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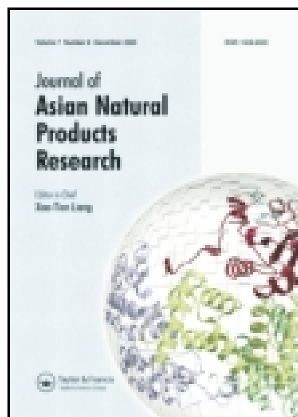

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A new flavonol glucoside from the aerial parts of Sida glutinosa

Niranjan Das [a] , Basudev Achari [b] , Yoshihiro Harigaya [c] & Biswanath Dinda [d]

[a] Department of Chemistry, Netaji Subhash Mahavidyalaya, Udaipur, 799120, South Tripura, India

[b] Department of Medicinal Chemistry, Indian Institute of Chemical Biology, Kolkata, 700032, India

[c] School of Pharmaceutical Sciences, Kitasato University, 5-9-1, Shirokane, Minato- ku, Tokyo, 108-8641, Japan

[d] Department of Chemistry, Tripura University, Suryamaninagar, Agartala, 799130, India

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A new flavonol glucoside from the aerial parts of *Sida glutinosa*

Niranjan Das[a], Basudev Achari[b], Yoshihiro Harigaya[c] and Biswanath Dinda[d]*

[a]*Department of Chemistry, Netaji Subhash Mahavidyalaya, Udaipur 799120, South Tripura, India;* [b]*Department of Medicinal Chemistry, Indian Institute of Chemical Biology, Kolkata 700032, India;* [c]*School of Pharmaceutical Sciences, Kitasato University, 5-9-1, Shirokane, Minato- ku, Tokyo 108-8641, Japan;* [d]*Department of Chemistry, Tripura University, Suryamaninagar, Agartala 799130, India*

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Phytochemical investigation on the dried aerial parts of *Sida glutinosa* has led to the isolation of a new flavonol glucoside, glutinoside (**1**), along with seven known compounds, 24(28)-dehydromakisterone A (**2**), 1,2,3,9-tetrahydropyrrolo[2,1-b]-quinazolin-3-amine (**3**), docosanoic acid, 1-triacontanol, campesterol, stigmasterol, and β-sitosterol. The structures of these compounds were elucidated by means of extensive spectroscopic techniques as well as GC/MS analysis (for sterols) and comparison with the literature data. All these seven known compounds are reported from this plant for the first time.

Keywords: *Sida glutinosa*; Malvaceae; flavonol glucoside; glutinoside

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1. Introduction

Sida glutinosa Roxb. syn *S. mysorensis* Wight & Arn. (Malvaceae) is an annual herb mostly distributed in waste places of south and eastern India, Burma to south-east Asia. In India, the roots and aerial parts of this plant and its sister species are used in traditional medicine for the treatment of pulmonary tuberculosis and rheumatism [1,2]. A literature survey indicated that no phytochemical work was reported on this plant. Phytochemical work on *n*-BuOH and CH_2Cl_2 soluble fractions of a methanol extract of *S. glutinosa* aerial parts led to the isolation of one new compound, glutinoside (**1**), and seven known compounds, 24(28)-dehydromakisterone A (**2**), 1,2,3,9-tetrahydropyrrolo[2,1-b]-quinazolin-3-amine (**3**), docosanoic acid, 1-triacontanol, campesterol, stigmasterol, and β-sitosterol. The structures of these compounds were elucidated by means of NMR (2D), MS, and other spectroscopic techniques, as well as comparison with the literature data.

2. Results and discussion

Air-dried aerial parts of *S. glutinosa* were extracted with MeOH at room temperature. The resulting extract was concentrated and suspended in water. The water layer was extracted with CH_2Cl_2, $CHCl_3$, and *n*-BuOH, successively. One part of *n*-BuOH soluble fraction was subjected to Diaion HP-20, silica gel, and basic alumina (Al_2O_3) chromatographies to get glutinoside (**1**) (Figure 1) and 24(28)-dehydromakisterone A (**2**). The other part of *n*-BuOH soluble fraction was churned with citric acid, and the aqueous filtrate was basified with NH_4OH and extracted

*Corresponding author. Email: dindabtu@gmail.com

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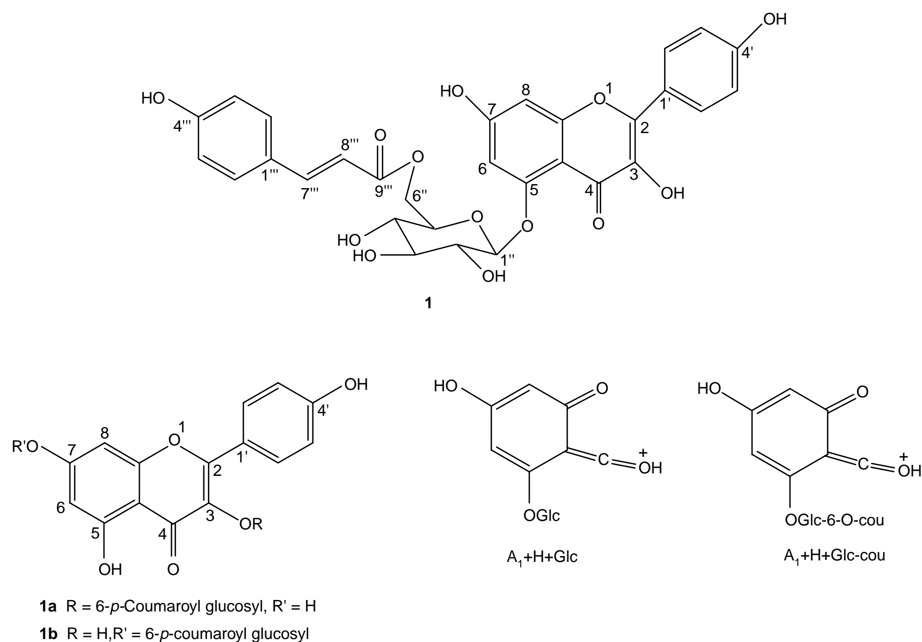

Figure 1. Structures of compounds **1**, **1a**, **1b**, and fragment ions in FAB-MS.

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with *n*-BuOH. The *n*-BuOH soluble part on silica gel chromatography afforded alkaloid (**3**). The CH_2Cl_2 soluble fraction on silica gel chromatography gave the aliphatics and sterols.

Glutinoside (**1**) was isolated as yellow crystals with mp 225°C. Its molecular formula was established as $C_{30}H_{26}O_{13}$ by observing pseudomolecular ion peak at *m/z* 617.1261 $[M + Na]^+$ in its HR-FAB-MS, in conjugation with ^1H and ^{13}C NMR spectral data. It showed UV absorption maxima in MeOH at 255, 322 sh, and 372 nm, typical of 3,4′,5,7-tetrahydroxy-flavone derivative [3]. The IR spectrum showed absorption bands for hydroxyl (3460 cm^{-1}), unsaturated ester (1695 and 1248 cm^{-1}), α,β-unsaturated ketone (1684 and 1655 cm^{-1}), aromatic ring (1628, 1607, and 1589 cm^{-1}), and glycoside linkage (3252 and 1067 cm^{-1}). The ^1H NMR spectrum (Table 1) showed the signals for a coumaroyl unit [δ 7.32 and 6.80 (each 2H, d, $J = 8.4$ Hz), 7.40 and 6.07 (each 1H, d, $J = 15.6$ Hz)], a β-glucopyranosyl moiety [δ 5.25 (1H, d, $J = 7.2$ Hz), 4.19 (1H, dd, $J = 11.4$, 6.6 Hz), 4.30 (1H, dd, $J = 11.4$, 1.5 Hz), and four methine protons in δ 3.19–3.47], and a kaempferol moiety [δ 6.14 and 6.32 (each 1H, d, $J = 1.8$ Hz), δ 8.00 and 6.83 (each 2H, d, $J = 8.4$ Hz)], suggesting that its flavonol glucoside structure coupled with a coumaroyl unit. The ^{13}C/DEPT-NMR spectra in CD_3OD displayed 30 carbon signals (Table 1), including two carbonyl carbons (δ 169.9 and 166.1), 22 aromatic and olefinic carbons (δ 95.0–163.2), and 6 sugar carbons (δ 104.0, 64.4–78.1). Among aromatic and olefinic carbons, two were assigned to olefinic methines at δ 114.9 and 146.7, six to aromatic methines, and the rest were quaternary carbons. These carbon resonances also supported the presence of one kaempferol, one glucose, and one coumaroyl moieties in the molecule [4,5]. The FAB-MS recorded a base ion at *m/z* 287 $[aglycone + H]^+$ corresponding to the molecular formula $C_{15}H_{10}O_6$ for the aglycone (kaempferol). In addition, strong mass ions at *m/z* 147 $[coumaroyl]^+$ and

Table 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectral data of **1** in CD_3OD (δ in ppm).

Position (H/C)	δ_H[a]	δ_C[b]	Position (H/C)	δ_H[a]	δ_C[b]
2	–	147.3	1″	5.25 (1H, d, $J = 7.2$)	104.0
3	–	135.3	2″	3.19 (1H, m)	76.0
4	–	169.9	3″	3.45 (1H, t, $J = 9.5$)	78.1
5	–	158.6	4″	3.34 (1H, m)[c]	71.9
6	6.14 (1H, d, $J = 1.8$)	100.1	5″	3.47 (1H, m)	75.9
7	–	163.2	6″	4.19 (1H, dd, $J = 11.4, 6.6$) 4.30 (1H, dd, $J = 11.4, 1.5$)	64.4
8	6.32 (1H, d, $J = 1.8$)	95.0	1‴	–	127.2
9	–	159.5	2‴, 6‴	7.32 (2H, d, $J = 8.4$)	131.4
10	–	105.8	3‴, 5‴	6.80 (2H, d, $J = 8.4$)	116.9
1′	–	122.9	4‴	–	161.7
2′, 6′	8.00 d (2H, d, $J = 8.4$)	132.4	7‴	7.40 (1H, d, $J = 15.6$)	146.7
3′, 5′	6.83 (2H, d, $J = 8.4$)	116.2	8‴	6.07 (1H, d, $J = 15.6$)	114.9
4′	–	161.4	9‴	–	166.1
7-OH	10.35 (1H, brs)[d]				

[a] Coupling constant (J) values in Hz in parentheses.
[b] Multiplicities were determined by DEPT.
[c] Multiplicity was not cleared due to overlapping with solvent peak.
[d] Observed in C_5D_5N.

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165 [coumaric acid + H]$^+$ supported the presence of a coumaroyl unit. The FAB-MS also recorded a mass ion at *m/z* 309 indicating the presence of coumaroyl glucose unit in the molecule. The additional mass ions at *m/z* 461 [A_1 + H + glucosyl coumaroyl]$^+$, 315 [A_1 + H + glucosyl]$^+$, and 299 [315–16]$^+$ indicated that coumaroyl glucose moiety was attached at ring A of the flavone. The above evidence revealed that **1** was 3,4′,5,7-tetrahydroxyflavone (kaempferol) with one glucose and one (*E*)-*p*-coumaroyl residues [5]. The acid hydrolysis of **1** afforded kaempferol, glucose and (*E*)-*p*-coumaric acid. The glucose was found to be D-series on the basis of its positive rotation as well as by comparing the retention time of the acetylated derivative of (+) 2-octyl glucoside with that of acetylated derivative (+) 2-octyl-D-glucoside [6]. The attachment of coumaroyl unit to C-6″ position of glucose moiety was assigned on the basis of downfield chemical shift of C-6″ carbon (δ_C 64.4) of glucose and upfield shift of coumaroyl ester carbonyl (δ_C 166.1). The attachment of glucose moiety to the hydroxyl group at the C-5 position of flavonol unit was deduced from the observation of a long-range HMBC correlation between the glucosyl anomeric proton H-1″ at δ_H 5.25 with C-5 at δ_C 158.6. The HMBC correlation of H-6″ at δ_H 4.30 with C-9‴ at δ_C 166.1 also supported the attachment of coumaroyl unit at C-6″ position of glucose unit (Figure 2). Moreover, the downfield resonance of anomeric carbon (δ_C 104.0) and the upfield resonances of anomeric proton (δ_H 5.25) and C-4, C-5, C-7, and C-9 carbons also corroborated this assignment [7]. The spectral data of this compound were very similar to that of tiliroside (**1a**) and buddlenoid A (**1b**) having the 6-*p*-coumaroyl-glucosyl group at C-3 and C-7 positions, respectively (Figure 1) [8,9]. The major difference in ^{13}C NMR spectra between this compound and **1a** or **1b** is the upfield chemical shift of C-4 at δ 169.9 and the downfield chemical shift of C-9 at δ 159.5. Possibly, C-5 glucosylation is responsible for this

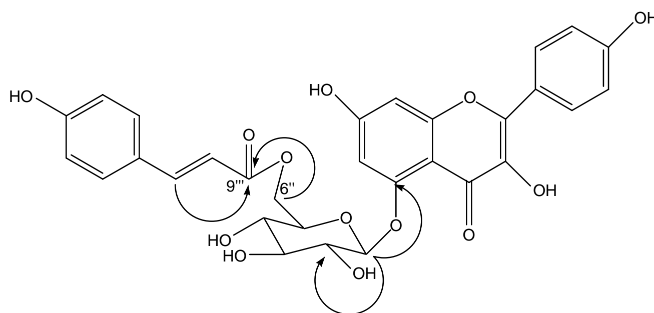

Figure 2. Key HMBC correlations of **1**.

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difference. Moreover, ^{1}H NMR spectrum of glutinoside in pyridine-d_5 gave a distinct hydroxyl signal at δ 10.35 (brs) for C-7 hydroxyl group. This indicated that C-5 hydroxyl group is not free. The hypsochromic shift of both bands I and II in UV spectrum at 248 nm (band II) and 353 nm (band I) on addition of NaOAc in MeOH solution of the compound indicated that C-5 hydroxyl is substituted. On the basis of the above evidence, the structure of glutinoside was elucidated as kaempferol-5-*O*-β-D-(6″-*O*-*trans*-coumaroyl)-glucopyranoside (**1**).

24(28)-Dehydromakisterone A (**2**) was isolated as light yellow crystals with mp 220°C, and was characterized by comparing its spectral data with the literature [10–12]. The detailed ^{1}H NMR and FAB-MS were not reported in the literature and hence are given in the experimental.

Alkaloid **3** was obtained as brown amorphous powder with mp 188–190°C from the basic butanol fraction of methanol extract by column chromatography (CC) through silica gel. It was identified as 1,2,3,9-tetrahydropyrrolo[2,1-b]-quinazolin-3-amine by comparing its spectral data with the literature [13–17]. The detailed ^{1}H NMR and EI-MS were not reported in the literature and, therefore, these are provided in the experimental.

From the petroleum ether extract, we have also isolated 1-triacontanol [18], $CH_3(CH_2)_{29}OH$, (M^+438), mp 86°C, docosanoic acid (behenic acid) [18], $CH_3(CH_2)_{20}COOH$, (M^+340) mp 82°C, and a sterol mixture, mp 110–113°C. The sterol mixture on GC/MS/MS analysis afforded three components, and comparison of the retention times and mass peaks of the components with the literature data revealed that these were β-sitosterol (68.2%), campesterol (16.6%), and stigmasterol (15.2%) [19].

3. Experimental

3.1 General experimental procedures

Melting points were determined by the use of Kofler-type electrical melting point apparatus and are uncorrected. UV spectra were recorded in MeOH on a PerkinElmer Lambda 25 spectrometer and IR spectra in KBr disk on a Shimadzu 8100 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were measured on a Varian 300, 400, and 600 spectrometer. Chemical shifts are given in δ values (ppm) relative to tetramethylsilane as an internal standard. EI-MS and FAB-MS were obtained by JEOL JMS 700 spectrometer. GC/MS/MS was carried out on a Hewlett-Packard 5890 GC interfaced with a triple-quadrupole mass spectrometer using DB-5 column. GC analysis of sugar was carried out on a Chemito GC using SP-1000 glass column. For CC, silica gel (mesh 60–120, Merck, Mumbai, India), Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan), and basic alumina (Al_2O_3) (Merck) were used. Silica gel G (Merck) was used for TLC.

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3.2 Plant material

The whole aerial parts of *S. glutinosa* were collected from Kalsi (Jolaibari), South Tripura in March 2008. The plant was identified by Dr B.K. Datta, taxonomist, Department of Botany, Tripura University. A voucher specimen (#BD/01/08) has been deposited in the National Herbarium, Botanical Survey of India, Botanical Garden, Howrah 711103.

3.3 Extraction and isolation

Air-dried powdered aerial parts of *S. glutinosa* (3.3 kg) were extracted three times with MeOH (6 l × 3) at room temperature for 1 week. The MeOH extract was concentrated under reduced pressure *in vacuo* to a gummy mass (106 g). The residue (90 g) was suspended in water (100 ml) and extracted three times with CH_2Cl_2, $CHCl_3$, and *n*-BuOH, successively.

One part of *n*-BuOH soluble fraction (10 g) was subjected to Diaion HP-20 CC and eluted with a stepwise gradient of H_2O/MeOH (100:0, 75:25, 50:50, 25:75, and 0:100) and 250 ml fraction being collected. The residues obtained from H_2O/MeOH (75:25 and 50:50) were mixed together, and were subjected to silica gel CC using CH_2Cl_2/$CHCl_3$ (different ratios) as eluates. The fractions eluted with $CHCl_3$ and $CHCl_3$/MeOH were further subjected to basic alumina CC, and the column was eluted with EtOAc and EtOAc/MeOH (95:5, 90:10, 80:20, 60:40, and 50:50), 250 ml each fraction being collected. EtOAc/MeOH (90:10) fraction gave a solid which on crystallization from $CHCl_3$/MeOH (80:20) gave 24(28)-dehydromakisterone A (**2**, 45 mg). The residue obtained from EtOAc/MeOH (50:50) fraction on crystallization from MeOH gave glutinoside (**1**, 35 mg).

Another part of *n*-BuOH soluble fraction (8 g) was churned with 5% aqueous citric acid for 6 h, and the aqueous filtrate was basified with dilute NH_4OH (~2 N) and extracted with *n*-BuOH. The *n*-BuOH soluble part was applied to silica gel CC and eluted with $CHCl_3$ and $CHCl_3$/MeOH (different ratios). The $CHCl_3$/MeOH (30:70) afforded alkaloid **3** (25 mg).

The CH_2Cl_2 soluble fraction (5 g) on silica gel CC afforded 1-triacontanol (25 mg), docosanoic acid (15 mg), and sterol mixture (50 mg).

3.3.1 *Glutinoside (kaempferol-5-*O*-β-*D*-(6″-*O-trans-*coumaroyl)-glucopyranoside,* **1***)*

Yellow crystals, mp 225°C. $[\alpha]_D^{24} -19.5$ ($c = 0.32$, MeOH). UV (MeOH) λ_{max} (nm): 255 (4.08), 322 sh (3.68), 372 (4.18). UV (+ NaOAc) λ_{max} (nm): 248, 321, 353 nm; IR (KBr) υ_{max} (cm^{-1}): 3460, 3252, 1695, 1684, 1655, 1628, 1607, 1589, 1501, 1360, 1294, 1182, 1067, 827; ^1H and ^{13}C NMR spectral data are given in Table 1. FAB-MS *m/z* (relative intensity): 617 $[M + Na]^+$(38), 595 $[M + H]^+$(13), 461 (4), 315 (9), 299 (14), 287 [aglycone + H]$^+$(100), 286 [aglycone]$^+$(72), 259 (16), 165 (28), 147 (75), 107 (50), 77 (59), 65 (22). HR-FAB-MS *m/z* (positive ion): 617.1261 $[M + Na]^+$ (calcd for $C_{30}H_{26}O_{13}Na$, 617.1266).

3.3.2 *24(28)-Dehydromakisterone A (***2***)*

Light yellow crystals, mp 220°C. UV (MeOH) λ_{max} (nm): 248 (3.61); ^1H NMR (300 MHz, CD_3OD): δ 1.78 (1H, brd, $J = 13.0$ Hz, H-1α), 1.46 (1H, brd, $J = 13.0$ Hz, H-1β), 3.85 (1H, ddd, $J = 11.5$, 4.0, 3.0 Hz, H-2α), 3.97 (1H, brq, $J = 2.5$ Hz, H-3α), 1.78 (1H, brs, H-4α), 1.73 (1H, brs, H-4β), 2.40 (1H, dd, $J = 12.5$, 5.0 Hz, H-5), 5.83 (1H, brs, H-7), 3.17 (1H, t-like, $J = 7.0$ Hz, H-9), 1.63 (1H, dd, $J = 11.0$, 7.5 Hz, H-11α), 1.82 (1H, brs, H-11β), 2.10 (1H, ddd, $J = 11.5$, 11.5, 4.5 Hz, H-12α), 1.88 (1H, brd, $J = 11.5$ Hz, H-12β), 1.59 (1H, m, H-15α), 2.02 (1H, m, H-15β), 1.73 (1H, brs,

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H-16), 1.97 (1H, m, H-16), 0.91 (3H, s, H_3-18), 0.98 (3H, s, H_3-19), 1.25 (3H, s, H_3-21), 3.61 (1H, brd, $J = 10.5$ Hz, H-22), 2.39 (1H, brd, $J = 13.5$ Hz, H-23), 2.14 (1H, dd, $J = 13.5$, 10.0 Hz, H-23), 1.39 (3H, s, H_3-26). 1.33 (3H, s, H_3-27), 5.15 (1H, brs, H-28), 4.97 (1H, brs, H-28). FAB-MS *m/z* (relative intensity): 515 $[M + Na]^+$(60), 493 $[M + H]^+$(53), 475 (60), 457 (100), 439 (40), 363 (33), 345 (35), 327 (13), 301 (33), 191 (27), 173 (47), 165 (47), 147 (33), 129 (40). HR-FAB-MS *m/z* (positive ion): 515.2998 $[M + Na]^+$ (calcd for $C_{28}H_{44}O_7Na$, 515.2984).

3.3.3 *Alkaloid (1,2,3,9-tetrahydropyrrolo [2,1-b]-quinazolin-3-amine,* ***3****)*

Amorphous powder, mp 188–190°C $[\alpha]_D^{20} -115.2°(c = 0.15$, MeOH). UV (MeOH) λ_{max} (nm): 226.11 (3.99) and 299.28 (3.88). ^1H NMR (400 MHz, CD_3OD): 3.27 (1H, ddd, $J = 12.5$, 7.0, 7.0 Hz, H_{ax}-1), 3.41 (1H, ddd, $J = 12.5$, 7.0, 4.0 Hz, H_{eq}-1), 1.93 (1H, dddd, $J = 13.0$, 8.0, 7.0, 7.0 Hz, H_{ax}-2), 2.37 (1H, dddd, $J = 13.0$, 7.0, 7.0, 4.0 Hz, H_{eq}-2), 4.58 (1H, dd, $J = 7.0$, 7.0 Hz, H-3), 6.99 (1H, dd, $J = 8.0$, 1.5 Hz, H-5), 7.13 (1H, ddd, $J = 8.0$, 7.5, 2.0 Hz, H-6), 6.94 (1H, brdd, $J = 8.0$, 7.5 Hz, H-7), 6.97 (1H, brdd, $J = 8.0$, 2.0 Hz, H-8), 4.61 (2H, brs, H_2-9); EI-MS *m/z* (relative intensity): 188 $[M + H]^+$(16), 187 $[M]^+$(100), 170 $[M-NH_3]^+$(75), 159 $[M-C_2H_4]^+$(90), 131 (92), 104 (77), 89 (74), 77 (80), 51 (31). HR-EI-MS *m/z* (positive ion): 188.1172 $[M + H]^+$ (calcd for $C_{11}H_{14}N_3$, 188.1188).

3.3.4 *Sterol mixture*

White solid, mp 110–113°C; GC/MS analysis: DB-5 column, using He gas and the following temperature conditions: isothermal 250°C, injector and separator temperatures 270°C; cholesterol was used as an internal standard.

3.4 Acid hydrolysis of glutinoside (1)

A solution of **1** (5.0 mg) in 2M HCl in MeOH (3 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was extracted with EtOAc. Kaempferol and *E-p*-coumaric acid were detected by TLC in the concentrated EtOAc extract. The aqueous layer was neutralized with Amberlite IRA-400 (OH form), and the resin was removed by filtration. The filtrate was concentrated. D-glucose was detected in the filtrate by co-TLC with standard sugar samples using silica gel with EtOAc–H_2O (3:1) and $CHCl_3$–MeOH–H_2O (55:45:10) and optical rotation study ($[\alpha]_D^{23} + 52.5$, H_2O).

3.5 GC analysis of sugar obtained from compound 1

A solution of sugar (0.25 mg) in (+) 2-octanol (1 ml) containing two drops of CF_3CO_2H was taken in an ampule and heated in an oil bath at 130°C for 12 h. The resulting mixture was concentrated to dryness *in vacuo*. The residue was dissolved in Ac_2O–pyridine (1:1, 0.8 ml) and kept at 100°C for 15 min, and then the solvent and the excess reagent were removed by concentration followed by distillation with anhydrous ethanol. The residue was dissolved in $CHCl_3$ and injected in a Chemito gas chromatograph having FID system and SP-1000 glass column. The carrier gas (N_2) flow rate was maintained at 0.5 ml per min and the temperature of injection port, column, and FID was 250, 230, and 280°C, respectively. The retention time was 8.02 min. It was almost the same as that of acetylated derivative of (+)-2-octyl-D-glucoside when run under identical conditions.

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