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Note

Synthesis and characterisation of methyl 2-*O*-(β-D-glucopyranosyl)-6-*O*-(α-L-rhamnopyranosyl)-α-D-glucopyranoside*

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The branched trisaccharide, 2-*O*-(β-D-glucopyranosyl)-6-*O*-(α-L-rhamnopyranosyl)-D-glucopyranose (**2**), is regarded as the sugar moiety of an anthocyanin pigment isolated from the fruits and flowers of certain *Begonia*, *Clivia*, *Rubus*, *Prunus*, and *Ribis* species[1,2]. The cyanidin-3-glycoside of **1** has been identified as one of the major pigments of Montmorency cherries (*Prunus cerasus*)[3]. It has also been isolated as quercetin and kaempherol glycosides from the flowers of potato (*Solanum tuberosum*)[4]. Synthesis of the methyl glycoside of **1**, methyl 2-*O*-(β-D-glucopyranosyl)-6-*O*-(α-L-rhamnopyranosyl)-α-D-glucopyranoside (**2**) is reported.

RESULTS AND DISCUSSION

The target methyl glycoside (**2**) was prepared in an overall yield of 21.8% starting from methyl 4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**3**, ref. 5).

Methyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**4**) was obtained from **3** (89%) utilising 90% aqueous trifluoroacetic acid[6] for debenzylidenation. Methyl 3,4-di-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-*O*-trityl-α-D-glucopyranoside (**5**) was obtained by treating **4** with chlorotriphenylmethane in pyridine, followed by acetylation.

Detritylation of **5** gave **6** (70% yield) which may be a generally useful intermediate in the synthesis of methyl 2-*O*-(β-D-glucopyranosyl)-6-*O*-(glycosyl)-α-D-glucopyranosides. Compound **8** was synthesised (63% yield) by condensation of **6** with 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide (**7**, ref. 7) under Helferich–Zirner conditions. Deacetylation was effected by transesterification with sodium methoxide[8] to yield the methyl glycopyranoside **2** in 76% yield.

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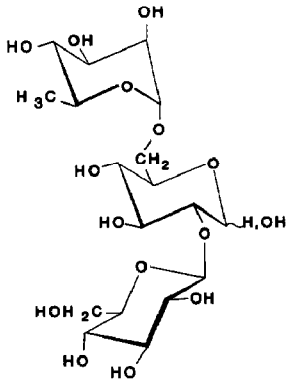

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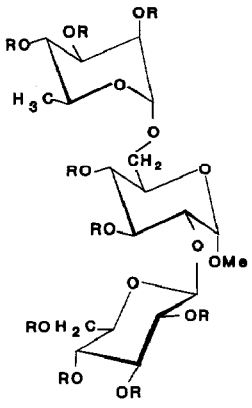
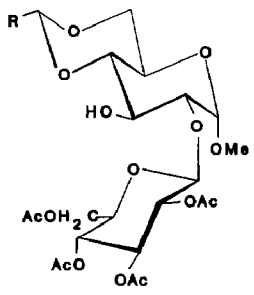
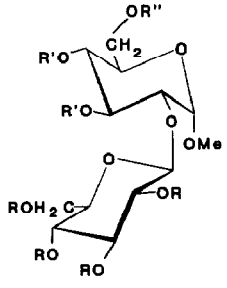


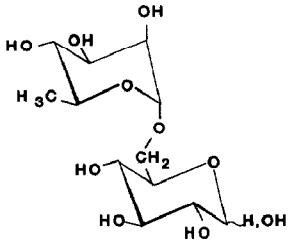
2 R = H
8 R = Ac



3 R = Phenyl



4 R = Ac, R' = H, R'' = H
5 R = Ac, R' = Ac, R'' = Trityl
6 R = Ac, R' = Ac, R'' = H
12 R = H, R' = H, R'' = H



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The structure of **2** was verified as follows: The methyl glucopyranoside (**2**) was permethylated[9], the product hydrolysed[10] with 2M HCl, and the hydrolysate analysed by g.l.c. after trimethylsilylation, according to the Kovats' retention-index system[11] (Table I). Samples of per-*O*-Me_3Si derivatives of 2,3,4-tri-*O*-methyl-L-rhamnopyranose (**9**), 2,3,4,6-tetra-*O*-methyl-D-glucopyranose (**10**), and 3,4-di-*O*-methyl-D-glucopyranose (**11**) were used as reference compounds. The close agreement observed between the retention indexes of derivatives obtained from **2** and the reference compounds supports the expected structure of **2**.

^{13}C-N.m.r. data for **2**, methyl 2-*O*-*β*-D-glucopyranosyl-*α*-D-glucopyranoside (**12**), and 6-*O*-*α*-L-rhamnopyransyl-D-glucopyranose (**13**) are summarised in Table II. Resonances in the spectrum of **2** were assigned by comparison with signals in the spectra of **12** and **13**. Resonances in the spectra of **12** and **13** were assigned according to the multiplicity (in the case of methoxyl and 6-glycosyl carbons) observed in the single-frequency proton off-beat decoupled spectra and by comparison with the spectra of suitable reference compounds in the literature[13–16]. The spectrum of **13**, complicated by the occurrence of both the *α*- and *β*-anomeric forms, is in close agreement with that[13] measured in D_2O.

TABLE I

G.l.c. retention indexes for the trimethylsilyl derivatives obtained from target and reference compounds

Compound	*Chromatographic conditions*		*Retention indexes*
	Temperature (°)	*Column*	
	110	1% OV1	1394.2
2	110	1% OV1	1535.5; 1552.4
	140	2.5% OV1	1719.8; 1781.1
2,3,4-Tri-*O*-methyl-L-rhamnopyranose (**10**)	110	1% OV1	1389.7
2,3,4,6-Tetra-*O*-methyl-α-D-glucopyranoside (**9**)	110	1% OV1	1537.6; 1550.3
3,4-Di-*O*-methyl-D-glucopyranose (**11**)	140	2.5% OV1	1719.6; 1781.6

TABLE II

^{13}C-N.m.r. data[a] for the methyl 2-*O*-β-D-glucopyranosyl-α-D-glucopyranoside (**12**), 6-*O*-α-L-rhamnopyranosyl-D-glucopyranose (**13**) and **2**

Compound	*Anomeric configuration*[b]	*Chemical shift*						
		C-1	C-2	C-3	C-4	C-5	C-6	OCH_3
12	α	98.78	81.44	72.24[a]	70.07	71.81[a]	60.73	54.25
	β	104.85	73.58	76.73	69.72	76.28	61.13	
13	α	92.06	72.09	72.92	70.25[a]	70.52[a]	66.89	
	β	96.67	74.61	76.46	70.25[a]	75.03	66.89	
	α″	100.43	70.25[b]	70.52[b]	71.91	68.14	17.75	
		100.29						
2	α	98.76	81.27	71.84[a]	70.09	70.99	66.37	54.27
	β	104.84	73.59	76.30	69.80	76.30	61.14	
	α″	100.50	70.31[b]	70.65[b]	71.98[a]	68.22	17.81	

[a] Chemical shifts in p.p.m. downfield from tetramethylsilane. Assignments marked with identical alphabetical characters may be reversed. [b] The unprimed, single-primed and double-primed anomeric prefixes refer to the α-D-glucopyranosidic (reducing D-glucopyranose in the case of **13**), β-D-glucopyranosyl and α-L-rhamnopyranosyl units in compounds **2**, **12**, and **13** respectively.

No conclusions could be made by ^{13}C-n.m.r. spectroscopy regarding the anomeric configuration of the rhamnopyranosyl linkage, as the anomeric carbon atoms in the spectra of α- and β-L-rhamnopyranosides exhibit practically identical chemical shifts[13,15]. This is attributed to the close proximity of three dipoles in the β-L-rhamnopyranosides and -rhamnopyranoses[16].

Anomeric configuration of glycosidic bonds may be assigned[12] on the basis of molecular rotation $[M_D]$. Calculated $[M]_D$ values of $+118.4°$ and $+453.6°$ were obtained for **8** and for the isomeric methyl 2-*O*-(β-D-glucopyranosyl)-6-*O*-(β-L-rhamnopy-

ranosyl)-α-D-glucopyranoside nona-acetate, respectively. The experimental $[M]_D$ value found for **8** (+96.9°) supports the α-L configuration for the rhamnosidic linkage.

EXPERIMENTAL

General methods. — Melting points were determined on a Kofler block. Optical rotations were determined with a Perkin–Elmer 241 polarimeter. ^{1}H-N.m.r. spectra were recorded at 60 MHz for solutions in $CDCl_3$. Chemical shifts are reported in p.p.m. downfield from Me_4Si. Gas–liquid chromatography was performed on a Hewlett–Packard 5831A gas chromatograph equipped with dual flame-ionisation detectors, operated in the single detector mode. Silanised glass columns (3 m × 2 mm i.d.) packed with OV-1 (Table II) on acid-washed, dimethylsilane-treated Chromosorb W (60–80 mesh) were utilised. Nitrogen was used as carrier gas at a flow rate of 15 mL/min. Injection and detector blocks were maintained at 270°. Hydrogen and air flow-rates to the detector were 42.5 and 255 mL/min, respectively. Retention indexes were determined relative to suitable *n*-alkanes. ^{13}C-N.m.r. spectra for solutions in Me_2SO-d_6 were recorded at 20 MHz using 5-mm tubes under the following conditions: acquisition time: 0.819 sec.; pulse delay: 0.000 sec.; pulse width: 6–9 × 10^{-6} sec.; number of data points: 8192–9000 depending on the sample; pulse angle: 45–67° depending on the sample; resolution: 1.221 Hz per data point.

Methylation of the target compound **2**. — Compound **2** (~ 50 mg) was methylated as described by Brimacombe[9].

Hydrolysis of the methylated target compound. — The methylation product of **2** was hydrolysed with 2M HCl[10], transferred to CHl_3 and dried *in vacuo*.

*2,3,4-Tri-*O*-methyl-*L*-rhamnopyranose* (**9**) *and 2,3,4,6-tetra-*O*-methyl-*D*-glucopyranose* (**10**). — Compounds **9** and **10** were obtained by methylation followed by hydrolysis[17].

Formation of Me_3Si derivatives. — The residue obtained after hydrolysis of the permethylated target compound, and also 3,4-di-*O*-methyl-D-glucopyranose (**11**), **9**, and **10** were silylated by using 5:1:1 pyridine–HMDS–TMCS[18]. The silylation products were transferred to *n*-hexane[19]. In the case of **11**, the available sample was extremely small and the silylation products were injected directly. Because of the severe tailing of pyridine observed on the 1% OV-1 column, a 2.5% OV-1 column was used.

*6-*O*-α-*L*-Rhamnopyranosyl-*D*-glucopyranose* (**13**). — Compound **13** was available from a previous synthesis; $[\alpha]_D^{20}$ −6.5° (*c* 1.5, MeOH)[20].

*Methyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyanosyl)-α-*D*-glucopyranoside* (**4**). — Compound **3** (ref. 5, 1.94 g, 3.2 mmol) was dissolved in 90% (v/v) aq. CF_3CO_2H (5 mL)[6]. After 20 min, diethyl ether (15 mL) was slowly added with stirring. The crystals

* The unprimed, single-primed and double-primed numbers refer to atoms in the α-D-glucopyranosidic, β-D-glucopyranosyl, and α-L-rhamnopyranosyl units, respectively.

were filtered off, dried in a vacuum desiccator over paraffin wax, and recrystallised from EtOAc (1.49 g, 89%), m.p. 162–163°, $[\alpha]_D^{17}$ +55.50° (*c* 1.9, $CHCl_3$).

Anal. Calc. for $C_{21}H_{32}O_{15}$: C, 48.09; H, 6.15. Found: C, 48.2; H, 6.05.

Methyl 3,4-di-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-6-O-trityl-α-D-glucopyranoside (**5**). — A solution of **4** (2.6 g, 5 mmol) and chlorotriphenylmethane (1.3 g, 3.6 mmol) in anhydrous pyridine (13 mL) were stirred for 12 h. Additional chlorotriphenylmethane (0.5 g, 1.8 mmol) was added, followed by Ac_2O (15 mL) after another 2 h. The mixture was heated to boiling point and allowed to cool. After 30 min, it was processed conventionally, and crystallised from MeOH to give **5** (2.9 g, 68%), m.p. 140–141°, $[\alpha]_D^{17}$ +71.10° (*c* 3, $CHCl_3$); ^{1}H-n.m.r.: δ 7.1–7.6 (m, 15 H, aromatic), 4.5–5.6 (m, 7 H), 4.1–4.4 (m, 2 H, Ha-6′*, Hb-6′), 3.6–4.1 (m, 3 H), 3.43 (s, 3 H, methoxyl), 2.8–3.25 (m, 2 H, Ha-6, Hb-6), 1.9–2.15 (m, 15 H, acetoxyl), 1.69 (s, 3 H, acetoxyl).

Anal. Calc. for $C_{44}H_{50}O_{17}$: C, 62.11; H, 5.92. Found: C, 62.2; H, 5.78.

Methyl 3,4-di-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**6**). — Compound **5** (1.0 g, 1.2 mmol) was dissolved in AcOH (18 mL) and cooled to ~10°. Hydrogen bromide [1.6 mL, 40% (m/v) in AcOH, ~8 mmol] was added and the mixture shaken vigorously for 45 sec. The mixture was processed conventionally and the product crystallised from EtOH to give **6** (0.5 g, 70%), m.p. 160–162°, $[\alpha]_D^{21}$ +49.7° (*c* 1.2, $CHCl_3$); ^{1}H-n.m.r.: δ 4.5–5.7 (m, 7 H), 4.1–4.6 (m, 2 H, Ha-6′, Hb-6′), 3.5–4.0 (m, 5 H), 3.42 (s, 3 H, methoxyl), 2.47 (s, 1 H, 6-OH), 1.98–2.12 (m, 18 H, acetoxyl).

Anal. Calc. for $C_{25}H_{36}O_{17}$: C, 49.34; H, 5.96. Found: C, 49.2; H, 6.12.

Methyl 3,4-di-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-6-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-D-glucopyranoside (**8**). — A mixture of **6** (0.5 g, 0.8 mmol), $Hg(CN)_2$ (0.32 g, 1.2 mmol) and $HgBr_2$ (0.45 g, 1.2 mmol) in MeCN (10 mL) was stirred while tri-*O*-acetyl-L-rhamnopyranosyl bromide (**8**, 0.46 g, 1.2 mmol) was added. After 45 min, the mixture was processed conventionally, and **8** crystallised from EtOH (0.46 g, 63.6%), m.p. 167–169°, $[\alpha]_D^{21}$ +11.0° (*c* 1.1, $CHCl_3$); ^{1}H-n.m.r.: δ 3.5–5.6 (m, 17 H), 3.43 (s, 3 H, methoxyl), 1.98–2.13 (m, 27 H, acetoxyl), 1.20 (d, 3 H, Ha-6″, Hb-6″, Hc-6″).

Anal. Calc. for $C_{37}H_{51}O_{24}$: C, 50.51: H, 5.84. Found: C, 50.6; H, 5.71.

Methyl 2-O-(β-D-glucopyranosyl)-6-O-(α-L-rhamnopyranosyl)-α-D-glucopyranoside (**2**). — Compound **2** was prepared by deacetylation of **8** (64 mg)[8] and was obtained as a pure (t.l.c., silica gel, 3:17 acetone–benzene) amorphous powder, which failed to crystallise from various solvents. (28 mg, 76%), $[\alpha]_D^{21}$ +13.1° (*c* 3, MeOH).

Methyl 2-O-β-D-glucopyranosyl-α-D-glucopyranoside (**12**). — Compound **12** was prepared by deacetylation of **4** (140 mg)[8]. The residue (81 mg, 85%) was crystallised from EtOH, m.p. 254–256°.

ACKNOWLEDGMENTS

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