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ISOLATION OF ISOLINARIINS A AND B, NEW FLAVONOID GLYCOSIDES FROM *LINARIA JAPONICA*

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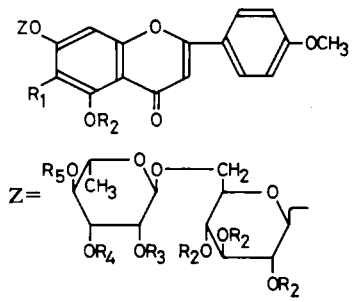
ABSTRACT.—Five flavonoid glycosides were isolated from *Linaria japonica* (Scrophulariaceae). Their structures were determined to be linarin [**1**], pectolinarin [**2**], linariin (4‴-*O*-acetylpectolinarin) [**3**], and two new flavonoid glycosides, isolinariin A (2‴-*O*-acetylpectolinarin) [**4**] and isolinariin B (3‴-*O*-acetylpectolinarin) [**5**]. The compounds were identified using spectroscopic evidence and the results of chemical conversions.

Linaria japonica Miq. (Scrophulariaceae) is a perennial herb which grows on sandy seashores and is used as a folk medicine due to its diuretic and purgative pharmacological activities. Early studies of this species by Takahashi *et al.* (1) and Morita *et al.* (2) revealed the presence of the flavonoid glycosides linarin [**1**], pectolinarin [**2**], linariin [**3**], and unranin. Two additional new flavonoid glycosides, isolinariins A [**4**] and B [**5**], were isolated, along with **1**, **2**, and **3**, on reinvestigation of the same species harvested in Tottori Prefecture. An MeOH extract, analyzed by hplc, showed that linariin and the isolinariins were present [ODS (Inertsil, 6 mm × 250 mm), 55% MeOH, 1.6 ml/min, 330 nm, 25°; linariin 16.8 min, isolinariin A 14.1 min, isolinariin B 9.0 min]. Thus, the isolinariins were not formed during the purification procedures. This paper deals with the determination of the structures of these compounds.

The five flavonoid glycosides were isolated from the MeOH extract of the whole plant biomass (see Experimental). The structures of **1–3** were determined mainly by comparison of their ^{13}C-nmr data with those reported for related compounds (3), and the assignments for compounds **2** and **3** are listed in Table 1.

Isolinariin A [**4**], $C_{31}H_{36}O_{16}$, was obtained as a pale yellow amorphous powder. Its mol wt was determined to be 664 by observation of a quasi-molecular ion peak in fabms at *m/z* 665 and cluster ion peaks at *m/z* 687 and 703 on the addition of NaI and KI, respectively. Its ir and uv spectra showed similar absorption maxima to those of linariin [**3**]. The ^{13}C- and ^{1}H-nmr spectra [AA′BB′ type coupling system at δ 7.144 and



	R_1	R_2	R_3	R_4	R_5
1	H	H	H	H	H
2	OMe	H	H	H	H
3	OMe	H	H	H	Ac
4	OMe	H	Ac	H	H
5	OMe	H	H	Ac	H
6	OMe	Ac	Ac	Ac	Ac

TABLE 1. ^{13}C-nmr Data for Pectolinarin [**2**], Linariin [**3**], and Isolinariins A [**4**] and B [**5**].[a]

Carbon	Compound			
	2	**3**	**4**	**5**
Aglycone				
C-2	163.99	163.93	164.01	163.90
C-3	103.30	103.28	103.28	103.14
C-4	182.27	182.27	182.31	182.34
C-5	152.12[b]	152.08[b]	152.14[b]	152.18[b]
C-6	132.61	132.66	132.69	132.55
C-7	152.46[b]	152.45[b]	152.48[b]	152.47[b]
C-8	94.26	94.37	94.38	94.38
C-9	156.45	156.34	156.40	156.31
C-10	105.83	105.79	105.87	105.18
C-1′	122.72	122.64	122.68	122.72
C-2′, -6′	128.34	128.31	128.37	128.34
C-3′, -5′	114.67	114.62	114.59	114.50
C-4′	162.31	162.34	162.30	162.28
7-OMe	60.25	60.25	60.24	60.23
4′-OMe	55.48	55.48	55.48	55.49
Glucose				
C-1″	100.31	100.09[c]	100.21	99.96[c]
C-2″	73.09	73.15	73.09	73.10
C-3″	76.38	76.39	76.35	76.35
C-4″	69.43	69.22	69.27	69.60
C-5″	75.68	75.30	75.41	75.80
C-6″	65.88	65.47	65.65	65.68
Rhamnose				
C-1‴	100.31	99.86[c]	97.10(−3.21)	99.85[c]
C-2‴	70.36[c]	70.28	72.03(+1.67)	67.77(−2.59)
C-3‴	70.70[c]	68.15(−2.55)[d]	68.56(−2.14)	74.25(+3.55)
C-4‴	71.93	73.76(+1.83)	72.12	68.73(−3.20)
C-5‴	68.24	65.68(−2.56)	68.24	68.30
C-6‴	17.68	17.13	17.49	17.61
CH_3CO-		20.76	20.65	20.77
MeCO-		169.86	169.57	169.75

[a]Measured at 100 MHz in DMSO-d_6.
[b,c]Assignments with the same superscripts may be interchanged.
[d]Figures in parentheses are Δδ **3**, **4** and **5** − **2**.

8.042, another two aromatic protons at δ 6.905 and 6.988, a singlet methyl signal of an acetyl group at δ 1.940 and a doublet methyl signal of a rhamnose moiety at δ 1.065] indicated that **4** is most probably a positional isomer of **3** as to an acetyl group. This assumption was further confirmed by the acetylation, which gave a compound, **6**, identical to the hexaacetate of linariin.

Although five OH groups that can be acetylated are present in the linariin skeleton to form isolinariin A [**4**], the one at the 5 position was excluded by the observation of a chelated hydroxyl proton signal at δ 12.939 in the ^{1}H-nmr spectrum of **4**. Three OH groups on the glucopyranosyl portion were also ruled out as candidates, since six of the glucopyranosyl carbon signals resonated at essentially the same frequencies as those of linariin (see Table 1). Finally, acylation-induced shift trends were applied to determine the position of the acetyl group, i.e., at either the 2‴ position or the 3‴ position (4). When the ^{13}C-nmr signals of the rhamnopyranose moiety were compared with those of pectolinarin [**2**], a significant downfield shift was observed at the 2‴ position (Δδ

+1.67 ppm), and upfield shifts were observed at both sides of carbon signals, i.e., $\Delta\delta$ −3.21 and −2.14 for the 1‴- and 3‴-carbons, respectively. This was indicative that the acetyl group was attached to the OH group at the 2‴ position. This conclusion was further supported by the fact that the doublet of doublet ^{1}H-nmr signal, which was caused by the acylation-induced lowfield shift and appeared at δ 4.915, could be assigned to the proton at the 2‴ position, since one of the coupling constants (J = 1.5 Hz) was the same as that of the anomeric proton of the rhamnopyranosyl moiety. The above-mentioned data led to the assignment of the structure of **4** as 2‴-*O*-acetylpectolinarin.

Isolinariin B [**5**], $C_{31}H_{36}O_{16}$, was crystallized from a mixture of MeOH and H_2O to give pale yellow needles. Its spectral data were similar to those of linariin [**3**] and isolinariin A [**4**], and the acetylation experiment also gave the same acetate as that from **3** or **4**. Thus, isolinariin B is a positional isomer as to an acetyl group, and the same discussion as that leading to the deduction of the structure of isolinariin A led to the conclusion that it is 3‴-*O*-acetyl pectolinarin, designated as **5** (see Table 1).

EXPERIMENTAL

INSTRUMENTATION.—^{1}H- (400 MHz) and ^{13}C- (100 MHz) nmr spectra were recorded on a JEOL JNM-GSX400 spectrometer with TMS (δ = 0) as an internal standard. Ir spectra were recorded on a Shimadzu IR-408 spectrophotometer, and uv spectra were measured with a Shimadzu UV-160A spectrophotometer. Optical rotations were measured with a Union Giken automatic digital polarimeter PM-101. Ms spectra were recorded on a JEOL JMS-SX102 mass spectrometer (eims at 25 eV). The droplet countercurrent chromatograph (dccc) (Tokyo Rikakikai Co., Tokyo) was equipped with 500 glass columns (40 cm length, 2 mm i.d.). The ascending method was used with $CHCl_3$–MeOH–H_2O–*n*-PrOH (45:60:40:10), and 5-*g* fractions were collected. The hplc system used was a Tosoh CCPM and UV-8000 assembly.

PLANT MATERIAL.—Whole plants of *L. japonica* were collected in late July 1990 in seashore areas of Tottori Prefecture, and a voucher specimen (90-LJ-Tottori) was deposited at the Department of Pharmacognosy, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine.

EXTRACTION AND PURIFICATION.—The air-dried plants (2.30 kg) were extracted with MeOH (15 liters × 2). On evaporation of the MeOH extract to 5 liters, 125 g of precipitate was obtained on filtration. The MeOH solution was adjusted to 95% aqueous MeOH by the addition of H_2O and then extracted with *n*-hexane (1.5 liters × 2, 35.0 g). The concentrated MeOH layer was suspended in 1.5 liters of H_2O and then extracted with EtOAc (1.5 liters × 2, 49.7 g) and *n*-BuOH (1.5 liters × 3, 151 g), successively.

A portion (20.0 g) of the precipitate was chromatographed on a Si gel column with $CHCl_3$ to $CHCl_3$-MeOH (3:1). The residue obtained from the 8% MeOH eluate was crystallized from MeOH to afford 3.20 g of **3** as pale yellow needles. Fractions rich in **1** and **2** (16%–25% MeOH eluate) were separated by dccc. Linarin [**1**] was obtained between fractions 90 and 100 (16 mg), and pectolinarin [**2**] between fractions 104 and 125 (145 mg).

The *n*-BuOH extract (150 g) was chromatographed on a column of a highly porous synthetic resin (Diaion HP-20; Nippon Rensui Co.) with stepwise increases of MeOH content in H_2O (20, 40, 60, 80, and 100%). Flavonoid glycosides were concentrated in the 80% aqueous MeOH eluate (12.0 g), which was then subjected to Si gel cc [$CHCl_3$ to $CHCl_3$-MeOH (3:1)]. Secondly, by dccc of the 10% MeOH eluate (700 mg out of 5.43 g), isolinariin B [**5**] was obtained in fractions 178–216 and was crystallized from MeOH/H_2O to give 37 mg of pale yellow needles. The mother liquid gave additional 165 mg of chromatographically pure **5**. In the following fractions, 280–330, linariin [**3**] and isolinariin A [**4**] were co-chromatographed. On concentration of these fractions, a considerable amount of linariin (204 mg) crystallized. Final purification of the mother liquid (173 mg) by hplc [Inertsil-ODS, MeOH-H_2O (11:9)] gave 138 mg of **4** as a pale yellow amorphous powder.

KNOWN COMPOUNDS ISOLATED.—Linarin [**1**]: colorless fine needles (MeOH); mp 265–268°; $[\alpha]^{20}_D$ −53.3° (c = 0.32, pyridine); ^{13}C nmr (DMSO-d_6) δ 17.71 (C-6‴), 55.48 (-OCH_3), 66.01 (C-6″), 68.24 (C-5‴), 69.51 (C-4″), 70.27 (C-2‴)[a], 70.66 (C-3‴)[a], 71.98 (C-4‴), 72.99 (C-2″), 75.58 (C-5″), 76.17 (C-3″), 94.70 (C-8), 99.57 (C-6)[b], 99.85 (C-1‴)[b], 100.44 (C-1″)[b], 103.74 (C-3), 105.38 (C-10), 114.63 (C_2-3′, -5′), 122.60 (C-1′), 128.38 (C_2-2′, -6′), 156.90 (C-5), 161.05 (C-4′)[c], 162.35 (C-9)[c], 162.88 (C-7)[c], 163.86 (C-2), 181.94 (C-4) (assignments with the same superscripts may be interchanged). Pectolinarin [**2**]: pale yellow crystals (MeOH); mp 276–278°; $[\alpha]^{20}_D$ −99.8° (c = 0.72,

pyridine); ^{13}C nmr see Table 1. Linariin [**3**]: pale yellow needles (MeOH); mp 252–254°; $[\alpha]^{20}D$ −87.8° (c = 0.74, pyridine); ^{1}H nmr (DMSO-d_6 containing a trace amount of D_2O) δ 4.711 (t, J = 9.8 Hz, H-4‴), 12.950 (s, 5-O*H*, without D_2O); ^{13}C nmr see Table 1.

ISOLINARIIN A [**4**].—Pale yellow powder: $[\alpha]^{20}D$ −105.4° (c = 0.72, pyridine); ir (KBr) ν max 3300, 1715, 1650, 1600, 1565, 1460, 1240, 1100–1000, 915, 835, 800 cm^{-1}; uv λ max (log ε) (MeOH) 213 (4.49), 277 (4.29), 329 (4.40) nm; λ max (+NaOAc) 212, 276, 328 nm; λ max (+$AlCl_3$) 211, 290 sh, 300, 357 nm; ^{1}H nmr (DMSO-d_6 containing a trace amount of D_2O) δ 1.065 (d, J = 6.2 Hz, H_3-6‴), 1.940 (3H, s, Ac), 3.784 (3H, s, -OMe), 3.862 (3H, s, -OMe), 4.601 (d, J = 1.5 Hz, H-1‴), 4.915 (dd, J = 1.5, 3.6 Hz, H-2‴), 5.173 (d, J = 7.5 Hz, H-1″), 6.905 (s, H-3), 6.988 (s, H-8), 7.144 (d, J = 9.2 Hz, H_2-3′, -5′), 8.042 (d, J = 9.2, H_2-2′, -6′), 12.939 (s, 5-O*H*, without D_2O); ^{13}C nmr see Table 1; fabms (glycerol) *m/z* $[MH]^+$ 665, $[M+Na]^+$ 687 (+NaI), $[M+K]^+$ 703 (+KI). Calcd for $C_{31}H_{36}O_{16}\cdot 2H_2O$, C 53.14, H 5.75; found C 53.46, H 5.68.

ISOLINARIIN B [**5**].—Pale yellow needles (MeOH/H_2O): mp 146–150°; $[\alpha]D$ −113.5° (c = 0.74, pyridine); ir (KBr) ν max 3300, 1720, 1650, 1600, 1560, 1100–1000, 830, 800 cm^{-1}; uv λ max (log ε) (MeOH) 214 (4.46), 278 (4.25), 328 (4.37) nm; λ max (+NaOAc) 213, 277, 328; λ max (+$AlCl_3$) 210, 291 sh, 301, 357 nm; ^{1}H nmr (DMSO-d_6 containing a trace amount of D_2O) δ 1.096 (d, J = 6.2 Hz, H_3-6‴), 1.750 (3H, s, Ac), 3.791 (3H, s, -OMe), 3.871 (3H, s, -OMe), 4.597 (d, J = 1.5 Hz, H-1‴), 4.705 (dd, J = 3.3, 9.9 Hz, H-3‴), 5.189 (d, J = 7.7 Hz, H-1″), 6.886 (s, H-3), 6.940 (s, H-8), 7.115 (d, J = 9.0 Hz, H_2-3′, -5′), 8.022 (d, J = 9.0, H_2-2′, -6′), 12.953 (s, 5-O*H*, without D_2O); ^{13}C nmr see Table 1; fabms (glycerol) *m/z* $[MH]^+$ 665, $[M+Na]^+$ 687 (+NaI), $[M+K]^+$ 704 (+KI). Calcd for $C_{31}H_{36}O_{16}\cdot 2.5H_2O$, C 52.46, H 5.82; found C 52.37, H 5.51.

ACETYLATION OF **3**, **4**, AND **5**.—Linariin (100 mg) was acetylated with a mixture of Ac_2O (1 ml) and pyridine (1 ml) at 60° overnight. Usual workup and crystallization from EtOH/H_2O gave 103 mg of colorless crystals. Compound **6**: mp 119–121°; $[\alpha]D$ −77.4° (c = 0.76, C_6H_6); ir (KBr) ν max 1750, 1630, 1610, 1510, 1360, 1250–1180, 1080–1030, 835 cm^{-1}; uv (MeOH) λ max (log ε) 209 (4.56), 225 (4.35), 260 (4.16), 320 (4.47) nm; ^{1}H nmr ($CDCl_3$) δ 1.893, 2.028, 2.051, 2.062, 2.073, 2.096 (alcoholic Ac × 6), 2.477 (phenolic Ac); ^{13}C nmr ($CDCl_3$) δ 17.35 (C-6‴), [20.61, 20.63 (×3), 20.75, 20.78, 21.01] (-OCO*C*H_3 × 7), 55.51 (4′-O*C*H_3), 61.88 (7-O*C*H_3), 66.31 (C-6″), 66.78 (C-5‴), 68.76 (C-4″)[a], 68.91 (C-3‴)[a], 69.42 (C-2‴)[a], 70.83 (C-2″)[b], 70.88 (C-4‴)[b], 72.34 (C-3″), 73.46 (C-5″), 98.00 (C-8), 99.20 (C-1‴), 103.25 (C-1″), 106.82 (C-3), 113.59 (C-10), 114.49 (C_2-3′, -5′), 123.60 (C-1′), 127.99 (C_2-2′, -6′), 140.58 (C-6), 142.62 (C-5), 153.31 (C-7)[c], 154.31 (C-9)[c], 162.39 (C-4′)[d], 162.52 (C-2)[d], [169.22, 169.41 (×2), 169.84 (×2), 170.00, 170.21] (-O*C*OMe × 7), 176.52 (C-4) (assignments with the same superscripts may be interchanged); fabms (*m*-nitrobenzyl alcohol) *m/z* $[MH]^+$ 917, $[M+Na]^+$ 939 (+NaI), $[M+K]^+$ 955 (+KI); eims *m/z* $[M-42)^+$ 874 (6), $[M-42\times 2]^+$ 832 (1), 314 (100), 299 (83), [Rham(OAc)$_3$ oxonium ion]$^+$ 273 (99), [273 − HOAc]$^+$ 213 (12), [213 − HOAc] 153 (99), [153 − CH_2=C=O] 111 (94). Isolinariins A [**4**] and B [**5**] (30 mg each) were also treated with Ac_2O and pyridine, and the same workup gave the corresponding acetates (30 mg and 28 mg, respectively). The physico-chemical properties of these acetates were essentially indistinguishable from those of the acetate of linariin.

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