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ALANGIFLAVOSIDE, A NEW FLAVONOL GLYCOSIDE FROM THE LEAVES OF *ALANGIUM PREMNIFOLIUM*

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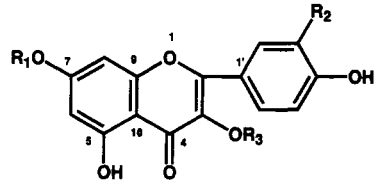
ABSTRACT.—A new flavonol with a tetrasaccharide unit named alangiflavoside [**4**] was isolated from the water-soluble fraction of a methanol extract of *Alangium premnifolium* leaves, along with three known flavonol glycosides. Structure determination was made based on spectroscopic and chemical evidence.

Phytochemical investigation of the *n*-BuOH-soluble fraction of a MeOH extract of the leaves of *Alangium premnifolium* Ohwi (Alangiaceae) afforded several kinds of megastigmane glycosides (1,2). Separation and purification of the H_2O-soluble fraction yielded four flavonol glycosides, three of which were found to be the known compounds kaempferol-3-*O*-β-D-(2″,6″-di-α-L-rhamnopyranosyl)glucopyranoside [**1**] (3), mauritianin [**2**] (4,5), and 3′-methoxykaempferol-3-*O*-β-D-(2″,6″-di-α-L-rhamnopyranosyl) galactopyranoside [**3**] (6). The structure of the remaining flavonol glycoside, named alangiflavoside [**4**], which has four sugar units, was elucidated in this study.



	R_1	R_2	R_3	
1	H	H	glc	(2″-1‴)rha (6″-1⁗)rha
2	H	H	gal	(2″-1‴)rha (6″-1⁗)rha
3	H	OCH_3	gal	(2″-1‴)rha (6″-1⁗)rha
4	glc (1⁗-6⁗)	H	gal	(2″-1‴)rha (6″-1⁗)rha

Alangiflavoside [**4**], $[\alpha]_D$ −93.9°, was obtained as a yellow amorphous powder whose elemental composition was determined to be $C_{39}H_{50}O_{24}$ by observation of a quasi-molecular ion in the hrfabms. The ir spectrum revealed the presence of hydroxyl groups (3350 cm^{-1}), aromatic rings (1600 and 1485 cm^{-1}), and a chelated ketone function (1650 cm^{-1}). The uv absorption maxima were similar to those of **2**. The ^{13}C- and ^1H-nmr spectra indicated the presence of four sugar moieties, three of which were expected to be two terminal α-rhamnopyranose units and one terminal β-glucopyranose unit. This was supported by the observation of oxonium ions of 6-deoxyhexose (*m/z* 273) and hexose (*m/z* 331) in the eims of the tetradecaacetate of alangiflavoside [**4**]. The remaining sugar was identified as galactose by glc analysis of the methanolysis product, showing the presence of rhamnose (rha), galactose (gal), and glucose (glc) in a 2:1:1 ratio. Signals attributable to the aglycone were essentially indistinguishable from those of kaempferol, as found in **2**. The ^{13}C-nmr chemical shifts of the C-2 and C-3 positions (δ_C 158.0 and 134.8) indicated that one of the sugar moieties must occupy the hydroxyl group at C-3 (7). The lack of a bathochromic shift on the addition of NaOAc in the uv spectrum indicated the absence of a free hydroxyl group at the C-

7 position. In a difference nOe experiment, significant enhancement of two aromatic proton signals at δ_H 6.47 (d, J=2 Hz) and 6.75 (d, J=2 Hz) (H-6 and H-8, respectively) on irradiation of the anomeric proton signal at δ_H 5.08 was evident, so that the hydroxyl group at the C-7 position must participate in the other sugar linkage. Because enzymatic hydrolysis of **4** by β-D-glucosidase yielded **2**, the structure of alangiflavoside was confirmed as the 7-*O*-β-D-glucopyranoside of mauritianin [**2**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded on a JEOL JNM GX-400 spectrometer at 400 MHz and 100 MHz, respectively; mass spectra were obtained on a JEOL JMS SX-102 spectrometer. Ir spectra were taken on a Shimadzu IR-408 spectrophotometer and uv on a Shimadzu UV-160A spectrophotometer. Specific optical rotations were determined using a Union Giken PM-101 digital polarimeter. For compound purification, the following were used: the highly porous synthetic resin, Diaion HP-20 (Mitsubishi Chemical Co., Ltd., Tokyo, Φ=80 mm, L=600 mm, H_2O-MeOH (19:1)→MeOH); Kieselgel 60 Si gel [(Merck), 70–230 mesh, $CHCl_3$→$CHCl_3$-MeOH; ODS, reversed-phase Si gel [Cosmosil, ODS 75 C_{18}-OPN (Nakarai Tesque, Kyoto), Φ=40 mm, L=250 mm, H_2O-MeOH (9:1, 1.5 liters)→H_2O-MeOH (3:7, 1.5 liters, fractions of 10 g collected]; droplet counter-current chromatography (dccc): 500 columns [Tokyo Rikakikai, Φ=2 mm, L=40 cm, $CHCl_3$-MeOH-H_2O-*i*-PrOH (9:12:8:2), fractions of 5 g being collected]; hplc, ODS [Inertsil (GL Science, Tokyo), Φ=20 mm, L=250 mm, H_2O-MeOH, flow rate 6 ml/min, detection at 254 nm]; gel filtration, Sephadex LH-20 [Pharmacia (Uppsala, Sweden), Φ=25 mm, L=1300 mm, MeOH, fractions of 8 g being collected]. Glc data were obtained on a Shimadzu GC-8A gas chromatograph with an FID detector, employing a Shimadzu CPB-20 column, 0.22 mm×25 m, layer thickness 0.25 μm, carrier gas: N_2 at 1.5 kg/cm^2.

PLANT MATERIAL.—Leaves of *A. premnifolium* were collected in Nakagami-gun, Okinawa, Japan in August 1990. A voucher specimen has been deposited at the Herbarium of the Department of Pharmacognosy, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine.

EXTRACTION AND ISOLATION.—The MeOH extract of leaves of *A. premnifolium* (5.72 kg) was concentrated to 3 liters and then 150 ml of H_2O was added to make a 95% aqueous MeOH solution. The solution was extracted with 3 liters of *n*-hexane and then the MeOH layer was concentrated to give a residue. The residue was suspended in H_2O (3 liters), and then extracted with EtOAc and *n*-BuOH, successively. On evaporation of the H_2O layer, 365 g of a H_2O-soluble material were obtained. The material was separated by Diaion HP-20 cc to give 22.6 g of a crude flavonol glycoside fraction in the 40% MeOH eluate. The residue of the eluate was separated over a Si gel column and then the residue (1.87 g) of the 25–30% MeOH eluate was further separated by reversed-phase cc to give two fractions (503 mg in fractions 83–106 and 125 mg in fractions 155–172). The former fraction was purified by dccc (67 mg in fractions 3–6) and finally purified by Sephadex LH-20 cc to yield 27 mg of **4** in fractions 31–34. The latter was subjected to dccc, followed by final purification of the residue (98 mg in fractions 8–13) by hplc (H_2O-MeOH, 7:3) to give three flavonol glycosides, namely, **2** (103 min, 55 mg), **1** (111 min, 24 mg), and **3** (122 min, 6 mg).

The known kaempferol-3-*O*-β-D-(2″,6″-di-α-L-rhamnopyranosyl)glucopyranoside [**1**], $[\alpha]^{24}D$ −79.6° (c=1.57, MeOH); kaempferol-3-*O*-β-D-(2″,6″-di-α-L-rhamnopyranosyl)galactopyranoside [**2**] (mauritianin), $[\alpha]^{24}D$ −100.0° (c=1.35, MeOH); and 3′-methoxykaempferol-3-*O*-β-D-(2″,6″-di-α-L-rhamnopyranosyl)-galactopyranoside [**3**], $[\alpha]^{21}D$ −20.4° (c=0.34, MeOH), were identified by comparison with literature data (3–6).

Alangiflavoside [**4**].—Yellow amorphous powder; $[\alpha]^{24}D$ −93.9° (c=0.61, MeOH); ir (KBr) ν max 3350, 2900, 1650, 1600, 1490, 1050 cm^{-1}; uv (MeOH) λ max δ (log ε) 209 (4.25), 245 (4.10), 266 (4.21), 347 (4.14) nm; (MeOH+AcONa) λ max (log ε) 210 (4.27), 245 (4.10), 266 (4.22), 347 (4.14) nm; (MeOH+MeONa) λ max (log ε) 212 (4.32), 250 (4.17), 268 (4.17), 389 (4.26) nm; (MeOH+$AlCl_3$) λ max (log ε) 211 (4.40), 275 (4.23), 302 (3.91), 354 (4.08), 400 (4.10) nm; ^{1}H nmr (CD_3OD) δ 0.99 (3H, d, J=6 Hz, H_3-6‴), 1.16 (3H, d, J=6 Hz, H_3-6⁗), 3.25 (1H, t, J=9 Hz, H-4⁗), 3.45–3.57 [8H, m, H-3″ (or 3⁗), 6″a, 4‴, 2⁗, 5‴, 2‴, 3⁗, 4⁗, and 5⁗], 3.65 (1H, t, J=6 Hz, H-5″), 3.70 (1H, dd, J=6 and 12 Hz, H-6⁗a), 3.70 (1H, dd, J=3 and 9 Hz, H-3‴ or 3″), 3.70 (1H, dd, J=5 and 10 Hz, H-6″b), 3.76 (1H, d, J=3 Hz, H-4″), 3.80 (1H, dd, J=3 and 10 Hz, H-3‴), 3.93 (1H, dd, J=2 and 12 Hz, H-6⁗b), 3.95 (1H, dd, J=8 and 10 Hz, H-2″), 4.00 (1H, dd, J=2 and 3 Hz, H-2‴), 4.07 (1H, qd, J=6 and 10 Hz, H-5‴), 4.52 (1H, br s, H-1‴), 5.08 (1H, d, J=7 Hz, H-1⁗), 5.22 (1H, d, J=1 Hz, H-1‴), 5.59 (1H, d, J=8 Hz, H-1″), 6.47 (1H, d, J=2 Hz, H-6), 6.75 (1H, d, J=2 Hz, H-8), 6.91 (2H, d, J=9 Hz, H-3′, H-5′), 8.10 (2H, d, J=9 Hz, H-2′, H-6′); ^{13}C-nmr data, see Table 1; negative-ion

TABLE 1. ^{13}C-Nmr Data for Alangiflavoside [**4**] (CD_3OD, 100 MHz).

Carbon	δ_c	Carbon	δ_c	Carbon	δ_c
2	158.0	1″	102.5	1‴″	100.9
3	134.8	2″	77.6	2‴″	74.8
4	179.6	3″	75.7	3‴″	78.4
5	162.8	4″	70.8	4‴″	71.3
6	100.9	5″	75.6	5‴″	77.9
7	164.5	6″	67.4	6‴″	62.5
8	95.8				
9	159.4	1‴,1‴″	101.5, 102.0		
10	107.7	2‴,2‴″	72.1, 72.3		
1′	122.9	3‴,3‴″	72.4, 72.4		
2′	132.4	4‴,4‴″	73.9, 74.1		
3′	116.3	5‴,5‴″	69.7, 69.9		
4′	161.5	6‴,6‴″	17.6, 18.0		
5′	116.3				
6′	132.4				

hrfabms found *m/z* 901.2580 $[M-H]^-$, $C_{39}H_{49}O_{24}$ requires 901.2614.

GLC ANALYSIS OF THE SUGAR PORTION.—About 2 mg of alangiflavoside [**4**] was treated with 5% HCl in MeOH at 95° for 3 h in a sealed tube. The reaction mixture was then neutralized by the addition of Ag_2CO_3. After filtering off the Ag_2CO_3, the solvent was evaporated and the residue dried *in vacuo*. The residue was then silylated with several drops of trimethylsilylimidazole at 60° for 15 min. H_2O and *n*-hexane (2 ml of each) were added, and then the separated *n*-hexane layer was evaporated and subjected to glc analysis (column temp. 160°). Alangiflavoside [**4**] (R_t data): rhamnose, 3.40 and 3.72 min; galactose, 5.70, 6.26, 6.58, and 8.21 min; glucose, 8.42 and 9.81 min. Standard sugars (R_t data): rhamnose, 3.39 and 3.71 min; galactose, 5.69, 6.26, 6.57, and 8.21 min; and glucose, 8.40 and 9.13 min.

ACETYLATION OF ALANGIFLAVOSIDE.—About 2 mg of alangiflavoside [**4**] were acetylated with 30 μl each of Ac_2O and pyridine at 50° for 12 h. The reagents were evaporated under a stream of N_2 with the occasional addition of MeOH, and the residue dried *in vacuo*. Tetradecaacetate, ^{1}H nmr ($CDCl_3$) δ 0.96 and 1.08 (3H each, each d, each J=6 Hz, H$_3$-6‴, H$_3$-6‴″), 1.92 (3H), 1.97 (3H), 2.02 (3H), 2.05 (3H), 2.068 (3H), 2.072 (3H), 2.073 (3H), 2.074 (3H), 2.081 (3H), 2.11 (6H), 2.14 (3H), 2.32 (3H), 2.48 (3H) (each s, CH_3CO-×14), 4.43 (1H, s), and 4.98 (1H, d, J=2 Hz) (H-1‴ and H-1‴″), 5.06 (1H, d, J=9 Hz, H-1‴″), 5.46 (1H, d, J=8 Hz, H-1″), 6.69 (1H, d, J=2 Hz, H-8), 7.01 (1H, d, J=2 Hz, H-6), 7.16 (2H, d, J=9 Hz, H-3′, H-5′), 8.06 (2H, d, J=9 Hz, H-2′, H-6′); fabms (*m*-nitrobenzyl alcohol) *m/z* 1491 $[M+H]^+$, 1513 $[M+Na]^+$ (+NaI), 1529 $[M+K]^+$ (+KI); eims *m/z* 1448 $[M-CH_2=C=O]^+$ (0.5), 791 [gal(OAc)$_2$rha(OAc)$_3$rha(OAc)$_3$ oxonium ion]$^+$ (8), 547 (9.1), 331 [glc(OAc)$_4$ oxonium ion]$^+$ (42), 286 (58), 273 [rha(OAc)$_3$ oxonium ion]$^+$ (100), 169 (53), 153 (56).

ENZYMATIC HYDROLYSIS OF ALANGIFLAVOSIDE [**4**] TO MAURITIANIN [**2**].—Alangiflavoside (15 mg) was incubated with 100 mg of β-glucosidase (emulsin) at 37° for 7 h. The reaction mixture was concentrated and purified by dccc to yield 8.4 mg (68%) of **2**, a yellow amorphous powder, $[\alpha]^{21}_D$ −88.0° (c=0.56, MeOH). Other spectroscopic data were indistinguishable from those of **2** (4).

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