国立科博専報, (45): 111–119, 2008年3月28日
Mem. Natl. Mus. Nat. Sci., Tokyo, (45): 111–119, March 28, 2008

New Flavonoid Chemotypes from *Asplenium normale* (Aspleniaceae) in Malaysia

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Abstracts. Seven *Asplenium normale* individuals in Malaysia were surveyed for flavonoid compounds. They were divided into two chemotypes, **H**- and **I**-types. The flavonoids were isolated by various chromatography and identified by TLC, HPLC, UV spectroscopic, LC-MS and NMR surveys. Two flavone *O*-glycosides, apigenin 7-*O*-rhamnosyl-(1→4)-rhamnoside (**1**) and apigenin 7-*O*-rhamnosyl-(1→4)-rhamnoside-4′-*O*-rhamnoside (**2**), and two flavone *C*-glycosides, vicenin-2 (**6**) and lucenin-2 (**7**), were contained in one chemotype (**H**-type). On the other hand, two flavonol *O*-glycosides, kaempferol 3-*O*-glucosylrhamnoside (**3**) and kaempferol 3,4′-di-*O*-glycoside (**4**) and a flavone *O*-glycoside, genkwanin 4′-*O*-glucosyl-(1→3)-rhamnoside (**5**), were found from another chemotype (**I**-type) together with **6** and **7**. In cases of Japanese *Asplenium normale* and related species, seven chemotypes have been reported. However, their chemotypes did not include flavonol *O*-glycosides and apigenin trirhamnoside. Apigenin 7-*O*-rhamnosyl-(1→4)-rhamnoside (**1**) and apigenin 7-*O*-rhamnosyl-(1→4)-rhamnoside-4′-*O*-rhamnoside (**2**) were reported in nature for the first time.

Key words: Apigenin *O*-glycosides, kaempferol *O*-glycosides, flavonoids, *Asplenium normale*, Malaysia, Aspleniaceae, chemotypes, chemotaxonomy.

Introduction

Asplenium normale D. Don (Aspleniaceae) (Fig. 1) is broadly distributed throughout East and Southeast Asia, Africa and Hawaii (Nakaike, 1992), and two related species, *A. boreale* (Ohwi ex Sa. Kurata) Nakaike and *A. shimurae* (H. Ito) Nakaike occur in the Sino-Japanese area (Ching & Iwatsuki, 1982). Another related *A. oligophlebium* Bak. including var. *iezimaense* (Tagawa) Tagawa, which is restricted to Iejima island, is endemic to Japan (Nakaike, 1992).

In the previous paper (Iwashina *et al.*, 1990), we have presented that *A. normale*, *A. boreale*, *A. shimurae* and *A. oligophlebium* have distinct flavonoid patterns, i.e., apigenin 7-*O*-dirhamnoside (**8**), luteolin 7-*O*-dirhamnoside (**9**) and genkwanin 4′-*O*-glucosylrhamnoside (**5**) in *A. normale*, apigenin 7,4′-di-*O*-rhamnoside (**10**), which has been misidentified as flavonoid **5** on account of their very similar chromatographic and UV spectral properties, in *A. boreale*, apigenin 7-*O*-glucosylrhamnoside (**11**) and luteolin 7-*O*-glucosylrhamnoside (**12**) in *A. shimurae*, and flavonoid **5** alone in *A. oligophlebium*. Of their glycosides, flavonoids **5**, **8**, **9** and **10** were new compounds in nature. In 1993, another new flavonoid, apigenin 7-*O*-rhamnoside-4′-*O*-glucosylrhamnoside (**13**) has been isolated and identified from *A. normale* by us (Iwashina *et al.*,

Fig. 1. *Asplenium normale* D. Don. Choyo Village, Kumamoto Pref., Japan, 3 Feb. 2001, photographed by T. Iwashina.

1993). Their flavonoid glycosides have completely been identified as apigenin 7-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-rhamnopyranoside (**8**), luteolin 7-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-rhamnopyranoside (**9**), genkwanin 4′-O-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranoside (**5**) and apigenin 7-*O*-α-L-rhamnopyranoside-4′-*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranoside (**13**) by ^{13}C NMR survey (Matsumoto *et al.*, 2003).

Among *A. normale* and related species, it was shown that *A. normale* is divided into seven chemotypes, i.e., **A**-type: flavonoids **5**, **8** and **9**; **B**-type: **5** alone; **C**-type: **13** alone; **D**-type: **8** and **9**; **E**-type: **8**, **9** and **13**; **F**-type: **5**, **8**, **9** and **13**; and **G**-type: **5** and **13** (Iwashina & Matsumoto, 1994). Two *C*-glycosylflavones, vicenin-2 (apigenin 6,8-di-*C*-glucoside, **6**) and luteolin 6,8-di-*C*-glycoside (**7**) were found in all chemotypes and related species, *A. shimurae*, *A. boreale* and *A. oligophlebium*. Another chemotype of *A. normale* was reported from Nepali individual, and consisted of flavonoid **10** and another apigenin 7,4′-*O*-glycoside (Iwashina & Matsumoto, 1990).

Malaysian *Asplenium normale* has been surveyed for flavonoids by Umikalsom *et al*. (1994). They reported two flavone *O*-glycosides, i.e. genkwanin 4′-*O*-glucosylrhamnoside and acacetin 7-*O*-glucosylrhamnoside, together with various uncharacterized kaempferol 3-*O*-glycosides and quercetin glycosides, and kaempferol 3,4′-*O*-glycosides from the species. Though flavone glycosides were reported from Japanese chemotypes of *A. normale*, flavonol glycosides have never been found until now. Thus, the individual of Malaysian *A. normale* was presumed as a new chemotype.

In this paper, we describe the detailed flavonoid characterization from two chemotypes of *Asplenium normale* in Malaysia.

Materials and Methods

Plant materials

Asplenium normale D. Don was collected in Malaysia through the project, "Biodiversity Inventory in the Western Region", by the National Museum of Nature and Science" in 2006 and 2007. Voucher specimens were deposited in the herbarium of the National Museum of Nature and Science, Tokyo (TNS). Collection sites and dates of seven plant materials are as follows. Climbing route (Summit Trail) from Headquarters, Mt. Kinabalu, ca. 1,800–2,000 m alt., Sabah, Malaysia, 31 Jan. 2007 (SB2007-1131); Nepenthes trail, Mesilau Nature Resort, Mt. Kinabalu, ca. 1,900–2,000 m alt., Sabah, Malaysia, 1 Feb. 2007 (SB2007-1153); Silau-Silau trail, around Headquarters, Mt. Kinabalu, 1,635 m alt., Sabah, Malaysia, 2 Feb. 2007 (SB2007-1176, 1177, 1178 and 1179); and Summit trail, Mt. Kinabalu, ca. 1,600–2,400 m alt., Sabah, Malaysia, 2 Feb. 2006 (M12).

Isolation of flavonoids

Total dry fronds (8.2 g) of six individuals, SB2007-1131, SB2007-1176, SB2007-1177, SB2007-1178, SB2007-1179 and M12, which are the same flavonoid composition, and an individual, SB2007-1153 (3.8 g), of *A. normale* were extracted with methanol, respectively. The concentrated extracts were applied to preparative paper chromatography using solvent systems: BAW (*n*-butanol/acetic acid/water=4 : 1 : 5, upper phase), 15% HOAc (acetic acid/water=15 : 85) and then BEW (*n*-butanol/ethanol/water=4 : 1 : 2.2). Isolated flavonoids were purified by Sephadex LH-20 (Pharmacia) column chromatography using solvent system: 70% methonol. Flavonoids **1**, **2** and **6** were obtained as pale yellow powders (yield, ca. 70 mg, 30 mg and 5 mg, respectively).

Thin layer chromatography (TLC) for flavonoids and paper chromatography (PC) for sugars

TLC of the flavonoid glycosides was performed on cellulose plastic plates (Merck) using solvent systems: BAW, 15%HOAc and BEW. PC of glycosidic sugars was performed using solvent systems: BBPW (*n*-butanol/benzene/pyridine/water=5 : 1 : 3 : 3) and BTPW (*n*-butanol/toluene/pyridine/water=5 : 1 : 3 : 3).

Acid hydrolysis

Flavonoid glycosides were hydrolyzed with 12% hydrochloric acid for 30 min on a boiling water bath. After cooling in water, the solution was shaken with diethyl ether, and whereby the aglycones in the ether phase and the sugars in the mother liquor were separated from each other.

High performance liquid chromatography (HPLC)

The HPLC survey of the isolated flavonoids and their aglycones was performed with a Shimadzu HPLC system using Senshu-Pack Pegasil ODS column [6.0 mm I.D.×150 mm L.] (Senshu Scientific Co., Ltd.), at a flow-rate of 10 ml min^{-1}, detection wavelength 190–350 nm, and eluents, acetonitrile/water/phosphoric acid (26 : 74 : 0.2, sol. I) or (15 : 85 : 0.2, sol. II).

Ultraviolet-visible spectra (UV)

UV spectra were recorded on a Shimadzu MPS-2000 multi purpose recording spectrophotometer according to Mabry *et al*. (1970).

Nuclear magnetic resonance (NMR) spectra and liquid chromatograph-mass spectra (LC-MS)

NMR spectra were measured in pyridine-d_5 at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR). LC-MS were measured on a Shimadzu LCMS-2010EV system using Senshu-Pack Pegasil ODS column [2.0 mm I.D.×150 mm L.] (Senshu Scientific Co., Ltd.), at a flow-rate of 0.2 ml min^{-1}, eluting with acetonitrile/water/formic acid (26 : 69 : 5), ESI^+ 4.5 kV and ESI^- 3.5 kV, 250°C, *m*/*z* 150–800.

Identification of flavonoids

The flavonoids were identified by UV spectroscopy, LC-MS, ^{1}H and ^{13}C NMR, characteri-

zation of acid hydrolysates (aglycones and sugars), and direct TLC and HPLC comparisons with authentic samples. TLC, UV, HPLC and LC-MS data of the isolated flavonoids were as follows.

Apigenin 7-*O*-rhamnosyl-(1→4)-rhamnoside (**1**). TLC: Rf 0.78 (BAW), 0.81 (BEW), 0.28 (15%HOAc); UV–dark purple, UV/NH_3–dark yellow. UV: λ max nm MeOH 267, 335; +NaOMe 274, 379 (inc.); +$AlCl_3$ 275, 299, 349, 375sh; +$AlCl_3$/HCl 276, 298, 342, 372; +NaOAc 255, 266, 389; +NaOAc/H_3BO_3 268, 341. HPLC: *t*R min 8.93 (sol. I). LC-MS: *m/z* 563 $[M+H]^+$, 561 $[M-H]^-$ (apigenin dirhamnoside), 271 $[M-glycosyl-H]^+$, 269 $[M-glycosyl-H]^-$ (apigenin). ^1H and ^{13}C NMR data will be described elsewhere (Iwashina *et al.*, in preparation).

Apigenin 7-*O*-rhamnosyl-(1→4)-rhamnoside-4′-*O*-rhamnoside (**2**). TLC: Rf 0.48 (BAW), 0.55 (BEW), 0.59 (15%HOAc); UV and UV/NH_3–dark purple. UV: λ max nm MeOH 269, 317; +NaOMe 286, 370 (dec.); +$AlCl_3$ 278, 299, 336, 374 sh; +$AlCl_3$/HCl 279, 298, 332, 369; +NaOAc 270, 311; +NaOAc/H_3BO_3 270, 320. HPLC: *t*R min 7.21 (sol. I). LC-MS: *m/z* 709 $[M+H]^+$, 707 $[M-H]^-$ (apigenin trirhamnoside), 563 $[M-monorhamnosyl+H]^+$, 561 $[M-monorhamnosyl+H]^-$ (apigenin dirhamnoside), 271 $[M-glycosyl+H]^+$, 269 $[M-glycosyl-H]^-$ (apigenin). ^1H and ^{13}C NMR data will be described elsewhere (Iwashina *et al.*, in preparation).

Kaempferol 3-*O*-glucosylrhamnoside (**3**). TLC: Rf 0.80 (BAW), 0.78 (BEW), 0.59 (15%HOAc); UV–dark purple, UV/NH_3–dark greenish yellow. UV: λ max nm MeOH 265, 342; +NaOMe 274, 322, 389 (inc.); +$AlCl_3$ 274, 303, 349, 387sh; +$AlCl_3$/HCl 274, 300, 343, 387sh; +NaOAc 274, 309, 381; +NaOAc/H_3BO_3 266, 344. HPLC: *t*R min 5.00 (sol. I). LC-MS: *m/z* 595 $[M+H]^+$, 593 $[M-H]^-$ (kaempferol glucosylrhamnoside), 287 $[M-glycosyl+H]^+$, 285 $[M-glycosyl-H]^-$ (kaempferol).

Kaempferol 3,4′-*O*-glycoside (**4**). TLC: Rf 0.93 (BAW), 0.90 (BEW), 0.34 (15%HOAc); UV and UV/NH_3–dark purple. UV: λ max nm MeOH 267, 323; +NaOMe 276, 387 (dec.); +$AlCl_3$ 276, 301, 343, 377sh; +$AlCl_3$/HCl 276, 300, 335, 375sh; +NaOAc 273, 314, 386sh; +NaOAc/H_3BO_3 267, 324. HPLC: *t*R min 7.11 (sol. I). LC-MS: *m/z* 563 $[M+H]^+$, 561 $[M-H]^-$ (kaempferol glycoside), 287 $[M-glycosyl+H]^+$ (kaempferol).

Genkwanin 4′-*O*-glucosyl-(1→3)-rhamnoside (**5**). TLC: Rf 0.73 (BAW), 0.81 (BEW), 0.42 (15%HOAc); UV and UV/NH_3–dark purple. UV: λ max nm MeOH 269, 318; +NaOMe 286, 373 (dec.); +$AlCl_3$ 279, 300, 338, 375sh; +$AlCl_3$/HCl 280, 299, 332, 370; +NaOAc 270, 317; +NaOAc/H_3BO_3 270, 321. HPLC: *t*R min 21.09 (sol. I). LC-MS: *m/z* 593 $[M+H]^+$, 591 $[M-H]^-$ (apigenin monomethyl ether glucosylrhamnoside).

Vicenin-2 (**6**). TLC: Rf 0.20 (BAW), 0.27 (BEW), 0.42 (15%HOAc); UV–dark purple, UV/NH_3–dark yellow. UV: λ max nm MeOH 273, 332; +NaOMe 283, 334, 400 (inc.); +$AlCl_3$ 279, 305, 353, 385sh; +$AlCl_3$/HCl 279, 304, 347, 384sh; +NaOAc 282, 336, 396; +NaOAc/H_3BO_3 276sh, 285, 321, 349, 408. HPLC: *t*R min 5.57 (sol. II). LC-MS: *m/z* 595 $[M+H]^+$, 593 $[M-H]^-$ (apigenin diglucoside).

Lucenin-2 (**7**). TLC: Rf 0.11 (BAW), 0.16 (BEW), 0.31 (15%HOAc); UV–dark purple, UV/NH_3–yellow. UV: λ max nm MeOH 257sh, 271, 350; +NaOMe 272, 337sh, 415 (inc.); +$AlCl_3$ 278, 426; +$AlCl_3$/HCl 263sh, 277, 299, 359, 386sh; +NaOAc 270sh, 282, 404; +NaOAc/H_3BO_3 270, 287sh, 430. HPLC: *t*R min 3.45 (sol. I). LC-MS: *m/z* 611 $[M+H]^+$, 609 $[M-H]^-$ (luteolin diglucoside).

Results and Discussion

Of seven individuals which were used as plant materials, six ones (SB2007-1131, 1176, 1177, 1178, 1179 and M12) were the same flavonoid composition (**H**-type, Fig. 2). On the other hand, one individual (SB2007-1153) showed the different flavonoid composition (**I**-type, Fig. 3). Seven flavonoids were isolated from the fronds of

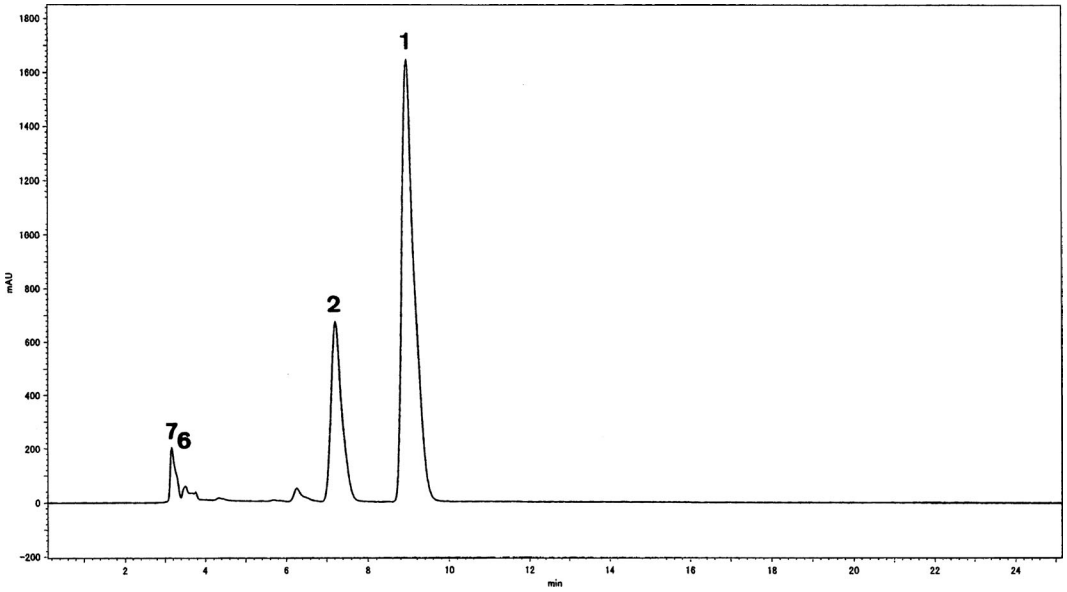

Fig. 2. HPLC chromatogram of crude extracts from chemotype (**H**-type) of *Asplenium normale*. **1**=apigenin 7-*O*-rhamnosyl-(1→4)-rhamnoside, **2**=apigenin 7-*O*-rhamnosyl-(1→4)-rhamnoside-4′-*O*-rhamnoside, **6**=vicenin-2 and **7**=lucenin-2.

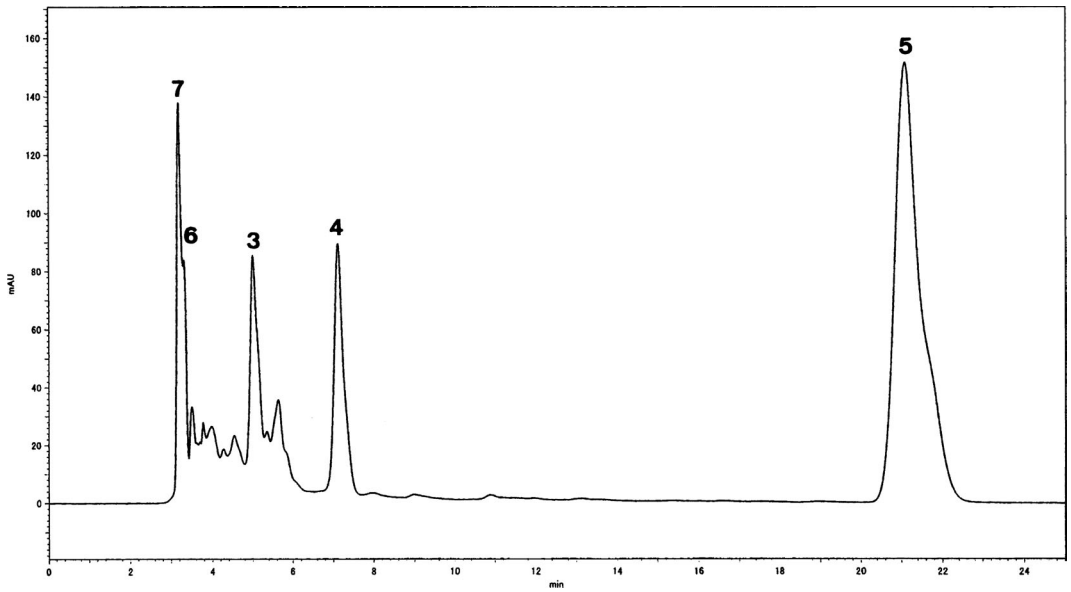

Fig. 3. HPLC chromatogram of crude extracts from chemotype (**I**-type) of *Asplenium normale*. **3**=kaempferol 3-*O*-glucosylrhamnoside, **4**=kaempferol 3,4′-*O*-glycoside, **5**=genkwanin 4′-*O*-glucosyl-(1→3)-rhamnoside, **6**=vicenin-2 and **7**=lucenin-2.

Malaysian *Asplenium normale*.

Flavonoid (**1**) was major component of **H**-type and obtained as pale yellow powder. Apigenin and rhamnose, which were characterized by direct HPLC and PC comparisons with authentic samples, were liberated by acid hydrolysis. It was

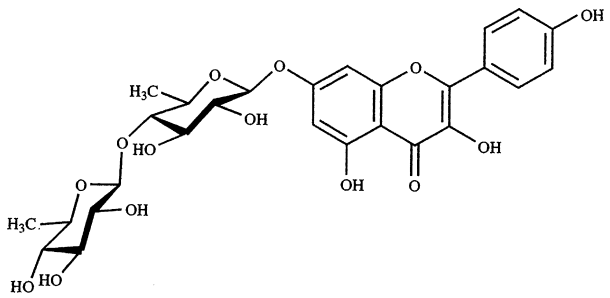

Fig. 4. Apigenin 7-*O*-rhamnosyl-(1→4)-rhamnoside (**1**).

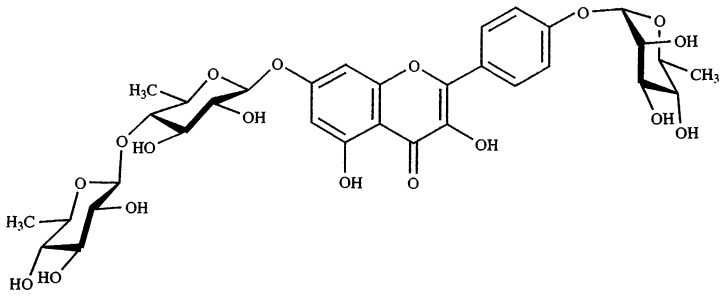

Fig. 5. Apigenin 7-*O*-rhamnosyl-(1→4)-rhamnoside-4′-*O*-rhamnoside (**2**).

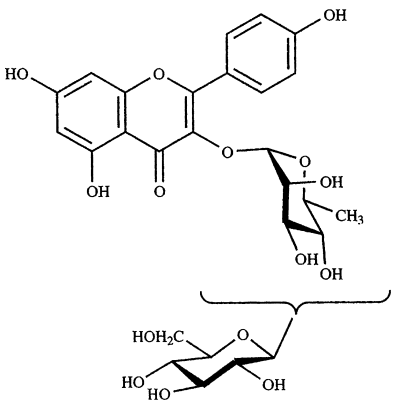

Fig. 6. Kaempferol 3-*O*-glucosylrhamnoside (**3**).

shown by UV spectral survey with various shift reagents according to Mabry *et al*. (1970) that rhamnose is attached to 7-position of apigenin. Since molecular ion peak, *m*/*z* 563 $[M+H]^+$ and 561 $[M-H]^-$, was indicated, it was shown that 2 mol rhamnose are present as glycosidic sugar. Thus, flavonoid (**1**) was characterized as apigenin 7-*O*-dirhamnoside. Such glycoside has been found in Japanese *A. normale* (Iwashina *et al.*, 1990) and identified as apigenin 7-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-rhamnopyranoside (**8**) by ^{13}C NMR (Matsumoto *et al.*, 2003). However, flavonoid (**1**) was elucidated by ^1H and ^{13}C NMR survey as apigenin 7-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranoside (Fig. 4), which was first reported as natural compound.

Flavonoid (**2**) was isolated from **H**-type and obtained as pale yellow powder. It also produced apigenin and rhamnose by acid hydrolysis. It was shown by LC-MS that 3 mol rhamnose were attached to apigenin. UV spectral survey indicated the presence of free 5-hydroxyl group, showing the attachment of glycosyl groups to 7- and 4′-positions of apigenin. Finally, flavonoid (**2**) was identified as apigenin 7-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranoside-4′-*O*-α-L-rhamnopyranoside (Fig. 5) by ^1H and ^{13}C NMR data. Apigenin trirhamnoside was reported in nature for the first time.

Flavonoid (**3**) was isolated from **I**-type of *A. normale*. UV spectral properties of the glycoside were those of flavonol, kaempferol, but not flavone, apigenin. Practically, kaempferol was liberated by acid hydrolysis as aglycone, together with glucose and rhamnose as glycosidic sugars. Since the presence of free 5-, 7- and 4′-hydroxyl

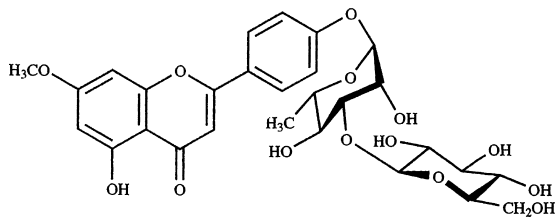

Fig. 7. Genkwanin 4′-*O*-glucosyl-(1→3)-rhamnoside (**5**).

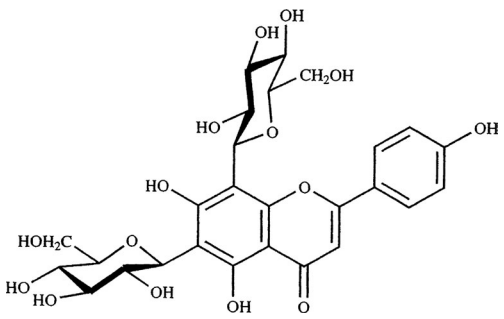

Fig. 8. Vicenin-2 (**6**).

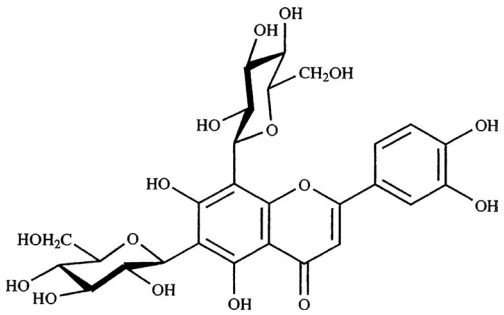

Fig. 9. Lucenin-2 (**7**).

groups was shown by UV spectral survey, the attachment of both the sugars to 3-position was indicated. Thus, flavonoid (**3**) was characterized as kaempferol 3-*O*-rhamnosylglucoside or 3-*O*-glucosylrhamnoside (Fig. 6). As such kaempferol 3-*O*-glycosides, kaempferol 3-*O*-rhamnosyl-(1→6)-glucoside (rutinoside) (Iwashina & Hatta, 1998), 3-*O*-rhamnosyl-(1→2)-glucoside (neohesperidoside) (Iwashina *et al.*, in press) and 3-*O*-glucosyl-(1→2)-rhamnoside (Takemura *et al.*, in preparation) have been reported. However, HPLC properties of flavonoid (**3**) did not agreed with those of their glycosides. Since fragment ion peak did not appeared by LC-MS, the glycoside was presumed as glucosylrhamnoside but not rhamnosylglucoside.

Flavonoid (**4**) was also isolated from **I**-type. The presence of free 5- and 7-hydroxyl and substituted 3- and 4′-hydroxyl groups was shown by UV spectral survey. Kaempferol was liberated by acid hydrolysis, but glycosidic sugar was not characterized for small amount of original glycoside. Thus, flavonoid (**4**) was characterized as kaempferol 3,4′-di-*O*-glycoside. Molecular ion peak, *m*/*z* 563 $[M+H]^+$ and 561 $[M-H]^-$ appeared, but it did not agree with other known kaempferol glycosides. A chemotype, which synthesizes not only flavones but also flavonols, has been reported from Malaysian *Asplenium normale* (collection site is recorded as Semenanjung) (Umikalsom *et al.*, 1994). Though acacetin 7-*O*-glucosylrhamnoside was not detected in this experiment, this chemotype may be the same one with that found by Umikalsom and co-wokers.

Flavonoid (**5**) was isolated from **I**-type, together with two kaempferol glycosides described above. Genkwanin (apigenin 7-methyl ether), glucose and rhamnose were liberated by acid hydrolysis. The attachment of sugars to 4′-hydroxyl group of genkwanin was shown by UV spectral survey using various shift reagents (Mabry *et al.*, 1970). Finally, flavonoid (**5**) was identified as genkwanin 4′-*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranoside (Fig. 7) by direct TLC and HPLC comparison with authentic sample obtained from Japanese *A. normale* (Iwashina *et al.*, 1990).

Flavonoid (**6**) was isolated from both chemotypes and obtained as pale yellow powder. UV and LC-MS properties of the compound were agreed with those of apigenin 6,8-di-*C*-glucoside. The glycoside was finally identified as

vicenin-2 (Fig. 8) by direct TLC and HPLC comparison with authentic sample, which isolated from the leaves of *Colocasia esculenta* (L.) Schott (Araceae) (Iwashina *et al.*, 1999). Vicenin-2 has been found in all Japanese chemotypes of *A. normale*.

Flavonoid (**7**) was also isolated from both chemotypes. It was shown by UV spectral survey that the presence of free 5-, 7-, 3′- and 4′-hydroxyl groups. Moreover, the compound could not be hydrolyzed by hot acid treatment, suggesting the compounds is *C*-glycosylflavonoid. Molecular ion peak, *m*/*z* 611 $[M+H]^+$ and 609 $[M-H]^-$ appeared by LC-MS, showing to be *C*-glycosylflavone attached 2 mol glucose. Thus, flavonoid (**7**) was identified as luteolin 6,8-di-*C*-glucoside, i.e., lucenin-2 (Fig. 9). Luteolin 6,8-di-*C*-glycoside has been reported from all Japanese chemotypes of *A. normale*. In this survey, it was first confirmed that their *C*-glycosylluteolin is lucenin-2.

In 1994, we have described seven chemotypes from Japanese *Asplenium normale* (Iwashina & Matsumoto, 1994). The occurrence of another chemotype was reported from Nepali population (Iwashina & Matsumoto, 1990). New chemotypes containing two new flavone *O*-glycosides or flavonol glycosides were found from Malaysian *A. normale* in this survey. As described in introduction, *A. normale* is broadly distributed throughout East and Southeast Asia, Africa and Hawaii (Nakaike, 1992). Other new chemotypes may be found from other area, e.g., China, Taiwan and so on.

Acknowledgements

The authors thank Dr. Junichi Kitajima, Laboratory of Pharmacognosy, Showa Pharmaceutical University for measurement of NMR spectra. We also thank Miss Aya Sugawara, JOCV Volunteer, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah for support of plant collection.

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マレーシア産ヌリトラノオ（チャセンシダ科）のフラボノイドの新化学型

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マレーシアのキナバル山周辺で採集された７個体のヌリトラノオのフラボノイドが分析された．それらのフラボノイド組成から，２種類の化学型の存在が明らかとなった．これらの化学型に含まれるフラボノイドを各種クロマトグラフィーで分離し，紫外可視吸収スペクトル (UV)，液体クロマトグラフ－質量スペクトル (LC-MS)，核磁気共鳴スペクトル (NMR) などを用いて同定を行った．その結果，ひとつの化学型（**H**-型）に含まれるフラボノイドはフラボンの *O*-配糖体である apigenin 7-*O*-rhamnosyl-(1→4)-rhamnoside (**1**)と apigenin 7-*O*-rhamnosyl-(1→4)-rhamnoside-4′-*O*-rhamnoside (**2**)，および *C*-グリコシルフラボンの vicenin-2 (**6**)と lucenin-2 (**7**)であった．これらのうち，前２者はこれまで自然界で報告されたことのない新規のフラボノイドであった．一方，他の化学型（**I**-型）に含まれていたフラボノイドはフラボノール配糖体の kaempferol 3-*O*-glucosylrhamnoside (**3**) と kaempferol 3,4′-*O*-glycoside (**4**)，それにフラボン配糖体の genkwanin 4′-*O*-glucosyl-(1→3)-rhamnoside (**5**) であった．なお，**H**-型に含まれていた２種類の *C*-グリコシルフラボンは，**I**-型にも共通に含まれていた．ヌリトラノオで発生する化学型については，これまでに日本産のものについて７種類，ネパール産のものについて１種類が報告されており，マレーシア産の２種類の化学型は，これらのいずれにも属さないものであることが判明した．