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IRIDOID GLUCOSIDES AND PHENYLPROPANOID GLYCOSIDES IN *AJUGA* SPECIES OF JAPAN

HIROKO SHIMOMURA, YUTAKA SASHIDA and KAZUNORI OGAWA

Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachiojishi, Tokyo, 192-03 Japan

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Key Word Index—*Ajuga ciliata* var. *villosior*; *A. decumbens*; *A. incisa*; *A. japonica*; *A. nipponensis*; *A. shikotanensis*; *A. yezoensis*; Labiatae; iridoid glucosides; 8-*O*-acetylharpagide; harpagide; reptoside; phenylpropanoid glycosides; verbascoside; desrhamnosylverbascoside; 2-*O*-(*p*-coumaroyl)-D-glucose.

Abstract—Iridoid glucosides, reptoside, 8-*O*-acetylharpagide, and harpagide, and phenylpropanoid glycosides, verbascoside, desrhamnosylverbascoside, and 2-*O*-(*p*-coumaroyl)-D-glucose have been isolated from seven species of *Ajuga* growing in Japan. No harpagide was originally contained in the living specimens. The iridoid glucoside pattern of each species could be correlated to the stem characteristics of that species.

INTRODUCTION

Some chemotaxonomical studies on the iridoid glucosides of *Ajuga* have been reported [1–3]. As a part of our continuous studies on the chemical constituents of *Ajuga* plants, we wish to report on the distribution of iridoid glucosides and phenylpropanoid glycosides in seven species of *Ajuga* of Japan.

RESULTS AND DISCUSSION

Nine species of the genus *Ajuga* are found in Japan [4]. We have investigated seven of them and isolated three iridoid glucosides, reptoside (**1**), 8-*O*-acetylharpagide (**2**), and harpagide (**3**) and three phenylpropanoid glycosides, verbascoside (**4**), desrhamnosylverbascoside (**5**), and 2-*O*-(*p*-coumaroyl)-D-glucose (**6**). The distribution of the iridoid glucosides and the phenylpropanoid glycosides are summarized in Table 1. From all the air-dried specimens containing **2**, **3** was isolated, but it was not originally contained in the living specimens (see Experimental).

The genus *Ajuga* is classified into several sections [5] and all the Japanese *Ajuga* species belong to the section Ajuga (= the section Bugula) [6]. Wu and Chen classified the section Ajuga of China into two subsections, Genevensis (dense verticillaster, 6 or more flowers, often forming spike-like racemes, erect stems, rarely stoloniferous) and Biflorae (verticils axillary, 2 or rarely 4 or more flowers, bracts similar sizes to cauline leaves, stoloniferous) [6]. According to the above-mentioned characteristics, all the Japanese species investigated belong to the subsection Genevensis. The first four of the seven species in Table 1 are characterized by their fascicular and ascending stems or their erect stems and creeping stems after the flowering period. These are usually below 20 cm high. *A. decumbens* has decumbent stems and no erect stems. *A. incisa* (30–50 cm high) and *A. ciliata* var. *villosior* (30–40 cm high) differ from the other Genevensis species with their erect stems and large sizes.

It is remarkable that the distribution of iridoid glucosides was obviously correlated with the characteristics of the stems within the seven Genevensis species examined. The distribution of **1** and **2** is considered to be applicable to subsectional classification of the section Ajuga.

From three of the seven species, **4** was obtained. *A. nipponensis* also contained **5** and **6**. This was the first time that **5** had been isolated from plants [7]. Recently, **5** has been isolated from the leaves of *Digitalis purpurea* [8]. The relationship between the phenylpropanoid glycoside distribution and taxonomy is not as clear as the case of the iridoid glucosides.

EXPERIMENTAL

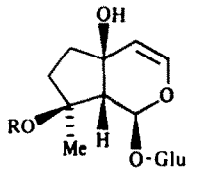
Plant materials. All specimens were collected in the flowering period. *A. nipponensis*, in June 1978 at Hachioji, Tokyo; *A. decumbens*, in June 1982 at Tsukui, Kanagawa; *A. incisa*, in June 1982 at Kiryu, Gumma; *A. japonica*, in June 1982 at Itsukaichi, Tokyo; *A. yezoensis*, in May 1983 at Agero, Niigata; *A. shikotanensis*, in July 1984 at Minamisaku, Nagano; *A. ciliata* var. *villosior*, in July 1984 at Minamisaku, Nagano. Specimens were deposited in the Harbarium of the Tokyo College of Pharmacy.

Isolation. The dried or fresh materials were extracted with MeOH. The concentrated extracts were divided between BuOH (or EtOAc) and H_2O. The H_2O layers were separated by silica gel column chromatography (Merck No. 9385, EtOAc–MeOH or $CHCl_3$–MeOH).

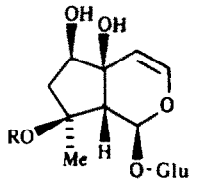
Reptoside (**1**). The data of **1** ($[\alpha]_D$, ^{1}H NMR, ^{13}C NMR, and IR) and pentaacetate of **1** (mp, $[\alpha]_D$, and ^{1}H NMR) were consistent with the data described in refs [9, 10].

8-O-*Acetylharpagide* (**2**) *and harpagide* (**3**). IR, $[\alpha]_D$, ^{1}H NMR, and ^{13}C NMR of **2** and **3** were in agreement with the reported data [10–12]. The peracetate of **2** or **3** was identified by comparison (mmp, TLC, and IR) with an authentic sample. Re-examination of the living specimens showed that **3** was not detectable, and it was gradually formed as the specimens were dried. Therefore, **3** resulted from deacetylation of **2** during the drying procedure.

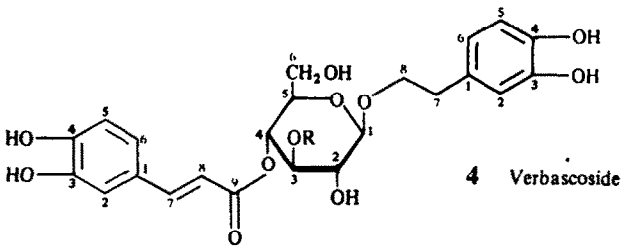
Verbascoside (**4**). IR, ^{1}H NMR, and ^{13}C NMR data were in agreement with refs [13–15]. Tetramethylether of **4** was identical with an authentic sample (IR and TLC).



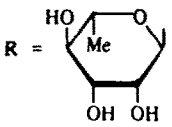
1 Reptoside



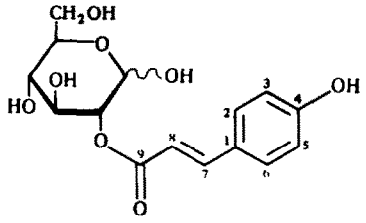
2 8-*O*-Acetylharpagide R = Ac
3 Harpagide R = H



4 Verbascoside

R = 

5 Desrhamnosylverbascoside R = H



6 2-(*p*-Coumaroyl)-D-glucoside

Table 1. Distribution of iridoid glucosides and phenylpropanoid glycosides in *Ajuga* of Japan

	Reptoside (1)	8-*O*-Acetyl harpagide (2)	Harpagide (3)*	Verbascoside (4)
A. nipponensis		+ (4.0)	+ (0.1)	+ (1.0)†
A. yezoensis		+ (1.8)	+ (1.0)	
A. japonica		+ (2.9)	+ (0.4)	+ (0.6)
A. shikotanensis		+ (1.0)	+ (0.4)	
A. decumbens	+ (0.1)	+ (1.3)	+ (0.3)	
A. incisa	+ (1.3)			+ (0.3)
A. ciliata var. *villosior*	+ (1.3)			

*No harpagide was detected from any of the fresh specimens.

†Desrhamnosylverbascoside (0.02) and 2-*O*-(*p*-coumaroyl)-D-glucose (0.02), in addition to verbascoside, were isolated from *A. nipponensis*. Yields (% of dry wt) are given in parentheses.

Desrhamnosylverbascoside (**5**). Amorphous powder. Secondary ion-MS *m/z*: 501 $[M+Na]^+$, 479 $[M+H]^+$. IR ν_{max}^{KBr} cm^{-1}: 3300, 1690, 1600, 1520, 1440. $[\alpha]_D^{20}$ −26.9° (MeOH, *c* 0.80). ^1H NMR (100 MHz, CD_3OD); δ 7.56 and 6.27 (2H, AB*q*, *J* = 16 Hz), 7.4–6.4 (6H), 4.33 (1H, *d*, *J* = 8 Hz), 4.2–3.0 (5H), 2.76 (2H, *t*, *J* = 7.6 Hz). ^{13}C NMR (25 MHz, CD_3OD); δ

glucose moiety [104.0 (1), 75.0 (2), 75.7 (3), 72.2 (4), 75.5 (5), 62.2 (6)], caffeic acid moiety [127.4 (1), 114.5 (2), 146.4 (3), 149.3 (4), 115.2 (5), 123.0 (6), 147.5 (7), 116.4 (8), 168.5 (9)]; 3,4-dihydroxy-β-phenethyl alcohol moiety [131.3 (1), 116.4 (2), 146.4 (3), 144.3 (4), 117.0 (5), 121.2 (6), 36.3 (7), 72.2 (8)]. ^{13}C NMR signals of C-3 and C-5 of the glucosyl moiety were shifted further upfield than those of methyl β-D-glucopyranoside (δ 78.3). The upfield shifts are due to β-effect from acyl group at C-4. The spectra agree with the predicted data for desrhamnosyl derivative of **4** [16]. Thus, **5** was determined as desrhamnosylverbascoside, 2-(2,3-dihydroxyphenyl)ethyl 4-*O*-caffeoyl-β-D-glucopyranoside.

2-O-(p-*Coumaroyl*)-D-*glucose* (**6**). White powder from EtOAc. IR ν_{max}^{KBr} cm^{-1}: 3480, 1700, 1600, 1440, 1280–1260. $[\alpha]_D^{20}$ + 27.3° (0.5 h) → + 51.8° (24 h) (MeOH, *c* 1.10). ^{1}H NMR (100 MHz, CD_3OD): δ 7.69 (7.66) and 6.38 (6.39), 7.46 (7.46) and 6.80 (6.80), 5.34, 4.0, 4.8–3.4. ^{13}C NMR (25 MHz, C_5D_5N); δ glucose moiety [α-anomer 91.3 (1), 75.6 (2), 72.1 (3), 72.3 (4), 73.3 (5), 62.6 (6); β-anomer 96.5 (1), 76.9 (2), 76.2 (3), 72.1 (4), 78.3 (5), 62.6 (6)]; *p*-coumaric acid moiety [α-anomer 126.0 (1), 116.5 (2 and 6), 130.4 (3 and 5), 160.9 (4), 145.2 (7), 115.1 (8), 167.4 (9); β-anomer 126.0 (1), 116.5 (2 and 6), 130.4 (3 and 5), 160.9 (4), 145.2 (7), 115.5 (8), 167.0 (9)]. The anomeric carbons are shifted upfield as a result of a β-effect from an adjacent *p*-coumarate group, which is attached to C-2 of the D-glucose.

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