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AMARELLOSIDE, A BITTER TRI-*O*-ACETYL TRI-*O*-BENZOYL TETRASACCHARIDE FROM *POLYGALA AMARELLA*

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Key Word Index—*Polygala amarella*; Polygalaceae; hexa-*O*-acyl tetrasaccharide; amarelloside; flavonoids; hydroxycinnamoyl esters.

Abstract—A very bitter compound, amarelloside, together with rutin, kaempferol-3-*O*-rutinoside and β-D-(1,3-disinapoyl)-fructofuranosyl-α-D-glucopyranoside have been isolated from the aerial parts of *Polygala amarella*. The structure of amarelloside has been established as β-D-(1,3,4-tri-*O*-benzoyl)-fructofuranosyl-{[β-D-(6-*O*-acetyl) glucopyranosyl(1 → 2)][β-D-glucopyranosyl(1 → 3)]}-α-D-(di-*O*-acetyl-4,6)glucopyranoside by NMR spectroscopy, FAB mass spectrometry and GC/MS of the partially methylated alditol acetates.

INTRODUCTION

Plants of the genus *Polygala* are widely distributed in America, Europe and Asia where they are used in traditional medicine for the treatment of parasitical diseases [1]. The presence of several hydroxycinnamoyl esters, saponins and phenolic glycosides has been reported in this genus [2], but *Polygala amarella*, one of the 33 European species, has not been previously studied. A preliminary investigation of the methanolic extract of the aerial parts of this plant led to the isolation of a very bitter tasting principle which we believed could provide a chemotaxonomical marker in the genus *Polygala*. We now report on the isolation and structure elucidation of this new hexa-*O*-acyl tetrasaccharide, which we have named amarelloside (**1**), together with the isolation and identification of two known flavonoids and one hydroxy cinnamoyl ester.

RESULTS AND DISCUSSION

Amarelloside (**1**) was obtained as a colourless amorphous powder which gives a positive reaction with Godin reagent (vanillin–H_2SO_4) and has a very bitter taste. Its negative FAB mass spectrum exhibits a strong $[M-H]^-$ ion at *m/z* 1103 indicating a M_r of 1104, in good agreement with the empirical formula $C_{51}H_{60}O_{27}$. The IR spectrum (KBr) indicates hydroxyl groups (3400 cm^{-1}, *br*), carbonyl groups (1730 cm^{-1}) and aromatic rings (1605, 1525, 1500 cm^{-1}). The UV spectrum (MeOH) exhibits typical absorptions of benzoyl derivatives at λ_{max} 203, 230 and 280 (sh) nm. Upon acid hydrolysis, glucose and fructose were identified by TLC and GC of their trimethylsilyl derivatives. The approach adopted here to determine the structure is to make assignments of the protons resonances as complete as possible via COSY experiments [3] and to support this analysis by assigning the ^{13}C NMR spectrum through heteronuclear $^1H/^{13}C$ shift correlated experiments [4, 5].

Analysis of the 300 MHz ^1H NMR spectrum revealed three acetyl protons signals as singlets at δ2.00, 1.98, 1.64, three orthobenzoyl protons signals as doublets at δ8.18, 8.12 and 7.96, the other benzoyl multiplets between δ7.80 and 7.40, two separate doublets at δ5.68 and 5.60, two separate triplets at δ5.12 and 4.42 and a crowded region between δ4.60 and 2.50. The 2D homonuclear ^1H NMR (COSY) spectrum at 300 MHz revealed the presence of four monosaccharide units by tracing all closed spin systems in the molecule (Table 1). This conclusion was supported by the presence of four anomeric carbon signals at δ104.24, 103.83, 103.77 and 92.45 in the ^{13}C NMR spectrum (Table 2). The identification of the four monosaccharides (A–D) was achieved by comparing their subspectra to those of the unsubstituted ones [6]. The COSY spectrum revealed the presence of three glucopyranosides. Two of them were characterized by the signals of anomeric protons at δ4.52 (*d*, $J = 7.5$ Hz) and 4.31 (*d*, $J = 7.2$ Hz) indicating the β anomeric configuration. The anomeric proton signal at δ5.6 (*d*, $J = 3.5$ Hz) was characteristic of one α-glucopyranoside. The fourth sugar was a β-fructofuranoside where the coupling constants were in good agreement with values reported for sucrose [7]. The sequence of the sugars residues and the position of the six acyl substituents were determined by establishing the connectivities of the proton resonances of each monosaccharide in the region δ2.8–5.8 of the 2D COSY spectrum [8] and the one-bond carbon–proton connectivities through heteronuclear carbon–proton shifts correlation [5]. By comparing the ^1H and ^{13}C NMR data of the four subunits with values reported for sucrose [7, 9, 10], 1,3-disinapoylsucrose [10], methylglycosides [6, 11], methyl *O*-acylglycosides [6, 12] and oligodisaccharides [13, 14], the protons and carbons of the backbone of amarelloside have been identified (Tables 1 and 2). The signals attributed to the protons A-1a, A-1b, A-3 and A-4 (δ4.58, 4.28, 5.68 and 4.62) appeared downfield (1–2 ppm) of those of the corresponding signals of sucrose and suggested a 1,3,4-trisubstitution of the fructo-

Table 1. ^{1}H NMR spectral data of compound **1** (300 MHz, $CDCl_3+CD_3OD$, TMS as int. standard)

H	Unit A β-fruf	Unit B α-glcp	Unit C β-glcp	Unit D β-glcp
		Glycosyl residues		
1	4.58 (−11.9)	5.60 (3.2)	4.52 (7.8)	4.31 (7.9)
1′	4.28	—	—	—
2		3.80 (9.8)	3.40 (8.4)	2.97 (8.2)
3	5.68 (8.2)	3.90 (9.4)	3.30 (8.5)	3.10 (9.0)
4	4.62 (7.3)	5.12 (9.7)	3.45 (9.0)	3.15 (9.1)
5	4.10 (7.3, 2.5)	4.30 (4.4, 4.4)	3.36 (1.7, 3.6)	2.81 (3.9, 1.8)
6	3.78 (−12)	4.11*	4.15 (−12.8)	3.80 (−11.8)
6′	3.60	4.09	3.95	3.45
	*H-6 and H-6′ are nearly equivalent, $J_{6,6'}$ is uncertain			
		Benzoyl residues		
		Bz 1	Bz 2	Bz 3
ortho	*d*	8.18 (8.3, 1.2)	8.12 (8.3, 1.3)	7.96 (8.2, 1.3)
meta	*t*	7.60 (7.5)	7.52 (7.5)	7.5 (7.5)
para	*t*	7.78	7.65	7.66
		Acetyl residues		
		Me-1	Me-2	Me-3
		2.01	1.98	1.65

furanosyl ring by acyl groups for unit A. The deshielded signals of the protons B-4 (δ5.12) and B-6 (δ4.11) suggested a 4,6-disubstitution of the α-glucopyranosyl ring by acyl groups for unit B. In addition, the deshielded signals (+0.5 ppm) of the protons B-2 (δ3.80) and B-3 (δ3.90) suggested a 2,3-disubstitution of α-glucopyranosyl by glycosidic residues for unit B. This conclusion is in good agreement with the ^{13}C NMR chemical shifts at δ80.76 (B-2) and 78.30 (B-3) appearing at lower field (−8 and −5 ppm respectively) than when these positions are simply hydroxylated. The value of the chemical shifts of the carbon B-3 (δ78.3) is lower than the corresponding value in the glucodisaccharide laminaribiose (δ84.2) [6, 14]. This observation must be the consequence of the γ-effect of the anomeric carbon of the glycosidic unit linked at B-2. The lower value at δ80.76 for the chemical shift of the carbon B-2 compared with the corresponding signal of sophorose (δ82.1) [14] must again be induced by the γ-effect of the two anomeric carbons of the glycosidic residues linked at the 1- and -3 positions on unit B. The investigation of the ^{1}H/^{1}H 2D-COSY and the ^{1}H/^{13}C heterocorrelated spectra was in favour of one glucosidic residue with free hydroxyls and one 6-*O*-acylglucosidic residue for the two last units of **1**. The chemical shifts and coupling constants values were very close to those of the corresponding values in various β-glucosides with a 6-free [6, 11] and with a 6-acylated position [8, 12].

The above data indicate that the amarelloside is a hexa-*O*-acyl branched tetrasaccharide with the following glycosidic sequence:

β-Glcp 1→3 α-Glcp 1→2 β-Fruf
2
↑
1
β-Glcp

This conclusion was corroborated by methylation analysis. The sample of amarelloside was methylated according to the Hakomori's method [15]. The strong nucleophilic conditions for deprotonation (sodium-methylsulphinylmethanide in DMSO) eliminate the acyl groups. The methylated tetrasaccharide obtained was hydrolysed and the partially methylated alditol monosaccharides analysed as their alditol acetates by GC/MS (see Experimental) [16]. The carbohydrates were identified as a 2,5-di-*O*-acetyl-1,3,4,6-tetra-*O*-methylhexitol, a 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol and a 1,2,3,5-tetra-*O*-acetyl-4,6-di-*O*-methylhexitol in a ratio 1:2:1. These results indicated the presence of one terminal fructose, two terminal glucose and one 1,2,3-linked glucose.

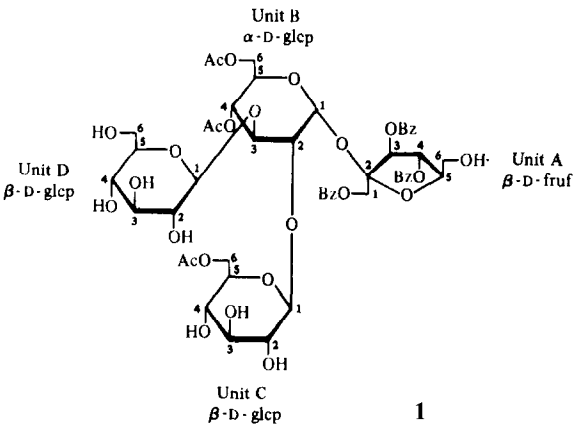
The above results established the interglycosidic linkages and the position of acylation for amarelloside. To localize the acyl groups (benzoyl or acetyl) on the acylation sites, a methylation analysis with very mild conditions of deprotonation was used. The permethylation was performed with methyl trifluoromethanesulphonate in trimethyl phosphate [17]; alkali-labile esters are stable under these conditions so that the substitutions of the carbohydrate residue by *O*-acyl groups can be localized. The methylated sample was hydrolysed and the released partially methylated monosaccharides analysed as their alditol acetates by GC/MS (see Experimental). The formation of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol, 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol, hexitolhexaacetate and 1,3,4-tri-*O*-benzoyl-2,5-di-*O*-acetyl-6-*O*-methylhexitol indicated, with the help of the homo- and heteronuclear 2D NMR experiments, the presence of a terminal β-glucopyranosyl (unit D), a terminal 6-*O*-acetyl β-glucopyranosyl (unit C), a 4,6-di-*O*-acetyl 1,2,3-linked α-glucopyranosyl (unit B) and a terminal 1,3,4-tri-*O*-benzoyl β-fructofuranosyl (unit A) residues. Assignment

Table 2. ^{13}C NMR data of compound **1** compared with values reported for reference compounds ($CDCl_3$, 100 MHz, TMS as int. standard)*

Unit	C	1	Sucrose	Laminaribiose	Sophorose
A	1	66.44	62.15		
	2	103.77	104.07		
	3	80.27	77.26		
	4	73.75	74.44		
	5	83.99	82.56		
	6	62.39	62.21		
B	1	92.45	91.76	91.70	91.37
	2	80.76	71.67	70.89	82.07
	3	78.30	72.96	84.54	71.64
	4	70.57	69.98	68.56	70.17
	5	69.10	72.83	71.80	71.72
	6	63.75	60.64	61.05	60.09
C	1	104.24		104.10	104.92
	2	74.35		73.79	73.81
	3	77.44		76.19	76.34
	4	70.57		70.14	70.10
	5	74.77		76.84	76.73
	6	63.60		60.95	61.17
D	1	103.83			
	2	74.56			
	3	77.32			
	4	70.39			
	5	74.05			
	6	62.79			

*The multiplicity of the signals have been confirmed by DEPT experiments and the attribution of the chemical shifts by using $^1H/^{13}C$ heterocorrelated 2D spectrum. For the reference substances, see refs [6, 7, 10, 11, 13].

For the benzoyl carbon atoms the following signals have been attributed: δ134.57, 134.33, 134.29 (C-1); 130.50, 130.45, 130.29 (C-2, C-6); 130.24, 130.18, 130.20 (C-3, C-5); 129.67, 129.65, 129.65 (C-4), 171.40, 170.97, 170.82 (C-7). For the acetyl carbon atoms: δ21.40, 21.37, 21.23 (Me-CO); 165.69, 166.04, 165.09 (Me-CO).



1

of the glucopyranosyl (unit D) and 6-*O*-acetylglucopyranosyl (unit C) residues to their (-2 or -3) positions on unit B was achieved by 2D correlation spectroscopy using the 2D-NOE technique, which establishes connectivities due to the dipole–dipole cross relaxation [18]. Inspection and comparison of the 2D-NOE with the 2D-COSY spectrum revealed through space interactions across the glycosidic bond observed for the following protons pairs: the anomeric proton of unit C (δ4.52) and the proton B-2 (δ3.80), the anomeric proton of unit D (δ4.31) and the proton B-3 (δ3.90), indicating the linkages C-1→B-2 and D-1→B-3. On the basis of the foregoing evidence, and if glucose and fructose are assumed to be members of the commonly found D-series, the structure of amarelloside is proposed to be β-D-(1,3,4-tri-*O*-benzoyl)-fructofuranosyl-{[β-D-(6-*O*-acetyl) glucopyranosyl(1→2)][β-D- glucopyranosyl(1→3)]}-α-D-(di-*O*-acetyl-4,6) glucopyranoside.

Analogous derivatives of β-D-fructofuranosyl(2→1)α-D-glucopyranoside (sucrose) characterized as mono-, di- or triesters with ferulic, sinapic or coumaric acid and as mixed esters of ferulic and acetic acid have been described in a few plants of the families Liliaceae [19, 20], Brassicaceae [21], Polygonaceae [22] and Polygalaceae [10]. Only 6,3′-diferuloyl sucrose and its acetyl derivatives have been isolated as bitter components from *Lilium speciosum* [23] and *Lilium longiflorum* [24]. In amarelloside (**1**), the basic structure of sucrose is substituted by two acetyl and three benzoyl residues via ester linkages and by one glucosyl and one 6-*O*-acetyl glucosyl residues via ether linkages. To our knowledge, the bitter compound **1** represents the first mixed ester of the tri-*O*-acetyl tri-*O*-benzoyl tetrasaccharide type, and as such represents a new natural compound.

Compound **2** was obtained as an amorphous yellow powder. Its spectroscopic data (UV, IR, ^1H NMR, ^{13}C NMR, FAB/MS) were identical with those described for (1,3-disinapoyl)-fructofuranosyl-α-D-glucopyranoside [10].

Compounds **3** and **4** were isolated and identified as rutin and kaempferol-3-*O*-rutinoside respectively by comparison with authentic samples (UV, IR, FAB/MS, ^1H NMR and TLC) [25].

EXPERIMENTAL

General. Mps: uncorr., IR: KBr disc. UV (λ_{max}^{MeOH}). ^1H NMR spectra (δ, TMS as internal standard, *J* Hz) were recorded at 300 MHz ($CDCl_3+CD_3OD$) and 500 MHz (Me_2CO-d_6) at room temp., using the selective 5 mm probehead. Standard microprograms of the Brucker library were used for the COSY-45 and NOESY measurements. ^{13}C NMR spectra were recorded at 100 MHz, $CDCl_3$ (int. standard: TMS) and 75 MHz ($CDCl_3+CD_3OD$) equiped with a dual probe using the same sample (in a 5 mm diameter NMR tube) utilized for protons studies.

GC/MS: system A: Kratos MS 80, on fused silica capillary column (25 m × 0.22 mm) with OV-1, gas flow rate for He: 0.8 ml/min., temp. program 120–250° (+5°/min.); system B: MAT 312 instrument, on a capillary column (30 m × 0.25 mm) with DB1, gas flow rate for He: 1 ml/min, temp. program: 150–260° (+5°/min.). TLC and HPTLC: precoated plate silica gel $60F_{254}$. Solvent systems: for compound **1**, (a) EtOAc–MeOH–H_2O (77:15:8); for flavonoids, (b) EtOAc–HCOOH–HOAc–H_2O (100:11:11:26); for monosaccharides, (c) $CHCl_3$–MeOH–H_2O (8:5:1). Spray reagents: for oligosaccharides, vanillin–H_2SO_4 (Godin) followed by heating; for flavonoids, Neu + PEG 400; for monosaccharides, diphenylamine–H_3PO_4 followed by heating. CC and flash CC: silica gel Merck, part. Size <0.063 mm, solvent systems (a) and (c). Prep. TLC: silica gel, 0.5 mm, (d) Me_2CO–CH_2Cl_2–H_2O (16:4:1).

Plant material. Polygala amarella was cultivated in the Munich Botanical Garden and provided by Dr G. Heubl. A sample is

deposited in the herbarium of the Institute of Pharmaceutical Biology, Munich.

Isolation and purification of **1–4**. Powdered air-dried aerial parts of *P. amarella* (500 g) were treated with 3 l petrol in a Soxhlet apparatus for 2 days, dried and extracted with 3 l MeOH (Soxhlet, 2 days). The MeOH extract was concd under red. pres. (89 g), dissolved in H_2O (1.5 l) and shaken successively with petrol, CH_2Cl_2, EtOAc and *n*-BuOH (3 × 400 ml for each solvent). Petrol extracts containing chlorophylls were discarded. CH_2Cl_2 and EtOAc extracts having the same composition on TLC (a) were collected together and conc *in vacuo* to afford a bitter residue (6.4 g). HPTLC: mixture of 6 Godin positive spots in the R_f region 0.3–0.7 (a). This extract was chromatographed over 300 g of silica gel (a) in two portions of 3.4 g each. Ten fractions were collected and the isolation of pure compound **1** (174 mg) was achieved by prep. TLC on silica gel (d) of the fractions II and III. The BuOH extract was conc to dryness to afford a brown residue (22 g). HPTLC: mixture of 8 Neu positive spots in the R_f region 0.19–0.40 (b). This extract was submitted to flash CC on silica gel (300 g). Elution with the solvent system EtOAc–MeOH–H_2O (21:4:3), yielded 7 fractions. Two of them (IV and V) were further separated by CC on silica gel (c) to afford pure compounds **3** (50 mg) and **4** (30 mg). The isolation of pure compound **2** (90 mg) was achieved by prep. TLC (CH_2Cl_2–MeOH, 1:1) of fraction V. Compounds were purified over a Sephadex LH-20 column prior to spectral analysis.

Acid hydrolysis. The compound (2 mg) was treated with 2M CF_3COOH–MeOH (1:1) at 100° in a sealed tube for 1 hr. After repeated evapns of the solvent, the residue was taken up in H_2O and extracted with Et_2O. The aglycone was identified in the Et_2O extracts of compounds **3** and **4** by TLC (silica gel, C_6H_5Me–HCOOEt–HCOOH, 5:4:1) as quercetin and kaempferol respectively. Sugars were identified in the aq. phase by TLC (c) as rhamnose and glucose for compounds **3** and **4**, glucose and fructose for compounds **1** and **2**. Then 1-(trimethylsilyl)imidazole (0.2 ml) was added and the reaction mixture was heated at 70° in a stoppered test tube for 1 hr and then analysed by GC [3% Silicone OV-17 on Shimalite W (80–100 mesh) column: 3 mm × 2 m; gas pressure 1.51 kg/cm² air; column temp. 120°].

Acetylation. The compound (2 mg) was dissolved in Ac_2O–pyridine (1:1) and stirred at room temp. for 36 hr. The soln was evapd with toluene and the crude extract purified on Sephadex LH 20 with MeOH.

Permethylation according to Hakomori's method and preparation of the partially methylated alditol acetates. In a 10 ml injection bottle containing an atmosphere of N_2, 5 mg of compound **1** was dissolved in DMSO and Na-methylsulphinylmethanide in DMSO (2 ml) added dropwise. The mixture was sonicated for 30 min at 25° in a Bransonic 12 ultrasound bath (60 W, 50 kHz) and kept at room temp. Me I (1.5 ml) was added to this soln under cooling, and the soln further sonicated at room temp. for 1 hr. The excess of Me I was distilled off at 40°, 5 ml H_2O added and the soln extracted (4 × 5 ml) with CH_2Cl_2. The CH_2Cl_2 phases were washed with H_2O and then evapd to dryness. The residue was hydrolysed with 90% HCOOH (1 ml) and 1M CF_3COOH (1 ml) for 2 hr at 100°, the soln evapd to remove the acid, the residue dissolved in H_2O (2 ml) and 25 mg $NaBD_4$ added. After standing at room temp. for 2 hr the mixture was acidified with HOAc to pH 3.5 and the solvent evapd to dryness. H_3BO_3 in the residue was removed by three cycles of codistillation with MeOH. The partially methylated alditols were acylated with Ac_2O (1 ml) for 1 hr at 100°. The Ac_2O was removed by codistillation in the presence of toluene, and the residue dissolved in CH_2Cl_2 (3 ml), washed with H_2O (3 × 1 ml) and then evapd to dryness. The mixture thus obtained was subjected to GC/MS (system A).

Permethylation using methyl trifluoromethanesulphonate in trimethyl phosphate. Compound **1** (5 mg) was dried over P_2O_5 and suspended by sonication in trimethyl phosphate (1 ml, Aldrich Chemical Co). To the clear soln 2,6-di-(terbutyl)pyridine (150 μl) and methyltrifluoromethanesulphonate (100 μl) were added and allowed to react for 2 hr at 50°. The soln was then distributed between $CHCl_3$ (5 ml) and H_2O (20 ml). The $CHCl_3$ layer was separated by centrifugation at 1700 g for 5 min, conc. by evapn and applied to a Sephadex LH-20 column (MeOH). The recovered methylated carbohydrate was then treated according the procedure described above. The partially methylated alditol acetates obtained were subjected to GC-MS analysis (system B).

β-D-(1,3,4-*tri*-O-*benzoyl*)-*Fructofuranosyl*-{[*β*-D-(6-O-*acetyl*)-*glucopyranosyl*(1 → 2)][*β*-D-*glucopyranosyl*(1 → 3)]}-α-D-(*di*-O-*acetyl*-4,6)*glucopyranoside*. Amarelloside (**1**), Amorphous powder; mp 137–142° with decompn; $[\alpha]_D^{25}$ −12° (MeOH; *c* 0.1); TLC (silica gel), (a): R_f 0.60; blue grey spots by spraying with Godin reagent; IR ν_{max}^{KBr} cm^{-1}: 3500–3300 (OH), 1725 (C=O ester), 1630 (C=C), 1600, 1450, 1380; UV λ_{max}^{MeOH} nm: 203, 230, 280 (sh); FABMS (negative ion, thioglycerol) *m/z*: 1103 $[M-H]^-$, 1061, 999, 957, 899, 795, 753, 691, 633, 591, 529, 467, 387, 367; ^1H NMR (300 MHz, $CDCl_3 + CD_3OD$): see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$): see Table 2. After GC-MS analysis of the partially methylated alditol acetates (methylation according to Hakamori's method) the following carbohydrates could be assigned for **1**: system A: terminal fructose (2,5-di-*O*-acetyl-1,3,4,6-tetra-*O*-methylhexitol, R_t = 2.31 min., *m/z* 43, 45, 71, 87, 101, 129, 145, 161, 205); terminal glucose (1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol, R_t = 3.19 min, *m/z* 43, 71, 75, 101, 117, 118, 129, 145, 161, 162, 205); 1,2,3-linked glucose (1,2,3,5-tetra-*O*-acetyl 4,6-di-*O*-methylhexitol, R_t = 5.45 min., *m/z* 43, 71, 85, 87, 101, 129, 161, 187, 201, 261). After methylation analysis (according to the method with methyl trifluoromethanesulphonate in trimethyl phosphate) the following carbohydrates could be assigned for **1**: system B: terminal glucose (R_t = 23.50 min.), terminal 6-*O*-acetylglucose (1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol, R_t = 25.80 min, *m/z* 23, 71, 75, 87, 99, 101, 102, 118, 129, 162, 173, 189, 233); 4,6-di-*O*-acetyl-1,2,3-linked-glucose (hexitolhexaacetate, R_t = 30.10 min, *m/z* 43, 70, 85, 103, 115, 128, 145, 171, 187, 217, 289, 290, 361, 362); terminal 1,3,4-tri-*O*-benzoyl-fructose (1,3,4-tri-*O*-benzoyl-2,5-di-*O*-acetyl-6-*O*-methylhexitol, R_t = 44 min, *m/z* 43, 45, 57, 71, 77, 101, 105, 129, 161, 190, 207, 250, 385).

β-D-(1,3-*disinapoyl*)-*Fructofuranosyl*-α-D-*glucopyranoside* (**2**). The experimental results were identical with those described in ref. [10].

Rutin (**3**) *and kaempferol*-3-*O*-*rutinoside* (**4**) were identified by comparison of UV, chromatographic properties and MS data with those of standard compounds [25].

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