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Tetrahydroisoquinoline-monoterpene glycosides from *Cephaelis acuminata*

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Abstract

From the dried roots of *Cephaelis acuminata*, five tetrahydroisoquinoline-monoterpene glycosides, 2-*O*-β-D-glucopyranosyldemethylalangiside, demethylisoalangiside, 6″-*O*-β-D-glucopyranosylipecoside, 6″-*O*-α-D-glucopyranosylipecoside and (4*R*)-4-hydroxyipecoside, were isolated. The structures of these glycosides were determined by spectroscopic and chemical means.

Keywords: *Cephaelis acuminata*; Rubiaceae; Roots; Structure elucidation; Tetrahydroisoquinoline-monoterpene glycosides

1. Introduction

Ipecac, the dried roots of *Cephaelis acuminata* Karsten or *C. ipecacuanha* A. Richard belonging to the family Rubiaceae, has been used as an emetic because of the emetic activity of ipecac alkaloids, emetine and cephaeline (Shamma, 1972). The crude drug is also known as a rich source of ipecoside (Bellet, 1954). We have previously investigated the roots of *C. acuminata* and isolated from the *n*-BuOH-soluble fraction several new tetrahydroisoquinoline-monoterpene glycosides, which were closely related to the ipecac alkaloids, and from the water-soluble fraction several new ipecac alkaloids (Itoh et al., 1991, 1994, 1999; Nagakura et al., 1993). In continuation of these studies, the water-soluble fraction of the same plant material was examined. As a result, the structure elucidation of five new tetrahydroisoquinoline-monoterpene glycosides is reported here.

2. Results and discussion

The water-soluble fraction of the dried roots of *C. acuminata* was separated by a combination of chromatographic procedures to afford five new glycosides **1**–**5**, along with the known glycosides, demethylalangiside (**6**) (Itoh et al., 1991), ipecoside (**7**) (Itoh et al., 1991), 7-dehydrologanin (Coscia et al., 1969), sweroside (Inouye et al., 1966), and 6′-*O*-β-apiofuranosylsweroside (Kumar et al., 2000). The last compound was isolated for the first time from this species.

The new glycoside **1** was isolated as an amorphous powder. It showed UV maxima at 206, 231, 242, and 280 nm and IR absorptions at 3384, 1653, 1575, 1514, and 907 cm^{-1}. The ^1H NMR spectrum of **1** exhibited a doublet for an olefinic proton at δ 7.41 ($J = 2.5$ Hz), singlets for two aromatic protons at δ 6.63 and 7.10, signals for a terminal vinyl group at δ 5.19 (*dd*, $J = 10.0$ and 2.0 Hz), 5.30 (*dd*, $J = 17.0$ and 2.0 Hz), and 5.52 (*dt*, $J = 17.0$ and 10.0 Hz), a doublet for an acetal proton at δ 5.49 (*d*, $J = 2.0$ Hz), and a doublet for an anomeric proton at δ 4.69 ($J = 8.0$ Hz), indicating the presence of a demethylalangiside (**6**) moiety in the molecule. The coupling constants between H_2-13 and H-13a ($J_{13\alpha,\ 13a} = 11.5$ Hz, $J_{13\beta,\ 13a} = 3.5$ Hz), the chemical shifts of C-6, 12a, and 13, and a negative Cotton effect at 239 nm indicated a *R*-configuration at C-13a (Itoh et al., 1995). Its HR–SIMS showed a pseudomolecular ion $[M-H]^-$ at *m*/*z* 652.2257, giving the molecular formula $C_{30}H_{39}NO_{15}$ which was $C_6H_{11}O_5$ more than that of **6**. The ^1H and ^{13}C NMR spectra suggested the residue to be a β-glucopyranosyl moiety. The location of the second glucosyl unit at C-2 of demethylalangiside moiety was determined by the interaction between H-1″ [δ 4.72 (*d*,

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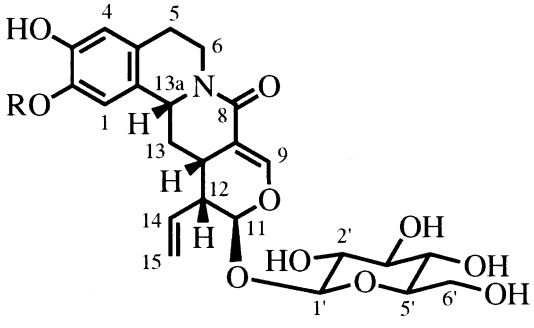
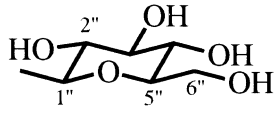
$J = 7.5$ Hz)] and C-2 in the HMBC spectrum, and a cross-peak between H-1″ and H-1 which was correlated with H-13a in its NOESY spectrum. Thus, glycoside **1** was characterized as 2-*O*-β-D-glucopyranosyldemethylalangiside. This is the first tetrahydroisoquinoline-monoterpene glycoside with a glucosylated hydroxyl group on the isoquinoline nucleus.

Glucoside **2** had the same molecular formula as demethylalangiside (**6**), and its ^{1}H and ^{13}C NMR spectra were closely related to those of **6**. However, the coupling constants between H_2-13 and H-13a ($J_{13\alpha,\ 13a} = 5.5$ Hz, $J_{13\beta,\ 13a} = 3.0$ Hz), the chemical shifts of C-6, 12a, and 13, and the negative Cotton effect at 225 nm indicated that **2** was the C-13a epimer of **6**, i.e. demethylisoalangiside (Itoh et al., 1995). Finally, glucoside **2** was identified with an authentic sample prepared from dopamine and secologanin (Itoh et al., 1995). This is the first instance of the isolation of **2** as a natural product and also the first occurrence from *C. acuminata* of a tetrahydroisoquinoline-monoterpene glucoside with the same stereochemistry as the ipecac alkaloids.

The next new glycoside **3** was also obtained as an amorphous powder. Its HR-SIMS measurement showed a pseudomolecular ion $[M-H]^-$ at m/z 726.2620, consistent with a molecular formula $C_{33}H_{45}NO_{17}$. Its ^{1}H NMR spectrum exhibited a doublet for an olefinic proton at δ 7.38 ($J = 1.5$ Hz), singlets for two aromatic protons at δ 6.48 and 6.50, signals for a terminal vinyl group at δ 5.43 (*dd*, $J = 10.0$, 2.0 Hz), 5.56 (*dd*, $J = 17.0$, 2.0 Hz), 5.77 (*dt*, $J = 17.0$, 10.0 Hz), a doublet for an acetal proton at δ 5.43 ($J = 3.5$ Hz), a doublet for an anomeric proton at δ 4.63 ($J = 8.0$ Hz), a singlet for a carbomethoxyl group at δ 3.66, and a singlet for an *N*-acetyl group at δ 2.19, demonstrating its close similarity to ipecoside (**7**). From the ^{1}H and ^{13}C NMR spectra, the structural difference between the glycosides was ascribed to the presence of an additional β-glucosyl moiety in **3**. The location of the second glucosyl unit at C-6″ of ipecoside (**7**) moiety was confirmed by the downfield shift of C-6″ and the upfield shift of C-5″ by glycosylation, as well as by the cross-peaks between H_2-6″ and C-1‴ and between H-1‴ and C-6″ in its HMBC spectrum. Accordingly, glycoside **3** was characterized as 6″-*O*-β-D-glucopyranosylipecoside.

The HR–SIMS measurement of glycoside **4** revealed the same molecular formula as **3**. Its ^{1}H and ^{13}C NMR spectral features were closely similar to those of **3**, suggesting that **4** also consisted of an ipecoside (**7**) moiety and another glucosyl unit. The attachment of the second glucosyl unit at C-6″ of ipecoside (**7**) moiety was confirmed by the glycosylation shift of C-6″ and C-5″, as well as its HMBC and NOESY correlations. The second glucosyl linkage was determined to be α from the coupling constant ($J_{1''',\ 2'''} = 3.5$ Hz) of the anomeric proton and the chemical shift of the anomeric carbon (Itoh et al., 1997). Thus, glycoside **4** was assigned the structure of 6″-*O*-α-D-glucopyranosylipecoside.



1: R= 

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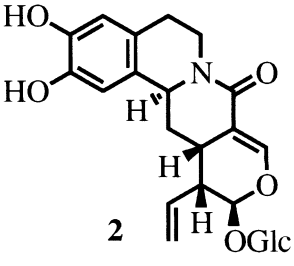

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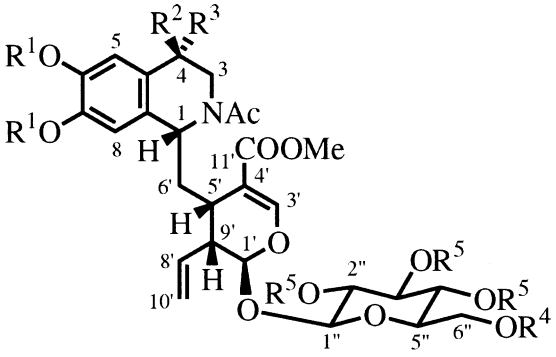

3: R^1=R^2=R^3=R^5=H, R^4=β-Glc

4: R^1=R^2=R^3=R^5=H, R^4=α-Glc

5: R^1=R^3=R^4=R^5=H, R^2=OH

7: R^1=R^2=R^3=R^4=R^5=H

8: R^1=Me, R^2=R^4=R^5=H, R^3=OH

9: R^1=Me, R^3=R^4=R^5=H, R^2=OH

10: R^1=Me, R^2=R^3=H, R^4=R^5=Ac

The last new glucoside **5** showed similar spectral features to those of ipecoside (**7**). However, its molecular formula, $C_{27}H_{35}NO_{13}$, obtained from HR–SIMS was one oxygen more than that of **7**. In its ^{13}C NMR spectrum, the C-4 signal was observed at δ 67.3 as an oxymethine carbon instead of a methylene carbon at δ 29.2 as in **7**. There were also marked differences in its ^{1}H NMR spectrum, where the H-4 signal appeared at δ 4.51 (*br t*, $J=2.0$ Hz) instead of methylene protons at δ 2.65 (*ddd*, $J=16.0$, 4.5 and 1.5 Hz) and 2.84 (*ddd*, $J=16.0$, 12.0 and 6.0 Hz) as in **7**. These spectroscopic data suggested the substitution of a hydroxyl group at C-4 of the ipecoside moiety in **5**. The stereochemistry of C-4 was determined by detailed NMR spectroscopic studies (Fig. 1). The NOESY correlations between H_a-3 at δ 4.02 and the *N*-acetyl group, and between H_b-3 at δ 3.74 and H-6′, suggested that H_a-3 and H_b-3 had β-equatorial and α-axial orientations, respectively. The coupling constants between H_2-3 and H-4 showed that H-4 adopted an equatorial position. Accordingly, the configuration of C-4 in **5** could be deduced to be *R*, supposing that **5** possessed the same absolute configuration of C-1 as ipecoside (**7**). Furthermore, the structure of **5** was ascertained by the preparation of (4*R*)-4-hydroxy-6,7-di-*O*-methylipecoside (**8**) and (4*S*)-4-hydroxy-6,7-di-*O*-methylipecoside (**9**) from 6,7-di-*O*-methylipecoside tetraacetate (**10**) (Itoh et al., 1991). 6,7-Di-*O*-methylipecoside tetraacetate (**10**) was treated with lead tetraacetate followed by deacetylation to afford **8** and **9**. The stereochemistry at C-4 of each derivative was established by the coupling constants between H_2-3 and H-4 and NOESY correlations (Fig. 1). Glucoside **8** was identified as the methylate of **5**. Accordingly, glucoside **5** was characterized as (4*R*)-4-hydroxyipecoside, which is the first example of a tetrahydroisoquinoline-monoterpene glucoside with a hydroxyl group at C-4.

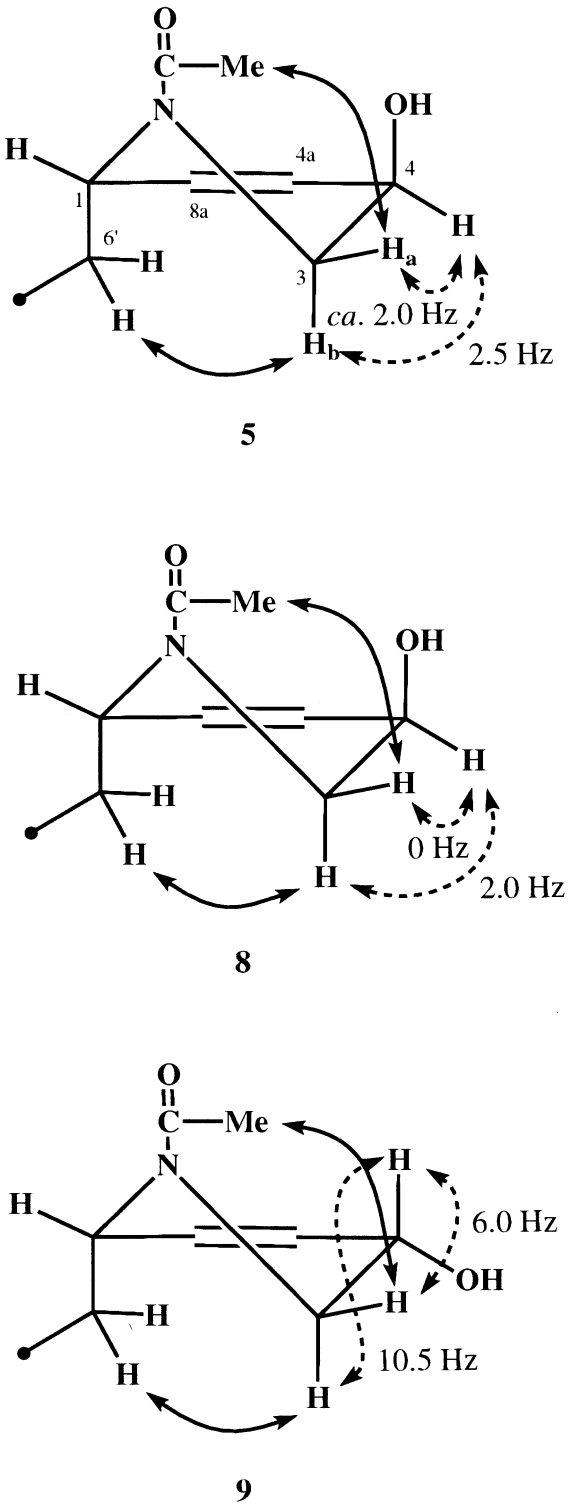

Fig. 1. Selected NOSEY correlations(↔) and ^{1}H–^{1}H coupling constants of **5**, **8** and **9**.

3. Experimental

3.1. General

UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and IR spectra on a Shimadzu FTIR-8200 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter and CD spectra on a Shimadzu-AVIV 62 A DS circular dichroism spectrometer. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a Varian VXR-500 spectrometer with TMS as an internal standard. MS and HR-MS were obtained with a Hitachi M-4100 mass spectrometer. Glycerol was used for SIMS and HR-SIMS as the matrix. MPLC was carried out with Wakogel LP-40 C18. TLC was performed on precoated Kieselgel $60F_{254}$ plates (Merck).

3.2. Plant material

The crude drug 'Ipecac' (roots of *Cephaelis acuminata*) was purchased from Nippon Funmatsu Yakuhin Co., Ltd., Osaka, Japan and identified by Dr. M.

Satake, National Institute of Health Sciences, Tokyo, Japan (Itoh et al., 1999). A voucher specimen (KPFY-863) is deposited in our laboratory.

3.3. Extraction and isolation

Dried roots (5 kg) of *C. acuminata* were extracted with hot MeOH and the MeOH extracts were evaporated in vacuo. The resulting residue (857 g) was resuspended in H_2O and extracted successively with $CHCl_3$ and *n*-BuOH. A part (101 g) of the residue (593 g) originating from H_2O layer was fractionated by DIAION HP-20 column chromotography: elution with H_2O–MeOH mixtures of indicated MeOH content gave 4 fractions, 1 (20%, 279 mg), 2 (25%, 623 mg), 3 (30%, 1.15 g), and 4 (40%, 2.22 g). Fr. 1 was purified by prep. HPLC (μBondasphere 5μ C18-100 Å, MeOH–H_2O, 7:3) and prep. TLC (AcOEt–benzene–EtOH, 4:1:1.5) to afford 7-dehydrologanin (1.5 mg) and sweroside (6.8 mg). Fr. 2 was submitted to Sephadex LH-20 CC (H_2O) and prep. TLC ($CHCl_3$–MeOH, 7:3, AcOEt–benzene–EtOH, 4:1:2) to give 7-dehydrologanin (4.4 mg), sweroside (4.9 mg), and 6′-*O*-β-apiofuranosylsweroside (25.7 mg). Fr. 3 was submitted to reversed-phase MPLC and elution with MeOH–H_2O (1:3), and was further purified by prep. HPLC (MeOH–H_2O, 7:13) to afford **1** (4.3 mg). Fr. 4 was also purified by reversed-phase MPLC, prep. HPLC (MeOH–H_2O, 2:3), and prep. TLC ($CHCl_3$–MeOH, 3:1) to afford **2** (6.1 mg), **3** (15.8 mg), **4** (12.0 mg), **5** (6.5 mg), **6** (6.4 mg), and **7** (794 mg).

3.4. 2-O-β-D-Glucopyranosyldemethylalangiside (*1*)

Amorphous powder. $[\alpha]_D^{27}$ −24° (*c* 0.34, MeOH). UV λ_{max}^{MeOH} nm (log ε): 206 (4.40), 231 (4.25), 242sh (4.18), 280 (3.66). CD λ_{max}^{MeOH} nm (Δ ε): 239 (−10.0). IR ν_{max}^{KBr} cm^{-1}: 3384, 1653, 1575, 1514, 907. ^1H NMR (CD_3OD) : δ 1.36 (1H, *td*, *J* = 13.0, 11.5 Hz, H-13), 2.41 (1H, *dt*, *J* = 13.0, 3.5 Hz, H-13), 2.64 (1H, *dt*, *J* = 16.0, 3.5 Hz, H-5), 2.71 (1H, *ddd*, *J* = 10.0, 5.5, 2.0 Hz, H-12), 2.73 (1H, *ddd*, *J* = 16.0, 11.0, 3.5 Hz, H-5), 2.88 (1H, *ddd*, *J* = 12.5, 11.0, 3.5 Hz, H-6), 3.19 (1H, *dd*, *J* = 9.0, 8.0 Hz, H-2′), 3.20 (1H, *dddd*, *J* = 13.0, 5.5, 3.5, 2.5 Hz, H-12a), 3.28 (1H, *dd*, *J* = 10.0, 9.0 Hz, H-4′), 3.33 (1H, *ddd*, *J* = 10.0, 6.0, 2.0 Hz, H-5′ or H-5″), 3.37 (1H, *dd*, *J* = 10.0, 9.0 Hz, H-4″), 3.38 (1H, *t*, *J* = 9.0 Hz, H-3′), 3.44 (1H, *ddd*, *J* = 10.0, 6.0, 2.0 Hz, H-5″ or H-5′), 3.46 (1H, *t*, *J* = 9.0 Hz, H-3″), 3.49 (1H, *dd*, *J* = 9.0, 7.5 Hz, H-2″), 3.67 (1H, *dd*, *J* = 12.0, 6.0 Hz, H-6′ or H-6″), 3.69 (1H, *dd*, *J* = 12.0, 6.0 Hz, H-6″ or H-6′), 3.90 (1H, *dd*, *J* = 12.0, 2.0 Hz, H-6′ or H-6″), 3.92 (1H, *dd*, *J* = 12.0, 2.0 Hz, H-6″ or H-6′), 4.69 (1H, *d*, *J* = 8.0 Hz, H-1′), 4.71 (1H, *dt*, *J* = 12.5, 3.5 Hz, H-6), 4.72 (1H, *d*, *J* = 7.5 Hz, H-1″), 4.75 (1H, *dd*, *J* = 11.5, 3.5 Hz, H-13a), 5.19 (1H, *dd*, *J* = 10.0, 2.0 Hz, H-15), 5.30 (1H, *dd*, *J* = 17.0, 2.0 Hz, H-15), 5.49 (1H, *d*, *J* = 2.0 Hz, H-11), 5.52 (1H, *dt*, *J* = 17.0, 10.0 Hz, H-14), 6.63 (1H, *s*, H-4), 7.10 (1H, *s*, H-1), 7.41 (1H, *d*, *J* = 2.5 Hz, H-9). ^{13}C NMR (CD_3OD): δ 27.9 (C-12a), 29.5 (C-5), 35.0 (C-13), 40.8 (C-6), 44.4 (C-12), 57.1 (C-13a), 62.7, 62.8 (C-6′, C-6″), 71.6 (C-4′, C-4″), 74.9 (C-2′, C-2″), 77.7 (C-3″), 78.0 (C-3′), 78.4 (C-5′, C-5″), 97.6 (C-11), 99.7 (C-1′), 104.4 (C-1″), 109.3 (C-8a), 116.2 (C-1), 116.8 (C-4), 120.5 (C-15), 129.2 (C-13a), 131.5 (C-4a), 133.9 (C-14), 145.8 (C-2), 147.2 (C-3), 148.8 (C-9), 166.1 (C-8). Negative ion SIMS *m*/*z*: 652 $[M–H]^-$, 490, 305, 162. HR–SIMS found 652.2257 $[M–H]^-$; $C_{30}H_{38}NO_{15}$ requires 652.2243. NOESY: H-1/H-1″; H-1/H-13 (δ 2.41); H-1/H-13a; H-12a/H-13a. HMBC: H-11 to C-1′; H-1′ to C-11; H-4 to C-5; H-1 to C-13a; H-1″ to C-2.

3.5. Demethylisoalangiside (*2*)

Amorphous powder, $[\alpha]_D^{27}$ −143 ° (*c* 0.24, MeOH). UV λ_{max}^{MeOH} nm (log ε): 234 (4.24), 287.5 (3.64). CD λ_{max}^{MeOH} nm (Δ ε): 225 (−7.8). IR ν_{max}^{KBr} cm^{-1}: 3423, 1653, 1558, 901. ^1H NMR (CD_3OD) : δ 1.95 (1H, *ddd*, *J* = 14.0, 12.5, 5.5 Hz, H-13), 2.34 (1H, *ddd*, *J* = 14.0, 4.5, 3.0 Hz, H-13), 2.61 (1H, *ddd*, *J* = 16.0, 5.0, 2.5 Hz, H-5), 2.66 (1H, *ddd*, *J* = 10.0, 5.5, 1.5 Hz, H-12), 2.89 (1H, *dddd*, *J* = 12.5, 5.5, 4.5, 2.5 Hz, H-12a), 2.90 (1H, *ddd*, *J* = 16.0, 11.5, 6.5 Hz, H-5), 3.05 (1H, *ddd*, *J* = 12.5, 11.5, 5.0 Hz, H-6), 3.06 (1H, *dd*, *J* = 9.0, 8.0 Hz, H-2′), 3.23 (1H, *dd*, *J* = 9.5, 9.0 Hz, H-4′), 3.28 (1H, *ddd*, *J* = 9.5, 5.5, 2.0 Hz, H-5′), 3.30 (1H, *t*, *J* = 9.0 Hz, H-3′), 3.65 (1H, *dd*, *J* = 12.0, 5.5 Hz, H-6′), 3.87 (1H, *dd*, *J* = 12.0, 2.0 Hz, H-6′), 4.61 (1H, *d*, *J* = 8.0 Hz, H-1′), 4.61 (1H, *ddd*, *J* = 12.5, 6.5, 2.5 Hz, H-6), 4.70 (1H, *br t*, *J* = 4.0 Hz, H-13a), 5.31 (1H, *dd*, *J* = 10.0, 2.0 Hz, H-15), 5.37 (1H, *dd*, *J* = 17.0, 2.0 Hz, H-15), 5.41 (1H, *d*, *J* = 1.5 Hz, H-11), 5.66 (1H, *dt*, *J* = 17.0, 10.0 Hz, H-14), 6.55 (1H, *s*, H-4), 6.68 (1H, *s*, H-1), 7.32 (1H, *d*, *J* = 2.5 Hz, H-9). ^{13}C NMR (CD_3OD): δ 24.4 (C-12a), 28.3 (C-13), 28.9 (C-5), 43.9 (C-6), 44.9 (C-12), 56.4 (C-13a), 62.7 (C-6′), 71.5 (C-4′), 74.5 (C-2′), 78.1 (C-3′), 78.3 (C-5′), 98.2 (C-11), 100.6 (C-1′), 109.4 (C-8a), 111.7 (C-1), 117.0 (C-4), 120.5 (C-15), 128.0 (C-4a), 129.1 (C-13b), 134.4 (C-14), 145.0, 145.7 (C-2, C-3), 148.7 (C-9), 166.6 (C-8). Negative ion SIMS *m*/*z*: 490 $[M-H]^-$, 162. HR–SIMS found 490.1718 $[M–H]^-$; $C_{24}H_{28}NO_{10}$ requires 490.1714.

3.6. 6″-O-β-D-Glucopyranosylipecoside (*3*)

Amorphous powder, $[\alpha]_D^{28}$ −149° (*c* 1.0, MeOH). UV λ_{max}^{MeOH} nm (log ε): 207 (4.52), 226 (4.19), 233 *sh* (4.15), 286.5 (3.60). IR ν_{max}^{KBr} cm^{-1}: 3355, 1697, 1626, 1526, 945. ^1H NMR: Table 1. ^{13}C NMR: Table 2. Negative ion SIMS *m*/*z*: 726 $[M–H]^-$, 564. HR–SIMS found 726.2620 $[M–H]^-$; $C_{33}H_{44}NO_{17}$ requires 726.2611. NOESY: H-6″ (δ 3.78)/H-1‴; H-1/H-8; H-5′/NAc; H-9′/H-10′ (δ 5.56); H-3 (δ 3.62–3.69)/H-6′ (δ 2.44); H-3 (δ 3.90)/NAc. HMBC: H-1‴ to H-6″; H_2-6″ to C-1‴; H-1″ to C-1′.

Table 1
^{1}H NMR spectral data of **3–5** and **7–9** in CD_3OD at 500 MHz

H	**3**			**4**			**5**		
1	5.55	*dd*	(11.5, 3.5)	5.56	*dd*	(11.5, 3.5)	5.64	*dd*	(12.0, 3.0)
3	3.62-3.69	*m*		3.62-3.72	*m*		3.74	*dd*	(14.5, 2.5)
3	3.90	*ddd*	(13.5, 6.0, 2.0)	3.89	*ddd*	(14.0, 6.0, 2.0)	4.02	*brd*	(14.5)
4	2.65	*ddd*	(16.0, 4.0, 2.0)	2.66	*ddd*	(16.0, 4.0, 2.0)	4.51	*brt*	(2.0)
4	2.83	*ddd*	(16.0, 12.0, 6.0)	2.83	*ddd*	(16.0, 11.5, 6.0)	-		
5	6.50[a], 6.64	*s*		6.52[a], 6.66	*s*		6.72	*s*	
8	6.48[a], 6.53	*s*		6.51[a], 6.54	*s*		6.46	*s*	
1′	5.43	*d*	(3.5)	5.40	*d*	(3.5)	5.41	*d*	(3.0)
3′	7.38	*d*	(1.5)	7.37[a], 7.44	*d*	(1.5)	7.38	*d*	(2.0)
5′	2.72	*m*		2.75	*m*		2.71	*dddd*	(12.0, 6.0, 2.5, 2.0)
6′	1.54	*ddd*	(14.5, 11.0, 3.5)	1.57	*ddd*	(14.5, 11.0, 3.5)	1.45	*ddd*	(14.5, 12.0, 3.0)
6′	2.44	*ddd*	(14.5, 11.5, 2.0)	2.42	*ddd*	(14.5, 11.5, 3.0)	2.45	*ddd*	(14.5, 12.0, 2.5)
8′	5.77	*dt*	(17.0, 10.0)	5.78	*dt*	(17.0, 10.0)	5.78	*dt*	(17.0, 10.0)
9′	3.15	*ddd*	(10.0, 6.0, 3.5)	3.13	*ddd*	(10.0, 5.5, 3.5)	3.28	*ddd*	(10.0, 6.0, 3.0)
10′	5.43	*dd*	(10.0, 2.0)	5.41	*dd*	(10.0, 2.0)	5.45	*dd*	(10.0, 2.0)
10′	5.56	*dd*	(17.0, 2.0)	5.54	*dd*	(17.0, 2.0)	5.58	*dd*	(17.0, 2.0)
1″	4.63[a], 4.67	*d*	(8.0)	4.65[a], 4.69	*d*	(8.0)	4.61	*d*	(8.0)
2″	3.18	*dd*	(9.0, 8.0)	3.21	*dd*	(9.0, 8.0)	3.16	*dd*	(9.0, 8.0)
3″	3.26–3.36	*m*		3.36	*t*	(9.0)	3.28–3.34	*m*	
4″	3.26–3.36	*m*		3.41	*t*	(9.0)	3.28–3.34	*m*	
5″	3.48	*ddd*	(9.5, 6.0, 2.0)	3.49	*ddd*	(9.0, 5.0, 2.0)	3.28–3.34	*m*	
6″	3.78	*dd*	(11.5, 6.0)	3.80–3.85	*m*		3.68	*dd*	(12.0, 5.0)
6″	4.17	*dd*	(11.5, 2.0)	3.94	*dd*	(11.0, 5.0)	3.89	*dd*	(12.0, 2.0)
COOMe	3.66[a], 3.69	*s*		3.67[a], 3.70	*s*		3.64	*s*	
NAc	2.17, 2.19[a]	*s*		2.17, 2.19[a]	*s*		2.25	*s*	
1‴	4.35, 4.39[a]	*d*	(8.0)	4.87, 4.89[a]	*d*	(3.5)			
2‴	3.22	*dd*	(9.0, 8.0)	3.40	*dd*	(9.5, 3.5)			
3‴	3.26–3.36	*m*		3.62–3.72	*m*				
4‴	3.26–3.36	*m*		3.31	*m*				
5‴	3.26–3.36	*m*		3.62–3.72	*m*				
6‴	3.62–3.69	*m*		3.62–3.72	*m*				
6‴	3.88	*dd*	(12.0, 1.5)	3.80–3.85	*m*				
6-OMe									
7-OMe									

H	**7**			**8**			**9**		
1	5.55	*dd*	(12.0, 3.5)	5.73	*dd*	(12.0, 3.0)	5.62	*dd*	(12.0, 2.5)
3	3.64	*ddd*	(14.0, 12.0, 4.5)	3.77	*dd*	(14.5, 2.0)	3.47	*dd*	(13.5, 10.5)
3	3.92	*ddd*	(14.0, 6.0, 1.5)	4.07	*br d*	(14.5)	4.07	*dd*	(13.5, 6.0)
4	2.65	*ddd*	(16.0, 4.5, 1.5)	4.60	*br s*		4.71	*dd*	(10.5, 6.0)
4	2.84	*ddd*	(16.0, 12.0, 6.0)	–			–		
5	6.50[a], 6.61	*s*		6.88	*s*		7.09	*s*	
8	6.45[a], 6.52	*s*		6.56	*s*		6.53	*s*	
1′	5.41	*d*	(3.0)	5.44	*d*	(3.0)	5.45	*d*	(3.0)
3′	7.38[a], 7.44	*d*	(1.5)	7.39	*d*	(2.0)	7.39	*d*	(2.0)
5′	2.71	*dddd*	(11.5, 6.0, 2.5, 1.5)	2.75	*m*		2.76	*m*	
6′	1.51	*ddd*	(14.5, 11.5, 3.5)	1.52	*ddd*	(14.5, 12.0, 3.0)	1.59	*ddd*	(14.5, 12.0, 2.5)
6′	2.51	*ddd*	(14.5, 12.0, 2.5)	2.50	*ddd*	(14.5, 12.0, 2.0)	2.62	*ddd*	(14.5, 12.0, 2.5)
8′	5.76	*dt*	(17.0, 10.0)	5.82	*dt*	(17.0, 10.0)	5.83	*dt*	(17.0, 10.0)
9′	3.23	*ddd*	(10.0, 6.0, 3.0)	3.26–3.36	*m*		3.24–3.37	*m*	
10′	5.43	*dd*	(10.0, 2.0)	5.47	*dd*	(10.0, 2.0)	5.47	*dd*	(10.0, 2.0)
10′	5.54	*dd*	(17.0, 2.0)	5.61	*dd*	(17.0, 2.0)	5.59	*dd*	(17.0, 2.0)
1″	4.61[a], 4.66	*d*	(8.0)	4.62	*d*	(8.0)	4.62	*d*	(8.0)
2″	3.16	*dd*	(9.0, 8.0)	3.17	*dd*	(9.0, 8.0)	3.17	*br t*	(8.5)
3″	3.27–3.36	*m*		3.26–3.36	*m*		3.24–3.37	*m*	
4″	3.27–3.36	*m*		3.26–3.36	*m*		3.24–3.37	*m*	
5″	3.27–3.36	*m*		3.26–3.36	*m*		3.24–3.37	*m*	
6″	3.67	*dd*	(12.0, 5.0)	3.67	*dd*	(12.0, 5.0)	3.67	*dd*	(12.0, 5.5)
6″	3.89	*dd*	(12.0, 2.0)	3.89	*dd*	(12.0, 2.0)	3.89	*dd*	(12.0, 2.0)
COOMe	3.65[a], 3.68	*s*		3.64	*s*		3.65	*s*	

(*continued on next page*)

Table 1 (*continued*)

H	7		8		9	
NAc	2.17, 2.19[a]	*s*	2.27	*s*	2.24	*s*
1‴						
2‴						
3‴						
4‴						
5‴						
6‴						
6‴						
6–OMe			3.82	*s*	3.81	*s*
7–OMe			3.78	*s*	3.77	*s*

[a] Represents signals due to the major rotomer.

Table 2
^{13}C NMR spectral data of **3–5** and **7–9** in CD_3OD at 125 MHz[a]

C	3	4	5	7[b]	8	9
1	50.6	50.5	49.9	50.3	50.1	50.7
3	41.2	41.2	48.1	41.1	48.0	47.5
4	29.2	29.2	67.3	29.2	67.2	66.2
4a	125.5	125.4	127.0	125.3	128.3	131.2
5	116.2	116.2	117.4	116.2	114.1	111.0
6	144.9a	144.9d	145.6g	145.0h	149.6	149.7
7	145.3a	145.3d	146.9g	145.3h	150.7	149.9
8	114.5	114.4	113.6	114.3	110.4	110.3
8a	130.3	130.3	131.1	130.3	132.2	131.6
1′	97.9	98.0	98.7	98.7	98.7	98.6
3′	152.9	152.8	153.0	153.0	153.1	153.0
4′	111.9	112.0	111.5	111.7	111.4	111.5
5′	27.6	27.6	27.4	27.3	27.5	27.5
6′	36.4	36.3	34.9	35.9	34.8	35.8
8′	135.8	135.6	135.9	135.9	136.4	136.3
9′	44.7	44.8	45.0	45.0	45.1	45.0
10′	120.5	120.6	120.5	120.4	120.2	120.2
11′	169.2	69.2	169.2	169.2	169.1	169.1
1″	99.8	100.0	100.5	100.5	100.5	100.4
2″	74.6	74.6	74.8	74.8	74.8	74.7
3″	78.0b	78.2	78.1	78.1i	78.1	78.1
4″	71.5c	71.5e	71.4	71.4	71.4	71.4
5″	77.4	76.8	78.2	78.2i	78.3	78.3
6″	70.0	67.8	62.7	62.7	62.7	62.7
COMe	172.4	172.4	173.5	172.3	173.5	172.4
COMe	21.5	21.5	21.7	21.4	21.7	21.4
COOMe	51.7	51.7	51.7	51.7	51.7	51.7
1‴	104.9	100.2				
2‴	75.2	73.7f				
3‴	78.1b	75.2				
4‴	71.7c	71.8e				
5‴	78.1b		73.8f			
6‴	62.9	62.7				
OMe					56.4	56.4
OMe					56.5	56.4

[a] Values with the same letter are interchangeable.
[b] We correct here ^{13}C NMR assignments of C-1, 3, 6′ in **7** (Itoh et al., 1991) based on 2D NMR experiments.

3.7. 6″-*O*-α-D-Glucopyranosylipecoside (*4*)

Amorphous powder, $[\alpha]_D^{27}$ −108° (*c* 0.24, MeOH). UV λ_{max}^{MeOH} nm (log ε): 207 (4.53), 225 (4.18), 234 *sh* (4.11), 287.5 (3.61). IR ν_{max}^{KBr} cm^{-1}: 3410, 1695, 1626, 1525, 945. ^{1}H NMR: Table 1. ^{13}C NMR: Table 2. Negative ion SIMS *m*/*z*: 726 [M–H]$^-$, 564. HR–SIMS found 726.2577 [M–H]$^-$; $C_{33}H_{44}NO_{17}$ requires 726.2611. NOESY: H-1/H-8; H-3 (δ 3.90)/NAc; H-3 (δ 3.61–3.70)/H-6′ (δ 2.44); H-5′/NAc; H-4 (δ 2.64)/H-5; H-1″/H-5″. HMBC: H-1″ to C-1′; H-6″ (δ 3.94) to H-1‴.

3.8. (4*R*)-4-Hydroxyipecoside (*5*)

Amorphous powder, $[\alpha]_D^{24}$ −140° (*c* 0.37, MeOH). UV λ_{max}^{MeOH} nm (log ε): 208 (4.50), 227 (4.17), 237 *sh* (4.09), 286 (3.53). IR ν_{max}^{KBr} cm^{-1}: 3390, 1697, 1624, 1526, 943. ^{1}H NMR: Table 1. ^{13}C NMR : Table 2. Negative ion SIMS *m*/*z*: 580 [M–H]$^-$, 323, 176, 149. HR–SIMS found 580.2050 [M–H]$^-$; $C_{27}H_{34}NO_{13}$ requires 580.2031. NOESY: H-3 (δ 3.74)/H-6′ (δ 2.45); H-3 (δ 4.02)/NAc; H-1′/H-1″; H-4 (δ 4.51)/H-5; H-1/H-8.

3.9. Preparation of 4-hydroxy-6,7-di-*O*-methylipecosides (*8* and *9*) from 6,7-di-*O*-methylipecoside tetraacetate (*10*)

To a solution of 6,7-di-*O*-methylipecoside tetraacetate (**10**) (255 mg) in acetic acid (10 ml) was added $Pb(OAc)_4$ (255 mg) and the mixture was stirred at 80 °C for 6 h. The reaction mixture was basified with saturated aq. $NaHCO_3$, and extracted with $CHCl_3$. The washed and dried organic layer was concentrated in vacuo. The resulting residue (235.7 mg) was dissolved in dry MeOH (9.0 ml) and treated with 0.1 N NaOMe (1.0 ml). After stirring for 2 h, 0.1 N NaOMe (0.5 ml) was added with further stirring for 35 min. The reaction mixture was neutralized by Amberlite IR-120 and evaporated in vacuo. The resulting residue was purified by prep. HPLC (MeOH–H_2O, 11:9) to afford (4*R*)-4-hydroxy-6,7-di-*O*-methylipecoside (**8**) (77.8 mg, 38%) and (4*S*)-4-hydroxy-6,7-di-*O*-methylipecoside (**9**) (16.5 mg, 8%). **8**: Colorless crystalline solid, mp 184–186 °C (MeOH). $[\alpha]_D^{27}$ −170 ° (*c* 1.0, MeOH). UV λ_{max}^{MeOH} nm (log ε): 232 (4.27), 279 *sh* (3.53), 283 (3.54), 288 *sh* (3.48). IR ν_{max}^{KBr} cm^{-1}: 3376, 1698, 1685, 1676, 1624, 1518, 941. ^{1}H NMR: Table 1. ^{13}C NMR: Table 2. Negative ion SIMS *m*/*z*: 608 [M–H]$^-$, 594, 446. HR–SIMS Found 608.2336 [M–H]$^-$; $C_{29}H_{38}NO_{13}$ requires 608.2344.

NOESY: H-3 (δ 4.07) / NAc; H-5′/NAc; H-3 (δ 3.77)/H-6′ (δ 2.50); H-1/H-8; H-1′/H-1″; H-4/H-5; H-8/H-6′ (δ 1.52). **9**: $[\alpha]_D^{26}$ −179° (*c* 1.0, MeOH). UV λ_{max}^{MeOH} nm (log ε): 231 (4.25), 283.5 (3.56), 289 *sh* (3.51). IR ν_{max}^{KBr} cm^{-1}: 3397, 1696, 1628, 1516, 939. ^1H NMR: Table 1. ^{13}C NMR: Table 2. Negative ion SIMS *m*/*z*: 608 $[M–H]^-$, 594, 446. HR–SIMS found 608.2369 $[M–H]^-$; $C_{29}H_{38}NO_{13}$ requires 608.2344. NOESY: H-3 (δ 3.47)/H-6′ (δ 2.62); H-3 (δ 4.07)/NAc; H-1/H-8; H-1″/H-3″; H-1′/H-1″.

3.10. Methylation of **5**

A methanolic solution of **5** (3.0 mg) was treated with CH_2N_2–Et_2O and the product was purified by prep. HPLC (MeOH–H_2O, 1:1) to give (4*R*)-4-hydroxy-6,7-di-*O*-methylipecoside (**8**) (2.5 mg). Its ^1H NMR data and retention time of HPLC analysis (μBondasphere 5μ C18-100 Å, MeOH–H_2O, 1:1) were identified with those of authentic sample derived from **10**. $[\alpha]_D^{22}$ −165 ° (*c* 0.13, MeOH). Negative ion SIMS *m*/*z* 608 $[M-H]^-$, 594, 446.

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