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Olfactory Studies on Enantiomeric Eremophilane Sesquiterpenoids

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Odor threshold concentrations, determined in the liquid phase as well as in the vapor phase, are reported for five enantiomeric pairs of sesquiterpene ketones, including (+)- and (−)-nootkatone and (+)- and (−)-α-vetivone. For these last two pairs comparative taste experiments were also carried out. Odor profiles for all compounds are presented.

Significant differences, both in odor strength and in odor character, have been found between all enantiomeric pairs and are particularly large for the nootkatones. (−)-Nootkatone has only a weak odor and lacks the characteristic grapefruit flavor of its dextrorotatory enantiomer.

In 1969 Naves reviewed the work done on enantiomeric forms of odorous substances and concluded that enantiomers can differ in odor quality as well as in odor strength. This conclusion, at that time perhaps a little premature, and questioned by Wright (1964), has since then been corroborated by some excellent investigations. Among these may be mentioned those on linalool by Ohloff and Klein (1962), on carvone by Russell and Hills (1969) and by Friedman and Miller (1971), and on derivatives of α- and β-pinene by Theimer and McDaniel (1971).

At present it seems generally accepted that enantiomers can differ in odor. Since this fact may well become of principal importance for the elucidation of the mechanism of olfaction, additional data may be useful. The present work was aimed at obtaining more information on the subject by studying the olfactive properties of the pure enantiomeric forms of nootkatone, α-vetivone, and some related compounds. The results are compared with recent work on (+)-nootkatone and some derivatives by Stevens et al. (1970).

EXPERIMENTAL

Synthesis. The synthesis of optically active nootkatone had thus far only been possible from the closely related sesquiterpenes (+)-valencene (Hunter and Brogden, 1965) and (+)-nootkatone (Erdtman and Topliss, 1957; Firmenich,

1969). Recently a stereoselective synthesis leading to optically active sesquiterpenoid ketones of the eremophilane series was developed in our laboratories (Gen et al., 1971). The levorotatory form of a tricyclic ketone, here referred to as "tricycloketone" (Figure 1), was prepared from (−)-sabinene and converted to (+)-8-dehydro-11,12-dihydronootkatone and (+)-nootkatone. This nootkatone was identical with the (+)-nootkatone prepared from (+)-valencene (Hunter and Brogden, 1965). (+)-α-Vetivone was obtained by acid-catalyzed isomerization of (+)-nootkatone. (+)-Tetrahydronootkatone was prepared by hydrogenation of (+)-nootkatone according to Erdtman and Hirose (1962). By starting the reaction sequence with (+)-sabinene the opposite enantiomeric forms of the compounds mentioned were obtained. Figure 1 shows the structural formulas of both series of compounds. Some physical constants are presented in Table I.

PURIFICATION. Since very small amounts of impurities can have a large influence on the odor, the utmost attention was paid to the purification of the compounds. Before being used in further synthetic steps, the key compounds, the tricycloketones, were repeatedly crystallized until the enantiomers had identical optical rotations of opposite sign (α^{20}D = +216° or −216°). The other crystalline materials, nootkatone and α-vetivone, were likewise purified by repeated crystallization (five times or more). Shortly before the sensory tests these compounds were crystallized twice. The liquid tetrahydro- and dehydrodihydronootkatones were
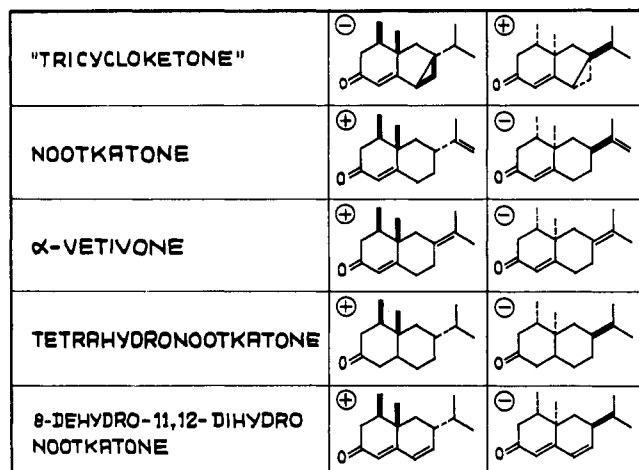
Figure 1. Structural formulas

Table I. Physical Constants

Substance	mp-bp	$(\alpha)^{20}_D$	λ_{max} uv
Tricycloketone	71–72° C	214–216° C (c 8, C_2H_5OH)	C_2H_5OH λ_{max} 265 nm (ϵ = 19,500)
Nootkatone	35–36° C	185–186° C (c 1.5, C_2H_5OH)	C_2H_5OH λ_{max} 237 nm (ϵ = 15,400)
α-Vetivone	50–51° C	239–241° C (c 1.5, C_2H_5OH)	C_2H_5OH λ_{max} 233 nm (ϵ = 13,300)
8-Dehydro-11,12-dihydro-nootkatone	124–126° C at 1 mm	210–211° C (c 3.0, $CHCl_3$)	C_2H_5OH λ_{max} 282.5 nm (ϵ = 17,800)
Tetrahydro-nootkatone	114–115° C at 1 mm	60–61° C (c 2.9, $CHCl_3$)	No strong absorption over 200 nm

purified by means of spinning band fractionation, followed by preparative gas–liquid chromatography.

Sensory Evaluation. Odor threshold concentrations in aqueous solution were determined by a method similar to that reported by Stevens *et al.* (1970). Our panel was an average group from our laboratory departments and consisted of 24 judges, who had not especially been selected or trained for the compounds under study. The compounds were tested in six or more concentrations. Each concentration was presented together with three samples of pure water in polyethylene squeeze bottles and in randomized order. A choice between the four bottles had to be made. This results in a chance level of correct responses of 25%. We defined threshold concentration as that concentration for which the level of correct responses is halfway between the 25 and the 100% level, *i.e.*, 62.5%.

Since the compounds are poorly soluble in water the samples were prepared by directly adding, by means of a hypodermic syringe, the appropriate amount, *e.g.*, 5 μl, of a standard solution containing 20% by weight of the substance in absolute ethanol or in the nearly odorless diethylene-glycol-monoethylether to 50 ml of water in the sample bottle (which in this example gives a concentration of 20 ppm). Thus ethanol concentrations were kept far below threshold level.

Threshold dilutions in the vapor phase were determined with the aid of an olfactometer of the type described by Randebrock (1970). The olfactometer room was air-con-
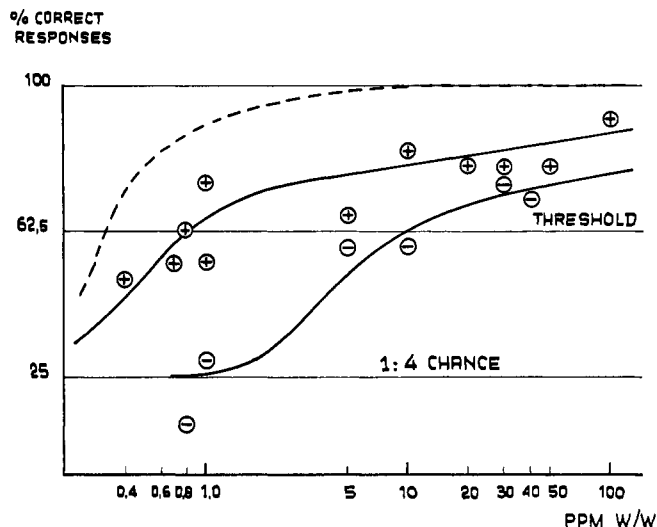
Figure 2. Odor threshold concentrations for (+)- and (−)-α-vetivone in aqueous solution

ditioned at 21° C and 60% relative humidity. A panel of six observers, experienced in olfactometry, was presented a series of dilutions of the saturated vapor of the compound under consideration. They were asked to assign an intensity rating to each dilution using a number between 9 and 0. Intensity rating 9 is given to the vapor of the undiluted substance as a reference. Intensity rating 1 corresponds to the dilution in which the odor is only just perceptible and represents by definition the threshold concentration. In order to reduce adaptation errors, the dilutions were presented to the observers in a random succession which was different for each observer. The observers were allowed to demand for previously judged concentrations and to correct assigned ratings.

According to Weber–Fechner's Law, a linear proportionality exists between intensity rating and the logarithm of the dilution. The threshold dilution is determined by the intersection of the plotted line with the intensity level of 1. We estimate the accuracy of the method at ±25%.

Odor character profiles of the five enantiomeric pairs were determined by asking six experienced perfumers to assign scale values to the most important odor aspects, such as fresh, green, sour, herbal, fruity, animal, erogenic, spicy, dusty, and woody.

RESULTS AND DISCUSSION

Figure 2 shows the results leading to the determination of the odor threshold concentrations in aqueous solution for (+)- and (−)-α-vetivone. The threshold of (+)-vetivone is found to be approximately 0.8 ppm. The lower value reported by Stevens *et al.* (0.175 ppm) may at least partly be explained by the fact that "panelists had been carefully screened for sensitivity and ability to detect again the odors of interest." If we select the most sensitive eight judges (30%) from our panel, we find threshold concentrations lowered by a factor between 2 and 6. In the case of (+)-α-vetivone we observed a response as indicated by the dotted line in Figure 2. Still, the threshold concentrations determined by our selected group remain somewhat higher for (+)-α-vetivone (0.3 ppm) and (+)-nootkatone (0.4 ppm) than those found by Stevens *et al.* (0.175 ppm in both cases). The threshold concentration of (−)-α-vetivone (approx. 10 ppm) is found to be significantly higher than that of its enantiomer. Differences of the same order of magnitude exist
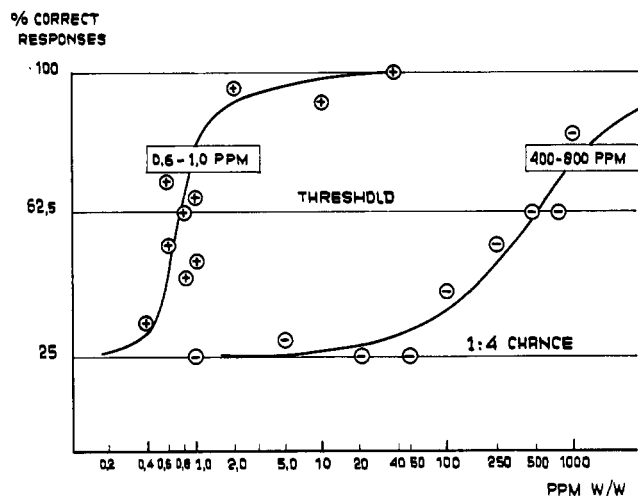
Figure 3. Odor threshold concentrations for (+)- and (−)-nootkatone in aqueous solution

Figure 4. Threshold concentrations (ppm)

SUBSTANCE		AQUEOUS SOLUTION		VAPOUR PHASE
		FULL PANEL	30% SELECTED	
NOOTKATONE	⊕	0.6-1.0	0.4	30
	⊖	400-800	100	~66,000
∝-VETIVONE	⊕	0.6-1.0	0.3	18
	⊖	6-15	3	36
TETRAHYDRONOOTKATONE	⊕	5-20	1	32
	⊖	30-70	15	−
8-DEHYDRO-11,12-DIHYDRO NOOTKATONE	⊕	5-10	1	33
	⊖	40-100	20	−
"TRICYCLOKETONE"	⊕	40-80	10	83
	⊖	80-120	40	140

between the enantiomeric forms of "tricycloketone," tetrahydro- and dehydrodihydronootkatone. A much larger difference is observed between the odor threshold concentrations of (+)- and (−)-nootkatone, *viz.*, 0.8 ppm *vs.* 600 ppm. (Figure 3).

A similar large difference is found between the threshold dilution levels of (+)- and (−)-nootkatone determined in the vapor phase. The threshold dilution of (+)-nootkatone is found to correspond with a concentration of 30 parts of saturated vapor per 1 million parts of air. For (−)-nootkatone this figure differs by a factor 2200 and is found at 66,000 ppm. At higher concentration levels this difference remains virtually unchanged. In an olfactometer experiment the saturated vapor of (+)-nootkatone had to be diluted 2000 times to match the odor intensity of the saturated vapor of (−)-nootkatone.

The threshold values found for the five enantiomeric pairs are summarized in Figure 4. In all cases odor threshold

concentrations for the (+)-forms are shown to be significantly lower than for the (−)-forms, with (+)- and (−)-nootkatone showing the most pronounced difference. It should be noted that the dextrorotatory "tricycloketone" is stereochemically related to the levorotatory isomers of the other members of the series. In the last column the vapor phase threshold dilutions are also given in ppm. Here the unit ppm means one volume of saturated vapor diluted a million times with air. Therefore a direct comparison of these threshold values with those determined for aqueous solutions is not relevant. If it is assumed that in both methods the partial vapor pressures at threshold level are equal, calculation learns that considerable deviations occur from the direct proportionality between vapor pressure and molar solute concentration in the aqueous phase, as predicted by Raoult's Law.

The low solubility of nootkatone and related compounds in water causes their partial vapor pressures in the headspace above the solution to be some orders of magnitude higher than expected. More detailed discussions of deviations from Raoult's Law in relation to odor enhancement or suppression are given by Haring (1971). For a comparison of odor and
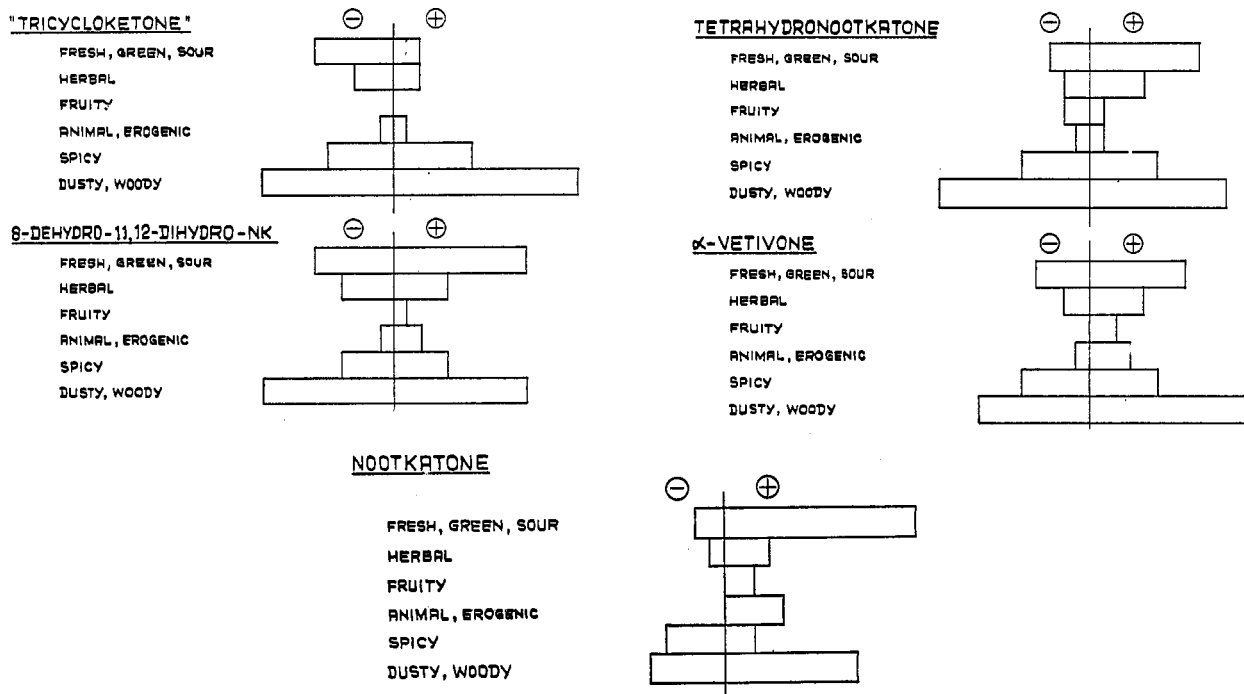

Figure 5. Odor profiles

taste thresholds (+)- and (−)-nootkatone and (+)- and (−)-α-vetivone have been tasted in a soft drink base by several experienced flavor chemists. (+)-Nootkatone was perceptible at a concentration of approximately 0.3 ppm and still had the flavor character of grapefruit. (−)-Nootkatone was perceptible at 40 ppm (much lower than the odor threshold value for aqueous solutions) and at that concentration tasted bitter and sour without any flavor impression. (+)-α-Vetivone was perceptible at 1.6 ppm and at that concentration is slightly reminiscent of grapefruit. It also has a strong woody flavor, not appreciated by flavor chemists. (−)-α-Vetivone, perceptible at approximately 8 ppm, is weaker than the (+)-form but has an even stronger woody note. The odor profiles are shown in Figure 5. Apparently the enantiomeric forms of the five sesquiterpenoid ketones do not only differ significantly in odor strength but in odor character as well. The largest differences are found again between (+)- and (−)-nootkatone.

ACKNOWLEDGMENT

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End of Part II on the Symposium on The Chemistry of Essential Oils and Related Products.

Volatile Components of Roasted Filberts

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Roasted filbert volatiles were isolated using the following techniques: steam distillation followed by solvent extraction; condensation of volatiles given off during steam distillation; and molecular distillation followed by fractionation using preparative gas chromatography. Extracts and fractions were analyzed by gc–ms using open tubular columns. 187 compounds are reported for the first time from roasted filbert volatiles including alkyl, alkenyl, oxygenated, and alicyclic pyrazines; pyrroles; pyridines; thiols, thiazoles, thiophenes, and sulfides; furans; phenols; acids; lactones; esters; terpenes; aromatic aldehydes, alkanals, 2-alkenals, and 2,4-alkadienals; aliphatic and olefinic alcohols and ketones; and aliphatic and aromatic hydrocarbons.

F ilberts (*Corylus avellana*) are a thick-shelled, sweet-flavored tree nut which may be consumed raw or, preferably, roasted. Little has been published concerning the flavor components of roasted filberts, except for the work of Sheldon (1969), who identified 41 compounds in the volatiles of roasted filberts. The use of modern analytical techniques, particularly the coupled gas chromatograph–mass spectrometer (gc–ms), has revealed the complexity of such heat-processed foods as coffee (Friedel *et al.*, 1971), cocoa (van Praag *et al.*, 1968) and peanut (Walradt *et al.*, 1971). Our objective, in a more detailed investigation of the roasted filbert volatiles, was to provide a qualitative comparison with other roasted products. Our gc–ms identifications were greatly assisted by the recent availability of additional reference data, particularly for the nitrogen-containing compounds (Friedel *et al.*, 1971; Pittet *et al.*, 1971). This paper reports the com-

pounds which were obtained from roasted filberts by four different isolation techniques and identified by gc–ms.

EXPERIMENTAL

The methods of isolation used in our work were (A) head-space analysis, (B) collection of volatiles emitted during roasting, (C) steam distillation, and (D) molecular distillation.

Roasting conditions were identical for all methods. Oregon-grown filberts were roasted in a solid-shell roaster (Probat-Werke, Germany) to 200°C and held for 5 min or until a medium to dark brown color was obtained.

(A) The headspace analysis was of uncondensed volatiles emitted during an atmospheric pressure steam distillation. Roasted filberts (1 kg) were ground to a fine slurry in an industrial Waring blender with 2 l. of deionized water and steam distilled for 20 min. The bulk of the water vapor was condensed in a cold water condenser (15°C) and collected in a wet ice cooled receiver. The uncondensed volatiles were passed into a 4-in. × ¹/₈-in. o.d. stainless steel tube packed with

International Flavors & Fragrances, Union Beach, New Jersey 07735.