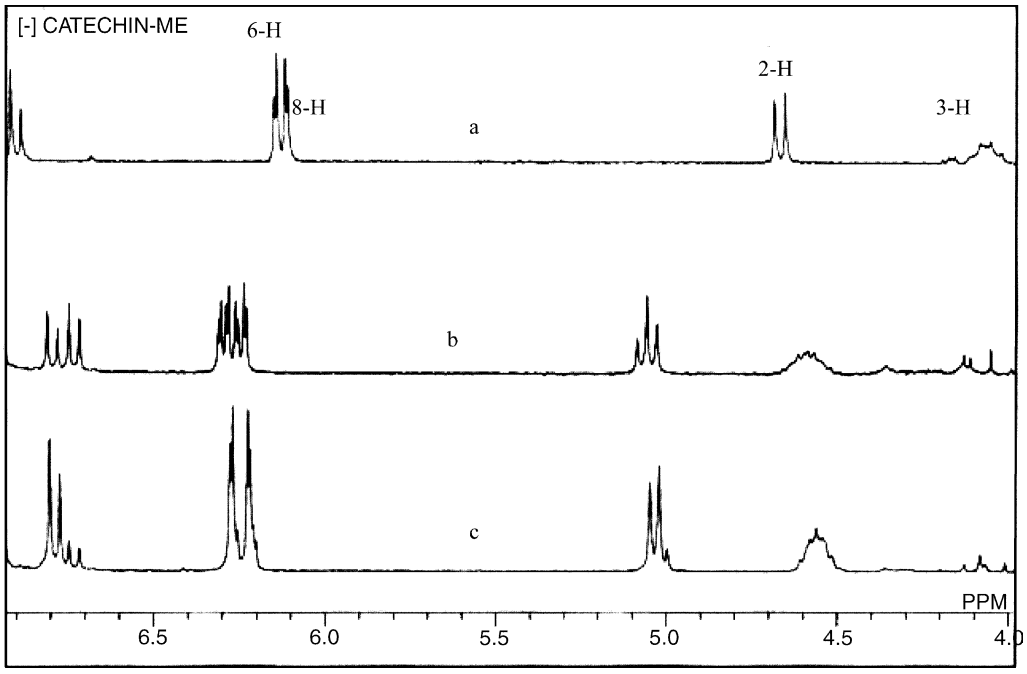


Fig. 4. Enantioselectivity of tetramethyl ether of catechin by ^{1}H NMR spectra recorded with $CDCL_3$ as solvent: (a) 1:1 mixture of **15** and the tetra-*O*-methyl ether of *ent*-catechin (**15a**) without $Rh_2(DTPA)_4$); (b) 1:1 mixture of **15** and **15a** in the presence of $Rh_2(DTPA)_4$); (c) 2:1 mixture of **15** and **15a** in the presence of $Rh_2(DTPA)_4$). The signals below 7.0 ppm overlapped with that of the chiral reagent while no significant chemical shift change for the signals of methoxyl groups above 4.0 ppm.

3.2. Plant material

A. hypogaea L. was taxonomically identified by Dr. Masaru Uchida of Tokiwa Phytochemical Co., Ltd., Chiba, Japan. A voucher specimen was deposited at the company under registration number AH-962001. Peanut skins were collected in December 1996.

3.3. Extraction and purification

The extraction procedure is the same as that in our previous report (Lou et al., 1999). Fractions 11 and 12 obtained by eluting with 50% acetone in water from a Toyopearl HW 40 column contained oligomeric proanthocyanidins as detected by UV measurement and TLC orange coloration with anisaldehyde-sulfuric acid reagent (Jacques et al., 1974). Fraction 11 (4.8 g) was further subjected to column chromatography using Sephadex LH-20 (50 g, 35×3.0 cm) and eluted with 50% methanol (1.2 l) (each 100 ml portion was collected as one eluant). The eluants, which have the same TLC coloration, were mixed together to give Fr.11-1 (1.1 g, from eluants 3–6) and Fr.11-2 (0.6 g, from eluants 8–10). Further separation of Fr.11-1 by preparative HPLC afforded compounds **1** (46 mg) and **2** (53 mg). The same method was used to separate Fr. 11-2 to give compounds **3** (88 mg), **4** (22 mg) and **5** (11 mg).

3.4. Epicatechin-(2β→O→7, 4β→6)-[epicatechin-(4β→8)]-catechin (*1*)

Off-white amorphous powder; m.p. 272 °C (decomp). $[\alpha]_D$ +86.2 (*c* 0.3, acetone). UV (MeOH) λ_{max}: 282nm. FAB-MS *m/z*: 865 (M^++1), Anal. Calcd. for $C_{45}H_{36}O_{18}\cdot 2H_2O$: C, 60.00; H, 4.47. Found: C, 60.09; H, 4.89. CD: $\Delta\varepsilon_{240}$ +26.18, $\Delta\varepsilon_{276}$ −0.524. For ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data see in Table 1.

3.5. Thiolytic degradation *1*

A mixture of **1** (32 mg), benzylthiol (1.2 ml) and acetic acid (1.2 ml) in ethanol (5 ml) was refluxed for 5 h with stirring under a nitrogen atmosphere. The reaction mixture, when concentrated in vacuo to give an oily residue, was subjected to chromatography with Sephadex LH-20 using 70% methanol in water as a mobile phase. Compounds **6** (**8** mg) and **7** (11 mg) were obtained.

3.6. Epicatechin-(2β→O→7, 4β→6)-catechin (*6*)

White needles (MeOH); mp. 272 °C (decomp), FAB-MS: *m/z*: 577 (M^++1). ^{1}H NMR (600 MHz, $CDC1_3$) and ^{13}C NMR (150 MHz, $CDC1_3$) data were the same as those previously reported (Lou et al., 1999).

Table 1
NMR spectroscopic data for compound **1**

Carbons		Correlated H	H coupled with C	H coupled with H
No	δ_C	δ_H		
2	101.8		H-3, H-4, H-12	
3	68.4	4.05, *d* (3. 8)	H-4	H-4
4	30.8	4.34, *d* (3.8)	H-3	H-3
5	155.9		H-3, H-7	
6	97.0	6.09, *d* (2.0)		H-8
7	158.9		H-6, H-8	
8	97.6	6.02, *d* (2.0)		H-6
9	155.7		H-8	
10	105.9		H-3, H-4	
11	132.0		H-4, H-12, H-14	
12	114.8	6.92, *d* (1.8)		H-16
13	146.5[a]		H-12	
14	146.6[a]		H-15	
15	117.0	6.69, *d* (8.2)	H-16	H-16
16	119.8	6.93, *d* (8.2, 1.8)	H-15	H-12, H-15
2′	82.4	5.07, *d* (7. 5)	H-3′, H-12′	H-3′
3′	68.8	4.20, *m*	H-2′, Hα-4′	H-4′, H2′
4′	26.8	2.76, *dd* (16.5, 4.0), H-α 2.57, *dd* (16.5, 4.6), H-β	H-3′, H-2′	H-3′
5′	151.9		H-4′	
6′	109.6			
7′	151.0		H-6′	
8′	111.7			
9′	152.8		H-8′	
10′	103.8		H-2^r, H-3′	
11′	133.5		H-2′, H-12′	
12′	116.9	6.88, *d* (2.0)		H-16′
13′	146.3*		H-12′	
14′	146.5*		H-15′	
15′	117.1	6.78, *d* (8.2)	H-16′	H-16′
16′	121.1	6.13, *dd* (8.2, 2.0)	H-15′	H-12′, H-15′
2″	78.0	5.34, *s*	H-3″, H-12″	H-3″
3″	74.5	3.78, br. *s*	H-2″, H-4″	H-2″, H-4″
4″	37.7	4.83, br. *s*	H-4″	H-3″
5″	158.6		H-4″, H-6″	
6″	97.6		H-6″, H-8″	
7″	158.2	5. 63, *d*, (2.2)		H-8″
8″	97.2		H-8″, H-4″	
9″	158.7	6.04, *d* (2.2)		H-6″
10″	105.2		H-4″, H-3″	
11″	134.0		H-2″, H-12″	
12″	116.7	6.94, *d* (2.0)	H-2″	H-16″
13″	145.5		H-12″	
14″	146.9		H-15″	
15″	116.8	6.69, *d* (8.2)	H-16″	H-16″
16″	121.2	6.57, *dd* (8.2, 2.0)	H-15″	H-12″, H-15″

[a] Data are interchangeable; data in parentheses are coupling constants.

3.7. 3′,4′,5,7-Tetra-O-methyl epicatechin (**9**)

Compound **7**, a white amorphous powder, was identified to be epicatechin-4-benzylthioether by measurement of its FAB-MS, *m/z*: 413 ($M^+ + 1$), in accordance with the formula $C_{22}H_{20}O_6S$. The thioether **7** (8 mg), dissolved in 1.5 ml of ethanol–acetic acid (10:1), was desulfurized with Raney nickel at 50 °C for 30 min. After the catalyst was removed by filtration, the reaction mixture was concentrated to dryness. The residue was dissolved in 1 ml MeOH, excess CH_2N_2 in Et_2O was added and the mixture was then stirred in an ice bath for 12 h. The methyl ether was formed,purified using silica gel (6 g) chromatography, and eluted with benzene-acetone (85:15). Compound **9** (5 mg) was obtained as a white powder, mp 132–135 °C (acetone), FAB-MS *m/z*: 347 ($M^+ + 1$). Its ^{1}H NMR data were the same as those of the 3′,4′,5,7-tetra-*O*-methyl ether of epicatech in (Fig. 3(a)). In the presence of the chiral reagent $Rh_2(DTPA)_4$, **9** was determined by measuring its ^{1}H NMR spectrum using with its enantiomer 3′,4′,5,7-tetra-*O*-methyl ether of *ent*-epicatechin (**9a**) (Figs. 3(b),(c)).

Table 2
NMR spectroscopic data for compound **2**

Carbons		δ_H	Carbons		δ_H
No	δ_C		No	δ_C	
2	100.3		2″	84.0	3.93, *d* (6.6)
3	67.2	3. 59, *d* (3.5)	3″	74.9	4.41, *d* (6.6)
4	29.1	4.03, *d* (3.8)	4″	38.5	4.40, *s*
5	154.4		5″	156.0*	
6	97.8	5.98, *d* (2.0)	6″	98.5	6.10, *s*
7	156.6		7″	155.7*	
8	98.6	5.84, *d* (2.0)	8″	109.7	
9	154.9*		9″	151.6	
10	105.4		10″	98.6	
11	132.6		11″	133.9	
12	116.0	6.89, *d* (1.8)	12″	116.0	7.11, *d* (1.8)
13	146.8**		13″	145.7	
14	146.6**		14″	146.0	
15	115.8	6.87, *d* (8.0)	15″	116.4	6.76, *d* (8.0)
16	120.3	6.72, *dd* (8.0, 1.8)	16″	120.4	6.72, *dd* (8.0, 1.8)
2′	77.7	5.78, *s*	2‴	80.2	4.92, *s*
3′	70.8	3.68, *s*	3‴	67.0	4.03, *m*
4′	38.0	4.46, *s*	4‴	23.7	2.53, br. *d* (16.2)
5′	157.8				2.08, *dd* (16.2,3.6)
6′	96.2	5.82, *s*	5‴	157.3*	
7′	153.3		6‴	95.6	6.02, *s*
8′	111.7		7‴	155.8	
9′	151.6		8‴	109.7	
10′	106.7		9‴	154.4	
11′	131.2		10‴	100.7	
12′	117.0	7.21, br. *s*	11‴	132.0	
13′	146.0		12‴	112.9	5.91, *d*(2.1)
14′	145.8		13‴	145.8	
15′	115.0	6.85, *d* (8.2)	14‴	145.7	
16′	121.5	6.81, br. *d* (8.2)	15‴	116.6	6.40, *d* (8.2)
			16‴	118.2	5.39, *dd* (8.2, 2.1)

Data with the same "*" or "**" are interchangeable; data in parentheses are coupling constants.

Table 3
Scavenging effect of **1**–**5** on DPPH radicals

Compound	**1**	**2**	**3**	**4**	**5**	EGG
IC_{50} μM	1.21±0.11	1.32±0.16	0.96±0.09	1.11±0.15	1.02±0.09	1.13±0.08

EGG, epigallocatechin gallate; used as a positive control

3.8. *Epicatechin-(2β→O→7, 4β→8) epicatechin-(4α→8)-catechin-(4α→8)-epicatechin (**2**)*

Off-white amorphous powder, m.p. 260 °C (decomp). $[\alpha]_D$ +27.6 (*c* 0.3, acetone). UV (MeOH) λ_{max}: 282 nm. FAB-MS *m/z*: 1153 (M^++1), Anal. Calcd. for $C_{60}H_{48}O_{24}\cdot 3H_2O$: C, 59.70; H, 4.38. Found: C, 59.62; H, 4.39. CD: $\Delta\varepsilon_{236}$ 5.39, $\Delta\varepsilon_{243}$ +5.22, $\Delta\varepsilon_{271}$ −3.27. For ^{1}H NMR (600 MHz, CD_3OD) δ and ^{13}C NMR (150 MHz, CD_3OD) δ data see in Table 2.

3.9. *Thiolytic degradation of **2***

A mixture of **1** (42 mg), benzylthiol (1.5 ml) and acetic acid (1.5 ml) in 5 ml of ethanol was refluxed for 1 h with stirring under a nitrogen atmosphere. The reaction mixture was concentrated in vacuo, and the obtained residue was subjected to preparative Rp-18 TLC (0.5 mm, Merck) with CH_3OH–THF–H_2O) (7:1:3), to give compounds **5** (3 mg), **8** (3 mg), **10** (2 mg), **11** (6 mg) and **12** (9 mg). Compound **5** was identified by direct comparison with an authentic sample. Compound **8** was converted by methylation to **9**, which was identified by the method described above. The FAB-MS of **10** at *m/z*: 987 and **11** at *m/z*: 699 were in accordance with the formulas $C_{52}H_{42}O_{18}S$ and $C_{37}H_{30}O_{12}S$, respectively.

3.10. *Desulfurization of **11***

Compound **11** (5 mg), dissolved in 1.0 ml of EtOH–AcOH (10:1), was desulfurized with Raney nickel at 50 °C for 30 min. After the catalyst was removed by filtra-

tion, the reaction mixture was concentrated to dryness. After purification on Sephadex LH-20 (20 ml bed volume) with 70% methanol, **13** (3 mg), m.p. 274 °C (decomp), FAB-MS *m/z*: 577 $[M+l]^+$, was obtained. ^1H NMR and ^{13}C NMR data were the same as those of proanthocyanidin A_2 (Lou et al., 1999).

3.11. 3′,4′,5,7-Tetra-O-methyl catechin (*15*)

Compound **12** (6 mg) was desulfurized using the method described previously for **7**, to give **14**. When **14** was methylated with CH_2N_2 and purified by prep-TLC, **15** (2 mg) was obtained, m.p. 124–125 °C (acetone), FAB-MS *m/z*: 347 $(M^+ + 1)$. The ^1H NMR data of **15** were the same as those of 3′,4′,5,7-tetra-O-methyl ether of catechin (Fig. 4(a)). In the presence of the chiral reagent $Rh_2(DTPA)_4$, the signals of H-2, H-3, H-6 and H-8 shifted to a lower field while that of H-16 at the B-ring shifted up-field (Fig. 4b). Compound **15** can be differentiated in its ^1H NMR spectrum by measuring it with its enantiomer, 3′,4′,5,7-tetra-*O*-methyl ether of *ent*-catechin (**15a**) (Fig. 4(c)).

3.12. Determination of the effects of *1–5* on DPPH radical

The test sample in solution was added to 1 ml of a methanolic solution of DPPH radical (final DPPH concentration was 20 mM). The mixture was shaken vigorously and left to stand for 30 min. The absorbance of the resulting solution was measured at 517 nm. The percent inhibition of each sample was calculated according to the equation (Yen and Duh, 1994)

$$\text{Inhibition}\% = 1 - (A_0 - A_S)/A_0,$$

where A_0 is the absorbance at 517 nm of the blank control, and A_S is the absorbance at 517 nm of the sample preparation. All tests and analyses were run in triplicate and averaged.

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