

Synthesis and Biological Evaluation of Some New Pyrazoline Derivatives from Sesquiterpene Lactones

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Abstract: In order to obtain structure-biological activity data, the synthesis of 13 new pyrazoline derivatives from eudesmanolides and guaianolides was undertaken. With this view for further promoting plant growth activity, carbon chain at C-13 was extended by treating 13- methyl derivative of dehydrocostus lactone with diazomethane and diazoethane to form substituted pyrazolines. In order to introduce methyl group at C-16 instead of C-13, dehydrocostus lactone was allowed to react with an ethereal solution of diazoethane. In order to prepare more compounds for biological screening, the same sequence of reactions were performed on alantolactone, isoalantolactone and isodehydrocostus lactone which form substituted pyrazolines. The structures of all the compounds were elucidated by spectroscopic techniques like IR, ¹H NMR and ¹³C NMR spectra. All the 13 compounds so obtained were subjected for biological evaluation as plant growth regulators. The results were fairly good over the parent compounds.

Key words: Saussurea lappa · Inula racemosa · Compositae · sesquiterpene lactones · pyrazoline · diazomethane and diazoethane

INTRODUCTION

Sesquiterpene lactones with an α -methylene- γ -lactone moiety such as dehydrocostus lactone 1 (isolated from the roots of Saussurea lappa) and alantolactone 2, isoalantolactone 3 (isolated from the roots of Inula racemosa) respectively are emerging as a group of plant growth regulators. This has been one of the main topics of research in our laboratory [5, 10, 12]. Many bioassay oriented studies [8, 11] have shown that pyrazoline of sesquiterpene lactones and their pyrolysed products exhibit enhanced bioactivity over the parent compounds. Decomposition of pyrazolines to give olefins and cyclopropanes has been of interest from both synthetic and mechanistic points of view [13,15]. Pyrazolines are the addends obtained by the 1,3- dipolar addition of diazoalkanes to conjugated olefins. Several references had been reported in literature regarding the pyrazolines [1,2,3,16]. But no reports have appeared in the literature regarding the formation of new pyrazoline derivatives from sesquiterpene lactones with an α -methylene- γ -lactone moiety such as dehydrocostus lactone 1, alantolactone 2 and isoalantolactone 3 and it

was hoped that this investigation would help to clarify the structural specificity required for enhancement for the growth activity of the terpenoid lactones. During our work, sesquiterpene lactones upon treatment with diazomethane followed by pyrolysis led to the formation of 13-Methyl derivatives which upon further treatment with diazomethane and diazoethane yielded substituted pyrazolines at C-13. In order to carry out carbon chain elongation at C-16; pyrazolines of sesquiterpene lactones were 3 prepared by treatment with diazoethane. To explain the reaction further for double bond isomerisation ^{4,7} the same sequence of reactions were performed on isodehydrocostus lactone which form substituted pyrazolines as it has already been reported that pyrazoline of isodehydrocostus lactone is a potent plant growth regulator ⁹. The main aim of this work was the evaluation of biological activity of new pyrazoline derivatives obtained from dehydrocostus lactone, alantolactone, isoalantolactone and isodehydrocostus lactone to review their potential for exploitation as plant growth regulators in terms of adventitious root initiation in the hypocotyl cuttings of Vigna radiata. An attempt was made to relate our results to the results of previously cited pyrazolines [6].

MATERIALS AND METHODS

Experimental: MPs: Uncorr; IR: KBr pellets; ¹H and ¹³C NMR: 300 MHz and 75.45 MHz, respectively, CDCl₃, TMS as int. standard. All chromatographic separations were performed on silica gel.

Plant Material: Roots of *Saussurea lappa* and *Inula racemosa* were procured from the Lahaul and Spiti region of India and plant specimen no. 1067 was deposited in the herbarium of the Department of Botany, Punjab Agricultural University, Ludhiana.

General Procedure for the Isolation of Compounds 1, 4, 5 and 6: Powdered costus roots (1 Kg) were packed in a glass column and eluted with petrol (40-60°). The total extract was evaporated under red pres to yield a semi-solid golden yellow material (25g). This was dissolved in the minimum quantity of petrol (40-60°) followed by CC on silica gel (2.0 Kg) and elution of the column with solvents of increasing polarity: petrol-Et₂O (20:1) when dehydrocostus lactone (1, 15.0 g) mp 60° and costunolide (7.0 g, mp 106°) were obtained. A solution of dehydrocostus lactone in ether (1,5.0 g) in ether containing 2-3 drops of triethylamine was reacted with excess of diazomethane. After keeping overnight, the evaporation of the solvent afforded a crystalline compound (5.4g) which was purified by crystallization. This was identified as the pyrazoline 4 of dehydrocostus lactone on the basis of comparison of its mp, mmp and IR spectrum with those of an authentic sample (mp 92°). The pyrazoline derivative (4,5.4g) of dehydrocostus lactone was heated at 120° in the oven the evolution of N₂ gas 12 stopped (45 minutes). The completion of the reaction was checked by TLC. The product mixture (5.4 g) was subjected to chromatography over silica gel (200 g) when it afforded two compounds 5 (2.5 g, mp 74°) and 6 (2.6 g, mp 70°). Compound 5 was identified as 13-methyl dehydrocostus lactone and compound 6 was identified as 11-spirocyclopropyl derivative of dehydrocostus lactone by comparison of its IR, NMR, mp and mmp with that of an authentic sample.

General Procedure for the Reaction of 13-methyl Dehydrocostus Lactone 5 with Diazomethane: A solution of 13-methyl dehydrocostus lactone (5, 1.0 g) in ether was reacted with excess of diazomethane. After keeping overnight, the evaporation of the solvent afforded a mixture (1.1 g) of two components (TLC) which were separated by column chromatography. The compounds obtained were compound 7 identified as 13-methyl

pyrazoline, mp & mmp 155°C in which -N=N is α placed w.r.t C-6 proton and compound 8 identified as 13-methyl pyrazoline of dehydrocostus lactone, mp & mmp 99°C in which -N=N is β placed w.r.t C-6 proton.

General Procedure for the Reaction of 13-methyl Dehydrocostus Lactone 4 with Diazoethane: A solution of 13-methyl dehydrocostus lactone (5, 1.0 g) in ether was reacted with excess of diazoethane. After keeping overnight, the evaporation of the solvent afforded a crystalline compound (1.2 g) which was purified by crystallisation. The compound 13 obtained was 9 identified as 13-ethyl pyrazoline of dehydrocostus lactone, mp & mmp 110°C.

General Procedure for the Reaction of Dehydrocostus Lactone 1 with Diazoethane: Dehydrocostus lactone (1,2.0 g) in ether was reacted with excess of diazoethane in ether medium containing 2-3 drops of triethylamine. After keeping overnight, 50% reaction was completed. More diazoethane was added. After 24 hrs, white crystalline product (10,2.4g) was obtained with mp 87°C.

Isolation of Compounds 2 and 3: Powdered *Inula racemosa* roots (1 Kg) were packed in a glass column and eluted with petrol (40-60°). The total extract was evaporated under red pres to yield a semi- solid golden yellow material (25g). This was dissolved in the minimum quantity of petrol (40- 60°) followed by CC on silica gel (2.0 Kg) and elution of the column with solvents of increasing polarity: petrol-Et₂O (20:1) when alantolactone (2, 14.0 g) mp 78° and isoalantolactone (3, 8.0 g) mp 111° were obtained.

The Reaction of Alantolactone 2 with Diazomethane: A solution of alantolactone (2, 4.0 g) in ether containing 2-3 drops of triethylamine was added in excess of ethereal solution of CH₂N₂. It was kept overnight. After completion of reaction (TLC), the solvent was evaporated which afforded crystalline compound (11,4.4g) identified as pyrazoline mp 119°C of alantolactone.

Pyrolysis of Pyrazoline 11 of Alantolactone: The pyrazoline derivative (11, 4.0 g) of alantolactone was heated at 120° in the oven till the evolution of N₂ gas stopped (45 minutes). The completion of the reaction was checked by TLC. The product mixture (3.8 g) was subjected to column chromatography over silica gel (300 g) when it afforded two products (12, 1.6 g) mp 78°C identified as 13- methylalantolactone and (13, 1.8 g) mp 75°C identified as 11-spirocyclopropyl derivative of alantolactone.

The Reaction of 13-methyl Alantolactone 12 with Diazomethane: A solution of 13-methyl alantolactone (12,700 mg) in ether was reacted with excess of diazomethane and the procedure followed was same as described in 3.7 to afford compound (14, 800 mg) identified as 13-methyl pyrazoline of alantolactone, mp 158°C.

The Reaction of 13-methyl Alantolactone 12 with Diazoethane: A solution of 13-methyl alantolactone (12,600 mg) in ether containing 2-3 drops of triethylamine was added an ethereal solution of C₂H₅N₂. It was kept overnight. The solvent was evaporated after the completion of reaction (checked by TLC). The compound obtained was 15 (750 mg) identified as 13-ethyl pyrazoline of alantolactone, mp 167°C.

The Reaction of Alantolactone 2 with Diazoethane: Alantolactone (2,2.0 g) in ether was reacted with excess of diazoethane in ether medium containing 2-3 drops of triethylamine. After keeping overnight, 50% reaction was completed. More diazoethane was added. After 24 hrs, white crystalline product (16,2.4g) was obtained with mp 121°C.

The Reaction of Isoalantolactone 3 with Diazomethane: A solution of isoalantolactone (3, 3.0 g) in ether containing 2-3 drops of triethylamine was added in excess of ethereal solution of CH₂N₂. It was kept overnight. After completion of reaction (TLC), the solvent was evaporated which afforded crystalline compound (17, 3.4g) identified as pyrazoline of isoalantolactone, mp 165°C.

Pyrolysis of Pyrazoline 17 of Isoalantolactone: The pyrazoline derivative (17, 3.0 g) of isoalantolactone was heated at 120° in the oven till the evolution of N₂ gas stopped (45 minutes). The completion of the reaction was checked by TLC. The product mixture (2.8 g) was subjected to column chromatography over silica gel (300 g) when it afforded two products (18, 1.2 g) mp 151°C identified as 13- methylisoalantolactone and (19, 1.3 g) mp 111°C identified as 11-spirocyclopropyl derivative of isoalantolactone.

The Reaction of 13-methyl Isoalantolactone 18 with Diazomethane: A solution of 13-methyl Isoalantolactone (18, 500mg) in ether was reacted with excess of diazomethane and the procedure followed was same as described in 3.9.3 to afford compound (20, 600 mg) identified as 13-methyl pyrazoline of isoalantolactone, mp 171°C.

The Reaction of 13-methyl Isoalantolactone 11 with Diazoethane: A solution of 13-methyl Isoalantolactone (11,550mg) in ether was reacted with excess of C₂H₅N₂ and the procedure followed was same as described in 3.9.1. The compound obtained was 21(700 mg) identified as 13-ethyl pyrazoline of isoalantolactone, mp 173°C.

The Reaction of Isoalantolactone 3 with Diazoethane: Isoalantolactone (3,2.0 g) in ether was reacted with excess of diazoethane in ether medium containing 2-3 drops of triethylamine. After keeping overnight, 50% reaction was completed. More diazoethane was added. After 24 hrs, white crystalline product (22,2.4g) was obtained with mp 109°C.

The Reaction of Dehydrocostus Lactone with Iodine/ benzene: To the solution of dehydrocostus lactone (1,3.0 g) in benzene (25 ml) was added iodine (catalytic) and refluxed for 12 hr. The reaction mixture was diluted with water and extracted with ether. Last traces of iodine from organic layer were removed by washing thoroughly with sodium thiosulphate and finally drying over sodium sulphate. Evaporation of the solvent afforded a thick brown liquid compound (23, 2.8 g) identified as isodehydrocostus lactone by comparison of its IR, ¹H NMR with those of an authentic sample.

The Reaction of Isodehydrocostus Lactone 23 with Diazomethane: A solution of isodehydrocostus lactone (23, 1.8 g) in ether was reacted with excess of diazomethane and the procedure followed was same as described in 3.9.3 to afford compound (24, 2.2 g) identified as pyrazoline of isodehydrocostus lactone mp 110°C.

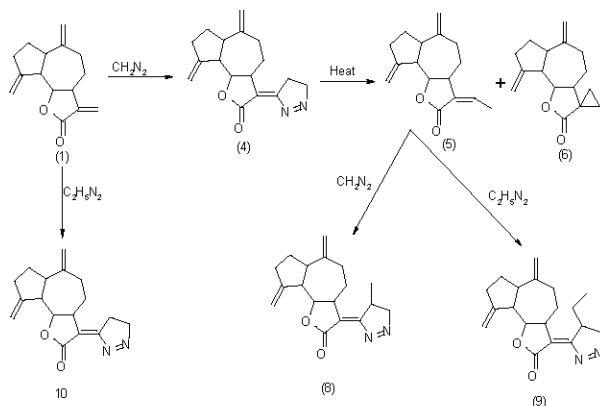
Pyrolysis of Pyrazoline 24 of Isodehydrocostus Lactone: The pyrazoline derivative (24, 2.2 g) of isodehydrocostus lactone was heated at 120° in the oven till the evolution of N₂ gas stopped (45 minutes). The completion of the reaction was checked by TLC. The product mixture (2.0 g) was subjected to column chromatography over silica gel (300 g) when it afforded two products (25, 900 mg) mp 81°C identified as 13- methylisodehydrocostus lactone and (26, 950 mg) mp 80°C identified as 11-spirocyclopropyl derivative of isodehydrocostus lactone.

The Reaction of 13-methylisodehydrocostus Lactone 25 with Diazomethane: A solution of 13-methylisodehydrocostus lactone (25, 450 mg) in ether containing 2-3 drops of triethylamine was reacted with an excess of diazomethane. The completion of the reaction

was checked by TLC. The evaporation of solvent afforded a crystalline compound (27, 550 mg) which was purified by crystallization. This was identified as 13-methyl pyrazoline of isodehydrocostus lactone, mp 151°C.

The Reaction of 13-methylisodehydrocostus Lactone 25 with Diazoethane: A solution of 13-methylisodehydrocostus lactone (25, 450 mg) in ether containing 2-3 drops of triethylamine was reacted with an excess of diazoethane. The completion of the reaction was checked by TLC. The evaporation of solvent afforded a crystalline compound (28, 600 mg) which was purified by crystallization. This was identified as 13-ethyl pyrazoline of isodehydrocostus lactone, mp 154°C.

The Reaction of Isodehydrocostus Lactone 23 with Diazoethane: A solution of isodehydrocostus lactone (23, 1.8 g) in ether containing 2-3 drops of triethylamine was reacted with an excess of diazoethane. After keeping overnight, 50% reaction was completed. More diazoethane was added. After 24 hrs, white crystalline product (29, 2.2 g) was obtained with mp 165°C which was identified to be C16-methyl pyrazoline of isodehydrocostus lactone.

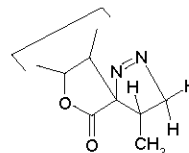


Scheme

RESULTS AND DISCUSSION

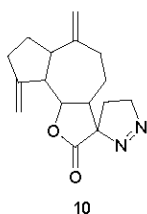
Sesquiterpene lactones having α -methylene- γ -lactone moiety are known to undergo 1,3 dipolar addition to diazomethane activated by electron attracting groups of which α -methylene- γ -lactone is a promising site. C16-guaianolide (5) was found to be much more active over the parent dehydrocostus lactone (1) to cause root formation in the hypocotyls cuttings of *Vigna radiata*. In order to learn the effect of placement of second methyl group at C-13 position on plant growth activity, the

compound (5) was reacted with diazomethane when it afforded two isomeric pyrazolines (7 & 8). The pyrazoline (7) mp 99°C, represents the unknown derivative in which $-N=N$ grouping of pyrazoline is cis placed w.r.t C-6 proton. This pyrazoline has C-13 isomeric relationship with pyrazoline (8) mp 155°C. The isomeric relationship between (7) & (8) could be proved from its ¹H NMR spectral features in which C-6 proton was considerably deshielded and appeared at 4.75 (hidden under the signals from 4.7-5.26) as compared with pyrazoline (8) in which C-6 proton appeared at δ 3.75. This is a sufficient proof that $-N=N$ is β -placed in compound (7) and it is α -placed in compound (8). The major pyrazoline (8) exhibited a secondary methyl doublet at δ 1.3 and each hydrogen of $-CH_2-N$ appeared as dd at 4.1 and 4.9 with coupling constant 10 and 18 Hz each. This might be due to vicinal coupling of $-N-CH_2$, a coupling constant of 18 Hz, which further splits by 10 Hz due to coupling with H present on carbon having the methyl in pyrazoline moiety.

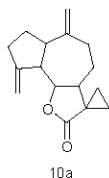


The same coupling of 10 Hz with both the hydrogens of $>CH_2$ is due to the fact that the dihedral angle of $>CH_2$ is almost bisected by the H of CH_3-C-H (almost fully staggered). This ¹H NMR is supported by ¹³C NMR which showed one methyl, five methines in both pyrazolines 7 & 8. Difference arises in chemical shift in which C-6 proton was deshielded and appeared at 80.00 as compared with pyrazoline (8) in which C-6 proton appeared at 75.00. Moreover, the deshielding of C13-H at 27.00 and C17-H at 18.70 in comparison with pyrazoline (8) in which signals of C13-H appeared at 25.34 and C17-H at 17.86. This proves that in pyrazoline (7) $-N=N$ grouping is cis placed w.r.t C-6 proton. In order to extend the carbon chain at C-13 position to relate structure with biological activity, 13-methyl dehydrocostus lactone (4) was treated with excess of ethereal solution of diazoethane to afford the pyrazoline (9) with mp 81°C. It showed IR (CHCl₃) bands: 1770 (γ -lactone), 1630 (C=C stretch), 1545 (N=N stretch), 1100 (C-N stretch), 850 (=C-H bend) cm⁻¹. Bands at 3080, 1600 and 850 cm⁻¹ are attributable to exocyclic double bonds whereas a medium intensity band at 1545 cm⁻¹ showed the presence of $-N=N$. The addition of diazoethane at Δ 11,13 position in 13-methyl dehydrocostus lactone was also confirmed by ¹H NMR signals supported by ¹³C NMR. It showed ¹H NMR

signals at δ 1.14(t, 3H, J=7.2 Hz, C₁₈-H), 3.78 (t, 1H, C6-H), 4.12 and 4.89 (dd, 1H each, J=10 & 18 Hz, C16-H's), 5.10 (bs, 2H, C14-H's), 5.25(bs, 2H, C15-H's). Lower chemical shift at δ 3.78 due to C6-H indicates that -N=N grouping is trans w.r.t. C6-H. Further evidence in favour of structure (9) is provided by ¹³C NMR having signals at δ 16.00 (C₁₈-q), 25.29(C₁₅-d), 25.47(C₁₇-t), 29.81(C₈-t), 30.20(C₉-t), 32.10(C₂-t), 35.89(C₃-t), 44.90(C₇-d), 51.00(C₅-d), 51.10(C₁-d), 77.00(C₆-d), 75.00(C₁₆-t), 100.50(C₁₁-s), 109.70(C₁₅-t), 110.50(C₁₄-t), 146.00(C₁₀-t), 150.40(C₄-s) and 170.89(C₁₂-s). All this confirms the structure (9) for this compound. In order to introduce the methyl group at C₁₆, dehydrocostus lactone (1) was treated with an excess of ethereal solution of diazoethane to afford pyrazoline (10) mp 87°C



¹H NMR showed all the spectral features for two exomethylene double bonds, a lactone moiety and a doublet for -CH₃ at δ 1.54. Taking into account the chemistry of the reaction, structure (10) may be suggested for the compound. The structure was further confirmed by ¹³C NMR which explained all the chemical shifts needed in this compound. The presence of -N=N- was evident by its decomposition pattern on heating which is a characteristic of such pyrazolines. In order to prove the stereochemistry of N=N at C-11 in pyrazoline (10), it was done by comparing this compound (10) with the structure of spirocyclopropyl derivative of dehydrocostus lactone (10a).



Looking into the structure of 10a, the methyl group at C₁₆ has to be trans w.r.t. the C-C bond between C₇-C₁₁. As this bond is β -oriented, therefore, methyl at C₁₆ has to be below the plane of cyclopropane ring¹⁴. Since this cyclopropane derivative is obtained from the decomposition of pyrazoline (10), having methyl trans to C₇-C₁₁ bond, hence it must also be trans in the original pyrazoline assuming biradical mechanism of decomposition. Similarly, pyrazoline of alantolactone (11)

and pyrazoline of isoalantolactone (17) on pyrolysis under thermal conditions gave the expected products (12, 13) and (18, 19). To explain this reaction further, the same sequence of reactions was performed on 13- methyl alantolactone (12) and 13-methyl isoalantolactone (18). The structures of pyrazoline adducts formed (14, 15, 16) and (20, 21, 22) were assigned by spectroscopic analysis (Table 1). Double bond migration is an important synthetic reaction in terpenoids. One such example is dehydrocostus lactone (1) which when refluxed with iodine for 12 hr gave a liquid compound (23). It has already been reported that diazomethane addend of isodehydrocostus lactone (24) is a potent plant growth regulator to cause adventitious root formation in the hypocotyl cuttings of *Vigna radiata*. Pyrazoline (24) decomposes to give 13-methyl isodehydrocostus lactone (25) and 11-spirocyclopropyl derivative (26). In order to prepare more compounds for biological screening, 13-methyl isodehydrocostus lactone (25) was treated with diazomethane to afford the pyrazoline (27), mp 151°C. Its ¹H NMR clearly confirms the stereostructure in (27) by displaying signals at δ 1.15(d, 3H, J=7 Hz, C17-H), 1.70 (bs, 3H, C15-H's), 3.90(t, 1H, J=8 Hz, C6-H), 5.10 (bs, 2H, C14-H's). The most interesting part is again of -CH₂- of pyrazoline which showed δ at 4.10 (dd, 1H, J=10 & 18 Hz) and 4.90 (dd, 1H, J=10 & 18 Hz). A comparison of chemical shift of C6-H in pyrazoline (27) that of the parent compound (23) was used to establish the stereochemistry of N=N at C-11. In isodehydrocostus lactone (23), C6-H appears as a triplet at δ 4.10 whereas the same signal in the corresponding substituted pyrazoline (27) is at δ 3.90 thereby suggesting a trans relationship between C6-H and -N=N. Further evidence in favour of structure (27) is provided by ¹³C NMR 10 having one methyl and six methines. Out of six methines, one (C-3) is vinylic carbon, giving doublet at δ 110.10 thereby suggesting here the endocyclic double bond at Δ 3,4 position, another methane (C-6) giving doublet at δ 75.00 again indicates that -N=N. To extend this idea of isomerisation, it was thought worthwhile to extend the carbon chain at C-13 position. So 13- methyl isodehydrocostus lactone (25) was treated with an an excess of ethereal solution of diazoethane to afford the pyrazoline (28) with mp 154°C. The compound (28) was identified as 13-ethyl pyrazoline of isodehydrocostus lactone by spectroscopic analysis (Table 1). In order to introduce the methyl at C-16, isodehydrocostus lactone (23) was treated with an ethereal solution of diazoethane to afford pyrazoline (29) with mp 165°C. ¹H NMR showed δ at 4.70 (m, 2H) due to C6-H and C16-H while in pyrazoline (29)

Table 1: Spectroscopic Data

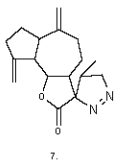
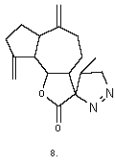
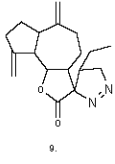
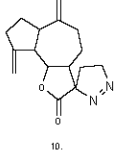
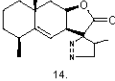
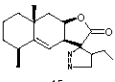
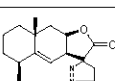
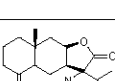
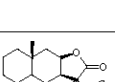
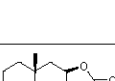
Compound	IR (cm ⁻¹)	¹ HNMR	¹³ CNMR
 7.	1770,1640, 1545,1230, 1000, 890	1.10(d, 3H, J=7Hz, C17 -H), 4.75(t, 1H, hidden under 4.1 5.26), 4.10 & 4.90 (dd, 1H each, J =10 &18 Hz,C16-H's), 5.10 (bs, 2H, C14- H's), 5.25 (bs, 2H, C15- H's)	18.70(C17-q),27.00(C13-d),29.80(C8-t),30.40(C9-t), 31.20 (C2-t), 36.44(C3-t),43.00(C7-d), 52.05 (C1-d), 52.49(C5-d), 80.00 (C6-d), 82.00 (C16-t), 100.30 (C11-s),109.66(C15-t),112.65(C14-t),147.00(C10-s), 150.03 (C4-s), 170.89(C12-s)
 8.	1760,1640, 1540,1230, 990, 900	1.30(d, 3H, J=7Hz, C17 -H), 3.75(t, 1H, J= 8Hz,C6-H), 4.10 &4.90 (dd, 1H each, J=10 &18 Hz,C16- H's), 5.10 (bs, 2H, C14-H's), 5.26 (bs, 2H, C15- H's)	17.86(C17-q),25.34(C13-d),29.98(C8-t),30.50(C9-t), 32.20 (C2-t), 36.00(C3-t), 45.00(C7-d), 51.05 (C1-d), 52.00(C5-d),75.00(C6-d), 81.50(C16-t),100.40(C11-s), 109.60(C15-t), 112.50(C14-t), 148.00(C10-s), 150.50 (C4-s),171.80 (C12-s)
 9.	3080,1770, 1630,1545, 1100,980&850	1.14(t, 3H, J=7.2Hz, C18 -H), 3.78 (t, 1H, C6-H), 4.12 & 4.89 (dd, 1H each, J=10 &18 Hz,C16-H's), 5.10 (bs, 2H, C14- H's), 5.25 (bs, 2H, C15- H's)	16.00(C18-q),25.29(C13-d),25.47(C17-t),29.81(C8-t), 30.20 (C9-t), 32.10(C2-t), 35.89(C3-t), 44.90(C7-d), 51.10(C1-d),51.00(C5-d),80.00(C6-d),81.49(C16-t), 100.50(C11-s), 109.70 (C15-t),110.50(C14-t),146.00 (C10-s), 150.40 (C4-s), 170.89 (C12-s)
 10.	3080,1770, 1600,1550, 1150 &850	1.54(d, 3H, J=7.29 Hz, C17 -H),4.77(m, 2H, C6-H & C16-H), 4.83 & 4.87 (bs, 1H each,C14-H's), 5.12 &5.30 (bs, 1H each, C15- H's)	19.35(C17-q), 25.84(C13-t), 30.09(C8-t),30.60(C9-t), 32.25(C2-t), 36.44(C3-t),47.26(C7-d), 52.05 (C1-d), 52.49(C5-d),84.72(C6-d),86.63(C16-d),100.57(C11-s), 109.66(C15-t), 112.65(C14-t), 149.00(C10-s), 151.03 (C4-s), 172.66(C12-s)
 14.	3085, 1750, 1630, 1540	1.15 (d, 3H, J= 7Hz,C17-H), 1.21 (d, 3H, J= 7.76 Hz, C15-H),1.25 (s, 3H, C14-H),4.10 & 4.90 (dd, 1Heach, J=10 & 18 Hz,C16-H's), 4.76 (m,1H, C8-H)	12.89(C17-q),16.80(C2-t),21.72(C15-q),27.65(C13-d), 28.51(C14-q), 32.70(C3-t), 38.00(C4-s),38.22 (C7-d), 41.78(C1-t), 41.90(C10-s), 42.60 (C9-t), 76.49(C8-d), 86.30 (C16-t), 102.10(C11-s),179.80(C6-d), 151.60 (C5-s),172.17(C12-s)
 15.	3070,1745, 1670, 1555	1.14 (t, 3H, J=7.2Hz,C18-H), 1.22 (d, 3H, J= 7.74 Hz, C15-H),1.27 (s, 3H, C14-H),4.10 & 4.90 (dd,1Heach, J=10 & 18Hz,C16-H's), 4.76 (m,1H, C8-H)	11.00(C18-q), 16.51(C2-t),17.12(C17-t),21.70(C15-q), 27.43(C13-d),28.43(C14-q),32.55(C3-t),38.01(C4-s), 38.20(C7-d),41.76(C1-t), 42.00(C10-s), 42.71 (C9-t), 76.40(C8-d),86.20(C16-t),102.15(C11-s),119.70(C6- d),151.50 (C5-s), 171.20(C12-s)
 16.	3080, 1740, 1610, 1550	1.23 (d, 3H, J= 7.76Hz, C15-H), 1.26 (s,3H, C14-H), 1.57(d,3H, N-CH-CH3, J=7.29 Hz), 4.76 (m,1H, C8-H), 5.51 (m,1H, C16-H)	13.27(C17-q),16.85(C2-t),22.64(C15-q),27.65(C13-t), 28.66(C14-q), 32.83(C3-t), 37.95(C4-s),38.20 (C7-d), 41.77(C1-t), 41.88(C10-s), 42.69 (C9-t), 76.47(C8-d), 86.66 (C16-d), 102.13(C11-s),119.83 (C6-d), 152.70 (C5-s), 173.27(C12-s)
 20.	3080,1770, 1685,1540, 1210 &880	1.10(s, 3H,C14-H),1.50 (d, 3H, C17-H,J=7.2 Hz), 4.50(m,1H,C8-H), 4.20&5.00 (dd, 1H each, J=10 & 18 Hz,C16- H's), 4.75 (bs, 1H each,C15-H's)	12.00(C17-q),17.50(C14-q),22.60(C2-t),26.10(C13-d), 27.90 (C6-t), 34.30(C3-t), 36.65 (C1-t), 41.00(C9-t), 41.20(C7-d), 42.00(C5-d),46.27(C10-s),78.00(C8-d), 86.01 (C16-t), 103.60(C11-s), 106.40 (C15-t), 149.01(C4-s),173.00(C12-s)
 21.	3080, 1765, 1675, 1,545, 1225 & 890	1.12(s, 3H,C14-H), 1.20(t, 3H, C18-H, J=7.29 Hz), 4.51(m,1H,C8-H), 4.10&4.90 (dd, 1Heach, J=10 &18 Hz,C16-H's), 4.76(bs, 1Heach, C15-H's)	11.09(C17-q),17.49(C14-q),22.61(C2-t),26.00(C13-d), 27.80(C6-t), 34.03(C3-t), 36.67 (C1-t),40.09(C9-t), 41.10(C7-d),42.01(C5-d),46.28(C10-s), 77.09(C8-d), 86.00 (C16-t), 103.50(C11-s),106.35 (C15-t), 149.02 (C4-s), 172.00(C12-s)
 22.	3085,1760, 1680,1550, 1200 &890	0.85(s, 3H, C14-H),1.55(d, 3H, J=7.29Hz, C17-H), 3.94(m,1H, C16-H), 4.52(m,1H, C8-H), 4.77(bs,1H each, C15-H's)	12.76 (C17-q),17.75(C14-q),22.69(C2-t),26.18(C13-t), 27.98(C6-t), 34.30(C3-t), 36.69 (C1-t),41.27(C9-t), 41.49(C7-d),42.23(C5-d),46.27(C10-s), 78.43(C8-d), 86.11 (C16-d), 103.64(C11-s),106.48 (C15-t), 149.18 (C4-s),173.38(C12-s)

Table 1: Continued

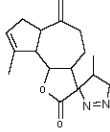
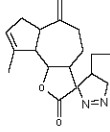
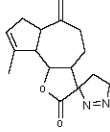
 27.	1770,1640, 1545,1230, 890 & 810	1.15(d, 3H, J=7 Hz,C17H),1.70(bris, 3HC15-H), 3.90(t, 1H, J=8 Hz, C6-H), 4.10 &4.90(dd,1H each, J=10 &18 Hz, C16-H's), 5.10(bs, 2H, C14-H's)	18.20(C17-q),25.00(C13-d),29.80(C8-t),30.40(C9-t), 31.20(C2-t), 43.00(C7-d), 52.05 (C1-d),52.49(C5-d), 75.00(C6-d), 82.00(C16-t), 100.40(C11-s), 109.60 (C15-q),110.10(C3-d),112.65(C14-t),147.00(C10-s), 151.03 (C4-s), 171.89(C12-s)
 28.	3080,1770, 1630,1545, 1100,980&900	1.14(t, 3H, J= 7.2 Hz,C18-H), 1.70(bs, 3H,C15-H), 4.15(t, 1H, J=8 Hz, C6-H), 4.10 &4.90(dd, 1H each, J=10&18 Hz, C16-H's),5.20(bs, 2H, C14-H's), 5.61(bris, 1H,C3-H)	16.00(C18-q), 25.29(C13-d), 25.47 (C17-t), 29.81 (C8-t), 30.20(C9-t), 32.10 (C2-t), 44.90(C7-d),51.00 (C5-d), 51.10(C1-d), 80.00(C6-d), 81.49 (C16-t), 100.50(C11-s), 108.89 (C15-q),109.00 (C3-d), 110.50 (C14 t),146.00(C10-s), 150.40 (C4-s), 170.89(C12-s)
 29.	3080,1770, 1600,1550, 1150 &850	1.54(d, 3H, J=7.20 Hz, C17-H), 1.70(bs, 3H, C15-H), 4.70(m, 2H, C6-H&C16-H), 5.20 (bs, 2H, C14- H's), 5.62(bris, 1H, C3-H)	19.35(C17-q),25.84(C13-t),30.09(C8-t),30.60(C9-t), 32.25 (C2-t),36.44(C3-t), 47.26(C7-d), 52.05 (C1-d), 100.57 (C11-s), 109.65(C15-q), 109.70(C3-d), 112.65(C14-t), 149.00(C10-s), 151.03 (C4-s), 172.50 (C12-s)

Table 2: Effect of 5, 10, 15, 20 mg/L concentrations of compounds on adventitious root formation in hypocotyl cuttings of Vigna radiata after 7 days.

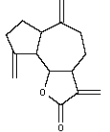
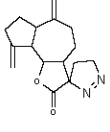
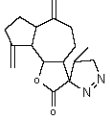
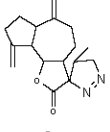
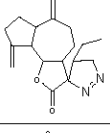
Compounds Number	Number of roots produced at concentrations(mg/L)			
	5	10	15	20
 1.	5.0±1.18	6.9±1.56	9.0±1.12	Toxic
 4.	9.3±2.1	10.2±1.9	11.7±1.65	7.5±3.2
 7.	8.0±1.0	9.4±1.98	10.1±1.3	8.4±0.3
 8.	10.50±1.56	11.50±1.9	12.4±1.51	9.0±1.7
 9.	9.0±2.0	10.0±1.8	11.5±1.7	7.0±3.1

Table 2: Continued

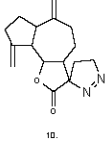
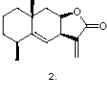
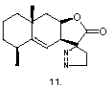
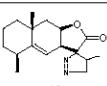
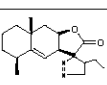
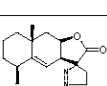
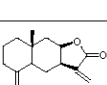
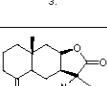
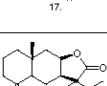
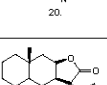
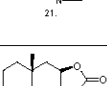
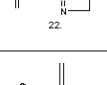
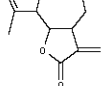
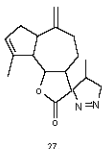
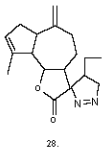
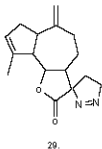
 10.	10.0±1.7	11.2±1.65	12.0±1.48	8.5±1.6
 2.	4.5±0.3	3.8±0.15	Toxic	Toxic
 11.	4.1±0.5	5.6±0.4	4.1±0.35	4.2±0.45
 14.	9.0±1.1	8.8±0.9	6.6±0.55	6.3±0.5
 15.	8.4±0.3	7.8±0.1	5.0±0.4	4.0±0.2
 16.	8.9±1.5	8.0±0.7	7.0±1.2	6.9±1.8
 3.	5.8±0.6	6.8±1.2	7.2±0.9	Toxic
 17.	6.0±0.5	7.6±0.4	9.1±0.35	10.2±0.45
 20.	9.1±2.0	10.0±1.75	11.3±1.6	8.0±3.4
 21.	7.9±0.5	9.1±0.4	10.0±0.35	9.2±0.45
 22.	8.9±1.9	10.1±1.75	11.0±1.4	7.5±3.1
 23.	6.0±1.22	7.9±2.11	9.5±2.32	10.50±1.56
 24.	10.5±1.20	12.4±1.51	17.12±1.47	9.4±1.98

Table 2: Continued

	11.5±1.9	13.5±1.6	18.12±2.0	11.5±1.7
27.				
	9.0±2.0	11.0±1.8	14.0±1.8	7.0±3.1
28.				
	11.0±1.8	13.0±1.7	17.9±1.9	10.7±1.4
29.				
IAA	8.1±0.72			

*Control Experiment, Water 4.6±0.5 (Mean±S.D.)

*P- Primordias

C6-H gives triplet at δ 3.90 (1 H, J=8 Hz) while C16-H's appeared as dd at 4.10 & 4.90 with coupling constant 10 & 18 Hz each. Similarly other signals are assigned on the basis of ^{13}C NMR data (Table 1).

Biological Testing: For the root initiation study on hypocotyl cuttings of *Vigna radiata*, seedlings were grown under continuous illumination. When the hypocotyls were 5-6 cm long, cuttings were made by excision 4 cm below the cotyledonary node leaving the cotyledonary leaves and apex intact. In all, four cones (5,10,15,20 mg/L) along with H₂O as control were tested. For all treatments 10 replicates were cultured in vials each containing 30 ml test soln. The final observations were recorded on day 8. The experiment was repeated ($\times 3$) at $27 \pm 2^\circ\text{C}$.

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