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Phenolic Compounds from Seeds of *Plantago ovata* and *P. psyllium*

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A new flavonoid, plantaovaside [quercetin 3-*O*-rutinoside 3'-*O*-β-apioside], along with a known flavonoid, rutin and two known phenylethanoids, acteoside and forsythoside B, have been isolated from the seeds of *Plantago ovata* Forskal (Plantaginaceae). Two known phenylethanoids, acteoside and forsythoside B, have been isolated from the seeds of *Plantago psyllium* L. The structures of the compounds were established by chemical and spectral evidence.

Keywords: *Plantago ovata*; *Plantago phyllium*; Plantaginaceae; seed; flavonoid; phenylethanoid

Plantago psyllium L. (Plantaginaceae) is cultivated in Spain and France for the seeds. The dried ripe seeds from this plant (Psyllium) are used in Europe as domulcents and in the treatment of chronic constipation, while the seeds from *P. ovata* Forskal (Ispaghula husk) are used for similar purposes in India and Pakistan.[1)]

In a previous paper,[2)] we reported on phenolic compounds from the seeds of *P. asiatica* L. and *P. depressa* Willd cultivated in China and Japan, and used as a diuretic and an anti-cough drug.

In this paper, we investigated phenolic compounds of the seeds of *P. ovata* and *P. psyllium*. Here we report on the isolation and structure elucidation of a new flavonoid from the seeds of *P. ovata*, along with a known flavonoid and two phenylethanoids from the seeds of *P. ovata* and *P. psyllium*.

The HPLC chromatogram of a methanol extract of the seeds of *P. ovata* showed the presence of four phenolic compounds, while that of *P. psyllium* showed the presence of two phenolic compounds (Fig. 1).

The methanol extract of the seeds of *P. ovata* was fractionated by successive extractions with diethyl ether, ethyl acetate and *n*-butanol. The ethyl acetate extract after the treatment described in Experimental furnished compound **1**. The *n*-butanol extract after the treatment described in Experimental furnished compounds **2**, **3** and **4**.

The methanol extract of the seeds of *P. psyllium* was treated in similar manner with that of *P. ovata*. The ethyl acetate extract furnished compound **1** and the *n*-butanol extract furnished compound **2**.

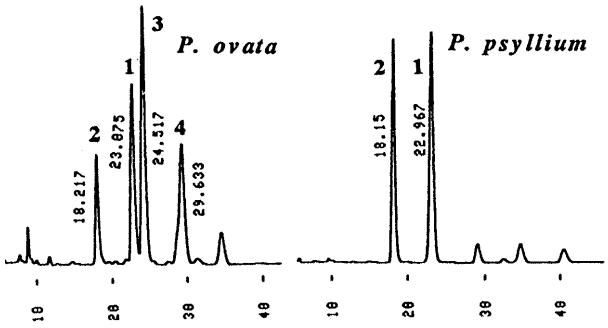
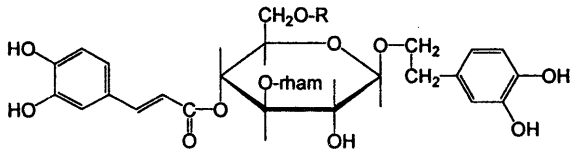
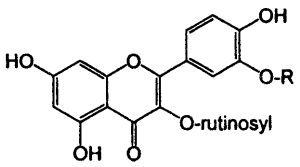


Fig. 1. HPLC Chromatograms of Phenolic Compounds in Methanol Extracts of *Plantago* Seeds



1 R=H
2 R=apiosyl



3 R=apiosyl
4 R=H

Table 1. ^{13}CNMR data for Compounds **1** and **2** (CD_3OD)

	1	2		1	2
Phenethyl moiety			Sugar moiety		
1	131.5	131.4	Glc-1	104.2	104.2
2	116.3	116.3	2	76.0	76.2
3	146.1	146.1	3	81.6	81.6
4	144.7	144.7	4	70.4	70.4
5	117.1	117.1	5	76.0	74.6
6	121.3	121.3	6	62.4	68.5
7	36.6	36.6	Rham-1	103.0	103.1
8	72.1	72.4	2	72.3	72.4
Caffeoyl moiety			3	72.0	72.1
1′	127.7	127.6	4	73.8	73.8
2′	115.2	115.2	5	70.6	70.9
3′	146.8	146.9	6	18.5	18.4
4′	149.8	149.8	Apio-1		111.1
5′	116.5	116.5	2		78.1
6′	123.2	123.2	3		80.6
7′	148.0	148.0	4		75.1
8′	114.7	114.7	5		65.6
9′	168.3	168.1			

Compounds **1** and **4** were characterized to be acteoside (**1**) and rutin (**4**) on the basis of ^{13}C NMR data and by comparison with respective authentic samples. Compound **2** was identified as forsythoside B by comparison of its spectral data with those reported in the literature.[3)]

Compound **3**, designated as plantaovaside, was obtained as a pale yellow powder, mp 210-213° C,

Table 2. ^{13}CNMR data for Compounds **3** and **4** (CD_3OD)

	3	4		3	4
Quercetin moiety			Sugar moiety		
2	158.5	158.5	Glc-1	103.7	104.7
3	135.3	135.6	2	75.8	75.7
4	179.4	179.4	3	78.3	78.2
5	163.1	163.0	4	71.6	71.4
6	100.0	99.9	5	77.3	77.2
7	166.1	166.0	6	68.6	68.6
8	94.9	94.8	Rham-1	102.3	102.4
9	158.6	159.3	2	72.4	72.1
10	105.7	105.6	3	72.1	72.2
1′	123.2	123.1	4	73.9	73.9
2′	119.6	116.0	5	69.7	69.7
3′	145.6	145.8	6	17.9	17.9
4′	151.6	149.8	Apio-1	109.8	
5′	116.7	117.7	2	78.1	
6′	125.5	123.5	3	80.5	
			4	75.6	
			5	64.9	

$[\alpha]_D$ -36.5° (MeOH). The positive ion FAB-mass spectrum of **3** displayed a quasimolecular ion at *m/z* 765 ($[M + Na]^+$), suggesting the molecular formula $C_{32}H_{38}O_{20}$. The UV spectrum of **3** in Band I showed absorption maxima at 356 nm, which showed a small hypsochromic shift from absorption maxima of **4** at 359 nm. The mild acid hydrolysis of **3** gave **4** and apiose. The ^{13}C NMR spectrum of **3** was correlated with that of **4**, indicating that **3** consists of rutin moiety and apiosyl moiety. A H-2' proton chemical shift of B-ring in ^{1}H NMR spectrum of **3** is shifted to downfield (δ 8.07), compared to that of **4** (δ 7.65). The apiosyl moiety in **3** was determined to be linked with the hydroxyl group at 3'-position of B-ring with the aid of NOE interaction between H-2' proton of B-ring and anomeric H-1 proton of apiosyl moiety. In the ^{1}H NMR spectrum, the coupling constant of 3 Hz for the signal due to the anomeric H-1 proton of apiosyl moiety in **3** was consistent with the values for β-apiosyl moity in **2** and β-apiosides such as methyl 2,3,5-tri-*O*-methyl β-apioside.[5)] In addition, the difference of the molecular rotation of -347° between **3** ($[M]_D$ -271°) and **4** ($[M]_D$ +76°) agreed with that

of -284° between **2** ($[M]_D$ -699°) having β-D-apiosyl moiety and **1** ($[M]_D$ -415°). These results verify the β-configuration of the glycoside linkage of apiosyl moiety in **3**. Consequently, the structure of **3** was established as quercetin 3-*O*-rutinoside 3'-*O*-β-apioside.

So far the isolation of plantagoside from the seeds of *P. japonica* is known as the first example of flavonoid from *Plantago* seeds.[4] Therefore, compounds **3** and **4** are the second example of the isolation of flavonoids from *Plantago* seeds.

The husk from the seed coat of *P. ovata* is now distributed as a dietary supplement on the market. The HPLC analysis of the husk also showed the presence of compounds **1** – **4** in a small amount.[2] So it is expected that further investigation on the biological effects of these phenolic compounds should be conducted from the viewpoint of the dietary supplement.

EXPERIMENTAL

General : All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured at 25° C on a JASCO DIP – 360. The ^1H and ^{13}C NMR spectra were recorded on a JEOL EX 400 and the chemical shifts were expressed on the δ (ppm) scale with TMS as an internal standard, FAB-MS on a JEOL JM-600H , UV spectra on a Shimadzu UV 210, and IR spectra on a Hitachi 270-30. TLC was performed by using precoated silica gel plate 60 F_{254} (Merck). Column chromatography was carried out on silica gel (Wakogel C-200, Wako) and Sephadex LH-20 (Pharmacia). HPLC was performed under the conditions : column : Develosil ODS-5 (4.6 x 250 mm), mobil phase : MeOH - H_2O - AcOH (80 : 300 : 20), flow rate : 1.0 ml/min., column temperature : 35° C, and detector : UV 330 nm.

Plant material : The seeds of *P. ovata* and *P. psyllium* were purchased from Chruterhusli, Basel, Switzerland, respectively. Voucher specimens have been deposited in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido.

Extraction and Isolation. The crushed seeds of *P. ovata* (500 g) were extracted with hot MeOH (1 l x 3). The extract was concentrated *in vacuo* at 40° C to one-fifth. H_2O was added and the suspension filtered. The filtrate was extracted successively with Et_2O, EtOAc and *n*-BuOH. The extracts were evaporated to dryness *in vacuo* at 40° C.

The EtOAc extract (1.2 g) was chromatographed on a silica gel column with 5 – 20 % EtOH-$CHCl_3$ gradient. Fractions (50 ml) were monitored by HPLC to give the crude fraction containing **1**. The crude fraction was chromatographed on Sephadex LH-20 with H_2O to give **1** (48 mg).

The *n*-BuOH extract (5.7 g) was chromatographed on a silica gel column with 5 – 20 % EtOH-$CHCl_3$ gradient. Fractions (50 ml) were monitored by HPLC to give the crude fraction containing **4**, crude fraction containing mixture of **1** and **3**, and crude fraction containing **2**, respectively. The crude fraction containing **4** was chromatographed on Sephadex LH-20 with H_2O to give **4** (43 mg). The crude fraction containing mixture of **1** and **3** was chromatographed on Sephadex LH-20 with H_2O, followed by preparative HPLC [solvent; H_2O – MeOH – CH_3CN (100 : 40 : 5)] to give **3** (9 mg). The crude fraction containing **2** was chromatographed on Sephadex LH-20 with H_2O to give **2** (34 mg).

The crushed seeds of *P. psyllium* (500 g) were treated in a similar manner to that of *P. ovata*. The EtOAc extract (0.9 g) gave **1** (74 mg). The *n*-BuOH extract (4.6 g) gave **2** (59 mg).

Acteoside (1) : Amorphous powder, $[\alpha]_D$ –66.5° (c=1.0, MeOH). FAB-MS *m/z* 647 $[M + Na]^+$. ^{13}C NMR (see Table 1).

IR, UV, ^1H and ^{13}C NMR data were consistent with those of an authentic sample.[6]

Forsythoside B (2) : Amorphous powder, $[\alpha]_D$ –92.5° (c=0.1, MeOH). FAB-MS *m/z* 779 $[M + Na]^+$. UV λ (MeOH) nm (log ε): 216 (4.28)sh, 248 (3.98)sh, 290 (4.00), 332 (4.10). IR ν (KBr) cm^{-1}: 3350 (OH), 1700 (CO), 1628 (C=C), 1600, 1518 (arom. C=C). ^1H

NMR (CD_3OD) δ; 1.10 (3H, *d*, *J*=6 Hz, rham CH_3), 2.77 (2H, *t*, *J*=7 Hz, H-7), 4.35 (1H, *d*, *J*=8 Hz, glc H-1), 4.91 (1H, *d*, *J*=3 Hz, apio H-1), 5.17 (1H, *d*, *J*=1.5 Hz, rham H-1), 6.23 (1H, *d*, *J*=16 Hz, H-8'), 7.55 (1H, *d*, *J*=16 Hz, H-7'). ^{13}C NMR (see Table 1).

These spectral data were consistent with those reported in the literature.[3)]

Plantaovaside (3) : Pale yellow powder, mp 210-213° C, $[\alpha]_D$ –36.5° (*c*=0.06, MeOH), FAB-MS *m/z* 765 $[M + Na]^+$. UV λ (MeOH) nm (log ε): 256 (4.39), 303 (3.89)sh, 356 (4.29). IR ν (KBr) cm^{-1}: 3400 (OH), 1654 (CO), 1606, 1508 (C=C). ^1H NMR (CD_3OD) δ : 1.07 (3H, *d*, *J*=6 Hz, rham CH_3), 4.58 (1H, *d*, *J*=1.5 Hz, rham H-1), 5.28 (1H, *d*, *J*=7.5 Hz, glc H-1), 5.67 (1H, *d*, *J*=3 Hz, apio H-1), 6.20 (1H, *d*, *J*=2 Hz, H-6), 6.40 (1H, *d*, *J*=2 Hz, H-8), 6.94 (1H, *d*, *J*=9 Hz, H-5'), 7.68 (1H, *dd*, *J*=2, 9 Hz, H-6'), 8.07 (1H, *d*, *J*=2 Hz, H-2'). ^{13}C NMR (see Table 2.).

Mild Acid Hydrolysis of 3. 5 mg of **3** was refluxed with 0.1 % H_2SO_4 soln. for 30 min. The solution was extracted with EtOAc. After the EtOAc soln. was washed with water, the solvent was evaporated. The presence of **4** in the residue was detected by HPLC analysis. The presence of apiose in the hydrolysate neutralized with $BaCO_3$ was detected by TLC [Solvent: *n*-BuOH – CH_3COOH – H_2O (4 : 1 : 1). The spots were detected by spraying the plate with aniline hydrogene phthalate reagent and heating.] in comparison with apiose in the hydrolysate of **2** treated in similar manner with that of **3**.

Rutin (4) : Pale yellow powder, mp 190-195° C. ^1H NMR (CD_3OD) δ : 1.11 (3H, *d*, *J*=6 Hz, rham CH_3), 4.51 (1H, *d*, *J*=1.5 Hz, rham H-1), 5.09 (1H, *d*, *J*=7.5 Hz, glc H-1), 6.19 (1H, *d*, *J*=2 Hz, H-6), 6.38 (1H, *d*, *J*=2 Hz, H-8), 6.86 (1H, *d*, *J*=9 Hz, H-5'), 7.62 (1H, *dd*, *J*=2, 9 Hz, H-6'), 7.65 (1H, *d*, *J*=2 Hz, H-2'). ^{13}C NMR (see Table 2).

IR, UV, ^1H and ^{13}C NMR data were consistent with those of an authentic sample which was purchased from Funakoshi (Tokyo, Japan).

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