Three New Steroidal Glycosides from the Roots of Cynanchum auriculatum

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Abstract: Three new steroidal glycosides, cyanoauriculosides F, G and H (1-3), were isolated from the roots of Cynanchum auriculatum (Asclepiadaceae) along with two known steroidal derivatives. On the basis of spectroscopic analysis and chemical methods, their structures were identified as 20-O-acetyl-8,14-seco-penupogenin-8-one 3-O-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside (1), 2′,3′-Z-gagaminine 3-O-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside (2), 17-O-acetyl-kidjoranin 3-O-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-digitoxopyranoside (3), gagaminine 3-O-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-digino-pyranosyl-(1→4)-β-D-cymaropyranoside (4) and wilfoside D1N (5).

Keywords: Cynanchum auriculatum; steroidal glycosides; cyanoauriculosides

1. Introduction

Cynanchum auriculatum is a famous traditional medicine widely used in south China for the prevention of hair graying, strengthening sinews and bones, and enhancing immunity [1]. In previous papers, we reported the isolation of five new C-21 steroidal glycosides, named cyanoauriculosides A-E,
from the roots of *C. auriculatum* [2]. Many C-21 steroidal glycosides isolated from *C. auriculatum* species have shown certain antitumor activities *in vitro* [3,4]. In a further phytochemical investigation of traditional Chinese medicinal plants to search for novel biologically active compounds, three new steroidal glycosides named cyanoauriculiosides F-H (1–3, Figure 1) were obtained from the roots of *Cynanchum auriculatum* (Asclepiadaceae), along with two known steroidal derivatives. All the structures were established on the basis of spectroscopic analysis and chemical methods.

**Figure 1.** Structures of new compounds 1–3.

2. Results and Discussion

Compound 1, obtained as a white amorphous powder, showed a positive reaction in the Libermann-Buchard and Keller-Killiani tests, indicating the presence of a steroidal skeleton with a 2-deoxysugar moiety [5]. Its molecular formula C₆₀H₉₀O₂₀ was deduced from the HRESIMS spectrum (*m/z* 1,165.5694 [M+Cl]⁻, calcd 1,165.5713). The ¹H-NMR and ¹³C-NMR data (Tables 1-2) suggested that
1 was a C-21 steroidal glycoside. The $^1$H-NMR spectrum showed three Me groups at $\delta_{\text{H}}$ 2.02 (s), 1.57 (3H, d, $J = 5.8$ Hz) and 1.31 (s), three oxygenated CH groups at $\delta_{\text{H}}$ 5.17 (1H, m), 4.03 (1H, m) and 3.85 (1H, m), one olefinic proton at $\delta_{\text{H}}$ 5.26 (1H, br s), one cinnamoyl group at $\delta_{\text{H}}$ 6.81 (1H, d, $J = 16.0$ Hz), 8.02 (1H, d, $J = 16.0$ Hz), 7.65 (2H, m) and 7.33 (3H, m) which was supported by the ion fragment at $m/z = 147$ [C$_9$H$_7$O$_2$]$^+$ arising from the aglycone moiety. From $^1$H-NMR, $^{13}$C-NMR, HSQC and HMBC data, one acetyl group was also identified by the observation of a proton signal at $\delta_{\text{H}}$ 1.96 (3H, s) and two carbon signals at $\delta_{\text{C}}$ 170.5, 21.6. Comparison of the $^{13}$C-NMR data of the aglycone portion of 1 with that of penupogenin [6,7], showed that the major difference between the two substances was the presence of an additional acetyl group in 1, and the fact that the chemical shift of C-20 in 1 was deshielded by ca. 6 ppm. This observation suggested that the extra acetyl group was located at C-20, which was supported by HMBC correlation between H-20 ($\delta_{\text{H}}$ 4.03, m) and C-10’ ($\delta_{\text{C}}$ 170.5). Additionally, the chemical shifts of 1 are different from those of penupogenin at C-7 (+8.1 ppm), C-9 (+13.2 ppm), C-14 (-7.4 ppm) and C-18 (+8.9 ppm), and the chemical shift of C-8 appears at $\delta$ 209.8. It could be further speculated that the hydroxy group at C-8 was oxidized into a carbonyl, which was supported by HMBC correlations between H-14 ($\delta_{\text{H}}$ 4.95, m) and C-18 ($\delta_{\text{C}}$ 20.3), and between H-9 ($\delta_{\text{H}}$ 2.21, m) and C-8 ($\delta_{\text{C}}$ 209.8). Thus, the aglycone of compound 1 was determined to be 20-0-acetyl-8,14-seco-penupogenin-8-one. In the NOESY spectrum, NOE correlations between H-9 ($\delta_{\text{H}}$ 2.21, m) and H-12 ($\delta_{\text{H}}$ 5.17, m) provided evidence for a $\beta$-linked 12-O-cinnamoyl group. Based on the literature [8], the stereochemistry of the C-14 hydroxyl group was assigned as $\beta$.

With respect to the glycosidic portion, it contained four anomeric C-atoms with signals at $\delta_{\text{C}}$ 96.5 (C1$^I$), 101.0 (C1$^II$), 99.5 (C1$^III$) and 99.1 (C1$^IV$), corresponding to anomeric H-atom signals at $\delta_{\text{H}}$ (H) 5.10 (overlap), 5.10 (overlap), 4.95 (overlap) and 5.05 (overlap), which indicated that there were four sugar moieties in 1. Acidic hydrolysis of 1 afforded a sugar mixture of cymarose and diginose, identified by TLC comparison with authentic samples. Comparing the $^{13}$C-NMR spectrum with that of penupogenin showed that the chemical shift of 1 are different from those of penupogenin at C-2 (-2.3 ppm), C-3 (+5.4 ppm) and C-4 (-4.4 ppm), due to glycosidation, therefore the sugar moiety was linked to the C (3)-O of the aglycone. Furthermore, HMBC correlations between H-C (1$^I$) at $\delta_{\text{H}}$ (H) 5.10 and C (3) at $\delta_{\text{C}}$ (C) 76.9 were observed. Signals of each sugar unit (Table 2) were assigned by the HSQC, HMBC and $^1$H-$^1$H COSY analyses and sugar moieties were identified as two $\beta$-D-cymaropyranosyls, one $\alpha$-L-diginopyranosyl and one $\alpha$-L-cymaropyranosyl. The sequence of the sugar chain was determined by the HMBC spectrum, in which distinct correlations between H-C (1$^I$) at $\delta_{\text{H}}$ (H) 5.10 and C (3) at $\delta_{\text{C}}$ (C) 76.9, between H-C (1$^{II}$) at $\delta_{\text{H}}$ (H) 5.10 and C (4$^I$) at $\delta_{\text{C}}$ (C) 82.4, between H-C (1$^{III}$) at $\delta_{\text{H}}$ (H) 4.95 and C (4$^{II}$) at $\delta_{\text{C}}$ (C) 74.6, and between H-C (1$^{IV}$) at $\delta_{\text{H}}$ (H) 5.05 and C (4$^{III}$) at $\delta_{\text{C}}$ (C) 82.4. Thus, compound 1 was determined to be 20-O-acetyl-8,14-seco-penupogenin-8-one 3-O-$\alpha$-L-cymaropyranosyl-(1$\rightarrow$4)-$\beta$-D-cymaropyranosyl-(1$\rightarrow$4)-$\alpha$-L-diginopyranosyl-(1$\rightarrow$4)-$\beta$-D-cymaropyranoside, and named cyanoauriculoside F.

Compound 2, obtained as a white amorphous powder, showed positive reactions in the Libermann-Buchard and Keller-Killiani tests, indicating again the presence of a steroidal skeleton with a 2-deoxysugar moiety [5]. Its molecular formula C$_{64}$H$_{91}$NO$_{20}$ was deduced from the HRESIMS spectrum ($m/z$ 1,216.6033 [M+Na]$^+$, calcd 1,216.6032). The $^1$H-NMR and $^{13}$C-NMR data (Tables 1-2) suggested that 2 was a C-21 steroidal glycoside.
Table 1. NMR data of the aglycone moieties of compounds 1-3 δ in ppm, J in Hz.*

| No | 1          | 2         | 3          |
|----|------------|-----------|------------|
|    | δC         | δH        | δC         | δH        | δC         | δH        |
| 1  | 39.5 1.09 (m), 1.82 (m) | 39.2 1.11 (m), 1.83 (m) | 39.0 1.10 (m), 1.82 (m) |
| 2  | 29.8 1.78 (m), 2.10 (m) | 29.8 1.75 (m), 2.03 (m) | 29.9 1.79 (m), 2.09 (m) |
| 3  | 76.9 3.85 (m) | 77.6 3.81 (m) | 77.7 3.84 (m) |
| 4  | 38.6 2.42 (m), 2.56 (m) | 38.2 2.40 (m), 2.52 (m) | 39.3 2.41 (m), 2.52 (m) |
| 5  | 140.5 | 139.1 | 139.3 |
| 6  | 118.9 5.26 (br s) | 119.3 5.31 (br s) | 119.2 5.30 (m) |
| 7  | 41.8 2.84 (m), 3.31 (m) | 33.6 2.30 (m), 2.45 (m) | 34.7 2.32 (m), 2.48 (m) |
| 8  | 209.8 | 74.4 |
| 9  | 57.2 2.21 (m) | 44.0 1.71 (m) | 44.6 1.72 (m) |
| 10 | 38.0 | 37.5 |
| 11 | 25.4 2.18 (m), 2.30 (m) | 25.1 2.15 (m), 2.29 (m) | 25.1 2.16 (m), 2.32 (m) |
| 12 | 74.6 5.17 (m) | 74.5 5.18 (m) | 73.7 5.18 (m) |
| 13 | 57.2 | 58.2 |
| 14 | 81.5 4.95 (m) | 88.8 | 89.6 |
| 15 | 34.2 2.11 (m) | 34.7 2.09 (m) | 33.9 2.12 (m) |
| 16 | 33.4 2.02 (m), 3.26 (m) | 33.8 2.03 (m), 3.23 (m) | 33.1 2.05 (m), 3.24 (m) |
| 17 | 87.7 | 92.5 |
| 18 | 20.3 2.02 (s) | 11.0 2.02 (s) | 10.8 2.04 (s) |
| 19 | 18.9 1.31 (s) | 18.0 1.33 (s) | 18.3 1.32 (s) |
| 20 | 76.8 4.03 (m) | 76.3 3.93 (m) | 210.2 |
| 21 | 14.9 1.57 (d, J = 5.8) | 15.3 1.54 (d, J = 6.0) | 27.9 2.49 (s) |
| 1′ | 167.8 | 166.0 |
| 2′ | 119.4 6.81 (d, J = 16.0) | 120.0 6.80 (d, J = 12.0) | 119.4 6.82 (d, J = 16.0) |
| 3′ | 145.7 8.02 (d, J = 16.0) | 144.6 7.96 (d, J = 12.0) | 145.1 7.99 (d, J = 16.0) |
| 4′ | 135.0 | 135.1 |
| 5′ | 128.8 7.65 (m) | 128.1 7.62 (m) | 128.7 7.64 (m) |
| 6′ | 129.3 7.33 (m) | 130.0 7.35 (m) | 129.4 7.37 (m) |
| 7′ | 130.7 7.33 (m) | 129.0 7.35 (m) | 130.8 7.37 (m) |
| 8′ | 129.3 7.33 (m) | 130.0 7.35 (m) | 129.4 7.37 (m) |
| 9′ | 128.8 7.65 (m) | 128.1 7.62 (m) | 128.7 7.64 (m) |
| 10′ | 170.5 | 170.6 |
| 11′ | 21.6 1.96 (s) | 21.2 2.06 (s) |
| 12′ | 151.6 | 9.59 (d, J = 1.6) |
| 13′ | 123.9 | 7.28 (d, J = 7.2) |
| 14′ | 137.4 | 8.40 (d, J = 7.8) |
| 15′ | 127.1 |
| 16′ | 153.7 | 8.79 (dd, J = 5.6, 1.6) |

* Compound 1 and 3: in C5D5N; compound 2: in CD3OD.
Table 2. NMR data of the sugar moieties of compounds 1-3 δ in ppm, J in Hz. *

| No     | 1         | 2         | 3         |
|--------|-----------|-----------|-----------|
|        | δC        | δH        | δC        | δH        | δC        | δH        |
| β-D-cym|           |           |           |           |           |           |
| 1\( I \) | 96.5      | 5.10 (overlap) | 96.0      | 5.23 (overlap) | 96.4      | 4.89 (d, J = 9.5) |
| 2\( I \) | 35.2      | 1.76 (m), 2.37 (m) | 35.2      | 1.73 (m), 2.30 (m) | 39.2      | 1.79 (m), 2.06 (m) |
| 3\( I \) | 77.5      | 3.88 (m)  | 77.6      | 3.88 (m)  | 67.2      | 4.64 (m)  |
| 4\( I \) | 82.4      | 3.44 (m)  | 82.3      | 3.46 (m)  | 83.5      | 3.51 (m)  |
| 5\( I \) | 69.2      | 4.18 (m)  | 69.3      | 4.19 (m)  | 68.6      | 4.27 (m)  |
| 6\( I \) | 18.8      | 1.49 (d, J = 6.1) | 18.5      | 1.50 (m)  | 18.6      | 1.51 (d, J = 6.0) |
| -OMe   | 57.2      | 3.50 (s)  | 57.2      | 3.46 (s)  |           |           |
| α-L-digin|           |           |           |           |           |           |
| 1\( II \) | 101.0     | 5.10 (overlap) | 100.9     | 5.14 (overlap) | 99.7      | 5.12 (d, J = 10.0) |
| 2\( II \) | 32.5      | 2.00 (m), 2.33 (m) | 32.4      | 1.98 (m), 2.32 (m) | 36.6      | 1.62 (m), 2.32 (m) |
| 3\( II \) | 73.8      | 4.01 (m)  | 73.8      | 3.98 (m)  | 70.7      | 3.85 (m)  |
| 4\( II \) | 74.6      | 3.82 (m)  | 74.7      | 3.79 (m)  | 80.0      | 3.23 (m)  |
| 5\( II \) | 67.5      | 4.23 (m)  | 67.4      | 4.11 (m)  | 69.8      | 3.64 (m)  |
| 6\( II \) | 17.9      | 1.40 (d, J = 6.3) | 17.8      | 1.40 (m)  | 18.7      | 1.33 (d, J = 7.5) |
| -OMe   | 55.4      | 3.40 (s)  | 55.3      | 3.41 (s)  |           |           |
| β-D-cym|           |           |           |           |           |           |
| 1\( III \)| 99.5     | 4.95 (overlap) | 99.3      | 5.05 (overlap) | 99.4      | 4.98 (d, J = 2.5) |
| 2\( III \)| 36.4     | 1.86 (m), 2.41 (m) | 36.3      | 1.86 (m), 2.41 (m) | 32.3      | 1.82 (m), 2.05 (m) |
| 3\( III \)| 77.9     | 3.87 (m)  | 77.6      | 3.86 (m)  | 77.9      | 3.85 (m)  |
| 4\( III \)| 82.4     | 3.44 (m)  | 82.3      | 3.42 (m)  | 73.4      | 3.62 (m)  |
| 5\( III \)| 69.5     | 4.21 (m)  | 69.3      | 4.21 (m)  | 65.1      | 4.48 (m)  |
| 6\( III \)| 18.7     | 1.25 (d, J = 6.1) | 18.3      | 1.26 (m)  | 18.7      | 1.50 (d, J = 6.0) |
| -OMe   | 58.3      | 3.51 (s)  | 58.2      | 3.46 (s)  | 56.8      | 3.37 (s)  |
| α-L-cym|           |           |           |           |           |           |
| 1\( IV \)| 99.1     | 5.05 (overlap) | 98.9      | 5.16 (m)  | 95.5      | 5.19 (d, J = 10.0) |
| 2\( IV \)| 32.2     | 1.89 (m), 2.33 (m) | 32.1      | 1.89 (m), 2.33 (m) | 36.6      | 3.37 (m)  |
| 3\( IV \)| 76.5     | 3.70 (m)  | 76.1      | 3.69 (m)  | 77.8      | 3.41 (m)  |
| 4\( IV \)| 73.3     | 3.59 (m)  | 73.2      | 3.61 (m)  | 82.4      | 3.31 (m)  |
| 5\( IV \)| 66.4     | 4.55 (m)  | 66.3      | 4.54 (m)  | 69.5      | 3.56 (m)  |
| 6\( IV \)| 18.5     | 1.37 (d, J = 6.3) | 18.1      | 1.37 (m)  | 18.7      | 1.35 (d, J = 6.5) |
| -OMe   | 56.7      | 3.37 (s)  | 56.5      | 3.37 (m)  |           |           |

* Compound 1 and 3: in C\(_2\)D\(_2\)N; compound 2: in CD\(_3\)OD; cym: cymaropyranosyl; digin: diginopyranosyl; glu: glucopyranosyl; digit: digitoxopyranosyl.
The $^1$H-NMR spectrum of 2 showed three Me groups at $\delta_H$ 2.02 (s), 1.54 (3H, d, $J = 6.0$ Hz) and 1.33 (s), three oxygenated CH groups at $\delta_H$ 3.81 (1H, m), 3.93 (1H, m) and 5.18 (1H, m), one olefinic proton at $\delta_H$ 5.31 (1H, br s), one cinnamoyl group at $\delta_H$ 6.80 (1H, d, $J = 12.0$ Hz), 7.96 (1H, d, $J = 12.0$ Hz), 7.62 (2H, m) and 7.35 (3H, m) which was supported by the ion fragment at $m/z = 147$ [C$_9$H$_{12}$O$_2$]$^+$ arising from the aglycone moiety. From $^1$H-NMR and $^{13}$C-NMR, one nicotinoyl group was supported by NOE correlations between H-21 ($\delta$ 2.49, s) and H-16 ($\delta$ 2.06, s), between $\delta$ 1.72, m) and H-12 ($\delta$ 5.18, m) gave evidence for a 12-O-cinnamoyl group that was $\beta$-linked. According to the literature [8], the stereochemistry of the C-14 hydroxyl group was assigned as $\beta$. The chemical shifts of C (13) and C (14) appear at $\delta$ 56.9 and 88.8, respectively. It could be deduced that the C/D ring junction was $cis$ compared with the same carbons at $\delta$ 41.6–42.7 and 58.7–59.2 for the trans form [8]. By comparing the spectroscopic data of the sugar moiety in 2 with those of 1, compound 2 was seen to possess the same sugar substitution pattern as that of 1. Thus, compound 2 was determined to be 2',3',Z-gagaminine 3-O-$\alpha$-L-cymaropyranosyl-(1$\rightarrow$4)-$\beta$-D-cymaropyranosyl-(1$\rightarrow$4)-$\alpha$-L-digaminopyranosyl-(1$\rightarrow$4)-$\beta$-D-cymaropyranoside, and named cyanoucariculicoside G.

Compound 3 was isolated as a white amorphous powder, and showed positive reactions in the Libermann-Buchard and Keller-Killiani tests, indicating the presence of another steroidal skeleton with a 2-deoxysugar moiety [5]. The molecular formula was established as C$_{65}$H$_{96}$O$_{23}$ according to the HRESIMS spectrum ($m/z$ 1,267.6268 [M+Na]$^+$, calcd. 1,267.6240). The $^{13}$C-NMR data of the aglycone portion of 3 were compared with those of kidjoranin [9], and showed that the major difference was the presence of an additional acetyl group [$\delta$(H) 2.06 (3H, s); $\delta$ (C) 170.6 (s), 21.2 (q)] in the structure of 3. Thus, the aglycone of compound 3 was proposed to be 17-O-acetylkidjoranin, which was supported by NOE correlations between H-21 ($\delta$ 2.49, s) and H-11' ($\delta$ 2.06, s), between H-16 ($\delta$ 2.05, 3.24, m) and H-11' ($\delta$ 2.06, s). The 17-$\alpha$ configuration was confirmed by the observation that the carbonyl carbon of the $\alpha$-linked methyl ketone at C-17 appears at $\delta$ 210.2, compared with $\delta$ 216 ppm for the $\beta$ configuration [10]. Thus, the 17-O-acetyl group was $\beta$-linked. In the NOESY spectrum, NOE correlations between H-9 ($\delta$ 1.71, m) and H-12 ($\delta$ 5.18, m) gave evidence for a 12-O-cinnamoyl group that was $\beta$-linked. The chemical shifts of C (13) and C (14) appear at $\delta$ 39.6–40.7 and 58.7–59.2, respectively. It could be deduced that the C/D ring junction was $cis$ compared with the same carbons at $\delta$ 41.6–42.7 and 58.7–59.2 for the trans form [8]. By comparing the spectroscopic data of the sugar moiety in 3 with those of 1, compound 2 was seen to possess the same sugar substitution pattern as that of 1. Thus, compound 2 was determined to be 2',3',Z-gagaminine 3-O-$\alpha$-L-cymaropyranosyl-(1$\rightarrow$4)-$\beta$-D-cymaropyranosyl-(1$\rightarrow$4)-$\alpha$-L-digaminopyranosyl-(1$\rightarrow$4)-$\beta$-D-cymaropyranoside, and named cyanoucariculicoside G.
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The sequence of the sugar chain was determined by HMBC spectrum, in which distinct correlations between H-C (1 II) at δ (H) 5.12 and C (4 I) at δ (C) 83.5, between H-C (1 III) at δ (H) 4.98 and C (4 II) at δ (C) 80.0, between H-C (1 IV) at δ (H) 5.19 and C (4 III) at δ (C) 73.4, and between H-C (1 V) at δ (H) 4.96 and C (4 IV) at δ (C) 82.4. Thus, compound 3 was determined to be 17-O-acetylkidjoranin-3-O-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-digitoxopyranoside, named cyanauriculoside H.

The known constituents were identified as gagaminine 3-O-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside (4) [6], and wilfoside D1N (5) [11] by comparison of their spectroscopic data with those reported in the literature.

3. Experimental

3.1. General

Column chromatography was carried using silica gel (200–300 mesh), and Thin-Layer Chromatography (TLC) was performed on silica gel GF254 from the Qingdao Haiyang Chemical Group Co., P. R. China. RP-18 silica gel was purchased from YMC CO., LTD., Japan. NMR spectra were run on a Bruker DRX-500 MHz spectrometer with TMS as internal standard. HRESIMS were measured on Micromass Q-Tof-Ultima mass spectrometer. The optical rotation was measured on a Jasco P-1020 polarimeter. HPLC was performed on an Ultimate 3000 apparatus using 5C18-MS-II column (ODS, 250 × 10 mm, 5 μm) and monitored by an UV detector.

3.2. Plant material

The roots of the C. auriculatum were collected from Jishou, Hunan Province, P. R. China, in September 2007, and identified by Prof. Ding-Rong Wan. The voucher specimen (07091201) was deposited in the Herbarium of College of Pharmacy, South Central University for Nationalities.

3.3. Extraction and isolation procedures

The aerial roots of C. auriculatum (4 kg) were powdered and extracted three times with 95% EtOH at room temperature (48, 48 and 24 h, 6 L × 3). The ethanolic extract (0.6 kg) was suspended in water (1.6 L) and then successively partitioned with petroleum ether (1.5 L × 3), CHCl3 (1.5 L × 3), EtOAc (1.5 L × 3) and n-BuOH (1.5 L × 3). The CHCl3 extract (195 g) was chromatographed on the silica column using gradient solvents of cyclohexane/EtOAc (100:0–0:100) and EtOAc/MeOH (100:0–0:100) to yield seven fractions (fr.1–fr.7). Fr.2 (4.3 g) was repeatedly chromatographed over a silica gel, then purified on a RP-C18 silica gel column to afford 1 (27 mg) and 5 (28 mg). Fr.4 (3.0 g) was subjected to CC (ODS, H2O/MeOH 9:1→1:9 gradient system) to afford 4 subfractions (Fr.4.1–Fr.4.4). Fr.4.1 was purified by semi-prep. HPLC (MeOH/H2O 80:20, 3 mL/min, tR 19.3 min) to yield 2 (30 mg). Fr.4.2 was purified by semi-prep. HPLC (MeOH/H2O 80:20, 3 mL/min, tR 23.7 min) to yield 4 (26 mg). Fr.4.3 was purified by semi-prep. HPLC (MeOH/H2O 80:20, 3 mL/min, tR 29.8 min) to yield 3 (32 mg).
3.4. Acid hydrolysis

A soln. of 1, 2, and 3 (each 5 mg) in MeOH was treated with 0.05 mol/L HCl, 4-dioxane 1:1 (1 mL) at 60 °C for 1.5 h, respectively. After removing dioxane, the soln. was extracted with EtOAc (3 × 2 mL). The aq. layer was neutralized by NaOH and concentrated under reduced pressure to give the sugar fraction. The presence of the monosaccharides in the hydrolysates of each compound was confirmed by TLC comparison with authentic samples. Cymarose was detected from compounds 1–3; diginose was detected from compounds 1 and 2; digitoxose was detected from compound 3. The Rf values of digitoxose, diginose and cymarose were 0.51, 0.66 and 0.76, respectively with CHCl3: MeOH (95:5), 0.07, 0.18 and 0.23, respectively with P.E.: Me2CO (3:1).

3.5. Physical data of new compounds

20-O-acetyl-8,14-seco-penupogenin-8-one 3-O-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside (1). White amorphous powder; UV λ_max (MeOH) nm (logε): 280 (4.35); [α]D^20 = + 19.2 (c 0.20, MeOH); For 1H-NMR and 13C-NMR spectroscopic data (in C5D5N), see Table 1 and 2; HRESIMS [M+Cl]^+ m/z 1,165.5694 (calcd. for C60H90O20: 1,165.5713).

2′,3′-Z-gagaminine 3-O-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside (2). White amorphous powder; UV λ_max (MeOH) nm (logε): 203 (4.32); [α]D^20 = + 21.6 (c 0.22, MeOH); For 1H-NMR and 13C-NMR spectroscopic data (in CD3OD), see Table 1 and 2; HRESIMS [M+Na]^+ m/z 1,216.6033 (calcd. for C64H91NO20Na: 1,216.6032).

17-O-acetyl-kidjoranin 3-O-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-digitoxopyranoside (3). White amorphous powder; UV λ_max (MeOH) nm (logε): 278 (4.37); [α]D^20 = - 60.2 (c 0.25, MeOH); For 1H-NMR and 13C-NMR spectroscopic data (in C5D5N), see Table 1 and 2; HRESIMS [M+Na]^+ m/z 1,267.6268 (calcd. for C65H96O23Na: 1,267.6240).

4. Conclusions

Twenty compounds were isolated from the dry roots *C.auriculatum Royle ex Wight*, including thirteen C-21 steroidal glycosides. The anti-tumour activity of these C-21 steroidal glycosides compounds has been studied. Further research on isolation and identification of more bioactive compounds will be helpful to understand this traditional medicine.

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Sample Availability: Samples of the compounds are available from the authors.

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