생 약 학 회 지
Kor. J. Pharmacogn.
29(2) : 75~78(1998)

Studies on the Constituents of the Herbs of *Ajuga multiflora* (I)

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Abstract – In the course of phytochemical studies for the aerial parts of *Ajuga multiflora*, one flavonoid and two iridoid glycosides were isolated and identified as apigenin (1), 8-*O*-acetylharpagide (2) and harpagide (3) on the basis of spectroscopic evidence.

Key words – *Ajuga multiflora*; Labiatae; apigenin; 8-*O*-acetylharpagide; harpagide.

The genus *Ajuga* have been used in folk medicine in various cultures and several interesting medicinal properties, such as antifebrile, anthelmintic, hypoglycaemic and vulnerary effects have been attributed to them. Traditionally, some *Ajuga* spp. have been used to treat inflammation in China. Many representatives of the genus *Ajuga* contain phytoecdysteroids, polyhydroxysteroids with a 5β-H-7-ene-6-one system and clerodane diterpenoids, exhibiting well established physiological activities in insects and also in mammals which might explain some of the successful applications of these plants in folk medicine.[1)] But, little phytochemical work have been done on *Ajuga multiflora*. We report herein the isolation and strutural elucidation of a flavonoid and two iridoid glycosides from this plant.

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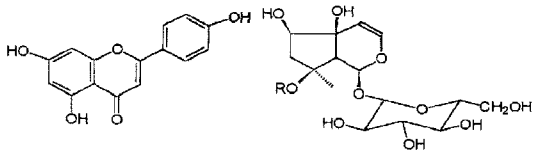
Experimental

General experimental procedures – The mps were taken on a Yanaco micro-melting point appratus and are uncorrected. The EI-MS and FAB-MS spectra were recorded on a JMS SX-102A and JMS HX-110/110A (JEOL) spectrometer. The ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AMX-300 spectrometer with TMS as an internal standard and chemical shifts are given in ppm. TLC chromatography was performed on precoated Kieselgel 60 F_{254} plates (Merck, 5715).

Plant material – The herbs of *A. multiflora* were collected in KyungBug province of Korea in the spring season of 1996 and authenticated by Prof. Kyu-Young Jung, Dept. of Plant Resources and Environment, Andong National University, Korea. The voucher speciman is deposited in our laboratory.

Extraction and isolation – The chopped herbs of *A. multiflora* (2.5 kg) were extracted with MeOH under reflux (three times, 12

h each). The combined MeOH extracts were evaporated under reduced pressure, to give a brown residue (302 g), which was partitioned with n-hexane, EtOAc, *n*-BuOH and water, successively. EtOAc (11 g), *n*-BUOH (50 g) and H_2O (30 g) extracts were chromatographed on silica gel with increasing concentration of MeOH in $CHCl_3$ and/or $CHCl_3$-MeOH-H_2O (8:2:0.5→7:3:1→52:28:8, each lower layer) as eluents. Compound 1 from EtOAc extract, compound 2 from *n*-BuOH extract and compound 3 from H_2O extract were isolated.



1

2 R= H_3C-CO-
3 R= H

Compound 1—A yellow amorphous powder from MeOH, mp > 300℃, $FeCl_3$, Mg/HCl tests: positive. EI-MS *m/z* (rel. int.) 270 $[M]^+$ (100.0), 269 $[M-H]^+$ (13.2), 242 $[M-CO]^+$ (18.4), 241 $[M-HCO]^+$ (6.0), 213 [241-CO]$^+$ (4.0), 153 [RDA fragment with A ring+H]$^+$ (22.0), 121 [RDA fragment with B ring]$^+$ (16.0). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 13.0 (1H, brs, C_5-OH), 7.93 (2H, d, *J*=8.8 Hz, H-2′ and 6′), 6.94 (2H, d, *J* =8.8 Hz, H-3′ and 5′), 6.79 (1H, s, H-3), 6.49 (1H, d, *J*=2.0 Hz, H-8), 6.20 (1H, d, *J*=2.0 Hz, H-6). ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ 163.7 (C-2), 102.8 (C-3), 181.7 (C-4), 161.4 (C-5), 98.8 (C-6), 164.1 (C-7), 93.9 (C-8), 157.3 (C-9), 103.7 (C-10), 121.1 (C-1′), 128.4 (C-2′, 6′), 115.9 (C-3′, 5′).

Compound 2—Amorphous powder from MeOH, mp 154~156℃. FAB-MS *m/z* (rel. int.) 429 $[M+Na]^+$ (31.16). ^{1}H-NMR (300 MHz, CD_3OD) δ 1.45 (3H, s, H-10), 1.94 (1H, dd, *J* =15.1, 4.5 Hz, H-7a), 2.01 (3H, s, OAc), 2.16 (1H, d, *J*=15.1 Hz, H-7b), 2.85 (1H, d, *J*=1.0 Hz, H-9), 3.16~3.41 (4H, m, H-2′~5′), 3.69 (2H, m, H-6, 6′a), 3.88 (1H, dd, *J*=11.8, 1.1 Hz, H-6′b), 4.58 (1H, d, *J*=7.9 Hz, H-1′), 4.91 (1H, dd, *J*=6.4, 1.6 Hz, H-4), 6.06 (1H, d, *J*= 1.0 Hz, H-1), 6.38 (1H, d, *J*=6.4 Hz, H-3). ^{13}C-NMR (75.5 MHz, CD_3OD) see Table I.

Table I. ^{13}C-NMR assignments of 8-*O*-acetylharpagide (2) and harpagide (3)[a]

Position	2	3
1	95.0 (CH)	93.7 (CH)
3	144.4 (CH)	144.0 (CH)
4	107.4 (CH)	108.9 (CH)
5	73.8 (C)	73.0 (C)
6	78.7 (CH)	78.8 (CH)
7	46.5 (CH_2)	47.7 (CH_2)
8	89.1 (C)	78.7 (C)
9	56.0 (CH)	60.1 (CH)
10	23.0 (CH_3)	25.4 (CH_3)
CH_3CO	173.8 (C)	
CH_3CO	22.7 (CH_3)	
1′	100.4 (CH)	99.9 (CH)
2′	75.0 (CH)	75.0 (CH)
3′	78.2 (CH)	78.0 (CH)
4′	72.2 (CH)	72.3 (CH)
5′	78.2 (CH)	78.6 (CH)
6′	63.4 (CH_2)	63.3 (CH_2)

[a]Assignments were carried out by means of DEPT, ^{1}H-^{1}H COSY and HMQC.

Compound 3—Amorphous powder from MeOH, mp 153-155℃. FAB-MS *m/z* (rel. int.) 387 $[M+Na]^+$ (100.0). ^{1}H-NMR (300 MHz, CD_3 OD) δ 1.26 (3H, s, H-10), 1.81 (1H, dd, *J*=13.7, 3.8 Hz, H-7a), 1.91 (1H, dd, *J*=13.7, 4.7 Hz, H-7b), 2.56 (1H, s, H-9), 3.22~3.42 (4H, m, H-2′~5′), 3.69 (2H, m, H-6, 6′a), 3.91 (1H, d, *J*=11.2 Hz, H-6′b), 4.59 (1H, d, *J*=7.9 Hz, H-1′), 4.96 (1H, d, *J*=6.2 Hz, H-4), 5.75 (1H, s, H-1), 6.33 (1H, d, *J*=6.2 Hz, H-3). ^{13}C-NMR (75.5 MHz, CD_3OD) see Table I.

Results and Discussion

After repeated column chromatography of each extract, three compounds, one flavonoid and two iridoid compounds, were iso-

lated from *A. multiflora*.

Compound 1 showed positive $FeCl_3$ and Mg/HCl tests and the molecular weight of 1 was decided as 270 by EI-MS, which exhibited the characteristic fragment ion peaks at *m/z* 153 and 121 by the *retro*-Diels Alder (RDA) fragmentation of flavonoid.[2] The ^{1}H-NMR spectrum of 1 exhibited typical signal pattern ascribable to flavone. That is, the siganals due to H-3, H-6 and H-8 were observed at δ 6.79 (1H, s), 6.20 (1H, d, *J*=2 Hz), 6.49 (1H, d, *J*=2 Hz) and the signals at δ 7.93 (2H, d, *J*=8.8 Hz) and 6.94 (2H, d, *J*=8.8 Hz) were resolved into AA'BB' system due to a *para*-substituted benzene ring. These data indicated that 1 was a 5,7,4'-trihydroxyflavone, apigenin, which was further identified by ^{13}C-NMR spectrum of 1.

Compound 2 was obtained as amorphous powder. The molecular weight of 2 was decided as 364 by FAB-MS spectrum, showing the pseudomolecular ion peak at *m/z* 387 $[M+Na]^+$. The ^{1}H- and ^{13}C-NMR spectra of 2 showed one tertiary methyl (δ 1.45; δ 23.0), one acetyl (δ 2.01; δ 22.7 and 173.8), and one disustituted double bond (δ 6.33 and 4.96; δ 144.4 and 107.4). The ^{13}C-NMR and DEPT spectra also showed an acetalic, a secondary carbinyl, a methylene, a methine, two quarternary carbons having an oxygen function, and the signals due to a glucose moiety. The β-glycosidic linkage was derived from the coupling constant (7.9 Hz) of anomeric proton.[3] In the ^{1}H-^{1}H COSY spectrum of 2, the acetalic proton (H-1) and a methylene prtons (H-7) were correlated with the signal at δ 2.85 (H-9) and a methine proton on an oxygen-bearing carbon (C-6), respectively. In the ^{1}H-NMR spectrum of 2, the signals of the methyl proton (H-10) and neighbouring protons (H-9 and H-1) were observed at rather low field region (δ 1.45, 2.85 and 6.06, respectively) due to the presence of the acetoxyl group at C-8.[4] On the basis of these findings, the structure of 2 was elucidated as 8-*O*-acetylharpagide.

Compound 3 exhibited the pseudomolecular ion peak at *m/z* 387 $[M+Na]^+$ in its FAB-MS spectrum. Compared to compound 2, compound 3 had a similar ^{1}H- and ^{13}C-NMR spectra except the absence of the acetyl group. In the ^{1}H-NMR spectrum of 3, the proton signals at C-9, C-10 and C-1, which lay at δ 2.85, 1.45 and 6.06, respectively in compound 2, were displaced to 2.56, 1.26 and 5.75, respectively. In the comparison of ^{13}C-NMR spectra of 3 and 2, the expected downfield shift (2.4 ppm) of methyl carbon signal (C-10, δ 25.4) was also observed.[5] From the above data, the structure of 3 was identified as harpagide and the spectral data were exactly consistent with literature.[6,7]

Acknowledgements

This research was partly supported by the research grant from Institute for Drug Research, Yeungnam University.

References

1. Camps, F. and Coll, J. (1993) Insect allelochemicals from *Ajuga* plants. *Phytochemistry* 32: 1361-1370.
2. Markham, K. R. (1982) Techniques of flavonoid identification. 87-90. Academic Press, London.
3. Shoji, N., Umeyama, A., Sunahara, N. and Arihara, S. (1992) Ajureptoside, a novel C_9 iridoid glucoside from *Ajuga reptans*. *J. Nat. Prod.* 55: 1004-1006.
4. Scarpati, M. L., Guiso, M. and Panizzi, L. (1965) Iridoids I. Harpagide acetate from *Melittis melissophyllum*. *Tetrahedron Letters* 3439-3443.
5. Chaudhuri, R. K., Afifi-Yazar, F. Ü., Sticher, O.

and Winkler, T. (1980) ^{13}C NMR spectroscopy of naturally occurring iridoid glucosides and their acylated derivatives. *Tetrahedron* 36: 2317-2326.

6. El-Naggar, L. and Beal, J. (1980) Iridoids. A review. *J. Nat. Prod.* 43: 649-707.
7. Lichti, H. and Wartburg, A. (1964) Zur konstitution von harpagosid. *Tetrahedron Letters* 835-843.

(Received March 6, 1998)