Structure–Odor Relationships for "Catty"-Smelling Mercapto Compounds in Humans

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Qualitative odor similarities of a series of mercapto ketones and mercaptans were determined on human subjects. *trans*-8-Mercapto-*p*-menthan-3-one, 4-mercapto-4-methyl-2-pentanone, 3-mercapto-3-methyl-2-pentanone, and *tert*-amyl mercaptan were selected from a group of 10 mercapto odorants and were tested in a confusion matrix subjected to factor correspondence analysis. The three mercapto ketones and surprisingly also the mercaptan were perceived as similar in odor quality. The tertiary mercapto amyl substructure appears to be the dominant odor quality determining feature of the "catty" note. The keto group can be in α- or β-position to the mercapto group but appears to be nonessential as in *tert*-amyl mercaptan. Similar structure–activity relationships had been found previously for the same volatiles in two behavioral experiments on rats measuring stress induction and odor similarity.

Minute traces of mercapto ketones can contribute both desirable flavor and off-flavor to several foods and beverages such as cheese, beer, and fruit juice (Table I). They and their derivatives are among the most powerful odorants known, with human thresholds in water down to 10^{-6}–10^{-8} ppm (Meilgard, 1975; Stoffelsma and Pypker, 1975; Demole and Enggist, 1982b; Rigaud et al., 1986). The similarity to the characteristic odor of tomcat urine associated with mercapto ketones was first reported by Pearce et al. (1967) for 4-mercapto-4-methyl-2-pentanone (a, Figure 1).

We have evaluated a group of mercapto odorants (Figure 1) with catty, tarry, sweaty, fruity, and gassy odor notes, for qualitative odor similarities on humans, using the method of confusion matrix. Our purpose was to find structure–odor relationships that would provide clues to which molecular features determine odor quality. Some of the same odorants had already been used previously for a structure–activity relationship (SAR) study in two behavioral experiments on rats. In the first one, it was observed that they could induce stress in male Wistar rats placed in an odorized open space (Vernet et al., 1984). A potential area repellent for rat pest control was suggested. In the second one, odor similarities were measured by a conditioned behaviorial response in an experimental context considered to be nonstressful (Fombon and Polak, 1987). SAR comparisons between these three experiments show a large degree of overlap (Table II).

METHODS

To obtain human judgments of qualitative odor similarity, we chose the confusion matrix method adapted from the descriptions by Koster (1975), Polak et al. (1978), and Polak (1983). This method is well suited to sets of odorants where quality differences are expected to be small.

Subjects were asked to identify from a set of unmarked samples all of the stimuli previously memorized by odor. The rank order of confusions (errors) was considered to correspond to the rank order of qualitative similarities.

In the present experiment, we have paid particular attention to odorant purity and stability and to approximate equal intensity matching of the odorants by each subject.

MATERIALS

Odorants. The 10 odorants used are given in Figure 1 and Table III. They consisted of nine sulfur-containing odorants including five rat stress-inducing ones (Vernet et al., 1984). Minty-smelling menthone (e), the non-sulfur analogue of b and f, was added as an easily discriminated control odorant. They were used in a preliminary screening experiment to select the five odorants used in the confusion matrix experiment (a–e).

Solvents. An odorless grade of diethylene glycol (DEG) from Prolabo, Paris, was used as solvent to prepare odorant predilutions. These were dosed into odorless commercial mineral water, pretreated with 6 mg/L of the odorless chelating agent, ethylenediaminetetraacetic acid tetrasodium salt (EDTA) (J. T. Baker Chemicals B.V., Deventer, Holland). This treatment was essential to complex traces of heavy metal ions capable of neutralizing the mercapto (thiol) odor. Dissolved EDTA did not change the odor background for humans.

Stimuli Concentrations. Those used are listed in Table IV. Odorants were prediluted in DEG (10^{-1}–10^{-4}%) and the resultant mixtures added to EDTA-treated water immediately prior to use each day. All concentrations were v/v in DEG or water. Headspace concentrations were not determined.

Stability of Stimuli. Because mercapto ketones and mercaptans were unstable and prone to oxidation to less intense odors in the presence of air and light and trace metals, they were stored at low temperature ($\leq$0 °C) as such or in DEG solutions.

Presentation of Stimuli. The odorants were presented in dilute 100-mL aqueous solutions, in coded sniff bottles (250-mL glass Erlenmeyers having a 30-mm inner diameter neck provided with a glass cover).

Subjects. Fifteen inexperienced subjects, 10 women (aged 21–31) and 5 men (aged 23–26), were selected by a qualitative and a quantitative test. The qualitative test consisted of matching a variety of unmarked appropriately diluted odorants with descriptive keywords. The quantitative test consisted of lining up aqueous serial dilutions of 1-butanol in order of increasing intensity. The selection of subjects was based on the Kramer rank order test (Kramer et al., 1974). Eleven subjects (4 male, 7 female) participated in the preliminary experiment and 8 subjects (2 male, 6 female) in the complete confusion matrix. Of these, 1 male and 3 female subjects participated in both tests.

EXPERIMENTAL SECTION

Preliminary Experiment. All 10 odorants (Figure 1) were tested with 11 subjects for qualitative proximity to 4-mercapto-4-methyl-2-pentanone (a) as the reference. For each subject, the concentrations were adjusted in 30%

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Table I. Occurrence of Mercapto Ketones and Derivatives Having Mercapto *tert*-Amyl Substructure

occurrence	odor description	reference	odorant[a]
beer	catty, ribes[b]	Cooser et al. (1980)	a
	sunstruck	Gunst and Verzele (1978)	3-methyl-2-butene-1-thiol
vegetables	catty	Aylward et al. (1967)	a
meat stores	catty	Patterson (1968)	a
buchu leaf oil	buchu	Lamparski and Schudel (1971)	b, f
	fruity-minty[c]	Sundt et al. (1971)	b, f
flavors	black currant	Fenaroli (1975)	b, f
	oniony, sweaty	Pypker and Stoffelsma (1975)	β-mercapto alcohols and esters
tobacco		Mookherjee (1982)	(methylthio)-β-damascone (methylthio)-β-damascenone
black currant bud oil	catty	Rigaud et al. (1986)	4-methoxy-2-methylbutane-2-thiol
air pollution	catty	Maarse and Ten Noever (1974)	c
cheese	catty	Steinholt and Swensen (1979)	a
grapefruit juice	green citrus (-) more fruity (+) more sulfury	Demole et al. (1982a,b)	1-p-menthene-8-thiol

[a] See Figure 1 for a–c and f. [b] Botanical name for black currant. [c] Trans (b) more black currant like than cis (f).

Table II. Comparison of Human and Rat Response to Mercapto Odorants (a–f) and Menthone (e)[a]

		odor similarity expt	
odorant	rat stress expt[b]	rats[c]	humans
a	+	+[d]	+
b	+	±	+
c	+	+	+
d	+	+	+
f	+	-	±
e	-	not tested	-

[a] Key: +, products discriminated as similar; -, products discriminated as dissimilar. [b] Vernet et al. (1984). [c] Fombon and Polak (1987). [d] Conditioning odorant.

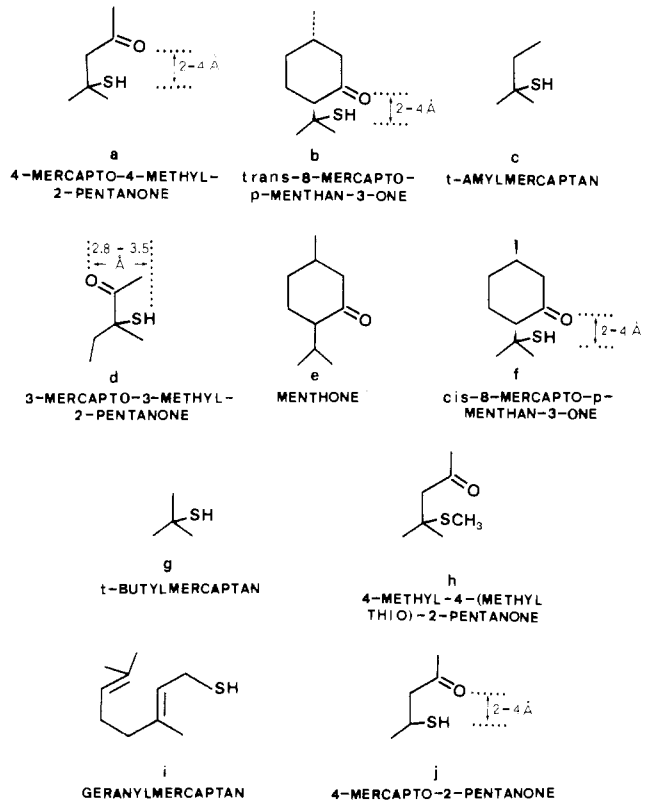

Figure 1. Structural formulas of odorants used. Estimated distances from Dreiding models (Å) between mercapto and keto groups are not on scale and show the range between eclipsed and staggered conformers.

steps in order to approximately equilibrate the intensities of the stimuli with that of a fixed concentration of the reference (a) at 5×10^{-5} ppm in water. (Concentrations used are indicated in Table IV.) In each of four daily 1-h sessions, subjects first became familiarized with the labeled reference odor (a) at the standard concentration as well as two other concentrations, 2.5×10^{-5} and 10^{-4} ppm, in water. Thus, any inconsistency in the intensity matching was reduced. Then, 5 unmarked samples of each of the 10 odorants were presented in random order at individual isointensity concentrations. For each sample, the question asked to the subject was whether this sample is qualitatively the same or different from the reference.

Among the 10 odorants tested (Table III), *trans*-8-mercapto-*p*-menthan-3-one (b), 3-mercapto-3-methyl-2-pentanone (d), and *tert*-amyl mercaptan (c) were most confused with (a) and were chosen for the confusion matrix. Menthone (e), which was discriminated best from the reference (a), was also retained to serve as a control.

Confusion Matrix Experiment. This required seven 1-h daily sessions, with eight subjects (A–H). In the first session, intensities were equilibrated as above, but at fixed concentration of reference odorant (a) of 10^{-4} ppm in water (concentrations used are indicated in Table IV).

The second session was a training session in which the five stimuli, labeled by a letter (a, b, etc.) were presented each at one concentration (Z). Subjects memorized the odor quality and then were asked to identify the odor of unmarked samples by their code letter. Two trials were given with correct code letter feedback by the experimenter. Confusion was tested in the five following sessions. Each session began with intensity reequilibration and quality retraining with feedback. Then, 15 unmarked samples were presented with the five odorants at three concentrations (Z, $^1/_2Z$, $2Z$). The samples were arranged at random in three sets of five samples. Subjects had to identify each sample without feedback and observed a 20-s pause between samples, a 2-min pause between sets of five samples, and a 10-min pause after 15 samples. The samples were then rearranged in another random order, and the protocol was repeated as above. Thus, in five sessions, each odorant was presented a total of 30 times per subject.

RESULTS

For the preliminary experiment, the group results of 11 subjects and 10 odorants are presented in Table V. While the reference 4-mercapto-4-methyl-2-pentanone (a) was identified 190/220 times, other odorants were frequently confused with it in the rank order shown. The mean error rate and range for the other mercapto odorants was 32 ± 9%. The reference (a) and the three odorants most confused with it (b–d) were chosen for the confusion matrix along with well-discriminated menthone (e) as a control.

The confusion matrix results for eight subjects are shown in Tables VI and VII. Table VI shows the group results

Table III

code	name[a]	source	estimated purity
a	4-mercapto-4-methyl-2-pentanone	Givaudan Research, Dubendorf, Switzerland	TLC: one spot
b	*trans*-8-mercapto-*p*-menthan-3-one	*b*	TLC: one spot
c	*tert*-amyl mercaptan [2-methyl-2-butanethiol]	Jansen Chimica, Beerse, Belgium[c]	GLC: 99.5%
d	3-mercapto-3-methyl-2-pentanone	PFW B. V., Amersfoort, Holland	TLC: one spot GLC: >99%
e	menthone [*p*-menthan-3-one]	Roure Bertrand, Argenteuil, France	GLC: 99.9% equal ratio of cis/trans
f	*cis*-8-mercapto-*p*-menthan-3-one	*b*	TLC: 99%, one impurity
g	*tert*-butyl mercaptan [2-methyl-2-propanethiol]	Jansen Chimica	GLC: >99%
h	4-methyl-4-(methylthio)-2-pentanone	Oril S.A., Paris, France	TLC: 99%
i	geranyl mercaptan [3,7-dimethyl-2,6-octadiene-1-thiol]	PFW, B.V.	GLC: 98% cis + trans isomers
j	4-mercapto-2-pentanone	PFW, B.V.[d]	TLC: >99%, one impurity

[a] *Chemical Abstracts* nomenclature has been added in brackets wherever it differs from common usage names used in the text. [b] The mixture of cis (+) and trans (−) isomers (ratio 40/60) was furnished by Givaudan. The isomers were separated and purified by M. Colin, ICSN at CNRS, Gif-sur-Yvette, France, according to an LLC procedure furnished by K. B. de Roos, Research Laboratory, PFW B.V., Amersfoort, Holland. Deactivated silica gel was used. [c] The technical grade (GLC 98.7%) was purchased from Aldrich, Beerse, Belgium, and purified by PFW, B.V., by preparative LLC and GLC to 99.5%. [d] Purified by M. Colin using the same procedure as for b and f.

Table IV. Concentrations Used in 10^{-3} μL/L Water (Equivalent to 0.001 ppm)

product	prelim expt	confusion matrix
a	0.05	0.1
f	2–10	–
b	5–50	1–3
h	2–7	–
d	4–8	7.5–16
c	0.2–0.8	0.06–0.7
g	0.01–0.025	–
i	0.2–0.08	–
e	2.5–12	110
j	100–200	–

Table V. Preliminary Experiment

code	name	responses[a]
a	4-mercapto-4-methyl-2-pentanone	190
d	3-mercapto-3-methyl-2-pentanone	150
b	*trans*-8-mercapto-*p*-menthan-3-one	127
c	*tert*-amyl mercaptan	95
f	*cis*-8-mercapto-*p*-menthan-3-one	63
g	4-mercapto-2-pentanone	43
h	*tert*-butyl mercaptan	35
i	4-methyl-4-(methylthio)-2-pentanone	27
j	geranyl mercaptan	23
e	menthone	6

[a] Number of times 10 odorants were identified as the reference odorant 4-mercapto-4-methyl-2-pentanone (a) by 11 subjects. Each odorant was presented a total of 220 times.

for eight subjects in the confusion matrix experiment and Table VII the individual results with sessions pooled. These tables do not take account of respectively between-subject variance and within-subject per session variance. In order to determine their contribution to odor similarity distances, the association patterns between subjects and responses were analyzed by multidimensional scaling. The factorial method of correspondence analysis was used; it is based on χ^2 distances in which both the variables and the subjects are represented by points in the same multidimensional space (Benzecri, 1980; Greenacre, 1984). The method has been used previously to analyze olfactory electrophysiological responses (Duchamp and Sicard, 1984a,b; Revial et al., 1978, 1983).

A table (not shown) was formed in which was horizontally presented each session per subject (40 rows: A1, A2, ..., H4, H5) and vertically presented the five possible combinations of one stimulus and one response for each of the five odorants (25 columns: aa, ab, ..., ed). The intersection of one row and one column was the number of responses for one session and one combination. Correspondence analysis applied to this table allowed the projection of responses and subject sessions on the plane containing the most information.

Table VI. Confusion Matrix[a]

odorants presented	responses a	b	c	d	e	total
a	*122*	54	37	18	9	240
b	53	*104*	35	39	9	240
c	25	34	*121*	49	11	240
d	24	39	40	*125*	12	240
e	6	6	10	13	*205*	240

[a] Group results for eight subjects and five odorants with each odorant presented 30 times per subject. See Figure 1 for odorant identification.

For clarity, the results have been separated into two superposable factor analysis diagrams rather than one. Figure 2A shows the response distribution, and Figure 2B shows each subject per session.

The diagram is interpreted as follows: If two stimulus response pairs are close together, its means that their response distribution, over 40 subject sessions, is similar. When parts A and B Figure 2 are reunited, the proximity of a subject session with a stimulus response pair means that they correspond. For example, E3 corresponds to ba, bc, cb, meaning that during the third session subject E tended to identify odorant b as either a or c, and odorant c as b.

On the positive side of axis 1 in Figure 2A is a cluster of equal stimulus–response pairs corresponding to good discrimination. As one moves radially outward from this cluster, one finds progressively unequal pairs (confusions), representing decreasing discrimination until the worst discrimination, represented by the few confusions with menthone.

For example, confusion bd being closest to the equal pair cluster dd suggests that stimulus b is the most similar in odor to odorant d, because the corresponding subject sessions showed the best discriminating ability. In the reverse confusion, db is further away, suggesting that similarity of d to b is not reciprocal. This information cannot be gleaned from the group results in Table VI showing equal group scores for bd and db.

The same areas in Figure 2B show the corresponding subject sessions. Thus, on the positive side of axis 1 one finds clustered the best discriminating subject sessions, and as one moves away from this cluster, discriminatory ability decreases. Subject C is the most discriminating, A and H are the poorest, and the others are in between.

Table VII. Total of Responses per Subject (Five Pooled Sessions per Subject)

	stimuli[a]																						
	a					b					c					d					e				
subject	aa	ab	ac	ad	ae	ba	bb	bc	bd	be	ca	cb	cc	cd	ce	da	db	dc	dd	de	ea	eb	ec	ed	ee
A	6	4	13	4	3	8	12	5	2	3	6	6	6	10	2	3	6	5	15	1	3	0	3	2	22
B	12	8	4	4	2	7	17	4	2	0	2	6	19	2	1	2	5	5	18	0	1	2	0	2	25
C	23	5	0	2	0	5	19	4	2	0	2	5	18	5	0	0	0	3	26	1	0	0	0	0	30
D	18	9	0	2	1	1	14	2	8	5	1	2	19	7	1	2	9	3	11	5	0	1	1	0	28
E	17	6	3	2	2	9	15	0	5	1	2	1	18	8	1	4	4	13	8	1	2	1	1	1	25
F	14	6	8	2	0	6	8	8	8	0	6	6	13	2	3	4	4	5	15	2	0	1	4	3	22
G	20	7	3	0	0	9	10	4	7	0	2	3	19	6	0	2	5	0	23	0	0	0	1	2	27
H	12	9	6	2	1	8	9	8	5	0	4	5	9	9	3	7	6	6	9	2	0	1	0	3	26

[a] Each stimulus is presented 30 times per subject. Response classes consist of correct answers (equal pairs aa, bb, ..., ee) and of confusions (unequal pairs, e.g. ab means that the stimulus a was identified as b).

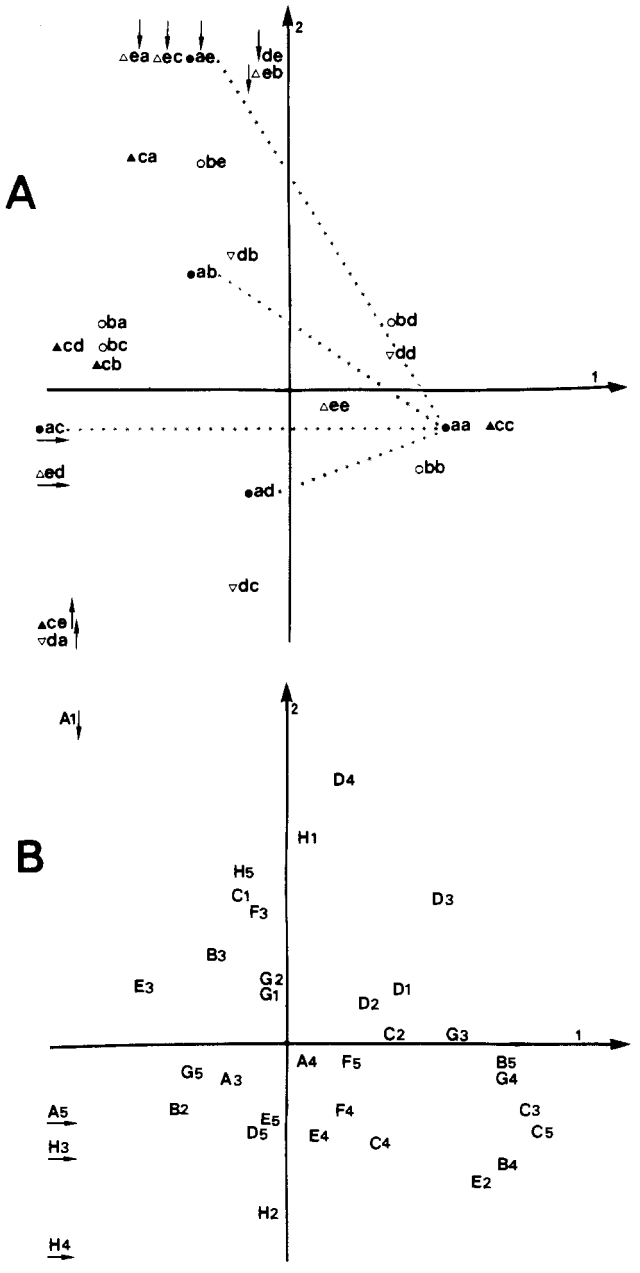

Figure 2. Factor analysis diagram of confusion matrix responses for eight subjects. (A) Variables (stimuli paired with responses) are represented by a first small letter as stimulus and a second small letter as response (aa, ab, ..., cc). Dotted lines connect only one equal pair (aa) to the confusions with a (unequal pairs). (B) Subjects were represented by capital letters associated with a corresponding session number (A1, A2, ..., H6). Arrows represent stimulus response pairs or subject sessions situated outside the figure limits.

Five either first (training) or second sessions out of 40 subject sessions were excluded from the analysis (A2, B1, E1, F1, F2) because they were incoherent with the other sessions of the same subject.

DICUSSION

Odor Similarities. Since menthone (e) was well discriminated, the observed confusions are not random. Mercapto ketones a, b, and d and mercaptan c were frequently confused among each other. However, no subject systematically confused one odorant with another. For most subjects, the sessions are not clustered together but widely dispersed (Figure 2B), indicating a change in judgments from session to session and difficulty in discriminating the mercapto odorants from one another.

Figure 2 differs from the confusion frequency Table VI in that it takes into account subjects' discriminating ability. A particular rank order of similarity among the mercapto odorants is not evident from Figure 2A, since most confusions (unequal pairs) are distributed asymmetrically and most are clustered in an equal-distance zone from the correct response cluster (equal pairs).

The results are those expected for odorants that are closely similar in odor quality but not identical. The same four odorants induced stress and showed odor similarities as well, in the rat experiments (Table II). Humans and rats seem to share similar structure–activity relationships.

Structure–Odor Relationships: Effects of Various Parameters. In the preliminary experiment, fruity–catty smelling *cis*-mercaptomenthanone (f) was recognized better than its more tarry–catty smelling trans isomer (b). Also, conversion of the mercapto group in reference ketone a into a methyl sulfide derivative i resulted in an odor that was 90% discriminated as different from a. Otherwise, the following comments are limited to the five confusion matrix odorants.

Size. Apparently size, in this particular case, is not a dominant odor quality determining factor. The tertiary mercapto amyl substructure $(C)_2CSHCC$ is the most characteristic feature shared by catty odorants a–d. The superimposability of a portion of the carbon skeletons of these compounds *and* the presence of a tertiary mercapto group appear to be sufficient for odor similarity.

The smaller sized, flexible, 5–6 carbon, open-chain compounds a, c, and d were often confused with the larger sized, more rigid, cyclic 10-carbon compound b.

Mercapto–Keto Distance. It made little difference in odor quality whether the mercapto group was α to the keto group, as in d, or β, as in a and b. Dreiding models show that the estimated distances between the keto and the α- or β-mercapto groups (Figure 1) do not appear to differ markedly either as eclipsed conformers (2.8 Å in d, 2.2 Å in a and b, with the mercapto isopropyl group of b in the

predominant equatorial position) or as staggered conformers (3.5 Å in d, 4.0 Å in a and b). α- and β-mercapto ketones might therefore both be present in conformations in which the functional groups overlap and accommodate the same receptor sites.

Possibly the catty and tarry odor quality can be maintained in mercapto ketones until a critical distance between the mercapto and ketone group is reached. For example, Ohloff et al. (1980) found that, for a flowery hydroxy aldehyde odor to be maintained in an odorant, the distance between the hydroxy and carbonyl groups should not exceed 3 Å. When the distance exceeded 3 Å, the compounds were odorless.

Odor similarity implies the activation of similar sets of receptors and hence receptor cells. The presence of a polar acetyl group in a small mercapto ketone such as $(CH_3)_2CHSHCH_2COCH_3$ (a), with its potential for nucleophilic bonding with receptor site, would be expected to activate different receptors than the corresponding nonpolar methylene group in mercaptan $(CH_3)_2CHSHCH_2CH_2CH_3$ (c).

Keto Group. Nevertheless, surprisingly, the keto group appears to be nonessential, provided the common skeletal feature is preserved, as in *tert*-amyl mercaptan (c).

Maarse et al. (1974) had already commented on the similarity of the catty note between mercaptan (c) and mercapto ketone (a). Since the odor similarity could be due to a trace contaminant in c, we took particular pains to purify the commercial quality of c as best as possible (99.5% content by capillary GLC). However, the odor quality did not change.

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