Sesquiterpene Lactone Glycosides, Eudesmanolides, and Other Constituents from *Carpesium macrocephalum*

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Original Paper

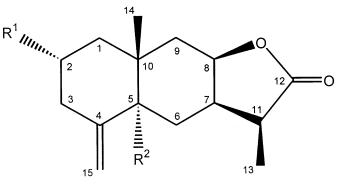
Abstract

Two new sesquiterpene lactone glycosides and two new eudesmanolides, along with twelve known compounds were isolated from seeds of *Carpesium macrocephalum*. The structures of these new compounds were elucidated as 2α-*O*-β-D-glucopyranosyl-5α, 11α*H*-eudesma-4(15)-en-12,8β-olide (**1**), 2α-*O*-β-D-glucopyranosyl-5α*H*-eudesma-4(15),11(13)-dien-12,8β-olide (**2**), 2α-acetoxy-5α-hydroxy-11α*H*-eudesma-4(15)-en-12,8β-olide (**3**) and 2α,5α-dihydroxy-11αH-eudesma-4(15)-en-12,8β-olide (**4**) by spectroscopic methods including 2D NMR techniques (^{1}H-^{1}H COSY, ^{1}H-^{1}H NOESY, HMQC, HMBC) and chemical transformations. Compounds **1**, **6**, **8**, **9** and **10** exhibited moderate antibacterial activity, while compound **4** showed appreciable cytotoxic activity against cultured SMMC-7721 (human hepatoma cell).

Key words
Carpesium macrocephalum · Compositae · eudesmanolide · sesquiterpene lactone glycosides · antibacterial activity · cytotoxic activity

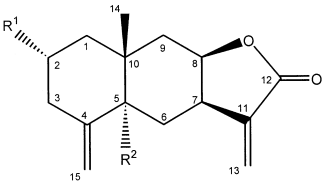
Introduction

The seeds of *Carpesium* (Compositae) species have been used as a vermifuge in Chinese traditional medicine. Sesquiterpene lactones have been reported as the most widespread main constituents in the genus [1], [2], [3], [4], [5], [6], [7], [8], [9]. *Carpesium macrocephalum* Franch. et Savat. has long been used as a Chinese folk medicine for its hemostatic and antipyretic properties [10]. Its chemical constituents have not been previously investigated. We describe herein the isolation and structural elucidation of four new compounds (**1 – 4**) and twelve known compounds (**5 – 16**) from the seeds of *Carpesium macrocephalum*, and the antibacterial activity test of ten compounds (**1**, **4**, **6**, **8 – 14**) and cytotoxic activity test of three compounds (**1**, **4** and **14**) (Figs. **1** and **2**).



	R^1	R^2
1	O-β-D-Glc	H
3	OAc	OH
4	OH	OH
5	OH	H
7	H	OH
11	H	H

Fig. **1**



	R^1	R^2
2	O-β-D-Glc	H
6	OH	H
8	H	OH
12	H	H

Fig. **2**

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Materials and Methods

Apparatus

Melting points were determined with a Kofler Melting point apparatus, and not corrected. Optical rotations were taken on a JASCO-20C automatic recording spectro-polarimeter. IR spectra were measured on a Nicolet 170SX FT-IR instrument. ^{1}H-NMR (400 MHz), ^{13}C-NMR (100 MHz) and 2D NMR spectra were recorded on a Bruker AM-400 FT-NMR spectrometer using TMS as internal standard. HRESI-MS was recorded on a Bruker APEX II, EI-MS on an HP 5988A GC/MS instrument and FAB-MS on a VG-ZAB-HS mass spectrometer. Silica gel (200 – 300 mesh) was used for column chromatography, and silica gel GF_{254} for TLC were supplied by the Qingdao Marine Chemical Factory in China.

Plant material

Carpesium macrocephalum Franch. et Savat. were collected in Tianshui, Gansu province, P. R. China, in September 1998. It was identified by Prof. Guoliang Zhang, Department of Biology, Lanzhou University. A voucher specimen (NO.9803) was deposited in the Institute of Organic Chemistry, Lanzhou University. Seeds were harvested, then air-dried.

Extraction and isolation

Air-dried seeds of *Carpesium macrocephalum* (980 g) were pulverized and extracted with methanol (3200 ml × 4 times, 7 days each time) at room temperature. The extract was evaporated giving a residue (68 g) which was column (6.0 × 75 cm) chromatographed over silica gel (200 – 300 mesh, 720 g) with a gradient of petroleum ether (60 – 90 °C)–acetone (1 : 0, 15 : 1, 7.5 : 1, 5 : 1, 2.5 : 1 and 1 : 1). Seven fractions A-G were collected according to TLC analysis. The eluent of 1 : 0 (5800 ml) was fraction A, which was purified by CC on silica gel (150 g) using petroleum ether–EtOAc (30 : 1 → 10 : 1, 680 ml each eluent) to yield **12** (12 mg, 20 : 1), **11** (21 mg, 20 : 1) and β-sitosterol **15** (10 : 1). Fraction B (15 : 1, 4800 ml) gave **8** (125 mg) by repeated recrystallization from EtOAc, and carabrone **9** (586 mg) was obtained from fraction C (7.5 : 1, 5800 ml). The residue of fraction B, further isolated over a silica gel column (30 g) with petroleum ether–Et_2O (2 : 1) yielded **7** (18 mg). The fraction D (7.5 : 1, 5800 – 15 600 ml) was purified using CC on silica gel (120 g) with petroleum ether–EtOAc (3 : 1, 1200 ml) to give carabrol **10** (1680 mg) and **3** (14 mg). Scopoletin **13** (22 mg), **6** (58 mg) and **5** (12 mg) were obtained from fraction E (5 : 1, 6800 ml) after CC on silica gel (120 g) with $CHCl_3$-CH_3OH (60 : 1, 1080 ml) and repeated recrystallization. Eluents of 5 : 1 (6800 – 11 000 ml) and 2.5 : 1 (8200 ml) were combined to yield fraction F, which was chromatographed on a silica gel column (100 g) with $CHCl_3$-CH_3OH (30 : 1 → 20 : 1, 590 ml each eluent) to obtain **4** (38 mg, 30 : 1) and **14** (50 mg, 20 : 1), which were purified by recrystallization (CH_3OH). Fraction G (1 : 1, 15 800 ml) afforded **16** by recrystallization. The residue was chromatographed on a silica gel column (160 g) and eluted with $CHCl_3$-CH_3OH (20 : 1 → 10 : 1, 500 ml each eluent) to afford **1** (56 mg, 10 : 1), which was recrystallized in CH_3OH, and **2** (7 mg, 10 : 1), which was purified by preparative TLC (GF_{254}, CH_2Cl_2-CH_3OH, 6 : 1, 180 ml two developments).

Acid hydrolysis of 1

Compound **1** (8 mg) dissolved in 2% HCl was heated in a 90 °C water-bath for 3 hours. After cooling, the reaction mixture was extracted with $CHCl_3$. The aqueous layer was neutralized with $NaHCO_3$ and concentrated under reduced pressure. The residue on a silica gel column (1 g) was first eluted with acetone, then with anhydrous ethanol to afford a pure sugar (1.8 mg). The sugar was confirmed as D-glucose by comparison with the authentic sample on PC and measuring its optical rotation.

Sugar of **1**: $[\alpha]_D^{20}$: + 48.0° (*c* 0.12, H_2O).

2α-(β-D-Glucopyranosyloxy)-5α,11αH-eudesma-4(15)-en-12,8β-olide (**1**): Colorless needles from CH_3OH, m. p. 206 – 208 °C. $[\alpha]_D^{20}$: + 17.9° (*c* 0.56, CH_3OH). IR (KBr): ν_{max} = 3577, 3389 (OH), 2936, 1778 (γ-lactone), 1646, 1460, 1360, 1266, 1166, 1079, 971, 902 cm^{-1}. HRMS: revealed *m/z* = 435.1996 $[M + Na]^+$, requires 435.1989. ^{1}H- and ^{13}C-NMR: see Tables **1**, **2**.

2α-(β-D-Glucopyranosyloxy)-5αH-eudesma-4(15),11(13)-dien-12,8β-olide (**2**): Colorless needles from CH_3OH, m. p. 176 – 178 °C. $[\alpha]_D^{25}$: + 13.0° (*c* 0.69, CH_3OH). IR (KBr): ν_{max} = 3574 (OH), 3408, 2932, 1768 (γ-lactone), 1650, 1600, 1454, 1349, 1170, 1081, 1039, 964, 895, 818 cm^{-1}. HRMS: revealed *m/z* = 428.2295 $[M + NH_4]^+$, requires 428.2279. ^{1}H- and ^{13}C-NMR: see Tables **1**, **2**.

2α-Acetoxy-5α-hydroxy-11αH-eudesma-4(15)-en-12,8β-olide (**3**): $[\alpha]_D^{25}$: + 77.0° (*c* 0.44, $CHCl_3$). IR (KBr): ν_{max} = 3482 (OH), 2962, 1756 (γ-lactone), 1702 (OAc), 1376, 1265, 1174, 1020, 964, 918, 881 cm^{-1}. HRMS: revealed *m/z* = 331.1515 $[M + Na]^+$, requires 331.1516. EI-MS: *m/z* (%) = 248 $[M - AcOH]^+$ (12), 230 $[248 - H_2O]^+$

Table **1** ^{1}H-NMR data of **1** and **2** (CD_3OD, TMS, δ, ppm)[a,b]

No.	*1*	*2*
1α	1.25 (br.dd, 12.5, 12.5)	1.24 (t, 12, 12)
1β	1.99 (ddd, 2, 5, 12.5)	2.00 (ddd, 2, 5, 12)
2β	3.87* (m)	3.85* (m)
3α	2.11* (t, 12.5, 12.5)	2.12* (t, 12, 12)
3β	2.79 (ddd, 2, 5, 12.5)	2.79 (ddd, 2, 5, 12)
4	–	–
5α	1.88 (br.d, 12.5)	1.94 (br.d, 13)
6α	1.63* (m)	1.82 (ddd, 2, 6, 13)
6β	1.06 (ddd, 12.5, 12.5, 12.5)	1.26 (ddd, 13, 13, 13)
7α	2.49 (m, 4, 7, 12.5)	3.10 (ddd, 4, 7, 13)
8α	4.52 (ddd, 2, 4, 4.5)	4.56 (ddd, 2, 4, 5)
9α	1.58 (dd, 4.5, 15.5)	1.63 (dd, 5, 15.5)
9β	2.16* (dd, 2, 15.5)	2.20 (br.d, 15.5)
10	–	–
11α	2.93 (dq, 7, 7)	–
12	–	–
13	1.18 (d, 7)	6.06, 5.70 (br.s)
14	0.78 (s)	0.80 (s)
15	4.89, 4.62 (br.s)	4.87, 4.59 (br.s)
1′	4.34 (d, 8)	4.34 (d, 8)
2′	3.12 (t, 8.2)	3.12 (t, 8.2)
3′	3.34* (t, 8.8)	3.34* (t, 8.8)
4′	3.30* (t, 8.8)	3.30* (t, 8.8)
5′	3.26* (m, 2, 5.8)	3.26* (m, 2, 5.8)
6′	3.85* (dd, 2, 11.8), 3.65 (dd, 5.8, 11.8)	3.85* (dd, 2, 11.8), 3.65 (dd, 5.8, 11.8)

[a] Signal multiplicity and coupling constants (Hz) are in parentheses.
[b] *Overlapping signals.

Table **2** ^{13}C-NMR (DEPT) and HMBC data of **1** and **2** (CD_3OD, TMS, δ, ppm)

No	^{13}C (DEPT) 1	HMBC (C/H) 1	^{13}C (DEPT) 2
1	48.5 t	C-1/H-14	48.4 t
2	76.0 d	C-2/H- 3, 1α, 1′	75.8 d
3	45.5 t	C-3/H-15	45.5 t
4	148.4 s	C-4/H-3, 5	148.1 s
5	46.9 d	C-5/H-3β, 9β, 15, 14	46.5 d
6	22.2 t	C-6/H-11	28.5 t
7	41.6 d	C-7/H-9β, 11, 13	41.5 d
8	79.6 d	C-8/H-9β	78.8 d
9	42.2 t	C-9/H-14	41.9 t
10	35.2 s	C-10/H-1α, 5, 9, 14	34.7 s
11	42.8 d	C-11/H-13	144.0 s
12	182.0 s	C-12/H-11, 13	173.0 s
13	9.5 q	C-13/H-11	120.9 t
14	19.1 q	C-14/H-1α	18.9 q
15	109.2 t	C-15/H-3	109.5 t
1′	102.9 d	C-1′/H-2′	102.9 d
2′	75.1 d	–	75.1 d
3′	78.1 d	C-3′/H-2′, 5′	78.1 d
4′	71.6 d	C-4′/H-3′	71.6 d
5′	77.9 d	C-5′/H-4′	77.9 d
6′	62.8 t	–	62.8 t

Table **3** ^{1}H-NMR data of **3** ($CDCl_3$, TMS, δ, ppm) and **4** (CD_3OD, TMS, δ, ppm)[a,b]

No	3	4
1α	1.92* (dd, 12, 12)	1.81* (dd, 12, 12)
1β	1.40* (dd, 4.5, 12.5)	1.40* (dd, 5, 12.5)
2β	4.98 (dddd, 4.5, 6, 11, 12)	3.79 (dddd, 5, 5.5, 11.5, 12)
3α	2.64* (ddd, 2, 11, 12)	2.59 (ddd, 2, 11.5, 12)
3β	2.58* (ddd, 2, 6, 12)	2.42 (ddd, 2, 5.5, 12)
4	–	–
5	–	–
6α	1.65 (dd, 6, 14)	1.69 (dd, 6, 13.8)
6β	1.56* (dd, 12, 14)	1.42* (dd, 12, 13.8)
7α	2.84* (dddd, 5, 6, 7, 12)	2.86 (dddd, 5, 6, 7, 12)
8α	4.54 (ddd, 2.4, 4.5, 5)	4.58 (ddd, 2, 4.6, 5)
9α	2.02 (dd, 4.5, 15)	2.02 (dd, 4.6, 15)
9β	1.83* (dd, 2.4, 15)	1.83* (dd, 2, 15)
10	–	–
11α	2.97 (dq, 7, 7)	2.97 (dq, 7, 7)
12	–	–
13	1.16 (d, 7)	1.16 (d, 7)
14	0.90 (s)	0.90 (s)
15	4.93, 4.80 (br.s)	4.93, 4.80 (br.s)
1′	–	
2′	2.04* (s)	

[a] Signal multiplicity and coupling constants (Hz) are in parentheses.
[b] *Overlapping signals.

(1), 233 (15), 204 (14), 149 (32), 123 (38), 109 (100), 95 (43), 94 (28), 91 (33), 81 (77), 69 (42), 55 (43), 41 (64). ^{1}H- and ^{13}C-NMR: see Tables **3**, **4**.

2α,5α-Dihydroxy-11αH-eudesma-4(15)-en-12,8β-olide (**4**): Colorless crystals from CH_3OH, m. p. 108 – 110 °C. $[\alpha]_D^{20}$: + 68.0° (*c* 0.74, CH_3OH). IR (KBr): ν_{max} = 3407, 3312 (OH), 2944, 1738 (γ-lactone), 1656, 1186, 1042, 964, 912, 878 cm^{-1}. HRMS: revealed *m/z* = 289.1413 $[M + Na]^+$, requires 289.1410. EI-MS: *m/z* (%) = 266 $[M]^+$ (1.6), 248 $[M - H_2O]^+$ (9.3), 230 $[248 - H_2O]^+$ (2.7), 182 (100), 155 (19), 137 (16), 109 (62), 94 (48), 91 (28), 81 (21), 69 (27), 55 (32), 41 (31). ^{1}H- and ^{13}C-NMR: see Tables **3**, **4**.

The $[\alpha]_D^{25}$ of known compounds **5 – 12**, **15** and **16** were: + 110.2° (*c* 0.74, $CHCl_3$); + 138.0° (*c* 1.19, $CHCl_3$); + 122.2° (*c* 0.50, $CHCl_3$); + 220.3° (*c* 1.41, $CHCl_3$); + 104.1° (*c* 0.61, $CHCl_3$); + 74.9° (*c* 1.43, $CHCl_3$); + 18.6° (*c* 0.70, $CHCl_3$); + 0.2° (*c* 1.43, $CHCl_3$); –37.0° (*c* 1.82, $CHCl_3$); –46.6° (*c* 1.12, C_5H_5N).

Table **4** ^{13}C NMR (DEPT) and HMBC data of **3** ($CDCl_3$, TMS, δ, ppm) and **4** (CD_3OD, TMS, δ, ppm)

No	^{13}C (DEPT) 3	HMBC (C/H) 3	^{13}C (DEPT) 4
1	40.0 t	C-1/H-3β, 9α, 14	45.1 t
2	69.2 d	C-2/H-1, 3	67.5 d
3	37.2 t	C-3/H-1β, 15	42.3 t
4	147.5 s	C-4/H-3, 15	151.1 s
5	73.3 d	C-5/H-1β, 3β, 6α, 9β, 14, 15	73.5 d
6	27.3 t	C-6/H-11	28.0 t
7	37.0 d	C-7/H-6, 9β, 11, 13	38.5 d
8	77.5 d	C-8/H-6α, 9β	79.9 d
9	35.4 t	C-9/H-1α, 14	36.8 t
10	37.1 s	C-10/H-6α, 9, 14	37.9 s
11	41.0 d	C-11/H-6β, 7, 13	42.1 d
12	179.1 s	C-12/H-11, 13	182.2 s
13	9.4 q	C-13/H-11	9.6 q
14	22.3 q	C-14/H-1α, 9	22.9 q
15	111.9 t	C-15/H-3	110.2 t
1′	170.4 s	C-1′/H-2, 2′	
2′	21.3 q	–	

Results and Discussion

From the methanol extracts of the seeds of *Carpesium macrocephalum* two new sesquiterpene lactone glycosides **1** and **2**, two new sesquiterpene lactones **3** and **4**, together with twelve known compounds: 11α,13-dihydroivalin **5** [11], ivalin **6** [11], 11α,13-dihydrotelekin **7** [4], telekin **8** [4], carabrone **9** [2], carabrol **10** [3], 5α,11αH-eudesma-4(15)-en-12,8β-olide **11** [12], 5αH-eudesma-4(15),11(13)-dien-12,8β-olide **12** [13], scopoletin **13** [14], 3,4′,5,7-tetrahydroxydihydroflavonol **14** [15], β-sitosterol **15** and sitogluside **16**. The known compounds **5 – 14** were identified by direct comparison of their spectral data (MS, ^{1}H-NMR and ^{13}C-NMR) with reported values in the literature, and compounds **15 – 16** by comparison with authentic samples. Compounds **3 – 12** are sesquiterpene lactones, characteristic for the genus. Compounds **1** and **2** are sesquiterpene lactone glycosides, and to our knowledge, previously unreported from the genus *Carpesium*.

Compound **1** was obtained as colorless needles, with molecular formula $C_{21}H_{32}O_8$ deduced from HRESI-MS (*m/z* = 435.1996 [M + Na]$^+$, calc. 435.1989). Its IR spectra showed the presence of a carbonyl group (1778 cm^{-1}), hydroxy groups (3389 cm^{-1}), double bond (1646 cm^{-1}) and a glycosidic linkage (1079 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra (Tables **1** and **2**) of **1** showed the typical signals for a β-glucopyranosyl, which was confirmed by PC after hydrolysis of **1**. Because the optical rotation of the pure sugar obtained after hydrolysis of **1** was +48.0°, the sugar was determined to have the D-configuration. The remaining signals indicated that the aglycone was a sesquiterpene lactone with one tertiary and one secondary methyl, a γ-lactone moiety, one terminal double bond, and two > CH–O– units. Moreover, these signals were similar to those of the know eudesmanolide, 2α-hydroxy-5α,11αH-eudesma-4(15)-en-12,8β-olide **1a** [11], with the obvious difference in chemical shifts of C-2 from 67.2 ppm in **1a** to 76.0 ppm in **1**, explicable by assuming the additional *O*-glucoside moiety is located at C-2. The doublet for H-1′ at δ = 4.34 (d, *J* = 8 Hz) and the resonance for C-1′ at δ = 102.9 indicated that the glucosidic bond of **1** had the β-configuration. Furthermore, structural elucidation was confirmed by ^{1}H-^{1}H COSY, HMQC and HMBC spectra. The attachment of glucose to the hydroxy at C-2 is deduced by the long-range coupling between H-1′ and C-2. The large coupling of H-2 ($J_{2\beta,1\alpha}$ = $J_{2\beta,3\alpha}$ = 12.5 Hz) confirmed that the 2-hydroxy group was α-oriented. Hence, compound **1** was identified as 2α-(β-D-glucopyranosyloxy)-5α,11αH-eudesma-4(15)-en-12,8β-olide.

The ^{1}H- and ^{13}C-NMR spectra of compound **2** (Tables **1** and **2**) were similar to those of **1** except that the signals of the 11β-oriented methyl γ-lactone group (δ_C = 182.0, C; δ_H = 2.93 dq, δ_C = 42.8, CH; δ_H = 1.18 d, δ_C = 9.5, CH_3) in **1** had been replaced by the signals of an α-methylene-γ-lactone group (δ = 173.0, C; δ = 144.0, C; δ_H = 6.06, 5.70, brs, δ_C = 120.9, CH_2). So compound **2** was determined as 2α-(β-D-glucopyranosyloxy)-5αH-eudesma-4(15),11(13)-dien-12,8β-olide.

From HRESI-MS, the molecular formulas of compounds **3** and **4** could be deduced as $C_{17}H_{24}O_5$ and $C_{15}H_{22}O_4$ on the basis of the quasi-molecular ion peaks at *m/z* = 331.1516 [M + Na]$^+$ and *m/z* = 289.1410 [M + Na]$^+$. Except for the additional signals of an acetyl group [C-1′ (C, δ_C = 170.4), C-2′ (CH_3, δ_C = 21.3, δ_H = 2.04 s)], the ^{1}H- and ^{13}C-NMR spectral data (Tables **3** and **4**) of compounds **3** were very similar to those of **4**, which suggested that compounds **3** and **4** have the same basic carbon skeleton: one tertiary and one secondary methyl, a γ-lactone moiety, a terminal double bond, one oxygenated quaternary carbon and two > CH–O– units.

Structural elucidation of **3** and **4** was based on ^{1}H- and ^{13}C-NMR (DEPT) spectra, including ^{1}H-^{1}H COSY, HMQC, and HMBC, which allowed the full assignment of all carbon and proton resonances. By ^{1}H-^{1}H COSY experiments, two partial structures: -CH_2-CH(-O-)-CH_2- and -CH_2-CH(CH-CH_3)-CH(-O-)-CH_2- were revealed. Together with ^{1}H-^{13}C long-range correlations (Table **4**): C-1/H-9,H-14; C-3/H-15; C-5/H-1,H-3,H-9,H-14,H-15; C-10/H-6; C-12/H-13, in an HMBC experiment, the whole planar structure was formed. Comparison of chemical shifts showed that H-2 (δ_H = 4.98, in **3**) was now downfield (δ_H = 3.79, in **4**), which implicated that the additional acetyl group is located at the C-2 hydroxy group. Moreover, this location of the acetyl group was confirmed by long-range coupling between H-2 and C-1′ in compound **3**. Finally, the stereochemistry was established by H^1-H^1 NOESY spectra. Clear NOE correlations (shown by arrows in Fig. **3**) between H-2β and H-14, H-8 and H-11, H-6β and H-13, H-14, as well as H-15 and H-6α, H-6β indicated that 14-CH_3, 13-CH_3 and H-2 had β-configurations, while H-7, 8, 11 and the hydroxy at C-5 were α-orientated. The shifts of H-14 (δ_H = 0.90 s) and H-15 (δ_H = 4.93 br.s, 4.80 br.s) signals were similar to those of the known methyl ester of 5α-hydroxycostic acid, which further confirmed an α-orientation of the 5-hydroxy [16]. Consequently, the two new eudesmanolides were characterized as 2α-acetoxy-5α-hydroxy-11αH-eudesma-4(15)-en-12,8β-olide **3** and 2α,5α-dihydroxy-11αH-eudesma-4(15)-en-12,8β-olide **4**.

Antibacterial activity

The compounds **1**, **4**, **6**, **8 – 14** were tested for their antibacterial activities against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* using the cup-plate technique in the nutrient agar media by measuring the inhibition zone in mm. The test was performed at 200 ppm concentration of each compound in a cup of 8 mm diameter. Positive control was choromycetin. From antibacterial activity data (Table **5**) it was found compounds **1**, **6**, **8**, **9** and **10** exhibited moderate activity against the microorganisms.

Cytotoxic activity

The compounds **1**, **4** and **14** were evaluated for their cytotoxic activity against cultured SMMC-7721 (human hepatoma) and HO-8910 (human ovarian carcinoma) cells using the MTT method. From cytotoxic activity data (Table **6**) it was found compound **4** shows appreciable cytotoxic activity against SMMC-7721.

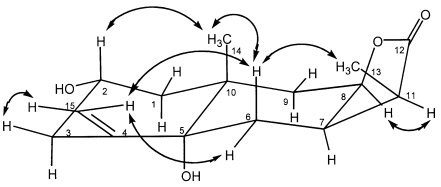

Fig. **3** Key NOEs observed for compound **4**.

Table **5** Antibacterial activity

Compound	B. subtilis	S. aureus	E. coli
1	+	++	++
4	–	+	+
6	++	+	+
8	+	++	+
9	++	+	+
10	++	+	+
11	–	+	+
12	+	+	+
13	+	+	–
14	+	+	+
Chloromycetin	+++	+++	+++

"–": Zone diameter of growth inhibition less than 10 mm, "+" equal to 11 – 13 mm, "++" equal to 14 – 15 mm, "+++" more than 15 mm.

Table **6** Cytotoxic activity

Compound	*SMMC-7721*	*HO-8910*
1	297.59 ± 1.80	> 400
4	70.28 ± 4.12	286.28 ± 22.4
14	> 400	> 400
Vinblastine	56.78 ± 4.08	46.57 ± 2.35

Half inhibition concentration IC_{50} ($\mu g\ ml^{-1}$).

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