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Five new C_{19}-diterpenoid alkaloids from *Aconitum carmichaeli*

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ABSTRACT

Five new aconitine-type C_{19}-diterpenoid alkaloids, namely, carmichaenine A–E (**1**–**5**), and six known diterpenoid alkaloids, namely, 14-benzoylneoline (**6**), neoline (**7**), 10-hydroxyneoline (**8**), neolinine (**9**), songoramine (**10**), and songorine (**11**), were isolated from the aerial parts of *Aconitum carmichaeli*. Their structures were determined by extensive spectroscopic methods, especially 2D NMR analyses. Compounds **8** and **9** were isolated for the first time from *A. carmichaeli*.

1. Introduction

As a botanical source for various pharmaceutically active components, the genus *Aconitum* (Ranunculaceae), represented by 208 species in China (Editorial Committee of Flora of China, 1979), has been commonly used as a traditional Chinese medicine for thousands of years. Phytochemical studies on *Aconitum* species have led to the discovery of various compounds (Hao et al., 2013), among which the best known type of medicinal compound is diterpenoid alkaloids, which are known for their complex chemical structures and noteworthy physiological effects (Tang et al., 2012; Vitalini et al., 2012; Wang et al., 2011; Qu et al., 2011).

As a continuation of our studies on medicinal plants of *Aconitum* species growing on the Yunnan-Tibet Plateau (Yang et al., 2007, 2008a,b), we herein report the isolation and structural elucidation of five new aconitine-type C_{19}-diterpenoid alkaloids, namely, carmichaenine A–E (**1**–**5**), together with six known diterpenoid alkaloids, namely, 14-benzoylneoline (**6**) (Wada et al., 1985), neoline (**7**) (Khetwal et al., 1994; Wada et al., 1985), 10-hydroxyneoline (**8**) (Takayama et al., 1990), neolinine (**9**) (Ross et al., 1987), songoramine (**10**) (Csupor et al., 2007), and songorine (**11**) (Csupor et al., 2007), from the aerial part of *Aconitum carmichaeli* (Fig. 1). Compounds **8** and **9** were isolated for the first time from *A. carmichaeli*.

2. Results and discussion

Carmichaenine A (**1**) was isolated as a white amorphous solid, with $[\alpha]_D^{18} = -8.0$. Its molecular formula was established as $C_{31}H_{43}NO_7$ on the basis of the HR-ESI–MS spectrum showing the $[M+H]^+$ peak at an m/z of 542.3115. The IR spectrum displayed characteristic absorption bands for hydroxyl groups (3431 cm^{-1}), an ester carbonyl group (1707 and 1280 cm^{-1}), and an aromatic ring (1585 and 1464 cm^{-1}). The ^1H NMR spectrum of **1** (Table 1) showed signals of a monosubstituted aromatic ring [δ_H 8.09 (2H, d, J = 7.4 Hz), 7.53 (1H, t, J = 7.4 Hz), and 7.42 (2H, t, J = 7.4 Hz)], three methoxyl groups [δ_H 3.32, 3.23 and 3.00 (each 3H, s)], and an *N*-ethyl group [δ_H 3.34 (2H, overlapped) and 1.45 (3H, t, J = 6.8Hz)]. The ^{13}C NMR spectrum (Table 2) clearly indicated one ester carbonyl (δ_C 165.8), one oxygenated carbon (δ_C 84.1), four oxygenated methine carbons (δ_C 71.1, 82.5, 74.3 and 80.9), one oxygenated methene carbon (δ_C 78.5), three methoxyl groups (δ_C 59.1, 58.4 and 56.6), and two carbons of an *N*-ethyl unit (δ_C 49.7 and 10.6). The ^1H–^1H COSY correlations (Fig. 2) revealed the presence of the following five structural fragments: $CH(OR)CH_2CH_2$ (fragment A), CH_3CH_2N (fragment B), CHCH(OR)CH (fragment C), $CHCHCH_2CHCH(OR)$ (fragment D), and $CH(OR)CH_2$ (fragment E). In the HMBC plot (Fig. 2), the correlations from H-1 to C-11 and from H-3 to C-4 identified fragment A as the C-1 to C-3 part of the

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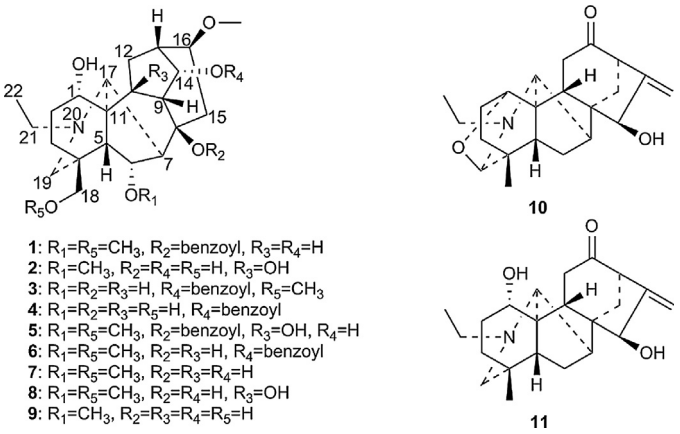

Fig. 1. Structures of compounds **1**–**6**.

molecule. Similarly, the correlations from H-5 to C-4 and C-11 as well as from H-7 to C-8 and C-17 confirmed that fragment C was identical to the C-5 to C-7 part of the molecule. The HMBC correlations from H-10 to C-8, C-11, and C-17 as well as H-9 to C-8 verified that fragment D constituted a five-membered ring. According to the correlations from H-15 to C-8, H-12 to C-16, and H-14 to C-16, fragment E could be identified as the C-15 to C-16 part of the molecule. The C_{19}-diterpene skeleton was finally established by HMBC correlations from H-1 to C-11, H-3 to C-4 and C-5, H-5, H-17, and H-18 to C-19, H-7 to C-9 and C-17, H-17 to C-10, and H-12 to C-11. For those substituent groups, HMBC correlations from H-2′/6′ to C-7′ confirmed the existence of a benzoate moiety. The locations of the three methoxyl groups were assigned as C-6, C-16 and C-18 based on the HMBC data. The substitution of C-14 with a hydroxyl group was determined by the chemical shift of H-14 at δ_H 4.22 because H-14 should resonate in the range δ_H 4.0–4.2 in the case of a hydroxyl group at C-14 but in the range δ_H 4.8–5.8 in the case of an acyl substitution at C-14 (Csupor et al., 2007; Hanuman and Katz, 1993). Then, the position of the benzoate group was determined to be at C-8, which can also be proven by the higher chemical shift (δ_c 84.1) of C-8.

The relative configuration of **1** was studied by means of a ROESY experiment (Fig. 2). As a reference point, the β stereochemistry of H-5 was used, which is characteristic for C_{19}-diterpenoid alkaloids. ROESY correlations of H-10 with H-1, H-9, H-13 and H-14 as well as H-9 with H-5 indicated β-oriented protons at these locations. On the other hand, no correlation between H-16 and H-13 can be observed, demonstrating the α-orientation of H-16. The correlations of H_α-15 with H-16 and H-17 and of H-19 with H-17 and H-22 suggested α-orientations of H_α-15, H-17 and H-19. The correlation of H-6 with H-7 and the coupling constant between H-5 and H-6 (J = 6.2 Hz) confirmed the β-positions of H-6 and H-7. ROESY correlations of H-16 with H_α-15, H-17 with H_α-12, H-5 with H_β-2, and H_α-2 with H_α-3 allowed the steric differentiation of protons H-2, H-3, H-12, and H-15.

Thus, compound **1** was determined to be an aconitine-type C_{19}-diterpenoid alkaloid, specifically, (1α,6α,14α,16β)-20-ethyl-1,14-dihydroxy-6,16-dimethoxy-4-(methoxymethyl) aconitan-8-yl benzoate.

Carmichaenine B (**2**) was isolated as a white amorphous solid, with $[\alpha]_D^{18}$ = +29.6. Its molecular formula was determined to be $C_{23}H_{37}NO_7$ on the basis of the HR-ESI–MS spectrum showing the $[M+H]^+$ peak at an m/z of 440.2643. The ^{1}H NMR spectrum of **2** (Table 1) showed signals of two methoxyl groups [δ_H 3.35 and 3.31 (each 3H, s)] and an *N*-ethyl group [δ_H 2.59 (1H, m), 2.52 (1H, m), and 1.13 (3H, t, J = 6.8)]. The ^{13}C NMR spectrum (Table 2) was quite similar to that of compound **1**, except that the signals of the benzoate group and one methoxyl group disappeared and the C-10 signal shifted from δ_C 43.5 in **1** to lower field (δ_C 83.5) in **2**, suggesting that an oxygen atom was connected with C-10. The two methoxyl groups were assigned to the same positions (6 and 16) as seen in **1**. Combined with the HMBC data, compound **2** was determined to be (1α,6α,10β,14α,16β)-20-ethyl-1,8,10,14-tetrahydroxy-4-(hydroxymethyl)-6,16-dimethoxyaconitane.

Carmichaenine C (**3**) was isolated as a white amorphous solid, with $[\alpha]_D^{18}$ = −5.0. Its molecular formula was determined to be $C_{30}H_{41}NO_7$ on the basis of the HR-ESI–MS spectrum showing the $[M+H]^+$ peak at an m/z of 528.2956. The ^{1}H NMR spectrum (Table 1) showed signals of a monosubstituted aromatic ring [δ_H 8.08 (2H, d, J = 7.2 Hz), 7.46 (1H, t, J = 7.2 Hz), and 7.59 (2H, t, J = 7.2 Hz)], two methoxyl groups [δ_H 3.29, and 3.31 (each 3H, s)], and a *N*-ethyl group [δ_H 2.93 (1H, m), 2.85 (1H, m), and 1.26 (3H, t, J = 6.8)]. The NMR signals (Tables 1 and 2) were also very similar to those of **1**. For compound **3**, the significant differences were that the H-14 signal shifted from δ_H 4.22 to lower field (δ_H 5.10), while the C-8 signal shifted from δ_C 84.1 to higher field (δ_C 75.5), suggesting that the benzoate group was substituted at C-14 (Shim et al., 2003). Moreover, the displacement of the methoxyl group at C-6 with a hydroxyl group could be ruled out by HMBC analysis. Therefore, compound **3** was determined to be (1α,6α,14α,16β)-20-ethyl-1,6,8-trihydroxy-16-methoxy-4-(methoxymethyl) aconitan-14-yl benzoate.

Carmichaenine D (**4**) was isolated as a white amorphous solid, with $[\alpha]_D^{18}$ = −2.0. Its molecular formula was determined to be $C_{29}H_{39}NO_7$ on the basis of the HR-ESI–MS spectrum showing the $[M+H]^+$ peak at an m/z of 514.2803. The ^{1}H NMR spectrum (Table 1) showed signals of a monosubstituted aromatic ring [δ_H 8.08 (2H, d, J = 7.8 Hz), 7.46 (1H, t, J = 7.8Hz), and 7.58 (2H, t, J = 7.8 Hz)], one methoxyl group [δ_H 3.29 (3H, s)], and a *N*-ethyl group [δ_H 2.64 (1H, m), 2.56 (1H, m), and 1.16 (3H, t, J = 7.1)]. The NMR signals (Tables 1 and 2) were very similar to those of **3**, except that a hydroxyl group replaced a methoxyl group, the position of which was assigned at C-18 by HMBC analysis. Therefore, compound **4** was determined to be (1α,6α,14α,16β)-20-ethyl-1,6,8-trihydroxy-4-(hydroxymethyl)-16-methoxyaconitan-14-yl benzoate.

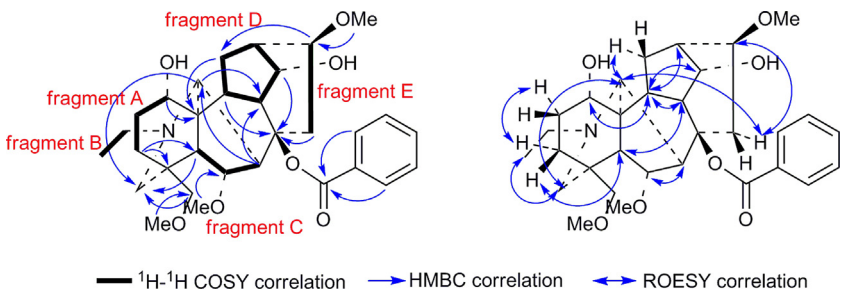

Fig. 2. Key ^{1}H–^{1}H COSY, HMBC and ROESY correlations of compound **1**.

Table 1
^{1}H NMR data (600 MHz) of compounds **1**–**5** (δ in ppm, J in Hz).

Position	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]
1	4.02 (br. s)	4.04 (br. s)	3.95 (br. s)	3.77 (br. s)	4.11 (br. s)
2	1.88 (m), 1.45 (m)	1.54 (m)	1.58 (m)	1.58 (m)	1.58 (m)
3	2.04 (m)	1.92 (m)	1.99 (m)	1.89 (m)	1.94 (m)
	1.69 (m)	1.54 (m)	1.74 (m)	1.59 (m)	1.61 (m)
5	2.28 (d, 6.2)	2.44 (d, 6.5)	2.15 (d, 6.6)	2.06 (d, 6.6)	2.63 (d, 5.9)
6	4.28 (d, 6.2)	4.16 (d, 6.5)	4.72 (d, 6.6)	4.66 (d, 6.6)	4.23 (d, 5.9)
7	3.45 (s)	1.96 (s)	2.01 (s)	1.91 (s)	3.27 (s)
9	2.58 (t, 5.4)	1.99 (d, 5.1)	2.44 (t, 6.0)	2.42 (t, 6.2)	2.38 (d, 5.0)
10	2.05 (m)	–	2.19 (m)	2.06 (m)	–
12	2.10 (m)	2.16 (d, 15.9)	2.21 (m)	2.18 (m)	2.21 (d, 15.8)
	1.94 (m)	1.84 (dd, 15.9, 8.0)	1.69 (m)	1.75 (m)	1.92 (dd, 15.8, 8.0)
13	2.32 (t, 4.9)	2.34 (m)	2.61 (m)	2.59 (m)	2.48 (m)
14	4.22 (t, 4.7)	4.55 (t, 5.0)	5.10 (t, 4.8)	5.09 (t, 4.8)	4.63 (t, 5.0)
15	2.92 (dd, 16.0, 8.3)	2.20 (d, 8.3)	2.31 (dd, 16.0, 8.3)	2.27 (dd, 14.3, 8.2)	2.92 (dd, 16.0, 8.3)
	2.48 (dd, 16.0, 6.3)		2.21 (dd, 16.0, 6.3)	2.21 (dd, 14.3, 6.2)	2.48 (dd, 16.0, 6.3)
16	3.59 (t, 8.3)	3.26 (t, 8.3)	3.36 (t, 8.3)	3.39 (t, 8.2)	3.31 (t, 8.3)
17	3.61 (s)	2.57 (s)	2.97 (s)	2.73 (s)	2.71 (s)
18	3.37 (d, 8.2)	3.78 (d, 10.2)	3.65 (d, 8.0)	3.80 (d, 10.2)	3.58 (d, 8.7)
	3.32 (d, 8.2)	3.60 (d, 10.2)	3.61 (d, 8.0)	3.69 (d, 10.2)	3.32 (d, 8.7)
19	3.17 (s)	2.73 (m)	3.16 (m)	2.81 (m)	2.79 (m)
		2.35 (m)	2.70 (m)	2.38 (m)	2.45 (m)
21	3.34 (overlapped)	2.59 (m)	2.93 (m)	2.64 (m)	2.78 (m)
		2.52 (m)	2.85 (m)	2.56 (m)	2.69 (m)
22	1.45 (t, 6.8)	1.13 (t, 6.8)	1.26 (t, 6.8)	1.16 (t, 7.1)	1.22 (t, 6.8)
2′/6′	8.09 (d, 7.4)	–	8.08 (d, 7.2)	8.08 (d, 7.8)	8.05 (d, 7.4)
3′/5′	7.42 (t, 7.4)	–	7.46 (t, 7.2)	7.46 (t, 7.8)	7.46 (t, 7.4)
4′	7.53 (t, 7.4)	–	7.59 (t, 7.2)	7.58 (t, 7.8)	7.58 (t, 7.4)
6-OMe	3.00 (s)	3.35 (s)	–	–	3.01 (s)
16-OMe	3.32 (s)	3.31 (s)	3.29 (s)	–	3.33 (s)
18-OMe	3.23 (s)	–	3.31 (s)	3.29 (s)	3.28 (s)

[a] In $CDCl_3$.
[b] In CD_3OD.

Carmichaenine E (**5**) was isolated as a white amorphous solid, with $[\alpha]_D^{18} = -2.6$. Its molecular formula was determined to be $C_{31}H_{43}NO_8$ on the basis of the HR-ESI–MS spectrum showing the $[M+H]^+$ peak at an m/z of 558.3065. The ^{1}H NMR spectrum (Table 1) showed signals of a monosubstituted aromatic ring [δ_H 8.05 (2H, d, J = 7.4Hz), 7.46 (1H, t, J = 7.4 Hz), and 7.58 (2H, t, J = 7.4 Hz)], three methoxyl groups [δ_H 3.01, 3.33, 3.28 (each 3H, s)], and a *N*-ethyl group [δ_H 2.78 (1H, m), 2.69 (1H, m), and 1.22 (3H, t, J = 6.8)]. The ^{1}H and ^{13}C NMR data were very similar to those of **2** (Tables 1 and 2), except that a benzoate group and a methoxyl group in **5** replaced two hydroxyl groups in **2**. Moreover, the C-8 signal shifted from δ_C 73.7 in **2** to lower field (δ_C 85.4) in **5**, indicating that the location of the benzoate group was at C-8. Thus, compound **5** was determined to be (1α,6α,10β,14α,16β)-20-ethyl-1,10,14-trihydroxy-6,16-dimethoxy-4-(methoxymethyl) aconitan-8-yl benzoate.

3. Experimental

3.1. General experimental procedures

The optical rotations were measured with a Jasco DIP-370 digital polarimeter. UV spectra were obtained in MeOH on Agilent 1200 series HPLC. IR spectra were recorded on a Bio-Rad-FTS-135 spectrometer. The NMR spectra were obtained with a Bruker Avance III 600 spectrometer. The HR-ESI–MS was recorded on Agilent 6230 TOF mass spectrometer. Column chromatography was performed on silica gel G, silica gel 254, silica gel 200–300 mesh (Qingdao Marine Chemical Factory) and Sephadex LH-20 (Amersham Pharmacia).

3.2. Plant material

A. carmichaeli was collected in November 2011 from the Yunnan province, China. The plant was identified by Mr. Yu Chen of the Kunming Institute of Botany, CAS, where a voucher specimen (No. BBP0415) has been deposited.

Table 2
^{13}C NMR data (150 MHz) of compounds **1**–**5** (δ in ppm, J in Hz).

Position	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]
1	71.1	71.2	72.8	73.8	70.9
2	27.4	31.6	29.5	30.5	31.4
3	28.7	29.6	29.0	29.7	30.1
4	38.4	39.8	39.1	40.0	38.9
5	43.5	42.2	45.6	47.1	41.3
6	82.5	84.2	72.9	73.8	84.8
7	49.5	53.8	58.2	57.7	49.6
8	84.1	73.7	75.5	75.9	85.4
9	45.7	57.8	45.4	45.8	56.6
10	43.5	83.5	44.7	44.8	83.3
11	50.5	55.6	51.3	51.2	55.9
12	28.9	42.2	30.4	30.7	41.8
13	39.7	42.4	39.4	39.6	42.3
14	74.3	75.0	77.5	78.0	74.1
15	37.2	43.9	42.1	42.4	40.3
16	80.9	83.7	83.9	84.2	83.7
17	64.6	64.6	64.5	64.2	64.2
18	78.5	70.2	80.3	70.2	80.6
19	56.6	58.4	58.8	58.2	58.3
21	49.7	49.2	49.7	49.1	49.5
22	10.6	13.4	11.8	13.4	12.9
1′	130.6	–	131.7	131.9	132.6
2′/6′	129.7	–	130.9	130.9	130.6
3′/5′	128.5	–	129.4	129.4	129.5
4′	133.2	–	134.1	134.0	134.1
7′	165.8	–	167.7	167.8	167.1
6-OMe	58.4	58.4	–	–	58.8
16-OMe	56.6	56.4	56.5	55.6	56.6
18-OMe	59.1	–	59.4	–	59.3

[a] In $CDCl_3$.
[b] In CD_3OD.

3.3. Extraction and isolation

The ground aerial parts (7.0 kg) of *A. carmichaeli* were extracted with 95% EtOH (100 L × 3 times) at room temperature. After removal of the solvent under reduced pressure, the EtOH extract was dissolved in 3% HCl (3 L). The acidic solution was alkalized with 25% aqueous NH_4OH to a pH of 10, extracted with $CHCl_3$ (3 L × 3 times) and then evaporated under vacuum to give crude alkaloids (250 g). The crude alkaloids underwent further chromatography on a silica gel column using gradient elution with petroleum ether/acetone (10:1, 6:1, 3:1, 1:1), acetone, and MeOH to give six main fractions (A–F). Chromatography of fraction D on a silica gel column eluted with $CHCl_3$/MeOH (200:1 → 10:1) produced five sub-fractions (D-1 to D-5). Fraction D-2 underwent chromatography on a silica gel column and was eluted with $CHCl_3$/MeOH (200:1 → 80:1) to provide compound **10** (28 mg). Fraction D-4 underwent chromatography on a silica gel column and was eluted with $CHCl_3$/MeOH (100:1 → 50:1) to provide compounds **1** (32 mg) and **6** (48 mg). Fraction E was separated on a silica column and eluted with $CHCl_3$/MeOH (1:0 → 100:1 → 0:1) to give seven sub-fractions (E-1 to E-7). Chromatography of fraction E-3 on a silica gel column using $CHCl_3$/MeOH (100:1 → 60:1) as the eluent provided compound **5** (108 mg). Fraction E-4 was purified on a silica gel column and eluted with $CHCl_3$/MeOH (80:1 → 60:1). Then, it was purified on a Sephadex LH-20 column and eluted with MeOH to afford compound **3** (105 mg). Fraction E-5 was separated on a silica gel column and eluted with $CHCl_3$/MeOH (80:1 → 60:1). Then, it was purified by preparative TLC developed with $CHCl_3$/MeOH (32:1) to afford compound **7** (200 mg). Fraction E-6 underwent chromatography on a silica gel column and was eluted with $CHCl_3$/MeOH (80:1 → 30:1) to afford compounds **8** (100 mg) and **11** (47 mg). Fraction F was separated on a silica column and eluted with $CHCl_3$/MeOH (20:1 → 0:1) to give six sub-fractions (F-1 to F-6). Fraction F-2 was separated on a silica gel column and eluted with $CHCl_3$/MeOH (20:1) to provide compounds **4** (74 mg) and **9** (31 mg). Fraction F-3 underwent chromatography on a silica gel column and was eluted with $CHCl_3$/MeOH (15:1) to provide compound **2** (147 mg).

3.3.1. Carmichaenine A (**1**)

Amorphous solid; $[\alpha]_D^{18} = -8.0$ (*c* 0.20, MeOH); IR (KBr) ν_{max} cm^{-1}: 3431, 1707, 1634, 1585, 1464, 1280, 1116; ^1H NMR ($CDCl_3$, 600 MHz) (Table 1); ^{13}C NMR ($CDCl_3$, 150 MHz) (Table 2); ESIMS *m/z* 542 $[M+H]^+$; HRESIMS *m/z* 542.3115 ($[M+H]^+$, calcd. for $C_{31}H_{44}NO_7$, 542.3118).

3.3.2. Carmichaenine B (**2**)

Amorphous solid; $[\alpha]_D^{18} = +29.6$ (*c* 0.20, MeOH); IR (KBr) ν_{max} cm^{-1}: 3423, 1633, 1103; ^1H NMR (CD_3OD, 600 MHz) (Table 1); ^{13}C NMR (CD_3OD, 150 MHz) (Table 2); ESIMS *m/z* 440 $[M+H]^+$; HRESIMS *m/z* 440.2643 ($[M+H]^+$, calcd. for $C_{23}H_{38}NO_7$, 440.2648).

3.3.3. Carmichaenine C (**3**)

Amorphous solid; $[\alpha]_D^{18} = -5.0$ (*c* 0.20, MeOH); IR (KBr) ν_{max} cm^{-1}: 3439, 1717, 1603, 1485, 1281, 1108; ^1H NMR (CD_3OD, 600 MHz) (Table 1); ^{13}C NMR (CD_3OD, 150 MHz) (Table 2); ESIMS *m/z* 528 $[M+H]^+$); HRESIMS *m/z* 528.2956 ($[M+H]^+$, calcd. for $C_{30}H_{42}NO_7$, 528.2961).

3.3.4. Carmichaenine D (**4**)

Amorphous solid; $[\alpha]_D^{18} = -2.0$ (*c* 0.20, MeOH); IR (KBr) ν_{max} cm^{-1}: 3426, 1719, 1603, 1483, 1280, 1109; ^1H NMR (CD_3OD, 600 MHz) (Table 1); ^{13}C NMR (CD_3OD, 150 MHz) (Table 2); ESIMS *m/z* 514 $[M+H]^+$); HRESIMS *m/z* 514.2803 ($[M+H]^+$, calcd. for $C_{29}H_{40}NO_7$, 514.2865).

3.3.5. Carmichaenine E (**5**)

Amorphous solid; $[\alpha]_D^{18} = -2.6$ (*c* 0.20, MeOH); IR (KBr) ν_{max} cm^{-1}: 3439, 1712, 1602, 1585, 1281, 1117; ^1H NMR (CD_3OD, 600 MHz) (Table 1); ^{13}C NMR (CD_3OD, 150 MHz) (Table 2); ESIMS *m/z* 558 $[M+H]^+$); HRESIMS *m/z* 558.3065 ($[M+H]^+$, calcd. for $C_{31}H_{44}NO_8$, 558.3067).

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