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Three New Flavonol C-Glycosides from *Sida cordifolia* Linn

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Three new flavonol C-glycosides: 3'-(3",7"-dimethyl-2",6"-octadiene)-8-C-β-D-glucosyl-kaempferol 3-O-β-D-glucoside (**1**), 3'-(3",7"-dimethyl-2",6"-octadiene)-8-C-β-D-glucosyl-kaempferol 3-O-β-D-glucosyl [1→4]-α-D-glucoside (**2**) and 6-(3"-methyl-2"-butene)-3'-methoxyl-8-C-β-D-glucosyl-kaempferol 3-O-β-D-glucosyl [1→4]-β-D-glucoside (**3**) have been isolated from 80% ethanolic extract of the aerial parts of *Sida cordifolia* Linn followed by partitioning with ethyl acetate. Structures were established by chemical and spectroscopic methods.

Keywords: Flavonol C-glycosides, *Sida cordifolia* Linn, Extracts, Characterization

INTRODUCTION

Sida cordifolia Linn is a herb belonging to the family Malvaceae. It grows to a height of ~3-5 feet and is extensively used as a common herbal drug in the Indian subcontinent. The roots, leaves, stems and seeds of *Sida cordifolia* Linn are used as traditional medicine against chronic dysentery, asthma and gonorrhea [1,2] in the subcontinent. The aqueous extract of the whole plant is specially used in treatment of rheumatism [2]. Earlier phytochemical investigations on the roots have shown the presence of ephedrine, vasicine, vasicinol, vasicinone and N-methyl tryptophan [3,4,5,6]. We report herein the isolation and characterization of three new flavonol C-glycosides (**1-3**) from the aerial parts of *Sida cordifolia* Linn. The isolation of the C-glycosides is being reported for the first time from the plant.

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EXPERIMENTAL

Apparatus

The UV and IR spectra were recorded on a Shimadzu UV-Vis spectrophotometer, Model UV 1601 PC and Shimadzu FTIR Model-8400, respectively. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker BPX-200 spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR spectra. The Chemical shift values are reported in ppm, relative to tetramethylsilane. The FAB mass spectra were obtained on a Varian MAT CH_5 instrument. The NMR and mass spectra were recorded in the Department of Chemistry of Ruhr-Universitat, Bochum, Germany. Column chromatography was performed on silica gel (200-400 mesh). Paper chromatography (PC) was done on Whatman no. 1 filter paper.

Plant Material

The aerial parts of *Sida cordifolia* Linn were collected

from the hilly region of the district of Chittagong situated in the south-eastern region of Bangladesh. For the experimental purposes, only the matured, sound, fresh and flowering plants were collected during the month of November 2002. The plant was identified in Bangladesh National Herbarium (BNH) by Mrs. B. Begum and a voucher specimen (Herbarium accession No. 31238) was deposited at BNH.

Extraction and Isolation

Air-dried plant material *Sida cordifolia* Linn (5.5 Kg) was successively extracted with $CHCl_3$ (3 × 72 h), MeOH (3 × 72 h) and 80% EtOH (3 × 72 h) respectively. The EtOH extract was concentrated to ~1.00 l under reduced pressure at a temperature < 40 ºC. The concentrated extract was then partitioned successively with *n*-hexane, DCM, EtOAc and *n*-BuOH. The EtOAc extract yielded a yellowish mass (5 g) on removal of the solvent. The extract was adsorbed onto silica gel and placed over a column of silica gel and eluted with $CHCl_3$-EtOAc-MeOH (1:1:1). The eluents were monitored by TLC and divided into three fractions. Fraction 1 (1-15) gave a pure compound **1** (50 mg). Fraction 2 (16-50) was a mixture of two major compounds. On repeated fractional crystallization from EtOAc-EtOH, two pure compounds **2** (60 mg) and **3** (75 mg) were isolated from fraction 2. Fraction 3 (51-70) gave a pure compound (80 mg) and was similar to the compound **3**.

Compound 1. m.p.: 226-228 ºC. UV λ_{max} (MeOH) nm: 268, 308, 330; +$AlCl_3$: 276, 305, 330; + $AlCl_3$/HCl: 279, 307, 330; NaOMe: 270, 321, 333, 349, 365, 377; NaOAc: 281, 310, 330. IR ν_{max} (KBr) cm^{-1}: 3397 (OH), 1652 (α,β-unsaturated ketone), 1602, 1260, 1076. ^{1}H NMR and ^{13} C NMR (DMSO-d_6): Table 1. FAB mass (positive ion mode): m/z (%) = 747.1 (5.2) $[M+H]^+$ ($C_{37}H_{46}O_{16}$), 585.2 (30.3) $[M-162+H]^+$, 465.1(12.5) $[M-162-120+H]^+$, 401.1 (90.1), 391.1 (48.2), 315.1(5.5), 273.3.1 (6.3), 257.2 (100), 239.2 (10.2), 193.4 (22.4), 185.0 (62.4), 163.1 (26.1), 95.1 (40.2).

Acid hydrolysis of 1. Compound **1** (30 mg) in a mixture of 5% HCl (3 ml) and MeOH (20 ml) was refluxed for 2 h. The reaction mixture was neutralized with 10% NaOH and extracted with EtOAc. On removal of the solvent, EtOAc extract yielded the aglycon $\mathbf{D_1}$ (18 mg). The aqueous part was concentrated to one fourth of its volume under reduced pressure. Examination of aqueous part on PC showed one spot corresponding to glucose. The aglycon $\mathbf{D_1}$ was obtained as light yellow amorphous solid, m.p.: 212-214 ºC. UV λ_{max} (MeOH) nm: 266, 302. IR ν_{max} (KBr) cm^{-1}: 3414 (OH), 1684 (α,β-unsaturated ketone), 1603, 1458 (aromatic ring). ^{1}H NMR and ^{13} C NMR (CD_3OD): Table 2. FAB mass (positive ion mode): m/z (%) = 585.2 (2.5) $[M_1+H]^+$ ($C_{31}H_{36}O_{11}$), 465.1 (7.2) $[M_1-120+H]^+$, 391.3 (38.4), 371.2 (24.4), 315.1 (28.1), 285.2 (42.2), 273.1 (15.2), 257.2 (100), 239.2 (30.1), 193.1 (34.5), 149.0 (28.1), 119.1 (23.1), 95.1 (46.1).

Compound 2. An amorphous yellow solid, melted at 261-262 ºC. UV λ_{max} (MeOH) nm: 272, 282, 330; +$AlCl_3$: 278, 308, 360; +$AlCl_3$/HCl: 279, 307, 330; NaOMe: 270, 321, 333, 349, 365, 377; NaOAc: 281, 310, 330. IR ν_{max} (KBr) cm^{-1}: 3550 (OH), 1625 (α,β-unsaturated ketone), 1601, 1514, 1269. ^{1}H NMR and ^{13}C NMR (DMSO-d_6): Table 1. FAB mass (positive ion mode): m/z (%) = 909.3 (4.1) $[M+H]^+$ ($C_{43}H_{56}O_{21}$), 747.1 (8.5) $[M-162+H]^+$, 585.2 (15.2) $[M-2\times162+H]^+$, 465.2 (32.1) [M-2×162-120+H], 401.1 (50.1), 391.4 (42.2), 325.2 (12.5), 315.3 (7.1), 273.1 (8.4), 257.1 (100), 235.5 (12.3), 163 (28.4).

Acid hydrolysis of 2. Compound **2** (30 mg) was dissolved in 5% HCl-MeOH and refluxed for 2 h. The mixture was then neutralized with 10% NaOH solution and extracted with EtOAc. Aglycon $\mathbf{D_1}$ (15 mg) was obtained from organic layer. Paper chromatographic analysis showed the presence of D-glucose and maltose in the aqueous part. The aqueous part was then subjected to further hydrolysis with 5% HCl-MeOH for 8 h. On examination on PC the resulting product showed only one spot corresponding to D-glucose. The aglycon obtained from compound **2** had similar m.p., UV, IR, ^{1}H NMR, ^{13}C NMR and FAB mass as $\mathbf{D_1}$.

Compound 3. Amorphous yellow solid, melted at 235-236 ºC. UV λ_{max} (MeOH) nm: 290, 305, 325; +$AlCl_3$: 279, 314, 335; +$AlCl_3$/HCl: 281, 307, 335; NaOMe: 284, 330, 356; NaOAc: 282, 305, 330. IR ν_{max} (KBr) cm^{-1}: 3550 (OH), 1668 (α,β-unsaturated ketone), 1585, 1445, 1269, 1170, 1124. ^{1}H NMR and ^{13}C NMR (DMSO-d_6): Table 1. FAB mass (positive ion mode): m/z (%) = 871.1 (3.1) $[M+H]^+$ ($C_{39}H_{50}O_{22}$), 709.3 (15.2) $[M-162+H]^+$, 547.1(24.1) $[M-2\times162+H]^+$, 427.2 (16.2) $[M-2\times162-120+H]^+$, 391.2 (16.3), 383.3 (16.4), 325.2, (22.5), 307.2 (4.5), 279.2 (7.3), 259.1 (6.1), 219.1 (36.5), 203.2 (8.4), 167.3 (28.3), 163.1(35.1), 149.0 (100), 91.0 (48.5).

Acid hydrolysis of 3. Compound **3** (30 mg) was refluxed in 5% HCl-MeOH for 2 h. The mixture was then neutralized

with 10% NaOH. The resulting mixture was extracted with EtOAc. Aglycon **D_2** (20 mg) was obtained from organic layer. The aqueous part was concentrated to one fourth of its volume and chromatographed on paper against reference sugar. The sugar component was identified as disaccharide. The aqueous part was further refluxed with 5% HCl-MeOH for 8 h and then worked up in the usual way. Examination on PC showed that the disaccharide remains unchanged. The disaccharide was then recovered from the aqueous part by preparative paper chromatography (PPC) and identified as cellobiose, m.p.: 249-250 ºC. The aglycon **D_2** was a light yellow amorphous solid, melted at 205-206 ºC. UV λ_{max} (MeOH) nm: 287, 302, 321; IR ν_{max} (KBr) cm^{-1}: 3384 (OH), 1682 (α,β-unsaturated ketone), 1603, 1452, (aromatic ring). ^1H NMR and ^{13}C NMR (CD_3OD): Table 2. FAB mass (positive ion mode): m/z (%) = 547.1 (2.5) $[M_1+H]^+$ ($C_{27}H_{30}O_{12}$), 427.1 (3.1) $[M_1-120+H]^+$, 391.2 (50.2), 329.1 (7.5), 307.2 (16.2), 279.1 (17.1), 259.1 (15.2), 219.1 (17.1), 203.1 (12.3), 167.0 (17.1), 163.2 (7.3), 149.0 (100), 91.1 (40.2).

RESULTS AND DISCUSSION

Compound **1** gave positive color reactions for flavonols [7]. It gave positive test for sugar [8] (Phenol-sulphuric acid). It also produced copious foams on shaking with water suggesting it to be a glycoside. Acid hydrolysis of compound **1** gave aglycon **D_1** and glucose. Compound **1** showed $[M+H]^+$ at m/z 747.1 in its FAB mass corresponding to molecular formula $C_{37}H_{46}O_{16}$. This is consistent with the FAB mass of aglycon **D_1** $[M_1+H]^+$ ($C_{31}H_{36}O_{11}$) at m/z 585.2 and its other mass fragments (Fig. 1). ^1H NMR and ^{13}C NMR spectra of compound **1** (Table 1) and aglycon **D_1** (Table 2) further supported the molecular formula $C_{37}H_{46}O_{16}$ for compound **1**.

The UV spectrum of compound **1** in methanol (λ_{max} 268, 308, 330 nm) and its changes after addition of shift reagents suggested the presence of free hydroxyl groups at C_5 and C_7 [9,10,11]. The IR spectrum of the compound showed intense absorption for O-H stretching at 3397 cm^{-1}. It also showed strong absorption at 1668 cm^{-1} for α,β-unsaturated ketone. ^1H NMR spectrum of compound **1** showed one proton singlet at δ 6.74 s (Table 1) indicating that ring A was trisubstituted [9]. The absence of an aromatic methine carbon signal in the ^{13}C NMR spectrum within the range 90.0-96.0 ppm suggested that

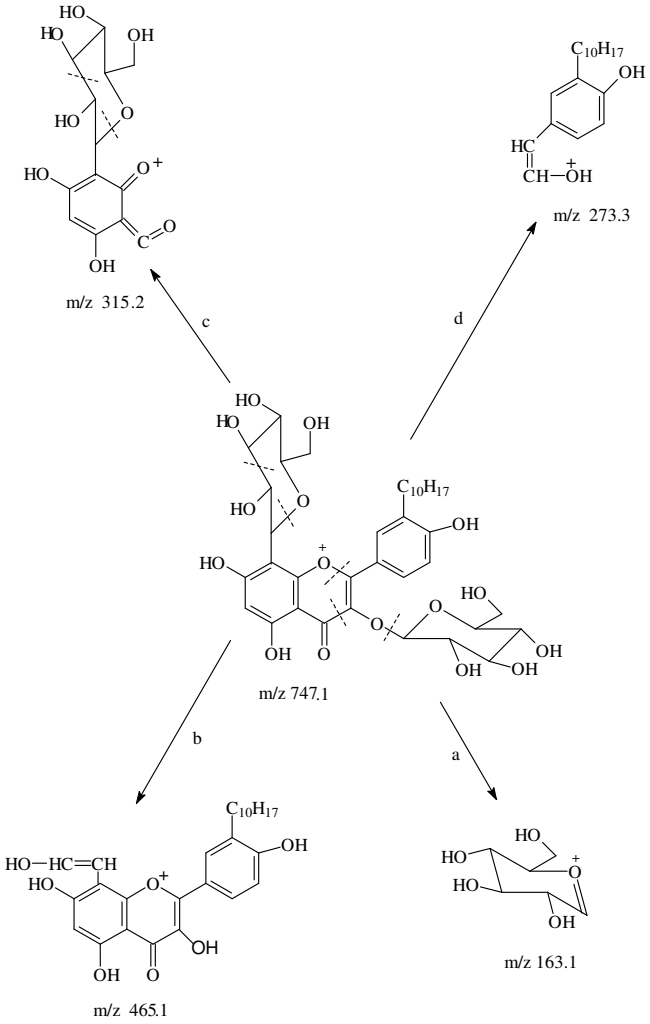


Fig. 1. Fragmentation of compound **1** during FAB.

C_8 of ring A is substituted [9]. Thus ring A is substituted at 5, 7 and 8 positions. Ring B showed a pattern of three one-proton signals at δ 7.25 d (C'_2-H), 6.84 d (C'_5-H) and 7.45 d, d (C'_6-H). Protons C'_2-H and C'_5-H appeared as doublets with J = 1.2 and 6.2 Hz, respectively, confirming *meta* and *ortho* coupling with C'_6-H. The proton at C'_6-H appeared as a dd (J = 1.2 and 6.2 Hz), as expected. The coupling of such pattern is the characteristic of 3',4' disubstituted flavonoids [9]. ^1H NMR of compound **1** showed two anomeric protons at δ 4.29 d and 5.48 d, indicating the presence of two sugar moieties. Acid hydrolysis of compound **1** afforded aglycon **D_1** and glucose

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Spectral Data of Compounds **1**, **2**, and **3** in DMSO-d_6

Compound **1**			Compound **2**			Compound **3**		
Carbon no.	^{13}C δ (ppm)	^{1}H δ (ppm)	Carbon no.	^{13}C δ (ppm)	^{1}H δ (ppm)	Carbon no.	^{13}C δ (ppm)	^{1}H δ (ppm)
2	157.65		2	157.76		2	158.15	
3	135.21		3	136.10		3	136.35	
4	178.76		4	178.53		4	177.64	
5	160.78		5	160.76		5	161.25	
6	99.87	H, 6.74 s	6	100.12	H, 6.75 s	6	120.65	
7	162.45		7	162.49		7	162.45	
8	105.76		8	105.60		8	105.26	
9	158.65		9	158.29		9	157.53	
10	107.98		10	107.52		10	107.47	
1'	122.54		1'	123.20		1'	122.36	
2'	132.73	H, 7.25 d	2'	132.65	H, 7.47 d	2'	132.34	H, 7.34 d
3'	120.94		3'	120.76		3'	161.53	
4'	160.63		4'	160.94		4'	160.53	
5'	116.74	H, 6.84 d	5'	115.90	H, 6.74 d	5'	116.23	H, 6.82 d
6'	132.84	H, 7.45 d,d	6'	132.29	H, 7.64 d,d	6'	132.64	H, 7.72 d,d
1"	50.64	2H, 2.78 d	1"	50.48	2H, 2.68 d	OCH_3	56.46	3H, 3.52 s
2"	120.64	H, 5.33 t	2"	120.95	H, 5.41 t	1"	50.76	2H, 2.90 d
3"	135.16		3"	136.20		2"	120.47	H, 5.31 t
4"	34.29	2H, 2.55 t	4"	35.75	2H, 2.55 t	3"	135.21	
5"	34.47	2H, 2.64 m	5"	34.54	2H, 2.75 m	4"	30.64	3H, 1.28 s
6"	120.05	H, 5.45 t	6"	120.43	H, 5.51 t	5"	30.46	3H, 1.28 s
7"	135.84		7"	135.21		1"'	72.85	H, 4.21 d
8"	30.67	3H, 1.28 s	8"	30.41	3H, 1.27 s	2"'	73.95	H, 3.81 m
9"	31.16	3H, 1.28 s	9"	31.20	3H, 1.27 s	3"'	77.53	H, 3.71 m
10"	31.15	3H, 1.28 s	10"	31.25	3H, 1.27 s	4"'	71.72	H, 3.62 m
1"'	72.74	H, 4.29 d	1"'	73.75	H, 4.28 d	5"'	82.56	H, 3.74 m
2"'	73.45	H, 3.53 m	2"'	73.72	H, 3.74 m	6"'	62.49	2H, 3.56 m
3"'	77.48	H, 3.83 m	3"'	77.69	H, 3.69 m	1""	105.34	H, 5.41 d
4"'	70.59	H, 3.57 m	4"'	70.78	H, 3.71 m	2""	74.86	H, 3.62 m
5"'	80.69	H, 3.74 m	5"'	81.43	H, 3.65 m	3""	76.73	H, 3.55 m
6"'	63.15	2H, 3.58 m	6"'	63.74	2H, 3.65 m	4""	70.87	H, 3.65 m
1""	104.37	H, 5.48 d	1""	103.84	H, 5.52 d	5""	79.93	H, 3.42 m
2""	73.47	H, 3.57 m	2""	74.52	H, 3.54 m	6""	62.45	2H, 3.74 m
3""	76.48	H, 3.57 m	3""	75.53	H, 3.53 m	1"""	105.73	H, 5.45 d
4""	70.84	H, 3.68 m	4""	70.64	H, 3.63 m	2"""	74.41	H, 3.54 d
5""	79.75	H, 3.48 m	5""	79.73	H, 3.54 m	3"""	76.93	H, 3.72 m
6""	63.95	2H, 3.76 m	6""	63.90	2H, 3.76 m	4"""	70.86	H, 3.42 m
			1"""	105.63	H, 5.51 d	5"""	79.74	H, 3.64 m
			2"""	74.64	H, 3.49 d	6"""	62.76	2H, 3.71 m
			3"""	75.73	H, 3.80 m			
			4"""	70.72	H, 3.50 m			
			5"""	79.42	H, 3.62 m			
			6"""	62.94	2H, 3.74 m			

Table 2. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Spectral Data of Aglycons $\mathbf{D_1}$ and $\mathbf{D_2}$ in CD_3OD

Aglycon $\mathbf{D_1}$			Aglycon $\mathbf{D_2}$		
Carbon no.	^{13}C δ (ppm)	^{1}H δ (ppm)	Carbon no.	^{13}C δ (ppm)	^{1}H δ (ppm)
2	157.76		2	158.10	
3	138.21		3	139.34	
4	178.53		4	177.64	
5	160.76		5	160.25	
6	99.97	H, 6.75 s	6	120.12	
7	161.49		7	162.22	
8	105.60		8	105.26	
9	158.95		9	157.53	
10	107.52		10	107.37	
1'	123.10		1'	122.44	
2'	132.15	H, 7.24 d	2'	132.45	H, 7.42d
3'	120.56		3'	161.60	
4'	160.54		4'	160.55	
5'	116.19	H, 6.80 d	5'	116.15	H, 6.81 d
6'	132.29	H, 7.47 d,d	6'	132.50	H, 7.84 d,d
1''	50.48	2H, 2.56 d	OCH_3	56.45	3H, 3.66 s
2''	120.95	H, 5.44 t	1''	50.65	2H, 2.57 d
3''	135.75		2''	120.36	H, 5.43 t
4''	34.75	2H, 2.27 t	3''	135.52	
5''	34.54	2H, 2.39 m	4''	30.62	3H, 1.28 s
6''	120.43	H, 5.35 t	5''	30.54	3H, 1.28 s
7''	135.21		1'''	72.65	H, 4.22 d
8''	30.41	3H, 1.28 s	2'''	73.85	H, 3.88 m
9''	31.57	3H, 1.28 s	3'''	77.45	H, 3.83 m
10''	31.46	3H, 1.28 s	4'''	71.81	H, 3.76 m
1'''	72.75	H, 4.29 d	5'''	80.60	H, 3.57 m
2'''	73.72	H, 3.89 m	6'''	62.56	2H, 3.72 m
3'''	77.69	H, 3.73 m			
4'''	70.78	H, 3.82 m			
5'''	80.43	H, 3.55 m			
6'''	63.74	2H, 3.67 m			

(paper chromatography). ^{13}C NMR (Table 2) of aglycon $\mathbf{D_1}$ showed a 3 ppm downfield shift (δ 138.21) for carbon C_3 than that (135.21) of in the glycoside itself, suggesting O-glycosidation at C_3 [11].

The site of glycosidation was further supported by HMBC correlations shown in Fig. 2. The correlation peak was observed between the anomeric proton C_1''''-H (δ 5.48) of glucose and C_3 (δ 135.21) of aglycon. Comparative analysis of ^{1}H NMR spectral data of aglycon $\mathbf{D_1}$ (Table 2) with that of compound **1** showed that the chemical shift of one anomeric proton at δ 4.29 remained unchanged suggesting that compound **1** to possesses a C-glycosidic linkage. It was supported by the downfield absorption (δ 105.76 ppm) of C_8 carbon of ring A from the usual range 90.00-96.00 ppm [9]. This was further supported by the HMBC correlation peak observed between the anomeric proton C_1'''-H (δ 4.29) of glucose and the carbons at 162.45, 105.76, and 158.65 ppm assigned to C7, C8, and C9 of the aglycon, respectively.

The ^{1}H NMR spectrum of compound **1** also showed two olefinic protons as triplets at 5.33 and 5.45 ppm, three methyl

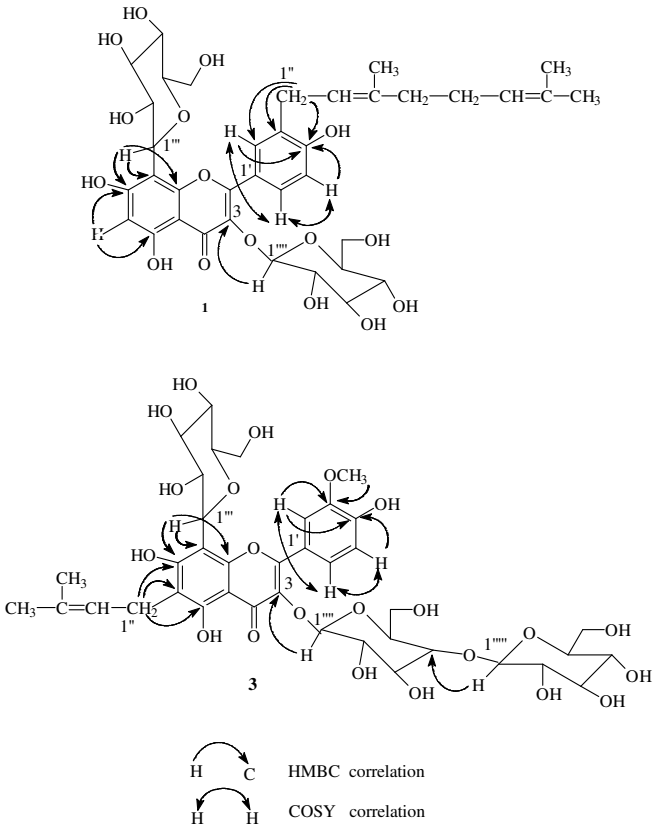


Fig. 2. Important HMBC and COSY correlations of compounds **1** and **3**.

proton (3 × CH_3) absorptions as a singlet at 1.28 ppm. These ^{1}H NMR absorptions along with the carbon content of the molecule suggests the presence of two isoprene units attached to the flavone nucleus.

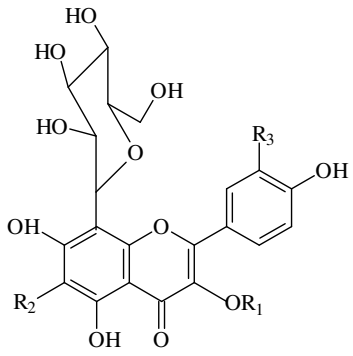
More important structural information is obtained from the mass spectral data of compound **1** which is summarized in Fig. 1. The FAB mass of the compound **1** gave four major product ions at m/z values of 163.1 (loss of a glucose unit, mode a) [11], 465.1 (removal of glucose unit followed by cleavage of ring D with a loss of 120 mass unit, mode a and b) [11,12] and 315.2 and 273.3 (loss of glucose unit followed by cleavage of flavonoid moiety, mode c and d) [11,13]. The FAB mass fragment at m/z 273.3 (mode d) in Fig. 1 together with two olefinic proton absorptions as triplets at 5.33, 5.45 ppm and three methyl proton absorptions as a singlet at 1.28 ppm in the ^{1}H NMR spectrum suggested that ring B contains the isoprene units. The site of the linkage is likely at C'$_3$, as shown by the 2D-COSY and HMBC connectivity (Fig. 2). Compound **1** is thus characterized as 3'-(3",7"-dimethyl-2",6"-octadiene)-8-C-β-D-glucosyl-kaempferol 3-O-β-D-glucoside.

Compound **2** was also a flavonol glycoside. It showed a molecular ion peak at m/z 909.3 $[M+H]^+$ corresponding to $C_{43}H_{56}O_{21}$. Compound **2** afforded the same aglycon **D_1** ($C_{31}H_{36}O_{11}$) and maltose after acid hydrolysis (5% HCl). FAB mass $[M_1+H]^+$ at 585.2 of aglycon and its ^{1}H NMR and ^{13}C NMR data (Table 2) supported the molecular formula ($C_{43}H_{56}O_{21}$) of compound **2.** The ^{13}C NMR spectral analysis confirmed the presence of 43 carbon atoms (Table 1) in the molecule **2**, 25 of which belonged to the nonsugar part. Compound **2** showed UV and IR spectra similar to those of compound **1**. Acid hydrolysis of compound **2** afforded the same aglycon **D_1** as obtained from the hydrolysis of compound **1**. Hydrolysis of the sugar part with 5% HCl for 2 h revealed the presence of maltose and glucose (PC). Further hydrolysis of the resulting mixture with fresh quantity of 5% HCl for 5 h showed the disappearance of maltose and only glucose could be detected (PC). The results suggested that initially maltose had undergone partial hydrolysis.

The presence of maltose was further confirmed by mass fragmentation of compound **2** which showed a mass peak at m/z 585.2 [M-2×162+H] (loss of 2 glucose units) [11]. Therefore compound **2** is characterized as 3'-(3",7"-dimethyl-2",6"-octadiene)-8-C-β-D-glucosyl-kaempferol 3-O-β-D-glycosyl [1→4]-α-D-glucoside, as shown in Fig. 3.

Compound **3** also gave positive test for flavonol glycoside. It showed a positive ion FAB mass at m/z 871.2 $[M+H]^+$ corresponding to $C_{39}H_{50}O_{22}$ supported by its ^{1}H NMR and ^{13}C NMR (Table 1). Compound **3** gave aglycon **D_2** and cellobiose after hydrolysis with 5% HCl. The positive ion FAB mass of aglycon **D_2** ($C_{27}H_{30}O_{12}$) at m/z 547.1 $[M_1+H]^+$ along with its other mass fragments and the NMR spectra (Table 2) supported the molecular formula ($C_{39}H_{50}O_{22}$) of compound **3**. UV and IR spectra of compound **3** were similar to those of compounds **1** and **2**.

The ^{1}H NMR spectrum of compound **3** (Table 1) revealed the presence of one methoxyl group appearing as a singlet at 3.52 ppm and in the ^{13}C NMR spectrum (Table 1) as one signal at 56.46 ppm. Two 3H (2 × CH_3) singlets at 1.28 ppm and one proton triplet at 5.31 ppm suggested the presence of



1 R_1 = glucose, R_2 = H, R_3 = $C_{10}H_{17}$

2 R_1 = glucosyl [1→ 4] glucose R_2 = H, R_3 = $C_{10}H_{17}$

3 R_1 = glucosyl [1→ 4] glucose R_2 = C_5H_9, R_3 = OCH_3

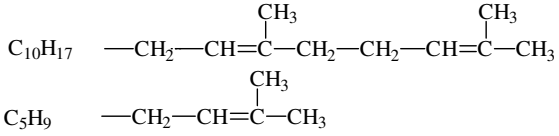


Fig. 3. Chemical structure of compound **2**.

an isoprene unit. ^{1}H NMR spectrum of compound **3** did not show any one proton singlet, as found for the A-ring proton of compounds **1** and **2**, indicating that the ring A of compound **3** is tetra substituted. The FAB mass peak at m/z 383 (mode c) together with one olefinic proton absorptions at 5.31 ppm and two methyl proton absorptions at 1.28 ppm, in the ^{1}H NMR spectrum confirmed that ring A was tetra substituted and the isoprene unit is attached to carbon C_6. This was also confirmed on the basis of carbon hydrogen correlations observed in the HMBC spectrum, between the signal at 2.90 ppm (C''_1-H) and the signals at 161.25 and 162.45 ppm (C_5 and C_7) Fig. 2.

Ring B of compound **3** showed absorption pattern of aromatic protons similar to those of compounds **1** and **2** (Table 1). FAB mass ion at m/z 167 (mode d) together with one methoxyl group absorption at 3.52 ppm in the ^{1}H NMR spectrum indicated that the methoxyl group was attached to ring B. This methoxyl group was placed on carbon C'_3 (δ 161.53) based on HMBC correlations of the methoxyl group with carbon 3'. The other prominent mass peaks were at 709.3 $[M-162+H]^+$ due to loss of glucose, 547.1 $[M-2\times162+H]^+$ due to loss of 2 glucose units, m/z 427.2 $[M-2\times162-120+H]^+$, due to loss of two glucose units followed by cleavage of ring D (mode b). Hydrolysis of compound **3** with 5% HCl afforded an aglycon $\mathbf{D_2}$ and a disaccharide (PC). The disaccharide remained unchanged even after further hydrolysis with 5% HCl for 8 h. The disaccharide was then recovered from aqueous part by preparative paper chromatography and identified as cellobiose, m.p.: 249-250 ºC [14]. Therefore, compound **3** is 6-(3''-methyl-2''-butene)-3'-methoxyl-8-C-β-D-glucosyl-kaempferol 3-O-β-D-glucosyl [1→4]-β-D-glucoside.

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