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Stigmastane derivatives and isovaleryl sucrose esters from *Vernonia guineensis* (Asteraceae)

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This paper is dedicated to the memory of the late Professor Johnson Foyere Ayafor, who died in a car accident near Yaoundé on 10 November 2000

Abstract

Vernoguinoside, 16β,22*R*;21,23*S*-diepoxy-3β-*O*-β-D-glucopyranosyloxy-21*S*,24-dihydroxy-5α-stigmasta-8,14-dien-28-one (**1**), a new stigmastane derivative, 16β,22*R*;21,23*S*-diepoxy-21*S*,24-dihydroxy-5α-stigmasta-8,14-diene-3,28-dione (**2**) and two new sucrose esters, 1′,3,3′,4′,6′-pentakis-*O*-(3-methylbutanoyl)-β-D-fructofuranosyl α-D-glucopyranoside (**3**) and 1′,2,3′,6,6′-pentakis-*O*-(3-methylbutanoyl)-β-D-fructofuranosyl α-D-glucopyranoside (**4**), have been isolated from the stem bark of *Vernonia guineensis*. The structures of the new compounds were determined on the basis of spectroscopic evidence.

Keywords: *Vernonia guineensis*; Asteraceae; Stigmastane derivatives; Isovaleryl sucrose esters

1. Introduction

Several *Vernonia* species are used widely in native cultures as folklore remedies for a variety of human ailments (Johri and Singh, 1997). They are a source of stigmastane glycosides possessing a $\Delta^{7,9(11)}$ or $\Delta^{8,14}$ C-29 steroid nucleus, different highly oxygenated side chains and a sugar moiety linked at C-3 of the aglycone (Jisaka et al., 1992, 1993; Igile et al., 1995; Ponglux et al., 1992; Sanogo et al., 1998). It should be noted that flavonoids, sesquiterpenoids, alkaloids, triterpenoids and cardiac glycosides have also been reported from this genus (Johri and Singh, 1997; Iwu, 1993; Toubiana, 1969). Recently we reported the isolation and characterisation of two new stigmastane derivatives, vernoguinosterol and vernoguinoside peracetate (Tchinda et al., 2002), from the stem bark of *Vernonica guineensis*. In the course of a search for further compounds from this plant we re-investigated the CH_2Cl_2–MeOH extract and isolated vernoguinoside (**1**), its 3-oxo derivative **2** and two new sucrose esters **3** and **4**.

2. Results and discussion

The FAB mass spectrum of **1** showed a quasi-molecular ion peak $M+H^+$ at *m*/*z* 649 which together with the ^{13}C NMR and DEPT 135 data (Table 1) is consistent with the molecular formula $C_{35}H_{52}O_{11}$. The ^{13}C NMR spectrum showed 35 signals, of which 29 were assigned to the stigmastane nucleus and six to the sugar, identified as a β-glucopyranose by its characteristic chemical shifts and coupling constants in the ^1H NMR spectrum. The attachment of the sugar moiety to C-3 of the aglycone was deduced from the correlation observed in the HMBC spectrum between the anomeric proton signal at δ 4.41 (H-1′) and C-3. The chemical shifts of C-2 (30.9), C-3 (79.1) and C-4 (35.8) confirmed the β-orientation of the glucopyranosyl moiety (Igile et al., 1995). The presence of the sugar core was further confirmed by the important peak observed at *m*/*z* 469 [M-glucose]$^+$ in the FAB mass spectrum. The steroidal nature of the aglycone part of **1** was indicated in the ^1H NMR spectrum by the angular methyl singlets at δ 0.98 (Me-18) and 1.04 (Me-19) and the characteristic H-3

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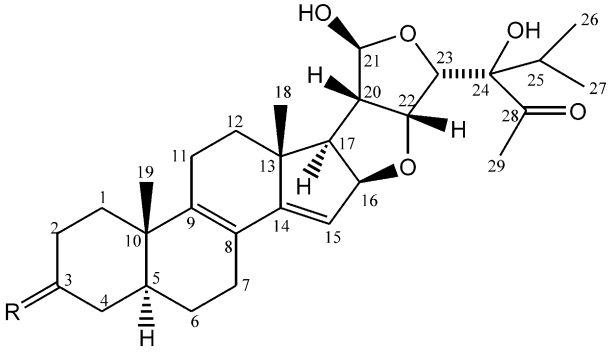
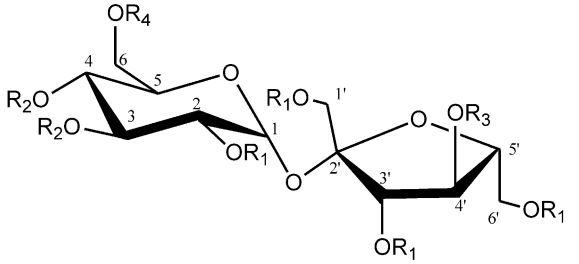
multiplet at δ 3.72. The UV spectrum showed an absorption maximum at 248 nm ($\varepsilon = 7900$) arising from the $\Delta^{8,14}$ diene chromophore (Jisaka et al., 1993; Igile et al., 1995). The ^{13}C NMR spectrum revealed a conjugated diene consisting of a trisubstituted carbon [δ 117.2 (C-15)] and three tetrasubstituted carbons [δ 158.1 (C-14), 145.5 (C-9) and 124.7 (C-8)]. The proton at δ 5.36 (H-15) showed cross peaks in the HMBC spectrum with C-8, C-13, C-14, C-16 and C-17 in agreement with the positioning of the diene in the sterol nucleus. The ^1H–^1H COSY spectrum of **1** clearly demonstrated the connectivities H-15/H-16/H-17 and H-21/H-20/H-22/H-23. Further analysis of the NMR data showed that the side chain was linked to the sterol nucleus through the C-17/C-20 bond and a C-16/C-22 ether linkage. The presence of the ether bond was indicated by the carbon chemical shifts of C-16 (δ_C 90.9) and C-22 (δ_C 87.8). In the side chain, an isopropyl group [δ_H 1.04 (d, $J = 6.8$ Hz, Me-26), 0.72 (d, $J = 6.8$ Hz, Me-27) and 2.20 (m, H-25)] was attached to a quaternary carbon atom at δ_C 83.6 (C-24) bearing a hydroxyl group, which remained unchanged upon acetylation. The methyl singlet (δ_H 2.25, s, Me-29) correlating in the HMBC spectrum to the carbonyl functionality at δ_C 213.6 (C-28) was part of the acetyl group which was found to be attached to C-24. The downfield proton H-21 (δ_H 5.28) bonded to a carbon at δ_C 105.7 (C-21) indicated the presence of a hemiacetal, which is a common feature of most *Vernonia* stigmastane derivatives (Jisaka et al., 1993; Igile et al., 1995; Ponglux et al., 1992). Compound **1** was acetylated and NMR data of the derivative obtained were identical to those of the acylated vernoguinoside obtained in the previous work (Tchinda et al., 2002).

The molecular formula $C_{29}H_{40}O_6$ of compound **2** was deduced from ^{13}C NMR data (Table 1) and from



1 R = H, β-OGlc
2 R = O



3 $R_1 = R_3 =$ -OCOCH$_2$CH(CH$_3$)$_2$, $R_2 = R_4 =$ H
3a $R_1 = R_3 =$ -OCOCH$_2$CH(CH$_3$)$_2$, $R_2 = R_4 =$ Ac
4 $R_1 = R_4 =$ -OCOCH$_2$CH

Table 1
NMR data of compounds **1** (CD_3OD) and **2** ($CDCl_3$)

Posit.	**1**				**2**[a]
	δ_C	δ_H	Mult. (J in Hz)	HMBC H→C)	δ_C
1	36.7	1.42	ov	C-3, C-5	38.5
		1.88	ov	C-10	
2	30.9	1.95	ov		36.8
		1.31	ov		
3	79.1	3.72	*m*		211.3[b]
4	35.8	1.85	ov		44.9
		1.32	ov		
5	42.6	1.42	*m*	C-3	43.0
6	30.9	2.09	*m*		26.9
		1.97	*m*		
7	26.7	1.59	ov		25.5
		1.49	ov		
8	124.7	–			124.3
9	145.5	–			142.4
10	38.5	–			37.2
11	23.5	2.30	*m*	C-8	22.6
12	37.7	1.85	ov	C-9, C-14, C-13	36.6
		1.38	ov		
13	46.5	–			45.5
14	158.1	–			156.2
15	117.2	5.36	*d* (2.6)	C-8, C-13, C-14, C-16, C-17	116.8
16	90.9	4.71	*dd* (6.2, 2.6)	C-14, C-15	89.8
17	61.8	2.48	*d* (6.2)	C-12, C-13, C-15, C-20, C-22, C-18	60.1
18	21.2	0.98	*s*	C-12, C-28, C-14, C-17	20.7
19	19.1	1.04	*s*	C-1, C-5, C-10	17.8
20	55.4	2.74	*d* (5.6)	C-13, C-16, C-17, C-23	54.0
21	105.7	5.28[c]	*d* (1.3)	C-17, C-20, C-23	104.6
22	87.8	4.74	*dd* (5.6, 2.5)	C-21, C-23	88.8
23	85.6	4.23	*d* (2.5)	C-24, C-28	84.1
24	83.6	–			82.8
25	33.9	2.20	*m*		33.1
26	17.2	1.04	*d* (6.8)	C-22, C-25, C-27	17.0
27	16.8	0.72	*d* (6.8)	C-22, C-25, C-26	16.6
28	213.6	–			211.5[b]
29	28.0	2.25	*s*		27.3
1′	102.7	4.41	*d* (7.7)	C-3	
2′	75.5	3.14	*t* (7.8)	C-1′, C-3′/C-5′	
3′	78.5	3.37	*dt* (7.1, 1.6)	C-4′	
4′	72.1	3.25	ov	C-3′/C-5′	
5′	78.2	3.30	*m*	C-3′	
6′	63.2	3.84	*d* (11.9)		
		3.65	*dt* (11.9, 2.5)		

ov, signal pattern unclear due to overlapping.

[a] Carbon assignments of **2** are based on DEPT, HMQC and HMBC data and by comparison with ^{13}C NMR data of related compounds.

[b] May be interchanged.

[c] Multiplicity not well resolved.

Table 2
NMR data of compounds **3** and **4**

Posito	**3**[a]			**3**[b]			**4**		
	δ_C	δ_H	Multi. *J* (Hz)	δ_C	δ_H	Multi. *J* (Hz)	δ_C	δ_H	mult. *J* (Hz)
1	9.19	5.59	*d* (3.7)	90.4	5.59	*d* (3.7)	90.8	5.53	*d* (3.7)
2	74.2	4.66	*dd* (10.2, 3.7)	72.4	4.74	*dd* (10.2, 3.7)	74.0	4.63	*dd* (10.2, 3.7)
3	72.6	3.82	ov	71.8	3.84	ov	72.6	3.74	*t* (9.5)
4	71.7	3.49	*t* (9.3)	70.9	3.60	*t* (9.3)	72.2	3.37	*t* (9.5)
5	74.9	3.88	*m*	72.9	3.91	*m*	72.5	4.04	*m*
6a	62.5	3.78	*m*	62.2	3.91	*m*	65.1	4.48	*dd* (11.8, 1.7)
6b		3.86	ov		3.84	ov		4.15	*dd* (11.8, 5.9)
1′a	64.9	4.12	*d* (11.9)	63.5	4.05	*d* (11.9)	65.7	3.91	*d* (11.6)
1′b		4.03	*d* (11.9)		4.00	*d* (11.9)		3.99	ov
2′	104.5	–		103.2	–		103.6		
3′	76.8	5.53	ov	75.2	5.52	*d* (7.7)	78.7	5.34	*d* (8.7)
4′	75.7	5.52	ov	74.3	5.44	*d* (7.7)	73.8	4.31	ov
5′	79.7	4.19	*m*	78.4	4.14	*dt* (3.8, 7.3)	81.3	3.97	*m*
6′a	64.9	4.38	*dd* (11.9, 6.7)	64.6	4.41	*dd* (11.9,7.4)	65.8	4.33	ov
6′b		4.32	*dd* (11.9, 4.3)		4.23	*dd* (11.9,3.8)		4.33	ov
C=O	174.6			173.4			175.0		
	174.5			173.3			174.7		
	173.9			172.5			174.5		
	173.8			172.4			174.3		
	173.6			172.3			174.2		
CH_2–	44.5	2.22–2.32	ov	43.4	2.17–2.29	ov	44.5	2.23–2.32	ov
	44.5			43.4			44.5		
	44.5			43.4			44.4		
	44.2			43.3			44.4		
	44.1			43.0			44.3		
–CH–	27.7	2.03–2.12	*m*	26.0	2.01–2.13	*m*	27.4	2.05–2.16	*m*
	27.6			26.0			27.3		
	27.6			25.5			27.2		
	27.6			25.5			27.2		
	27.4			25.4			26.9		
$–CH_3$	23.1–23.3	0.94–0.98	ov	22.1–22.2	0.91–0.96	ov	23.1–23.2	0.87–0.92	ov
–OH					4.05				
					3.46				
					3.22				

Ov, overlapping signals.
[a] NMR data of compound **3** recorded in CD_3OD.
[b] NMR data of compound **3** recorded in $CDCl_3$. Assignments are based on ^1H and ^{13}C NMR, DEPT 135, ^1H–^1H COSY, HMQC and HMBC experiments.

FABMS data which showed a quasi-molecular peak ion $[M+H]^+$ at *m*/*z* 485. Its ^{13}C NMR data were very similar to those of vernoguinoside except for the absence of the multiplet at 3.72 ppm (H-3) and the presence of an additional carbonyl function at δ_C 211.5 (or 211.3). Careful analysis of the NMR data including HMBC, HMQC and ^1H–^1H COSY spectra and comparison with related compounds (Tchinda et al., 2002) allowed the assignment of all the carbons of the steroid framework. These studies enabled us to assign structure **2** to the compound, which has been given the trivial name vernoguinosterone.

The FAB mass spectrum of compound **3** showed a pseudo-molecular ion peak $[M+Na]^+$ at *m*/*z* 785 in agreement with the molecular formula $C_{37}H_{62}O_{16}$. Compound **3** was identified as a disaccharide from its ^{13}C NMR and DEPT 135 spectra which contained twelve signals in the region $60<\delta<105$ ppm (Wahlberg et al., 1986) (Table 2). A comparison of its ^1H chemical shifts and coupling constants and the ^{13}C NMR data with the corresponding values for sucrose (Pfeffer et al., 1979) and derivatives (Nishida et al., 1986; Garegg et al., 1988) strongly suggested that **3** was a sucrose derivative. The ^1H–^1H COSY spectrum clearly showed the connectivities H-1/ H-2/ H-3/ H-4/ H-5 /2H-6 and H-3′/ H-4′/ H-5′/ 2H-6′. The ^{13}C NMR spectrum also exhibited signals at δ 174.6, 174.5, 173.9, 173.8 and 173.6 consistent with the presence of five ester groups. In the high field region of the ^1H NMR spectrum, three groups of overlapping signals, corresponding to methylene,

methine and methyl protons, were observed at δ_H 2.22–2.32, 2.03–2.12 and 0.94–0.98, respectively. The cross-peaks observed in the ^1H–^1H COSY and HMBC spectra indicated the connectivity CH_3–CH–CH_2. Empirical calculations based on the integration of proton signals in the ^1H NMR spectrum indicated a total of 10 methyl, five methine and five methylene groups with the last two correlating to ester carbonyls in the HMBC spectrum. From the above data we concluded that compound **3** had a sucrose moiety esterified by five units of isovaleric acid (Kim et al., 2000; Yamamoto et al., 1993). This was further confirmed by the base peak at *m*/*z* 85, corresponding to the 3-methylbutanoyl ion, observed in the EI and FAB mass spectra. Moreover, the fragment ion at *m*/*z* 499 ascribable to the fructofuranosy moiety underwent sequential loss of three isovaleroyl units. The ester positions were assigned on the basis of the HMBC spectrum which showed cross-peaks between H-2, 2H-1′, H-3′, H-4′, 2H-6′ and the ester carbonyls. Thus this sugar derivative has structure **3**. On acetylation it afforded a triacetate which showed characteristic methyl singlets at δ 1.90, 1.96 and 2.03 and a total of eight ester carbonyl signals in its ^{13}C NMR spectrum.

Compound **4** displayed the same pseudo-molecular ion peak $[M+Na]^+$ at *m*/*z* 785 as compound **3**, corresponding to the proposed molecular formula $C_{37}H_{62}O_{16}$. The NMR data of **4** (Table 2) were similar to those of **3** but the two compounds differed in the positions of esterification. The chemical shifts of H-6a (δ_H 3.78, m) and H-6b (δ_H 3.86, ov) in **3** shifted to δ_H 4.48 (dd, $J=11.8$, 1.7 Hz) and 4.15 (dd, $J=11.8$, 5.9 Hz) in compound **4**, indicating that there was an ester attached to C-6. Furthermore, the change of H-4 from δ_H 5.52 (H-4′) in **3** to δ_H 4.31 in **4** suggested the loss of the isovaleroyl moiety at C-4′. The cross-peaks observed in the HMBC spectrum between 2H-6 and the ester carbonyl at δ_C 175.1 confirmed the attachment of an isovaleryl unit at C-6. Further NMR and mass spectra data led to the conclusion that compound **4** was the 1′,2,3′,6,6′-pentakis-*O*-sucrose derivative.

To the best of our knowledge, this is the first time sucrose esters have been reported in the genus *Vernonia*. However, some have been isolated from Greek tobacco (Wahlberg et al., 1986). Pentaisovaleryl sucrose esters have also been reported from *Euphorbia lathyris* (Kim et al., 2000) and *Atractylodes japonica* (Yamamoto et al., 1993).

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on an AA series automatic Polaar-2000 polarimeter. Melting points were determined by means of a Reichert apparatus and are uncorrected. The UV spectra were obtained with a Shimadzu 3101 PC instrument and the IR spectra determined with a Jasco FT-IR 410 apparatus. ^1H (400.6 MHz) and ^{13}C (100.13 MHz) NMR spectra were recorded in $CDCl_3$ (with its signals at δ_H 7.25 and δ_C 77.0 ppm as standard reference) or in CD_3OD (with its signals at δ_H 3.21 and δ_C 49.4 ppm as standard reference) with a Brüker DPX 400 apparatus using the XWIN NMR software (version 2.6) package for data acquisition and processing. The mass spectra (70 eV) were recorded with a JOEL JMS 700 apparatus. Column chromatography was run on Merck silica gel 60 and sephadex LH-20. Prep. TLC was performed on silica gel PF_{254} plates (20×20 cm, 0.5 cm thick) while analytical tlc was carried out on silica gel 60 GF_{254} pre-coated plates with detection by spraying with 50% H_2SO_4 followed by heating at 100 °C. MPLC was carried out using a chromatotron connected to an FMI lab pump (flow rate 10 ml/mn), Model QD, the plates being prepared with silica gel 60 PF_{254} containing $CaSO_4$.

3.2. Plant material

The stem bark of *V. guineensis,* Benth was collected in July 1999 at Bafoussam, West Province, Cameroon. A voucher specimen (BUD 0407) has been deposited at the Botany Department of the University of Dschang.

3.3. Extraction and isolation

The stem bark of *V. guineensis* (4 kg) was air-dried, ground and extracted with a mixture of CH_2Cl_2–MeOH (1:1) at room temperature for 48 h to give a dark brown extract (330 g) after concentration under reduced pressure. The conc. extract was further treated with a mixture $CHCl_3$–MeOH–H_2O (2:2:1) to give a brown gummy chloroform extract (90 g) which was chromatographed on a silica gel column eluting with hexane–EtOAc and EtOAc–MeOH mixtures of increasing polarity. The important fractions were obtained from the column with hexane–AcOEt [(3:2) (fraction A); (1:1) (fraction B)], pure AcOEt (fraction C) and AcOEt–MeOH (98:2) (fraction D). Fraction A was further chromatographed on a silica gel column eluting with $CHCl_3$/acetone. Two pooled fractions were obtained with the mixture 95:5. The first fraction was purified on a silica gel prep. TLC plate using hexane–AcOEt (1:1) as eluent to afford compound **2** (5 mg) while the second fraction was subjected to chromatotron separation eluting with $CHCl_3$–acetone (95:5) to give compound **4**. Chromatography of fraction B on a silica gel column using $CHCl_3$–acetone (95:5) as eluent furnished a fraction which was further purified through the chromatotron with the mixtures $CHCl_3$–acetone (9:1) and then

(4:1) to afford vernoguinosterol (5 mg) and compound **3** (300 mg), respectively. Sitosterol glycoside was obtained from fraction C. Fraction D was passed through a silica gel column eluting with $CHCl_3$–MeOH in increasing polarity. The fraction obtained with the mixture (94:6) was further purified through the chromatotron using $CHCl_3$–MeOH as eluent and yielded **1** (150 mg).

*3.3.1. 16β,22R;21,23S-diepoxy-3β-O-β-D-glucopyranosyloxy-21S,24-dihydroxy-5α-stigmasta-8,14-dien-28-one (**1**)*

Yellowish powder from acetone; mp 187–188 °C; $[\alpha]_D$ −80 (*c* 0.7 MeOH); UV (MeOH) λ_{max} (log ε) 248 (3.88) nm; IR (KBr) γ_{max} 3429, 2925, 2873, 1628, 1357, 1076, 1020 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) see Table 1. FABMS *m/z* 649 $[M+Na]^+$ (53), 631(38), 605(20), 554 (6), 469 (65), 432 (62), 431 (24), 360 (3), 337 (8), 295 (19), 253 (92), 244 (4), 224 (8), 220 (5), 143 (52), 113 (77), 86 (100), 72 (82).

*3.3.2. 16β,22R;21,23S-diepoxy-21S,24-dihydroxy-5α-stigmasta-8,14-diene-3,28-dione (**2**)*

Colourless oil, $[\alpha]_D$ +26.6 (*c* 0.3 CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 360 (2.90); 244 (4.31), 224 (4.72) nm; IR (KBr) γ_{max} 3440, 2925, 1709, 1632, 1375, 1029 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 0.75 (3H, *d*, *J* = 6.8 Hz, Me-27),0.88 (3H, *d*, *J* = 6.8 Hz, Me-26), 0.90 (3H, s, Me-18), 1.12 (3H, *s*, Me-19), 2.21 (3H, *s*, Me-29), 2.38 (1H, *d*, *J* = 6.2 Hz, H-17), 2.76 (1H, *d*, *J* = 5.5 Hz, H-20), 4.43 (1H, 2.4, H-23), 4.75 (1H, *dd*, *J* = 2.6, 6.2 Hz, H-16), 4.80 (1H, *dd*, *J* = 2.4, 5.5 Hz, H-22), 5.32 (1H, *s*, H-21), 5.37 (1H, *d*, *J* = 2.6 Hz, H-15); ^{13}C NMR (125 MHz, $CDCl_3$) see Table 1. FABMS *m/z* 485$[M+H]^+$ (7), 484 [M^+, $C_{29}H_{40}O_6$, not observed], 467 $[M+1-H_2O]$ (5), 414 (6), 391 (30), 351 (9), 329 (12), 279 (47), 258 (14), 245 (15), 205 (11), 176 (25), 149 (100), 136 (35).

*3.3.3. 1′,2,3′,4′,6′-Pentakis-O-(3-methylbutanoyl)-β-D-fructofuranosyl α-D-glucopyranoside (**3**)*

Yellowish oil; $[\alpha]_D^{20}$ +2.71 (*c* 0.7 CH_2Cl_2); IR ν_{max} (CH_2Cl_2) 3436, 2960, 2873,1735, 1467, 1370, 1294, 1161, 1069, 1023 cm^{-1}; ^1H NMR (400 MHz, CD_3OD and $CDCl_3$) and ^{13}C NMR (125 MHz, CD_3OD and $CDCl_3$), see Table 2; FABMS *m/z* 785 $[M+Na]^+$ (24); EIMS (70 ev) 762 [M+, not observed], 747 $[M-CH_3]$ (0.04), 729 $[M-CH_3-H_2O]$ (0.03), 499 (48), 456 (4), 415 (5), 401 (14), 331 (15), 295 (56), 247 (69), 229 (14), 211 (55), 127 (17), 109 (19), 85 (100), 57 (73), 43 (12), 41 (15).

*3.3.4. 1′,2,3′,6,6′-Pentakis-O-(3-methylbutanoyl)-β-D-fructofuranosyl α-D-glucopyranoside (**4**)*

Colourless oil; $[\alpha]_D^{20}$ +15.0 (*c* 0.45 CH_2Cl_2); IR ν_{max} (CH_2Cl_2) 3495, 2975, 1745, 1400, 1375,1365, 1250, 1150, 1050 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) see Table 2; FABMS *m/z* 785 (16) $[M+Na]^+$ (17), 762 [M^+, $C_{37}H_{62}O_{16}$, not observed]. EIMS (70 ev) 415 (59), 399 (2), 331 (43), 313 (15), 295 (12), 229 (15), 211 (25), 127 (11), 109 (24), 85 (100), 57 (43), 44 (14).

*3.3.5. Acetylation of **3***

A solution of of **3** (75 mg) in a mixture of Ac_2O–pyridine (1:1) (6 ml) was kept at room temperature for 8 h. Then, 10% $CuSO_4$ (6 ml) was added to the reaction mixture. Work-up as usual afforded an oily mixture which was purified by gel permeation through sephadex, eluting with hexane–CH_2CH_2 (1:1) to afford 50 mg of the corresponding triacetate, 3,4,6-tris-*O*-acetyl-1′,2,3′,4′,6′-pentakis-*O*-(3-methylbutanoyl)-β-D-fructofuranosyl α-D-glucopyranoside (**3a**), as a yellowish oil: $[\alpha]_D^{20}$ + 7.1 (*c* 0.28 CH_2Cl_2); IR ν_{max} (CH_2Cl_2) 2961, 1746, 1595, 1369, 1240, 1096, 1030 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.84–0.94 (30H, overlapping signals, 5×(CH_3)$_2$CHCH$_2$–), 1.90 (3H, *s*, –OCOCH_3), 1.96 (3H, *s*, –OCOCH_3), 2.03 (3H, *s*, –OCOCH_3), 1.96–2.14 (5H, overlapping signals, 5×(CH$_3$)$_2$*CH*CH$_2$–), 2.28–2.90 (10H, overlapping signals, 5×(CH$_3$)$_2$CH*CH*$_2$–), 3.93 (1H, d, *J* = 11.9 Hz, H-1′a), 4.00 (1H, *d*, *J* = 11.9 Hz, H-1′b), 4.08 (*m*, H-5′), 4.12 (1H, ov, H-6a), 4.21 (1H, ov, H-6b), 4.23 (1H, *m*, H-5), 4.28 (2H, ov, 2H-6′), 4.89 (1H, dd, *J* = 3.7, 10.4 Hz, H-2), 4.98 (1H, *t*, *J* = 9.7 Hz, H-4), 5.33 (1H, *t*, *J* = 9.9 Hz, H-3), 5.38 (1H, *d*, *J* = 7.5 Hz, H-4′), 5.49 (1H, *J* = 7.6 Hz, H-3′), 5.57 (1H, *d*, *J* = 3.7 Hz, H-1). ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.6 (*q*, 3×-OCOCH$_3$), 21.1, 21.2, 21.2, 21.2, 21.3 (*q*, 5×(CH_3)$_2$CHCH$_2$), 24.5–24.7 (*d*, 5×(CH$_3$)$_2$*CH*CH$_2$–), 41.8–42.0 (*t*, 5×(CH$_3$)$_2$CH*CH*$_2$–), 60.9 (*t*, C-6), 62.1 (*t*, C-1′), 62.7 (*t*, C-6′), 67.4 (*d*, C-4), 68.5 (*d*, C-5), 68.8 (*d*, C-2), 72.6 (*d*, C-3), 73.8 (*d*, C-4′), 76.2 (*d*, C-3′), 77.4 (C-5′), 88.5 (C-1), 101.9 (C-2′), 168.5, 168.8, 169.7, 170.7, 170.8, 170.9, 170.9, 171.6 (s, 3×–*O*C*O*CH$_3$; s, 5×(CH$_3$)$_2$CHCH$_2$O*C*O–); FABMS *m/z* 911 $[M+Na]^+$ (10), 888 [M^+, $C_{43}H_{68}O_{19}$, not observed], 499 (26), 373 (14), 295 (23), 211(38), 169 (24), 85 (100), 59 (44).

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