



Chemistry of Khusinol: A Sesquiterpenoid Isolated from Vetiver Oil

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Vetiver essential oil was obtained from roots of *Vetiveria zizanioides* by hydrodistillation method. The unique feature of oil is the presence of antipodal terpenoids of which khusinol may be mentioned as representative. Khusinol, a white crystalline solid (m.p. 87 °C) isolated by column chromatography from vetiver oil was subjected to chemical transformations to afford khusinol oxides, cadina-4 α ,10 β -diol and *trans*-calamenene derivatives. The structures of khusinol and its derivatives were elucidated using spectroscopic techniques.

Keywords: Vetiver oil, Sesquiterpenoid, Khusinol, Cadina-4 α ,10 β -diol, *trans*-Calamenene.

INTRODUCTION

Vetiveria zizanioides L. commonly known as *khus* grass belonging to family poaceae is the major source of well known essential oil obtained by hydrodistillation of the fragrant roots. It is only grass cultivated worldwide for the root essential oil used in perfumery and aromatherapy [1-3]. It grows abundantly as a wild cultivated and semi wild plant in several tropical countries of Asia, Africa and Australia. Khusimol, α -vetivone and β -vetivone are the major constituents of vetiver oil extracted throughout the world [4-8]. The vetiver oil of commerce coming from Jawa and Reunion islands is dextrorotatory in nature while all the north Indian oil are laevorotatory. One of the important points of difference between highly priced variety of north Indian (Bharatpur variety) and the oil coming from foreign countries is the absence of important terpenoid vetivone in Indian oil [9,10]. The extensive chemical studies of north Indian vetiver oil revealed the presence of several sesquiterpenoids like khusinol, khusol, khusimol, khusilic acid and khusilal. Isolation of khusinol from vetiver oil and its transformation products are reported.

EXPERIMENTAL

Perkin Elmer, Model RX-1 FT-IR spectrophotometer. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz): Bruker AC (400 MHz) or mentioned otherwise as solutions (in CDCl_3) using TMS as an internal reference.

Fractionation of vetiver oil: Vetiver oil (100 g) was fractionated by chromatography over silica gel (1.5 kg) into three fractions using *viz.* petroleum ether, benzene and acetone as eluting solvents. Benzene fraction was further mixed with a

solution of semicarbazide hydrochloride (20 g) in water (200 mL) and ethanol (40 mL). The mixture was kept at 0 °C for 24 h with regular shaking. The semicarbazone was obtained in the form of yellow cake. The crude semicarbazone (30 g) obtained was chromatographed as such over silica gel (1 kg). Elution with petroleum ether afforded a complicated mixture of hydrocarbons (5 g) as evidenced by thin layer chromatography. Elution with benzene afforded a fraction containing mixture of compounds from which khusinol was isolated using extensive column chromatography.

Isolation of khusinol: Khusinol rich fraction of the oil (10 g) was subjected to column chromatography over silica gel (600 g). The column was eluted using petroleum ether: CH_2Cl_2 in order of increasing polarity. Khusinol (**1**, 2.5 g), a white crystalline solid with m.p. 87 °C was isolated using petroleum ether: CH_2Cl_2 (20 %) as eluting solvent.

Reaction of khusinol with perbenzoic acid: A solution of khusinol (1.0 g) in CH_3Cl (20 mL) was treated with 1 mol of perbenzoic acid. The reaction was allowed to complete and checked by TLC. After the completion of reaction, the reaction mixture was diluted with water, washed with sodium thiosulfate, NaHCO_3 solution, finally with water and then dried over anhydrous Na_2SO_4 . Evaporation of solvent afforded a thick yellow liquid (0.80 g). The crystalline compound (**2**, 0.5 g) with m.p. 113 °C and (**3**, 0.2 g) with m.p. 82 °C were obtained by chromatography over silica gel using petroleum ether and CH_2Cl_2 as solvents of increasing polarity. However, khusinol on treatment with excess of perbenzoic acid yielded a pure crystalline compound (**4**) with m.p. 105 °C.

Reaction of khusinol with *t*-butyl hydroperoxide in presence of vanadium oxyacetylacetonate: To a solution of

khusinol (1 g) in CH_2Cl_2 (20 mL) was added $\text{VO}(\text{acac})_2$ (5 mg) and the green reaction mixture was stirred at room temperature. A solution of *t*-butyl hydroperoxide (TBHP) (3 mL, 70 %) was added drop-wise. The colour of reaction mixture changed to brown. It was further stirred for 15 min at the same temperature. The reaction was allowed to stand for 24 h. After completion of reaction, mixture was diluted with water, extracted with CH_2Cl_2 and dried over anhydrous Na_2SO_4 . Evaporation of the solvent under reduced pressure gave a thick liquid yellow liquid (0.82 g) chromatographed over silica gel yielded the product (**2**, 0.7 g) with m.p. 113 °C.

Reaction of khusinol with N-bromosuccinimide/NaOH:

To a solution of khusinol (1 g) in acetone (4 mL) and N-bromosuccinimide (0.98 g) in water (1 mL), 0.04 g of silica was added and the reaction mixture was stirred at room temperature. To the bromodryrin of khusinol formed, aqueous NaOH was added with constant stirring. At the end of reaction, mixture was diluted with 50 mL of water and extracted with diethyl ether, washed with water and dried over anhydrous Na_2SO_4 . Evaporation of the solvent under reduced pressure gave a mixture of two products **5** and **6** separated by chromatography over silica gel.

Reaction of khusinol with mercuric acetate: To a solution of khusinol (0.5 g) in 1 mL of water and 2 mL of tetrahydrofuran, mercuric acetate (300 mg) was added in a round bottom flask. The reaction mixture was refluxed for 0.5 h and 10 mL each of 20 % aqueous sodium hydroxide and 3 N NaBH_4 were added with constant stirring. The reaction mixture was cooled down to room temperature and mercury was allowed to coalesce. The upper tetrahydrofuran layer was extracted with dichloromethane (3×25 mL) and dried over anhydrous Na_2SO_4 . The evaporation of solvent afforded a white crystalline compound (**7**) with m.p. 210 °C.

Reaction of khusinol with hydrochloric gas: A slow stream of dry hydrochloric acid gas was bubbled through a solution of khusinol (0.5 g) in diethyl ether (20 mL) at 0 °C till the solution was saturated. The reaction mixture was diluted with water and thoroughly extracted with diethyl ether (3×50 mL). The mixture was finally dried over sodium sulfate. Evaporation of the solvent yielded a thick brownish liquid with b.p. 105 °C (**8**, 0.46 g).

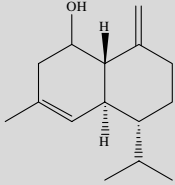
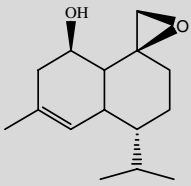
Reaction of khusinol with iodine: In a round bottom flask, khusinol (**1**, 0.5 g) in benzene (20 mL) was refluxed with catalytic amount of metallic iodine for 6 h. The progress of reaction was monitored by TLC. After the completion of reaction, the reaction mixture was diluted with water and extracted with diethyl ether. The diethyl ether layer was washed over sodium thiosulfate to remove iodine and dried over sodium sulfate. Removal of solvent gave a single product b.p. 105 °C (**8**, 0.4 g). Structure of khusinol (**1**) and its derivatives (**2-8**) were identified on the basis of IR, ^1H NMR and ^{13}C NMR spectral data (Table-1).

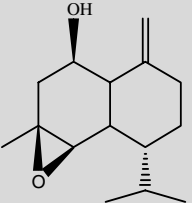
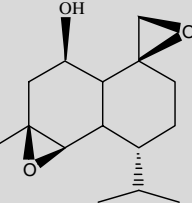
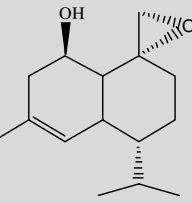
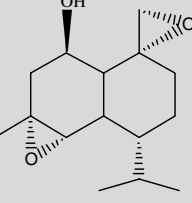
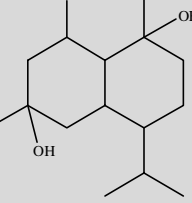
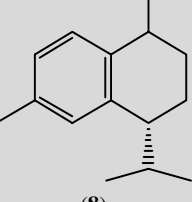
RESULTS AND DISCUSSION

North Indian vetiver oil obtained from S.H. Kelkar & Company, Mumbai. was yellowish-brown in colour having strong smell. The pH, specific gravity and refractive index of vetiver oil were 4.0, 1.004 and 1.494, respectively. The essential oil was soluble in acetone, benzene, methanol, dichloromethane and insoluble in water. Khusinol (m.p. 87 °C) was isolated by column chromatography and chemical modifications of two double bonds present in khusinol are reported. The derivatives of khusinol (**1**) synthesized *via* reaction with perbenzoic acid, vanadium oxyacetylacetonate/*t*-butyl hydroperoxide, N-bromosuccinimide, mercuric acetate/ NaBH_4 , hydrochloric gas and iodine-benzene afforded khusinol mono epoxides (**2**, **3**, **5**), khusinol diepoxides (**4**, **6**), cadina-4 α ,10 β -diol (**7**) and (-)-*trans*-calamenene (**8**) as products, respectively.

Khusinol on treatment with $\text{VO}(\text{acac})_2$ /*t*-butyl hydroperoxide resulted in the formation of monoepoxide (**2**) similar in all aspects with the monoepoxide formed with perbenzoic acid. Vanadium oxyacetylacetonate is a stereoselective reagent known for epoxidation of allylic or homoallylic alcohols. Thus the epoxide formed will be of same stereochemistry as that of the hydroxyl group present at homoallylic position. However, indirect epoxidation of khusinol with 1 mol of N-bromosuccinimide and sodium hydroxide system yielded a monoepoxide (**2**) and diepoxide (**5**) with stereochemistry opposite to that formed with perbenzoic acid and $\text{VO}(\text{acac})_2$ /*t*-butyl hydroperoxide. Reaction with hydrochloric gas and iodine resulted in aromatization of ring (**8**).

TABLE-1
SPECTROSCOPIC DATA OF KHUSINOL (**1**) AND ITS DERIVATIVES (**2-8**)

Compounds	IR (ν_{max} , cm^{-1})	^1H NMR	^{13}C NMR
 (1)	3389, 2964, 2872, 2850, 1650, 1675, 1446, 1074 and 908	0.67 (d, 3H, $J = 6.96$ Hz, C_{12}), 0.85 (d, 3H, $J = 6.96$ Hz, C_{13}), 1.63 (s, 3H, C_{15}), 3.98-4.04 (m, 1H, C_2), 4.80 and 4.71 (d each, 1H, $J = 0.5$ Hz, C_{14}) and 5.47 (brs, 1H, C_5)	52.37 (C_1), 67.26 (C_2), 38.46 (C_3), 132.21 (C_4), 121.54 (C_5), 45.49 (C_6), 47.28 (C_7), 27.33 (C_8), 37.29 (C_9), 103.77 (C_{10}), 26.69 (C_{11}), 21.56 (C_{12}), 15.08 (C_{13}), 150.95 (C_{14}) and 23.74 (C_{15})
 (2)	3460, 3064, 2958, 2930, 2870, 1446, 1069 and 1047	0.76 (3H, d, $J = 6.92$ Hz, C_{12}), 0.93 (3H, d, $J = 6.92$ Hz, C_{13}), 1.7 (3H, s, C_{15}), 2.66 (1H, d, $J = 3.52$ Hz, C_{14}), 3.18 (1H, d, $J = 3.2$ Hz, C_{14}), 3.69-3.75 (1H, m, C_2) and 5.44 (1H, brs, C_5)	46.82 (C_1), 68.45 (C_2), 38.83 (C_3), 132.69 (C_4), 120.24 (C_5), 42.27 (C_6), 46.17 (C_7), 26.52 (C_8), 35.13 (C_9), 64.85 (C_{10}), 23.48 (C_{11}), 21.44 (C_{12}), 14.93 (C_{13}), 49.94 (C_{14}) and 23.70 (C_{15})

Compounds	IR ($\nu_{\max}$, cm^{-1})	^1H NMR	^{13}C NMR
 (3)	3462, 2958, 2940, 2865, 1422, 1069 and 1047	0.69 (3H, d, J = 5.28 Hz, C_{12}), 0.55 (3H, d, J = 5.28 Hz, C_{13}), 1.07 (3H, s, C_{15}), 2.69 (1H, d, J = 11.52 Hz, C_5), 3.52-3.62 (m, 1H, C_2), 4.45 and 4.59 (1H, d, J = 0.5 Hz, C_{14})	44.20 (C_1), 60.00 (C_2), 37.24 (C_3), 46.85 (C_4), 66.01 (C_5), 37.00 (C_6), 44.39 (C_7), 26.73 (C_8), 29.66 (C_9), 150.54 (C_{10}), 27.26 (C_{11}), 21.36 (C_{12}), 15.26 (C_{13}), 104.73 (C_{14}) and 24.52 (C_{15})
 (4)	3436, 2953, 2927, 2872, 1419, 1070 and 1043	0.84 (3H, d, J = 6.8 Hz, C_{12}), 0.99 (3H, d, J = 3.2 Hz, C_{13}), 1.34 (3H, s, C_{15}), 2.64 (1H, d, J = 4Hz, C_{14}), 2.98 (1H, s, C_5), 3.13(1H, d, J = 4Hz) and 3.46-3.52 (1H, m, C_2)	43.54 (C_1), 65.01 (C_2), 41.26 (C_3), 57.08 (C_4), 59.42 (C_5), 26.61 (C_6), 41.53 (C_7), 24.49 (C_8), 37.51 (C_9), 67.63 (C_{10}), 34.95 (C_{11}), 15.28 (C_{12}), 21.37 (C_{13}), 49.65 (C_{14}) and 23.53 (C_{15})
 (5)	3430, 2966, 2927, 2908, 2861, 1425, 1047 and 875	0.77 (3H, d, J = 6.96 Hz, C_{12}), 0.95 (3H, d, J = 6.96 Hz, C_{13}), 1.68 (3H, s, C_{15}), 2.68 (1H, d, J = 3.6 Hz, C_{14}), 3.19 (1H, d, J = 3.2 Hz, C_{10}), 3.69-3.76 (1H, m, C_2) and 5.45(1H, brs, C_5)	46.82 (C_1), 68.50 (C_2), 38.84 (C_3), 132.77 (C_4), 120.27 (C_5), 42.30 (C_6), 46.17 (C_7), 26.55 (C_8), 35.15 (C_9), 64.95 (C_{10}), 23.52 (C_{11}), 21.47 (C_{12}), 14.95 (C_{13}), 50.03 (C_{14}) and 23.72 (C_{15})
 (6)	3431, 2957, 2926, 2870, 1427, 1046 and 835.	0.85 (3H, dd, J = 6.92 Hz, C_{12}), 0.98 (3H, dd, J = 6.96 Hz, C_{13}), 1.32 (3H, s, C_{15}), 2.63 (1H, d, J = 3.52 Hz, C_{14}), 2.94 (1H, s, C_5), 3.09 (1H, d, J = 3.0 Hz, C_{14}) and 3.50-3.53 (1H, m, C_2)	46.45 (C_1), 64.71 (C_2), 42.68 (C_3), 59.21 (C_4), 59.45 (C_5), 26.60 (C_6), 43.89 (C_7), 23.81 (C_8), 38.41 (C_9), 66.71 (C_{10}), 34.89 (C_{11}), 15.23 (C_{12}), 21.47 (C_{13}), 49.86 (C_{14}) and 23.27 (C_{15})
 (7)	3435, 2875, 1427, 1230 and 835	0.75 (3H, d, J = 6.88Hz, C_{12}), 0.88 (3H, d, J = 7.20 Hz, C_{13}), 1.22 (3H, s, C_4) and 1.26 (3H, s, C_{15})	54.17 (C_1), 68.82 (C_2), 48.02 (C_3), 70.80 (C_4), 39.67 (C_5), 15.16 (C_6), 45.50 (C_7), 21.00 (C_8), 42.72 (C_9), 74.08 (C_{10}), 29.72 (C_{11}), 21.51 (C_{12}), 26.25(C_{13}), 33.66 (C_{14}), and 31.99 (C_{15})
 (8)	2957, 2928, 2869, 1613, 1498, 1462 and 814	0.97 (3H, d, J = 6.96 Hz, C_{12}), 1.26 (3H, d, J = 6.96 Hz, C_{13}), 1.55 (3H, d, J = 6.88 Hz), 2.55 (3H, s, C_{15}) and 7.18- 7.38 (3H, m)	140.04 (C_1), 127.00 (C_2), 126.43 (C_3), 134.62 (C_4), 128.94 (C_5), 140.23 (C_6), 44.05 (C_7), 22.55 (C_8), 31.11 (C_9), 32.17 (C_{10}), 32.76 (C_{11}), 21.37 (C_{12}), 17.57(C_{13}), 21.52 (C_{14}) and 21.76 (C_{15})

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