Three New Paeonidanin-Type Monoterpene Glycosides from *Paeonia suffruticosa* ANDR.

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Three new paeonidanin-type monoterpene glycosides, named suffrupaeonidanins A–C, were isolated as minor components from the root cortex of *Paeonia suffruticosa* ANDR. Their structures were elucidated by a combination of 1D- and 2D-NMR, and mass spectral techniques.

Introduction. – *Paeonia*, the only genus in the Paeoniaceae family, has *ca.* 35 species distributed in the warm temperate regions of the Eurasia mainland and America [1]. The root cortex of *Paeonia suffruticosa* ANDR. is a famous traditional Chinese medicine known for its immunoregulatory [2], anticoagulant, anti-inflammatory, analgesic, and sedative activities, and its efficacy in the treatment of cardiovascular diseases and dysmenorrhea [3]. Previous phytochemical investigations of *P. suffruticosa* resulted in the isolation of phenolic constituents [4], monoterpenes [5], flavones [6], triterpenes [7], and resveratrol trimers [8]. The plants of *Paeonia* species are rich in biologically active monoterpene compounds possessing a unique 'cage-like' pinane skeleton. To the best of our knowledge, *ca.* 22 monoterpene glycosides were isolated from *P. suffruticosa*, for example, paeoniflorin, oxypaeoniflorin, and benzoylpaeoniflorin [9]. Paeonidanins A and B, whose aglycon structure is different from the common monoterpenes in this genus, were isolated from *Paeonia albiflora* and showed strong inhibitory activity on nitric oxide production in lipopolysaccharide-activated N9 microglia [10]. With the aim to isolate structurally interesting and bioactive monoterpene glycosides, we investigated the alcohol extract of *P. suffruticosa*. This led to the isolation of three new paeonidanin-type glycosides, suffrupaeonidanins A–C[1]) (**1**–**3**, resp.; see *Fig. 1*). Herein, we describe the isolation and structure elucidation of these new paeonidanin-type glycosides.

Results and Discussion. – Compound **1** was obtained as an optically active white powder. The molecular formula $C_{31}H_{34}O_{13}$ was established on the basis of HR-ESI-MS (m/z 637.1898 ($[M+Na]^+$)), which implies 15 degrees of unsaturation in the molecule. The IR absorptions at 3413 and 1713 cm^{-1} revealed the presence of OH and C=O groups. The ^{1}H-NMR spectrum of **1** (*Table 1*) showed nine aromatic H-atom signals, indicating the presence of two aryl groups. A set of five H-atom signals (δ(H) 8.02 (*d*, $J = 7.8$ Hz, 2 H), 7.52 (*t*, $J = 7.6$ Hz, 2 H), and 7.65 (*t*, $J = 7.6$ Hz, H)) is assignable to a Bz

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

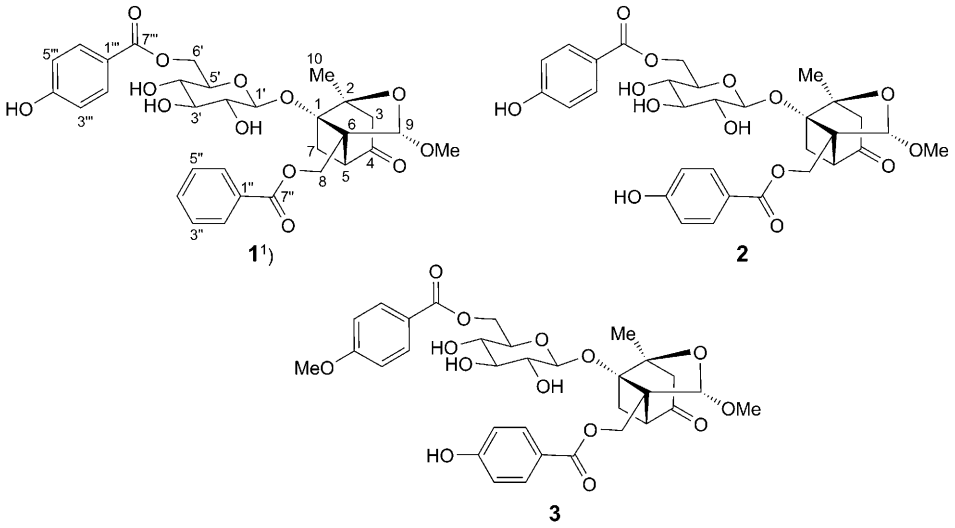

Fig. 1. *Suffrupaeonidanins A – C* (**1** – **3**), *isolated from* Paeonia suffruticosa ANDR.

Table. *^{1}H-NMR Data of Compounds* **1** – **3**. δ in ppm, J in Hz.

	1[a])[c])	**2**[b])[c])	**3**[a])[c])
H_a–C(3)	2.34 (*d*, *J* = 18.2)	2.37 (*d*, *J* = 17.6)	2.33 (*d*, *J* = 17.6)
H_b–C(3)	2.58 (*d*, *J* = 18.2)	2.53 (*d*, *J* = 18.0)	2.51 (*d*, *J* = 18.0)
H–C(5)	2.86 (*d*, *J* = 7.6)	2.86 (*d*, *J* = 7.2)	2.82 (*d*, *J* = 7.6)
H_a–C(7)	2.00 (*d*, *J* = 11.2)	1.86 (*d*, *J* = 10.4)	1.95 (*d*, *J* = 11.2)
H_b–C(7)	3.01 (*dd*, *J* = 11.2, 7.6)	2.86 (*dd*, *J* = 10.4, 7.2)	2.97 (*dd*, *J* = 11.2, 7.4)
H_a–C(8)	4.70 (*d*, *J* = 12.0)	4.60 (*d*, *J* = 12.0)	4.63 (*d*, *J* = 11.6)
H_b–C(8)	4.75 (*d*, *J* = 12.0)	4.71 (*d*, *J* = 12.0)	4.69 (*d*, *J* = 11.6)
H–C(9)	5.05 (*s*)	5.01 (*s*)	5.01 (*s*)
Me(10)	1.34 (*s*)	1.28 (*s*)	1.32 (*s*)
H–C(1′)	4.73 (*d*, *J* = 8.0)	4.58 (*d*, *J* = 7.6)	4.71 (*d*, *J* = 7.6)
H–C(2′)	3.31 – 3.35 (*m*)	3.26 – 3.29 (*m*)	3.30 – 3.34 (*m*)
H–C(3′)	3.45 – 3.50 (*m*)	3.38 – 3.42 (*m*)	3.45 – 3.48 (*m*)
H–C(4′)	3.39 – 3.45 (*m*)	3.30 – 3.34 (*m*)	3.38 – 3.43 (*m*)
H–C(5′)	3.66 – 3.70 (*m*)	3.56 – 3.59 (*m*)	3.50 – 3.56 (*m*)
H_a–C(6′)	4.43 (*dd*, *J* = 11.6, 7.2)	4.04 (*dd*, *J* = 11.2, 7.2)	4.45 (*dd*, *J* = 11.2, 7.2)
H_b–C(6′)	4.62 (*dd*, *J* = 11.6, 2)	4.54 (*m*)	4.67 (*m*)
H–C(2″,6″)	8.02 (*d*, *J* = 7.8)	7.83 (*d*, *J* = 9.2)	7.99 (*d*, *J* = 9.2)
H–C(3″,5″)	7.52 (*t*, *J* = 7.6)	6.78 (*d*, *J* = 9.2)	6.91 (*d*, *J* = 9.2)
H–C(4″)	7.65 (*t*, *J* = 7.6)		
H–C(2‴,6‴)	7.91 (*d*, *J* = 8.6)	7.84 (*d*, *J* = 8.9)	7.89 (*d*, *J* = 8.8)
H–C(3‴,5‴)	6.89 (*d*, *J* = 8.6)	6.75 (*d*, *J* = 8.9)	7.00 (*d*, *J* = 8.8)
MeO–C(9)	3.25 (*s*)	3.24 (*s*)	3.24 (*s*)
MeO–C(4‴)			3.88 (*s*)

[a]) In (D_6)acetone. [b]) in CD_3OD. [c]) Measured at 400 Hz.

rest, and the other four aromatic H-atoms (δ(H) 7.91 (*d*, *J* = 8.6 Hz, 2 H) and 6.89 (*d*, *J* = 8.6 Hz, 2 H)) belong apparently to a 4-hydroxybenzoyl group. An anomeric resonance at δ(H) 4.73 (*d*, *J* = 8.0 Hz, H–C(1′)) and the six H-atom signals in the area of δ(H) 4.62 ~ 3.31 suggested the presence of a β-glucopyranosyl moiety. The occurrence of Bz and Glc groups is a typical feature of the monoterpene glycosides from the genus *Paeonia*. Besides the C-atoms of the above three units, there were other eleven C-atom signals in the ^{13}C-NMR spectrum of **1** ascribable to the monoterpene aglycone, *i.e.*, signals for a ketonic C=O group at δ(C) 204.6 (C(4)), two CH_2 at δ(C) 49.2 (C(3)) and 27.1 (C(7)), an O–CH_2 at δ(C) 62.9 (C(8)), a tertiary Me at δ(C) 20.8 (C(10)), a CH linked to the C=O group at δ(C) 47.7 (C(5)), an O–CH at δ(C) 107.0 (C(9)), a MeO at δ(C) 55.5, and three quaternary C-atoms at δ(C) 88.2 (C(1)), 86.8 (C(2)), and 63.8 (C(6)), assigned by 1D-NMR and HMQC experiments. The ^{1}H,^{1}H-COSY plot of **1** showed the signals of a CH_2CH fragment at δ(H) 2.00 and 3.01 (CH_2(7)) and 2.86 (H–C(5)). This CH_2CH fragment and another CH_2 at δ(H) 2.34 and 2.58 (CH_2(3)) were linked to the ketonic C=O at δ(C) 204.6 (C(4)) as indicated by the HMBCs of CH_2(7), H–C(5), and CH_2(3) with C(4). Thus, the aglycone of **1** appeared to contain a $CH_2C(=O)CHCH_2$ fragment. Careful analysis of the NMR spectra revealed that the structure of **1** is similar to that of paeonidanin [11], except for one more 4-hydroxybenzoyl moiety in **1**. A notable HMBC (see *Fig. 2*) of H_a–C(6′) at δ(H) 4.43 (*dd*, *J* = 11.6, 7.2 Hz) with the C=O C-atom of this 4-hydroxybenzoyl group at δ(C) 166.3 (C(7‴)) indicated that the 4-hydroxybenzoyl group is located at C(6′) of the Glc unit. The location of the β-glucopyranosyloxy moiety at C(1) was established unambiguously by the HMBC cross-peak between H–C(1′) and C(1). Furthermore, the HMBCs of H–C(2″,6″) and H–C(8) with C(7″) (δ(C) 166.8) showed that the BzO was connected to C(8) of the monoterpene.

Paeonidanin-type compounds and the C(9) epimer of paeonidanin, 9-epi-paeonidanin, were found to have distinctly different optical rotations (– 34° to – 42.6° *vs.* – 98°) [10][12]. The optical rotation of **1** is close to that of paeonidanin-type compounds, suggesting that compound **1** may have the same configuration at C(9) as paeonidanin. In an NOE-enhancement experiment of compound **1**, intensive NOEs were observed in the signals of Me(10), H–C(9), H–C(3), and H–C(5) on irradiation of the MeO–C(9) signal at δ(H) 3.25, indicating an *endo*-side orientation of the MeO–C(9) group. Since compound **1** can be considered as derived from paeoniflorin, the other chiral centers of **1** are likely to have the same absolute configuration as paeoniflorin. Based on the above spectral analyses, compound **1** was elucidated as {(1*R*,3*R*,6*S*,8*S*,9*S*)-1-{[6-*O*-(4-hydroxybenzoyl)-β-D-glucopyranosyl]oxy}-8-methoxy-6-methyl-4-oxo-7-oxatricyclo[4.3.0.0^{3,9}]non-9-yl}methyl benzoate, named suffrupaeonidanin A (see *Fig. 1*).

Compound **2** had the molecular formula $C_{31}H_{34}O_{14}$, as determined by HR-ESI-MS (*m*/*z* 653.1848 ([*M* + Na]$^+$)). Comparison of the spectroscopic data of **2** and **1** revealed similarities, except that the signals of the benzoyl moiety of **1** were replaced by the signals of a 4-hydroxybenzoyl group in **2**. In the ^{1}H-NMR spectrum (*Table 1*) of **2**, signals for a second 4-hydroxybenzoyl group are seen at δ(H) 7.83 (*d*, *J* = 9.2 Hz, H–C(2″,6″)) and 6.78 (*d*, *J* = 9.2 Hz, H–C(3″,5″)). The HMBCs (*Fig. 2*) of H–C(2″,6″) and H–C(8) with C(7″) at δ(C) 168.3 confirmed that this 4-hydroxybenzoyl group was connected with C(8) of the monoterpene. Thus, compound **2** was deduced to be {(1*R*,3*R*,6*S*,8*S*,9*S*)-1-{[6-*O*-(4-hydroxybenzoyl)-β-D-glucopyranosyl]

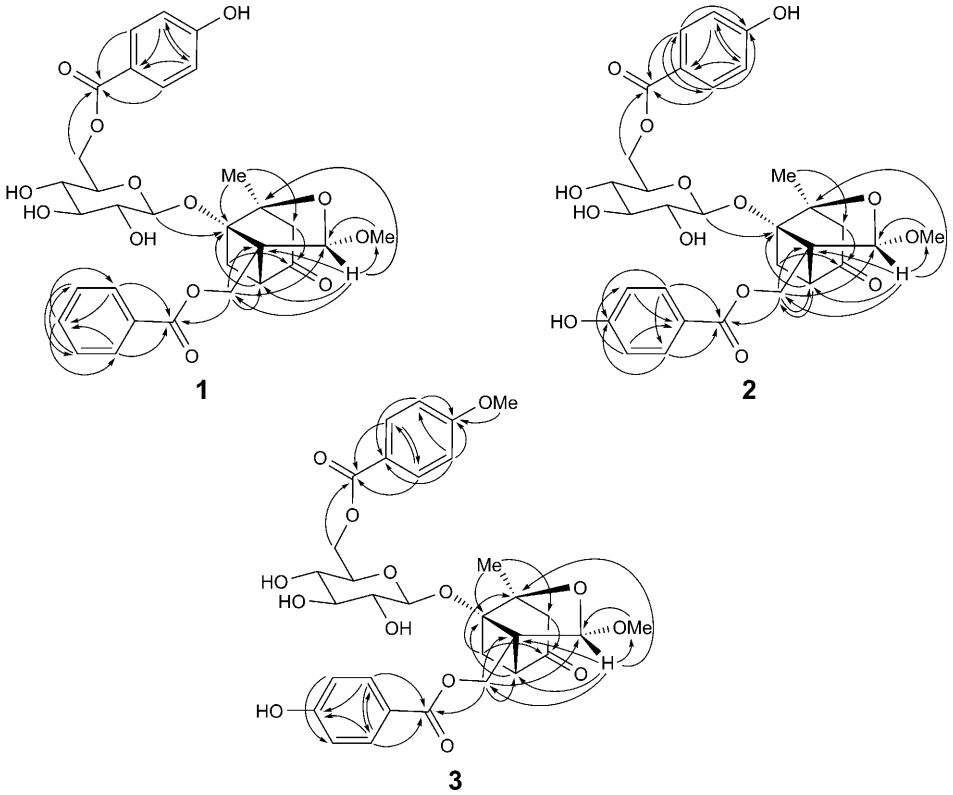

Fig. 2. *Major HMBCs* (H → C) *of compounds* **1**–**3**

oxy}-8-methoxy-6-methyl-4-oxo-7-oxatricyclo[4.3.0.0^{3,9}]non-9-yl}methyl 4-hydroxybenzoate, named suffrupaeonidanin B.

Compound **3** was isolated as a white amorphous powder, whose molecular formula was inferred as $C_{32}H_{36}O_{14}$ by HR-ESI-MS (*m*/*z* 667.2018 ([*M* + Na]$^+$)). The NMR data of **3** were very similar to that of **2**, except for an additional MeO group in **3**. The HMBC data (*Fig. 2*) revealed long-range correlations of this MeO group, H–C(3‴), and H–C(5‴) with C(4‴), indicating the presence of a 4-methoxybenzoyloxy unit. Further, the notable correlations of H–C(2‴,6‴) and H_a–C(6′) with C(7‴) at δ(C) 165.4 established the attachment of the methoxybenzoyloxy group to C(6′) of the Glc group, and consequently, of the 4-hydroxybenzoyloxy group to C(8) of the monoterpene. Based on the above spectral analyses, compound **3** was determined as {(1*R*,3*R*,6*S*,8*S*,9*S*)-8-methoxy-1-{[6-*O*-(4-methoxybenzoyl)-*β*-D-glucopyranosyl]oxy}-6-methyl-4-oxo-7-oxatricyclo[4.3.0.0^{3,9}]non-9-yl]methyl 4-hydroxybenzoate, named suffrupaeonidanin C.

The observed optical-rotation values for compounds **2** and **3** were $[\alpha]_D^{20} = -22.9$ and -27.7, respectively, similar to that of **1** ($[\alpha]_D^{20} = -16.2$). Therefore, these new compounds were assumed to have the same absolute configuration as **1**.

Both C(9) OH and MeO derivatives of paeonidanin are known in the literatures [11][13]. It can not be ruled out that the MeO groups at C(9) were artifacts formed by reaction of the corresponding hemiacetal with MeOH on account of the use of MeOH as solvent during the isolation procedure.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Inc.*, P. R. China), *Lichroprep RP-18* gel (40–63 μm; *Merck*, Germany), *Sephadex LH-20* (*GE-Healthcare Bio-Science AB*, Sweden). Semi-prep. HPLC: *Agilent-1100* system with UV detector and *RP-18* semi-prep. column (250 × 10 mm, 5 μm; *YMC Corporation*, Japan). M.p.: *X4* apparatus (*Beijing Tiandiyu Co.*); uncorrected. Optical rotation: *PE-243* spectrometer (*Perkin-Elmer*, USA). UV: *Cintro-20* spectrometer (Australia); λ_{max} (log ε) in nm. IR: *Nicolet-Manga* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *JNM-ECA-400* spectrometer at 400 (^1H) and 100 (^{13}C) MHz; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Micromass-LCT* spectrometer; in *m/z* (rel. %).

Plant Material. The root cortex of *P. suffruticosa* ANDR. was purchased in July 2007 from *Beijing Tong Ren Tang Co., Ltd*., Beijing, P. R. China, and authenticated by Mr. *Qi-Yun Ma* at the Beijing Institute of Pharmacology and Toxicology. A voucher specimen (No. 20071020) has been deposited with the Laboratory of Phytochemistry, Beijing Institute of Pharmacology and Toxicology.

Extraction and Isolation. Dry powdered root barks of *P. suffruticosa* ANDR. (5 kg) were repeatedly (3 ×) extracted with 95% EtOH (8 l) under reflux for 1 h. A dark brown mass (624 g) was obtained after evaporation of the solvent. This residue was suspended with H_2O (0.5 l) and then extracted with AcOEt (3 × 1.5 l). The AcOEt extract (334 g) was subjected to CC (SiO_2 (500 g), $CHCl_3$/MeOH 9.5 : 0.5 → 8 : 2): *Fractions A – F. Fr. D* (25 g) was subjected to CC (SiO_2 (300 g), petroleum ether/acetone 8.5 : 1.5): *Frs. D1 – D4. Fr. D2* (7.9 g) was refractionated by CC (*RP-18* gel, MeOH/H_2O 4 : 6 → 7 : 3): *Fr. D2.1 – D2.4. Fr. D2.3* (2.4 g) was repeatedly subjected to CC (*Sephadex LH-20*, MeOH) to give an enriched fraction, a part (0.12 g) of which was submitted to semi-prep. HPLC (58% MeOH/H_2O, 3 ml/min, 258 nm) to afford **1** (14 mg, t_R 25 min) and **3** (7 mg, t_R 80 min). Separation of *Fr. E* (9 g) by CC (*RP-18* gel, MeOH/H_2O 4 : 6 → 7 : 3) followed by semi-prep. HPLC (MeOH/MeCN/H_2O 4 : 1 : 11, 3 ml/min, 258 nm) afforded compound **2** (10 mg, t_R 31 min).

Suffrupaeonidanin A (=*{(1*R,*3*R,*6S,8S,9S*)*-1-{[6-*O*-*(4-Hydroxybenzoyl)-β-*D*-glucopyranosyl]oxy}-8-methoxy-6-methyl-4-oxo-7-oxatricyclo[4.3.0.0^{3,9}]non-9-yl}methyl Benzoate*; **1**): White powder. M.p. 133–135°. $[\alpha]_D^{20} = -16.2$ (*c* = 0.45, MeOH). UV (MeOH): 202 (3.73), 231 (3.68), 257 (3.70). IR (KBr): 3413, 2946, 1713, 1560, 1270. ^{1}H-NMR: *Table.* ^{13}C-NMR ((D$_6$)acetone, 100 MHz): 88.2 (C(1)); 86.8 (C(2)); 49.2 (C(3)); 204.6 (C(4)); 47.7 (C(5)); 63.8 (C(6)); 27.1 (C(7)); 62.9 (C(8)); 107.0 (C(9)); 20.8 (C(10)); 99.5 (C(1′)); 74.7 (C(2′)); 77.8 (C(3′)); 71.5 (C(4′)); 74.8 (C(5′)); 64.9 (C(6′)); 131.1 (C(1″)); 130.1 (C(2″,6″)); 129.4 (C(3″,5″)); 134.0 (C(4″)); 166.8 (C(7″)); 122.7 (C(1‴)); 132.6 (C(2‴,6‴)); 116.1 (C(3‴,5‴)); 162.9 (C(4‴)); 166.3 (C(7‴)); 55.5 (*Me*O–C(9)). HR-ESI-MS: 637.1898 ([*M* + Na]$^+$, $C_{31}H_{34}NaO_{13}^+$; calc. 637.1897).

Suffrupaeonidanin B (=*{(1*R,*3*R,*6S,8S,9S*)*-1-{[6-*O*-*(4-Hydroxybenzoyl)-β-*D*-glucopyranosyl]oxy}-8-methoxy-6-methyl-4-oxo-7-oxatricyclo[4.3.0.0^{3,9}]non-9-yl}methyl 4-Hydroxybenzoate*; **2**): White powder. M.p. 160–162°. $[\alpha]_D^{20} = -22.9$ (*c* = 0.38, MeOH). UV (MeOH): 204 (4.44), 257 (4.37). IR (KBr): 3419, 2986, 1723, 1569, 1278. ^{1}H-NMR: *Table.* ^{13}C-NMR (CD$_3$OD, 100 MHz): 87.4 (C(1)); 86.0 (C(2)); 48.3 (C(3)); 203.8 (C(4)); 46.3 (C(5)); 65.1 (C(6)); 26.3 (C(7)); 62.0 (C(8)); 106.2 (C(9)); 20.0 (C(10)); 98.7 (C(1′)); 74.0 (C(2′)); 77.1 (C(3′)); 70.9 (C(4′)); 74.1 (C(5′)); 63.8 (C(6′)); 121.8 (C(1″)); 133.4 (C(2″,6″)); 117.1 (C(3″,5″)); 165.4 (C(4″)); 168.3 (C(7″)); 121.5 (C(1‴)); 133.3 (C(2‴,6‴)); 117.1 (C(3‴,5‴)); 165.8 (C(4‴)); 168.5 (C(7‴)); 54.7 (*Me*O–C(9)). HR-ESI-MS: 653.1848 ([*M* + Na]$^+$, $C_{31}H_{34}NaO_{14}^+$; calc. 653.1846).

Suffrupaeonidanin C (= *[(1*R,*3*R,*6*S,*8*S,*9*S*)*-8-Methoxy-1-{[6-*O*-*(4-methoxybenzoyl)-β-*D*-glucopyranosyl]oxy}-6-methyl-4-oxo-7-oxatricyclo[4.3.0.0*^{3,9}*]non-9-yl]methyl 4-Hydroxybenzoate*; **3**). White powder. M.p. 140–143°. $[\alpha]_D^{20} = -27.7$ ($c = 0.26$, MeOH). UV (MeOH): 205 (3.15), 257 (3.04). IR (KBr): 3403, 2912, 1702, 1565, 1273. ^{1}H-NMR: *Table.* ^{13}C-NMR ((D$_6$)acetone, 100 MHz): 89.0 (C(1)); 87.9 (C(2)); 49.1 (C(3)); 209.7 (C(4)); 48.9 (C(5)); 63.1 (C(6)); 27.8 (C(7)); 63.7 (C(8)); 108.0 ((C(9)); 21.1 (C(10)); 100.3 (C(1′)); 75.5 (C(2′)); 78.3 (C(3′)); 72.6 (C(4′)); 75.9 (C(5′)); 65.3 (C(6′)); 121.1 (C(1″)); 131.4 (C(2″,6″)); 115.4 (C(3″,5″)); 162.4 (C(4″)); 165.4 (C(7″)); 122.5 (C(1‴)); 131.8 (C(2‴,6‴)); 113.8 (C(3‴,5‴)); 163.2 (C(4‴)); 165.8 (C(7‴)); 55.1 (*Me*O–C(9)); 55.1 (*Me*O–C(4‴)). HR-ESI-MS: 667.2018 ($[M+Na]^+$, $C_{32}H_{36}NaO_{14}^+$; calc. 667.2003).

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