A new sesquiterpene lactone from yacon leaves

Ying Yuan, Khin Khin Win Aung, Xiao-Ku Ran, Xiao-Tong Wang, De-Qiang Dou and Feng Dong

College of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian, PR China; Technology Development Department, Zhen-Ao Co.Ltd, Dalian, PR China; Pharmaceutical Research Department, Ministry of Science and Technology, Yangon, Myanmar

ABSTRACT
The chemical constituents of 60% EtOH extract of yacon leaves were separated to yield a new compound, together with four known compounds, which were isolated for the first time from yacon. The new compound was characterised and named as chlorodalin (1) on the basis of NMR (1D and 2D), HR-MS and other spectral methods. The cytotoxic activities of 1–5 were evaluated on two human tumour cell lines and the new compound showed significant cytotoxic activity.

1. Introduction
Yacon, Smallanthus sonchifolius (Peoepp. & Endl.) H. Robinson belonging to family Asteraceae (Kakuta et al. 1992) is an indigenous plant of the Andes of South America (Lachman et al. 2003). It was firstly introduced into China from Japan as an anti-diabetic herbal tea (Genta et al. 2010) in the early 1990s. Currently, anti-diabetes, antioxidant and antitumour actions of yacon leaves were evaluated (Valentova et al. 2003; Genta et al. 2010; Raga et al. 2010; de Moura et al. 2012; Russo et al. 2015). Up to now, ent-kaurenic acids, phenolic acids, diterpenes, lignans and sesquiterpene lactones were isolated from yacon leaves (Kakuta et al. 1992; Jiřovský et al. 2003; Dou et al. 2008, 2010; Mercado et al. 2010; Xiang et al. 2010a; Lv et al. 2012; Oliveira et al. 2013). Previously, the active constituents of yacon leaves were explored for anti-diabetic effect and a series of smaditerpenic acids possessing α-glucosidase inhibitory action were discovered (Dou et al. 2008; Xiang et al. 2010b). The constituents of yacon leaves...
were further investigated to furnish a new compound 1, together with four known compounds 2, 3, 4 and 5, which were obtained for the first time from this plant (Figure 1). This paper deals with the structure elucidation of the new compound and their cytotoxic activity.

2. Results and discussion

Compounds 1–5 were isolated from the 60% ethanol extract of yacon leaves using silica gel and ODS column chromatography and purified by preparative HPLC. The chemical structures were elucidated on the basis of spectral data analysis.

Compound 1 was isolated as a white crystal with molecular formula of C_{23}H_{29}O_{9}Cl determined from the HR-MS at \( m/z \) 485.1571 [M + H]^+. The \(^{13}\)C NMR spectrum of 1 displayed 23 carbon signals, of which the low-field signals at \( \delta \) 173.3, 170.2, 169.0 and 165.8 were assignable to four carbonyl functions. Three double bonds were recognised from the presence of six olefinic signals at \( \delta \) 148.4, 138.2, 133.7, 130.7, 126.6 and 122.3 in the \(^{13}\)C NMR spectrum of 1. The \(^1\)H NMR spectrum showed two olefinic methines \( \delta \) 4.96 (1H, d, \( J = 10.4 \) Hz, H-5), 7.01 (1H, dd, \( J = 7.7, 10.2 \) Hz, H-1) and two olefinic methylenes 5.76 (1H, d, \( J = 3.1 \) Hz, H-13a), 6.28 (1H, d, \( J = 3.4 \) Hz, H-13b). Furthermore, five methyl signals at \( \delta \) 1.31 (3H, s, H-5'), 1.49 (3H, d, \( J = 6.8 \) Hz, H-4'), 1.99 (3H, s, H-17), 2.00 (3H, d, \( J = 1.0 \) Hz, H-18) and 3.81 (3H, s, H-15) could be observed in the \(^1\)H NMR spectrum of 1. In the HMBC of 1, the carbonyl signal at \( \delta \) 170.2 (C-16) correlated with methyl protons \( \delta \) 1.99 (H-17) and 5.41 (H-9); the carbonyl signal at \( \delta \) 165.8 (C-14) correlated with 5.41 (H-9) and 3.81 (H-15) due to the carbomethoxy group; the carbonyl signal at \( \delta \) 173.3 (C-1') correlated with the methyl protons \( \delta \) 1.31 (H-5') and 6.65 (H-8). Meanwhile, correlations could be observed between signal at \( \delta \) 169.0 (C-12) and exocyclic olefinic methylene protons 5.76 (H-13a), 6.28 (H-13b); \( sp^2 \) carbons signal at \( \delta \) 133.7 (C-11) and 2.79 (H-7); 75.0 (C-6) and 2.79 (H-7) confirmed the \( \alpha \)-methylene-\( \gamma \)-lactone moiety.

Figure 1. Structures of compounds 1–5 isolated from yacon leaves.
to be $C_6$-$C_7$-$C_{11}$-$C_{12}$. On the other hand, the strongly negative cotton effect exhibited by 1 ($\lambda_{\text{max}}$ 226 nm, $\Delta\varepsilon$ −91.3) suggested cis fusion of the $\gamma$-lactone ring (Herz & Bhat 1970). In addition, from the HMBC of 1, correlations could also be observed between $\delta$ 138.2 (C-10) and two protons at $\delta$ 2.46 (H-2a), 2.69 (H-2b); 130.7 (C-4) and H-2, H-3 indicated the chain of $C_{10}$-$C_1$-$C_2$-$C_3$-$C_4$-$C_5$. Thus, the mother skeleton was determined as shown in Figure 1. When compared 1 with uvedalin (Bardón et al. 2001), the $^{13}$C NMR of 1 was superimposable to those of uvedalin except for the side chains at C-8 and the $^1$H NMR spectrum of 1 was almost identical to those of chlorohydrin (Ali et al. 1972) due to the side chain at C-8 of 1. In addition, the chlorin substituent at C-3’ of 1 induced signals at H-3’ and C-3’ to the lower field region. The MS spectrum of 1 showed a strong peak at $m/z$ 273 [M–CH$_3$COO–CH$_3$OCO–OCOC(CH$_3$)(OH)CH(Cl)CH$_3$]+ and ion peak at $m/z$ 425 revealed the loss of (CO)OCH$_3$ group from the molecular ion. Peak at $m/z$ 333 is related to the loss of 3-chloro-2-hydroxy-2-methylbutanoic acid. The stereochemistry of compound 1 was further supported by the NOE experiments. NOE correlations observed between $\delta$ 7.01 (H-1) and 3.81 (H-15) as well as 5.12 (H-6) and 2.00 (H-18) established the geometry of the both double bonds at C$_1$ and C$_{10}$, C$_4$ and C$_5$ as E type. NOE correlations were observed between H-7 and H-8 as well as H-6, H-9, H-3’ and H-5’, which further revealed the absolute configurations of C-2’ and C-3’ of 1 as S and S, respectively. Thus, the structure of compound 1 was elucidated as shown (Figure 1) and named as chlorodalin.

Compound 1 is the uvedalin chloride resulted in the opening of epoxy group of uvedalin and most possibly, it was transformed from uvedalin in yacon leaves during the isolation process with chlorinated solvents or by hydrolysis in storage over one year, although the leaves were stored in a dry and dark place. So fresh leaves were adopted and immediately extracted ultrasonically with methanol for UPLC/TOF-MS analysis, indicating that compound 1 naturally occurred in the fresh yacon leaves.

Evaluating the cytotoxic activity of the five compounds, compound 1 showed significant cytotoxicity against two human cancer cell lines (Table 1).

### 3. Experimental

#### 3.1. Instruments and chemicals

Optical rotations were recorded on a JASCO P-2000 (Japan) digital polarimeter. CD was measured on MOS-450 (France). HR-MS was obtained on a Waters Xevo Q-TOF (USA). NMR spectra were recorded on a Bruker ARS 500 NMR spectrometer (Germany). Preparative HPLC separations were performed on HITACHI 7100 equipped with a YMC-Pack ODS-A, 10 × 250 mm column (Japan), using a flow rate of 2.0 mL/min at a column temperature of 25 °C, and

| Compound | IC$_{50}$ (μM) | Bel-7402 | HGC-27 |
|----------|---------------|----------|--------|
| 1        | 17.616        | 11.225   |
| 2        | 306.711       | 243.163  |
| 3        | 299.151       | 245.919  |
| 4        | 286.341       | 249.201  |
| 5        | 250.979       | 225.986  |
| 5-Fu     | 40.001        | 56.367   |
detection was performed with a VWD detector. Column chromatography was performed on silica gel (Marine Chemical Factory, Qingdao, China) and ODS (Fuji, Japan). TLC was conducted on silica gel GF254 (Marine Chemical, Factory, Qingdao, China). Analytical grade MeOH was purchased from Damao (Tianjin, China).

3.2. Plant material
Leaves of *S. sonchifolius* were collected from Dalian (10 September 2013), China and identified by Prof. Tingguo Kang, College of Pharmacy, Liaoning University of Traditional Chinese Medicine. A voucher specimen (Batch No. 20131001) has been deposited at the Pharmacognosy Laboratory, College of Pharmacy, Liaoning University of TCM.

3.3. Extraction and isolation
The shade-dried leaves of yacon (2.0 kg) were extracted twice with 60% EtOH (20 L) at 60 °C for 2 h. Temperature below 60 °C was controlled at any step of the isolation process to avoid hydrolysis. The extract was concentrated by a rotary evaporator under reduced pressure. Once evaporated, it was suspended in water and then partitioned in a CH$_2$Cl$_2$–H$_2$O mixture. The CH$_2$Cl$_2$ fraction (20 g, evaporated under reduced pressure) was applied to silica gel normal phase chromatography using gradient mixtures of CH$_2$Cl$_2$–MeOH (100:0–5:1) to afford six major fractions, respectively. Fraction 2 (CH$_2$Cl$_2$–MeOH, 50:1) was further chromatographed on silica gel with CH$_2$Cl$_2$–MeOH (10:1) to furnish Fr.2–4, which was further purified with preparative HPLC with 45% MeOH to yield compound 1 (98 mg). The water fraction (93 g, freeze-dried) was subjected to column chromatography over silica gel and eluted with CH$_2$Cl$_2$–MeOH in gradient (20:1–0:20) to afford 10 fractions. Fraction 4 (CH$_2$Cl$_2$–MeOH, 10:1) was further chromatographed on ODS with 15% MeOH to furnish Fr.4–1, which was further purified with Rp-18 preparative HPLC with 9 and 25% MeOH to give compounds 3 (10.0 mg) and 4 (22.8 mg), respectively. Fraction 7 (CH$_2$Cl$_2$–MeOH, 5:1) was rechromatographed on ODS (10% MeOH) followed by preparative HPLC with 10% MeOH to furnish compound 2 (42.8 mg). Fraction 8 (CH$_2$Cl$_2$–MeOH, 1:1) was partitioned in n-BuOH–H$_2$O for two times, and the n-BuOH extract was further separated by ODS with 30% MeOH elution, and then, it was further purified by preparative HPLC with 21% MeOH eluting to yield compound 5 (15.4 mg).

3.4. Cytotoxicity
The *in vitro* cytotoxicity of the five compounds against two human cancer cell lines (Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China) was investigated by the MTT colorimetric method. 5-Fu (5-fluorouracil), a known anticancer agent, was used as positive control. The human cancer cell lines were grown in HyClone's modified 1640 medium containing 10% foetal bovine serum and cultivated in humidified incubators (SANYO, Japan). Afterwards, the cells were seeded in 96-well plates and cultivated for 12 h, and then treated with compounds of five concentrations (12.5, 25, 50, 100 and 200 μM) for 48 h. Then, 10 μL of MTT (5 mg/mL) was added to each well, and the cells were incubated for additional 4 h. Then, DMSO (100 μL per well) was added to dissolve the formazan crystals. Absorbance was measured at 492 nm by enzyme immunoassay instrument (Caretium, KC-100, Shenzhen, China).
chlorodalin (1): white crystal; HR-MS m/z 485.1571 [M + H]^+ (Calcd C_{23}H_{30}O_{9}Cl, 485.1578). [α]_{D}^{20} = −11.28° (c 0.266, CH₂Cl₂); CD (c 0.03 mg/mL, CH₂Cl₂) λ max (Δε) 226 (−91.3) nm; 13C NMR (CDCl₃, 125 MHz) δ: 173.3 (C-1'), 170.2 (C-16), 169.0 (C-12), 165.8 (C-1), 138.2 (C-10), 133.7 (C-11), 130.7 (C-4), 126.6 (C-5), 122.3 (C-13), 76.8 (C-2'), 75.0 (C-6), 72.3 (C-8), 71.0 (C-9), 61.7 (C-3'), 52.3 (C-15), 50.9 (C-7), 36.9 (C-3), 26.1 (C-2), 23.0 (C-5'), 21.0 (C-17), 18.0 (C-4'), 16.9 (C-18); 1H NMR (CDCl₃, 500 MHz) δ: 7.01 (1H, dd, J = 7.7 and 10.2 Hz, H-1), 6.65 (1H, dd, J = 0.9 and 8.3 Hz, H-8), 6.28 (1H, d, J = 3.4 Hz, H-13b), 5.76 (1H, d, J = 3.1 Hz, H-13a), 5.41 (1H, dd, J = 8.4 and 0.9 Hz, H-9), 5.12 (1H, td, J = 10.0 and 6.5 Hz, H-6), 4.96 (1H, d, J = 10.4 Hz, H-5), 4.15 (1H, q, J = 6.8 Hz, H-4'), 3.81 (3H, s, H-15), 3.19 (s, OH), 2.79 (1H, m, H-7), 2.69 (1H, m, H-2b), 2.46 (1H, m, H-2a), 2.40 (1H, m, H-3b), 2.05 (1H, d, J = 11.9 Hz, H-3a), 2.00 (3H, s, H-18), 1.99 (3H, s, H-17), 1.49 (3H, d, J = 6.8 Hz, H-4'), 1.31 (3H, s, H-5').

[2R- (1R*, 2α, 4α)]4- (2,4-dihydroxy-2,6,6-trimethylcyclohexylidene)-3-buten. (2): oil-like solid; HR-MS m/z 225.1500 [M + H]^+ (Calcd C_{13}H_{21}O_{3}, 225.1491). [α]_{D}^{27} = −128° (c 0.121, MeOH); CD (c 0.60 mg/mL, Dioxane) λ max (∆ε) 215 (+4.8), 240 (+2.3), 290 (+4.8) nm; 13C NMR (CD₃OD, 125 MHz) δ: 211.4 (C-7), 200.8 (C-9), 119.7 (C-6), 101.2 (C-8), 73.5 (C-3), 72.4 (C-5), 47.3 (C-4), 47.2 (C-2), 37.0 (C-1), 32.1 (C-12), 30.6 (C-13), 29.3 (C-11), 26.6 (C-10); 1H NMR (CD₃OD, 500 MHz) δ: 5.84 (1H, s, H-8), 4.96 (1H, br.t, J = 11.9 Hz, H-3), 2.48 (1H, br. d, J = 11.9 Hz, H-4a), 2.22 (1H, br. d, J = 11.9 Hz, H-2a), 2.20 (3H, s, H-10), 1.55 (1H, t, J = 11.9 Hz, H-4b), 1.49 (1H, t, J = 11.9 Hz, H-2b), 1.42 (3H, s, H-11), 1.39 (3H, s, H-13), 1.17 (3H, s, H-12). This is the first time to report the data of 13C NMR and 1H NMR of compound 2 and its CD showed opposite to that of grasshopper keton (Shinde et al. 2007). The NMR data of C/H 2,3,4 are the only difference compared with grasshopper keton (Baumeler et al. 1990; Shinde et al. 2007).

Adenine (3): white powder; 13C NMR (DMSO-d₆, 125 MHz) δ: 155.2 (C-6), 152.3 (C-2, 4), 139.3 (C-8), 117.4 (C-5); 1H NMR (DMSO-d₆, 500 MHz) δ: 12.76 (1H, s, H-9), 8.10 (1H, s, H-2), 8.08 (1H, s, H-8), 7.05 (1H, s, H-10). The NMR spectra data of compound 3 were agreed with the data of Adenine in the reference (Lim et al. 2006).

Benzyl-O-β-D-glucopyranoside (4): pale yellow oil; 13C NMR (CD₃OD, 125 MHz) δ: 139.1 (C-1), 129.2 (C-2), 129.2 (C-3), 128.7 (C-4), 103.3 (C-1'), 78.1 (C-3'), 78.0 (C-5'), 75.1 (C-2'), 71.8 (C-4'), 71.7 (C-7), 62.8 (C-9). The NMR spectra data of compound 4 were agreed with the data in the reference (Wen et al. 2007).

(3S)-1, 2, 3, 4-tetrahydro-β-carboline-3-carboxylic acid (5): white powder, 13C NMR (DMSO-d₆, 125 MHz) δ: 169.8 (C-10), 136.2 (C-8a), 127.5 (C-9a), 126.0 (C-5a), 121.4 (C-7), 118.80 (C-6), 117.7 (C-5), 111.2 (C-8), 106.0 (C-4a), 55.8 (C-3), 40.0 (C-1), 22.6 (C-4); 1H NMR (DMSO-d₆, 500 MHz) δ: 10.94 (1H, s, H-9), 7.45 (1H, d, J = 7.8 Hz, H-8), 7.34 (1H, d, J = 8.0 Hz, H-5), 7.09 (1H, t, J = 7.4 Hz, H-7), 7.00 (1H, t, J = 7.5 Hz, H-6), 4.26 (2H, dd, J = 15.6 and 26.3 Hz, H-1), 3.85 (1H, m, H-3), 3.18 (1H, m, H-4a), 2.89 (1H, m, H-4b). The NMR spectra data of compound 5 agreed with its data in the reference (Li et al. 2007).

Supplementary material
Supplementary material relating to this article is available online, alongside the CD and HR-MS of compound 1 and 2, and the NMR data of compounds 1–5.

Disclosure statement
None of the authors declare any conflict of interests in this study.
Funding
This work was supported by Program for Liaoning Innovate Research Team in University [grant number LT2013020] and General Program [grant number L2013364].

References
Ali E, Ghosh Dastidar PP, Pakrashi SC. 1972. Studies on Indian medicinal plants-XXVIII: sesquiterpene lactones of Enhydra fluctuans Lour. Structures of enhydrin, fluctuanin and fluctuadin. Tetrahedron. 28:2285–2298.
Bardón A, Cardona L, Cartagena E, Catalán CAN, Pedro JR. 2001. Melampolides from Enydra anagallis. Phytochemistry. 57:125–130.
Baumeler A, Brade W, Haag A, Eugster CH. 1990. Synthese von enantiomerereninen ‘grasshopper’-ketonen und verwandten verbindungen [Synthesis of optically pure grasshopper ketone, its diastereoisomers, and related compounds]. Helvetica Chimica Acta. 73: 700–715. German.
de Moura NA, Nelci CA, Caetano BFR, Sivieri K, Urbano LH, Cabello C, Rodrigues M. 2012. Protective effects of yacon (Smallanthus sonchifolius) intake on experimental colon carcinogens. Food Chem Toxicol. 50:2902–2910.
Dou DQ, Tian F, Qiu YK, Kang TG, Dong F. 2008. Structure elucidation and complete NMR spectral assignments of four new diterpenoids from Smallanthus sonchifolius. Magn Reson Chem. 46:775–779.
Dou DQ, Tian F, Qiu YK, Xiang Z, Xu BX, Kang TG, Dong F. 2010. Studies on chemical constituents of the leaves of Smallanthus sonchifolius (yacon): structures of two new diterpenes. Nat Prod Res. 24:40–47.
Genta SB, Cabrera WM, Mercado MI, Grau A, Catalán CA, Sánchez SS. 2010. Hypoglycemic activity of leaf organic extracts from Smallanthus sonchifolius: constituents of the most active fractions. Chem Biol Interact. 185:143–152.
Herz W, Bhat SV. 1970. Isolation and structure of two new germacranolides from Polymnia uvedalia (L.) L. J Organomet Chem. 56:2605–2611.
Jirovský D, Horáková D, Kotoček M, Valentová K, Ulrichová J. 2003. Analysis of phenolic acids in plant materials using HPLC with amperometric detection at a platinum tubular electrode. J Sep Sci. 26:739–742.
Kakuta H, Seki T, Hashidoko Y, Mizutani J. 1992. Ent-kaurenerfic acid and its related compounds from glandular trichome exudates and leaf extracts of Polymnia sonchifolia. Biosci Biotechnol Biochem. 56:1562–1564.
Lachman J, Fernández EC, Orsák M. 2003. Yacon [Smallanthus sonchifolius (Poepp. Et Endl.) H. Robinson] chemical composition and use – a review. Plant Soil Environ. 49:283–290.
Li GQ, Deng ZW, Li J, Fu HZ, Lin WH. 2004. Chemical constituents from starfish Asterias rollestoni. J Chin Pharm Sci. 13:81–85.
Lim SS, Jung YJ, Hyun SK, Lee YS, Choi JS. 2006. Rat lens aldose reductase inhibitory constituents of Nelumbo nucifera Stamens. Phytother Res. 20:825–830.
Lv J, Liao Z, Wang L, Yang GY, Yang YL. 2012. Extraction of lignin from the leaves of Smallanthus sonchifolius and its antioxidant activity. Asian J Chem. 24:312–314.
Mercado MI, Coll Araoz MV, Grau A, Catalan CA. 2010. New alicyclic diterpenic acids from yacon (Smallanthus sonchifolius) leaves. Nat Prod Commun. 5:1721–1726.
Oliveira RB, Daniela CA, Adriana S, Thais GH, Lucia FH, Anna CP, Costa D, Fernando B. 2013. Topical anti-inflammatory activity of yacon leaf extracts. Rev Bras Farmacogn. 23:497–505.
Raga DD, Alimboyoguen AB, del Fierro RS, Ragasa CY. 2010. Hypoglycaemic effects of tea extracts and ent-kaurenic acid from Smallanthus sonchifolius. Nat Prod Res. 24:1771–1782.
Russo D, Malafronte N, Frescura D, Imbrenda G, Faraone I, Milella L, Fernandez E, De Tommasi N. 2015. Antioxidant activities and quali-quantitative analysis of different Smallanthus sonchifolius [(Poepp. and Endl.) H. Robinson] landrace extracts. Nat Prod Res. 29:1673–1677.
Shinde PB, Kim MA, Son BW, Lee CO, Jung JH. 2007. Apocarotenoids from an association of two marine sponges. Nat Prod Sci. 13:365–368.
Valentova K, Cvak L, Muck A, Ulrichova J, Simanek V. 2003. Antioxidant activity of extracts from the leaves of Smallanthus sonchifolius. Eur J Nutr. 42:61–66.
Wen P, Han HY, Wang RW, Wang NL, Yao XS. 2007. C-glycosylflavones and aromatic glycosides from *Campylotropis hirtella*. Asian J Tradit Med. 2:149–153.
Xiang Z, Gai K, Dou DQ, Chen GR, Kang TG, Shi YY, Li XT, Dong F. 2010a. A new hexenol glycoside from leaves of *Smallanthus sonchifolius*. Nat Prod Res. 24:1592–1597.
Xiang Z, He F, Kang TG, Dou DQ. 2010b. Anti-diabetes constituents in leaves of *Smallanthus sonchifolius*. Nat Prod Commun. 5:95–98.