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IRIDOID MONO- AND DIESTERS OF D-GLUCOPYRANOSE FROM *LINARIA JAPONICA*

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Key Word Index—*Linaria japonica*; Scrophulariaceae; iridoid; iridolinarosides A–D.

Abstract—From whole plants of *Linaria japonica*, four iridoid esters of D-glucopyranose were isolated. Iridolinaroside A is an iridoid ester on the 1-position of β-D-glucopyranose, while iridolinarosides B–D are esters between two units of the same iridoid moiety and one molecule of D-glucopyranose. Since two iridoid moieties were esterified onto non-anomeric hydroxyl groups, iridolinarosides B–D were isolated as an anomeric mixture.

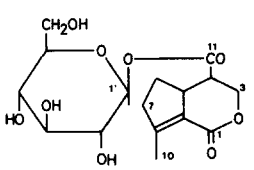
INTRODUCTION

The isolation of flavonoid glycosides [1], iridoid glucosides [2] and a diterpenoid [3] from *Linaria japonica* Miq. were reported earlier. From the same plants harvested in the western part of Japan, new flavonoid glycosides [4], phenylethanoids [5] and iridoid glucosides [6, 7] have been isolated. Further investigation on the plant revealed the presence of several iridoid esters of D-glucopyranose.

RESULTS AND DISCUSSION

The *n*-butanol-soluble portion of a methanol extract of *L. japonica* was fractionated by several types of chromatography to afford four compounds, named iridolinarosides A–D.

Iridolinaroside A (**1**), $[\alpha]_D$ −65.3°, was obtained as an amorphous powder, whose composition was determined to be $C_{16}H_{22}O_9$ by HR-FAB-mass spectrometry. The IR spectrum indicated the presence of hydroxyl (~ 3350 cm^{-1}), ester (1740 cm^{-1}) and α,β-conjugated ester (1695 and 1635 cm^{-1}) groups. The UV absorption at 240 nm was due to the α,β-unsaturated ester function. The ^{13}C NMR spectrum consisted of 16 signals. While five signals were attributed to C-1–C-5 of β-D-glucopyranoside, the anomeric carbon signal, resonating at δ_C 95.7, was indicative of the participation of this carbon in an ester linkage. The highly deshielded chemical shift of an anomeric proton signal (δ_H 5.47) was in accordance with this assumption and its coupling constant (J = 8.1 Hz) suggested the β mode of linkage. The other 10 signals, consisted of one methyl, three methylenes, one of which had an oxygen substituent, two methines, two carbonyl groups and a tetrasubstituted double bond, and were very similar to those of the iridoid portion (**X** in the formulae; a free acid form, **6**, corresponding to **X** is named 7-deoxyiridolactonic acid) of iridolinarin C (see Table 1) [7]. Two-dimensional NMR spectroscopy, including a long-range ^{13}C-^1H COSY experiment which indicated that a lactone ring was formed between C-1 and C-3 [the carbon signal at δ_C 166.3 (C-1) crossed the proton signal at δ_H 4.45 (one of the H_2-3)] confirmed the presence of the 7-deoxyiridolactonic acid moiety in iridolinaroside A. Thus, iridolinaroside A is an ester between 7-deoxyiridolactonic acid and the anomeric hydroxyl group of β-D-glucopyranose (**1**).



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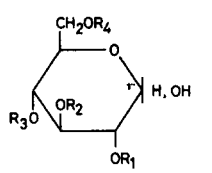

2 R_1, R_4 = H, R_2, R_3 = X

3 R_2, R_4 = H, R_1, R_3 = X

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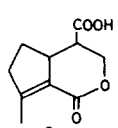
5 R_1, R_2, R_3, R_4 = H



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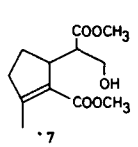

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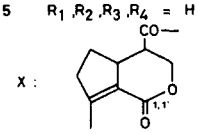

X :

Iridolinaroside B (**2**), $[\alpha]_D$ −14.6°, was obtained as an amorphous powder. HR-FAB-mass spectrometry gave the molecular formula $C_{26}H_{32}O_{12}$ (M_r 536). Its IR absorption maxima were essentially the same as those of iridolinaroside A. The UV absorption was also similar to that of iridolinaroside A, while the absorption intensity was nearly two times stronger (ε 14 400) than that of iridolinaroside A (ε 7900). The ^1H NMR

Table 1. ^{13}C NMR data for iridolinarosides A–D (**1**–**4**), D-glucose (**5**) and 7-deoxyiridolactonate (**X**)

C	**1**		**2**		**3**		**4**			**X***
1	166.3	1α, 1β 1′α, 1′β	165.9 (35), 166.2 (27)	165.9 (28) 166.3 (23)	166.2 (53), 166.4 (28)	166.4 (41)	166.2 (42), 166.5 (42)	166.4 (26)	1″	166.2
3	70.5	3α, 3β 3′α, 3′β	70.7 (72), 70.8 (115)	70.8 (119) 70.8 (95)	70.8 (103), 70.9 (83)	70.8 (77) 71.0 (116)	70.9 (91), 70.9 (116)	70.9 (153)	3″	70.8
4	43.6	4α, 4β 4′α, 4′β	44.01 (135), 44.2 (90)	44.1 (93) 44.2 (103)	43.5 (100), 44.0 (158)	43.9 (92)	43.7 (73), 44.1 (70),	43.8 (142) 44.2 (136)	4″	44.0
5	46.0	5α, 5β 5′α, 5′β	45.9 (146),	46.0 (172)	46.0 (121), 46.1 (179)	46.1 (106)	46.0 (307)		5″	46.0
6	28.0	6α, 6β 6′α, 6′β	27.8 (129), 28.1 (90)	28.1 (109)	27.8 (163), 28.0 (106)	27.8 (94)	28.1 (160),	28.2 (158)	6″	28.2
7	39.3	7α, 7β 7′α, 7′β	39.3 (120), 39.3 (115)	39.3 (147) 39.3 (120)	39.3 (282),		39.2 (248),	39.3 (139)	7″	39.2
8	162.3	8α, 8β 8′α, 8′β	162.5 (70), 163.0 (48)	162.9 (57)	162.2 (65), 162.5 (69),	162.2 (51) 162.60 (55)	162.0 (54), 162.1 (77),	162.1 (50) 162.3 (47)	8″	162.0
9	123.9	9α, 9β 9′α, 9′β	123.7 (57), 123.8 (41)	123.7 (39) 123.8 (52)	123.9 (36), 123.9 (61),	123.9 (49) 124.0 (64)	124.0 (27), 124.1 (70)	124.0 (103)	9″	124.0
10	16.6	10α, 10β 10′α, 10′β	16.8 (304)		16.6 (105), 16.7 (208)	16.7 (96)	16.7 (160), 16.8 (106)	16.7 (81)	10″	16.7
11	171.4	11α, 11β 11′α, 11′β	171.1 (47), 172.1 (40),	171.1 (60) 172.3 (42)	171.6 (48), 171.8 (58),	171.7 (68) 172.3 (69)	172.3 (50), 172.6 (75)	172.5 (105)	11″	172.7
									α	5†β
1†	95.74	1″α, 1″β	93.72 (100),	98.20 (69)	90.94 (100),	96.21 (76)	93.96 (100),	98.30 (59)	93.00 (100),	98.09 (121)
2†	73.87	2″α, 2″β	70.87 (101),	74.42 (71)	75.80 (107),	76.71 (54)	72.12 (98),	74.57 (64)	73.76 (124),	76.22 (142)
3†	78.94	3″α, 3″β	75.64 (80),	77.59 (64)	70.58 (108),	73.96 (73)	76.81 (88),	78.70 (52)	74.81 (101),	78.00 (142)
4†	71.10	4″α, 4″β	71.79 (87),	70.93 (77)	72.80 (90),	72.61 (67)	69.70 (113),	69.67 (71)	71.79 (124),	71.64 (147)
5†	78.17	5″α, 5″β	70.13 (100),	75.21 (74)	69.88 (99),	75.80 (107)	70.60 (100),	75.20 (66)	72.95 (143),	77.93 (156)
6†	62.36	6″α, 6″β	61.62 (70),	61.79 (60)	62.11 (78),	62.25 (62)	64.40 (87),	64.50 (52)	62.70 (89),	62.79 (96)

Figures in the parentheses are signal intensities, which were normalized to that of the 1″-position of α-glucopyranose (100%).
*Data taken from ref. [7].
†Assigned by 2D-INADEQUATE experiment.

signals were almost unassignable owing to their extreme complexity, except for an isolated doublet signal at δ5.12 (J = 3.7 Hz), which was assigned to an anomeric proton from an α-anomer of glucopyranose. Although the ^{13}C NMR signal pattern showed some resemblance to that of iridolinaroside A, each group of signals consisted of two, three or four close signals. Among them, the two signals at δ_C 93.7 and 98.2 appeared without any closely stacked signal. Thus, these were considered to be anomeric carbon signals of α and β anomers of glucopyranose, respectively. On alkaline methanolysis of iridolinaroside B, two compounds were isolated, D-glucopyranose (**5**) and 7-deoxyiridolactonic acid dimethyl ester (**7**). These results suggested that iridolinaroside B was an anomeric mixture of D-glucose to which two units of 7-deoxyiridolactonic acid (**6**) were connected through ester linkage. The positions of the esterified hydroxyl groups were determined by application of ^{13}C NMR acylation-induced shift trends [8]. Since the anomeric carbon signals (δ_C 93.7 and 98.2) of **2** resonated at almost the same frequencies as those of the unsubstituted D-glucopyranose (see Table 1), the 2-position was excluded as an esterification site. At the same time, the 6-position was ruled out as a binding position, since no downfield shift was observed. As a result, the 3- and 4-positions were the only possible positions that could be esterified. Therefore, the structure of iridolinaroside B is 3,4-bis-7-deoxyiridolactonic acid ester of D-glucopyranose (**2**).

Iridolinarosides C (**3**) and D (**4**) were obtained as needles (mp 174–176° and 110–112°, respectively). These compounds have the same composition as iridolinaroside B (**2**), and their IR and UV spectra are essentially the same as those of **2**. Thus, iridolinarosides C and D were considered to be analogous compounds to **2**, namely positional isomers with respect to the ester moieties.

In the ^{13}C NMR spectrum of **3**, the signals for the 6-position of glucose were unaffected, whereas the anomeric carbon signals were obviously shifted upfield by δ 2.06 (α) and 1.88 (β) ($\Delta\delta_5 - \delta_3$). Thus, iridolinaroside C had to be a 2,3- or 2,4-disubstituted derivative. The two carbon signals at δ_C 78.00 and 77.93, which were observed in unsubstituted glucopyranose (see Table 1), and were assigned to those of the 3- and 5-positions of the β-anomer, were considered to be shifted upfield, since the signal that resonated at δ 76.7 was the most deshielded signal of the sugar portion of **3**, except for those of the anomeric carbon. This implied that another 7-deoxyiridolactonic acid moiety must be located at the 4-position. Therefore, the structure of iridolinaroside C was concluded to be the 2,4-bis-7-deoxyiridolactonic acid ester of D-glucopyranose (**3**).

In the ^{13}C NMR spectrum of **4**, the signals of the anomeric carbons were unaffected, whereas the signals of the 6-positions showed downfield shifts of approximately 1.7 ppm. Thus, iridolinaroside D was assumed to be a 3,6- or 4,6-disubstituted derivative. The pair of signals at δ_C 69.7 and 69.7, which were finally assigned to the 4-positions of the glucose moiety, were considered to have shifted upfield. In general, acylation-induced upfield shifts for adjacent carbon atoms are in the range of 1 ~ 3 ppm. On going from unsubstituted glucose (**5**) to **4**, the only possible positions whose calculated upfield shifts did not exceed the limitation were for the 4-positions. The other esterification site of the glucose moiety in **4** seemed most likely to be at the 3-position. Thus the signals of the 4-position (δ_C 71.79 and 71.64) of **5** were affected, showing shifts up to δ_C 69.7 (− 2.09) and 69.67 (− 1.97), respectively. Therefore, the structure of iridolinaroside D is 3,6-bis-7-deoxyiridolactonic acid ester of D-glucopyranose (**4**).

Concerning the stabler forms of glucopyranose, it is well known that all hydroxyl substituents except for anomeric ones tend to be orientated in the equatorial directions to minimize the steric hindrance of substituents. Even for anomeric hydroxyl groups, the case is the same in that an equatorially oriented β-form is thermodynamically dominant in solution (α:β = 9:11, **5** in Table 1). Large values of the α- to β-form ratio in iridolinarosides B–D indicate the influence of the repulsion of large substituents on the ratio of anomeric isomers.

EXPERIMENTAL

^{1}H and ^{13}C NMR: 400 and 100 MHz, respectively; DCCC: 500 glass columns (Φ = 2 mm, L = 40 cm. The ascending method was used with $CHCl_3$–MeOH–H_2O– *n*–PrOH (9:12:8:2), and 5 g frs were collected and numbered according to their order of elution with the mobile phase.

Isolation and purification. Whole plants of *L. japonica* were collected in late July 1990 from seashore areas of Tottori Prefecture. A voucher specimen (90-LJ-Tottori) is deposited at the Herbarium of the Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine.

The isolation and a part of the purification procedures were described in the previous paper [6]. The 40% MeOH eluate (frs 5 and 6) obtained on Diaion HP-20 CC (10.95 g) was sepd by silica gel (450 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (2 l), $CHCl_3$–MeOH (39:1, 2 l; 19:1, 3 l; 9:1, 9 l; 17:3, 6 l; 4:1, 3 l), 500 ml frs being collected]. The residue (1.14 g) of the 10% MeOH eluate was subjected to 3 runs of DCCC. Iridolinaroside A (**1**) was concentrated in frs 84–118 (645 mg) and then this fraction was further purified by reversed phase CC [ODS, Cosmosil (Φ = 50 mm, L = 250 mm), 10% aq. MeOH (1 l) → 50% aq. MeOH (1 l), linear gradient, 10 g frs being collected]. The residue of frs 71–87 (197 mg) was finally purified by prep. HPLC [ODS, Inertsil (Φ = 20 mm, L = 250 mm), 20% aq. MeOH] to give **1** 60 mg.

The 60% MeOH eluate (frs 8 and 9) obtained on Diaion HP-20 CC (14.30 g) was separated by silica gel (450 g) CC with increasing amounts of MeOH in

$CHCl_3$ [$CHCl_3$ (2 l), $CHCl_3$–MeOH (99:1, 3 l; 49:1, 3 l, 24:1, 3 l; 47:3, 6 l; 49:1, 6 l; 90:1, 6 l; 7:1, 3 l, 17:3, 3 l; 4:1, 3 l), 500 ml frs being collected]. The residue from frs 23–25 (317 mg) was purified by prep. HPLC [ODS, Inertsil, (Φ = 20 mm, L = 250 mm), MeOH–H_2O (2:3), 6 ml min^{-1}]. Two peaks which appeared at 44 and 48 min were collected. Although these peaks were collected separately, they were found to be interconvertible, when analysed for their purity by analytical HPLC [ODS, Inertsil, (Φ = 6 mm, L = 250 mm), MeOH–H_2O (2:3), 1.6 ml min^{-1}, R_t = 14.4 and 15.6 min]. This compound was named iridolinaroside B (183 mg, **2**). HPLC analysis of the residue (frs 20–22, 92 mg) obtained on silica gel CC gave three peaks [ODS, Inertsil, (Φ = 6 mm, L = 250 mm), MeOH–H_2O (2:3), 1.6 ml min^{-1}, R_t = 11.5, 13.6 and 15.8 min], which were separated by prep. HPLC (conditions as above). The first peak was named iridolinaroside C (16 mg, **3**), and the interconvertible second and third peaks were named iridolinaroside D (17 mg, **4**).

Iridolinaroside A (**1**). Amorphous powder, $[\alpha]_D^{15}$ − 65.3° (MeOH; c = 1.13), IR ν_{max}^{KBr} cm^{-1}: 3350, 2900, 1740, 1695, 1635, 1400, 1255, 1155, 1070; UV λ_{max}^{MeOH} nm (ε): 240 (7900); ^{13}C NMR: Table 1; ^1H NMR (CD_3OD): δ1.87 (H, *ddd*, J = 9.9, 12.6, 19.8 Hz, H-7a); 2.15 (3H, *t*, J = 0.9 Hz, H_3-10), 2.18 (H, *m*, H-7b), 2.42 (H, *br dd*, J = 9.9, 18.0 Hz, H-6a), 2.55 (H, *m*, H-6b), 3.13 (H, *m*, H-4), 3.41 (H, *m*, H-5), 3.40 (H, *t*, J = 9.2 Hz, H-3H), 3.66 (H, *dd*, J = 5.1, 12.0 Hz, H-6′a), 3.83 (H, *dd*, J = 1.8, 12.0 Hz, H-6′b), 4.45 (H, *dd*, J = 3.5, 11.8 Hz, H-3a) 4.53 (H, *dd*, J = 2.8, 11.8 Hz, H-3b), 5.47 (H, *d*, J = 8.1 Hz, H-1′); FAB-MS (positive centroid) m/z: 359 $[M + H]^+$, 381 $[M + Na]^+$ (+ NaI), 397 $[M + K]^+$ (+ KI); HR-FAB-MS (negative centroid) m/z: 357.1151 $[M - M]^-$ ($C_{16}H_{21}O_9$ requires 357.1186).

Iridolinaroside B (**2**). Amorphous powder, $[\alpha]_D^{15}$ − 14.6° (MeOH: c 1.57, after being dissolved in MeOH for 24 hr), IR ν_{max}^{KBr} cm^{-1}: 3400, 2925, 1725, 1635, 1260, 1055, 1045, 765, 740; UV λ_{max}^{MeOH} nm (ε): 239 (14 400); ^{13}C NMR: Table 1; ^1H NMR (CD_3OD): δ4.97 (*t*, J = 9.1 Hz, H-3″β), 5.02 (*dd*, J = 7.3, 8.3 Hz, H-4″α), 5.08 (*t*, J = 9.3 Hz, H-4″β), 5.12 (*d*, J = 3.7 Hz, H-1″α), 5.29 (*t*, J = 9.3 Hz, H-3″α); HR-FAB-MS (positive centroid) m/z (rel. int.): 537.1945 (39) $[M + H]^+$ ($C_{26}H_{33}O_{12}$ requires 559.1972), 519.1837 $[M - OH]^+$ (100, oxonium ion) ($C_{26}H_{31}O_{11}$ requires 519.1863).

Iridolinaroside C (**3**). Needles, mp 174–176° (MeOH), $[\alpha]_D^{15}$ − 72.2° (MeOH, c 1.07, after being dissolved in MeOH for 24 hr), IR ν_{max}^{KBr} cm^{-1}: 3350, 2900, 1725, 1700, 1635, 1405, 1255, 1180, 1150, 1095, 1055, 1035, 765; UV λ_{max}^{MeOH} nm (ε): 240 (13 900); ^{13}C NMR: Table 1; ^1H NMR (CD_3OD): δ4.74 (*dd*, J = 8.0, 9.7 Hz), 4.91 (*dd*, J = 9.5, 10.1 Hz), 5.25 (*d*, J = 3.7 Hz, H-1″α); HR-FAB-MS (positive centroid) m/z (rel. int.): 537.1897 (100) $[M + H]^+$ ($C_{26}H_{33}O_{12}$ requires 559.1972), 519.1856 $[M - OH]^+$ (25, oxonium ion) ($C_{26}H_{31}O_{11}$ requires 519.1863).

Iridolinaroside D (**4**). Needles, mp 110–112° (MeOH), $[\alpha]_D^{15}$ − 34.4° (MeOH; c 1.13, after being dissolved in MeOH for 24 hr), IR ν_{max}^{KBr} cm^{-1}: 3400, 2900, 1715, 1635, 1055, 1155, 1090, 1030, 760; UV λ_{max}^{MeOH} nm (ε): 239 (14 300); ^{13}C NMR: Table 1; ^1H NMR (CD_3OD): δ4.93 (*t*, J = 9.5 Hz, H-3″α), 5.07 (*d*, J = 3.7 Hz, H-1″α), 5.20 (*t*, J = 9.7 Hz, H-3″β); HR-FAB-MS (positive centroid) m/z (rel. int.): 537.1878 (100) $[M + H]^+$ ($C_{26}H_{33}O_{12}$ requires 559.1972), 519.1887 $[M - OH]^+$ (33, oxonium ion) ($C_{26}H_{31}O_{11}$ requires 519.1863).

Alkaline methanolysis of iridolinaroside B (**2**). Iridolinaroside B (35 mg) was treated with 10 ml of 0.1 M methanolic NaOH under an N_2 atmosphere for 3 hr at 25°. Purification of the resultant material by silica gel CC [Φ = 15 mm, L = 200 mm, C_6H_6–$CHCl_3$ (3:7), 100 ml; $CHCl_3$, 100 ml; $CHCl_3$–MeOH (4:1), 100 ml; $CHCl_3$–MeOH (7:3), 300 ml, 12 ml frs being collected] gave 10.0 mg (28%) of 7-deoxyiridolactonic acid dimethyl ester (**7**) and 11.9 mg (89%) of D-glucose (**5**). 7-Deoxyiridolactonic acid dimethyl ester (**7**), $[\alpha]_D^{25}$ + 13.5° (MeOH; c 0.67), UV λ_{max}^{MeOH} nm (ε): 231 (8500); ^{13}C NMR (CD_3OD): δ16.6 (C-10), 26.3 (C-6), 40.2 (C-7), 47.5 (C-5), 51.5 (MeO- on C-1), 52.1 (MeO- on C-11), 52.1 (C-4), 60.8 (C-3), 129.2 (C-9), 159.0 (C-8), 167.6 (C-1), 176.1 (C-11); other physical data for 7-deoxyiridolactonic acid dimethyl ester were indistinguishable from those reported [7]. D-Glucose, $[\alpha]_D^{25}$ + 24.1° (H_2O; c 0.79, after being dissolved in H_2O for 24 hr).

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