

Sesquiterpene Lactones from *Cichorium intybus*

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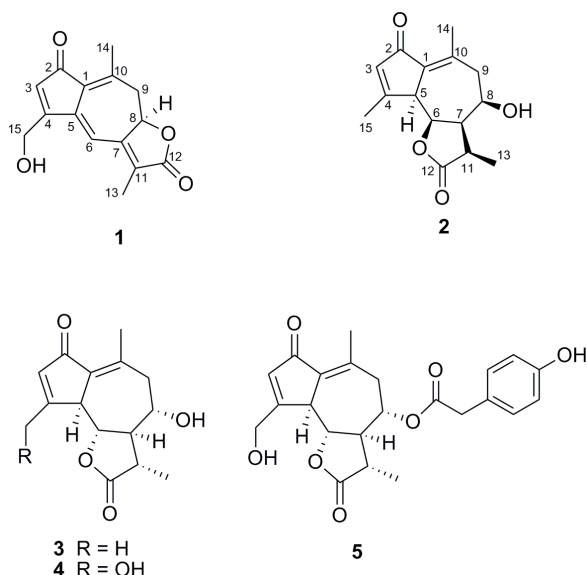
Z. Naturforsch. 2011, 66b, 729–732; received April 20, 2011

Two new sesquiterpene lactones (guaianolides), 15-hydroxytaraxacin (**1**) and 6,8,11-*epi*-desacetylmatricarin (**2**), along with three known compounds, desacetylmatricarin (**3**), 11 β ,13-dihydrolactucin (**4**), and 11 β ,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 (guaianolides) were isolated and identified by spectroscopic data analyses as 15-hydroxytaraxacin (**1**), and 6,8,11-*epi*-desacetylmatricarin (**2**), desacetylmatricarin (**3**), 11 β , 13-dihydrolactucin (**4**), and 11 β ,13-dihydrolactucopicrin (**5**).



Results and Discussion

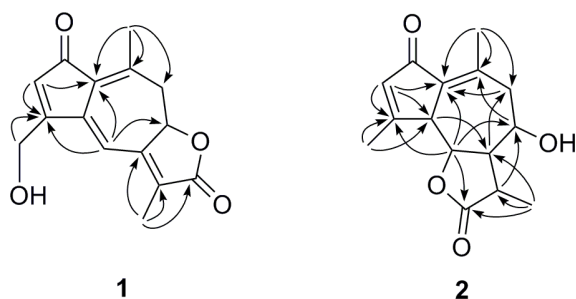
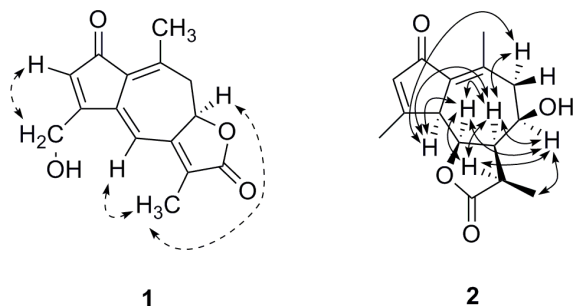
Compound **1** was isolated as a yellow solid $\{[\alpha]_{\text{D}}^{27} = -153 (c = 0.1, \text{MeOH})\}$. The EI-MS exhibited an $[M]^+$ ion at $m/z = 258$, and its molecular formula, $\text{C}_{15}\text{H}_{14}\text{O}_4$, was established by HR-EI-MS (found $m/z = 258.0896$, calcd. 258.0892), which 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 ^1H NMR spectrum (Table 1) showed resonances for two tertiary vinyl methyl groups [$\delta = 1.98$ (s, H_3 -13) and 2.52 (s, H_3 -14)], an oxy-methylene unit [$\delta = 4.76$ (2H, d, $J = 5.7$ Hz, H_2 -15)], a hydroxyl group [$\delta = 1.83$ (t, $J = 5.7$ Hz, OH-15)], an oxy-methine moiety [$\delta = 5.08$ (br. d, $J =$

Table 1. ^1H (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_2	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 [$\delta = 2.97$ (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 [$\delta = 6.49$, (s, H-3) and 6.46 (s, H-6)]. In agreement with the ^1H NMR spectral data, the ^{13}C NMR spectrum (Table 1) showed resonances assigned to two tertiary methyl groups [$\delta = 8.9$ (CH_3 -13) and 22.2 (CH_3 -14)], two methylene units [$\delta = 59.0$ (CH_2 -15) and 41.3 (CH_2 -9)], an oxy-methine carbon [$\delta = 76.8$ (CH-8)], four double bonds [$\delta = 128.7$ (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 [$\delta = 193.7$ (C-2)], and a conjugated ester carbonyl carbon atom [$\delta = 173.3$ (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, H₃-13 and C-7, C-11, C-12, H₃-14 and C-1, C-9, C-10, and H₂-15 and C-4. The COSY spectrum showed long range correlations between H-3 and H₂-15, H₃-13 and H-6, H-8, and H₃-14 and H₂-9 in addition to vicinal correlations (Fig. 2). The ^1H 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 [$\delta_{\text{C}} = 59.0$ (C-15), $\delta_{\text{H}} = 4.76$ (2H, d, $J = 5.7$ Hz, H₂-15), 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 $\delta_{\text{C}} = 163.3$ and 140.9, respectively, through HMBC correlations (Fig. 1). They seem to be wrongly reported as $\delta_{\text{C}} = 143.7/143.4$ for C-4 and $\delta_{\text{C}} = 161.5/160.6$ for C-5 in taraxacin and related compounds [11]. The ^1H and ^{13}C NMR chemical shifts of

Fig. 1. Key HMBC interactions of **1** and **2**.Fig. 2. ^1H - ^1H 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, $\text{C}_{15}\text{H}_{18}\text{O}_4$, 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 [$\delta = 2.41$ (s, H₃-14) and 2.29 (s, H₃-15)], a secondary methyl group [$\delta = 1.42$ (d, $J = 6.8$ Hz, H₃-13)], an olefinic methine proton [$\delta = 6.14$, (s, H-3), and two oxy-methine protons [$\delta = 3.80$ (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 [$\delta = 197.9$ (C-2)], an ester carbonyl carbon of a lactone [$\delta = 180.0$ (C-12)], two double bonds [$\delta = 134.6$ (C-1), 136.0 (CH-3), 173.4 (C-4), and 148.5 (C-10)], two oxy-methine units [$\delta = 82.9$ (CH-6) and 76.1 (CH-8)], three methyl groups [$\delta = 15.6$ (CH₃-13), 21.5 (CH₃-14), and 19.9 (CH₃-15)], a methylene unit [$\delta = 46.2$ (CH₂-9)], and three methine carbon atoms [$\delta = 52.5$ (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, H₃-15 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 11 β , 13-dihydrolactucin [13], 11 β ,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 ($\text{IC}_{50} > 30 \mu\text{M}$) in contrast to doxorubicin ($\text{IC}_{50} = 0.912 \pm 0.12 \mu\text{M}$).

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 GF₂₅₄ 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. EI-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. H₂O 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 (CHCl₃-MeOH, 92:8) to yield **4** (20.7 mg). Compound **5** (28.3 mg) was purified by preparative TLC (CHCl₃-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 (CHCl₃-MeOH, 86 : 14). The *n*-BuOH-soluble part was subjected to VLC over diion (30 × 4 cm) [dist. H₂O (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), CH₂Cl₂-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 sub-fractions (B1–B10). Fr. B2 (eluted with CH₂Cl₂-MeOH, 1 : 0, 1.7 g) was subjected to CC [silica gel (36 × 3 cm), CH₂Cl₂-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): $\lambda_{\max} = 350, 299, 256$ nm. – IR (KBr): $\nu = 3383, 1742, 1684,$

1635 cm⁻¹. – ¹H and ¹³C 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 C₁₅H₁₄O₄, [M]⁺).

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

$[\alpha]_D^{27} = +15.67$ ($c = 0.5$, MeOH). – UV (MeOH): $\lambda_{\max} = 256$ nm. – IR (KBr): $\nu = 3529, 1761, 1682, 1612, 1232$ cm⁻¹. – ¹H and ¹³C 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 C₁₅H₁₈O₄, [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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- [1] R. Sharma, *Medicinal plant of India-an encyclopedia*, Daya publishing press, Delhi, **2003**, pp. 57–58.
- [2] M. I. A. Mammon, N. M. Shahani, G. M. Syed, *Glimpses of the medicinal plants of sindh*, Sindh Agriculture University Press, Tandojam, **1988**, p. 25.
- [3] M. Daniel, *Medicinal plants chemistry and properties*, Oxford and IBH press, New Delhi, **2006**, p. 84.
- [4] R. Norbaek, K. Nielsen, T. Kondo, *Phytochemistry* **2002**, *60*, 357–359.
- [5] W. Kisiel, K. Zielinska, *Phytochemistry* **2001**, *57*, 523–527.
- [6] M. Abdalla, E. Lakany, A. Maha, A. Ela, M. Abdul-Ghani, M. Hattem, *Nat. Prod. Sci.* **2004**, *10*, 69–73.
- [7] J. W. Timmermans, T. M. Slaghek, M. Iizuka, W. V. Ende, J. D. Roover, A. Laere, *J. Carbohydr. Chem.* **2001**, *20*, 375–395.
- [8] N. I. Khar'k, F. K. Khim, *Chem. Nat. Comp.* **1973**, *9*, 115–115.
- [9] V. G. Dem'yanenko, L. I. Dranik, *Chem. Nat. Comp.* **1971**, *7*, 104–104.
- [10] W. Kisiel, K. Michalska, *Fitoterapia* **2002**, *73*, 544–546.
- [11] V. U. Ahmad, S. Yasmeen, Z. Ali, M. A. Khan, M. I. Choudhary, F. Akhtar, G. A. Miana, M. Zahhid, *J. Nat. Prod.* **2000**, *63*, 1010–1011.
- [12] M. Martinez, A. Munoz-Zamora, P. Joseph-Nathan, *J. Nat. Prod.* **1988**, *51*, 221–228.
- [13] T. M. Sarg, A. A. Omar, S. M. Khafagy, M. Grenz, F. Bohlmann, *Phytochemistry* **1982**, *21*, 1163–1163.
- [14] Y. F. Han, G. X. Cao, X. J. Gao, M. Xia, *Food Chem.* **2010**, *120*, 1083–1088.
- [15] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55–63.
- [16] Y.-L. Chen, Y.-H. Lan, P.-W. Hsieh, C.-C. Wu, S.-L. Chen, C.-T. Yen, F.-R. Chang, W.-C. Hung, Y.-C. Wu, *J. Nat. Prod.* **2008**, *71*, 1207–1212.