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HIGHLY OXYGENATED ISOFLAVONES FROM *IRIS JAPONICA*

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Key Word Index—*Iris japonica*; Iridaceae; irisjaponins A and B; isoflavones.

Abstract—Two new isoflavones, irisjaponins A and B, have been isolated along with seven known isoflavones from the aerial parts of *Iris japonica*. Their structures have been determined by spectroscopic analyses and comparison of ^{13}C NMR data with those of their congener, as 5,7-dihydroxy-6,2′,3′,4′,5′-pentamethoxyisoflavone and 5,7-dihydroxy-6,2′,3′,4′-tetramethoxyisoflavone.

INTRODUCTION

The rhizomes of *Belamcanda chinensis*, called 'She-gan' in China, have been used in a Chinese traditional drug for the treatment of throat ailments [1] and a number of isoflavones have already been isolated [2,3]. In the course of our studies on biologically active substances in *B. chinensis* [4, 5], we have noted the occurrence of a different plant species named She-gan in Japan, which is *Iris japonica* [6]. This coincidence prompted us to reinvestigate the chemical constituents of the aerial parts of *I. japonica*, resulting in the isolation of two new isoflavones **1** and **2** designated irisjaponins A and B, respectively, along with seven known isoflavones. In the present paper, we report the isolation and structural elucidation of the new isoflavones.

RESULTS AND DISCUSSION

A methanol extract of the aerial parts of *I. japonica* was fractionated by repeated column chromatography on silica gel and Sephadex LH-20 to give two new isoflavones, irisjaponin A (**1**) and irisjaponin B (**2**), together with the previously reported iristectorigenin A (**3**) [6], junipegenin B (**4**) [7], tectorigenin (**5**) [8], irisoridon (**6**) [8], 7-*O*-methylorobol (**7**) [9], iriskumaonin methyl ether (**8**) [8] and irisolone methyl ether (**9**) [8, 10].

Irisjaponin A (**1**) had a molecular formula $C_{20}H_{20}O_9$ which was established by HREI mass spectrometry (*m/z* 404.1118 $[M]^+$). Its IR spectrum showed absorptions attributable to hydroxyl groups (3360 cm^{-1}), which were converted into two acetyl groups, a conjugated carbonyl group (1657 cm^{-1}) and an aromatic moiety (1620 and 1584 cm^{-1}). The ^1H NMR spectrum (Table 1) of **1** contained five methoxyl signals at δ_H 3.75, 3.84, 3.93, 3.96 and 4.04, two singlet aromatic signals at δ_H 6.54 and 6.63, in addition to a low-filed singlet signal at δ_H 7.90, characteristic of the H-2 of isoflavone. Addition of $AlCl_3$ induced a bathochromic shift (263 → 270 nm) in the UV spectrum, indicating the presence of a hydroxyl group at the C-5 position on isoflavone. These spectral data indicated that **1** is isoflavone substituted with five methoxyl and two hydroxyl groups. The presence of 5,7-dihydroxyl-6-methoxyl substituents, typical of isoflavones commonly found in *Iris* species [11], was suggested, as since one (δ_H 6.54) of the two singlet aromatic signals shifted to high field due to being surrounded with two oxygen atoms and showed no NOE interactions with any of the five methoxyl signals. In fact, the ^{13}C NMR data for the A and C rings of **1** corresponded to those of junipegenin B (**4**) (Table 2) except for C-3 and, in the HMBC spectrum, the H-8 signal showed distinct cross-peaks with the C-7 and 9 carbon signals, and the C-6 carbon signal, which correlated with the methoxyl signal at δ_H 4.04. The four remaining methoxyl groups must therefore be linked to the C-2′,3′,4′, and 5′ positions from the following spectral evidence. The singlet aromatic proton signal at δ_H 6.63 showed HMBC correlations with the C-3 (δ_C 120.0) and C-2′ (δ_C 149.4) signals, the latter of which in turn correlated with the methoxyl signal at δ_H 3.84, irradiation of which resulted in a NOE enhancement of the H-2 signal. Accordingly, irisjaponin A (**1**) was assigned as 5,7-dihydroxy-6,2′,3′,4′,5′-pentamethoxyisoflavone.

Irisjaponin B (**2**) had a molecular formula $C_{19}H_{18}O_8$ (*m/z* 374.0998 $[M]^+$) indicating a loss of a methoxyl group from compound **1**. The IR and UV displayed absorptions assignable to a 5,7-dihydroxylated isoflavone. The presence of two hydroxyl groups was supported by the formation of the diacetate **2a** from **2**. The

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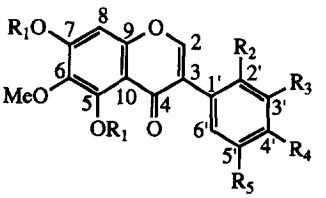

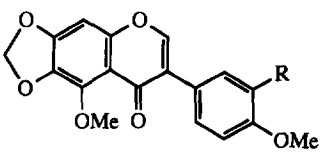

1 $R_1 = H, R_2 = R_3 = R_4 = R_5 = OMe$
1a $R_1 = Ac, R_2 = R_3 = R_4 = R_5 = OMe$
2 $R_1 = R_5 = H, R_2 = R_3 = R_4 = OMe$
2a $R_1 = Ac, R_2 = R_3 = R_4 = OMe, R_5 = H$
3 $R_1 = R_2 = R_5 = H, R_3 = OH, R_4 = OMe$
4 $R_1 = R_2 = R_5 = H, R_3 = R_4 = OMe$
5 $R_1 = R_2 = R_3 = R_5 = H, R_4 = OH$
6 $R_1 = R_2 = R_3 = R_5 = H, R_4 = OMe$
7 $R_1 = R_2 = R_5 = H, R_3 = R_4 = OH$

8 R = OMe
9 R = H

Table 1. ^{1}H NMR spectral data of compounds **1**, **2** and **4** (400 MHz, in $CDCl_3$)

H	1	2	4
2	7.90 *s*	7.85 *s*	7.89 *s*
8	6.54 *s*	6.53 *s*	6.53 *s*
2′	—	—	7.10 *d* (2.0)
5′	—	6.74 *d* (8.6)*	6.94 *d* (8.3)
6′	6.63 *s*	7.02 *d* (8.6)	7.03 *dd* (8.3, 2.0)
6-OMe	4.04 *s*	4.04 *s*	4.04 *s*
2′-OMe	3.84 *s*	3.84 *s*	—
3′-OMe	3.96 *s*	3.91 *s*	3.93 *s*
4′-OMe	3.93 *s*	3.89 *s*	3.92 *s*
5′-OMe	3.75 *s*	—	—

*Coupling constants (J in Hz) given in parentheses.

Table 2. ^{13}C NMR spectral data of compounds **1**, **2** and **4** (100 MHz, in $CDCl_3$)

C	1	2	4
2	152.5	152.5	152.6
3	120.0	120.3	121.3
4	181.2	181.3	181.3
5	154.8	154.3	154.0
6	130.4	130.3	130.4
7	155.2	155.0	155.2
8	93.3	93.2	93.2
9	153.5	153.5	153.4
10	106.5	106.5	106.5
1′	118.6	117.0	121.3
2′	149.4	151.5	112.3
3′	147.3	142.3	148.9
4′	143.5	154.3	149.3
5′	145.9	107.2	111.2
6′	108.9	125.8	123.3
6-OMe	56.2	56.1	56.0
2′-OMe	60.9	60.8	—
3′-OMe	61.2	60.9	60.9
4′-OMe	61.3	61.2	60.9
5′-OMe	61.2	—	—

^{1}H NMR spectrum of **2** indicated the presence of four methoxyl groups and two aromatic protons (J = 8.6 Hz) at δ_H 6.74 and 7.02, suggesting a loss of a methoxyl group at the C-5′ position in irisjaponin A. The ^{13}C NMR data (Table 2) for **2** were almost identical to those of **1**, except for the ^{13}C NMR data assignable to C-1′ ~ C-6′. In addition, one of the *ortho*-coupled aromatic proton signals (δ_H 6.74) showed a NOE enhancement only upon irradiation of the methoxyl signal at δ_H 3.89 among the four methoxyl groups, indicating a substituted array of methoxyl groups at the C-2′,3′ and 4′ positions. Thus, the structure of irisjaponin B (**2**) was 5,7-dihydroxy-6,2′,3′,4′-tetramethoxyisoflavone.

The present study indicated that *B. chinensis* [2, 3] and *I. japonica*, collectively called She-gan, elaborate characteristic 5,6,7-trioxygenated isoflavones. The main differences between the two species are the degree of oxygenation of the B ring and variable *O*-substituents of the 5,6,7-trihydroxyl groups.

EXPERIMENTAL

^{1}H (400 MHz) and ^{13}C (100 MHz): $CDCl_3$ unless otherwise indicated, TMS as int. standard. CC: silica gel (Merck, 70–230 mesh) and Wakogel (C-300). TLC: precoated silica gel 60 F254 and RP-8 F254 plates. Spots were visualized by UV (254 nm) and 2% $CeSO_4$ in H_2SO_4 after heating.

Plant material. Aerial parts of *Iris japonica* were collected in Tokushima, Japan, and a voucher specimen has been deposited in the herbarium of our institute.

Extraction and isolation. Dried and powdered aerial parts (1 kg) were immersed in MeOH at room temp. for 1 month. The MeOH extract was evapd *in vacuo* to give a crude extract (50 g), which was fractionated by CC on silica gel (Merck) using a CH_2Cl_2–EtOAc gradient into 4 frs: fr. 1 (10 g, CH_2Cl_2); fr. 2 (4 g, CH_2Cl_2–EtOAc, 4:1); fr. 3 (0.7 g, CH_2Cl_2–EtOAc, 1:1); fr. 4 (40 g, EtOAc). Fr. 1 (10 g) was further fractionated on Sephadex LH-20 using CH_2Cl_2–*n*-hexane (4:1) to give frs 5–8. Fr. 7 (631 mg) was purified by repeated silica gel CC (C-300,

-1(i) *n*-hexane–EtOAc, 3:1; (ii) CH_2Cl_2–EtOAc, 3:1) to give irisjaponin A (**1**) (26 mg) and irisjaponin B (**2**) (12 mg). Junipegenin B (**4**), irisoridon (**6**), iriskumaonin Me ether (**8**) and irisolone Me ether (**9**) were isolated from fr. 1, and iristectorigenin A (**3**), tectorigenin (**5**) and 7-*O*-methylorobol (**7**) from fr. 4.

Irisjaponin A (**1**). Amorphous.HREIMS *m/z* (rel. int.): 404.1118 $[M]^+$ (100, $C_{20}H_{20}O_9$ requires 404.1108). EIMS *m/z* (rel. int.): 404 $[M]^+$ (100), 389 $[M-15]^+$ (36), 373 (33). UV λ_{max}^{EtOH} nm (ε): 263 (28300); $\lambda_{max}^{EtOH+AlCl_3}$ nm: 270. IR ν_{max}^{FT} cm^{-1}: 3360 (OH), 1657 (C=O), 1620 and 1584 (aroma.). ^{13}C and ^1H NMR: Tables 1 and 2.

5,7-Di-O-acetylirisjaponin A (**1a**). Irisjaponin A (**1**) (2.5 mg) was acetylated with Ac_2O–pyridine in the usual way to yield, after silica gel CC (CH_2Cl_2–EtOAc, 5:1), the diacetate (**1a**) (2.7 mg) as a gum. EIMS *m/z* (rel. int.): 488 $[M]^+$ (12), 446 $[M-42]^+$ (60), 404 (100), 389 (45), 373 (40). IR ν_{max}^{FT} cm^{-1}: 1778 (OCOMe), 1630 (C=O). ^1H NMR: δ2.39 (3H, *s*), 2.45 (3H, *s*), 3.71 (3H, *s*), 3.83 (3H, *s*), 3.87 (3H, *s*), 3.91 (3H, *s*), 3.94 (3H, *s*), 6.57 (1H, *s*), 7.20 (1H, *s*), 7.86 (1H, *s*).

Irisjaponin B (**2**). Amorphous. HREIMS *m/z* (rel. int.): 374.0998 $[M]^+$ (100, $C_{19}H_{18}O_8$ requires 374.1002). EIMS *m/z* (rel. int.): 374 $[M]^+$ (100), 359 $[M-15]^+$ (40), 343 (33). UV λ_{max}^{EtOH} nm (ε): 265 (36000); $\lambda_{max}^{EtOH+AlCl_3}$ nm: 271. IR ν_{max}^{FT} cm^{-1}: 3364 (OH), 1655 (C=O), 1626 and 1597 (aroma.). ^{13}C and ^1H NMR: Tables 1 and 2.

5,7-Di-O-acetylirisjaponin B (**2a**). Irisjaponin B (**1**) (2 mg) was acetylated with Ac_2O–pyridine in the usual way to yield, after silica gel CC (CH_2Cl_2–EtOAc, 5:1), the diacetate (**1a**) (2.2 mg) as a gum. EIMS *m/z* (rel. int.): 458 $[M]^+$ (20), 416 $[M-42]^+$ (100), 374 (67), 359 (26), 343 (19). IR ν_{max}^{FT} cm^{-1}: 1770 (OCOMe), 1660 (C=O). ^1H NMR: δ2.43 (3H, *s*), 2.44 (3H, *s*), 3.78 (3H, *s*), 3.87 (3H, *s*), 3.88 (3H, *s*), 3.89 (3H, *s*), 6.72 (1H, *d*, J = 8.6 Hz), 6.98 (1H, *d*, J = 8.6 Hz), 7.19 (1H, *s*), 7.84 (1H, *s*).

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