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Chemical modifications of dehydrocostus lactone from *Saussurea lappa* and the study of structure-activity relationship

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Various chemical analogues of dehydrocostus lactone have been prepared by using various chemo- and regio-selective reagents. The biological activity of the products as plant growth regulators has revealed that the extension of carbon chain length at C_{13} to a limited extent has marked effect on biological activity.

Keywords: Dehydrocostus lactone, sesquiterpenes, structure-activity relationship, biological activity

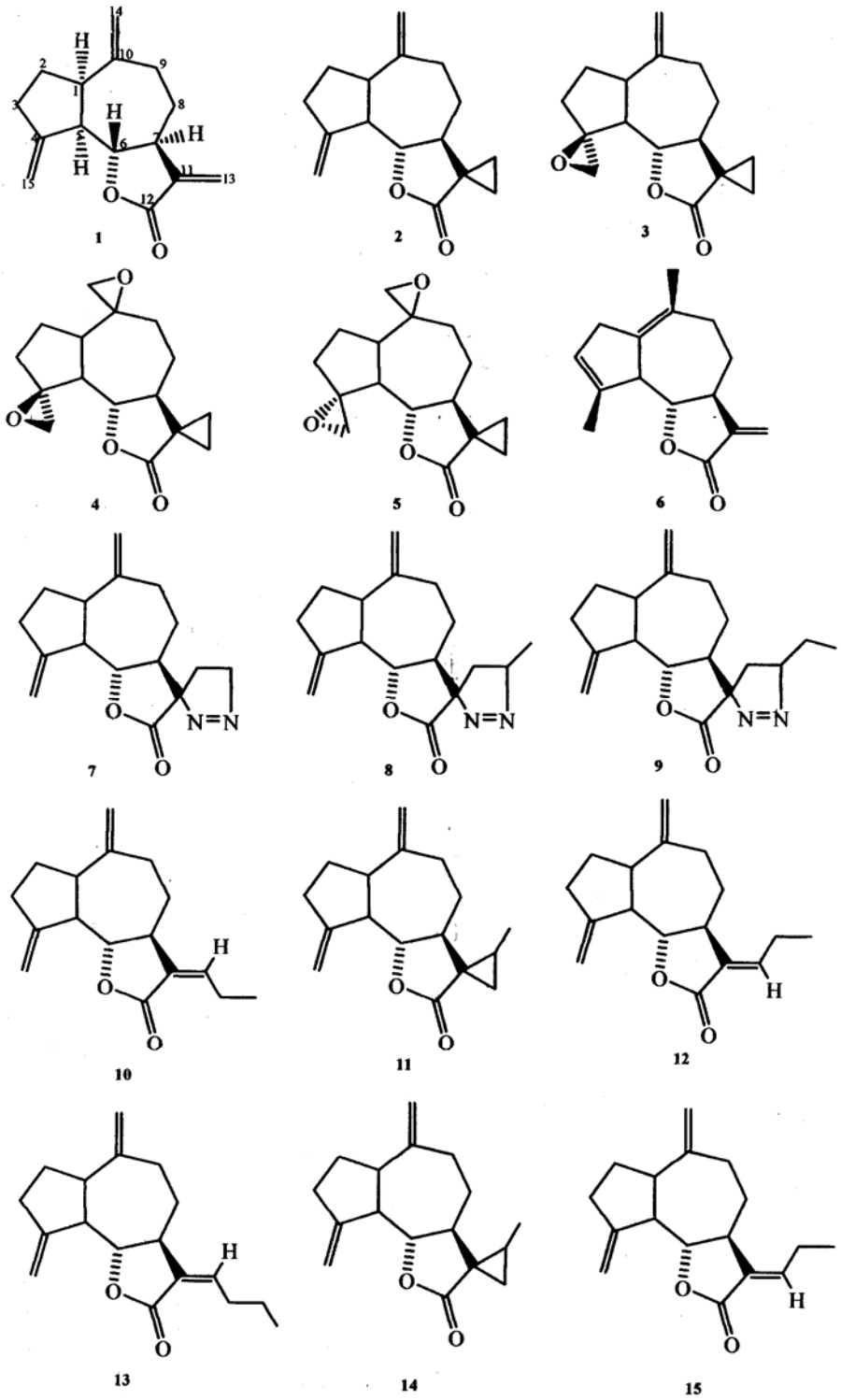
IPC: Int.Cl.[8] A 61 K

Sesquiterpene lactones with α-methylene-γ-lactone moiety fused on various skeletons are a rapidly expanding group of natural products that represent a rich source of biologically active compounds. These are an example of molecular diversity, with recognized potential in drug discovery[1] and a wide spectrum of biological activities like anti-cancer[2], anti-inflammatory[3], anti-tumor[4], anti-bacterial[5] and as molluskicidal agents[6]. They are particularly known for their potential as plant growth regulators[7]. Some of them have been successfully employed as markers in biochemical systematic studies. The oil from the roots of *Saussurea lappa* is known to possess some such properties and is a rich source of sesquiterpenoids. These properties were earlier ascribed solely to the presence of α-methylene-γ-lactone moiety. However, this idea was later modified as other structural features have also been found to be significant. Thus, chemical and stereochemical modifications on these compounds have marked effect on plant growth regulatory activity. Taking these views into consideration, an effort has been made to provide different reaction conditions in order to get biologically active compounds, which can lead to the successful establishment and further clarification of the structure-activity relationship.

Results and Discussion

The oil obtained from roots of *Saussurea lappa* is a rich source of sesquiterpenoids, the major one being dehydrocostus lactone. It was obtained from the powdered costus roots by soxhlet extraction in petroleum ether followed by refrigeration. The solidified material was purified by column chromatography over silica gel to afford pure crystalline dehydrocostus lactone **1**, m.p. 60°C (**Scheme I**). It was chemically modified through a series of reactions including epoxidation, double bond isomerization and synthesis of pyrazolines and their subsequent pyrolysis. 11-Spirocyclopropyl derivative **2** of the dehydrocostus lactone prepared by the established method[8], was subjected to reaction with perbenzoic acid in order to explore some biologically active epoxides. This reaction afforded a product mixture of three components that were purified by column chromatography to yield a monoepoxide **3** and two diepoxides **4** and **5**. The stereochemistry of the epoxy ring in compounds **4** and **5** was assigned on the basis of comparison of chemical shift differences for C_{15} oxirane protons in them with those reported in other compounds[9]. Double bond migration reaction of **1** was attempted under microwave irradiated conditions with the aim to isomerise both the *exo*-methylene C=Cs to the *endo* position. The reaction mixture on chromatography afforded the isomerised product **6**.

It has been established that γ-lactones in which a trisubstituted C=C or a cyclopropane ring is conjugated with the lactone carbonyl are more active than the parent α-methylene-γ-lactone[10]. It was thus



Scheme I

concluded on the basis of these findings that the increase in activity was due to the introduction of an additional carbon atom at C_{13}. This may be attributed to the fact that the increase in lipophilic character resulted in enhancement of activity. Hence, reactions were carried out with the aim to increase the length of the side chain at C_{13} and prepare the higher homologues. The method employed to achieve C-alkylation at conjugated C=C to lactone moiety was synthesis of pyrazolines using diazoethane and diazopropane to

Table I — δ values in ^{1}H and ^{13}C NMR spectra of the synthesized compounds

Compd	Mol Formula	^{1}H NMR ($CDCl_3$)	^{13}C NMR ($CDCl_3$)
3	$C_{16}H_{20}O_3$	0.65-1.15 (4H, C_{11}-cyclopropyl-H), 2.70 (1H, d, *J* = 4Hz, C_{15}-H), 3.1 (1H, d, *J* = 4Hz, C_{15}-H), 4.15 (1H, t, *J* = 9Hz, C_6-H), 4.95 (2H, brs, C_{14}-H)	34.5(t), 25.5 (t), 61.7 (s), 52.2 (t), 53.5 (d), 43.2 (d), 148.5 (s), 109.2 (t), 36.0 (t), 27.8 (t), 40.5 (d), 32.5 (s), 15.3 (t), 15.1(t), 171.2 (s), 78.2 (d).
4	$C_{16}H_{20}O_4$	0.5-0.85 (4H, m, C_{11}-cyclopropyl-H), 2.48 (1H, d, *J* = 4Hz, C_{15}-H), 2.76 (2H, m, C_{14}-H), 3.19 (1H, d, *J* = 4Hz, C_{15}-H), 4.12 (1H, t, *J* = 9Hz, C_6-H)	34.5 (t), 21.1 (t), 41.0 (d), 41.2 (d), 61.5 (s), 52.0 (t), 61.7 (s), 52.6 (t), 32.5 (t), 23.4 (t), 57.5 (d), 84.9 (d), 32.5 (s), 15.0 (t), 15.3 (t), 171.1 (s).
5	$C_{16}H_{20}O_4$	0.5-0.9 (4H, m, C_{11}-cyclopropyl-H), 2.58 (2H, dd, *J* = 2Hz, C_{14}-H), 2.8 (1H, d, C_{15}-H), 3.28 (1H, d, *J* = 4Hz, C_{15}-H), 4.14 (1H, t, *J* = 9Hz, C_6-H)	34.5 (t), 21.1 (t), 41.0 (d), 41.2 (d), 58.1 (s), 54.8 (t), 61.7 (s), 52.6 (t), 32.5 (t), 23.4 (t), 57.5 (d), 84.9 (d), 32.5 (s), 15.0 (t), 15.3 (t), 171.1 (s).
6	$C_{15}H_{18}O_2$	1.49 (3H, s, C_{14}-H), 1.93 (3H, s, C_{15}-H), 4.15 (1H, t, *J* = 8Hz, C_6-H), 4.92 (1H, s), 5.3-5.7 (3H, m, C_3-H and C_9-H), 6.1 (1H, d, *J* = 4Hz), 6.18 (1H, d, *J* = 4Hz, C_{13}-H)	40.0 (t), 125.0 (d), 145.3 (s), 21.2 (q), 53.0 (d), 140.5 (s), 131.0 (s), 33.5 (t), 31.4 (t), 51.0 (d), 84.5 (d), 17.5 (q), 139.2 (s), 120.5 (t), 170.0 (s) .
8	$C_{17}H_{22}O_2N_2$	1.54 (3H, d, *J* = 7.29Hz, C_{17}-H), 2.91 (1H, brs, C_7-H), 4.71-4.90 (2H, m, C_6-H & C_{16}-H), 4.83 (1H, d, *J* = 4Hz, C_{14}-H), 4.87 (1H, d, *J* = 4Hz, C_{15}-H), 5.29 (1H, d, *J* = 4Hz, C_{15}-H)	37.5 (t), 37.0 (t), 150.8 (s), 109.1 (t), 57.1 (d), 50.8 (d), 148.5 (s), 109.0 (t), 36.2 (t), 25.0 (t), 35.2 (d), 80.2 (d), 106.1 (s), 48.7 (t), 52.4 (d), 19.9 (q), 172.0 (s).
9	$C_{17}H_{22}O_2N_2$	1.14 (3H, d, *J* = 7.29Hz, C_{18}-H), 2.90 (1H, brs, C_7-H), 4.73-4.90 (3H, m, C_{14}-H & C_{16}-H), 5.12 (1H, brs, C_{15}-H), 5.29 (1H, brs, C_{15}-H)	37.5 (t), 37.0 (t), 150.8 (s), 109.1 (t), 50.8 (d), 148.5 (s), 109.0 (t), 36.2 (t), 28.3 (t), 59.2 (d), 9.0 (q), 172.7 (s) .
10	$C_{17}H_{22}O_2$	1.03 (3H, t, *J* = 7.6Hz, C_{17}-H), 3.86 (1H, t, *J* = 8Hz, C_6-H), 4.76 (1H, s, C_{14}-H), 4.84 (1H, s, C_{14}-H), 5.03 (1H, s, C_{15}-H), 5.28 (1H, s, C_{15}-H), 5.95 (1H, dt, *J* = 2Hz;7Hz, C_{13}-H)	37.5 (t), 37.0 (t), 150.8 (s), 109.1 (t), 50.8 (d), 148.5 (s), 109.0 (t), 57.0 (d), 35.8 (t), 32.0 (t), 48.5 (d), 85.0 (d), 169.8 (s), 130.1 (s), 141.5 (d), 20.4 (t), 14.3(q).
11	$C_{17}H_{22}O_2$	0.81-0.92 (3H, m, C_{11}-cyclopropyl-H), 1.40 (3H, d, *J* = 6Hz, C_{17}-H), 3.98 (1H, t, *J* = 7Hz, C_6-H), 4.76 (1H, s, C_{14}-H), 4.85 (1H, s, C_{14}-H), 5.05 (1H, s, C_{15}-H), 5.25 (1H, s, C_{15}-H)	37.5 (t), 37.0 (t), 150.8 (s), 109.1 (t), 50.8 (d), 148.5 (s), 109.0 (t), 57.5 (d), 36.1 (t), 28.2 (t), 38.0 (d), 82.9 (d), 31.5 (s), 15.0 (d), 20.5 (q), 32.4 (t), 172.0 (s).
12	$C_{17}H_{22}O_2$	1.1 (3H, t, *J* = 7.5Hz, C_{17}-H), 4.0 (3H, t, *J* = 8Hz, C_6-H), 4.78 (1H, s, C_{14}-H), 4.86 (1H, s, C_{14}-H), 5.24 (1H, s, C_{15}-H), 5.29 (1H, s, C_{15}-H), 6.74 (1H, dt, *J* = 3Hz;7.5Hz, C_{13}-H)	37.5 (t), 37.0 (t), 150.7 (s), 109.1 (t), 109.0 (t), 148.5 (s), 50.8 (d), 57.0 (d), 85.0 (d), 41.9 (d), 31.5 (t), 36.5 (t), 130.1 (s), 141.5 (d), 20.4 (t), 14.3 (q), 169.8 (s).
13	$C_{17}H_{24}O_2$	0.93 (3H, t, *J* = 7.5Hz, C_{18}-H), 3.90 (1H, t, *J* = 9Hz, C_6-H), 4.76 (1H, s, C_{14}-H), 4.86 (1H, s, C_{14}-H), 5.06 (1H, d, *J* = 1.5Hz, C_{15}-H), 5.25 (1H, d, *J* = 1.5Hz, C_{15}-H), 6.02 (1H, dt, *J* = 2.7Hz; 7.5Hz, C_{13}-H)	37.5 (t), 37.0 (t), 109.0 (t), 148.5 (s), 50.8 (d), 57.0 (d), 150.6 (s), 109.4 (t), 85.2 (d), 48.5 (d), 31.5 (t), 36.5 (t), 129.0 (s), 142.5 (d), 29.6 (t), 23.0 (t), 14.2 (q), 170.2 (s).
14	$C_{17}H_{24}O_2$	0.86-0.9 (3H, m, C_{11}-cyclopropyl-H), 0.90 (3H, t, *J* = 7.5Hz, C_{18}-H), 3.98 (1H, t, *J* = 9Hz, C_6-H), 4.76 (1H, s, C_{14}-H), 4.86 (1H, s, C_{14}-H), 5.06 (1H, d, *J* = 1.5Hz, C_{15}-H), 5.25 (1H, d, *J* = 1.5Hz, C_{15}-H)	37.5 (t), 37.0 (t), 109.0 (t), 148.5 (s), 50.8 (d), 57.0 (d), 150.9 (s), 109.0 (t), 50.5 (d), 85.0 (d), 38.5 (d), 27.8 (t), 36.8 (t), 29.0 (s), 22.5 (d), 28.5 (t), 11.6 (q), 29.7 (t), 171.9 (s).
15	$C_{17}H_{24}O_2$	0.97 (3H, t, *J* = 7.5Hz, C_{18}-H), 3.99 (1H, t, *J* = 9Hz, C_6-H), 4.7 (1H, s, C_{14}-H), 4.85 (1H, s, C_{14}-H), 5.06 (1H, d, *J* = 1.2Hz, C_{15}-H), 5.3 (1H, d, *J* = 1.2Hz, C_{15}-H), 6.75 (1H, dt, *J* = 3Hz; 9Hz, C_{13}-H)	37.5 (t), 37.0 (t), 109.0 (t), 148.5 (s), 50.8 (d), 57.0 (d), 150.7 (s), 109.5 (t), 85.0 (d), 41.9 (d), 31.5 (t), 36.5 (t), 129.0 (s), 142.5 (d), 29.6 (t), 23.0 (t), 14.2 (q), 170.2 (s).

yield the pyrazoline products **7-9**, whose authenticity was confirmed by comparison of the IR spectral data with the known sample[11]. These pyrazolines upon pyrolysis gave the desired compounds **10-15** through the intermediacy of singlet and triplet diradicals. The structural pairs **10**, **12** and **13**, **15** show similar ^{1}H NMR data (**Table I**). As is clear from the table, *Z*-isomer **10** showed chemical shift at δ 5.95 for C_{13} in contrast to 6.74 in case of the *E*-isomer **12**. Similarly, for compounds **13** and **15**, the chemical shifts for C_{13} were observed at δ 6.02 and 6.75. These observations were further corroborated by a detailed study of ^{13}C

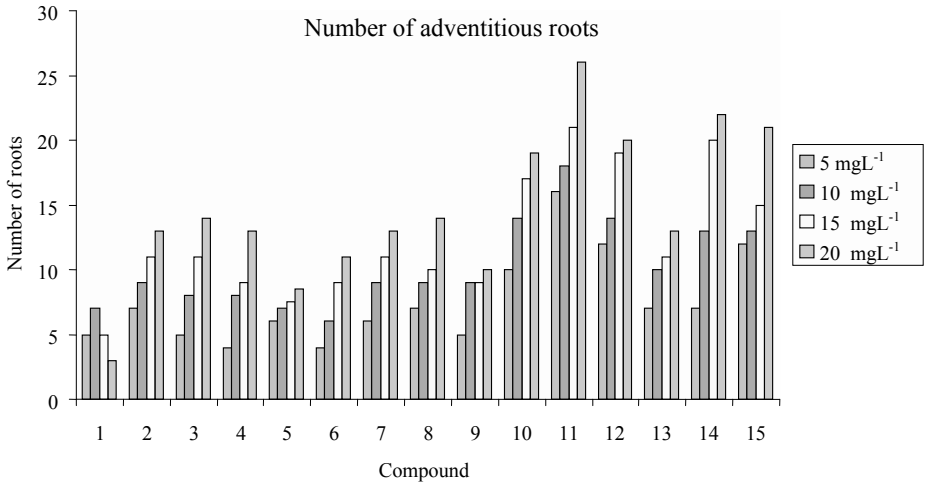


Figure 1 — Effect of different concentrations of the test compounds on initiation of adventious roots in *Vigna radiata*

NMR spectra which affirm these two pairs of compounds to be geometrical isomers. The isomers showed almost same carbon shifts except for C_{16}, which exhibited signals at δ 29.8 in compound **10** and at 20.0 in case of compound **12**. Similarly, for compounds **13** and **15**, signals for C_{16} appeared at δ 30.4 and 22.1 respectively, thereby showing the proximity of this carbon to the carbonyl oxygen.

All the compounds thus obtained were subjected to bioactivity analysis by root initiation studies in *Vigna radiata*. The testing was done at four concentrations (5, 10, 15 and 20 mg L^{-1}) with ten replicated at each concentration. As is seen from the figures, the derivatized compounds have been found to be more active to cause root initiation in stem cuttings of *Vigna radiata* as compared to their parent counterparts, and this activity has been found to increase with increase in concentration (**Figure 1**).

Among the tested compounds, compound **11** has been found to be most effective in promoting induction of adventitious roots. It is clear from the figure that the pyrolysed products **10-15** show significant enhancement in the growth of roots. Compounds **12** and **15** are more active in comparison to their geometrical isomers **10** and **13** respectively. But the difference is not very significant. Thus, it may be proposed that the mechanism of action is associated with the alkylating ability of the α-methylene-γ-lactones because they undergo Michael-type reaction with the biological nucleophiles such as L-cysteine or thiol containing enzymes. The increase in number of roots is significantly more at a higher concentration in case of compound **15** as compared to that in **12**.

Among the compounds **2**, **11** and **14**, the introduction of cyclopropyl ring has led to increase in activity and the effect is further ameliorated with the addition of a methyl group. This may be attributed to an increase in lipophilic character, which seems to be responsible for the growth promoting effects due to increased uptake of the bioactive compound by the plants. However, a decrease in the effectiveness of compound **14** in comparison to **11** can be correlated with the fact that a further increase in chain length has a retarding effect on the activity of these compounds. In case of compounds **7**, **8** and **9**, the increase in activity is visible but not to a significant extent. The possible reason for this may be the probable decomposition of the pyrazolines within the plant system to inactive compounds[12]. Among the epoxides **3**, **4** and **5**, the low activity may be attributed to the increase in polarity with the addition of oxygen atoms, which results in increase in hydrophilic character thus, leading to decrease in activity.

Conclusion

The root initiation studies reveal that the derivatization of **1** has ameliorated the biological activity. This enhancement has been found to correspond to the increase in length of the carbon chain at C_{13}.

Experimental Section

The melting points were determined in open capillaries on a Buchi B-545 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded with Varian EM 360 (300 MHz) and ^{13}C NMR with Bruker AC 300F (300MHz) spectrometers as

solutions (in $CDCl_3$) using TMS as internal reference and chemical shift values are expressed in δ units. Compounds were routinely checked for their purity on silica gel 60 F_{254} TLC plates and their spots were visualized by exposure to iodine vapours or by charring the plates with 5% H_2SO_4-MeOH reagent. Microwave induced reaction was carried out in a LG domestic oven MS-194W.

Reaction of 2 with perbenzoic acid. To the solution of **2** (1.2 g) in chloroform (10.0 mL), was added slowly an excess of solution of perbenzoic acid in chloroform. The reaction mixture was kept at 0°C for 24 hr and washed thoroughly with an aqueous solution of sodium bicarbonate till alkaline. It was then neutralized by washing with water. Further extraction with ether yielded a mixture which on chromatography afforded pure compounds **3**, **4** and **5** with m.p. 115°C, 122°C and 149°C respectively.

Double bond isomerization reaction. Compound **1** (2.0 g) was dissolved in minimum quantity of solvent. The solution was adsorbed on silica gel (60.0 g) and irradiated with microwaves at 800W power for 6 min., keeping a time lag of two minutes between the consecutive 1 min. irradiations. The silica gel was then cooled and eluted through a column with dichloromethane. The evaporation of the solvent yielded a mixture, which was chromatographed on silica gel to afford the pure isomerised product **6**.

Preparation and pyrolysis of pyrazolines of 1. The solution of **1** (2.5 g) in ether was treated with excess of diazomethane/diazoethane/diazopropane and allowed to stand still for 2 hr. The evaporation of the solvent yielded white crystalline compounds **7**/**8**/**9**. For pyrolysis, these pyrazolines (**7-9**, 2.0 g in each case) were heated at 110°C for 2 hr. The reaction mixture after pyrolysis was chromatographed over silica gel (60.0 g) to yield the pyrolyzed products **10-15**].

Root initiation studies

The root initiation studies were carried out on hypocotyls cuttings of *Vigna radiata* (var. ML_{613}). Seedlings were grown under continuous illumination at 27±2°C. After 7 days, when the hypocotyls were 5-6 cm long, cuttings were made by excision 4 cm below the cotyledonary node keeping the cotyledonary leaves and apex intact. Testing was done at four concentrations (5, 10, 15 and 20 mg L^{-1}) using water as control. For all compounds and each concentration, ten replicates were cultured in vials containing 20 mL test solution. The final observations were recorded on the 10^{th} day.

References

1 Abreu P M & Branco P S, *J Braz Chem Soc,* 14, **2003**, 289.
2 Douglas K A, *Molecules*, 5, **2000**, 285.
3 Cho J Y & Baik K U, *Europ J Pharmacol*, 398, **2000**, 399.
4 Cho J Y & Park J, *Planta Medica*, 64, **1998**, 594.
5 Yoo H D, Zeng L, Lee C M, Goering M G & Hu J F, *J Nat Prod*, 68, **2005**, 122.
6 Fronczek F R, Vargas D, Fischer N H & Hoastettman K, *J Nat Prod*, 47, **1984**, 1036.
7 Chhabra B R, Gupta S, Jain M & Kalsi P S, *Phytochem*, 49, **1998**, 801.
8 Kalsi P S, Kaur P & Chhabra B R, *Phytochem*, 18, **1979**, 1877.
9 Appendino G, Gariboldi P & Belliardo F, *Phytochem*, 25, 1986, 2163.
10 Kalsi P S, Kaur B, Singh B, Dhillon R S & Talwar K K, *Indian J Chem*, 23B, **1984**, 70.
11 Kalsi P S, Chhabra B R, Chhabra A & Wadia M S, *Tetrahedron*, 35, **1979**, 1993.
12 The study of the decomposition of pyrazolines in plant system is underway and will be reported elsewhere after completion of the work (B R Chhabra & R R Setia : unpublished).