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Note

Cyanogenic and non-cyanogenic glycosides from *Manihot esculenta* (Euphorbiaceae)

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A novel cyanogenic glycoside, 2-((6-O-(β-D-apiofuranosyl)-β-D-glucopyranosyloxy)-2-methylbutanenitrile, **1**, three novel non-cyanogenic glycosides, (2*S*)-((6-O-(β-D-apiofuranosyl)-β-D-glucopyranosyloxy) butane, **2**; 2-((6-O-(β-D-apiofuranosyl)-β-D-glucopyranosyloxy) propane, **3**, ethyl β-D-glucopyranoside, **4**, two known cyanogenic glycosides, (*R*)-2-(β-D-Glucopyranosyloxy)-2-methyl butanenitrile (lotaustralin), **5**, 2-(β-D-Glucopyranosyloxy)-2-methylpropane nitrile (linamarin), **6** have been isolated from ethanolic extract of the fresh rootcortex of *Manihot esculenta.* Lotaustralin and linamarin and two flavonoid glycosides, kaempferol-3-O-rutinoside, **7** and quercetin-3-O-rutinoside, **8** have been isolated from the methanol extract of the fresh leaves of the same plant.

Keywords: *Manihot esculenta*, Euphorbiaceae, cassava, roots, leaves, cyanogenic, non-cyanogenic, glycosides, flavonoids

Cassava meal is a major energy source for both humans and domestic animals in tropical countries[1]. Investigation of the root cortex of *Manihot esculenta* has led to the isolation of four new glycosides, two known cyanogenic glycosides and known flavonoid glycosides. X-ray crystallographic structures of the acetylated derivatives of compounds **2** and **4** have been determined[2].

Results and Discussion

The concentrated ethanolic extract of the root was partitioned in CH_2Cl_2:MeOH:H_2O (6:4:1) and it separated into two layers – upper and lower. Column chromatography of the crude material from the upper layer over silica gel gave **4**, **5** and **6**, and a mixture containing isobutyl cyanogenic glycoside **1**, isobutyl glycoside, **2** and isopropylglycoside, **3** (**Scheme I**).

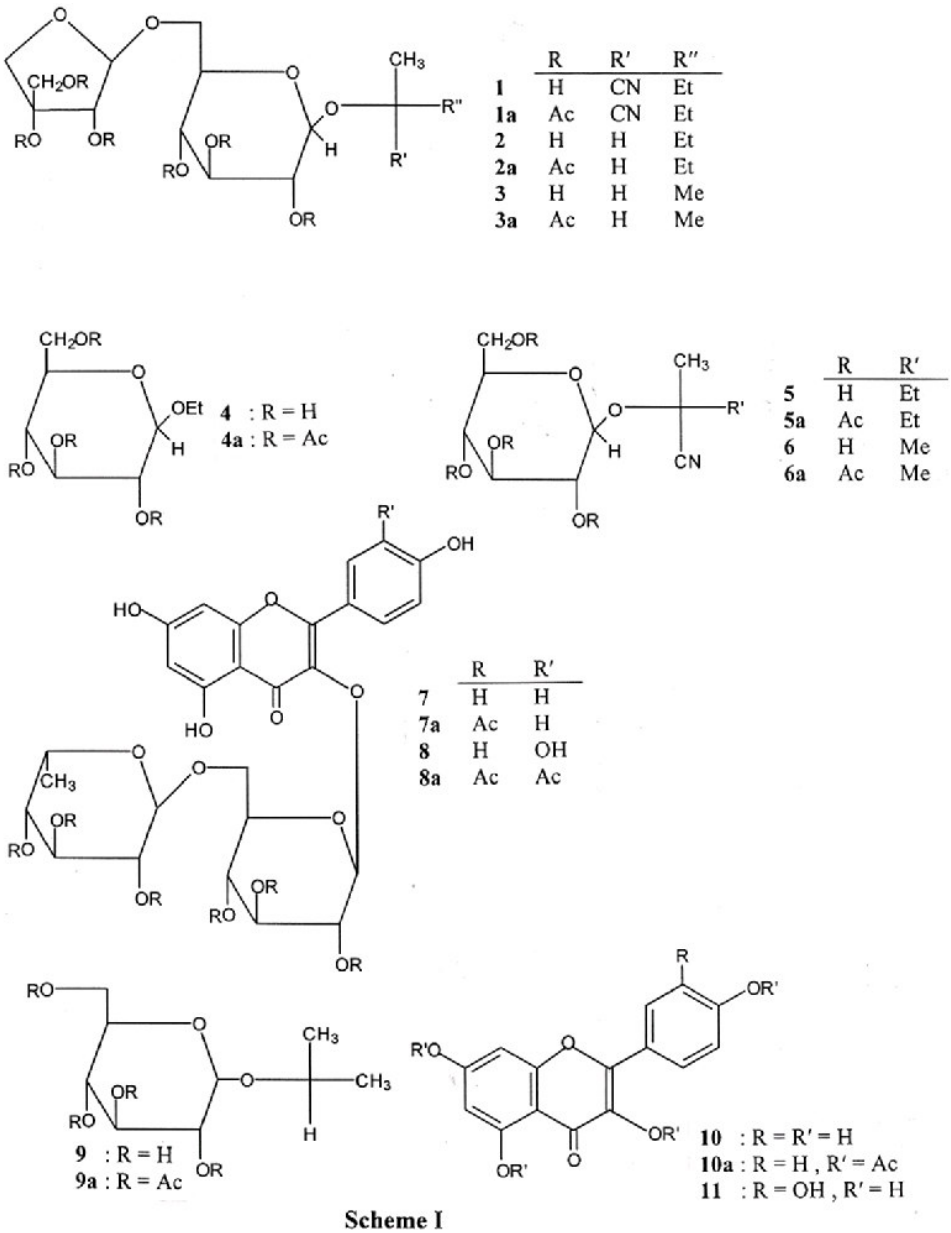
Acetylation of the mixture gave a mixture of the acetate derivatives which were resolved by column chromatography over silica gel. Deacetylation of the isobutyl glycoside acetate with methanolic K_2CO_3 yielded isobutyl glycoside **2**.

CIMS of **2** (NH_3) gave a pseudo molecular ion peak at *m/z* 386 $(C_{15}H_{28}O_{10} + NH_4)^+$ which agreed with 15 carbon signals in the ^{13}C NMR spectrum. The fragment peak at *m/z* 312 $(C_{11}H_{19}O_9+NH_3)^+$ corresponded to loss of an isobutoxy group (C_4H_9O). The ^1H NMR spectrum of **2** showed signals from two anomeric protons as two doublets at δ 4.32 (*J* = 7.5 Hz) and δ 5.02 (*J* = 1.5 Hz) which were assigned to β-D-glucose and β-D-apiose respectively. The aglycone, isobutoxy group, was indicated by the signals of two methyl groups appearing as a doublet at δ 1.24 (*J* = 6.2 Hz) and a triplet at 0.93 (*J* = 7.0 Hz), a methine proton at δ 3.72 (sextet, *J* = 7.0 Hz) and two methylene protons which gave two quintets at δ 1.47 (*J* = 7.0 Hz) and 1.62 (*J* = 7.0 Hz). The ^{13}C NMR spectrum of **2** showed signals for two anomeric carbons (δ 104.0 and 111.6). The peak at δ 70.3(t) which showed a significant glycosidation shift indicates the linkage of the terminal apiose to the glucosyl moiety at C-6.

Acetylation of **2** gave hexaacetate **2a** whose ^1H NMR spectrum showed two anomeric proton signals at δ 4.54 (d, *J* = 8.0 Hz) and 5.05 (br,s) which were assigned to those of β-D-glucose and β-D-apiose respectively. The assignments for all individual sugar signals were made through selective single frequency proton-decoupling experiments. The upfield occurrences of the resonances of the H-6′a and H-6′b (δ 3.61 and 3.67, no acetylation shift) indicated that the glycosidic linkage was at C-6 of glucose. Furthermore, selective irradiation of the apiose anomeric proton, H-1″, gave NOE enhancements of the signals from H-6′a, 3.5% and H-6′b, 2% as well as of the signal from H-2″, 3.5%. The structure of **2** was deduced based on the foregoing evidence.

The acetate derivative of compound **2a** was obtained as colourless needles suitable for X-ray crystallographic analysis. The single crystal X-ray analysis confirmed the structure of **2a** and indicated the (*S*)- configuration at C-2: An ORTEP projection of the structure is shown in **Figure 1**. The structure of **2** is, therefore, (2*S*)-((6-O (β-D-apiofuranosy1) -β-D-glucopyranosyl) - β-D- glucopyranosyl) butane.

The ^1H NMR spectrum of the acetate derivative **1a** was similar to that of **2a** except that the chemical



Scheme I

shifts of C-2-CH_3, (H-3)$_2$ and H-4 were shifted downfield by 0.35, 0.4 and 0.18 ppm respectively compared with **2a**. This may be attributed to the presence of the nitrile group on C-2 in **1a**. Furthermore, C-2-CH_3 resonated as a singlet and the (H-3)$_2$ resonance was less complex.

The ^{13}C NMR spectrum of **3** was similar to that of **2** except for the presence of one carbon less than that of **2** (δ 20-30-region). The two anomeric carbon signals appeared at δ 102.9 and 111.4 which were ascribed to those of β-D-glucose and β-D-apiose respectively. The CIMS of **3** exhibited a pseudo-molecular ion peak at *m/z* 372 $[M+NH_4]^+$; the EIMS of **3** showed peaks at *m/z* 295 $[M-59]^+$, 221 $[M-133]^+$ and 133 $[M-59-162]^+$ corresponding to losses of isopropoxy, pentose and isopropoxy and hexose groups respectively. Acetylation of **3** gave hexaacetate **3a**. The two anomeric proton signals appeared at δ 4.54 (d, *J* = 7.5 Hz) and 5.05 (br,s). That the aglycone is isopropoxy group was indicated by the signals of two methyl groups appearing as two doublets at δ 1.13 (*J* = 6.0 Hz) and 1.21 (*J* = 6.0 Hz) and the septet signal of a methine proton at δ 3.91. A double quantum-filtered ^{1}H-^{1}H 2D correlation spectrum (DQFCOSY) provided assignments of all individual sugar signals. These were confirmed by

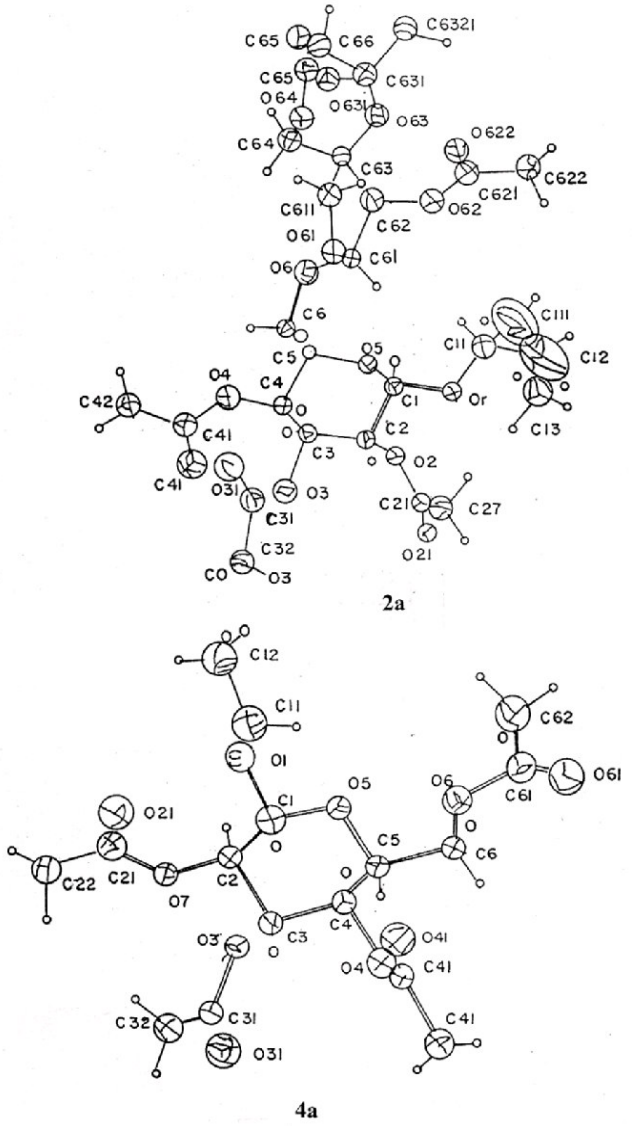


Figure 1 — Single molecules of compounds **2a** and **4a**. Thermal ellipsoids (2090) are shown for non-hydrogen atoms; hydrogen atoms have arbitrary radius of 0.1 Å. Crystallographic skeletal numbering is shown.

selective single frequency proton-decoupling experiments. In addition, NOE enhancements were observed between the CH_3 signal at δ 1.13 and H-1′ (δ 4.54) (26%) between H-2 (δ 3.90) and H-1′ (5.5%) and between (H-6′)$_2$ and H-1″ (6%). The glycoside **3**, therefore, was characterized as 2-(6-O-((β-D-apio-furanosyl)-β-D- glucopyranosyl)oxy) propane.

The ^{13}C NMR spectrum of **4** showed eight carbon signals. An anomeric carbon signal appeared at δ 104.2. The sugar moiety of **4** was identified as β-D-glucose. The CIMS of **4** showed a pseudomolecular ion peak at *m/z* 226 $[M+NH_4]^+$ together with a fragment ion at *m/z* 180 $[(M+NH_3)–45]^+$ corresponding to a loss of an ethoxy group. The ^{1}H NMR spectrum exhibited the signals of a methyl group at δ 1.25 (t, *J* = 6.5 Hz) and two methylene protons at δ 3.63 (dq, *J* = 9.0, 6.5 Hz) and 3.61 (dq, *J* = 9.0, 6.5 Hz) corresponding to the presence of an ethoxy group in **4**. Acetylation of **4** provided the acetate **4a**. The ^{1}H NMR spectrum of **4a** showed signals for an anomeric proton at δ 4.51 (d, *J*= 7.0 Hz), a methyl group at δ 1.20 (t, *J* = 6.5 Hz) and one methylene group at δ 3.58 (dq, *J* = 9.0, 6.5 Hz) and 3.91 corresponding to an ethoxy group. By selective single frequency proton – decoupling experiments assignments of individual protons of the glucose moiety were confirmed. The glycoside, **4**, therefore, was identified as ethyl β-D- glucopyranoside. A single crystal X-ray analysis of **4a** confirmed its structure. An ORTEP projection of **4a** is shown in **Figure 1**. Compound **4** may be an artefact since ethanol was used as the solvent for extraction.

Compounds **5** and **6** were identified by comparing their spectral data with those reported previously[3-6]. Acetylation of **5** and **6** gave the acetate derivatives **5a** (ref. 3,4) and **6a** (ref. 3-6) respectively.

The methanol extract of the fresh leaves of *M. esculenta* gave **5**,**6**,**7** and **8** (**Scheme I**). Acetylation of **7** and **8** gave the acetate derivatives **7a** and **8a** respectively. The flavonoid glycosides **7** and **8** were identified by comparison of their spectral data with those of the same compounds reported previously[7-9] (**Table I**).

Experimental Section

All melting points are uncorrected. UV-Vis: MeOH; ^{1}H NMR: $CDCl_3$, 400MHz; Decoupling experiments: $CDCl_3$ + C_6D_6; Optical rotations: $CHCl_3$, Me_2CO and H_2O; TLC: precoated PF_{254} plates (Merck); Column chromatography carried out over silica gel (70-230 mesh, Merck). Compounds were identified by comparison of ^{1}H NMR, IR and melting points.

Extraction and Isolation

Fresh cassava root cortex (2.5 kg) was ground in boiling 95% EtOH in a waring blender, filtered and the ethanolic extract evaporated to give a brown solid (100 g) which was then extracted with CH_2Cl_2: MeOH: H_2O (lower phase, 6:4:1). Additional water was added to produce two layers. The upper layer, on evaporation, gave a brown solid (25 g). The brown

solid was column chromatographed over silica gel (1.7 kg) eluting with a gradient of CH_2Cl_2:MeOH:H_2O (lower phase, 20:3:1, 3 L), (10:3:1, 4 L), (7:3:1, 7 L), (6.5:3.5:1, 7 L). Fractions were combined on the basis of their behaviour on TLC and were evaporated to give compounds **5** and **6** as solids (0.13 and 2.36 g respectively), compound **4** as a slightly yellow semi-solid (0.89 g), and a mixture of compounds **1** and **2** as a slightly yellow semi-solid (0.33 g) and compound **3** as a slightly yellow solid (4.28 g). Fresh leaves (6 kg) were ground in boiling MeOH in a blender. After filtration, the extract was evaporated to dryness and the residue washed with several portions of hexane to remove chlorophyll and other hexane-soluble compounds. The solution which was then brown was evaporated to give a dark brown solid (222 g) which was then extracted with CH_2Cl_2:MeOH:H_2O (lower phase) (6:4:1). Water was added to separate the layers. The upper layer, on evaporation, gave a brown solid (80 g) which was column chromatographed over silica gel (1.8 kg) and eluted with a gradient of CH_2Cl_2: MeOH:H_2O (lower phase, 20:3:1, 20 L), (10:3:1, 9 L), (7:3:1, 13 L). The TLC of the eluate showed the presence of compounds **5**, **6**, **7** and **8**. Concentration of the eluate gave a yellow-brown solid (24 g) which was rechromatographed over silica gel (1.65 kg) eluting with a gradient of CH_2Cl_2:MeOH:H_2O (lower phase, 10:3:1, 300 mL), (7:3:1, 3.4 L). Successive fractions were combined on the basis of their behaviour on TLC and evaporated to give a mixture of **5** and **6** as a slightly yellow solid (6.2 g), compound **7**, a yellow solid (0.5 g) and compound **8**, a yellow solid (0.1 g).

The mixture of compounds 1 and 2

This was difficult to resolve with column chromatography. ^{1}H NMR spectrum of the mixture indicated compound **2** to be the major constituent.

Acetylation of the mixture

The mixture (263 mg) was acetylated with Ac_2O (2 mL) in pyridine (3 mL) at RT for 2 days to give a mixture of acetates, **1a** and **2a** which was recrystallised from EtOAc-hexane as colourless granules. The mixture was resolved over silica gel column with EtOAc-hexane (3.5:6.5) mixture as eluent to give **la** (12 mg) and **2a** (248 mg). **1a** was recrystallized from EtOAc-hexane as colourless needless. m.p. 175-76.5°C; $[\alpha]_D^{25}$ -38.2° (*c* 0.26, $CHCl_3$); IR ($CHCl_3$): 3000, 2975, 2240, 1750, 1415, 1365, 1220, 1050 cm^{-1}; ^{1}H NMR: δ 1.05 (3H, t, *J* =

Table I — ^{13}C NMR spectral data for compounds 2-6 (D_2O, DSS as internal standard)

Carbon	2	3		4		5		6
Aglycone								
1	22.54		23.57*	-		25.79		28.39*
2	72.38		75.52		68.48		78.28*	73.95
3	30.88		24.92*	16.79		35.54		29.09*
4	11.32		-		-		10.40	
CN	-		-		-		123.63	123.57
Glucose								
1′	104.01	102.88	104.20	101.14	100.98			
2′	77.14*	75.52		75.47		75.36		74.87
3′	81.37*	78.23*	78.23		78.61*	78.12*		
4′	72.38		72.16		72.05		72.10	71.56
5′	78.39		77.04*	78.23		78.12*	77.63*	
6′	70.26		70.10		63.22		63.17	62.79
Apiose								
1″	111.53	111.38	-		-		-	
2″	79.46*	78.93*	-		-		-	
3″	81.86		81.70		-		-	-
4″	76.12		75.95		-		-	-
5″	66.20		66.04		-		-	-

*Assignments may be interchanged between the carbons in the same column.

7.5 Hz, H-4), 1.54 (3H, s, CH_3), 1.87 (2H, m, H-3), 2.0, 2.02, 2.05, 2.053, 2.08, 2.12 (3H each, all s, 6 × OAc), 3.55 (1H, m, H-5′), 3.72 (2H, m, H-6′a and H-6′b), 4.15 (1H, d, *J* = 9.0 Hz, H-4″a), 4.23 (1H, d, *J* = 9.0 Hz, H4″b), 4.53 (1H, d, *J* = 12.5 Hz, H-5″a), 4.79 (1H, d, *J* = 12.5 Hz, H-5″b), 4.83 (1H, d, *J* = 8.0 Hz, H-1′), 4.96 (1H, t, *J* = 9.5 Hz, H-4′), 4.98 (lH, dd, *J* = 9.5, 8.0 Hz,H-2′), 5.03 (lH, brs, H-l″), 5.25 (1H, t, *J* = 9.5 Hz, H-3′), 5.36 (1H, brs, H-2″).

Compound **2a**, recrystallized from EtOAc-hexane as colourless needless. m.p.143-44°C; Found: C, 52.4; H, 6.5, $C_{27}H_{40}O_{16}$ requires C, 52.3; H, 6.5%. $[\alpha]_D^{25}$ -63.2° (*c* 0.07, Me_2CO); IR ($CHCl_3$): 2950, 1745, 1400, 1360, 1230, 1020 cm^{-1}; ^1H NMR: δ 0.87 (3H, t, *J* = 7.5 Hz, H-4), 1.19 (3H, d, *J* = 7.5 Hz, H-1), 1.44 (1H, m, H-3a), 1.49 (1H, m, H-3b), 1.99, 2.022, 2.024, 2.03, 2.08, 2.11 (3H each, all s, 6 × OAc), 3.61 (3H, overlapping, H-5′, H-6′a and H-2), 3.67 (1H, m, H-6′b), 4.14 (1H, d, *J* = 11.0 Hz, H-4″a), 4.22 (1H, d, *J* = 11.0 Hz, H-4″b), 4.54 (1H, d, *J* = 8.0 Hz, H-1′), 4.55 (1H, d, *J* = 12.5 Hz, H-5″a), 4.77 (1H, d, *J* = 12.5 Hz, H-5″b), 4.91 (1H, t, *J* = 9.5 Hz, H-4′), 4.93 (1H, dd, *J* = 9.5, 8.0 Hz, H-2′), 5.05 (1H, brs, H-1″), 5.19 (1H, t, *J* = 9.5 Hz, H-3′), 5.34 (1H, brs, H-2″); CIMS: *m/z* (%) 638 $[M+NH_4]^+$ (100), 596 $[(M+H)-25]^+$(1), 259 $[M-361]^+$(1); EIMS: *m/z* (%) 361 $[M-259]^+$(0.5), 259$[M-361]^+$(43), 73 $[M-547]^+$(4), 43 $[M-577]^+$(100).

Deacetylation of 2a

A solution of **2a** (160 mg) in a satd. solution of K_2CO_3 in dry MeOH (3 mL) was heated under reflux for 1.5 hr. The reaction mixture was cooled, diluted with H_2O and evaporated. The aq. solution was extracted with *n*-BuOH satd. with H_2O. Evaporation gave a solid residue which was purified by column chromatophy over silica gel eluting with 5% MeOH in CH_2Cl_2 to give **2**, colourless solid (72 mg), m.p. 114-16°C. Anal. Found: C, 47.9; H, 8.1. C_{15} H_{28} O_{10}.½H_2O requires C, 47.7; H, 7.8%. $[\alpha]_D^{25}$ -64.9° (*c* 0.09,H_2O); IR ($CHCl_3$): 3300(br), 2870, 1050 cm^{-1}. ^1H NMR: δ 0.93 (3H, t, *J* = 7.0 Hz, H -4), 1.24 (3H, d, *J* = 6.2 Hz, H-1), 1.47 (1H, quartet, *J* = 7.0 Hz, H-3a), 1.62 (1H, q, *J* = 7.0 Hz, H-3b), 3.30 (1H,t, *J* = 7.5 Hz, H-4′), 3.41-3.44 (3H overlapping, H-2′, H3′ and OH), 3.72 (1H, sextet, *J* = 7.0 Hz, H-2), 3.82-3.93 (4H, overlapping, H-4″a, H-4″b, H-5″a and H-5″b), 3.96 (1H, dd, *J* = 11.0, 1.5 Hz, H-6′b), 4.25 (1H, brs, OH), 4.32 (1H, d, *J* = 7.5 Hz, H-1′), 4.36 (2H, brs, 2 × OH), 4.67 (1H, brs, OH), 5.02 (1H, d, *J* = 1.5 Hz, H-1″); CIMS: *m/z* (%) 386 $[M+NH_4]^+$(100), 312 $[(M+NH_3)-73]^+$(1); EIMS: *m/z* (%) 295 $[M-73]^+$(1), 163 $[(M+H)-206]^+$(10), 73$[M-295]^+$(75), 57$[M-331]^+$(100).

Compound 3

Compound **3** was purified by column chromatography over silica gel eluting with CH_2Cl_2:MeOH:H_2O (lower layer, 6.5:3.5:1) to give colourless solid (4.28 g), m.p. 119-20°C. Anal. Found: C, 46.2; H, 7.6. $C_{14}H_{26}O_{10}$.½H_2O requires C, 46.3; H,7.5%. $[\alpha]_D^{25}$ -82.7° (*c* 0.59, H_2O). IR ($CHCl_3$): 3400(br), 2925, 2870, 1460, 1370, 1050 cm^{-1}; ^1H NMR: δ 1.20 (3H, d, *J* = 5.6 Hz, CH_3), 1.25 (3H, d, *J* = 5.6 Hz, CH_3), 4.31 (1H, d, *J* = 7.5 Hz, H-1′), 5.01 (1H, d, *J* = 1.8 Hz, H-1″); CIMS: *m/z* (%) 372 $[M+NH_4]^+$(100), 312 $[M-60]^+$(1), 116 $[M-238]^+$(1); EIMS: *m/z* (%) 295 $[M-59]^+$(1), 221 $[M-133]^+$(2), 133 $[M-221]^+$(65), 43 $[M-311]^+$(100).

Acetylation of 3

Compound **3** (100 mg) was acetylated with Ac_2O (1 mL) and pyridine (2 mL) at RT for 35 hr to give the hexaacetate, **3a** (142 mg) which was recrystallized from EtOAc-hexane as colourless needles. m.p. 142-43°C. Anal. Found: C, 51.5; H, 6.3. $C_{16}H_{38}O_{16}$ requires C, 51.5; H, 6.3%. $[\alpha]_D^{25}$ -58.2° (*c* 0.41, acetone); IR ($CHCl_3$): 3013, 2975, 2875, 1745, 1380, 1235, 1040 cm^{-1}; ^1H NMR: δ 1.13 (3H, d, *J* = 6.0 Hz, CH_3), 1.21 (3H, d, *J* = 6.0 Hz, CH_3), 1.995, 2.023, 2.029, 2.033, 2.08, 2.11 (3H each, all s, 6 × OAc), 3.64 (3H, m, H-6′a, H-6b, H-5′), 3.91 1H, septet *J* = 6.0 Hz, H-2), 4.15 (1H, d, *J* = 9.0 Hz, H-4″a), 4.22 1H, d, *J* = 9.0 Hz, H-4″b), 4.34 (1H, d, *J* = 7.5 Hz, H-1′), 4.56 (1H, d, *J* = 11.0 Hz, H-5″a), 4.76 (1H,d,*J* = 11.0 Hz, H-5″b), 4.91 (1H, dd, *J* = 8.5 Hz, 7.5 Hz, H-2′), 4.91 (1H, t, *J* = 8.5 Hz, H-4′), 5.05 (1H, brs, H-1″), 5.19 (1H, t, *J* = 8.5 Hz, H-3′), 5.28 (1H, brs,H-2″); CIMS: *m/z* (%) 624 $[M+NH_4]^+$(100), 259 $[M-347]^+$(1); EIMS: *m/z* (%) 331 $[M-275]^+$(10), 275 $[M-331]^+$(40), 259 $[M-347]^+$(3), 43 $[M-563]^+$(100).

Partial hydrolysis of 3

A solution of **3** (201 mg) in 1% H_2SO_4 in 50% aq. EtOH (5 mL) was heated at 60-68°C for 6 hr. The aqueous solution was neutralized with Na_2CO_3, filtered and evaporated to give a crude residue which was column chromatographed over silica gel eluting with CH_2Cl_2:MeOH:H_2O (20:3:1, 15:3:1, 10:3:1, lower layer) as eluent to give isopropyl-β-D-glucopyranoside, **9** (79 mg). $[\alpha]_D^{25}$ -41.1° (*c* 0.11,

H_2O); IR ($CHCl_3$): 3400(br), 2860, 1460, 1380, 1160, 1120, 1143, 1305 cm^{-1}.

Acetylation of 9

Compound **9** (45 mg) was acetylated with Ac_2O (0.05 mL) and pyridine (1 mL) at RT for 2 hr. to give the acetate derivative, **9a** (53 mg) which was recrystallized from hexane as colourless needles, m.p. 138-40ºC. Anal. Found: C, 5.25; H, 6.8. $C_{18}H_{26}O_{10}$ requires C, 52.3; H, 6.7%. $[\alpha]_D^{25}$ -26.4º, (*c* 0.18, $CHCl_3$); IR ($CHCl_3$): 3015, 2975, 2850, 1750, 1380, 1235, 1040 cm^{-1}; ^1H NMR: δ 1.14 (3H, d, *J* = 5.6 Hz, CH_3), 1.23 (3H, d, *J* = 5.6 Hz, CH_3), 2.0, 2.01, 2.03, 2.08 (3H each, all s, 4 × OAc), 3.68 (1H, ddd, *J* = 9.3, 5.0, 2.0 Hz, H-5′), 3.92 (1H, septet, *J* = 5.6 Hz, H-2), 4.13 (1H, dd, *J* = 11.5, 2.5 Hz, H-6′a), 4.25 (1H ,dd, *J* = 11.5, 4.0 Hz, H-6′b), 4.55 (1H, d, *J* = 7.5 Hz, H-1′), 4.94 (1H, dd, *J* = 13,7.5 Hz, H-2′), 5.07 (1H, t, *J* = 9.3 Hz, H-4′), 5.21 (1H, t, *J* = 9.3 Hz, H-3′).

Compound 4

Compound **4** was purified by column chromatography over silica gel eluting with 3% MeOH in CH_2Cl_2 to give colourless semi-solid $[\alpha]_D^{25}$ - 30.9° (*c* 0.32,H_2O) [(lit, Ferguson, 1932). $[\alpha]_D^{25}$ - 36.7º]; IR (Neat): 3400(br), 2875, 2825, 1060 cm^{-1}; ^1H NMR: δ 1.25 (3H, t, *J* = 6.5 Hz, H-2), 3.23-3.28 (2H, overlapping, H-5′ and OH), 3.43-3.46 (2H, overlapping, H-2′ and H-3′), 3.56 (1H, t, *J* = 6.0 Hz, H-4′), 3.63 (1H, dq, *J* = 9.0, 6.5 Hz, H-la), 3.79 (1H,m, H-6′a), 3.84 (1H, m, H- 6′b), 3.96 (1H, dq, *J* = 9.0, 6.5 Hz,H-1b), 4.31 (1H, d, *J* = 7.0 Hz,H-1′), 4.21, 4.53, 4.58 (1H each, all d, *J* = 3.0, 2.0, 3.0 Hz, 3 × OH); CIMS: *m/z* (%) 226 $[M+NH_4]^+$(100), 208 $[M]^+$(3), 180 $[(M+NH_3)-45]^+$(33), 163 $[M-45]^+$(4).

Acetylation of 4

A solution of **4** (40 mg) in pyridine (1.5 mL) and Ac_2O (1 mL) was stirred at RT under N_2 for 3.5 hr. The acetate, **4a**, was obtained as a colourless solid (65 mg) which was purified over silica gel eluting with 1% MeOH in CH_2Cl_2 to give a colourless solid which was recrystallized from EtOAc-hexane as colourless rhombic crystals. m.p. 106-07°C (lit; Ferguson, 1932, colourless needles, m.p. 106.8°C). Anal. Found: C, 51.5; H, 6.4. $C_{16}H_{24}O_{10}$ requires C, 51.1; H, 6.4%. $[\alpha]_D^{25}$ -23.6° (*c* 0.11, Me_2CO) (lit[7]; $[\alpha]_D^{25}$ -22.7º); IR ($CHCl_3$): 3000, 2975, 2875, 1750, 1435, 1380, 1245, 1035 cm^{-1}; ^1H NMR: δ 1.20 (3H, t, *J* = 6.5 Hz, H-2), 2.01, 2.02, 2.05, 2.09 (3H, each, all s, 4 × OAc), 3.58 (1H, dq, *J* = 9.0, 6.5 Hz, H-la), 3.69 (1H, ddd, *J* = 9.0, 4.0, 2.0 Hz, H-5′), 3.91 (1H, dq, *J* = 9.0, 6.5 Hz, H-1b), 4.14 (1H, dd, *J* = 11.0, 2.0 Hz, H-6′a), 4.27 (1H, dd, *J* = 11.0, 4.0 Hz, H-6′b), 4.51 (1H, d, *J* = 7.0 Hz, H-1), 4.98 (1H, dd, *J* = 9.0, 7.0 Hz, H-2′), 5.09 (1H, t, *J* = 9.0 Hz, H-4′), 5.20 (1H, t, *J* = 9.0 Hz, H-3′); CIMS: *m/z* (%) 394 $[M+NH_4]^+$(100), 352 $[(M+1)-25]^+$(1).

Compound 5

Compound **5** (127 mg) was purified by column chromatography over silica gel (12.7 g) eluting with CH_2Cl_2–MeOH-H_2O (10:3:1 and 7:3:1, lower phase) to give colourless solid which recrystallized from EtOAc-hexane as colourless granules, m.p. 125-26°C (lit[3] 123.5-24.5°C); $[\alpha]_D^{25}$ -17.4° (*c* 0.22, H_2O) (lit[3]. -19.15°) (*c* 1.0). The IR, ^1H NMR and MS were consistent with structure.

Acetylation of 5

A mixture of **5** (20 mg), dry pyridine (0.5 mL) and Ac_2O (1.5 mL) was stirred, at RT for 1 hr. The acetate, **5a**, was obtained as a colourless solid (33 mg) which crystallized from EtOAc-hexane as colourless needles. m.p. 118-19°C (lit[2,3]; 116-16.5°C). Anal. Found: C, 53.2; H, 6.4; N, 3.2. Calcd. for $C_{19}H_{27}NO_{10}$: C, 53.1; H, 6.3; N, 3.3%. $[\alpha]_D^{25}$ - 9.6º (*c* 0.5, Me_2CO, lit[4]., -2.88° (*c* 2.08, ($CHCl_3$)). IR, ^1H NMR and MS data were consistent with the structure.

Compound 6

Compound **6** (2.37 g) was purified by column chromatography over silica gel (236 g) eluting with CH_2Cl_2:MeOH:H_2O (7:3:1, lower phase) to give **6** as a colourless solid which was recrystallized from EtOAc-hexane as colourless granules, m.p. 146-148°C (lit[3,5,6]., 140-41°C; 143-45°C; 139-41°C) respectively.

$[\alpha]_D^{25}$ - 22.2º (*c* 0.35, H_2O, lit[5]., 28.5° (*c* 0.39). IR, ^1H NMR and MS were identical with those of authentic sample.

Acetylation of compound 6

Compound **6** (100 mg) was acetylated with Ac_2O and pyridine to give tetraacetate, **6a** (158 mg) which recrytallized from EtOAc-hexane as colourless

needles, m.p. 142-43°C (lit[3,5,6]., 140-41°C; 140-41°C, 138-39°C respectively). $[\alpha]_D^{25}$ - 11.2 (*c* 0.2, Me_2CO, lit[5].; 10.55°); IR, ^1H NMR and MS data of this compound were identical with those of an authentic sample.

Compound 7

Compound **7** (2.3 g) was purified by column chromatography over silica gel (160 g) eluting with CH_2Cl_2:MeOH:H_2O (7, 3, l, lower phase) to give a yellow solid which recrystallized from MeOH as yellow granules; m.p. 178-83°C (lit[7]; 185-90°C). $[\alpha]_D^{25}$ - 4.9° (*c* 0.4, MeOH). IR, UV-VIS, ^1H NMR and MS data were consistent with the structure.

Acetylation of compound 7

A solution of **7** (20 mg) in pyridine (0.5 mL), 4-dimethyl aminopyridine (0.3 g) and Ac_2O (1.5 mL) was stirred at RT overnight. The acetate derivative, **7a**, was obtained as a solid (31 mg), m.p. 110°C. $[\alpha]_D^{25}$ - 60.2º (*c* 2.23, $CHCl_3$). IR, and ^1H NMR were consistent with the structure.

Acid hydrolysis of 7

A solution of the glycoside (67 mg) in 1% H_2SO_4 in 50% aqueous EtOH (4 mL) was refluxed for 8 hr. After removal of EtOH, the residue was partitioned between H_2O - *n*-BuOH. The *n*-BuOH layer was evaporated to give the crude flavonoid as a yellow residue (90 mg) which was separated over a column of silica gel (10 g) eluting with CH_2Cl_2:MeOH:H_2O (20:3:1, lower layer) to give **10** (kaempferol) as a yellow solid (25 mg). Recrystallization from MeOH yielded yellow granules, m.p. 268°C (lit[11]., 276-78°C). The structure was consistent with the IR, ^1H NMR and MS data.

Acetylation of 10

A solution of **10** (10 mg) in pyridine (0.5 mL), 4-dimethyl aminopyridine (0.3 g) and Ac_2O (1 mL) was stirred overnight at RT. The acetate, **10a**, was obtained as a brown solid (15 mg), m.p. 100°C, resolidifies, remelts at 173-74°C (dec.), lit[7], 120°C, resolidifies, remelts at 178-80°C (dec.) IR, ^1H NMR and MS data were consistent with the structure.

Compound 8

Compound **8** was purified by column chromatography over silica gel eluting with CH_2Cl_2:MeOH:H_2O (6.5:3.5:1, lower phase) to give a yellow solid which crystallized from MeOH as yellow granules. m.p. 192-94°C (lit[10], 214-15°C dec.). $[\alpha]_D^{25}$ +5.8° (*c* 0.27, EtOH), (lit[10], 13.82°. IR, UV, ^1H NMR and MS data were consistent with structure.

Acetylation of 8

A solution of **8** (10 mg) pyridine (0.5 mL), 4-dimethyl aminopyridine (0.3 g) and Ac_2O (1.5 mL) was stirred at RT for 2 hr. The decaacetate, **8a**, was obtained as a brown solid (15.3 mg). $[\alpha]_D^{25}$ - 53.5° (*c* 0.43, $CHCl_3$). IR and ^1H NMR were consistent with the structure.

Acid hydrolysis of 8

Compound **8** (47 mg) in 5% HCl in 50% aq. EtOH (1.5 mL) was refluxed for 2 hr. After evaporating EtOH, the residue obtained was partitioned between H_2O-*n*-BuOH. The BuOH layer, on evaporation, gave crude flavonoid fractions as a yellow residue (120 mg) which was separated over a silica gel column (10 g) eluting with CH_2Cl_2: MeOH:H_2O (20:3:1, lower phase) to give **11**, quercetin, after crystallizing from MeOH as yellow granules. m.p.>300°C (lit[8], 313-14°C). The IR and ^1H NMR data were identical with those of an authentic sample.

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