Sesquiterpene Lactones from *Cichorium intybus*

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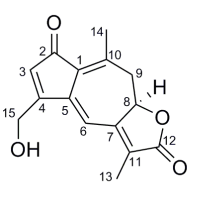
Two new sesquiterpene lactones (guaianolides), 15-hydroxytaraxacin (**1**) and 6,8,11-*epi*-desacetylmatricarin (**2**), along with three known compounds, desacetylmatricarin (**3**), 11β,13-dihydrolactucin (**4**), and 11β,13-dihydrolactucopicrin (**5**), were isolated from the aerial parts of *Cichorium intybus* L.

Key words: *Cichorium intybus*, Asteraceae, Sesquiterpene Lactones, 15-Hydroxytaraxacin, 6,8,11-*epi*-Desacetylmatricarin

Introduction

Cichorium intybus L., a small to medium size perennial herb, belongs to the family Asteraceae and is known as Kasini in Urdu (Pakistan) and Chicory in English [1, 2]. Traditionally, the plant is used in the treatment of fever, vomiting, diarrhea, alexiferic, liver disorder, gout, and rheumatism [3]. Previously, anthocyanins [4], sesquiterpene lactones [5, 6], fructans [7], flavonoids [8], and coumarins [9, 10] have been reported from it. To search for new natural therapeutics, the aerial parts of *C. intybus* were investigated. Two new (**1** and **2**) and three known (**3** – **5**) sesquiterpene lactones (guainolides) were isolated and identified by spectroscopic data analyses as 15-hydroxytaraxacin (**1**), and 6,8,11-*epi*-desacetylmatricarin (**2**), desacetylmatricarin (**3**), 11β, 13-dihydrolactucin (**4**), and 11β,13-dihydrolactucopicrin (**5**).



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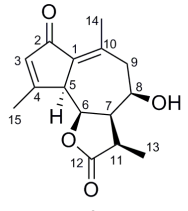

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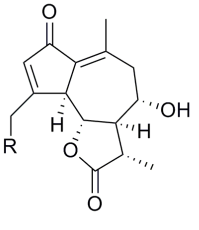

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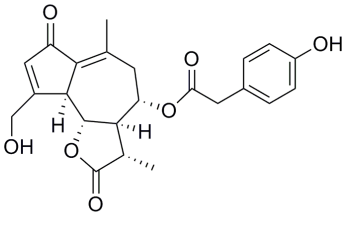
4 R = OH



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Results and Discussion

Compound **1** was isolated as a yellow solid $\{[\alpha]_H^{27} = -153$ (c = 0.1, MeOH)$\}$. The EI-MS exhibited an $[M]^+$ ion at *m/z* = 258, and its molecular formula, $C_{15}H_{14}O_4$, was established by HR-EI-MS (found *m/z* = 258.0896, calcd. 258.0892), which indicated eight degrees of unsaturation. The IR spectrum showed characteristic absorptions of hydroxyl (3383 cm^{-1}), γ-lactone (1742), and α,β-unsaturated carbonyl (1684 cm^{-1}) functions. The absorption maxima at 350, 299, and 256 nm in the UV spectrum indicated extended conjugations. The ^{13}C NMR spectrum displayed 15 resonances which were identified by a DEPT experiment as two methyl, two methylene, three methine, and eight quaternary carbons. The ^{1}H NMR spectrum (Table 1) showed resonances for two tertiary vinyl methyl groups [δ = 1.98 (s, H_3-13) and 2.52 (s, H_3-14)], an oxy-methylene unit [δ = 4.76 (2H, d, J = 5.7 Hz, H_2-15)], a hydroxyl group [δ = 1.83 (t, J = 5.7 Hz, OH-15)], an oxy-methine moiety [δ = 5.08 (br. d, J =

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (100 MHz) spectroscopic data of compounds **1** – **3**.

No.	**1** in $CDCl_3$		**2** in CD_3OD		**3** in CD_3OD	
	δ_H, mult. (*J* in Hz)	δ_C, mult.	δ_H, mult. (*J* in Hz)	δ_C, mult.	δ_H, mult. (*J* in Hz)	δ_C, mult.
1	–	128.7, C	–	134.6, C	–	134.2, C
2	–	193.7, C	–	197.9, C	–	198.0, C
3	6.49, s	132.1, CH	6.14, s	136.0, CH	6.14, s	135.9, CH
4	–	163.3, C	–	173.4, C	–	173.5, C
5	–	140.9, C	3.62, d (10.0)	52.5, CH	3.59, d (10.0)	52.7, CH
6	6.46, s	111.2, CH	3.80, t (10.0)	82.9, CH	3.71, t (10.0)	83.1, CH
7	–	154.4, C	2.38, dt (12.0, 10.0)	60.7, CH	2.18, dt (12.0, 10.0)	62.6, CH
8	5.08, br. d (13.0)	76.8, CH	4.30, dt (2.5, 10.0)	76.1, CH	3.64, dt (2.0, 10.0)	70.1, CH
9	2.97, dd (16.5, 3.5)	41.3, CH_2	2.87, dd (13.5, 2.5)	46.2, CH_2	2.81, dd (13.5, 10.0)	49.6, CH_2
	2.78, dd (16.5, 13.0)		2.82, dd (13.5, 10.0)		2.36, dd (13.5, 2.0)	
10	–	148.8, C	–	148.5, C	–	148.9, C
11	–	124.7, C	2.74, dq (12.0, 6.8)	42.1, CH	2.63, dq (12.0, 7.0)	42.3, CH
12	–	173.3, C	–	180.0, C	–	180.2, C
13	1.98, d (1.5)	8.9, CH_3	1.42, d (6.8)	15.6, CH_3	1.37, d (7.0)	15.8, CH_3
14	2.52, s	22.2, CH_3	2.41, s	21.5, CH_3	2.40, s	21.8, CH_3
15	4.76, d (5.7)	59.0, CH_3	2.29, s	19.9, CH_3	2.29, s	19.9, CH_3
OH	1.83, t (5.7)	–				

13.0 Hz, H-8)], a methylene unit [δ = 2.97 (1H, dd, *J* = 16.5, 3.5 Hz, H-9a) and 2.78 (1H, dd, *J* = 16.5, 13.0 Hz, H-9b], and two olefinic methine protons [δ = 6.49, (s, H-3) and 6.46 (s, H-6)]. In agreement with the ^{1}H NMR spectral data, the ^{13}C NMR spectrum (Table 1) showed resonances assigned to two tertiary methyl groups [δ = 8.9 (CH_3-13) and 22.2 (CH_3-14)], two methylene units [δ = 59.0 (CH_2-15) and 41.3 (CH_2-9)], an oxy-methine carbon [δ = 76.8 (CH-8)], four double bonds [δ = 128.7 (C-1), 132.1 (CH-3), 163.3 (C-4), 140.9 (C-5), 111.2 (CH-6), 154.4 (C-7), 148.8 (C-10), and 124.7 (C-11)], a conjugated carbonyl carbon [δ = 193.7 (C-2)], and a conjugated ester carbonyl carbon atom [δ = 173.3 (C-12)]. Key HMBC correlations (Fig. 1) were observed between H-3 and C-1, C-4, H-6 and C-1, C-4, C-8, H_3-13 and C-7, C-11, C-12, H_3-14 and C-1, C-9, C-10, and H_2-15 and C-4. The COSY spectrum showed long range correlations between H-3 and H_2-15, H_3-13 and H-6, H-8, and H_3-14 and H_2-9 in addition to vicinal correlations (Fig. 2). The ^{1}H and ^{13}C NMR chemical shift assignments of compound **1** (Table 1) are close to those of taraxacin (**6**) [11] with the exception that the hydroxymethyl resonances [δ_C = 59.0 (C-15), δ_H = 4.76 (2H, d, *J* = 5.7 Hz, H_2-15), and 1.83 (1H, t, *J* = 5.7 Hz, OH-15)] in **1** have replaced the methyl group resonances in taraxacin. Moreover, the carbon resonances for C-4 and C-5 were assigned as δ_C = 163.3 and 140.9, respectively, through HMBC correlations (Fig. 1). They seem to be wrongly reported as δ_C = 143.7/143.4 for C-4 and δ_C = 161.5/160.6 for C-5 in taraxacin and related compounds [11]. The ^{1}H and ^{13}C NMR chemical shifts of

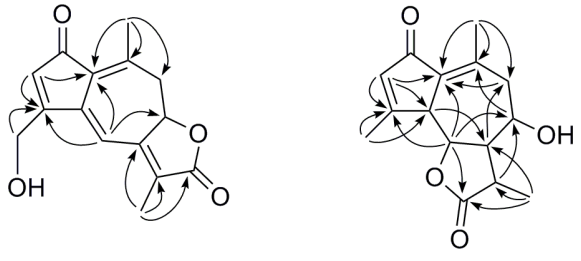

Fig. 1. Key HMBC interactions of **1** and **2**.

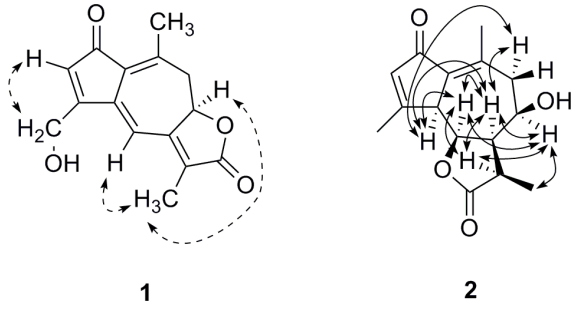

Fig. 2. ^{1}H-^{1}H long range interactions of **1** and key NOESY correlations of **2**.

CH-8 and CH_2-9, the coupling constants of H-8, H-9a and H-9b, as well as the negative optical rotation were found to be similar to those of taraxacin [11], which consequently supported an *R* configuration at C-8 as in taraxacin. Finally, the structure of **1** was established as 15-hydroxytaraxacin.

Compound **2** was obtained as a colorless crystalline solid. The molecular formula, $C_{15}H_{18}O_4$, was deter-

mined by HR-EI-MS (found *m/z* = 262.1204 $[M]^+$, calcd. 262.1205). The IR spectrum showed hydroxyl (3529 cm^{-1}), lactone carbonyl (1761 cm^{-1}) and α,β-unsaturated ketone (1682 cm^{-1}) functions. The ^{13}C NMR spectrum of **2** displayed 15 characteristic resonances of a sesquiterpene, which were resolved by DEPT NMR experiment as three methyl, a methylene, six methine, and five quaternary carbons. The ^1H NMR spectrum (Table 1) showed resonances for two vinyl tertiary methyl groups [δ = 2.41 (s, H_3-14) and 2.29 (s, H_3-15)], a secondary methyl group [δ = 1.42 (d, J = 6.8 Hz, H_3-13)], an olefinic methine proton [δ = 6.14, (s, H-3), and two oxy-methine protons [δ = 3.80 (t, J = 10.0 Hz, H-6) and 4.30 (dt, J = 10.0, 2.5 Hz, H-8)], in addition to resonances in the aliphatic region for three methine protons and a methylene group. The ^{13}C NMR spectrum (Table 1) showed characteristic resonances of a conjugated carbonyl carbon [δ = 197.9 (C-2)], an ester carbonyl carbon of a lactone [δ = 180.0 (C-12)], two double bonds [δ = 134.6 (C-1), 136.0 (CH-3), 173.4 (C-4), and 148.5 (C-10)], two oxy-methine units [δ = 82.9 (CH-6) and 76.1 (CH-8)], three methyl groups [δ = 15.6 (CH_3-13), 21.5 (CH_3-14), and 19.9 (CH_3-15)], a methylene unit [δ = 46.2 (CH_2-9)], and three methine carbon atoms [δ = 52.5 (CH-5), 60.7 (CH-7), and 42.1 (CH-11)]. The ^1H and ^{13}C NMR chemical shifts were assigned based on COSY, HMQC, and HMBC spectra (Fig. 1). Compound **2** and desacetylmatricarin (**3**) [12] exhibit the same planar structure and similar ^1H NMR spectral data. However, the NOESY spectrum of **2** did not support the stereochemistry of desacetylmatricarin (**3**) [12]. The NOESY interactions between H-5 and H-6, H-7, H-9a, H-6 and H-5, H-7, H-8, H-11, H-7 and H-5, H-6, H-8, H-9a, H-11, and H-8 and H-6, H-7, H-11, H_3-15 in **2** (Fig. 2) imply that the protons at all stereogenic centers were oriented in the same plane. The carbon resonance of C-8 was shifted about 6 ppm downfield in **2** compared to the one in desacetylmatricarin (**3**) (Table 1) [12] suggesting an α-orientation of H-8 in **2** rather than a β-orientation in **3**. The protons at other chiral carbons were according assigned to be α-oriented. In summary, the structure of **2** was elucidated as 6,8,11-*epi*-desacetylmatricarin.

The known compounds were identified as 11β,13-dihydrolactucin [13], 11β,13-dihydrolactucopicrin [14], and desacetylmatricarin [12] *via* comparison of their spectral data with those reported earlier.

The isolated compounds were evaluated for antiproliferative effects against two tumor cell lines, PC-3 (prostate cancer cell), and Hela (cervical cancer cell) using the MTT assay [15, 16] and were found to be inactive ($IC_{50} > 30\ \mu M$) in contrast to doxorubicin ($IC_{50} = 0.912 \pm 0.12\ \mu M$).

Experimental Section

General

Column chromatography (CC) and vacuum liquid chromatography (VLC) were performed on silica gel (70 – 230 mesh, E. Merck). Thick layer chromatography was performed on pre-coated silica gel GF_{254} preparative plates (20 × 20, 0.5 mm, E. Merck). NMR spectra were recorded on Bruker AM-300, AM-400, and AMX-500 spectrometers. UV and IR spectra were recorded on Hitachi U-3200 and Shimadzu FTIR-8900 spectrometers. Optical rotations were measured on a Glan-Taylor Prism instrument. El-MS and HR-EI-MS were obtained on Finnigan MAT-112 and MAT-113 spectrometers.

Plant material

The plant material was collected from Gilgit, Pakistan in July, 2007 and identified by the taxonomist at the Department of Botany, University of Karachi, where a voucher specimen (No. 1036, general herbarium # 71320) was deposited.

Extraction and isolation

The powder of shade-dried aerial parts of *Cichorium intybus* (9.5 kg) was soaked in 95 % EtOH (30 L) for 7 d. The solvent was evaporated under reduced pressure. The extract (418 g) was suspended in dist. H_2O and extracted with hexanes, EtOAc, and *n*-BuOH, to yield hexanes- (190 g), EtOAc- (45 g), and *n*-BuOH- (132 g) soluble parts. The EtOAc extract was subjected to vacuum liquid chromatography (VLC) [silica gel (20 × 14 cm), hexanes-EtOAc (10 %, 20 %, 30 % … 100 %, 1.0 L each), and EtOAc-MeOH (5 %, 10 %, 20 %, 30 %, 1.0 L each)] to obtain 14 fractions (1 – 14). Fractions 5 (1.8 g) and 6 (1.7 g) were subjected to column chromatography (CC) [silica gel (40 × 2 cm), hexanes-EtOAc (10 %, 20 %, 30 %. … 100 %, 100.0 mL each), and EtOAc-MeOH (5 %, 10 %, 20 %, 30 %, 100.0 mL each)] to obtain 5 (5A – 5E) and 7 (6A – 6F) fractions, respectively. Fraction 5A (eluted with hexanes-EtOAc, 2 : 8 – 0 : 1, 1.8 g) was subjected to preparative TLC ($CHCl_3$-MeOH, 92 : 8) to yield **4** (20.7 mg). Compound **5** (28.3 mg) was purified by preparative TLC ($CHCl_3$-MeOH, 92 : 8) from fraction 6B (eluted with hexanes-EtOAc, 75 : 25 – 70 : 30, 1.9 g). Fraction 7 (3.9 g) was subjected to CC [silica gel (36 × 3 cm), hexanes-EtOAc (10 %, 20 %, 30 % … 100 %, 100.0 mL each), and EtOAc-MeOH (5 %, 10 %, 20 %, 30 %, 100.0 mL each)] to get 6 fractions (7A – 7F). Compound **2**

(20.7 mg) were purified from Fr. 7C (eluted with EtOAC-MeOH, 9 : 1, 150.5 mg) by preparative TLC ($CHCl_3$-MeOH, 86 : 14). The *n*-BuOH-soluble part was subjected to VLC over diion (30 × 4 cm) [dist. H_2O (100, 1.0 L), (1 : 1, 1.0 L), (1 : 2, 1.0 L), and MeOH (100, 1.0 L)] to obtain 4 fractions (A – D). Fr. B (10.9 g) was subjected to CC [silica gel (36 × 3 cm), CH_2Cl_2-MeOH (1 : 0, 1 : 49, 1 : 19, 7 : 93, 1 : 9, 3 : 17, 1 : 4, 1 : 3, 6 : 13, and 3 : 2, 500 mL each)] to obtain 10 subfractions (B1 – B10). Fr. B2 (eluted with CH_2Cl_2-MeOH, 1 : 0, 1.7 g) was subjected to CC [silica gel (36 × 3 cm), CH_2Cl_2-MeOH, increasing order of polarity] to obtain compound **3** (19.7 mg) and compound **1** (12.4 mg).

*15-Hydroxytaraxacin (**1**)*

$[\alpha]_D^{27}$ = −153 (*c* = 0.1, MeOH). – UV/Vis (MeOH): λ_{max} = 350, 299, 256 nm. – IR (KBr): ν = 3383, 1742, 1684, 1635 cm^{-1}. – ^1H and ^{13}C NMR spectral data: Table 1. – MS ((+)-EI): *m/z* (rel. int. %) = 258 (100), 240 (5), 229 (30), 215 (9), 201(18). – HRMS ((+)-EI): *m/z* = 258.0896 (calcd. 258.0892 for $C_{15}H_{14}O_4$, $[M]^+$).

*6,8,11-Epi-desacetylmatricarin (**2**)*

$[\alpha]_D^{27}$ = +15.67 (*c* = 0.5, MeOH). – UV (MeOH): λ_{max} = 256 nm. – IR (KBr): ν = 3529, 1761, 1682, 1612, 1232 cm^{-1}. – ^1H and ^{13}C NMR spectral data: Table 1. – MS ((+)-EI): *m/z* (rel. int. %) = 262 (22), 244 (25), 216 (4), 198 (15), 171(36), 159. – HRMS ((+)-EI): *m/z* = 262.1204 (calcd. 262.1205 for $C_{15}H_{18}O_4$, $[M]^+$).

In vitro cytotoxicity assay

The cell growth inhibition effect of compounds was determined by using the MTT assay [15, 16].

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