Chemical and Chemotaxonomical Studies on *Dicranopteris* Species[1)]

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Clerodane glycosides and flavonoids in *Dicranopteris pedata* and three varieties of *D. linearis* were investigated. All the ferns contained a new glycoside, (6*S*,13*S*)-6-[6-*O*-acetyl-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyloxy]-13-[α-L-rhamnopyranosyl-(1→4)-β-D-fucopyranosyloxy]-cleroda-3,14-diene, as a chemical marker of this group. Flavonoids were limited to flavonol 3-*O*-glycosides. The ferns and isolated flavonoids are as follows; *D. pedata*: afzelin, quercitrin. *D. linearis* var. *brevis*: afzelin, quercitrin. *D. linearis* var. *tenuis*: quercitrin, isoquercitrin. *D. linearis* var. *sebastiana*: astragarin, isoquercitrin, rutin, kaempferol 3-*O*-(4-*O*-*p*-coumaroyl-3-*O*-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.

Key words *Dicranopteris pedata*; *Dicranopteris linearis* var. *brevis*; *Dicranopteris linearis* var. *tenuis*; *Dicranopteris linearis* var. *sebastiana*; clerodane glycoside; flavonol 3-*O*-glycoside

The chemical studies of *Dicranopteris pedata*, a Gleicheniaceous fern in Japan, showed the presence of afzelin (**2**),[2)] quercitrin (**3**),[2)] protocatechuic acid,[3)] shikimic acid[3)] *p*-β-rutinosyloxystyrene[3)] and proanthocyanidins.[4)] Recently, clerodane-type glycosides which might be common to Gleicheniaceous ferns were also isolated from the rhizoma of this fern.[5)]

From a chemotaxonomical point of view, flavonoids and clerodane-type glycosides in the fronds of *D. pedata* (HOUTT.) NAKAIKE [=*D. linearis* (BURM. f.) UND. =*D. dichotoma* (THUMB.) BERNH. =*D. linearis* (BURM. f.) UND. var. *dichotoma* (THUNB.) HOLTT. REINWARDT.] in Japan, *D. linearis* (BURM. f.) UND. var. *sebastiana* PANIGRAHI & DIXIT., *D. linearis* (BURM. f.) UND. var. *brevis* MANICKAM & IRUDAYARAJ. and *D. linearis* (BURM. f.) UND. var. *tenuis* MANICKAM & IRUDAYAJ. in India were investigated comparatively.

Concerning clerodane-type glycosides, all the ferns contained a considerable amount of a new compound, **1**, which may be a chemical marker of this group. Compound **1**, a colorless amorphous solid, $[\alpha]_D$ −72° (*c*= 1.0, MeOH), was formulated as $C_{46}H_{76}O_{20}$ from high resolution fast atom bombardment mass spectrum (HR-FAB-MS). By ^{13}C–^{1}H correlation spectroscopy (COSY) and ^{1}H–^{1}H COSY, the presence of two rhamnopyranosyl groups, one fucopyranosyl, one glucopyranosyl and one acetyl group were revealed (Table 1). The remaining signals indicated that the aglycone was the same as that of the glycosides isolated from the rhizoma of this fern[5)] and also from the fronds of *Trichomanes reniforme*.[6)] On methanolysis with anhydrous Na_2CO_3 in MeOH, **1** gave a deacetyl compound, **1a**, a colorless amorphous solid, $[\alpha]_D$ −80° (*c*=0.5, MeOH) . On acid hydrolysis, **1a** gave L-rhamnose, D-fucose and D-glucose, but the aglycone was degraded in this condition. On enzymatic hydrolysis with a glycosidase mixture of turbo and crude hesperidinase, **1a** gave a monorhamnoside of the aglycone, **1b**, a colorless amorphous solid, $[\alpha]_D$ −80° (*c*=1.0, MeOH). The physical properties and spectral data were practically the same as those of (6*S*,13*S*)-13-hydroxy-6-α-L-rhamnopyranosyloxycleroda-3,14-diene.[6)]

The sequence of the sugar moiety of **1** was determined by nuclear Overhauser effect correlation spectroscopy (NOESY), where the correlations between H-6 of the aglycone and the anomeric proton of the rhamnosyl group (A), between H-4 of the rhamnosyl group (A) and the anomeric proton of the glucosyl group, between H_3-16 of the aglycone and the anomeric proton of the fucosyl group and between H-4 of the fucosyl group and the anomeric proton of the rhamnosyl group (B) were observed.

The position of the acetyl group was determined to be

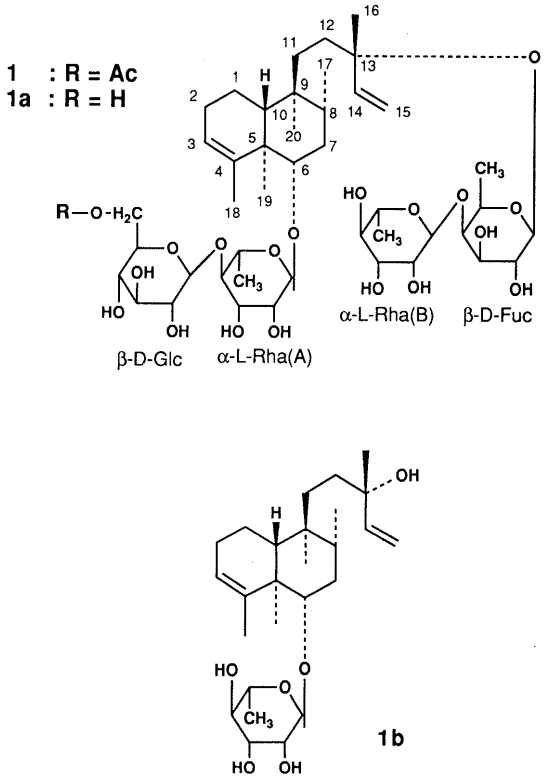

Chart 1

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Table 1. NMR Data for **1** in C_5D_5N (J in Hz)

	Aglycone moiety	
	^{13}C	^1H
1	17.8	
2	26.7	
3	123.0	5.06 (1H, br s)
4	143.1	
5	43.9	
6	86.3	3.34 (1H, dd, 11.0, 4.4)
7	35.0	
8	34.1	
9	38.0	
10	45.6	
11	31.9	
12	35.0	
13	80.3	
14	144.6	6.18 (1H, dd, 17.5, 11.3)
15	114.9	5.24 (1H, dd, 11.3, 1.1)
		5.38 (1H, dd, 17.5, 1.1)
16	22.7	1.54 (3H, s)
17	15.8	0.72 (3H, d, 6.6)
18	22.8	1.72 (3H, s)
19	16.1	1.03 (3H, s)
20	17.9	0.62 (3H, s)
	Sugar moiety	
	^{13}C	^1H
Fucosyl		
1	99.8	4.70 (1H, d, 7.7)
2	72.2	4.24 (1H, dd, 7.7, 10.1)
3	76.0	4.10 (1H, dd, 10.1, 3.0)
4	77.8	4.16 (1H, d, 3.0)
5	70.5	3.72 (1H, q, 6.5)
6	18.0	1.47 (3H, d, 6.5)
Rhamnosyl (A)		
1	103.2	5.33 (1H, s)
2	72.0	4.49 (1H, NC)
3	72.6	4.50 (1H, NC)
4	85.0	4.35 (1H, t, 9.2)
5	68.1	4.20 (1H, dq, 9.2, 4.8)
6	17.9	1.73 (3H, d, 4.8)
Rhamnosyl (B)		
1	103.0	6.24 (1H, s)
2	72.3	4.85 (NC)
3	72.6	4.48 (NC)
4	73.8	4.25 (1H, t, 9.2)
5	70.1	4.40 (1H, dq, 9.2, 6.2)
6	18.4	1.62 (3H, d, 6.2)
Glucosyl		
1	106.5	5.16 (1H, d, 8.6)
2	76.1	4.08 (1H, t, 8.6)
3	78.2	4.15 (1H, t, 8.6)
4	71.4	3.97 (1H, t, 8.6)
5	75.1	3.85 (1H, ddd, 8.6, 10.0, 6.7)
6	64.4	4.72 (1H, dd, 6.7, 1.5)
		4.83 (1H, dd, 10.0, 1.5)
Acetyl		
CH_3	20.6	1.95 (3H, s)
CO	170.6	

NC: coupling patterns were not confirmed because of overlapping signals.

at C-6 of the glucosyl group by comparison of the ^{13}C-NMR data. The differences in chemical shifts between **1** and **1a**, δ_{1-1a}: −3.5 (C-5 of glucosyl), +1.8 (C-6 of glucosyl), are ascribable to acetylation at C-6 of the glucosyl group of **1**.[7] The downfield shifts of the H_2-6 signals of the glucosyl group in the ^1H-NMR spectrum of **1**, δ4.72 and 4.83, also supported the above determination.

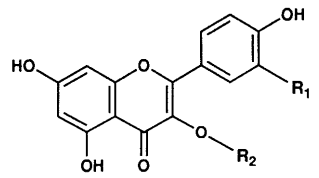
Thus, the structure of **1** was determined to be (6*S*,13*S*)-6-[6-*O*-acetyl-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyloxy]-13-[α-L-rhamnopyranosyl-(1→4)-*β*-D-fucopyranosyloxy]-cleroda-3,14-diene.

Concerning flavonoids, all the ferns were revealed to contain only flavonol 3-*O*-glycosides. Both *D. pedata* and *D. linearis* var. *brevis* contained afzelin (**2**) and quercitrin (**3**), showing the close relationship between the two. *D. linearis* var. *tenuis* contained quercitrin (**3**) and isoquercitrin (**5**). On the other hand, *D. linearis* var. *sebastiana*

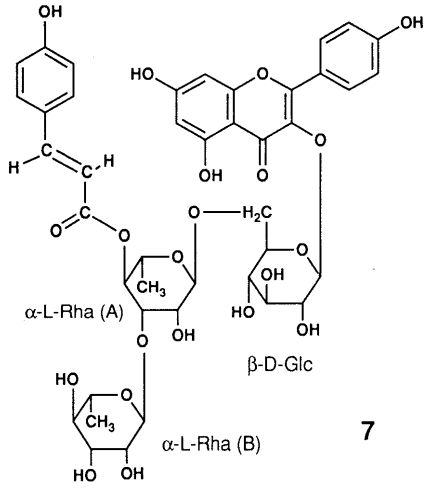
Table 2. NMR Data for **7** in DMSO-d_6 (J in Hz)

	^{13}C	^1H
Kaempferol moiety		
2	156.6	
3	133.0	
4	177.5	
5	161.1	
6	98.7	6.18 (1H, d, 1.9)
7	164.3	
8	93.5	6.31 (1H, d, 1.9)
9	156.7	
10	103.7	
1′	120.8	
2′, 6′	130.7	7.54 (2H, d, 8.6)
3′, 5′	114.9	6.92 (2H, d, 8.6)
4′	159.8	
p-Coumaroyl		
1	127.2	
2, 6	131.6	7.99 (2H, d, 8.6)
3, 5	116.9	6.83 (2H, d, 8.6)
4	161.4	
7	147.5	7.55 (1H, d, 15.8)
8	114.7	6.19 (1H, d, 15.8)
9	168.7	
Glucosyl		
1	101.1	5.39 (1H, d, 6.9)
2	74.2	3.2 (1H, NC)
3	76.3	3.2 (1H, NC)
4	69.4	3.1 (1H, NC)
5	75.2	3.3 (1H, NC)
6	66.6	3.4 (1H, NC), 3.7 (1H, NC)
Rhamnosyl (A)		
1	102.2	4.56 (1H, br s)
2	70.6	3.41 (1H, br s)
3	76.1	3.64 (1H, dd, 9.8, 2.7)
4	71.9	4.91 (1H, t, 9.8)
5	65.9	3.56 (1H, dq, 9.8, 6.1)
6	16.9	0.77 (3H, d, 6.1)
Rhamnosyl (B)		
1	100.4	4.47 (1H, br s)
2	69.7	3.69 (1H, br s)
3	70.3	3.4 (1H, NC)
4	71.7	3.13 (1H, t, 9.3)
5	68.4	3.60 (1H, dq, 9.3, 6.1)
6	17.5	1.02 (3H, d, 6.1)

NC: coupling patterns were not confirmed because of overlapping signals.



2 : R_1 = H, R_2 = α-L-rhamnosyl
3 : R_1 = OH, R_2 = α-L-rhamnosyl
4 : R_1 = H, R_2 = β-D-glucosyl
5 : R_1 = OH, R_2 = β-D-glucosyl
6 : R_1 = OH, R_2 = β-rutinosyl



7

Chart 2

contained astragarin (**4**), isoquercitrin (**5**), rutin (**6**) and a new glycoside (**7**).

Compound **7**, a yellow amorphous solid, $[\alpha]_D$ −42° (c=1.0, MeOH) was formulated as $C_{42}H_{46}O_{21}$ from the HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra revealed the presence of a kaempferol moiety, a *p*-coumaroyl moiety, a hexose moiety and two 6-deoxyhexose moieties (Table 2). On acid hydrolysis, **7** gave kaempferol, D-glucose, L-rhamnose and *p*-coumaric acid. The ^{13}C-NMR data of the kaempferol moiety and the UV spectra with various shift reagents (see Experimental)[8] indicated **7** to be a 3-*O*-glycoside of kaempferol.

The ^{13}C-NMR data of the sugar moiety were assigned by ^{13}C–^{1}H COSY, long range ^{13}C–^{1}H COSY as shown in Table 2. The sequence of the sugar moiety was determined by NOESY, which indicated a correlation between H-6 of the glucosyl group and the anomeric proton of the rhamnosyl group (A) and between H-3 of the rhamnosyl group (A) and the anomeric proton of the rhamnosyl group (B). The esterified position was determined to be C-4 of the rhamnosyl group (A) based on the downfield shift of the corresponding proton signal, 4.91 (1H, t, J=9.8 Hz), in the ^{1}H-NMR spectrum.

Thus, the structure of **7** was determined to be kaempferol 3-*O*-(4-*O*-*p*-coumaroyl-3-*O*-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.

Experimental

The instruments, materials and experimental conditions were the same as described in Part LXXXVII of this series.[6]

Isolation The air-dried fronds (400 g) of *D. pedata* collected in October at Mt. Shibi in Kagoshima prefecture, Japan, were extracted twice with 2 l of MeOH under reflux for 6 h. The extracts and 5 l of MeOH were passed over a column of activated charcoal (50 g) to obtain fraction M. The column was eluted with a mixture of MeOH and $CHCl_3$ (7 : 3, 5 l) to obtain fraction C–M. Fraction M was chromatographed on silica gel using $CHCl_3$ and MeOH, and the fractions containing **1** were collected and rechromatographed on Sephadex LH-20 with 90% MeOH to obtain **1** (165 mg). Fraction C–M was repeatedly chromatographed on Sephadex LH-20 using aqueous MeOH and on silica gel using a mixture of $CHCl_3$, MeOH and H_2O (6 : 4 : 1) to obtain **2** (75 mg) and **3** (52 mg).

The air-dried fronds of *D. linearis* var. *sebastiana* (450 mg) collected in October at Kothyar Hills, South India, were extracted and separated in the same manner as *D. pedata*. Compound **1** (330 mg) was obtained from the fraction M and compounds **4** (15 mg), **5** (7 mg), **6** (63 mg) and **7** (44 mg) were obtained from the fraction C–M.

The air-dried fronds of *D. linearis* var. *brevis* (400 mg) collected in August at Palni Hills, South India, were extracted and separated in the same manner as *D. pedata*. Compound **1** (280 mg) was obtained from the fraction M. Compounds **2** (60 mg) and **3** (56 mg) were obtained from the fraction C–M.

The air-dried fronds of *D. linearis* var. *tenuis* (350 g) collected in September at Thenmalai, South India, were extracted and separated in the same manner as *D. padata*. Compound **1** (398 mg) was obtained from the fraction M and compounds **3** (180 mg) and **5** (106 mg) were obtained from the fraction C–M.

Compound 1 A colorless amorphous solid, $[\alpha]_D$ −72° (c=1.0, MeOH). HR-FAB-MS (negative mode) m/z: 947.483 $[M-H]^-$, Calcd for $C_{46}H_{75}O_{20}$: 947.485.

Compound 7 Yellow amorphous solid, $[\alpha]_D$ −42° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm: 267 (log ε 4.56), 317(4.68), $\lambda_{max}^{MeOH+MeONa}$: 275, 375, $\lambda_{max}^{MeOH+AlCl_3}$: 275, 398, $\lambda_{max}^{MeOH+AlCl_3+HCl}$: 275, 400, $\lambda_{max}^{MeOH+NaOAc}$: 275, 367, $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$: 268, 382. IR ν_{max}^{KBr} cm^{-1}: 3400, 1650, 1600, 1510, 1350, 1170, 1060, 1030, 830. HR-FAB-MS (negative) m/z: 885.248 $[M-H]^-$, Calcd for $C_{42}H_{45}O_{21}$: 885.245. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.99 (2H, d, J=8.6 Hz), 7.55 (1H, d, J=15.8 Hz), 7.54 (2H d, J=8.6 Hz), 6.92 (2H, d, J=8.6 Hz), 6.83 (2H, d, J=8.6 Hz), 6.31 (1H, d, J=1.9 Hz), 6.19 (1H, d, J=15.8 Hz), 6.18 (1H, d, J=1.9 Hz), 5.39 (1H, d, J=6.9 Hz), 4.56 (1H, br s), 4.47 (1H, br s), 3.73—3.11 (14H), 1.02 (3H, d, J=6.1 Hz), 0.77 (3H, d, J=6.4 Hz).

Alkaline Methanolysis of 1 A mixture of **1** (80 mg) and anhydrous Na_2CO_3 (1 g) in MeOH (20 ml) was stirred under reflux for 1 h. The mixture was filtrated and the filtrate was chromatographed on silica gel using a mixture of MeOH and $CHCl_3$ (1 : 4) to obtain **1a** (57 mg).

Compound 1a A colorless amorphous solid, $[\alpha]_D$ −80° (c=0.5, MeOH). ^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.65 (3H, s, H_3-20), 0.72 (3H, d, J=6.4 Hz, H_3-17), 1.07 (3H, s, H_3-17), 1.49 (3H, d, J=6.4 Hz, H_3-6 of fucosyl), 1.56 (3H, s, H_3-16), 1.65 (3H, d, J=6.1 Hz, H_3-6 of rhamnosyl B), 1.69 (3H, d, J=6.1 Hz, H_3-6 of rhamnosyl A), 1.74 (3H, s, H_3-18), 3.37 (1H, dd, J=4.5, 11.1 Hz, H-6), 4.73 (1H, d, J=7.6 Hz, H-1 of fucosyl), 4.89 (1H, br s, H-3), 5.25 (1H, d, J=7.6 Hz, H-1 of glucosyl), 5.26 (1H, d, J=11.0 Hz, H-15), 5.35 (1H, s, H-1 of rhamnosyl A), 5.40 (1H, d, J=17.7 Hz, H-15), 6.21 (1H, dd, J=11.0, 17.7 Hz, H-14), 6.30 (1H, s, H-1 of rhamnosyl B). ^{13}C-NMR (C_5D_5N) δ: 17.9 (C-1), 26.8 (C-2), 123.0 (C-3), 143.2 (C-4), 44.0 (C-5), 86.3 (C-6), 35.1 (C-7), 34.2 (C-8), 38.1 (C-9), 45.7 (C-10), 32.0 (C-11), 35.1 (C-12), 80.4 (C-13), 144.7 (C-14), 115.0 (C-15), 22.8 (C-16), 15.9 (C-17), 22.8 (C-18), 16.3 (C-19), 18.0 (C-20), 99.9 (Fuc-1), 72.4 (Fuc-2), 76.1 (Fuc-3), 77.9 (Fuc-4), 70.6 (Fuc-5), 18.1 (Fuc-6), 103.3 (Rha A-1), 72.2 (Rha A-2), 72.8 (Rha A-3), 85.1 (Rha A-4), 68.3 (Rha A-5), 18.1 (Rha A-6), 103.2 (Rha B-1), 72.4 (Rha B-2), 72.7 (Rha B-3), 73.9 (Rha B-4), 70.2 (Rha B-5), 18.6 (Rha B-6), 106.9 (Glc-1), 76.6 (Glc-2), 78.6 (Glc-3), 71.5 (Glc-4), 78.6 (Glc-5), 62.6 (Glc-6). FAB-MS (negative) m/z: 843 $[M-H]^-$.

Acid Hydrolysis of 1a Compound **1a** (20 mg) was hydrolyzed with 3% HCl (7 ml) under reflux for 1 h. The reaction mixture was concentrated under reduced pressure and chromatographed on silica gel using $CHCl_3$ and MeOH to obtain sugar fractions. The sugar fractions were collected and subjected to HPLC (ODS, 20%H_2O/MeOH) to yield D-fucose (1.4 mg, $[\alpha]_D$ +75°), L-rhamnose (2.2 mg, $[\alpha]_D$ +10°) and D-glucose (1.8 mg, $[\alpha]_D$ +40°). Their trimethylsilyl ethers were identified with authentic samples on GLC.

Enzymatic Hydrolysis of 1a A solution of **1a** (30 mg), a glycosydase mixture of turbo (100 mg, Seikagaku Kogyo Co., Ltd.) and crude

hesperidinase (100 mg) in 0.05 M citrate buffer (pH 4.0, 30 ml) was stirred at 40 °C for 17 h. The reaction mixture was extracted with *n*-BuOH (100 ml). The extract was washed with water and concentrated and chromatographed on silica gel using $CHCl_3$ and MeOH to get **1b** (7 mg).

Compound 1b A colorless amorphous solid, $[\alpha]_D$ −40° (c=0.5, MeOH). ^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.71 (3H, s, H_3-20), 0.79 (3H, d, J=6.7 Hz, H_3-17), 1.08 (3H, s, H_3-19), 1.50 (3H, s, H_3-16), 1.67 (3H, d, J=5.2 Hz, H_3-6 of rhamnosyl), 1.78 (3H, d, J=1.2 Hz, H_3-18), 2.20 (1H, dt, J=2.5, 13.8 Hz, H-7), 3.44 (1H, dd, J=4.6, 11.0 Hz, H-6), 5.13 (1H, br s, H-3), 5.18 (1H, dd, J=2.1, 10.7 Hz, H-15), 5.37 (1H, d, J=1.2 Hz, H-1 of rhamnosyl), 5.57 (1H, dd, J=2.1, 17.3 Hz, H-15), 6.16 (1H, dd, J=10.7, 17.3 Hz, H-14). ^{13}C-NMR (C_5D_5N) δ: 17.9 (C-1), 26.9 (C-2), 122.9 (C-3), 143.6 (C-4), 44.1 (C-5), 86.3 (C-6), 35.3 (C-7), 34.3 (C-8), 38.0 (C-9), 46.0 (C-10), 32.5 (C-11), 35.8 (C-12), 72.5 (C-13), 147.2 (C-14), 111.3 (C-15), 28.7 (C-16), 16.0 (C-17), 22.9 (C-18), 16.3 (C-19), 18.3 (C-20), 103.7 (Rha-1), 72.7 (Rha-2), 74.0 (Rha-3), 73.0 (Rha-4), 70.0 (Rha-5), 18.2 (Rha-6). FAB-MS m/z: 451 $[M-H]^-$.

Acid Hydrolysis of 7 Compound **7** (25 mg) was hydrolyzed with 3% HCl (7 ml) under reflux for 1.5 h. Precipitated yellow needles (kaempferol, mp 275—276 °C, 4.1 mg) were filtered off and washed with water. The filtrate was concentrated under reduced pressure and chromatographed on silica gel using a mixture of $CHCl_3$ and MeOH (4:1) to obtain L-rhamnose (3.1 mg, $[\alpha]_D$ +10°), D-glucose (0.8 mg, $[\alpha]_D$ +40°) and *p*-coumaric acid (0.3 mg). Their trimethylsilyl ethers were identified with authentic samples on GLC. Kaempferol was identified with an authentic sample.

References and Notes

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