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CYANOGENIC AND NON-CYANOGENIC GLYCOSIDES FROM *MANIHOT ESCULENTA*

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Key Word Index—*Manihot esculenta*; Euphorbiaceae; cassava; roots; leaves; cyanogenic glycosides; non-cyanogenic glycosides; flavonoids.

Abstract—In addition to lotaustralin and linamarin, a novel cyanogenic glycoside, 2-((6-*O*-(β-D-apiofuranosyl)-β-D-glucopyranosyl)oxy)-2-methylbutanenitrile, two novel non-cyanogenic glycosides, (2*S*)-((6-*O*-(β-D-apiofuranosyl)-β-D-glucopyranosyl)oxy)butane and 2-((6-*O*-(β-D-apiofuranosyl)-β-D-glucopyranosyl)oxy)propane, and a simple non-cyanogenic glycoside, ethyl β-D-glucopyranoside, were isolated from an ethanolic extract of the fresh root cortex of *Manihot esculenta*. From a methanolic extract of the fresh leaves of this species lotaustralin and linamarin, and two flavonoid glycosides, kaempferol-3-*O*-rutinoside and quercetin-3-*O*-rutinoside were isolated.

INTRODUCTION

Cassava, *Manihot esculenta*, is a major source of dietary energy for human and domestic animals in many tropical countries [1]. Chemical investigation of the root cortex of this plant has led to the isolation of four new glycosides, 2-((6-*O*-(β-D-apiofuranosyl)-β-D-glucopyranosyl)oxy)-2-methylbutanenitrile (**1**), (2*S*)-((6-*O*-(β-D-apiofuranosyl)-β-D-glucopyranosyl)oxy)butane (**2**), 2-((6-*O*-(β-D-apiofuranosyl)-β-D-glucopyranosyl)oxy)propane (**3**), ethyl β-D-glucopyranoside (**4**) and two known cyanogenic glycosides, lotaustralin (**5**) and linamarin (**6**). Together with compounds **5** and **6**, two known flavonoid glycosides, kaempferol-3-*O*-rutinoside (**7**) and quercetin-3-*O*-rutinoside (**8**) were isolated from the fresh leaves of the plants. X-ray crystallographic structures of the acetate derivatives of **2** and **4** have been determined.

RESULTS AND DISCUSSION

The concentrated ethanol extract of fresh cassava root cortex was separated into two layers by addition of CH_2Cl_2–MeOH–H_2O (6:4:1). Column chromatography of the material in the upper layer on silica gel gave lotaustralin (**5**), linamarin (**6**), ethyl glucoside (**4**), a mixture of *iso*butyl cyanogenic glycoside (**1**), and *iso*butyl glycoside (**2**), and *iso*propyl glycoside (**3**).

Acetylation of the mixture of **1** and **2** gave a mixture of acetate derivatives, **1a** and **2a**, which was separated by column chromatography on silica gel. Deacetylation of **2a** with methanolic K_2CO_3 gave the *iso*butyl glycoside **2**. CI mass spectrometry (NH_3) of **2** showed a pseudomolecular ion peak at *m/z* 386 $(C_{15}H_{28}O_{10} + NH_4)^+$ which was in good agreement with 15 carbon signals in the ^{13}C NMR spectrum. A fragmentation peak at *m/z* 312 $(C_{11}H_{19}O_9 + NH_3)^+$ corresponded to loss of an *iso*butoxyl group (C_4H_9O). The ^1H NMR spectrum of **2** exhibited signals from two anomeric protons as two doublets at δ4.32 (J = 7.5 Hz) and δ5.02 (J = 1.5 Hz) which were assigned to those of β-D-glucose and β-D-apiose, respectively. The aglycone *iso*butoxyl group was indicated by the signals of two methyl groups appearing as a doublet at δ1.24 (J = 6.2 Hz) and a triplet at δ0.93 (J = 7.0 Hz); a methine proton resonated at δ3.72 (sextet, J = 7.0 Hz) and two methylene protons gave rise to two quintets at δ1.47 (J = 7.0 Hz) and 1.62 (J = 7.0 Hz). The ^{13}C NMR spectrum of **2** exhibited signals for two anomeric carbons (δ104.0 and 111.6). The peak at δ70.3

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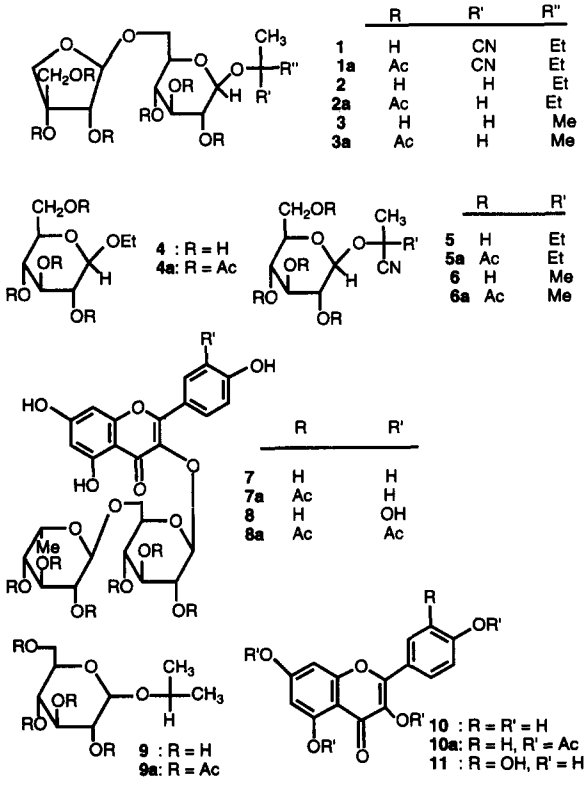

Table 1. ^{13}C NMR spectral data for compounds **2**–**6** (D_2O, DSS as internal standard)

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	11.31	—	—	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″	111.55	111.38	—	—	—
2″	79.15*	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.

(*t*), which showed a significant glycosidation shift, is indicative of the linkage of the terminal apiose to the glucosyl moiety at C-6.

Acetylation of **2**, in the usual manner, provided the hexaacetate **2a**. The ^{1}H NMR spectrum of **2a** showed two anomeric proton signals at δ4.54 (*d*, J = 8.0 Hz) and 5.05 (*br s*), which were assigned to those of β-D-glucose and β-D-apiose, respectively. Through selective single frequency proton-decoupling experiments, assignments for all individual sugar signals were made. The occurrence upfield of the resonances of the H-6′a and H-6′b (δ3.61 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 (H1″) 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%). Based on this evidence, the structure **2** was deduced.

The acetate derivative **2a** was obtained as colourless needles which were suitable for X-ray crystallographic analysis. The single crystal X-ray analysis confirmed the structure **2a** and indicated the (*S*)-configuration at C-2; an ORTEP projection of the structure is shown in Fig. 1. The structure of **2** is therefore (2*S*)-((6-*O*-(β-D-apiofuranosyl)-β-D-glucopyranosyl)oxy)butane.

The ^{1}H NMR spectrum of the acetate derivative **1a** was similar to that of **2a** except that the chemical shifts of C-2–CH_3, $(H\text{-}3)_2$, and $(H\text{-}4)_3$ were shifted downfield by 0.35, 0.4, and 0.18 ppm, respectively, compared with **2a**. This may due to the presence of the nitrile group on C-2 in **1a**. Furthermore, C-2–CH_3 resonated as a singlet and the $(H\text{-}3)_2$ 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** (δ20–30 region). The two anomeric carbon signals appeared at δ102.9 and 111.4 which were ascribed to those of β-D-glucose and β-D-apiose, respectively. The CI mass spectrum of **3** exhibited a pseudomolecular ion peak at *m/z* 372 $[M + NH_4]^+$; the EI mass spectrum of **3** showed peaks at *m/z* 295 $[M - 59]^+$, 221 $[M - 133]^+$ and 133 $[M - 59 - 162]^+$, corresponding to losses of *iso*propoxyl, pentose and *iso*propoxyl, and hexose groups, respectively. Acetylation of **3** by the standard procedure gave the hexaacetate **3a**. The two anomeric proton signals appeared at δ4.54 (*d*, J = 7.5 Hz) and 5.05 (*br s*). The aglycone *iso*propoxyl group was indicated by the signals of two methyl groups appearing as two doublets at δ1.13 (J = 6.0 Hz) and 1.21 (J = 6.0 Hz) and the septet signal of a methine proton at δ3.91. A double quantum-filtered ^{1}H–^{1}H 2D correlation spectrum (DQFCOSY) provided assignments of all individual sugar signals. These were confirmed by selective single frequency proton-decoupling experiments. In addition, NOE enhancements were observed between the CH_3 signal at δ1.13 and H1′ (δ4.54) (2.6%), between H2 (δ3.90) and H1′ (5.5%), and between $(H6')_2$ and H1″ (6%). Therefore, the glycoside **3** was characterized as 2-((6-*O*-(β-D-apiofuranosyl)-β-D-glucopyranosyl)oxy)propane.

The ^{13}C NMR spectrum of **4** showed eight carbon signals. An anomeric carbon signal appeared at δ104.2. The sugar moiety of **4** was identified as β-D-glucose. The CI mass spectrum 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

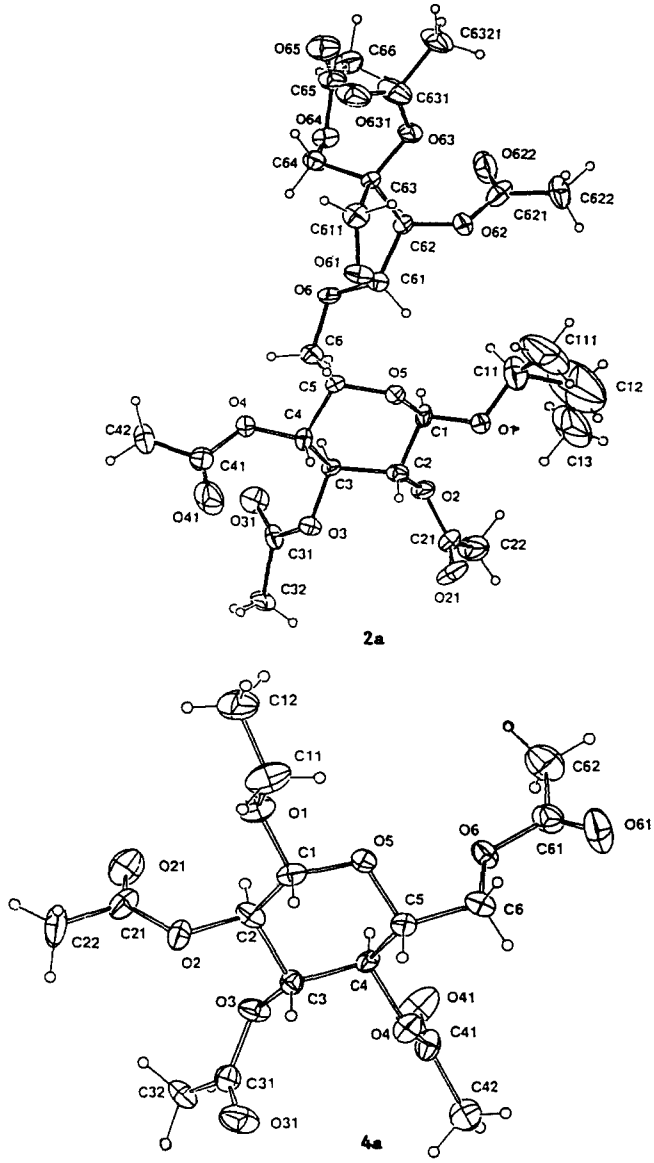

Fig. 1. Single molecules of compounds **2a** and **4a**. Thermal ellipsoids (2090) are shown for non-hydrogen atoms; hydrogen atoms have an arbitrary radius of 0.1 Å. Crystallographic skeletal numbering is shown.

of an ethoxyl group. The ^{1}H NMR spectrum exhibited the signals of a methyl group at δ1.25 (*t*, J = 6.5 Hz) and two methylene protons at δ3.63 (*dq*, J = 9.0, 6.5 Hz) and 3.96 (*dq*, J = 9.0, 6.5 Hz) corresponding to the presence of an ethoxyl group in **4**. Acetylation of **4** provided the tetraacetate **4a**. The ^{1}H NMR of **4a** showed signals for an anomeric proton at δ4.51 (*d*, J = 7.0 Hz), a methyl group at δ1.20 (*t*, J = 6.5 Hz) and one methylene group at δ3.58 (*dq*, J = 9.0, 6.5 Hz) and 3.91 (*dq*, J = 9.0, 6.5 Hz) corresponding to an ethoxyl group. By selective single frequency proton-decoupling experiments, assignments of all individual protons of the glucose moiety were confirmed. Therefore, the glycoside **4** was identified as ethyl β-D-glucopyranoside. A single crystal X-ray analysis of **4a** confirmed the structure. An ORTEP projection of **4a** is shown in Fig. 1. As ethanol was used as the solvent for the extraction, it is probable that **4** is an artefact.

Lotaustralin **5** and linamarin **6** were identified by comparison their spectral data with data reported proviously [2–5]. Acetylation of **5** and **6** by standard procedures gave the acetate derivatives **5a** [2, 3] and **6a** [2–5] respectively.

The methanol extract of the fresh leaves *M. esculanta* gave lotaustralin **5**, linamarin **6**, nicotiflorin **7** and rutin **8**. Acetylation of **7** and **8** by standard procedures gave the acetate derivatives, **7a** and **8a**, respectively. The flavonoid glycosides **7** and **8** were identified by comparison of their spectral data with data reported previously [6–8].

EXPERIMENTAL

Unless otherwise stated, analyses were carried out by the Scientific and Technological Research Equipment Center, Chulalongkorn University, Bangkok, Thailand. Mps: uncorr. UV: MeOH. ^{1}H NMR: $CDCl_3$, 400 MHz; decoupling experiments: $CDCl_3 + C_6D_6$. Optical rotations: $CHCl_3$, Me_2CO and H_2O. TLC: precoated PF254 plates (Merck). CC: silica gel 70–230 mesh (Merck). Compounds were identified by comparison of ^{1}H NMR, IR and mmp.

Extraction and isolation. Fresh cassava root cortex (2.5 kg) was ground in boiling 95% EtOH in a Waring blender. The filtrate was evaporated to give a dark-brown solid (100 g) which was extracted with CH_2Cl_2–MeOH–H_2O (6:4:1). Additional H_2O was added as necessary to produce two layers. The upper layer was evaporated to give a brown solid (25 g). The brown solid was chromatographed on a column of silica gel (1.7 kg) and was eluted 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)). Successive frs were combined on the basis of their behaviour on TLC and 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), a mixt. of compounds **2** and **1**, 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 chlorophylls and other hexane-sol. compounds. The brown residue was evaporated to give a dark brown solid (222 g) which was then extracted with CH_2Cl_2–MeOH–H_2O (6:4:1). Additional H_2O was added as necessary to separate the layers. The upper layer was evaporated to give a brown solid (80 g) which was chromatographed on a column of 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). At this stage, TLC of the eluent showed the presence of four compounds, lotaustralin **5**, linamarin **6**, nicotiflorin **7** and rutin **8**. Removal of solvent gave a yellow–brown solid (24 g) which was rechromatographed on a column of silica gel (1.65 kg). The column was eluted with a gradient of CH_2Cl_2–MeOH–H_2O (lower phase) (10:3:1 (300 ml), 7:3:1 (3.4 l)). Successive frs were combined on the basis of their behaviour on TLC and evaporated to give a mixt. of **5** and **6** as a slightly yellow solid (6.2 g), compound **7** as a yellow solid (0.5 g) and compound **8** as a yellow solid (0.1 g).

2-((6-*O*-(β-D-*Apiofuranosyl*)-β-D-*glucopyranosyl*)*oxy*)-2-*methylbutanenitrile* (**1**) *and* (2*S*)-((6-*O*-(β-D-*apiofuranosyl*)-β-D-*glucopyranosyl*)*oxy*)*butane* (**2**). Attempts to separate the mixt. of **1** and **2** were unsuccessful. ^{1}H NMR indicated that compound **2** was the major component.

Acetylation of **1** *and* **2**. The mixt. of compounds **1** and **2** (263 mg) was acetylated with Ac_2O (2 ml) in pyridine (3 ml) at room temp. for 2 days to give a mixt. of acetate derivatives **1a** and **2a** which was recrystallized from EtoAc–hexane as colourless granules.This mixt. was separated on a column of silica gel with EtOAc–hexane (3.5:6.5) to give the respective acetate derivatives **1a** (12 mg) and **2a** (248 mg). Compound **1a** was recrystallized from EtOAc–hexane as a colourless needle, mp 175–176.5°. $[\alpha]_D^{25}$ − 38.2° (*c* 0.26, $CHCl_3$). ν_{max} $CHCl_3$ cm^{-1}: 3000, 2975, 2240, 1750, 1415, 1365, 1220, 1050. ^{1}H NMR: δ 1.05 (3H, *t*, *J* = 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, H-4″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 (1H, *dd*, *J* = 9.5, 8.0 Hz, H-2′), 5.03 (1H, *br s*, H-1″), 5.25 (1H, *t*, *J* = 9.5 Hz, H-3′), 5.36 (1H, *br s*, H-2″). Compound **2a** was recrystallized from EtOAc–hexane as colourless needles, mp 143–144°. 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). ν_{max} cm^{-1}: 2950, 1745, 1400, 1360, 1230, 1020. ^{1}H 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, *br s*, H-1″), 5.19 (1H, *t*, *J* = 9.5 Hz, H-3′), 5.34 (1H, *br s*, H-2″). CI MS *m/z* (rel. int.): 638 $[M + NH_4]^+$ (100), 596 $[(M + H) - 25]^+$ (1), 259 $[M - 361]^+$ (1). EI MS *m/z* (rel. int.): 361 $[M - 259]^+$ (0.5), 259 $[M - 361]^+$ (43), 73 $[M - 547]^+$ (4), 43 $[M - 577]^+$ (100).

Deacetylation of compound **2a**. A soln of compound **2a** (160 mg) in a satd soln of K_2CO_3 in dry MeOH (3 ml) was heated under reflux for 1.5 hr. The reaction mixt. was cooled, dild with H_2O and evaporated. The aq. soln was extracted with *n*-BuOH satd with H_2O. Removal of solvent gave a solid residue which was purified by CC using silica gel with 5% MeOH in CH_2Cl_2 as eluent, to give compound **2** as a colourless solid (72 mg), mp 114–116°. Found: C, 47.9; H, 8.1. $C_{15}H_{28}O_{10}.1/2\ H_2O$ requires C, 47.7; H, 7.8%. $[\alpha]_D^{25}$ − 64.9° (*c* 0.09, H_2O). ν_{max} cm^{-1}: 3300 (*br*), 2870, 1050. ^{1}H 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, *quintet*, *J* = 7.0 Hz, H-3a), 1.62 (1H, *quintet*, *J* = 7.0 Hz, H-3b), 3.30 (1H, *t*, *J* = 7.5 Hz, H-4′), 3.41–3.44 (3H, overlapping, H-2′, H-3′ and OH), 3.63 (4H, overlapping, H-5′, H-6′a, H-2″ 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, *br s*, OH), 4.32 (1H, *d*, *J* = 7.5 Hz, H-1′), 4.36 (2H, *br s*, 2 × OH), 4.67 (1H, *br s*, OH), 5.02 (1H, *d*, *J* = 1.5 Hz, H-1″). CI MS *m/z* (rel. int.): 386 $[M + NH_4]^+$ (100), 312 $[(M + NH_3) - 73]^+$ (1). EI MS *m/z* (rel. int.): 295 $[M - 73]^+$ (1), 163 $[(M + H) - 206]^+$ (10), 73 $[M - 295]^+$ (75), 57 $[M - 331]^+$ (100).

2-[[6-*O*-(β-D-*Apiofuranosyl*)-β-D-*glucopyranosyl*]-*oxy*]*propane* (**3**). Compound **3** was purified by CC using silica gel and CH_2Cl_2–MeOH–H_2O (6.5:3.5:1, lower layer) as eluent, to give a colourless solid (4.28 g), mp 119–120°. Found: C, 46.2; H, 7.6. $C_{14}H_{26}O_{10}.1/2\ H_2O$ requires C, 46.3; H, 7.5%. $[\alpha]_D^{25}$ −82.7° (*c* 0.59, H_2O). ν_{max} cm^{-1}: 3400 (*br*), 2975, 2925, 2870, 1460, 1370, 1050. ^1H NMR: δ1.20 (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″). CI MS *m/z* (rel. int.): 372 $[M + NH_4]^+$ (100), 312 $[M - 60]^+$ (1), 116 $[M - 238]^+$ (1). EI MS *m/z* (rel. int.): 295 $[M - 59]^+$ (1%), 221 $[M - 133]^+$ (2), 133 $[M - 221]^+$ (65), 43 $[M - 311]^+$ (100).

Acetylation of compound (**3**). Compound **3** (100 mg) was acetylated with Ac_2O (1 ml) and pyridine (2 ml) at room temp. for 35 hr to give the hexaacetate **3a** (142 mg) which was recrystallized from EtOAc–hexane as a colourless needle, mp 142–143°. Found: C, 51.5; H, 6.3. $C_{26}H_{38}O_{16}$ requires C, 51.5; H, 6.3%. $[\alpha]_D^{25}$ −58.2° (*c* 0.41, acetone). ν_{max} cm^{-1}: 3013, 2975, 2875, 1745, 1380, 1235, 1040. ^1H NMR: δ1.13 (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-6′b, 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), 4.54 (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, *br s*, H-1″), 5.19 (1H, *t*, *J* = 8.5 Hz, H-3′), 5.28 (1H, *br s*, H-2″). CI MS *m/z* (rel. int.): 624 $[M + NH_4]^+$ (100), 259 $[M - 347]^+$ (1). EI MS *m/z* (rel. int.): 331 $[M - 275]^+$ (10), 275 $[M - 331]^+$ (40), 259 $[M - 347]^+$ (20), 43 $[M - 563]^+$ (100).

Partial hydrolysis of compound (**3**). A soln of compound **3** (201 mg) in 1% H_2SO_4 in 50% aq. EtOH (5 ml) was heated at 60–68° for 6 hr. The aq. soln was neutralized with Na_2CO_3, filtered and evaporated to give a crude residue which was chromatographed on a column of silica gel using CH_2Cl_2–MeOH–H_2O (20:3:1, 15:3:1 and 10:3:1, lower layer) as eluents to give *iso*propyl β-D-glucopyranoside **9** (79 mg). $[\alpha]_D^{25}$ −41.1° (*c* 0.11, H_2O), ν_{max}^{Nujol} cm^{-1}: 3400 (*br*), 2860, 1460, 1380, 1160, 1120, 1045, 1305.

Acetylation of compound **9**. Compound **9** (45 mg) was acetylated with Ac_2O (0.5 ml) and pyridine (1 ml) at room temp. for 2 hr to give the acetate derivative **9a** (80 mg) which was recrystallized from hexane as colourless needles, mp 138–140°. Found: C, 52.5; H, 6.8. $C_{17}H_{26}O_{10}$ requires C, 52.3; H, 6.7%. $[\alpha]_D^{25}$ −26.4° (*c* 0.28, $CHCl_3$). $\nu_{max}^{CHCl_3}$ cm^{-1}: 3015, 2975, 2850, 1750, 1385, 1235, 1040. ^1H NMR: δ1.14 (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, 2.5 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, 5.0 Hz, H-6′b), 4.55 (1H, *d*, *J* = 7.5 Hz, H-1′), 4.94 (1H, *dd*, *J* = 9.3, 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′).

Ethyl-β-D-glucopyranoside (**4**). Compound **4** was purified by CC using silica gel with 3% MeOH in CH_2Cl_2, to give a colourless semi-solid. $[\alpha]_D^{25}$ −30.9° (*c* 0.32, H_2O) [lit. [8] $[\alpha]_D$ −36.7°]. ν_{max}^{neat} cm^{-1}: 3400 (*br*), 2875, 2825, 1060. ^1H NMR: δ1.25 (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-1a), 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). CI MS *m/z* (rel. int.): 226 $[M + NH_4]^+$ (100), 208 $[M]^+$ (3), 180 $[(M + NH_3) - 45]^+$ (33), 163 $[M - 45]^+$ (4).

Acetylation of compound (**4**). A soln of compound **4** (40 mg) in pyridine (1.5 ml) and Ac_2O (1 ml) was stirred at room temp under N_2 for 3.5 hr. After work-up, the acetate derivative **4a** was obtained as a colourless solid (65 mg). Acetate **4a** was purified on a column of silica gel using 1% MeOH in CH_2Cl_2 to give a colourless solid which was recrystallized from EtOAc–hexane as a colourless rhombic crystals, mp 106–107° (lit. [8] colourless needles, mp 106.8°). (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. [8] $[\alpha]_D$ −22.7°). ν_{max} cm^{-1}: 3000, 2975, 2875, 1750, 1435, 1380, 1245, 1035. ^1H NMR: δ1.20 (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-1a), 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′). CI MS *m/z* (rel. int.): 394 $[M + NH_4]^+$ (100), 352 $[(M + 1) - 25]^+$ (1). Spectral data (IR, ^1H NMR) of ethyl β-D-glucopyranoside and its acetate have not been reported previously.

(R)-2-(β-D-*Glucopyranosyloxy*)-2-*methylbutanenitrile* (*lotaustralin*) (**5**). Compound **5** (127 mg) was purified by CC using silica gel (12.7 g) and CH_2Cl_2–MeOH–H_2O (10:3:1 and 7:3:1, lower phase) as eluent, to give a colourless solid which was recrystallized from EtOAc–hexane as colourless granules, mp 125–126° (lit. [2] 123.5–124.5°). $[\alpha]_D^{25}$ −17.4° (*c* 0.22, H_2O) (lit. [2] −19.15°) (*c* 1.0). IR, ^1H NMR and MS data consistent with structure.

Acetylation of compound (**5**). A mixt. of compound **5** (20 mg), dry pyridine (0.5 ml) and Ac_2O (1.5 ml) was stirred at room temp for 1 hr. After work-up, the acetate derivative **5a** was obtained as a colourless solid (33 mg) which was recrystallized from EtOAc–hexane as colourless needles, mp 118–119° (lit. [2] 116–116.5°). Found: C, 53.2; H, 6.4; N, 3.2. Calc. for $C_{19}H_{27}NO_{10}$: C, 53.1; H, 6.3; N, 3.3%. $[\alpha]_D^{25}$ −9.6° (*c* 0.5, Me_2CO) (lit. [3] −2.88° (*c* 2.08, $CHCl_3$)). IR, ^1H NMR and MS data consistent with the structure.

2-(β-D-*Glucopyranosyloxy*)-2-*methylpropanenitrile* (*linamarin*) (**6**). Compound **6** (2.37 g) was purified by CC using silica gel (236 g) and CH_2Cl_2–MeOH–H_2O (7:3:1, lower phase) to give compound **6** as a colourless solid

which was recrystallized from EtOAc–hexane as colourless granules, mp 146–148° (lit. [2, 4, 5] 140–141°, 143–144°, 139–141°). $[\alpha]_D^{25}$ − 22.2° (*c* 0.35, H_2O) [lit. [4] − 28.5° (*c* 0.39)]. IR, ^{1}H NMR and MS data identical to those of an authentic sample.

Acetylation of compound (**6**). Compound **6** (100 mg) was acetylated with Ac_2O and pyridine to give the tetraacetate **6a** (158 mg) which was recrystallized from EtOAc–hexane as colourless needles, mp 142–143° (lit. [2, 4, 5] 140–141°, 140–141°, 138–139°). $[\alpha]_D^{25}$ − 11.2° (*c* 0.2, Me_2CO) (lit. [5] − 10.55°). IR, ^{1}H NMR and MS data identical to those of an authentic sample.

Kaempferol-3-O-rutinoside (*nicotiflorin*) (**7**). Compound **7** (2.3 g) was purified by CC using silica gel (160 g) and CH_2Cl_2–MeOH–H_2O (7:3:1, lower phase) as eluent to give **7** as a yellow solid, which was recrystallized from MeOH to give compound **7** as yellow granules, mp 178–183° (lit. [6] 185–190°). $[\alpha]_D^{25}$ − 4.9° (*c* 0.4, MeOH). IR, UV, ^{1}H NMR and MS data consistent with the structure.

Acetylation of compound (**7**). A soln of compound **7** (20 mg) in pyridine (0.5 ml), 4-dimethylaminopyridine (0.3 g) and Ac_2O (1.5 ml) was stirred at room temp. overnight. After work-up, the acetate derivative **7a** was obtained as a solid (31 mg), mp 110°. $[\alpha]_D^{25}$ − 60.2° (*c* 2.23, $CHCl_3$). IR and ^{1}H NMR data consistent with the structure.

Acid hydrolysis of compound (**7**). A soln of the glycoside **7** (67 mg) in 1% H_2SO_4 in 50% aq. 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). This was separated on a column of silica gel (10 g) which was eluted with CH_2Cl_2–MeOH–H_2O (20:3:1, lower layer) to give kaempferol **10** as a yellow solid (25 mg). Recrystallization from MeOH yielded yellow granules, mp 268° (lit. [9] 276–278°). IR, ^{1}H NMR and MS data consistent with the structure.

Acetylation of kaempferol (**10**). A soln of kaempferol **10** (10 mg) in pyridine (0.5 ml), 4-dimethylaminopyridine (0.3 g) and Ac_2O (1 ml) was stirred at room temp overnight. After work-up, the acetate derivative **10a** was obtained as a brown solid (15 mg), mp 100° resolidifies, remelts at 173–174° (dec.). [lit. [6] 120° resolidifies, remelts at 178–180° (dec.)]. IR, ^{1}H NMR and MS data consistent with the structure.

Quercetin-3-O-rutinoside (*rutin*) (**8**). Compound **8** was purified by CC using silica gel and CH_2Cl_2–MeOH–H_2O (6.5:3.5:1, lower phase) as eluent to give a yellow solid, which was recrystallized from MeOH as yellow granules, mp 192–194° (lit. [10] 214–215° dec.). $[\alpha]_D^{25}$ + 5.8° (*c* 0.27, EtOH) (lit. [10] + 13.82°). IR, UV, ^{1}H NMR and MS data consistent with the structure.

Acetylation of compound (**8**). A soln of compound **8** (10 mg), pyridine (0.5 ml), 4-dimethylaminopyridine (0.3 g) and Ac_2O (1.5 ml) was stirred at room temp for 2 hr. After work-up, the decaacetate **8a** was obtained as a brown solid (15.3 mg). $[\alpha]_D^{25}$ − 53.5° (*c* 0.43, $CHCl_3$). IR and ^{1}H NMR consistent with the structure.

Acid hydrolysis of compound (**8**). Compound **8** (47 mg) in 5% HCl in 50% aq. EtOH (1.5 ml) was refluxed for 2 hr. After the removal of EtOH, the residue was partitioned between H_2O–*n*-BuOH. The *n*-BuOH layer was evaporated to give the crude flavonoid fr. as a yellow residue (120 mg). This was separated on a silica gel column (10 g) which was eluted with CH_2Cl_2–MeOH–H_2O (20:3:1, lower phase) to give quercetin **11**, after recrystallization from MeOH, as yellow granules, mp > 300° (lit. [7] 313–314°). IR and ^{1}H NMR identical with those of an authentic sample.

Structural determination of compounds (**2a** *and* **4a**). Unique room temp (~ 295 K) diffractometer data sets were measured (monochromatic MoK_α radiation, $\lambda = 0.7107_3$ Å) yielding N independent reflections, N_0 ($I > 3\sigma(I)$) being considered 'observed' and used in the full matrix least squares refinements without absorption correction after solution of each structure by direct methods. Anisotropic thermal parameters were refined for C, O; (x, y, z, U_{iso}) H were included constrained at estimated values. Conventional residuals R, R_w on $|F|$ at convergence are quoted, statistical reflection weights derivatives of $\sigma^2(I) = \sigma^2(I_{diff}) + 0.0004\sigma^4(I_{diff})$ being used. Neutral atom complex scattering factors were employed, chirality being assumed from the chemistry. Computation used the XTAL 2.2 program system implemented in Ref. [11]. Pertinent results are given in the Figs and deposited material (atom coordinates and thermal parameters, molecular non-hydrogen geometries, structure factor amplitudes). Specific details are as follows. **2a**: $C_{27}H_{40}O_{16}$, $M_r = 620.6$. Orthorhombic, space group $P2_12_12_1$ (D_2^4, No. 19), $a = 7.582$ (2), $b = 11.963$ (3), $c = 36.120$ (8) Å, $V = 3253$ (1) Å^3. D_c ($Z = 4$) = 1.27 g cm^{-3}; F (000) = 1320. $\mu_{Mo} = 1.1$ cm^{-1}; specimen: 0.42 × 0.27 × 0.25 mm. $2\theta_{max} = 45°$; $N = 2466$, $N_0 = 1500$; $R = 0.076$, $R_w = 0.083$.

Abnormal features/variations in procedure. Data were weak and diffuse and limited in scope with consequent adverse precision. In view of the long *c* axis and wide line width, data was measured by an ω-scan procedure; even so, a number of reflections were obviously affected adversely by profile overlap problems and were deleted from the refinement. **4a**: $C_{16}H_{24}O_{10}$. $M_r = 376.4$. Orthorhombic, space group $P2_12_12_1$ (D_2^4, No. 19), $a = 17.131$ (8), $b = 15.740$ (7), $c = 7.282$ (3) Å, $V = 1963$ (1) Å^3. D_c ($Z = 4$) = 1.27 g cm^{-3}; F(000) = 800. μ_{Mo} = 1.1 cm^{-1}; specimen: cuboid, ~ 0.2 mm. $2\theta_{max} = 50°$; $N = 2015$, $N_0 = 969$; $R = 0.063$, $R_w = 0.035$.

Abnormal features/variations in procedure. Again, weak and limited data were limiting factors on the precision of the determination. The 'observed' reflection threshold was set at $I > 2\sigma(I)$.

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