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Synthesis and Biological Study of Some New Derivatives of Sesquiterpene Lactones Isolated from Medicinal Plants

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Abstract: *To introduce a methyl group at C-16 instead of C-13, dehydrocostus lactone was allowed to react with an ethereal solution of diazoethane. To obtain structure-biological activity data, thirteen new pyrazoline derivatives from eudesmanolides and guaianolides were synthesised. The carbon chain at C-13 was extended by treating a 13-methyl derivative of dehydrocostus lactone with diazomethane and diazoethane to form substituted pyrazolines. The same sequence of reactions was performed on alantolactone, isoalantolactone and isodehydrocostus lactone, which formed substituted pyrazolines. The structure of each compound was elucidated by spectroscopic techniques including IR, ^{1}H NMR and ^{13}C NMR spectra. All thirteen generated compounds were subjected to biological evaluation as plant growth regulators. The results demonstrate an overall increase in effectiveness compared to the parent compounds.*

Keywords: *saussurea lappa*, *inula racemosa*, composite, sesquiterpene lactones, pyrazoline, diazomethane and diazoethane

1. INTRODUCTION

Sesquiterpene lactones with an α-methylene-γ-lactone moiety such as dehydrocostus lactone 1 (isolated from the roots of *Saussurea lappa*), alantolactone 2 and isoalantolactone 3 (isolated from the roots of *Inula racemosa*) are emerging as a group of plant growth regulators.[5,10,12] Many bioassay oriented studies[8,11] have shown that pyrazoline, of sesquiterpene lactones and their pyrolysed products, exhibits enhanced bioactivity over the parent compounds. The 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 from the 1,3-dipolar addition of diazoalkanes to conjugated olefins. Several references had been reported in literature regarding the pyrazolines.[1,2,3,16] However, no reports have demonstrated 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. The aim of this investigation is to help clarify the

structural specificity required for the enhancement of the growth activity of the terpenoid lactones.

In our study, the treatment of sesquiterpene lactones with diazomethane followed by pyrolysis resulted in the formation of 13-Methyl derivatives that, upon further treatment with diazomethane and diazoethane, yielded substituted pyrazolines at C-13. To carry out carbon chain elongation at C-16, pyrazolines of sesquiterpene lactones were prepared by treatment with diazoethane. To explain double-bond isomerisation,[4,7] the same reaction sequence was performed on isodehydrocostus lactone, which forms substituted pyrazolines. It has already been reported that pyrazolines from isodehydrocostus lactone are potent plant growth regulators.[9] The main aim of this work was to evaluate the biological activity of new pyrazoline derivatives obtained from dehydrocostus lactone, alantolactone, isoalantolactone and isodehydrocostus lactone; and 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 reported pyrazolines.[6]

2. MATERIALS AND METHOD

Experimental:
MPs: Uncorr.; IR: KBr pellets; 1H and ^{13}C NMR: 300 MHz and 75.45 MHz, respectively, $CDCl_3$, TMS as international standard. All chromatographic separations were performed on a 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, Jamia Hamdard University, New Delhi.

2.1 General Procedure for the Isolation of Compounds 1, 4, 5 and 6

1 kg of powdered costus roots was packed into a glass column and eluted with petrol at 40–60°C. The total extract was evaporated under red pres to yield 25 g of semi-solid, golden yellow material. This was dissolved in the minimum quantity of petrol at 40–60°C and was then applied on a 2.0 kg silica gel and an elution of the column with solvents of increasing polarity. We then obtained 15.0 g of dehydrocostus lactone (compound 1, mp 60°C) and 7.0 g of costunolide (mp 106°C). 5.0 g solution of dehydrocostus lactone in ether (compound 1) containing 2–3 drops of triethylamine was reacted with excess diazomethane.

After keeping overnight, the evaporation of the solvent resulted in 5.4 g of crystalline compound, which was then purified by crystallisation. This was identified as pyrazoline 4 of dehydrocostus lactone by comparison of its melting point and infrared spectrum with those of an authentic sample (mp 92°C). 5.4 g of pyrazoline derivative (compound 4) of dehydrocostus lactone was heated to 120°C in the oven for 45 min until the evolution of N_2 gas stopped. The completion of the reaction was checked by TLC (thin layer chromatography). The product mixture weighing 5.4 g was subjected to chromatography over 200 g of silica gel, which subsequently produced 2.5 g of compound 5 (mp 74°C) and 2.6 g of compound 6 (mp 70°C). Compound 5 was identified as 13-methyl dehydrocostus lactone, and compound 6 was identified as an 11-spirocyclopropyl derivative of dehydrocostus lactone by comparison of its IR (infrared), NMR (nuclear magnetic resonance) and of the melting point with an authentic sample.

2.2 General Procedure for the Reaction of 13-Methyl Dehydrocostus Lactone 5 with Diazomethane

1.0 g solution of 13-methyl dehydrocostus lactone (compound 5) in ether was reacted with excess diazomethane. After keeping overnight, the evaporation of the solvent resulted in a 1.1 g mixture of two components (verified by TLC) that were separated by column chromatography. The compounds obtained were compound 7, identified as 13-methyl pyrazoline, mp 155°C in which -N=N is α placed with respect to C-6 proton, and compound 8, identified as 13-methyl pyrazoline of dehydrocostus lactone, mp 99°C in which -N=N is β placed with respect to C-6 proton.

2.3 General Procedure for the Reaction of 13-Methyl Dehydrocostus Lactone 4 with Diazoethane

1.0 g solution of 13-methyl dehydrocostus lactone (compound 5) in ether was reacted with excess diazoethane. After keeping overnight, the evaporation of the solvent resulted in 1.2 g of crystalline compound which was purified by crystallisation. The resulting compound, compound 9, was identified as 13-ethyl pyrazoline of dehydrocostus lactone, mp and mmp 110°C.

2.4 General Procedure for the Reaction of Dehydrocostus Lactone 1 with Diazoethane

2.0 g of dehydrocostus lactone (compound 1) in ether was reacted with excess diazoethane in ether medium containing 2–3 drops of triethylamine. After keeping overnight, 50% of the reaction was complete. More diazoethane was added. After 24 h, 2.4 g of white crystalline product (compound 10) was obtained with mp 87°C.

2.5 Isolation of Compounds 2 and 3

1 kg of powdered *Inula racemosa* roots was packed into a glass column and eluted with petrol at 40–60°C. The total extract was evaporated under red pres to yield 25 g of semi-solid, golden yellow material. This material was then dissolved in the minimum quantity of petrol at 40–60°C, and was then applied on 2.0 kg of silica gel and an elution of the column with solvents of increasing polarity. 14 g of alantolactone (compound 2), mp 78°C and 8.0 g of isoalantolactone (compound 3), mp 111°C were then obtained.

2.6 The Reaction of Alantolactone 2 with Diazomethane

4.0 g solution of alantolactone (compound 2) in ether, containing 2–3 drops of triethylamine, was added in the excess ether solution of CH_2N_2. It was kept overnight. After the completion of the reaction (verified by TLC), the solvent was then evaporated, which resulted in 4.4 g of crystalline compound (compound 11) identified as pyrazoline, mp 119°C, of alantolactone.

2.7 Pyrolysis of Pyrazoline 11 from Alantolactone

4.0 g of pyrazoline derivative (compound 11) of alantolactone was heated to 120°C in the oven for 45 min until the evolution of N_2 gas stopped. The completion of the reaction was checked by TLC. The product mixture of 3.8 g was subjected to column chromatography over 300 g of silica gel, producing two products. The first was 1.6 g of 13-methyl alontolactone (compound 12, mp 78°C) and the second was 1.8 g of 11-spirocyclopropyl derivative of alantolactone (compound 13, mp 75°C).

2.8 The Reaction of 13-Methyl Alantolactone 12 with Diazomethane

700 mg solution of 13-methyl alantolactone (compound 12) in ether was reacted with excess diazomethane and the same procedure as in 2.7 was followed to produce 800 mg of compound 14, identified as 13-methyl pyrazoline of alantolactone, mp 158°C.

2.9 The Reaction of 13-Methyl Alantolactone 12 with Diazoethane

600 mg solution of 13-methyl alantolactone (compound 12) in ether containing 2–3 drops of triethylamine was added to an ethereal solution of $C_2H_5N_2$. It was kept overnight. The solvent was evaporated after completion of the reaction (checked by TLC). The compound obtained, weighing 750 mg (compound 15) was identified as 13-ethyl pyrazoline of alantolactone, mp 167°C.

2.10 The Reaction of Alantolactone 2 with Diazoethane

2.0 g of alantolactone (compound 2) in ether was reacted with the excess diazoethane in ether medium containing 2–3 drops of triethylamine. After being kept overnight, 50% of the reaction was complete. More diazoethane was added. After 24 h, 2.4 g of white crystalline product (compound 16) was obtained with mp 121°C.

2.11 The Reaction of Isoalantolactone 3 with Diazomethane

3.0 g solution of isoalantolactone (compound 3) in ether containing 2–3 drops of triethylamine was added in to an excess ethereal solution of CH_2N_2, which was then kept overnight. After completing the reaction (verified by TLC), the solvent was evaporated, which resulted in 3.4 g of crystalline compound (compound 17), identified as pyrazoline of isoalantolactone, mp 165°C.

2.12 Pyrolysis of Pyrazoline 17 of Isoalantolactone

3.0 g of pyazoline derivative (compound 17) of isoalantolactone was heated to 120°C in an oven for 45 min until the evolution of N_2 gas had stopped. Completion of the reaction was checked by TLC. The product mixture of 2.8 g was subjected to column chromatography over 300 g of silica gel, producing two products. The first product was 1.2 g of 13-methyl isoalantolactone (compound 18, mp 151°C), and the second was 1.3 g of 11-spirocyclopropyl derivative of isoalantolactone (compound 19, mp 111°C).

2.13 The Reaction of 13-Methyl Isoalantolactone 18 with Diazomethane

500 mg solution of 13-methyl isoalantolactone (compound 18) in ether was reacted with excess diazomethane and the same procedure as in 2.9 was followed to produce 600 mg compound identified as 13-methyl pyrazoline of isoalantolactone, mp 171°C (compound 20).

2.14 The Reaction of 13-Methyl Isoalantolactone 11 with Diazoethane

550 mg solution of 13-methyl isoalantolactone (compound 11) in ether was reacted with an excess of $C_2H_5N_2$, and the same procedure as in 2.9 was followed. The compound obtained, weighing 700 mg (compound 21) was identified as 13-ethyl pyrazoline of isoalantolactone, mp 173°C.

2.15 The Reaction of Isoalantolactone 3 with Diazoethane

2.0 g of isoalantolactone (compound 3) in ether was reacted with excess diazoethane in ether medium containing 2–3 drops of triethylamine. After being kept overnight, 50% of the reaction was complete. More diazoethane was then added, and after 24 h, 2.4 g of white crystalline product (compound 22) was obtained with mp 109°C.

2.16 The Reaction of Dehydrocostus Lactone with Iodine/Benzene

To 3.0 g solution of dehydrocostus lactone (compound 1) in benzene (25 ml), iodine was added (catalytic) and refluxed for 12 h. The reaction mixture was diluted with water and extracted with ether. The last traces of iodine from the organic layer were removed by washing thoroughly with sodium thiosulphate and finally dried over sodium sulphate. Evaporating the solvent resulted in 2.8 g thick brown liquid compound (compound 23) identified as isodehydrocostus lactone by comparision of its IR and ^{1}H NMR with an authentic sample.

2.17 The Reaction of Isodehydrocostus Lactone 23 with Diazomethane

1.8 g solution of isodehydrocostus lactone (compound 23) in ether was reacted with excess diazomethane and the same procedure as in 2.9 was followed to produce 2.2 g compound (compound 24) identified as pyrazoline of isodehydrocostus lactone with mp 110°C.

2.18 Pyrolysis of Pyrazoline 24 of Isodehydrocostus Lactone

2.2 g of pyrazoline derivative (compound 24) of isodehydrocostus lactone was heated to 120°C in an oven for 45 min until the evolution of N_2 gas stopped. The completion of the reaction was checked by TLC. The product mixture of 2.0 g was subjected to column chromatography over 300 g of silica gel, producing two compounds. The first compound was a 900 mg of 13-methyl isodehydrocostus (compound 25, mp 81°C), and the second compound was 11-spirocyclopropyl derivative of isodehydrocostus lactone (compound 26, mp 80°C).

2.19 The Reaction of 13-Methyl Isodehydrocostus Lactone 25 with Diazomethane

450 mg solution of 13-methyl isodehydrocostus lactone (compound 25) in ether containing 2–3 drops of triethylamine was reacted with excess diazomethane. The completion of the reaction was verified by TLC. The evaporation of solvent produced 550 mg of crystalline compound (compound

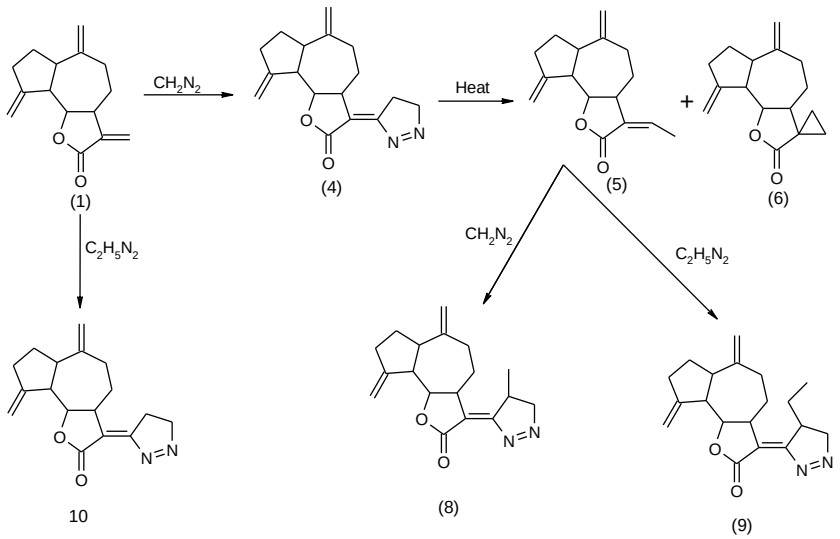
27), which was purified by crystallisation. This was identified as 13-methyl pyrazoline of isodehydrocostus lactone with mp 151°C.

2.20 The Reaction of 13-Methyl Isodehydrocostus Lactone 25 with Diazoethane

450 mg solution of 13-methyl isodehydrocostus lactone (compound 25) in ether containing 2–3 drops of triethylamine was reacted with excess diazoethane. The completion of the reaction was verified by TLC. The evaporation of solvent produced 600 mg of crystalline compound (compound 28), which was purified by crystallisation. This was identified as 13-ethyl pyrazoline of isodehydrocostus lactone with mp 154°C.

2.21 The Reaction of Isodehydrocostus Lactone 23 with Diazoethane

1.8 g solution of isodehydrocostus lactone (compound 23) in ether containing 2–3 drops of triethylamine was reacted with an excess of diazoethane. After keeping overnight, 50% of the reaction was complete. More diazoethane was added. After 24 h, 2.2 g of white crystalline product (compound 29) was obtained with mp 165°C, which was identified to be C16-methyl pyrazoline of isodehydrocostus lactone.



Scheme-1

3. RESULTS AND DISCUSSION

Sesquiterpene lactones having an 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 (compound 5) was found to be much more active over the parent dehydrocostus lactone (compound 1) to promote root formation in the hypocotyl cuttings of *Vigna radiata*.

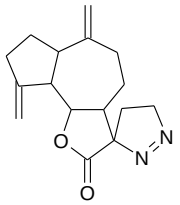
To determine the effect of the second methyl group at the C-13 position on plant growth activity, compound 5 was reacted with diazomethane and produced two isomeric pyrazolines (compound 7 and 8). Pyrazoline 7, with mp 99°C, represents the unknown derivative in which -N=N grouping of pyrazoline is cis-placed with respect to C-6 proton. This pyrazoline has a C-13 isomeric relationship with pyrazoline 8 (mp 155°C). The isomeric relationship between compounds 7 and 8 could be shown by ^{1}H NMR spectral features in which the C-6 proton was considerably deshielded and appeared at 4.75 (hidden under the signals from 4.7–5.26) compared to pyrazoline 8 in which the C-6 proton appeared at δ3.75. This is sufficient proof that -N=N is β-placed in compound 7, which is then α-placed in compound 8.

The major pyrazoline (compound 8) exhibited a secondary methyl doublet at δ1.3, and each hydrogen of -CH_2-N appeared as dd at 4.1 and 4.9 with a coupling constant of 10 and 18 Hz, respectively. 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 the 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 ^{1}H NMR is supported by ^{13}C NMR which showed one methyl and five methines in both pyrazolines 7 and 8.

A difference arose in the chemical shift in which a C-6 proton was deshielded and appeared at 80.00, compared to pyrazoline 8 in which a C-6 proton appeared at 75.00. Moreover, the deshielding of C13-H at 27.00 and C17-H at 18.70 compared to pyrazoline 8 in which signals of C13-H appeared at 25.34, and C17-H appeared at 17.86. This proves that in pyrazoline 7, -N=N grouping is cis-placed with respect to C-6 proton. To extend the carbon chain at the C-13 position to relate structure with biological activity, 13-methyl dehydrocostus lactone (compound 4) was treated with excess ethereal solution of diazoethane, which produced the pyrazoline (compound 9) with mp 81°C. It showed the following IR ($CHCl_3$) bands: 1770 (γ-lactone), 1630 (C=C stretch), 1545 (N=N stretch), 1100 (C-N stretch), and 850 (=C-H bend) cm^{-1}. Bands at 3080, 1600 and 850 cm^{-1} are attributable to exocyclic double bonds, whereas a

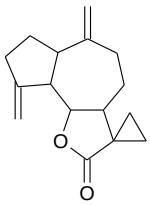
medium intensity band at 1545 cm^{-1} showed the presence of -N=N. The addition of diazoethane at Δ11, the 13 position in 13-methyl dehydrocostus lactone was also confirmed by ^{1}H NMR signals supported by ^{13}C NMR. It showed ^{1}H NMR signals at δ1.14 (t, 3H, J=7.2 Hz, C18-H), 3.78 (t, 1H, C6-H), 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).

A lower chemical shift at δ3.78 due to C6-H indicates that -N=N grouping is trans with respect to C6-H. Further evidence in favour of structure 9 is provided by ^{13}C NMR, having signals at δ16.00 (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), 51.00 (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 of these confirm the structure (compound 9) for this compound. To introduce the methyl group at C16, dehydrocostus lactone (compound 1) was treated with an excess of an ether solution of diazoethane to produce pyrazoline (compound 10) with mp 87ºC.



10

^{1}H NMR showed all of the spectral features for two exomethylenic double bonds, a lactone moiety and a doublet for -CH_3 at δ1.54. Taking into account the chemistry of the reaction, structure 10 may be suggested for the compound. The structure was further confirmed by ^{13}C NMR, which explained all of the chemical shifts needed in this compound. The presence of -N=N was evident by its decomposition pattern upon being heated, which is characteristic of such pyrazolines. To prove the stereochemistry of N=N at C-11 in pyrazoline, compound 10 was compared to the structure of spirocyclopropyl derivative of dehydrocostus lactone (10a).



10a

Looking into the structure of 10a, the methyl group at C_{16} has to be trans with respect to the C-C bond between C_7-C_{11}. As this bond is β-oriented, the methyl group at C_{16} has to be below the plane of the cyclopropane ring.[14] Because this cyclopropane derivative is obtained from the decomposition of pyrazoline (compound 10), having methyl trans to the C_7-C_{11} bond, it must also be trans in the original pyrazoline, assuming a biradical mechanism of decomposition. Similarly, pyrazoline of alantolactone (compound 11) and pyrazoline of isoalantolactone (compound 17) on pyrolysis under thermal conditions gave the expected products, which are compounds 12, 13 and 18, 19, respectively.

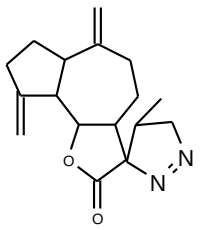
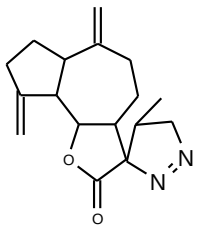
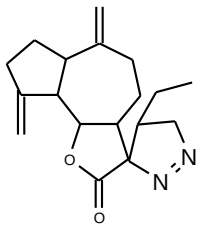
To explain this reaction further, the same sequence of reactions was performed on 13-methyl alantolactone (compound 12) and 13-methyl isoalantolactone (compound 18). The structures of pyrazoline adducts formed (compound 14, 15, and 16) as well as 20, 21 and 22, and were assigned by spectroscopic analysis (Table 1). Double-bond migration is an important synthetic reaction in terpenoids. One such example is dehydrocostus lactone (compound 1) that when refluxed with iodine for 12 h, gave a liquid compound (compound 23). It has already been reported that the diazomethane addend of isodehydrocostus lactone (compound 24) is a potent plant growth regulator that promotes adventitious root formation in the hypocotyl cuttings of *Vigna radiata*. Pyrazoline (compound 24) decomposes to give 13-methyl isodehydrocostus lactone (compound 25) and an 11-spirocyclopropyl derivative (compound 26). To prepare more compounds for biological screening, 13-methyl isodehydrocostus lactone (compound 25) was treated with diazomethane to produce pyrazoline (compound 27) with mp 151°C. Its ^{1}H NMR clearly confirms the stereostructure (compound 27) by displaying signals at δ1.15 (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).

Again, the most interesting part is that the -CH2- of the pyrazoline showed the δ at 4.10 (dd, 1H, J=10 & 18 Hz) and 4.90 (dd, 1H, J=10 & 18 Hz). A comparison of the chemical shift of C6-H in pyrazoline (compound 27) to that of the parent compound (compound 23) was used to establish the stereochemistry of N=N at C-11. In isodehydrocostus lactone (compound 23), C6-H appears as a triplet at δ4.10, whereas the same signal in the corresponding substituted pyrazoline (compound 27) is at δ3.90, thereby suggesting a trans relationship between C6-H and -N=N. Further evidence in favour of structure 27 is provided by ^{13}C NMR 10, having one methyl and six methines. Out of six methines, one (C-3) is a vinylic carbon, giving a doublet at δ110.10, thereby suggesting the endocyclic double bond at the Δ3,4 position. Another methane (C-6) giving a doublet at δ75.00 again indicates that -N=N. To illustrate this idea of isomerisation, the carbon chain at the C-13 position was extended. So 13-methyl isodehydrocostus lactone (compound 25) was treated with excess ethereal solution of diazoethane to produce the pyrazoline (compound 28) with mp 154°C.

The compound 28 was identified as 13-ethyl pyrazoline of isodehydrocostus lactone by spectroscopic analysis (Table 1).

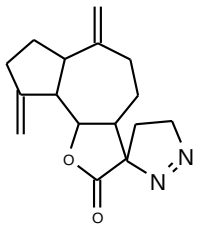
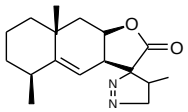
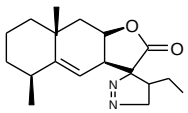
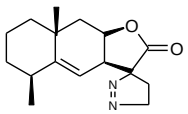
To introduce the methyl at C-16, isodehydrocostus lactone (compound 23) was treated with an ether solution of diazoethane to produce pyrazoline (compound 29) with mp 165°C. ^{1}H NMR showed a δ at 4.70 (m, 2H) due to C6-H and C16-H, whereas pyrazoline (compound 29) C6-H gives a triplet at δ 3.90 (1 H, J=8 Hz), and C16-H's appeared as dd at 4.10 and 4.90 with a coupling constant of 10 and 18 Hz, respectively. Similarly, other signals are assigned on the basis of 13C NMR data (Table 1).

Table 1: Spectroscopic Data.

Compound	IR (cm^{-1})	^{1}HNMR	^{13}CNMR
 7.	1770, 1640, 1545, 1230, 1000, 890	1.10 (d, 3H, J=7 Hz, 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=7 Hz, C17-H), 3.75 (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), 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=7.2 Hz, 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)

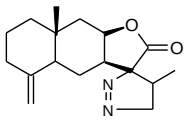
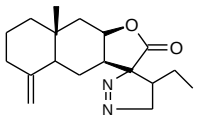
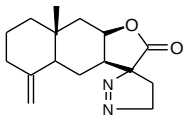
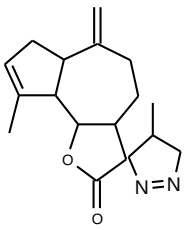
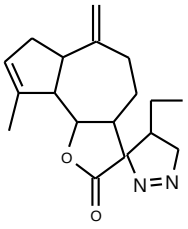
(*continued*)

Table 1: (*continued*)

Compound	IR (cm^{-1})	^{1}HNMR	^{13}CNMR
 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=7 Hz, C17-H), 1.21 (d, 3H, J=7.76 Hz, C15-H), 1.25 (s, 3H, C14-H), 4.10 & 4.90 (dd, 1H each, 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 (C11s), 179.80 (C6-d), 151.60 (C5-s), 172.17 (C12-s)
 15.	3070, 1745, 1670, 1555	1.14 (t, 3H, J=7.2 Hz, C18-H), 1.22 (d, 3H, J=7.74 Hz, C15-H), 1.27 (s, 3H, C14-H), 4.10 & 4.90 (dd, 1H each, J=10 & 18 Hz, 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= 7.76 Hz, 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)

(*continued*)

Table 1: (*continued*)

Compound	IR (cm^{-1})	^{1}HNMR	^{13}CNMR
 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, 1H each, J=10 &18 Hz, C16-H's), 4.76 (bs, 1H each, 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=7.29 Hz, 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)
 27.	1770, 1640, 1545, 1230, 890 & 810	1.15 (d, 3H, J=7 Hz, C17H), 1.70 (brs, 3H, C15-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 (brs, 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)

(*continued*)

Table 1: (*continued*)

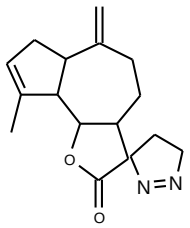
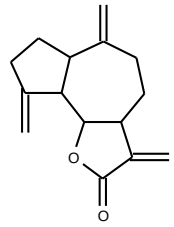
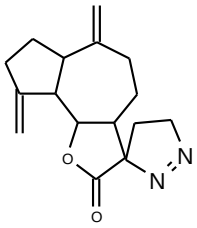
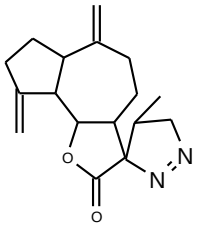
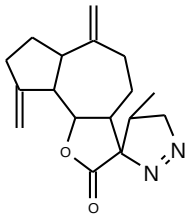
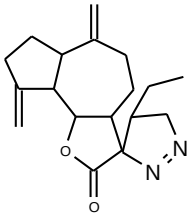
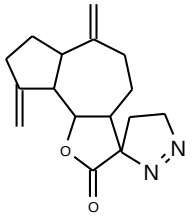
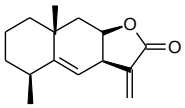
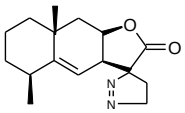
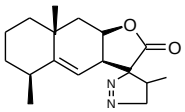
Compound	IR (cm^{-1})	^{1}HNMR	^{13}CNMR
 29.	3080, 1770, 1600, 1550, 1150 & 850	1.54 (d, 3H, J=7.20 Hz, C17-H), 1.70 (bs, 3H, C15-H), 4.70 (m, 2H, C6-H & C16-H), 5.20 (bs, 2H, C14-H's), 5.62 (brs, 1H, C3-H)	19.35 (C17-q), 25.84 (C13-t), 30.09 (C8-t), 30.60 (C9-t), 32.25 (C2-t), 36.44 (C3-t), 47.26 (C7-d), 52.05 (C1-d), 100.57 (C11-s), 109.65 (C15-q), 109.70 (C3-d), 12.65 (C14-t), 149.00 (C10-s), 151.03 (C4-s), 172.50 (C12-s)

Table 2: Effect of 5, 10, 15, and 20 mg/L of each compound on adventitious root formation in hypocotyl cuttings of Vigna radiata after seven days.

Compound Number	Number of roots produced for each concentration (mg/L)			
	5	10	15	20
 1.	5.2 ± 1.18	5.8 ± 1.56	7.0 ± 1.12	Toxic
 4.	8.3 ± 2.1	10.2 ± 1.7	11.7 ± 1.65	7.4 ± 3.2
 7.	8.1 ± 1.0	9.2 ± 1.18	10.1 ± 1.5	8.5 ± 0.4

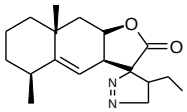
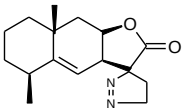
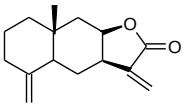
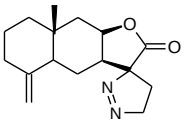
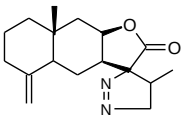
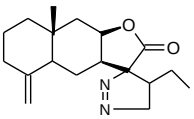
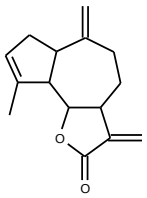
(*continued*)

Table 2: (*continued*)

Compound Number	Number of roots produced for each concentration (mg/L)			
	5	10	15	20
 8.	10.5 ± 1.56	12.5 ± 1.9	13.4 ± 1.41	9.0 ± 1.7
 9.	9.0 ± 2.0	10.0 ± 1.8	11.5 ± 1.7	7.0 ± 3.1
 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

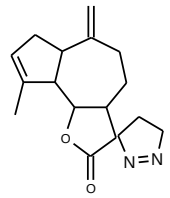
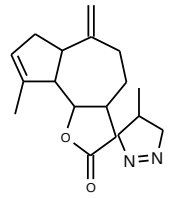
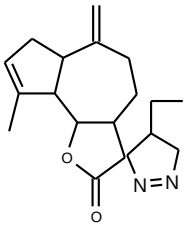
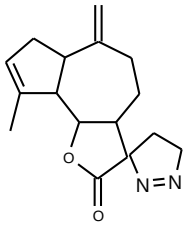
(*continued*)

Table 2: (*continued*)

Compound Number	Number of roots produced for each concentration (mg/L)			
	5	10	15	20
 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.	7.8 ± 0.5	9.1 ± 0.4	10.0 ± 0.25	9.2 ± 0.45
 23.	6.0 ± 1.22	7.9 ± 2.11	9.5 ± 2.32	10.50 ± 1.56

(*continued*)

Table 2: (*continued*)

Compound Number	Number of roots produced for each concentration (mg/L)			
	5	10	15	20
 24.	10.4 ± 1.20	11.4 ± 1.51	16.12 ± 1.47	8.4 ± 1.18
 27.	11.5 ± 1.9	13.5 ± 1.6	18.12 ± 2.0	11.5 ± 1.7
 28.	9.0 ± 2.0	11.0 ± 1.8	14.0 ± 1.8	7.0 ± 3.1
 29.	11.0 ± 1.8	13.0 ± 1.7	17.9 ± 1.9	10.7 ± 1.4
IAA			8.1 ± 0.72	

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

4. 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 total, four concentrations (5, 10, 15, 20 mg/L) of each compound, along with H_2O as a control, were tested. For all treatments, ten replicates were cultured in vials, each containing 30 mL of test solution. The final observations were recorded on day eight. The experiment was repeated in triplicate at 27 ± 2°C.

5. CONCLUSION

Each compound was analysed for its biological potential as a plant growth regulator. Compounds were tested at four concentrations (5, 10, 15 and 20 mg/L), and the results were compared to a control (distilled water) (Table 2). We found that substituted pyrazolines that have a methyl group at C-13 and C-16 promote an appreciable increase in root generation when compared to control and parent compounds.

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