Flavonol Glycosides with α-Glucosidase Inhibitory Activities and New Flavone *C*-Diosides from the Leaves of *Machilus konishii*

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Seventeen flavonoids, five of which are flavone *C*-diosides, **1**–**5**, were isolated from the BuOH- and AcOEt-soluble fractions of the leaf extract of *Machilus konishii*. Among **1**–**5**, apigenin 6-*C*-β-D-xylopyranosyl-2″-*O*-β-D-glucopyranoside (**2**), apigenin 8-*C*-α-L-arabinopyranosyl-2″-*O*-β-D-glucopyranoside (**4**), and apigenin 8-*C*-β-D-xylopyranosyl-2″-*O*-β-D-glucopyranoside (**5**) are new. Both **4** and **5** are present as rotamer pairs. The structures of the new compounds were elucidated on the basis of NMR-spectroscopic analyses and MS data. In addition, the ^{1}H- and ^{13}C-NMR data of apigenin 6-*C*-α-L-arabinopyranosyl-2″-*O*-β-D-glucopyranoside (**3**) were assigned for the first time. The isolated compounds were assayed against α-glucosidase (type IV from *Bacillus stearothermophilus*). Kaempferol 3-*O*-(2-β-D-apiofuranosyl)-α-L-rhamnopyranoside (**12**) was found to possess the best inhibitory activity with an IC_{50} value of 29.3 μM.

Introduction. – *Machilus konishii* HAYATA (Lauraceae) is a medium to large-sized evergreen tree endemically distributed in the central to southern parts of Taiwan [1]. Recently, we isolated eleven compounds, including five epicatechin oligomers [2], from a leaf extract of this plant. In continuation of this work, the BuOH- and AcOEt-soluble fractions were further investigated to search for other constituents active against the α-glucosidase. This enzyme plays a key role in the digestion of dietary carbohydrates in humans, and some inhibitors such as acarbose and miglitol have been used in the clinic to improve postprandial glucose control in type-II diabetic patients [3].

Results and Discussion. – The EtOH extract of the dried leaves of *M. konishii* was divided into fractions soluble in CH_2Cl_2, AcOEt, BuOH, and H_2O by liquid-liquid partitioning procedure [1]. The BuOH- and AcOEt-soluble fractions were separated on *Sephadex LH-20* columns and further purified by centrifugal partition chromatography (CPC), *RP-18 Lobar* CC, and semi-preparative HPLC on *RP-18* columns to yield compounds **1**–**17**.

The ^{1}H-NMR spectra of **1**–**5** (*Table 1*), **10**, and **15** showed typical signals for the flavone apigenin [4], a *singlet* for H–C(3) (δ(H) 6.62; for **10**) in addition to the *AX* (δ(H) 6.18 for H–C(6) and 6.42 for H–C(8), $J = 2.0$; for **10**) and *AA′XX′* systems (δ(H) 7.24 (H–C(3′)/H–C(5′)) and 7.93 (H–C(2′)/H–C(6′)), $J = 8.9$; for **10**). The ^{1}H-NMR spectra of **6**–**9**, **11**, **14**, **16**, and **17** displayed typical signals for the flavonol quercetin [5][6], a *meta*-coupled *AX* system for H–C(6) and H–C(8) (δ(H) 6.20 and 6.40 (each d, $J = 2.0$); for **8**), and an *ABX* system for H–C(2′) (δ(H) 7.83 (d, $J = 2.1$); for **8**), H–C(5′) (δ(H) 6.85 (d, $J = 8.5$); for **8**) and H–C(6′) (δ(H) 7.58 (dd, $J = 8.5, 2.1$); for

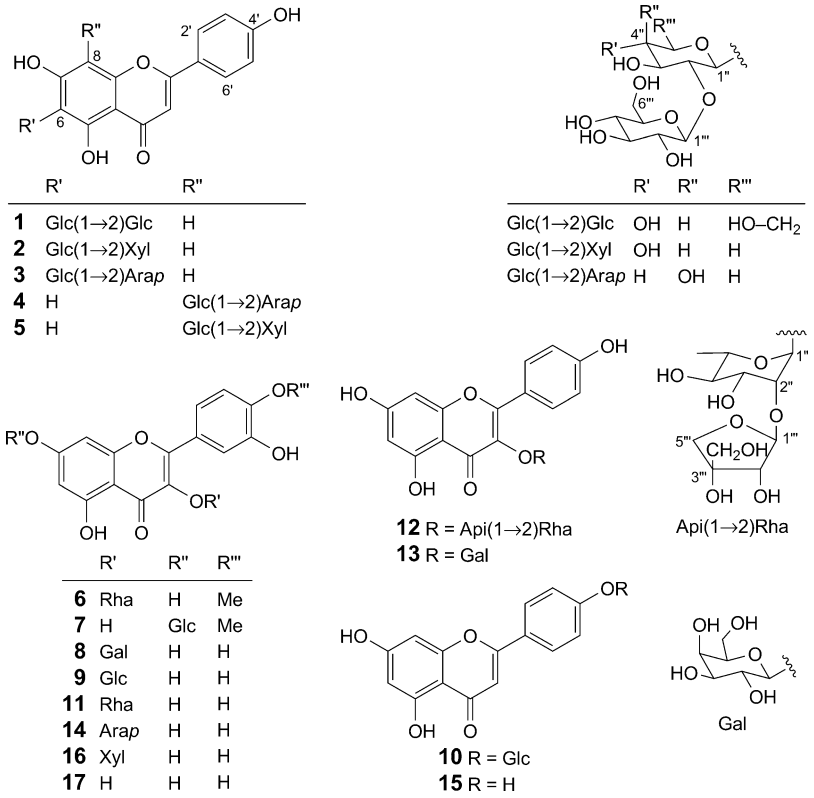

Fig. 1. *Structures of compounds* **1**–**17**

8). The ^{1}H-NMR spectra of **12** and **13** exhibited typical signals for the flavonol kaempferol [6], a *meta*-coupled *AX* system for H–C(6) and H–C(8), and an *AA′XX′* system for the H-atoms of ring *B*. Compounds **15** and **17** are simple flavone and flavonol, resp., and were identified as apigenin [4] and quercetin [5].

Compounds **6**–**11**, **13**, **14**, and **16** are flavonoid monoosides, while compound **12** is a flavonoid dioside, as deduced from the ESI-MS, and ^{1}H- and ^{13}C-NMR data. Most of the glycons were identified by the characteristic H-atom signals in the sugar unit (CD_3OD; Tables S1–S3 in *Supplementary Material*[1]), *e.g.*, δ(H) 5.15 (*d*, $J = 7.8$, H–C(1″)) and 3.84 (br. *d*, $J = 3.0$, H–C(4″)) of the β-D-galactopyranosyl (Gal) moiety of **8** [5][6]; δ(H) 5.24 (*d*, $J = 7.6$, H–C(1″)) and 3.42 (*t*, $J = 9.0$, H–C(4″)) of the β-D-glucopyranosyl (Glc) moiety of **9** [5][6]; δ(H) 5.36 (*d*, $J = 1.6$, H–C(1″)) and 0.91 (*d*, $J = 5.6$, H–C(6″)) of the α-L-rhamnopyranosyl (Rha) moiety of **6** [7]; δ(H) 5.16 (*d*, $J = 7.3$, H–C(1″)) and 3.41 (*t*, $J = 8.7$, H–C(3″)) of the β-D-xylopyranosyl (Xyl) moiety of **16** [5]; δ(H) 5.15 (*d*, $J = 6.5$, H–C(1″)) and 3.64 (*dd*, $J = 8.4$, 3.2, H–C(3″)) of the α-L-arabinopyranosyl (Ara*p*) moiety of **14** [5][6]; and δ(H) 5.11 (*d*, $J = 2.6$, H–C(1″)) and 3.68 and 3.82 (2 *d*, $J = 9.7$, CH_2(4″)) of the β-D-apiofuranosyl (Api) moiety of **12** [6].

[1]) *Supplementary Material* is available from the corresponding author.

The ^{13}C-NMR data (CD_3OD) were supportive for the glycon determination (*cf. Table 2*).

On the basis of these analyses and optical rotations, compounds **8**–**14** and **16** were identified as quercetin 3-*O*-*β*-D-galactopyranoside (**8**) [5][6], quercetin 3-*O*-*β*-D-glucopyranoside (**9**) [5][6], quercetin 3-*O*-*α*-L-rhamnopyranoside (**11**) [5][6], quercetin 3-*O*-*α*-L-arabinopyranoside (**14**) [5][6], and quercetin 3-*O*-*β*-xylopyranoside (**16**) [5], apigenin 4′-*O*-*β*-D-glucopyranoside (**10**) [6][8], and kaempferol 3-*O*-(2-*β*-D-apiofuranosyl)-*α*-L-rhamnopyranoside (**12**) [6], and kaempferol 3-*O*-*β*-D-galactopyranoside (**13**) [6]. With the aid of NOESY for the positional assignment of the MeO groups, compounds **6** and **7** were identified as 4′-*O*-methylquercetin 3-*O*-*α*-L-rhamnopyranoside [7] and 4′-*O*-methylquercetin 7-*O*-*β*-D-glucopyranoside [9], respectively.

Compounds **1**–**5** (*Fig. 1*) are flavone *C*-glycosides as evidenced by the ESI-MS, and ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*, resp.). The ^{1}H-NMR spectra of **1**–**5** (CD_3OD) showed one *AA′XX′* system for H–C(3′)/H–C(5′) (δ(H) 6.92 for **2**) and H–C(2′)/H–C(6′) (δ(H) 7.84 for **2**) (*d*, $J = 8.9$), two *singlet*s for H–C(3) (*ca.* δ(H) 6.60) and H–C(8) (*ca.* δ(H) 6.48 for **1**–**3**) or H–C(6) (*ca.* δ(H) 6.24; for **4** and **5**), and a *doublet* for an anomeric H-atom at *ca.* δ(H) 4.90 ($J \approx 10$), suggesting **1**–**3** to be apigenin 6-*C*-glycosides, while **4** and **5** to be apigenin 8-*C*-glycosides [10]. The ^{1}H- and ^{13}C-NMR data (CD_3OD) of **1** showed the signals of two *β*-Glc moieties as resolved by COSY and HSQC analyses (*Tables 1* and *2*). The HMBC spectrum of **1** showed correlation δ(H) 4.94 (Glc H–C(1))/δ(C) 109.1 (C(6)), 162.7 (C(5)), and 164.9 (C(7)), indicating that the Glc I was *C*-linked to C(6) of the apigenin moiety. The correlation δ(H) 4.39 (Glc II H–C(1))/δ(C) 81.7 (Glc I C(2)) evidenced the Glc(1 → 2)Glc linkage. Compound **1** thus was identified as the known isovitexin 2″-*O*-*β*-glucopyranoside [11].

Compounds **2**–**5** were obtained as yellowish solids and had the same molecular formula of $C_{26}H_{28}O_{14}$, as established by negative-ion-mode HR-ESI-MS and ^{13}C-NMR data. Subtraction of the molecular formula from the apigenin moiety left a $C_{11}H_{19}O_9$ residue, corresponding to a hexosyl and a pentosyl unit. The ^{1}H-NMR spectrum of **2** showed signals for two anomeric H-atoms at δ(H) 4.84 (*d*, $J = 10.2$) and 4.37 (*d*, $J = 6.8$), resp. The COSY spectrum displayed two sugar H-atom spin systems, one as δ(H) 4.84 (*d*, H–C(1″)) ↔ 4.88 (derived from the HSQC spectrum, *i.e.*, δ(H) 4.88/δ(C) 81.7) ↔ 3.59 (*t*) ↔ 3.68 (*ddd*) ↔ 3.30 and 3.95 (*dd*) (CH_2(5″)), and the other as δ(H) 4.37 (*d*, H–C(1‴)) ↔ 3.07 (*dd*) ↔ 3.24 (*t*) ↔ 3.18 (*t*) ↔ 2.87 (*dt*) ↔ 3.30 (*dd*) and 3.39 (*dd*) (CH_2(6‴)). Analysis of the coupling constants (*Table 1*) indicated that these two monosaccharide residues were *β*-xylopyranosyl and *β*-glucopyranosyl, respectively. The HMBC spectrum of **2** showed correlation δ(H) 4.84 (Xyl H–C(1))/δ(C) 109.0 (C(6)), 162.8 (C(5)), and 165.1 (C(7)) (*Fig. 2*), suggesting the xylosyl residue to be linked to C(6) of the apigenin moiety. The correlation δ(H) 4.37 (Glc H–C(1))/δ(C) 81.7 (Xyl C(2)) evidenced the Glc(1 → 2)Xyl linkage. The NOESY spectrum of **2** showed mutual NOE relationships among δ(H) 4.37 (Glc H–C(1)), 2.87 (Glc H–C(5)), and 3.24 (Glc H–C(3)) (*Fig. 2*), confirming the presence of the *β*-glucopyranosyl residue. Therefore, compound **2** was established as apigenin 6-*C*-*β*-xylopyranosyl-2″-*O*-*β*-glucopyranoside.

The ^{1}H- and ^{13}C-NMR spectra of **3** were similar to those of **2** except for the replacement of the signals attributed to the xylosyl residue by those of another pentosyl

Table 1. *^{1}H-NMR* (600 MHz) *Data of Compounds* **1**–**5**. In CD_3OD; δ in ppm, J in Hz.

Position	**1**	**2**	**3**	**4a**	**4b**	**5a**	**5b**
3	6.57 (*s*)	6.60 (*s*)	6.61 (*s*)	6.65 (*s*)	6.59 (*s*)	6.60 (*s*)	6.60 (*s*)
6				6.24 (*s*)	6.24 (*s*)	6.24 (*s*)[a])	6.22 (*s*)[a])
8	6.47 (*s*)	6.48 (*s*)	6.50 (*s*)				
2′, 6′	7.80 (*d*, *J* = 8.8)	7.84 (*d*, *J* = 8.9)	7.85 (*d*, *J* = 8.8)	8.19 (*d*, *J* = 8.2)	7.88 (*d*, *J* = 8.0)	7.84 (*d*, *J* = 7.9)	7.89 (*d*, *J* = 8.0)
3′, 5′	6.90 (*d*, *J* = 8.8)	6.92 (*d*, *J* = 8.9)	6.92 (*d*, *J* = 8.8)	6.94 (*d*, *J* = 8.5)	6.94 (*d*, *J* = 8.5)	6.95 (*d*, *J* = 8.3)	6.95 (*d*, *J* = 8.3)
1″	4.94 (*d*, *J* = 9.9)	4.84 (*d*, *J* = 10.2)	4.83 (*d*, *J* = 8.9)	4.93 (*d*, *J* = 10.1)[b])	5.04 (*d*, *J* = 9.6)[b])	5.01 (*d*, *J* = 9.6)	4.96 (*d*, *J* = 10.7)
2″	overlapped	4.88[b])	overlapped	4.76 (*t*, *J* = 9.3)	4.39 (*t*, *J* = 9.3)	4.40 (*t*, *J* = 9.0)	4.30 (*d*, *J* = 9.5)
3″	3.68 (*t*, *J* = 8.9)	3.59 (*t*, *J* = 9.0)	3.81 (*dd*, *J* = 9.1, 2.7)	3.85 (*dd*, *J* = 9.1, 3.0)[a])	3.92 (br. *d*, *J* = 7.5)[a])	3.68 (*t*, *J* = 9.3)[a])	3.66 (*t*, *J* = 9.4)[a])
4″	3.50 (*t*, *J* = 9.4)	3.68 (*ddd*, *J* = 10.4, 9.2, 5.4)	3.96 (*d*, *J* = 3.1)	4.06 (*d*, *J* = 3.5)[a])	4.02 (br. *s*)	3.73 (*dd*, *J* = 10.0, 5.6)[a])	3.81–3.86 (*m*)
5″a	3.39–3.43 (*m*)	3.30 (*t*, *J* = 9.2)	3.70 (*d*, *J* = 12.2)	3.76 (*d*, *J* = 12.4)	3.83 (*d*, *J* = 12.3)	3.38 (*t*, *J* = 11.0)	3.38 (*t*, *J* = 11.0)
5″b		3.95 (*dd*, *J* = 11.1, 5.3)	3.97 (*dd*, *J* = 10.0, 3.1)	4.07 (br. *d*, *J* = 10.5)	4.07 (br. *d*, *J* = 10.5)	4.03 (*dd*, *J* = 10.9, 4.5)	4.08 (*dd*, *J* = 10.0, 4.4)
6″a	3.72 (*dd*, *J* = 12.1, 5.2)						
6″b	3.89 (*dd*, *J* = 12.1, 2.1)						
1‴	4.39 (*d*, *J* = 6.3)	4.37 (*d*, *J* = 6.8)	4.34 (*d*, *J* = 7.8)	4.17 (*d*, *J* = 7.6)	4.28 (*d*, *J* = 7.6)	4.31 (*d*, *J* = 8.6)	4.20 (*d*, *J* = 7.6)
2‴	3.08 (*t*, *J* = 8.0)	3.07 (*dd*, *J* = 9.0, 8.0)	3.06 (*dd*, *J* = 9.2, 7.9)	2.99 (*t*, *J* = 8.3)	2.89 (*d*, *J* = 8.5)	2.97 (*t*, *J* = 8.6)	2.99 (*t*, *J* = 8.7)
3‴	3.25 (*t*, *J* = 9.6)	3.24 (*t*, *J* = 9.0)	3.23 (*t*, *J* = 9.1)	3.10 (*t*, *J* = 9.0)	3.17 (*d*, *J* = 10.8)	3.20 (*t*, *J* = 9.2)[a])	3.12 (*t*)
4‴	3.17 (*t*, *J* = 9.4)	3.18 (*t*, *J* = 9.0)	3.13 (*t*, *J* = 9.2)	3.19 (*d*, *J* = 8.6)	2.97 (*t*, *J* = 8.8)	3.09 (*t*, I = 9.7)	3.13 (*t*)
5‴	2.89 (*dd*, *J* = 9.4, 3.0)	2.87 (*dt*, *J* = 9.3, 3.2)	2.88 (*dt*, *J* = 9.7, 3.6)	2.64 (br. *d*, *J* = 9.7)	2.87 (*d*, *J* = 7.9)	2.86 (*dt*, *J* = 9.5, 3.2)[a])	2.70 (*dt*, *J* = 8.9, 3.2)[a])
6‴a	3.36–3.40 (*m*)	3.31[b])	3.34[b])	3.22 (*d*, *J* = 11.0)	3.30[b])	3.37[b])	3.28[b])
6‴b		3.39 (*dd*, *J* = 11.4, 4.0)		3.41 (*dd*, *J* = 11.5, 2.5)[a]	3.35		

[a]) Data obtained at 278 K. [b]) Data obtained from HMQC, and J values from 1D-TOCSY.

Table 2. *^{13}C-NMR* (150 MHz) *Data of Compounds* **1**–**5**. In CD_3OD; δ in ppm.

Position	**1**	**2**	**3**	**4a**	**4b**	**5a**	**5b**
2	166.1	166.2	166.2	166.7	166.3	166.3	166.4
3	103.8	103.9	103.9	103.3	103.9	104.0	104.0
4	184.0	184.1	184.1	184.2	184.1	184.3	184.1
5	162.7	162.8	162.9	162.8	162.8	162.7/162.8[a])	162.7/162.8[a])
6	109.1	109.0	109.2	99.6/100.8[b])	99.6/100.8[b])	100.5	99.6
7	164.9	165.1	165.4	165.3	165.3	164.9	165.0
8	95.1	94.9	95.4	104.9	104.6	104.3	105.0
9	158.7	158.8	158.9	158.5	157.2	157.8	158.2
10	105.2	105.2	105.1	105.5	105.3	105.4	105.6
1′	123.1	123.2	123.2	123.0	123.6	123.7	123.7
2′, 6′	129.4	129.5	129.5	130.6	129.8	129.6	129.8
3′, 5′	117.0	117.0	117.0	117.1	117.1	117.0	117.0
4′	162.7	162.8	162.9	162.8	162.8	162.8	162.7
1″	73.3	74.1	74.4	74.6	75.73	74.5	75.5
2″	81.7	81.7	79.9	80.4	80.8	82.3	81.6
3″	80.0	80.1	76.0	76.7	75.66	80.0	80.2
4″	71.6	71.3	70.7	71.0	70.4	71.1	71.8
5″	82.5	71.5	71.9	72.7	72.0	71.8	71.8
6″	62.9						
1‴	106.2	106.4	106.1	106.4	105.9	106.3	105.8
2‴	76.0	76.0	76.0	75.79	76.1	76.1	75.8
3‴	77.8	77.9	77.9	77.75	77.84	77.9	77.7
4‴	70.9	70.9	71.1	70.7	71.6	71.1	71.1
5‴	77.5	77.6	77.5	77.2	77.2	77.6	77.1
6‴	62.2	62.2	62.5	61.9	62.9	62.4	62.2

[a]), [b]) Data could not be assigned to individual rotamers.

residue, whose anomeric H-atom signal appeared at δ(H) 4.83 (*d*, $J = 8.9$; *Table 1*), as obtained from an HSQC correlation δ(H) 4.83/δ(C) 74.4 (Fig. S14[1])). The combination of COSY and TOCSY spectroscopic analyses suggested the following coupling relationship for the H-atoms of this pentosyl moiety: δ(H) 4.88 (*dd*, H–C(2″)) ↔ 3.81 (*dd*) ↔ 3.96 (*ddd*) ↔ 3.70 and 3.97 (*dd*) (CH_2(5″)) besides those for the glucosyl

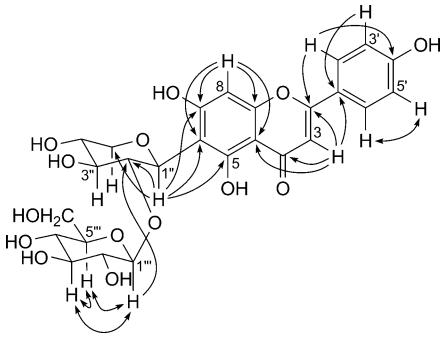

Fig. 2. *Key HMBCs and NOESY correlations of compound* **2** (CD_3OD, 600 MHz)

moiety, *i.e.*, δ(H) 4.34 (H–C(1‴)) ↔ 3.06 (*dd*) ↔ 3.23 (*t*) ↔ 3.13 (*t*) ↔ 2.88 (*dt*) ↔ 3.34 (*m*) (CH_2(6‴)). The coupling constants of the pentosyl H-atoms (*Table 1*) indicated that H–C(2), H–C(3), and H–C(4) were axially, axially, and equatorially oriented, respectively, thus evidencing the presence of an α-arabinopyranosyl residue. The NOESY spectrum, showing mutual NOE relationship among δ(H) 4.83 (Ara H–C(1)), 3.81 (Ara H–C(3)), and 3.70 (Ara H_α–C(5)), and between δ(H) 3.81 (Ara H–C(3)) and 3.96 (Ara H–C(4)), supported the structure of this residue. The Ara*p* residue was *C*-linked to C(6) of the apigenin moiety as evidenced by the HMBCs of δ(H) 4.83 (Ara H–C(1)) to δ(C) 109.2 (C(6)), 162.8 (C(5)), and 165.4 (C(7)), besides δ(C) 71.9 (Ara C(5)), 76.0 (Ara C(3)), and 79.9 (Ara C(2)). Another key HMBC of δ(H) 4.34 (Glc H–C(1)) to δ(C) 79.9 (Ara C(2)) indicated an ether linkage between Glc C(1) and Ara*p*-C(2). Accordingly, compound **3** was established as apigenin 6-*C*-α-arabinopyranosyl-2″-*O*-β-glucopyranoside, *i.e.*, isomollupentin 2″-*O*-β-glucopyranoside. Although this compound had been reported from *Cerastium arvense* with its structure having been elucidated based on MS analysis [12], its ^{1}H- and ^{13}C-NMR data had not been reported. These data (*Tables 1* and *2*) were assigned in this study by analysis of 2D-NMR spectra as indicated above.

Both compounds **4** and **5** exist as two HPLC inseparable rotamers, as revealed by the ^{1}H- and ^{13}C-NMR spectra (CD_3OD), each showing two-set signals (*Tables 1* and *2*, and Figs. S18, S19, S26, and S27[1])). The equilibrium between these two rotamers of each compound was supported by strong exchange of peaks of the equivalent H-atoms in the TOCSY and NOESY spectra (Figs. S23, S24, S31, and S32[1])). For example, the NOESY spectrum of **4** showed cross-correlation due to chemical exchange between the corresponding protons H–C(2′)/H–C(6′), δ(H) 7.88 for **4b** and 8.19 for **4a** (*Fig. 3*), indicating them to be rotamers [13]. This phenomenon, created by rotational hindrance at the C(sp^3)-C(sp^2) glycosyl–flavone linkage, has been reported for flavones containing 8-*C* diosides such as apigenin 8-*C*-neohesperidoside [13]. The ^{1}H- and ^{13}C-NMR signals of **4** and **5** were assigned on the basis of their 1D- and 2D-NMR spectra (*Tables 1* and *2*), recorded at 300 K and/or 278 K. The H-atom signals at δ(H) 7.88/8.19 (*d*, H–C(2′,6′)), 6.94 (*d*, H–C(3′,5′)), 6.59/6.65 (*s*, H–C(3)), and 6.24 (*s*, H–C(6)), and the C-atom signals at δ(C) 184.1/184.2 (C(4)), 162.8 (C(5), C(4′)), and 165.3 (C(7)) of compound **4** were consistent with the corresponding signals in an apigenin 8-*C*-glycoside [13]. The ratio of the rotamers, **4a**/**4b**, is *ca.* 1.00 : 0.94, based on the H-atom signal integration of H–C(2′)/H–C(6′) (δ(H) 8.19 (**4a**); δ(H) 7.88 (**4b**)) in the ^{1}H-NMR spectrum recorded at 278 K (Fig. S18[1])). The H-atom signals of the glycon part of **4**, assigned by a combination of COSY, TOCSY, and selective 1D-TOCSY analyses (Figs. S20, S24, and S25[1])), were similar to those of **3** (*Table 1*). Thus, the α-Ara*p* residue was *C*-linked to C(8) of the apigenin moiety, which was confirmed by the HMBC of δ(H) 4.93 (*d*, $J = 10.1$, Ara*p* H–C(1))/δ(C) 104.9 (C(8)), 165.3 (C(7)), and 158.5 (C(9)) for **4a**. The *O*-linkage of Glc(1 → 2)Ara*p* was supported the HMBC δ(H) 4.76 (Ara*p* H–C(2))/δ(C) 106.4 (Glc C(1)) for **4a**. These data established **4** as apigenin 8-*C*-α-arabinopyranosyl-2″-*O*-β-glucopyranoside.

The rotamer ratio **5a**/**5b** was *ca.* 0.69 : 1.00, based on the H-atom signal integration for the H–C(2′)/H–C(6′) (δ(H) 7.84 (**5a**); δ(H) 7.89 (**5b**)) in the ^{1}H-NMR spectrum recorded at 278 K (Fig. S26[1])). The glycon H-atoms' signals of **5**, assigned from combination of COSY, TOCSY, and selective 1D-TOCSY analyses, were similar to

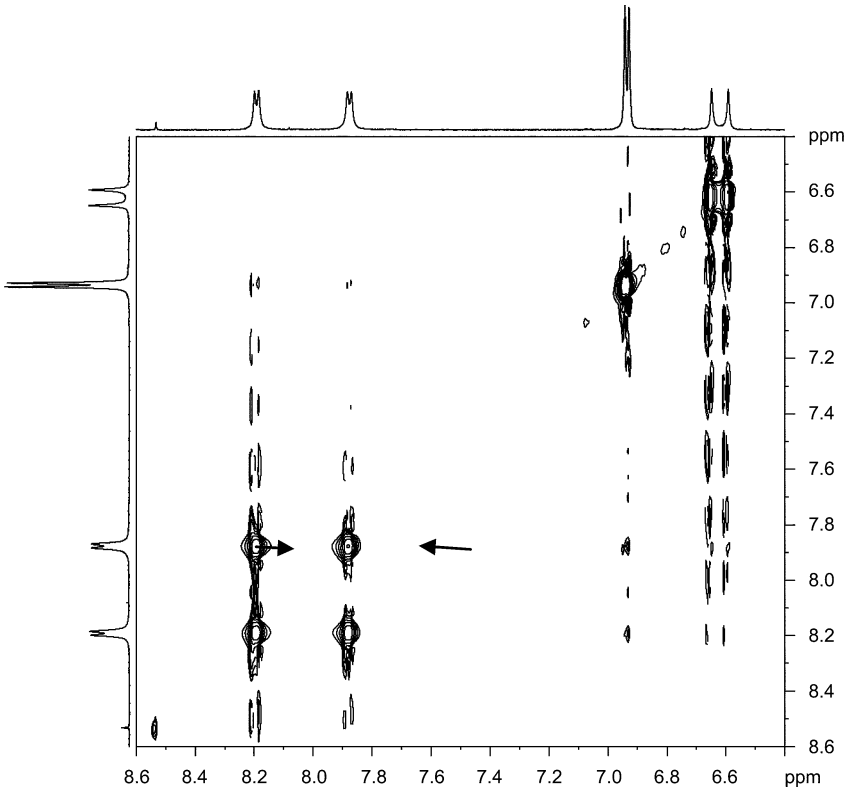

Fig. 3. *The lower-field section of NOESY spectrum of* **4**. The arrows indicate the exchanged cross-peaks between H–C(2′) and H–C(6′) of **4a** and **4b**.

those of **2** (*Table 1*). For **5a**, the following spin system was observed corresponding to a Xyl residue, δ(H) 5.01 (Xyl H–C(1), *d*) ↔ 4.40 (*t*) ↔ 3.68 (*t*) ↔ 3.73 (*dd*) ↔ 3.38 (*t*)/4.03 (*dd*) (Xyl CH_2(5)). The signal of the Xyl H–C(1) at δ(H) 5.01 (*d*, $J = 9.6$ (**5a**)) and the HMBC of this H-atom to C(7) (δ(C) 164.9), C(8) (δ(C) 104.3), and C(9) (δ(C) 157.8), in addition to the Xyl C(2) (δ(C) 82.3), indicated the Xyl residue to be *C*-linked to C(8) of the apigenin moiety. Another key HMBC of Xyl H–C(2) (δ(H) 4.40)/Glc C(1) (δ(C) 106.3) revealed an *O*-glycosidic linkage of Glc(1 → 2)Xyl. These data thus established **5** as apigenin 8-*C*-β-xylopyranosyl-2″-*O*-β-glucopyranoside.

Since HMBC and NOESY correlations of **2**–**5** are similar, a representative depiction for **2** is shown in *Fig. 2*.

Compounds **8**, **9**, **11**, and **14** exhibited levorotatory optical rotations ($[\alpha]_{25}^{D}$ ($c = 0.10$, MeOH) of −10, −10, −130, and −53, resp.), which were consistent with those reported. The glycon moieties in these compounds were identified as β-D-galactopyranosyl [14], β-D-glucopyranosyl [15], α-L-rhamnopyranosyl [14], and α-L-arabinopyranosyl [16], respectively. Quercetin 3-*O*-β-xylopyranoside (**16**) showed a dextrorotatory optical rotation ($[\alpha]_{25}^{D} = +10$ ($c = 0.10$, MeOH)), but no comparison was possible, since its optical rotation and the configuration of the xylosyl residue have not been yet

reported. GC Analysis of the trimethylsilylated thiazolidine derivatives of the monosaccharides, obtained from hydrolysis of the glycosides **1** and **16**, by a standard method [17], indicated the terminal monosaccharide residue in compounds **1** and the glycon in **16** to be D-glucopyranosyl and D-xylopyranosyl, respectively. Being from the same plant material, the absolute configurations of the composed monosaccharides in the diosides **1**–**5** are assumed the same as that in the monoosides, *i.e.*, D-glucose, D-xylose, and L-arabinose.

Eight isolated compounds, **1**, **6**, **10**–**14**, **16**, and **17**, were tested against α-glucosidase (type IV from *Bacillus stearothermophilus*) in a spectrophotometric assay [18]. 3-*O*-α-L-Rhamnosyl-4′-*O*-methylquercetin (**6**; IC_{50} 82.9 ± 21.7 μM), kaempferol 3-*O*-(2-β-D-apiofuranosyl)-α-L-rhamnopyranoside (**12**; IC_{50} 30.3 ± 6.1 μM), and quercetin (**17**; IC_{50} 112.8 ± 14.2 μM) showed significant activities. The other compounds exhibited < 50% inhibition at 100 μg/ml.

Recently, we reported the isolation of three apigenin *C*-6-diosides and one apigenin *C*-8-dioside together with two quercetin 3-*O*-monoosides and apigenin 4′-*O*-glucoside from *M. japonica* [19]. The current study on *M. konishii* led to patterns of flavonoid glycosides similar to those from *M. japonica*, indicating potential chemotaxonomic significance for the genus *Machilus*.

Experimental Part

General. HPLC: *Agilent 1100* system, *Phenomenex Prodigy ODS-3* (250 × 10 mm, 5 μm), detection at 280 nm. CH_2Cl_2, $CHCl_3$, AcOEt, BuOH, MeOH, and hexane (ACS grade), and THF (HPLC grade; *Mallinckrodt Baker Inc.*, USA), PrOH (ACS grade), MeCN (HPLC grade), and (D_4)MeOD (99.8%; *Merck Co.*, DE-Darmstadt), deionized H_2O (*Barnstead water purification system*, *Barnstead International*, Dubuque, IA, USA). Centrifugal partition chromatography (CPC): *Sanki CPC* (*model LLB-M*, 110 ml; *Sanki Engineering Ltd.*, Kyoto, Japan). TLC: Silica gel *60 F_{254}* (*Merck*, DE-Darmstadt). Optical rotations: *JASCO DIP-370 polarimeter*; in MeOH. UV (MeOH): λ_{max} nm (log ε), *Hitachi U-2001* spectrophotometer. CD Spectra (MeOH): *JASCO J-720* spectropolarimeter; λ in nm. ^{1}H-, ^{13}C-, and 2D-NMR spectra: *Bruker AV-400* and *AVIII-600* spectrometers; δ in ppm; in CD_3OD; δ(H) 3.30 and δ(C) 49.0 ppm; *J* in Hz. MS: *Esquire-2000 ion trap* (ESI-MS) and *MicrOTOF orthogonal ESI-TOF* (HR-ESI-MS) mass spectrometers (*Bruker*, *Daltonics*, DE-Bremen). Bioassay system: *SPECTRAmax PLUS* (*Molecular Devices*, California, USA); *p*-nitrophenyl α-D-glucopyranoside, α-glucosidase type IV from *B. stearothermophilus* from *Sigma–Aldrich Co.*, Germany, and K_2HPO_4 and KH_2PO_4 from *Merck*, DE-Darmstadt.

Plant Material. The leaves of *M. konishii* HAYATA were collected in September 2007 in *Fu-shan Research Center*, *Taiwan Forestry Research Institute*, Yilan county, Taiwan. A voucher specimen (NTUSP200709 MP) was authenticated by Mr. *Jer-Tone Lin*, former Associate Researcher, *Taiwan Forestry Research Institute*, and was deposited with the herbarium of that institute.

Extraction and Isolation. The powdered, dried leaves (4.0 kg) were extracted with 95% EtOH (1 × 20 l; 3 × 18 l) at r.t. to give the EtOH extract (816.3 g) after evaporation under reduced pressure. The EtOH extract (2 × 150.0 g) suspended in H_2O (1.2 l) was partitioned with CH_2Cl_2 (3 × 1.2 l), AcOEt (1.2 l), and BuOH (3 × 1.2 l). The org. layers were condensed to dryness under reduced pressure at 45° to give CH_2Cl_2-, AcOEt-, and BuOH-soluble fractions (91.7, 14.7, and 62.5 g, resp.).

Part of the BuOH-soluble fraction (20.00 g), which showed 99% inhibition against α-glucosidase at 100 μg/ml, was fractionated on a *Sephadex LH-20* column (2.6 l, MeOH) to give eight fractions, *Frs. B-1*–*B-8*, combined based on TLC (SiO_2) analysis.

Fr. B-2 (1.92 g) was separated by CC (*Sephadex LH-20*; MeOH/H_2O 1:1; 180 ml) to give four subfractions, *Fr. B-2-1*–*B-2-4*. *Fr. B-2-2* (974.4 mg) was subjected to CC (*RP-18 Lobar* (type *B* (*Merck*); 27–30% $MeOH_{aq}$; flow rate, 2 ml/min) to give **1** (472.2 mg). *Fr. B-2-3* (143.5 mg) was purified by CC

(*RP-18 Lobar* (type *A*); 35% $MeOH_{aq}$; flow rate, 1 ml/min) to give seven subfractions, *Frs. B-2-3-1 – B-2-3-7*, of which *Frs. B-2-3-2* and *B-2-3-4* consisted of **1** (38.3 mg) and **2** (10.2 mg), resp., and *Fr. B-2-3-6* (12.5 mg) yielded **3** (t_R 31.1 min; 4.9 mg), **4** (t_R 38.4 min; 1.9 mg), and **5** (t_R 41.4 min; 2.2 mg) after semi-prep. HPLC (*RP-18*; 18% THF_{aq}; flow rate, 2.4 ml/min).

Fr. B-3 (1.28 g) was separated by CPC (lower layer of $CHCl_3$/MeOH/H_2O/PrOH (9 : 12 : 8 : 1; flow rate, 1.5 ml/min) to furnish six subfractions, *Frs. B-3-1 – B-3-6*. *Frs. B-3-2* (6.9 mg) and *B-3-3* (6.4 mg) were further purified by semi-prep. HPLC (*RP-18* column; with 45 and 30% $MeOH_{aq}$, resp.; flow rate, 2.4 ml/min) to afford **6** (t_R 167.4 min; 1.2 mg; *Fr. B-3-2*) and **7** (t_R 177.8 min; 0.3 mg; *Fr. B-3-3*).

Fr. B-5 (0.92 g) was submitted to CC (*RP-18 Lobar* (type *B*); 20% MeOH in H_2O; flow rate, 2 ml/min) to give five subfractions, *Frs. B-5-1 – B-5-5*. *Fr. B-5-4* (55.4 mg) was further separated by semi-prep. HPLC (*RP-18*; 9% $MeCN_{aq}$; flow rate, 2.4 ml/min) to afford **8** (t_R 206.4 min, 1.2 mg) and **9** (t_R 235.6 min, 1.7 mg). *Fr. B-5-5* (15.5 mg) gave **10** (t_R 163.8 min, 2.5 mg;) and **11** (t_R 176.0 min, 1.2 mg) after semi-prep. HPLC (*RP-18*; 30% $MeOH_{aq}$; flow rate, 2.4 ml/min).

The AcOEt-soluble fraction (14.00 g), showing 89% inhibition against α-glucosidase at 100 µg/ml, was subjected to CC (*Sephadex LH-20*; MeOH, 2.6 l) to give nine fractions *Frs. E-1 – E-9*), combined by TLC (silica gel) analysis. *Fr. E-3* (0.46 g) was separated by CC (*RP-18 Lobar* (type *B*); MeOH/H_2O from 20 : 80 to 100 : 0; flow rate, 2 ml/min) to give **12** (6.3 mg). *Fr. E-4* (0.33 g) was separated by CPC, (lower layer of $CHCl_3$/MeOH/H_2O/PrOH 9 : 12 : 8 : 1; flow rate, 1.5 ml/min) to furnish **13** (4.5 mg) and an additional crop of **6** (26.8 mg). *Fr.E-6* (2.2 g) gave an additional crop of **8** (87.6 mg) upon recrystallization from MeOH. The residue, obtained after evaporation of the mother liquid, was separated by a further CPC (same conditions as described above) to give **14** (40.1 mg) and an additional crop of **8** (34.2 mg). Under similar CPC conditions as described for *Fr. E-4*, *Fr. E-7* (1.10 g) gave 19 subfractions, *E-7-1 – E-7-19*) and afforded **15** (2.3 mg; *Fr. E-7-4*), **16** (46.7 mg; *Fr. E-7-17*), and **14** (170.7 mg; *Fr. E-7-19*), and *Fr. E-9* (1.1 g) gave **17** (13.8 mg).

Apigenin 6-C-β-D-*Xylopyranosyl-2″*-O-β-D-*glucopyranoside* (=*(1S)-1,5-Anhydro-1-[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H-*chromen-6-yl]-2*-O-β-D-*glucopyranosyl*-D-*xylitol*; **2**). Yellowish amorphous solid. $[\alpha]_D^{22} = -62.5$ ($c = 0.40$, MeOH). UV: 273 (4.37), 337 (4.20). CD: $[\theta]_{212}$ +4740, $[\theta]_{218}$ −7880, $[\theta]_{228}$ −3600, $[\theta]_{273}$ +5860. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS: 587 ($[M+Na]^+$; pos.), 563 ($[M-H]^-$; neg.). HR-ESI-MS (neg.): 563.1392 ($[M-H]^-$, $C_{26}H_{27}O_{14}^-$; calc. 563.1401).

Apigenin 8-C-α-L-*Arabinopyranosyl-2″*-O-β-D-*glucopyranoside* (=*(1S)-1,5-Anhydro-1-[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H-*chromen-8-yl]-2*-O-β-D-*glucopyranosyl*-L-*arabinitol*; **4**). Yellowish amorphous solid. $[\alpha]_D^{22} = +50$ ($c = 0.10$, MeOH). UV: 220 (5.30), 267 (4.80), 273 (4.78), 335 (4.30). CD: $[\theta]_{212}$ −5180, $[\theta]_{217}$ −176720, $[\theta]_{222}$ +123610, $[\theta]_{226}$ −12640, $[\theta]_{232}$ +16710, $[\theta]_{241}$ +2640, $[\theta]_{269}$ −12310, $[\theta]_{326}$ +9530. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HMBC (CD_3OD, 600 MHz): H–C(3)/C(2), C(4), C(10), C(1′); H–C(6)/C(4), C(5), C(7), C(8), C(10); H–C(2′) and H–C(6′)/C(2), C(4′); H–C(3′) and H–C(5′)/C(1′), C(4′); H–C(1″)/C(7), C(8), C(9), C(2″); H–C(2″)/C(1″), C(3″), C(1‴); H–C(4″)/C(3″); CH_2(5″)/C(4″); H–C(1‴)/C(2″); H–C(2‴)/C(1‴), C(3‴); H–C(3‴)/C(2″), C(4‴); H–C(4‴)/C(5‴), C(6‴); H–C(5‴)/C(1‴), C(3‴). ESI-MS: 587 ($[M+Na]^+$), 563 ($[M-H]^-$). HR-ESI-MS: 563.1394 ($[M-H]^-$, $C_{26}H_{27}O_{14}^-$; calc. 563.1406).

Apigenin 8-C-β-D-*Xylopyranosyl-2″*-O-β-D-*glucopyranoside* (=*(1S)-1,5-Anhydro-1-[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H-*chromen-8-yl]-2*-O-β-D-*glucopyranosyl*-D-*xylitol*; **5**). Yellowish amorphous solid. $[\alpha]_D^{22} = -10$ ($c = 0.10$, MeOH). UV: 217 (5.32), 231 (5.32), 244 (5.32), 250 (5.32), 270 (5.21), 331 (4.54). CD: $[\theta]_{213}$ −60000, $[\theta]_{219}$ +106580, $[\theta]_{229}$ −10900, $[\theta]_{235}$ +48770, $[\theta]_{241}$ −62310, $[\theta]_{250}$ −46130, $[\theta]_{254}$ +10180, $[\theta]_{258}$ −124990, $[\theta]_{262}$ +124730, $[\theta]_{271}$ −24930, $[\theta]_{286}$ −8200, $[\theta]_{325}$ +4280. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HMBC (CD_3OD, 600 MHz): H–C(3)/C(2), C(4), C(10), C(1′); H–C(6)/C(4), C(5), C(7), C(8), C(10); H–C(2′) and H–C(6′)/C(2), C(4′); H–C(3′) and H–C(5′)/C(1′), C(4′); H–C(1″)/C(7), C(8), C(9), C(2″); H–C(2″)/C(1″), C(3″), C(1‴); H–C(3″)/C(2″); H_a–C(5″)/C(1″), C(3″), C(4″); H–C(1‴)/C(2‴); H–C(2‴)/C(1‴), C(3‴); H–C(3‴)/C(4‴); H–C(4‴)/C(3‴). ESI-MS: 587 ($[M+Na]^+$), 563 ($[M-H]^-$). HR-ESI-MS: 563.1391 ($[M-H]^-$, $C_{26}H_{27}O_{14}^-$; calc. 563.1406).

Apigenin 6-C-α-L-*Arabinopyranosyl-2″*-O-β-D-*glucopyranoside* (=*(1S)-1,5-Anhydro-1-[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H-*chromen-6-yl]-2*-O-β-D-*glucopyranosyl*-L-*arabinitol*; **3**). Yellowish

amorphous solid. $[\alpha]_D^{22} = +10$ ($c = 0.20$, MeOH). UV: 215 (4.37), 272 (4.18), 338 (4.18). CD (MeOH): $[\theta]_{212}$ +7500, $[\theta]_{224}$ −6010, $[\theta]_{271}$ +19810, $[\theta]_{320}$ −5150. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS: 587 ($[M+Na]^+$), 563 ($[M-H]^-$). HR-ESI-MS: 563.1391 ($[M-H]^-$, $C_{26}H_{27}O_{14}^-$; calc. 563.1406).

Sugar Analysis. Acid hydrolyses of **1** (30.0 mg) and **16** (2.0 mg) were carried out according to standard procedures (4N HCl/MeOH 1:1; reflux, 3 h). After cooling, the mixture was neutralized with 2N $NaOH_{aq}$ and evaporated under reduced pressure to yield a residue, which was separated by CC (*Sephadex LH-20*) to yield *quercetin* (**17**) [5] and xylose from **16**, and the *C*-glycoside isovitexin (14.8 mg) [10] and glucose (10.5 mg) from **1**. The absolute configurations of the glycons were determined by GC analysis of trimethylsilylated thiazolidine derivatives, which were prepared as described in [17]. Conditions for GC: cap. column, *DB-5* (0.25 mm × 30 m); oven temp. program, 180–300° at 5°/min; injection temp., 250°; carrier gas, He at 0.8 ml/min. Under such conditions, the derivatives of the acid hydrolysates of **1** and **16** had t_R values of 17.263 min and 13.766 min, resp. Under the same conditions, the t_R values for the trimethylsilylated thiazolidine derivatives of the standard D/L-glucose and D/L-xylose were 17.278/17.553 min and 13.766/14.195 min, resp.

Assay for α-Glucosidase Activity. The inhibitory activity against α-glucosidase was determined by a slight modification of the method reported in [18]. To each well of 96-well microtiter plate were added 20 µl of α-glucosidase (3 U/ml in phosphate-buffered saline (PBS), pH 6.5), 40 µl of PBS (pH 6.5), 10 µl of the sample (concentrations: 1000, 100, 10 µg/ml in 10% MeOH), and H_2O (20 µl). The mixture was incubated at 37° for 15 min, then *p*-nitrophenyl α-D-glucopyranoside (10 µl, 7.5 mM in PBS) was added, and the mixture was incubated for additional 10 min. The absorbance (A) of each well was measured at 405 nm with a microplate spectrophotometer. The inhibition activity was calculated by the equation: Inhibition $[\%] = [1 - (A_{sample}/A_{control})] \times 100\%$. The positive control was acarbose (*Bayer*) with an IC_{50} value of 0.040 ± 0.001 µM against the same enzyme. The assay for each compound concentration was undertaken in triplicate, and the IC_{50} values were expressed as mean ± standard deviation.

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