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Three new steroidal glycosides from roots of *Reineckia carnea*

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Two new spirostanols and a new furostanol, reinocarnoside A (**1**), B (**2**) and C (**3**), were isolated from the roots of *Reineckia carnea*, together with two known compounds, (25S)-1β,3β,4β-trihydroxyspirostan-5β-yl-*O*-β-D-glucopyranoside (**4**), kitigenin-5β-*O*-β-D-glucopyranoside (**5**). The structures of three new compounds were elucidated by spectroscopic methods including 1-D NMR, 2-D NMR and MS spectrums, and their anticancer activities were evaluated by MTT method.

Keywords: *Reineckia carnea*; steroidal glycosides; structural elucidation; anticancer activity; NMR

1. Introduction

Reineckia carnea (Andr.) Kunth, an evergreen medicinal herb, is a monotypic genus of *Reineckia* from family Liliaceae (syn. yudaicao, guanyincao, etc. in Chinese), and *R. carnea* is only distributed in China and Japan. In China, it is mainly distributed around Yangzi valley, and is an important medical plant to Miao nationality for haemostasia, relieving cough, clearing away the lung-heat, detoxification and relieving the inflammation of the pharynx (Fu, 2002). During the investigation of new anti-inflammatory and anticancer activity metabolites from the plants around Yangzi valley, we found the crude extracts from two plants, *Tupistra chinensis* Baker and *R. carnea*, showed strong anti-inflammatory activity *in vitro*, and we have isolated many new furostanols with anti-inflammatory and anticancer activity from the plant of *T. chinensis* (Guo et al., 2009; Xu et al., 2007; Zou, Wang, Du, Li, & Tu, 2006; Zou, Wang et al., 2007; Zou, Wu et al., 2007; Zou et al., 2009). Investigation of bioactive constituents from the dried rhizomes of *R. carnea*, has resulted in the isolation of three new compounds, reinocarnoside A (**1**), B (**2**) and C (**3**), together with two known compounds, (25S)-1β,3β,4β-trihydroxyspirostan-5β-yl-*O*-β-D-glucopyranoside (**4**), kitigenin-5β-*O*-β-D-glucopyranoside (**5**). In this article, the structural elucidation and anticancer activity of reinocarnoside A (**1**), B (**2**) and C (**3**) from *R. carnea* are reported (Figure 1).

2. Results and discussion

A total of 1.5 kg dried rhizomes of *R. carnea*, collected in Changyang of Hubei Province, were powdered and extracted with 70% EtOH, and the 70% EtOH extract was partitioned

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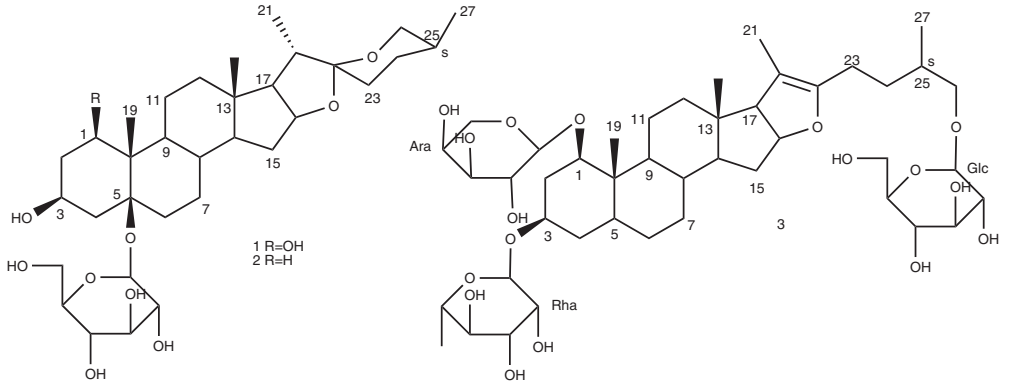

Figure 1. The structures of compounds **1**–**3**.

successively with petroleum ether, ethyl acetate and *n*-butanol. The *n*-butanol fraction was purified by repeated column chromatography, including silica gel, Sephadex LH-20 column, reverse phase silica gel chromatography and HPLC to afford three new and two known compounds.

Reinocarnoside A (**1**), which was obtained as white powder, possessed the molecular formula of $C_{33}H_{54}O_{10}$, which was determined on the basis of HR–ESI–MS data with $m/z[M+H]^+$ 611.7838 and $[M+Na]^+$ 633.7656 (Calcd 611.7835 and 633.7650, respectively) and NMR data. It gave a positive Liebermann–Burchard reaction and negative Ehrlich reaction (Wu, 2007), which suggested that **1** was spirostanol-type compound. Compound **1** was hydrolysed with 2 mol/L HCl to afford D-glucose by PC test only. The ^{1}H-NMR spectrum showed two singlet methyl signals and two doublet methyl signals at δ 0.80 (s, 18-CH_3, 3H), 1.10 (s, 19-CH_3, 3H), 1.16 (d, $J = 7.6$ Hz, 21-CH_3, 3H) and 1.06 (d, $J = 7.8$ Hz, 27-CH_3, 3H), respectively. In ^{13}C-NMR spectrum of compound **1**, the carbon resonance at δ 109.7 (C-22) obviously was the hemiketal carbon signal. These characteristic proton and carbon signals displayed that the aglycone of compound **1** might be a spirostanol-type structure. The ^{13}C-NMR and DEPT experiments disclosed that there were 4 quaternary carbons, 14 methines, 11 methenes and 4 methyls in compound **1**. Except for the carbon signals at δ 66.8 (C-3), 80.9 (C-16), 62.1 (C-17) and 64.7 (C-26), there were seven oxygenated carbons signals at δ 71.9 (C-1), 87.6 (C-5), 74.4 (Glu-2), 77.1 (Glu-3), 70.0 (Glu-4), 76.8 (Glu-5), 61.1 (Glu-6), in addition, the anomeric carbon signal at δ 95.6 (Glu-1), implying that a glucose existed and two substituted hydroxyl groups in the aglycone in reinocarnoside A (**1**). Comparing the ^{13}C-NMR data of A, B, C, D and E rings from reinocarnoside A to the reference compound tupichigenin E (Pan, Chang, Wei, & Wu, 2003), we found that they were exactly similar, besides glycosidation shift and deuterated solvent effects. Thus, the aglycone of reinocarnoside A was determined as spirostanol-1β,3β,5β,26-tetraol. The configuration of anomeric proton [δ_H 4.95 (d, $J = 7.8$ Hz, 1H), δ_C 95.6] from glucose was confirming by the coupling constant of anomeric proton and acid hydrolysis. The coupling constant of anomeric proton from glucose was 7.8 Hz, which indicated that the anomeric hydroxyl group was β configuration, and only β-D-glucose was test in acid hydrolysis procedure by PC. The linkage of β-D-glucose to the aglycone of reinocarnoside A was proved by the HMBC and HMQC experiments, and there was a cross-correlation signal from δ 4.95 (d, $J = 7.8$ Hz, Glu-1H, 1H) to δ 87.6 (C-5) in HMBC spectrum. The absolute configuration of 27-Me was determined as *S*, because of the difference of chemical shifts between H-26a and H-26b

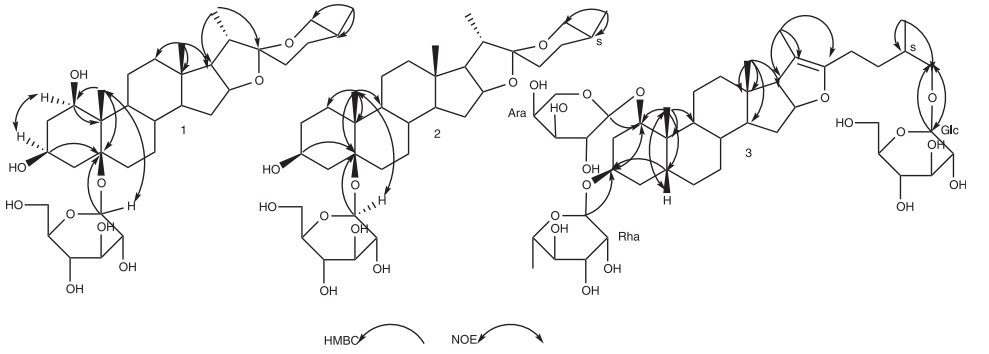

Figure 2. The key HMBC and NOE correlations of the new compounds **1**–**3**.

and the proton shift of 27-Me (Agrawal, 2005). Therefore, the structure of compound **1** was identified as (25S)-spirostanol-1β,3β,5β,26-tetraol-5β-D-glucopyranoside (Figure 2).

Reinocarnoside B (**2**) was obtained as white powder and its molecular formula was $C_{33}H_{54}O_9$, which was determined on the basis of HR–ESI–MS data with m/z $[M+H]^+$ 595.7844 and $[M+Na]^+$ 617.7662 (Calcd 595.7840 and 617.7666, respectively) and NMR data. It gave a positive Liebermann–Burchard reaction and negative Ehrlich reaction (Wu, 2007), which displayed that **2** was spirostanol-type compound. The ^{1}H-NMR spectrum showed two singlet methyl signals and two doublet methyl signals at δ 1.37 (s, 18-CH_3, 3H), 1.00 (s, 19-CH_3, 3H), 1.43 (d, $J = 7.6$ Hz, 21-CH_3, 3H) and 1.32 (d, $J = 6.5$ Hz, 27-CH_3, 3H), respectively. In ^{13}C-NMR spectrum of compound **2**, a carbon resonance at δ 110.4 (C-22) was the hemiketal carbon signal apparently. These characteristic proton and carbon signals suggested that the aglycone of **2** might be spirostanol-type structure. The ^{13}C-NMR and DEPT experiments showed that there were 4 quaternary carbons, 13 methines, 12 methenes and 4 methyls in compound **2**. Except for the carbon signals at δ 66.5 (C-3), 81.8 (C-16), 64.2 (C-17) and 67.6 (C-26), there were six oxygenated carbons signals at δ 90.2 (C-5), 75.2 (Glu-2), 79.4 (Glu-3), 71.2 (Glu-4), 78.8 (Glu-5), 63.8 (Glu-6), in addition, the anomeric carbon signal at δ 102.3 (Glu-1), implying that a glucose existed and one substituted hydroxyl group in the aglycone of reinocarnoside B (**2**). Comparing the NMR data of reinocarnoside B (**2**) to those of reinocarnoside A (**1**), we found that they were exactly similar, besides only one additional oxygenated carbon signal in compound **1,** which indicated that **2** was a analogue of compound **1**. Further comparing the NMR data of **1** and **2** carefully, the aglycone of compound **2** was identified as spirostanol-3β,5β,26-triol. Similarly, the results of the acid hydrolysis procedure and analysis of NMR data showed the structure of compound **2** was (25S)-spirostanol-3β,5β,26-triol-5β-D-glucopyranoside.

Compound **3** was obtained as a white amorphous powder. Its positive Molish reaction, Liebermann–Burchard reaction and Ehrlich reaction indicated its furostanol-type skeleton (Wu, 2007). The molecular formula of $C_{44}H_{72}O_{19}$ was established by HR–ESI–MS, which showed a quasi-molecular ion peak at m/z 874.0408 (Calcd for $[M+H]^+$ 874.0402) and 895.4663 (Calcd for $[M+Na]^+$ 895.4667). The ^{13}C-NMR spectrum and DEPT experiment indicated the presence of 44 carbon signals in compound **3**, including 5 methyls, 12 methenes, 23 methines and 4 quaternary carbons. Five methyl groups of compound **3** were observed at δ 0.90 (s, 3H) (H-18), 1.27 (s, 3H) (H-19), 1.64 (s, 3H) (H-21), 1.03 (d, $J = 6.0$ Hz, 3H) (H-27) and 1.71 (d, $J = 6.5$ Hz, 3H) (Rha-6), indicating that there was a 6-desose in compound **3**. Four protons of oxygenated methines or methylenes were found at δ 4.13 (m, 1H), 4.63 (m, 1H), 4.98 (m, 1H), 3.48 (t, $J = 9.2$ Hz, 1H), 4.10 (dd, $J = 10.0$, 9.2 Hz, 1H) in the

aglycone moiety. The above proton signals were correlated to their corresponding carbons at δ 83.4 (C-1), 78.6 (C-3), 85.7 (C-16) and 75.2 (C-26) in HMQC spectrum of compound **3**, respectively. These characteristic proton and carbon resonance signals, combined with the two sp^2 carbon signals at δ_C 153.1 (C-22) and 105.0 (C-20), suggested that the aglycone of compound **3** might be a 20(22)-en-furostanol-type steroid.

A total of 17 carbon signals of two hexoses and a pentaose were observed at δ 101.9 (Ara-1), 73.2 (Ara-2), 73.6 (Ara-3), 69.8 (Ara-4), 68.9 (Ara-5); 98.6 (Rha-1), 71.9 (Rha-2), 71.1 (Rha-3), 73.3 (Rha-4), 69.4 (Rha-5), 17.9 (Rha-6) and 104.8 (Glu-1), 74.3 (Glu-2), 78.2 (Glu-3), 71.8 (Glu-4), 77.8 (Glu-5), 62.9 (Glu-6) in the ^{13}C-NMR spectrum. In the HMQC spectrum, the three anomeric protons at δ 4.61 (d, $J = 7.0$ Hz, 1H), 5.44 (br s, 1H) and 4.25 (d, $J = 8.0$ Hz, 1H) were crossed with three anomeric carbons at δ 101.9, 98.6 and 104.8, respectively. Acid hydrolysis of **3** with 2.0 mol/L HCl afforded to three monosaccharides, β-D-glucose, α-L-rhamnose and β-D-arabinose, which can be tested by PC. The HMBC correlations from the anomeric proton of arabinose [4.61 (d, 7.0H z, 1H) (Ara-1H)] to 83.4 (C-1) and from 4.13 (m, 1H) (H-1) to the anomeric carbon of arabinose 101.9 (C-1, Ara), from 5.44 (br s, 1H) (H-1, Rha) to 78.6 (C-3) and from 4.63 (m, 1H) (H-3) to 98.6 (C-1, Rha) and from 4.25 (d, $J = 8.0$ Hz, 1H) (H-1, Glc) to 75.2 (C-26) and from 3.48 (t, $J = 9.2$ Hz, 1H) (H-26a) to 104.8 (C-1, Glc) disclosed that the glycosidation positions were at C-1, C-3 and C-26 in the aglycone of compound **3**, respectively. Moreover, the positions of glucose linked to the aglycone were confirmed by the correlations of NOE NMR experiments, from 4.13 (m, 1H) (H-1) to [4.61 (d, $J = 7.0$ Hz, 1H) (H-1, Ara)], from 4.63 (m, 1H) (H-3) to 5.44 (br s) (H-1, Rha) and from 3.49 (t, $J = 9.2$ Hz, 1H) (H-26a) to 4.25 (d, $J = 8.0$ Hz, 1H) (H-1, Glc). The configurations of anomeric protons from glucose and arabinose were determined as β by the coupling constants, and the configuration of anomeric protons from rhamnose was α by the broad singlet peak type in proton NMR spectrum. The configuration of aglycone moiety was determined by comparing the NMR data of aglycone from **3** to (25S)-26-*O*-β-D-glucopyranoyl-furost-1β,3β,26-triol-3-*O*-β-D-glucopyranoside (Zou et al., 2009). The absolute configuration of C-25 was proved by proton chemical shifts of H-26 and 27-Me (Agrawal, 2005). Thus, the structure of **3** was identified as (25S)-26-*O*-β-D-glucopyranoyl-20(22)-en-furost-1β,3β,5β,26-tetraol-1-*O*-β-D-arabinopyranoside-3-*O*-α-L-rhamnopyranoside.

Compounds **4** and **5** were determined as (25S)-1β,3β,4β-trihydroxyspirostan-5β-yl-3-*O*-β-D-glucopyranoside, kitigenin-5β-*O*-β-D-glucopyranoside (**5**) by comparing their NMR data to the references (Kanmoto, Mimaki, Nikaido, Koike, & Ohmoto, 1994; Shen et al., 2003).

The anticancer activity of isolated compounds **1**–**5** were evaluated by MTT method, and the results showed that compounds **1–4** displayed cytotoxic activity to A549 cell line in different levels, but showed no significant cytotoxic activity to Caski, HepG 2 and MCF-7 cell lines (Table 1).

Table 1. The results of compounds **1**–**5** inhibiting cancer cell lines.[a]

Cell line	Compound **1**	Compound **2**	Compound **3**	Compound **4**	Compound **5**
A549	20	50	40	30	>100
Caski	50	>100	70	>100	>100
HepG 2	>100	>100	80	>100	>100
MCF-7	>100	>100	>100	>100	>100

Notes: [a]The inhibiting activity to cancer cell lines were recoded with IC_{50} and the units of IC_{50} was $\mu g\,mL^{-1}$.

3. Experimental

3.1. *General experimental procedure*

Column chromatography was carried out with silica gels (Qingdao Ocean Chemical Company, 200–300 meshes). TLC was performed with percolated silica gel GF-254 plates. The detection of spots was done at UV 254 nm or by spraying with 95% H_2SO_4. IR spectrum was recorded on Nicolet FT360 spectrometer. Optical rotations were measured on a Perkin Elmer 241 spectropolarimeter using a 10-cm cell tube. Mass spectra (ESI–MS, HRESI–MS) were measured using a fast atom bombardment mode on aVG AUTO Spec-300 mass spectrometer and using an electron spraying mode on a Finnigan-MAT LCQ DECA XP plus mass spectrometer, respectively, and ions were given in m/z. The ^{1}H-, ^{13}C- and 2-D-NMR spectrum were recorded on a Bruker 400 spectrometer in methanol-d_4 using tetramethylsilane as an internal standard. Chemical shifts were given in ppm and scalar coupling reported in hertz.

3.2. *Plant material*

The rhizomes of *R. carnea* were collected from Changyang in Hubei Province in July 2006 and identified by Dr Faju Chen and Yubing Wang. A voucher specimen (no. 2006071802) was deposited at Hubei Key Laboratory of Natural Products Research and Development, China Three Gorges University, Yichang, China.

3.3. *Extraction and isolation*

The dry roots of *R. carnea* (1.5 kg) were powdered and refluxed with 70% ethanol (6 L × 5), and the extract was evaporated *in vacuo* to yield a residue, which was freeze-dried to afford a powder (250 g). Then, it was suspended in water and extracted with petroleum-ether, ethyl acetate and *n*-butanol successively. The *n*-butanol extract (95 g) was dissolved in water (2.0 L), and subjected to macroporous resin (AB-8) column chromatography eluting with a gradient ethanol–water system (100% water → 100% ethanol). The 70% ethanol eluate (5 g) was purified by repeated Rp-C18 silica gel column chromatography eluted with a gradient acetonitrile–water system (100% water → 60% acetonitrile) to give 12 fractions. Fractions 5 and 10 were combined, respectively, and re-purified by Sephadex LH-20 gel chromatography. Fractions 15 (0.5 g) was further purified by repeated preparative HPLC eluted with gradient solvent (45% → 80% acetonitrile/water (V/V) within 30 min, 2.0 mL/min, detection at UV 203 nm), and then repeated semi-preparative HPLC eluted with gradient solvent (45% → 60% acetonitrile/water (V/V) within 30 min, 1.5 mL/min, detection at UV 203 nm) to afford compounds **3** (15.5 mg, $t_R = 15$ min) and **2** (10.8 mg, $t_R = 25$ min).

Fractions 20 (0.2 g) was further purified by repeated preparative HPLC eluted with gradient solvent (45% → 70% acetonitrile/water (V/V) within 35 min, 1.5 mL/min, detection at UV 203 nm), and then repeated semi-preparative HPLC eluted with gradient solvent (45% → 70% acetonitrile/water (V/V) within 35 min, 1.5 mL/min, detection at UV 203 nm) to give compounds **1** (30 mg, $t_R = 25$ min), **4** (20 mg, $t_R = 15$ min) and **5** (10 mg, $t_R = 10$ min).

3.4. *Acid hydrolysis*

A total of 2.0 mg of **1**, **2** and **3** were hydrolysed with 2.0 mol/L HCl, respectively. The hydrolysed products were tested by paper chromatography.

3.5. *Inhibiting cancer cell line assay*

The cytotoxic activity to A549, Caski, HepG 2 and MCF-7 were screened by MTT method.

3.5.1. *Compound* ***1***

White powder, m.p. 176–177°C, $[\alpha]_D^{20}$ −80.2° (c 0.09, CH_3OH); IR (kBr, υ_{max}): 3430, 2921, 1556 and 1320 cm^{-1}; HR–ESI–MS data with *m/z* $[M+H]^+$ 611.7838 and $[M+Na]^+$ 633.7656 (Calcd 611.7835 and 633.7650, respectively); ^1H (400 MHz, CD_3OD-d_4): 4.15 (H-1, m, 1H), 2.57 (H-2a, m, 1H), 2.05 (H-2b, m, 1H), 4.41 (H-3, m, 1H), 2.35 (H-4a, m, 1H), 2.19 (H-4b, m, 1H), 1.94 (H-6a, m, 1H), 1.47 (H-6b, m, 1H), 1.45 (H-7a, m, 1H), 1.01 (H-7b, m, 1H), 1.65 (H-8, m, 1H), 1.15 (H-9, m, 1H), 1.35 (H-11, m,2H), 0.98 (H-12a, m, 1H),1.69 (H-12b, m, 1H), 1.04 (H-14, m, 1H), 1.97 (H-15a, m, 1H), 1.43 (H-15b, m, 1H), 4.50 (H-16, m, 1H), 1.76 (H-17, m, 1H), 1.10 (H-18, s, 3H), 0.80 (H-19, s, 3H), 2.25 (H-20, m, 1H), 1.16 (H-21, d, 7.6, 3H), 1.92 (H-23a, m, 1H), 1.85 (H-23b, m, 1H), 1.72 (H-24a, m, 1H), 1.42 (H-24b, m, 1H), 1.86 (H-25, m, 1H), 3.47 (H-26a, dd, 7.8, 9.3, 1H); 4.07 (H-26b, m, 1H), 1.06 (H-27, d, 6.5, 3H), 4.95 (Glu H-1, d, 7.8, 1H), 4.03 (Glu H-2, m, 1H), 4.25 (Glu H-3, m, 1H), 4.28 (Glu H-4, m, 1H), 3.95 (Glu H-5, m, 1H), 4.54 (Glu H-6a, m, 1H), 4.30 (Glu H-6b, m, 1H); ^{13}C (100 MHz, CD_3OD-d_4): 71.9 (C-1), 25.6 (C-2), 66.8 (C-3), 27.9 (C-4), 87.6 (C-5), 31.2 (C-6), 24.1 (C-7), 34.2 (C-8), 43.8 (C-9), 42.8 (C-10), 20.9 (C-11), 39.6 (C-12), 40.3 (C-13), 55.8 (C-14), 26.4 (C-15), 80.9 (C-16), 62.1 (C-17), 15.5 (C-18), 13.3 (C-19), 42.1 (C-20), 15.0 (C-21), 109.7 (C-22), 25.4 (C-23), 23.8 (C-24), 27.1 (C-25), 64.7 (C-26), 16.1 (C-27), 95.6 (Glu-1), 74.4 (Glu-2), 77.1 (Glu-3), 70.0 (Glu-4), 76.8 (Glu-5), 61.1 (Glu-6).

3.5.2. *Compound* ***2***

White powder, m.p. 180–181°; $[\alpha]_D^{20}$ −85.2°(c 0.08, CH_3OH); IR (kBr, υ_{max}): 3435, 2920, 1560 and 1323 cm^{-1}; HRESI–MS, *m/z*$[M+H]^+$ 595.7844 and $[M+Na]^+$ 617.7662 (Calcd 595.7840 and 617.7666, respectively); ^1H (400 MHz, CD_3OD-d_4): 1.69 (H-1a, m, 1H), 0.98 (H-1b, m, 1H), 2.58 (H-2a, m, 1H), 2.03 (H-2b, m, 1H), 4.39 (H-3, m, 1H), 2.37 (H-4a, m, 1H), 2.16 (H-4b, m, 1H), 1.96 (H-6a, m, 1H), 1.48 (H-6b, m, 1H), 1.47 (H-7a, m, 1H), 1.00 (H-7b, m, 1H), 1.66 (H-8, m, 1H), 1.17 (H-9, m, 1H), 1.38 (H-11, m, 2H), 0.99 (H-12a, m, 1H), 1.70 (H-12b, m, 1H), 1.02 (H-14, m, 1H), 1.95 (H-15a, m, 1H), 1.45 (H-15b, m, 1H), 4.48 (H-16, m, 1H), 1.77 (H-17, m, 1H), 1.37 (H-18, s, 3H), 1.00 (H-19, s, 3H), 2.25 (H-20, m, 1H), 1.43 (H-21, d, 7.6, 3H), 1.90 (H-23a, m, 1H), 1.88 (H-23b, m, 1H), 1.70 (H-24a, m, 1H), 1.43 (H-24b, m, 1H), 1.84 (H-25, m, 1H), 3.45 (H-26a, dd,7.8, 9.3, 1H); 4.09 (H-26b, m, 1H), 1.32 (H-27, d, 6.5, 3H), 4.88 (Glu H-1, d, 7.8, 1H), 4.00 (Glu H-2, m, 1H), 4.27 (Glu H-3, m, 1H), 4.25 (Glu H-4, m, 1H), 3.96 (Glu H-5, m, 1H), 4.57 (Glu H-6a, m, 1H), 4.32 (Glu H-6b, m, 1H); ^{13}C (100 MHz, CD_3OD-d_4): 29.3 (C-1), 39.4 (C-2), 66.5 (C-3), 40.1 (C-4), 90.2 (C-5), 47.2 (C-6), 26.4 (C-7), 35.5 (C-8), 45.6 (C-9), 41.8 (C-10), 21.2 (C-11), 40.6 (C-12), 41.6 (C-13), 56.2 (C-14), 34.5 (C-15), 81.8 (C-16), 64.2 (C-17), 18.0 (C-18), 16.5 (C-19), 38.8 (C-20), 16.8 (C-21), 110.4 (C-22), 31.9 (C-23), 26.2 (C-24), 31.5 (C-25), 67.6 (C-26), 19.4 (C-27), 102.3 (Glu-1), 75.2 (Glu-2), 79.4 (Glu-3), 71.2 (Glu-4), 78.8 (Glu-5), 63.8 (Glu-6).

3.5.3. *Compound* ***3***

White amorphous powder; m.p. 201–202°, $[\alpha]_D^{20}$ −98.0°(c 0.1, CH_3OH); IR (kBr, υ_{max}): 3428, 2925, 1559 and 1318 cm^{-1}; HR–ESI–MS *m/z* 874.0408 (Calcd for $[M+H]^+$874.0402) and 895.4663 (Calcd for $[M+Na]^+$ 895.4667); ^1H-NMR (400 MHz, CD_3OD-d_4): 4.13 (H-1, m, 1H), 2.58 (H-2a, br d, 14.4, 1H), 2.06 (H-2b, br d, 14.4, 1H),

4.63 (H-3, m, 1H), 1.93 (H-4a, m, 1H), 1.75 (H-4b, m, 1H), 2.40 (H-5, m, 1H), 1.29 (H-6a, m, 1H), 1.16 (H-6b, m, 1H), 1.72 (H-7a, m, 1H), 0.99 (H-7b, m, 1H), 1.60 (H-8, m, 1H), 1.20 (H-9, m, 1H), 1.31 (H-11a, m, 1H), 1.21 (H-11b, m, 1H), 1.72 (H-12a, m, 1H), 1.07 (H-12b, dd, 5.5, 10.8, 1H), 1.08 (H-14, m, 1H), 2.00 (H-15a, m, 1H), 1.44 (H-15b, m, 1H), 4.98 (H-16, m, 1H), 1.96 (H-17, m, 1H), 0.90 (H-18, s, 3H), 1.27 (H-19, s, 3H), 1.64 (H-21, s, 3H), 1.89 (H-23a, m, 1H), 1.45 (H-23b, m, 1H), 2.23 (H-24a, m, 1H), 2.18 (H-24b, m, 1H), 1.94 (H-25, m, 1H), 3.48 (H-26a, t, 9.2, 1H), 4.10 (H-26b, dd, 10.1, 9.2, 1H), 1.03 (H-27, d, 6.0, 3H), 4.61 (Ara H-1, d, 7.0, 1H), 3.59 (Ara H-2, m, 1H), 3.70 (Ara H-3, m, 1H), 4.03 (Ara H-4, m, 1H), 3.49 (Ara H-5a, m, 1H), 3.98 (Ara H-5b, m, 1H), 5.44 (Rha H-1, br s, 1H), 4.60 (Rha H-2, br s, 1H), 4.46 (Rha H-3, m, 1H), 4.27 (Rha H-4, t, 7.8, 1H), 4.83 (Rha H-5, m, 1H), 1.71 (Rha H-6, d, 6.5, 1H), 4.25 (Glc H-1, d, 8.0, 1H), 3.98 (Glc H-2, m), 1H, 26 (Glc H-3, m, 1H), 4.25 (Glc H-4, m, 1H), 3.98 (Glc H-5, m, 1H), 4.40 (Glc H-6a, m, 1H), 4.57 (Glc H-6b, m, 1H); ^{13}C (100 MHz, CD_3OD-d_4): 83.4 (C-1), 27.7 (C-2), 78.6 (C-3), 35.1 (C-4), 36.5 (C-5), 27.1 (C-6), 27.3 (C-7), 34.5 (C-8), 44.6 (C-9), 43.2 (C-10), 22.4 (C-11), 40.3 (C-12), 41.0 (C-13), 56.0 (C-14), 32.8 (C-15), 85.7 (C-16), 65.8 (C-17), 17.3 (C-18), 14.9 (C-19), 105.0 (C-20), 11.9 (C-21), 153.1 (C-22), 32.1 (C-23), 24.1 (C-24), 33.7 (C-25), 75.2 (C-26), 17.2 (C-27), 101.9 (Ara-1), 73.2 (Ara-2), 73.6 (Ara-3), 69.8 (Ara-4), 68.9 (Ara-5), 98.6 (Rha-1), 71.9 (Rha-2), 71.1 (Rha-3), 73.3 (Rha-4), 69.4 (Rha-5), 17.9 (Rha-6), 104.8 (Glc-1), 74.3 (Glc-2), 78.2 (Glc-3), 71.8 (Glc-4), 77.8 (Glc-5), 62.9 (Glc-6).

4. Conclusions

In conclusion, five steroidal compounds, including three new compounds – reinocarnoside A, B and C, were isolated from the roots of *R. carnea,* and the structure of reinocarnoside A, B and C were identified as (25S)-spirostanol-1β,3β,5β,26-tetraol-5β-D-glucopyranoside (**1**), (25S)-spirostanol-3β,5β,26-triol-5β-D-gluco-pyranoside (**2**) and (25S)-26-*O*-β-D-gluco-pyranosyl-20(22)-en-furost-1β,3β,5β,26-tetraol-1-*O*-β-D-arabinoside-3-*O*-α-L-rhnosamno-side (**3**) by NMR and MS spectrums. The anticancer activity of isolated compounds **1**–**5** was evaluated by MTT method, and the results showed that compounds **1–4** displayed cytotoxic activity to A549 cell line in different levels, but showed no significant cytotoxic activity to Caski, HepG 2 and MCF-7 cell lines.

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References

Agrawal, P.K. (2005). Assigning stereodiversity of the 27-Me group of furostane-type steroidal saponins via NMR chemical shifts. *Steroids, 70*, 715–724.

Fu, S.X. (2002). *Flora of hubei province* (Wuhan Botanical Garden, Chinese Academy Science, pp. 15–16). Wuhan: Science and Technology of Hubei Press.

Guo, Z.Y., Zou, K., Wang, J.Z., Liu, C., Tang, Z.C., & Yang, C.Y. (2009). Structural elucidation and NMR spectral assignment of three new furostanol saponins from the roots of *Tupistra chinensis*. *Magnetic Resonance in Chemistry, 47*, 613–616.

Kanmoto, T., Mimaki, Y., Nikaido, Y., Koike, K., & Ohmoto, T. (1994). Recurvosides A – E, new polyhydroxylated steroidal saponins from *Nolina recurvata* stems. *Chemical and Pharmaceutical Bulletin, 42*, 926–931.

Pan, W.B., Chang, F.R., Wei, L.M., & Wu, Y.C. (2003). New flavans, spirostanol sapogenins, and a pregnane genin from tupistra chinensis and their cytotoxicity. *Journal of Natural Products, 66*, 161–168.

Shen, P., Wang, S.L., Liu, X.K., Yang, C.R., Cai, B., & Yao, X.S. (2003). Steroidal Saponins from Rhizomes of *Tupistra wattii* HOOK. f. *Chemical and Pharmaceutical Bulletin, 51*, 305–308.

Wu, L.J. (2007). *Natural medicinal chemistry* (5th ed., pp. 310–349). Beijing: People's Medical Press.

Xu, L.L., Zou, K., Wang, J.Z., Wu, J., Zhou, Y., Dan, F.J., & Yang, J. (2007). New polyhydroxylated furostanol saponins with inhibitory action against NO Production from Tupistra chinensis rhizomes. *Molecules, 12*, 2029–2037.

Zou, K., Wang, J.Z., Du, M., Li, Q., & Tu, G.Z. (2006). A pair of diastereoisomeric steroidal saponins from cytotoxic extracts of tupistra chinensis rhizomes. *Chemical and Pharmaceutical Bulletin, 54*, 1440–1442.

Zou, K., Wang, J.Z., Guo, Z.Y., Du, M., Wu, J., Zhou, Y., . . . , Liu, C. (2009). Structural elucidation of four new furostanol saponins from *Tupistra chinensis* by 1D and 2D NMR spectroscopy. *Magnetic Resonance in Chemistry, 47*, 87–91.

Zou, K., Wang, J.Z., Wu, J., Zhou, Y., Liu, C., Dan, F.J., . . . , Yang, J. (2007). Furostanol saponins with inhibitory action against COX-2 production from Tupistra Chinensis rhizomes. *Chinese Chemical Letters, 18*, 1239–1242.

Zou, K., Wu, J., Du, M., Liu, C., Tang, Z.C., & Wang, J.Z. (2007). Diastereoisomeric saponins from the rhizomes of *Tupistra chinensis*. *Chinese Chemical Letters, 18*, 65–68.