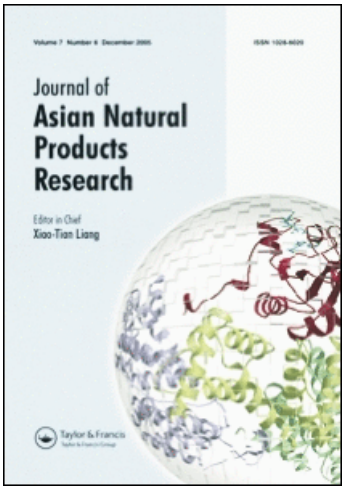

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Further flavonol and iridoid glycosides from *Ajuga remota* aerial parts

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Five new iridoid glycosides characterised as 6-keto-8-acetylharpagide (**1**), 6,7-dehydro-8-acetylharpagide (**2**), 7,8-dehydroharpagide (**3**), 8-acetylharpagide-6-*O*-β-glucoside (**4**), harpagide-6-*O*-β-glucoside (**5**) together with three flavonol glycosides, myricetin 3-*O*-rutinoside-4′-*O*-rutinoside (**6**), myricetin 3-*O*-rutinoside-3′-*O*-rutinoside (**7**) and isorhamnetin 3-*O*-rutinoside-7-*O*-rutinoside-4′-*O*-β-glucoside (**8**) have been isolated from the aerial parts of *Ajuga remota*. Also isolated were two known compounds ajugarin IV and ajugarin V. Their structures were established using spectroscopic methods including UV, IR, FAB-MS, HR-MS, 1D and 2D NMR techniques.

Keywords: *Ajuga remota*; Labiatae; Iridoid glycosides; Flavonol glycosides

1. Introduction

In our continuation with the chemical study of *Ajuga remota* Benth. (Labiatae), we herein report the isolation of five new iridoid glycosides, 6-keto-8-acetylharpagide (**1**), 6,7-dehydro-8-acetylharpagide (**2**), 7,8-dehydroharpagide (**3**), 8-acetylharpagide-6-*O*-β-glucoside (**4**), harpagide-6-*O*-β-glucoside (**5**), together with three flavonol glycosides, myricetin 3-*O*-rutinoside-4′-*O*-rutinoside (**6**), myricetin 3-*O*-rutinoside-3′-*O*-rutinoside (**7**), isorhamnetin 3-*O*-rutinoside-7-*O*-rutinoside-4′-*O*-β-glucoside (**8**) and two known compounds, ajugarin IV and ajugarin V [1,2].

2. Results and discussion

Chromatographic separation of the aqueous methanolic extract from the aerial parts of *Ajuga remota* afforded five new iridoid glycosides (**1–5**) and three flavonol glycosides (**6–8**) together with known compounds ajugarins IV and V. Their structures (figure 1) were established mainly by spectroscopic methods.

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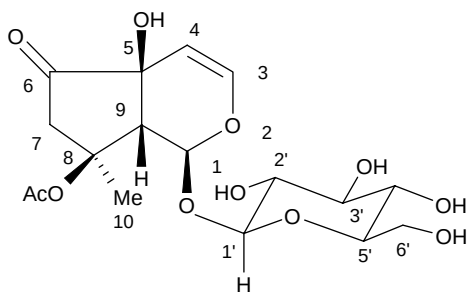

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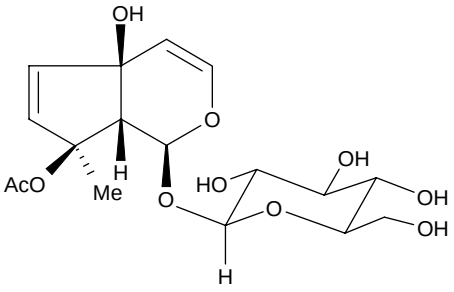

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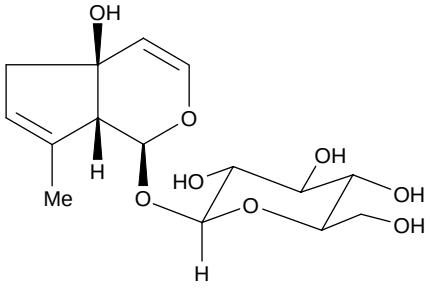

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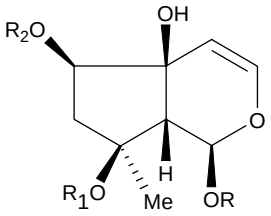
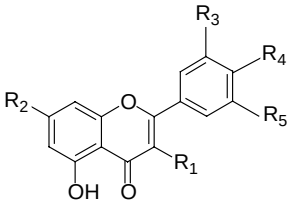


4 R = R_2 = Glc, R_1 = Ac

5 R = R_2 = Glc, R_1 = H



6 R_1 = O-rhamnosyl-(1‴→6″)-glucoside, R_4 = O-rhamnosyl-(1⁗→6⁗⁗)-glucoside, R_2 = R_3 = R_5 = OH

7 R_1 = O-rhamnosyl (1⁗→6″)-glucoside, R_2 = R_4 = R_5 = OH, R_3 = O-rhamnosyl-(1⁗⁗→ 6‴)-glucoside.

8 R_1 = O-rhamnosyl-(1‴→ 6″)-glucoside, R_2 = O-rhamnosyl-(1⁗→6⁗⁗)-glucoside, R_3 = OMe, R_4 = O-glucose, R_5 = OH.

Figure 1. The structures of compounds **1**–**8**.

Compound **1**, colourless platelets, $[\alpha]_D^{25} + 46^0$ gave a HR-MS molecular ion peak at *m/z* 404.1319 $[M]^+$, consistent with the formula $C_{17}H_{24}O_{11}$. Its ^{1}H NMR data were similar to those of 8-acetylharpagide (**9**) [3,4] except for the presence of a keto group, confirmed by ^{13}C NMR at δ 213.1. Acid hydrolysis yielded glucose as the sugar residue identified by TLC and PC co-chromatography with authentic sample. The large coupling constant of the anomeric proton (d, $J = 7.5$ Hz) indicated that glucose unit was present in the β-configuration.

The combined interpretation of the ^{1}H NMR and ^{13}C NMR was aided by the 2D HMQC spectrum which allowed association of all the protons with the relevant carbon signals, and by

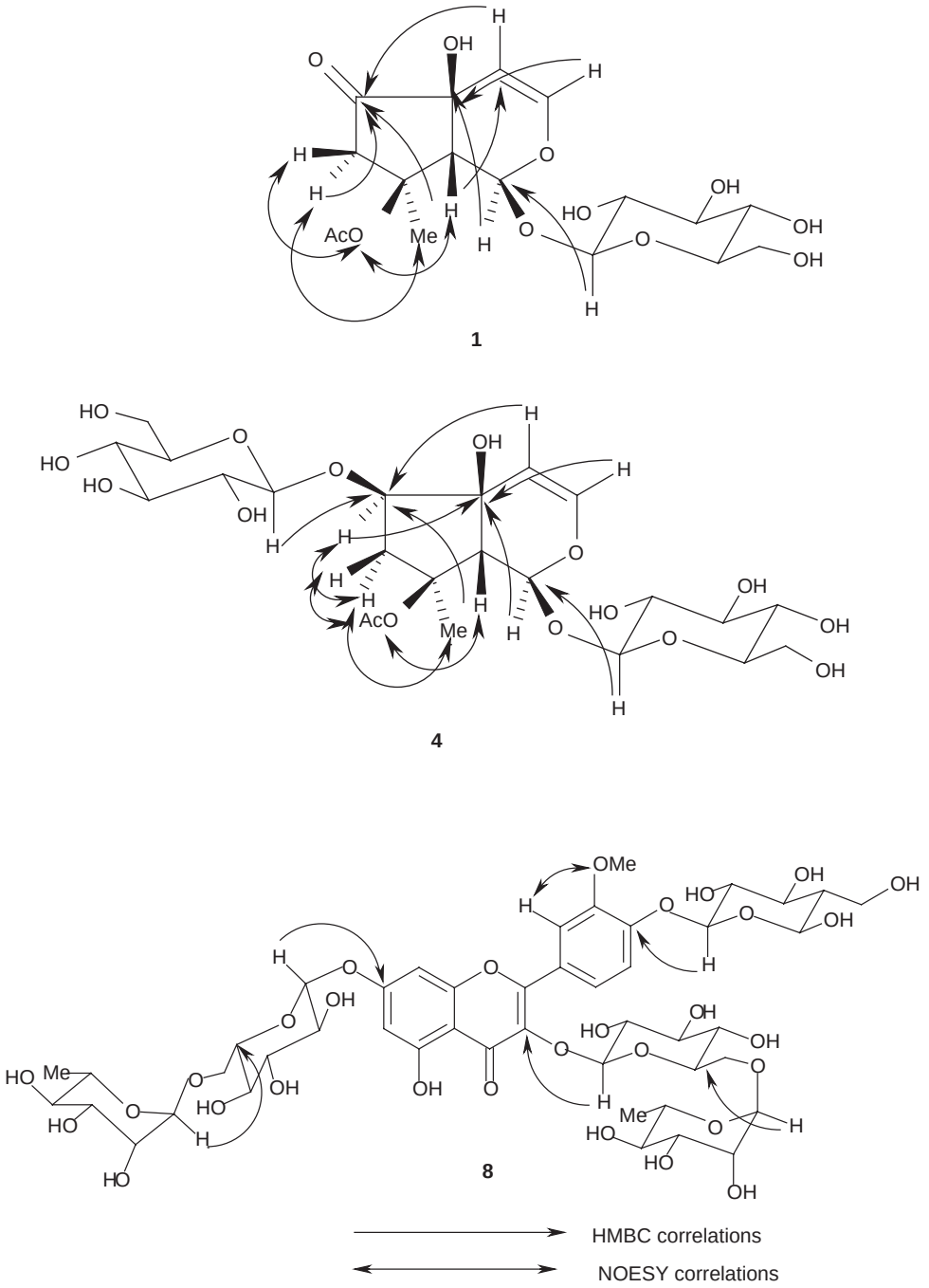


Figure 2. Significant HMBC and NOESY correlations for compounds **1**, **4** and **8**.

the 2D HMBC spectrum, which was vital in interconnecting the various spin systems. The positions of the acetoxy and keto groups as well as the glucose on the aglycone were established from HMQC and HMBC experiments (figure 2). In this way, it was proved that the glucose unit was attached glycosidically to C-1. Similarly, the presence of key HMBC

correlations between H-7 (δ 2.40), H-9 (δ 2.90) and C-8 (δ 87.3); between H-9, H-1′ and C-1 (δ 95. 4); between H-3 (δ 5.50), H-4 (δ 5.10) and C-6 (δ 213.1) unambiguously confirmed that compound **1** is 6-oxo-derivative of 8-acetylharpagide, a fact further confirmed by NOESY plots (figure 2). Thus, on the basis of spectroscopic data, compound **1** was characterised as 6-keto-8-acetylharpagide.

Compound **2** afforded ^{1}H NMR features which are also similar to those of 8-acetylharpagide (**9**) [4,5] except for additional double bond as evidenced by the presence of δ 6.02 (d, $J = 9.0$, 1.6 Hz, H-6) and 5.84 (d, $J = 9.0$, 3.3 Hz, H-7), with corresponding ^{13}C NMR peaks at δ 128.7 and 133.6, respectively. This together with the molecular ion peak at *m/z* 388.1370 in HR-MS established the disubstituted double bonds which are not conjugated as supported by UV $\lambda_{max} = 214$ nm (log ε 3.0). Acid hydrolysis yielded glucose as the sugar residue identified by TLC and PC co-chromatography with authentic sample. The ^{1}H–^{1}H COSY spectrum combined with ^{1}H, ^{13}C and 2D HMQC and HMBC spectra revealed the following subunits: —C (OH)—CH═CH—O—C (O—)—CH— and —CH—CH═CH—CH—, which are consistent with iridoid skeleton [4,6]. The coupling constant ($J = 2.0$ Hz) between H-9 and H-1 showed that the latter is axial [3], a fact confirmed by the non NOESY correlation between the two protons. Based on the above evidence, compound **2** was concluded to be 6,7-dehydro-8-acetylharpagide.

Compound **3** afforded an ion peak at *m/z* 346.1264 corresponding to a molecular formula of $C_{15}H_{22}O_9$. Its ^{1}H NMR spectrum revealed the presence of two olefinic bonds at δ 5.50 (d, $J = 6.7$ Hz, H-3), 4.90 (dd, $J = 6.7$, 2.4 Hz, H-4) and 5.70 (d, $J = 6.3$ Hz, H-7), oxymethine protons at δ 5.34, ($J = 1.8$ Hz, H-1) and 4.30, ($J = 5.1$ Hz, H-6) and a vicinal methyl group at δ 1.40. In addition, there was a peak at δ 4.65 (d, $J = 7.6$ Hz) attributable to the anomeric proton of a β-linked glucose, a fact supported by acid hydrolysis followed by TLC and PC co-chromatography with authentic sample. The position of attachment of the sugar moieties to the aglycone was concluded from HMBC correlation between the anomeric proton (δ 4.65) and the aglycone C-1 (δ 96.0). Comparison of the ^{1}H NMR and ^{13}C NMR of **3** with those of 6,7-dehydro-8-acetylharpagide (**2**) and 8-acetylharpagide (**9**) revealed the presence of an extra double bond between C-7 and C-8 but no acetyl group. Decoupling experiments showed the extra olefinic protons were part of an isolated —C (OH)—CH═CH (CH_3)—CH— system analogous to the C-6 to C-9 region in aucubin previously isolated from *Pedicularis striata* subsp. arachnoids [6]. The ^{1}H–^{1}H COSY, HMBC and HMQC experiments aided the assignments of ^{1}H NMR and ^{13} C NMR signals while the disposition of both H-6 and H-1 as α was based on non NOESY correlations between H-1 and H-9; and between H-9 and H-6. Thus, on the basis of spectroscopic data, **3** was deduced to be 7,8-dehydroharpagide.

Compound **4**, colourless platelets, showed HR-MS ion $[M]^+$ peak at *m/z* 568.2003 in accordance with the molecular formula of $C_{23}H_{36}O_{16}$. Its IR spectrum revealed significant absorptions attributable to free hydroxyl (3540 cm^{-1}) and an ester (1740 cm^{-1}) groups. Furthermore, the presence of an acetyl group was deduced from the ^{1}H NMR spectrum (δ 2.04). The ^{1}H NMR spectrum of **4** closely resembled that of 8-acetylharpagide (**9**) [3] except for the presence of additional sugar unit as revealed by anomeric proton peak at δ 5.01 ($J = 7.5$ Hz) with a corresponding carbon atom at δ 101.0 (C-1″). This was confirmed by ^{13}C NMR spectrum which displayed a total of 23 carbon signals sorted out into 2 methyl, 15 methine, 3 methylene and 3 quaternary carbon atoms by DEPT. Acid hydrolysis gave glucose as the sugar residue identified by TLC and PC co-chromatography with authentic

samples. Thus, compound **4** contained two glucose units and the large coupling constants of their anomeric protons ($J = 7.6$ Hz and $J = 7.5$ Hz) indicated that they were present in β-configurations [7]. Complete assignment of the ^{1}H NMR and ^{13}C NMR signals was achieved using HMQC and HMBC experiments and was further supported by NOESY (figure 2). This led to confirmation that the two glucose units were glycosidically at positions C-1 and C-6 of the aglycone while the acetyl group was at C-8. Thus, compound **4** was concluded to be 8-acetylharpagide 6-*O*-β-glucoside.

Compound **5**, isolated using preparative HPLC, afforded ^{1}H NMR data similar to those of **4** with notable exception being the absence of acetyl group. The HR-MS ion $[M]^+$ peak at *m/z* 526.1976 gave a molecular formula of $C_{21}H_{34}O_{15}$ and was confirmed by ^{13}C NMR spectrum which exhibited 21 carbon signals; multiplicity assignments from DEPT experiments revealed 15 methine, 3 methylene, one methyl and 2 quaternary carbon atoms. Acid hydrolysis yielded glucose identified by co-chromatography with authentic samples. Careful examination of ^{1}H NMR spectrum revealed characteristic doublets at δ 6.20 (H-3) and 5.95 (H-4), respectively and decoupling experiments indicated the olefinic protons to be part of an HO—C—CH═CH—O— system analogous to the C-5 to C-3 region in **5** [3,4,6], thus C-4 and C-3 were unsubstituted. Furthermore, the presence of OH at C-5 was evidenced by a singlet at δ 3.14 (H-9) and the absence of long-range coupling to H-3. Accordingly, the ^{1}H NMR decoupling experiments in combination with HMQC and HMBC correlations confirmed the two glucose units to be at C-6 and C-1, respectively. The relatively shielded position of the methyl at δ 1.23 suggested the placement of the OH group at C-8, a fact confirmed by the HMBC correlations between H-6 (δ 5.0), H-1 (δ 5.36) and C-8 (δ 73.4). Thus, compound **5** was concluded to be harpagide 6-*O*-β-glucoside.

Compound **6**, obtained as a yellow amorphous powder on acid hydrolysis, yielded rhamnose, glucose and myricetin identified by TLC and PC after comparison with authentic samples. The myricetin structure was also confirmed by the downfield part of the ^{1}H NMR spectrum that showed a singlet at δ 7.20 (two protons) and two meta coupled doublets at δ 6.32 and 6.24, respectively [8]. Its FAB-MS spectrum showed a molecular ion peak $[M + H]^+$ at *m/z* 935 ($C_{39}H_{50}O_{26} + H$), which together with other significant fragments at *m/z* 789 $[M + H\text{-}146]^+$ (loss of rhamnose), 643 $[M + H\text{-}2 \times 146]^+$ (loss two rhamnoses), 481 $[M + H\text{-}2 \times 146\text{–}162]^+$ (loss of two rhamnoses and glucose unit) and 319 $[M + H\text{-}2 \times 146\text{-}2 \times 162]^+$ (loss of two rhamnoses and two glucoses), suggested that **6** is a myricetin tetraglycoside [9]. This was supported by the ^{1}H NMR spectrum signals representing the four anomeric protons at δ 5.50 (d, $J = 7.7$ Hz), 5.30 (d, $J = 7.8$ Hz), 4.62 (d, $J = 1.6$ Hz) and 4.56 (d, $J = 1.2$ Hz). Both the FAB-MS and the ^{1}H NMR results pointed out that the two rhamnoses were present as terminal sugars (rhamnosylglucose biosides) [10,11]. The localisation of the sugar moieties on the aglycone was provided by the ^{13}C NMR (table 2), which was in complete agreement with those reported for myricetin 3-*O*-galactoside-4′-*O*-rhamnoside [8,12]. Support for this was shown by HMBC correlations between the glucose anomeric protons at δ 5.50 with carbon at δ 134.7 (C-3) and between the other one at δ 5.30 with carbon at δ 138.7 (C-4′), and the UV spectrum and its changes after addition of shift reagents [12,13]. The terminal rhamnosyl units were linked to the two primary glucose units through $1 \rightarrow 6$ bonds as evidenced by downfield shifts of approximately + 6.0 ppm at δ 68.8 and δ 67.4, respectively [14], a fact further corroborated by HMBC correlations of the rhamnosyls H-1 to the various glucosyls C-6. Thus, compound **6** was myricetin 3-*O*-rutinoside-4′-*O*-rutinoside.

Compound **7,** analysed for $C_{39}H_{50}O_{26}$, *m/z* 934.2591 $[M]^+$, exhibited UV absorption maxima at 260 and 354 nm in MeOH and with shift reagents ($AlCl_3$, $AlCl_3$/HCl, NaOAc/H_3BO_3, NaOAc, NaOMe) it showed free OH groups at C-5, C-7 and an orthodihydroxyl system in the ring B, suggesting glycosylation at C-3 [12,15]. A careful comparative analysis of the effect of NaOMe upon the aglycone and **7** suggested the presence of a sugar moiety in ring B [12]. Furthermore, the glycoside was fairly stable to NaOMe while the aglycone rapidly decomposed and this was attributable to the presence of alkali sensitive 3′,4′,5′-hydroxylation in the latter [12,13]. On account of these data, **7** was suspected to be myricetin 3,3′-di-*O*-glycoside.

The identity of the aglycone was evidenced as myricetin on the basis of the fragment ion at *m/z* 318 (100%) in EI-MS, a fact supported by ^{1}H NMR typical data of the aglycone [8]. Acid hydrolysis of the compound gave rhamnose, glucose and myricetin confirmed by co-chromatography with authentic samples. The ^{1}H NMR spectrum indicated the presence of two glucosyls [at δ 5.40 (d, $J = 7.8$ Hz) and 5.26 (d, J = 7.6 Hz)] and two rhamnosyls [4.56 (d, $J = 1.2$ Hz) and 4.30 (d, $J = 1.4$ Hz)], respectively. The rhamnosyl anomeric protons at δ 4.56 and 4.30 suggested sugar–sugar linkage [16]. The localisation of the primary glucoses on the aglycone was aided by HMBC correlations between H-1″ (δ 5.40) with C-3 (δ 133.5) and between glucosyl H-1‴′ (δ 5.26) with C-3′ (δ 147.2), indicating that the rhamnosylglucose biosides were at C-3 and C-3′ of the aglycone [15]. In the ^{13}C NMR spectrum, both the C-6 (glc) were downfield shifted at δ 66.6 and 67.4, indicating glycosylation of the glucose units by the rhamnosyls on C-6 hydroxyls, a fact corroborated by the HMBC correlations between the C-6 (glc) and the H-1 (rha), thus confirming the inter linkage points between the four sugar moieties. Therefore, based on the above chemical and spectroscopic consideration, **7** was characterised as myricetin 3-*O*-rutinoside-3′-*O*-rutinoside.

Compound **8**, a yellow amorphous powder, afforded a molecular ion peak at *m/z* 1095 $[M + H]^+$ ($C_{46}H_{62}O_{30} + H$) in the FAB-MS. Its UV spectrum in MeOH and with customary shift reagents [12,13] suggested that **8** is a hydroxycinnamoyl flavonoid glycoside with a free hydroxyl group at C-5, a fact further evidenced by the ^{1}H NMR peak at δ 12.70, D_2O exchangeable. Acid hydrolysis gave rhamnose, glucose and isorhamnetin (identified by TLC and PC co-chromatography with authentic samples). The chemical shift assignments of the ^{1}H NMR and ^{13}C NMR confirmed the aglycone as isorhamnetin (3′-*O*-methylquercetin) [17], a fact corroborated by the NOESY cross peaks between H-2′ (δ 7.76) and 3′-OMe (δ 3.60) group and further supported by MS peak at *m/z* 317 $[M + H\text{-}2 \times 146\text{-}3 \times 162]^+$ (loss of two rhamnoses and three glucoses) (figure 3). The anomeric proton signals above δ 5.0 indicated the presence of three aglycone–sugar linkages [12,18], as confirmed by the HMBC correlations between δ 5.45 with δ_c 163.2 (C-7); between δ 5.53 with δ_c 134.5 (C-3) and δ 5.35 with δ 148.3 (C-4′), thus leading to a conclusion that **8** is isorhamnetin bearing primary glucoses at C-3, C-7 and C-4′ positions. The sugar–sugar linkages were assigned using ^{13}C NMR spectrum on the basis of the downfield shifts for two glucose C-6 at δ 67.2 and 68.1 ppm, respectively [19], confirmed by HMBC experiments (figure 2). Therefore, compound **8** was characterised as isorhamnetin 3-*O*-rutinoside-7-*O*-rutinoside-4′-*O*-β-glucoside.

3. Experimental

3.1 General experimental procedures

UV and IR spectra were recorded using Beckmann DU-65 spectrophotometer and Perkin–Elmer FTIR 600 series, respectively. The FAB-MS data were measured on a VG ZABSPEC

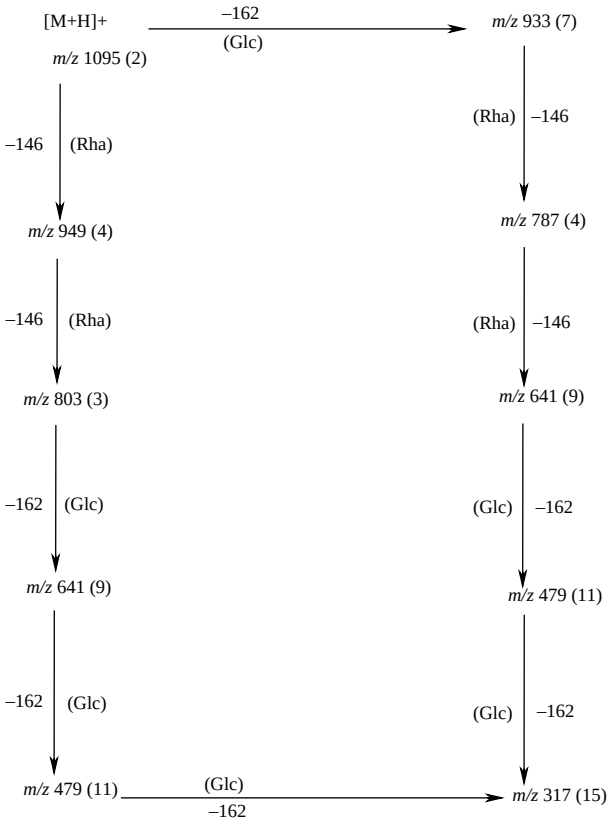


Figure 3. Fragmentation pattern of compound **8** in FAB-MS.

instrument. The NMR data were taken in DMSO-d_6 and DMSO-d_6/$CDCl_3$ mixture on a Bruker NMR Advance Ultrashield TM spectrometer operating at 500 and 125 MHz. EI-MS data were measured using 70 eV MAT 8200 A Varian Bremen instrument. Preparative high performance liquid chromatography (HPLC) was performed on a JASCO Labor and Daten Technik Deutschland GmBH equipment (RP-C18, 250 × 20 mm, 7 μm JASCO Kromasil 100). Silica gel for TLC was deactivated with 2% oxalic acid.

3.2 Plant material

The aerial parts of *Ajuga remota* were collected around Chepkoilel Campus, Moi University, Eldoret, in February 2001. Voucher specimens (No. 2001/2 NMUH) were identified after comparison with authentic samples at the National Museum Herbarium.

3.3 Extraction and isolation

The powdered dried leaves (approx. 3 kg) were first extracted with CH_2Cl_2 for one week and then with aqueous MeOH (70%) at room temperature for two weeks. Concentration of the latter extract under reduced pressure afforded a dark green residue (108 g). A portion of the extract (100 g) was preadsorbed on silica gel and then fractionated, starting with CH_2Cl_2/MeOH gradient and elution concluded with MeOH, fractions of 20 ml each being collected. A total of 281 fractions were collected and their homogeneity determined by TLC

(eluent: CH_2Cl_2/MeOH, 19:1, 9:1, 3:2 and 2:1; n-BuOH/HOAc/H_2O, 4:5:1) and those showing similar TLC profiles were combined into four major pools (I–IV).

Pool I (fractions 1–91, 30 g) upon further purification by repeated low pressure chromatography using CH_2Cl_2/MeOH (99:1) followed by the same solvent system in the ratio 19:1, collecting 10 ml each afforded ajugarin IV (70 mg), ajugarin IV (65 mg) [1,2] and **1** (85 mg). Pool II (fractions 92–188, 18 mg) was similarly purified as described for the above case using CH_2Cl_2/MeOH (19:1) followed by the same solvent system in the ratios 9:1 and 19:3 to give a further **1** (15 mg), **2** (34 mg) and a mixture of **3** and **4**. The mixture was further purified by reverse phase HPLC using gradient elution of acetonitrile–H_2O (starting with 75% acetonitrile and 25% H_2O and finishing with 100% H_2O; mobile flow rate, 12 ml/min and pressure of 6.1 mPa; time per run was 50 min), injecting 10 μl each time to give 75 mg and 45 mg, respectively. Pool III (fractions 189–220, 24 mg) was found to contain one major compound contaminated with minor impurities and was similarly purified by preparative HPLC as in the above case to give **5** in 60 mg. Pool IV (fractions 221–281, 26 g) mainly from methanol elution, yielded a gummy material after evaporation *in vacuo* and was further subjected to repeated flash chromatography using CH_2Cl_2/MeOH (4:1, 7:3, 1:1) and MeOH, collecting 10 ml each to give **6** (85 mg) and a mixture of **7** and **8**, which were purified by preparative HPLC using MeOH–H_2O (9:1) affording 45 mg and 67 mg, respectively.

3.3.1 6-Keto-8-acetylharpagide (1). Colourless platelets from CH_2Cl_2/MeOH (19:1), mp >250°C, $[\alpha]_D^{25}$ + 46 (*c* 1.0, MeOH); UV (MeOH) λ_{max} 208 (log ε 3.2) nm; IR (KBr) ν_{max} 3540 (OH), 3350 (OH), 1740, 1700, 1650, 1470, 1350, 1030–1010, 920, 870 cm^{-1}; ^1H NMR ($CDCl_3$ + drop DMSO-d_6) δ: 5.50 (d, *J* = 6.1 Hz, H-3), 5.40 (d, *J* = 1.8 Hz, H-1), 5.10 (dd, *J* = 6.1, 2.0 Hz, H-4), 2.90 (d, *J* = 1.6 Hz, H-9), 2.40 (d, *J* = 6.3 Hz, H-7a), 2.25 (d, *J* = 6.3 Hz, H-7b), 1.90 (s, OAc), 1.50 (s, Me-10); glucose: 4.70 (d, *J* = 7.5 Hz, H-1′), 3.80 (m, H-6′a), 3.68 (m, H-6′b), 3.52 (m, H-5′), 3.43 (m, 2′), 3.36 (m,, H-4′), 3.29 (m, H-3′); ^{13}C NMR ($CDCl_3$ + drop DMSO-d_6) data, see table 1; FAB-MS: *m/z* 405 $[M + H]^+$, 163 $[Glc]^+$; HR-MS *m/z*: 404.1319 (calcd for $C_{17}H_{24}O_{11}$, 404.1241).

3.3.2 6,7-Dehydro-8-acetylharpagide (2). Colourless platelets, mp >250°C; $[\alpha]_D^{25}$ − 19 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 214 (log ε 3.0) nm; IR (KBr) ν_{max} 3450–3100 (OH), 2920, 2850, 1735, 1644, 1470, 1050, 890 cm^{-1}; ^1H NMR ($CDCl_3$ + drop DMSO-d_6) δ: 6.02 (d, *J* = 9.0, 1.6 Hz, H-6), 5.84 (d, *J* = 9.0, 3.3 Hz, H-7), 5.70 (d, *J* = 6.2, Hz, H-3), 5.40 (d, *J* = 2.0 Hz, H-1), 5.21 (dd, *J* = 6.4, 2.2 Hz, H-4), 3.21 (d, *J* = 1.8 Hz, H-9), 2.02 (s, OAc), 1.37 (s, Me-10); glucose: 4.50 (d, *J* = 7.4 Hz, H-1′), 3.70 (m, H-6′a), 3.62 (m, H-6′b), 3.55 (m, H-3′), 3.48 (m, H-2′), 3.33 (m,, H-5′), 3.20 (m, H-4′); ^{13}C NMR ($CDCl_3$ + drop DMSO-d_6) data, see table 1; FAB-MS *m/z*: 389 $[M + H]^+$, 225 $[M + H\text{-}162]^+$, 163 $[glc + H]^+$;HR-MS *m/z*: 388.1370 (calcd for $C_{17}H_{24}O_{10}$, 388.1291).

3.3.3 7,8-Dehydroharpagide (3). Isolated as amorphous colourless powder from CH_2Cl_2/MeOH, mp 241–244°C; $[\alpha]_D^{25}$ − 108 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 223 (log ε 2.9) nm; IR (KBr) ν_{max} 3500–3100 (OH), 2928, 2850, 1640, 1470, 1030–1010, 980 cm^{-1}; ^1H NMR ($CDCl_3$ + drop DMSO-d_6) δ: 5.70 (d, *J* = 6.3 Hz, H-7), 5.50 (d, *J* = 6.7, Hz, H-3), 5.34 (d, *J* = 1.8 Hz, H-1), 4.90 (dd, *J* = 6.7, 2.4 Hz, H-4), 4.30 (d, *J* = 5.1 Hz, H-6), 3.00 (d, *J* = 3.1 Hz, H-9), 1.40 (s, Me-10); glucose: 4.65 (d, *J* = 7.6 Hz, H-1′), 3.68 (m, H-6′a), 3.60 (m, H-2′), 3.58 (m, H-6′b), 3.53 (m, H-3′), 3.49 (m, 5′), 3.40 (m,, H-4′),

Table 1. ^{13}C NMR of compounds **1**–**5** and **9**.

Carbon	***1***	***2***	***3***	***4***	***5***	***9***
1	95.4	94.8	96.0	94.80	95.7	95.3
2						
3	145.0	143.8	144.2	145.3	146.0	145.5
4	109.0	107.3	108.6	108.4	107.8	107.7
5	73.7	76.0	74.6	76.0	75.1	75.0
6	213.1	133.6	78.4	75.9	76.6	79.8
7	49.8	128.7	119.5	48.6	48.3	47.4
8	87.3	90.5	134.6	91.2	73.4	89.8
9	55.9	47.0	56.9	56.4	55.5	56.0
10	22.9	23.0	21.8	23.4	21.4	23.9
1′	99.4	99.0	101.3	101.0	100.4	100.1
2′	73.5	74.4	73.7	74.7	74.0	75.4
3′	77.3	76.9	76.4	76.8	76.1	76.5
4′	71.4	70.4	70.0	69.8	69.9	74.4
5′	76.9	77.6	77.1	76.5	76.8	77.8
6′	61.5	62.1	61.5	61.0	61.6	63.0
1″				99.9	101.0	
2″				74.0	73.6	
3″				76.7	76.5	
4″				69.4	70.4	
5″				76.5	77.0	
6″				60.2	60.9	
OAc	171.2	169.8		170.3		170.0
	24.6	25.4		23.8		25.0

3.30 (m, H-3′); ^{13}C NMR ($CDCl_3$ + drop DMSO-d_6) data: see, table 1; FAB-MS *m/z*: 347 $[M + H]^+$, 225 $[M + H\text{-}162]^+$; HR-MS *m/z*: 346.1264 (calcd for $C_{15}H_{22}O_9$, 346.1186).

3.3.4 8-Acetylharpagide-6-*O*-β-glucoside (4). Colourless amorphous powder from MeOH–CH_2Cl_2, mp >250°C; $[\alpha]_D^{25}$ − 85 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 218 (log ε 2.8) nm; IR (KBr) ν_{max} 3540 (OH), 2930, 2850, 1740, 1645, 1470, 1040–1010, 980 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 6.45 (d, *J* = 6.4, Hz, H-3), 6.35 (d, *J* = 1.5 Hz, H-1), 6.04 (dd, *J* = 6.4, 2.0 Hz, H-4), 4.90 (d, *J* = 4.5 Hz, H-6), 3.21 (d, *J* = 2.2 Hz, H-9), 2.20 (m, H-7a), 2.10 (m, H-7b), 2.04 (s, OAc), 1.47 (s, Me-10); 1-*O*-glucose: 4.80 (d, *J* = 7.6 Hz, H-1′), 3.55 (m, H-6′a), 3.46 (m, H-2′), 3.41 (m, H-6′b), 3.38 (m, H-3′), 3.30 (m, 5′), 3.25 (m, H-4′); 6-*O*-glucose: 5.01 (d, *J* = 7.5 Hz, H-1″), 3.58 (m, H-6″a), 3.53 (m, H-2″), 3.44 (m, H-6″b), 3.40 (m, H-3″), 3.28 (m, 5″), 3.18 (m, H-4″); ^{13}C NMR (DMSO-d_6) data, see table 1; FAB-MS *m/z*: 569 $[M + H]^+$, 407$[M + H\text{-}162]^+$, 389 $[M + H\text{-}162\text{-}H_2O]^+$, 163 $[glc]^+$; HR-MS *m/z*: 568.2003 (calcd for $C_{23}H_{36}O_{16}$, 568.1996).

3.3.5 Harpagide 6-*O*-β-glucoside (5). Colourless amorphous powder from MeOH/CH_2Cl_2, mp >250°C; $[\alpha]_D^{25}$ − 57 (*c* 1.0, MeOH); UV (MeOH) λ_{max} 226 (log ε 2.4) nm; IR (KBr) ν_{max} 3550–3100 (OH), 2920, 2850, 1650, 1475, 1020–1000, 950 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 6.20 (d, *J* = 6.7 Hz, H-3), 5.36 (d, *J* = 1.1 Hz, H-1), 5.94 (dd, *J* = 6.7, 1.2 Hz, H-4), 5.00 (d, *J* = 5.6 Hz, H-6), 3.14 (d, *J* = 1.6 Hz, H-9), 2.18 (m, H-7a), 2.08 (m, H-7b), 1.23 (s, Me-10); 1-*O*-glucose: 4.60 (d, *J* = 7.2 Hz, H-1′), 3.56 (m, H-6′a), 3.50 (m, H-2′), 3.47 (m, H-6′b), 3.39 (m, H-3′), 3.29 (m, 5′), 3.20 (m, H-4′); 6-*O*-glucose: 4.84 (d, *J* = 7.8 Hz, H-1″), 3.68 (m, H-6″a), 3.54 (m, H-2″), 3.48 (m, H-6″b), 3.44 (m, H-3″), 3.36 (m, 5″), 3.14 (m, H-4″); ^{13}C NMR (DMSO-d_6) data, see table 1; FAB-MS *m/z*: 527 $[M + H]^+$,

509 $[M + H\text{-}H_2O]^+$, 365$[M + H\text{-}162]^+$, 347 $[M + H\text{-}162\text{-}H_2O]^+$; HR-MS *m/z*: 526.1976 (calcd for $C_{21}H_{34}O_{15}$, 526.1901).

3.3.6 Myricetin 3-*O*-rutinoside-4′-*O*-rutinoside (6). Yellow amorphous powder from MeOH/H_2O, mp 231–233°C; $[\alpha]_D^{25}$ − 139 (*c* 0.6, MeOH); UV (MeOH) λ_{max} 266, 352; + $AlCl_3$: 267, 301, 344; + $AlCl_3$/HCl: 267, 300, 344; + NaOMe: 276, 372; + NaOAc: 272, 350; + NaOAc/H_3BO_3: 267, 344 nm; IR (KBr) ν_{max} 3500–3100 (OH), 1680 (αβ-unsaturated C═O), 1650, 1480, 1375, 1030–1010 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 12.70 (s, OH-5, D_2O exchang.), 7.20 (s, H-2′ and H-6′), 6.32 (d, $J = 2.0$ Hz, H-8), 6.24 (d, $J = 2.0$ Hz, H-6); 3-*O*-glucose: 5.50 (d, $J = 7.7$ Hz, H-1″), 3.76 (m, H-2″), 3.62 (m, H-3″), 3.50 (m, H-6″a), 3.40 (m, H-4″), 3.35 (m, $J = 8.2$, H-6″$_b$), 3.25 (m, H-5″); 6″-*O*-rhamnose: 4.56 (d, $J = 1.2$ Hz, H-1‴), 3.67 (m, H-2‴), 3.47 (m, H-3‴), 3.39 (m, H-5‴), 3.20 (m, H-4‴), 1.1 (d, $J = 6.2$ Hz, Me-6‴); 4′-*O*-glucose: 5.30 (d, $J = 7.8$ Hz, H-1⁗), 3.83 (m, H-2⁗), 3.72 (m, H-6⁗a), 3.53 (m, H-6⁗b), 3.45 (m, H-3⁗), 3.30 (m, H-4⁗), 3.26 (m, H-5⁗); 6⁗-*O*-rhamnose: 4.62 (d, $J = 1.6$ Hz, H-1⁗⁗), 3.49 (m, H-2⁗⁗), 3.61 (m, H-3⁗⁗), 3.24 (m, H-4⁗⁗), 3.54 (m, H-5⁗⁗), 1.1 (d, $J = 6.5$ Hz, Me-6⁗⁗); ^{13}C NMR (DMSO-d_6) data, see table 2; EI-MS (70 eV): *m/z* (%) 318 (100), 244 (10), 153 (4), 136 (20); FAB-MS *m/z*: 935 $[M + H]^+$, 789 $[M + H\text{-}146]^+$, 643 $[M + H\text{-}2 \times 146]^+$, 481 $[M + H\text{-}2 \times 146\text{–}162]^+$, 319 $[M + H\text{-}2 \times 146\text{-}2 \times 262]^+$, 309 $[Glc + Rha + H]^+$, 163 $[Glc + H]^+$, 147 $[Rha + H]^+$153, 137; HR-MS *m/z* 934.2901 (calcd for $C_{39}H_{50}O_{26}$, 934.2823).

3.3.7 Myricetin 3-*O*-rutinoside-3′-*O*-rutinoside (7). Obtained as a yellow amorphous powder, mp >250°C; $[\alpha]_D^{25}$ − 113 (*c* 0.6, MeOH); UV (MeOH) λ_{max} 260, 354; + $AlCl_3$: 274, 412; + $AlCl_3$/HCl: 271, 398; + NaOMe: 270, 396; + NaOAc: 272, 374; + NaOAc/H_3BO_3: 261, 371 nm; IR (KBr) ν_{max} 3500 (OH), 1680 (αβ-unsaturated C═O), 1650, 1605, 900. 860 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 12.65 (s, OH-5, D_2O exchang.), 10.70 (br s, OH-7, D_2O exchang.), 9.20–8.50 (br s, OH-4′, D_2O exchang.), 7.30 (s, H-2′ and H-6′), 6.40 (d, $J = 2.0$ Hz, H-8), 6.23 (d, $J = 2.0$ Hz, H-6); 3-*O*-glucose: 5.40 (d, $J = 7.8$ Hz, H-1″), 3.75 (m, H-6″a), 3.68 (m, H-2″), 3.56 (m, H-6″$_b$), 3.44 (m, H-3″), 3.30 (m, H-4″), 3.28 (m, H-5″); 6″-*O*-rhamnose: 4.56 (d, $J = 1.2$ Hz, H-1‴), 3.70 (m, H-2‴), 3.69 (m, H-5‴), 3.51 (m, H-3‴), 3.25 (m, H-4‴), 0.94 (d, $J = 6.3$ Hz, Me-6‴); 3′-*O*-glucose: 5.26 (d, $J = 7.6$ Hz, H-1⁗), 3.75 (m, H-6⁗a), 3.58 (m, H-6⁗$_b$), 3.50 (m, H-2⁗), 3.44 (m, H-3⁗), 3.40 (m, H-4⁗), 3.28 (m, H-5⁗), 3.23 (m, H-4⁗), 3.21 (m, H-5⁗); 6⁗-*O*-rhamnose: 4.30 (d, $J = 1.4$ Hz, H-1⁗⁗), 3.60 (m, H-2⁗⁗), 3.38 (m, H-3⁗⁗), 3.19 (m, H-2⁗⁗), 3.09 (m, H-5⁗⁗), 1.03 (d, $J = 6.5$ Hz, Me-6⁗⁗); ^{13}C NMR (DMSO-d_6) data, see table 2; EI-MS (70 eV): *m/z* (%) 318 (100), 286 (2), 153 (5), 137 (10); FAB-MS *m/z* 935 $[M + H]^+$, 789 $[M + H\text{-}146]^+$, 643 $[M + H\text{-}2 \times 146]^+$, 481 $[M + H\text{-}2 \times 146\text{–}162]^+$, 319 $[M + H\text{-}2 \times 146\text{-}2 \times 162]^+$, 309 $[Glc + Rha + H]^+$, 163 $[Glc + H]^+$, 153, 147 $[Rha + H]^+$, 137; HR-MS *m/z* 934.2591 (calcd for $C_{39}H_{50}O_{26}$, 934.2512).

3.3.8 Isorhamnetin 3-*O*-rutinoside-7-*O*-rutinoside-4′-*O*-β-glucoside (8). Yellow amorphous powder from MeOH-H_2O with mp ~250°C; $[\alpha]_D^{25}$ − 19 (*c* 1.5, MeOH); UV (MeOH) λ_{max} 266, 301, 355; + $AlCl_3$: 266, 314, 352, 400; + $AlCl_3$/HCl: 267, 309, 357, 402; + NaOMe: 269, 365, 403; + NaOAc:280, 317, 375; + NaOAc/H_3BO_3: 280, 314, 355 nm; ^{1}H NMR (DMSO-d_6) δ: 12.70 (s, OH-5, D_2O exchang.), 7.76 (d, $J = 2.4$ Hz, H-2′), 7.65 (d, $J = 7.6$, 2.6 Hz, H-6′), 6.80 (d, $J = 7.5$ Hz, H-5′), 6.40 (dd, $J = 2.2$ Hz, H-8),

Table 2. ^{13}C NMR of compounds **6**–**8**.

Carbon	***6***	***7***	***8***
1			
2	156.4	156.6	156.2
3	134.7	133.5	134.5
4	177.8	177.7	177.5
5	162.5	161.8	160.6
6	99.8	98.9	99.2
7	164.3	164.0	163.2
8	94.8	93.7	94.7
9	157.8	157.0	156.9
10	104.6	104.1	105.8
1′	121.8	122.0	122.1
2′	108.0	108.1	114.0
3′	146.6	147.2	149.0
4′	138.7	137.9	148.3
5′	146.6	147.2	116.0
6′	108.0	108.1	122.6
1″	105.4	104.5	106.1
2″	74.3	75.4	74.3
3″	76.4	77.1	77.5
4″	69.7	70.1	69.9
5″	73.6	76.0	76.5
6″	68.8	66.6	67.2
1‴	99.8	99.0	100.1
2‴	71.60	70.7	70.3
3‴	70.0	70.1	70.5
4‴	72.0	72.3	72.0
5‴	68.2	68.6	69.0
6‴	17.8	17.9	18.2
1⁗	101.5	102.5	103.0
2⁗	74.2	74.6	75.2
3⁗	76.7	76.7	77.0
4⁗	70.2	69.3	70.5
5⁗	75.9	76.2	76.9
6⁗	67.4	67.4	68.10
1⁗′	99.9	100.1	99.3
2⁗′	70.0	70.6	70.7
3⁗′	70.9	70.1	71.2
4⁗′	72.4	71.9	72.3
5⁗′	68.8	69.0	69.5
6⁗′	18.0	18.3	18.2
1⁗″			101.1
2⁗″			74.4
3⁗″			77.7
4⁗″			70.0
5⁗″			76.4
6⁗″			61.3
3′-OMe	56.8		57.7

6.21 (d, $J = 2.2$ Hz, H-6), 3.60 (s, OMe-3); 3-*O*-glucose: 5.53 (d, $J = 7.7$ Hz, H-1″), 3.70 (m, H-6″$_a$), 3.65 (m, H-3″), 3.51 (m, H-2″), 3.48 (m, H-6″$_b$), 3.45 (m, H-5″), 3.30 (m, H-4″); 6″-*O*-rhamnose: 4.46 (d, $J = 1.0$ Hz, H-1‴), 3.65 (m, H-3‴), 3.51 (m, H-2‴), 3.45 (m, H-5‴), 3.22 (m, H-4‴), 1.03 (d, $J = 6.4$ Hz, Me-6‴); 7-*O*-glucose: 5.45 (d, $J = 7.5$ Hz, H-1⁗), 3.57 (m, H-6⁗a), 3.61 (m, H-5⁗), 3.50 (m, H-4⁗), 3.33 (m, H-6⁗b), 3.29 (m, H-2⁗), 3.27 (m, H-3⁗); 6⁗-*O*-rhamnose: 4.50 (d, $J = 1.3$ Hz, H-1⁗′), 3.48 (m, H-3⁗′), 3.40 (m, H-5⁗′), 3.25 (m, H-2⁗′), 3.18 (m, H-4⁗′), 1. 0 (d, $J = 6.4$ Hz, Me-6⁗′); 4′-O-glucose: 5.35 (d, $J = 7.4$ Hz, H-1⁗″), 3.75 (m, H-6⁗″a), 3.63 (m, H-6⁗″$_b$), 3.60 (m, H-2⁗″), 3.52 (m, H-4⁗″), 3.44 (m, H-5⁗″), 3.32 (m, H-3⁗″); ^{13} C NMR data, see table 1; EI-MS (70 eV):

m/z (%) 316 (100), 153 (13), 151 (6), 43 (20); FAB-MS *m/z* 1095 $[M + H\text{-}146]^+$, 803 $[M + H\text{-}2 \times 146]^+$, 641 $[M + H\text{-}162\text{-}2 \times 146]^+$, 479 $[M + H\text{-}2 \times 162\text{-}2 \times 146]^+$, 317 $[M + H\text{-}2 \times 146\text{-}3 \times 162]^+$, 309 $[Glc + Rha + H]^+$, 163 $[Glc + H]^+$, 147 $[Rha + H]^+$; HR-MS *m/z* 1094.3326 (calcd for $C_{46}H_{62}O_{30}$, 1094.3248).

3.3.9 8-Acetylharpagide (9). Colourless crystals from CH_2Cl_2-MeOH mixture, mp 227–229°C; UV (MeOH) λ_{max} 201 ($\log \varepsilon$ 3.1) nm; IR (KBr) ν_{max} 3500–3100 (OH), 1730 (ester), 1670, 1040–1010 (C—O) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ: 5.40 (d, $J = 6.2$ Hz, H-3), 5.43 (d, $J = 1.0$ Hz, H-1), 4.86 (dd, $J = 6.2$, 1.8 Hz, H-4), 4.02 (d, $J = 4.2$ Hz, H-6), 2.86 (d, $J = 1.4$ Hz, H-9), 2.24 (d, $J = 15.7$ Hz, H-7β), 2.03 (dd, $J = 15.7$, 4.6 Hz, H-7α), 1.97 (s, OAc-8), 1.39 (s, Me-10); 1-*O*-glucose: 4.65 (d, $J = 7.6$ Hz, H-1′), 3.98 (dd, $J = 12.4$, 2.4 Hz, H-6′a), 3.77 (dd, $J = 12.4$, 6.1 Hz, H-6′b), 3.54 (m, H-3′), 3.48 (m, H-5′), 3.40 (m, H-4′), 3.30 (m, H-2′); ^{13}C NMR ($CDCl_3$ + DMSO-d_6) data, see table 1; FAB-MS *m/z* 407 $[M + H]^+$, 346, 317, 297, 209, 184, 167, 163 $[Glc + H]^+$, 157, 149, 137, 113, 97, 85, 73, 61, 43, 41.

3.4 Acid hydrolysis

Compounds **1**–**8**, each 15 mg in a mixture of 8% HCl (2 ml) and MeOH (20 ml), were separately heated under reflux for 2 h. The reaction mixtures were reduced under pressure to dryness, dissolved in H_2O (3 ml) and neutralised with NaOH. The neutralised products were then subjected to TLC (eluent: EtOAc/MeOH/H_2O/HOAc, 6:2:1:1) and PC (eluent: n-BuOH/HOAc/H_2O, 4:5:1). The chromatograms were sprayed with aniline hydrogen phthalate followed by heating at 100°C. The sugars were identified after comparison with authentic samples.

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