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Phytochemical investigation of aerial parts of *Pluchea lanceolata* C.B.Clarke

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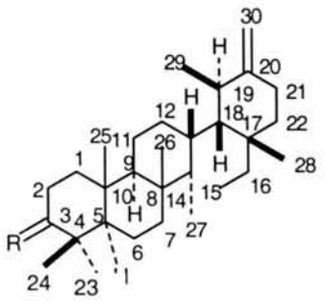
Six new chemical constituents, a seco-ursane-type triterpene, two sesquiterpenic esters, an alkyl δ-lactone, a cyclohexyl substituted alkanoic acid and a sesterterpenic ester have been isolated from the aerial parts of *Pluchea lanceolata* and their structures were established as 14, 15-seco-urs-18βH-20 (30)-en-3β-yl acetate **1**, 4, 8-dimethyldodeca-7Z-en-yl-9'α, 10'α-dihydroxy undecan-1'-oate **2**, α-dotriaconta-29, 32-olide **3**, 4'α- (2''-ketobutyl cyclohexyl)-20α-eincosan-14α-ol-1-oic acid **4**, 5, 9, 13, 17-tetramethyl-18-en-8α-ol nonadecanoic acid octanyl ester **5** and 3, 7, 11-trimethyl dodeca-10-en-yl-*n*-hexadecan-9'α-ol-1'-oate **6** by spectral data analyses and chemical evidences.

Pluchea lanceolata C.B. Clarke. (Family: Asteraceae), commonly known as "Rasna', is an erect undershrub growing in Indian sandy or saline soils of Punjab, upper Gangetic plain, Delhi, Rajasthan and Gujarat. The plant is used for the treatment of rheumatoid arthritis. Flavonoids[1], pluchine[2], chromenone[3], monoterpene[3], pentacyclic triterpene[4-7], sterols[6,7], aliphatic constituent[6,8] and phenolic acid[9]have been reported from this plant. We describe herein the isolation and structural elucidation of seco-ursane-type triterpene, two sesquiterpenic esters, an alkyl δ-lactone, a cyclohexyl substituted alkanoic acid and a sesterterpenic ester from the aerial parts of the plant.

Results and Discussion

Compound **1**, designated plucheaursenyl acetate, obtained in benzene eluants, gave positive in Liebermann-Burchard test and had a molecular formula of $C_{32}H_{54}O_2$ (M^+, m/z 470) on the basis of mass and ^{13}C NMR spectral data. It indicated six double bond equivalents, four of them were interpreted in the seco-carbon framework of the β-amyrin type triterpene, one in olefinic linkage and one in acetyl group. Its IR spectrum exhibited ester group (1745 cm^{-1}) and unsaturation (1605 cm^{-1}) absorption bands. The mass spectrum of **1** was distinctive of pentacyclic triterpenes of amyrin series in which rings A, B, C and D were saturated. The characteristic ion peaks at m/z 250, 220 $[C_{8,14}\text{-}C_{9,11}$ fission$]^+$, 190$[C_{8\text{-}14}\text{-}C_{11,12}$ fission$]^+$, 218[M-190-AcOH]$^+$, 206 $[C_{11,12}\text{-}C_{8,14}$ fission$]^+$ and 190[250-AcOH]$^+$ were generated due to fission of ring C. The ion fragments at m/z 410[M-AcOH]$^+$ and 395[410-Me]$^+$ disclosed the existence of acetoxyl group in the molecule.The ion peaks at m/z 83$[C_{3,4}$ - $C_{5,10}$ - $C_{8,9}$ fission$]^+$, 155$[C_{2,3}$ - $C_{5,10}$ -$C_{8,9}$ fission$]^+$, 142$[C_{1,10}$ - $C_{4,5}$ fission$]^+$, 122$[C_{5,6}$ - $C_{9,10}$ fission - AcOH$]^+$, 136$[C_{6,7}$ - $C_{9,10}$ fission - AcOH$]^+$ and 150$[C_{7,8}$ - $C_{9,10}$ fission - AcOH$]^+$ indicated that the rings A and B were saturated and the acetoxyl group was present in ring A which was placed at C-3 on biogenetic grounds. The ion fragments at m/z 151$[C_{13}$ - C_{18} fission$]^+$, 136[151-Me]$^+$, 125[151-C_2H_5]$^+$ and 82$[C_{18,19}$- $C_{17,22}$ fission$]^+$ supported C_8-C_{14} seco nature of the molecule and exocyclic methylene group in ring E. The ^1H NMR spectrum of **1** displayed a two-proton doublet at δ 4.68 (J=14.7 Hz) assigned to C-30 exocyclic methylene protons and a one-proton double-doublet for C-3 carbinol proton at δ 4.47 and its coupling constants of 5.9 and 10.0 Hz indicated its α-orientation. Five three-proton signals at δ 2.04, 1.25, 1.03, 0.97 and 0.92 were associated with acetyloxy group, C-23, C-28, C-24 and C-25 tertiary methyl groups, respectively. The C-26 and C-27 methyl groups appeared as a six-proton singlet at δ 0.84. A three-proton doublet at δ 0.87 (*J*=6.5 Hz) assigned to C-29 secondary methyl group and a double doublet at δ 2.09 (*J*=4.3, 8.5 Hz) due to C-18 methine proton supported the ursane-type carbocyclic framework of the molecule. The remaining methine and methylene protons resonated between δ 2.52-1.17. More

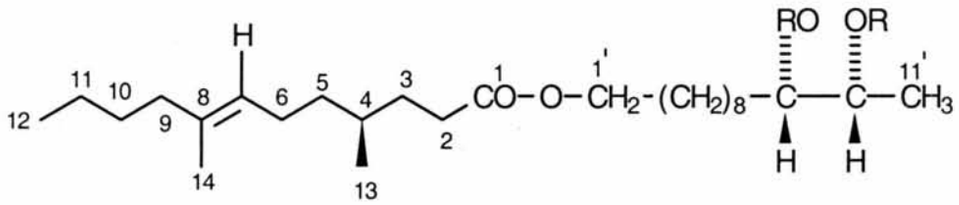


1 R = α-H, β-OAc
1a R = α-H, β-OH
1b R = O

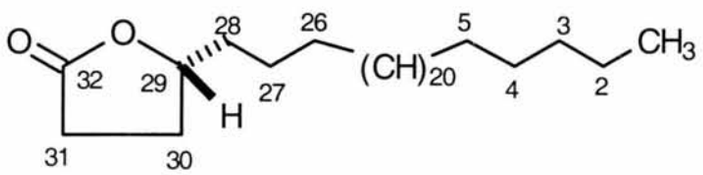
compelling evidence for the structure **1** was provided by a study of its ^{13}C NMR spectrum which showed the presence of 32 carbon atoms. The signals at δ 170.80 and 21.46 supported the presence of acetoxyl group. The C-3 carbinol carbon appeared at δ 81.01. Two downfield signals at δ 154.65 and 107.11 were assigned to C-20 and C-30 olefinic carbons. Deviation of C-14 and C-15 signals to δ 27.94 and 18.18 confirmed C_{14}-C_{15} seco ring D of the molecule. The carbon signal at δ 55.45 for C-18 also supported ursane type carbon framework. The assignments of the carbon chemical shifts were made by comparison with the δ values of the corresponding carbon atoms in the other ursane type molecules[10,11]. Conclusive evidence for the structure of **1** was derived from the results of chemical reactions. Alkaline hydrolysis of **1** yielded a free alcohol (**1a**). Oxidation of **1a** with Jones reagent gave a keto derivative (**1b**) which responded positively to Zimmermann test indicating the presence of 3-oxo groups[12]. The sodium borohydride reduction of **1b** regenerated the parent alcohol **1** confirming the equatorial orientation of the hydroxyl group in **1a** and, hence, the acetoxy group in **1.** These findings led to establish the structure of **1** as 14, 15-seco-18βH-urs-20 (30)-en-3β- yl acetate.

Biogenetic evidence suggesting the structure of **1** is provided by the recent isolation of a similar triterpene containing $\Delta^{20(30)}$ olefinic linkage[11]. To the best of our knowledge, this is the first report of a naturally occurring seco-pentacyclic triterpene with an exocyclic double bond at $\Delta^{20(30)}$; its isolation may be significant in chemotaxonomic study.

Compound **2**, named plucheasesquiterpenyl undecanoate, was obtained as colourless amorphous powder from benzene eluants. It had IR absorption bands at 3400 (OH), 1740 (ester-CO), 1600 (C=C) and 715 cm^{-1} (long aliphatic chain) and possessed a molecular ion peak at m/z 426 corresponding to a desmethylsesquiterpene ester, $C_{26}H_{50}O_4$. It has two double equivalents, one was adjusted in olefinic linkage and the other in ester carbonyl group. The prominent ion peaks at m/z 57[C_8-C_9 fission]$^+$, 97[C_6 - C_7 fission]$^+$, 125[C_4 - C_5 fission]$^+$, 153 and 273[C_3 - C_4 fission]$^+$ indicated the existence of olefinic linkage at C-7. Generation of ion fragments at m/z 209 and 217 due to cleavage of CO–O linkage suggested that the compound was a C-14 desmethylsesquiterpenic acid esterified with trihydroxy dodecane. Cleavage of $C_{8'}$ - $C_{9'}$ and $C_{9'}$ - $C_{10'}$ linkages yielded ion peaks at m/z 351 and 381, respectively, supporting the hydroxyl groups at C-9′ and C-10′. In addition to these ion peaks, the mass spectrum exhibited C_nH_{2n+1}, C_nH_{2n} and C_nH_{2n-1} ions in higher abundance for lower fragments, most of the fragments were separated by 14 mass unit and decrease in abundance with increasing molecular weight of long straight chain hydrocarbon. The existence of more intense clusters of peaks corresponding to C_nH_{2n-1} (m/z 83, 97, 111, 125 etc.) in comparison to that corresponding to C_nH_{2n+1} (m/z 85, 99, 113, 127 etc.) also supported the unsaturated nature of the molecule. The ^1H NMR spectrum of **2** displayed a one-proton deshielded double-doublet at δ 5.34 Hz (*J*=4.6, 15.1 Hz) assigned to H-7. Two one-proton triple-doublets at δ 4.31 (*J*=3.8, 4.4, 1.9) and 4.12 (*J*=5.8, 5.8, 1.2 Hz) were ascribed to oxygen-substituted C-1′ methylene protons. The C-9′ and C-10′ hydroxyl methine proton appeared as one-proton multiplets at δ 3.99 ($w_{1/2}$=10.4 Hz) and 3.63 ($w_{1/2}$=6.5 Hz) respectively, and their low half-widths suggested α-orientation of the hydroxyl groups. The C-2 methylene group adjacent to ester carbonyl group resonated as double doublets at δ 2.80 (*J*=6.9, 13.2 Hz) and 2.73 (*J*=6.2, 13.2 Hz). Two one-proton multiplets at δ 2.34 and 2.27 were associated to C-6 methylene protons attached to C-7 vinylic carbon. A three-proton broad singlet at δ 1.67 was accounted to C-14 methyl function attached to the vinylic carbon. Two three-proton doublets at δ 1.30 (*J*=6.5 Hz) and 0.84 (*J*=6.2 Hz) were associated with C-11′ and C-13 secondary methyl groups. A three-proton triplet at δ 0.93 (*J*=6.0 Hz) was attested the presence of C-12 primary methyl group. The ^{13}C NMR spectrum of **2** displayed signals for an ester (δ 170.1), two carbinol (δ 71.7, 70.1) and two olefinic



2 R = H
2a R = Ac

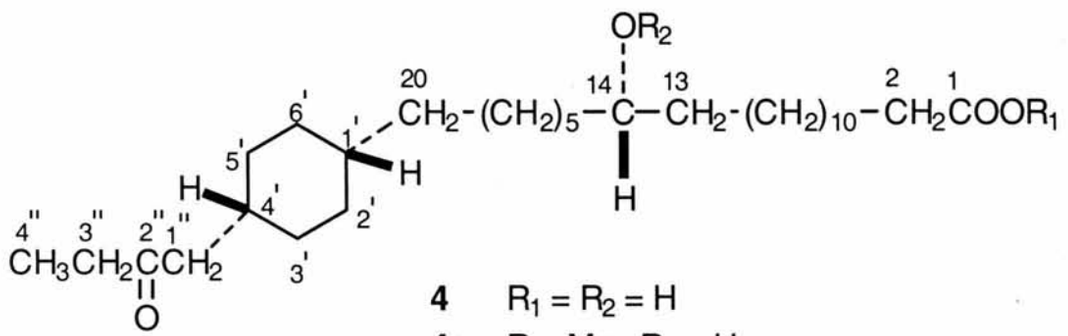


3

(δ 145.2, 123.5) carbons. Acetylation of **2** with acetic anhydride and pyridine yielded a diacetyl product (**2a**). On the basis of foregoing account the new natural product **2** was characterized as 4, 8-dimethyl dodeca-7Z-en-yl-9′α, 10′α-dihydroxy undecan-1′-oate. This is a new desmethyl-sesquiterpenic ester and the presence of a sesquiterpene is being reported for the first time from *P. lanceolata*.

Compound **3**, designated pluchealactone, was obtained as light grey amorphous powder from chloroform eluants. Its IR spectrum demonstrated the presence of γ-lactone (1765 cm^{-1}) and long aliphatic chain (750, 740 cm^{-1}). It had molecular ion peak at m/z 478 in its mass spectrum consistent with the molecular formula of a saturated γ-lactone $C_{32}H_{62}O_2$. The spectrum showed important ion peaks at m/z 449[M-C_2H_5]$^+$, 57[C_4-C_5 fission]$^+$, 421[M-$C_{11}H_9$]$^+$, 85[C_{28}-C_{29}fission]$^+$, 407[M-C_5H_{11}, C_5-C_6 fission]$^+$, 393[407-CH_2]$^+$, 379[393-CH_2]$^+$, 365[379-CH_2]$^+$, 351[365-CH_2]$^+$, and 337[351-CH_2]$^+$. In addition, mass spectrum showed C_nH_{2n+1}, C_nH_{2n} and C_nH_{2n-1} ions in higher abundance for lower fragments. The most of the fragments were separated by 14 mass units and decreased in abundance with increasing molecular weight of long straight chain hydrocarbon. The absence of [M-Me]$^+$ ion suggested its straight chain nature, whereas the presence of [M$^+$+1] ion arose due to unsymmetrical nature. More intense clusters of peaks corresponding to C_nH_{2n-1} (e.g. m/z 83, 97, 111, 125, 139, etc.) in comparison to C_nH_{2n+1} (m/z 85, 99, 113, 127, 141, etc.) supported the cyclic nature of the compound. From these information it was clear that the compound **3** contained a C_{24}aliphatic chain attached to the lactone ring. The ^{1}H NMR spectrum of **3** displayed a one-proton multiplet at δ 3.63 assigned to H-29 oxygen-substituted methine proton supporting the attachment of the aliphatic chain at C-29 and it half width of 9.6 Hz indicated α-orientation of the side chain. Two one-proton triple doublets at δ 2.34 and 2.12 were ascribed to C-31 methylene protons adjacent to the carbonyl group of the lactone ring. A three-proton triplet at δ 0.87 with coupling interaction of 5.81 Hz was attributed to C-1 primary methyl protons. The remaining methylene protons resonated between δ 1.57-1.00. The absence of any signal beyond δ 3.63 ruled out the secondary or tertiary olefinic linkage and saturated nature of the molecule. On the basis of these spectral data the structure of **3** was deduced as α-dotriaconta-29, 32-olide. This is new γ-lactone and the presence of a lactonic compound is being reported from *P. lanceolata* for the first time.

Compound **4,** namely lanceolatoic acid, was obtained as colourless amorphous powder from the chloroform eluants. It gave effervescence with sodium bicarbonate solution and a positive 2, 4-dinitrophenylhydrazine test suggesting a keto acid. It had characteristic IR absorption bands at 3500 (OH),



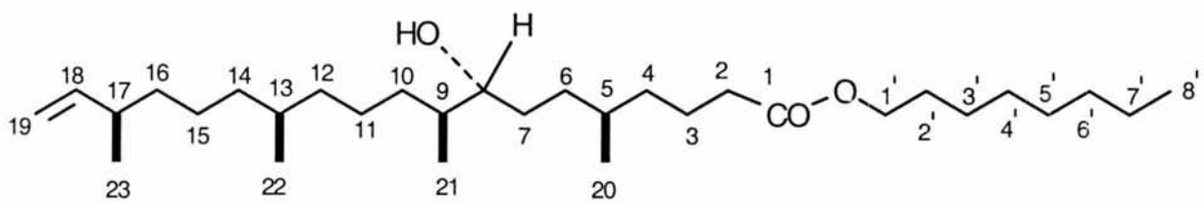
4 $R_1 = R_2 = H$

4a $R = Me$, $R_2 = H$

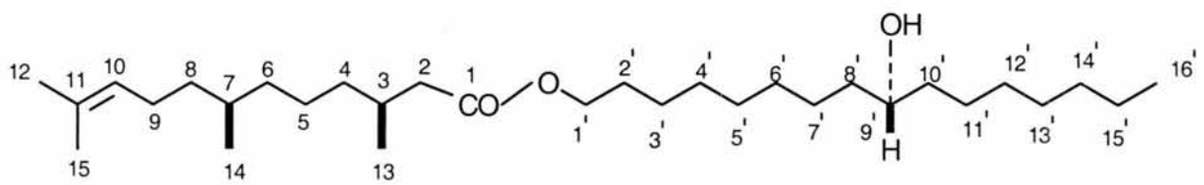
4b $R_1 = H$, $R_2 = Ac$

3100 (COOH), 1710 (CO), 1695 (COOH) and 765 (long aliphatic chain) cm^{-1}. The molecular ion peak at m/z 480 suggested the molecular formula as $C_{30}H_{56}O_4$. It had three degrees of unsaturation; one each was adjusted in carbonyl group, carboxylic group and a cyclic ring. The location of the carbonyl group was deduced to be at C-28 from the prominent α and β-fission ions at m/z 57 and 71. The ion peaks at m/z 82[$C_{4'}$-$C_{1''}$and C_{20}-$C_{1'}$ fission]$^+$, and 153[C_{20}-$C_{1'}$ fission]$^+$ suggested the existence of a cyclohexane ring between C-20 and C-1″ carbons. The ion peaks at m/z 237[C_{14}-C_{15} fission]$^+$, 223 [237-CH_2]$^+$, 209[223-CH_2]$^+$, 195[209-CH_2]$^+$, 181[195-CH_2]$^+$, 166[237-$C_2H_5COCH_2$]$^+$, 267[C_{13}-C_{14} fission]$^+$ and 213[M-267]$^+$ supported the presence of the hydroxyl group at C-14. The elimination of CH_2COOH moiety from the molecular ion peak generated an ion peak at m/z 421. The absence of an [M-15]$^+$ ion suggested its straight chain whereas the presence of an [M+1]$^+$ was characteristic for its unsymmetrical nature. The mass spectrum also showed C_nH_{2n+1}, $C_{2n}H_{2n}$ and C_nH_{2n-1} ions in higher abundance for lower fragments. The spectrum also showed separation of the fragments by 14 mass units and decreasing in abundance with increasing molecular weight of long straight chain hydrocarbon. The more intense clusters of peaks corresponding to C_nH_{2n-1} (m/z 83, 97, 111, 125, etc.) in comparison to that corresponding to C_nH_{2n+1} (m/z 85, 99, 113, 127, 141 etc.) also supported the cyclic nature of the compound. The ^{1}H NMR spectrum of **4** showed one-proton multiplet at δ 3.48 assigned to C-14 hydroxyl methine proton and its half-width of 9.4 Hz indicated α-orientation of the hydroxyl group. Two multiplets at δ 2.06 and 2.03, integrating two protons each, were ascribed to C-1″ and C-3″ methylene protons adjacent to the C-28 carbonyl group. A two-proton multiplet at δ 1.67 was assigned to H-1′ and H-4′ methine protons and its half-width of 9.1 Hz suggested β-orientation of these protons. A three-proton triplet at δ 0.84 (J=6.4 Hz) was associated with the C-4″ terminal primary methyl group. The remaining methylene and methine protons appeared between δ 1.54-1.15. The absence of any signal beyond δ 3.48 supported the saturated nature of the molecule. The ^{13}C NMR spectrum of **4** showed signals for one carbonyl (δ 202.1), one carboxylic (δ 163.5), and one carbinol (δ 71.6) carbons. Methylation of **4** with diazomethane yielded a monomethyl ester **(4a).** A monoacetyl derivative **(4b)** was obtained when **4** was treated with a mixture of acetic anhydride and pyridine. On the basis of the above data the structure of compound **4** was formulated as 4′α-(2″-ketobutyl cyclohexyl) 20α-eicosan-14α-ol-1-oic acid. This is a new natural product reported from a natural or synthetic origin for the first time.

Compound **5**, named plucheasesterterpenyl ester, was obtained as colourless flakes from the chloroform-methanol (95:5) eluants. Its IR spectrum exhibited absorption bands for hydroxyl group (3450 cm^{-1}), ester group (1735 cm^{-1}), unsaturation (1640 cm^{-1}) and aliphatic chain (720, 705 cm^{-1}). Its mass spectrum showed a molecular ion peak at m/z 480 corresponding to a nordimethylsesterterpenoic ester $C_{31}H_{60}O_3$. It had two double bond equivalent; one of them was adjusted in the olefinic linkage and the other in the ester group. The mass spectrum of **5** displayed important ion peaks at m/z 453[$C_{29}H_{57}O_3$, C_{17}-C_{18} fission, M-CH_2=CH]$^+$, $C_{16}H_{31}O_2$,



5



6

411[$C_{26}H_{51}O_3$]$^+$, and 69[C_5H_9]$^+$ [C_{15}-C_{16} fission]$^+$, 255[$C_{16}H_{31}O_2$, C_7-C_8 fission]$^+$, 351[$C_{23}H_{43}O_2$]$^+$ and 129[$C_8H_{17}O$]$^+$ [CO-O fission]$^+$ suggesting the existence of the olefinic linkage at the terminal carbon, the hydroxyl group at C-8 and *n*-octanol esterified with C-19 norditerpene. The spectrum showed C_nH_{2n+1}, C_nH_{2n} and C_nH_{2n-1} ions in higher abundance for lower fragments due to aliphatic chain. The ^{1}H NMR spectrum of **5** showed a one-proton downfield multiplet at δ 5.34 due to H-18 and two one-proton each deshielded doublets at δ 5.14 (*J*=7.8 Hz) and 5.06 (*J*=11.2 Hz) assigneable to C-19 terminal methylene group. Two one-proton each triple-doublets at δ 3.63 (*J*=5.2, 6.5, 2.3 Hz) and 3.58 (*J*=6.1, 6.5, 9.2 Hz) were assigned to C-1′ oxygen-substituted methylene group. A one-proton multiplet at δ 3.52 was attributed to C-8 carbinol proton and its half-width of 8.4 Hz indicated its α-orientation of the hydroxyl group. A three-proton triplet at δ 0.87 (*J*=5.8 Hz) was ascribed to C-8′ primary methyl group. Four secondary methyl group protons appeared as three-proton doublets at δ 0.93 (*J*=6.5 Hz, Me-23), 0.83 (*J*=6.5 Hz, Me-20), 0.78 (*J*=6.0 Hz, Me-22) and 0.69 (*J*=6.5 Hz, Me-21). The remaining methylene and methine groups appeared between δ 2.34-1.15. The ^{13}C NMR spectrum of **5** exhibited important signals for ester (δ 171.7, 62.6), one hydroxymethine (δ 73.2) and two olefinic (δ 123.5, 109.3) carbons. Acetylation of **5** with acetic anhydride and pyridine yielded a monoacetyl derivative **5a**. Alkaline hydrolysis of **5** gave a mono carboxylic acid. On the basis of these findings the structure of **5** was established as 5, 9, 13, 17-tetramethyl-18-en-8α-ol-nonadecanoic acid octanyl ester. This is a new nordimethylsesterterpenic ester and the presence of a sesterterpene is being reported for the first time in *Pluchea lanceolata*.

Compound **6**, a sesquiterpenic ester, named plucheasesquiterpenyl hexadecanoate was obtained as colourless flakes from chloroform-methanol eluants (95:5). Its IR spectrum showed the presence of hydroxyl (3450 cm^{-1}) and ester (1740 cm^{-1}) groups. Its mass spectrum exhibited a molecular ion peak at m/z 480 consistent with the molecular formula $C_{31}H_{60}O_3$. It indicated two degrees of unsaturation, one of them was adjusted to the ester group and another one to an olefinic linkage. In its mass spectrum most of the ion peak were separated by 14 mass unit and the intense peaks corresponding to C_nH_{2n+1} and C_nH_{2n-1} were observed. The presence of a distinct M-15 peaks indicated methyl branchings. The ion peak at m/z 55 [C_9-C_{10} fission]$^+$, and the subsequent ions at m/z 69, 83, 111, 125 139, 153, 181 and 195 suggested the presence of the olefinic linkage at Δ^{10}. The ion peaks at m/z 223 and 257 arose due to cleavage of the ester group. An ion peak at m/z 129 generated due to cleavage of $C_{8'}$-$C_{9'}$ linkage indicating the existence of the hydroxyl group at C-9′.The ^{1}H NMR spectrum of **6** displayed a downfield one-proton triplet at δ 5.36 (J =5.4 Hz) assigned to C-

10 olefinic proton. Two one-proton each triple doublets at δ 4.28 (*J*= 8.1, 5.7, 2.3 Hz) and 4.15 (*J*= 5.7, 6.6, 2.3 Hz) were attributed to 6 C-1′ methylene protons substituted to ester oxygen. A one-proton multiplet at δ 3.64 was ascribed to H-9′ carbinol proton and its half-width of 6.6 Hz indicated α-orientation of the hydroxyl group. A six-proton broad singlet at δ 1.58 was associated with C-12 and C-15 methyl functionalities attached to C-11′vinylic carbon. The C-13, C-14 and C-16′ methyl protons appeared as doublets at δ 0.88 and 0.85 with coupling interactions of 7.5 Hz and as a triplet at δ 1.02 (*J*= 5.5 Hz), respectively. Two one-proton double doublets at δ 2.33 (J =2.3, 9.5 Hz) and 2.29 (*J*= 7.2, 9.5 Hz) were associated with C-2 methylene group adjacent to the carbonyl group. The C-9 methylene protons adjacent to the olefinic linkage appeared as one-proton multiplets at δ 2.07 and 2.01. The remaining methylene and methine protons resonated at δ 2.79 (1H) and 1.68 - 1.25. Acid hydrolysis of **6** yielded an acyclic sesquiterpenic acid and a C-16 diol. Based on these evidences the structure of **6** has been established as 3, 7, 11-trimethyl dodeca-10-en-yl-*n*-hexadecan-9′α-ol-1′-oate. This is a new sesquiterpenic ester isolated from a natural source.

Experimental Section

Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$on Bruker DRX-300 at 300 M Hz and 100 M Hz, respectively, using TMS as an internal standard; mass spectra on Jeol D-300 EI/CI) system; UV spectra on Bechman DU-64 model; and IR spectra on Hitachi model-270. Purity of the compounds was checked by TLC over silica gel G (Merck). The spots were visualized by exposure to iodinevapours, UV radiation and by spraying with perchloric acid and ceric sulphate solution.

Plant material. The aerial parts of *P. lanceolata* were collected from the cultivated species grown in the Herbal Garden of the Jamia Hamdard, New Delhi.

Extraction and Isolation of metabolites. The air-dried and coarsely powdered plant (2kg) was extracted exhaustively with ethanol (95%) in a soxhlet apparatus and the ethanolic extract was concentrated to get a dark brown viscous mass (210 g). It was dissolved in minimum amount of methanol and adsorbed on silica gel to form a slurry. The air-dried slurry was loaded to silica gel column prepared in petroleum ether. The column was eluted with petroleum ether benzene, chloroform and methanol in order of increasing polarity to isolate in following compounds:

Plucheaursenyl acetate 1 : Elution of the column with benzene afforded colourless amorphous powder of **1**, recrystallized from chloroform-methanol (1:1). 317 mg (0.01585% yield) R_f 0.051 (chloroform-petroleum ether, 70 : 30)**;** m.p. 192-194 °C; UV (MeOH) : 216 nm. (log ε 4.5); IR : 2955, 2880, 1745, 1605, 1475, 1385, 1260, 1040, 1025, 995, 895 cm^{-1}; ^{1}H NMR : δ 4.68 (2H, d, *J*=14.7 Hz, H_2-30), 4.47 (1H, dd, *J* = 5.9, 10.0 Hz, H-3α), 2.52 (1H, m, H-19), 2.44 (1H, dddd, *J* = 4.5, 11.6, 10.5, 5.4, H_2- 21α), 2.41 (1H, dddd, *J* = 5.5, 11.6, 10.5, 12.6 Hz, H-21β), 2.3 (1H, dddd, *J* = 6.5, 8.9, 11.6, 12.6, H-1b), 2.23 (1H, dddd, *J* = 3.3, 3.2, 8.0, 12.1, H-7a), 2.12 (1H, dddd, *J* = 5.3, 9.4, 5.8, 13.1 Hz, H-7b), 2.09 (1H, dd, *J* = 4.3, 8.5 Hz, H-18β), 2.04 (3H, brs, OAc), 1.94 (1H, d, *J*=10.5 Hz, H-14β), 1.86 (1H, m, H-2α), 1.81 (1H, m, H-2β), 1.74 (1H, m, H-12β), 1.68 (1H, m, H-12α), 1.64 (1H, dd, *J*=4.5, 8.6 Hz, H-5α), 1.59 (2H, m, H-16α, H-13β), 1.55 (3H, m, H-6α, H-6β, H-16β), 1.46 (1H, t, *J*=4.2 Hz, H-9α), 1.41 (1H, m, H-22β), 1.37 (1H, dddd, *J*=4.8, 8.7, 8.5, 3.4, H-1α), 1.32 (1H, m, H-11α), 1.25 (3H, brs, Me-23), 1.17 (2H, m, H-11β, H-22α), 1.03 (3H, brs, Me-28), 1.00 (3H, t, *J*=6.5 Hz, Me-15), 0.97 (3H, brs, Me-24), 0.92 (3H, brs, Me-25), 0.87 (3H, d, *J*=6.5 Hz, Me-29), 0.84 (6H, brs, Me-26, Me-27). EIMS m/z (rel.int.) 470 $[M]^+$ ($C_{32}H_{54}O_2$) (14.3), 410. (14.1), 395 (4.3), 250 (8.7), 220 (20.7), 218 (4.4), 206 (22.4), 204 (14.9), 192 (34.2), 190 (56.0), 162 (14.2), 155 (4.8), 151 (12.0), 150 (12.7), 142 (5.5), 136 (30.1), 122 (31.2), 110 (33.1), 95 (37.6), 83 (20.1), 82 (36.2), 69 (55.3), 57 (78.8), 43 (100).^{13}C NMR δ 38.87 (C-1), 26.65 (C-2), 81.01 (C-3), 38.29 (C-4), 50.40 (C-5), 19.47 (C-6), 34.00 (C-7), 40.92 (C-8), 48.66 (C-9), 37.80 (C-10), 21.31 (C-11), 25.61 (C-12), 39.16 (C-13), 27.94 (C-14), 18.18 (C-15), 26.15 (C-16), 38.44 (C-17), 55.45 (C-18), 39.38 (C-19), 154.65 (C-20), 34.52 (C-21), 37.05 (C-22), 29.69 (C-23), 16.49 (C-24), 14.72 (C-25), 15.89 (C-26), 23.69 (C-27), 25.49 (C-28), 25.49 (C-29), 107.11 (C-30), 170.80, 21.46 (OAc).

Alkaline hydrolysis of 1. Compound **1** (50 mg) was refluxed with 2*M* alcoholic KOH solution (10 mL) for 2 hr. The reaction mixture was neutralized with dilute HCl, extracted with chloroform (3 × 10

mL), the organic phase washed with water (2 × 10 ml), dried (Na_2SO_4) and evaporated to get plucheaursenol (**1a**), 27 mg, m.p. 173-74°C; IR: 3450 cm^{-1}.

Jones oxidation of 1a. Compound **1a** (20 mg) was dissolved in Me_2CO (5 mL), cool to 4°C and Jones reagent (2 mL) was added till the persistence of a brown colour. Water (10 mL) was added and the reaction mixture extracted with chloroform (3 × 10 mL). After usual work-up plucheaursenone **1b** (12 mg) was obtained, m.p.147-48°C; IR: 1710 cm^{-1}.

$NaBH_4$ reduction of 1b. Compound **1b** (10 mg) was dissolved in methanol (5 mL) and $NaBH_4$ (2 mg) was then added with stirring (1hr). After dilution with water, the mixture was extracted with chloroform and the chloroform layer was washed with water, dried (Na_2SO_4) and evaporated to obtain plucheaursenol (**1a,** 8 mg), mp-161-62°.

Plucheasesquiterpenyl ester 2: Elution of the column with benzene gave colourless amorphous powder of **2**, recrystallized from chloroform-methanol (1:1). 483 mg (0.02415 % yield). R_f 0.61 (chloroform : petroleum ether; 70:30.), m.p. 60-62 °; UV : 210 nm (log ε 3.5); IR: 3400, 2925, 2855, 1740, 1600, 1465, 1380, 1265, 1170, 1100, 1055, 715, cm^{-1}; ^1H NMR δ 5.34 (1H, dd, *J*=4.6, 15.1 Hz, H-7), 4.31 (1H, ddd, *J*=3.8, 4.4, 1.9 Hz, H_2-1′a), 4.12 (1H, ddd, *J*=5.8, 5.8, 1.2 Hz, H_2-1′b), 3.99 (1H, m, $w_{1/2}$=10.4 Hz, H-9′), 3.63 (1H, m, $w_{1/2}$=6.5 Hz, H-10′), 2.80 (1H, dd, *J*=6.9, 13.2 Hz, H_2-2a), 2.73 (1H, dd, *J*=6.2, 13.2 Hz, H_2-2b), 2.43 (2H, brs, H_2-9), 2.34 (1H, m, H_2-6a), 2.27 (1H, m, H_2-6b), 1.81 (1H, brs, H-4), 1.67 (3H, brs, Me-14), 1.59 (2H, brs, H_2-3), 1.30 (3H, d, *J*=6.5 Hz, Me-11′), 1.25 (16H, brs, 8×CH_2), 1.07 (2H, m, CH_2), 1.03 (2H, m, CH_2), 1.01 (2H, m, CH_2), 0.93 (3H, t, *J*=6.0 Hz, Me-12), 0.84 (3H, d, *J*=6.2 Hz, Me-13); EIMS m/z (rel.int.): 426[M]$^+$ ($C_{26}H_{50}O_4$) (8.4), 411 (3.9), 408 (3.8), 394 (3.7), 381 (1.1), 351 (2.5), 273 (3.8), 259 (4.9), 253 (3.1), 239 (3.1), 225 (3.3), 217 (19.7), 209 (5.4), 207 (12.8), 201 (4.7), 187 (24.3), 181 (8.1), 173 (11.8), 167 (13.0), 155 (8.6), 153 (16.0), 141 (10.0), 127 (11.3), 125 (32.5), 113 (13.2), 111 (52.8), 99 (21.2), 97 (77.2), 83 (78.3), 71 (86.1), 57 (100), 43 (50.2). ^{13}C NMR: δ 170.1 (C-1), 145.2 (C-8), 123.5 (C-7), 71.7 (C-9′), 70.1 (C-10′), 62.81 (C-1′), 45.3 (C-4), 41.2 (CH_2) 39.5 (CH_2) 31.6 (CH_2), 29.5 (10 × CH_2), 28.8 (CH_2) 25.1 (CH_2) 15.2 (C-14), 14.7 (C-13), 13.6 (C-12), 12.7 (C-11′).

Acetylation of 2. Compound (15 mg), was added to a mixture of Ac_2O (5 mL) and pyridine (1 mL), heated for 1 hr and left overnight. Water (10 mL) was added and extracted with chloroform (3×10 mL). The organic phase was washed with water, dried (Na_2SO_4) and evaporated to get diacetate (**2a,** 13 mg), m.p. 56-57°; IR: 1725, 1735 cm^{-1}.

Pluchealactone 3. Elution of column with chloroform furnished light grey amorphous powder of **3**, recrystallized from chloroform-methanol (1:1), 519 mg (0.02595% yield). R_f 0.41 (chloroform : petroleum ether; 9:1), m.p. 55-57°C; UV : 240 nm (log ε 3.5); IR: 2965, 2880, 1765, 1480, 1405, 1395, 1285, 1260, 1195, 750; 740 cm^{-1}; ^1H NMR : δ 3.63 (1H, m, $w_{1/2}$=9.6 Hz, H-29β), 2.34 (1H, ddd, *J*=10.4, 13.4, 7.4, Hz, H-31a), 2.12 (1H, ddd, *J*=7.4, 13.0, 9.5, Hz, H-31b), 1.57 (2H, m, H_2-30), 1.42 (2H, m, H_2-28), 1.25 (48H, brs, 24×CH_2), 1.15 (2H, m, H_2), 1.00 (2H, m, H_2), 0.87 (3H, t, *J*=5.81 Hz, Me-1). EIMS m/z (rel.int.) 478[M]$^+$ ($C_{32}H_{62}O_2$) (5.1), 449 (5.0), 421 (5.2), 407 (3.5), 393 (3.8), 379 (1.3), 365 (3.6), 351 (3.2), 337 (3.0), 323 (3.1), 309 (3.6), 295 (3.8), 281 (3.9), 267 (4.3), 253 (4.4), 239 (4.1), 225 (5.2), 218 (10.5), 211 (4.8), 197 (6.2), 189 (15.6), 183 (6.2), 181 (13.0), 169 (7.7), 155 (8.9), 15.3 (15.6), 141 (9.3), 127 (11.3), 113 (14.3), 111 (50.6), 99 (60.3), 97 (70.1), 85 (61.3), 83 (85.2), 71 (82.1), 69 (63.5), 57 (100), 43 (60.5).

Lanceolatoic acid 4. Elution of column with chloroform gave white amorphous powder of **4**, recrystallized from methanol. 286 mg (0.0143% yield).R_f 0.81 (chloroform : petroleum ether : methanol; 80:20:1). m.p. 50-52°;. UV : 225 nm (log ε 3.5); IR (KBr) : 3500, 3100, 2955, 2845, 1710, 1695, 1608, 1470, 1410, 1385, 1290, 1260, 1230, 1180, 1155, 1090, 1000, 865, 765 cm^{-1}; ^1H NMR : δ 3.48 (1H, m, $w_{1/2}$=9.4 Hz, H-14), 2.06 (2H, m, H_2-1″) 2.03 (2H, m, H_2-3″), 1.67 (2H, m, $w_{1/2}$=9.1 Hz, H-1′ H-4′), 1.54 (10H, brs, 5×CH_2), 1.36 (2H, m, CH_2) 1.25 (30H, brs, 15×CH_2), 1.15 (2H, m, CH_2), 0.84 (3H, t, *J* = 6.4 Hz, Me-4″). EIMS m/z (rel. Int.) 480[M]$^+$ ($C_{30}H_{56}O_4$) (1.5), 421 (9.9), 393 (4.0) 267 (3.2), 237 (11.1), 223 (9.7), 213 (8.4), 209 (12.7) 195 (15.6), 181 (15.2), 166 (12.7), 157 (3.2) 153 (14.6), 139 (19.6), 125 (32.2), 111 (50.3) 97 (80.3), 85 (48.3), 82 (77.5), 71 (77.8), 69 (68.9) 57 (100), 55 (48.1), 43 (56.2). ^{13}C NMR : δ 202.1 (C-2″), 163.5 (C-1), 71.6 (C-14), 45.3 (C-1′), 43-1 (C-4′), 37.3 (C-2), 32.8 (C-1″), 29.1 (C-3″), 28.5 (18 × CH_2), 25.7 (CH_2). 25.3 (2 × CH_2), 13-5 (C-4″).

Methylation of 4. Compound (10 mg) was dissolved in solvent ether (10 mL), an etherea

solution of diazomethane (CH_2N_2) was added to it and left overnight. The solvent was removed to get methyl lanceolatoate **4a**, m.p. 57-58°.

Acetylation of 4. Compound (10 mg), was heated with a mixture of Ac_2O (5 mL) and pyridine (1 mL) for 1hr. After usual work-up acetyl lanceolatoic acid **4b** was obtained, m.p. 60-61°C, IR: 3250, 1725, 1690 cm^{-1}.

Plucheasesterterpenyl ester 5. Elution of the column with chloroform-methanol (95:5) afforded colourless flakes of **5,** recrystallized from chloroform-methanol (1:1). 293 mg (0.01465% yield). R_f 0.51 (chloroform : methanol; 9:1); m.p. 73-75°C; UV : 218 nm (log ε 5.1); IR: 3450, 2955, 2875, 1735, 1640, 1465, 1060, 720, 705 cm^{-1}. ^1H NMR δ 5.34 (1H, m, H-18), 5.14 (1H, d, *J*=7.8 Hz, H-19a), 5.06 (1H, d, *J*=11.2 Hz, H-19b), 3.63 (1H, ddd, *J*=5.2, 6.5, 2.3 Hz, H-1′a), 3.58 (1H, ddd, *J*=6.1, 6.5, 9.2 Hz, H-1′b), 3.52 (1H, m, $W_{1/2}$=8.4 Hz, H-8), 2.34 (1H, m, H-17), 2.28 (1H, m, H_2-2a), 2.02 (1H, m, H_2-2b), 1.87 (1H, m, H-9), 1.82 (1H, m, H-13), 1.58 (1H, m, H-5), 1.53 (2H, m, CH_2), 1.47 (2H, brs, CH_2), 1.25 (26H, brs, 13×CH_2), 1.15 (2H, m, CH_2), 0.90 (3H, d, *J*=6.5 Hz, CH_3-23), 0.87 (3H, t, *J*=5.8 Hz, CH_3-8′), 0.83 (3H, d, *J*=6.5 Hz, CH_3-20), 0.78 (3H, d, *J*=6.0 Hz, CH_3-22), 0.69 (3H, d, *J*=6.5 Hz, CH_3-21). EIMS m/z (rel.int.) : 480 ($C_{31}H_{60}O_3$) $[M]^+$ (7.6), 465 (4.3), 453 (10.3), 451 (28.6), 437 (4.0), 425 (11.1), 411 (11.3), 409 (4.0), 397 (5.8), 395 (3.8), 381 (3.5), 351 (5.4), 255 (16.3), 253 (4.8), 241 (7.3), 227 (6.3), 199 (7.8), 195 (3.6), 185 (15.8), 171 (9.4), 167 (8.1), 157 (9.1), 153 (9.2), 139 (15.1), 129 (31.7), 125 (21.5), 113 (10.2), 111 (15.3), 99 (11.3), 97 (74.3), 85 (50.1), 83 (87.3), 71 (86.5), 69 (80.1), 57 (100), 55 (82.6), 43 (91.3)[13] C NMR 171.7 (C-11), 123.5 (C-18), 109.3 (C-19), 73.2 (C-8), 62.6 (C-1′), 45.1 (C-17), 42.8 (C-13), 41.3 (C-9), 39.5 (C-5), 31.7 (CH_2), 28.7 (15 × CH_2), 26.2 (CH_2) 17.3 (C-8), 15.2 (C-20), 14.3 (C-21), 14.1 (C-22), 13.8 (C-23).

Alkaline hydrolysis of 5. Compound **5** (25 mg) was refluxed with 2*M* alcoholic KOH solution for 2hr. The reaction mixture was neutralized with dilute HCl, extracted with chloroform (3×10 mL), dried over Na_2SO_4 and evaporated to obtain plucheasesterterpe-noic acid, m.p. 93-95°C; IR: 3500, 3350, 1690 cm^{-1}, formed effervescence with $NaHCO_3$ solution.

Acetylation of 5. Compound (10 mg) was heated with a mixture of Ac_2O (5mL) and pyridine (1mL) for 2hr. After usual work-up a monoacetyl derivative (**5a**) was obtained, m.p. 68-69°; IR: 1735, 1725 cm^{-1}.

Plucheasesquiterpenyl hexadecanoate 6. Fractions eluted with chloroform-methanol (95 : 5) gave colourless lustrous flakes of **6,** recrystallized from chloroform-methanol (1:1). 372 mg (0.0186 % yield).R_f 0.735 (chloroform : petroleum ether; 70 :30); m.p. 60-61°;UV (MeOH): 218 nm (log ε 3.2) IR (KBr): 3450, 2955, 2870, 1740, 1470, 1375, 1255, 1230, 1160, 702 cm^{-1}:^1H NMR δ 5.36 (1H, t, *J* = 5.4 Hz, H-10), 4.28 (1H, ddd, *J* = 8.1, 5.7, 2.3 Hz, H-1′ a), 4.15 (1H, ddd, *J* = 5.7, 6.6, 2.3 Hz, H-1′ b), 3.64 (1H, m, $w_{1/2}$=6.6 Hz, H-9′), 2.79 (1H, m, H-3), 2.33 (1H, d, J =2.3, 9.5 Hz, H-2a), 2.29 (1H, dd, *J* = 7.2, 9.5 Hz, H-2b), 2.07 (1H, m, H-9a), 2.01 (1H, m, H-9b), 1.68 (1H, brs, H-7), 1.58 (6H, brs, Me-12, Me-15), 1.30 (6H, brs, 3×CH_2), 1.25 (28H, brs, 14×CH_2), 1.02 (3H, t, *J*=5.5 Hz, Me-16′), 0.88 (3H, d, *J*=7.5 Hz, Me-13), 0.85 (3H, d, *J* = 7.5 Hz, Me-14).EIMS m/z (rel. int.) 480$[M]^+$ ($C_{31}H_{60}O_3$) (12.8), 465 (6.3), 451 (11.6), 423 (3.6), 257 (3.8), 241 (4.9), 223 (6.5), 195 (9.0), 185 (13.6), 181 (13.7), 167 (17.3), 153 (23.6), 139 (31.8), 129 (31.7), 125 (43.7), 111 (13.6), 97 (24.3), 83 (21.6), 71 (22.5), 69 (18.7), 57 (100), 55 (14.3), 43 (52.1).

Alkaline hydrolysis of 6. Compound **6** (10 mg) was heated with alcoholic 0.1*M* NaOH solution for 2 hr.The solution was extracted with chloroform (3 × 10 mL), basified with dilute HCl to *p*H 4.0 and re-extracted with chloroform (2 × 10 mL). After evaporation of the solvent sesquiterpenic acid was obtained, mp 75-76°C; IR: 3300, 1690 $cm^{1.}$ The first chloroform extraction on evaporation gave alkane-diol, mp 68-69°C, IR: 3450, 3400 cm^{-1}.

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