Three New Limonoids from *Melia toosendan*

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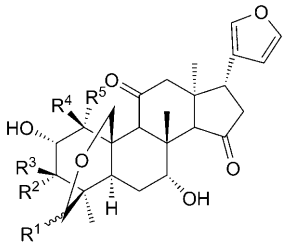
In order to find new and biologically active compounds, the constituents of the root bark of *Melia toosendan* were investigated, and three new limonoids, 29-[(2-methylbutanoyl)oxy]-2α-hydroxyamoorastatone (**1**), 1,3-*epi*-29-[(2-methylbutanoyl)oxy]-2α-hydroxyamoorastatone (**2**), and 1,3-*epi*-29-[(2-methylpropanoyl)oxy]-2α-hydroxyamoorastatone (**3**), were isolated from the root bark of *Melia toosendan*. Their structures were elucidated by means of extensive spectroscopic studies.

Introduction. – Limonoids are highly oxidized tetranortriterpenoids. Many experimental results indicate that most limonoids display substantial anticancer actions [1–3]. *Meliaceae* plants are a rich source of limonoids. A typical plant, *Melia toosendan* SIEB. ET ZUCC., the Chinaberry tree, has long been recognized as an insecticidal and medicinal plant in China. Recently, researchers pay close attention to limonoids, and more and more limonoids have been isolated from *Melia toosendan* since 1975 [4–7]. Our search for new bioactive limonoids from the bark of *Melia toosendan* collected in the Sichuan Province in China has now furnished three new limonoids, 29-[(2-methylbutanoyl)oxy]-2α-hydroxyamoorastatone (**1**), 1,3-*epi*-29-[(2-methylbutanoyl)-oxy]-2α-hydroxyamoorastatone (**2**), and 1,3-*epi*-29-[(2-methylpropanoyl)oxy]-2α-hydroxyamoorastatone (**3**). Here, we report the isolation and structural elucidation of these new compounds.



	R^1	R^2	R^3	R^4	R^5
1	EtCH(Me)COO	AcO	H	H	OH
2	EtCH(Me)COO	H	AcO	OH	H
3	Me_2CHCOO	H	AcO	OH	H

Results and Discussion. – Compound **1** was obtained as a white, amorphous powder. The optical rotation was determined to be $[\alpha]_D^{16} = -24.36$ ($c = 0.220$, MeOH), and the *Ehrlich* test showed a positive result. The molecular formula was determined as $C_{33}H_{44}O_{11}$ by HR-ESI-MS, which showed a $[M+Na]^+$ ion peak of m/z 639.2792 ($C_{33}H_{44}NaO_{11}^+$; calc. 639.2776), indicating twelve degrees of unsaturation. The IR spectrum revealed the presence of OH (3423.7 cm^{-1}), CO (1726.4 cm^{-1}), and an AcO group (1245.6, 1055.1 cm^{-1}), as well as a furan ring (874.4 cm^{-1}). The ^{1}H-NMR signals (*Table 1*) of three olefinic H-atoms at δ(H) 6.43 (*dd*, $J = 1.7, 0.8$, H–C(22)), 7.45 (br. *s*, H–C(21)), and 7.48 (*t*, $J = 1.6$, H–C(23)) indicated that the compound possessed a furan ring. These H-atom signals correlated with the C-atom signals (*Table 2*) at δ(C) 111.7, 141.9, and 144.4, respectively, and with a quaternary C-atom signal at δ(C) 123.7 in the HMQC spectrum. Additionally, the correlations of H–C(21) with C(20), and H–C(22) with C(20) and C(23) in the HMBC spectrum (*Fig. 1*) supported the conclusion of the presence of a furan ring. Furthermore, the correlation in the HMBC of H–C(17) with C(20) and C(22) confirmed that the furan ring is linked to C(17).

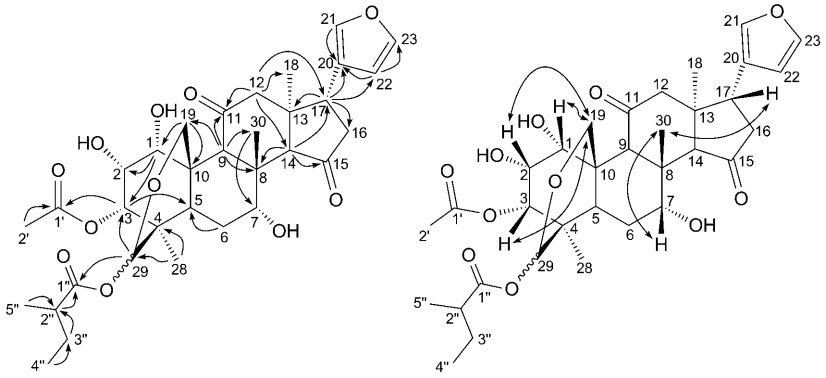


Fig. 1. *Key HMBC* (H → C) *and key ROESY* (H ↔ H) *correlations of* **1**

The ^{1}H-NMR spectrum suggested five Me groups at δ(H) 0.80 (*s*), 1.00 (*s*), 1.07 (*s*), 0.94 (*t*, $J = 7.4$), and 1.17 (*d*, $J = 7.0$), and one AcO group at δ(H) 2.11 (*s*). Combined with the HMQC spectrum, the ^{13}C-NMR spectrum (*Table 2*) suggested five O-bearing CH groups at δ(C) 95.3, 74.6, 76.4, 67.7, and 70.0, and one CH_2O group at δ(C) 65.8. The ^{13}C-NMR spectrum also showed two CO signals at δ(C) 213.6 and 220.2, and two ester CO signals at δ(C) 172.9 and 176.6. The HMBC interactions of H–C(2′) with C(1′), H–C(3″/5″) with C(2″), H–C(4″) with C(3″), and H–C(2″) with C(1″) suggested the presence of an AcO and a (2-methylbutanoyl)oxy group (*Fig. 1*). Furthermore, the correlations of H–C(3) with C(1′) and H–C(29) with C(1″) in the HMBC spectrum confirmed that the AcO group was linked to C(3), while the (2-methylbutanoyl)oxy group was at C(29).

These spectral data, together with the twelve degrees of unsaturation, suggested that **1** is a tetracyclic limonoid having a C(19)/C(29) acetal bridge, as meliatoxin B_1 [8].

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

Position	**1**[a])	**2**[b])	**3**[b])
1	4.06 (*d*, *J* = 4.6)	4.18–4.21 (*m*)	4.19 (*dd*, *J* = 3.8, 1.4)
2	4.49 (*t*, *J* = 4.8)	5.70 (*t*, *J* = 3.8)	5.70 (*t*, *J* = 3.8)
3	5.48 (*d*, *J* = 4.4)	4.02–4.06 (*m*)	4.05 (*d*, *J* = 1.5)
5	2.68–2.77 (*m*)	2.78 (*dd*, *J* = 13.9, 3.2)	2.79 (*dd*, *J* = 15.4, 3.1)
6	2.10–2.18 (*m*),	2.16 (*dd*, *J* = 14.1, 2.0),	2.11–2.16 (*m*),
	1.68–1.77 (*m*)	1.74 (*dt*, *J* = 14.2, 3.6)	1.74 (*dt*, *J* = 14.3, 3.2)
7	4.01 (*dd*, *J* = 3.2, 1.6)	4.02–4.06 (*m*)	4.00–4.03 (*m*)
9	3.59 (*s*)	3.58 (*s*)	3.61 (*s*)
12	2.60 (*d*, *J* = 16.3),	2.61 (*d*, *J* = 16.4),	2.61 (*d*, *J* = 16.4),
	2.38 (*d*, *J* = 16.3)	2.32 (*d*, *J* = 16.3)	2.32 (*d*, *J* = 16.3)
14	3.34 (*s*)	3.35 (*s*)	3.34 (*s*)
16	2.64–2.78 (*m*),	2.70 (*ddd*, *J* = 18.9, 12.1, 1.6),	2.69 (*ddd*, *J* = 18.9, 12.1, 1.6),
	2.44–2.56 (*m*)	2.49 (*dd*, *J* = 18.9, 8.3)	2.49 (*dd* , *J* = 18.9, 8.2)
17	3.27 (*s*)	3.29 (*t*, *J* = 3.9)	3.29 (*t*, *J* = 4.0)
18	1.00 (*s*)	0.99 (*s*)	0.99 (*s*)
19	4.55 (*d*, *J* = 13.4),	4.48 (*d*, *J* = 12.9),	4.48 (*dd*, *J* = 12.9, 1.1),
	4.29 (*d*, *J* = 12.7)	4.32 (*d*, *J* = 12.9)	4.32 (*d*, *J* = 12.9)
21	7.45 (br. *s*)	7.43 (*d*, *J* = 0.7)	7.43 (*d*, *J* = 0.7)
22	6.43 (*dd*, *J* = 1.7, 0.8)	6.42 (*dd*, *J* = 1.8, 0.8)	6.42 (*dd*, *J* = 1.8, 0.8)
23	7.48 (*t*, *J* = 1.6)	7.47 (*t*, *J* = 1.6)	7.47 (*t*, *J* = 1.6)
28	0.80 (*s*)	0.96 (*s*)	0.96 (*s*)
29	5.75 (*s*)	5.79 (*s*)	5.78 (*s*)
30	1.07 (*s*)	1.08 (*s*)	1.08 (*s*)
2′	2.11 (*s*)	2.12 (*s*)	2.12 (*s*)
2″	2.41–2.48 (*m*)	2.42 (*q*, *J* = 7.0)	2.56–2.63 (*m*)
3″	1.64–1.75 (*m*),	1.63–1.69 (*m*),	1.18 (*d*, *J* = 1.1)
	1.50–1.69 (*m*)	1.50–1.67 (*m*)	
4″	0.94 (*t*, *J* = 7.4)	0.92 (*t*, *J* = 7.5)	1.16 (*d*, *J* = 1.1)
5″	1.17 (*d*, *J* = 7.0)	1.15 (*d*, *J* = 7.0)	

[a]) Measured at 300 MHz in CD_3OD. [b]) Measured at 500 MHz in CD_3OD.

In the ROESY spectrum (*Fig. 1*), correlations of H–C(1), H–C(2), and H–C(3) with CH_2(19) were observed. Thus, the configurations of H–C(1), H–C(2), and H–C(3) were determined as β, β, and β, respectively. The ^{1}H- and ^{13}C-NMR spectra, in combination with HMQC, HMBC, and ROESY data, established the structure of compound **1** as 29-[(2-methylbutanoyl)oxy]-2α-hydroxyamoorastatone, a 29-*O*-substituted amoorastatone [9] derivative.

Compound **2** was obtained as a white amorphous powder. The optical rotation was determined to be $[\alpha]_D^{16} = -53.26$ ($c = 0.155$, MeOH), and it also showed a positive *Ehrlich* test result. The molecular formula was determined as $C_{33}H_{44}O_{11}$ by HR-ESI-MS, which showed the $[M+Na]^+$ ion peak of m/z 639.2764 ($C_{33}H_{44}NaO_{11}^+$; calc. 639.2776), indicating twelve degrees of unsaturation. The IR spectrum revealed the presence of OH (3431.6 cm^{-1}), CO (1726.7 cm^{-1}), and an AcO group (1245.1, 1055.0 cm^{-1}), as well as a furan ring (874.4 cm^{-1}).

Comparison of the NMR data of compound **1** and **2** (*Tables 1* and *2*) showed that only in the ring *A* the C- and H-atom signals were different. The HMBC interactions of

Table 2. *^{13}C-NMR Data of Compounds* **1**–**3**. δ in ppm.

Position	**1**[a])	**2**[b])	**3**[b])
1	74.6 (*d*)	74.4 (*d*)	74.4 (*d*)
2	67.7 (*d*)	72.3 (*d*)	72.3 (*d*)
3	76.4 (*d*)	74.3 (*d*)	74.3 (*d*)
4	41.7 (*s*)	42.6 (*s*)	42.7 (*s*)
5	29.1 (*d*)	27.9 (*d*)	27.9 (*d*)
6	24.2 (*t*)	24.4 (*t*)	24.3 (*t*)
7	70.0 (*d*)	70.0 (*d*)	70.0 (*d*)
8	46.6 (*s*)	46.4 (*s*)	46.4 (*s*)
9	52.2 (*d*)	52.1 (*d*)	52.2 (*d*)
10	43.6 (*s*)	44.2 (*s*)	44.2 (*s*)
11	213.6 (*s*)	213.5 (*s*)	213.6 (*s*)
12	51.7 (*t*)	51.8 (*t*)	51.8 (*t*)
13	45.2 (*s*)	45.1 (*s*)	45.1 (*s*)
14	61.8 (*d*)	61.8 (*d*)	61.8 (*d*)
15	220.2 (*s*)	220.0 (*s*)	220.1 (*s*)
16	44.7 (*t*)	44.7 (*t*)	44.7 (*t*)
17	42.5 (*d*)	42.6 (*d*)	42.6 (*d*)
18	28.1 (*q*)	28.1 (*q*)	28.1 (*q*)
19	65.8 (*t*)	65.2 (*t*)	65.3 (*t*)
20	123.7 (*s*)	123.7 (*s*)	123.7 (*s*)
21	141.9 (*d*)	141.8 (*d*)	141.9 (*d*)
22	111.7 (*d*)	111.6 (*d*)	111.7 (*d*)
23	144.4 (*d*)	144.3 (*d*)	144.4 (*d*)
28	18.8 (*q*)	19.6 (*q*)	19.5 (*q*)
29	95.3 (*d*)	95.6 (*d*)	95.6 (*d*)
30	20.2 (*q*)	20.2 (*q*)	20.2 (*q*)
1′	172.9 (*s*)	172.1 (*s*)	172.2 (*s*)
2′	21.0 (*q*)	21.0 (*q*)	21.0 (*q*)
1″	176.6 (*s*)	176.7 (*s*)	177.2 (*s*)
2″	42.4 (*d*)	42.4 (*d*)	35.4 (*d*)
3″	27.7 (*t*)	27.7 (*t*)	19.1 (*q*)
4″	11.8 (*q*)	11.7 (*q*)	19.1 (*q*)
5″	16.9 (*q*)	16.7 (*q*)	

[a]) Measured at 75 MHz in CD_3OD. [b]) Measured at 125 MHz in CD_3OD.

H−C(1) with C(2) and C(3), H−C(3) with C(5), H−C(29) with C(3), and Me(28) with C(4) (*Fig. 2*) indicated that the constitution of ring *A* was the same as in compound **1**. In the ROESY spectrum (*Fig. 2*), correlations of H−C(1) with H−C(9), H−C(2) with CH_2(19), and H−C(3) with H−C(28) were observed. Thus, the configurations of H−C(1), H−C(2), and H−C(3) were determined to be α, β, and α, respectively. The ^{1}H- and ^{13}C-NMR spectra, in combination with HMQC, HMBC, and ROESY data, established the structure of compound **2** as 1,3-*epi*-29-[(2-methylbutanoyl)oxy]-2α-hydroxyamoorastatone.

Compound **3** was obtained as a white amorphous powder. The optical rotation was determined to be $[\alpha]_D^{16} = -44.62$ ($c = 0.100$, MeOH), and the *Ehrlich* test showed a positive result. The molecular formula was determined to be $C_{32}H_{42}O_{11}$ by HR-ESI-MS,

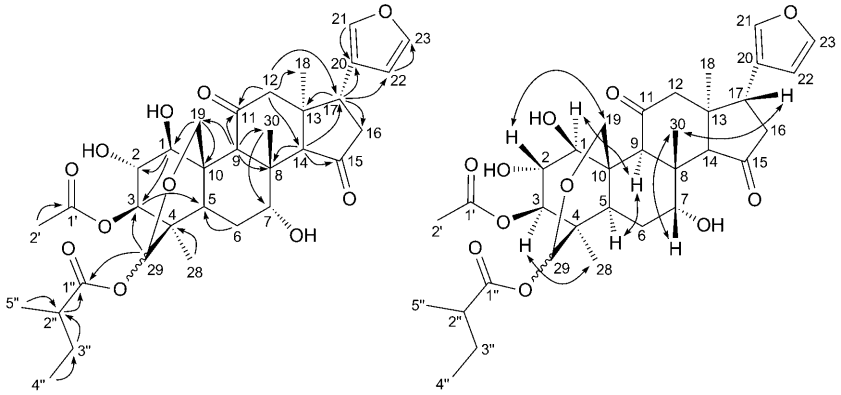


Fig. 2. *Key HMBC* (H → C) *and key ROESY* (H ↔ H) *correlations of* **2**

which showed the $[M+Na]^+$ ion peak of *m/z* 625.2595 ($C_{32}H_{42}NaO_{11}^+$; calc. 625.2619), indicating twelve degrees of unsaturation. The IR spectrum revealed the presence of OH (3415.2 cm^{-1}), CO (1728.7 cm^{-1}), and an AcO group (1249.7, 1056.3 cm^{-1}), as well as a furan ring (876.3 cm^{-1}). The NMR data of compounds **2** and **3** (*Tables 1* and *2*) indicated that compound **3** possessed one less C-atom signal. The ^{13}C-NMR spectrum of **3** showed an ester CO signal at δ(C) 177.2. The HMBC interaction of H−C(3″) and H−C(4″) with C(2″) and of H−C(2″) with C(1″) suggested the presence of an (2-methylpropanoyl)oxy unit (*Fig. 3*). Furthermore, the correlation of H−C(29) with C(1″) in the HMBC spectrum confirmed that the (2-methylpropanoyl)oxy unit is connected to C(29).

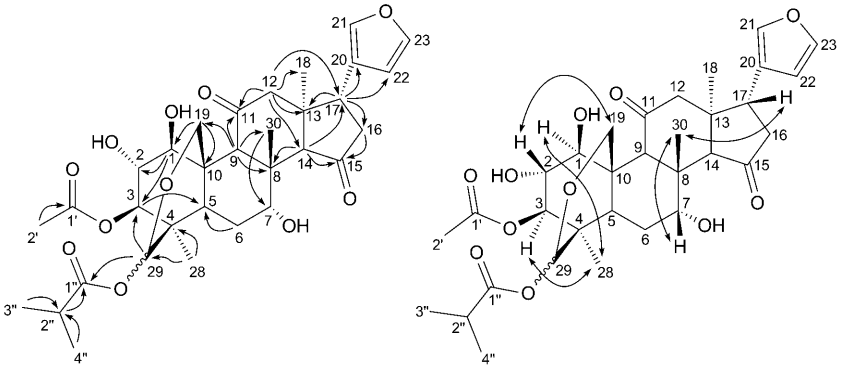


Fig. 3. *Key HMBC* (H → C) *and key ROESY* (H ↔ H) *correlations of* **3**

In the ROESY spectrum (*Fig. 3*), correlations of H−C(1) with Me(28), H−C(2) with CH_2(19), and H−C(3) with Me(28) were observed. Thus, the relative configurations of H−C(1), H−C(2), and H−C(3) were determined to be α, β, and α, respectively. The ^{1}H- and ^{13}C-NMR spectra data, along with those of HMQC,

HMBC, and ROESY, established the structure of compound **1** as 1,3-*epi*-29-[(2-methylpropanoyl)oxy]-2α-hydroxyamoorastatone.

Experimental Part

General. Column chromatography (CC): Silica gel (SiO_2, 100–200, 200–300 mesh, *Qingdao Haiyang Chemical Co. Ltd.*), *C18* reversed-phase silica gel (150–200 mesh, *Merck*), and *Sephadex LH-20* gel (*Amersham Biosciences*). TLC: precoated silica gel *GF254* plates were from *Qingdao Marine Chemical Plant*, Qingdao, P. R. China. Prep. HPLC: *Waters 600* instrument and a *Waters 2487 Dual* λ absorbance detector (USA), on a 19 mm × 300 mm i. d., 6 µm, *Prep Nova-Pak® HR C18* column (*Waters*, USA); the flow rate and detected wavelength were adjusted to 15 ml/min and 210 nm, resp. IR Spectra (KBr): *Bruker v33* spectrometer; in cm^{-1}. Optical rotations: *Jasco P1020* digital polarimeter (Japan). ^{1}H-, ^{13}C-, and 2D-NMR Spectra: with TMS as internal reference on *Bruker AV-300* and *AV-500* spectrometers (Switzerland); δ in ppm, *J* in Hz. ESI-MS and HR-ESI-MS (70 eV): in the positive-ion mode, on an *Agilent LC/TOF* MS spectrometer (USA); in *m/z*.

Plant Material. The bark of this medicinal plant was purchased from Ding Town, Kai County, Sichuan Province of China. The plant was identified as *Melia toosendan* (Meliaceae). A voucher specimen has been deposited with the Herbarium of China Pharmaceutical University, Nanjing, P. R. China (ref. No. 20060612).

Extraction and Isolation. The air-dried bark (30.0 kg) was cut into small pieces, macerated in EtOH at r.t. for one night, and was then refluxed in EtOH (60 l × 3) for 3, 1.5, and 1.5 h, resp. The filtered soln. was concentrated *in vacuo* to give a brown residue (1.9 kg), which was partitioned between H_2O and petroleum ether (60–90°; PE), AcOEt, and BuOH successively. The concentrated AcOEt extract (250.0 g) was next subjected to column chromatography (CC) on SiO_2, eluting with PE/AcOEt (1:0, 3:1, 1:1, 0:1) to afford 40 fractions (*Frs. 1–40*). *Fr. 36* (29.2 g) was repeatedly subjected to CC (SiO_2; $CHCl_3$/MeOH 8:1, 1:1, 0:1), *Sephadex LH-20* ($CHCl_3$/MeOH 1:1), reversed-phase SiO_2 (MeOH/H_2O 75:25), and prep. HPLC (32–36% MeCN, gradient elution) to afford compounds **1** (6.4 mg), **2** (6.1 mg), and **3** (5.5 mg).

29-[(2-Methylbutanoyl)oxy]-2α-hydroxyamoorastatone (=*(1*R*,2*R*,3*S*,4*R*,7*R*,8*S*,10*S*,13*S*,17*R*)-3-(Acetyloxy)-17-(furan-3-yl)hexadecahydro-1,2,7-trihydroxy-4,8,13-trimethyl-11,15-dioxo-4,10-(methanooxymethano)cyclopenta[*a*]phenanthren-20-yl 2-Methylbutanoate*; **1**). White amorphous powder. $[\alpha]_D^{16} = -24.36$ ($c = 0.220$, MeOH). IR (KBr): 3424, 2937, 1726, 1246, 1055, 874, 603. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. The key correlations of HMBC and ROESY are presented in *Fig. 1*. ESI-MS (pos.): 638.9 ($[M+Na]^+$). HR-ESI-MS (pos.): 639.2792 ($[M+Na]^+$, $C_{33}H_{44}NaO_{11}^+$; calc. 639.2776).

1,3-epi-*29-[(2-Methylbutanoyl)oxy]-2α-hydroxyamoorastatone* (=*(1*S*,2*R*,3*R*,4*R*,7*R*,8*S*,10*S*,13*S*,17*R*)-3-(Acetyloxy)-17-(furan-3-yl)hexadecahydro-1,2,7-trihydroxy-4,8,13-trimethyl-11,15-dioxo-4,10-(methanooxymethano)cyclopenta[*a*]phenanthren-20-yl 2-Methylbutanoate*; **2**). White amorphous powder. $[\alpha]_D^{16} = -53.26$ ($c = 0.155$, MeOH). IR (KBr): 3432, 2937, 1727, 1245, 1055, 874, 603. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. The key correlations of HMBC and ROESY are presented in *Fig. 2*. ESI-MS (pos.): 638.9 ($[M+Na]^+$). HR-ESI-MS (pos.): 639.2764 ($[M+Na]^+$, $C_{33}H_{44}NaO_{11}^+$; calc. 639.2776).

1,3-epi-*29-Isobutyroyloxy-2α-hydroxyamoorastatone* (=*(1*S*,2*R*,3*R*,4*R*,7*R*,8*S*,10*S*,13*S*,17*R*)-3-(Acetyloxy)-17-(furan-3-yl)hexadecahydro-1,2,7-trihydroxy-4,8,13-trimethyl-11,15-dioxo-4,10-(methanooxymethano)cyclopenta[*a*]phenanthren-20-yl 2-Methylpropanoate*; **3**). White amorphous powder. $[\alpha]_D^{16} = -44.62$ ($c = 0.100$, MeOH). IR (KBr): 3415, 2936, 1729, 1250, 1056, 876, 611. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. The key correlations of HMBC and ROESY are presented in *Fig. 3*. ESI-MS (pos.): 624.5 ($[M+Na]^+$). HR-ESI-MS (pos.): 625.2595 ($[M+Na]^+$, $C_{32}H_{42}NaO_{11}^+$; calc. 625.2619).

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