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Triterpenoidal saponins from the aerial parts of *Zygophyllum coccineum* L. and *Zygophyllum fabago* L.

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Two new triterpenoid saponins 3-O-[β-D-2′-O-sulphonyl quinovopyranosyl]-decarboxy quinovic acid-28-O[β-D-glucopyranosyl ester (**1**), 3-O-[β-D-2′-O-sulphonyl quinovopyranosyl]-quinovic acid-27-O-[β-D-glucopyranosyl] ester (**2**) and two known saponins 3-O-[β-D-quinovopyranosyl]-quinovic acid (**3**), 3-O-[β-D-2′-O-sulphonyl quinovopyranosyl]-quinovic acid-28-O-[β-D-glucopyranosyl] ester (**4**) have been isolated from the aerial parts of *Zygophyllum coccineum* L. The known compounds **3**, **4** were also isolated from the aerial of *Zygophyllum fabago* L. The structures of the isolated compounds were elucidated by spectral studies.

1. Introduction

Zygophyllum coccineum L. is used in folk medicine as part of a drug against rheumatism, gout and hypertention [1], and is also used as a diuretic, antihelmentic and antidiabetic agent [2].

Reviewing the current literature, nothing has been done on the isolation of the active constituents of *Zygophyllum fabago* L., while some saponins have been isolated from the roots of *Zygophyllum coccineum* L. [2, 3] and the aerial parts of the same plant [4]. This was the motivation to investigate the natural products of the aerial parts of *Zygophyllum coccineum* L. and *Zygophyllum fabago* L. In this report we present the isolation of the two new tritreponoid saponins **1**, **2** and two known saponins **3**, **4**.

2. Investigations results and discussion

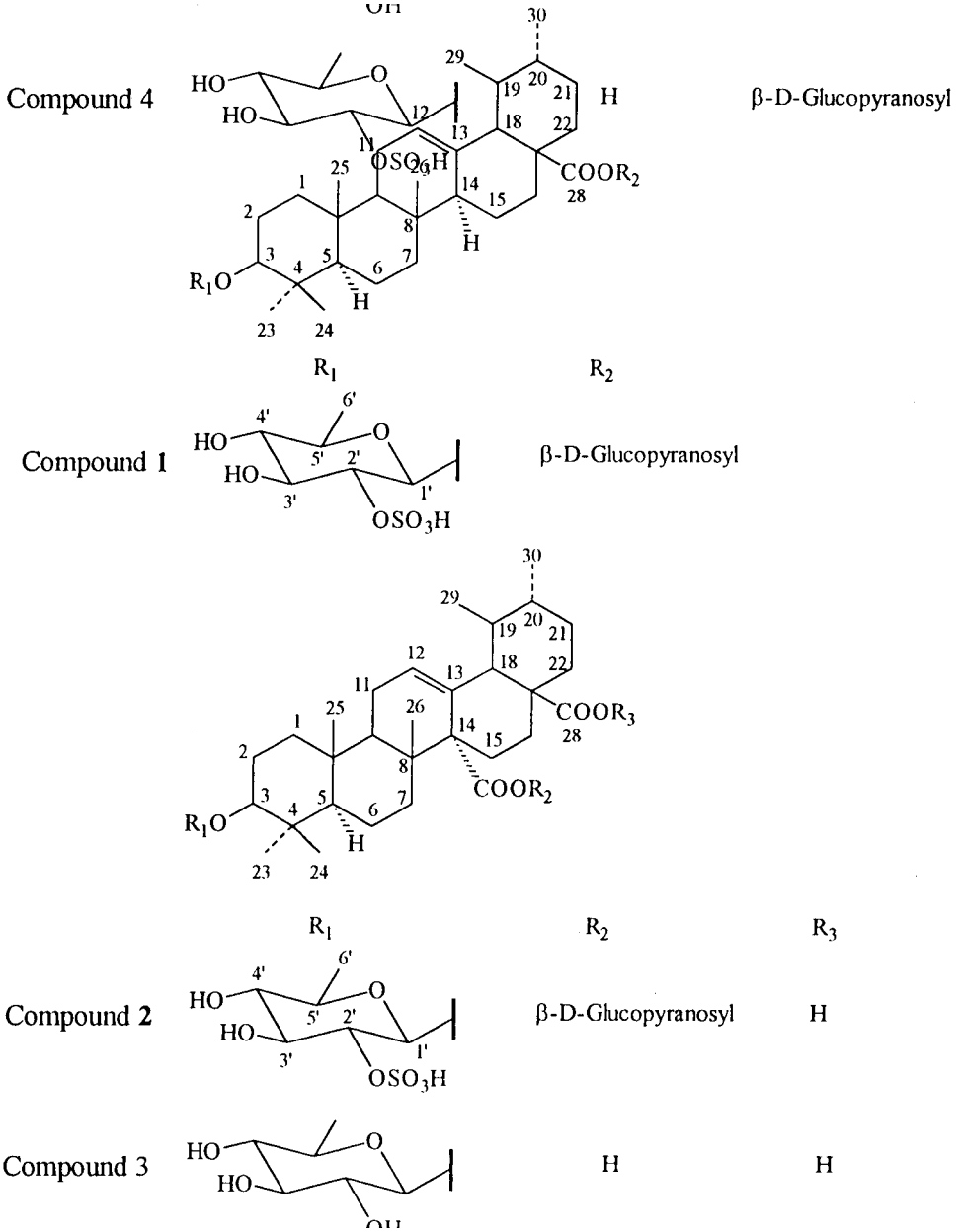
Compound 1 was purified by repeated column chromatography. The ^{13}C NMR spectrum, summarized in the Table, showed 41 carbon resonance signals, indicating the presence of two sugar moieties with a triterpenoid aglycone. Also the occurance of two anomeric signals at δ 4.71 (1 H, d, J = 7.6 Hz, H-1′) and 6.35 (1 H, d, J = 8.1 Hz, H-1″) and the doublet at δ 1.57 due to a methyl group, in the ^1H NMR spectrum of **1** indicated the presence of a β-D-quinovopyranose and a β-D-glucopyranose unit [2]. The negative ion FABMS of **1** showed a [M−H] ion peak at m/z 829 and ^{13}C NMR. Thus, we were able to propose $C_{41}H_{66}O_{15}S$ as the molecular formula. The ^1H NMR of compound **1** indicated the presence of four tertiary and two secondary methyl groups at δ 0.86 (3 H-25), 1.13 (3 H-24), 1.19 (2 H-26), 1.27 (3 H-23) and doublets at δ 0.76 (3 H, d, J = 7.4 Hz, H-29), 1.16 (3 H, d, J = 7.5 Hz, H-30). The signal at δ 5.97 (3 H, d, J = 6.1 Hz) was attributed to the olefinic proton H-12. The doublets of two anomeric proton signals at δ 4.71 (J = 7.5 Hz), 6.35 (J = 8.1 Hz) and the doublet at δ 1.57 (J = 6.1 Hz), due to a methyl group, indicated the presence of a β-D-quinovopyranose and a β-D-glycopyranose unit [2]. The chemical shifts of the H-1 sugar signals at δ 4.71 and 6.35 indicated a glycosylation of **1** with quinovose at C-3 and glucose at C-27 or C-28 [2]. In ^{13}C NMR, the presence of only one signal at 176.5 (due to O=C−O) together with the ion mass spectrum at m/z 442 of the aglycone indicates that the aglycone is decarboxy quinovic acid with C-28 (O=C−O).

The presence of the carboxy group at C-17 as well as the glucose ester at position C-28 was established by compar-

Table: ^{13}C NMR spectral data for compounds 1–4

C	1	2	3	4
1	39.6	39.9	39.4	39.4
2	26.6	27.0	26.8	26.6
3	89.4	91.3	88.5	89.3
4	40.2	40.2	40.1	40.1
5	55.8	55.8	55.8	55.7
6	18.4	19.3	18.7	18.4
7	37.5	37.8	37.5	37.4
8	40.2	40.9	40.1	40.1
9	47.3	48.0	47.3	47.1
10	37.0	37.8	37.0	37.3
11	23.4	23.9	23.4	23.3
12	129.6	130.9	128.9	129.4
13	133.3	133.3	134.1	133.2
14	56.8	56.9	56.8	56.7
15	25.5	25.8	25.5	25.4
16	26.2	26.4	26.0	26.5
17	48.9	*	48.7	48.8
18	54.7	55.3	54.9	54.6
19	37.5	38.3	37.7	36.9
20	39.1	40.1	39.3	39.1
21	30.3	31.1	30.7	30.1
22	36.5	37.0	37.0	36.3
23	28.1	28.5	28.1	28.0
24	17.0	17.0	17.0	16.9
25	16.6	16.9	16.5	16.5
26	19.2	19.2	18.9	19.0
27	–	178.0	178.0	178.0
28	176.5	180.9	180.0	176.0
29	18.1	18.0	18.2	18.1
30	21.1	21.4	21.3	21.1
Sugar carbons				
1′	103.9	104.0	106.6	103.7
2′	81.0	82.1	75.9	81.0
3′	78.3	77.6	78.4	77.8
4′	76.1	76.9	76.6	76.3
5′	72.3	72.6	72.6	72.1
6′	18.4	18.0	18.6	18.6
1″	95.7	95.6		95.5
2″	74.2	74.1		74.1
3″	78.9	98.5		78.9
4″	71.3	71.4		71.1
5″	79.2	78.5		78.9
6″	62.4	62.7		62.2

Compounds **1**, **3**, **4** in C_5D_5N and **2** in CD_3OD
* Signal masked by CD_3OD peaks



ison of ^{1}H NMR and ^{13}C NMR spectra with those of compounds previously isolated [3, 5]. Providing further confirmation for the presence of a glucose ester at position C-28 and quinovose ether linkage at C-3 was achieved by HMBC where a correlation peak existed between the anomeric protons at δ 6.35 (H-1 of glucose) and the signal at δ 176.5 (C-28 carboxyl group) and between the signal at δ 4.71 (H-1 of quinovose) and the signal at δ 89.4 (C3 of aglycone). In ^{13}C NMR, the downfield signal at δ 81.0 assigned for C-2 of the sugar moiety indicated the presence of a OSO_3H group attached to the C-2 oxygen of the sugar moiety [5]. The OSO_3H moiety is characterized by the fragment ions at m/z 97 $[SO_4H]$ and 80 $[SO_3]^-$ in the FABMS. The downfield shifts of the H-2′ ($\Delta\delta + 1.02$) and C-2′ ($\Delta\delta + 5.1$) compared with those of compound **3** providing further confirmation for the presence of the sulphate group in position C-2′ of the quinovose. Acid hydrolysis of **1** with 10% sulfuric acid yielded decarboxy quinovic acid (the ^{13}C NMR resonances of the aglycone were consistent with those of the decarboxy quinovic acid aglycone), glucose and quinovose. The monosaccharides were detected by TLC. The determination of the D- or L-form of the sugars was achieved by GLC of the trimethylsilylated derivatives in comparison with authentic samples. From the above evidence, the structure of **1** was determined to be 3-O-[β-D-2′-O-sulphonyl-quinovopyranosyl] – decarboxy quinovic acid – 28-O-[β-D-glucopyranosyl]ester.

Compound **2** was purified through column chromatography using chloroform-methanol gradient elution. The ^{13}C NMR spectrum of **2** showed two anomeric signals which appeared at 104.0 and 95.6 indicating the presence of two

sugar moieties. The anomeric carbon signal at δ 95.6 showed that one sugar residue was bonded to the aglycone by an ester bond [6]. The ^{13}C NMR signals appeared at δ 95.6 (CH), 74.1 (CH), 78.5 (CH), 71.4 (CH), 78.5 (CH) and 62.7 (CH_2) and were assigned to C-1–C6, of glucose [7, 8]. The olefinic resonance of the glycoside at δ 133.3 and 130.9 corresponding to quaternary and methine carbons suggested the urs-12-ene skeleton with a carboxylic group at C-27 [9], also the carbonyl carbons at δ 180.9 and 178.0 showed the presence of one unsubstituted carboxylic group at C-17 and linked one at C-14 [3]. Since the unsubstituted carboxylic group at C-17 appears at δ 180.9 and the unsubstituted carboxylic group at C-14 appears at δ 179.2 the upfield shift of C-27 signal ($\Delta\delta - 1.2$) in comparison with the data of a saponin previously isolated [5], indicated that the C-27 was substituted.

The ^1H NMR spectrum of **2** showed the existence of four tertiary and two secondary methyl groups characterized by signals at δ 0.83 (3 H-25), 0.87 (3 H-24), 0.96 (3 H-26), 1.03 (3 H-23) and the doublets at δ 0.91 (3 H, d, J = 6 Hz, H-30) and 1.25 (3 H, d, J = 6.2 Hz, H-29). The doublets of two anomeric proton signals at δ 4.42 (1 H, d, J = 7.6 Hz, H-1′) and at δ 5.36 (1 H, d, J = 8.0 Hz, H-1″) indicated the presence of two sugar moities and the doublet at (3 H, δ, J = 6.1 Hz) due to a methyl group indicated the presence of a β-D-quinovopyrnose and a β-D-glycopyranose unit. The chemical shifts of H-1 sugar signals at δ 4.42 and 5.36 indicated the glycosylation of **2** with quinovose at C-3 (glycoside) and glucose at C-27 or C-28 (glucopyranosyl ester). In the ^{13}C NMR of **2**, the signal of C-12 is downfield ($\Delta\delta + 1.0$) and the signal of C-13 upfield shifted ($\Delta\delta - 1.3$) in comparison with the ^{13}C NMR spectrum of a previously isolated compound with glycosylation at C-28 [5]. The chemical shifts of the C-12 signal (δ 130.9) and the C-13 signal (δ 133.3) were in agreement with glycosylation at C-27, the signal of C-12 and C-13 would have been expected at δ 128.9 and 135.4, respectively [10]. The HMBC showed a correlation peak between the anomeric protons at δ 4.42 (H-1′ of quinovose) and the signal at δ 91.3 (C-3 of the aglycone), and between the signal at δ 5.36 (H-1″ of the glucose) and the signal at δ 178 (C-27 carboxyl group). This provides further evidence for the structure of compound **2**. The downfield signal at δ 82.1 assigned for C-2 of the sugar moiety indicated the presence of a group attached to the C-2 oxygen of the sugar moiety, the remaining of the signals of the sugar in **2** are identical with the data reported for 2′-O-sulphonyl quinovopyranosyl [5]. Acid hydrolysis yielded glucose and quinovose (detected by TLC) and the aglycone quinovic acid (the ^{13}C NMR resonances of the aglycone were consistence with quinovic acid aglycone). The D- or L-form of the sugars of **2** were investigated by GLC and using authentic samples, which showed the presence of D-glucose and D-quinovose. From the above data, the structure of **2** was determined to be 3-O-[β-D-2′-O-sulphonyl quinovopyranosyl]-quinovic acid-27-O-[β-D-glucopyranosyl] ester.

Compound **3** was purified by repeated column chromatography using a chloroform-methanol gradient as eluent. The ^1H NMR and ^{13}C NMR spectra of **3** showed the presence of quinovic acid as aglycone. One anomeric proton signal at δ 4.69 (1 H, d, J = 7.7 Hz, H-1′) indicated the presence of one monosaccharide bonded as the glycoside. The signal at δ 6.0 (1 H, m) was attributed to the olefinic proton H-12. And the douplet at δ 1.64 due to a methyl group indicated the presence of a β-D-quinovopyranose. The ^1H NMR spectrum also showed the existence of four tertiary and two secondary methyl groups characterized by the signals at δ 0.89 (3 H-25), 0.94 (3 H-24), 1.1 (3 H-26), 1.14 (3 H-23) and the doublets at δ 0.80 (3 H, d, J = H-30), 1.23 (3 H, d, J = 6.3 Hz, H-29). The chemical shifts of the H-1 sugar signal at δ 4.69 indicated the glycosylation of compound **3** with quinovose at C-3 (glycoside). In ^{13}C NMR spectrum of **3** (Table) showed the signals at δ 128.9 and 134.1 corresponding to quaternary and methine carbons, the carbonyl carbons at δ 178.0 and 180.0 showed the presence of two unsubstituted carboxylic groups at C-14 and C-17, respectively. The rest of ^{13}C NMR spectrum was consistent with the data reported for 3-O-substituted quinovic acid [3]. Acid hydrolysis yielded D-quinovose and the aglycone quinovic acid (the ^{13}C NMR resonances of the aglycone were consistence with the aglycone of compound **2**). From the above evidence and the presence of 3-O-quinovose (^{13}C NMR and ^1H NMR), the structure of **3** was determined to be 3-O-[β-D-quinovopyranosyl]-quinovic acid. This compound was previously isolated from *Zygophyllium dumosum* [2].

Compound **4** was purified by repeated column chromatography. The ^{13}C NMR data is summerized in the Table, showed 42 carbon resonances, indicating the presence of two sugar moieties with a triterpenoid aglycone. Two anomeric carbon signals at δ 103.7, 95.5 indicating the presence of two sugar moieties. The anomeric carbon signal at δ 95.5 showed that one sugar residue was bonded to the aglycone by an ester bond [6]. The ^1H NMR spectrum (400 MHz, pyridine-d_5) of **4** showed the existance of four tertiary and two secondary methyl groups characterized by the signals at δ 0.80 (J = 5.9 Hz, 3 H-30), 1.20 (J = 6.2 Hz, 3 H, 3 H-29). The signals at δ 5.96 (1 H, m) was attributed to the olefinic proton H-12. The doublets of two anomeric proton signals at δ 4.74 (1 H, d, J = 7.6 Hz, H-1′), 6.26 (1 H, d, J = 8.1 Hz, H-1″) and the doublet at δ 1.56 (3 H, d, J = 5.9 Hz, H-6′), due to a methyl group, indicated the presence of a β-D-quinovopyranose and a β-D-glucopyranose unit [2]. The chemical shifts of the H-1 sugar signals at δ 4.74 and 6.26 indicating the glycosylation of **4** with quinovose at C-3 (glycoside) and glucose at C-27 or C-28 (glucopyranosyl ester) [2]. The position of the glucose moiety linked in ester form was identified at C-28 of the quinovic acid on the bases of ^1H- and ^{13}C NMR spectral data which matched with those reported for the 28-O-β-D-glucopyranosyl ester of quinovic acid [6]. The downfield signal at δ 81.0 for C-2 of the sugar moiety indicated the presence of a group attached to the C-2 oxygen of the sugar moiety. The ^{13}C NMR resonance of quinovose for **4** matched well with the signal of **1** indicating the same sugar having a SO_3H-group. The acid hydrolysis afforded D-glucose and D-quinovose (detected by TLC and GLC) and quinovic acid as aglycone (the ^{13}C NMR resonances of the aglycone were consistence with quinovic acid aglycone of compound **2**). The above evidence led us to conclude that the structure of **4** is 3-O-[β-D-2′-O-sulphonylquinovopyranosyl]-quinovic acid-28-O-[β-D-glucopyranosyl] ester. This compound **4** has been isolated previously from *Zygophyllium* species [3, 5].

3. Experimental

3.1. Equipment and methods

M.p.'s are uncorrected. ^1H NMR and ^{13}C NMR spectra were run in pyridine-d_5 and CD_3OD using a Brucker AM-400 and AM-500 (Germany) for recording. Negative ion FABMAS were recorded on a JEOL JMS 600 spectrometer. CC was performed on Merck Silica gel (70–230 mesh). TLC was performed on Silica gel plates (0.25 mm, $CHCl_3$–MeOH–H_2O,

14 : 7 : 1) (system I) the spots were sprayed with 10% methanolic H_2SO_4. GLC analysis was performed on a Perkin−Elmer, PE Nelson Model 1022 using a fused silica capillary column (30 m × 0.32 mm I.d.) with N_2 as carrier gas (30 ml/min), temp. program: 160–210 °C (1 °C/min) followed by 210 °C for 15 min.

3.2. Plant material

The aerial parts of *Zygophyllum coccineum* L. and *Zygophyllum fabago* L. were obtained from North Saini near the Red Sea coast and kindly identified by prof. Abed El-Aziz Faeid, Faculty of Science, Assiut University, Assiut.

3.3. Extraction and isolation

Z. coccineum dried powdered aerial parts (10 kg) were extracted with petrol, EtOAc and Methanol. The MeOH extract was evaporated at reduced pressure to afford a gummy residue (400 g) which was partitioned between EtOAc and H_2O. The water layer was extracted with n-BuOH. The n-BuOH layer was separated and evaporated under reduced pressure to afford a crude saponin mixture (100 g). The saponin mixture was chromatographed on a silica gel column using a gradient of MeOH in $CHCl_3$ as eluent, to afford 4 saponin fractions. Each fraction was purified by repeated CC to afford the pure saponins **1**, **2**, **3** and **4**.

Dried powdered aerial parts (5 kg) of *Z. fabago* L. were extracted with petrol, EtOAc and MeOH. The methanolic residue was partitioned as described for *Z. coccineum*. The butanolic fraction gave a crude saponin mixture (35 g). The saponin mixture was chromatographed on a silica gel column using a gradient of MeOH in $CHCl_3$ as eluent. Purification of each fraction was achieved by repeated CC to a afford the pure saponins **3** and **4**.

3.4. Acid hydrolysis of glycosides 1–4

Acid hydrolysis of compounds **1**–**4**, 10 mg of each glycoside was separately hydrolysis with 1 M H_2SO_4 for 90 min after completion, the solution in each case was shaken with $CHCl_3$. The recovered quinovic acid aglycone was identified in the case of compounds **2**, **3** and **4** by comparison with literature data [11, 12] and the decarboxy quinovic acid aglycone in case of compound **1** [4, 13] and direct authentication in both above cases. The aquous layer was neutralized with $KHCO_3$ and the sugars were identified by direct TLC comparison with authentic sugars ($CHCl_3$−MeOH−AcOH−H_2O, 8 : 3 : 5 : 2) after spraying with sugar reagent.

3.5. Preparation for TMSi ethers of the component sugars 1–4

Each compound **1**–**4** (20 mg) was treated by the procedure described in the literature [14]. The corresponding trimethylsilylated ethers were investigated by GLC, their retention time values were determined and showed a good agreement with the trimethylsilylated reference compounds.

3.6. Compound characterization

Compound **1** was obtained as colourless amorphous powder (40 mg), m.p. 230–232 °C, $R_f = 0.80$ in system I, ^{1}H NMR (pyridine-d_5); δ 5.97 (1 H, m, H-12), 1.27 (3 H, s, H-23), 1.13 (3 H, s, H-24), 0.86 (3 H, s, H-25), 1.19 (3 H, s, H-26), 0.76 (3 H, d, J = 7.6 Hz, H-29), 1.16 (3 H, d, J = 7.5 Hz, H-30), 4.71 (1 H, d, J = 7.6 Hz, H-1′), 6.35 (1 H, d, J = 8.1 Hz, H-1) and quinovose Me 1.57 (3 H, d, J = 6.1 Hz, H-6′), FABMS, m/z 829 (M−H), 667 (M−H-glucose), 543 [M−H-glucose-(COO + SO_3)] and 397 [M−H-glucose-(COO + SO_3)-quinovose], ^{13}C NMR: Table.

Compound **2** was obtained as colourless amorphous powder (19 mg), m.p. 278–280 °C, $R_f = 0.68$ in system 1, ^{1}H NMR (CD_3 OD): δ 5.61 (^{1}H, m, H-12), 0.83 (3 H, s, H-23), 1.03 (3 H, s, H-24), 0.96 (3 H, s, H-25), 0.87 (3 H, s, H-26), 0.91 (3 H, d, J = 6 Hz, H-30), 1.25 (1 H, d, J = 6.2 Hz, H-29), 4.42 (1 H, d, J = 7.6 Hz, H-1′), 5.36 (1 H, d, J = 8.0 Hz, H-1″) and 1.57 (3 H, d, J = 6.1 Hz, H-6′) methyl group of quinovose, ^{13}C NMR: Table.

Compound **3** was obtained as colourless amorphous powder (28 mg), m.p. 224–226 °C, $R_f = 0.87$ in system 1, H NMR (pyridine-d_5); δ 6.0 (^{1}H, m, H-12), 1.14 (3 H, s, H-23), 0.94 (3 H, s, H-24), 0.89 (3 H, s, H-25), 1.10 (3 H, s, H-26), 0.80 (3 H, d, J = 5.9 Hz, H-30), 1.23 (1 H, d, J = 6.1 Hz, H-29), 4.69 (1 H, d, J = 7.5 Hz, H-1′) and 1.64 (3 H, d, J = 6.0 Hz, H-6″) methyl group of quinovose. ^{13}C NMR: Table.

Compound **4** was obtained as colourless amorphous powder (30 mg), m.p. 265–267 °C, $R_f = 0.61$ in system 1, H NMR (pyridine-d_5); δ 5.96 (1 H, m, H-12), 0.85 (3 H, s, H-25), 1.11 (3 H, s, H-24), 1.17 (3 H, s, H-26), 1.26 (3 H, s, H-23), 0.80 (3 H, d, J = 5.9 Hz, H-30), 1.20 (3 H, d, J = 6.2 Hz, H-29), 4.74 (1 H, d, J = 7.6 Hz, H-1′), 6.26 (1 H, d, J = 8.1 Hz, H-1″) and 1.56 (3 H, d, J = 5.9 Hz, H-6′) methyl group of quinovose, ^{13}C NMR: Table.

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