Essential Oils and Their Constituents. Isolation of Aromatic Sesquiterpenes from Reunion Vetiver Oil

ISAAC MIZRAHI* and ISHWAR C. NIGAM†

Abstract The occurrence of three aromatic sesquiterpene hydrocarbons in Réunion vetiver oil is reported for the first time. One of these was identified as α-calacorene. The others were found to be new compounds. Spectral and gas chromatographic characteristics as well as the results of hydrogenation and dehydrogenation experiments on the new sesquiterpenes, called A and B, are recorded. Sesquiterpene A may be tentatively represented as a dehydrocurcumene (VI), while sesquiterpene B is a cadalene-type hydrocarbon.

Keyphrases ☐ Sesquiterpenes, aromatic—Réunion vetiver oil ☐ α-Calacorene—isolation, identity ☐ Column chromatography—separation ☐ IR spectrophotometry—identity, structure ☐ UV spectrophotometry—identity, structure ☐ GLC—separation, structure

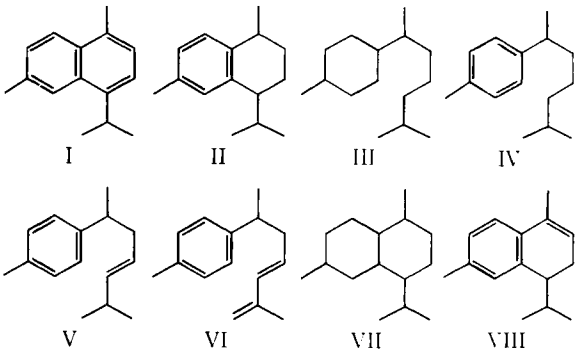
Several sesquiterpene hydrocarbons possessing mono-, bi-, and tricyclic structures have been isolated from oil of vetiver (1–7). The present communication describes, for the first time, the isolation of aromatic sesquiterpene hydrocarbons from Réunion vetiver oil.

The hydrocarbon fraction, obtained by rectifying the oil and collecting a forerun boiling up to 144° at 16 mm., was purified by chromatography on alumina and refractionated (reflux ratio 20:1). Successive cuts (2 ml. each) were examined by gas chromatography and IR spectroscopy. The gas chromatograms of Fractions No. 16 to 22 indicated marked compositional similarities and IR spectra suggested the presence of aromatic components. Repeated column chromatography on alumina and preparative scale gas chromatography on columns of silicone nitrile and Reoplex led to the isolation of an alicyclic hydrocarbon (peak No. 1, Fig. 1), which appeared from its IR spectrum and rotation to be the *dextro*-rotatory isomer of γ_2-cadinene (2), and three aromatic compounds (peak Nos. 2, 3, and 4) whose characterization is described below. Two of the aromatic constituents (peak Nos. 2 and 3) proved to be hitherto unknown compounds and have been tentatively designated as sesquiterpene A and sesquiterpene B, respectively.

CHARACTERISTICS OF COMPOUNDS

Peak No. 2—Sesquiterpene A—Its IR spectrum (Fig. 2a) showed characteristic aromatic absorptions at 1,495, 1,585, and 1,605 cm.$^{-1}$. In addition, strong bands were observed at 885 and 1,645 cm.$^{-1}$ (terminal methylene group) and at 1,672 cm.$^{-1}$ (second olefinic linkage). Absence of a doublet in 1,380 cm.$^{-1}$ region suggested the absence of an isopropyl group. A broad UV absorption band in the 228–238 mμ region (ϵ = 3,165) indicated the presence of conjugated double bonds. Lack of strong absorption in the 240–250 mμ region, on the other hand, ruled out double bond-phenyl ring conjugation, *cf.*, *e.g.*, spectral data of styrene and its alkyl derivatives (8–11).

When the compound was subjected to dehydrogenation by means of reaction gas chromatography (12, 13), cadalene (I, 15%) and an unidentified compound (45%) were obtained. The IR spectrum of the latter product resembled closely that of calamenene (II, 14) but its UV spectrum lacked the strong band exhibited by calamenene at 228 mμ. The yield of cadalene (15%) was lower than that (25–35%) obtained when cadinenes are reacted similarly.



Partial hydrogenation of the sesquiterpene yielded bisabolane isomers (III), dihydrocurcumene (IV), and a new aromatic hydrocarbon. The latter exhibited characteristic IR absorption bands at 1,510 cm.$^{-1}$ (phenyl ring), 1,390, and 1,372 cm.$^{-1}$ (isopropyl group), and 960 cm.$^{-1}$ (*trans* disubstituted double bond). Its UV spectrum showed characteristic aromatic absorptions between 254 and 272 mμ. Absence of strong absorption between 240 and 250 mμ suggested absence of conjugation between the double bond and the benzene ring. The side chain double bond is, however, conjugated to the other double bond (*viz.*—terminal methylene group) in sesquiterpene A (see above). Hence, the new aromatic hydrocarbon obtained by partial dehydrogenation of A may be assigned Structure V and sesquiterpene A may be represented as a dehydrocurcumene, VI.

The formation of cadalene during dehydrogenation of sesquiterpene A appears to be the result of ring closure, a phenomenon observed during the dehydrogenation of certain monocyclic sesquiterpenes (15–18).

Peak No. 3—Sesquiterpene B—Its IR spectrum (Fig. 2b) exhibited characteristic absorptions at 1,605 and 1,495 cm.$^{-1}$ (aromatic ring), at 1,640 and 885 cm.$^{-1}$ (terminal methylene group), and at

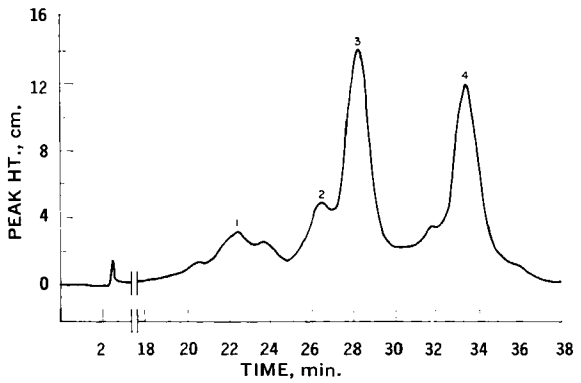


Figure 1—*Gas chromatogram of a typical fraction containing aromatic hydrocarbons. Column: silicone nitrile.*

Table I—Gas Chromatographic Characteristics of Sesquiterpenes

Peak No.[a]	Constituent	Relative Retention Time[b] Reoplex 400	Silicone Nitrile
1	+γ_2-Cadinene	—	1.47
2	Sesquiterpene A	1.05	1.75
3	Sesquiterpene B	1.28	1.87
4	α-Calacorene	1.78	2.20

[a] See Fig. 1. [b] Reference standard: naphthalene (retention time: on Reoplex 400, 17.2 min.; silicone nitrile, 15.3 min.).

840 and 820 cm.$^{-1}$ (trisubstituted ethylenic linkage). The UV absorption spectrum showed strong absorptions at 228, 234, and 244 mμ and a weak band at 274 mμ, (aromatic ring conjugated with at least one double bond). On subjecting the substance to dehydrogenation gas chromatography (12–13) cadalene (I, 50%) was obtained, while hydrogenation yielded cadinane isomers (VII). Thus, sesquiterpene B possesses a cadalenic structure. On the basis of its spectral characteristics, it appears to be a new compound.

Peak No. 4—α-Calacorene—This constituent exhibited characteristic aromatic absorptions throughout the IR (1,885, 1,608, 1,562, and 1,491 cm.$^{-1}$) and UV regions (266, 227, 220, and 213 mμ). On gas chromatographic dehydrogenation it yielded cadalene (43%). Comparison of the UV and IR spectra of the substance with those published in the literature (14) proved it to be α-calacorene (VIII).

EXPERIMENTAL

Fractional distillations were carried out employing a precise fractionation assembly (Todd). IR spectra were determined in carbon tetrachloride solution (using a Perkin-Elmer Model 221 instrument). UV spectra were recorded in ethanol solution, by means of a spectrophotometer (Beckman DB).

Gas Chromatographic Analysis—Two instruments operated under the conditions described below, were employed:

(1) Burrell Kromo-Tog K2; column, Reoplex 400 (20%) prepared as described before (19); temperature, 160° for vetiver oil fractions and isolates, 220° for products of dehydrogenation; carrier gas, helium (75 ml./min.).

(2) Aerograph A-700; column, aluminum tube 3.66 m. × 0.93 cm. (12 ft. × 3/8 in.); packing, silicone nitrile (10%) on 60-80 mesh acid-washed diatomite aggregate;[1] temperature 170°; carrier gas, helium, 120 ml./min.

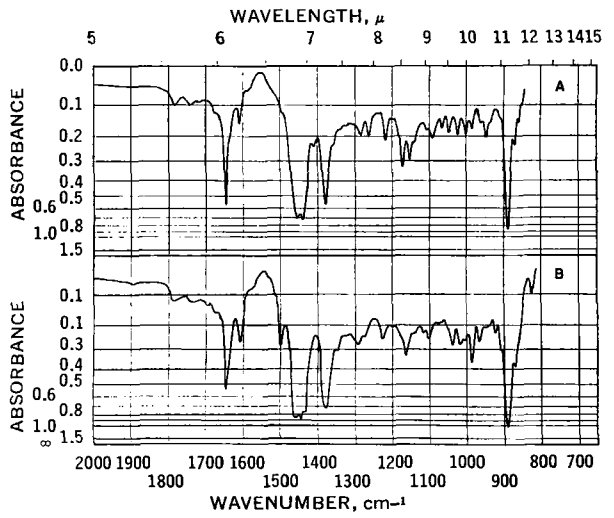


Figure 2—*IR spectra of new aromatic hydrocarbons: A, sesquiterpene A; B, sesquiterpene B.*

[1] Chromosorb W, Johns-Manville Products Corp., New York, N. Y.

Table II—Products of Dehydrogenation Gas Chromatography

Product	Retention Time, min.	% Sesquiterpene A	% Sesquiterpene B	% α-Calacorene
Cadalene	13.0	15	50	43
1,6-Dimethylnaphthalene[a]	8.6	4	7	13
Unidentified Aromatic Hydrocarbon[b]	5.4	45	—	—
Unreacted and uncharacterized material	—	36	43	44

[a] Identified by retention time only. Also found in dehydrogenation products from several sesquiterpenes (20, 21). [b] Spectral characteristics: IR absorptions at 1,606, 1,572, 1,492, 1,475, 1,455, 1,375, 1,361, 1,258, 1,160, 1,135, 1,087, 1,018, 945, and 868 cm.$^{-1}$. Strong UV bands at 214–218 mμ and weak absorption at 258, 267, and 275 mμ.

Hydrocarbon Fraction—Réunion vetiver oil (219 g.) was subjected to fractional distillation under reduced pressure (16 mm., reflux ratio of 10:1). The condensate (54 g.), collected up to 144°, was chromatographed on alumina (Woelm, grade I; 450 g.) and the hydrocarbon fraction was eluted with *n*-hexane (500 ml.).

Isolation of Constituents—The hydrocarbon fraction was refractionated (reflux ratio of 20:1) and 23 fractions (2 ml. each) were collected. Fractions 16 to 22 (b_{14} 124–130°) gave similar gas chromatograms (Fig. 1) and their IR spectra showed marked absorptions at 1,495 and 1,605 cm.$^{-1}$. They were combined and chromatographed repeatedly on active alumina (grade I) to obtain fractions enriched with individual constituents. *n*-Hexane, benzene and mixtures of the two were used as eluants. Some of the isolated products polymerized rapidly even when stored in cold under nitrogen. The constituents were isolated by gas chromatography on silicone nitrile column and purified further employing Reoplex 400 column. Their retention data are recorded in Table I.

Dehydrogenation of Sesquiterpenes—A reactor packed with 1 g. of catalyst (5% platinum on alumina) was attached to the inlet of the gas chromatographic column (Reoplex 400). Both the reactor and the column were maintained at 220°. Samples of sesquiterpene hydrocarbons (1–3 mg.) were injected and reaction products characterized by their retention times as well as IR and UV spectra. Experimental results are given in Table II.

Hydrogenation of Sesquiterpenes—Samples (5–10 mg.) were dissolved in glacial acetic acid (2 ml.) and stirred with Adams catalyst (5 mg.) in an atmosphere of hydrogen. Partial hydrogenations were carried out by discontinuing reaction as soon as absorption of hydrogen slowed down. Products recovered after evaporation of solvent were analyzed by gas chromatography on a silicone nitrile column. The following results were obtained.

Sesquiterpene A—The hydrogenation mixture was composed of isomeric bisabolanes (33%; relative retention times: 0.59 and 0.63; reference, curcumene), dihydrocurcumene (28%; relative retention time: 0.81) and an unidentified compound (27%; relative retention time: 0.88) exhibiting weak UV absorptions at 254, 258, 264, 266, and 272 mμ, and characteristic IR bands at 1,510, 1,460, 1,445, 1,390, 1,372, 1,267, 1,167, 1,087, 1,025, 990, and 960 cm.$^{-1}$.

Sesquiterpene B—Its reaction products generated two peaks in the gas chromatogram at relative retention times 0.30 (18%) and 0.35 (82%) (reference, curcumene). These were identified as cadinane isomers.

REFERENCES

(1) N. T. Anh and M. Fetizon, *Am. Perfumer*, **80**, 40(1965).

(2) C. C. Kartha, P. S. Kalsi, A. M. Shaligram, K. K. Chakravarti, and S. C. Bhattacharya, *Tetrahedron*, **19**, 241(1963).

(3) K. Morikawa and Y. Hirose, *J. Chem. Soc. Japan*, **88**, 795 (1967).

(4) J. A. Marshall, N. H. Anderson, and P. C. Johnson, *J. Am. Chem. Soc.*, **89**, 2748(1967).

(5) J. A. Marshall and P. C. Johnson, *ibid.*, **89**, 2750(1967).

(6) I. C. Nigam, H. Komae, G. A. Neville, C. Radecka, and S. K. Paknikar, *Tetrahedron Letters*, **1968**, 2497.

(7) R. Sakuma and Y. Yoshikoshi, *Chem. Commun.*, **1968**, 42.

(8) C. G. Overberger and D. Tanner, *J. Am. Chem. Soc.*, **77,** 369(1955).
(9) Y. Hirschberg, *ibid.*, **71,** 3241(1949).
(10) E. A. Braude and C. J. Timmons, *J. Chem. Soc.*, **1950,** 2000.
(11) W. G. Young, M. Kosmin, R. Y. Mixer, and T. W. Campbell, *J. Am. Chem. Soc.*, **74,** 608(1952).
(12) I. C. Nigam, *J. Chromatog.*, **24,** 188(1966).
(13) I. Mizrahi and I. C. Nigam, *ibid.*, **25,** 230(1966).
(14) J. Pliva, M. Horak, V. Herout, and F. Šorm, "Die Terpene, Sammlung der Specktren und Physikalischen Konstanten, Teil I: Sesquiterpene," Akademie Verlag, 1960.
(15) L. Ruzicka, J. Meyer, and M. Mingazzini, *Helv. Chim. Acta*, **5,** 345(1922).
(16) J. L. Simonsen, "The Terpenes," vol. 3, University Press, Cambridge, Mass., 1952, p. 20.
(17) R. D. Batt and S. N. Slater, *J. Chem. Soc.*, **1949,** 838.
(18) P. S. Kalsi, K. K. Chakravarty, and S. C. Bhattacharya, *Tetrahedron*, **18,** 1165(1962).
(19) I. C. Nigam and L. Levi, *Can. J. Chem.*, **40,** 2083(1962).
(20) I. C. Nigam and L. Levi, *J. Chromatog.*, **17,** 466(1965).
(21) C. F. Seidel, P. H. Muller, and H. Schinz, *Helv. Chim. Acta*, **27,** 738(1944).

ACKNOWLEDGMENT AND ADDRESSES

Received September 6, 1968, from *the Research Laboratories, Food and Drug Directorate, Ottawa, Ontario, Canada*

Accepted for publication March 5, 1969.

* National Research Council of Canada Postdoctorate Fellow, 1964, on leave of absence from Instituto Nacional de Tecnologia Agropecuaria, Buenos Aires, Argentina.

The authors are grateful to Fritzsche Brothers Inc., New York, NY, for the gift of a genuine sample of Réunion vetiver oil.

† Deceased. All correspondence concerning this manuscript should be directed to Dr. David W. Hughes, Department of National Health and Welfare, Tunney's Pasture, Ottawa 3, Ontario, Canada.

DRUG STANDARDS

Quantitative Separation of Progestins and Estrogens from Anovulatory Formulations

ANGEL ALVAREZ FERNÁNDEZ and VICTORIANO TORRE NOCEDA

Abstract □ The quantitative separation of progestins and estrogens, from orally administered anovulatory formulations, by gel filtration on synthetic polysaccharide (SephadexLH -20), and their direct determination by UV spectrometry is described.

Keyphrases □ Progestins, estrogens in dosage forms—quantitative determination □ Column chromatography—separation □ UV spectrophotometry—analysis □ GLC—analysis

A general method is described for the separation of progestins and estrogens from orally administered anovulatory formulations, on synthetic polysaccharide[1] with methanol–water (17:3) as eluant.

The procedure permits the separation of progestins (norethindrone, norethynodrel, megestrol acetate, norgestrel, chlormadinone acetate, and ethynodiol diacetate) from estrogens (ethynyl estradiol, mestranol, estradiol, and estradiol benzoate) in the variable proportions which are usually encountered in commercial anovulatories.

Excipients such as polyvinylpyrrolidone, magnesium stearate, lactose, starch, and talc do not inhibit the separation.

[1] Sephadex LH-20, Pharmacia Fine Chemicals Inc., New Market, N. J.

Methyltestosterone and prednisolone acetate may also be separated by this method from the estrogens cited.

In a formulation containing lynestrenol, mestranol, and α-tocopherol acetate, the progestin-estrogen separation was incomplete; however, the technique quantitatively separates α-tocopherol acetate from mestranol and each could be determined by UV spectrophotometry.

The orally administered anovulatory agents, which have been widely accepted in increasing numbers during these past years, are generally composed of estrogen-progestin mixtures of variable proportions and the quantitative determination of the estrogen is rendered difficult due to its low content and the interference of the progestins. The estrogenic agents principally used are ethynyl estradiol and its methyl ether (mestranol).

The progestational agents found in these formulations correspond to two principal groups. The members of one group are characterized by the absence of the methyl group on C_{19} and are designated as nor-compounds.

The second group consists of substances containing the basic progesterone nucleus with different types of substitutions.

In 1965 Schulz (1) described a method for the determination of mestranol by GLC and he compared the