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BIOGENETIC IMPLICATIONS OF THE ANTIPODAL SESQUITERPENES OF VETIVER OIL*

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Abstract—Oil of vetiver samples of Haiti, Reunion, and North Indian origin have been examined by GLC. The North Indian variety (Khus Oil) is distinctly different and represents a chemically distinct race of *Vetiveria zizanioides* (Gramineae) or perhaps a distinct species. The biogenetic implications of the antipodal sesquiterpenes isolated from vetiver oil varieties are reevaluated in light of these results.

INTRODUCTION

OIL OF vetiver is undoubtedly one of the most complex of the essential oils and is unique in containing both cadinane and eudesmane sesquiterpenes of the rare antipodal configuration. Of these khusol (VIIa), khusinol (VIIb), khusitone (VIIc), (−)-γ_2-cadinene (VIII), and laevojuneol (X) have been isolated from North Indian variety of the oil.[1] Constituents of the more typical oils (Haiti, Java, Reunion, Congo, Zambia) are veticadinol (IX,[1] relative and absolute stereochemistry unknown), the vetivones (V, VI),[2] other sesquiterpenes of the nootkatane type (II,[3a, 4] III[5]), and a number of representatives of the zizaane structure (I).[3] The recent structure revisions for α-vetivone[6] (isonootkatone,[6b] V) and β-vetivone [6βH-vetispira-2,8(11)-dien-4-one, VI][7a] indicate that the various "vetivane" sesquiterpenes may also be derived from antipodal (or 4,10-*epi*) eudesmane precursors. Thus the vetivones can be considered as derived from a eudesmane such as IV by migration of the C-10 CH_3 (→V) or ring carbon

* Part I in a projected series on vetiver oil constituents.

[1] The structures, physical constants, and references for these substances appear in G. OURISSON, S. MUNAVALLI and C. EHRET, *International Tables of Selected Constants*, Vol. 15 (Sesquiterpenoids), Pergamon Press, Oxford (1966).

[2] A. ST. PFAU and PL. A. PLATTNER, *Helv. Chim. Acta* **22**, 202 (1939); **23**, 768 (1940); Y. R. NAVES and E. PERROTTET, *Helv. Chim. Acta* **24**, 3 (1941).

[3] A number of sesquiterpenes previously assigned to different classes have now been correlated with zizaene: (a) N. HAMAYAMA, F. KIDO, R. SAKUMA, H. UDA and A. YOSHIKOSHI, *Tetrahedron Letters* 6099 (1968); (b) F. KIDO, H. UDA, A. YOSHIKOSHI, *Tetrahedron Letters* 1247 (1968); (c) I. C. NIGAM, H. KOMAE, G. A. NEVILLE, C. RADECKA and S. K. PAKNIKAV, *Tetrahedron Letters* 2497 (1968); and (d) R. SAKUMA and A. YOSHIKOSHI, *Chem. Commun.* 41 (1968).

[4] S. TAKAHASHI, *Chem. Pharm. Bull.* **16**, 2449 (1968).

[5] The major hydrocarbon of the Reunion oil, β-vetivenene, previously assigned a hydroazulenic structure,[1] has now been assigned structure III. The degradative studies for this compound and a number of related "vetivanes" will be published soon.

[6] (a) K. ENDO and P. DEMAYO, *Chem. Commun.* **89** (1967); (b) J. A. MARSHALL and N. H. ANDERSEN, *Tetrahedron Letters* 1611 (1967).

[7a] J. A. MARSHALL, N. H. ANDERSEN and P. C. JOHNSON, *J. Am. Chem. Soc.* **89**, 2748 (1967); J. A. MARSHALL and P. C. JOHNSON, *J. Am. Chem. Soc.* 2750 (1967).

[7b] The skeleton of β-vetivone is designated as vetispirane (or agarospirane). The sesquiterpene alcohol, agarospirol[8] (a stereoisomer of hinesol?), of unknown stereochemistry, has been assigned this skeleton.

C-9 (→VI). Unfortunately none of the sesquiterpenes from these oils has established stereochemistry at the isopropyl group; in fact vetiver oil constituents (including a number of new ones of as yet undetermined structure)[5] generally have an sp^2-center at the attachment of the isopropyl group and thus one cannot distinguish the origin (totally antipodal or 4,10-*epi*-precursors) of these constituents.*

Although the stereochemical implications of Ruzicka's biogenetic postulates have found wide acceptance, little attention has been paid to any possible implications of absolute stereochemistry correlations.[8] Eudesmanes, guaianes, and various sesquiterpenes related to these by Wagner–Meerwein rearrangements have generally been considered as further transformation products of *trans,trans*-farnesyl pyrophosphate whereas bisabolanes and cadalanes were derived either directly or circuitously from *cis,trans*-farnesyl pyrophosphate. As yet, no inter-relation of these two series has appeared and thus there is no explanation for the generally observed correlation in absolute configuration of sesquiterpenes of different classes from a single plant source. It is our contention that possible explanations should be considered and that the study of an exceptional case (oil of vetiver) might be the most direct route to an understanding of the chemical basis for the rule.

RESULTS AND DISCUSSION

A biogenetic scheme (an extension of that recently proposed by Hirose *et al.*)[9] which can serve as a working hypothesis is shown on Chart 2. In this scheme the sesquiterpene types are correlated to proposed ten-member ring intermediates which could be interconvertible via hydride shifts or *cis,trans* olefin isomerization. Entry into this system could occur at cyclodecadienyl cations *A*, *B*, *C* or *D* by the appropriate, enzymic cyclization of a nerolidol or farnesol derivative. Thus a single enzymic cyclization (in which one asymmetric center is established) can account for all of these sesquiterpene types and thus for the observed correlation in absolute stereochemistry. One possible modification of this scheme is suggested by a number of absolute stereochemistry determinations in the literature and by our conviction that the 7β-isopropyl group is the feature of singular importance in assessing the absolute configurational class of a sesquiterpene. These observations are: that (−)-α-copaene and (+)-α-ylangene consistently co-occur in essential oils (an obvious exception to the absolute configurational homogeneity rule if the current structure, as used in Chart 2, of (+)-α-ylangene is correct); and that both (+)-ϵ-bulgarene[12a] and (−)-γ-amorphene[12b] afford S(+)-isopropylsuccinic acid (also obtained from cadinanes and muurolanes having a 7β-isopropyl group) on vigorous oxidation. The modification suggested by these observations is

* It should be noted that hinesol (which has been converted to the antipode of natural β-vetivone[10a]) is a congenor of β-eudesmol in attractytol.[10b]

[8] An excellent recent review of sesquiterpene biogenesis covers the earlier work as well as adding to it: W. PARKER, J. S. ROBERTS and R. RAMAGE, *Quart. Rev.* **21**, 331 (1967).

[9] Y. OHTA, K. OHARA and Y. HIROSE, *Tetrahedron Letters* 4181 (1968).

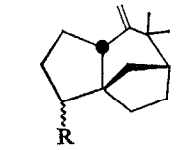
[10] (a) I. YOSIOKA and T. KIMURA, *Chem. Pharm. Bull.* **13**, 1430 (1965); (b) I. YOSIOKA, S. TAKAHASHI, H. HIKINO and Y. SASAKI, *Chem. Pharm. Bull.* **7**, 319 (1959).

[11a] The difference i.r. spectra can be used to identify the source of vetiver oil.[11b] That of Khus Oil shows a lack of vetivones and the absorption bands are those of khusilal.

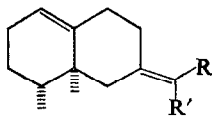
[11b] G. S. K. RAO, I. C. NIGAM, J. C. BARTLET and L. LEVI, *Toilet Goods Assoc. Scientific Section, Proc.* **38**, 5 (1962).

[12a] R. VLAHOV, M. HOLUB and V. HEROUT, *Coll. Czech. Chem. Commun.* **32**, 822 (1967).

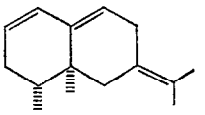
[12b] O. MOTL, M. ROMANUK and V. HEROUT, *Coll. Czech. Chem. Commun.* **31**, 2025 (1966).



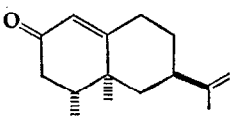
*(Ia) R = β-CH_3
(Ib) R = β-CH_2OH
(Ic) R = β-CO_2H
(Id) R = α-CO_2H



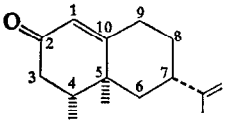
*(IIa) R′ = CH_2OH; R = CH_3
(IIb) R = CO_2H; R′ = CH_3



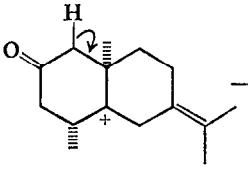
(III)
"β-Vetivenene"



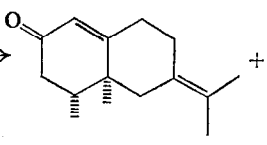
Nootkatone



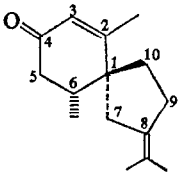
7-*epi*-Nootkatone



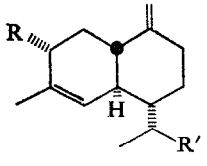
(IV)



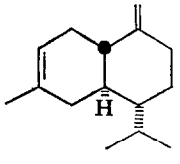
(V)
"α-Vetivone"



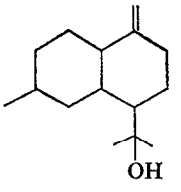
(VI)
"β-Vetivone"



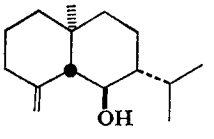
* (VIIa) R = H; R′ = H, CH_2OH
(VIIb) R = OH; R′ = CH_3
(VIIc) R = H, R′ = O



(VIII)
(−)-γ_2-Cadinene



(IX)
Veticadinol



(X)
Laevojuneol

*(Ia) = Zizaene
(Ib) = Khusimol
(Ic) = Zizanoic acid
(Id) = Epizizanoic acid

(IIa) = Bicyclovetivenol
(IIb) = Isovalencenic acid

(VIIa) = Khusol
(VIIb) = Khusinol
(VIIc) = Khusitone

CHART I. OIL OF VETIVER COMPONENTS.

the elimination of the step involving two 1,2 H-shifts that inverts the isopropyl center. This modification strengthens the basis for an absolute configurational homogeneity rule, since a single enzymic cyclization can account for all of the sesquiterpene types having a 7β-isopropyl group.

The unique constituents of oil of vetiver suggest the following lines of inquiry. (1) A search for totally antipodal cadinanes and eudesmanes in *typical* vetiver oils, (2) a search for

Note added in proof—A correlation of (+)-α-ylangene and (−)-α-copaene appeared after this communication was submitted (Y. OHTA and Y. HIROSE, *Tetrahedron Letters* 1601 (1969)). Both sesquiterpenes can now be regarded as having a β-isopropyl group.

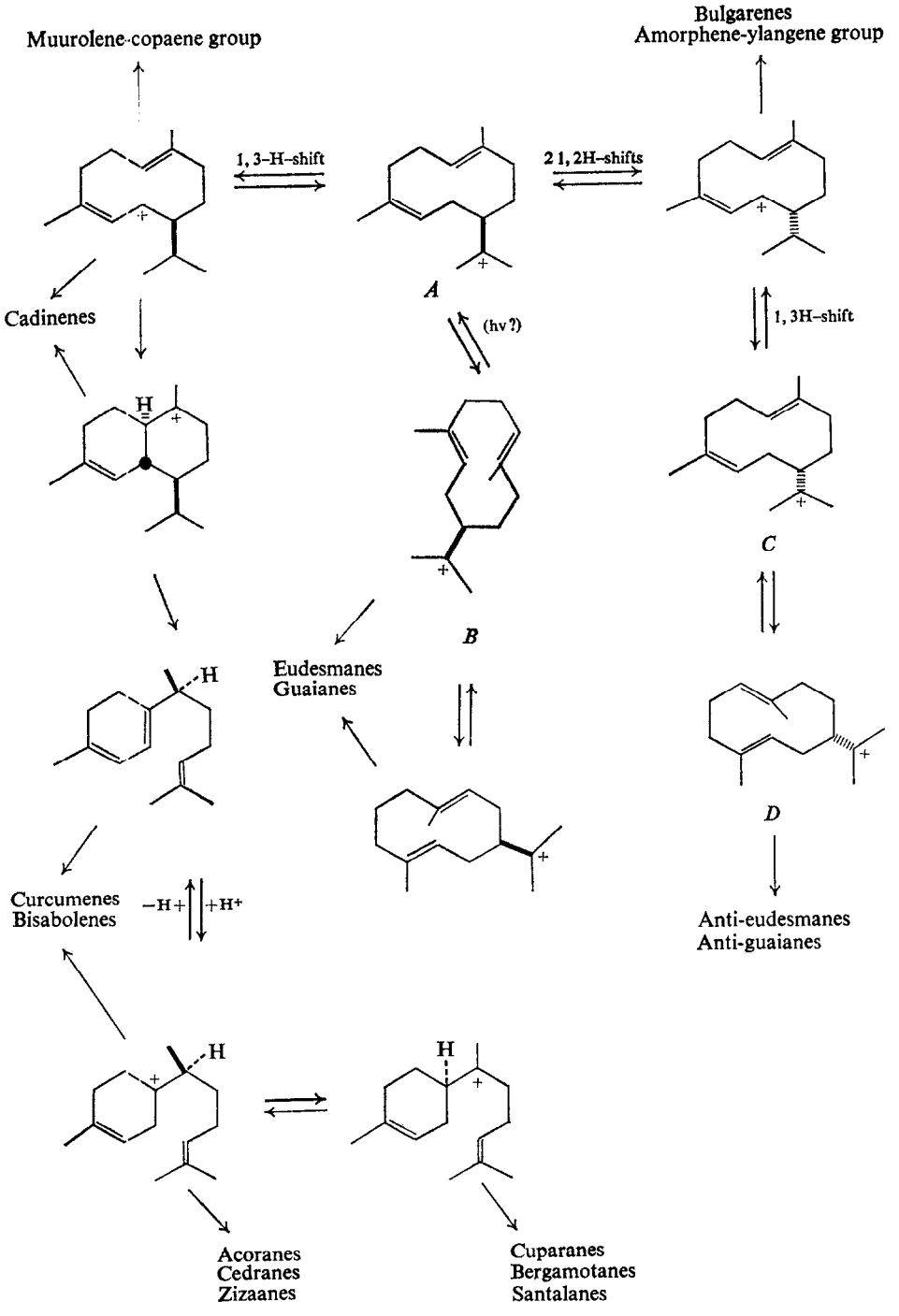


CHART 2. BIOGENETIC PATHWAYS (FOR "NORMAL" SESQUITERPENE TYPES).

bulgarenes or amorphenes, and (3) an explanation for the unusual predominance of constituents having an sp^2 center at the point of attachment of the isopropyl group.

Concerning the first point—the known constituents of the Khus Oil cannot be used in the argument until they have been shown to be typical of vetiver oils. To this end we have examined a number of vetiver oils of Haiti and Reunion origin as well as a sample of the North Indian variety of the oil. Comparison with a previous study of vetiver oil variation with geographic origin suggests that our samples were typical of those from these producing districts.[11] The figure shows three representative chromatograms: A, the total oil of Reunion

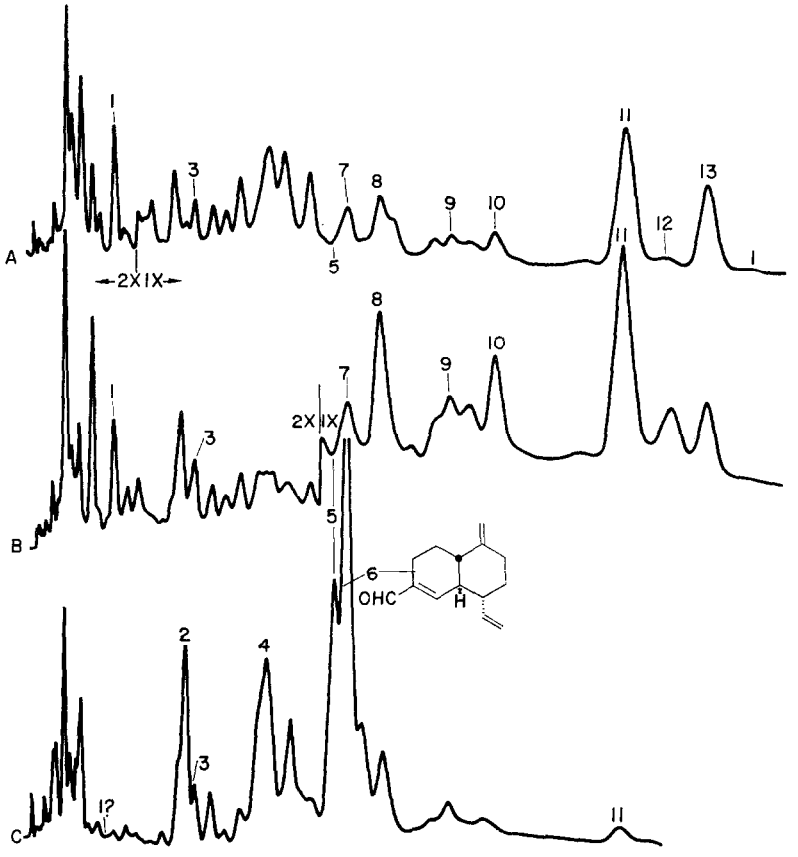


FIG. 1. GLC TRACES OF: A, REUNION VETIVER OIL; B, HAITI VETIVEROL FRACTION; C, KHUS OIL. The numbered constituents are: (1) β-vetivenene, (2) bharatpurol-I[13], (3) laevojuneol, (4) bharatpurol-II,[13] (5) khusinol, (6) khusilal, (7) a mixture of at least three alcohols, not khusilal, (8) vetiverol-d,[13] (9) vetiverol-g,[13] (10) vetiverol-c,[13] (11) β-vetivone and/or khusimol, (12) "nootkatone", (13) α-vetivone.

origin; B, the main fraction of Haiti oil after the removal of ketones with Girard Reagent-T; and C, total Khus Oil. Although there were minor differences between all the samples—the Haiti oil contains a larger proportion of higher-boiling polar alcohols—only Khus Oil was

[13] Vetiverol-c, vetiverol-d, vetiverol-g, bharatpurol-I, and bharatpurol-II are new sesquiterpene alcohols presently under investigation.

significantly different. It appeared to contain no vetivones and its major constituent, khusilal, is not found in the typical oils. The strong laevorotation of the North Indian vetiver oils is in fact due to the large amount of khusilal in these oils. The different i.r. spectra confirms the importance of khusilal in the North Indian oils.[11] The antipodal cadinane alcohols—khusinol and khusol (at greater retention time than shown on the traces pictured)—found in Khus Oil are absent from the Haiti and Reunion oils. The typical oils did have a peak that appeared to be laevojuneol but we have not yet isolated this component. The peak (in Khus Oil) at the retention time of β-vetivone is probably khusimol* or some other alcohol, as TLC did not show a spot for vetivone.† In addition, β-vetivenene (III)[5] is a major hydrocarbon constituent in the Reunion and Haiti oils but not in the North Indian oil. Thus there is no evidence for the presence of antipodal cadinene derivatives in the typical oils and no evidence for nootkatene related materials in Khus Oil. Previous data[11b] and that presented above suggest that the North Indian vetiver is sufficiently distinct to constitute another species.

In light of these results the constituents of a single variety of vetiver must be studied in detail prior to any further discussion of sesquiterpene biogenesis in vetiver, and this is in progress.

Concerning the third line of inquiry, the vetivones themselves seem to offer a clue. The fresh oils (Haiti and Reunion) contain four isomeric vetivones (α-, β-, and two unidentified) but no peak which corresponds to nootkatone (by GLC at 200°). However, after extensive column chromatography or preparative GLC, these samples contain nootkatone, α- and β-vetivone and only one unidentified vetivone. In addition GLC analysis at 225° or with higher injection port temperatures shows nootkatone in all samples. We wish to suggest that one of these vetivones is 7-epi-nootkatone which owes its instability to the 1,3-diaxial $CH_3/C(CH_3)=CH_2$ interaction and that this might be the explanation for the predominance of sesquiterpenes with an sp^2 center at the isopropyl group in this oil.

EXPERIMENTAL

Analytical and preparative GLC was carried out on an F & M Scientific 700 Laboratory Chromatograph fitted with a sensitive (WX filaments) thermal conductivity detector. The analytical columns used were 16–50 ft × 0·125 in. with a stationary phase loading of 0·5–3 per cent on silanized Chromasorb-G. Preparative GLC columns were 12–16 ft × 0·375–0·50 in. with a stationary phase loading of 6–8 per cent on Chromasorb G or W. TLC was performed on commercial precoated plates (250-μm layers) of Merck alumina or silica incorporating an inorganic indicator. Column chromatography was performed on acid-wash Merck alumina, Woelm basic alumina (activity I, for hydrocarbons), or Mallinckrodt Silicar CC-7 (100–200 mesh).

Separation and Examination of Vetiver Oil Samples

Vetiver oil samples were fractionated through a small column affording: (*a*) the hydrocarbon fraction, b.p. 120–140° (12 mm); (*b*) the lower vetiverols, b.p. 90–110° (0·2 mm); (*c*) the vetiverol fraction,‡ b.p. 111–115° (0·2 mm); and (*d*) the vetivone fraction,§ b.p. 116–125° (0·2 mm).

The whole oils and the various fractions were examined by GLC on Carbowax 20-M and silicone oil columns at 130–225°. β-vetivenene,‖ α-vetivone,‖ and β-vetivone‖ could be obtained in pure form by direct preparative GLC[6b] of the appropriate fractions.

Direct preparative GLC of the vetiverol fractions was not as successful. Prior chromatography on acidic alumina using a hexane–CH_2Cl_2 gradient gave enriched fractions of vetiverols c, d and g, bharatpurol-I and -II, and khusimol suitable for further purification by GLC.[13] Khusimol (Ib) was identified by i.r., and NMR spectroscopy and by conversion to zizaene (Ia).

* Khusimol occurs at the same retention time as β-vetivone on Carbowax 20M columns.

† The vetivones quench fluorescence and show a number of characteristic colors with various visualizing reagents (see Experimental).

‡ Also contains vetivones. These can be removed by treatment with Girard Reagent T.

§ Also contains vetiverols.

‖ Identified by u.v., i.r., and NMR spectral comparison with the literature values.

The whole oils, and various fractions, were also examined by TLC. On silica (two developments with CH_2Cl_2) the following R_fs were obtained: sesquiterpenes hydrocarbons, 0·84; sesquiterpenes oxides, 0·63–0·80; tertiary vetiverols, 0·46–0·58; major vertiverols, 0·30 and 0·40; α- and β-vetivone, 0·33. On alumina or with CH_2Cl_2–THF (20:1) as the developing solvent the vetivones run much faster than the vetiverols. The plates were viewed under u.v. light (to locate the vetivones) and then permanently visualized by spraying with 3% $Cu(OAc)_2$ in 15% aq. H_3PO_4 followed by heating on a hot plate. This visualizing reagent gives characteristic colors for various types of sesquiterpenes: red → indigo for hydrocarbons, yellow → green for conjugated ketones red-violet or blue-violet for sesquiterpene alcohols.

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