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Isolation and Characterization of Flowers of *Rosa damascena*

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In the present study, three compounds were isolated and characterized from ethyl acetate fraction of alcoholic extract of the flowers of *Rosa damascena*. The compounds cedr-6-en-12-ol-14-oic acid, *n*-cosan-5β-ol and 7-hydroxy-4-(3'-methyl butanoxyl)coumarin-3-en-2-one were reported.

Key Words: *Rosa damascena*, Flowers, Isolation.

INTRODUCTION

Rosa damascena (F. Rosaceae) commonly known as Gulab-ke-phul (in Hindi) is well represented in India[1] and other countries. The floweres are red, pink or white, on slender glandular-hispid and prickly pedicles, sweet and scented[2]. The plant is mild astringent, carminative, refrigerant and cardiac tonic[3]. Thirty two compounds have been characterized by GC-MS and relative retention time in the oil of *Rosa damascena*, cultivated in Kangra Valley of Himachal Pradesh, which are hexanol, heptanal, α-pinene, β-pinene, myrcene, β-phenylethyl alcohol, *cis*-rose oxide, *trans*-rose oxide, terpinene-4-ol, α-terpineol, citronellol, β-phenylethyl acetate, geraniol, citral, eugenol, geranyl acetate, methyl acetate, β-caryophyllene, α-cadinene, docosane, heptadecane, farnesol, tridecane, 9-eicosane, nonadecane, heptacosane, eicosane, tetradecanol, henicosane, pentacosane and octadecane. The major components were citronellol (40 %), geraniol (14.49 %), nonadecane (12.30 %), heneicosane (6.69 %), and phenylethyl alcohol(4.06%)[4]. Three flavonol glycosides were isolated from the flowers of *Rosa damascena* and characterized as kaempferol 3-O-α-L-arabinofuranoside, kaempferol-3-β-D-(6''-*p*-coumaroyl) glucopyranoside and kaempferol 3-O-α-L-rhamnopyranoside[5]. In the present study, flowers of the plant have been chemically characterized.

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EXPERIMENTAL

Melting points were determined on V. Scientific melting point apparatus. The IR spectra were recorded on Rx-1 Perkin Elmer FTIR. UV spectra were measured on UV-1601, UV-Visible spectrophotometer, Shimadzu. ^{1}H NMR and ^{13}C NMR were run on Gemini 200 MHz spectrophotometer and dpX 300 in DMSO, respectively. Mass spectra (EIMS) were obtained on a Jeol JMS-Dx 300 instrument.

Extraction and isolation: Authenticated floweres of *Punica granatum* was purchased from the local market, New Delhi. The dried floweres (3 kg) were powdered and extracted by maceration with absolute alcohol (15l) for 7 d. After filteration the alcoholic extract was concentrated to one-fourth of its volume by rotary evaporator. It was acidified with 10 % sulphuric acid (100 mL), warmed on waterbath for 30-45 min and fractionated with ethyl acetate (each 500 mL X5)[6] and the soluble fraction (225 g) was subjected to column chromatography over silica gel G activated at 110°C for 1 h. The elution was carried out successfully with 100 mL portions each of toluene alone, toluene-chloroform graded mixtures (95:5, 90:10... upto 100 % chloroform); chloroform-ethyl acetate and ethyl acetate-methanol similar graded mixtures[7]. The fractions were combined based on their TLC pattern and individual compounds were further purified by preparative thin layer chromatography on silica gel G (for TLC)[8] in the presence toluene-ethyl acetate-ethanol (3:3:4) as mobile phase; afforded three compounds, compound **R1** (120 mg), **R2** (92 mg) and **R3** (400 mg).

Compound **R1** (cedr-6-en-12-ol-14-oic acid): Colourless, crystalline mass; R_f 0.81 in chloroform-ethyl acetate (4.5:5.5); m.p. 56-57°C; IR ν_{max} 3450, 3420, 2926, 2854, 1717, 1630, 1463, 1376, 1245, 1175, 1096, 1032, 795 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.23 (1H, brm, H-7), 4.00 (1H, d, J=7.07 Hz, $H_{2\text{-}12a}$), 3.55 (1 H, d, J=13.26 Hz, $H_{2\text{-}12b}$), 2.48(1H, dd, J=1.98, 4.12 Hz, H-11β), 2.26(1H, d, J=4.46 Hz, H-4β), 1.98 (1H, d, J=2.5 Hz, H-1), 1.48 (2H, brm, H_2-9), 1.21 (9H, brs, XCH_2, Me-13), 0.86 (3H, d, J=5.25 Hz, Me-15), 3.41 (1H,OH), 2.23 (1H,COOH); ^{13}C NMR (DMSO-d_6): δ 29.01(C-1), 28.67 (C-2), 22.07(C-3), 33.26 (C-4), 33.49(C-5), 131.71(C-6), 129.59(C-7), 51.10(C-8), 27.21(C-9), 26.56(C-10), 31.28(C-11), 60.01(C-12), 24.41(C-13), 176.35(C-14), 13.88 (C-15); EIMS m/z (rel.int): 250$(M)^+$ ($C_{15}H_{22}O_3$) (3.5), 248(7.1), 204 (7.6), 189 (7.7), 183(5.9), 180(5.6), 178(4.1), 169(8.9), 165(7.2), 163(9.3), 158(9.1), 155(22.6), 153(13.3), 150 (29.6), 143(9.6), 139(10.1), 138(14.3), 137(16.1), 136(37.6), 125(13.7), 124(13.3), 121(23.6), 113(10.5), 111(19.3), 110(20.6), 109(15.9), 107(17.2), 104(18.3), 95(34.9), 85(20.2), 97(36.5), 83(44.5), 81(38.1), 72(39.3), 69(52.7), 67(33.2), 56(100).

Compound **R2** (*n*-cosan-5β-ol): Colourless, amorphous powder; R_f 0.85 in ethyl acetate-methanol (5:5); m.p. 73-74°C; IR ν_{max} (KBr): 3437, 2920, 2840, 1598, 1402, 1098, 794, 725, cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.54 (1H, brm, $w_{1/2}$=16.3 Hz, H-5), 1.25 (34H, brs, 17 X CH_2), 0.83 (3H, t, J=6.2 Hz, Me-1), 0.79 (3H, t, J=6.0 Hz, Me-20),3.21 (1H,OH); EIMS m/z (rel.int: 298 $(M)^+$ ($C_{20}H_{42}O$) (6.9), 283(7.5), 255(10.3), 241(5.31), 213(7.8), 211(6.6), 199(6.7), 185(11.2), 155(12.6), 149(19.6), 129(16.7), 111(15.8), 99(16.1), 97(27.2), 87(13.6), 85(73.2), 83(27.1), 71(74.3), 57(100), 43(93.8).

Compound **R3** (7-hydroxy-4-(3'-methyl butanoxyl)coumarin-3-en-2-one): Pale yellow crystals; R_f 0.68 in toluene-ethyl acetate-ethanol (3:3:4); m.p. 157-159°C; IR ν_{max} (MeOH): 3436, 2924, 2854, 1710, 1612, 1530, 1451, 1377, 1231, 1044 cm^{-1} ; ^1H NMR (DMSO-d_6) δ 6.95 (1H, d, J=7.4 Hz, H-5), 6.89 (1H, dd, J=7.4, 3.0 Hz, H-6), 6.83 (1H, d, J=3.0 Hz, H-8), 6.81 (1H, brs, H-3), 3.65 (1H, d, J=9.3 Hz, H2-1'a), 3.55 (1H, d, J=9.3 Hz, H2-1'b), 2.48 (1H, m, H-3'), 1.28 (2H, m, H2-2'), 1.21 (6H, brs, Me-4', Me-5'); ^{13}C NMR (DMSO-d_6): δ 167.60(C-2), 128.87(C-3), 165.40(C-4), 115.44(C-4a), 138.52(C-5), 119.41(C-6), 165.38(C-7), 108.83(C-8), 145.51(C-8a), 62.70(C-1'), 29.0(C-2'), 47.04(C-3'), 15.11(C-4'), 14.0(C-5'); EIMS m/z (rel.int): 248 $(M)^+$ ($C_{14}H_{16}O_4$) (4.67), 206 (10.1), 203(29.3), 184(32.6), 170(52.3), 153(100), 126(36.1), 107(32.3), 85(53.4), 55(65.6), 43(78.9).

RESULTS AND DISCUSSION

Column chromatography of the alcoholic extract led to the isolation of three compounds.

Compound **R1**, designated as rosacedrenoic acid, was obtained as a colourless crystalline mass from chloroform-methanol (1:1) eluents. It gave effervescence with sodium bicarbonate solution and decolourized bromine water indicated the presence of a carboxylic group and unsaturation in the molecules. Its IR spectrum demonstrated the presence of the hydroxyl group (3450 cm^{-1}), carboxylic acid (3420, 1717 cm^{-1}) and unsaturation (1630 cm^{-1}). Its mass spectrum displayed a molecular ion peak at m/z 250 assigning to the cedrene type sesquiterene molecular formula $C_{15}H_{22}O_3$. The important ion fragments appearing at m/z 72 [$C_{3,4}$-C_{18} fission)$^+$, 165,85 [C4,5-C1,8 fission]$^+$, 121[165-CO_2]$^+$, 153, 97[C4,5-C8,9 fission]$^+$, 66[97-CH_2OH]$^+$, 109[153-CO_2]$^+$, 138[135-Me]$^+$, 111,139[$C_{4,5}$-$C_{10,11}$ fission]$^+$, 110[135-Me]$^+$, 81[125-CO_2]$^+$ and 143,107[$C_{4,5}$-$C_{6\text{-}11}$ fission]$^+$ supported the existence of the hydroxylmethylene group at C-1 and methyl and carboxylic group at the ion peak at m/z 56[$C_{8,9}$-$C_{6,11}$ fission]$^+$, 81,169[$C_{8,9}$-$C_{8,7}$-$C_{5,6}$ fission]$^+$, 67,183[$C_{9,10}$-$C_{7,8}$-$C_{5,6}$ fission]$^+$, 204[M-HCOOH]$^+$, 189[204-Me]$^+$, 158[189-Me]$^+$, 136[169-H_2O-Me]$^+$ and 150[183-Me-H_2O]$^+$ suggested the location of the vinylic linkage at Δ^6 and another methyl group at C-11.

^{1}H NMR spectrum of **R1** displayed a deshielded one-proton broad signal at δ 5.23 assigned to vinylic H-7. Two one-proton doublets at δ 4.00 (J=7.07 Hz) and 3.55 (J=13.26 Hz) were ascribed oxygenated C-12 methylene protons. Two one-proton doublets at δ 2.26 (J=4.46 Hz) and 1.98 (J=2.5 Hz) and a one-proton double doublet at δ 2.48(J=1.98, 4.12 Hz) were accounted to methylene H-4β, H-1α and 11α protons, respectively. A three-proton doublet at δ 0.86 was associated with C-15 *sec*-methyl protons. A nine-proton broad signal at δ 1.21 was ascribed to three methylene and L-13 methyl protons. A two-proton broad signal at δ 1.48 was due to methylene $H_{2\text{-}9}$.

^{13}C NMR spectrum of **R1** exhibited important signals for vinylic carbons at δ 131.71 (C-6) and 129.59 (C-7) oxygenated methylene carbon at δ 60.01(C-12), carboxylic carbon at δ 176.35 (C-14) and methyl carbons at δ 24.41 (C-13) and 13.88 (C-15). Acetylation of **R1** with acetic anhydride and pyridine yielded a monoacetyl product. Methylation of **R1** with diazomethane formed a monomethoxy derivative.

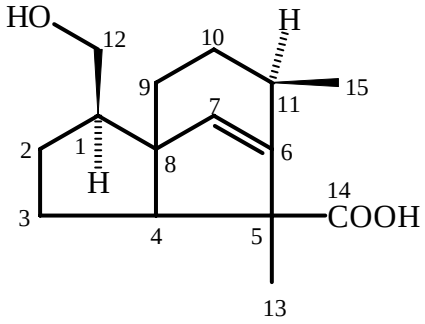
On the basis of these spectral studies, the structure of **R1** has been established as cedr-6-en-12-ol-14-oic acid.

Compound **R2**, an aliphatic alcohol, was obtained from the chloroform eluents. It did not react with tetranitromethane solution and bromine water indicating saturated nature of the molecule. Its IR spectrum displayed characteristic absorption bands for hydroxyl group (3437 cm^{-1}) and long aliphatic chain (794,725 cm^{-1}). The mass spectrum of **R2** had a molecular ion peak at m/z 298 corresponding to a saturated aliphatic alcohol $C_{20}H_{12}O$. The spectrum displayed C_nH_{2n} and C_nH_{2n-1} fragments and most of the fragments were separated by 14 mass units. The lower fragments were in higher abundance than that higher molecular weight fragments. The abundance of C_nH_{2n+1}, fragments (e.g. 57, 71, 85, 99, *etc*.) in comparison to C_nH_{2n-1} fragments (e.g. 55, 69, 83, 97, 111, 125, *etc*.) supported saturated and cyclic nature of the molecules. Generation of prominent ion peaks at m/z 241, 57 $(C_4\text{-}C_5$ fission$)^+$ and 211, 87 $(C_6\text{-}C_7)$ fission$)^+$ indicated the location of hydroxyl group at C-5.

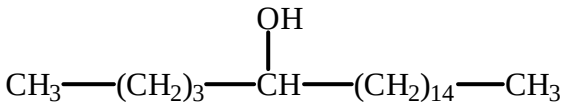
^{1}H NMR spectrum of **R2** showed one proton broad multiplet at δ 3.54 with half width of 16.3 Hz assigned to C-5 carbinol proton. Two-three proton triplet at δ 0.83 (J=6-2 Hz) and 0.79 (J=6.0 Hz) were accounted to terminal C-1 and C-2 primary methyl protons respectively. A 3 proton broad signal was associated with the methylene protons. The absence of any signal beyond δ 3.54 supported the saturated nature of the molecule. On the basis of this evidence the structure of **R2** has been characterized as *n*-cosan-5β-ol.

Compound **R3**, named rosacoumarin, was obtained as pale yellow crystalline mass. Its ultraviolet spectrum exhibited characteristic absorption

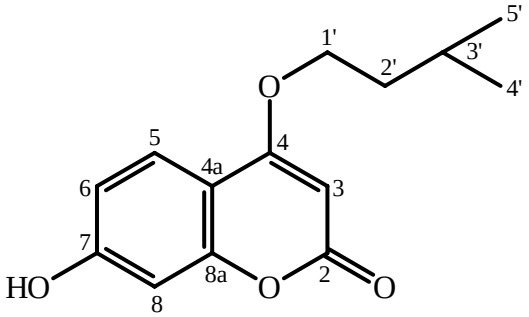
maxima at 320 nm and IR absorption bands for hydroxyl group (3436 cm^{-1}), carbonyl group (1710 cm^{-1}) and unsaturation (1612, 1530 cm^{-1}). Its mass spectrum displayed a molecular ion peak at m/z 248 corresponding to a molecular formula $C_{14}H_{16}O_4$. ^1H NMR of **R3** showed a one-proton ortho-coupled doublet at δ 6.95 (J=7.4 Hz) assigned to H-5. A one-proton ortho-meta-coupled double doublet at δ 6.89 (J=7.4, 3.0 Hz), a one-proton meta coupled proton broad signal at δ 6.81 ascribed to H-6, H-8 and H-3, respectively. Two one-proton doublet at δ 3.65 (J=9.3 Hz) and 3.55 (J=9.3 Hz) were associated with C-1' oxygenated methylene protons. A six-proton broad signal at δ 1.21 was accounted to C-4' and C-5' methyl protons. A two proton multiple at δ 2.48 was attributed to C-3' methylene proton.



Cedr-6-en-12-ol-14-oic acid (**R1**)



n-Cosan-5β-ol (**R2**)



7-Hydroxy-4-(3'-methyl butanoxyl)-coumarin-3-en-2-one (**R3**)

^{13}C NMR spectrum of **R3** exhibited signals for carboxyl carbons (δ 167.60), aromatic carbons in the range δ 165.40-108.83, oxygenated methylene carbon (δ 62.7) and methyl carbons (δ 15.11, 14.0).

On the basis of these information, the structure of **R3** has been determined as 7-hydroxy-4-(3'-methyl butanoxyl)coumarin-3-en-2-one.

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