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STEREOSTRUCTURE OF NORKHUSINOLOXIDE, A NEW ANTIPODAL C_{14} TERPENOID FROM VETIVER OIL. CONFIRMATION OF STEREOSTRUCTURAL FEATURES BY BIOLOGICAL EVALUATION, A NEW TOOL FOR PREDICTION OF STEREOSTRUCTURE IN CADINANES

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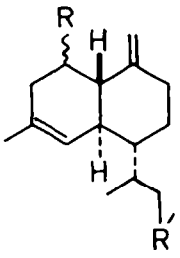
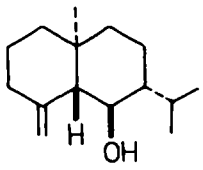
Abstract - From the alcoholic fraction of North Indian vetiver oil (Vetiveria zizanioides) a new C_{14} terpenoid, norkhusinol-oxide has been isolated. Stereostructure has been assigned to it on the basis of chemical correlation coupled with spectral data. This stereostructure was further confirmed on the basis of the comparison of its plant growth activity with that displayed by khusinol oxide of known stereostructure. It is the first report where biological evaluation has been used as a tool to confirm stereostructure of a naturally occurring terpenoid.

North Indian vetiver oil (Vetiveria zizanioides) is one of the most complex essential oils and provides a rich source of both cadinane as well as eudesmane sesquiterpenoids belonging to the antipodal types[1-4] and typical of these are khusol 1, khusinol 2 and laevo-junenol 3.



1 R=H, R'=OH
2 R=β-OH, R'=H
4 R=α-OH, R'=H



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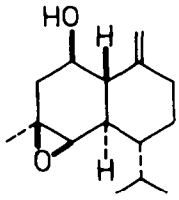

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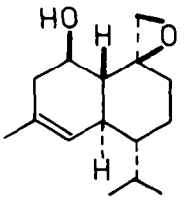
Further work from our laboratory reported the isolation of several known and new antipodal terpenoids related with khusinol and prominent among these are epikhusinol[5] 4 and isokhusinoloxide 5, the latter being isomeric with naturally occurring khusinoloxide 6. The oil has been shown to be a rich source of several interesting C_{14}-compounds like khusilal[7] and khusitone[8]. The occurrence of khusitene 7 with normal stereochemical features[9] in the oil is of particular biogenetic significance. Work from our laboratory had also recorded the isolation[10] of several aldehydes, ketones and alcohols from vetiver oil, whose structures have not been elucidated due to paucity of the materials.

Based on our recent work depicting relation between the structure of a terpenoid and its biological activity,[11-18] we set forth to isolate compounds from

the alcoholic fraction of vetiver oil.

RESULTS AND DISCUSSION

North Indian vetiver oil (Vetiveria zizanioides) was subjected to extensive column chromatography on alumina, silica gel followed by silica gel/$AgNO_3$. The oil was thus separated into fractions rich in hydrocarbons, carbonyl compounds and alcohols. In the present paper work on the alcoholic fraction of the oil is described. This fraction was subjected to further extensive chromatography and at each stage the fractions were tested for their biological activity[11] as root initiators on the hypocotyl cuttings of Phaseolus aureus. This process led to the isolation of khusol 1, khusinol 2, khusinodiol 8, (+)-α-cadinol 9, cadina-4 $^{\alpha}$, 10 β-diol 10, from the biologically inactive fractions while this procedure provided an opportunity to isolate a biologically potent fraction from which khusinol oxide 6 could be isolated as the major component. The fractions free from khusinoloxide retained the biological activity from which a new epoxy alcohol was isolated. We propose to name it norkhusinoloxide because of its structural relationship with khusinol 2.



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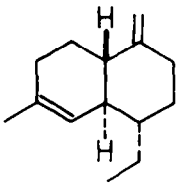

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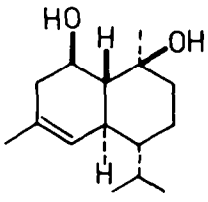

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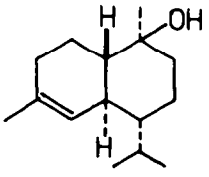

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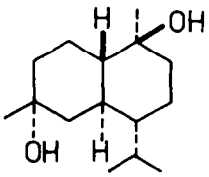

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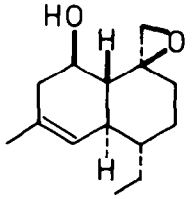

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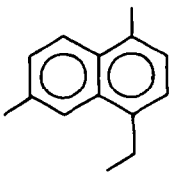
Norkhusinoloxide 11, ($C_{14}H_{22}O_2$)mp 76°; $[\alpha]_D^{20}$ - 120°, positive TNM test, end absorption in UV, showed in its IR spectrum bands due to hydroxyl group (3460 cm^{-1}) end methylenic group (3080, 1645 and 890 cm^{-1}) and a trisubstituted double bond (835 cm^{-1}). The ^1H NMR spectrum with signals at δ (ppm) 0.95 (t, 3H, J=7Hz, $-CH_2-\underline{C}H_3$), 1.62 (s, 3H, $-\dot{C}=\dot{C}-CH_3$), 5.5 (bs, 1H, $-\dot{C}=CH-$), 3.7 (bm, 1H, $W_{\frac{1}{2}}$ 20 Hz, $-\underline{C}HOH$), [2.25(d, 1H, J=4Hz); 2.8 (dd, 1H, J=2, 4Hz) $CH_2-C<$ (O)]



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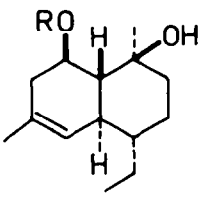
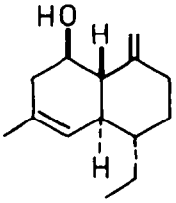


13 R=H
14 R=Ac



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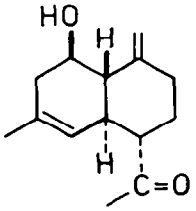

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Norkhusinoloxide on dehydrogenation afforded 1,6-dimethyl-4-ethyl naphthalene 12. Reduction of norkhusinoloxide with LAH afforded a diol 13 ($C_{14}H_{24}O_2$) mp 87°. The diol on acetylation afforded the corresponding hydroxy ester 14 which on dehydration followed by hydrolysis afforded the monol 15 as the major product. The formation of exo-olefin as the major product of dehydration of the tertiary alcohol indicates that the tertiary hydroxyl group is equatorially oriented and th erefore in norkhusinoloxide the epoxy group is β -oriented. The monol 15 on reaction with p-toluene sulphonyl chloride in pyridine at room temperature gave the corresponding tosyl derivative which on LAH reduction afforded a hydrocarbon mixture. This on chromatographic separation on silica gel/$AgNO_3$ afforded among other products(-)-khusitene $C_{14}H_{22}$, $[\alpha]_D^{20}$- 120° of known stereostructure as the major product. The obtention of khusitene, therefore, establishes the stereochemistry at C-1, C-6 and C-10 as shown in 11. Recently we have established the stereostructure of khusitoneol,[18] another C_{14} antipodal keto alcohol from North Indian vetiver oil as shown in 16. Khusitoneol 16 on Wolff Kishner reduction afforded an alcohol identical in all respects with a sample prepared from norkhusinoloxide. These data, therefore, confirm the stereostructure of norkhusinoloxide as shown in 11. Norkhusinoloxide matched in its biological activity to cause root initiation in the hypocotyl cuttings of Phaseolus aureus[11] with khusinoloxide 6. This further confirmed the position and relative stereochemistry of the hydroxyl, ring juncture hydrogen and epoxy groups cis(B) placed as is so in khusinoloxide 6. This confirmation of stereochemistry based on biological activity may prove in future a new tool in structure elucidation.

EXPERIMENTAL

Mps are uncorr. NMR spectra were measured in $CDCl_3$ with TMS as internal standard.

Isolation of Norkhusinoloxide - Vetiver oil (0.5 kg) was chromatographed over alumina (grade III, 5 kg) and was eluted with hexane, benzene followed by ether. From the benzene fraction (100 g), carbonyl compounds were eliminated as their semicarbazones. The residual fraction (85g) was chromatographed on silica gel coated with $AgNO_3$(20%, 2.5 kg). Four fractions were collected by eluting the column with benzene-ether (19:1); (9:1); (8:2) followed by ether. Each fraction was further subjected to extensive chromatography followed by preparative TLC for the isolation of different compounds. Known terpenoids were identified from their mmp and comparison of IR spectra with authentic samples.

Fraction 1 (35g, Biologically inactive) afforded khusinol 2 and α -cadinol 9

Fraction 2 (20 g, Biologically inactive) afforded khusol 1 and other unknown alcohols.

Fraction 3 (15g, Biologically active) afforded khusinoloxide 6, isokhusinoloxide 5, norkhusinoloxide 11, $C_{14}H_{22}O_2$(Found:C, 75.60; H, 9.92$C_{14}H_{22}O_2$ requires: C, 75.63, H, 9.97%) mp 76°,$[\alpha]_D^{20}$- 120° alongwith other new epoxy alcohols.

Fraction 4 (10g, Biologically inactive) afforded khusinodiol 8, Cadina-4α, 10β -diol 10 alongwith other compounds.

Dehydrogenation of 11 50 mg of 11 was sealed under vacuum in a glass tube with 50 mg of Pd/C (5%) and heated at 200° for 12 hr. PLC of the product afforded 30 mg of 1,6-dimethyl-4-ethyl naphthalene 12. TNB derivative, mp 135° undepressed on admixture with an authentic specimen.

Diol 13 Reduction of 600 mg of 11 with LAH yielded 550 mg of 13, $C_{14}H_{24}O_2$ mp 87°. IR (γ, cm^{-1}): 3400, 1660, 830. ^1H NMR (δ, ppm) 0.90 (t_J 3H, $-CH_2-\underline{CH_3}$), 1.34 (s, 3H, $-C(OH)\underline{CH_3}$), 1.65(s, 3H,$-\dot{C}=\dot{C}-\underline{CH_3}$), 5.52(bs, 1H, -C=CH-), 3.65(bm, 1H, $W_{\frac{1}{2}}$ 20Hz, $-\underline{CH}OH$). (Found: C, 74.90; H, 10.80. $C_{14}H_{24}O_2$ requires: C,74.95;H,10.78%).

Acetylation of 13 Acetylation of 500 mg of 13 with Ac_2O/Py at room temperature gave hydroxy ester 14 (550 mg). IR (γ, cm^{-1}); 3400, 1735, 1660, 840. ^1H NMR (δ, ppm) 0.92 (t, 3H, J=7Hz, $-CH_2-\underline{CH_3}$), 1.34 (s, 3H, $-C(OH)\underline{CH_3}$), 1.60(s,3H,-C=C-$\underline{CH_3}$), 4.35 (bm, 1H, $W_{\frac{1}{2}}$ 20 Hz, $-\underline{CH}O$ Ac), 5.5(bs, 1H, -C=CH-). (Found: C,72.18; H, 9.82. $C_{16}H_{26}O_3$ requires: C, 72.14; H, 9.84%).

Dehydration of diolmonoacetate 14 Diol monoacetate (500 mg) was dissolved in a mixture of pyridine-benzene (2:1, 5 ml) and a solution of $POCl_3$(1 ml) in benzene (2 ml) was added dropwise at room temperature. After 30 min under vigorous stirring the product (480 mg) was hydrolysed with alcoholic KOH and after usual work up a crude crystalline alcohol (15, 350 mg) was obtained. It was purified by further crystallization from pet.ether, mp 86°. IR (γ, cm-1): 3500, 1640, 892, 830. ^1H NMR (δ, ppm) 0.92 (t, 3H, J=7Hz, $-CH_2-\underline{CH_3}$), 1.62(s, 3H, $-C=C-\underline{CH_3}$)

3.72(bm, 1H, $W_{\frac{1}{2}}$ 20 Hz, -CHOH), 5.5(bs, 1H,-C=CH-),4.4, 4.7(s, 1H each, >C=CH_2). (Found: C, 81.56; H, 10.72. $C_{14}H_{22}O$ requires: C, 81.50; H, 10.75%).

(-)-Khusitene from 15 Monol 15, (0.3 g) on reaction with p-toluene sulphonyl chloride (0.4 g) and pyridine (5 ml) afforded a crude tosylate which on LAH reduction at reflux for 10 hr. followed by chromatography of the product on silica gel/$AgNO_3$ afforded pure(-)-khusitene $[\alpha]_D^{20}$ -120°, superimposable IR spectrum on (+)-khusitene.

Synthesis of 15 from khusitoneol Khusitoneol (0.2 g) in ethanol (2 ml),diethylene glycol (6 ml) and hydrazine hydrate (1 ml) under the conditions of Wolff Kishner reduction afforded a product which on chromatography afforded 15 (120 mg) mp and mmp with an authentic sample 86°.

Comparison of plant growth activity of norkhusinoloxide with khusinol oxide The root initiation studies on hypocotyl cuttings of Phaseolus aureus were done according to the standard procedure[11] The effect of different concentrations of compounds on the number of roots is given below.

	Number of roots			
Treatment (mg/l)	10	20	30	40
Khusinoloxide	6.5 ± 1.6	14.2 ± 1.8	16.9 ± 1.8	24.3 ± 1.6
Norkhusinoloxide	7.0 ± 1.3	14.0 ± 1.0	18.0 ± 0.8	25.0 ± 1.2

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