Isolation of monoterpene ester, chromenone and steroidal lactone from Pluchea lanceolata roots

Ramidi Ramachandram & Mohd. Ali*
Faculty of Pharmacy, Jamia Hamdard (Hamdard University), P.O. Hamdard Nagar,
New Delhi 110062, India
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Three new chemical constituents, a monoterpene ester, plucheachromenone, and plucheasterolide, have been isolated from the roots of Pluchea lanceolata and their structures established as 4-isopropylcyclohex-1-en-7-(2'-oxy-2'-methyl butyl)-oate, 2,2-dimethyl-7-acetyl-8-hydroxychromenone, and ergost-5,22-diene-3β-ol-20,28-olide, respectively by spectral data and chemical evidences.

Pluchea lanceolata C.B. Clarke (Family Asteraceae), commonly known as "Rasana", is an erect undershrub growing in Indian sandy or saline soils of Punjab, upper gangetic plain, Rajasthan and Gujarat. The plant is used for the treatment of rheumatoid arthritis. Flavonoids, pluchine, pentacyclic triterpenes, sterols and aliphatic constituents have been reported from the aerial parts of the plant. We describe herein the isolation and structural elucidation of a new monoterpene ester, a chromenone and a steroidal lactone from the roots of the plant.

Results and Discussion

Compound 1 was assigned the molecular formula C_{15}H_{26}O_2 based on its El-mass spectral data. Its IR spectrum showed absorption bands for an ester (1738 cm⁻¹) and an olefinic linkage (1630 cm⁻¹). The H-NMR spectrum of 1 displayed a one-proton downfield double doublet at δ 5.12 (J=9.5, 4.0 Hz) assigned to vinylic H-2. A six-proton broad singlet at δ 1.44 was ascribed to two equivalent tertiary C-1' and C-5' methyl groups. Two doublets at δ 0.90 and 0.84, each with J=6.5Hz, integrating for three protons, were associated with C-9 and C-10 methyls. The C-4' primary methyl group appeared as a three-proton triplet at δ 1.09 (J=6.5Hz). The remaining methine and methylene protons resonated between δ 2.28-1.49. The mass spectrum of 1 exhibited ion fragments of diagnostic importance at m/z 123 [C₅H₇ fission]⁺, 108 [123-Me]⁺, 115 [C₇H₁₅COO]⁻, 151[C₁₀H₁₅O]⁻, 121[136-Me]⁺, 87[C₃H₆]⁺, 71[C₅H₈]⁻ and 56 [71-Me]⁺, suggesting a menthene type framework with 2-methyl-2-butoxy moiety. Appearance of a prominent fragment corresponded to an isopropyl residue (m.u. 43). The C NMR spectrum of 1 showed important signals for olefinic carbon at δ 133.81 (C-1) and 120.93 (C-2), ester group carbons at δ 172.61 (C-7) and 73.19 (C-2') and methyl carbons at δ 17.23, 17.20, 27.31, 19.28 and 26.11. Alkaline hydrolysis of 1 yielded an acid which gave effervescences with sodium bicarbonate solution and showed IR absorption bands for carboxylic group.
On the basis of these evidences the structure of monoterpene ester 1 was formulated as 4-isopropylcyclohex-1-en-7-(2'-oxy-2'-methyl butyl)octoate.

Compound 2, designated as pluchearchromone, was assigned the molecular formula C_{19}H_{26}O_{5} based on its EI-mass and ^{13}C NMR spectra. Its IR spectrum showed the characteristic bands for hydrogen-bonded hydroxyl group (3336 cm⁻¹), carbonyl group (1695 cm⁻¹), and aromatic ring (1608, 1480, 938, 887 cm⁻¹). The UV spectrum of 2 exhibited absorption maxima at 202, 266, 310 nm indicating the presence of a conjugated system of a 2H-chromene. A six-proton broad signal of geminal methyl protons at δ 1.44 and a pair of olefinic protons of AB system at δ 5.58 (d, J=10.05 Hz) and 6.71 (d, J=10.05 Hz) in the ^{1}H NMR spectrum of 2 suggested that the compound possessed a 2,2-dimethylchromene skeleton. The characteristic aromatic hydroxyl proton bonded to a peri-carbonyl group appeared at δ 12.97. The absence of another pair of ortho-, ortho-coupled protons of AB system at δ 6.33 (d, J=8.85 Hz) and 7.51 (d, J=8.85 Hz), assigned to H-5 and H-6, respectively, indicated the location of the hydroxyl group at C-8 (ref. 10). A three-proton signal at δ 2.52 was typical of a methyl ketone and it was placed at C-7. Absence of any signal between δ 5.58-2.52 ruled out the existence of a primary or secondary carbinol proton. The chromone structure of 2 was supported by the ^{13}C NMR spectrum showing signals at δ 28.3 (C-11 and C-12 methyls), 77.69 (C-2), 113.82 (C-4) and 128.19 (C-3). The absence of any signal near δ 99.90, as observed in the ^{13}C NMR spectrum of sarolactone, also supported the presence of a nine-carbon-unsaturated side chain with δ-lactone structure. The ion fragments at m/z 83[C_{23}H_{33}O_{7}C_{6}H_{5}fission], 72 [C_{22}H_{27}O_{5}C_{6}H_{5}fission], 54 [C_{23}H_{33}O_{7}C_{6}H_{5}fission], 69[C_{23}H_{33}O_{7}], 201[M-83, SC]⁻, 124[C_{23}H_{33}O_{7}C_{6}H_{5}fission], 106 [124-H_{2}O], 149[302-SC], 138[C_{23}H_{33}O_{7}C_{6}H_{5}fission], 302 [M-124], 120 [138-H_{2}O], 288 [M-138]⁻ and 135 [288-SC]⁻ supported the existence of the hydroxyl group in ring-A, placed at C-3 on the basis of biogenetic considerations, and the trisubstituted olefinic linkage at C-5. The saturated nature of ring-C was inferred from the ion fragments appearing at m/z 164[C_{24}H_{34}O_{11}C_{6}H_{5}fission], 146 [164-H_{2}O], 192[C_{24}H_{34}O_{11}C_{6}H_{5}fission], 174 [192-H_{2}O], 177 [177-H_{2}O], 234[M-192]⁻ and 81 [234-SC]⁻. The ^{13}C NMR spectral data of 3 showed the existence of 28 carbon atoms in the molecule. The signals at δ 164.80, 139.31, 129.98, and 121.61 were associated with the unsaturated carbons at C-5, C-22, C-23 and C-6, respectively. The oxygen-substituted carbons resonated at δ 171.68 (C-3), 81.59(C-20) and 163.95 (C-28). The assignments of the carbon chemical shifts were made by comparison with the δ values of the corresponding carbon atoms with the carboxyclic framework of other steroids, namely lawsaritol, β-sitosterol and pluchiol. Acetylation of the compound 3 with acetic anhydride-pyridine mixture at room temperature afforded a monoacetaate 3a, thus confirming the presence of one acetylable hydroxyl group. Oxidation of 3 with Jones' reagent formed a 3-oxo.
derivative 3b which responded positively to the Zimmermann test\textsuperscript{13} for 3-ketosteroids. On this basis the structure of compound 3 has been formulated as ergost-5, 22-diene-3β-ol-20, 28-olide.

**Experimental Section**

Melting points are uncorrected. \textsuperscript{1}H and \textsuperscript{13}C NMR spectra were recorded in CDCl\textsubscript{3} at 300 MHz and 75 MHz respectively using TMS as internal standard; mass spectra on JEOL-JMS-D300/JMA-2000 system; UV spectra on Beckman DU-64 model; and IR spectra on Perkin-Elmer-882 model. Purity of the compounds was checked by TLC over Silica gel G (Merck). The spots were visualized by exposure to I\textsubscript{2} vapours, UV radiation and by spraying with perchloric acid and ceric sulphate solution.

**Plant material**

The roots of *P. lanceolata* were procured from the Khari Bawli market, Delhi and identified in the Department of Botany by Dr Khari Bawli market, Delhi and identified in the herbarium of the department. The voucher of the sample is deposited in the herbarium of the Department.

**Isolation of metabolites**

The air dried and coarsely powdered roots (1.0 kg) of *P. lanceolata* were extracted exhaustively with ethanol (95\%) in a Soxhlet apparatus. The ethanolic extract was concentrated and adsorbed on silica gel to form a slurry. The air dried slurry was subjected to silica gel column prepared in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol in order of increasing polarity to isolate the following compounds.

**Monoterpene ester 1.** Elution of the column with petroleum ether-chloroform (3:1) afforded colourless crystals of 1, R\textsubscript{f} 0.878. (chloroform-benzene-methanol, 4:3:1), yield 250 mg (0.025\%), mp 103-104\°; UV (MeOH): 202, 266, 310 nm (log ε 4.3, 6.7, 3.8); IR (KBr): 3336, 2975, 2924, 1695, 1608, 1480, 1427, 1371, 1268, 1205, 1165, 966, 938, 887, 826 cm\textsuperscript{-1}; \textsuperscript{1}H NMR: δ 12.97 (1H, D\textsubscript{2}O exchangeable, br s, OH), 7.51 (1H, d, J = 8.85 Hz, H-6), 6.71 (1H, d, J = 10.05 Hz, H-4), 6.33 (1H, d, J = 8.85 Hz, H-5), 5.58 (1H, d, J = 10.05 Hz, H-3), 2.52 (3H, br s, Me-14), 1.44 (6H, br s, Me-11, Me-12). \textsuperscript{13}C NMR: δ 77.69 (C-2), 128.19 (C-3), 113.82 (C-4), 108.28 (C-5), 101.18 (C-6), 131.64 (C-7), 159.59 (C-8), 131.64 (C-9), 115.7 (C-10), 28.29 (C-11), 28.29 (C-12), 202.72 (C-13), 26.14 (C-14), EIMS: m/z (rel. int.) 218 [M\textsuperscript{+} (C\textsubscript{10}H\textsubscript{14}O\textsubscript{3}) (96.4), 203 (96.2), 201 (12.8), 188 (5.3), 186 (100), 175 (36.3), 171 (2.5), 164 (1.8), 169 (75.6), 160 (48.6), 158 (27.1), 150 (10.2), 145 (12.8), 137 (3.4), 133 (35.5), 131 (40.3), 128 (47.5), 117 (12.5), 107 (5.3), 105 (12.3), 102 (47.8), 94 (59.7), 90 (39.1), 76 (72.1), 68 (4.8), 54 (28.3), 43 (98.7).

**Acetylation of compound 2.** A mixture of 2 (10 mg), Ac\textsubscript{2}O (3 mL) and pyridine (1 mL) was heated on a water-bath for 1 hr. After usual work-up, monoacetyl product 2a was obtained, mp 95-96\°, IR (KBr): 1725, 1690 cm\textsuperscript{-1}.

**Plucaherrnemonone 2.** Fractions eluted with petroleum ether-chloroform (1:1) gave light yellow coloured crystals of 2. R\textsubscript{f} 0.709 (chloroform-benzene-methanol, 4:3:1), yield 250 mg (0.025\%), mp 103-104\°; UV (MeOH): 202, 266, 310 nm (log ε 4.3, 6.7, 3.8); IR (KBr): 3336, 2975, 2924, 1695, 1608, 1480, 1427, 1371, 1268, 1205, 1165, 966, 938, 887, 826 cm\textsuperscript{-1}; \textsuperscript{1}H NMR: δ 12.97 (1H, D\textsubscript{2}O exchangeable, br s, OH), 7.51 (1H, d, J = 8.85 Hz, H-6), 6.71 (1H, d, J = 10.05 Hz, H-4), 6.33 (1H, d, J = 8.85 Hz, H-5), 5.58 (1H, d, J = 10.05 Hz, H-3), 2.52 (3H, br s, Me-14), 1.44 (6H, br s, Me-11, Me-12). \textsuperscript{13}C NMR: δ 77.69 (C-2), 128.19 (C-3), 113.82 (C-4), 108.28 (C-5), 101.18 (C-6), 131.64 (C-7), 159.59 (C-8), 131.64 (C-9), 115.7 (C-10), 28.29 (C-11), 28.29 (C-12), 202.72 (C-13), 26.14 (C-14), EIMS: m/z (rel. int.) 218 [M\textsuperscript{+} (C\textsubscript{10}H\textsubscript{14}O\textsubscript{3}) (96.4), 203 (96.2), 201 (12.8), 188 (5.3), 186 (100), 175 (36.3), 171 (2.5), 164 (1.8), 169 (75.6), 160 (48.6), 158 (27.1), 150 (10.2), 145 (12.8), 137 (3.4), 133 (35.5), 131 (40.3), 128 (47.5), 117 (12.5), 107 (5.3), 105 (12.3), 102 (47.8), 94 (59.7), 90 (39.1), 76 (72.1), 68 (4.8), 54 (28.3), 43 (98.7).

**Hydrolysis of 1.** Compound 1 (20 mg) was heated with 0.5 N ethanolic KOH (5 mL) for 30 min. Water (10 mL) was added to the reaction mixture, acidified with dilute HCl and extracted with CHCl\textsubscript{3} (3 x 10 mL). The CHCl\textsubscript{3} phase was washed with water, dried over Na\textsubscript{2}SO\textsubscript{4}, and evaporated to get 4-isopropylcyclohex-1-en-7-oic acid, mp 101-02\°, IR: 3250, 1690, 1610 cm\textsuperscript{-1}.

**Plucaherrnemonone 3.** Elution of the column with CHCl\textsubscript{3}-MeOH (95:5) furnished colourless crystals of compound 3. R\textsubscript{f} 0.782 (chloroform-benzene-methanol, 4:3:1), yield 150 mg (0.015\%), mp 106-107\°; IR (KBr): 3458, 2949, 2861, 1740, 1626, 1459, 1374, 1255, 1169, 968 cm\textsuperscript{-1}; \textsuperscript{1}H NMR: δ 5.36 (1H, dd, J = 6.50, 3.00 Hz, H-6), 5.14 (1H, d, J = 15.15 Hz, H-22), 5.02 (1H, dd, J = 8.50, 15.15 Hz, H-23), 3.49 (1H, br m, w\textsubscript{i/2} = 22.0 Hz, H-3 α), 1.25 (3H, br s, Me-21), 1.00 (3H, br s, Me-...
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19), 0.84 (3H, d, J = 6.5 Hz, Me-27), 0.78 (3H, d, J = 6.5 Hz, Me-26), 0.69 (3H, d, J = 6.5 Hz, Me-26), 0.69 (3H, brs, Me-18), 13C NMR δ 37.80 (C-1), 31.58 (C-2), 71.68 (C-3), 42.24 (C-4), 140.80 (C-5), 121.61 (C-6), 31.89 (C-7), 29.70 (C-8), 50.15 (C-9), 31.58 (C-10), 21.08 (C-11), 39.69 (C-12), 42.24 (C-13), 56.86 (C-14), 24.35 (C-15), 28.24 (C-16), 55.95 (C-17), 12.03 (C-18), 19.39 (C-19), 0.59 (C-20), 18.79 (C-21), 139.31 (C-22), 129.98 (C-23), 42.24 (C-24), 29.13 (C-25), 21.22 (C-26), 19.00 (C-27), 163.95 (C-28); ElMS : m/z (rel.int.) 426 [M+ (C2sH42O) (2.3), 411 (3.0), 396 (12.5), 393 (3.11), 302 (10.0), 288 (19.8), 273 (15.1), 271 (22.5), 258 (5.0), 255 (42.5), 234 (2.3), 231 (12.4), 212 (21.2), 201 (5.0), 197 (8.8), 192 (7.5), 177 (10.0), 174 (2.4), 165 (10.2), 164 (2.5), 159 (37.6), 149 (16.5), 146 (27.5), 138 (3.7), 135 (25.1), 124 (9.1), 120 (29.9), 109 (37.5), 106 (40.2), 83 (100), 81 (68.3), 72 (22.3), 69 (57.5), 54 (89.9).

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References