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Acylated 5,7,2′,6′-oxygenated flavone glycosides from *Andrographis alata* ☆

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Abstract

Five acylated 5,7,2′,6′-oxygenated flavone glycosides along with the known 5,2′,6′-trihydroxy-7-methoxyflavone-2′-*O*-β-D-glucopyranoside have been isolated from the whole plant of *Andrographis alata*. The structures of the compounds were established from spectral (mainly 1D and 2D NMR) and chemical studies.

Keywords: *Andrographis alata*; Acanthaceae; Flavone glycosides; Acylation; Structure elucidation; Spectral and chemical studies

1. Introduction

Andrographis species are widely distributed in different parts of India and are associated with various important medicinal properties (Kartikar and Basu, 1975; Chopra et al., 1980). Earlier chemical investigations on several *Andrographis* species afforded mainly flavonoids and diterpenoids (Kleipool, 1952; Govindachari et al., 1969; Balmain and Connolly, 1973; Gupta et al., 1983; Damu et al., 1998a,b, 1999; Reddy et al., 2003; Rao et al., 2004). Some of these constituents have been identified as promising immunostimulant (Puri et al., 1993), antileukemic (Matsuda et al., 1994) and anti-HIV agents (Singh et al., 2005). However, a little investigation has been carried out on *Andrographis alata* Nees (Damu et al., 1998a,b). We have now isolated five new acylated 5,7,2′,6′-oxygenated flavone glucosides, **1**–**5** along with 5,2′,6′-trihydroxy-7-methoxyflavone-2′-*O*-β-D-glucopyranoside (**6**) (Damu et al., 1998a) from the plant. Here, we report the isolation and characterization of the constituents. The occurrence of such acylated flavone glucosides is rare in nature.

2. Results and discussion

The $CHCl_3$–MeOH (1:1) extract of the whole plant of *A. alata* was subjected to column chromatography over silica gel to obtain six flavone glucosides **1**–**6** among which compound **6** is known. The original extract showed (TLC) the presence of these compounds. All of these compounds were isolated as light yellow solids. The unknown flavonoids, **1**–**5** were subjected to acid hydrolysis to afford the same aglycone, 5,2′,6′-trihydroxy-7-methoxyflavone along with D-glucose. Compounds **1**–**3** on alkaline hydrolysis produced the identical flavonoid, 5,2′,6′-trihydroxy-7-methoxyflavone-2′-*O*-β-D-glucopyranoside (**6**) and on acetylation the identical hexa-acetyl flavonoid **7**.

The UV absorption maxima in MeOH (ca. 257, 298 nm) and those with shift reagents suggested that **1**–**4** are 5,7,2′,6′-oxygenated flavone with a free 5-hydroxy group (shift with $AlCl_3$) and a protected 7-hydoxy group (no shift with NaOAc), while **5** is also a flavone of similar structural pattern but here both the 5- and 7-hydroxy groups are

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protected (Damu et al., 1998a,b; Mabry et al., 1970). The IR spectra of all the compounds indicated the presence of hydroxyl, flavone carbonyl and ester groups. The ^{1}H and ^{13}C NMR spectra data (Tables 1 and 2, respectively) revealed the compounds to be flavone glucosides containing acetyl groups in the glucose unit and with similar flavone moieties. The assignment of protons and carbons was made with the help of 2D NMR (COSY, HSQC, HMBC and ROESY) experiments (Fig. 1).

Compound **1** analyzed for $C_{24}H_{24}O_{12}$ from the mass spectrum ($[M+H]^+$ at m/z 505 in FABMS), elemental analysis and ^{1}H and ^{13}C NMR spectra (indicating the presence of 24 protons and 24 carbons). The ^{1}H NMR spectrum of **1** was similar to that of the known flavone glucoside, **6** (direct comparison) except for an additional signal (δ 1.76, 3H, *s*) for an acetoxyl group. The spectrum of **1** showed the presence of a methoxyl (δ 3.86, 3H, *s*) and two aromatic hydroxyl groups (one is chelated: δ 12.86, *brs* and other is non-chelated: δ 10.11, *brs*). The presence of three consecutive spin-coupled protons in an aromatic ring (δ 6.68, 1H, *dd*, $J = 8.0$, 2.0 Hz, H-3′; 7.28, 1H, *t*, $J = 8.0$ Hz, H-4′ and 6.64, 1H, *dd*, $J = 8.0$, 2.0 Hz, H-5′), two *meta*-coupled doublets (δ 6.61 and 6.40, 1H each, *d*, $J = 2.0$ Hz, H-8 and H-6, respectively) along with a singlet (δ 6.18, 1H, H-3) in the aromatic region was also evident from the spectrum. These signal patterns resembled those of 5,7,2′,6′-tetraoxygenated flavones (Zhou et al., 1997). The values of the protons were assigned with the help of ^{1}H–^{1}H COSY and ROESY experiments. The ROESY experiment also indicated the position of the methoxyl group at C-7 as this group showed NOE correlation with H-6 as well as with H-8. The anomeric proton of the sugar moiety appeared at δ 5.04 (1H, *d*, $J = 7.8$ Hz, H-1″). The detailed analysis of the positions and coupling constants of the protons of this moiety (Table 1) indicated it to be β-glucopyranoside having an acetyl group. The ^{1}H–^{1}H COSY experiment showed a clear correlation between the anomeric proton (H-1″) with a downfield shifted proton (δ 4.61, *dd*, $J = 9.0$, 7.8 Hz) suggesting the position of the acetoxy group at C-2″. The presence of three hydroxyl groups at C-3″, 4″ and 6″ was also observed (Table 1). As the ^{1}H NMR spectrum of **1** showed the presence of only one non-chelated hydroxyl group the linkage of the sugar moiety would reasonably be through C-2′ hydroxy group.

The ^{13}C NMR spectrum of **1** was also found to be similar to that of **6** but the former contained the signals for an additional acetyl group (δ 168.6 and 20.3) (Table 2). The HMBC spectrum showed the correlation between H-1″ and C-2′ (δ 156.3) confirming the attachment of the sugar residue with OH-2′ of **1**. In the sugar part the correlation between H-2″ and –CO-group of the acetyl function was also evident. The structure of **1** was thus conclusively established as 5,2′,6′-trihydroxy-7-methoxyflavone-2′-*O*-β-D-(2″-*O*-acetyl) glucopyranoside.

Compound **2** analyzed for $C_{26}H_{26}O_{13}$ from its mass spectrum ($[M+H]^+$ at m/z 547 in FABMS), elemental

Table 1
^{1}H NMR spectral data (δ in ppm) of compounds **1**–**5**[a,b]

Proton	1	2	3	4	5
H-3	6.18(*s*)	6.20(*s*)	6.37(*s*)	6.31(*s*)	6.30(*s*)
H-6	6.40(*d*, 2.0)	6.32(*d*, 2.0)	6.30(*d*, 2.0)	6.40(*d*, 2.0)	6.39(*d*, 2.0)
H-8	6.61(*d*, 2.0)	6.50(*d*, 2.0)	6.48(*d*, 2.0)	6.61(*d*, 2.0)	6.54(*d*, 2.0)
H-3′	6.68(*dd*, 8.0, 2.0)	6.84(*dd*, 8.0, 2.0)	6.92(*dd*, 8.0, 2.0)	6.74(*dd*, 8.0, 2.0)	6.84(*dd*, 8.0, 2.0)
H-4′	7.28(*t*, 8.0)	7.32(*t*, 8.0)	7.36(*t*, 8.0)	7.24(*t*, 8.0)	7.36(*t*, 8.0)
H-5′	6.64(*dd*, 8.0, 2.0)	6.78(*dd*, 8.0, 2.0)	6.78(*dd*, 8.0, 2.0)	6.66(*dd*, 8.0, 2.0)	6.78(*dd*, 8.0, 2.0)
H-1″	5.04(*d*, 7.8)	5.18(*d*, 7.8)	5.20(*d*, 7.8)	4.96(*d*, 7.8)	5.06(*d*, 7.8)
H-2″	4.61(*dd*, 9.0, 7.8)	4.84(*dd*, 9.0, 7.8)	3.52(*brdd*, 9.0, 7.8)	3.18(*ddd*, 9.0, 7.8, 6.0)	3.40–3.34(*m*)
H-3″	3.50–3.41(*m*)	3.66(*brt*, 9.0)	5.02(*t*, 9.0)	3.30(*td*, 9.0, 6.0)	3.52(*brt*, 9.0)
H-4″	3.22(*td*, 9.0, 6.0)	3.54(*brt*, 9.0)	3.58(*brt*, 9.0)	3.22(*td*, 9.0, 6.0)	3.40–3.34(*m*)
H-5″	3.50–3.41(*m*)	3.82(*ddd*, 9.0, 6.0, 2.0)	3.83(*ddd*, 9.0, 6.0, 2.0)	3.62(*brdd*, 9.0, 6.0)	3.70(*ddd*, 9.0, 6.0, 2.0)
H_a-6″	3.72(*brdd*, 12.0, 6.0)	4.44(*dd*, 12.0, 2.0)	4.41(*dd*, 12.0, 2.0)	4.34(*brd*, 12.0)	4.42(*dd*, 12.0, 2.0)
H_b-6″	3.52(*ddd*, 12.0, 9.0, 6.0)	4.23(*dd*, 12.0, 6.0)	4.22(*dd*, 12.0, 6.0)	4.05(*dd*, 12.0, 6.0)	4.21(*dd*, 12.0, 6.0)
H-2‴	–	–	–	5.90(*d*, 14.0)	–
H-3‴	–	–	–	6.92(*m*)	–
H-4‴	–	–	–	1.88(*d*, 7.0)	–
OMe-7	3.86(*s*)	3.88(*s*)	3.84(*s*)	3.88(*s*)	3.86(*s*)
OH-5	12.86(*brs*)	12.94(*brs*)	12.92(*brs*)	12.92(*brs*)	–
OH-2′	–	–	–	–	9.12–8.86(*brs*)
OH-6′	10.11(*brs*)	9.12(*brs*)	9.16(*brs*)	10.21(*brs*)	9.12–8.86(*brs*)
OH-2″	–	–	4.74–4.62(*brs*)	5.20(*d*, 6.0)	4.56–4.28 (*brs*)
OH-3″	5.23(*d*, 6.0)	4.72(*brs*)	–	5.24(*d*, 6.0)	4.56–4.28(*brs*)
OH-4″	5.21(*d*, 6.0)	4.68(*brs*)	4.74–4.62(*brs*)	5.12(*d*, 6.0)	4.56–4.28(*brs*)
OH-6″	4.64(*t*, 6.0)	–	–	–	–
OAc-2″	1.76(*s*)	1.84(*s*)	–	–	–
OAc-3″	–	–	1.98(*s*)	–	–
OAc-6″	–	2.02(*s*)	2.03(*s*)	–	2.03(*s*)

[a] The spectra of **1** and **4** were run in DMSO-d_6 while those of **2**, **3** and **5** in acetone-d_6.
[b] Multiplicity and coupling constant (J in Hz) are in parenthesis; the assignments were made on the basis of ^{1}H–^{1}H COSY, ROESY and HMBC data.

Table 2
^{13}C NMR spectral data (δ in ppm) of compounds **1**–**5**[a,b]

Carbon	1	2	3	4	5
C-2	161.5	163.1	163.1	161.6	157.6
C-3	112.1	113.5	113.8	112.5	113.9
C-4	182.0	183.3	183.4	182.0	183.3
C-4a	104.9	106.4	106.4	105.0	107.9
C-5	161.2	162.1	162.3	161.3	162.2
C-6	97.9	98.6	98.6	97.8	98.8
C-7	165.1	166.4	166.5	165.1	166.6
C-8	92.2	93.1	93.2	92.5	93.2
C-8a	158.4	159.9	159.8	158.3	157.3
C-1′	110.5	111.9	111.7	112.5	112.2
C-2′	156.3	157.1	157.4	156.5	157.6
C-3′	105.3	107.0	107.8	106.0	107.9
C-4′	132.2	133.0	133.1	132.0	133.0
C-5′	109.8	111.2	111.2	109.8	111.1
C-6′	155.7	157.1	157.4	156.0	157.3
C-1″	98.2	99.9	102.1	100.7	102.3
C-2″	73.0	73.7	72.7	73.1	74.5
C-3″	73.7	75.4	78.5	76.4	77.9
C-4″	69.7	71.3	69.4	69.8	71.2
C-5″	77.2	75.0	74.9	73.7	75.1
C-6″	60.5	64.0	63.9	63.1	64.2
C-1‴	–	–	–	165.3	–
C-2‴	–	–	–	122.1	–
C-3‴	–	–	–	145.2	–
C-4‴	–	–	–	17.6	–
OMe-7	56.0	56.3	56.4	56.0	56.3
–OCO*Me*-2″	20.3	20.6	–	–	–
–OCO*Me*-3″	–	–	20.7	–	–
–OCO*Me*-6″	–	20.7	21.0	–	20.7
–O*C*OMe-2″	168.3	169.4	–	–	–
–O*C*OMe-3″	–	–	170.7	–	–
–O*C*OMe-6″	–	170.9	170.9	–	171.1

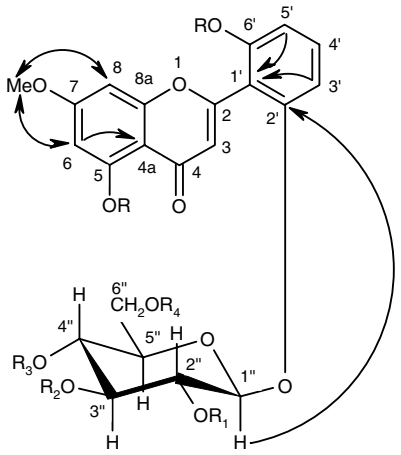
[a] The spectra of **1** and **4** were run in DMSO-d_6 and those of **2**, **3** and **5** in acetone-d_6.
[b] The assignments were made on the basis of HSQC and HMBC data.

analysis and ^{1}H and ^{13}C NMR spectra (indicating the presence of 26 protons and 26 carbons). The ^{1}H NMR and ^{13}C NMR spectral data (Tables 1 and 2) suggested that the structure of **2** was similar to that of **1** but the former contains two acetyl groups instead of one in **1**. This is also supported by a comparison of the molecular formula of **2** with that of **1**. The ^{1}H NMR spectrum of **2** also showed that two acetyl groups were present in the sugar residue (Table 1). The anomeric proton, H-1″ resonated as δ 5.18 (*d*, $J = 7.8$ Hz) and two acetyl groups at δ 2.02 and 1.84 (3H each, *s*). The ^{1}H–^{1}H COSY experiment clearly indicated the positions of the two acetoxyl groups at C-2″ and C-6″ as H-1″ was correlated with the downfield shifted proton at H-2″ (δ 4.84, *dd*, $J = 9.0$, 7.8 Hz) while H-5″ (δ 3.82, *ddd*, $J = 9.0$, 6.0, 2.0 Hz) with two downfield shifted protons H_2-6″ (δ 4.44, 1H, *dd*, $J = 12.0$, 2.0 Hz; δ 4.23, 1H, *dd*, $J = 12.0$, 6.0 Hz). The ^{13}C NMR spectrum depicted two acetyl groups (δ 170.9, 169.4, 20.7, 20.6) (Table 2). The HMBC spectrum clearly showed the correlations between H-1″ and C-2′, H-2″ and –CO– of an acetyl group and H-6″ and –CO– of other acetyl group. The structure of **2** was thus definitively settled as 5,2′,6′-trihydroxy-7-methoxyflavone-2′-*O*-β-D-(2″,6″-di-*O*-acetyl) glucopyranoside.

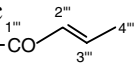
Compound **3** analyzed for $C_{26}H_{26}O_{13}$ from its mass spectrum ($[M+H]^+$ at *m*/*z* 547 in FABMS), elemental analysis and ^{1}H and ^{13}C NMR spectra (indicating the presence of 26 protons and 26 carbons). A comparison of the ^{1}H and ^{13}C NMR spectra of **3** and also of its molecular formula with those of **2** suggested that the structures of both the compounds are similar but the position of an acetoxyl groups in the glucose residue was different. The ^{1}H NMR spectrum also suggested the β-glucopyranoside structure of the sugar residue in **3** containing two acetyl groups (δ 2.03 and 1.98, 3H each, *s*). The ^{1}H–^{1}H COSY experiment revealed the presence of the two acetoxy groups at C-3″ and C-6″. The deshielded H-3″ (δ 5.02, 1H, *t*, $J = 9.0$ Hz) showed correlation with H-2″ (δ 3.52, *brdd*, $J = 9.0$, 7.8 Hz) and H-4″ (δ 3.58, *brt*, $J = 9.0$ Hz) while two other deshielded protons (H_2-6″) (δ 4.41, 1H, *dd*, $J = 12.0$, 2.0 Hz; δ 4.22, 1H, *dd*, $J = 12.0$, 6.0 Hz) showed correlation with each other and with H-5″ (δ 3.83, *ddd*, $J = 9.0$, 6.0, 2.0 Hz). The ^{13}C NMR spectrum of **3** also indicated the presence of two acetyl groups (δ 170.9, 170.7, 21.0, 20.7). The positions of these two acetyl groups were again confirmed from the HMBC experiment which showed the correlations of the carbonyl functions of these groups (δ 170.9 and 170.7) with H-3″ and H_2-6″, respectively. The HMBC spectrum also revealed that the anomeric proton (δ 5.20, 1H, *d*, $J = 7.8$ Hz, H-1″) of the sugar residue was related to C-2′ (δ 157.4) indicating the linkage of this sugar moiety with the hydroxyl group at C-2′. The structure of **3** was thus conclusively arrived at 5,2′,6′-trihydroxy-7-methoxyflavone-2′-*O*-β-D-(3″,6″-di-*O*-acetyl) glucopyranoside.

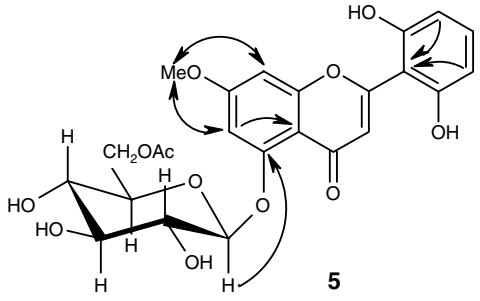
Compound **4** analyzed for $C_{26}H_{26}O_{12}$ from its mass spectrum ($[M+H]^+$ at *m*/*z* 531 in FABMS), elemental analysis and ^{1}H and ^{13}C NMR spectra (showing the presence of 26 protons and 26 carbons). The ^{1}H NMR and ^{13}C NMR spectral data (Tables 1 and 2) assigned by 2D NMR experiments suggested that the flavone part of **4** (which is similar to that of **1**–**3**) contains two hydroxyl groups at C-5 and C-6′ and a methoxyl group at C-7. However, in the sugar residue the former contains a *trans*-crotonyl group (δ 6.92, 1H, *m*; 5.90, 1H, *d*, $J = 14.0$ Hz and 1.88, 3H, *d*, $J = 7.0$ Hz in the ^{1}H NMR spectrum and δ 165.3, 145.2, 122.1 and 17.6 in the ^{13}C NMR spectrum) instead of the acetyl groups present in the other flavonoids. This *trans*-crotonyl group was reasonably placed at C-6″ as two downfield shifted protons (δ 4.34, *brd*, $J = 12.0$ Hz and 4.05, *dd*, $J = 12.0$, 6.0 Hz) at this position showed correlation with H-5″ (δ 3.62, *brdd*, $J = 9.0$, 6.0 Hz) in the ^{1}H–^{1}H COSY experiment and also with the acetyl carbonyl group (δ 165.3) in the HMBC spectrum. The HMBC experiment also showed the correlation between H-1″ (δ 4.96, *d*, $J = 7.8$ Hz) and C-2′ (δ 156.5) indicating the attachment of the sugar residue with OH-2′. The structure of **4** was thus suggested as 5,2′,6′-trihydroxy-7-methoxyflavone-2′-*O*-β-D-(6″-*O*-*trans*-crotonyl) glucopyranoside.

Compound **5** analyzed for $C_{24}H_{24}O_{12}$ from its mass spectrum ($[M+H]^+$ at *m*/*z* 505 in FABMS), elemental analysis and ^{1}H and ^{13}C NMR spectra (indicating the



1 - 4, 6, 7

1 $R_1 = Ac, R = R_2 = R_3 = R_4 = H$
2 $R_1 = R_4 = Ac, R = R_2 = R_3 = H$
3 $R = R_1 = R_3 = H, R_2 = R_4 = Ac$
4 $R = R_1 = R_2 = R_3 = H, R_4 =$ 
6 $R = R_1 = R_2 = R_3 = R_4 = H$
7 $R = R_1 = R_2 = R_3 = R_4 = Ac$



5

Fig. 1. Selected HMBC (→) and ROESY (↔) correlations.

presence of 24 protons and 24 carbons). The molecular formula of the compound is similar to that of **1**. However, unlike the compounds **1**–**4** the UV spectrum of **5** using shift reagents suggested that the hydroxy groups at C-5 and C-7 of this flavonoid are protected (Damu et al., 1998a,b). The ^1H spectrum revealed the presence of two non-chelated hydroxyl groups (δ 9.12–8.86, 2H, *brs*) and a methoxyl group (δ 3.86, 3H, *s*) along with six aromatic protons (Table 1) in the flavone unit. The spectrum also indicated the presence of β-glucopyranoside moiety containing an acetyl group (δ 2.03, 3H, *s*). It is interesting that here in the aromatic ring B the chemical shifts of 2′ and 6′ and of 3′ and 5′ are somewhat different. The ROESY experiment suggested the presence of the methoxyl group at C-7 as H-6 (δ 6.39, 1H, *d*, $J = 2.0$ Hz) and H-8 (δ 6.54, 1H, *d*, $J = 2.0$ Hz) were related to this group. The ^{13}C NMR spectrum clearly showed the characteristic signals for 24 carbons present in **5** containing an acetyl group (δ 171.1, 20.7) in the sugar residue (Table 2). The HMBC spectrum revealed the attachment of this sugar part with OH-5 as the anomeric proton, H-1″ (δ 5.06, 1H, *d*, $J = 7.8$ Hz) was related to C-5 (δ 162.2). The acetoxyl group was placed at C-6″ as two downfield shifted protons (δ 4.42, 1H, *dd*, $J = 12.0$, 2.0 Hz; δ 4.21, 1H, *dd*, $J = 12.0$, 6.0 Hz) attached to this carbon was related with H-5″ (δ 3.70, 1H, *ddd*, $J = 9.0$, 6.0, 2.0 Hz) in the ^1H–^1H COSY experiment and with carbonyl group of the acetyl function in the HMBC experiment. The structure of **5** was thus concluded as 5,2′,6′-trihydroxy-7-methoxyflavone-5-*O*-β-D-(6″-*O*-acetyl) glucopyranoside.

It is interesting to note that all the isolated flavonoids are oxygenated at 2′ and 6′ in the ring B. This is quite unusual in flavonoids. The sugar part in each molecule is derived from D-glucose and in all the new molecules this moiety is acylated.

3. Experimental

3.1. General

Melting points were measured in a Büchi-510 apparatus and are uncorrected. The spectra were recorded with the following instruments: UV, GBC Cintra 10e spectrophotometer; IR, Perkin–Elmer spectrum RX 1 FT-IR; NMR, Varian Gemini-200 MHz and mass spectra on VG-Micromass 7070H and Finnigan-MAT 1020 instruments. For the HMBC experiments the delay was 1.5 s and the mixing time for the ROESY experiments was 300 ms Optical rotations were measured on a JASCO DIP-360 polarimeter. Column chromatography was performed with silica gel (BDH, 100–200 mesh) and TLC with silica gel GF_{254}. PC was carried out on Whatman No. 1.

3.2. Plant material

The whole plants of *A. alata* were collected from Tirumala hill, Andhra Pradesh, in September, 2004 and identified by Professor C. Rajugopal, Department of Botany, Osmania University, Hyderabad. A voucher specimen (No. CP-AP-3) is preserved in our laboratory and another voucher specimen (IICP-301104) in IICT herbarium.

3.3. Extraction and isolation

Air dried whole plants (6 kg) were powdered and extracted with MeOH (12 l) for 120 h at room temperature. The extract was concentrated to afford a greenish mass (30.3 g). The residue (30 g) was subjected to column chromatography over silica gel, the column being eluted with solvents of increasing polarity using hexane and EtOAc. The fraction eluted with EtOAc was rechromatographed with a mixture of $CHCl_3$ and MeOH as eluent. The following compounds were eluted according to the increasing order of polarity: compound **2** (37 mg), compound **3** (48 mg), compound **4** (33 mg), compound **1** (45 mg),

compound **5** (28 mg) and compound **6** (34 mg) (the compounds were eluted with 1–3% MeOH in $CHCl_3$).

3.4. 5,2′,6′-Trihydroxy-7-methoxyflavone-2′-O-β-D-(2″-O-acetyl) glucopyranoside (*1*)

Light yellow solid, m.p. 204–205 °C (MeOH), $[\alpha]_D^{25} - 42.2$(c 0.1, MeOH); UV λ_{max} (MeOH) nm (log ε): 295 (4.46), 257 (4.21); +NaOAc: 297, 257; +$AlCl_3$: 341, 263; IR ν_{max} (KBr) cm^{-1}: 3450, 1725, 1650, 1626, 1462; ^1H and ^{13}C NMR: Tables 1 and 2; FABMS: m/z 505 $[M+H]^+$. Anal. Calc. for $C_{24}H_{24}O_{12}$: C, 57.14; H, 4.76%. Found: C, 57.08; H, 4.72%.

3.5. 5,2′,6′-Trihydroxy-7-methoxyflavone-2′-O-β-D-(2″,6″-di-O-acetyl) glucopyranoside (*2*)

Light yellow solid, m.p. 126–128 °C (MeOH), $[\alpha]_D^{25} - 20.0$(c 0.1, MeOH); UV λ_{max} (MeOH) nm (log ε): 296 (4.38), 259 (4.17); +NaOAc: 296, 258; +$AlCl_3$: 341, 259; IR ν_{max} (KBr) cm^{-1}: 3404, 1736, 1658, 1459; ^1H and ^{13}C NMR: Tables 1 and 2; FABMS: m/z 547 $[M+H]^+$. Anal. Calc. for $C_{26}H_{26}O_{13}$: C, 57.14; H, 4.76%. Found: C, 57.23; H, 4.81%.

3.6. 5,2′,6′-Trihydroxy-7-methoxyflavone-2′-O-β-D-(3″,6″-di-O-acetyl) glucopyranoside (*3*)

Light yellow solid, m.p. 140–142 °C (MeOH), $[\alpha]_D^{25} - 16.2$(c 0.1, MeOH); UV λ_{max} (MeOH) nm (log ε): 296 (4.56), 258 (4.22); +NaOAc: 297, 258; +$AlCl_3$: 347, 237; IR ν_{max} (KBr) cm^{-1}: 3493, 1727, 1654, 1624, 1460; ^1H and ^{13}C NMR: Tables 1 and 2; FABMS: m/z 547 $[M+H]^+$. Anal. Calc. for $C_{26}H_{26}O_{13}$: C, 57.14; H, 4.76%. Found: C, 57.22; H, 4.72%.

3.7. 5,2′,6′-Trihydroxy-7-methoxyflavone-2′-O-β-D-(6″-O-trans-crotonyl) glucopyranoside (*4*)

Light yellow solid, m.p. 236–238 °C (MeOH), $[\alpha]_D^{25} - 4.8$(c 0.1,MeOH); UV λ_{max} (MeOH) nm (log ε): 298 (4.47), 258 (4.19); +NaOAc: 297, 257; +$AlCl_3$: 364, 263; IR. ν_{max} (KBr) cm^{-1}: 3446, 1720, 1653, 1461; ^1H and ^{13}C NMR: Tables 1 and 2; FABMS: m/z 531 $[M+H]^+$. Anal. Calc. for $C_{26}H_{26}O_{12}$: C, 58.87; H, 4.91%. Found: C, 58.95; H, 4.86%.

3.8. 5,2′,6′-Trihydroxy-7-methoxyflavone-5-O-β-D -(6″-O-acetyl) glucopyranoside (*5*)

Light yellow solid, m.p. 218–219 °C (MeOH), $[\alpha]_D^{25} - 36.1$(c 0.1, MeOH); UV λ_{max} (MeOH) nm (log ε): 340 (4.38), 257 (4.14); +NaOAc: 341, 258; +$AlCl_3$: 341, 262; IR ν_{max} (KBr) cm^{-1}: 3367, 1729, 1656, 1615, 1459; ^1H and ^{13}C NMR: Tables 1 and 2; FABMS: m/z 505 $[M+H]^+$. Anal. Calc. for $C_{24}H_{24}O_{12}$: C, 57.14; H, 4.76%. Found: C, 56.98; H, 4.81%.

3.9. Acid hydrolysis of *1–5*

Compounds **1**–**5** (10 mg each) were separately dissolved in 2 N HCl in MeOH (5 ml) and refluxed at 100 °C for 2 h. Each reaction mixture was diluted with water (10 ml) and extracted with EtOAc (3 × 10 ml). The extract was concentrated in each case and the residue on crystallization from MeOH afforded the same aglycone, 5,2′,6′-trihydroxy-7-methoxyflavone (6 mg), m.p. 211–212 °C, whose spectral (IR, ^1H NMR and MS) properties were identical to those reported earlier (Damu et al., 1998a). The sugar in each aqueous layer was identified as D-glucose by co-PC using the solvent system, *n*-BuOH–HOAc–H_2O, 4:1:5 (Damu et al., 1999).

3.10. Alkaline hydrolysis of *1–3*

Compounds **1**–**3** (10 mg each) were separately dissolved in 1% aqueous KOH solution (5 ml) and refluxed for 2 h. Each reaction mixture was acidified with 1 N HCl and extracted with Et_2O (3 × 5 ml) followed by *n*-BuOH (3 × 5 ml). The *n*-BuOH extract was concentrated in each case and the residue on crystallization from MeOH produced the same flavone glucoside **6** (8 mg), m.p. 139–140 °C, identified from spectral (IR, ^1H NMR and MS) values which are similar to those reported earlier (Damu et al., 1998a) and also by direct comparison with naturally occurring sample.

3.11. Acetylation of compounds *1–3*

Compounds **1**–**3** (5 mg) were separately treated with Ac_2O (1 ml) and pyridine (0.1 ml) for 24 h at room temperature. Usual work-up yielded the same hexaacetyl acetate **7** (5 mg), m.p. 182–183 °C (MeOH), having spectral (IR, ^1H NMR and FABMS) data identical to those reported earlier (Damu et al., 1998a).

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