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New epoxy alantolactone from *Inula racemosa*

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ABSTRACT

An investigation of *Inula racemosa* afforded, in addition to four known sesquiterpene lactones, a new epoxy alantolactone isolated from *Inula racemosa*. Their structures have been characterized using different spectral data and chemical correlation. © 2008 Trade Science Inc. - INDIA

KEYWORDS

Inula racemosa;
Compositeae;
Lactones;
GC/MS analysis.

INTRODUCTION

Inula is a large genus of about sixty species distributed throughout Europe, Africa and Asia. The plants are herbs, rarely shrubs, leaves radical and alternate. Genus Inula is represented in India by twenty species[1]. However three species occur in Kashmir, *Inula racemosa*, *Inula royleana* and *Inula grandi*. *Inula racemosa* is a stout herb upto 5 feet tall found in north western Himalayas at an altitude of 5000 to 14000 feet. Fresh roots of the species have a strong aromatic odour. They are used in Kashmir as an adulterant of kuth (sassurea lappa C.B.Clarke)

Sesquiterpene lactones are the chief constituents in the genus Inula[2-14] which possess antiseptic, expectorant, and diuretic[15] properties. The other isolated compounds are Alkaloids[16-18], Diterpenoids[19-20], Seteroids [21], and Terpenoids[22-23].

Roots of *Inula racemosa* were exhaustively extracted with light petroleum. The extract was vacuum dried, and five compounds were isolated by repeated column chromatography of the extract. Epoxy alanto lactone was obtained as colorless crystalline solid. The GC/MS of this compound showed molecular ion peak at M/Z 246 corresponding to molecular formula $C_{15}H_{18}O_3$. The compound showed UV absorption maxima at 211 nm. The molecular formula suggests its sesquiterpene nature with index of unsaturation to be seven. Out of the remaining four un-saturated sites three are accounted for in the form of α:β un-saturated γ-lactone and an exocyclic methylene, this also accommodates two out of three oxygen function. While the remaining lone oxygen function as well as one un-saturated site is accommodated in the form of oxirane ring. Which stands further confirmed by its PMR.. The IR spectrum of compound **(1)** showed the presence of γ-

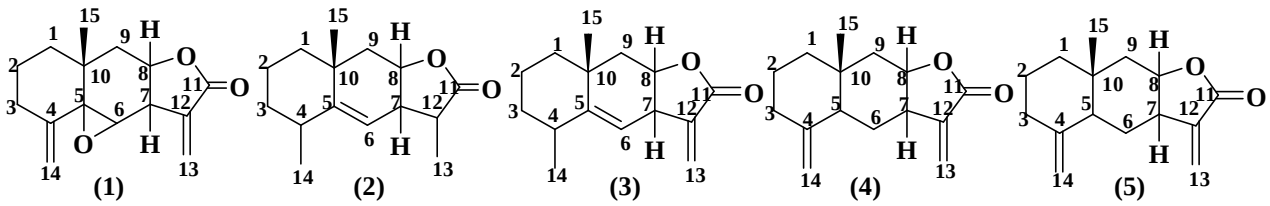

TABLE 1: PMR of compound-(1) (200 MHz, $CDCl_3$)

δ	No. of protons	Multiplicity	Proton assignment
0.83	3H	s	Me-15
2.36	1H	ddd	H-7
2.97	1H	d	H-6
2.17	2H	dd	H-9
4.48	1H	m	H-8
5.58	2H	dd	H-14
6.13	2H	Br,s	H-13

TABLE 2: ^{13}C NMR of compound (1) (200 MHz, $CDCl_3$)

Carbon	δc	Carbon	δc
1	41.3	9	42.1
2	22.6	10	34.2
3	36.7	11	170.5
4	148.9	12	142.2
5	162.2	13	119.9
6	142.2	14	106.6
7	40.4	15	17.6
8	76.7	-	-

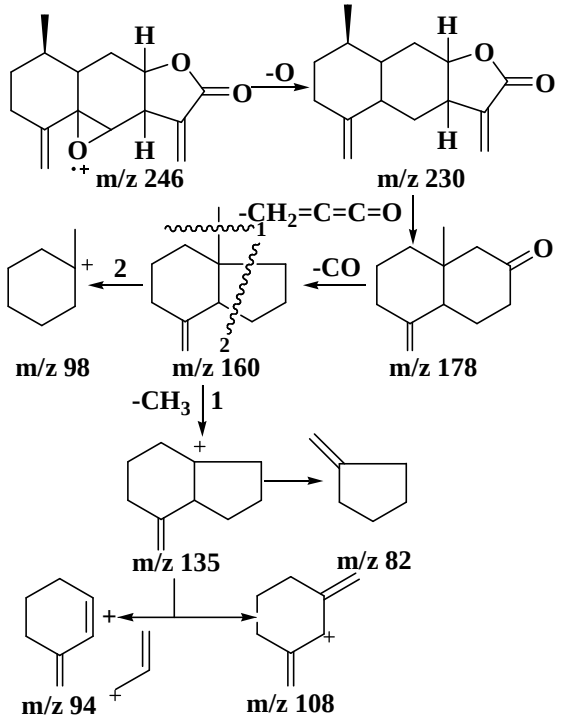

Figure 1: Mass fragmentation of compound (1)

lactone moiety by exhibiting a prominent band at 1752 cm^{-1}, its IR spectrum also showed the presence of trisubstituted double bonds by exhibiting bands at 1440,1262,880cm^{-1}.

The presence of an epoxide ring at C-5, C-6 and its stereo-chemistry was clear from its ^1H NMR spectrum which exhibits one proton doublet at δ2.97. The compound was hydrolysed with dilute. H_2SO_4 when it gave a diol which was directly converted into a diacetate by treatment with Ac_2O-C_5H_5N at room temperature. This was indicative of the presence of epoxide on carbons one of which must be tertiary one

The PMR spectrum of compound **(1)** revealed the presence of two tri-substituted double bonds by displaying signals at δ 6.13, 5.58, assigned for C-13 and C-14 protons.

The IR and PMR data was further substantiated by ^{13}C NMR (TABLE 2) resonance signals at δc 106.6, 170.5 and 119.9 which were attributed to C-14, C-11 and C-13 respectively. The other ^{13}C NMR signals appeared at δc 162.2, 142.2 attributed to C-5, C-12 respectively.

The compound **(1)** on spectral analysis showed M^+ at M/Z 246 analyzed for $C_{15}H_{18}O_3$. The spectrum showed a diagnostic fragment ion at m/z 230 $[M-16]^+$ indicative of the presence of an epoxide. In addition to this, the mass spectrum contains some diagnostic fragments as well at, M/Z 178 $(M-CH_2=C=C=O)^+$, 160 $(M-CO)^+$, 135 $(M-Me)^+$, 108 and 82 as well after RDA fragmentation

Melting points were determined on Kofler block, melting point apparatus, and are uncorrected. IR spectra were recorded on Perkin Elmer-350 Spectrometer. NMR spectra recorded on FT-NMR 90 MHz (JEOL-90) 200 MHz, Brucker NMR, using tetramethy-lsilane as internal standard and $CDCl_3$ as solvent. ^{13}C NMR, APT, DEPT (90º) experiments were done on a Brucker Instrument. TLC was carried out on silica gel-G layers (BDH, 0.3 mm). The plates were activated at 110-120º for 30 min. and then stored in a desicator. Column chromatography was carried on 60-120 mesh silica gel (BDH). The analytical samples were dried in vacuum at 35º over P_2O_5 for 25 hrs. All the reactions were carried out under anhydrous conditions, unless stated otherwise. All the solvents used were of AR grade.

Isolation of compound (1)

The plant material was collected from Aharbal Shopian of Kashmir Valley and identified by Dr. A.R. Naqshi, Reader, Dept.of Taxonomy, University of Kashmir and voucher specimen has been deposited in the Phytochemistry Laboratory, CORD, University of Kashmir, Srinagar.

2 kgs of *Inula racemosa* (root) were powdered and defatted with 15 litre of Pet. ether (60-80º). Defat-

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ted plant material was subjected to extraction with 12 litre of methanol in Soxhlet apparatus for about 48 hours. Methanol extract, so obtained, was vacuum dried to give a residue (95g), which was fractionated by column chromatography using silica gel (mesh size 60-120). Elution of column was carried out separately using 3% ethyl acetate in pet ether (A), 6% ethyl acetate in pet ether (B), and 10% ethyl acetate in pet ether (C).

(A). Fractionation with 3% ethyl acetate in pet ether

Fractions (1-15) upto 280 ml collected with 3% EtOAc in pet ether revealed the presence of intricate mixture and was further subjected to re-column chromatography over silica gel. Elution of column was carried out with increasing polarity of ethyl acetate in pet ether with 2% and 4%. Fractions upto (1-12) 120 ml of 2% ethyl acetate showed presence of spot on TLC. The volume of the fraction was reduced to 15 ml by distillation. Crystallization of the fraction using 2 ml ethyl acetate and 25 ml of pet ether as solvent yielded white crystalline needles (95 mg) labeled as Compound-1 m p 112-13ºC. Next fractions (13-32) up to 150 ml of 4% ethyl acetate revealed the presence of an interesting spot on TLC. The volume of the fraction was reduced to 15 ml by distillation. Crystallization of the compound using 25 ml pet ether and 2 ml ethyl acetate, yielded 90 mg white crystals labeled as Compound-2 m p 130-32ºC.

Hydrolysis

The compound-**(1)** was hydrolysed with dilute H_2SO_4 to give a diol which was directly converted into a di-acetate by treatment with Ac_2O-C_5H_5N at room temperature.

Hydrogenation of compound (2)

Catalytic hydrogenation of compound-**(2)** over 5% Pd/c in EtOAc was carried out at room temperature and atmospheric pressure. After the reaction was complete, catalyst was removed by filtration and the filtrate vacuum dried leaving behind white solid, which on crystallization from Pet ether and ethyl acetate yielded the hydrogenated product.

LAH-reduction of compound (2)

To a stirred solution of the compound-**(2)** (50 mg in dry ether) added LAH (5 mg) in dry ether slowly at room temperature and monitored by TLC. The solution was diluted with dry and distilled EtOAc in order to remove excess of LAH. The solution was filtered and the filtrate dried leaving behind white solid which on crystallization from pet ether and EtOAc yielded a hydrogenated compound.

(B). Fractionation with 6% ethyl acetate in pet ether

Fractions (16-40) of volume 450 ml from bomb column were pooled and volume was reduced to 30 ml by distillation. This fraction revealed the presence of intricate mixture and further separated by re-column chromatography. Column elution was carried out by increasing polarity of ethyl acetate in pet ether from 2% and 4%. Fractions (1-45) up to volume of 30 ml of 2% ethyl acetate did not show the presence of any spot on TLC and were discarded. Next fractions (46-75) up to volume of 350 ml of 4% ethyl acetate shows single spot on TLC and volume was reduced to 15 ml by distillation. The compound was crystallized by using 2 ml of ethyl acetate and 25 ml of pet ether as the solvent which yielded a compound 75 mg white crystals labeled as Compound **(3)** melting point 78-80ºC.

Hydrogenation of compound- (3)

Catalytic hydrogenation with same catalyst and reaction conditions as in compound **(2)** gave hydrogenated product.

LAH reduction of compound (3)

LAH reduction of the hydrogenated product of compond-3 under similar conditions as in compound **(2)** gave same product as in compound **(2)**.

(C). Fractionation with 10% ethyl acetate in pet ether

Fractions (41-75) of volume 850 ml from bomb column were pooled and volume was reduced to 20 ml by distillation. This fraction revealed the presence of intricate mixture and further tried to separate them by re-column chromatography. Column elution was carried out with increasing polarity of ethyl acetate in pet ether from 5% to 10%. Fraction (1-35) up to volume of 320 ml of 5% ethyl acetate revealed the presence of spot on TLC and volume was reduced to 15 ml by

distillation. Crystallization of the compound using 2 ml of ethyl acetate and 25 ml of pet ether yielded 120 mg of white crystals labeled as compound-**(4)** m.p. 110-11ºC.

Hydrogenation of compound (4)

Catalytic hydrogenation of compound **(4)** with same catalyst and reaction conditions as in compound **(2)** gave same product as in compound **(2)**.

Next fraction (36-85) up to volume of 550 ml of 10% ethyl acetate shows single spot on TLC and volume was reduced to 15 ml by distillation. The compound was crystallized by using 2 ml of ethyl acetate and 25 ml of pet ether as the solvent, yielded a compound 100 mg white crystals labeled as compund-5 melting point 171-72ºC.

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