Eight new C_{19}-diterpenoid alkaloid arabinosides, named aconicarmichosides E–L (1–8), were isolated from an aqueous extract of the lateral roots of Aconitum carmichaelii (Fu Zi). Their structures were determined by spectroscopic and chemical methods including 2D NMR experiments and acid hydrolysis. Compounds 1–8, together with the previously reported four neoline 14-O-arabinosides from the same plant, represent the only examples of glycosidic diterpenoid alkaloids so far. At a dose of 1.0 mg/kg (i.p.), as compared with the black control, compounds 1, 2, and 4–6 exhibited analgesic effects with >65.6% inhibitions against acetic acid-induced writhing of mice. Structure–activity relationship was also discussed.

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1. Introduction

The lateral and principle roots of the poisonous plant *Aconitum carmichaelii* Debx. (Ranunculaceae), named “Fu Zi” and “Wu Tou” in Chinese, respectively, are important traditional Chinese medicines used for the treatment of rheumatalgia, neuralgia, arthrythmia, acardianeuria, and inflammations1-4. Considerable chemical and pharmacological studies have previously been reported, along with isolation of more than a hundred compounds from various extracts of different parts of *A. carmichaelii*5-12. Among the reported chemical constituents, diterpenoid alkaloids were recognized as active components, especially the lipophilic diesterified aconitane-type C19-diterpenoid alkaloids identified as the main toxic constituents. In addition, the previous reports showed that toxicity of these medicines was dramatically reduced by processing and decocting because contents of the toxic diesterified aconitane-type alkaloids were remarkably decreased by the treatments13-16. However, in the chemical studies, organic solvents, such as benzene, chloroform, methanol, and ethanol, were applied for extracting the raw and processed plant materials2. Chloroform, methanol, and ethanol were applied for extracting the ever-increasing medicinal plants2. Therefore, as part of our program to consider chemical and NMR spectroscopic data (Experimental Section 4.3.1, and Table 1) due to hydroxyl groups. The (+)-HR-ESI-MS and NMR spectroscopic data (Experimental Section 4.3.1, and Table 1) indicated that *I* had the molecular formula C29H32NO10. The 1H NMR spectrum of *I* in CD3OD showed resonances characteristic for an aconite-type C19-diterpenoid alkaloid, including an N-CH2CH3 unit at δH 3.30 and 3.25 (1H each, m, H2-20) and 1.43 (3H, t, J = 7.5 Hz, H2-21) and three methoxy groups at δH 3.40 (s, OMe-16), 3.35 (s, OMe-6), and 3.32 (s, OMe-18); four oxymethines at δH 4.33 (brd, J = 7.0 Hz, H-6), 4.19 (brs, H-1), 4.14 (dd, J = 5.0 and 4.5 Hz, H-14), and 3.35 (m, H-16); one nitrogen-bearing methine at δH 3.27 (brs, H-17); an oxymethylene at δH 3.57 and 3.50 (1H each, d, J = 8.0 Hz, H2-18); and a nitrogen-bearing methylene at δH 3.39 and 3.06 (1H each, d, J = 12.5 Hz, H3-19); as well as partially overlapped resonances due to four aliphatic methylenes (H2-2, H2-3, H2-12, and H2-15) and five aliphatic methines (H-5, H-7, H-9, H-10, and H-13) between δH 1.50 and 2.42. Additionally the spectrum showed signals diagnostic for a penrose moiety, consisting of four oxygen-bearing methylenes at δH 5.11 (d, J = 5.5 Hz, H-1′), 4.16 (dd, J = 8.0 and 5.5 Hz, H-2′), 4.02 (dd, J = 8.0 and 7.0 Hz, H-3′), and 3.76 (m, H-4′), and an oxygen-bearing methylene at δH 3.75 (m, H-5′a) and 3.65 (dd, J = 12.5 and 6.5 Hz, H-5′b). The 13C NMR and DEPT spectra showed 29 carbon signals corresponding to the above units and three quaternary carbons including an oxygen-bearing carbon at δC 74.6 (C-8). Comparison of the spectroscopic data with those of aconicarmichosides A-D18 indicated that *I* was an isomer of neoline β-1-arabinofuranoside. Specifically compared with the NMR spectroscopic data of aconicarmichoside D18, the resonances of H-1, H-2a, H-5, and H-15 and C-1, C-1′, and C-17 in *I* were remarkably deshielded by ΔδH > 0.15, whereas H-2b, H-3b, H-9, and H-13 and C-2 and C-14 were shielded by ΔδH < -0.15. These differences suggested that location of the hydroxyl and arabinosylxyloxy at C-1 and C-14 in aconicarmichoside D was exchanged in *I*, which was proved by 2D NMR data analysis as below.

The proton and corresponding hydrogen-bond carbon resonances in the NMR spectra of *I* were unambiguously assigned (Table 1) by analysis of the HSQC spectroscopic data. In the 1H–1H COSY spectrum of *I*, homonuclear vicinal coupling correlations of H-1/H2-2/H-3, H-5/H-6/H-7, H-14/H-9/H-10/H2-12/H-13/H-14, H-2b/H-15/H-16, and H-20/H-21 demonstrated the presence of five spin systems separated by the quaternary carbons and/or heteroatoms in the aglycone moiety (Fig. 2, thick lines). The HMBC spectrum showed two- and three-bond correlations (Fig. 2, red arrows) from H2-3 to C-4; from H-5 to C-4, C-18, and C-19; from H2-18 to C-3, C-4, C-5, and C-19; and from H2-19 to C-4, C-5, and C-15, indicating that the quaternary C-4 connected to C-3, C-5, C-18, and C-19. The HMBC correlations from H-7 to C-8, C-9, and C-15 and from H-9 to C-7, C-8, and C-15, together with their chemical shifts, revealed that the oxygen-bearing quaternary C-8 was linked by C-7, C-9 and C-15. The HMBC correlations from H-1 to C-3, C-10, and C-11; from H-5 to C-10, C-11, and C-17; from H-10 to C-1, C-5, C-11, and C-17; and from H-17 to C-5, C-6, C-10, and C-11 revealed a linkage of the quaternary C-11 to C-1, C-5, C-10 and C-17. The connection between C-13 and C-16 was confirmed by the HMBC correlations from H-12 to C-14 and C-16, from H-13 to C-15, from H-14 to C-16, and from H-16 to C-12 and C-14, though the vicinal coupling correlation between H-16 and H-13 and H-12 were undistinguishable in the 1H–1H COSY spectrum due to an overlap of the H-13 and H-15 resonances. In addition, the HMBC correlations from H-6 to C-17; from H-17 to C-6, C-8, C-19, and C-20; from H2-19 to C-17 and C-20; from H-20 to C-17 and C-19; together their chemical shifts, demonstrated that C-17 connected with C-7 and via the nitrogen atom to both C-19 and C-20. The HMBC correlations from OCH3-6 to C-6, from OCH3-16 to C-16, and from OCH3-18 to C-18 located the three methoxy groups at the corresponding carbons. In addition, the 1H–1H COSY cross-peaks of H-1/H2-2/H-3/H-4/H-5/H-6 and the HMBC correlations from H-1′ to C-1, C-3′, and C-4′ and from H-4′ to C-1′ proved that there was an arabinofuranosylxyloxy at C-1 of *I*. The coupling constant values of J1′,2′; (5.5 Hz), J2′,3′; (8.0 Hz), and J3′,4′; (7.0 Hz) confirmed the β-configuration of the arabinofuranosylxyloxy. The hydroxyl group must be located at C-8 according to the chemical shift of this carbon and the molecular formula requirement of *I*. Thus, the planar structure of *I* was proved as shown. In the ROEY′ spectrum of *I*, the correlations between H-1 with H-10 and H-12a, between H-3a and H2-18, between H-5 with H-10 and H-18, between H-6 and H-9, between H-10 with H-12a and H-14, and between H-14 with H-9 and H-13 demonstrated that
these hydrogens were oriented on the same side of the ring system. Meanwhile, the ROESY correlations between H-3b with H-19b, between H-16 and H-12b, and between H-17 with H-12b and H-21 revealed that these hydrogens were oriented on the other side of the ring system. Additionally, the NOE correlations between H-1’ with H-1 and H-2a further supported location of the sugar unit at C-1. Comparing with those of aconicarmichosides 3, 4 and 6, the 1H NMR spectroscopic data and the specific rotation values with those of the authentic samples (isotalatizidine 14-α-L-arabinofuranoside and named aconicarmichoside E).

Compound 2, a colorless gum with \( [\alpha]_{D}^{20} = -24.8 \) (c 0.63, MeOH), has the molecular formula C_{29}H_{47}NO_{11} as determined by (+)-HR-ESI-MS and NMR spectroscopic data (Experimental Section 4.3.2, and Tables 1 and 2). Comparison of the NMR spectroscopic data of 2 and 1 demonstrated that 2 had one less methoxy group than 1. Especially the chemical shifts of proton and carbon resonances for the pentose moiety in the two compounds were completely different. This suggests that 2 is a demethoxy analogue of 1 with simultaneous change in the sugar moiety. Subsequent analysis of 2D NMR spectroscopic data (Figs. 2 and 3) confirmed that the aglycone of 2 was 6-demethoxynorine (isotalatizidine). Particularly the \( ^{1}H-^{1}H \) COSY cross-peaks of H-1/H-2/H-3/H-4/H-5/H-6 and the HMBC correlations from H-1’ to C-5’ and from H-5’ to C-1’, together with the coupling constant values of \( J_{1'-2'} \) (6.6 Hz) and \( J_{3'-4'} \) (3.6 Hz), verified that the pentosyl in 2 was α-arabinopyranosyl. Meanwhile, the HMBC correlations from H-1’ to C-14 and from H-14 to C-1’ located the α-arabinopyranosyl at C-14 in 2. Furthermore, from the acid hydrolysis of 2, isotalatizidine \( ([\alpha]_{D}^{20} + 23.4 \) (c 0.09, MeOH)) and \( \beta \)-arabinose \( ([\alpha]_{D}^{20} + 106.3 \) (c 0.10, H_{2}O)) were isolated and identified by comparison of the \( ^{1}H \) NMR spectroscopic data and the specific rotation value with those of the authentic samples (isotalatizidine, \( [\alpha]_{D}^{20} + 18.4 \) (c 0.04, MeOH); \( \beta \)-arabinose, \( [\alpha]_{D}^{20} + 113.3 \) (c 0.29, H_{2}O))\(^{18} \). Therefore, the structure of compound 2 was determined as isotalatizidine 14-O-α-L-arabinopyranoside and named aconicarmichoside F.

Compound 3, a colorless gum with \( [\alpha]_{D}^{20} + 28.3 \) (c 0.14, MeOH), showed spectroscopic data similar to those of 2 (Experimental Section 4.3.3 and Tables 1 and 2), except that the H-9 and H-14 and C-13 resonances in 3 were significantly shielded by \( \Delta \delta_{H} = -0.13 \) and \( -0.09 \) and \( \Delta \delta_{C} = -2.1 \), respectively, whereas H-13 and C-9 were deshielded by \( \Delta \delta_{H} = +0.09 \) and \( \Delta \delta_{C} = +2.0 \). Especially the anomeric hydrogen and carbon resonances were changed significantly from \( \delta_{H} = 4.36 \) (d, \( J = 6.6 \) Hz) and \( \delta_{C} = 103.1 \) in 2 to \( \delta_{H} = 4.95 \) (d, \( J = 3.0 \) Hz) and \( \delta_{C} = 100.3 \), while the NMR data of arabinosyl in 3 were in good agreement with those of \( \beta \)-arabinopyranosyl in neolide 14-O-β-D-arabinopyranoside (aconicarmichoside B) isolated from the same extract\(^{18} \). This indicates that 3 is an isomer of 2 with replacement of α-L-arabinopyranosyl by \( \beta \)-D-arabinopyranosyl. The deduction was further confirmed by 2D NMR spectroscopic data of 3 (Figs. 2 and 3) as well as by isolation and identification of isotalatizidine and \( \beta \)-arabinose from the acid hydrolysate of 3. Therefore, the structure of compound 3 was determined as isotalatizidine 14-O-β-D-arabinopyranoside and named aconicarmichoside G.

Compound 4 was obtained as a colorless gum with \( [\alpha]_{D}^{20} = -43.7 \) (c 0.40, MeOH). The spectroscopic data of 4 indicate that it is another isomer of 2 and 3 differing only in the arabinosyl moiety. The NMR spectroscopic data showed that the anomeric hydrogen and carbon of arabinosyl had more deshielded chemical shifts at \( \delta_{H} = 5.05 \) (H-1’) and \( \delta_{C} = 109.2 \) (C-1’) than those in 2 and 3. Meanwhile, the H-1’ resonance of 4 appeared as a broad singlet instead of the doublets of 2 and 3. In particular the chemical shifts and coupling pattern of the arabinosyl moiety in 4 were well consistent with those of α-arabinofuranosyl in aconicarmichoside C (neolide 14-O-α-L-arabinofuranoside)\(^{18} \), indicating that an α-L-arabinofuranosyl in 4 replaced the \( \beta \)-arabinopyranosyls in 2 and 3. This was verified by the \( ^{1}H-^{1}H \) COSY cross-peaks of H-1/H-2/H-3/H-4/H-5/H-6 and the HMBC correlations from H-1’ to C-4’ and from H-4’ to C-1’ (Fig. 2). In addition, the HMBC correlations from H-1’ to C-14 and from H-14 to C-1’ confirmed location of the sugar unit in 4. Therefore, the structure of compound 4 was determined and named aconicarmichoside H.

Compound 5, a colorless gum with \( [\alpha]_{D}^{20} = -7.7 \) (c 0.15, MeOH), has the molecular formula C_{28}H_{45}NO_{9} as determined by (+)-HR-ESI-MS combined with NMR spectroscopic data. Comparison of the NMR spectroscopic data of 5 and 1 (Tables 1 and 2) demonstrated that the methylene (CH_{2}-15) of the aglycone in 1...
Table 1  The $^1$H NMR spectroscopic data (δ) for compounds 1–8.

| No. | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | 4.19 brs | 4.05 brs | 4.06 brs | 4.06 brs | 3.94 brs | 3.99 brs | 3.57 brs | 3.52 brs |
| 2a  | 2.00 m   | 1.68 m   | 1.68 m   | 1.68 m   | 1.58 m   | 1.62 m   | 1.93 m   | 1.91 m   |
| 2b  | 1.51 m   | 1.62 m   | 1.62 m   | 1.62 m   | 1.55 m   | 1.59 m   | 1.44 m   | 1.38 m   |
| 3a  | 2.02 m   | 1.93 m   | 1.93 dd (15.0, 5.0) | 1.94 m   | 1.97 m   | 2.02 dd (14.4, 6.0) | 1.88 dd (15.0, 4.8) | 1.96 dd (15.0, 4.8) |
| 3b  | 1.55 dt (15.5, 4.0) | 1.81 m   | 1.82 dt (3.4, 15.0) | 1.83 dt (15.0, 6.0) | 1.77 dt (15.0, 6.0) | 1.82 dt (6.0, 14.4) | 1.60 dt (4.8, 15.0) | 1.58 dt (4.8, 15.0) |
| 5   | 2.42 d (7.0) | 2.05 m   | 2.07 m   | 2.05 m   | 2.29 d (7.5) | 2.35 d (6.6) | 2.04 brs | 2.35 d (6.6) |
| 6a  | 4.33 brd (7.0) | 2.10 m   | 2.08 m   | 2.07 m   | 4.26 brd (7.5) | 4.23 brd (6.6) | 2.05 m   | 4.29 brd (6.6) |
| 6b  | 1.84 m   | 1.84 m   | 1.84 m   | 1.85 m   | 1.84 m   | 1.84 m   | 1.85 m   | 1.85 m   |
| 7   | 2.20 brs | 2.28 brd (8.4) | 2.28 brd (8.4) | 2.25 brd (8.4) | 2.44 brs | 2.50 brs | 2.19 brd (7.8) | 2.11 brs |
| 9   | 2.14 dd (6.5, 5.0) | 2.40 dd (5.4, 4.8) | 2.27 dd (6.0, 4.8) | 2.29 dd (4.8, 6.0) | 2.34 dd (5.5, 4.5) | 2.27 dd (6.0, 4.8) | 2.36 t (5.4) | 2.34 dd (6.6, 4.8) |
| 10  | 2.18 m   | 2.14 m   | 2.16 m   | 2.15 m   | 2.09 m   | 2.15 m   | 2.12 m   | 2.12 m   |
| 12a | 2.10 m   | 2.14 m   | 2.14 m   | 2.15 m   | 2.10 m   | 2.15 m   | 2.11 m   | 2.11 m   |
| 12b | 1.66 dd (14.5, 4.5) | 1.47 m   | 1.50 dd (13.8, 4.2) | 1.46 m   | 1.53 m   | 1.58 m   | 1.24 m   | 1.24 m   |
| 13  | 2.25 m   | 2.35 dd (6.6, 4.8) | 2.44 dd (7.2, 4.8) | 2.37 dd (6.6, 4.8) | 2.31 dd (6.0, 4.5) | 2.39 dd (6.6, 4.8) | 2.31 dd (6.0, 4.8) | 2.31 t (6.0, 4.8) |
| 14  | 4.14 dd (5.0, 4.5) | 4.24 t (4.8) | 4.15 t (4.8) | 4.09 t (4.8) | 4.13 t (4.5) | 4.02 t (4.8) | 4.20 t (4.8) | 4.18 t (4.8) |
| 15a | 2.28 dd (15.0, 9.0) | 2.24 dd (15.0, 9.0) | 2.28 dd (15.0, 9.0) | 2.28 dd (15.0, 9.0) | 2.45 d (7.0) | 4.23 d (6.6) | 2.17 dd (13.8, 6.6) | 2.20 dd (13.8, 8.4) |
| 15b | 2.25 dd (15.0, 6.0) | 2.18 dd (15.0, 6.0) | 2.15 dd (15.0, 6.0) | 2.11 dd (15.0, 6.0) | 2.95 brd (7.0) | 2.99 brd (6.6) | 3.23 m | 3.22 t (8.4) |
| 16  | 3.35 m   | 3.32 m   | 3.30 m   | 3.30 m   | 2.95 brd (7.0) | 2.99 brd (6.6) | 3.23 m | 3.22 t (8.4) |
| 17  | 3.27 brs | 3.29 brs | 3.26 brs | 3.25 brs | 3.28 brs | 3.29 brs | 3.21 brs | 3.19 brs |
| 18a | 3.57 d (8.0) | 3.21 d (9.0) | 3.22 d (9.0) | 3.22 d (9.0) | 3.48 s | 3.53 s | 3.15 d (9.0) | 3.48 d (8.4) |
| 18b | 3.50 d (8.0) | 3.15 d (9.0) | 3.17 d (9.0) | 3.17 d (9.0) | 3.48 s | 3.53 s | 3.10 d (9.0) | 3.45 d (8.4) |
| 19a | 3.39 d (12.5) | 2.98 d (12.6) | 2.99 d (12.6) | 2.98 d (12.6) | 3.40 d (12.0) | 3.44 d (13.2) | 2.90 d (12.6) | 3.38 d (12.0) |
| 19b | 3.06 d (12.5) | 2.88 d (12.6) | 2.89 d (12.6) | 2.89 d (12.6) | 2.93 d (12.0) | 2.98 d (13.2) | 2.85 d (12.6) | 2.93 d (12.0) |
| 20a | 3.30 m   | 3.30 dq (12.0, 7.2) | 3.30 m   | 3.30 m   | 3.27 m   | 3.30 m | 2.98 m | 3.02 m |
| 20b | 3.25 m   | 3.15 dq (12.0, 7.2) | 3.15 m   | 3.14 m   | 2.98 m | 3.02 m | 3.07 dq (12.6, 7.2) | 3.10 dq (13.2, 7.2) |
| 21  | 1.43 t (7.5) | 1.37 t (7.2) | 1.36 t (7.2) | 1.35 t (7.2) | 1.38 t (7.5) | 1.42 t (7.2) | 1.30 t (7.2) | 1.31 t (7.2) |
| 21’ | 3.30 s   | 3.30 s | 3.30 s | 3.30 s | 3.30 s | 3.30 s | 3.30 s | 3.30 s |

Data were measured in CD$_3$OD at 500 MHz for 1 and 5 and at 600 MHz for 2–4 and 6–8, respectively. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on $^1$H–$^1$H COSY, HSQC, and HMBC experiments.
was substituted by an oxymethine [δ_H 4.25 (d, J = 7.0 Hz) and δ_C 79.6] in 5, while the chemical shift of C-16 in 5 was deshielded significantly by Δδ_C +9.4. This revealed that the aglycone in 5 was 15α-hydroxyneoline (fuziline). Additionally, the chemical shifts and coupling patterns of the arabinosyl moiety in 5 were completely different from those of β-arabinofuranosyl in 1, but were well consistent with those of α-arabinopyranosyl in 2. Accordingly, compound 5 was elucidated as a fuziline α-arabinopyranoside. The deduction was further verified by 2D NMR data analysis of 5 (Figs. 2 and 3). Especially the 1H–1H COSY cross-peaks between H-15 and H-16 and the HMBC correlations from H-15 to C-16 and C-8 and from H-16 to C-12, C-14, and C-15, together with their chemical shifts, confirmed the location of the hydroxy group at C-15. The HMBC correlations from H-1′ to Table 2

| No. | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
|-----|----|----|----|----|----|----|----|----|
| 1   | 77.3 | 72.2 | 72.1 | 72.2 | 72.5 | 72.1 | 82.5 | 82.3 |
| 2   | 22.6 | 28.7 | 28.7 | 28.7 | 29.5 | 29.0 | 22.2 | 22.2 |
| 3   | 28.8 | 26.2 | 26.2 | 26.2 | 28.8 | 28.4 | 25.8 | 28.0 |
| 4   | 39.1 | 38.8 | 38.8 | 38.8 | 38.8 | 39.8 | 39.3 | 38.7 |
| 5   | 44.2 | 40.7 | 40.8 | 40.8 | 44.0 | 43.6 | 40.5 | 43.7 |
| 6   | 82.7 | 26.0 | 25.9 | 25.9 | 84.1 | 83.6 | 84.1 | 82.9 |
| 7   | 55.1 | 47.6 | 48.2 | 48.0 | 49.2 | 49.9 | 47.4 | 55.0 |
| 8   | 74.6 | 75.2 | 74.9 | 75.0 | 79.4 | 78.9 | 75.2 | 74.7 |
| 9   | 48.0 | 43.9 | 45.9 | 44.6 | 46.8 | 46.8 | 43.9 | 45.2 |
| 10  | 45.3 | 44.8 | 44.7 | 44.6 | 45.4 | 44.7 | 45.0 | 45.2 |
| 11  | 52.1 | 50.6 | 50.5 | 50.5 | 51.6 | 51.0 | 51.3 | 52.1 |
| 12  | 31.0 | 30.5 | 29.8 | 30.2 | 31.6 | 30.8 | 30.4 | 30.7 |
| 13  | 41.3 | 41.0 | 38.9 | 41.1 | 41.0 | 40.9 | 40.9 | 41.0 |
| 14  | 76.0 | 81.5 | 81.5 | 80.7 | 82.3 | 81.0 | 81.4 | 81.6 |
| 15  | 41.8 | 42.3 | 42.3 | 42.4 | 79.6 | 79.4 | 42.3 | 42.0 |
| 16  | 83.6 | 84.1 | 83.8 | 84.2 | 93.0 | 93.1 | 84.0 | 83.9 |
| 17  | 66.7 | 65.4 | 65.6 | 65.5 | 64.7 | 64.2 | 64.5 | 63.8 |
| 18  | 80.0 | 79.8 | 79.0 | 78.9 | 80.3 | 79.8 | 78.7 | 79.7 |
| 19  | 58.6 | 57.8 | 57.8 | 57.8 | 59.4 | 58.9 | 58.1 | 59.3 |
| 20  | 50.9 | 50.1 | 50.1 | 50.1 | 51.0 | 50.5 | 50.1 | 50.1 |
| 21  | 10.3 | 10.6 | 10.7 | 10.7 | 11.3 | 10.9 | 10.6 | 10.7 |
| OCH3-1 | 56.3 | 56.0 |
| OCH3-6 | 58.5 |
| OCH3-16 | 56.5 | 56.5 | 56.6 | 56.5 | 58.0 | 57.6 | 56.5 | 56.5 |
| OCH3-18 | 59.5 | 59.6 | 59.6 | 59.6 | 59.9 | 59.5 | 59.6 | 59.5 |
| 1′  | 97.7 | 103.1 | 100.3 | 109.2 | 103.9 | 109.4 | 103.1 | 103.2 |
| 2′  | 77.9 | 72.2 | 70.7 | 81.7 | 72.7 | 82.0 | 72.2 | 72.2 |
| 3′  | 75.8 | 74.7 | 71.5 | 79.1 | 75.2 | 79.1 | 74.7 | 74.6 |
| 4′  | 84.0 | 69.8 | 70.2 | 87.7 | 70.4 | 87.5 | 69.8 | 69.7 |
| 5′  | 63.6 | 67.2 | 64.8 | 63.4 | 67.9 | 63.4 | 67.2 | 67.1 |

aData were measured in CD3OD at 125 MHz for 1 and 5 and at 150 MHz for 2–4 and 6–8, respectively. The assignments were based on 1H–1H COSY, HSQC, and HMBC experiments.

Figure 2 Main 1H–1H COSY (thick lines) and three-bond HMBC (arrows, from 1H to 13C) correlations of compounds 1–8.
C-14, from H2-5’ to C-1’, and from H-14 to C-1’ located α-L-arabinopyranosyl at C-14 in 5. The NOESY correlations supported that the aglycone configuration was identical to fuziline. The configuration was further confirmed by isolation and identification of fuziline and L-arabinose from the acid hydrolysate of 5. Therefore, the structure of compound 5 was determined as fuziline 14-O-α-L-arabinopyranosyl and named aconicarmichoside I.

Compound 6 was obtained as a colorless gum with [α]D20 +19.8 (c 0.12, MeOH). As compared, the NMR spectroscopic data for the aglycone of 6 were well consistent with those of 5, whereas the data for the pentosyl were almost overlapped with those of 6 (Tables 1 and 2). Thus, 6 was elucidated as the isomer of 5 with replacement of the α-L-arabinopyranosyl by an α-L-arabinofuranosyl. This was proved by analysis of the 2D NMR spectroscopic data of 6 (Figs. 2 and 3). Therefore, the structure of compound 6 was determined as fuziline 14-O-α-L-arabinofuranosyl and named aconicarmichoside J.

Compound 7, a colorless gum with [α]D20 +50.9 (c 0.43, MeOH), has the molecular formula C29H47NO9 as indicated from (+)-HR-ESI-MS and NMR spectroscopic data. The NMR spectroscopic data of 7 were similar to those of 2, except for resonances attributable to an additional methoxy group (δH4 3.32 and δC 56.3). In addition, as compared with those of 2, the H-1 and C-1 and C-2 resonances of 7 were significantly shifted by ΔδH +0.48 and ΔδC +10.3 and +6.5, respectively. These differences suggest that 7 is the 1-O-methyl ether of 2, e.g., the aglycone of 7 is 1-O-methylisotatalizidine (tatalizamine). The suggestion was proved by 2D NMR spectroscopic data of 7 (Figs. 2 and 3). Especially the HMBC correlations from OCH3-1 to C-1, from OCH3-16 to C-16, and from OCH3-18 to C-18 positioned the three methoxy groups in 7, while the HMBC correlations from H-1’ to C-14 and from H-14 to C-1’ located α-L-arabinopyranosyl at C-14. Hence, the structure of compound 7 was determined as talatizidine 14-O-α-L-arabinopyranosyloside and named aconicarmichoside K.

Compound 8 was obtained as a colorless gum with [α]D20 +5.5 (c 0.11, MeOH). Its spectroscopic data indicated that this compound was another C10-diterpenoid alkaloid α-L-arabinopyranosyloside. Comparison the NMR spectroscopic data between 8 and 7 demonstrated the presence of one more methoxy group (δH 3.35 and δC 58.6) in 8 and replacement of one methylene (CH2-6) in 7 by an oxymethine (δH 4.29 and δC 82.9) in 8. In addition, the H-5 resonance was changed from the broad singlet at δH 2.04 in 7 to a doublet at δH 2.35 (J = 6.6 Hz), and the H-18a, H-18b, and H-19a and C-7 resonances were significantly deshielded by ΔδH +0.33, +0.35, and +0.48 and ΔδC +7.6, respectively. This indicated that 8 was the 6-methoxy analogue of 7, which was supported by comparison of the NMR spectroscopic data of 8 with the reported data for the aglycone (6-methoxytalatizamine, chasmanine) and further confirmed by 2D NMR spectroscopic data analysis (Figs. 2 and 3). Therefore, the structure of compound 8 was determined as chasmanine 14-O-α-L-arabinopyranosyloside and named aconicarmichoside L.

Although the aglycones and sugar have positive specific rotations except for talatizamine ([α]D20 +4.4 (c 1.0, CHCl3))51, the β-L-arabinosides 1 and 3 have the positive data, whereas the α-L-arabinosides 2 and 4–8 have the larger negative data. This is consistent with that previously observed for neoline L-arabinosides48, suggesting that the C-1’ configuration of L-arabinosyl plays a decisive role in the specific rotations. Accordingly, the specific rotation data are valuable to preliminarily assign the configurations of the C10-diterpenoid alkaloid arabinosides. This was supported by acid hydrolysis of the neoline L-arabinoside isomers as well as of 2, 3, and 5. Because the absolute configuration of the co-occurring aglycones, including neoline52–54, isotalatizidine 55, fuziline50, talatizamine,51,52,55 and chasmanine51–54, were previously determined by chemical transformation and/or X-ray crystallography, the structures as shown in Fig. 1 represent the absolute configurations of 1–8. Additionally, based on the splitting pattern of H-1 in the 1H NMR spectra (Table 1), the ring A in 1–8 has a boat conformation in CD3OD52,53, which is consistent with that of the aglycones in crystals53,54,55.

Because the ring A conformation in solution may be affected by the presence of acid in the samples and because trifluoroacetic acid (TFA) was used in the isolation procedure, compounds 1–8 were suspected to be obtained as trifluoroacetates57–59. Although the 13C NMR spectra of 1–8 did not display the corresponding resonances of TFA, the presence of TFA in the samples were confirmed by the 19F NMR spectra. Furthermore, using benzene (C6H6) and hexafluorobenzene (C6F6) as internal standards, ratios of the alkaloid and TFA in these samples were estimated to be 1:0.8 to 1:3.1 (Supplementary Information Figs. S107–S122). To preliminarily investigate the influence of TFA on the ring conformations, the NMR spectra of 2 and 7 in pyridine-d5 were acquired. As shown in Figs. 4 and 5, comparing with those of the same samples in CD3OD, intensity and resolution of some resonances especially for the ring A and N-Et moieties in 7 were significantly decreased or disappeared in pyridine-d5. However, this phenomenon was not observed for 2. The results demonstrated that (a) the different conformations of the ring A and N-Et moieties in 7 were relatively slowly transformed during the time scale of the NMR detection under the basic condition of pyridine-d5, (b) TFA

Figure 3 Main NOE correlations (pink dashed double arrows) of compounds 1–8.
Based on clinic application of “Fu Zi”, the analgesic effects of compounds 1, 2, and 4–8 were evaluated using acetic acid-induced writhing assay except for 3 due to limitation of the small sample amount. As shown in Table 3 and Fig. 6, at doses of 1.0, 0.3, and 0.1 mg/kg (i.p.), as compared with the vehicle group, 1, 2, and 4–6 exhibited significant reduction of writhing of mice in a dose-dependent manner. However, compounds 7 and 8 showed weak activity with <20% inhibition of writhes at the high dose of 1.0 mg/kg (i.p.). This result indicated that the structural change of the L-arabinosyl moieties in 1, 2, and 4–6 and the absence of the methoxy group at C-6 in 2 and 4 had little influence on the analgesic activity. Whereas, methylation of the hydroxyl group at C-1 (7 and 8) significantly decreased the activity.

3. Conclusions

Eight new aconitine-type C19-diterpenoid alkaloid L-arabinosides (1–8) were isolated and characterized from the lateral roots of A. carmichaelii (Fu Zi). These compounds, together with four neoline 14-O-arabinosides from the same extract, are the only glycosidic diterpenoid alkaloids, though around a thousand C20-, C19-, and C18-diterpenoid alkaloids have been isolated from nature. This finding not only adds diversity of the bioactive diterpenoid alkaloids, but also provides a solid evidence for the occurrence of glycosidation of the diterpenoid alkaloids in plant. The analgesic effects of 1, 2, and 4–6 supports clinic application of the traditional herbal medicine, and provides candidates for new drug development. In addition, the structure–activity relationship observed in this study directs a rational path for structural modification of the C19-diterpenoid alkaloids to improve their biological and pharmaceutical properties.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on P-2000 polarimeter (JASCO, Tokyo, Japan). IR spectra were recorded on a Nicolet 5700 FT-IR microscope instrument (FT-IR microscope transmission) (Thermo Electron Corporation, Madison, WI, USA). NMR spectra were obtained at 500 MHz or 600 MHz for H NMR, 125 MHz or 150 MHz for C NMR, and 470 MHz for F NMR respectively, on Inova 500 or SYS 600 (Varian Associates Inc., Palo Alto, CA, USA), or Bruker 600 NMR, Bruker 500 NMR (Bruker Corp, Karlsruhe, Germany) spectrometer in MeOD-d4, D2O, or pyridine-d5 with TMS or solvent peaks as references. ESI-MS and HR-ESI-MS data were obtained on Agilent 1100 Series LC-MS-D-Trap-SL and Agilent 6520 Accurate-Mass Q-TOFL CMS spectrometers (Agilent Technologies, Ltd., Santa Clara, CA, USA), respectively. Column chromatography (CC) was performed with macroporous adsorbent resin (HPD-100, Cangzhou Bon Absorber Technology Co., Ltd., Cangzhou, China), MCI gel (CHP 20P, 75–150 μm, Mitsubishi Chemical Inc., Tokyo, Japan), silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), or CHP 20P (Mitsubishi Chemical Inc., Tokyo, Japan). HPLC separation was performed on a system consisting of an Agilent ChemStation for LC system, an Agilent 1200 pump, and an Agilent 1100 single-wavelength absorbance detector (Agilent Technologies, Ltd.) or a Smartline RI detector (Knauer, Berlin, Germany) detector, using containing in the sample of 7 should play an important role to stabilize the conformation in CD3OD; (c) an intramolecular hydrogen bonding between 1-OH and nitrogen atom would be the key fact to stabilize the conformation of 2, since 2 and 7 differ only in replacement of 1-OH in 2 by 1-OCH3 in 7 and since the conformation of 2 was unchanged in the two solvents. This, together with our previous observations, indicates that acids and bases may cause conformational and/or structural changes of the diterpenoid alkaloids. However, the changes are highly dependent upon the chemical structures of the diterpenoid alkaloids. Because TFA was undoubtedly introduced during the experimental procedure, the structure assignments of the diterpenoid alkaloids 1–8 were unambiguous though the samples contained the different amounts of TFA and possible formation of trifluoroacetates and/or equilibration of dissociation in solution could not be excluded. Notably, having alkali properties, in hydrophilic bio-systems the diterpenoid alkaloids would interact with the endogenous acidic and basic biomolecules or microenvironments to increase solubility, bioavailability, and transportsations as well as to play biological functions. This deserves further investigations on a case by case basis.
The H₂O extract was concentrated to 120 L under reduced pressure without further purification. The lateral root of *A. carmichaelii* was powdered and extracted with H₂O (3 × 150 mL × 6 h) at 40 °C. The H₂O extract was concentrated to 120 L under reduced pressure, subjected to chromatography over a macroporous adsorbent resin (HPD-110, 19 kg) column (200 cm × 20 cm), and eluted successively with H₂O (50 L), 30% EtOH (120 L), 50% EtOH (120 L), and 95% EtOH (100 L) to afford the corresponding fractions A–D. After removal of the solvent, fraction C (3.5 kg) was chromatographed over MCI gel (CHP 20 P) with successive elution using H₂O (10 L), 30% EtOH (30 L), 50% EtOH (20 L), and 95% EtOH (10 L) to give fraction C1–C4. Fraction C2 (600 g) was chromatographed over MCI gel (CHP 20 P), with successive elution using H₂O (10 L), 30% EtOH (30 L), 50% EtOH (20 L), and 95% EtOH (10 L), to yield corresponding subfractions C2-1–C2-4. Fraction C2-1 (200 g) was dissolved in H₂O (500 mL), basified to pH 10 with concentrated ammonium hydroxide (25 mL), then extracted with EtOAc (500 mL × 4). The aqueous layer was acidified to pH 4 with 6 mol/L HCl (66 mL), and partitioned with n-butanol (500 mL × 3). Evaporation of the aqueous phase under reduced pressure yielded C2-1-C (32 g). Fraction C2-1-C was subjected to CC over silica gel, eluting with a gradient of CHCl₃–MeOH (1:1) to afford C2-1-C-1–C2-1-C-6. Fraction C2-1-C-4 (4 g) was separated by CC over Sephadex LH-20 (MeOH) to yield C2-1-C-4-1–C2-1-C-4-6, of which C2-1-C-4-4 (2 g) was further fractionated by CC over Sephadex LH-20 (50% MeOH in H₂O) to give C2-1-C-4-4-1–C2-1-C-4-4-6. Separation of C2-1-C-4-4-1 (340 mg) by reverse phase C-18 silica gel (10–90% MeOH in H₂O) afforded C2-1-C-4-4-1-1–C2-1-C-4-4-1-5, of which C2-1-C-4-4-1-1 (66 mg) was separated by CC over silica gel, eluting with a gradient of EtOAc–MeOH–H₂O (8:3:1–8:4:1), to obtain C2-1-C-4-4-1-1-1 and C2-1-C-4-4-1-1-2. Purification of C2-1-C-4-4-1-1-1 (30.6 mg) by reversed phase HPLC (Ultimate XB-phenyl semi-preparative column, 28% MeOH in H₂O, containing 0.1% TFA, 2 mL/min) obtained 7 (3 mg, τᵣ = 45 min). Fraction C2-1-C-4-4-1-3 (20 mg) was isolated by HPLC using the same column (40% MeOH in H₂O containing 0.2% TFA, 2 mL/min) to obtain 8 (3.4 mg, τᵣ = 27 min). Fraction C2-1-C-5 (13.0 g) was separated by CC over Sephadex LH-20 (H₂O) to give C2-1-C-5-1–C2-1-C-5-6, of which C2-1-C-5-4 (4 g) was further fractionated by CC over Sephadex LH-20 (H₂O) to afford C2-1-C-5-4-1–C2-1-C-5-4-6. Fraction C2-1-C-5-4-6 (1.1 g) was separated by CC over reverse phase C-18 silica gel (0–50% MeOH in H₂O) to give C2-1-C-5-4-6-1–C2-1-C-5-4-6-5, of which C2-1-C-5-4-6-3 (53.7 mg) was purified by reversed phase HPLC (30% MeOH in H₂O, containing 0.1% TFA, 2 mL/min) to yield 2 (10.4 mg, τᵣ = 45 min).

Fraction C2-2 (200 g) was separated by CC over Sephadex LH-20 (CHCl₃–MeOH, 1:1) yielded C2-2-1–C2-2-8. Fraction C2-2-4 (9.5 g) was chromatographed over silica gel (150 g) eluting with a

| Group          | Reagent       | Dose (mg/kg) | Number of writhing | Percent inhibition (%) |
|---------------|--------------|-------------|-------------------|------------------------|
| Vehicle group | Normal saline| –           | 38.9 ± 5.58       | –                      |
| Positive group| Morphine     | 0.3         | 18.3 ± 1.60       | 65.47                  |
| Test group    | 1            | 0.1         | 20.1 ± 3.28       | 43.15                  |
|               |              | 0.3         | 12.8 ± 2.44       | 63.63                  |
|               |              | 1.0         | 7.80 ± 2.58       | 78.34                  |
|               | 2            | 0.1         | 31.4 ± 4.75       | 19.23                  |
|               |              | 0.3         | 14.3 ± 3.54       | 63.34                  |
|               |              | 1.0         | 12.3 ± 2.07       | 68.39                  |
|               | 4            | 0.1         | 15.3 ± 5.54       | 60.56                  |
|               |              | 0.3         | 15.7 ± 3.06       | 59.61                  |
|               |              | 1.0         | 13.4 ± 2.76       | 65.59                  |
|               | 5            | 0.1         | 25.67 ± 4.32      | 27.49                  |
|               |              | 0.3         | 12.8 ± 2.98       | 63.84                  |
|               |              | 1.0         | 11.7 ± 3.09       | 67.04                  |
|               | 6            | 0.1         | 11.2 ± 4.35       | 68.36                  |
|               |              | 0.3         | 13.1 ± 2.68       | 62.87                  |
|               | 7            | 1.0         | 29.0 ± 2.07       | 20.01                  |
|               | 8            | 1.0         | 29.4 ± 3.85       | 13.82                  |

~Not applicable. Data are expressed as mean ± SEM, n = 10.

**P < 0.01.

***P < 0.001 compared to model group.

Table 3: Experimental data for the analgesic effect of compounds 1, 2, and 4–8.
gradient of petroleum ether-Me2CO-diethylamine (5:2:1-2:2:1) to give C2-2-4-1–C2-2-4-7. Fraction C2-2-4-6 (2.65 g) was separated by CC over silica gel, eluting with a gradient of CHCl3 (saturated with ammonia water)–MeOH (20:1–5:1), to give C2-2-4-6-1–C2-2-4-6-11, of which C2-2-4-6-1 (1.5 g) was further fractionated by CC over reversed phase C-18 silica gel (30%–50% MeOH in H2O) to give C2-2-4-6-6–C2-2-4-6-6-3. Fraction C2-2-4-6-6-1 (1.2 g) was subjected to preparative TLC [CHCl3 (saturated with ammonia water)–MeOH, 5:1] to yield C2-2-4-6-6-1 and C2-2-4-6-6-2. Further separation of C2-2-4-6-6-1 (0.9 g) by reversed phase HPLC (15% MeCN in H2O containing 0.1% TFA, 2 mL/min) yielded C2-2-4-6-6-1-1–C2-2-4-6-6-1-1-5, of which C2-2-4-6-6-1-1-2 (250 mg) was separated by HPLC (10% MeCN in H2O containing 0.1% TFA, 2 mL/min) to yield C2-2-4-6-6-1-1-2–C2-2-4-6-6-1-1-2-5. Fraction C2-2-4-6-6-1-1-2-4 (38 mg) was isolated by HPLC (25% MeOH in H2O containing 0.1% TFA, 2 mL/min) yield 4 (6.5 mg, Rg = 70 min). Fraction C2-2-4-6-6-1-1-2-5 (20 mg) was separated by HPLC (30% MeOH in H2O containing 0.1% TFA, 2 mL/min) to afford 5 (1.8 mg, Rg = 48 min). Fraction C2-2-4-6-7 (110 mg) was separated by preparative TLC [CHCl3 (saturated with ammonia water)–MeOH (5:1)] to yield C2-2-4-6-7-1–C2-2-4-6-7-3, of which C2-2-4-6-7-1 (20 mg) was isolated by HPLC (19% acetonitrile in H2O containing 0.1% TFA, 2 mL/min) to yield 6 (1.5 g, Rg = 19 min). Fraction C2-2-4-6-8 (110 mg) was separated by HPLC (40% MeOH in H2O containing 0.1% TFA) to yield C2-2-4-6-8-1–C2-2-4-6-8-11, of which C2-2-4-6-8-3 (76 mg) was fractionated by HPLC (40% MeOH in H2O containing 0.1% TFA) to afford C2-2-4-6-8-3–C2-2-4-6-8-3-4. Compound 3 (1 mg, Rg = 35 min) was isolated by HPLC (40% MeOH in H2O, containing 0.1% TFA, 2 mL/min) and 5 (3 mg, Rg = 46 min) from C2-2-4-6-8-4 (65 mg) by HPLC (37% MeOH in H2O, containing 0.1% TFA, 2 mL/min).

4.3.1. Aconicarmichoside E (1) Colorless gum; [α] D 23 +28.3 (c 0.14, MeOH); IR ν max 3408, 1655, 1403, 1194, 1143, 1011, 802, 724 cm⁻¹; ¹H NMR (CD3OD, 600 MHz) spectroscopic data, see Table 1; 13C NMR (CD3OD, 150 MHz) spectroscopic data, see Table 2; (+)-ESI-MS m/z 540.3182 [M + H]+ (Calcd. for C28H46NO9, 540.3167).

4.3.2. Aconicarmichoside F (2) Colorless gum; [α] D 23 +28.3 (c 0.63, MeOH); IR ν max 3408, 1655, 1403, 1194, 1143, 1011, 802, 724 cm⁻¹; ¹H NMR (CD3OD, 600 MHz) spectroscopic data, see Table 1; 13C NMR (CD3OD, 150 MHz) spectroscopic data, see Table 2; (+)-ESI-MS m/z 540 [M + H]+; (+)-HR-ESI-MS m/z 540.3182 [M + H]+ (Calcd. for C28H46NO9, 540.3167).

4.3.3. Aconicarmichoside G (3) Colorless gum; [α] D 23 +28.3 (c 0.14, MeOH); IR ν max 3408, 1655, 1403, 1194, 1143, 1011, 802, 724 cm⁻¹; ¹H NMR (CD3OD, 600 MHz) spectroscopic data, see Table 1; 13C NMR (CD3OD, 150 MHz) spectroscopic data, see Table 2; (+)-ESI-MS m/z 562 [M + Na]+, 540 [M + H]+; (+)-HR-ESI-MS m/z 540.3182 [M + H]+ (Calcd. for C28H46NO9, 540.3167).

4.3.4. Aconicarmichoside H (4) Colorless gum; [α] D 23 +28.3 (c 0.40, MeOH); IR ν max 3371, 2946, 1679, 1439, 1204, 1137, 842, 802, 724 cm⁻¹; ¹H NMR (CD3OD, 600 MHz) spectroscopic data, see Table 1; 13C NMR (CD3OD, 150 MHz) spectroscopic data, see Table 2; (+)-ESI-MS m/z 562 [M + Na]+, 540 [M + H]+; (+)-HR-ESI-MS m/z 540.3181 [M + H]+ (Calcd. for C28H46NO9, 540.3167).

4.3.5. Aconicarmichoside I (5) Colorless gum; [α] D 23 +7.7 (c 0.15, MeOH); IR ν max 3334, 2944, 1678, 1436, 1293, 1202, 1136, 1010, 954, 840, 801, 724 cm⁻¹; ¹H NMR (CD3OD, 500 MHz) spectroscopic data, see Table 1; 13C NMR (CD3OD, 125 MHz) spectroscopic data, see Table 2; (+)-ESI-MS m/z 586 [M + H]+; (+)-HR-ESI-MS m/z 586.3239 [M + H]+ (Calcd. for C28H46NO9, 586.3222).

4.3.6. Aconicarmichoside J (6) Colorless gum; [α] D 23 +19.8 (c 0.12, MeOH); IR ν max 3358, 2924, 2853, 1678, 1468, 1427, 1311, 1204, 1137, 842, 802, 723 cm⁻¹; ¹H NMR (CD3OD, 600 MHz) spectroscopic data, see Table 1; 13C NMR (CD3OD, 150 MHz) spectroscopic data, see Table 2; (+)-ESI-MS m/z 586 [M + H]+; (+)-HR-ESI-MS m/z 586.3228 [M + H]+ (Calcd. for C28H46NO9, 586.3222).

4.3.7. Aconicarmichoside K (7) Colorless gum; [α] D 23 +50.9 (c 0.43, MeOH); IR ν max 3363, 3074, 2926, 1685, 1446, 1381, 1257, 1203, 1136, 1087, 1012, 950, 925, 880, 837, 801, 721 cm⁻¹; ¹H NMR (CD3OD, 600 MHz) spectroscopic data, see Table 1; 13C NMR (CD3OD, 150 MHz) spectroscopic data, see Table 2; (+)-ESI-MS m/z 554 [M + H]+; (+)-HR-ESI-MS m/z 554.3335 [M + H]+ (Calcd. for C29H48NO14, 554.3324).

4.3.8. Aconicarmichoside L (8) Colorless gum; [α] D 23 +5.5 (c 0.11, MeOH); IR ν max 3366, 2942, 1684, 1444, 1378, 1202, 1139, 1078, 1010, 953, 844, 801, 724 cm⁻¹; ¹H NMR (CD3OD, 600 MHz) spectroscopic data, see Table 1; 13C NMR (CD3OD, 150 MHz) spectroscopic data, see Table 2; (+)-ESI-MS m/z 584 [M + H]+; (+)-HR-ESI-MS m/z 584.3437 [M + H]+ (Calcd. for C30H52NO16, 584.3429).

**Figure 6** Analgesic effects of compounds 1, 2, and 4–8, and morphine against acetic acid-induced writhing of mice.
4.4. Acid hydrolysis of 2, 3, and 5

Compounds 2, 3, and 5 (2–5 mg, each) were separately dissolved in acetonitrile (0.1 mL), and hydrolyzed in 2 mol/L HCl (2.0 mL) at 95 °C for 3 h. After evaporation under reduced pressure, the residue was chromatographed by CC over Sephadex LH-20 (MeOH) to yield aglycone (0.8–2.3 mg) and sugar (0.5–1.6 mg). By comparison of the 1H NMR spectroscopic data (Supplementary Information Figs. S25–S29, S42, S43, and S68–S70) and specific rotation with those of authentic diterpenoid alkaloid samples previously isolated in this study and commercially available sugar samples, the aglycones were identified as isotalatizidine \([\psi_{D}^0 +23.4 (c 0.09, MeOH) +21.5 (c 0.05, MeOH)]\) from 2 and 3 and fusilizine \([\psi_{D}^0 +8.2 (c 0.04, MeOH)]\) from 5, respectively, while the sugar from the three compounds was identified as 1-arabinose \([\psi_{D}^0 +96.6 to +106.3 (c 0.05–0.10, H_2O)]\).

4.5. Acetic acid-induced writhing test

An acetic acid-induced writhing method was adopted for the evaluation of analgesic activity. Briefly, ICR female mice were randomly divided into five groups (10 mice per group), and pre-treated intraperitoneally with normal saline (the vehicle group), morphine (0.3 mg/kg, the positive control group), and each compound (1.0 mg/kg, 0.3 mg/kg, and 0.1 mg/kg, the three test groups), respectively. 30 min later, mice were treated by intraperitoneal injection of 1.0% v/v acetic acid solution (0.1 mL/kg). The number of writhing was recorded for 15 min. The analgesic effects of the test compounds and positive control were respectively expressed by decreasing the number of writhes compared to normal saline. Percent inhibition was calculated using formula as below:

\[
\text{Percent inhibition (%) = } \left( \frac{W_m - W_i}{W_m} \right) \times 100
\]

where \(W_m\) is the number of writhing of the vehicle group, and \(W_i\) is the number of writhing of test group or positive group. The results (Fig. 6 and Table 3) showed that compounds 1, 2, 4–6 significantly reduced the writhes induced by acetic acid in a dose-dependent manner.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aspbs.2018.03.009.

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