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Phytochemistry 67 (2006) 830–837
PHYTOCHEMISTRY
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Flavonol and iridoid glycosides of *Ajuga remota* aerial parts

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Received 29 July 2005; received in revised form 2 January 2006
Available online 9 March 2006

Abstract

Six flavonol glycosides characterised as myricetin 3-*O*-α-rhamnosyl-(1‴ → 2″)-α-rhamnoside-3′-*O*-α-rhamnoside, 5′-*O*-methylmyricetin 3-*O*-[α-rhamnosyl (1‴ → 2″)][α-rhamnosyl (1‴′ → 4″)]-β -glucoside-3′-*O*-β-glucoside, 5′-*O*-methylmyricetin 3-*O*-α-rhamnosyl (1‴ → 2″)-α-rhamnoside 3′-*O*-β-galactoside, kaemferol 3-*O*-rutinoside-7-*O*-rutinoside, myricetin 3-*O*-rutinoside-3′-*O*-α-rhamnoside, myricetin 3-*O*-β-glucosyl (1‴ → 2″)-β-glucoside-4′-*O*-β-glucoside together with two iridoid glycosides identified as 6,8-diacetylharpagide and 6,8-diacetylharpagide-1-*O*-β-(3′,4′-di-*O*-acetylglucoside) have been isolated from extract of *Ajuga remota* aerial parts. Also isolated from the same extract were known compounds; kaempferol 3-*O*-α-rhamnoside, quercetin 3-*O*-β-glucoside, quercetin 3-*O*-rutinoside, 8-acetylharpagide, ajugarin I and ajugarin II.

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

1. Introduction

Ajuga remota Benth (Labiatae) is a short-lived perennial plant widely distributed in various parts of Kenya (Beentje, 1994; Dale and Greenway, 1961). The plant is commonly used in indigenous system of medicine as antibacterial and anti-malarial (Kokwaro, 1976). Therefore, *A. remota* has been subjected to many chemical investigations (Cantrell et al., 1999; Ley et al., 1983; Kubo et al., 1976, 1980; Kubo et al., 1983a,b; Kuria et al., 2002). In the present work, the defatted aqueous methanolic extract of the plant aerial parts was subjected to phytochemical investigation leading to the isolation of eight novel compounds; two iridoid glycosides (**1** and **2**) and six new flavonol glycosides (**3**–**8**). Together with these were known compounds; 8-acetylharpagide (**9**), kaempferol 3-*O*-α-rhamnoside (**10**), quercetin 3-*O*-β-glucoside (**11**), quercetin 3-rutinoside (**12**), ajugarin I (**13**) and ajugarin II (**14**) (Kuria et al., 2002; Manguro Arot et al., 2003, 2004). Their structures were established by spectroscopic means and also by comparison with closely related compounds.

2. Results and discussion

Compound **1**, colourless crystals exhibited IR absorption bands due to hydroxyl (3500 cm^{-1}), acetate (1734 cm^{-1}) and a double bond (1650 cm^{-1}). Its ^{1}H NMR data were similar to those of 8-acetylharpagide (**9**) (Belofsky et al., 1989; Kuria et al., 2002) except for the presence of an additional acetoxy group as evidenced by a singlet at δ 2.10. This was further supported by ^{13}C NMR spectrum, which displayed a total of 18 carbon atoms, their multiplicity assignments using DEPT established the presence of three methyl, two methylene, 10 methine and four quaternary carbons. The foregoing evidences in combination with HRMS $[M]^{+}$ peak at *m*/*z* 448.1581 ($C_{19}H_{28}O_{12}$) confirmed 6,8-diacetylharpagide. Acid hydrolysis yielded glucose as the sugar residue

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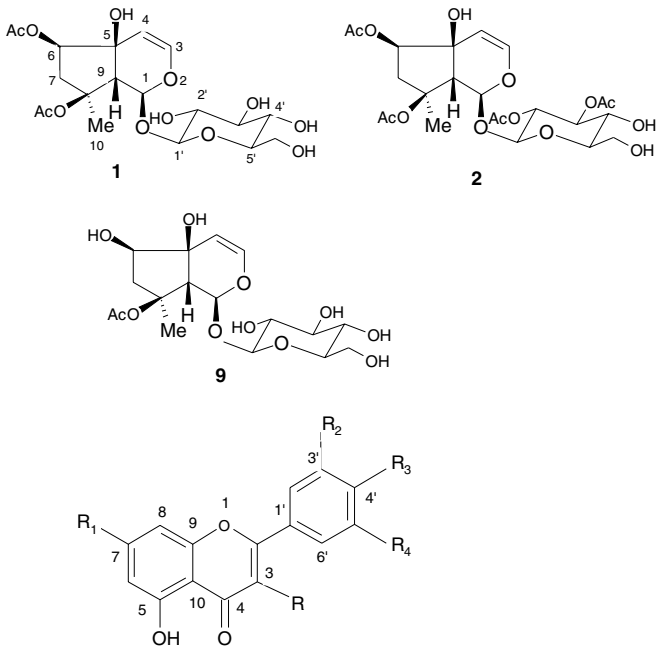
doi:10.1016/j.phytochem.2006.01.005



3 R = *O*-α-rhamnosyl-(1‴→2″)-α-rhamnoside, R_2 = *O*-α-rhamnoside, $R_1 = R_3 = R_4$ = OH.

4 R = *O*-[α-rhamnosyl-(1‴→2″)][α-rhamnosyl-(1⁗→4″)]-β-glucoside, R_2 = *O*-β-glucoside, R_4 = OMe, $R_1 = R_3$ = OH.

5 R = *O*-α-rhamnosyl- (1‴→2″)-α-rhamnoside, R_2 = *O*-β-galactoside, R_4 = OMe, $R_1 = R_3$ = OH.

6 R = *O*-rutinoside, R_1 = *O*-rutinoside, R_3 = OH, $R_2 = R_4$ = OH.

7 R = *O*-rutinoside, R_2 = *O*-α-rhamnoside, $R_1 = R_3 = R_4$ = OH.

8 R = *O*-β-glucosyl-(1‴→2″)-β-glucoside, R_3= *O*-β-glucoside, $R_1 = R_2 = R_4$ = OH.

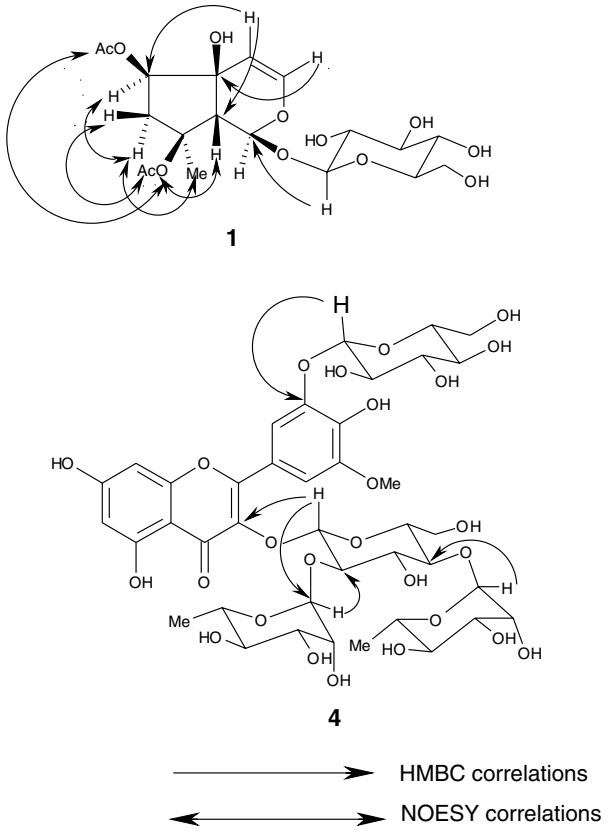


Fig. 1. Significant correlations in the HMBC and NOESY spectra of compounds **1** and **4**.

identified by co-chromatography with authentic sample. The large coupling constant of the anomeric proton (*d*, $J = 7.6$ Hz) indicated that glucose unit was present in the β-configuration.

The positions of the acetoxy groups and the primary glucose on the aglycone were established from HMQC and HMBC experiments, which indicated one-bond H, C- and two or three-bond H, C-correlations, respectively (Fig. 1). In this way, it was proved that the glucose unit was attached glycosidically to C-1 while the two-acetoxy groups were at C-6 and C-8 of the aglycone, respectively, a fact that was further evidenced by NOESY correlations as outlined in Fig. 1. Thus, on the basis of spectroscopic data, compound **1** was concluded to be 6,8-diacetylharpagide.

Compound **2** on acid hydrolysis afforded glucose as the sugar residue (identified by TLC and PC co-chromatography with authentic sample). Its NMR depicted data resembling those of **1** except for additional two acetoxy groups (Takeda et al., 1987; Shimomura et al., 1987). Accordingly, the ^{13}C NMR spectrum displayed 23 signals including, as determined from DEPT spectrum five methyl, two methylene, 10 methine and six quaternary carbon atoms, which together with the HRMS molecular peak at *m*/*z* 532.1992 $[M]^+$ ($C_{23}H_{32}O_{14}$) confirmed that the compound is harpagide tetraacetate. Careful examination of the ^{1}H NMR data indicated that C-5 was hydroxylated as evidenced by a doublet at δ 2.84 (H-9) and the absence of long range coupling to H-3. On the other hand, the unshielded C-8 methyl at δ 1.36 and the presence of an acetoxy group at δ 2.04 suggested the placement of the acetate group at C-8 (Belofsky et al., 1989; Shimomura et al., 1987). Also from ^{1}H NMR decoupling experiments, the relatively low field doublet at δ 5.30 assigned to H-6 indicated that the OH group at C-6 was acetylated. In fact, the chemical shift values for the aglycone were in good agreement with those of 6,8-diacetylharpagide (**1**). This led to the conclusion that the two remaining acetyl groups were attached to the sugar residue, a fact corroborated by diagnostic downfield shifts for H-2′ (δ 5.02) and H-3′ (δ 5.10) and further confirmed by ^{13}C NMR downfield shifts for the C-3′ (+2.3 ppm) and C-2′ (+2.4 ppm) and the up field shifts for the C-1′ (−2.0 ppm) and C-4′ (−2.2 ppm) in comparison with homologous carbons of 8-acetylharpagide (Kuria et al., 2002). The proton–proton correlation spectrum also aided in further confirming the substitution pattern on the glucose moiety; whereby the peaks at δ 5.02 (H-2′) was shown to couple with a multiplet at δ 5.10 (H-3′), the latter in turn was further coupled to another multiplet at δ 3.50 (H-4′). The H-1″ collapsed to a singlet when H-2′ (δ 5.02) was irradiated. Thus, **2** was concluded to be 6,8-diacetylharpagide 1-*O*-(2′,3′-diacetyl-β-glucoside).

Isolated along with the iridoid glycosides from the same extract were flavonol glycosides (**3**–**8**).

Compound **3** UV spectrum in MeOH and its changes after addition of shift reagents (Mabry et al., 1970; Markham, 1982) suggested the presence of free hydroxyl groups at C-5, C-7, C-4′ and C-5′. Acid hydrolysis afforded rhamnose and myricetin (identified by TLC and PC co-chromatography with authentic samples). In the positive ion FAB mass spectrum, **3** exhibited a $[M+H]^+$ peak at *m/z* 757 consistent with the formula $C_{33}H_{40}O_{20}$, which together with other prominent fragments at *m/z* 611 $[M+H-146]^+$, 465 $[M+H-2\times146]^+$, 319 $[M+H-3\times146]^+$ suggested that the compound is myricetin triglycoside. This was supported by the ^{1}H NMR spectrum three anomeric protons at δ 5.20 (*d*, $J=1.2$ Hz), 5.05 (*d*, $J=1.1$ Hz) and 4.30 (*d*, $J=1.1$ Hz). Both the FAB-MS and ^{1}H NMR results indicated that two rhamnose units were attached to myricetin while the third was present as a terminal one. The locations of the sugar moieties on myricetin was provided by the ^{13}C NMR (Table 2) in comparison with myricetin, which showed the shielding of both C-3 (δ 133.6) and C-3′ (δ147.0) and the deshielding of C-4 (δ 177.7), C-2 (δ 156.5), C-2′ (δ 107.1) and C-4′ (δ 137.8) (Arya et al., 1992; Mahmoud et al., 2001), confirmed by HMBC correlations between the anomeric proton at δ 5.20 with the carbon at δ 133.6 (C-3) and between the anomeric proton at δ 5.05 with the carbon at δ 147.0 (C-3′). The ^{13}C NMR, the ^{1}H-^{13}C COSY and HMBC studies were used in determining the rhamnose–rhamnose linkage and the sequence of the bioside. In the ^{13}C NMR spectrum, the primary rhamnose at C-3 was shown to be linked to a terminal one through $1''' \rightarrow 2''$ bond on the basis of rhamnosyl C-2″ downfield shift at δ 80.4 (Braca et al., 1999; Cimanga et al., 1997). The ^{1}H–^{13}C COSY studies correlated the rhamnosylated carbon at δ 80.4 with the C-2″ linked proton (δ 3.90). The HMBC spectrum confirmed the foregoing evidences by exhibiting a cross peak between C-2″ (δ_C 80.4 and H-1″ (δ 4.30). Based on the above evidences, compound **3** was characterized as myricetin 3-*O*-α-rhamnosyl ($1''' \rightarrow 2''$)-α-rhamnoside-3′-*O*-α-rhamnoside.

Compound **4** on acid hydrolysis afforded rhamnose and glucose as sugar residues (similarly identified by TLC and PC co-chromatography with authentic samples). Its ^{1}H NMR and UV depicted data typical of 5′-*O*-methylmyricetin (Arya et al., 1992; Braca et al., 1999; Mabry et al., 1970) containing two glucosyl and two rhamnosyl residues, a fact supported by the HRMS $[M]^+$ peak at *m/z* 948.2747 ($C_{40}H_{52}O_{26}$). Accordingly, the ^{13}C NMR spectrum displayed 40 signals (Table 2), which were resolved into 3 methyl, 2 methylene, 24 methine and 11 quaternary carbon atoms by DEPT. The linkage positions of sugar units to the aglycone were substantiated by HMBC spectrum, in which H-1″ (δ 5.40) of glucose and H-1′′′′′ (δ 5.20) of another glucose correlated with C-3 (δ 133.1) and C-3′ (δ 149.5) of the aglycone through three bond H,C-couplings, respectively. Similarly, the anomeric proton signals of rhamnoses at δ 4.50 ($J=1.4$ Hz, H-1′′′) and 4.60 ($J=1.2$ Hz, H-1′′′′) exhibited three bond H, C-cross peaks with C-2″ (δ 83.2) and C-4″ (δ 82.1) carbon resonances of glucose, respectively (Fig. 1). Thus, the spectral data indicated the presence of [α-rhamnosyl ($1''' \rightarrow 2''$)][α-rhamnosyl ($1'''' \rightarrow 4''$)]-β-glucosyl and glucosyl at position C-3 and C-3′ of the aglycone, respectively. This was further supported by the similarities of the relevant ^{1}H, ^{13}C and HMBC data which were in agreement with those reported for kaempferol 7-*O*-[α-rhamnosyl ($1''' \rightarrow 2''$)][α-rhamnosyl ($1'''' \rightarrow 4''$)]-β-glucosyl (Cimanga et al., 1997; Markham et al., 1992; Curir et al., 2001). From the above spectroscopic data, the structure of **4** was deduced to be 5′-*O*-methylmyricetin 3-*O*-[α-rhamnosyl ($1''' \rightarrow 2''$)][α-rhamnosyl ($1'''' \rightarrow 4''$)]-β-glucoside-3′-*O*-β-D-glucoside.

Compound **5**, a yellow powder exhibited IR absorption bands due to hydroxyl (3500 cm^{-1}), α,β-unsaturated ketone (1650 cm^{-1}) and C–O bond (1090–1030 cm^{-1}). Its ^{1}H NMR spectrum exhibited A and B ring signals typical of 5′-*O*-methylmyricetin 3,3′-di-*O*-substituted glycosides (Arya et al., 1992; Mizuno et al., 1992). This was further supported by UV spectrum in MeOH and with diagnostic customary shift reagents (Mabry et al., 1970), suggesting free OH groups at C-5, C-7 and C-4′, confirmed by ^{1}H NMR peaks at δ 12.60 (OH-5), 10.50 (OH-7 and 8.40 (OH-4′), all D_2O exchangeable. Acid hydrolysis afforded rhamnose and galactose as the sugar residues as inferred by TLC and PC co-chromatography with authentic samples. The positive FAB-MS molecular ion peak at *m/z* 787 $[M+H]^+$ ($C_{34}H_{42}O_{21}$) together with other prominent peaks 641 $[M+H-146]^+$, 479 $[M+H-146–162]^+$, 333 $[M+H-2\times146–162]^+$ were consistent with the presence of two rhamnoses and a galactose in 5′-*O*-methylmyricetin residue, thus the fragmentation sequence suggested the presence of a terminal rhamnose. The ^{1}H and ^{13}C NMR chemical shift values indicated that the primary rhamnose and galactose are linked to the aglycone at C-3 and C-3′, respectively, a conclusion confirmed by the HMBC correlations; between rhamnose H-1″ (δ 5.40) with C-3 (δ 134.6) and between galactose H-1′′′′ (δ 5.10) with C-3′ (δ 147.1). The interglycosidic linkage and the sequence of the rhamnosylrhamnosyl bioside was determined using ^{1}H, ^{13}C NMR, ^{1}H–^{13}C COSY and HMBC.

In the ^{1}H NMR spectrum, the anomeric protons of an inner and terminal rhamnoses were observed at δ 5.40 ($J=1.2$ Hz) and 4.60 (*d*, $J=1.0$ Hz), respectively; in the ^{1}H–^{13}C COSY, a proton signal at δ 4.30 in a multiplet assigned to H-2″ of primary rhamnose correlated with the C-2″ carbon (δ 81.7); the HMBC experiments confirmed the rhamnose moieties linkage by showing correlation between the signal at δ 81.7 (C-2″) of inner rhamnose with terminal rhamnose anomeric proton (δ 4.60). Hence, **5**, was concluded to be 5′-*O*-methylmyricetin 3-*O*-rhamnosyl ($1''' \rightarrow 2''$)-α-rhamnoside-3′-*O*-D-galactoside.

Compound **6** afforded UV data which were in accord with 3,7-di-*O*-substituted flavonol (Mabry et al., 1970), a fact supported by the ^{13}C NMR spectrum data typical of kaempferol 3,7, di-*O*-substituted glycosides (Norbaek

et al., 1999). Acid hydrolysis released kaempferol, glucose and rhamnose identified by co-chromatography with authentic samples. Four ^{1}H NMR anomeric proton signals observed at δ 5.50 (δ, $J = 7.6$ Hz), 5.38 (*d*, $J = 7.5$ Hz), 4.60 (*d*, $J = 1.1$ Hz) and 4.45 (*d*, $J = 1.0$ Hz), together with two other doublets at δ 1.13 and 1.11 assigned to rhamnose methyls indicated that the compound is kaempferol tetraglycoside, possibly with the rhamnoses as terminal sugars. The presence of two methyl, two methylene, 26 methine and 9-quaternary carbon atoms in the DEPT, in combination with the HRMS molecular ion peak at *m/z* 902.2701[M]$^+$established $C_{39}H_{50}O_{24}$ formula, thus confirming kaempferol tetraglycoside with two glucose and two rhamnose units. The rhamnosyl sugars were linked to glucose through $1 \rightarrow 6$ bond as evidenced by the glucosyls C-6 downfield shifts of approximately +6.0 ppm, a fact further corroborated by the HMBC correlations of the rhamnosyls H-1 to the various glucosyl C-6. The data indicated that compound **6** is kaempferol 3-*O*-rutinoside-7-rutinoside.

Compound **7**, analysed for $C_{33}H_{40}O_{21}$, HRMS *m/z* 772.2154 [M]$^+$, exhibited UV absorption maxima at 260 and 354 nm in methanol and with shift reagents ($AlCl_3$, $AlCl_3$–HCl, NaOAC–H_3BO_3, NaOMe) it showed free OH groups at C-5, C-7 and an *ortho*-dihydroxyl system in the ring B suggesting glycosylation at C-3. A careful comparative analysis of the effect of NaOMe upon the aglycone and the glycoside suggested the presence of a sugar moiety in ring B (Mabry et al., 1970). Furthermore, the glycoside was fairly stable to NaOMe while the aglycone rapidly decomposed and this could be attributed to the presence of alkali sensitive 3′,4′,5′-hydroxylation on the aglycone (Mabry et al., 1970). On account of these data, compound **7** was suggested to be myricetin 3,3′-di-*O*-glycoside. The identity of the aglycone was evidenced as myricetin on the basis of EIMS (70 eV) fragment ion at *m/z* 318 (100%), a fact further supported by the ^{1}H NMR singlet at δ 7.30 (2H) and two meta-coupled doublets at δ 6.30 and 6.16, respectively.

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 rhamnosyls and a glucosyl at δ 5.24 (*d*, $J = 1.3$ Hz), 4.50 (*d*, $J = 1.4$ Hz) and 5.40 (*d*, $J = 7.7$ Hz), respectively. The relatively high field rhamnosyl proton at δ 4.50 suggested that it is not attached directly to the aglycone, thus there is sugar–sugar linkage (Mahmoud et al., 1989). The localisation of the sugars on the aglycone was provided by HMBC as evidenced by cross peaks between glucosyl H-1″ (δ 5.40) with C-3 (δ 133.5), and between rhamnosyl H-1‴′ (δ 5.24) with C-3′ (δ 147.7). Therefore, the rhamnosylglucosyl bioside was at C-3 of the aglycone. The position of interglycosidic linkage was provided by ^{13}C NMR down field shift of C-6″ at δ 67.7, a fact confirmed by HMBC experiments. Thus, based on the above chemical and spectroscopic considerations, **7** was characterised as myricetin 3-*O*-rutinoside-3′-*O*-α-rhamnoside.

Compound **8**, a yellow amorphous powder from aqueous MeOH, on acid hydrolysis yielded glucose as the sugar residues (identified by TLC and PC after comparison with authentic samples). Its positive ion FAB-MS molecular ion peak *m/z* 805 $[M + H]^+$ ($C_{33}H_{40}O_{23}$), together with other significant fragments at *m/z* 643 $[M - 162 + H]^+$, 481 $[M - 2 \times 162 + H]^+$, and 319 $[M - 3 \times 162 + H]^+$ suggested that the compound is myricetin triglycoside. This was further supported by the ^{1}H NMR spectrum diagnostic signals for the three anomeric protons at δ 5.36 (*d*, $J = 7.5$ Hz), 5.16 (*d*, $J = 7.2$ Hz), 4.63 (*d*, $J = 7.4$ Hz) and the aglycone peaks at δ 7.28 (*s*, H-2′ and H-6′), 6.36 (*d*, $J = 2.1$ Hz, H-8) and 6.20 (*d*, $J = 2.1$ Hz, H-6). Both the FAB-MS and ^{1}H NMR results indicated that glucose residue is a terminal sugar, glucosylglucose bioside. The UV spectrum in MeOH and its changes after addition of shift reagents suggested that free OH groups are at positions C-5, C-7, C-3′ and C-5′, a fact corroborated by the ^{1}H NMR spectrum peaks at δ 12.57 (OH-5) and 10.45 (*s*, OH-7, D_2O exchangeable), 9.30–8.20 (OH-3′, 5′), both disappeared upon D_2O addition. Further evidence for the localization of the sugar moieties on the aglycone was provided by the ^{13}C NMR (Table 2) of the compound in comparison with myricetin, which showed the shielding of both C-3 (δ 134.0) and C-4′ (δ 139.3) and deshielding for C-4 (δ 177.1), C-2 (δ 156.2), C-3′ (δ 145.0) and C-5′ (δ 146.0), thus indicating glycosylation sites were at C-3 and C-4′. Support for this was provided by the HMBC correlations between the aglycone carbon at δ 139.3 (C-4′) and the glucose anomeric proton at δ 5.16 (H-1‴′), and between glucose anomeric proton at δ 5.36 (H-1″) with the carbon at δ 134.0 (C-3). The ^{13}C and ^{1}H–^{13}C COSY studies gave unambiguous correlations which were used in determining the interglycosidic linkage. In the ^{13}C NMR spectrum the inner glucose was shown to be linked to the terminal one through $1''' \rightarrow 2''$ bond on the basis of rhamnosyl C-2″ downfield shift at δ 83.2, indicating the bioside is glucosyl($1''' \rightarrow 2''$)-glucoside. The ^{1}H–^{13}C COSY studies confirmed the correlation between the identified protons with their carbon signals. The glucosylated carbon at δ 83.2 correlated unambiguously with C-2″ glucose proton at δ 3.46. In the NOESY spectrum, H-2″ (δ 3.46) of glucose unit showed cross peak with the anomeric proton of terminal glucose unit at δ 4.63. The HMBC correlation spectrum confirmed the foregoing evidences and correlation peaks between δ_H 5.36 and δ_C 134.0 (C-3); between the anomeric proton δ_H 4.63 and δ_C 83.2 (C-2″) and between δ_H 5.16 with signal at δ_C 139.3 (C-4′) were observed. This was further supported by the near identity of the relevant ^{1}H, ^{13}C NMR and HMBC which were in good agreement with kaempferol 3-*O*-β-glucosyl($1 \rightarrow 2$)-glucoside-7-*O*-β-glucoside (Nielsen et al., 1998). Based on the above evidences compound **8** was characterised as myricetin 3-*O*-β-glucosyl-($1''' \rightarrow 2''$)-β-glucoside-4′-*O*-β-glucoside.

3. Experimental

3.1. General experimental procedures

The UV and IR data were recorded on Hewlett–Packard Array 8452 A spectrophotometer and Perkin–Elmer FT-IR 600 series, respectively. The FAB mass spectra were obtained on a VG ZABSPEC instrument. The NMR data were taken in DMSO-d_6 and $CDCl_3$-DMSO-d_6 on a Bruker NMR Advance Ultrashield TM spectrometer operating at 500 and 125 MHz. Preparative high-performance liquid chromatography (HPLC) was done on a Bischoff instrument connected to 785 A programmable monitor 8252 dual pen recorder. EIMS and CIMS data were obtained on a 70 eV MAT 8200 A Varian Bremen instrument. Silica gel for column and TLC plates were impregnated with 2% oxalic acid solution.

3.2. Plant material

The aerial parts of *Ajuga remota* were collected in Doonholm estate, Embakasi division, Nairobi in the month of August 2002. A voucher specimen (No. 2002/8 NMU) was identified after comparison with authentic sample.

3.3. Extraction and isolation

Powdered dry material approximately 5 kg was extracted in the cold with MeOH (7.5 l × 3) for a period of one and a half weeks. The resulting extracts were combined and solvent reduced under pressure to give a dark green residue (350 g), which was suspended in H_2O and then successively defatted with dichloromethane. The H_2O layer was freeze dried affording a dark green solid material 105 g. A portion of this (100 g) was pre-adsorbed on silica gel and applied on a 400 g silica gel packed column, elution done sequentially with CH_2Cl_2–MeOH (5% increments of MeOH) and finally with MeOH. Two hundred and seventy three fractions (each 20 ml) were sampled and their composition monitored by TLC (eluent: CH_2Cl_2–MeOH, 9:1 and 1:1), with those showing similar TLC profiles grouped into five pools (I–V). Pool I (fractions 1–25, 17.6 g) was further purified by repeated flash chromatography using eluent CH_2Cl_2–MeOH (19:1) followed by the same solvent system (9:1) to afford ajugarin I (**13**) 600 mg, ajugarin II (**14**) 150 mg, 8-acetylharpagide (**9**) 100 mg, 6,8-di-*O*-acetylharpagide (**1**) 75 mg and 6,8-di-*O*-acetylharpagide 1-*O*-(2′,3′-di-*O*-acetylglucoside) (**2**) 52 mg. Fractions 26–78 (Pool II, 15.5 g) was similarly subjected to repeated flash chromatography; eluent CH_2Cl_2–MeOH (9:1) followed by the same solvent in the ratio 4:1, collecting 20 ml each to give kaempferol 3-*O*-α-rhamnoside (**10**) 85 mg, quercetin 3-*O*-β-glucoside (**11**) 105 mg.

Pool III (fractions 79–163, 6.7 g) upon further purification as described above using CH_2Cl_2–MeOH (4:1) followed by the same solvent in the ratio 7:3, collecting 10 ml each gave quercetin 3-rutinoside (**12**) 100 mg and myricetin 3-*O*-α-rhamnosyl (1‴ → 2″)-α-rhamnoside-3′-*O*-α-rhamnoside (**3**) 63 mg. Fractions 163–225 (Pool IV, 8.4 g) contained mainly two components which were resolved using flash chromatography; eluent CH_2Cl_2–MeOH (2:1) into 5′-*O*-methylmyricetin 3-*O*-[α-rhamnosyl (1‴ → 2″)][α-rhamnosyl (1⁗ → 4″)]-β-glucoside-3′-*O*-β-glucoside (**4**) 55 mg and 5′-*O*-methylmyricetin 3-*O*-α-rhamnosyl (1‴ → 2″)-α-rhamnoside-3′-*O*-β-galactoside (**5**) 81 mg. Pool V (fraction 226–273, 20 g) mainly from methanol elution was further subjected to medium pressure chromatography with CH_2Cl_2–MeOH mixture of increasing polarity of the latter to give 100 fractions of 20 ml each. The eluates 20–88 were found to contain three components which were purified by preparative HPLC reverse phase using MeOH–H_2O (9:1) followed by crystallization in aqueous MeOH to give kaempferol 3-*O*-rutinoside-7-*O*-rutinoside (**6**) 43 mg, myricetin 3-*O*-rutinoside-3′-*O*-α-rhamnoside (**7**) 37 mg and myricetin 3-*O*-β-glucosyl- (1‴ → 2″)-glucoside-4′-*O*-β-glucoside (**8**) 51 mg, respectively.

3.4. 6,8-diacetylharpagide (*1*)

Amorphous colourless powder, m.p. 166–168 °C $[\alpha]_D^{25}$ + 65° (MeOH, c 0.5). UV λ_{max} (MeOH) nm: 203 (log ε 3.5). IR ν_{max} (KBr) cm^{-1}: 3500 (OH), 1734 (acetate), 1650, 1030–1010 (C–O). ^1H NMR (DMSO-d_6 + $CDCl_3$) δ: 6.53 (*d*, *J* = 6.6 Hz, H-3), 5.0 (*dd*, *J* = 6.6, 1.7 Hz, H-4), 5.54 (*d*, *J* = 1.0 Hz, H-1), 5.23 (*d*, *J* = 4.5 Hz, H-6), 2.86 (*m*, H-9), 2.20 (*d*, *J* = 15.6 Hz, H-7β), 2.10 (*s*, OAc-6), 2.00 (*dd*, *J* = 15.6, 4.0 Hz, H-7α), 1.98 (*s*, OAc-8), 1.35 (*s*, Me-10), 1-*O*-glucose: 4.70 (*d*, *J* = 7.6 Hz, H-1′), 3.94 (*dd*, *J* = 13.0, 1.8 Hz, H-6′a), 3.80 (*dd*, *J* = 13.6, 5.5 Hz, H-6′b), 3.60 (*m*, H-3′), 3.52 (*m*, H-5′), 3.39 (*m*, H-4′), 3.28 (*m*, H-2′). ^{13}C NMR (DMSO-d_6 + $CDCl_3$) data: see Table 1. FABMS: *m/z* 449 $[M + H]^+$, 389 $[M + H - HOAc]^+$, 329 $[M + H - 2 \times HOAc]^+$, 297, 210, 184, 168, 155, 149, 137, 113, 97, 85. HRMS *m/z*: 448.1581 $[M]^+$, calc. for $[C_{19}H_{28}O_{12}]^+$, 448.1503.

3.5. 6,8-diacetylharpagide 1-O-β-(2′,3′-diacetylglucoside) (*2*)

Colourless crystals from CH_2Cl_2–MeOH mixture, m.p. 174–176 °C; $[\alpha]_D^{25}$ + 92° (CH_2Cl_2, c 1.5). UV λ_{max} (MeOH) nm: 210 ((log ε 2.76). IR ν_{max} (KBr) cm^{-1}: 3500–3200 (OH), 1732 (acetate), 1650, 1025–1010 (C–O). ^1H NMR ($CDCl_3$ + DMSO-d_6) δ: 6.44 (*d*, *J* = 6.3 Hz, H-3), 5.56 (*d*, *J* = 1.1 Hz, H-1), 5.30 (*d*, *J* = 4.3 Hz, H-6), 4.94 (*dd*, *J* = 6.3, 1.3 Hz, H-4), 2.84 (*d*, *J* = 1.8 Hz, H-9), 2.30 (*d*, *J* = 15.5 Hz, H-7β), 2.14 (*dd*, *J* = 15.5, 1.2 Hz, H-7α), 2.15 (*s*, OAc-6), 2.04 (*s*, OAc-8), 1.36 (*s*, Me-10), 1-*O*-glucose: 5.10 (*t*, *J* = 9.0, H-3′), 5.02 (*t*, *J* = 8.8 Hz, H-2′), 4.60 (*d*, *J* = 7.8 Hz, H-1′), 4.01 (*dd*, *J* = 12.4, 2.2 Hz, H-6′a), 3.81 (*dd*, *J* = 12.4, 5.4 Hz, H-6′b), 3.55 (*m*, H-5′), 3.50 (*m*, H-4′), 2.01 (*s*, OAc-2′), 1.95 (*s*, OAc-3′). ^{13}C NMR ($CDCl_3$ + DMSO-d_6) data: see Table 1. FABMS positive ion mode *m/z* 533 $[M + H]^+$, 473

Table 1
^{13}C NMR of compounds **1**, **2** and **9**

Carbon	1	2	9
1	95.1	95.4	95.3
2			
3	144.2	143.7	145.5
4	108.0	107.2	107.7
5	74.5	73.3	75.0
6	79.1	80.0	79.8
7	47.6	46.9	47.4
8	90.7	89.0	89.8
9	55.9	55.2	56.0
10	23.5	22.8	23.9
1′	98.1	98.9	100.1
2′	77.9	74.0	75.4
3′	78.8	76.6	76.5
4′	72.6	71.2	74.4
5′	77.4	76.9	77.8
6′	62.8	62.4	63.0
AcO	170.1	169.8	170.0
	170.4	170.0	
		170.1	
		171.0	
Me-Ac	24.8	25.0	25.4
	25.2	25.4	
		24.9	
		25.0	

$[M + H - HOAc]^+$, 413 $[M + H - HOAc]^+$, 261, 209, 184, 167, 155, 149. HRMS *m/z*: 532.1992 $[M]^+$, calc. for $[C_{23}H_{32}O_{14}]^+$, 532.1924.

3.6. Myricetin 3-*O*-α-rhamnosyl (1‴ → 2″)-α-rhamnoside-3′-*O*-α-rhamnoside (*3*)

Pale yellow amorphous powder from aqueous methanol. UV λ_{max} (MeOH) nm: 260, 351; +$AlCl_3$: 272, 430; +$AlCl_3$/HCl: 270, 400; +NaOMe: 270, 396; +NaOAc: 269, 372; +NaOAc/H_3BO_3: 260, 371. IR ν_{max} (KBr) cm^{-1}: 3500 (OH), 1650 (α,β-unsaturated CO), 1605, 900, 860, 770. ^{1}H NMR ($CDCl_3$ + DMSO-d_6) *δ*. 12.50 (*s*, OH-5, D_2O exchangeable), 10.50 (*s*, OH-7, D_2O exchangeable), 9.00–8.50 (br *s*, OH-4′ and OH-5′, D_2O exchangeable), 7.26 (*d*, *J* = 1.9 Hz, H-2′ and H-6′), 6.42 (*d*, *J* = 2.2 Hz, H-8), 6.26 (*d*, *J* = 2.2 Hz, H-6), 3-*O*-rhamnose: 5.20 (*d*, *J* = 1.2 Hz, H-1″), 3.90 (*m*, H-2″), 3.75 (*m*, H-3″), 3.65 (*m*, H-5″), 3.45 (*m*, H-4″), 1.10 (*d*, *J* = 5.9 Hz, Me-6″); 2″-*O*-rhamnose: 4.30 (*d*, *J* = 1.1 Hz, H-1‴), 3.77 (*m*, H-2‴), 3.60 (*m*, H-3‴), 3.54 (*m*, H-5‴), 3.40 (*m*, H-4‴), 1.05 (*d*, *J* = 6.1 Hz, Me-6‴); 3′-*O*-rhamnose: 5.05 (*d*, *J* = 1.1 Hz, H-1⁗), 3.85 (*m*, H-2⁗), 3.71 (*m*, H-3⁗), 3.56 (*m*, H-5⁗), 3.30 (*m*, H-4⁗), 0.93 (*d*, *J* = 6.0 Hz, Me-6⁗). ^{13}C NMR ($CDCl_3$ + DMSO-d_6) data: see Table 2. EIMS (70 eV): *m/z* (%) 318 (100), 289 (11), 245 (2), 216 (8), 153 (10), 136 (15), 108 (3). FABMS positive ion *m/z*: 757 $[M + H]^+$, 611 $[M + H - Rha]^+$, 465 $[M + H - 2Rha]^+$, 319 $[M - 3Rha]^+$, 293 $[Rha + Rha + H]^+$, 165, 146 $[Rha]^+$, 137. HRMS *m/z*: 756.3013 $[M]^+$, calc. for $[C_{33}H_{40}O_{20}]^+$, 756.2935.

Table 2
^{13}C NMR of compounds **3**–**8**

Carbon	3	4	5	6	7	8
2	156.5	156.5	156.3	156.8	157.0	156.2
3	133.6	133.1	134.6	133.8	133.5	134.0
4	177.7	176.9	177.8	177.7	178.3	177.1
5	161.5	160.7	161.1	160.8	161.4	162.9
6	98.8	98.1	99.4	99.2	98.9	99.9
7	164.4	163.6	163.8	163.5	163.6	164.6
8	93.7	92.8	94.7	93.9	94.1	94.7
9	156.6	156.7	156.8	156.9	157.6	158.0
10	104.0	130.3	105.1	105.6	106.3	105.5
1′	122.2	119.3	121.0	122.0	122.4	121.8
2′	107.1	107.2	107.1	132.0	105.5	108.5
3′	147.0	149.5	147.1	115.8	147.7	145.0
4′	137.8	136.2	137.8	159.0	138.8	139.3
5′	148.7	146.8	146.8	116.0	149.1	146.0
6′	107.4	107.1	107.3	131.2	108.5	108.0
1″	102.6	101.1	101.3	103.7	105.2	103.8
2″	80.4	83.2	81.7	74.0	75.0	83.2
3″	70.3	76.2	70.5	76.6	77.1	77.7
4″	67.9	82.1	71.9	70.1	69.9	71.1
5″	72.1	75.8	69.8	77.5	76.9	77.8
6″	17.7	62.1	17.8	67.9	67.7	62.4
1‴	98.3	99.3	98.7	100.3	98.4	100.0
2‴	70.4	70.1	70.3	70.5	69.3	75.8
3‴	70.6	67.6	70.8	71.8	70.0	76.9
4‴	72.1	72.4	72.0	72.1	70.2	69.9
5‴	68.1	70.4	68.8	69.8	68.6	77.1
6‴	17.6	18.0	17.5	17.8	17.6	61.2
1⁗	100.8	98.8	104.3	104.5	99.1	102.1
2⁗	70.0	70.1	75.5	74.6	69.8	74.4
3⁗	70.3	67.7	77.1	76.8	70.6	76.6
4⁗	71.6	73.1	67.8	69.6	71.3	70.2
5⁗	68.3	70.6	78.4	77.0	71.0	77.4
6⁗	17.5	17.7	62.1	68.0	17.7	60.9
1⁗′		102.7		103.5		
2⁗′		74.3		73.7		
3⁗′		77.0		76.3		
4⁗′		70.2		70.1		
5⁗′		76.1		76.6		
6⁗′		60.1		60.2		
OMe		57.6	56.8			

3.7. 5′-*O*-Methylmyricetin 3-*O*-[α-rhamnosyl (1‴ → 2″)]-[α-rhamnosyl ((1⁗′ → 4″)]-β-glucoside-3′-*O*-β-glucoside (*4*)

Yellow amorphous powder. UV λ_{max} (MeOH) nm: 254, 300, 358; +$AlCl_3$: 270, 309, 362, 402; +$AlCl_3$/HCl: 270, 310, 363; +NaOMe: 267, 326, 402; +NaOAc: 267, 321, 424; +NaOAc/H_3BO_3: 267, 363. IR ν_{max} (KBr) cm^{-1}: 3450 (OH), 1650 (α,β-unsaturated CO), 1605, 1580, 900, 860, 770. ^{1}H NMR (DMSO-d_6) *δ*: 12.70 (*s*, OH-5, D_2O exchangeable), 10.45 (*s*, OH-7, D_2O exchangeable), 8.50 (br *s*, OH-4′, D_2O exchangeable), 7.40 (*d*, *J* = 2.0 Hz, H-2′), 7.35 (*d*, *J* = 2.0 Hz, H-6′), 6.40 (*d*, *J* = 2.4 Hz, H-8), 6.24 (*d*, *J* = 2.4 Hz, H-6), 3.80 (*s*, OMe); 3-*O*-glucose: 5.40 (*d*, *J* = 7.8 Hz, H-1″), 3.94 (*m*, H-6″a), 3.82 (*m*, H-4″), 3.68 (*m*, H6″b), 3.52 (*m*, H-2″), 3.46 (*m*, H-3″), 3.35 (*m*, H-5″); 2″-*O*-rhamnose: 4.50 (*d*, *J* = 1.4 Hz, H-1‴), 3.73 (*m*, H-2‴), 3.60 (*m*, H-3‴), 3.54 (*m*, H-5‴), 3.10 (*m*, H-4‴), 1.08 (*d*, *J* = 6.2 Hz, Me-6‴); 4″-*O*-rhamnose: 4.60 (*d*, *J* = 1.2 Hz, H-1⁗′), 3.64 (*m*, H-2⁗′), 3.52 (*m*, H-3⁗′),

3.43 (*m*, H-5‴′), 3.10 (*m*, H-4‴′), 0.80 (*d*, $J = 6.3$ Hz, Me-6‴′); 3′-*O*-glucose: 5.20 (*d*, $J = 7.7$ Hz, H-1⁗), 3.80 (*m*, H-2⁗), 3.70 (*m*, H-6⁗a), 3.60 (*m*, H-6⁗b), 3.50 (*m*, H-4⁗), 3.45 (*m*, H-3‴′), 3.20 (*m*, H-5⁗). ^{13}C NMR (DMSO-d_6) δ: see Table 2. EIMS (70 eV): *m*/*z* (%) 332 (100), 321 (8), 293 (6), 279 (10), 164 (12), 153 (14), 137 (45), 124 (13). FAB-MS positive ion mode *m*/*z*: 949 $[M + H]^+$, 803 $[M + H - 146]^+$, 657 $[M + H - 2 \times 146]^+$, 495 $[M + H - 2 \times 146–162]^+$, 455 [Glu + Rha + Rha + H]$^+$, 333 $[M + H - 2 \times 146 - 2 \times 162]^+$, 309 [Glu + Rha + H]$^+$, 163 [Glu + H]$^+$, 147 [Rha + H]$^+$. HRMS *m*/*z*: 948.2747 $[M]^+$, calc. for $[C_{40}H_{52}O_{26}]^+$, 948.2669.

3.8. 5′-*O*-Methylmyricetin 3-*O*-α-rhamnosyl (1‴ → 2″)-α-rhamnoside-3′-*O*-β-galactoside (*5*)

Pale yellow powder, m.p. 240–241 °C. UV λ_{max} (MeOH) nm: 254, 266, 301, 358; +$AlCl_3$: 269, 310, 366, 402; +$AlCl_3$/HCl: 270, 310, 366, 402; +NaOMe: 267, 330, 402, 420; +NaOAc: 267, 320, 423; +NaOAc/H_3BO_3: 267, 360. IR ν_{max} (KBr) cm^{-1}: 3500 (OH), 1650 (α,β-unsaturated CO), 1605, 1090–1030, 960, 770. ^{1}H NMR (DMSO-d_6) δ: 12.60 (*s*, OH-5, D_2O exchangeable), 10.50 (*s*, OH-7, D_2O exchangeable), 8.40 (*s*, OH-4′, D_2O exchangeable), 7.50 (*s*, H-2′ and H-6′), 6.50 (*d*, $J = 2.2$ Hz, H-8), 6.30 (*d*, $J = 2.4$ Hz, H-6), 3.65 (*s*, OMe); 3-*O*-rhamnose: 5.40 (*d*, $J = 1.2$ Hz, H-1″), 4.30 (*m*, H-2″), 3.73 (*m*, H-3″), 3.50 (*m*, H-4″), 3.41 (*m*, H-5″), 1.40 (*d*, $J = 6.3$ Hz, Me-6″); 2″-*O*-rhamnose: 4.60 (*d*, $J = 1.0$ Hz, H-1‴), 3.90 (*m*, H-2‴), 3.62 (*m*, H-3‴), 3.55 (*m*, H-5‴), 3.32 (*m*, H-4‴), 1.16 (*d*, $J = 6.2$ Hz, Me-6‴); 3′-*O*-galactose: 5.10 (*d*, $J = 7.7$ Hz, H-1⁗), 3.96 (*m*, H-2⁗), 3.80 (*m*, H-5⁗), 3.56 (*m*, H-6⁗a), 3.45 (*m*, H-6⁗b), 3.35 (*m*, H-3⁗), 3.20 (*m*, H-4⁗).

^{13}C NMR (DMSO-d_6) data: see Table 2. EIMS (70 eV): *m*/*z* (%) 322 (100), 292 (5), 280 (10), 165 (9), 153 (15), 137 (9). FAB-MS positive ion *m*/*z*: 787 $[M + H]^+$, 641 $[M + H - 146]^+$, 495 $[M + H - 2 \times 146]^+$, 479 $[M + H - 146–162]^+$, 333 $[M + H - 2 \times 146–162]^+$, 293 [Rha + Rha + H]$^+$, 163 [Gal + H]$^+$, 147 [Rha + H]$^+$. HRMS *m*/*z*: 786.2220 $[M]^+$, calc. for $[C_{34}H_{42}O_{21}]^+$, 786.2141.

3.9. Kaempferol 3-*O*-rutinoside-7-*O*-rutinoside (*6*)

A yellow amourphous powder, m.p. > 250 °C. UV λ_{max} (MeOH) nm: 263, 310, 345; +$AlCl_3$: 269, 302, 350, 396; +$AlCl_3$/HCl: 268, 301, 349, 395; +NaOMe: 270, 304, 337, 380; +NaOAc: 266, 386; +NaOAc/H_3BO_3: 267, 350. IR ν_{max} (KBr) cm^{-1}: 3450 (OH), 1670 (α,β-unsaturated CO), 1610, 1580, 970, 860, 760. ^{1}H NMR (DMSO-d_6) δ: 12.65 (*s*, OH-5, D_2O exchangeable), 8.50 (*s*, OH-4′, D_2O exchangeable), 8.01 (*d*, $J = 9.0$ Hz, H-2′ and H-6′), 6.90 (*d*, $J = 9.2$ Hz, H-3′ and H-5′), 6.32 (*d*, $J = 2.0$ Hz, H-8), 6.18 (*d*, $J = 2.0$ Hz, H-6); 3-*O*-glucose: 5.38 (*d*, $J = 7.5$ Hz, H-1″), 3.84 (*m*, H-2″), 3.77 (*m*, H-6″a), 3.63 (*m*, H-6″b), 3.74 (*m*, H-6″a), 3.61 (*m*, H-3″), 3.57 (*m*, H-6″b), 3.51 (*m*, H-5″), 3.30 (*m*, H-4″); 6″-*O*-rhamnose: 4.60 (*d*, $J = 1.1$ Hz, H-1‴), 3.76 (*m*, H-3‴), 3.60 (*m*, H-2‴), 3.40 (*m*, H-5‴), 3.26 (*m*, H-4‴), 1.13 (*d*, $J = 6.1$ Hz, Me-6‴); 7-*O*-glucose: 5.50 (*d*, $J = 7.6$ Hz, H-1⁗), 3.86 (*m*, H-6⁗a), 3.70 (*m*, H-6⁗b), 3.62 (*m*, H-4⁗), 3.56 (*m*, H-5⁗), 3.41 (*m*, H-2‴′), 3.33 (*m*, H-3‴′), 6‴-*O*-rhamnose: 4.45 (*d*, $J = 1.0$ Hz, H-1⁗), 3.67 (*m*, H-3⁗), 3.58 (*m*, H-2⁗), 3.43 (*m*, H-5⁗), 3.30 (*m*, H-4⁗), 1.11 (*d*, $J = 6.3$ Hz, Me-6⁗). ^{13} C NMR (DMSO-d_6) data: see Table 2. EIMS (70 eV): *m*/*z* (%) 286 (100), 285 (6), 257 (7), 253 (40), 243 (13), 125 (50). FAB-MS positive ion mode *m*/*z*: 903 $[M + H]^+$, 757 $[M + H - 146]^+$, 611$[M + H - 2 \times 146]^+$, 449 $[M + H - 2 \times 146–162]^+$, 309 [Rha + Glc + H]$^+$, 287 $[M + H - 2 \times 146 - 2 \times 162]^+$, 309 [Rha + Glc + H]$^+$, 162 [Glc]$^+$, 146[Rha]$^+$. HRMS *m*/*z*: 902.2701 $[M]^+$, calc. for $[C_{39}H_{50}O_{24}]^+$, 902.2621.

3.10. Myricetin 3-*O*-rutinoside-3′-*O*-α-L-rhamnoside (*7*)

Yellow amorphous powder; UV λ_{max} (MeOH) nm: 260, 354; +$AlCl_3$: 270, 430; +$AlCl_3$/HCl: 270, 400; +NaOMe: 271, 396; +NaOAc: 269, 372; +NaOAc/H_3BO_3: 260, 370. IR ν_{max} (KBr) cm^{-1}: 3550 (OH), 1650 (α,β-unsaturated CO), 1605, 900, 970, 860, 780. ^{1}H NMR (DMSO-d_6) δ: 12.50 (*s*, OH-5, D_2O exchangeable), 10.50 (*s*, OH-5, D_2O exchangeable), 9.50-8.50 (br *s*, OH-4′ and OH-5′, D_2O exchangeable), 7.30 (*d*, $J = 1.9$ Hz, H-2′ and H-6′), 6.30 (*d*, $J = 2.2$ Hz, H-8), 6.16 (*d*, $J = 2.2$ Hz, H-6); 3-*O*-glucose: 5.40 (*d*, $J = 7.7$ Hz, H-1″), 3.86 (*m*, 6″a), 3.76 (*m*, H-2″), 3.65 (*m*, H-6″b), 3.57 (*m*, H-3″), 3.46 (*m*, H-4″), 3.34 (*m*, H-5″); 6″-*O*-rhamnose: 4.50 (*d*, $J = 1.4$ Hz, H-1‴), 3.91 (*m*, H-2‴), 3.83 (*m*, H-3‴), 3.66 (*m*, H-5‴), 3.56 (*m*, H-4‴), 1.00 (*d*, $J = 6.2$ Hz, Me-6‴); 3′-*O*-rhamnose: 5.24 (*d*, $J = 1.3$ Hz, H-1⁗), 3.82 (*m*, H-2⁗), 3.71 (*m*, H-3⁗), 3.54 (*m*, H-5⁗), 3.30 (*m*, H-4⁗), 0.98 (*d*, $J = 6.0$ Hz, Me-6⁗). ^{13}C NMR (DMSO-d_6) data: see Table 2. EIMS (70 eV): *m*/*z* (%) 318 (100), 287 (3), 265 (10), 244 (15), 153 (16), 136 (11). FAB-MS positive ion *m*/*z*.773 $[M + H]^+$, 627 $[M + H - 146]^+$, 481 $[M + H - 2 \times 146]^+$, 465 $[M + H - 146–162]^+$, 319 $[M + H - 2 \times 146–162]^+$, 293 [Rha + Rha + H]$^+$, 163 [Gal + H]$^+$, 147 [Rha + H]$^+$. HRMS *m*/*z*: 772.2154 $[M]^+$, calc. for $[C_{33}H_{40}O_{21}]^+$, 772.2073.

3.11. Myricetin 3-*O*-β-glucosyl-(1‴ → 2″)-glucoside-4′-*O*-β-glucoside (*8*)

Yellow amorphous powder from aqueous MeOH. UV λ_{max} (MeOH) nm: 267, 350; +$AlCl_3$: 266, 302, 342; +$AlCl_3$/HCl: 267, 301, 341; +NaOMe: 276, 372; +NaOAc: 270, 352; +NaOAc/H_3BO_3: 268, 344. IR ν_{max} (KBr) cm^{-1}: 3550 (OH), 1680 (α,β-unsaturated CO), 1605, 900, 1090-1010, 960, 760. ^{1}H NMR (DMSO-d_6) δ: 12.57 (*s*, OH-5, D_2O exchangeable), 10.45 (*s*, OH-7, D_2O exchangeable), 9.30–8.20 (br *s*, OH-3′ and OH-5′, D_2O exchangeable), 7.28 (*s*, H-2′ and H-6′), 6.36 (*d*, $J = 2.1$ Hz, H-8), 6.20 (*d*, $J = 2.1$ Hz, H-6); 3-*O*-glucose: 5.36 (*d*, $J = 7.5$ Hz, H-1″), 3.75 (*m*, 6″a), 3.59 (*m*, H-6″b), 3.46 (*m*, H-2″), 3.40 (*m*, H-3″), 3.36 (*m*, H-4″), 3.28 (*m*, H-5″), 2″-*O*-rhamnose: 4.63 (*d*, $J = 7.4$ Hz, H-1‴), 3.69 (*m*, H-6‴a), 3.57 (*m*,

H-6‴b), 3.55 (*m*, H-2‴), 3.47 (*m*, H-3‴), 3.38 (*m*, H-4‴), 3.26 (*m*, H-5‴), 4′-*O*-glucose: 5.16 (*d*, $J = 7.2$ Hz, H-1‴′), 3.67 (*m*, H-6‴′a), 3.54 (*m*, H-6‴′b), 3.49 (*m*, H-2‴′), 3.32 (*m*, H-4‴′), 3.24 (*m*, H-5‴′). ^{13}C NMR (DMSO-d6) data: see Table 2. EIMS (70 eV): *m/z* (%) 318 (100), 289 (10), 244 (8), 153 (21), 137 (45). FAB-MS (positive ion mode) *m/z*: 805 $[M + H]^+$, 643 $[M + H - 162]^+$, 481 $[M + H - 2 \times 162]^+$, 325 $[Glc + Glc + H]^+$, 319 $[M + H - 3 \times 162]^+$, 162 $[Glc]^+$, 146 $[Rha]^+$, 167,137. HRMS *m/z*: 804.1854 $[M]^+$, calc. for $[C_{33}H_{40}O_{23}]^+$, 804.1777.

3.12. 8-Acetylharpagide (**9**)

Colourless crystals from CH_2Cl_2–MeOH mixture, m.p. 227–229 °C; UV λ_{max} (MeOH) nm: 201 ($\log \varepsilon$ 3.1). IR ν_{max} (KBr) cm^{-1}: 3500–3100 (OH), 1730 (ester), 1670, 1040–1010 (C–O). ^{1}H 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. FABMS: *m/z* 407 $[M + H]^+$, 346, 317, 297, 209, 184, 167, 163 $[Glc + H]^+$, 157, 149, 137, 113, 97, 85, 73, 61, 43, 41.

3.13. Acid hydrolysis

Compounds (**1**–**9**), each in a mixture of 8% HCl (2 ml) and MeOH were separately refluxed for 2 h. The reaction mixtures were reduced in vacuo to dryness, dissolved in H_2O (2 ml) and neutralized with NaOH. The neutralized products were subjected to TLC analysis (eluent: EtOAc–MeOH–H_2O–HOAc, 6:2:1:1) and PC (eluents: *n*-BuOH–HOAc–H_2O (4:5:1) and C_6H_6–*n*-BuOH–H_2O–pyridine, 1:5:3:3). The chromatograms were sprayed with aniline hydrogen phthalate followed by heating. The sugars were identified after comparison with authentic samples.

Acknowledgements

The authors are grateful to Alexander von Humboldt foundation for provision of fellowship to L. Manguro. Prof. Thornston Bach of Technische Universitaet Muenchen is sincerely thanked for hosting L. Manguro in his laboratory.

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