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Acylated 2-Hydroxythymol 3-*O*-diglycosides from *Melampodium divaricatum*

Juliane Hüther[a], Claus M. Passreiter[a,*], Victor Wray[b], Günter Willuhn[a]

[a]*Institut für Pharmazeutische Biologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstrasse 1, D-40225 Düsseldorf, Germany*
[b]*Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-38124 Braunschweig, Germany*

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Abstract

From the aerial parts of *Melampodium divaricatum* (Rich. in Pers.) DC, Asteraceae, four new doubly and eight new triply acylated 2-hydroxythymol 3-*O*-β-D-fucopyranosyl-(1→3)-β-D-fucopyranosides (seven compounds) and 2-hydroxythymol 3-*O*-β-D-fucopyranosyl-(1→3)-β-D-quinovopyranosides (five compounds) were isolated and their structures established by spectroscopic methods. The acids involved in the acylation of the sugar moieties are angelic, isovaleric, 2-methylbutyric, and acetic acid.

Keywords: Melampodium divaricatum; Asteraceae; Heliantheae; Hydroxythymol glycosides

1. Introduction

Melampodium divaricatum (Rich. in Pers.) DC, Asteraceae, tribe Heliantheae, is a widespread plant in Central America. In Guatemala the leaves are used in folk medicine for the treatment of malaria and stomach pains, and the whole plant is used against influenza (Girón, Freire, Alonzo, & Càceres, 1991). In Mexico embolism is treated with an infusion of the whole plant, taken orally or placed as a poultice on the chest (Martinez Alfaro, 1984). It is also used as a remedy for wounds of the eyelid and as antidysenteric and anticonvulsive, taken orally and/or rectally (del Amo, 1980; Morton, 1981; Martinez, 1990).

Previous investigations of the aerial parts of the plant led to the identification of the sesquiterpene lactone mikanokryptin (Herz & Kalyanaraman, 1975), several sesquiterpenes (Bohlmann & Le Van, 1977; Hubert & Wiemer, 1985), the diterpene kolavenol (Hubert & Wiemer, 1985), the phenyl propanoid eugenol (Bohlmann & Le Van, 1977), two flavonoid glycosides (Bohm & Stuessy, 1991) and two coumarin derivatives (Borges-del-Castillo, Martinez-Martir, Rodriguez-Luis, Rodriguez-Ubis, & Vázquez-Bueno, 1984). In 1977, the identification of a variety of acylated 2-hydroxythymol 3-*O*-glycosides was reported, including six β-D-quinovopyranosides (is 6-deoxy-β-D-glucopyranosides), two β-D-fucopyranosides (is 6-deoxy-β-D-galactopyranosides) and two β-D-fucopyranosyl-(1→3)-β-D-quinovopyranosides (Bohlmann & Le Van, 1977).

The present paper deals with the isolation and spectroscopic structure elucidation of 12 further acylated 2-hydroxythymol 3-*O*-diglycosides from the aerial parts of *M. divaricatum*, among which the compounds **1**–**7** have bound the hitherto unknown disaccharide β-D-fucopyranosyl-(1→3)-β-D-fucopyranoside.

2. Results and discussion

Column chromatography (CC) of the methanol soluble part of the dichloromethane extract on Sephadex LH-20 with methanol afforded one fraction rich in triply acylated 2-hydroxythymol diglycosides (compounds **1**–**5** and **8**–**9**) and one immediately subsequent fraction rich in the analogous doubly acylated diglycosides **6**, **7**, **11** and **12**. The compounds were isolated from the fractions by repeated CC on silica gel and by MPLC on RP 18.

The structure of the diglycoside **1** clearly followed from its mass, ^{1}H and ^{13}C NMR spectra, including DEPT, 2-D COSY and 2-D HMBC experiments. The CIMS (NH_3) revealed the quasi-molecular ion as base peak at m/z 724 $[M+NH_4]^+$, indicating a molecular mass of 706. The molecular ion was absent from the EIMS and the first fragment ion at m/z 541 $[M\text{-}165]^+$ resulted from the loss of hydroxythymol. Further fragmentation was typical of a disaccharide from two acylated deoxyhexoses

* Corresponding author. Tel.: +49-211-8114172; fax: +49-211-8111923; e-mail: passreit@uni-duesseldorf.de

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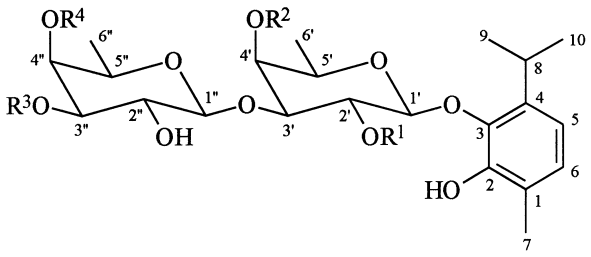
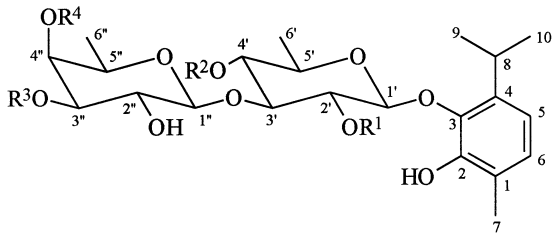


	R^1	R^2	R^3	R^4
1	ival	ang	H	ang
2	ival	ang	ang	H
3	ang	ang	H	ang
4	ang	ang	ang	H
5	ang	ac	H	ang
6	H	ang	ang	H
7	H	ac	ang	H

Structure 1.



	R^1	R^2	R^3	R^4
8	ang	ang	ang	H
9	ang	ang	H	ac
10	ang	ang	ac	H
11	H	ang	H	2-mebut
12	H	ang	H	ival

Structure 2.

(Bohlmann & Le Van, 1977), one of which was esterified with an unsaturated and a saturated C_5-acid (*m/z* 313 $[C_4H_7CO–C_6H_9O_4–OCC_4H_9]^+$, 295 $[313\text{-}H_2O]^+$, 213 $[313\text{-}C_4H_7COOH]^+$, 211 $[313\text{-}C_4H_9COOH]^+$, 85 $[C_4H_9CO]^+$, 83 $[C_4H_7CO]^+$) and the other one with an unsaturated C_5-acid (*m/z* 229 $[C_6H_9O_4–OCC_4H_7]^+$, 129 $[229\text{-}C_4H_7COOH]^+$).

In accordance with the calculated molecular formula $C_{37}H_{54}O_{13}$, the ^{13}C NMR spectrum showed 37 signals (see Table 1). Ten of them, including four quaternary and two methine carbon signals in the aromatic shift range, resulted from the aglycone 2-hydroxythymol. Its presence clearly followed from the ^1H NMR data (Table 2), which corresponded to those previously reported for this thymol derivative (Bohlmann & Le Van, 1977). Moreover, the assignments of these carbons were confirmed by the HMBC spectrum. Twelve further signals confirmed the presence of a disaccharide from two 6-deoxy hexoses with the anomeric methine carbon signals at 103.4 and 103.8 ppm and the methyl carbon signals at 16.3 and 16.6 ppm, respectively. As expected from the mass spectra, the remaining 15 signals were due to two angeloyl and one isovaleryl ester groups, which were identified by comparison of their ^{13}C and ^1H NMR shift values with those previously reported in the literature (Herz & Kumar, 1980; Joseph-Nathan, Wesener, & Günther, 1984) (see Table 2).

The assignments of the ^1H and ^{13}C NMR signals of the carbohydrate moiety were made on the basis of detailed COSY and HMBC experiments and by comparison with the data of similar glycosides and the known glycosidation and acylation shifts (Bohlmann & Le Van, 1977; Agrawal, Mahmood, & Thakur, 1989; Agrawal, 1992). The proton signals clearly showed that the disaccharide consisted of two *β*-D-fucose moieties from the coupling constants $^3J_{1',2'}=8.2$ Hz, $^3J_{2',3'}=10.1$ Hz and $^3J_{3',4'}=3.8$ Hz, and $^3J_{1'',2''}=7.6$ Hz, $^3J_{2'',3''}=9.5$ Hz and $^3J_{3'',4''}=3.2$ Hz, respectively (see Table 2). The HMBC spectrum showed a correlation between the anomeric proton of the terminal fucose at δ 4.32 ppm (H-1″) and the signal at δ 77.7 ppm (C-3′) of the other fucose, thus demonstrating C-3′ as the linkage site. In agreement with this, the signals of H-3′ and C-3′ are shifted to lower field. The attachment of the disaccharide to the aglycone at C-3 followed from a long range C-H-correlation between the signal of the anomeric proton at δ 4.73 ppm (H-1′) and the carbon signal at δ 141.1 ppm (C-3 of the aglycone). The presence of three acyl substituents at the sugar moieties at 2′-*O*, 4′-*O*, and 4″-*O* was evident from the acylation induced downfield shifts of the signals of H-2′ (δ 5.53 ppm), H-4′ (δ 5.42 ppm), and H-4″ (δ 5.25 ppm), respectively, which corresponded to those reported for 2,4-di-*O*-acylfucopyranoside (Bohlmann & Le Van, 1977). The binding sites of the respective acyl groups were confirmed by the HMBC spectrum, which showed correlations between the carboxyl carbons of the isovaleryl group with H-

Table 1
^{13}C NMR spectral data of compounds **1**–**12**. (125 MHz: **1**, **5**, **6**; 75 MHz: **2**, **4**, **8**–**11**; 100 MHz: **3**, **7**, **12**; $CDCl_3$, TMS as int. standard (**1**–**6**, **8**))

C	1	2	3	4	5	6	7	8	9	10	11	12
1	123.6	123.6	123.6	123.7	123.7	123.0	123.0	123.7	123.7	123.7	123.0	123.1
2	147.9	147.9	147.9	148.0	147.9	147.4	147.4	147.9	147.9	147.9	147.2	147.3
3	141.1	141.1	141.3	141.3	141.5	142.2	142.4	141.2	141.2	141.2	142.2	142.3
4	139.5	139.6	139.5	139.6	139.6	140.5	140.5	139.5	139.5	139.5	140.4	140.4
5	116.4	116.4	116.1	116.2	116.2	116.0	116.1	116.3	116.2	116.2	116.2	116.1
6	127.7	127.7	127.6	127.7	127.6	127.4	127.4	127.8	127.8	127.8	127.4	127.4
7	15.8	16.0	15.8	16.1	15.9	15.7	15.8	15.9[a]	15.9	15.9	15.7	15.7
8	25.5	25.6	25.7	25.8	25.8	25.8	25.9	25.8	25.8	25.8	25.8	25.8
9	24.2[a]	24.2[a]	24.0[a]	24.1[a]	24.1[a]	24.0[a]	24.1[a]	24.2[b]	24.2[a]	24.2[a]	24.0[a]	24.0[a]
10	23.4[a]	23.5[a]	22.6[a]	22.7[a]	22.6[a]	23.7[a]	23.9[a]	22.9[b]	22.8[a]	22.8[a]	23.8[a]	23.8[a]
Fuc								*Qui*				
1′	103.4	103.4	103.6	103.7	103.7	105.9	106.0	103.5	103.5	103.5	105.2	105.2
2′	70.9	71.0	70.4	70.4	70.2	71.0	70.9[b]	72.6	72.6	72.6	74.4	74.3
3′	77.6	77.2	78.2	77.8	78.3	77.4	78.4	79.8	79.9	79.9	84.6	85.3
4′	72.0	72.3	71.9	72.2	72.0	71.8	72.2	72.8	72.9	72.9	72.8	73.1
5′	70.7	70.7	70.8	70.7	70.7	70.1	70.1	71.0	71.0	71.0	70.9	71.0
6′	16.2	16.0[b]	16.3	16.3	16.2	16.2	16.2[c]	17.5	17.5	17.5	17.4	17.4
Fuc								*Fuc*				
1″	103.8	104.1	104.3	104.5	104.4	102.6	102.9	104.3	103.9	104.3	104.5	104.3
2″	71.6[d]	69.0	71.6	69.0	71.6	68.4	68.8	68.8	71.3	68.6	72.6	72.6
3″	72.0	75.1	71.9	75.1	71.8	75.7	75.9	75.3	72.1	75.5	72.4	72.4
4″	71.7[d]	69.8	71.6	69.8	72.0	69.9	69.9	69.7	71.8	69.8	72.2	72.3
5″	69.7	70.6	69.7	70.7	69.7	71.2	71.2[b]	70.5	69.6	70.5	69.7	69.8
6″	16.5	16.3	16.4	16.3	16.5	16.2	16.1[c]	15.8	15.9	15.8	16.0[b]	16.1[b]
R^1												
1	172.4	172.3	166.5	166.5	166.5			166.3	166.3	166.3		
2	43.0	43.0	126.8	126.7	126.6			126.5	126.5	126.4		
3	24.9	25.0	141.0	141.2	141.4			141.8	142.0	142.0		
4	22.6	22.7[c]	16.0[b]	16.1[b]	16.1			16.1[a]	15.9[b]	15.9[b]		
5	22.6	22.6[c]	20.4	20.9[c]	20.8[b]			20.7[c]	20.6[c]	20.6[c]		
R^2												
1	167.0	167.4	166.9	167.3	170.0	168.3	171.6	166.2	166.3	166.2	166.6	166.5
2	127.6	127.7	127.7	127.6	20.5	127.4	21.0	127.5	127.8	127.5	127.4	127.5
3	138.6	139.0	138.2	139.1		139.7		139.9[d]	138.9	140.0	139.6	139.5
4	16.0[b]	15.9[b]	16.0[b]	15.9[b]		16.0		16.0[a]	16.1[b]	16.0[b]	15.9[b]	15.9[b]
5	20.6[c]	20.8[d]	20.7	20.6[c]		20.7[b]		20.5[c]	20.5[c]	20.5[c]	20.5	20.6
R^3												
1		167.6		167.6		167.8	167.9	167.6		170.8		
2		127.5		127.5		127.3	127.2	127.5		21.2		
3		139.2		139.1		140.0	140.0	139.1[c]				
4		15.8[b]		15.9[b]		16.0	16.0	15.9[a]				
5		20.5[d]		20.6[c]		20.5[b]	20.5	20.5[c]				
R^4												
1	167.8		167.7		167.8				171.1		177.6	173.9
2	127.2		127.3		127.3				20.7		41.3	43.3
3	139.6		139.3		139.5						26.7	25.9
4	15.9[b]		15.9[b]		16.1						11.6	22.4
5	20.8[c]		20.5		20.6[b]						16.7	22.4

[a,b,c,d] Assignments interchangeable.

2′ and the two angeloyl groups with H-4′ and H-4″, respectively. Thus, the structure of **1** is 2-hydroxythymol 3-*O*-(4-*O*-angeloyl-*β*-D-fucopyranosyl)-(1→3)-(4-*O*-angeloyl-2-*O*-isovaleryl)-*β*-D-fucopyranoside.

Compound **2** was shown to be an isomer of **1**, since its mass spectra ($CI(NH_3)$ and EI) exhibited a molecular mass of 706 and a fragmentation pattern analogous to **1**. Consistently, the ^{13}C and ^{1}H NMR spectra (Tables 1–2) were similar to those of **1**, except for the signals of C-3″ and C-4″ (δ 75.1 instead of 71.7 ppm and 69.8 instead of 72.1 ppm, respectively) and the corresponding protons H-3″ and H-4″ (δ 4.80 instead of 3.74 ppm and 3.86 instead of 5.25 ppm, respectively) of the terminal fucose moiety, which clearly indicated that the angeloyloxy substituent in **2** is attached to C-3″ instead of C-4″. This was additionally confirmed by a correlation between H-3″

Table 2
^{1}H NMR spectral data of compounds **1**–**7**. (500 MHz: **1**, **5**, **6**; 300 MHz: **2**, **4**; 400 MHz: **3**, **7**; $CDCl_3$, TMS as int. standard (**1**–**6**))

H	1	2	3	4	5	6	7
5	6.70 d	6.70 d	6.66 d	6.67 d	6.67 d	6.69 d	6.68 d
6	6.94 d	6.94 d	6.91 d	6.92 d	6.92 d	6.92 d	6.90 d
7	2.21 s	2.21 s	2.21 s	2.21 s	2.21 s	2.21 s	2.19 s
8	3.32 m	3.29 m	3.21 m	3.20 m	3.20 m	3.63 m	3.61 m
9	1.24 d[a]	1.24 d[a]	1.19 d[a]	1.19 d[a]	1.19 d[a]	1.17 d[a]	1.16 d[a]
10	1.12 d[a]	1.12 d[a]	1.00 d[a]	0.99 d[a]	1.00 d[a]	1.16 d[a]	1.15 d[a]
Fucose							
1′	4.72 d	4.74 d	4.78 d	4.78 m	4.75 d	4.52 d	4.47 d
2′	5.53 dd	5.53 dd	5.66 dd	5.66 dd	5.66 dd	4.01 m	4.03 dd
3′	3.89 dd	3.95 dd	3.95 dd	4.01 dd	3.90 dd	3.97 m	3.90 dd
4′	5.42 d	5.45 d	5.46 d	5.48 d	5.35 d	5.30 d	5.21 d
5′	3.78 m	3.80 m	3.82 q	3.85 m	3.78 q	3.73 q	3.67 m
6′	1.23 d	1.24 d	1.26 d	1.27 d	1.24 d	1.21 d	1.19 d
Fucose							
1″	4.32 d	4.40 d	4.31 d	4.39 d	4.30 d	4.66 d	4.64 d
2″	3.52 dd	3.69 m	3.50 dd	3.69 m	3.53 dd	3.93 dd	3.92 m
3″	3.74 m	4.80 dd	3.70 m	4.78 m	3.72 m	4.89 dd	4.86 dd
4″	5.25 d	3.86 d	5.22 d	3.85 m	5.25 d	3.88 d	3.87 d
5″	3.74 m	3.71 m	3.70 m	3.69 m	3.71 m	3.80 q	3.77 q
6″	1.19 d	1.32 d	1.17 d	1.31 d	1.18 d	1.36 d	1.34 d
R^1							
2	2.37 dd, 2.27 m	2.37 dd, 2.26 dd					
3	2.15 m	2.15 m	6.22 m	6.20 m[b]	6.25 m		
4	1.01 d[b]	1.00 d[b]	2.03 m	2.04 m	2.06 dd		
5	1.00 d[b]	0.99 d[b]	1.97 m	1.97 m	1.97 t		
R^2							
2					2.19 s		2.19 s
3	6.17 m	6.16 m	6.14 m	6.20 m[b]		6.18 m	
4	2.08 dd	2.10 m	2.03 m	2.04 m		2.04 m	
5	2.00 t	2.00 m	2.00 m	2.01 m		1.98 t	
R^3							
3		6.16 m		6.12 m[b]		6.18 m	6.16 m
4		2.00 m		2.04 m		2.04 m	2.01 m
5		1.92 m		1.91 m		1.95 t	1.93 t
R^4							
3	6.16 m		6.14 m		6.18 m		
4	2.00 m		2.03 m		2.02 dd		
5	1.91 t		1.90 m		1.93 t		

[a,b] Assignments interchangeable.*J* (Hz) **1**–**7**: 5, 6 = 7.9; 8, 9 = 6.8; 8, 10 = 6.9; 1′, 2′ = 6.9–8.2; 2′, 3′ = 9.7–10.1; 3′, 4′ = 2.5–3.8; 4′, 5′ = < 1; 5′, 6′ = 6.4; 1″, 2″ = 7.5–7.9; 2″, 3″ = 9.5–10.2; 3″, 4″ = 2.5–3.3; 4″, 5″ = < 1; 5″, 6″ = 6.5; ang: 3, 4 = 6.9–7.6; 3, 5 = 1.3; 4, 5 = 1.3; ival: 2a, 2b = 16.3; 2a, 3 = 6.8; 2b, 3 = 7.4; 3, 4 = 6.8; 3, 5 = 6.3.

and the carboxyl carbon signal of the angeloyl ester group at δ 167.6 ppm in the HMBC spectrum.

As found for compounds **1** and **2**, the isolated isomeric compounds **3** and **4** differed markedly in TLC and HPLC retention. The CIMS (NH_3) and EIMS revealed a molecular mass of 704 for both, differing from **1** and **2** by two mass units. The fragmentation patterns of both compounds were similar to those of **1** and **2** with the only difference being the presence of a third unsaturated C_5-acid ester group at the disaccharide instead of the isovaleryl ester. This was verified by the ^{13}C and ^{1}H NMR spectra (Tables 1 and 2), which displayed the signals of a third angeloyl group, located at C-2′ from the HMBC spectrum.

The crystalline compound **5** differed from **3** only by the presence of an acetyl ester group at C-4′ instead of the angeloyl substituent, which followed from the comparison of its spectroscopic data (CIMS(NH_3), EIMS, ^{13}C and ^{1}H NMR) with those of **3** (see Tables 1–2).

Compound **6** (molecular mass 622) was identified in the same way as the 2′-*O*-deangeloyl derivative of **4**. Accordingly, the signal of H-2′ was at higher field, indicating a free hydroxyl group at this position (see Table 2). Compound **7** with a molecular mass of 582 was shown to be acylated at the same positions as **6**, differing only by the presence of an acetate instead of the angelic acid ester at C-4′ (see Tables 1–2).

The acylated 2-hydroxythymol 3-*O*-diglycosides **1**–**7** are new natural compounds. Moreover, the disaccharide *β*-D-fucopyranosyl-(1→3)-*β*-D-fucopyranoside has not been described previously.

In contrast to **1**–**7**, the disaccharide found in compounds **8**–**12** consisted of the 6-deoxy hexoses *β*-D-quinovose (is 6-deoxy-glucose) and *β*-D-fucose (is 6-deoxy-galactose), which clearly followed from the coupling constants $^{3}J_{3',4'} = 9.4$ and $^{3}J_{3'',4''} = 3.3$ Hz, respectively. The spectroscopic data (Tables 1 and 3), including HMBC experiments, showed that the disaccharide was identical with *β*-D-fucopyranosyl-(1→3)-*β*-D-quinovopyranoside, which has already been found in acylated 2-hydroxythymol 3-*O*-diglycosides isolated from *M. divaricatum* (Bohlmann & Le Van, 1977).

Compound **8** was shown by its CIMS(NH_3) and EIMS to possess the same molecular mass (704) and a similar fragmentation pattern to that of compound **4**, though it differed markedly in TLC and HPLC retention. The NMR data (Tables 1 and 3) were remarkably similar to those of **4**, except for the chemical shift value of H-4′ (δ 5.01 instead of 5.48 ppm) and $^{3}J_{3',4}$. Thus, **8** is the novel compound 2-hydroxythymol 3-*O*-(3-*O*-angeloyl-*β*-D-fucopyranosyl)-(1→3)-(2,4-di-*O*-angeloyl)-*β*-D-quinovopyranoside.

The minor compounds **9** (R_f 0.12) and **10** (R_f 0.22) were isolated as a mixture (3:1), and were not further separated. The CIMS(NH_3) of the mixture exhibited only one quasi molecular ion at *m*/*z* 682 $[M+NH_4]^+$, indicating a molecular mass of 664 for both compounds. Again no molecular ion was found in the EIMS. The first fragment ion at *m*/*z* 459 was in accordance with a disaccharide from two 6-deoxyhexoses esterified with two unsaturated C_5-acids and one acetic acid. The fragment ions clearly indicated, that in both compounds one of the 6-deoxyhexoses carried two C_5-acyl groups and the other the acetyl group. This was confirmed from the ^{1}H and ^{13}C NMR spectra of the mixture, including COSY and HMBC experiments. The NMR data were closely related to those of **8** (see Tabs. 1 and 3). The only difference between **10** and **8** was the occurrence of the ^{1}H and ^{13}C resonances for a 3″-*O*-acetyl group instead of a 3″-*O*-angeloyl group, whereas compound **9** additionally differed in the position of the acetoxy group at C-4″ instead of C-3″, which followed from the chemical shift values of H-3″ and H-4″ (see Tabs. 1 and 3). Interestingly, the isomeric compounds **9** and **10** showed the same TLC behavior as the analogous pairs of isomers **1**/**2** and **3**/**4**, respectively, which similarly only differed in the position of acylation of the hydroxyl groups at C-3″ or C-4″ of the terminal fucose. In each case the compound with the acylated hydroxyl group at C-4″ showed a significantly lower R_f value than its isomer esterified at C-3″.

The CIMS(NH_3) and EIMS of **11** and **12** revealed a molecular mass of 624 and similar fragmentation patterns for both compounds, which was in agreement with a doubly acylated 2-hydroxythymol diglycoside. In detail, the fragmentation indicated that the disaccharide of both compounds consisted of two monoacylated 6-deoxy hexoses, bearing an unsaturated and a saturated C_5-acid ester group. In agreement with this, the ^{1}H and ^{13}C NMR spectra of both compounds displayed the signals of one angeloyl ester group. Additionally, the spectra showed the signals of a 2-methylbutyryl ester group (**11**) and of a 3-methylbutyryl (is isovaleryl) ester group (**12**) (Séquin, 1981). The positions of the angeloyl ester at C-4′ and of the saturated C_5-acid ester at C-4″ were determined by comparison of the ^{1}H NMR signals with those of **8**–**10**, and additionally confirmed by a HMBC experiment for **11**.

The acylated 2-hydroxythymol 3-*O*-diglycosides **8**–**12** are reported here for the first time. The two analogous diglycosides with a 2,4-*O*-diangeloyl and a 3,4-*O*-diangeloyl substitution at the fucopyranosyl moiety, respectively, which have already been reported for *M. divaricatum* (Bohlmann & Le Van, 1977) were not detected in the present plant material.

3. Experimental

3.1. Plant material

M. divaricatum (Rich. in Pers.) DC. cv. Medallion was cultivated in the New York Botanical Garden, Bronx NY and collected in summer 1994 at the flowering stage. A voucher specimen was deposited at the herbarium of the Institute for Pharmaceutical Biology at the Heinrich-Heine-University of Düsseldorf, Reg. No. 147.

3.2. Extraction and isolation

Dried and powdered aerial parts (1025 g) of *M. divaricatum* were extracted with CH_2Cl_2 and the extract (43 g) macerated five times with 100 ml MeOH. Gel chromatography of the soluble material (21 g) with Sephadex LH-20/MeOH gave a fraction containing the triply acylated glycosides **1**–**5** and **8**–**9** (7.0 g) and an immediately

Table 3
^{1}H NMR spectral data of compounds **8**–**12**. (300 MHz: **8**–**11**; 400 MHz: **12**; $CDCl_3$, TMS as int. standard (8))

H	**8**	**9**	**10**	**11**	**12**
5	6.67 d	6.65 d	6.65 d	6.68 d	6.68 d
6	6.92 d	6.91 d	6.91 d	6.90 d	6.91 d
7	2.21 s	2.20 s	2.20 s	2.19 s	2.20 s
8	3.15 m	3.14 m	3.14 m	3.59 m	3.58 m
9	1.19 d[a]	1.18 d[a]	1.18 d[a]	1.16 d[a]	1.17 d[a]
10	0.99 d[a]	0.97 d[a]	0.97 d[a]	1.14 d[a]	1.15 d[a]
Quinovose					
1′	4.75 d	4.74 d	4.74 d	4.52 d	4.53 d
2′	5.59 dd	5.58 dd	5.58 dd	3.96 dd	3.97 dd
3′	4.05 dd	4.01 dd	4.02 dd	3.77 dd	3.76 m
4′	5.01 dd	4.99 dd	4.99 dd	4.93 dd	4.96 dd
5′	3.68 m	3.68 m	3.68 m	3.49 m	3.49 m
6′	1.33 d	1.32 d	1.31 d	1.24 d	1.25 d
Fucose					
1″	4.32 d	4.23 d	4.27 d	4.51 d	4.51 d
2″	3.68 m	3.49 m	3.68 m	3.65 dd	3.70 m
3″	4.72 dd	3.61 dd	4.63 dd	3.77 dd	3.76 m
4″	3.81 s	5.09 d	3.75 s	5.07 d	5.06 s
5″	3.61 m	3.61 m	3.57 m	3.69 m	3.70 m
6″	1.20 d	1.06 d	1.23 d	1.06 d	1.10 d
R^1					
3	6.24 m	6.25 m	6.25 m		
4	2.01 m	2.04 m	2.04 m		
5	1.98 t	1.96 t	1.96 t		
R^2					
3	6.12 m	6.09 m	6.09 m	6.10 dq	6.11 m
4	2.01 m	1.99 m	1.99 m	1.99 dd	2.00 m
5	1.89 t	1.88 t	1.88 t	1.87 t	1.87 t
R^3					
2			2.12 s		
3	6.12 m				
4	2.01 m				
5	1.89 t				
R^4					
2		2.09 s		2.47 m	2.30 dd, 2.26 dd
3				1.70 m, 1.48 m	2.12 m
4				0.91 t	0.97 d
5				1.16 d	0.97 d

[a]Assignments interchangeable. *J* (Hz) **8**–**12**: 5, 6 = 7.9; 8, 9 = 6.8; 8, 10 = 6.9; 1′, 2′ = 8.1; 2′, 3′ = 9.1–9.5; 3′, 4′ = 9.4; 4′, 5′ = 9.5; 5′, 6′ = 6.2; 1″, 2″ = 7.6; 2″, 3″ = 9.7–10.2; 3″, 4″ = 2.7–3.5; 4″, 5″ = < 1; 5″, 6″ = 6.4; ang: 3, 4 = 7.2; 3, 5 = 1.4; 4, 5 = 1.5; 2-mebut: 2, 3b = 6.7; 2, 5 = 6.9; 3, 4 = 7.4; ival: 2a, 2b = 14.6; 2a, 3 = 7.3; 3, 4 = 6.7; 3, 5 = 6.6.

following fraction rich in the doubly acylated glycosides **6**, **7**, **11**, and **12** (5.1 g). Separation of these fractions by repeated CC on silica gel with toluene–EtOAc (3:2 and 9:1, respectively, with increasing amounts of EtOAc in each case), CH_2Cl_2–EtOAc or *n*-pentane–Et_2O mixtures of various polarities as well as final purification by MPLC on RP 18 gave 29 mg **1**, 13 mg **2** (in mixture with **1**), 87 mg **3**, 11 mg **4**, 20 mg **5**, 25 mg **6**, 6 mg **7**, 13 mg **8**, 7 mg of a mixture of **9** and **10**, 62 mg **11**, and 4 mg **12**.

3.3. Chromatography

MPLC: RP 18 (LiChroprep 15–25 μm), 450 × 10 mm, isocratic 70% MeOH, flow 5 ml min^{-1}, detection: UV

225 nm. HPLC: RP 18 (Hypersil ODS, 5 μm), 125 × 4.6 mm, flow 1.8 ml min^{-1}: 80% MeOH (8 min), to 100% MeOH (8–11 min); detection: UV 225 and 275 nm. TLC: silica gel 60 F_{254}, toluene–EtOAc (2:3); detection: anisaldehyde–H_2SO_4, compounds **1**–**12** brown.

3.4. 2-Hydroxythymol 3-O-(4-O-angeloyl-β-D-fucopyranosyl)-(1→3)-(4-O-angeloyl, 2-O-isovaleryl)-β-D-fucopyranoside (**1**)

$C_{37}H_{54}O_{13}$, colorless gum. TLC: R_f 0.32; HPLC: R_t 6.50 min. IR, ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1750 (shoulder, OCOR), 1720, 1650 (C=CCO_2R); CIMS (NH_3), 100 eV, *m/z* (rel. int.): 724 $[M+NH_4]^+$ (100), 642 $[M+NH_4-C_4H_6CO]^+$ (2), 541 $[M-C_{10}H_{13}O_2]^+$ (8); EIMS, 70 eV, *m/z* (rel. int.): 541 $[M-C_{10}H_{13}O_2]^+$ (6), 341 [541-2 × $C_4H_7COOH]^+$ (7), 313 [541−228 ($C_6H_9O_4$–OCC_4H_7)]$^+$ (18), 229 [$C_6H_{10}O_4$–$OCC_4H_7]^+$ (7), 213 [313−$C_4H_7COOH]^+$ (9), 211 [313-$C_4H_9COOH]^+$ (19), 166 $[C_{10}H_{14}O_2]^+$ (8), 151 [166-$CH_3]^+$ (16), 129 [229-$C_4H_7COOH]^+$ (13), 111 [211-$C_4H_7COOH]^+$ (32), 85 $[C_4H_9CO]^+$ (12), 83 $[C_4H_7CO]^+$ (100), 57 [85-CO]$^+$ (38), 55 [83-CO]$^+$ (44). ^{13}C NMR: Table 1; ^{1}H NMR: Table 2.

3.5. 2-Hydroxythymol 3-O-(3-O-angeloyl-β-D-fucopyranosyl)-(1→3)-(4-O-angeloyl, 2-O-isovaleryl)-β-D-fucopyranoside (**2**)

$C_{37}H_{54}O_{13}$, colorless gum. TLC: R_f 0.57; HPLC: R_t 6.19 min. CIMS (NH_3), 100 eV, *m/z* (rel. int.): 724 $[M+NH_4]^+$ (100), 642 $[M+NH_4-C_4H_6CO]^+$ (5), 640 $[M+NH_4-C_4H_8CO]^+$ (7), 541 $[M-C_{10}H_{13}O_2]^+$ (5); EIMS, 70 eV, *m/z* (rel. int.): 541 $[M-C_{10}H_{13}O_2]^+$(7), further fragmentation analogous to that of **1**. ^{13}C NMR: Table 1; ^{1}H NMR: Table 2.

3.6. 2-Hydroxythymol 3-O-(4-O-angeloyl-β-D-fucopyranosyl)-(1→3)-(2,4-di-O-angeloyl)-β-D-fucopyranoside (**3**)

$C_{37}H_{52}O_{13}$, colorless gum. TLC: R_f 0.27; HPLC: R_t: 5.34 min. IR, ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1720, 1645 (C=CCO_2R); CIMS (NH_3), 100 eV, *m/z* (rel. int.): 722 $[M+NH_4]^+$ (100), 640 $[M+NH_4-C_4H_6CO]^+$ (4), 539 $[M-C_{10}H_{13}O_2]^+$ (4); EIMS, 70 eV, *m/z* (rel. int.): 539 $[M-C_{10}H_{13}O_2]^+$ (22), 339 [539-2 × $C_4H_7COOH]^+$ (5), 311 [539−228 ($C_6H_9O_4$–OCC_4H_7)]$^+$ (20), 229 [$C_6H_{10}O_4$–$OCC_4H_7]^+$ (31), 211 [311-$C_4H_7COOH]^+$ (21), 166 $[C_{10}H_{14}O_2]^+$ (6), 151 [166-$CH_3]^+$ (16), 129 [229-$C_4H_7COOH]^+$ (15), 111 [211-$C_4H_7COOH]^+$ (36), 83 $[C_4H_7CO]^+$ (100), 55 [83-CO]$^+$ (63). ^{13}C NMR: Table 1; ^{1}H NMR: Table 2.

3.7. 2-Hydroxythymol 3-O-(3-O-angeloyl-β-D-fucopyranosyl)-(1→3)-(2,4-di-O-angeloyl)-β-D-fucopyranoside (**4**)

$C_{37}H_{52}O_{13}$, colorless gum. TLC: R_f 0.56; HPLC: R_t 4.89 min.; CIMS (NH_3), 100 eV, *m/z* (rel. int.): 722 $[M+NH_4]^+$ (100), 640 $[M+NH_4-C_4H_6CO]^+$ (4), 539 $[M-C_{10}H_{13}O_2]^+$ (8); EIMS, 70 eV, *m/z* (rel. int.): 539 $[M-C_{10}H_{13}O_2]^+$ (8), further fragmentation analogous to that of **3**. ^{13}C NMR: Table 1; ^{1}H NMR: Table 2.

3.8. 2-Hydroxythymol 3-O-(4-O-angeloyl-β-D-fucopyranosyl)-(1→3)-(4-O-acetyl, 2-O-angeloyl)-β-D-fucopyranoside (**5**)

$C_{34}H_{48}O_{13}$, colorless needles, mp 149°. TLC: R_f 0.20; HPLC: R_t 2.89 min. IR, ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1750 (OCOR), 1715, 1645 (C=CCO_2R); CIMS (NH_3), 100 eV, *m/z* (rel. int.): 682 $[M+NH_4]^+$ (100), 600 $[M+NH_4-C_4H_6CO]^+$ (3) 499 $[M-C_{10}H_{13}O_2]^+$ (2); EIMS, 70 eV, *m/z* (rel. int.): 499 $[M-C_{10}H_{13}O_2]^+$ (2), 299 [499-2 × $C_4H_7COOH]^+$ (2), 271 [499−228 ($C_6H_9O_4$–OCC_4H_7)]$^+$ (4), 229 [$C_6H_{10}O_4$–$OCC_4H_7]^+$ (15), 211 [271-$CH_3COOH]^+$ (4), 166 $[C_{10}H_{14}O_2]^+$ (3), 151 [166-$CH_3]^+$ (7), 129 [229-$C_4H_7COOH]^+$ (9), 111 [211-$C_4H_7COOH]^+$ (27), 83 $[C_4H_7CO]^+$ (100), 55 [83-CO]$^+$ (61), 43 $[CH_3CO]^+$ (44). ^{13}C NMR: Table 1; ^{1}H NMR: Table 2.

3.9. 2-Hydroxythymol 3-O-(3-O-angeloyl-β-D-fucopyranosyl)-(1→3)-(4-O-angeloyl)-β-D-fucopyranoside (**6**)

$C_{32}H_{46}O_{12}$, colorless gum. TLC: R_f 0.51; HPLC: R_t 3.24 min. IR, ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1710, 1645 (C=CCO_2R); CIMS (NH_3), 100 eV, *m/z* (rel. int.): 640 $[M+NH_4]^+$ (100); 457 $[M-C_{10}H_{13}O_2]^+$ (2); EIMS, 100 eV, *m/z* (rel. int.): 457 $[M-C_{10}H_{13}O_2]^+$ (5), 257 [457-2 × $C_4H_7COOH]^+$ (5), 229 [$C_6H_{10}O_4$–$OCC_4H_7]^+$ (40), 211 [229-$H_2O]^+$ (14), 166 $[C_{10}H_{14}O_2]^+$ (13), 151 [166-$CH_3]^+$ (23), 129 [229-$C_4H_7COOH]^+$ (45), 111 [211-$C_4H_7COOH]^+$ (41), 83 $[C_4H_7CO]^+$ (100), 55 [83-CO]$^+$ (68). ^{13}C NMR: Table 1; ^{1}H NMR: Table 2.

3.10. 2-Hydroxythymol 3-O-(3-O-angeloyl-β-D-fucopyranosyl)-(1→3)-(4-O-acetyl)-β-D-fucopyranoside (**7**)

$C_{29}H_{42}O_{12}$, colorless gum. TLC: R_f 0.33; HPLC: R_t 1.91 min. IR, ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1750 (shoulder, OCOR), 1720, 1645 (C=CCO_2R); CIMS (NH_3), 100 eV, *m/z* (rel. int.): 600 $[M+NH_4]^+$ (100); EIMS, 70 eV, *m/z* (rel. int.): 417 $[M-C_{10}H_{13}O_2]^+$ (7), 229 [$C_6H_{10}O_4$–$OCC_4H_7]^+$ (78), 211 [229-$H_2O]^+$ (49), 189 [417−228 ($C_6H_9O_4$–OCC_4H_7)]$^+$ (54), 171 [189-$H_2O]^+$ (16), 166 $[C_{10}H_{14}O_2]^+$ (41), 151 [166-$CH_3]^+$ (53), 129 [229-$C_4H_7COOH]^+$ (72), 111 [211-$C_4H_7COOH]^+$ (74), 83 $[C_4H_7CO]^+$ (100), 55 [83-CO]$^+$ (75), 43 $[CH_3CO]^+$ (78). ^{13}C NMR: Table 1; ^{1}H NMR: Table 2.

3.11. 2-Hydroxythymol 3-O-(3-O-angeloyl-β-D-fucopyranosyl)-(1→3)-(2,4-di-O-angeloyl)-β-D-quinovopyranoside (**8**)

$C_{37}H_{52}O_{13}$, colorless gum. TLC: R_f 0.67; HPLC: R_t 7.53 min. IR, ν_{max}^{KBr} cm^{-1}: 3460 (OH), 1725, 1650 (C=CCO_2R);

CIMS (NH_3), 100 eV, *m/z* (rel. int.): 722 $[M+NH_4]^+$ (100), 640 $[M+NH_4-C_4H_6CO]^+$ (5), 539 $[M-C_{10}H_{13}O_2]^+$ (5); EIMS, *m/z* (rel. int.): 539 $[M-C_{10}H_{13}O_2]^+$ (25), further fragmentation analogous to that of **3**. ^{13}C NMR: Table 1; ^1H NMR: Table 3.

*3.12. 2-Hydroxythymol 3-O-(4-O-acetyl-β-D-fucopyranosyl)-(1→3)-(2,4-di-O-angeloyl)-β-D-quinovopyranoside (**9**) and 2-Hydroxythymol 3-O-(3-O-acetyl-β-D-fucopyranosyl)-(1→3)-(2,4-di-O-angeloyl)-β-D-quinovopyranoside (**10**) in a mixture of 3:1*

$C_{34}H_{48}O_{13}$, colorless gum. TLC: R_f 0.12 (**9**), 0.22 (**10**); HPLC: R_t 5.50 min. IR, ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1725, 1645 ($C=CCO_2R$); CIMS (NH_3), 100 eV, *m/z* (rel. int.): 682 $[M+NH_4]^+$ (100), 640 $[M+NH_4-CH_2CO]^+$ (5), 600 $[M+NH_4\text{-}C_4H_6CO]^+$ (2), 499 $[M-C_{10}H_{13}O_2]^+$ (3); EIMS, 70 eV, *m/z* (rel. int.): 499 $[M-C_{10}H_{13}O_2]^+$ (18), 311 [499–188 $(C_6H_9O_4-OCCH_3)]^+$ (9), 293 $[311\text{-}H_2O]^+$ (13), 211 $[311\text{-}C_4H_7COOH]^+$ (8), 193 $[293\text{-}C_4H_7COOH]^+$ (30), 189 $[C_6H_{10}O_4-OCCH_3]^+$ (8), 171 $[189\text{-}H_2O]^+$ (16), 166 $[C_{10}H_{14}O_2]^+$ (6), 151 $[166\text{-}CH_3]^+$ (16), 129 $[229\text{-}C_4H_7COOH]^+$ (25), 111 $[211\text{-}C_4H_7COOH]^+$ (33), 83 $[C_4H_7CO]^+$ (100), 55 $[83\text{-}CO]^+$ (75), 43 $[CH_3CO]^+$ (52). ^{13}C NMR: Table 1; ^1H NMR: Table 3.

*3.13. 2-Hydroxythymol 3-O-(4-O-(2-methylbutyryl)-β-D-fucopyranosyl)-(1→3)-(4-O-angeloyl)-β-D-quinovopyranoside (**11**)*

$C_{32}H_{48}O_{12}$, colorless needles, mp 154°; TLC: R_f 0.26; HPLC: R_t 8.30 min. IR, ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1720, 1645 ($C=CCO_2R$); CIMS (NH_3), 100eV, *m/z* (rel. int.): 642 $[M+NH_4]^+$ (100), 560 $[M+NH_4-C_4H_6CO]^+$ (1), 459 $[M-C_{10}H_{13}O_2]^+$ (2); EIMS, 70 eV, *m/z* (rel. int.): 459 $[M-C_{10}H_{13}O_2]^+$ (79), 257 $[459\text{-}C_4H_9COOH-C_4H_7COOH]^+$ (43), 231 $[C_6H_{10}O_4-OCC_4H_9]^+$ (69), 229 $[C_6H_{10}O_4-OCC_4H_7]^+$ (43), 213 $[231\text{-}H_2O]^+$ (32), 211 $[229\text{-}H_2O]^+$ (24), 166 $[C_{10}H_{14}O_2]^+$ (36), 151 $[166\text{-}CH_3]^+$ (48), 129 $[229\text{-}C_4H_7COOH,\ 231\text{-}C_4H_9COOH]^+$ (56), 111 $[211\text{-}C_4H_7COOH,\ 213\text{-}C_4H_9COOH]^+$ (78), 85 $[C_4H_9CO]^+$ (90), 83 $[C_4H_7CO]^+$ (100), 57 $[85\text{-}CO]^+$ (94), 55 $[83\text{-}CO]^+$ (93). ^{13}C NMR: Table 1; ^1H NMR: Table 3.

*3.14. 2-Hydroxythymol 3-O-(4-O-isovaleryl-β-D-fucopyranosyl)-(1→3)-(4-O-angeloyl)-β-D-quinovopyranoside (**12**)*

$C_{32}H_{48}O_{12}$, colorless gum; TLC: R_f 0.21; HPLC: R_t 8.71 min. IR, ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1725, 1650 ($C=CCO_2R$); CIMS (NH_3), 100 eV, *m/z* (rel. int.): 642 $[M+NH_4]^+$ (100), 560 $[M+NH_4-C_4H_6CO]^+$ (1), 459 $[M-C_{10}H_{13}O_2]^+$ (2); EIMS, 70 eV, *m/z* (rel. int.): 459 $[M-C_{10}H_{13}O_2]^+$ (39), further fragmentation analogous to that of **11**. ^{13}C NMR: Table 1; ^1H NMR: Table 3.

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