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Olfaction and Molecular Shape. Chirality as a Requisite for Odor

Ernst T. Theimer,* Takao Yoshida, and Erich M. Klaiber

Although it has been established that chiral isomers can differ in odor, the number of cases reported in which the differences are small seems inconsistent with the logical assumption of optically active olfactory receptors. The observation that several terpenoid cyclohexanones in racemic form possess the urinous odor led to a program of synthesis of a group of these compounds in optically active form and the evaluation of their odors. At low concentrations all the compounds were either odorless or possessed the same characteristic urinous odor. However, the odor strength varied greatly with isomeric configuration although the high incidence of urinous anosmia known for *d*-androstenone and *cis*-(*tert*-butylcyclohexyl)isohexanone prevailed, suggesting the same olfactory receptor mechanism. Ratios of odor strength as high as 20:1 and one case of urine odorless vs. odorous between chiral isomers were observed. Liquid isomers were found to be stronger than solid isomers. At very high concentrations all the compounds had woody type odors with no observed cases of anosmia. An hypothesis for odor perception to account for all these observations is proposed.

Elucidation of the mechanism of olfaction remains for the future. Even the functioning of the olfactory cells, the first step in the perception of odors by animals, is still only a vaguely understood process. However, studies during the past two decades on the relationship of molecular shape to quality of odor have established useful criteria and led to a number of working hypotheses (Amoore, 1952, 1962, 1963; Beets, 1957; Davies, 1953; Theimer and Davies, 1967).

One particularly baffling aspect of structure–odor relationships has been difficulty in defining clearly the role of chirality. In contrast to large differences in other biological interactions between body tissues and dextro and levo forms of the same compounds, for example, in drug effectiveness and in taste perception, the differences between odors based solely on chirality have been so small that they have often been explained away as due to impurities only. This has been a roadblock on the way to a better understanding of the primary process of odor detection, for it has raised doubts about what would otherwise have been an a priori conclusion, namely, that the initial odor stimulus at the receptor cell results from an odor-molecule/receptor interaction on the olfactory epithelium. Since the epithelium, like all biological tissue, must have chirality, it should be able to differentiate between *d* and *l* isomers.

Fortunately, this ability to discriminate has now been established unequivocally by several discreet methods (Theimer and McDaniel, 1971; Friedman and Miller, 1971), but the relatively small differences in odor strength between chiral isomers has remained a disturbing element.

The present study was undertaken to try to shed light on the reasons for the usually slight effect of chirality and to demonstrate that chirality can actually be dominant in determining odor strength as well as odor quality.

If we accept the existence of specialized receptors on the olfactory epithellium (Amoore, 1952, 1962, 1963; Beets, 1957; Davies, 1953; Theimer and Davies, 1967), we must conclude that an olfactory stimulus requires the entering molecules to have the proper shape in addition to other obvious properties such as volatility and suitable free-energy parameters. But the shape of a molecule as "sensed" by a receptor may depend not only upon its chemical structure but upon its particular conformation at the moment of contact and upon the attitude with which it presents itself to the receptor. Since intramolecular or spinning motion is rapid relative to intermolecular or translatory motion, the attitude of the molecule will not be important, since repositioning will permit effective interaction when this is at all possible. Similarly, the molecule will present itself in all its various conformations which can explain the similarity in odor between most chiral pairs studied to date.

Since chiral isomers are identical in all respects except spatial shape, they can, *if flexible*, assume conformations which are all but undistinguishable as far as the olfactory sensor is concerned. Such flexibility can be expected from acyclic molecules with many degrees of freedom. Molecular models show clearly the similarity in shape between, for example, the *d* and *l* forms of citronellol and of citronellal. The monocyclic carvones, more rigid, can be distinguished by odor quality, and their odor thresholds are also very different (Leitereg et al., 1971), as are also those of *d*-Nootkatone, the "Grapefruit ketone" and its enantiomer whose odor is far weaker and woody rather than fruity (Haring et al., 1972). On the other hand, *d*-

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The Research Laboratories of International Flavors and Fragrances, Inc., Union Beach, New Jersey 07735.
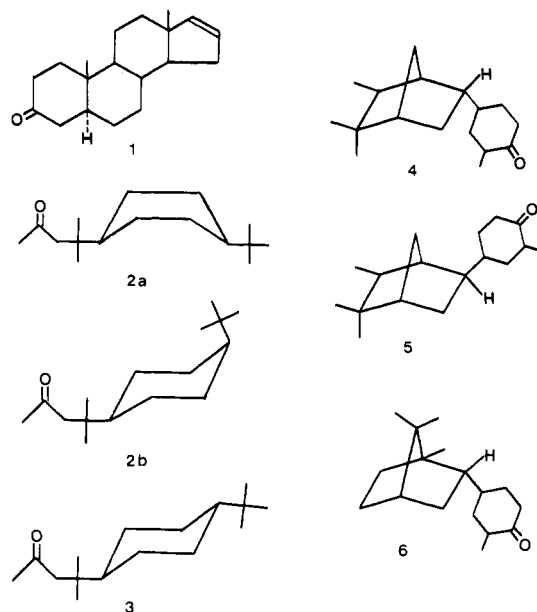
Figure 1. Important compounds in the study of urinous odor/structure relationships.

and l-camphor have substantially the same odor, although these rigid molecules should be easily distinguishable. Clearly there are other considerations.

The explanation may be in the type of odor perception involved. If there were a specific receptor site for every molecular shape which invaded the nose (clearly unlikely, if not impossible), isomers would all have similar odor thresholds. However, it appears there are two types of odors, primary and general. The former are the result of interaction at specific multiplicity of sites; the latter are a summation of interactions at a multiplicity of sites. These two types of odor perception can be distinguished by the following characteristics: primary: (1) relatively low threshold due to highly specialized reception or "fine tuning"; (2) incidence of specific anosmia in otherwise normal and even hypersensitive subjects, due to absence of a specific site in a generally functional system [for background on cases of specific anosmia, see Amoore (1967) and Guillot (1948, 1958)]; generalized: (1) relatively high threshold due to "broad spectrum" stimulation at multiple sites; (2) no specific anosmia.

Based on these criteria the "urinous" odor is a classical primary, with by far the highest incidence of specific anosmia known. The prototype for this odor is d-androstenone (1) (Figure 1), a rigid molecule, whose l isomer would not fit on the same receptor. Unfortunately the l isomer is not available for testing.

However, other molecular species have been found whose odors and anosmias are so similar to d-androstenone that it can be postulated that olfactive interactions at the same site are involved. One of these compounds, cis-4-(4-tert-butylcyclohexyl)-4-methylpentan-2-one (2) has already been described (Beets and Theimer, 1970). The structure of 2, which had not yet been confirmed, has now been established as cis by an alternate synthesis as well as by the synthesis of the trans isomer 3 which is a substantially odorless crystalline material in conformity with its rigid structure. The lack of chirality of 2, however, makes it useless for the present study. On the other hand, there is a series of terpene-derived cyclohexanones whose molecules contain a plurality of chiral centers and these compounds also possess the primary urinous odor.

It was the object of the present research to prepare the optical isomers of this series and to determine struc-
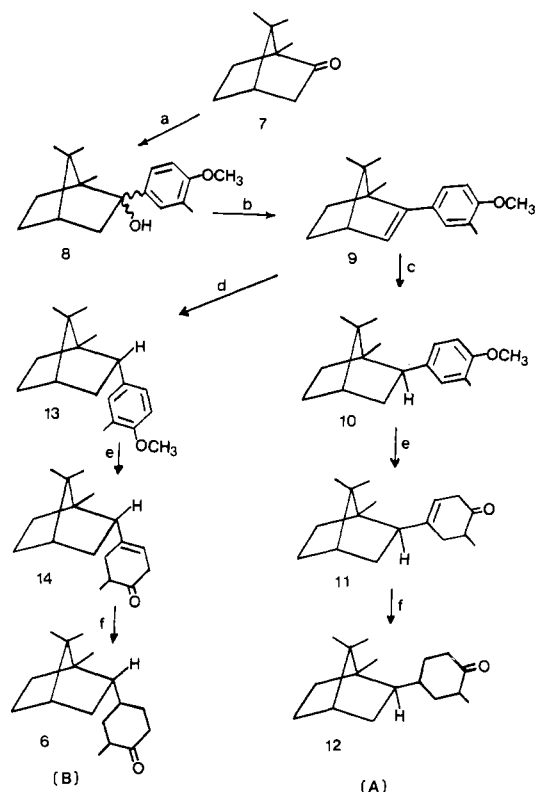

Figure 2. Synthetic routes for endo- and exo-4-(2-bornyl)-2-methylcyclohexanones. Reagents: (a) p-bromomagnesium O-methylanisole, (b) BF_3OEt_2, (c) H_2/Ra Ni, (d) Na/liquid NH_3, (e) Li/Liquid NH_3-t-BuOH, (f) H_2/Pd–C.

ture–odor relationships therein. The incentive for studying these compounds was the observation that racemic 2-methyl-4-(5,5,6-exo-trimethyl-2-exo-norbornyl)cyclohexanone (5) had a powerful urinous odor. (Although the known racemic 5 produced from camphene and o-cresol followed by hydrogenation and oxidation has been described as useful in perfumery (Kheifits et al., 1961; Saunders, 1967), its urinous odor has apparently escaped notice.)

DISCUSSION

Syntheses were first carried out using d- and l-camphor (7) to produce bornyl and isobornyl isomer pairs (6 and 12).

Next, racemic 5,5,6-exo-trimethyl-2-norbornanone (15) and optically active 15 were prepared and converted by novel synthetic routes to both the endo- and exo-2-methyl-4-(5,5,6-exo-trimethyl-2-norbornyl)cyclohexanones (4 and 5).

Isobornylmethylcyclohexanones. The synthetic routes, from camphor (7), reported in part (Erman, 1964), described in detail in the experimental part, are shown in Figure 2. d-Camphor (d-7) was optically pure, but the available l isomer (l-7) (structure was confirmed spectrometrically) contained about 17% d. Attempts to upgrade the optical purity by the method of Woodward et al. (1941) failed. Therefore, l-camphor (l-7) was used as obtained. Apart from the usual problems associated with obtaining satisfactory yields, no major difficulties were encountered. As expected, catalytic hydrogenation of 9 gave predominantly the exo isomers (10), since the steric hindrance by the methyls in the bornyl system led to H introduction mainly from the less hindered side. Conversely, chemical reduction of 9 gave predominantly the endo isomers (13). The endo isomers were upgraded by repeated crystallization resulting in a final product con-
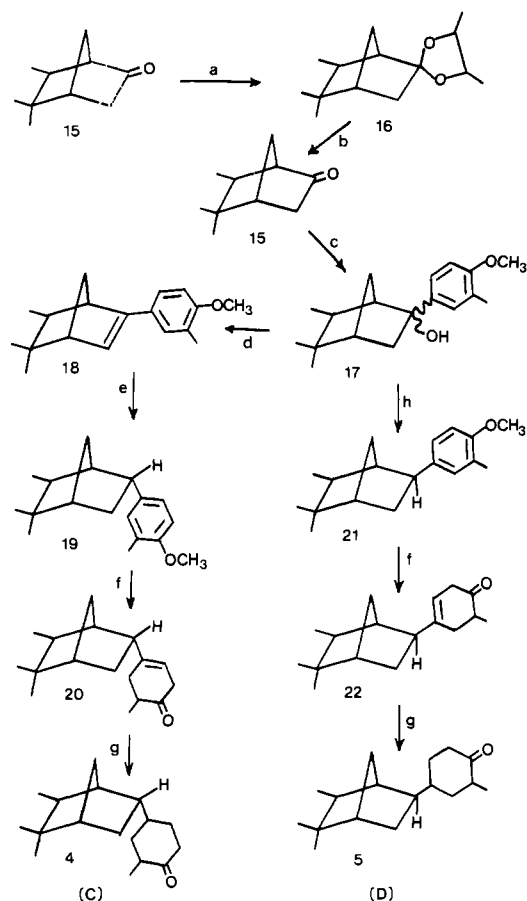
Figure 3. Synthetic routes for endo-and exo-2-methyl-4-(5,5,6-exo-trimethyl-2-norbornyl)cyclohexanones. Reagents: (a) l-2,3-butanediol-PTS, (b) H⁺, (c) p-bromomagnesium O-methylanisole, (d) BF₃·OEt₂, (e) H₂/Pd–C or Na/liquid NH₃, (f) Li/liquid NH₃-t-BuOH, (g) H₂/Pd–C, (h) Ra Ni/n-PrOH.

taining 95% endo isomer. These materials thus contain 5% exo isomer, which has no bearing on the results, since the end products from exo isomer are urine-odorless.

The four products, d-12, l-12, d-6, l-6, are themselves isomer mixtures involving stereochemistry of the cyclohexanone ring as well as possibly the cyclohexanone methyl. (In all cases the use of the terms l and d in describing the urinous compounds refers to the original starting materials and not to the (+) or (−) value of the optical rotation, [α]$_D$. The two nomenclatures may not coincide; for example, d-2-methyl-4-(5,5,6-exo-trimethyl-2-exo-norbornyl)cyclohexanone is in fact levorotatory.) In the case of the exo products, 12, each consisted of a liquid and a solid portion which were separately odor evaluated. No spectral differences were observed between liquid and solid, thus, no absolute structure assignments within the cyclohexanone ring itself were possible.

5,5,6-exo-Trimethyl-2-norbornylmethylcyclohexanones. The same synthetic routes, A and B (Figure 2), used to prepare 12 and 6 in the camphor series were carried out with racemic 5,5,6-exo-trimethyl-2-norbornanone (dl-15) as starting material to produce 4 and 5 (Figure 3). However, in this case, route A as well as B gave exclusively 4 (endo), none of the desired 5 (exo) being produced. This was not surprising when 18 was hydrogenated to 19, but unexpectedly, chemical reduction of 18 also gave 19 exclusively, no 21 being detected in the reduction product (route C). The "underside" of the 2 carbon in 18 is apparently so blocked that reduction of 18 will always give only endo product. Therefore route D, the direct hydrogenolysis of 17, was studied and conditions

were found which yielded up to 77% 21. These conditions for hydrogenolysis are critical and permit no deviation from the experimental conditions described. The separation of 21 and 19 was accomplished by preparative high performance liquid chromatography. Since this is perhaps the first instance of exo–endo separation in this way, the procedure is described in detail elsewhere (Yoshida et al., 1977).

The routes to pure racemic 4 and 5 having been established, it remained to carry out the same reactions on d- and l-15 separately.

Resolution of 5,5,6-exo-Trimethyl-2-norbornanone (15). The intended method to resolve 15 by means of l-menthydrazide (Woodward et al., 1941), which has been reported to work well on racemic camphor, failed. In spite of repeated painstaking crystallizations of the menthydrazones, no separations were observed. The method may not be generally applicable.

Gas chromatographic separation of the d,l- and l,l-butylene glycol ketals of camphor on a small scale using a highly polar liquid phase has been described (Corey and Mitra, 1962). This method was modified and upscaled successfully to produce the optical isomers of 15 in 4–5 g amounts as required for the subsequent multistep synthesis. The procedure involves a number of innovations. Although it was in general successful, the method gave only the d,l-16 in substantially pure form. The l,l-16, which eluted first, still contained 15% d,l-16 due to the inherent peak overlap which could not be avoided. The effect of the d-isomer content was considered in the odor evaluations. The final products, d- and l-4, consisted of solid and liquid phases which were separated before evaluation.

Odor Evaluations. The odor studies were done with groups comprising both lay and expert subjects. Solutions of the compounds in odorless mineral oil, on filter paper in plastic cups, were presented in pairs or groups in random order (Theimer, 1974).

The materials when presented in this manner had thresholds from 100 to 100 000 ppm concentration. No bias in the ability to perceive the urinous odor was apparent based either on expertise or on sex, the latter in contrast to some previous reports (Le Magnen, 1948, 1952). In assembling a panel of subjects suitable for evaluating the urinous odor, individuals first had to be screened for anosmia (Amoore, 1967; Guillot, 1948, 1958), as it was already known that about one-half of a randomly selected sample of the population could be expected to be anosmic. The individuals selected were those able to smell the standard used in the tests, a 1% solution of d-androstenone in mineral oil. The results of this preliminary screening:

Urinous anosmia	
Subjects tested	110
Total anosmia	50
Partial anosmia	6

agree well with the usual 50% incidence of urinous anosmia, which held with remarkable consistency throughout. The partial anosmics were those who could smell the steroid but not the other compounds in the program. Considering that the steroid is about ten times as strong as most of the other compounds tested, the number of partial anosmics is rather small. It is not unreasonable to postulate that some individuals have fewer such receptors, but are not totally bereft. At the other end of the scale are those (ca. 5%) who could detect 10 ppm steroid solutions suggesting a Gaussian distribution for the odor threshold, but there were insufficient data for a true curve. Most of the panel, however, could detect 100 ppm of the steroid and 1000 ppm and 1% solutions of the other

Table I. Relative Urinous Strength

Compound	Rel urinous strength
d-Androstenone (1)	1000
cis-4-(4-*tert*-Butylcyclohexyl)-4-methylpentan-2-one (2)	100
trans-4-(4-*tert*-Butylcyclohexyl)-4-methylpentan-2-one (3)	1(?)[a]
2-Methyl-4-(5,5,6-*exo*-trimethyl-2-*exo*-norbornyl)cyclohexanone	
dl-5	90
d-5	200
l-5	10[b]
2-Methyl-4-(5,5,6-*exo*-trimethyl-2-*endo*-norbornyl)cyclohexanone	
dl-4	20
d-4, liquid	40
d-4, solid	10
l-4, liquid	4
l-4, solid	2
4-(2-*exo*-Bornyl)-2-methylcyclohexanone	
d-12, liquid	0
d-12, solid	0
l-12, liquid	0
l-12, solid	0
4-(2-*endo*-Bornyl)-2-methylcyclohexanone	
d-6	1
l-6	0

[a] See discussion. [b] Calculated for the optically pure isomer by subtracting the odor contribution due to the 15% d component of known odor strength.

Table II. Relative Strength Test: *dl*-2-Methyl-4-(5,5,6-*exo*-trimethyl-2-norbornyl)cyclohexanone (*dl*-5 and *dl*-4)

Evaluations	No. of judgments	
	1000 ppm	1%
exo-(*dl*-5) > *endo*-(*dl*-4)	10.5	13
endo-(*dl*-4) > *exo*-(*dl*-5)	0.5	0

stronger and weaker compounds as expected.

Using as panel individuals who were osmatic, a series of comparisons resulted in a semiquantitative urine odor strength grading for all the compounds of the program (Table I). Although the numerical values for strength are approximations only, they are in proper sequence and lead to interesting structure–odor strength correlation. Using the steroid as standard, the relative strength of the *cis*-*tert*-butyl ketone 2 was established by averaging the results of comparisons using 10, 100, 1000, 10 000 ppm solutions of each. Most ratings placed the ketone at or near the odor strength of the next lower steroid concentration, making the value of 100 obvious for strength relative to 1000.

The value for the racemic exo and racemic endo 5,5,6-*exo*-trimethyl-2-norbornyl derivatives was determined similarly by comparison with 2. The other 5,5,6-*exo*-trimethyl-2-norbornyl compounds were then rated against each other, as were the bornyl and isobornyl derivatives. Whether *trans*-*tert*-butyl ketone 3 has any urinous odor is open to question. The neat crystals are substantially odorless, but solutions do smell. This may, however, be due to traces of cis compound still remaining even in the triply recrystallized product.

The convincing nature of the relative strength evaluations is evident from Tables I, II, III, and IV. The subjective nature of odor perception make some aberrations inevitable, but the differences are seen to be beyond question when the results are considered statistically. It can be seen that there is no doubt regarding the greater strength of d vs. l, and of the liquid, presumably axial, methyl isomers over the more rigid solid equatorial methyl isomers. In the comparisons involving racemic vs. optically

Table III. Relative Strength Test: 2-Methyl-4-(5,5,6-*exo*-trimethyl-2-*endo*-norbornyl)cyclohexanone (4)

Evaluations	No. of subjects	No. of judgments
d-4 > *l*-4	38	54
l-4 > *d*-4	4	5
d-4, solid > *l*-4, solid	16	24
l-4, solid > *d*-4 solid	2	2
liquid > solid (both *d* and *l*)	14	
solid > liquid (both *d* and *l*)	2	
d-4 > *dl*-4 > *l*-4	19	21
dl-4 > *d*-4 > *l*-4	11	11
l-4 > *dl*-4 > *d*-4	1	1

Table IV. Odor Tests for 6 and 12[a]

Evaluations	No. of subjects
Only *d*-*endo*-(*d*-6) = urinous	25
All other ratings	9

[a] All subjects were presented with all four isomers (*d*-6, *l*-6, *d*-12, *l*-12).

active material, the relative weakness of the l is evident. On the other hand, d was not strikingly superior to racemic. However, remembering that racemic is half d, and therefore half as strong even excluding any synergism, the two to one choice of d is about what might be expected. The racemic material used in the test was not merely a mixture made up of d and l, but the product of an independent synthesis starting with racemic 5,5,6-*exo*-trimethyl-2-norbornanone (15).

The number of judgments (Table IV) confirms that even the lowly rating of 1 on the scale of (Table I) for compound *d*-6 has strong significance as compared with the zero rating for the other three isomers (*l*-6 and *d*- and *l*-12). This is perhaps the first case in which chirality is the sole differentiating cause between odorous and odorless molecules. Almost three out of four subjects without prompting picked this one isomer of the four as the only one with urinous character. The other judgments included all those negative, equivocal, and doubtful.

In analyzing the significance of the results, let us begin with the assumption that a specific type of chiral receptor such as an enzyme active portion of the olfactory epithelium is able to accomodate the molecule *d*-androstenone (1) of known absolute configuration. Since *d*-androstenone (1) is a steroid occurring naturally in urine, the odor of which has biological significance in some species, such a receptor can be expected to evolve by normal evolutionary processes, and survive, even if no longer important in humans.

The geometry of the specific receptor can be inferred from a particular feature of the *d*-androstenone molecule such as the distance from the carbonyl function to the bulky quaternary position seven carbons removed.

Any other molecule which can fulfill the requirements of proper shape, bulk, volatility, rate of desorption, etc., should also trigger a urinous sensation. However, unless such a molecule is rigid, it will exist in various conformations only a part of which can simulate the steroid molecule. Therefore, no other class of compounds can be expected to be as effective as *d*-androstenone (1) as a urinous odorant. If the molecule is rigid, it will be either weak or inactive at the urinous receptor site, since its inflexible different shape will not fit. These conclusions are in agreement with the urinous odor strength of each of the 16 compounds of the present study, being roughly proportional to the fraction of each molecular species which can be expected to reside in the necessary configuration. In addition, of course, the molecules must fulfill

all the other requirements mentioned previously.

Molecular models confirm the correspondence of shape of the proper conformer to that of the steroid, taking into account odor strength as a function of "flexibility".

In the case of the *tert*-butyl ketones 2 and 3, the trans isomer 3 surely resides almost exclusively in the stable diequatorial chair form, which does not imitate the steroid and has very little, if any urinous odor. Its conformational homogeneity is indicated also by the sharpness of the NMR peaks of the ring protons and by the higher melting point of the compound. In contrast, the cis compound 2, a liquid, shows broad NMR absorptions for the ring protons, as expected because of the transitions between diequatorial boat 2a and equatorial axial chair conformations 2b, neither of which is "comfortable". Rapid interchange between these states must be taking place, for low-temperature NMR fails to "freeze" a conformation, the peaks being merely broadened.

The same argument holds for all the compounds studied. Those which are more flexible and thus can assume proper conformations have urinous odors. They are invariably liquids. Those which are less flexible are solids and have weak or no urinous odor. Of course, even the flexible molecules if they do not also have the proper molecular dimensions, have little or no odor.

Of particular interest is the matter of chirality. Of the bornyl–isobornyl series compounds, only one of the four has a urinous odor, albeit relatively weak. This is the *endo* bornyl *d*-6, the corresponding endo isomer *l*-6 having no urinous odor. The *endo*-trimethylnorbornyl compounds *d*-4 and *l*-4 have weaker odors than the exo compounds *d*-5 and *l*-5 but are stronger than the bornyl compounds. The d isomer 4 is stronger than the l isomer 4 in both the solid and liquid pairs (the liquids being stronger than the solids), showing that the receptor is indeed chiral, as expected. In contrast, the nonurinous odors associated with these compounds, perceptible at higher threshold, exhibit no dependence upon chirality, being the result of an odor sensation resulting from a multiplicity of diverse molecule–site interactions. These nonurinous odors, usually described as woody-camphoraceous, are perceived by all subjects, whether or not they are urinous anosmics.

Most conclusive is the greater than 20:1 ratio of odor strength between the chiral isomers of 5. An independent evaluation by a somewhat different technique gave a 30:1 strength ratio between these chiral isomers (Amoore et al., 1977). The d isomer 5, which is shown by molecular models to approach *d*-androstenone (1) most closely in molecular shape, has by far the strongest urinous odor of any surrogate for the steroid. Even more significant is the relative weakness of urinous odor in the *l* isomer 5, proving that as had been postulated, *molecular shape due to chirality is equally as important as other molecular geometry in determining odor strength*. Reference was made earlier to a feature of the *d*-androstenone molecule, namely the intramolecular distance between the functional group and the C-13 quaternary group. The validity of the hypothesis that other molecular species must be able to simulate the shape of the steroid, at least in some of their reasonable conformers, was confirmed with molecular models.

Even with these models it is not easy to isolate the essential feature, but careful study reveals that the closer a molecule approaches *d*-androstenone in carbonyl to quaternary distance, the stronger is its urinous odor. Particularly important in this regard is the *absolute* configuration of the chiral isomers. These are therefore discussed in detail later.

SUMMARY

The significant factors revealed by the odor comparisons are: (1) One-half of all randomly selected subjects (±2%) were anosmic to all the compounds although otherwise normal in odor perception. (2) "Urinous anosmia" was equally distributed among professionals (perfumers) and lay personnel. (3) Any one subject could smell either all or none of the compounds in the series of Table I. (4) Comparison of molecular structures using Dreiding models show an excellent correlation between odor strength and similarity in shape to *d*-androstenone. (5) In all cases of isomers where phase separation occurred, the liquid isomer was stronger than the solid isomer, although these were indistinguishable by NMR. (6) At high concentrations, the "urinous odorless" camphor derivatives became odorous to all subjects even the urinous anosmics, but the perception was "woody-camphoraceous" in all cases.

It is possible to combine all the above observations to provide a compelling argument for the following conclusion: Urinous quality as exemplified by the naturally occurring *d*-androstenone is a primary odor resulting from an interaction of odorvector molecules at specific chiral receptor sites present in only one-half the human population.

Since the specific urinous anosmia holds for all the compounds, they must of necessity be perceived by the same mechanism, which is most easily explained by a specialized receptor site.

d-Androstenone, being a rigid molecule with uniform conformation, would have a high probability of stimulative interaction with a "tailor-made" site. All other molecules would a priori have a lower probability of such stimulative interaction, that is, be apparently weaker in urinous odor, assuming roughly equal population of molecules at the olfactory epithelium, as would be expected with compounds which are all ketones of approximately equal volatility. Obviously the closer in shape a molecule of another species is to *d*-androstenone, the higher is its probability of reacting at the specific site.

The tendency to crystallize is related to conformational homogeneity, since the molecules are then oriented into the same pattern. But such homogeneity is a negative factor for urinous odor strength of potential surrogates, since it precludes the molecules assuming a *d*-androstenone-like conformation required to react at the receptor site. It is for this reason that the compounds which are conformationally less homogeneous, and are therefore liquids, have a greater probability of reacting at the site, which means that they are stronger in urinous odor.

A good example of the liquid–solid, strong–weak, disoriented–oriented isomer pair is 2 and 3. Superficially these cis and trans isomers are similar in structure (Beets and Theimer, 1970), and it might at first seem surprising that the cis isomer has a strong odor whereas the trans isomer is substantially odorless. Models provide an explanation. The cis isomer 2 can be either in the equatorial–axial (2b) chair form, the diequatorial boat form (2a) or in transition between these. None of these conformations being outstandingly preferred, the molecules are constantly changing, as can be seen from the broad peak for the cyclohexyl ring protons in the NMR spectrum. As the molecule changes from diequatorial to equatorial–axial, the equatorial ring protons become axial, and the two peaks blend into one. This change is very rapid, for even at liquid nitrogen temperatures the conformations cannot be frozen and the peak merely broadens instead of resolving. In short, the cis isomer can behave like a chameleon. The opposite is true for the trans isomer which
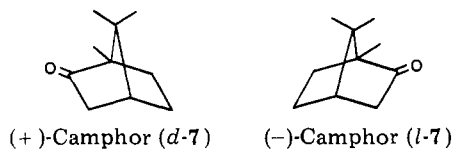
(+)-Camphor (d-7)   (−)-Camphor (l-7)

Figure 4.

d-endo-6   l-endo-6

d-exo-12   l-exo-12

Figure 5.

Figure 6. CD curves of (+)- and (−)-5,5,6-exo-trimethyl-2-norbornanones.

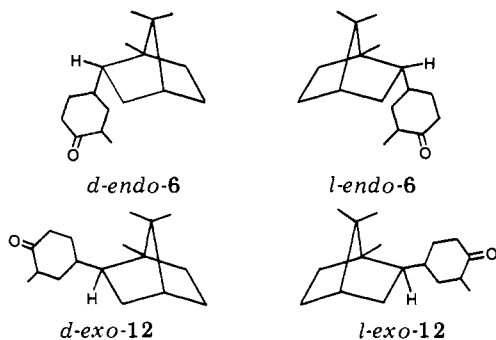**Figure 7.** Relationship between Octant rule and absolute structure of 1(R),4(R),6(S)-5,5,6-exo-trimethyl-2-norbornanone.

is in the crystalline conformationally homogeneous diequatorial chair form since all other conformations would be strained and thus less favored. The probability of **3** assuming a d-androstenone-like shape is thus extremely low. Similar though less dramatic, conditions apply to all the other isomer pairs.

The change in odor characteristics with increase in concentration can be explained by additional types of molecule–receptor interaction in which nonspecific and/or sites with different specialization are involved. These require higher threshold concentrations, but can involve many species of molecules, leading to the large number of "woody-camphoraceous" odored molecules, the perception of which is the result of a combination of molecule–site interactions, not requiring the specialized urinous receptor.

STRUCTURAL ASSIGNMENT

d- and l-Camphor (d-7 and l-7). These absolute configurations are shown in Figure 4 (see Crabbe, 1965).

4-(2-exo-Bornyl)-2-methylanisole (10). In NMR this isomer has methyl signals at δ 0.71 (one methyl) and 0.79 (two methyls superimposed due to upfield shift of the bridgehead methyl signals by the aromatic ring shielding effect) and the C-2 proton signal at δ 2.81 as a triplet due to the steric effect of the bridgehead methyl and the aromatic ring which forces the C-2 proton under the six-membered ring resulting in two dihedral angles of ∼20° and ∼140° with the C-3 protons, at which the coupling constants become about equal (∼8 Hz). Also, since catalytic hydrogenation occurs from the less hindered side of the molecule, exo isomer should result (see also: Erman, 1962; Flautt and Erman, 1963).

4-(2-endo-Bornyl)-2-methylanisole (13). In NMR this isomer has three methyl signals at δ 0.71, 0.91, and 1.01, and the C-2 proton signal at δ 2.95 as a quartet (each peak splits into doublet possibly due to the long-range coupling) with two coupling constants of 12 and 6 Hz caused by dihedral angles of ∼0 and ∼120° between the C-2 proton and the two C-3 protons (see also: Erman, 1962; Flautt and Erman, 1963).

4-(2-endo-Bornyl)-2-methylcyclohexanone (6) and 4-(2-exo-Bornyl)-2-methylcyclohexanone (12). From the results discussed above the absolute configurations for these optically active compounds were determined as shown in Figure 5.

These structures leave unresolved only the configuration of the methyls on cyclohexanone ring. Since many 2-methylcyclohexanones epimerize at room temperature via
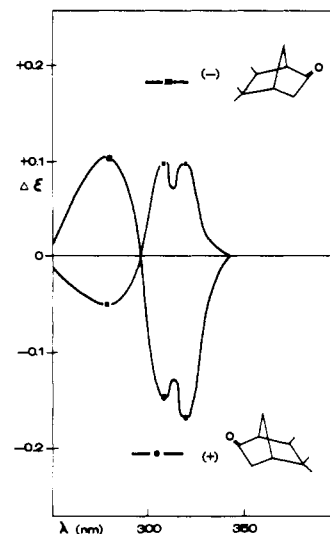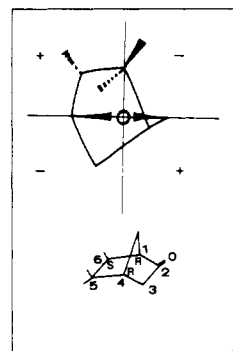
their enols, any attempt at further resolution would appear useless.

d- and l-5,5,6-exo-Trimethyl-2-norbornanone (d-15 and l-15). The CD curves for (−)-15 and (+)-15 in hexane are shown in Figure 6. (−)-Ketone (l-15) (85% pure) has positive maxima at 307 and 318 nm and a negative maximum at 279 nm. The curve, considering the solvent effect reported in the literatures (Crabbe, 1965; Coulombeau, 1966), shows the positive cotton effect in the n-π* transition of carbonyl, hence the assignment of the configuration as 1R, 4R, and 6S.

2-Methyl-4-(5,5,6-exo-trimethyl-2-endo-norbornyl)anisole (19). In NMR this isomer has methyl signals at δ 0.68 (doublet, J = 8 Hz) and 0.76 (singlet, two methyls). This high field shift (compare with exo isomer 21), especially for the C-5 endo-methyl, results from the shielding effect of the aromatic ring. A broad multiplet at δ 3.1 for the benzylic proton (C-2) results from three coupling constants with protons on C-1 and C-3. Both catalytic hydrogenation and Birch reduction of 18 gave this isomer, which could be expected to be endo isomer by hydride attack from the less hindered exo side.

2-Methyl-4-(5,5,6-exo-trimethyl-2-exo-norbornyl)-anisole (21). In NMR this isomer has methyl signals at δ 0.89 (doublet, J = 8 Hz) and two singlets at δ 0.90 and 1.04. The signal for one benzylic proton (C-2) at δ 2.68 is a triplet (J = 8 Hz) since its coupling constant is ∼0 with the C-1 proton (dihedral angle ∼90°) leaving coupling with the two C-3 protons only.

2-Methyl-4-(5,5,6-exo-trimethyl-2-endo-norbornyl)cyclohexanone (4) and 2-Methyl-4-(5,5,6-

d(−)-endo-4   l(+)-endo-4
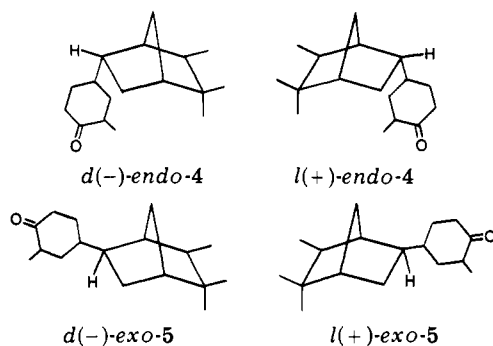
d(−)-exo-5   l(+)-exo-5

Figure 8.

exo-trimethyl-2-exo-norbornyl)cyclohexanone (5). From the results discussed above, the absolute configurations for these optically active compounds were determined as shown in Figure 8. Here also the methyls on the cyclohexanone ring are indeterminate.

EXPERIMENTAL SECTION

NMR spectra were taken in CDCl₃ solution on a Varian HA-100 spectrometer. Chemical shifts are reported in ppm relative to the internal standard Me_4Si ($\delta = 0$). IR spectra were measured on a Beckman IR4 spectrometer. Mass spectra were obtained on CEC 21-103C and MS-902 spectrometers. Melting points and boiling points are uncorrected. The CD data for all compounds are shown in Table V.

d-Camphor (d-7) and l-Camphor (l-7). These were obtained from commercial sources (Eastman Organic Chemicals Co. and J. Manheimer, Inc., respectively). Optical purity was 100% d ($[\alpha]^{25}_D$ +48°) and 83% l ($[\alpha]^{25}_D$ −36°), respectively. Attempts to improve the optical purity of l-camphor were unsuccessful.

l-2,3-Butanediol. This material, supplied by Dr. R. Teranishi of the USDA and by Dr. C. T. Bishop of NRC of Canada, was purified by fractionation: α^{25}_D −12.8° (neat), n^{20}_D 1.4310.

2-Methyl-4-bromanisole. To a solution of o-methylanisole (244 g, 2.0 mol) in acetic acid (900 mL) was added bromine (320 g, 2.0 mol) at 8–10 °C over 2.5 h, followed by 1 h of stirring. The white crystalline product was collected by filtration, washed with cold water, and recrystallized from water: yield, 311 g (77%); mp 67–68 °C; NMR δ 1.80 (3 H, s), 3.28 (3 H, s), 6.10 (1 H, d, $J = 5$ Hz), 6.73 (12 H, m); IR (neat, KBr) 1590 (arom), 1245, 1025 (-OCH₃), 800 (arom) cm⁻¹.

d-4-[2-(2-Oxybornyl)]-2-methylanisole (d-8) and d-4-[2-(2-Bornylenyl)]-2-methylanisole (d-9). To a mixture of magnesium (29.2 g, 1.2 mol) and THF (100 mL) was added a solution of 2-methyl-4-bromoanisole (201 g, 1.0 mol) in THF (500 mL) at 40 ∼ 50 °C during 1.5 h. After stirring at 40 ∼ 50 °C for 1 h, a solution of d-camphor (d-7) (152.2 g, 1.0 mol) in THF (200 mL) was added under reflux. The usual workup gave 260 g of crude oil. Sublimation removed unreacted camphor, leaving a mixture of d-8 and d-9. After standing 2 days, most of the d-8 had precipitated, leaving 51.5 g (20%) of crude d-9 which was purified by column chromatography using silicic acid (650 g) and ether–hexane (1:2) as an eluent to give 44 g (17%) of d-9 as a colorless oil: NMR δ 0.78 (3 H, s), 0.86 (3 H, s), 1.06 (3 H, s), 1.06 (3 H, s), 1.1∼2.0 (4 H, m), 2.17 (3 H, s), 2.31 (1 H, t, $J = 3$ Hz), 3.74 (3 H, s), 5.84 (1 H, d, $J = 3$ Hz), 6.6 7.2 (3 H, m); IR (neat NaCl) 1600 (arom), 1245, 1130, 1040 (-OCH₃) 805 (arom) cm⁻¹; MS m/e 256; $[\alpha]^{25}_D$ −148.2° (EtOH).

d-4-(2-exo-Bornyl)-2-methylanisole (d-10). d-4-[2-(2-Bornylenyl)]-2-methylanisole (d-9) (41.0 g, 0.16 mol)

Table V. CD Data

(+)-Camphor and derivatives			(−)-Camphor and derivatives		
Compd	nm	Δε	Compd	nm	Δε
d-7	313	+1.01sh	l-7	312	−0.58sh
	303	+1.51m		303	−0.86m
	293	+1.32sh		295	−0.76sh
	201	+0.40!		199	−0.21!
d-8	282	+0.12m	l-8	282	−0.05m
	276	+0.10		257	+0.34m
	255	−0.06m		232	+2.14m
	229	−2.46m		210	−1.51!
	209	+2.89!			
d-9	258	−7.92m	l-9	259	+6.28m
	214	−2.37?m		217	+1.51m
d-13	283	−0.11m	l-13	287/283	+0.09m
	267	−0.12m		270	+0.12m
	227	−0.82m		227	+0.87m
d-10	282	−0.62m	l-10	287	+0.33sh
	271	−0.67m		282	+0.40m
	234	+0.50m		270	+0.50m
	210	−3.28!		235	−0.59m
				213	+1.75!
d-14	347	−0.04sh	l-14	382	+0.03sh
	298	−0.24m		362	+0.06sh
	238	+0.51m		344	+0.07sh
	208	−1.52!		298	+0.23m
				237	−0.64m
				226	+0.80!
d-11	346	+0.05sh	l-11	320	−0.11sh
	322	+0.12sh		300	−0.24m
	310	+0.26sh		223	+0.82sh
	300	+0.29m		211	+1.05!
	222	−1.95sh			
	207	−2.56!			
d-6	314	+0.12sh	l-6	314	−0.10sh
	305	+0.21sh		304	−0.18sh
	297	+0.24m		295	−0.19m
	211	−0.17		288	−0.16sh
				210	+0.11!
d-12	317	+0.04sh	l-12	306	−0.05m
	306	+0.06m		278	+0.03m
	296	+0.06m		230	−0.05m
	274	−0.03m		214	+0.27sh
	211	−1.64sh		205	+0.50!
	203	−2.46!			
(+)-5,5,6-exo-Trimethyl-2-norbornanone and derivatives			(−)-5,5,6-exo-Trimethyl-2-norbornanone and derivatives		
d-15	318	−0.17m	l-15	318	+0.10m
	308	−0.15m		307	+0.10m
	280	+0.11m		279	−0.15m
d-18	305	−0.42m	l-18	308	+0.41m
	268	+2.40m		270	−1.76m
	222	+4.94m		222	−3.52m
d-21	308	−0.01m	l-21	308	+0.01m
	280	+0.18?m		303	+0.01m
	259	−0.33m		284	+0.33sh
	226	+1.60m		279	+0.38m
	215	0.00!		228	−1.71m
				214	−0.71!
d-19	286	−0.16m	l-19	287	+0.11m
	268	−0.18m		268	+0.15m
	229	+0.84m		232	−0.77m
d-22	308	−0.03sh	l-22	309	+0.02sh
	301	−0.04m		301	+0.02m
	240	+0.01m		240	−0.01m
	218	−0.06!		218	+0.12!
d-20	295	+0.25m	l-20	296	−0.16m
	223	−0.38m		215	+0.54m
d-5	313	+0.03sh	l-5	313	−0.03sh
	304	+0.06m		303	−0.05m
	296	+0.06m		294	−0.06m
	215	+0.41!		215	−0.36!
d-4	317	−0.05sh	l-4	317	+0.02sh
	307	−0.07m		308	+0.04m
				215	−0.05!

was hydrogenated in methanol (400 mL) using Raney nickel (40.0 g) at room temperature under a hydrogen pressure of 1000 psi. Filtration of catalyst and removal of solvent gave 39 g (95%) of crude product which on recrystallization from ethanol gave d-10 (86% exo isomer, 14% endo isomer).

Repeated crystallization from methanol gave exo isomer of 95% purity: mp 51.8 ∼ 53.2 °C; NMR δ 0.72 (3 H, s), 0.79 (6 H, s), 1.2 ∼ 1.9 (7 H, m), 2.16 (3 H, s), 2.81 (1 H, t, J = 8 Hz), 3.76 (3 H, s), 6.6 ∼ 7.1 (3 H, m); IR (neat, NaCl) 1600 (arom); 1250, 1130 (-OCH$_3$) cm^{-1}; MS m/e 258; $[\alpha]^{25}_D$ -60.0° (EtOH).

In the same manner l-9 gave l-10: mp 47.8 ∼ 49.8 °C; $[\alpha]^{25}_D$ +50.7° (EtOH).

d-4-(2-exo-Bornyl)-6-methyl-3-cyclohexanone (d-11). To d-4-(2-exo-bornyl)-2-methylanisole (d-10) (20.0 g, 0.078 mol) in THF (340 mL), liquid ammonia (700 mL), and tert-butyl alcohol (340 mL), lithium (10 g, 1.45 g-atom) was added over 30 min at -35 °C. After 5 h stirring at -35 °C, ethanol (200 mL) was added during 30 min, and the ammonia was evaporated. Usual workup gave 21.5 g of pale-yellow oil. This was dissolved in ether (350 mL) and treated with 10% aqueous solution of oxalic acid (400 g) for 20 h at room temperature. Workup and column chromatography on alumina gave 12.6 g (66%) of d-11: NMR δ 0.78 (s), 0.82 (s), 0.90 (s), 1.06 (d, J = 6 Hz), 1.0 ∼ 2.2 (m), 2.88 (2 H, broad s), 5.50 (1 H, broad s); IR (neat, NaCl) 1720 (C=O) cm^{-1}; MS m/e 246; $[\alpha]^{25}_D$ -54.5° (EtOH).

In the same manner l-10 gave l-11: $[\alpha]^{25}_D$ +36.8° (EtOH).

d-4-(2-exo-Bornyl)-2-methylcyclohexanone (d-12). Hydrogenation of d-4-(2-exo-bornyl)-6-methyl-3-cyclohexenone (d-11) (10.5 g, 0.043 mol) in ethanol (150 mL) with 10% palladium on charcoal (2.0 g) at room temperature and atmospheric pressure of hydrogen for 14 h gave crude d-12, which was purified by column chromatography using silicic acid to give 10.3 g (97%) of d-12 (semisolid): NMR δ 0.82 (s), 0.86 (s) 0.9 ∼ 2.5 (m); IR (neat, NaCl) 1720 cm^{-1} (C=O), m/e 248; $[\alpha]^{25}_D$ -50.0° (EtOH).

In the same manner l-11 gave l-12: $[\alpha]^{25}_D$ +25.0° (EtOH)

d-4-(2-endo-Bornyl)-2-methylanisole (d-13). To a solution of d-4-(2-bornylenyl)-2-methylanisole (d-9) (77.5 g, 0.3 mol) in ether (200 mL) and liquid ammonia (1400 mL), lithium (16.5 g, 2.4 g-atom) was added portionwise over 30 min at -35 °C. After stirring for 30 min at -35 °C, workup as usual gave 77.3 g of crude d-13, which was recrystallized from cold acetone to give 64.2 g (82%) of pure d-13: NMR δ 0.71 (3 H, s), 0.91 (3 H, s), 1.01 (3 H, s), 1.2 ∼ 2.2 (7 H, m), 2.21 (3 H, s), 2.95 (1 H, quartet, J = 12, 6 Hz with splitting by 2 Hz for each peak), 3.78 (3 H, s), 6.7 ∼ 7.1 (3 H, m); IR (neat, NaCl) 1600 (arom), 1250, 1145, 1040 (-OCH$_3$) cm^{-1}; MS m/e 258; mp 89.0-90.8 °C; $[\alpha]^{25}_D$ -39.0° (EtOH).

In the same manner, l-9 gave l-13: mp 90.0 ∼ 92.4 °C; $[\alpha]^{25}_D$ +40.0° (EtOH).

d-4-(2-endo-Bornyl)-6-methyl-3-cyclohexenone (d-14). The method described for d-11 from d-10 yielded 61% d-14 from d-13: NMR δ 0.82 (3 H, s), 0.86 (3 H, s), 0.91 (3 H, s), 1.07 (3 H, d, J = 7 Hz), 1.0 ∼ 2.8 (13 H, m), 2.92 (2 H, broad s), 5.50 (1 H, broad s); IR (neat, NaCl) 1715 (C=O) cm^{-1}; MS m/e 246; $[\alpha]^{25}_D$ -35.0° (EtOH).

In the same manner l-13 gave l-14: $[\alpha]^{25}_D$ +24.2° (EtOH).

d-4-(2-endo-Bornyl)-2-methylcyclohexanone (d-6). Hydrogenation of d-4-(2-endo-bornyl)-6-methyl-3-cyclohexenone (d-14) as described for the preparation of d-12

gave crude d-6 in a yield of 97%. This was purified via its semicarbazone (mp 197.0 ∼ 198.8 °C) d-6; NMR δ 0.82 (s), 0.85 ∼ 2.5 (m); IR (neat, NaCl); 1720 (C=O) cm^{-1}; MS m/e 248; $[\alpha]^{25}_D$ +44.2° (EtOH).

In the same manner l-14 gave l-6: $[\alpha]^{25}_D$ -31.8° (EtOH).

dl-5,5,6-exo-Trimethyl-2-norbornanone (dl-15). In a 100-gal glass-lined reactor were charged camphanyl guaiacol (11.1 kg), benzene (50 kg), and water (190 kg), and the mixture was warmed to 30 °C. Potassium permanganate (11.1 kg) was added over 12 h at 30 °C, followed after 2 h stirring by 50% sulfuric acid (2.5 kg) over 2 h. The reaction mixture was heated to drive off benzene-water azeotrope, the water layer being returned to the reactor. When 48 kg of the benzene layer had been recovered, the mass was adjusted to pH 4 with 50% sulfuric acid and refluxing was resumed. From a total 57 kg of benzene layer recovered, there was obtained after stripping of solvent 611 g of a mixture of dl-15 and camphor (3:2 ratio). This yielded 144 g pure dl-5,5,6-exo-trimethyl-2-norbornanone (dl-15) via GLC (F&M Prepmaster) separation. Similarly dl-15 was also obtained from camphanyl phenol: mp 67.4 ∼ 70.8 °C; NMR δ 0.96 (3 H, d, J = 7 Hz), 1.01 (6 H, s), 1.4 ∼ 2.4 (7 H, m); IR (neat, NaCl) 1745 (C=O) cm^{-1}; MS m/e 152.

dl-5,5,6-exo-Trimethyl-2-norbornanone l-2,3-Butanediol Ketal (d,l- and l,l-16). Ketalization was accomplished by 5 h reflux in a Dean-Stark apparatus of dl-5,5,6-exo-trimethyl-2-norbornanone (dl-15) (15.2 g, 0.1 mol), l-2,3-butanediol (9.0 g, 0.1 mol), p-toluenesulfonic acid (200 mg), and cyclohexane (100 mL). Usual workup and distillation gave d,l- and l,l-16, 19.6 g (87%): bp 63-75 °C (0.8 mmHg); NMR δ 0.82 (3 H, d, J = 7 Hz), 0.85 (3 H, s), 0.97 (3 H, s), 1.14 ∼ 1.28 (6 H, m), 1.3 ∼ 2.0 (7 H, m), 3.4 ∼ 3.7 (2 H, m); IR (neat, NaCl) 1105 (C-O-C) cm^{-1}; MS m/e 224.

Optical Resolution of dl-5,5,6-exo-Trimethyl-2-norbornanone (dl-15). The method described for the separation of the analogous ketals of camphor on a small scale (Corey, 1962) was applied successfully to the preparative scale optical resolution of the dl-5,5,6-exo-trimethyl-2-norbornanone l-2,3-butanediol ketal using an F&M Prepmaster. Conditions: column, $^3/_8$ in. × 24 ft stainless steel; liquid phase, 20% 1,2,3-tri-(2-cyanoethoxy)propane; solid phase, 60/80 mesh Chromosorb WAW; column temperature, 110 °C; injection port temperature, 200 °C; detector temperature, 200 °C; carrier gas (He) flow rate, 250 cm^3/min. One hundred twenty-five injections (200 μL each) gave l,l-16 (4.7 g, 85% pure, $[\alpha]^{25}_D$ -18.0°) and d,l-16 (3.2 g, 99% pure, $[\alpha]^{25}_D$ -3.3°).

Sample size and temperature were limited by the sensitivity of the liquid phase to overloading and to high temperature. Although each injection required 2 h to elute, the total time required was brought within tolerable limits by overlapping the injections which did not affect the separation.

Hydrolysis of Ketal (d,l-16 and l,l-16). The ketal (d,l-16) (4.2 g, 19 mmol) in methanol (40 mL) was hydrolyzed with 5% sulfuric acid (16 mL). The usual workup gave d-5,5,6-exo-trimethyl-2-norbornanone (d-15) [2.7 g, 95% yield; $[\alpha]^{25}_D$ +11.6° (hexane)].

In the same way l-15, $[\alpha]^{25}_D$ -8.1° (hexane), was obtained.

d-2-Methyl-4-(2-oxy-5,5,6-exo-trimethyl-2-(exo- and endo)-norbornyl)anisole (d-17). To magnesium (0.78 g, 32 mmol) and THF (5 mL) was added a solution of 2-methyl-4-bromoanisole (5.37 g, 27 mmol) in THF (20 mL) at 40 °C during 40 min. After stirring at 50 °C for 1.5 h, a solution of d-15 (2.70 g, 18 mmol) in THF (20 mL)

was added at 45 °C over 1 h and the reaction mixture was stirred at 45 °C for 1.5 h. The usual workup gave 5.24 g of crude product which was purified by column chromatography using Florisil (150 mL) and ether–hexane (1:20) as an eluent: yield, 2.00 g (62%); NMR δ 0.91 (3 H, s), 0.91 (3 H, d, J = 8 Hz), 1.13 (3 H, s), 1.2 $\sim$ 2.5 (8 H, m), 2.22 (3 H, s), 3.78 (3 H, s), 6.7 $\sim$ 7.3 (3 H, m) ppm; IR (neat, KBr) 3530, 3430 (-OH), 1600 (arom) cm^{-1}.

In the same way, dl-17 (78% yield) and l-17 (71% yield) were obtained.

d-2-Methyl-4-(5,5,6-exo-trimethyl-2-norbornyl-enyl)anisole (d-18). Treatment of d-17 with boron trifluoride etherate in ether at room temperature gave d-18 in a yield of 85%: NMR δ 0.82 (3 H, s), 1.02 (3 H, s), 1.04 (3 H, d, J = 6 Hz), 0.9 $\sim$ 1.6 (3 H, m), 1.7 $\sim$ 1.9 (1 H, m), 2.18 (3 H, s), 2.34 (1 H, broad s), 2.72 (1 H, s), 3.78 (3 H, s), 6.18 (1 H, d, J = 3 Hz), 6.7 $\sim$ 7.2 (3 H, m) ppm; IR (neat, KBr) 1600 (arom), 1250 (-OCH$_3$), 800 (arom) cm^{-1}; MS m/e 256.

In the same manner, l-17 gave l-18.

d-2-Methyl-4(5,5,6-exo-trimethyl-2-$endo$-nor-bornyl)anisole (d-19). Both hydrogenation over Raney nickel and modified Birch reduction of d-18 as described for the preparation of 10 and 13 gave d-19 exclusively: NMR δ 0.68 (3 H, d, J = 8 Hz), 0.76 (6 H, s), 1.10 $\sim$ 2.20 (7 H, m), 2.23 (3 H, s), 3.1 (1 H, m), 3.80 (3 H, s), 6.6 $\sim$ 7.2 (3 H, m) ppm; IR (neat KBr) 1600 (arom), 1250, 1140, 1040 (-OCH$_3$), 860 (arom) cm^{-1}; MS m/e 258.

d-6-Methyl-4-(5,5,6-exo-trimethyl-2-$endo$-nor-bornyl)-3-cyclohexenone (d-20). In the same manner described for the preparation of d-14, the modified Birch reduction followed by hydrolysis of d-19 gave d-20: NMR δ 0.83 (3 H, s), 0.86 (3 H, d, J = 7 Hz), 0.87 (3 H, s), 1.08 (3 H, d, J = 7 Hz), 1.0 $\sim$ 2.7 (11 H, m), 2.90 (2 H, broad s), 5.50 (1 H, broad s); IR (neat, NaCl) 1720 (C=O) cm^{-1}; MS m/e 258.

d-2-Methyl-4-(5,5,6-exo-trimethyl-2-$endo$-nor-bornyl)hexanone (d-4). In the same manner described for the preparation of d-6, the hydrogenation of d-20 over 10% palladium on carbon gave d-4; NMR δ 0.82 (3 H, d, J = 7 Hz), 0.85 (3 H, s), 0.97 (3 H, d, J = 7 Hz), 0.98 (3 H, s) 1.0 $\sim$ 2.6 (16 H, m) ppm; IR (neat, NaCl) 1720 (C=O) cm^{-1}; MS m/e 260; [α]$^{25}_D$ -25.8° (acetone).

In the same manner, l-20 gave l-4: [α]$^{25}_D$ +14.7 (acetone).

2-Methyl-4-(5,5,6-exo-trimethyl-2-(exo- and $endo$)-norbornyl)anisole (d-21 and d-19). A mixture of d-2-methyl-4-(2-oxy-5,5,6-exo-trimethyl-2-norbornyl)-anisole (d-17) (3.0 g, 11 mmol), freshly prepared Raney nickel (30 g) and 1-propanol was refluxed for 5 h. Filtration and solvent stripping gave 2.70 g (99% pure) (94% yield) of a mixture of d-21 and d-19 (ratio 77:23). In the same way, l-21 and l-19 (68% yield) (ratio 71:29), and dl-21 and dl-19 (63% yield) (ratio, 65:35) were prepared. The exo and endo isomers were separated by HPLC.

Separation of d-2-Methyl-4-(5,5,6-exo-trimethyl-2-(exo- and $endo$)-norbornyl)anisole (d-21 and d-19). Separation of d-21 and d-19 was accomplished via preparative high-performance liquid chromatography. Apparatus and conditions: column $^3/_8$ in. × 3.3 ft stainless steel; pump Model 6000 (Water Associates); Packing 37 Porasil T (Water Associates); detector UV-254 (Varian Aerograph); injector loop valve injector (Disc Instruments); collector valve three-way valve (Valco Instruments); eluent spectroscopic grade hexane; flow rate, 10 mL/min. Partial recycling technique was used for complete separation of d-21 and d-19. The details of this procedure will be reported (Yoshida, 1977). By this method both exo and endo

isomers were obtained in 99+% purity: d-21, NMR δ 0.89 (3 H, d, J = 8 Hz), 0.90 (3 H, s), 1.04 (3 H, s), 1.2 $\sim$ 1.9 (7 H, m), 2.68 (1 H, t, J = 8 Hz), 3.80 (3 H, s), 6.6 $\sim$ 7.3 (3 H, m) ppm; IR (neat, KBr) 1600 (arom), 1250, 1140, 1040 (-OCH$_3$), 800 (arom) cm^{-1}; MS m/e 258.

d-2-Methyl-4-(5,5,6-exo-trimethyl-2-exo-nor-bornyl)-4-cyclohexenone (d-22). A solution of d-2-methyl-4-[5,5,6-exo-trimethyl-2-norbornyl)anisole (d-21) (1.6 g, 6.2 mmol) in THF (48 mL) was added to liquid ammonia (90 mL) at -35 °C over 5 min, followed by lithium pieces (1.3 g, 0.19 g-atom) over 10 min. After 15 min of stirring at -40 $\sim$ -35 °C, $tert$-butanol (17.7 g) was added at -30 °C over 20 min. Stirring was continued at this temperature for 1.5 h and usual workup gave 1.5 g of oil which was dissolved in THF (20 mL) and heated under reflux with 30% aqueous acetic acid for 6 h. Usual workup gave 1.2 g (78% yield of d-22: NMR δ 0.87 (3 H, d, J = 8 Hz), 0.88 (3 H, s), 0.96 (3 H, s), 1.12 (3 H, d, J = 7 Hz), 1.0 $\sim$ 2.7 (11 H, m), 2.85 (2 H, broad s), 5.34 (1 H, broad s) ppm; IR (neat, NaCl) 1720 (C=O) cm^{-1}.

d-2-Methyl-4-(5,5,6-exo-trimethyl-2-exo-nor-bornyl)cyclohexanone (d-5). Hydrogenation of d-2-methyl-4-(5,5,6-exo-trimethyl-2-exo-norbornyl)-4-cyclo-hexenone (d-22) (1.2 g, 4.9 mmol) in ethanol (20 mL) at room temperature and atmospheric pressure with 10% palladium on carbon (100 mg) gave crude product (1.0 g) purified by column chromatography using 30 g of Florisil to give 0.9 g (75%) of d-5: NMR δ 0.86 (3 H, d, J = 8 Hz), 0.88 (3 H, s), 0.93 (3 H, s), 1.01 (3 H, d, J = 7 Hz), 1.0 $\sim$ 2.6 (16 H, m) ppm; IR (neat, NaCl) 1720 (C=O) cm^{-1}; MS m/e 248 [α]$^{25}_D$ +60.9 °C (acetone).

In the same manner l-22 gave l-5: [α]$^{25}_D$ -52.4° (acetone).

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Distribution, Movement, Persistence, and Metabolism of N-Nitrosoatrazine in Soils and a Model Aquatic Ecosystem

Philip C. Kearney,* James E. Oliver, Charles S. Helling, Allan R. Isensee, and Arnold Kontson

[ring-^{14}C]-N-Nitrosoatrazine (II) [2-chloro-4-(N-nitroso-N-ethylamino)-6-(isopropylamino)-s-triazine] was prepared by nitrosating [ring-^{14}C]atrazine (I) [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] at 0 °C with dinitrogen tetroxide and purified by silica gel column chromatography. The mobility of II, as measured by soil TLC, was only slightly less than that of I in five soils of differing texture and organic matter content. When II was held in the dark, it degraded to I in ecosystem water (33% degraded after 9 days and 62% after 18 days). Based on ^{14}C, bioaccumulation ratios (BR) for II in fish were 22 after 9 days and 31 after 18 days. For I, BR values were 17 and 16 after 9 and 18 days, respectively. No formation of II from I was detected (a) after 1, 2, or 3 months in either of two soils treated with 2 ppm I and 0, 100, or 1000 ppm N (as NH$_4$NO$_3$), or (b) after 1 month in a soil treated with 2 ppm I and 100 ppm N (NH$_4$NO$_3$) maintained at pH's 2.5, 3.5, or 4.5. Transient formation of II from I was observed in an acidic soil amended with 100 ppm N as sodium nitrite. The limit of detection was about 10 ppb for nitrosoatrazine under the experimental conditions described.

The possible formation of N-nitroso derivatives of pesticides or their metabolites in the environment is a subject of current interest. Pesticides N-nitrosated under in vitro and in vivo conditions in the laboratory have included the fungicide ziram (zinc dimethyldithiocarbamate), the insecticides carbaryl (1-naphthyl methylcarbamate) and propoxur (o-isopropoxyphenyl methylcarbamate), and the herbicides benzthiazuron [N-(2-benzothiazolyl)-N'-methylurea], simazine [2-chloro-4,6-bis(ethylamino)-s-triazine], and atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] (Eisenbrand et al., 1975). Wolfe et al. (1976) prepared N-nitrosoatrazine [2-chloro-4-(N-nitroso-N-ethylamino)-6-(isopropylamino)-s-triazine] by nitrosating atrazine with aqueous nitrous acid. Little is known about the environmental formation or stability of N-nitrosoatrazine. The extensive use of atrazine in crop production programs utilizing heavy application of N fertilizers has raised questions about the possibility of its N-nitrosation in soils.

This paper describes the synthesis of [ring-^{14}C]-N-nitrosoatrazine, the extent of N-nitrosoatrazine formation from [ring-^{14}C]atrazine in soils under various N and pH regimes, and the stability and distribution of [ring-^{14}C]-N-nitrosoatrazine and atrazine in soils and in a model aquatic ecosystem.

METHODS AND MATERIALS

Synthesis of [^{14}C]-N-Nitrosoatrazine. Anhydrous sodium acetate (50 mg) and a solution of dinitrogen tet-

Agricultural Environmental Quality Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705.

roxide (N$_2$O$_4$, 0.25 mmol) in methylene chloride (0.4 mL) were combined at -78 °C, then a solution of atrazine (4.9 mg) plus [ring-^{14}C]atrazine (45 μCi, ~0.7 mg) in methylene chloride (4 mL) was added. The mixture was stirred and warmed to 0 °C, maintained at 0 °C for 0.5 h, then filtered through a column of Florisil in a Pasteur pipet. The solvent was evaporated and was replaced with a solution of acetic acid (6 μL) in benzene (2 mL). The resulting solution was warmed gently 15 min, then was concentrated somewhat and added to a column of silica gel (5 g). The column was eluted with benzene (15 mL), then with benzene-ethyl acetate (95:5, v/v). The first 18 mL of the latter was discarded, then the bulk of N-nitrosoatrazine was collected in the next 15 mL. This fraction was evaporated to dryness to give 3 mg of [ring-^{14}C]-nitrosoatrazine (22 μCi) as a yellow solid. Since N-nitrosoatrazine is very sensitive to light (Wolfe et al., 1976), all operations, including analyses, were carried out in a darkened room.

Soils Studies. *Nitrosoatrazine Formation in the Presence of Nitrate.* Two soils, Matapeake loam and Monmouth fine sandy loam, were utilized to study the possible formation of N-nitrosoatrazine. Ammonium nitrate was added to two 1-kg portions of each air-dried soil at rates equivalent to 100 and 1000 ppm N (i.e., 0.286 and 2.86 g/kg soil); a third portion of each soil received no NH$_4$NO$_3$. [ring-^{14}C]Atrazine (1 μCi) was also added to give a final concentration of 2 ppm. The soils were wetted to 75% field moisture capacity and stored in the dark. Core samples of the soil (~100 g) were taken after 1, 2, and 3 months, shake extracted with benzene-ethyl acetate (1:3) overnight, and again with methanol (2 h). Extracts were filtered, an aliquot removed for liquid scintillation