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Chemical composition of *Inula cuspidata* C.B. Clarke

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Thymyl isobutyrate, thymol, thymyl isovalerate, 8α-hydroxy presilphiperfolene and intermedeol have been isolated from steam volatile extract of *Inula cuspidata* and identified from their spectral data, synthesis and chemical modification of major constituents. Sharp qualitative and quantitative variations among the constituents of leaf, flower and roots of *Inula cuspidata* are noticed.

Keywords: *Inula cuspidata,* thymol, thymyl isobutyrate, thymyl isovalerate, 8α-hydroxy presilphiperfolene, intermedeol

The genus *Inula*, one of the smallest and most widely distributed genera of the family Asteraceae, is comprised of 20 species in India and several of these are reported to possess medicinal properties and used in folk medicines, as tonic, stomachic, diuretic, diaphoretic, anti-inflammatory, bactericidal, hepatoprotective, antitumor and carminative[1-4]. Four among these *viz*., *I. cappa* DC., *I. cuspidata* C.B. Clarke, *I. nervosa* Wall., and *I. rubricaulis* Clarke have been reported to grow in and around Nainital. *Inula cuspidata* (C.B. Clarke) is an erect shrub and is usually found growing on steep, rocky or precipitous ground[5,6].

Literature reports show sesquiterpene lactones as characteristic components of the genus *Inula*[7-15]. Thymol derivatives have also been noticed in some species[15-19]. Five germacranolides, closely related to ineupatorolide, a hydroxygermacrene and two isomeric acetylenic sulphoxides were isolated from aerial parts of *Inula cuspidata*[20]. The essential oil from the leaves of *Inula cuspidata* showed antifungal activity against pathogenic fungi[21]. To the best of the knowledge, there is no earlier report on roots and flower constituents of *Inula cuspidata*.

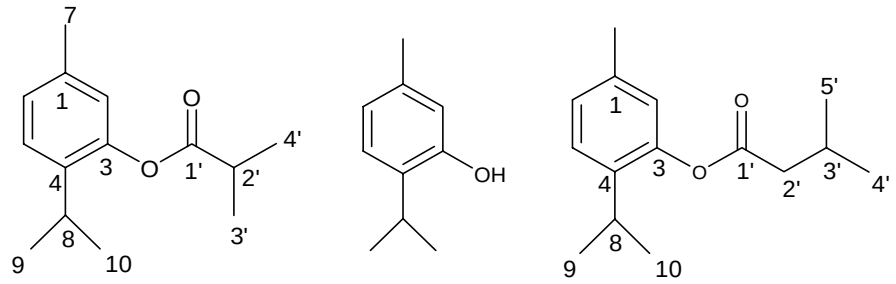
Results and Discussion

The CC and HPLC of organic phase extracts of the steam distillates of the leaves, flowers and roots of *I. cuspidata* C.B. Clarke afforded five compounds **1-5** (**Figure 1**).

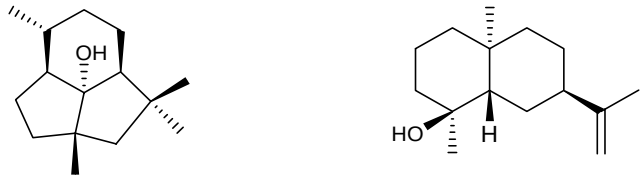
The compound **1** was obtained as solid. The EI-MS of compound **1** displayed molecular ion peak at *m/z* 220 (M^+) corresponding to molecular formula $C_{14}H_{20}O_2$. The ^{13}C and DEPT NMR assignments showed 14 signals attributed to five CH_3, five CH and four quaternary carbons. The IR spectrum showed the presence of an acetoxyl group (1756 cm^{-1}) with ^{13}C NMR resonance at δ 175.5. Further, in ^1H NMR two doublets at δ 6.98 and 7.17 (1H, *J*=8.1 Hz each) and one singlet at 6.78 (1H) indicated the compound **1** to be a thymol derivative with isobutyl ester chain represented by a doublet at δ 1.18 (6H, *J*=6.9 Hz) and one septet at δ 2.80 (1H, *J*=6.9 Hz) in its ^1H NMR spectrum. Compound **1** was identified as thymyl isobutyrate[22,23] which was confirmed by its reduction into thymol **2** and also by its synthesis from thymol (**Scheme I**). Thymyl isobutyrate derivatives have previously been reported as major constituents of *Arnica*[24-26], *Eupatorium cannabinum*[27] and *Pulicaria odora*[28].

The compound **2** has molecular formula $C_{10}H_{14}O$ (EI-MS, M^+ at *m/z* 150). The ^{13}C and DEPT NMR spectra of compound **2** showed 10 carbon resonances with three CH_3, four CH and three quaternary carbons. The spectral data of compound **2** are consistent with thymol[22,29,30].

Compound **3**, a colourless liquid, has molecular ion peak at *m/z* 222 corresponding to molecular formula of $C_{15}H_{26}O$, another fragment ion at *m/z* 204 due to loss of H_2O (M^+-18), characteristic of a sesquiterpene alcohol. The ^{13}C NMR (DEPT) spectrum of compounds **3** showed four CH_3, five CH_2, three CH and three quaternary carbons. Absence of olefinic carbons showed compound **3** to be a tricyclic



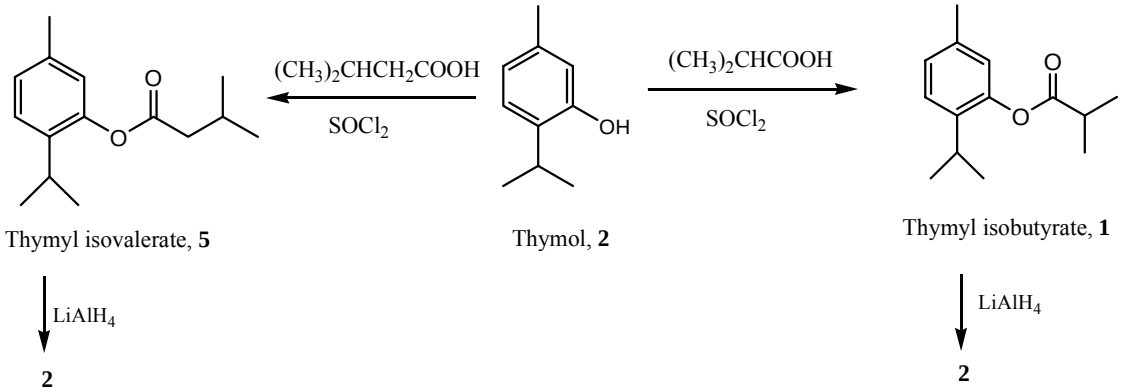
Thymyl isobutyrate **1** Thymol **2** Thymyl isovalerate **5**



8α-Hydroxy presilpheperfolene **3** Intermedeol **4**

Major constituents of *I. cuspidata*

Figure 1



Scheme I

sesquiterpene alcohol. On the basis of its ^{1}H and ^{13}C spectral data, compound **3** was identified as 8α-hydroxy presilphiperfolene. It has been previously isolated from *Eriophyllum* species and *Inula cuspidata*[20,31].

Compound **4** displayed molecular ion peak at *m/z* 222 in its EI-MS for $C_{15}H_{26}O$ and fragment ion peak at *m/z* 204 (loss of H_2O) suggesting compound **4** to be a sesquiterpene alcohol. The ^{13}C NMR (DEPT) showed the presence of three CH_3, seven CH_2, two CH and three quaternary carbons. The NMR data of compound **4** are comparable with intermedeol, a diastereomer of eudesm-11-en-4-ol[32,33].

Compound **5,** molecular ion peak at *m/z* 234 ($C_{15}H_{22}O_2$), showed mass spectral similarity with **1** with an additional carbon as CH_2, possibly in the side chain. The ^{1}H NMR signals at δ 1.08 (6H, *d*, *J*=6.6 Hz), 2.29 (1H, *m*) and 2.46 (2H, *d*, *J*=7.2 Hz) account for the isovaleryl as ester moiety. The ^{1}H NMR signals at δ 1.19 (6H, *d*, *J*=6.9 Hz) and 2.98 (1H, *septet*, *J*=6.9 Hz) account for isopropyl group attached to the benzene ring. The ring protons appeared at δ 7.02 and 7.17 (1H, each, *d*, *J*=7.8Hz) along with 6.79 (1H, *s*). The spectral data suggest the compound to be thymyl isovalerate which is supported by the ^{13}C, DEPT and HSQC and HMBC NMR experiments.

Table I — Major constituents of *Inula cuspidata*

Compd	% Content in the extract*		
	Leaf	Flower	Root
Thymol **2**	-	-	5.1
Isoledene	0.1	6.5	-
(*E*)-β-Farnesene	2.6	1.0	0.2
Seychellene	t	10.3	-
Germacrene D	3.8	0.7	0.2
Thymyl isobutyrate **1**	-	-	87.3
Cubebol	3.5	4.0	t
δ-Cadinene	2.7	2.6	t
Thymyl isovalerate **5**	-	-	3.8
Germacrene D-4-ol	4.5	0.4	-
8α-Hydroxy-presilphiperfolene **3**	43.1	37.1	-
Intermedeol **4**	2.1	2.0	-

* % Composition on FID response in GC of steam volatile extracts

This was further confirmed by its synthesis and reduction to thymol (**Scheme I**).

The comparison of GC and GC-MS results of the extracts from leaves, flowers and roots have revealed presence of 8α-hydroxy presilphiperfolene (43.1%), germacrene D-4-ol (4.5%), germacrene D (3.8%), cubebol (3.5%), δ-cadinene (2.7%), (*E*)-β-farnesene (2.6%) and intermedeol (2.1%) in leaf extract while the flower extract showed 8α-hydroxy presilphiperfolene (37.1%), seychellene (10.3%), *iso*-ledene (6.5%), cubebol (4.0%) and intermedeol (2.0%) as major constituents. On the contrary, the root extract was marked by the dominant presence of thymol and its derivatives (96.2%), represented by thymyl isobutyrate (87.3%; **1**), thymol (5.1%; **2**) and thymyl isovalerate (3.8%; **5**). It is interesting to note that 8α-hydroxy presilphiperfolene is present (37.1 to 43.1%) in the aerial parts (leaves and flowers) while the root extract was marked by almost exclusive presence of thymyl derivatives. Dominant presence of thymyl derivatives (96.2%) in the root extract of *Inula cuspidata* makes it a good source of thymyl derivatives.

Materials and Methods

Plant material

The leaves, flowers and roots of *I. cuspidata* were collected from Nainital. The plant was identified at BSI, Dehradun. The voucher specimen (No. Chem./ DST/IC) has been deposited in the Phytochemistry Laboratory, Chemistry Department, Kumaun University, Nainital.

Extraction and isolation

The plant materials (leaf, flower and root: 2 kg each) were subjected to steam distillation for 2 hr separately obtaining 5 L water distillate each. The distillates were saturated with NaCl and extracted with hexane/dichloromethane. The organic phase was then dried over anhydrous Na_2SO_4 and the solvent distilled off in a thin film rotary vacuum evaporator at 30°C. The extracts were obtained as yellow oils. Fractionation carried out on silica gel CC (230-400 mesh, Merck) with gradient elution from hexane to 15% Et_2O in hexane gave five compounds **1-5**, which were purified by Water's HPLC using μ-porosil column (250 mm × 7.8 mm), 2.0 mL/min flow rate, RI detector in an attenuation of 32X at 2000 psi using 5.0-15.0% Et_2O in hexane.

General experimental procedures

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker-Avance DRX 300 MHz and 75 MHz instrument using TMS as internal standard at 25°C. HPLC analysis were run on Water's 440 model equipped with RI detector and μ-porosil column

(250 mm × 7.8 mm) using varying concentration of Et_2O in hexane at a flow rate of 2.0 mL/min. The GC analysis was run on Nucon 5765 gas chromatograph (Rtx-5 column, 30 m × 0.32 mm i.d., FID), split ratio 1:48, N_2 flow of 4 kg/cm^2 and on ThermoQuest Trace GC 2000 interfaced with Finnigan MAT PolarisQ Ion Trap Mass spectrometer fitted with a Rtx-5 (Restek Corp.) fused silica capillary column (30 m × 0.25 mm; 0.25 µm film coating). The column temperature was programmed 60°C-210°C at 3°C /min using He as carrier gas at 1.0 mL/min. The injector temperature was 210°C, injection size 0.1µL prepared in *n*-hexane, split ratio 1:40. MS were taken at 70 eV with a mass scan range of 40-450 amu. The identification was done on the basis of Relative Retention Index (RRI), MS Library search (NIST and WILEY), by comparing with the MS literature data[34] and by NMR (^{1}H and ^{13}C NMR) of major isolates. The results are given in **Table I**.

Synthesis and reduction of 1

To isobutyric acid (0.060 g, 0.00068 mol) was added 0.5 mL thionyl chloride drop wise (**Scheme I**). The resulting reaction mixture was refluxed for 2 hr. After completion of reaction, the resulting mixture was concentrated to dryness under vacuum to obtain acid chloride (0.068 g, Yield: 95%). In 2.0 mL acid chloride solution (prepared in anhydrous Et_2O), triethylamine (0.129 g, 0.0013 mol) and thymol (0.105 g, 0.0007 mol) were added and the resulting reaction mixture was stirred for 5 hr under magnetic stirring. After completion of the reaction, the reaction mixture was extracted with DCM (3 × 20 mL). The organic layer was washed with water and brine solution. The organic layer was finally dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to obtain thymyl isobutyrate **1** (0.116 g, yield: 84.0%).

To a solution of $LiAlH_4$ (0.030 g, 0.0009 mol) in anhydrous Et_2O (3.0 mL) compound **1** (0.100 g, 0.00045 mol) was added at 0°C and stirred carefully for 2 hr. After completion of the reaction the reaction mixture was quenched with 5 mL NH_4Cl solution and extracted with Et_2O (3 × 20 mL) and washed with water and brine solution. The ethereal layer was dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to obtain thymol **2** (0.048 g, yield: 70.5%).

Synthesis and reduction of 5

To isovaleric acid (0.06 g, 0.0005 mol), 0.5 mL thionyl chloride was added drop-wise and follows the procedure as given for **1** (**Scheme I**)**.** Thymyl isovalerate **5** (0.110 g, yield: 81%) was obtained which was further reduced to thymol **2** (0.046 g, yield: 71.8%).

Thymyl isobutyrate 1: White solid; IR: 2968, 1756, 1621, 1504, 1461, 1386, 1233, 1129, 910, 817 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.18 (6H, *d*, *J*=6.9 Hz, H-3′/4′), 1.32 (6H, *d*, *J*=7.2 Hz, H-9/10), 2.28 (3H, *s*, H-7), 2.80 (1H, *sept*, *J*=6.9 Hz, H-2′), 2.97 (1H, *sept*, *J*=6.9 Hz, H-8), 6.78 (1H, *s*, H-2), 6.98 (1H, *d*, *J*=8.1 Hz, H-6), 7.17 (1H, *d*, *J*=8.1 Hz, H-5); ^{13}C NMR ($CDCl_3$, 75 MHz, DEPT): δ 136.8 (C-1), 126.1 (C-2), 147.9 (C-3), 136.3 (C-4), 126.8 (C-5), 122.6 (C-6), 20.6 (C-7), 26.8 (C-8), 22.5 (C-9), 22.5 (C-10), 175.5 (C-1′), 34.1 (C-2′), 18.9 (C-3′/4′); EIMS ($C_{14}H_{20}O_2$, 70 eV): *m/z* (%) 65 (1.0), 77 (2.2), 79 (2.6), 91 (6.0), 105 (3.8), 107 (5.0), 121 (2.7), 133 (9.2), 135 (100.0), 150 (48.8), 220 [M$^+$] (5.8).

Thymol 2: White solid; IR: 3464, 3361, 2963, 2871, 1708, 1623, 1584, 1512, 1347, 831, 730 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.16 (6H, *d*, *J*=9.0 Hz, H-9/10), 2.19 (3H, *s*, H-7), 3.08 (1H, *m*, H-8), 4.56 (1H, ArO*H*), 6.50 (1H, *s*, H-2), 6.65 (1H, *d*, *J*=9.0 Hz, H-6), 7.01 (1H, *d*, *J*=9.0 Hz, H-5); ^{13}C NMR ($CDCl_3$, 75 MHz, DEPT): δ 136.3 (C-1), 116.1(C-2), 152.9 (C-3), 131.8 (C-4), 126.3 (C-5), 121.7 (C-6), 20.9 (C-7), 26.8 (C-8), 22.7 (C-9), 22.7 (C-10); EIMS ($C_{10}H_{14}O$, 70 eV): *m/z* (%) 65 (3.4), 77 (6.1), 79 (8.6), 91 (23.8), 105 (13.0), 107 (26.2), 115 (22.8), 117 (5.1), 121 (2.5), 133 (2.3), 135 (100.0), 150 [M$^+$] (29.0).

8α-Hydroxy presilphiperfolene 3: Colourless liquid; IR: 3616, 3023, 1545, 1455, 1377, 1159, 1118 cm^{-1}; EIMS ($C_{15}H_{26}O$, 70 eV): *m/z* (%.) 77 (16.0), 81 (23.9), 91 (43.7), 95 (39.4), 105 (49.8), 119 (73.6), 121 (19.4), 133 (61.5), 149 (34.0), 161 (48.7), 175 (17.3), 189 (58.7), 205 (5.3), 204 (25.0), 207 (100.0), 222 [M$^+$] (1.0).

Intermedeol 4: Colourless liquid; IR: 3634, 3092, 2936, 2865, 1457, 1384, 896 cm^{-1}; $[\alpha]_D^{22}$ + 15.4° (C, 6.05, $CHCl_3$); EIMS ($C_{15}H_{26}O$, 70 eV): *m/z* (%) 67 (23.7), 91 (28.7), 93 (29.1), 95 (21.9), 105 (45.5), 119 (34.8), 121 (15.9), 133 (51.9), 147 (27.6), 161 (100.0), 175 (25.7), 189 (80.2), 205 (11.5), 204 (53.2), 207 (1.5), 222 [M$^+$] (0.2).

Thymyl isovalerate 5: Colourless liquid; IR: 3068, 2955, 2850, 1752, 1628, 1520, 1465, 1386, 1380, 1235, 817, 755 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.08 (6H, *d*, *J*=6.6 Hz, H-4′/5′), 1.19 (6H, *d*, *J*=6.9 Hz, H-9/10), 2.29 (1H, *m*, H-3′), 2.31 (3H, *s*, H-7), 2.46

(2H, *d*, *J*=7.2 Hz, H-2'), 2.98 (1H, *sept*, *J*=6.9 Hz, H-8), 6.79 (1H, *s*, H-2), 7.02 (1H, *d*, *J*=7.8 Hz, H-6), 7.17 (1H, *d*, *J*=7.8 Hz, H-5);); ^{13}C NMR ($CDCl_3$, 75 MHz, DEPT): δ 137.45 (C-1), 123.14 (C-2), 148.49 (C-3), 136.77 (C-4), 126.7 (C-5), 127.26 (C-6), 21.05 (C-7), 27.52 (C-8), 23.32 (C-9), 23.32 (C-10), 171.85 (C-1′), 43.8 (C-2′), 26.12 (C-3′), 22.77 (C-4′/5′); EIMS ($C_{15}H_{22}O_2$, 70 eV): *m/z* (%) 57 (3.3), 77 (3.5), 79 (3.7), 91 (9.3), 105 (5.0), 107 (5.3), 115 (4.5), 133 (11.9), 135 (100.0), 150 (59.2), 151 (6.4), 234 [M^+] (5.8).

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