separated by GLC. Each of these derived compounds was subjected to the purification procedure described above.

The enantiomeric pairs were trapped from the final GLC purification step in specially designed glass-packed tubes. These were then mounted in a three-channel micro-olfactometer designed to accommodate small samples collected from gas chromatographs (*7*). The olfactometer was linked through a heated stainless steel line to a gas chromatograph by which the stimuli concentrations were monitored. The odorant stimuli, at a concentration of 180 ng per milliliter of nitrogen carrier gas, were evaluated by a panel of trained judges. The sample presentation followed the general format for Duo-Trio difference testing (*8*). The judges were presented via the olfactometer, as described (*7*), a labeled standard of one enantiomer along with two coded samples—the same compound as the reference, and the other enantiomer—in all possible combinations. They were then asked to pick the coded sample which more closely resembled the reference standard. The judges were also asked to describe the odors of the compounds tested. In addition to the eight odorant stimuli (four enantiomeric pairs) isolated as described above, the two enantiomers of carvone obtained by inversion synthesis (*1, 9*) were also purified in an identical manner and presented to the panel. The results are summarized in Table 1.

The enantiomers subjected to sensory analysis, whether they were isolated from natural products or synthesized chemically, had significantly different odors. After preliminary sessions, round table discussions were held to evaluate odor qualities of sample standards such as caraway oil and spearmint oil. The judges' descriptions of the odors for compounds reported in our study were consistently indicative of different odor qualities. Compounds **1**, **2**, and **3** (Fig. 1) were described as having the odor of caraway; compounds **5**, **6**, and **7** were described as having the odor of spearmint. The judges had difficulty describing the odors of **4** and **8** (akin to musty and woody), but had no difficulty distinguishing between the enantiomeric pairs. Compounds **1**, **2**, and **3**, which all have a caraway odor, have similar geometry, as do compounds **5**, **6**, and **7**, which have a spearmint odor. Compounds **4** and **8** differ from the other compounds both in geometry and odor quality. These observations lend definite support to stereochemical considerations of odor perception. The experimental data (on the compounds investigated) indicate different physiological responses to isomers differing only in their steric enantiomorphism. These data do not support a vibrational theory of odor (*10*), as differences in vibrational and rotational internal energies would be identical for the isomeric pairs.

Table 1. Odor comparisons between enantiomeric stereoisomers. Numbers in parentheses are percentages.

Comparison	Evaluations (No.)	Correct (No.)	Adj. χ^2
2 versus 6	75	68 (89)	24.0*
1 versus 5	56	34 (82)	13.6*
3 versus 7†	60	55 (92)	20.0*
4 versus 8†	42	36 (86)	11.9*
2 versus 6‡	65	58 (89)	20.8*

* Highly significant ($P < .001$). † See (*11*).
‡ Samples supplied as per (*9*).

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9. The samples of carvone were obtained after the other components described in the text had been analyzed. The same panel of judges was convened, and identical experimental conditions were used for the enantiomers sent by Prof. Friedman.
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11. A trace of the corresponding tautomeric stereoisomer for each of the *cis*- and *trans*-dihydrocarvones was generated by the GLC conditions, but in trace amounts that did not affect the sensory judgments as these were below threshold levels of detection.

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Odor Incongruity and Chirality

Abstract. *The enantiomers* R-(−)- *and* S-(+)-*carvone are the organoleptic constituents in oil of spearmint and caraway, respectively. Their odor distinctiveness has been unambiguously demonstrated by chemical interconversion, independent synthesis, and resolution. Odor differences between other chiral isomers were also firmly established.*

Theories attempting to explain the basis for the human perception of odor have revolved about vibrational energy levels, intermolecular interaction, and molecular size and shape (*1–7*). None of these specifically examines the possible effect of chirality in odor. This is not unexpected since, for over 50 years, this has been an area of controversy (*5*, p. 134; *3*, p. 144; *6*, p. 30; *8–11*). Unfortunately, claims that enantiomers have different odors have been discredited by invective or because the enantiomers may have been contaminated with impurities (*9*; *10*; *7*, p. 30).

Inasmuch as most life processes are chiral-dependent, a priori, odor perception could indeed be responsive to chiral differences. The absence of a definitive investigation to clarify this point, utilizing appropriate model compounds together with modern synthetic and analytical techniques, is inexplicable since results would be of utmost importance in formulating more accurate concepts of odor perception.

"Optically pure" *R*-(−)- and *S*-(+)-carvone are commercially available (*12*), naturally occurring α,β-unsaturated terpenoid ketones. The *R*-(−) isomer is the organoleptic principle in oil of spearmint and has a strong, clear, sharp spearmint odor (*13*). The *S*-(+) isomer is found in oil of caraway and has the odor associated with caraway (*13*). These odor incongruences are discernible by most people [apparently 8 to 10 percent of the population (this study) suffer from carvone anosmia; that is, carvone odor blindness. This is an unambiguous example of specific chiral anosmia (*14*)]. In spite of this, these odor differences were ignored or claimed (*5, 3, 6, 8–11*) to be a result of minute amounts of all-powerful (low threshold), albeit unknown, impurities. Fortunately, carvone is unusual in that it lends itself to simple chemical manipulations. By extending the chemical sequence developed by Wharton and Bohlen (*15*) and applied to carvone by Klein and Ohloff (*16*), it is possible to interconvert (*17*) in high overall yield both *R*-(−)- or *S*-(+)-carvone into their enantiomers, *S*-(+)- and *R*-(−)-carvone, respectively (scheme 1) (*18*). In addition to providing a convenient pathway for chiral inversion, this chemical sequence filters out many classes of possible impurities. For example, (i) only α,β-unsaturated ketones or aldehydes are attacked by alkaline H_2O_2; (ii) only primary or secondary (not tertiary) allylic alcohols are oxidizable to aldehydes or ketones with manganese dioxide; and, in addition, (iii) *trans*-carveol (**3**) was further purified by way of its solid 3,5-dinitrobenzoate. Those impurities that could conceivably survive the sequence (Fig. 1) were shown to be absent by gas-liquid partition chromatography and odor evaluation.

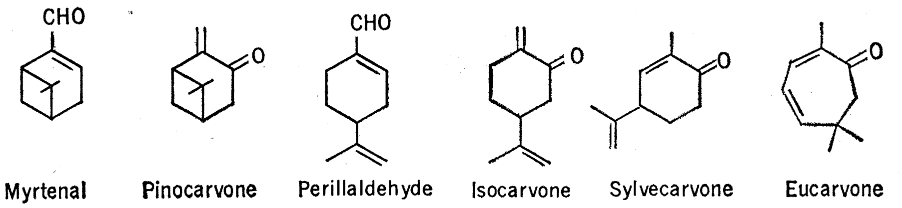


Fig. 1. Possible terpenoid impurities.

Highly purified commercial *R*-(−)- and *S*-(+)-carvone [$\mathbf{1}_{\mathrm{c}}$-*R*-(−) and $\mathbf{1}_{\mathrm{c}}$-*S*-(+)] (*19*) were converted to their respective enantiomers and back again (inversion cycles) (Eqs. 1 and 2).

$$\mathbf{1}_{\mathrm{c}}\text{-}S\text{-}(+) \rightarrow \mathbf{1}_{\mathrm{i}}'\text{-}R\text{-}(-) \rightarrow \mathbf{1}_{\mathrm{i}}''\text{-}S\text{-}(+) \tag{1}$$

$$\mathbf{1}_{\mathrm{c}}\text{-}R\text{-}(-) \rightarrow \mathbf{1}_{\mathrm{i}}'\text{-}S\text{-}(+) \rightarrow \mathbf{1}_{\mathrm{i}}''\text{-}R\text{-}(-) \tag{2}$$

In all cases the carvones from the interconversion cycles

$$\mathbf{1}_{\mathrm{i}}'\text{-}R\text{-}(-),\ \mathbf{1}_{\mathrm{i}}'\text{-}S\text{-}(+),\ \mathbf{1}_{\mathrm{i}}''\text{-}S\text{-}(+),\ \text{and}\ \mathbf{1}_{\mathrm{i}}''\text{-}R\text{-}(-)$$

respectively, had identical physical properties (*20*) except that the *R*-(−) enantiomers consistently had an odor different from that of the *S*-(+) enantiomer [that is, always: *R*-(−), spearmint; *S*-(+), caraway] and equal and opposite specific rotations (Table 1). Thus, with a change of chirality there was a corresponding reproducible change in odor (*21, 22*)

[caraway]

$$\mathbf{1}\text{-}S\text{-}(+) \equiv \mathbf{1}_{\mathrm{c}}\text{-}S\text{-}(+) = \mathbf{1}_{\mathrm{i}}'\text{-}S\text{-}(+) = \mathbf{1}_{\mathrm{i}}''\text{-}S\text{-}(+)$$

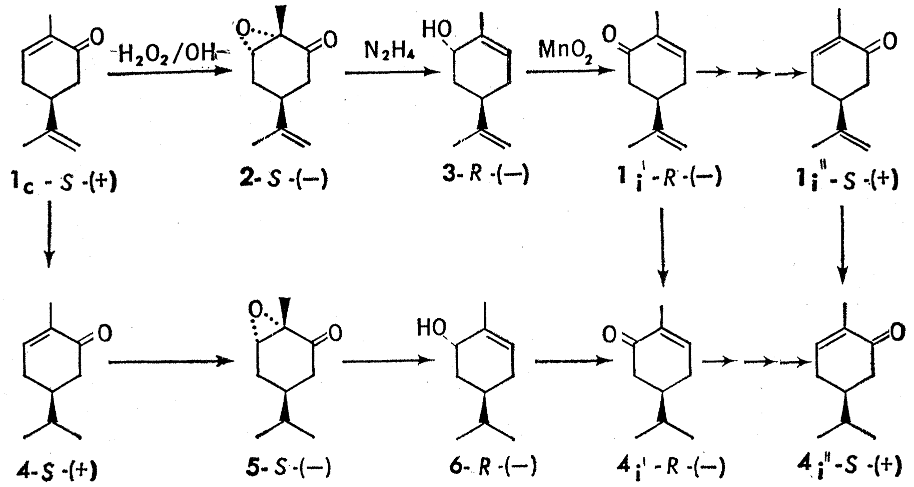
$$\mathbf{1}\text{-}R\text{-}(-) \equiv \mathbf{1}_{\mathrm{c}}\text{-}R\text{-}(-) = \mathbf{1}_{\mathrm{i}}'\text{-}R\text{-}(-) = \mathbf{1}_{\mathrm{i}}''\text{-}R\text{-}(-)$$

[spearmint]

Further evidence for this was obtained by two independent partial syntheses:

1) Naturally occurring *R*-(+)-limonene [**7**-*R*-(+)], from orange oil, and *S*-(−)-limonene [**7**-*S*-(−)], from lemon oil, were converted by way of their nitroso chlorides to the synthetic carvones $\mathbf{1}_{\mathrm{s}}$-*R*-(−) and $\mathbf{1}_{\mathrm{s}}$-*S*-(+), respectively, a previously developed sequence (*23*) (scheme 2). The intermediate solid **8**-*R*-(−) and **8**-*S*-(+) oximes are easily purified.

2) The photosensitized oxygenation of both *R*-(+)- and *S*-(−)-limonene (*24*) provided *cis*-*R*-(−)- and *cis*-*S*-(+)-carveol [**9**-*R*-(−) and **9**-*S*-(+), respectively] which were subsequently oxidized with MnO_2 to carvone (scheme 2). The products from either synthetic route [that is, $\mathbf{1}_{\mathrm{s}}$-*R*-(−) and $\mathbf{1}_{\mathrm{s}}$-*S*-(+)] exhibited the expected odor dependence on chirality and were identical in all respects to the corresponding enantiomers prepared by way of the interconversion scheme. Additional evidence for the absolute odor differences came from the resolution of the diastereomeric mixture of (+,−) and (−,−) carvone menthhydrazones (*25*). Partial resolution (75 percent) was achieved by repeated recrystallization from 95 percent ethanol. These diastereoisomers were individually hydrolyzed to give the carvones $\mathbf{1}_{\mathrm{r}}$-*S*-(+) and $\mathbf{1}_{\mathrm{r}}$-*R*-(−) (enantiomeric purity 88 percent), which exhibited the

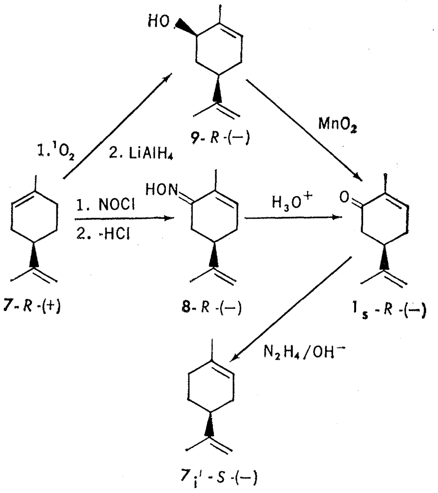


Scheme 1. Interconversion sequence.

Table 1. Dependence of odor on chirality. Specific rotation was measured as a 2 percent solution in ethanol; a Perkin-Elmer model 141 polarimeter (± 0.2°) was used. Odor was evaluated as described (*9*).

Compound*	Specific rotation $[\alpha]_D^{25}$			
	R-(−)	*S*-(+)	*R*-(+)	*S*-(−)
$\mathbf{1}_{\mathrm{c}}$	61.8 (spearmint)	61.6 (caraway)		
$\mathbf{1}_{\mathrm{i}}'$	62.0 (spearmint)	62.1 (caraway)		
$\mathbf{1}_{\mathrm{i}}''$	62.5 (spearmint)	62.5 (caraway)		
$\mathbf{1}_{\mathrm{s}}$	60.5 (spearmint)	59.9 (caraway)		
2			87.2 (faint floral)	86.7 (faint caraway)
2′			87.0 (faint floral)	86.1 (faint caraway)
3	212.5 (minty)	213.5 (musty)		
3′	212.1 (minty)	212.0 (musty)		
4	57.8 (spearmint)	58.3 (caraway)		
$\mathbf{4}_{\mathrm{i}}'$	58.8 (spearmint)	58.4 (caraway)		
$\mathbf{4}_{\mathrm{i}}''$	58.5 (spearmint)	58.1 (caraway)		
5			98.8 (strong floral)	99.5 (caraway-like)
6	192.1 (minty)	191.5 (musty)		
7			120.5 (orange)	119.2 (lemon)
8	39.5 (none)	38.8 (none)		
10	34.8 (musty)	34.3 (fecal)		

* Within the limits of analytical detection (capillary gas-liquid phase chromatography, nuclear magnetic resonance, mass spectroscopy, and so forth) the compounds tested were "pure." Character of odor is given in parentheses.



Scheme 2. Partial syntheses of carvone.

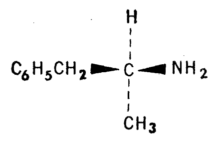
odor of spearmint and caraway, respectively (Table 1).

Unexpectedly, it was found that if the terminal double bond in carvone is hydrogenated, the resulting "carvotanacetones" **4**-*S*-(+) and **4**-*R*-(−) have exactly the same odors as their precursors ($\mathbf{1}_c$-*S*-(+) and $\mathbf{1}_c$-*R*-(−), respectively. Thus when both **4**-*S*-(+) and **4**-*R*-(−) were subjected to the interconversion sequence previously described (scheme 1) each was converted in good yield to its chiral partner: **4**-*R*-(−) and **4**-*S*-(+), respectively. Not surprisingly these two enantiomers exhibited identical physical properties, except for sign of rotation and odor (Table 1). If the internal conjugated double bond is removed, a new chiral center is created. The organoleptic properties of these isomers are discussed by Russell and Hills (*22*).

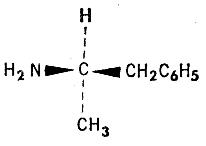
As part of our study, we sought and found several pairs of enantiomers which exhibited markedly different odors. For example, different odors were found for the enantiomeric *trans*-carvone epoxides **2**-*S*-(−) and **2**-*R*-(+) as well as the *trans*-carveols **3**-*R*-(−) and **3**-*S*-(+). Also, **5**-*S*-(−) and **6**-*R*-(−) have quite different odors from their respective enantiomers: **5**-*R*-(+) and **6**-*S*-(+).

The difference in odor between naturally occurring *R*-(+)- and *S*-(−)-limonene(**7**) (that is, orange and lemon, respectively) has been attributed to impurities. It is interesting to note, however, that *synthetic R*-(+)- and *S*-(−)-limonene [prepared easily from *S*-(+)- and *R*-(−)-carvone, respectively, scheme 2] also exhibit these characteristic odors.

A less esthetically pleasing system but one which nevertheless further demonstrates odor incongruity and chirality is the sympathomimetic pair *R*-(−)- and *S*-(+)-amphetamine (α methylphenethylamine). *RS*-(∓)-Amphetamine (**10**) was resolved with (+)-tartaric acid by way of the bitartrate salts to give enantiomers **10**-*R*-(−) and **10**-*S*-(+) which were further purified with (−)-tartaric acid. These enantiomers also exhibit an easily discernible odor dependence on chirality: *R*-(−): musty; *S*-(+): fecal (*26*).



10-*S*-(+)



10-*R*-(−)

Lest the impression be created that all enantiomers possess different odors, it should be stated that there are many examples to the contrary (*d*- and *l*-camphor, or *d*- and *l*-2-octanol). However, it can be concluded that enantiomers *can* have quite different odors, and it is anticipated that this additional parameter will play a useful role in the formulation of a unified theory of odor. Thus, it would appear that the vibrational theory of odor (*27*) is incompatible with our results. However, while the stereochemical theory of odor can be modified to accommodate the effect of chirality on odor, this dependence would suggest that, instead of rigidly defined receptor sites, an undefined chiral interaction between protein and substrate is involved (*28*).

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14. This specific anosmia has not been observed previously. To date more than 60 examples of specific anosmia have been compiled [*2*, table 40, p. 164; also see R. I. Henkin, *Life Sci.* **5**, 1031 (1966); M. Guillot, *Compt. Rend. Acad. Sci.* **226**, 1307 (1948)].
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17. The conversion of *R*-(—)-carvone to its enantiomer has been reported [V. Honwad, E. Sescovic, A. Rao, *Indian J. Chem.* **5**, 234 (1967)]. However, their claim that *R*-(—)-carvone gives a 1 : 1 mixture of *cis*- and *trans*-carvone epoxides is in disagreement with our results and those of Klein and Ohloff, inasmuch as the epoxidation is completely stereospecific [see J. Katsuhara, *Bull. Chem. Soc. Japan* **42**, 2391 (1969); D. L. Coffen and D. G. Korzan, *J. Org. Chem.* **36**, 390 (1971)].
18. *Numbering System*: Bold-faced Arabic numbers refer to individual structures without designating chirality; *R* or *S* refers to absolute configuration; (+) or (—) refers to sign of rotation at 589 nm; subscripts c (commercial), i (via interconversion), s (synthetic), and r (resolved) refer to the source of the compound; the prime notation is used to indicate the number of interconversion cycles that have been effected on the compound. For example, $\mathbf{1}_i''$-*S*-(+) represents *S*-(+)-carvone which was prepared from commercial material via two inversion cycles.
19. Each enantiomer was distilled through a 1.5-m spinning band column with a reflux-to-takeoff ratio of 100 : 1. Gas-liquid partition chromatography was carried out on a column (0.3 cm by 6 m, 20 percent Carbowax 20M on 60/80 mesh Chromosorb W) with a Varian Aerograph 1200 flame-ionization chromatograph.
20. Proton magnetic resonance, infrared, ultraviolet, and mass spectra, and index of refraction.
21. Initial evaluation of odor was based on the comments of graduate students and secretaries at Case Western Reserve University. These results were subsequently confirmed through the efforts of Dr. E. T. Theimer of International Flavor and Fragrances, where a professional panel of perfumers evaluated the odors of compounds $\mathbf{1}_c$-*S*-(+), $\mathbf{1}_i'$-*R*-(—), $\mathbf{1}_i''$-*S*-(+), $\mathbf{1}_c$-*R*-(—), $\mathbf{1}_i'$-*S*-(+), $\mathbf{1}_i''$-*R*-(—), **3**-*R*-(—), and **3**-*S*-(+). Further confirmation on the odors of compounds $\mathbf{1}_i''$-*S*-(+) and $\mathbf{1}_i''$-*R*-(—) was obtained from a sensory panel analysis carried out by Professor G. Russell on an olfactometer of his design (*22*). We thank Dr. Theimer and his co-workers, and Professor Russell for their analytical assistance (see *7*).
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25. Prepared from *dl*-carvone and *l*-menthhydrazide [R. Kapp and A. Knoll, *J. Am. Chem. Soc.* **65**, 2061 (1943)]; (+, —)-carvone methhydrazide: $[\alpha]_D^{25} = +94.9$ (1.0, ethanol), m.p. 155°C; (—, —)-carvone menthhydrazide: $[\alpha]_D^{25} = -196$ (1.0, ethanol), m.p. 167°C.
26. These odor differences were known by people involved in the manufacture of *R*-(—)- and *S*-(+)-**10** for several decades. Unfortunately, this knowledge was not disseminated since it was not deemed significant.
27. The vibrational theory is based on the supposition that odors can be correlated with far infrared absorption patterns (*4*). Inasmuch as enantiomers have identical infrared spectra in all regions, the basic supposition becomes untenable.
28. The stereochemical theory (*2*) was modified to include a type of asymmetry at certain odor sites in order to explain the differences in odor among the diastereomeric (not enantiomeric) menthols. The chirality of this asymmetry was never delineated. The data reported here require either chiral, enantiomeric, or totally different receptor sites.

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