Two New Diterpenoids from *Callicarpa pedunculata*

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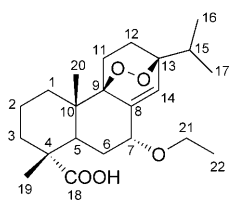
Two new diterpenoids, pedunculatic acid A (=(4*R*,5α,7α)-7-ethoxy-9β,13β-dioxyabiet-8(14)-en-18-oic acid; **1**) and pedunculatic acid B (=(4*S*,5α,12β)-8β,14β-epoxy-12-hydroxy-11-oxototaran-19-oic acid; **2**), together with three known sesquiterpenoids, were isolated from the Chinese medicinal herb *Callicarpa pedunculata* R. BROWN. Their structures were elucidated by spectroscopic analyses, including 1D- and 2D-NMR, and by high-resolution mass spectrometry.

1. Introduction. – *Callicarpa pedunculata* R. BROWN (Verbenaceae), a deciduous frutex of genus *Callicarpa*, is widely distributed in the southern part of China. It has been used for the treatment of hemorrhage, and as an anti-inflammatory and antibacterial drug [1]. In previous papers, we reported the structures of four types of diterpenoids, flavones, and triterpenoids [2] [3]. Our continuing phytochemical investigation of this plant has now led to the isolation of two new diterpenoids, pedunculatic acid A (**1**) and pedunculatic acid B (**2**), together with three known sesquiterpenoids, (1β,6α)-eudesm-4(14)-ene-1,6-diol [4], (9β)-caryolane-1,9-diol [5], and (−)-clovane-2β,9α-diol [5]. Their isolation and structure determination are presented herein.



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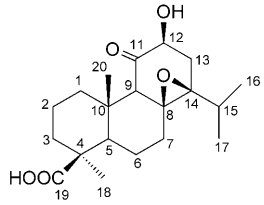

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2. Results and Discussion. – The aerial parts of *C. pedunculata* were extracted with 95% EtOH. The $CHCl_3$-soluble part of the EtOH extract was purified by successive column chromatography on silica gel, *RP-18* gel, and *Sephadex LH-20* to afford the above compounds.

Compound **1**, obtained as a colorless oil, had the molecular formula $C_{22}H_{34}O_5$ (six degrees of unsaturation), based on HR-ESI-MS ($[M+Na]^+$ at *m/z* 401.2300 (calc. 401.2304)). The IR spectrum showed absorptions for OH (3450), COOH (1697), and C=C (1640 cm^{-1}) functions.

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Table 1. *^{1}H- and ^{13}C-NMR Data of* **1**. At 400/100 or 500/125 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz.

Atom	δ(C)	δ(H)	HMBC (^{1}H → ^{13}C)
H_α–C(1)	33.2 (*t*)	1.58–1.61 (*m*)	C(3), C(5), C(20)
H_β–C(1)		1.87–1.89 (*m*)	C(3), C(5), C(20)
H_α–C(2)	17.4 (*t*)	1.23–1.24 (*m*)	C(1), C(4), C(10)
H_β–C(2)		1.59–1.62 (*m*)	C(1), C(4), C(10)
H_α–C(3)	37.0 (*t*)	1.64–1.65 (*m*)	C(1), C(4), C(5), C(19)
H_β–C(3)		1.67–1.68 (*m*)	C(4), C(5), C(19)
C(4)	46.8 (*s*)		
H–C(5)	38.3 (*d*)	2.29–2.32 (*m*)	C(3), C(4), C(6), C(9), C(18), C(19), C(20)
H_α–C(6)	28.4 (*t*)	1.61–1.64 (*m*)	C(4), C(7), C(8), C(10)
H_β–C(6)		2.01–2.13 (*m*)	C(4), C(7), C(8), C(10)
H–C(7)	71.8 (*d*)	4.39 (*ddd*, J=2.3, 3.6, 14.0)	C(5),C(6), C(8), C(14), C(21)
C(8)	144.7 (*s*)		
C(9)	81.7 (*s*)		
C(10)	38.5 (*s*)		
H_α–C(11)	23.7 (*t*)	2.02–2.03 (*m*)	C(8), C(10), C(13)
H_β–C(11)		1.59–1.61 (*m*)	C(8), C(10)
H_α–C(12)	26.6 (*t*)	2.00–2.02 (*m*)	C(13), C(14)
H_β–C(12)		1.45–1.50 (*m*)	C(13), C(14)
C(13)	79.9 (*s*)		
H–C(14)	129.2 (*d*)	6.48 (*d*, J=2.3)	C(7), C(9), C(13), C(15)
H–C(15)	32.2 (*d*)	1.89–1.91 (*m*)	C(12), C(13), C(14), C(16), C(17)
H–C(16)	17.4 (*q*)	0.96 (*d*, J=1.9)	C(13), C(15)
H–C(17)	17.1 (*q*)	0.97 (*d*, J=1.9)	C(13), C(15)
C(18)	183.7 (*s*)		
H–C(19)	17.4 (*q*)	1.29 (*s*)	C(3), C(4), C(5), C(18)
H–C(20)	18.5 (*q*)	1.05 (*s*)	C(1), C(5), C(9)
H_α–C(21)	64.3 (*t*)	3.46 (*dd*, J=2.0, 7.1)	C(7), C(22)
H_β–C(21)		3.57 (*dd*, J=2.0, 7.1)	C(7), C(22)
H–C(22)	15.4 (*q*)	1.19 (*t*, J=1.7, 7.5)	C(21)

The ^{13}C-NMR (DEPT) spectrum of **1** (*Table 1*) revealed 22 signals: five Me, seven CH_2 (containing an oxygenated one), and four CH groups (including an oxygen-bearing and an olefinic one), and six quaternary C-atoms (including a COOH, two oxygenated, and one olefinic C-atom). The ^{1}H-NMR spectrum of **1** (*Table 1*) showed signals for an i-Pr group (δ(H) 0.96, 0.97 (2*d*, J=1.9 Hz each, 2×3 H); 1.89–1.91 (*m*, 1 H)). From these spectroscopic data and by comparison with the spectra of diterpenoids previously isolated from this plant [2], compound **1** was assigned an abietane structure. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** with those of '9β,13β-epidioxyabiet-8(14)-en-18-oic acid' [6] indicated the same abietane skeleton, except that **1** had an additional EtO group (δ(C) 64.3 (*t*), 15.4 (*q*)). The ^{13}C-NMR data of **1** exhibited a downfield-shifted C(7)-atom, and upfield shifted C(6)- and C(8)-atoms, respectively, compared to the above congener. This suggested that the EtO group was located at C(7), as corroborated by HMBC correlations between H–C(7) (δ(H) 4.39) and C(5) (δ(C) 38.3), C(6) (24.8), C(8) (144.7), C(14) (129.2), and C(21) (64.3) (*Table 1*).

The relative configuration of **1** was derived by a ROESY experiment (*Fig. 1*). The α-orientation of the 7-EtO group was apparent from the ROESY correlation between H_β–C(7) and both H_β–C(6) and Me(20). From these data, the structure of compound **1** was elucidated as (4*R*,5α,7α)-7-ethoxy-9β,13β-dioxyabiet-8(14)-en-18-oic acid, and named *pedunculatic acid A*.

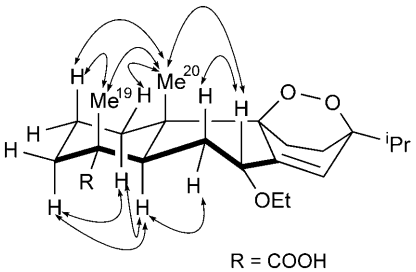
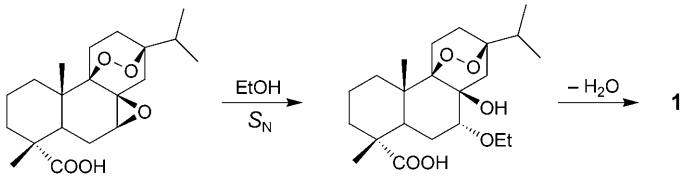


Fig. 1. *Key ROESY correlations of* **1**

Since EtO groups are very rare in natural products, we considered the possibility that **1** was an artifact produced during extraction and isolation. A possible precursor of **1** might be the epoxide **3** (*Scheme*), which, by nucleophilic addition of EtOH, could yield the intermediate **4**. The latter might then eliminate H_2O to afford **1**. Unfortunately, we were unable to corroborate this hypothesis because we could not identify any potential precursors.

Scheme. *Putative Pathway to Account for the Formation of* **1**



3 **4**

Compound **2**, a colorless oil, had the molecular formula $C_{20}H_{30}O_5$, as confirmed by EI-MS (M^+ at m/z 350) and HR-ESI-MS ($[M+Na]^+$ at m/z 373.1983 (calc. 373.1991)), which corresponds to six degrees of unsaturation. The IR spectrum showed absorptions for OH (3443), C=O (1725), and COOH (1696 cm^{-1}) groups.

The ^{13}C-NMR (DEPT) spectrum of **2** (*Table 2*) exhibited 20 signals: four Me, six CH_2, and four CH (including an oxygenated one), and six quaternary C-atoms (including one C=O, one COOH, and two oxygen-bearing C-atoms). The ^{1}H-NMR spectrum (*Table 2*) showed signals for an i-Pr group (δ(C) 0.96, 0.98 (2*d*, J=7.1 Hz, 2×3 H); 1.68–1.70 (*m* (1 H)). This suggested that **2** was a diterpene. The ^{1}H,^{1}H-COSY and HMQC plots indicated the following partial structures: CH_2–CH_2–CH_2, CH–CH_2–CH_2, CH–CH_2, and Me_2CH. In the HMBC experiment (*Table 2*), correlations were observed between both the Me(17) and Me(16) H-atoms at δ(H) 0.96 and 0.98, respectively, and C(16,17), C(15), and C(14) at δ(C) 18.1, 34.5, and 62.0; between the methine H-atom H–C(15) and the carbon signals for Me(16,17), C(13) (δ(C) 35.0), C(14), and C(8) (δ(C) 68.1); between the methine H-atom H–C(12) (δ(H) 5.61) and C(13), C(14)), C(9) (δ(C) 62.3), and C(11) (δ(C) 210.0); and between the H-atom H–C(9) (δ(H) 3.94) and H–C(20) (δ(C) 21.9), C(14), 68.1 (C(8)), C(12) (δ(C) 72.8), and C(11). From these data, the partial structure **2a** was derived (*Fig. 2*).

The following further HMBC correlations were observed: H_β–C(1) at δ(H) 2.17–2.20 with C(20) (δ(C) 21.9), C(3) (35.8), C(5) (48.8), and C(9) (62.3); between H–C(5) at δ(H) 3.50 and C(6) (δ(C) 21.5), C(20) (21.9), C(18) (28.0), C(7) (29.6), C(1) (31.5), C(3) (35.8), C(10) (43.4), C(4) (48.1), and C(19) (180.7); and between the H-atoms of Me(18) at δ(H) 1.27 and C(3), C(4), and C(19). This led

Table 2. *^{1}H- and ^{13}C-NMR Data of* **2**. At 400/100 or 500/125 MHz, resp. in C_5D_5N; δ in ppm, J in Hz.

Atom	δ(C)	δ(H)	HMBC (^{1}H → ^{13}C)
H_α–C(1)	31.5 (*t*)	2.06–2.09 (*m*)	C(5), C(9)
H_β–C(1)		2.17–2.20 (*m*)	C(3), C(5), C(9), C(20)
H_α–C(2)	20.0 (*t*)	1.43 (*d*, *J*=3.8)	C(1), C(3), C(4), C(10)
H_β–C(2)		2.04–2.06 (*m*)	C(1), C(3), C(4), C(10)
H_α–C(3)	35.8 (*t*)	2.25–2.27 (*m*)	C(1), C(5), C(19)
H_β–C(3)		2.27–2.30 (*m*)	C(1), C(5), C(19)
C(4)	48.1 (*s*)		
H–C(5)	48.8 (*d*)	3.50 (*dd*, *J*=10.3, 10.6)	C(1), C(3), C(4), C(6), C(7), C(10), C(18), C(19), C(20)
H_α–C(6)	21.5 (*t*)	2.17–2.20 (*m*)	C(4), C(5), C(7)
H_β–C(6)		2.40–2.42 (*m*)	C(4), C(5), C(7), C(8), C(10)
H_α–C(7)	29.6 (*t*)	0.87 (*d*, *J*=13.3)	C(5), C(6), C(9)
H_β–C(7)		1.61–1.68 (*m*)	C(5), C(9), C(14)
C(8)	68.1 (*s*)		
H–C(9)	62.3 (*d*)	3.94 (*s*)	C(5), C(7), (8), C(11), C(12), C(14), C(20)
C(10)	43.4 (*s*)		
C(11)	210.0 (*s*)		
H–C(12)	72.8 (*d*)	5.61 (*dd*, *J*=2.3, 8.6)	C(9), C(11), C(13), C(14)
H_α–C(13)	35.0 (*t*)	1.62–1.68 (*m*)	C(8), C(15)
H_β–C(13)		2.42–2.45 (*m*)	C(8), C(11), C(15)
C(14)	62.0 (*s*)		
H–C(15)	34.5 (*d*)	1.68–1.70 (*m*)	C(13), C(14), C(16), C(17)
H–C(16)	18.1 (*q*)	0.98 (*d*, *J*=7.1)	C(14), C(15), C(17)
H–C(17)	18.1 (*q*)	0.96 (*d*, *J*=7.1)	C(14), C(15), C(16)
H–C(18)	28.0 (*q*)	1.27 (*s*)	C(3), C(4), C(5), C(19)
C(19)	180.7 (*s*)		
H–C(20)	21.9 (*q*)	1.46 (*s*)	C(1), C(5), C(9)

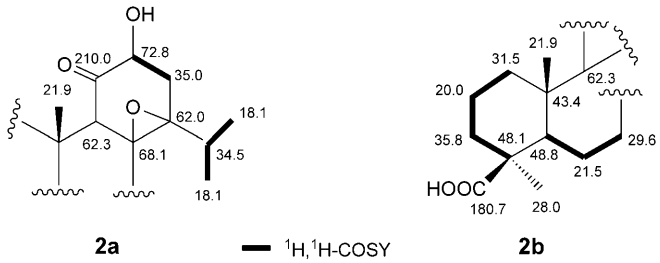


Fig. 2. *Fragment structures of* **2** *identified by HMBC and COSY experiments* (see text)

to the fragment **2b** (*Fig. 2*). Finally, the HMBC correlations between H–C(9) at δ(H) 3.94 and the C-atoms C(20), C(7), C(1), and C(5) at δ(C) 21.9, 29.6, 31.5, and 48.8, respectively, allowed the combination of the two fragments to compound **2**.

The relative configuration of **2** was deduced from a ROESY experiment (*Fig. 3*). The NOE of Me(18) at δ(H) 1.27 with H–C(5) at δ(H) 3.50 indicated the α-position for Me(18) and the β-position for the COOH group at C(19). The β-position of the 12-OH group was inferred from the NOE between H–C(12) at δ(H) 5.61 and H–C(9) at δ(H) 3.94. The NOEs between H–C(9) at δ(H) 3.94 and H–C(15), Me(16), and Me(17) at δ(H) 1.68, 0.98, and 0.96, respectively, indicated α-position for the i-Pr group and β-position for the 8,14-epoxy moiety.

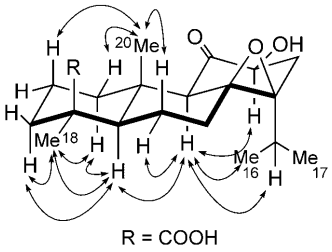


Fig. 3. *Key ROESY correlations of* **2**

From the above data, the structure of compound **2** was identified as (4*S*,5α,12β)-8β,14β-epoxy-12-hydroxy-11-oxototaran-19-oic acid, which was named *pedunculatic acid B*. Since compound **2** represents already the *fifth* type of diterpenoid skeleton isolated from this plant, *C. pedunculata* seems to represent a special case in chemotaxonomic terms.

The authors are grateful to the analytical group of the *State Key Laboratory of Phytochemistry and Plant Resources* in West China, *Kunming Institute of Botany*, *Chinese Academy of Sciences*, for spectroscopic and spectrometric experiments.

Experimental Part

General. TLC: silica-gel plates; visualization by spraying with 10% H_2SO_4 in EtOH, followed by heating. Column chromatography (CC): silica gel (200–300 mesh, 10–40 μm; *Qingdao Marine Chemical, Inc.*), *Lichroprep RP-18* (43–63 μm; *Merck*), or *Sephadex LH-20* (*Pharmacia*). M.p.: *XRC-1* apparatus; uncorrected. Optical rotations: *Jasco DIP-370* digital polarimeter. IR Spectra: *Bio-Rad FTS-135* spectrometer; KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AM-400* and *Bruker DRX-500* instruments; at 400/100 or 500/125 MHz, resp.; δ in ppm rel. to Me_4Si, *J* in Hz. EI-MS: *VG AutoSpec-3000* mass spectrometer; in *m/z*. ESI- and HR-ESI-MS: *API Qstar Pulsar* instrument.

Plant Material. Callicarpa pedunculata R. BROWN was collected in Guiyang, Guizhou Province, P. R. China, in August, 2000. A voucher specimen (KUN No. 0300993) was deposited at the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The dried and powdered aerial parts of *C. pedunculata* (6.0 kg) were extracted with 95% EtOH at reflux for 8 h (3×). After removal of the solvent under vacuum, the resulting residue was partitioned between H_2O and $CHCl_3$, and then between H_2O and BuOH. The $CHCl_3$ extract (300 g) was subjected to MPLC (1 kg SiO_2; petroleum ether (PE), then PE/AcOEt 10:1, 5:1, 4:1, 3:1, 1:1, and 0:1): seven fractions (Fr.). *Fr. 3* (10.5 g) was subjected to repeated MPLC (1. SiO_2, PE/AcOEt 8:1 and 5:1; 2. *RP-18*, MeOH/H_2O 6:4) to afford the three known compounds *(1β,6α)-eudesm-4(14)-ene-1,6-diol* (13 mg) [4], *(9β)-caryolane-1,9-diol* (11 mg) [5], and *(−)-clovane-2β,9α-diol* (85 mg) [5]. *Fr. 3* (9.0 g) was subjected to repeated MPLC (SiO_2; PE/AcOEt 6:1 and 4:1) and CC (*Sephadex LH-20*; MeOH) to provide **1** (14 mg). *Fr. 4* (15.5 g) was subjected to repeated MPLC (SiO_2; PE/AcOEt 4:1 and 2:1) and CC (*Sephadex LH-20*; MeOH) to afford **2** (9 mg).

Pedunculatic Acid A (=*(4*R*,5α,7α)-7-Ethoxy-9β,13β-dioxyabiet-8(14)-en-18-oic Acid*; **1**). Colorless oil. $[\alpha]_D^{26} = -11.2$ ($c = 1.3$, $CHCl_3$). IR (KBr): 3450, 2962, 2937, 2875, 1697, 1640, 1465, 1388, 1279, 1104. ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 378 (10, M^+), 346 (45, $[M-O_2]^+$), 300 (100, $[M-O_2-EtOH]^+$), 285 (27), 255 (80), 245 (47), 239 (91), 211 (50), 199 (80), 193 (90), 159 (70), 123 (92), 109 (83), 81 (61), 67 (47), 55 (50). HR-ESI-MS: 401.2300 ($[M+Na]^+$, $C_{22}H_{34}NaO_5^+$; calc. 401.2304).

Pedunculatic Acid B (=*(4*S*,5α,12β)-8β,14β-Epoxy-12-hydroxy-11-oxototaran-19-oic Acid*; **2**). Colorless oil. $[\alpha]_D^{26} = -92.9$ ($c = 1.9$, $CHCl_3$). IR (KBr): 3443, 2969, 2943, 1725, 1696, 1465, 1261, 1172, 1156,

1066, 915. ^{1}H- and ^{13}C-NMR: see *Table 2*. EI-MS: 350 (2, M^+), 332 (17, $[M-H_2O]^+$), 317 (20), 289 (11), 268 (22), 253 (18), 243 (31), 237 (100), 215 (10), 207 (20), 191 (20), 178 (20), 165 (31), 107 (32), 91 (22), 69 (17), 55 (31). HR-ESI-MS: 373.1983 ($[M+Na]^+$, $C_{20}H_{30}NaO_5^+$; calc. 373.1991).

(1β,6α)-Eudesm-4(14)-ene-1,6-diol [4]. Colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.67 (*s*, Me(15)); 0.83 (*d*, *J* = 7.0, Me(12)); 0.92 (*d*, *J* = 7.0, Me(13)); 3.38 (*dd*, *J* = 4.6, 11.5, H–C(1)); 3.68 (*dd*, *J* = 5.7, 9.7, H–C(6)); 4.71 (br. *s*, H_b–C(14)); 4.99 (br. *s*, H_a–C(14)). ^{13}C -NMR (100 MHz, $CDCl_3$): 146.2 (*s*, C(4)); 107.8 (*t*, C(14)); 78.9 (*d*, C(1)); 67.0 (*d*, C(6)); 55.8 (*d*, C(5)); 49.3 (*d*, C(7)); 41.6 (*s*, C(10)); 36.2 (*t*, C(9)); 35.0 (*t*, C(2)); 31.8 (*t*, C(3)); 25.9 (*d*, C(11)); 21.1 (*q*, C(13)); 18.1 (*t*, C(8)); 16.1 (*q*, C(12)); 11.5 (*q*, C(15)). EI-MS: 238 (5, M^+), 220 (63), 202 (34), 189 (39), 177 (71), 165 (20), 159 (72), 149 (80), 135 (42), 121 (90), 107 (100), 93 (65), 81 (57), 69 (21), 55 (22).

(9β)-Caryolane-1,9-diol [5]. Colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.89 (*s*, Me(15)); 0.96 (*s*, Me(13)); 0.98 (*s*, Me(14)); 3.40 (br. *s*, H–C(9)). ^{13}C-NMR (100 MHz, $CDCl_3$): 72.2 (*d*, C(9)); 70.7 (*s*, C(1)); 42.4 (*d*, C(5)); 42.4 (*t*, C(12)); 39.3 (*s*, C(8)); 38.1 (*d*, C(2)); 35.4 (*t*, C(7)); 35.1 (*s*, C(4)); 34.0 (*t*, C(3)); 33.4 (*t*, C(11)); 30.5 (*q*, C(14)); 28.1 (*t*, C(10)); 26.6 (*q*, C(15)); 20.8 (*q*, C(13)); 20.4 (*t*, C(6)). EI-MS: 238 (3, M^+), 220 (23, $[M-H_2O]^+$), 202 (35, $[M-2\,H_2O]^+$), 179 (40), 162 (48), 149 (100), 127 (68), 123 (100), 109 (66), 93 (45), 81 (49), 71 (42), 67 (24), 57 (34).

(−)-Clovane-2β,9α-diol [5]. Colorless plates ($CHCl_3$/MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): 0.86 (*s*, Me(13)); 0.96 (*s*, Me(15)); 1.03 (*s*, Me(14)); 3.34 (br. *s*, H–C(9)); 3.80 (*dd*, *J* = 5.8, 10.1, H–C(2)). ^{13}C-NMR (100 MHz, $CDCl_3$): 80.8 (*d*, C(2)); 75.1 (*d*, C(9)); 50.5 (*d*, C(5)); 47.5 (*t*, C(3)); 44.1 (*s*, C(1)); 37.1 (*s*, C(4)); 35.5 (*t*, C(12)); 34.7 (*s*, C(8)); 33.1 (*t*, C(7)); 31.4 (*q*, C(14)); 28.3 (*q*, C(15)); 26.3 (*t*, C(11)); 26.0 (*t*, C(10)); 25.4 (*q*, C(13)); 20.6 (*t*, C(6)). EI-MS: 238 (21, M^+), 220 (39, $[M-H_2O]^+$), 205 (24), 202 (13), 182 (34), 170 (77), 164 (100), 153 (50), 149 (44), 135 (41), 121 (26), 107 (29), 93 (28), 81 (27), 69 (20).

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