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Flavonoid glucosides and dicaffeoylquinic acids from flowerheads of *Buphthalmum salicifolium*[1]

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Abstract

From flowerheads of *Buphthalmum salicifolium* the new acylated flavonol glycosides quercetagetin 7-*O*-(6″-*O*-isobutyryl)-β-glucoside, quercetagetin 7-*O*-(6″-*O*-isovaleryl)-β-glucoside, quercetagetin 7-*O*-(6″-*O*-2-methylbutyryl)-β-glucoside, patuletin 7-*O*-(6″-*O*-isobutyryl)-β-glucoside and the new acylated caffeoylquinic acid derivative, 1-isobutyryl-3,5-dicaffeoylquinic acid were isolated, as well as ten common flavonoid glycosides together with three sesquiterpenes, 10-acetoxy-8,9-epoxythymolisobutyrate and *S*-(+)-dehydrovomifoliol. All structures were mainly established on the basis of UV-, MS- and NMR (^{1}H, ^{13}C, ^{1}H–^{1}H COSY, HMQC and HMBC) spectroscopic data.

Keywords: *Buphthalmum salicifolium*; Asteraceae; Flavonoid glucosides; Acylated flavonols; Dicaffeoylquinic acids

1. Introduction

In some regions of Austria ethanolic extracts from flowerheads of the Asteraceae *Buphthalmum salicifolium* L. are sometimes declared as Arnica tincture and used against inflammation, sprain and contusion. However, studies on the chemistry of this plant are scanty with only two reports on its polyacetylenic constituents (Bohlmann & Berger, 1965; Bohlmann & Zdero, 1971). In this paper we report on the isolation and identification of a new and two known caffeoylquinic acid derivatives, of 14 flavonoid glycosides as well as of five lipophilic constituents. The four acylated flavonol glycosides are described for the first time.

2. Results and discussion

The dichloromethane extract afforded the sesquiterpenes 1,9-*trans*-caryophyllenoxide, 1β,6α-dihydroxyeudesmen and (−)-10β-hydroxyoplopan-4-on, as well as 10-acetoxy-8,9-epoxythymolisobutyrate and *S*-(+)-dehydrovomifoliol. The structures of these known compounds were established by co-chromatography with authentic samples and comparison of their spectroscopic data with those reported in the literature (Kato et al., 1977; Willuhn, Junior, & Wendisch, 1986; Ohmoto, Ikeda, Nomura, Shimizu, & Saito, 1987; Piers & Gavai, 1990; Nishiya, Kimura, Takeya, & Itokawa, 1992).

From the methanolic extract eleven known flavonoids were isolated. Comparative TLC, UV, DCI-MS, ^{1}H NMR and partly ^{13}C NMR analysis led to the identification of eriodictyol 3′-*O*-β- and 7-*O*-β-glucoside, of the 3-*O*-β-glucosides of quercetin, kaempferol, isorhamnetin and patuletin as well as the 7-*O*-β-glucosides of luteolin, patuletin, quercetagetin and 6-hydroxy-kaempferol (Bacon et al., 1978; Ulubelen,

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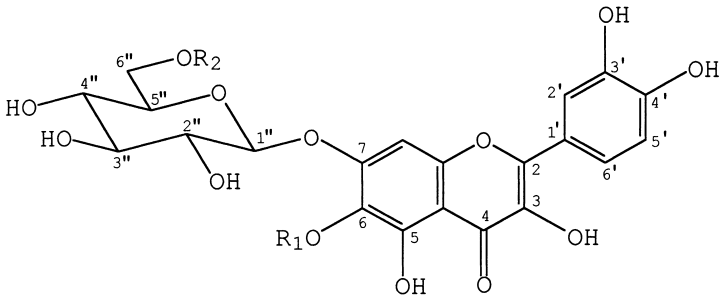
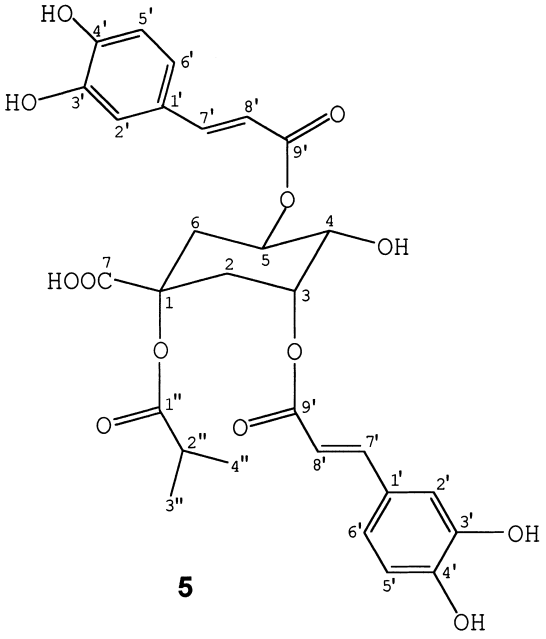


	R_1	R_2
1	H	isobutyryl
2	H	isovaleryl
3	H	2-methylbutyryl
4	CH_3	isobutyryl



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Kerr, & Mabry, 1980; Shen & Theander, 1985; Skibinski, Merfort, & Willuhn, 1994; Nair, Gunasegaran, Krishnan, Bayet, & Voirin, 1995).

In addition, the new 7-*O*-(6″-*O*-isobutyryl)-β-glucoside (**1**), 7-*O*-(6″-*O*-isovaleryl)-β-glucoside (**2**) and 7-*O*-(6″-*O*-2-methylbutyryl)-β-glucoside (**3**) of quercetagetin were isolated. These acyl-derivatives **1**–**3** were obtained as an unseparable mixture. The UV-spectra of compounds **1**–**3** in methanol and after addition of the diagnostic shift reagents provided evidence for a 3′,4′-dihydroxyflavonol with a substituted 7-OH group (Mabry, Markham, & Thomas, 1970). Acid hydrolysis on a TLC plate gave glucose (Merfort & Wendisch, 1987). The DCI mass spectra (NH_3) (negative ion mode) gave M^- peaks at *m/z* 550 and 564. The ion at *m/z* 480 was obtained after the loss of the acyl moiety. The intensive fragment ion at *m/z* 318 derived from the aglycone. In the positive ion DCI mass spectrum (NH_3) ions at *m/z* 565 and 551, due to the pseudomolecular ions $[M+H]^+$, and a fragment ion at *m/z* 319 from the protonated aglycone were observed. Additionally, ions resulting from the acylated sugar moiety at *m/z* 250 $[(C_3H_7CO\text{–hexose})\text{–}H_2O+NH_4]^+$ and 232 $[250\text{-}H_2O]^+$, respectively, at *m/z* 264 $[(C_4H_9CO\text{–hexose})\text{–}H_2O+NH_4]^+$ and 246 $[264\text{-}H_2O]^+$ were found. The loss of the acyl moiety gave ions at

Table 1
^{1}H-NMR data of compounds **1**–**3** (500/*200 MHz, CD_3OD, TMS as internal standard)

H	**1**–**3**			**4***
8	6.76 s			6.83 s
2′	7.72 d (J=1.8)			7.63 d (J=2.3)
5′	6.88 d (J=8.3)			6.88 d (J=7.5)
6′	7.58 dd (J=1.8, 8.3)			7.60 dd (J=2.3, 7.5)
1″	4.99 d (J=7.3)			5.15 d (J=7.3)
2″	3.61 m			
3″	3.61 m			3.20–3.71 m[a]
4″	3.37 m			
5″	3.77 m			
6a″	4.46 dd (J=1.7, 11.8)			4.45 dd (J=1.7, 11.8)
6b″	4.17 dd (J=7.9, 11.8)			4.20 dd (J=7.9, 11.8)
OCH_3				3.89 s
	1	**2**	**3**	**4***
2‴	2.59 sept (J=7.0)	2.15 d (J=7.1)	2.40 m (J=7.5)	2.59 sept (J=7.0)
3‴	1.03 d (J=7.0)	1.91 m (J=6.6, 6.7, 7.1)	1.40 m 1.20 m	1.03 d (J=7.0)
4‴ CH_3	0.93 d (J=7.0)	0.69 d (J=6.7)	0.63 t (J=7.0)	0.94 d (J=7.0)
5‴ CH_3		0.64 d (J=6.6)	1.02 m (J=7.5)	

[a] H-2″–5″.

m/z 197 and 180. The ^{1}H NMR spectrum of the mixture of compounds **1**–**3** was very similar to that of quercetagetin 7-*O*-β-glucoside (Nair et al., 1995) except for a downfield-shift of the H-6″ protons indicating esterification at C-6″. Moreover, the ^{1}H NMR spectrum revealed characteristic shift values and a signal pattern of an isobutyryl, an isovaleryl and a 2-methylbutyryl moiety (see Table 1) (Bohlmann, Jakupovic, Ahmed, & Schuster, 1983). The number and characteristic shifts of the ^{13}C signals indicated the presence of quercetagetin as aglycone, bound to glucose in the pyranose form (Nair et al., 1995) as well as esterification of the 6″-hydroxyl by isobutyric, isovaleric and 2-methylbutyric acid, respectively (Chari, Jordan, Wagner, & Thies, 1977; Herz & Kumar, 1981) (see Table 2). The HMBC spectrum finally confirmed acy-

Table 2
^{13}C-NMR data of compounds **1**–**3** (50 MHz, CD_3OD, TMS as internal standard)

C-atoms of the flavonoid glycoside	**1**–**3**	C-atoms of the acyl moiety	**1**	**2**	**3**
2	149.0	1‴	177.5	174.8	178.5
3	137.2	2‴	35.2	44.0	42.2
4	178.8	3‴	19.4	26.7	27.7
5	146.8	4‴	19.4	22.4	11.6
6	130.8	5‴		22.4	17.1
7	150.2				
8	95.0				
9	152.7				
10	106.5				
1′	121.8				
2′	116.3				
3′	146.3				
4′	148.9				
5′	116.5				
6′	124.0				
1″	102.0				
2″	74.6				
3″	77.2				
4″	71.9				
5″	75.7				
6″	65.1				

lation at 6″-hydroxyl. The full assignment of all signals was performed by heteronuclear 2D NMR spectroscopy (HMQC).

Compound **4** was identified as patuletin 7-*O*-(6″-*O*-isobutyryl)-β-glucoside. A pseudomolecular ion was obtained at *m/z* 565 $[M+H]^+$ by DCIMS in the positive mode. Further fragment peaks were observed at *m/z* 333 resulting from the aglycone and at 250, 232, 197 as well as 180 from the acylated sugar moiety. Therefore, it could be deduced that compound **4** differed from **2** only by its aglycone. The DCMS of the negative ions confirmed the molecular weight of 564 and the weight of the aglycone (*m/z* 332). The UV spectra in methanol and after the addition of diagnostic reagents were nearly identical to those of patuletin 7-*O*-glucoside (Ulubelen et al., 1980), but the TLC behaviour was different. The ^{1}H NMR spectrum was also similar to that of patuletin 7-*O*-glucoside except for the signals of the sugar moiety (Ulubelen et al., 1980). The chemical shifts for the sugar protons agreed well with those of β-glucose esterified at its C-6-OH group by isobutyric acid as shown with compound **1**.

Furthermore, besides the well known 5-caffeoylquinic acid (chlorogenic acid) and 1,5-dicaffeoylquinic acid the novel 1-isobutyryl-3,5-dicaffeoylquinic acid (**5**) was isolated. The UV-spectra of compound **5** in MeOH and after addition of $AlCl_3$ and $AlCl_3$/HCl indicated the presence of a caffeic acid derivative. The DCIMS (NH_3, positive ion mode) exhibited a M^+ peak at *m/z* 586. Sequential loss of the isobutyryl and the caffeoyl moieties led to fragment ions at *m/z* 516, 354 and 192 (quinic acid). Intensive fragment ions also appeared at *m/z* 424 and 262 corresponding to the sucessive loss of two caffeoyl moieties. In the negative ion DCI (NH_3) mass spectrum no molecular ion, but sequential loss of isobutyric acid and a caffeoyl moiety was observed due to signals at *m/z* at 498 and 336. Ions at *m/z* at 424 and 406 arose by fragmentation of a caffeoyl moiety and H_2O.

The ^{1}H NMR spectrum exhibited signals for two caffeoyl moieties, one isobutyryl moiety and a quinic acid (see Section 3). The proton shifts of H-3 ($\delta = 5.42$) and H-5 ($\delta = 5.45$) of the quinic acid moiety were closely related to a 3,5 diesterified derivative (Timmermann et al., 1983; Merfort, 1992; Agata, Goto, Hatano, Nishibe, & Okuda, 1993). All 29 carbons of the ^{13}C NMR spectrum were fully assigned by a HMQC experiment (see Section 3). The carbon signals of quinic acid agreed to those reported for 3,5-dicaffeoylquinic acid, except for combine that from C-1 (Timmermann et al., 1983). The downfield shift of 6.8 ppm to $\delta = 81.3$ ppm indicated that also the hydroxy group at C-1 must be esterified. The HMBC experiment showed that the hydroxy groups at C-3 and C-5 were esterified by caffeic acid, because of the cross peaks arising from the carbonyls of the caffeic acids ($\delta = 168.7$, 168.8) and H-3, resp. H-5. Therefore, the third acyl moiety isobutyric acid must be bound to the 1-OH.

Our phytochemical study shows that the flavonoid pattern found in flowerheads from *B. salicifolium* is quite different from that in flowers of *Arnica montana* (Merfort, Willuhn, & Jerga, 1990). In *A. montana* flavones and flavonols, mainly methoxylated at C-6, are found, whereas flavonols are the dominating flavonoids in *B. salicifolium*. The only detected flavone is the common luteolin 7-*O*-glucoside. With the exception of the new flavonol glycosides **1**–**4** and 6-hydroxy-kaempferol-7-glucoside all other isolated flavonoids occur often in nature (Harborne & Williams, 1988). The above mentioned kaempferol derivative has been isolated in species from the genus *Tetragonotheca* (Bacon et al., 1978), in *Neurolaena lobata* (Kerr, Mabry, & Yoser, 1981) and in *Eupatorium glandulosum* (Nair et al., 1995) up to now.

All acylated flavonol glucosides are here described for the first time. It should be noted that, to the best of our knowledge, this is the first report on flavonoid glycosides acylated with isobutyric and isovaleric acid. Moreover, no other report on caffeoylquinic acid derivatives additionally acylated with an aliphatic acid exists.

3. Experimental

3.1. General

UV spectra were recorded on a Beckmann DB-G spectrophotometer at room temperature. MS spectra (direct inlet) were taken on an Incos 50 (Finnigan MAT) with NH_3 as reagent gas. NMR spectra were recorded at 500 MHz (^{1}H) and 125 MHz (^{13}C) on a Bruker DRX 500, at 300 MHz (^{1}H) and 75 MHz (^{13}C) on a Varian VXR 300 and at 200 MHz (^{1}H) and 50 MHz (^{13}C) on a Bruker AC 200, with TMS as int. standard. Chemical shifts are quoted in δ.

3.2. Plant material

Buphthalmum salicifolium L. was cultivated at the botanical garden of the Heinrich-Heine-University, Düsseldorf and collected from August to September in 1992. A voucher specimen is deposited at the Institute of Pharmaceutical Biology, Albert-Ludwigs-Universität, Freiburg (Germany).

3.3. Extraction and isolation

Powdered, air-dried flowerheads of *B. salicifolium* (1020 g) were exhaustively extracted in a soxhlet apparatus with CH_2Cl_2 followed by MeOH. The CH_2Cl_2

extract (51 g) was separated by CC on Sephadex LH-20 with MeOH. One fraction (3.97 g) was further fractionated by CC on silica gel using increasingly polar mixtures of CH_2Cl_2, EtOAc and MeOH. The fraction (0.25 g) obtained by elution with CH_2Cl_2 and CH_2Cl_2–EtOAc (9:1) was subjected to prep. TLC with *n*-pentane–Et_2O (32:68) yielding 1,9-*trans*-caryophyllenoxide (4 mg) and 10-acetoxy-8,9-epoxy-thymolisobutyrate (3 mg). Separation of the CH_2Cl_2–EtOAc (8:2, 0.03 g) and of the CH_2Cl_2–EtOAc fraction (7:3, 0.36 g) was performed by MPLC on Lichroprep RP-18 and a MeOH–water gradient affording 1β,6α-dihydroxyeudesmen (2 mg), (−)-10β-hydroxyoplopan-4-on (5 mg) and *S*-(+)-dehydrovomifoliol (2 mg).

49.5 g of the methanolic extract (total 208 g) were subjected to CC on Sephadex LH-20 using MeOH yielding 12 fractions. Fraction 3 (0,27 g) was fractionated by CC on cellulose with mixtures of petrol–EtOAc (3:7 and 2:8, saturated with H_2O). Subfraction 2 was separated on Sep-Pak® RP_{18} cartridges using a MeOH–H_2O gradient. Further purification was achieved by subsequent CC on Polyclar with CH_2Cl_2–MeOH–EtOAc–acetone (20:10:5:1) and Sephadex LH-20 with cyclohexane–CH_2Cl_2–MeOH (7:4:3) and CH_2Cl_2–MeOH (4:2). Thus, compound **4** (1.5 mg) was obtained. Performance of MPLC with subfractions 3, 4 and 6 on Sep-Pak® RP18 cartridges using a MeOH–H_2O gradient afforded kaempferol 3-*O*-β-glucoside (9 mg), isorhamnetin 3-*O*-β-glucoside (12 mg) and patuletin 3-*O*-β-glucoside (7 mg). Separation of fraction 4 by CC on cellulose with mixtures of petrol–EtOAc (3:7 and 2:8, saturated with H_2O) followed by MPLC on Sep-Pak® RP_{18} cartridges as described above yielded eriodictyol 3′-*O*-β-glucoside (1 mg) and eriodictyol 7-*O*-β-glucoside (8 mg). Fraction 5 was chromatographed on cellulose in the same manner as mentioned for fraction 4. MPLC on Sep-Pak® RP_{18} cartridges was done with the respective subfractions 4 and 9 using a MeOH–H_2O gradient and the mixture of compounds **1**–**3** (31 mg) as well as patuletin 7-*O*-β-glucoside (61 mg) were obtained. CC on Polyclar with CH_2Cl_2–MeOH–EtOAc–acetone (20:10:5:1) of subfraction 5 yielded quercetin 3-*O*-β-glucoside (7 mg). Luteolin 7-*O*-β-glucoside (2 mg) was isolated from subfraction 11 by CC on Sephadex LH-20 with MeOH. Fraction 7 afforded compound **5** (131 mg) by CC on cellulose (conditions see fraction 4) and on Sephadex LH-20. 6-Hydroxykaempferol 7-*O*-β-glucoside (2 mg) was isolated from fraction 8 (64 mg) by CC on Sephadex LH-20 with MeOH followed by MPLC on Sep-Pak® RP_{18} cartridges (see above). Quercetagetin 7-*O*-β-glucoside was obtained from fraction 9 (0.97 g) by CC on Sephadex LH-20 with MeOH (80%). Detection of chlorogenic acid and 1,5-dicaffeoylquinic acid followed from subfraction 2 by HPLC and TLC analysis.

*3.4. Quercetagetin 7-O-(6″-O-isobutyryl)-β-glucoside, quercetagetin 7-O-(6″-O-isovaleryl)-β-glucoside, quercetagetin 7-O-(6″-O-2-methylbutyryl)-β-glucoside (**1**–**3**)*

Yellow amorphous powder. DCIMS (NH_3): positive ions *m/z* (rel. int.): 565 $[M$ (**2** and **3**)$+H]^+$ (1), 551 $[M$ (**1**)$+H]^+$ (5), 336 $[\text{aglycone}+NH_4]^+$ (7), 319 $[\text{aglycone}+H]^+$ (62), 264 $[(C_4H_9CO\text{–hexose})–H_2O+NH_4]^+$ (24), 250 $[(C_3H_7CO\text{–hexose})–H_2O+NH_4]^+$ (100), 246 $[264\text{-}H_2O]^+$ (16), 232 $[250\text{-}H_2O]^+$ (49), 197 $[\text{hexose}–H+NH_4]^+$ (14), 180 $[\text{hexose}–H_2O+NH_4]^+$ (61), 162 $[180\text{-}H_2O]^+$ (23). DCIMS (NH_3): neg. ions *m/z* (rel. int.): 564 $[M$ (**2** and **3**)$]^-$ (14), 550 $[M$ (**1**)$]^-$ (42), 480 [M-acyl]$^-$ (7), 318 [aglycone]$^-$ (100). UV λ_{max}^{MeOH} (nm) 357, 270 sh, 257; +NaOMe 372 dec, 350 sh, 295, 245; +$AlCl_3$ 452, 282; +$AlCl_3$–HCl 422, 395, 272; +NaOAc 365 dec, 285 sh, 257; +NaOAc–H_3BO_3 369, 276 sh, 266. ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

*3.5. Patuletin 7-O-(6″-O-isobutyryl)-β-glucoside (**4**)*

Yellow amorphous powder. DCIMS (NH_3): positive ions *m/z* (rel. int.): 565 $[M+H]^+$ (6), 333 $[\text{aglycone}+H]^+$ (75), 264 $[(C_4H_9CO\text{–hexose})–H_2O+NH_4]^+$ (52), 250 $[(C_3H_7CO\text{–hexose})–H_2O+NH_4]^+$ (84), 246 $[264\text{-}H_2O]^+$ (27), 232 $[250\text{-}H_2O]^+$ (69), 197 $[\text{hexose}–H+NH_4]^+$ (26), 180 $[\text{hexose}–H_2O+NH_4]^+$ (84), 162 $[180\text{-}H_2O]^+$ (81), 108 (100). *m/z* (rel. int.): DCIMS: neg. ions *m/z* (rel. int.): 564 $[M]^-$ (36), 332 [aglycone]$^-$ (100). UV λ_{max}^{MeOH} nm 368, 259; +NaOMe 435 dec, 274, 250 sh; +$AlCl_3$ 454, 350 sh, 315 sh, 274; +$AlCl_3$–HCl 424, 375 sh, 325 sh, 270. ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

*3.6. 1-Isobutyryl-3,5-dicaffeoylquinic acid (**5**)*

Light-yellow, amorphous powder. DCIMS (NH_3, positive ion mode) *m/z* (rel. int.): 586 $[M]^+$ (1), 568 $[M–H_2O]^+$ (<1), 516 $[M–C_3H_7CO+H]^+$ (2), 424 [M–caffeoyl+H]$^+$ (11), 354 [516-caffeoyl+H]$^+$ (16), 262 [M-2 caffeoyl+2 H]$^+$ (64), 198 (69), 192 [354-caffeoyl+H]$^+$ (84), 182 (52), 163 [caffeoyl]$^+$ (100). DCIMS (NH_3, neg. ion mode) *m/z* (rel. int.): $[M]^-$ absent, 498 $[M–C_3H_7COOH]^-$ (7), 424 [M-caffeoyl+H]$^-$ (3), 406 $[424\text{-}H_2O]^-$ (30), 336 [498-caffeoyl+H]$^-$ (67), 179 [caffeic acid–H]$^-$ (100), 163 [caffeoyl]$^-$ (35). UV λ_{max}^{MeOH} nm 328, 300 sh, 242; +NaOMe 373, 310 sh, 261; +$AlCl_3$ 362, 310 sh, 262; +$AlCl_3$–HCl 330, 310 sh, 242, +NaOAc 375 sh, 332, 298; +NaOAc+H_3BO_3 351, 300 sh, 252. ^{1}H-NMR (500 MHz, CD_3OD): two caffeoyl moities: 7.62 and

7.61 (1H, d, $J=15.8$, H-7′), 7.07 and 7.06 (1H, d, $J=1.9$, H-2′), 6.97 and 6.96 (1H, dd, $J=1.9$ and 8.2, H-6′), 6.80 and 6.79 (1H, d, $J=8.2$, H-5′), 6.33 and 6.31 (1H, d, $J=15.8$, H-8′); quinic acid: 5.45 (1H, ddd, $J=3.8$, 9.5, 10.7, H-5), 5.42 (1H, m, $J \approx 3.0$, 3.2, 3.8, H-3), 3.97 (1H, dd, $J=3.8$, 9.5, H-4), 2.76 (1H, dd, $J= \approx 3.0$, 15.8, H-2β), 2.64 (1H, dd, $J=3.8$, 13.3, H-6α), 2.35 (1H, dd, $J=3.2$, 15.8, H-2α), 2.02 (1H, dd, $J=10.7$, 13.3, H-6β); isobutyric acid: 2.56 (1H, sept, $J=6.9$, H-2″), 1.27 and 1.07 (3H each, d, $J=6.9$, H-3″ and 4″). ^{13}C-NMR (125 MHz, CD_3OD): 177.5 (C-1″), 174.5 (C-7) 168.8 and 168.7 (2 × C-9′), 149.7 and 149.7 (2 × C-4′), 147.3 and 147.3 (2 × C-7′), 146.9 and 146.8 (2 × C-3′), 127.8 and 127.7 (2 × C-1′), 123.1 and 123.1 (2 × C-6′), 116.6 and 116.5 (2 × C-5′), 115.3[3] and 115.3 (2 × C-8′), 115.1[3] and 115.1 (2 × C-2′), 81.3 (C-1), 73.3 (C-3), 72.1 (C-4), 71.2 (C-5), 37.3 (C-6), 35.3 (C-2″), 34.0 (C-2), 19.5 and 18.8 (C-3″ and C-4″).

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[3] Assignment may be interchangeable.