NEW FLAVOID GLYCOSIDES OF *HELICTERES ANGUSTIFOLIA*[1]

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Abstract-Two new flavonoid glycosides, takakin 8-*O*-β-D-glucuronide 6"-methyl ester (2), takakin 8-*O*-β-D-glucuronide 2"-sodium sulfate (3) together with a known flavonoid glycoside, takakin 8-*O*-β-D-glucuronide were isolated from the root bark of *Helicteres angustifolia* (Sterculiaceae). The structures of these compounds were established on the basis of spectroscopic and chemical evidence.

In the previous report,[2] four sesquiterpenoid quinones have been isolated from the root bark of *Helicteres angustifolia* L. In this paper, we describe the isolation and structural elucidation of two new flavonoid glycosides, takakin 8-*O*-β-D-glucuronide 6"-methyl ester (2) and takakin 8-*O*-β-D-glucuronide 2"-sodium sulfate (3) from the *n*-butanol extract of the root bark of this plant. Compound (3) is a novel sulfated flavonoid glycoside which the sulfate group is linked to the flavone through the sugar substituent. The uv spectra of these compounds exhibited typical absorption of 5,7,8,4'-tetraoxygenated flavones.[3] The coupling constants of the anomeric protons of the sugar moieties of **1**, **2** and **3** support the β-anomer of those compounds.

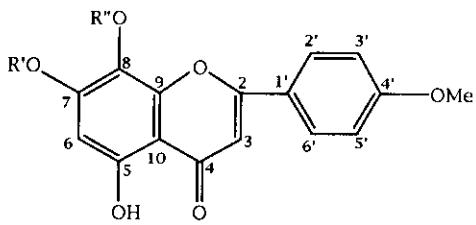
Compound (2), pale yellow needles, $[\alpha]_D$ + 9.7°(c 2.90, DMSO), mp 276-278°C, showed ir absorptions of hydroxy (3280 cm^{-1}), ester (1740 cm^{-1}) and aromatic groups (1580, 1515, 1430 cm^{-1}). The

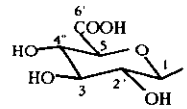
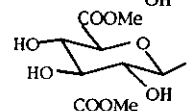
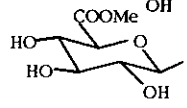
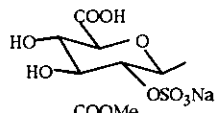
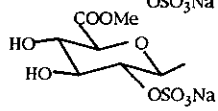
high-resolution negative ion FAB ms of **2** showed an ion peak at *m/z* 489.1080 ([M-H]$^-$, $C_{23}H_{21}O_{12}$). The ^{1}H- and ^{13}C-nmr spectra of **2** were similar to those of **1** except that the former showed a carboxymethyl group at δ 3.56 (^{1}H-nmr) and δ 51.9 (^{13}C-nmr). Methylation of **2** with etheral diazomethane gave a methyl ether (**2a**), which showed a molecular ion peak at *m/z* 504 M$^-$ in negative ion FAB ms. The ^{1}H-nmr spectrum of **2a** showed a signal at δ 12.85 (1H, s, 5-OH) and an additional aryl methoxy group at δ 3.86 (3H, s, 7-OMe), in comparison with those of **2**. The uv spectrum of **2a** in MeOH showed absorptions at λ_{max}(MeOH):344 sh, 322, 303 and 273 nm; unchanged on addition of NaOAc. These facts indicated that **2a** was 7-*O*-methyl ether of **2**. On controlled acid hydrolysis of **2** with 0.1 N trifluoroacetic acid(TFA), **1** was obtained. On methanolysis, a crystalline product was obtained which was identical to takakin (**2b**).[5] These facts indicated that **2** was a methyl ester of **1**. Consequently, the structure of **2** was determined as takakin 8-*O*-β-D-glucuronide 6"-methyl ester.

Compound (**3**), yellow needles, $[\alpha]_D$ -18.8° (c 5.40, H_2O), mp 255-257°C (decomp.), showed ir absorptions of hydroxy (3500 cm^{-1}), carboxyl (3500-2500, 1730 cm^{-1}), conjugated carbonyl (1660 cm^{-1}), aromatic groups (1580, 1520, 1440 cm^{-1}) and a strong broad band due to S=O group (1245 cm^{-1}).[6] The high-resolution negative ion FAB ms of **3** showed an ion peak at *m/z* 577.0244 ([M-H]$^-$, $C_{22}H_{18}O_{15}NaS$). On controlled acid hydrolysis of **3** with 1 N TFA, **1** was obtained. These facts confirmed the presence of a sulfate group with a Na$^+$ as counter ion. The sulfate group was linked to the sugar moiety on the basis of the following facts. Analysis of the ^{13}C-nmr spectral data (Table 1) of **1** and **3**, the carbon signals due to aglycone moiety of **3** showed almost the same ^{13}C-nmr chemical shifts as those of **1**, however the carbon siginals due to the sugar moiety of **3** showed significant shift in comparison with those of **1** (Table 1). The uv spectrum of **3** showed no bathochromic shift on addition of conc. HCl that revealed no sulfate group to be linked to aglycone moiety of **3**.[6] Methylation of **3** with etheral diazomethane afforded **3a** which showed an ion peak at *m/z* 583 [M-Na]$^-$ in FAB ms. The ^{1}H-nmr spectrum of **3a** showed a signal at δ 12.85 due to 5-OH, signals at δ 3.85, 3.84 (each 3H, s) due to two aryl methoxy protons(7-OMe, 4'-OMe) and the signal at δ 3.49(3H, s) due to carboxymethyl protons. These facts confirmed the sulfate group to be not linked to 5- or 7-position of the aglycone moiety of **3**. The chemical shifts of H-2" was confirmed by following double resonance experiments.

Table 1 ^{13}C-Nmr Spectral Data of Compounds (1), (2), (2b) and (3) (DMSO-d_6, δ)

Carbon	1	2	2b	3
2	163.6(s)	163.4(s)	163.2(s)	163.7(s)
3	103.2(d)	103.1(d)	103.1(d)	103.3(d)
4	182.0(s)	181.9(s)	182.1(s)	182.0(s)
5	157.1(s)	156.9(s)	153.0(s)	157.3(s)
6	99.1(d)	99.0(d)	98.7(d)	100.3(d)
7	157.5(s)	157.4(s)	153.5(s)	160.1(s)
8	125.4(s)	125.1(s)	128.2(s)	124.7(s)
9	149.4(s)	149.3(s)	145.5(s)	149.7(s)
10	103.6(s)	103.6(s)	103.2(s)	103.2(s)
1'	122.8(s)	122.7(s)	123.1(s)	123.0(s)
2',6'	128.9(d)	128.7(d)	128.4(d)	129.5(d)
3',5'	114.7(d)	114.5(d)	114.5(d)	115.1(d)
4'	162.5(s)	162.3(s)	162.3(s)	162.6(s)
sugar moiety				
1"	106.5(d)	106.1(d)		101.6(d)
2"	71.5(d)	71.5(d)		78.7(d)
3"	73.9(d)	73.8(d)		72.7(d)
4"	75.3(d)	75.1(d)		76.0(d)
5"	76.2(d)	75.7(d)		76.6(d)
6"	170.1(s)	169.4(s)		174.0(s)
4'-O<u>C</u>H$_3$	55.6(q)	55.5(q)	55.5(q)	55.8(q)
CO<u>O</u>C<u>H</u>$_3$		51.9(q)		



	R'	R"
1	H	
2	H	
2a	Me	
2b	H	H
3	H	
3a	Me	

Irradiation of the signals at δ 4.40 (1H, t, J = 8.0 Hz, H-2"), collapsed the signals at δ 4.98 (1H, d, J = 7.2 Hz, H-1") and the signals at δ 3.50 (1H, t, J= 8.0 Hz, H-3") to a singlet and a doublet, respectively. Irradiation of the signals at δ 4.98 only collapsed the signals at δ 4.40 to a doublet. The sulfate group of **3** was linked to 2"-OH on the basis of following sulfation shifts.[6] First, the signals at δ 4.40 due to H-2" of **3** showed downfield shift by 0.93 ppm in comparison with the signals at δ 3.47 due to H-2" of **1**, assigned by ^{1}H-^{1}H COSY spectrum based on the correlation between H-2" and anomeric proton (δ 4.81). Second, the ^{13}C-nmr spectral data (Table 1) of **3** showed that the signal at δ 78.7 due to C-2" carbon atom showed downfield shift by 7.2 ppm, while the signals at δ 101.6 and 72.7 due to C-1" and C-3" carbon atoms showed upfield shift by 4.9 and 1.2 ppm, respectively, in comparison with those of **1**. Assignments of carbon signals of compound (**3**) (Table 1) were made with the aid of ^{1}H-^{13}C COSY spectrum and DEPT experiments. Therefore, the structure of compound (**3**) was determined as takakin 8-*O*-β-D-glucuronide 2"-sodium sulfate.

EXPERIMENTAL

Mps were uncorrected. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. Ir spectra were taken on a Perkin Elmer 781 infrared spectrophotometer. Uv spectra were measured on a Hitachi 200 spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM-400 spectrometer. FAB ms were recorded on a JMS-HX-110 spectrometer with thioglycerol as matrix.

Extraction and Isolation. Dried powdered root bark of *H. angustifolia* (3.8 Kg) was extracted with $CHCl_3$ (3 x 5 l) under reflux for 6 h. The residue (3.5 Kg) was extracted with MeOH (5 x 5 l) under reflux for 6 h. The concentrated MeOH extract (201 g) was suspended in water. The suspension was extracted with EtOAc and *n*-butanol, successively. The *n*-butanol soluble fraction (35 g) was chromatographed on a silica gel column with $CHCl_3$-MeOH-H_2O (65:35:10, lower layer) as eluent and 250 ml were collected for each fraction. Fractions 7-9 (1.2 g) were collected and chromatographed on a Sephadex LH-20 column with H_2O-EtOH (1:1) as eluent to afford takakin 8-*O*-β-D-glucuronide (**1**, 16 mg) and takakin 8-*O*-β-D-glucuronide 6"-methyl ester (**2**, 32 mg). Fractions 15-33 (3.6 g) were collected and chromatographed on a Sephadex LH-20 column with H_2O-EtOH (1:1) as eluent to afford takakin

8-*O*-β-D-glucuronide 2"-sodium sulfate (3, 220 mg).

Takakin 8-O-β-D-glucuronide 6"-methyl ester (2). Yellow needles (MeOH), mp 276-278°C, $[\alpha]_D^{27}$ + 9.7° (c 2.90, DMSO). Uv λ_{max} (MeOH) nm (*log* ε): 345 *sh*, 324 (4.35), 303 (4.32), 274 (4.38); λ_{max} (MeOH+NaOMe) nm (log ε): 371 (4.18), 312 sh, 302 (4.41), 282 (4.54); λ_{max} (MeOH+$AlCl_3$) *nm*(log ε): 392 (4.14), 343 (4.34), 307 (4.32), 282 (4.28), 265 sh; unchanged on addition of HCl; λ_{max} (MeOH+NaOAc) nm (log ε): 369 (4.19), 312 sh, 302 (4.42), 282 (4.54). Ir υ_{max} (KBr) cm^{-1}: 3280, 2920, 1740, 1660,1605, 1580,1560, 1515, 1430, 1365, 1250, 1190, 1170, 1120, 1080, 1040, 1015, 840. Negative ion FAB ms *m/z* : 489 $[M-H]^-$, 299 $[M-C_7H_{11}O_6]^-$. High-resolution FAB ms *m/z* : 489.1080($[M-H]^-$, Calcd for $C_{23}H_{21}O_{12}$: 489.1033). Eims *m/z* (rel. int.) 300(100), 167 (60), 133 (27). ^{1}H-Nmr(DMSO-d_6) : δ 12.71 (1H, s, 5-OH), 8.12 (2H, d, J= 8.8 Hz, H-2',6'), 7.03 (2H, d, J= 8.8 Hz, H-3',5'), 6.88 (1H, s, H-3), 6.28 (1H, s, H-6), 4.84 (1H, d, J= 8.0 Hz, H-1"), 3.92 (3H, s, OMe), 3.56(3H, s, OMe), 3.5-3.32(m). ^{13}C-Nmr : see Table 1.

Takakin 8-O-β-D-glucuronide 2"-sodium sulphate (3). Yellow needles(MeOH), mp 255-257°C, $[\alpha]_D^{27}$ -18.8° (c 5.40, H_2O). Uv λ_{max}(MeOH) nm(log ε): 342 sh, 326(4.38), 301(4.34), 273(4.40); λ_{max} (MeOH+NaOMe) nm (log ε): 379 (4.21), 312 sh, 301 (4.44), 282 (4.56), λ_{max} (MeOH+$AlCl_3$) nm (log ε): 392(4.23), 346 (4.44), 308 (4.38), 281 (4.36), 264 (4.21); unchanged on addition of HCl; λ_{max} (NaOAc) nm (log ε): 372 (4.20), 312 sh, 301 (4.42), 282 (4.56). Ir υ_{max} (KBr) cm^{-1}: 3500-2500, 1730, 1660, 1610, 1580, 1520, 1440, 1365, 1245 (broad and strong, S=O), 1180, 1100, 1085, 1030, 1000, 840. Negative ion FAB ms *m/z* 577 $[M-H]^-$, 555 $[M-Na]^-$, 475 $[M-SO_3Na]^-$, 299 $[M-C_6H_9O_9NaS]^-$. High-resolution FAB ms *m/z* : 577.0244 ($[M-H]^-$, Calcd for $C_{22}H_{18}O_{15}NaS$: 577.0225). Eims *m/z*(rel. int.): 300(100), 167(50), 133(30). ^{1}H-Nmr(DMSO-d_6): δ 12.68(1H, s, 5-OH), 8.29 (2H, d, J= 8.8 Hz, H-2',6'), 7.09 (2H, d, J= 8.8 Hz, H-3',5'), 6.83 (1H, s, H-3), 6.19 (1H, s, H-6), 4.98 (1H, d, J= 7.2 Hz, H-1"), 4.40 (1H, t, J= 8.0 Hz, H-2"), 3.83 (3H, s, OMe), 3.64 (1H, t, J= 8.0 Hz, H-4"), 3.56 (1H, d, J= 8.0 Hz, H-5"), 3.50 (1H, t, J= 8.0 Hz, H-3"). ^{13}C-Nmr : see Table 1.

Acid Hydrolysis of Takakin 8-O-β-D-glucuronide (**1**). A solution of **1** (1 mg) in 3% hydrochloric acid (3 ml) was heated on a boiling water bath for 6 h. The mixture was evaporated *in vacuo*. The residue was dissolved in 0.5 ml of dry pyridine and the trimethylsilyl ethers were prepared by addition of 0.4 ml of hexamethyldisilazane and 0.2 ml of trimethylchlorosilane, successively. The mixture was evaporated *in vacuo*, 0.5 ml of *n*-heptane was added. The insoluble material was filtered off. The filtrate was shown to contain TMS-ether of D-glucuronic acid by glc[packed glass column, 3% OV-101 on Chromosorb W-HP 80-100 mesh, 2 mm x 2 m; column temperature, 150-250°C at 10°C/min; carrier gas, N_2; t_R 5.77, 6.32 min].

Methylation of **2**. A solution of **2** (5.0 mg) in MeOH(10 ml) was treated with etheral CH_2N_2. Work-up in the usual manner gave a yellow needles (**2a**, 3.5 mg), mp 220-222°C. Uv λ_{max} (MeOH) nm (log ε) : 344 sh, 322(4.40), 303(4.36), 273(4.42); unchanged on addition of NaOAc; λ_{max}(MeOH+NaOMe) nm (log ε): 357(4.15), 298(4.47), 282(4.45); λ_{max}(MeOH+$AlCl_3$) nm(log ε): 393(4.25), 344(4.46), 308 (4.41), 280 (4.40); unchanged on addition of HCl. Negative ion FAB ms *m/z*: 504 M^-, 314 $[M-C_7H_{10}O_6]^-$. ^{1}H-Nmr (DMSO-d_6): δ 12.85 (1H, s, 5-OH), 8.20 (2H, d, J= 8.8 Hz, H-2',6'), 7.07 (2H, d, J= 8.8 Hz, H-3',5'), 6.9(1H, s, H-3), 6.58 (1H, s, H-6), 4.87 (1H, d, J=7.6 Hz, H-1"), 3.86 (3Hx2, s, OMe), 3.45 (3H, s, OMe), 3.50-3.30(m).

Controlled acid hydrolysis of **2**. A solution of **2**(4.0 mg) in 0.1N trifluoroacetic acid (10 ml) was heated under 80°C for 3 h. The reaction mixture was extracted with *n*-butanol. The extract was concentrated to dryness, then chromatographed on a Sephadex LH-20 column with H_2O-EtOH (1:1) as eluent to give a yellow needles (2.2 mg), which was identical to **1** by direct comparision (mp, tlc, ^{1}H-nmr).

Methanolysis of **2**. Compound (2) (10.0 mg) was reacted with 10% HCl methanolic solution (10 ml) under reflux for 4 h. Work-up in the usual manner gave a crystalline product (4.0 mg) which was identical to takakin (**2b**),[5] mp 264-267°C(decomp.). Uv λ_{max}(MeOH) nm(log ε): 350 sh, 302 (4.33), 284 (4.32); λ_{max} (MeOH+NaOMe) nm (log ε) : 364 (3.90), 315 sh, 278 (4.37), 256 (4.24); λ_{max} (MeOH+$AlCl_3$) nm(log ε): 350 sh, 325(4.35), 296(4.28); λ_{max} (MeOH+$AlCl_3$+HCl) nm (log ε): 345 (4.25), 314 (4.30), 290 (4.23); λ_{max} (MeOH+NaOAc) nm (log ε): 296 (4.34). Negative ion FAB

ms *m/z* : 299 $[M-H]^-$. ^{1}H-Nmr(DMSO-d_6): δ 12.34 (1H, s, 5-OH), 8.12 (2H, d, J= 8.8 Hz, H-2',6'), 7.12 (2H, d, J= 8.8 Hz, H-3',5'), 6.83 (1H, s, H-3), 6.28(1H, s, H-6), 3.85(3H, s, OMe). ^{13}C-Nmr : see Table 1.

Controlled acid hydrolysis of **3**. A solution of **3** (9.0 mg) in 1 N trifluoroacetic acid (10 ml) was heated under 80°C for 8 h. A product was crystallized from the aqueous solution appearing as yellow needles (6.2 mg). It was identical to **1** by direction comparision(mp, tlc, ir, ^{1}H-nmr).

Methylation of **3**. A solution of **3** (7.0 mg) in MeOH (35 ml) was treated with etheral CH_2N_2 (4 ml). Work-up in the usual manner gave **3a** (4.0 mg), mp > 300°C. Uv λ_{max} (MeOH) nm (log ε) : 344 sh, 324(4.38), 302(4.35), 273(4.40); unchanged on addition of NaOAc; λ_{max} (MeOH+NaOMe) nm (log ε): 375(4.07), 302(4.47), 293(4.45); λ_{max} (MeOH+$AlCl_3$) nm (log ε) : 398 (4.20), 345 (4.42), 309 (4.37), 281 (4.35); unchanged on addition of HCl. Negative ion FAB ms *m/z*: 583 $[M-Na]^-$, 314 $[M-C_7H_9O_9NaS]^-$. ^{1}H-Nmr(DMSO-d_6): δ 12.85(1H, s, 5-OH), 8.21(2H, d, J= 8.8 Hz, H-2',6'), 7.09 (2H, d, J= 8.8 Hz, H-3',5'), 6.90 (1H, s, H-3), 6.56 (1H, s, H-6), 5.05 (1H, d, J= 7.6 Hz, H-1"), 4.19(1H, t, J= 8.0 Hz, H-2"), 3.85 (3H, s,OMe), 3.84 (3H, s, OMe), 3.49 (3H, s, OMe), 4.0-3.3 (m).

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