

Isolation and Characterization of Two New Compounds from Genus *Sonchus*

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ABSTRACT

Two new compounds Erucaic acid (1) and sonchusnoate (2) were isolated from the whole plant of *Sonchus eruca* and *Sonchus asper* respectively. Both species belong from genus *Sonchus*. The structures of the compounds were elucidated through extensive spectroscopic analysis including EIMS, HREIMS, ¹H-NMR, ¹³C-NMR and including advance techniques like NMR ¹H-¹H COSY, HMBC, and NOESY.

Key words: *S. eruca*, *S. Asper*, Erucaic acid and Sonchusnoate

INTRODUCTION

Genus *Sonchus* belong to the family Asteraceae. It is the largest family of angiosperms comprises of over 1535 genera and 23000 species, distributed in three subfamilies and 17 tribes. The number of genera is rather increasing as more than 10 genera are described each year and several are resurrected from or reduced to synonymy ¹.

The genus *Sonchus* L. (Asteraceae) has a worldwide distribution except for Central and South America ². It is commonly considered to be related to *Aetheorhiza* Cass., *Reichardia* Roth and *Launaea* Cass. ³⁻⁴, but it is a more isolated and distinct genus ⁴⁻⁵. *Sonchus* is represented with seven taxa in Turkey ⁶. Some of which are used as a food in Turkey ⁷. The genus *Sonchus*, representing 8 species in China, has long been used as folk medicine for the treatment of fever, stasis, and inflammation, as well as for detoxication and mobilization of blood circulation ⁸. Genus *sonchus* showed a marked reduction in its relative growth

rate at elevated levels of ozone ⁹. Flowers are perfect and generally self-incompatible. Perennial sowthistle can reproduce by seed and vegetatively is pollinated by insects including honeybees and other bees, hover flies, and blister beetles ¹⁰. Pollen grains of Asteraceae may be resolved into two major groups i.e., lophate and non-lophate grains ¹¹. Within the genus *Sonchus*, probably rising from *Launaea* ¹². The most primitive taxa, included in the subg. *Origosonchus* Boulos, show pollen characters very similar to those of *Launaea* ¹²⁻¹³.

RESULTS AND DISCUSSION

Compound 1 (Fig 1) was isolated as a colorless powder from the chloroform soluble part of the methanolic extract of *S. eruca* was subjected to silica gel gradient column chromatography (CC) using solvent system of hexane/chloroform (1: 9).

The HR-EI-MS of 1 showed a molecular ion peak at *m/z* 472.23 which was in agreement with the molecular formula C₃₀H₄₆O₄ showing seven

degrees of un-saturation. The molecular formula of the compound 1 was further confirmed by the exact measurement of various mass fragment ions. In addition to the molecular ion peak, other prominent peaks appeared at m/z 190.10, 246.17, 201.175. The IR spectrum showed hydroxyl (3525 cm^{-1}), carboxylic (1715 cm^{-1}) and C=C (1660 cm^{-1}) functionalities.

The ^1H NMR of 1 was very informative in establishing its basic triterpenoidal skeleton having signals for three secondary methyl which were resonated at $\delta = 1.05$ (3H, d, H-26), $\delta = 1.66$ (3H, d, H-27) and $\delta = 1.05$ (3H, d, H-30) while signals at $\delta = 0.94$ (3H, s H-23), and $\delta = 0.95$ (3H, s, H-24) and one along with tertiary methyl at $\delta = 1.76$ (3H, s, H-25). One methyl attached with olefinic carbon

Table 1: ^1H NMR Data of Erucaic acid (1)

Proton on Carbon	Chemical Shift (δ)	Proton on Carbon	Chemical Shift (δ)
1	1.36 (d)	16	2.02 (m)(2H, $J = 3.0\text{Hz}$)
2	3.68 (m)	17	-
3	3.52 (d)(1H, d, $J = 6.11\text{Hz}$).	18	2.76 (d)(1H, d, $J = 11.2\text{Hz}$)
4	-	19	1.05 (m)
5	1.43 (dd)(1H, $J = 13.6, 4.0\text{Hz}$)	20	0.96 (m)
6	1.10 (dd)(2H, $J = 11.6, 4.7\text{Hz}$)	21	1.00 (m)
7	1.23 (m)	22	1.08 (t)(2H, $J = 3.5\text{Hz}$)
8	1.55 (m)	23	0.94(3H, s)
9	1.36 (dd)(1H, $J = 10.2, 3.7\text{Hz}$)	24	0.95(3H, s)
10	-	25	1.76(3H, s)
11	2.34 (d)(1H, $J = 12.2\text{Hz}$)	26	1.05(3H, s)
12	-	27	1.66(3H, s, H-27)
13	-	28	1.71(3H, s)
14	1.49 (m)	29	-
15	1.31 (m)	30	1.05(3H, s, H-30)

Table 2: ^{13}C NMR Data of Erucaic acid (1).

C. No	Multiplicity(DEPT)	^{13}C NMR(δ)	C. No	Multiplicity(DEPT)	^{13}C NMR(δ)
1	CH_2	27.4	16	CH_2	35.5
2	CH	77.2	17	C	48.0
3	CH	80.9	18	CH	50.3
4	C	42.8	19	CH	48.6
5	CH	39.3	20	CH_2	38.2
6	CH_2	29.9	21	CH_2	39.4
7	CH	42.2	22	CH_2	40.0
8	CH	47.4	23	CH_3	21.3
9	CH	46.3	24	CH_3	27.9
10	C	40.8	25	CH_3	18.0
11	C	27.3	26	CH_3	16.4
12	C	150.0	27	CH_3	23.4
13	C	154.4	28	CH_3	25.3
14	C	109.3	29	C	171.0
15	CH_2	34.2	30	CH_3	14.7

resonated at $\delta = 1.71$ (3H, s, H-28) where as the signal at $\delta = 3.68$ (m) was owed to proton (H-2) while another signal at $\delta = 3.52$ (d, $J = 6.11$ Hz) was due to another carbinylic proton (H-3). The characteristic signals for double bond in ^{13}C NMR at $\delta = 150.0$ and 154.0 (C-12, C-13), suggested compound 1 to be an oleanane triterpenoids with carboxylic functionality ($\delta = 181.3$) [14]. The β -orientations of hydroxyl groups at C-2 and C-3 were determined through chemical shift and coupling constant of H-2 ($J = 4.9, 9.7$ Hz) and H-3 ($J = 6.11$ Hz).

The ^{13}C NMR assignments of various carbon atoms were confirmed through DEPT analysis, which showed 30 signals, which were substantiated through DEPT experiment into seven methyl, seven methylenes, ten methines and six quaternary carbons. The position of hydroxyl group and quaternary carbon was further confirmed through HMBC correlations (Fig. 2). The proton at $\delta 3.52$ (H-3) showed correlation to the carbons at $\delta 42.82$ (C-4), $\delta 21.33$ (C-23), and $\delta 27.95$ (C-24). The proton at $\delta 1.01$ (H-25) showed correlation with carbon at $\delta 40.85$ (C-10), and $\delta 39.39$ (C-5), having the methyl group at C-10. (H-7) methane proton (δ

1.23) showed correlation with carbon at $\delta 29.9$ (C-6). The proton at $\delta 2.76$ (H-18) showed correlation with carbon at $\delta 14.7$ (C-30). Similarly, the proton at $\delta 0.96$ (H-20) showed correlations with carbon at $\delta 14.7$ (C-30).

The NOESY for H-C (2)/H-C (3), H-C (3)/Me (23), H-C (5)/H-C (9), H-C (9)/H-C (18) and H-C (18)/H-C (19) (Fig 3) indicated that these protons have same orientation (α). On the other hand, an NOE for H-C (8)/Me (26) suggested their position on the β side and the magnitude of the coupling constants was indicative for a chair confirmation of ring E¹⁵. From this data compound 1 was found new and was trivially named to be Erucaic acid. Proton and ^{13}C NMR Data are given in Table 1 and Table 2.

Compound 2 (Fig 4) was obtained as light yellow solid from ethyl acetate soluble fraction of methanolic extract of *Sonchus asper* eluted with methanol: chloroform (1: 9). This compound was UV active. The EI-MS of 2 exhibited the molecular ion peak at m/z 292.1120. The base peak appeared at m/z 76 (100 %). The molecular formula was

Table 3: ^1H NMR Data of Compound Sonchusnoate (2)

Proton on Carbon	Chemical Shift(δ)	Multiplicities	Coupling Constant(J) (Hz)
H-2, H-6	7.07	d	8.3
H-3, H-5	6.75	d	8.3
H-2''	4.23	t	4.01
H-3''	2.85	m	7.09
H-1'	2.27	t	7.0
H-2'	1.23	M	-
H-6'	2.13	T	6.9

Table 4: ^{13}C NMR Data of Compound Sonchusnoate (2)

C.No	(DEPT)	^{13}C NMR(δ)	C.No	(DEPT)	^{13}C NMR(δ)	C.No	(DEPT)	^{13}C NMR(δ)
1	C	152.1	1''	C	173.0	1'	CH_2	39.63
2	CH	130.1	2''	CH_2	64.87	2'	CH_2	32.37
3	CH	117.3	3''	CH_2	34.35	3'	CH_2	29.16
4	C	136.3	4''	CH_2	33.34	4'	CH_2	24.98
5	CH	119.3	5''	CH_2	31.94	5'	CH_2	22.70
6	CH	130.1	6''	CH_3	14.70	6'	CH_2	62.08

determined through HR- EI- MS as $C_{18}H_{28}O_3$ (calcd. m/z 292.1118). In addition to the molecular ion peak, other prominent peaks appeared at m/z 115, 71 and 101.

The 1H -NMR spectrum of **2** showed a triplet with coupling constant (7.0 Hz) at δ 2.27 which was directly attach with aromatic ring. Similarly, another triplet with coupling constant (4.01 Hz) at δ 4.23 was due to oxygenated methylene.

The 1H - and ^{13}C - NMR chemical shifts of methylene (δ 4.23 and 64.8) revealed its attachment with an oxygen atom. The appearance of two doublets at aromatic ring (δ =7.07 and 6.52) with the same coupling constant (J = 8.3 Hz) indicated a para substitution pattern.

The doublets at δ 7.07 was due to carbons at δ 130.0 which was deduced through HMQC experiments and considered as methines with the help of DEPT experiments. The broad band ^{13}C -NMR spectrums of **2** indicated 18 signals due to which were resolved through DEPT experiments into 1 methyl, 4 methines, 10 methylenes and 3 quaternary carbons. The methine signal at 130.0 was assigned to the *ortho* positions (C-2, C-6). The C-3 and C-5 were routinely given by methines at δ 115.3 having protons at δ 6.75. The quaternary signal at δ 154.1 was assigned to C-1 by comparing with reported data ¹⁶. Another quaternary signal at δ 146.3 revealed its attachment to a hydrocarbon chain containing terminal hydroxyl group and assigned to C-4. The downfield quaternary carbon

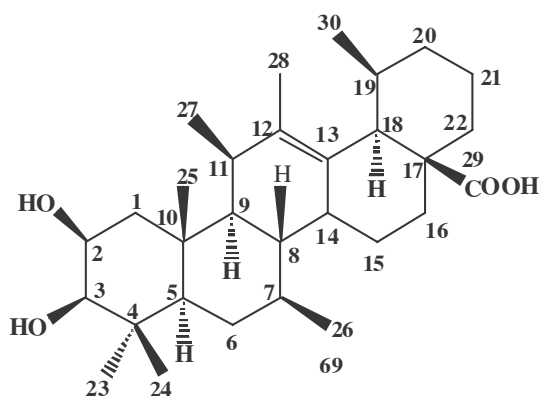


Fig. 1: erucaic acid

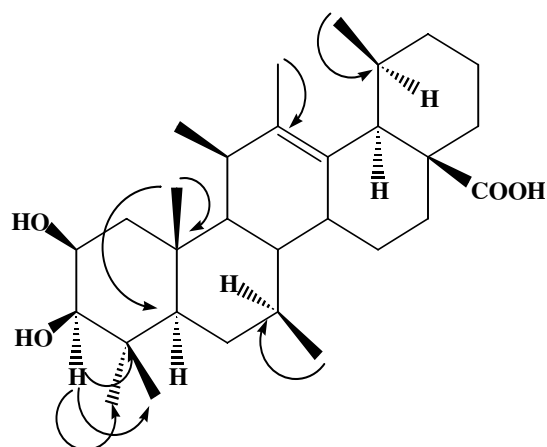


Fig. 2: HMBC Colrelation of Compound 1

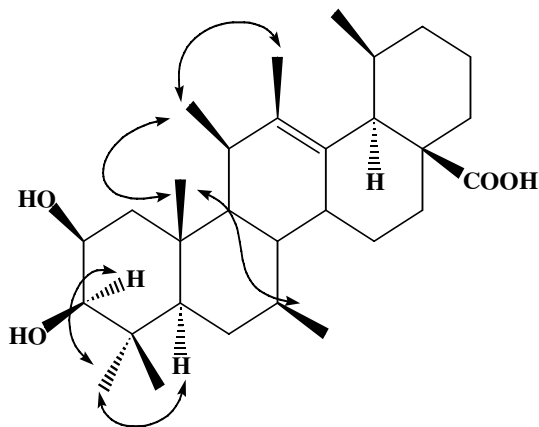


Fig. 3: Important NOESY Colrelation of 1

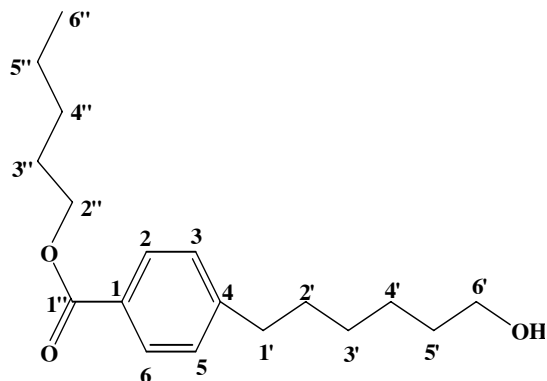


Fig. 4: Sonchusnoate

(C-1") at δ 173.01 was due to a carbonyl group. The signals at δ 64.87 and 34.35 in the ^{13}C -NMR spectrum were ascribed to (C-2") and (C-3"). This discussion led to the presence of the following fragments in the molecule.

All the chemical shifts were confirmed through EI, ^1H , ^{13}C NMR and HMBC techniques and partial comparison with the reported data¹⁶⁻¹⁸. The discussion so far led to deduce 1 was found new and was trivially named to be Sonchusnoate. Proton and ^{13}C NMR Data are given in Table 3 and Table 4.

MATERIALS AND METHOD

Plants Material

Both plants *S. eruca* and *S. asper* were collected at Parachinar Kurram agency, KPK Pakistan, in July, 2008, and were identified by plant taxonomist Mohammad Siraj Assistant professor, Govt: Post Graduate College (Jahan zeb college swat. The voucher specimens of plants *S. eruca* (SEZ1) and *S. asper* (SEZ2) were deposited in the herbarium of botany department Kohat University of Science and Technology Kohat (KUST).

Extraction and Purification of Compound 1

The whole plant, including roots (3 kg) of *S. eruca* was crushed and extracted x 3 with methanol (20 L each) at room temperature. The resulting methanol extract (155 g) was suspended in water and successively portioned to provide n-hexane (19 g), chloroform (57 g), ethyl acetate (40 g) and butanol (7 g) fractions. The chloroform (57 g) was subjected to silica gel column (90 x 850 nm, 500 mL each part). The column was eluted with solvent of increasing polarity hexane- CHCl_3 (9:1 up to 1:9), CHCl_3 - CHCl_3 -MeOH (9:1 up to 1:9) and MeOH to give three fractions. The fraction 3 (300 mg) was then subjected to silica gel (flash silica

250-400 mesh) column chromatography using hexane - CHCl_3 (1: 9) to afforded a compounds 1, provisionally named Erucaic acid. Erucaic acid White powder, 50 mg. IR (KBr): OH (3525 cm^{-1}), carboxylic (1715 cm^{-1}) and C=C (1660 cm^{-1}) functionalities. ^1H NMR (300 MHz, CDCl_3) Table 1. ^{13}C NMR (75 MHz, CDCl_3) Table 2

Extraction and Purification of Compound 2

The whole plant, including roots (5 kg) of *S. asper* was crushed and extracted three times with methanol (20 L each) at room temperature. The resulting methanol extract (190 g) was suspended in water & successively portioned to provide n-hexane (34 g), chloroform (70 g) and ethyl acetate (47 g) fractions. The chloroform (70 g) was subjected to silica gel column chromatography. The column was eluted with solvent of increasing polarity hexane-chloroform, chloroform, chloroform-methanol and methanol to give five fractions 1-5. The fraction 2 (350 mg) was then subjected to silica gel (flash silica 230-400 mesh) column chromatography using methanol: chloroform (1:9) to afforded a new powder like compound 2, provisionally named sonchusnoate. The compound was found ceric sulfate active. Sonchusnoate White powder, 15 mg. ^1H NMR (300 MHz, CDCl_3) Table 3. ^{13}C NMR (75 MHz, CDCl_3) Table 4.

CONCLUSIONS

New Constituents, Erucaic acid (1) and sonchusnoate (2) were isolated from the whole plant of *S. eruca* and *S. asper* respectively. The isolation of the new compounds are a new addition to the molecular diversity of *S. eruca* and *S. asper*

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