

Sesquiterpenolides from *Inula racemosa* and their Chemical Transformations

RAMANDEEP KAUR, K.K. CHAHAL* and URVASHI BHARDWAJ

Department of Chemistry, Punjab Agricultural University Ludhiana-141 004, India

*Corresponding author: E-mail: drkkchahal@pau.edu

Received: 18 August 2016;

Accepted: 28 October 2016;

Published online: 30 December 2016;

AJC-18201

Inula racemosa roots are rich source of sesquiterpene lactones. Soxhlet extraction of roots using chloroform followed by column chromatography on silver nitrate impregnated silica gel led to the isolation of two sesquiterpene lactones namely alantolactone and isoalantolactone. These sesquiterpene lactones were subjected to chemical transformations using different reagents (Mg/methanol, CHBr₃/KOH, LiAl[OC(CH₃)₃]₃ and NaBH₄) to afford modifications on their α -methylene- γ -lactone moiety. The structures of isolated compounds and their transformation products were elucidated using spectroscopic techniques.

Keywords: *Inula racemosa*, Sesquiterpene lactones, Alantolactone, Isoalantolactone.

INTRODUCTION

Sesquiterpenelactones a class of chemical compounds made up of three isoprene units, containing lactone ring are the main compounds present in wide varieties of plants. *Inula racemosa* commonly known as *Pushkarmula* is a perennial herb with a range of medicinal properties [1]. It is distributed all over East Asia, Europe and Africa and in India this herb has its distribution from temperate to alpine belts of Kashmir and Himachal Pradesh. The chemical profiling of the roots of this herb showed the presence of eudesmanolide group of sesquiterpenelactones. The different sesquiterpene lactones present in this herb are: alantolactone, isoalantolactone, inunal, alloalantolactone, isoalloalantolactone and inunolide *etc.* [2-5] along with sitisterol, daucosterol, inunolide, aplotexene and phenylacetone [6]. The nor-eudesmane-type sesquiterpenoids were also reported [7]. The α,β -unsaturated γ -lactone is the most common group in sesquiterpene lactones. The isolation of two isomeric lactones-alantolactone and isoalantolactone from roots of *I. racemosa* and their chemical transformations on exocyclic methylene α,β -unsaturated γ -lactone moiety are reported.

EXPERIMENTAL

Melting points reported were determined in open capillaries and are uncorrected. FT-IR spectra were measured in CHCl₃ solution or nujol on a Perkin Elmer, Model RX-1 FT-IR spectrophotometer. ¹H NMR and ¹³C NMR spectra were recorded with Bruker AC (400 MHz) or mentioned otherwise as solutions

(in CDCl₃) using TMS as an internal reference. FTIR, ¹³C NMR and ¹H NMR spectroscopic analysis was recorded at Central Instrumentation Laboratories (CIL), Panjab University, Chandigarh. The chemical shifts are expressed in δ (ppm) values.

Soxhlet extraction of powdered roots of *I. racemosa*:

The roots (250 g) of *I. racemosa* dried, powdered and subjected to Soxhlet extraction using chloroform (1 L) as the solvent. The chloroform extract was distilled to yield yellow oil (5 g).

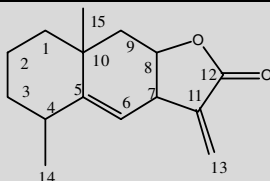
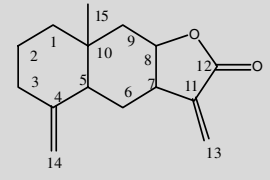
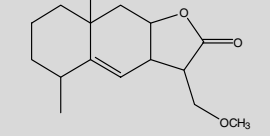
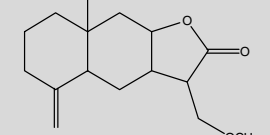
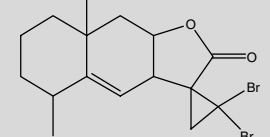
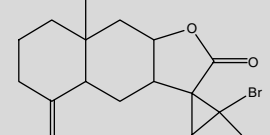
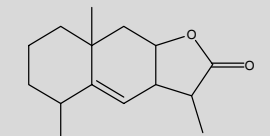
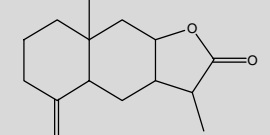
Preparation of silver nitrate impregnated silica gel:

To acetonitrile (20 mL) was added silver nitrate (20 g) and silica gel (200 g) in small proportions and mixed thoroughly. Excess of acetonitrile was evaporated and silica gel impregnated with silver nitrate was filled in the brown bottle and kept in oven for 24 h for activation. The impregnated silica gel was cooled and used further.

Isolation of pure compounds: Extract of two batches (10 g) was subjected to column chromatography using silver nitrate impregnated silica gel. The column was eluted with solvents of increasing polarity (hexane:dichloromethane) and alantolactone (**1**) having melting point 78 °C was obtained in hexane fraction and isoalantolactone (**2**) having m.p. 108 °C in 5 % hexane: dichloromethane. The structures of the compounds were confirmed using spectroscopic data (Table-1).

General procedure for reaction of lactones (alantolactone and isoalantolactone) with Mg/methanol: To dry methanol (50 mL), alantolactone (0.5 g) and active Mg turnings (0.5 g) were added with constant stirring under dry conditions. A mild exothermic reaction was observed after 3 h with the evolution of

TABLE-1
 SPECTROSCOPIC DATA OF ALANTOLACTONE (1), ISOALANTOLACTONE (2) AND THEIR DERIVATIVES (3-8)

Structure	IR (cm ⁻¹)	¹ H NMR (δ)	¹³ C NMR (δ)
 (1)	1745, 1665, 1462, 1375, 1262, 890 and 810	1.09 (d, 3H, <i>J</i> = 7.6 Hz, C ₁₄ -H _s), 1.19 (s, 3H, C ₁₅ -H _s), 3.57-3.61 (m, 1H, C ₇ -H), 4.81-4.84 (m, 1H, C ₈ -H), 5.16 (d, 1H, <i>J</i> = 4.12 Hz, C ₆ -H), 6.19 (d, 1H, <i>J</i> = 1.87 Hz, C ₁₃ -H), 5.63 (d, 1H, <i>J</i> = 1.72 Hz, C ₁₃ -H)	39.46 (C ₁), 22.56 (C ₂), 37.56 (C ₃), 32.86 (C ₄), 148.94 (C ₅), 118.83 (C ₆), 41.70 (C ₇), 76.45 (C ₈), 42.63 (C ₉), 32.69 (C ₁₀), 139.84 (C ₁₁), 170.45 (C ₁₂), 121.63 (C ₁₃), 2 16.74 (C ₁₄), and 28.58 (C ₁₅).
 (2)	1760, 1648, 1470, 1262, 890, 880 and 815	0.82 (s, 3H, C ₁₅ -H _s), 2.95-3.01 (m, 1H, C ₇ -H), 4.44 (d, 1H, <i>J</i> = 1.4 Hz, C ₁₄ -H) and 4.77 (d, 1H, <i>J</i> = 1.52 Hz, C ₁₄ -H), 4.48-4.51 (m, 1H, C ₈ -H), 5.59 (d, 1H, <i>J</i> = 1.52 Hz, C ₁₃ -H) and 6.13 (d, 1H, <i>J</i> = 1.52 Hz, C ₁₃ -H).	36.81 (C ₁), 17.68 (C ₂), 40.52 (C ₃), 148.98 (C ₄), 46.15 (C ₅), 22.69 (C ₆), 41.35 (C ₇), 76.85 (C ₈), 42.18 (C ₉), 32.28 (C ₁₀), 142.22 (C ₁₁), 170.68 (C ₁₂), 120.08 (C ₁₃), 106.41 (C ₁₄) and 27.48 (C ₁₅).
 (3)	1750, 1660, 1450, 1370, 1262, 1138, 1109, 890 and 810	1.04 (d, 3H, <i>J</i> = 3.84 Hz, C ₁₄ -H _s), 1.15 (s, 3H, C ₁₅ -H _s), 2.91- 2.95 (m, 1 H, C ₇ -H), 3.29 (s, 3 H, -OCH ₃), 3.57 (dd, <i>J</i> = 9.16 Hz, <i>J</i> = 3.57 Hz, C ₁₃ -H), 3.67 (dd, <i>J</i> = 9.16 Hz, <i>J</i> = 5.12 Hz, C ₁₃ -H), 4.79-4.82 (m, 1H, C ₈ -H), 5.10 (d, 1H, <i>J</i> = 3.64, C ₆ -H.	38.75 (C ₁), 22.67 (C ₂), 37.89 (C ₃), 32.90 (C ₄), 148.90 (C ₅), 119.96 (C ₆), 42.85 (C ₇), 76.74 (C ₈), 42.00 (C ₉), 32.72 (C ₁₀), 50.43 (C ₁₁), 177.94 (C ₁₂), 72.43 (C ₁₃), 16.87 (C ₁₄), 28.86 (C ₁₅) and 59.21 (C ₁₆).
 (4)	1770, 1660, 1470, 1380, 1140, 1110, 895 and 730	0.80 (s, 3H, C ₁₅ -H _s), 2.93-2.95 (m, 1H, C ₇ -H), 3.46 (dd, <i>J</i> = 10.12 Hz, <i>J</i> = 3.2 Hz, C ₁₃ -H), 3.62 (dd, <i>J</i> = 10.12 Hz, <i>J</i> = 6.12 Hz, C ₁₃ -H), 4.47 (d, 1H, <i>J</i> = 1.4 Hz, C ₁₄ -H) and 4.73 (d, 1H, <i>J</i> = 1.52 Hz, C ₁₄ -H), 4.45 (m, 1H, C ₈ -H), 3.27 (s, 3 H, -OCH ₃).	37.21 (C ₁), 19.65 (C ₂), 40.52 (C ₃), 149.24 (C ₄), 47.45 (C ₅), 23.62 (C ₆), 40.25 (C ₇), 76.25 (C ₈), 41.26 (C ₉), 33.75 (C ₁₀), 44.78 (C ₁₁), 170.59 (C ₁₂), 75.30 (C ₁₃), 106.41 (C ₁₄) 27.48 (C ₁₅) and 59.30 (C ₁₆).
 (5)	1767, 1640, 890 and 688	1.05 (D, 3H, <i>J</i> = 7.6, C ₁₄ -H _s), 1.19 (s, 3H, C ₁₅ -H _s), 1.92 and 2.18 (D, 1H each, <i>J</i> = 8 Hz, C ₁₃ -H _s), 3.16-3.18 (m, 1H, C ₇ -H), 4.95-4.98 (m, 1H, C ₈ -H), 4.91 (d, 1H, <i>J</i> = 3.52 Hz, C ₆ -H).	40.64 (C ₁), 22.83 (C ₂), 38.10 (C ₃), 32.95 (C ₄), 151.38 (C ₅), 115.29 (C ₆), 41.26 (C ₇), 76.79 (C ₈), 42.02 (C ₉), 32.83 (C ₁₀), 42.65 (C ₁₁), 171.84 (C ₁₂), 27.27 (C ₁₃), 16.80 (C ₁₄), 28.54 (C ₁₅) and 29.66 (C ₁₆).
 (6)	1762, 1643, 890 and 650	0.86 (s, 3H, C ₁₄ -H _s), 1.6-1.8 (m, 2H, C ₁₃ -H _s), 2.59-2.64 (m, 1H, C ₇ -H), 4.80-4.82 (m, 1H, C ₈ -H), 4.79 (d, 1H, C ₁₅ -H, <i>J</i> = 1.28), 4.45 (d, 1H, C ₁₅ -H, <i>J</i> = 1.32).	41.23 (C ₁), 22.64 (C ₂), 36.70 (C ₃), 148.85 (C ₄), 46.43 (C ₅), 23.74 (C ₆), 42.77 (C ₇), 76.73 (C ₈), 42.09 (C ₉), 25.67 (C ₁₀), 42.68 (C ₁₁), 172.11 (C ₁₂), 34.64 (C ₁₃), 17.85 (C ₁₄), 106.69 (C ₁₅) and 28.97 (C ₁₆).
 (7)	1728, 1599, 1456 and 890	1.24 (d, 3H, <i>J</i> = 7.12 Hz, C ₁₃ -H _s), 1.26 (s, 3H, C ₁₅ -H _s), 1.14 (d, 1H, <i>J</i> = 7.64 Hz, C ₁₄ -H _s), 2.40-2.54 (m, 1H, C ₁₁ -H), 3.02-3.07 (m, 1H, C ₇ -H _s), 4.74-4.77 (m, 1H, C ₈ -H), 5.18 (d, 1H, <i>J</i> = 3.24, C ₆ -H).	38.76 (C ₁), 23.07 (C ₂), 38.52 (C ₃), 33.05 (C ₄), 150.61 (C ₅), 115.56 (C ₆), 42.92 (C ₇), 76.98 (C ₈), 40.38 (C ₉), 32.91 (C ₁₀), 42.25 (C ₁₁), 179.33 (C ₁₂), 10.73 (C ₁₃), 16.88 (C ₁₄) and 28.75 (C ₁₅).
 (8)	1756, 1640 and 889	0.83 (s, 3H, C ₁₅ -H _s), 1.22 (d, 3H, <i>J</i> = 7.16, C ₁₃ -H _s), 2.79-2.82 (m, 1H, C ₁₁ -H), 2.95-3.01 (m, 1H, C ₇ -H), 4.44-4.51 (m 1H, C ₈ -H), 4.77 (bs, 2H, C ₁₄ -H _s).	40.50 (C ₁), 22.69 (C ₂), 36.80 (C ₃), 148.96 (C ₄), 46.50 (C ₅), 34.82 (C ₆), 42.24 (C ₇), 76.84 (C ₈), 41.75 (C ₉), 27.47 (C ₁₀), 41.57 (C ₁₁), 179.46 (C ₁₂), 9.29 (C ₁₃), 106.59 (C ₁₄) and 17.79 (C ₁₅)

hydrogen gas. Unreacted metal was then separated and the mixture was added to dilute HCl (150 mL) to get clear solution. The pH was adjusted around 8-9 by adding ammonia solution. The reaction mixture was then diluted with water and extracted with dichloromethane (4 × 20 mL). The organic layer was dried (anhydrous sodium sulfate) and evaporation of solvent

under vacuum afforded a mixture (0.44 g) which on chromatography over silica gel yielded pure compound 13-methoxydihydroalantolactone (**3**, 0.34 g) with m.p. 82 °C. Isoalantolactone was subjected to reaction using same method to yield 13-methoxydihydroisoalantolactone (**4**, 1.6 g) with m.p. 91 °C.

General procedure for reaction of lactones (alantolactone and isoalantolactone) with CHBr_3 : CHBr_3 (15 mL) was treated with aqueous KOH (10 mL, 50 %) and phase transfer catalyst (TEBAC, 100 mg), stirred and treated with alantolactone (2, 1.0 g). Stirring was continued at room temperature for 5 h. The reaction mixture was diluted with water and extracted with ethyl acetate (3×50 mL) and dried over anhydrous sodium sulfate. Solvent was removed under vacuum. The crystalline pure compound (**5**, 0.9 g) was obtained having m.p. 58°C . Isoalantolactone on reaction with bromoform (CHBr_3) under similar reaction conditions afforded a product (**6**, 0.8 g) with m.p. 149°C .

General procedure for reaction of lactones (alantolactone and isoalantolactone) with NaBH_4 : Alantolactone (**1**, 1 g) was dissolved in methanol (20.0 mL) in round bottomed flask. To this, sodium borohydride (1:2 molar ratio) was added. The reaction mixture was stirred for 25 min. The extent of completion of the reaction was monitored (TLC). After completion of reaction few drops of acetic acid were added and the reaction mixture was extracted with water and then with dichloromethane (3×50 mL). The organic layer was dried (anhydrous sodium sulfate) and excess of solvent was distilled off to get pure crystals of compound, which was identified as dihydroalantolactone (**7**, 0.7 g) with m.p. 85°C . When similar reaction was carried out using isoalantolactone dihydroisoalantolactone (**8**, 0.8 g) was obtained as a product with m.p. 172°C .

General procedure for reaction of lactones (alantolactone and isoalantolactone) with $\text{LiAl}[\text{OC}(\text{CH}_3)_3]_3$: Alantolactone, **1** (2.0g) was dissolved in dry THF (20.0mL) and lithium tri-*tert*-butoxyaluminumhydride (1.0 g) was added. The reaction mixture was stirred for 15 min, the progress of reaction was monitored by TLC. After completion of reaction, THF was removed under vacuum and few drops of glacial acetic acid were added and the reaction mixture was extracted with water and finally with diethyl ether (3×50 mL). Evaporation of solvent afforded pure crystals of dihydroalantolactone

(**7**, 1.8 g). Isoalantolactone give dihydroisoalantolactone (**8**, 1.9 g) under similar reaction conditions.

RESULTS AND DISCUSSION

The isolation of two isomeric lactones-alantolactone and isoalantolactone was carried out using silver nitrate impregnated silica gel and the chemical modifications were carried out with alantolactone and isoalantolactone. Reactions were carried out using different reagents such as Mg/methanol, CHBr_3/KOH in presence of phase transfer catalyst-TEBAC (triethylbutylammonium chloride), $\text{LiAl}[\text{OC}(\text{CH}_3)_3]_3$ and NaBH_4 . The products obtained on reaction with Mg/methanol are 13-methoxydihydro derivatives (**3** and **4**) formed *via* Michael addition of CH_3OH in the presence of base (Mg). The reaction with CHBr_3/KOH in the presence of phase transfer catalyst resulted into dibromocarbene derivatives (**5** and **6**) of alantolactone and isoalantolactone. The use of strong but bulky reducing agent $\text{LiAl}[\text{OC}(\text{CH}_3)_3]_3$ resulted into selective reduction of conjugated double bond yielding dihydroderivatives (**7** and **8**) of alantolactone and isoalantolactone, respectively. These products were similar to those obtained with sodium borohydride but the yield was more although the amount of reagent used was less in case of $\text{LiAl}[\text{OC}(\text{CH}_3)_3]_3$. The spectroscopic data of all the compounds is presented in Table-1.

REFERENCES

1. P.D. Lokhande, K.P. Gawai, K.M. Kodam, B.S. Kuchekar, A.R. Cheabukswar and S.C. Jagdale, *Res. J. Med. Plant*, **1**, 7 (2007).
2. P.S. Kalsi, R. Goyal, K.K. Talwar and B.R. Chhabra, *Phytochemistry*, **28**, 2093 (1989).
3. B. Kaur and P.S. Kalsi, *Phytochemistry*, **24**, 2007 (1985).
4. P. Bhandari and R.P. Rastogi, *Indian J. Chem.*, **22B**, 286 (1983).
5. R.K. Raghvan, K.R. Ravindranath, G.K. Trivedi, S.K. Paknikar and B.S. Bhattacharya, *Indian J. Chem.*, **7**, 310 (1969).
6. K.H. Wang, H.T. Liu, Y.K. Zhao, X.G. Chen, Y. Hu, Y.C. Song and X. Ma, *Talanta*, **52**, 1001 (2000).
7. L.W. Xu and Y.P. Shi, *J. Asian Nat. Prod. Res.*, **13**, 570 (2011).