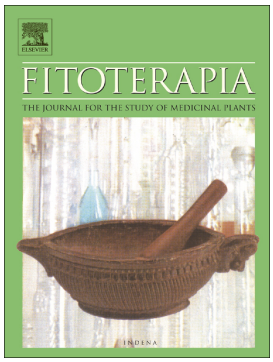

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Bioactive triterpenoid saponins from the tubers of *Hemsleya amabilis* Diels

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ABSTRACT

Five new cucurbitane-type triterpenoid saponins Xuedanosides A-E (**1**-**5**) were isolated from the medicinal plant *Hemsleya amabilis* Diels by silica gel column, octadecylsilyl (ODS) column, and pre-HPLC techniques. Their structures were determined by spectroscopic analysis and examined alongside existing data from prior studies. Separated compounds were evaluated for their cytotoxic activity in HeLa, HCT-8, MCF-7 and HepG2 human cancer cell lines, and compounds **1** and **2** showed significant effects against HeLa cells with IC_{50} values of 3.21 and 8.57 μM, respectively.

Keywords: *Hemsleya amabilis* Diels*;* cucurbitane-type triterpenoid saponins; cytotoxic activity

1. Introduction

Hemsleya amabilis Diels, a member of the Cucurbitaceae family, is mainly distributed in the tropical and subtropical regions of China [1]. The roots of *Hemsleya amabilis*, usually called "xue dan" in China, have been an important traditional Chinese medicine for the treatment of bacillary dysentery, inflammation, ulcers, jaundice, and tuberculosis [2][3]. Among the isolated structures, the main chemical constituents of this species are triterpenoid saponins, including oleanane glycosides and cucurbitane-type glucosides [4], and triterpenoid saponins show *in vitro* or *in vivo* pharmacological effects [5]. In clinical applications, the drugs with the main chemical component of hemsleyadin possess hepatoprotective, antiinflammatory, purgative, cardiovascular, antimicrobial, antihelmintic, and antifertility effects and have been sold on the market,[6][7]. With the purpose of finding new biologically active natural products from this genus, the 95% EtOH extract of *H. amabilis* was examined, and 5 new cucurbitane-type triterpenoids, named Xuedanosides A-E (**1**-**5**) were obtained (Figure 1). In this paper, we report the structure elucidation of the new cucurbitane-type triterpenoids and their cytotoxic activity.

2. Experiments

2.1. General experimental procedures

Optical rotation data were acquired with a Perkin-Elmer 341 digital polarimeter. UV data were obtained with a Shimadzu UV2550 spectrometer. IR data were documented with a FTIR-8400S spectrometer. NMR spectra were acquired with a Bruker AV Ⅲ 600 NMR spectrometer (chemical shift values are shown as δ values with TMS as the internal standard). HRESIMS was conducted with the LTQ-Obitrap XL spectrometer. HPLC separation was performed with a Lumiere K-1001 pump, a Lumiere K-2501 single λ absorbance detector, and a Kromasil (250 × 10 mm) semipreparative column loaded with C_{18} (5 μm). C-18 reversed-phase silica gel (40-63 μm, Merck, Darmstadt, Germany) and silica gel (100～200 and 300～400 mesh, Qingdao Marine Chemical plant, Qingdao, People's Republic of China) were

implemented for CC, and precoated silica gel GF_{254} plates (Zhi Fu Huang Wu Pilot Plant of Silica Gel Development, Yantai, China) were used for TLC. All of the solvents we used were of analytical grade (Beijing Chemical Plant, China).

2.2. Plant material

The entire plants of *H. amabilis* Diels were collected in Nanning, Guangxi Province, People's Republic of China, in October 2017 and were verified by Prof. Xiao-Lei Zhou. A voucher specimen (CS171024) was submitted to the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences.

2.3 Extraction and isolation

The tubers of *H. amabilis* (5.0 kg) were removed under refluxing three times and extracted with 95% EtOH three times (each time for 1 h. Removal of the EtOH under reduced pressure yielded the extract (314 g). The residue was suspended in H_2O (1.5 L) and partitioned with petroleum ether (3 × 1 L), EtOAc (3 × 1 L), and *n*-BuOH (3 × 1 L), successively. Part of the EtOAc extract (200 g) was subjected to silica gel chromatography, with a gradient system of CH_2Cl_2-MeOH (from 1:0 to 0:1) to produce 10 fractions (Fr. A-J). Fraction C (20.6 g) was eluted after HW-40C column chromatography with MeOH-H_2O (30:70; 60:40; 70:30; 80:20; 90:10; 100:0, v/v) giving six subfractions (Fr. C1-C6). Fraction C3 (962 mg) was chromatographed by semipreparative HPLC using MeOH-H_2O (60:40, v/v) to yield compounds **1** (8.6 mg, t_R = 16.0 min) and **2** (6.3 mg, t_R = 20.9 min). Fraction E (10.9 g) was loaded on an ODS C_{18} column eluted with MeOH-H_2O (30:60; 60:40; 70:30; 80:20; 90:10; 100:0, v/v) to give six subfractions (Fr. E1–E6). Fraction E2 (413 mg) was chromatographed by semipreparative HPLC using MeOH-H_2O (65:35, v/v) to yield compound **3** (7.1 mg, t_R = 30.2 min). Fraction G (7.0 g) was fractioned by MCI-gel column chromatography and eluted with MeOH-H_2O (30:60; 60:40; 70:30; 80:20; 90:10; 100:0, v/v) to give six subfractions (Fr. G1–G6). Fraction G4 (193 mg) was chromatographed by preparative HPLC using MeOH-H_2O (75:25, v/v) to yield compounds **4** (5.9 mg, t_R = 22.3 min) and **5** (8.1 mg, t_R = 35.2 min).

*2.3.1. Xuedanoside A (**1**)*

$C_{38}H_{60}O_{14}$, White amorphous powder; $[\alpha]_D^{22}$ + 106.7 (c = 0.1, MeOH); IR (KBr)

3449-3382, 1666, 1664, 1376 cm^{-1}; and ^{1}H and ^{13}C-NMR (pyridine-d5); see (Tables 1 and 2); HR-ESI-MS m/z 763.8746 [M + Na]$^{+}$ (calculated for $C_{30}H_{46}O_8Na^{+}$ 763.5000).

*2.3.2. Xuedanoside B (**2**)*

$C_{38}H_{60}O_{14}$, White amorphous powder; $[\alpha]_D^{22}$ + 112.1 (c = 0.1, MeOH); IR (KBr) 3422-3400, 1660, 1648, 1076 cm^{-1}; ^{1}H and ^{13}C-NMR (pyridine-d5); see (Tables 1 and 2); HR-ESI-MS m/z 763.8746 [M + Na]$^{+}$ (Calculated for $C_{31}H_{50}O_7Na^{+}$ 763.5000).

*2.3.3. Xuedanoside C (**3**)*

$C_{38}H_{60}O_{13}$, White amorphous powder; $[\alpha]_D^{22}$ + 60.5 (c = 0.5, MeOH); IR (KBr) 3434-3395, 1694, 1643, 1290 cm^{-1}; and ^{1}H and ^{13}C-NMR (pyridine-d5); see (Tables 1 and 2); HR-ESI-MS m/z 747.8752 [M + Na]$^{+}$ (Calculated for $C_{30}H_{48}O_8Na^{+}$ 747.4018).

*2.3.4. Xuedanoside D (**4**)*

$C_{36}H_{58}O_{11}$, White amorphous powder; $[\alpha]_D^{22}$ + 83.8 (c = 0.1, MeOH); IR (KBr) 3410-3300, 1663, 1638, 1363, 1216 cm^{-1}; and ^{1}H and ^{13}C-NMR (pyridine-d5); see (Tables 1 and 2); HR-ESI-MS m/z 689.8391 [M + Na]$^{+}$ (Calculated for $C_{30}H_{46}O_4Na^{+}$ 689.3973).

*2.3.5. Xuedanoside E (**5**)*

$C_{36}H_{56}O_{11}$, White amorphous powder; $[\alpha]_D^{22}$ + 86.0 (c = 0.1, MeOH); IR (KBr) 3464-3366, 1645, 1262, 1110 cm^{-1}; and ^{1}H and ^{13}C-NMR (pyridine-d5); see (Tables 1 and 2); HR-ESI-MS m/z 687.8232 [M + Na]$^{+}$ (Calculated for $C_{30}H_{50}O_4Na^{+}$ 687.3334).

2.4. Cytotoxicity assays

The cytotoxicity of compounds **1**-**5** was assessed in HeLa, HCT-8, MCF-7 and HepG2 human cancer cell lines by the MTT method. The cells were incubated in DMEM supplemented with 10% fetal bovine serum and cultured at a density of 1.2×10^4 cells/mL in a 96-well microtiter plate. Five different concentrations of each agent dissolved in dimethyl sulfoxide (DMSO) were then placed in the wells. Each concentration was evaluated three times. After incubation in a 5% CO_2 incubator at 37℃ for 48 h, 10 μL of MTT (4 mg/mL) was placed into each well, and the cells were incubated for an additional 4 h. The media were removed, and DMSO (200 μL) was

added into the wells. The absorbance was documented with a microplate reader at a wavelength of 570 nm. The experiments were conducted a minimum of three times.

*2.5. Acid hydrolysis of compounds **1-5***

Each compound (3.0 mg) was heated in 3 mol/L CF_3COOH (4 mL) for 3 h in a water bath. Each mixture was then extracted with EtOAc. The aqueous layer was evaporated to dryness with ethanol in vacuo at 50°C until the solution is neutral . The residues were determined in comparison with D-Glucose using TLC (eluted with $CHCl_3$:MeOH:H_2O = 3:2:0.2, and visualized with ethanol-5% H_2SO_4 spraying). Furthermore, the absolute configurations of the sugar residues were determined by gas chromatography. In this method, L-cysteine methyl ester hydrochloride (0.06 mol/L) and hexamethyldisilazane-trimethylchlorosilane (HMDS-TMCS, 3:1) were added to the aqueous phase for derivatization. The solution was then centrifuged and the precipitate was removed. After these processes, n-hexane was used to extract the derivative and analyzed by GC. D-Glucose (t_R = 26.7 min) was detected by comparison with the authentic monosaccharide.

3. Results and discussion

Compound **1** was acquired as a white amorphous powder with $[\alpha]_D^{22}$+106.7 (*c* 0.1, MeOH), and the Molish and Liebermann-Burchard reactions were positive. The UV and IR spectra revealed the absorption of hydroxyl groups (3407 cm^{-1}) and carbonyl groups (1664 cm^{-1}). The HRESIMS spectrum demonstrated a quasi-molecular ion at *m/z* 763.8746 (Calculated for $C_{38}H_{60}NaO_{14}^+$, 763.5000), which in comparison with the NMR data, confirmed the molecular formula was $C_{38}H_{60}O_{14}$, accounting for 9 indices of hydrogen deficiency. The NMR spectra (Tables 1 and 2) of compound **1** showed the characteristic signals of a triterpenoid saponin. Its ^{1}H NMR spectrum showed the presence of 9 angular methyl signals at δ_H 1.24 (s), 1.37 (s), 1.48 (s), 1.48 (s), 1.50 (s), 1.51 (s), 1.57 (s), 1.71 (s), and 1.91 (s); 9 oxygenated methines at δ_H 3.62 (m), 3.88 (m), 4.07 (m), 4.18 (m), 4.30 (m), 4.35 (m), 4.48 (m), 4.91 (m), and 5.34 (t, *J* = 6.0 Hz); and one olefinic proton signal at δ_H 6.23 (d, *J* = 6.0 Hz). The ^{13}C NMR spectrum showed 38 signals isolated by APT investigations into 9 angular methyls δ_C 19.8, 20.7, 22.2, 22.6, 23.2, 25.5, 25.9, 26.4, and 26.5; 6 methylenes; 13 methines (1 sp^2 methines

δ_C 123.2 and 9 oxygenated methines δ_C 66.5, 70.8, 71.8, 76.3, 79.0, 79.0, 81.0, 83.5, and 107.0); and 10 quaternary carbons (1 sp^2 carbons δ_C 144.8; 3 carbonyl carbon δ_C 170.6, 213.8, and 215.5; and 2 oxygenated carbon δ_C 80.5 and 82.0), of which 32 were assigned to the typical cucurbitacin formations [8] and the remaining 6 were ascribed to the sugar moiety. The glucose signals at δ_C 107.0 (d), 76.3 (d), 79.0 (d), 71.8 (d), 79.0 (d), and 62.9 (t), as well as the signal of C-2 being shifted downfield by 12.1 ppm to δ_C 83.5 (d) in **1**, were observed, which indicated the glucopyranosyl moiety being linked at C-2 in **1**. This deduction was confirmed by the HMBC correlations from the anomeric proton signal at δ_H 5.34 (d, J = 6.0 Hz, H-1') to δ_C 83.5 (d, C-2). The coupling value (J = 6.0 Hz) of the anomeric proton suggested the presence of a *β*-glucopyranosyl moiety. The NMR data of **1** were similar to those of 2-O-β-D-glucopyranosyl cucurbitacin [9], except for the additional hydroxyl group at C-7 in **1.** This observation was confirmed by the ^{13}C NMR data (δ_C 66.5) and HMBC correlations from δ_H 6.23 (H-6) to δ_C 66.5 (C-7) and the proton signal at δ_H 4.48 (m, H-7) to C-5 (δ_C 144.8), C-6 (δ_C 123.2), and C-9 (δ_C 49.0). In the HMBC spectrum, the correlations from δ_H 4.35 (H-2) to δ_C 81.0 (C-3), δ_H 1.87 (H-15) to δ_C 70.8 (C-16), and δ_H 2.90 (H-17) to δ_C 80.5 (C-20) confirmed the presence of other hydroxyl groups at C-3, C-16, and C-20. Similarly, the correlations from δ_H 6.23 (H-6) to δ_C 144.8 (C-5), δ_H 3.11 (H-12) to δ_C 213.8 (C-11), and δ_H 3.32 (H-23) to δ_C 35.8 (C-24) and 215.5 (C-22) suggested that the olefinic group was at C-5 and the carbonyl groups were at C-11, and C-22. Furthermore, the HMBC correlations from δ_H 2.43 (H-24) and 1.91 (-OAc) to δ_C 82.0 (C-25) confirmed the acetoxyl group at C-25. Examinations of its ^1H-^1H COSY and HSQC spectra advanced the establishment of fragments C-10-C-1-C-2-C-3, C-6-C-7-C-8, C-15-C-16-C-17, and C-23-C-24. The chemical changes of C-2 and C-3 and the coupling constant between H-2 and H-3 implied a 2,3-*cis*-diol structure on ring A [10]. The corresponding configuration of the tetracyclic system of **1** was established with a NOESY experiment, taking into consideration cucurbitacins' biogenesis, and by contrasting the coupling constant pattern with that documented in the literature for comparable compounds [11][12]. The NOE (Figure 3) improvements between H-2 and H-10 and H-3 and H-19

suggested OH-2 was *β*-oriented and OH-3 was *α*-oriented, respectively. The ^{3}J coupling constant (J = 6.0 Hz) also substantiated the antiperiplanar link between H-2 and H-3. The cross-peaks observed between H-7/H_3-28 supported a *β*-orientation of the OH group at C-7. Similarly, NOE correlations between H-8/H_3-19 and H-8/H_3-18 and H_3-18/H-16 corroborated the *β*-orientation of these protons. Thus, the structure of **1** was determined to be 3*α*, 7*β*, *16α*, 20*β*- tetrahydroxycucurbita- 5 (E)- diene- 11, 22- dione- 25- O- acetate- 2- O- β- D- glucopyranoside, and named Xuedanoside A.

Compound **2** was obtained as an amorphous white powder with $[\alpha]_D^{22}$+60.5 (*c* 0.1, MeOH), and its molecular formula was established as $C_{38}H_{60}O_{14}$ from the molecular ion peak at *m/z* 763.8746 $[M + Na]^+$ in the HRESMS. Its ^{1}H and ^{13}C NMR data (Tables 1 and 2) were close to those of **1**, which were isomeric, with the exception of the presence of the sugar group at C-24 (δ_C 80.3), hydroxyl group at C-25 (δ_C 77.0) and acetoxyl group at C-16 (δ_C 75.2) in **2** instead of the sugar group at C-2 (δ_C 83.5), hydroxyl group at C-16 (δ_C 70.8) and acetoxyl group at C-25 (δ_C 82.0) in **1**, and the lack of a hydroxyl group at C-7 (δ_C 24.4). In the HMBC spectrum, the sugar moiety was located at C-24 on the basis of the correlation between the proton signal at δ_H 4.44 (H-24) and anomeric carbon at δ_C 95.6, and the signal for C-24 revealed a powerful downfield shift to *δ* 80.3 (+44.5 ppm). Similarly, the correlations from δ_H 3.02 (H-17) and 2.22 (-OAc) to C-16 (δ_C 75.2) confirmed the acetoxyl group at C-16. the correlations of H-24 (δ_H 4.44), H-26 (δ_H 1.38) and H-27 (δ_H 1.36) with the upfield carbon C-25 (δ_C 77.0) (compared with C-25 in **1**) implied the hydroxyl group at C-25. Furthermore, in comparison to **1**, the signal for C-7 revealed a powerful upfield shift to *δ* 24.4 (-42.1 ppm), while the hydroxyl group was absent. Taken along with ^{1}H-^{1}H COSY, HSQC, HMBC, and NOE spectra, the structure of compound **2** was determined to be 2*β*, 3*α*, 20*β, 25*- tetrahydroxycucurbita- 5(E)- diene- 11,22- dione- 16- O- acetate- 24- O- β- D- glucopyranoside and named Xuedanoside B.

Compound **3** was separated as a white amorphous powder with $[\alpha]_D^{22}$+112.1 (*c* 0.1, MeOH). Its molecular formula was determined to be $C_{38}H_{60}O_{13}$ by HRESIMS (observed *m/z* 747.8752 $[M + Na]^+$), necessitating nine degrees of unsaturation. Its ^{1}H and ^{13}C NMR data (Tables 1 and 2) were close to those of **2,** with the exception of the

presence of the sugar group at C-25 (δ_C 77.0) in **3** instead of the sugar group at C-24 (δ_C 80.3) in **2**. In the HMBC spectrum, the sugar moiety was located at C-25 on the basis of the correlation between the proton signal at δ_H 4.95 (H-1') and oxygenated carbon at δ_C 77.0 (C-25), and the signal for C-25 revealed a powerful downfield shift to δ 83.0 (+5 ppm). Taken along with ^{1}H-^{1}H COSY, HSQC, HMBC, and NOE spectra, the structure of compound **3** was determined to be 2*β*, 3*α*, 20*β*- trihydroxycucurbita- 5 (E)- diene- 11, 22- dione- 16- O- acetate- 25- O- β- D- glucopyranoside, and named Xuedanoside C.

Compound **4** was separated as a white amorphous powder with $[\alpha]_D^{22}$+112.1 (*c* 0.1, MeOH). Its molecular formula was determined to be $C_{36}H_{58}O_{11}$ by HRESIMS (observed *m/z* 689.8391 $[M + Na]^+$), necessitating eight degrees of unsaturation. Its ^{1}H and ^{13}C NMR data (Tables 1 and 2) were close to those of **1**, with the exception of the presence of the sugar group at C-3 (δ_C 87.6) and hydroxyl group at C-25 (δ_C 77.0) in **4** instead of the sugar group at C-2 (δ_C 83.5) and acetoxyl group at C-25 (δ_C 82.0) in **1**, and the lack of a hydroxyl group at C-7 (δ_C 24.4). In the HMBC spectrum, the sugar moiety was located at C-3 on the basis of the correlation between the proton signal at δ_H 3.64 (H-3) and the anomeric carbon at δ_C 107.8, and the signal for C-3 revealed a powerful downfield shift to δ 87.6 (+6.6 ppm). Similarly, the correlations of H-24 (δ_H 2.25), H-26 (δ_H 1.37) and H-27 (δ_H 1.37) with the upfield carbon C-25 (δ_C 69.5) (compared with C-25 in **1**) implied the hydroxyl group at C-25. Furthermore, in comparison to **1**, the signal for C-7 revealed a powerful upfield shift to δ 24.6 (-41.9 ppm), while the hydroxyl group was absent. Taken along with ^{1}H-^{1}H COSY, HSQC, HMBC, and NOE spectra, the structure of compound **4** was determined to be 16*α*, 20*β, 25*- trihydroxycucurbita- 5 (E)- diene- 11, 22- dione- 3- O- β- D- glucopyranoside and it was named Xuedanoside D.

Compound **5** was separated as a white amorphous powder with $[\alpha]_D^{22}$+112.1 (*c* 0.1, MeOH). Its molecular formula was determined to be $C_{36}H_{56}O_{11}$ by HRESIMS (observed *m/z* 687.8232 $[M + Na]^+$), necessitating nine degrees of unsaturation. The IR spectrum revealed strong bands at 3633-3421 cm^{-1}, which is typical of the hydroxyl group. The ^{1}H NMR (Tables 1 and 2) displayed signals due to 7 tertiary

methyl groups at δ_H 1.21 (s), 1.24 (s), 1.26 (s), 1.31 (s), 1.41 (s), 1.53 (s), and 1.99 (s); 9 oxygenated methines at δ_H 3.37 (d, J = 9.0 Hz), 3.94 (m), 4.12 (m), 4.21 (m), 4.24 (m), 4.29 (m), 4.98 (m), 5.12 (m), and 5.19 (m); and 2 olefinic proton signals at δ_H 5.68 (d, J = 6.0 Hz) and δ_H 6.68 (d, J = 6.0 Hz). The ^{13}C NMR spectrum of **5** revealed 36 carbon signals corresponding to 7 angular methyls, 7 methylenes (2 oxygenated methylenes), 14 methines (2 sp^2 methines and 9 oxygenated methines), and 8 quaternary carbons (2 sp^2 carbons, 1 carbonyl carbon, and 1 oxygenated carbon). The NMR data of **1** were similar to those of Xuedanencins B, except for the additional glucopyranosyl moiety at C-3 in **5** which was confirmed by the HMBC correlations from the anomeric proton signal at δ_H 5.19 (d, J = 8.4 Hz, H-1') to δ_C 94.3 (d, C-3). The ^{1}H and ^{13}C NMR spectra of **5** substantiated the substructure for another ring created by the cyclization of the side chain by an ether linkage [13][14]. Additionally, a ^{13}C-^{1}H long-rang correlation signal between H-16 and C-23 showed that C-16 and C-23 were connected via an ether bond to create a pyranoid structural element. In the HMBC, ^{13}C-^{1}H long-range correlation signals at H-23/C-25 and H_3-26 and H_2-27/C-24 put the olefinic bond between C-24 and C-25, and an OH on one of the terminal methyl groups. The NOE cross-peaks at H-24/H-16 and H_3-28/H-17 suggested that H-23/H-17 was α-oriented. The NOE cross-peaks at H-2/H-10, H-3/H-1α, H_3-18/H-16, and H_3-21/H-23 showed that OH-2, Oglc-3, H-16, and OH-20 were β-oriented and OH-3 was α-oriented. The NOE correlation between H_2-27 and H-24 showed the *E* configuration of the olefinic bond; therefore, **5** was typified as 2β, 20β, 26- trihydroxycucurbita- 1*6*α- *23*α- epoxy- 5, 24 (E)- diene- 11- one- 3- O- α- D- glucopyranoside, and was named Xuedanoside E.

Compounds **1-5** were evaluated to establish their cytotoxic activity according to the MTT procedure with HeLa, HCT-8, MCF-7 and HepG2 human cancer cell lines. The outcomes of their cytotoxic activity are shown in Table 3 (see Tables). As it can be seen, compounds **1** and **2** showed significant cytotoxic effects against HeLa cells with IC_{50} values of 3.21 and 8.57 μM, while compounds **3-5** had moderate effects. Compound **1** showed moderate effects against HepG2 cells with IC_{50} values of 18.62 μM. Compound **5** exhibited moderate cytotoxicity against HCT-8 cells with IC_{50}

values of 19.36 μM. All compounds demonstrated mild or no effects against MCF-7 cells. These above results indicated that the isolates could selectively enhance the cytotoxicity against the tested HeLa cells line to a certain degree. Furthermore, cucurbitacins were prepared to establish the structure–activity relationships for cytotoxicity against the HeLa cells.

Conflict of interest

We declare that we have no financial and personal relationships with individuals or organizations that can inappropriately influence our work, and there is no professional or personal interest of any kind in any product, service and/or company that could be construed as influencing the results presented in, or the review of, the present manuscript.

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Table 1. ^{1}H NMR Spectroscopic Data (600 MHz) for Compounds 1–5 (δ_H in ppm, *J* in Hz).

Position	1	2	3	4	5
1	2.67 (1H, m) 1.75 (1H, m)	2.40(1H, m) 1.52(1H, m)	2.42(1H, m) 1.52(1H, m)	1.63(1H, m) 1.49(1H, m)	2.61(1H, m) 1.43(1H, m)
2	4.35 (1H, m)	4.03(1H, m)	4.10(1H, m)	2.43(1H, d, 12.0) 1.86(1H, m)	4.21(1H, m)
3	3.62 (1H, d, 6.0)	3.42 (1H, d, 9.0)	3.42(1H, m)	3.64(1H, s)	3.37(1H, d, 9.0)
4					
5					
6	6.23 (1H, d, 6.0)	5.72(1H, d, 6.0)	5.73(1H, d, 6.0)	5.50(1H, d, 12.0)	5.68(1H, d, 6.0)
7	4.48 (1H, m)	2.28(1H, m) 1.78(1H, m)	2.29(1H, m) 1.79(1H, m)	2.16(1H, m) 1.79(1H, m)	2.26(1H, m) 1.82(1H, m)
8	2.88 (1H, m)	1.86(1H, d, 7.8)	1.88(1H, m)	1.81(1H, m)	1.93(1H, m)
9					
10	2.52 (1H, s)	2.63(1H, d, 14.4)	2.64(1H, m)	2.52(1H, d, 14.4)	2.42(1H, d, 14.4)
11					
12	3.11 (1H, d, 18.0) 2.73 (1H, d, 12.0)	3.21(1H, d, 14.4) 2.79(1H, d, 14.4)	3.18(1H, d, 12.0) 2.75(1H, d, 14.4)	3.20(1H, d, 14.4) 2.75(1H, d, 14.4)	3.08(1H, d, 14.4) 2.61(1H, d, 14.4)
13					
14					
15	2.17 (1H, m) 1.87 (1H, m)	2.03(1H, m) 1.51(1H, m)	2.02(1H, m) 1.44(1H, m)	1.84(1H, m) 1.68(1H, m)	1.88(1H, m) 1.57(1H, m)
16	4.91 (1H, m)	5.96(1H, t, 6.0)	5.90(1H, t, 12.0)	4.87(1H, m)	5.12(1H, m)
17	2.90 (1H, m)	3.02(1H, d, 7.2)	3.05(1H, m)	2.97(1H, d, 6.0)	2.12(1H, d, 14.4)
18	1.24 (3H, s)	1.17(3H, s)	1.15(3H, s)	1.20(3H, s)	1.26(3H, s)
19	1.71 (3H, s)	1.16(3H, s)	1.20(3H, s)	1.15(3H, s)	1.24(3H, s)
20					
21	1.57 (3H, s)	1.69(3H, s)	1.57(3H, s)	1.59(3H, s)	1.41(3H, s)
22					1.95(1H, m) 1.74(1H, d, 13.8)
23	3.32 (1H, m) 3.06 (1H, m)	3.17(1H, m) 3.09(1H, m)	3.40(1H, m) 3.27(1H, m)	3.50(1H, m) 3.27(1H, m)	4.98(1H, m)
24	2.43 (1H, m) 2.32 (1H, m)	4.44(1H, d, 7.8)	2.21(1H, m) 2.11(1H, m)	2.25(1H, m) 2.19(1H, m)	6.68(1H, d, 6)
25					
26	1.48(3H, s)	1.38(3H, s)	1.44(3H, s)	1.37(3H, s)	1.99(3H, s)
27	1.48(3H, s)	1.36(3H, s)	1.44(3H, s)	1.37(3H, s)	4.60(1H, m) 4.50(1H, m)
28	1.50(3H, s)	1.26(3H, s)	1.27(3H, s)	1.48(3H, s)	1.31(3H, s)
29	1.37(3H, s)	1.31(3H, s)	1.33(3H, s)	1.09(3H, s)	1.53(3H, s)
30	1.51(3H, s)	1.50(3H, s)	1.50(3H, s)	1.54(3H, s)	1.21(3H, s)
OAc-25	1.91(3H, s)	2.22(3H, s)	2.14(3H, s)		

Glc					
1`	5.34(1H, d, 6.0)	4.98(1H, d, 6.0)	4.95(1H, d, 7.8)	4.89(1H, d, 6)	5.19(1H, d, 8.4)
2`	4.07(1H, t, 6)	3.55(1H, t, 1.8)	3.93(1H, m)	3.98(1H, m)	4.12(1H, m)
3`	4.18 (1H, t, 6)	4.17(1H, m)	4.20(1H, m)	4.22(1H, d, 6)	4.29(1H, m)
4`	4.30 (1H, m)	4.02(1H, m)	4.19(1H, m)	4.21(1H, m)	4.24(1H, m)
5`	3.88 (1H, m)	4.16(1H, m)	3.88(1H, m)	3.96(1H, m)	3.94 (1H, m)
6`	4.50 (1H, m)	4.52(1H, d, 12)	4.46(1H, m)	4.55(1H, d, 12)	4.56(1H, d, 12)
	4.38 (1H, d, 12.0)	4.33(1H, m)	4.32(1H, m)	4.39 (1H, m)	4.35(1H, m)

Table 2. ^{13}C NMR (150MHz, in pyridine-d_5) spectral data of compounds 1-5.

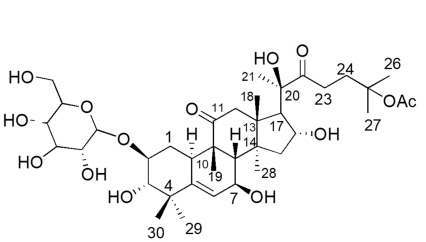
Position	**1**	**2**	**3**	**4**	**5**
1	33.6	35.0	35.0	22.5	34.3
2	83.5	71.4	71.4	29.0	71.6
3	81.0	81.8	81.8	87.6	94.3
4	43.2	43.3	44.3	42.5	42.9
5	144.8	142.8	142.9	141.7	142.3
6	123.2	119.0	119.0	118.9	119.8
7	66.5	24.4	24.4	24.6	24.6
8	53.5	43.0	43.0	43.6	43.4
9	49.0	48.5	48.5	49.2	49.3
10	35.5	34.8	34.8	36.3	34.3
11	213.8	212.9	213.1	213.7	213.4
12	49.8	49.1	49.1	49.6	49.1
13	48.4	49.4	49.4	49.7	49.1
14	50.7	50.9	51.0	51.6	49.6
15	46.9	44.2	43.3	46.9	42.1
16	70.8	75.2	75.1	70.8	71.3
17	59.3	55.8	55.7	59.2	56.5
18	20.7	20.7	20.5	20.7	20.5
19	22.2	20.8	20.8	20.8	21.0
20	80.5	80.4	80.3	80.6	72.8
21	25.5	25.4	25.5	25.9	30.6
22	215.5	213.1	216.4	216.6	47.1
23	32.6	40.1	33.0	33.2	70.9
24	35.8	80.3	36.6	38.9	129.3
25	82.0	77.0	77.0	69.5	139.0
26	26.5	19.4	27.6	30.5	22.2
27	26.4	19.3	27.4	30.4	61.2
28	19.8	22.9	19.4	19.5	21.5
29	23.2	26.7	19.3	28.8	25.7
30	25.9	26.0	26.0	26.3	23.7
OAc-25	170.6	171.1	171.0		
	22.6	21.8	21.8		
Glc					

1`	107.0	95.6	99.3	107.8	107.7
2`	76.3	82.5	75.7	75.9	76.7
3`	79.0	75.3	79.2	79.2	79.2
4`	71.8	80.7	72.2	72.2	72.2
5`	79.0	72.8	78.6	78.7	79.1
6`	62.9	63.1	63.3	63.4	63.2

Table 3. The IC_{50} values of compounds 1–5 against cancer cell lines.

Compounds	IC_{50}			
	HeLa	**HCT-8**	**MCF-7**	**HepG2**
1	3.21 ± 0.13	24.72 ± 0.52	＞50	18.62 ± 167
2	8.57 ± 0.34	＞50	＞50	＞50
3	12.86 ± 0.25	36.24± 1.27	＞50	＞50
4	18.37 ± 0.35	＞50	＞50	＞50
5	10.29 ± 0.44	19.36± 0.92	＞50	37.44 ± 2.16
Doxorubicin[b]	1.08 ± 0.06	3.27 ± 0.64	7.55 ± 0.17	4.28 ± 0.40

[a] Value present mean ± SD of triplicate experiments. [b] Positive control substance.



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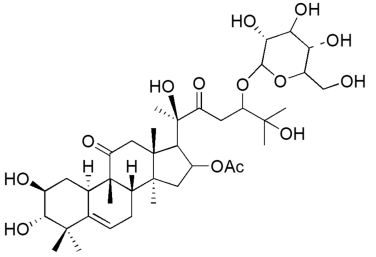

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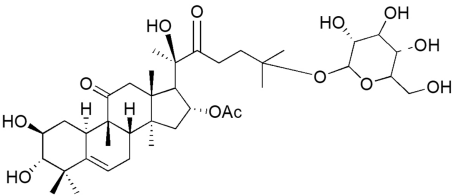

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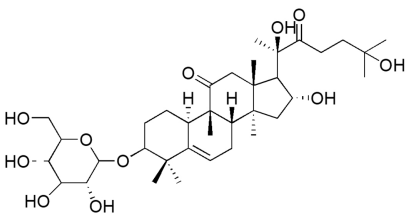

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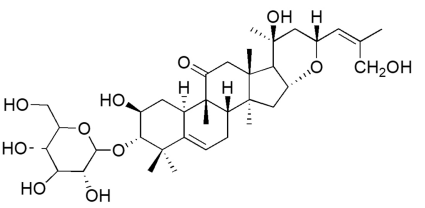

5

Figure 1

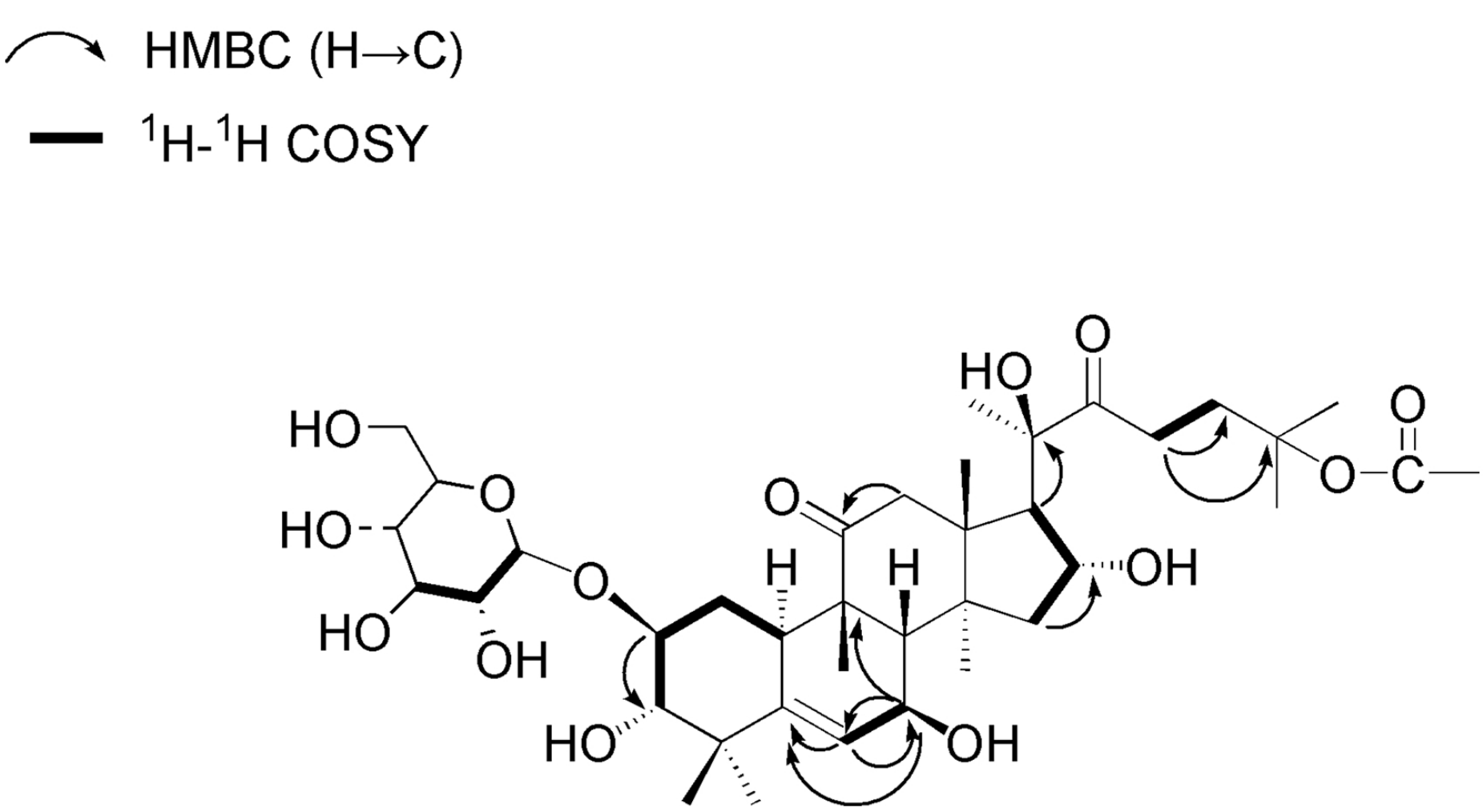

Figure 2

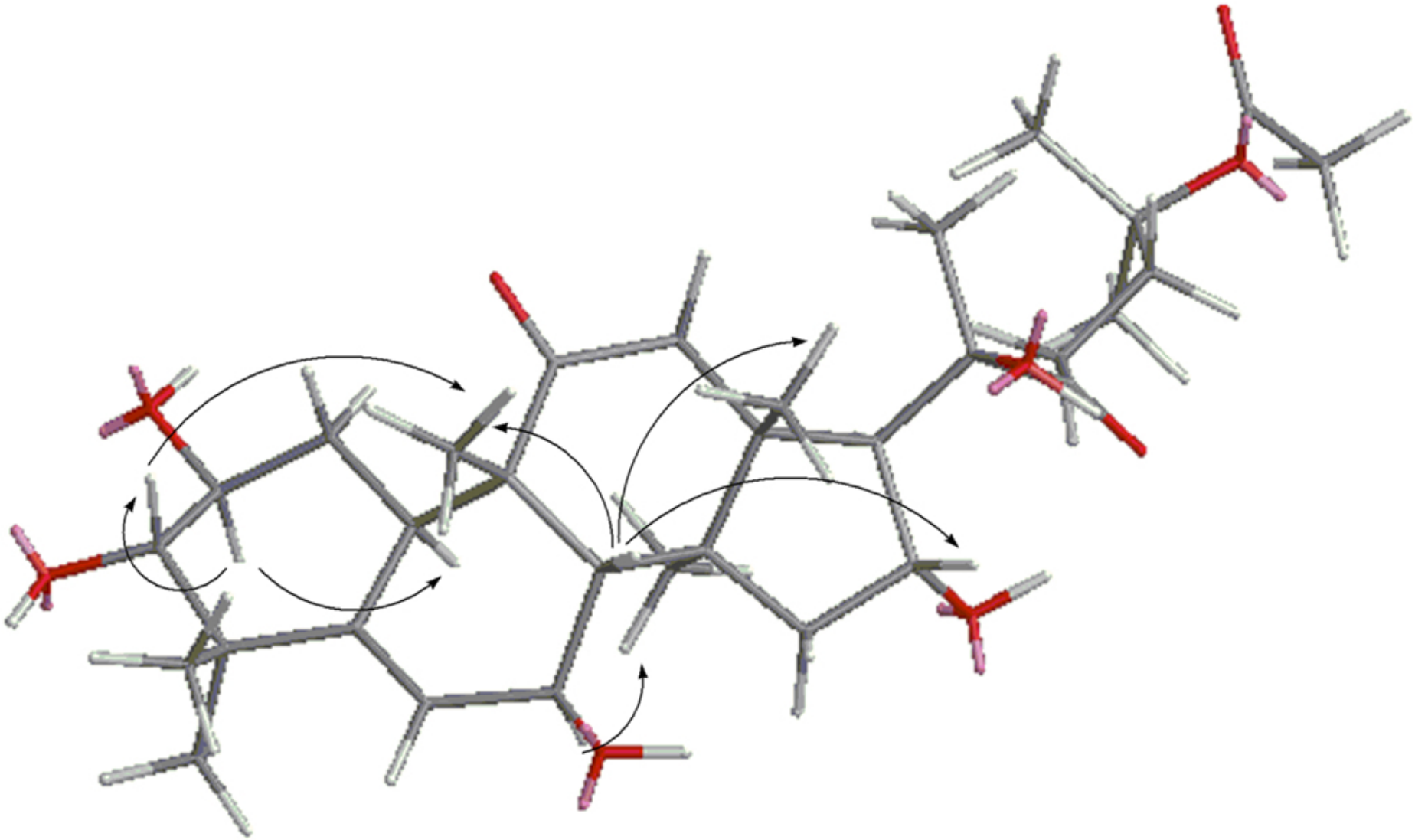

Figure 3