Application of the Computer Automated Structure Evaluation Methodology to a QSAR Study of Chemoreception. Aromatic Musky Odorants

Gilles Klopman* and Dmitri Ptchelintsev

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106

Eighty-seven nitro-free aromatic musks and 65 of their odorless structural analogs were studied using the computer automated structure evaluation (CASE) methodology. A QSAR equation relating the strength of musky odor to 23 structural descriptors and $(\text{Log } P)^2$ was obtained. Lipophilicity appears to be less important than structural features for the strength of musky odor. A hierarchical Multi-CASE analysis identified nine structural determinants responsible for the musky smell as well as seven dearomatizing fragments. This allowed speculations about the spatial requirements for interaction between a musk molecule and a hypothetical musky odor receptor. In random testing the (Multi-)-CASE model was able to predict a priori 9 of 10 musky odorants and 8 of 9 odorless chemicals correctly. Forty-six nitrated musks were analyzed. Overlap was found between the structural requirements for musky odor in nitrated and nitro-free musks.

INTRODUCTION

Three major goals are being pursued by applied chemoreception expert panels in the flavor and fragrance industry (Boelens et al., 1983): (i) prediction of the olfactory properties of given odorants, (ii) rational design of new odorants with predicted olfactory quality, and (iii) elucidation of the primary processes in the mechanism of perception. In the course of this pursuit, many attempts have been made over the years to correlate the structures of odorants with the olfactory impressions they invoke. The nature of these efforts ranged from a multiparameter regression analysis using physicochemical parameters (Wolkowski et al., 1977) and gas chromatographic models (Nachbar and Morton, 1981) to molecular connectivity studies (Kier et al., 1977) and computer-assisted pattern recognition (Jurs et al., 1986). Despite those studies, the area of structure–activity studies in olfaction remains rather confusing. Greenberg reported that the hydrophobic properties were the major determinants of the olfactory properties of molecules which he studied and indicated only poor correlation between odor and structural features (Greenberg, 1979). For other groups of odorants both the molecular weight and the partition coefficient were shown to be insigificant, while structural parameters appeared to be more important (Boelens et al., 1983). Even so, the explicit structural features easily traceable back to the molecules in which they are embedded still remain desirable but elusive in olfactory SAR. As to the importance of lipophilicity, in the 1980s there were speculations that the receptors for taste and smell were probably located on the surface of the cells and, therefore, the overall lipophilicity might not be an especially important property after all (Tute, 1983). This opinion is of interest in light of the most recent experimental evidence that the perception and recognition of odor is mediated via the G-protein coupled transmembrane protein receptors (Buck and Axel, 1991). The structural specificity of the odorant–receptor interaction translates into a specific nature of the olfactory impression.

In any case, without contesting the significance of previous attempts in olfactory QSAR, we clearly see that the selection of parameters in each particular case remains a problem. We, therefore, felt that the computer automated structure evaluation (CASE) methodology developed in our laboratory could provide a promising alternative approach to QSAR in chemoreception. The CASE methodology is an automated computer expert system capable of correlating biological activity with a number of automatically generated and selected parameters including molecular structure, hydrophobicity, and, since recently, molecular weight and water solubility. This methodology has been described on a number of occasions (Klopman, 1984) and has been successfully used since then in a number of SAR studies (Klopman and Srivastava, 1989). Besides evaluating the significance of hydrophobicity, molecular weight, and water solubility, the CASE analysis of structure–activity relationships affords ample and easily interpretable structural descriptors pertinent to activity.

A group of aromatic nitro-free musks was used in this study. The odor of musk has long been an important note in many fragrant compositions. The natural sources of musky aroma include both rare animal and plant species. Natural musks are macrocyclic lactones and ketones with 15–17-carbon chains linked in a ring structure. However, it is by no means a recent discovery that chemicals of other structural types successfully imitate the odor of natural musks. In the 19th century it was discovered that some of the nitrated derivatives of benzene (I) had a musky odor (Baur, 1891).

In over a hundred years, the family of synthetic musks grew significantly to include nitrated derivatives of tetralin and indan (II). At the same time, the limited stability of the nitro musks and their tendency to light-induced discoloration sustained an interest in more stable non-nitro alternatives. Impressive synthetic programs including the ones at Givaudan Corp. (USA) and International Flavors and Fragrances, Inc., resulted in the advent of a large class of non-nitro musks. The latter includes nitro-free derivatives of benzene (I), tetralin and indan (II), isochroman (III) and some of its hydrogenated relatives (IX), acenaphthene (IV), coumarin (V), naphthindanone (VI), hydrindacene (VII), and hydrindacenone (VIII) (Figure 1).

Besides being commercially important, musks also possess a very distinct odor which can hardly be confused with any other smell. In fact, musk is believed to be one

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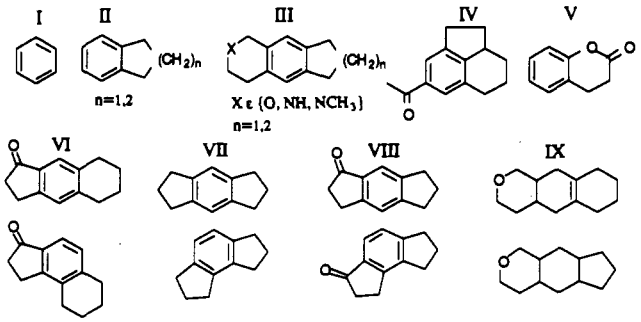

Figure 1. Structural types of the nitro-free aromatic musks used in the CASE analysis of musky odor–structure relationship.

Table I. Structure of the Database Used in the (Multi-)CASE Analysis of Structure–Musky Odor Relationships in Aromatic Musks

structural type	moderate/ strong	weak	odorless/ nonmusk
benzene	4	3	11
indan	16	14	4
tetralin	10	5	42
coumarine	1	1	0
acetyltetramethyl-*s*-hydrindacene	0	1	1
acetyltetramethyl-*as*-hydrindacene	1	1	0
acenaphthene	1	0	2
poly(alkylhydrindacenone)	8	2	6
poly(alkyltetrahydronaphthindanone)	9	1	5
isochroman	12	7	6
total	62	35	75

of the primary human odors (Jennings-White, 1985). Therefore, musks present both an important and the most misclassification-proof set of molecules for an SAR study in olfaction.

We identified several structural determinants we believe are responsible for the musky odor or lack of such in chemicals of structural types I–IX (Figure 1). They were used to speculate about the structural requirements for musky smell in aromatic nitro-free musks and suggest some spatial characteristics of the hypothetical "musky" receptor. (Log P)^2 was selected as a parameter in the overall QSAR equation, indicating that lipohilicity does play a role in muskiness. The QSAR equation yielded a good retrofit of the experimental data. The model was successfully used to predict a priori the musky odor or lack of such in 20 chemicals not included in the training set. We also analyzed a group of nitro musks and identified an overlap in odorphoric structures between nitro and nitro-free musks.

DATABASE AND METHODOLOGY

All sensory data for this study were obtained from a comprehensive review of the literature on the chemistry of aromatic musks compiled by T. Wood of Givaudan Corp. (Wood, 1970). A total of 172 chemicals included 97 musky odorants of 10 different structural types along with 75 of their close structural but nevertheless odorless analogs. The former group of 97 musks was subdivided into two categories—"weak" and "moderate/strong" musks based on their odor intensity as reported in the review papers. It should be emphasized that a musk referred to as weak has the same musky odor quality as a moderate/strong musk; it is only on the changes in odor thresholds, not in odor quality, that we base the usage of the descriptive measures of odor. As a result, the database contained 35 weak musks and 62 musks of moderate and strong intensity (Table I). Twenty compounds were picked randomly and removed from the database to be submitted later for a priori predictions.

The same source was used to compile a database of 46 nitrated aromatic derivatives—34 compounds with strong/moderate musky odor, 5 weak musks, and 7 odorless compounds.

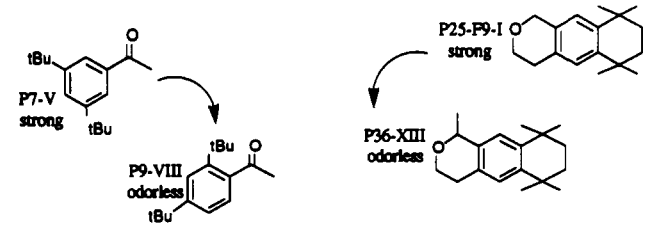

Figure 2. Minute structural changes produce a dramatic impact on the strength of musky odor.

The required input consists of the molecular structure encoded with the Klopman line notation (KLN) code (Klopman and McGonigal, 1981) and biological activity values represented by the CASE indices. On the basis of the CASE activity scale, we assigned activity values of 1 to all nonmusk and/or odorless chemicals, 2 to all weak/faint musks, 3 to all chemicals reported as moderate musks, and 4 to strong musks.

Once the database has been entered, it is submitted to the analysis. The latter starts by fragmenting each molecule into chains of 2–10 heavy atoms with attached hydrogens. A fragment is labeled as *inactive* if it has been derived from an inactive molecule and *active* if it originates from an active molecule. Simultaneously, the program calculates the molecular weight, partition coefficient, and water solubility for each compound. These parameters along with all automatically generated structural features constitute a pool of descriptors subjected to the statistical evaluation. Fragments encountered randomly in both active and inactive molecules are regarded as irrelevant to activity. Each substructural descriptor with distribution significantly skewed toward the active part of the database is assumed to be contributing to the activity. Accordingly, if a descriptor occurs predominantly in the odorless molecules, it is believed to quench the activity. A fragment is significant if its binomial distribution has less than 15% probability of being due to chance. The descriptors associated with the subsets of musky molecules are called biophores. Significant descriptors from the inactive part of the database are called biophobes. It is postulated that the presence of a biophore in a molecule is a prerequisite for activity. Alternatively, the presence of a biophobe will most likely render a compound inactive.

RESULTS AND DISCUSSION

Analysis of Nitro-Free Aromatic Musks. Even upon first examination, it appears that the intensity of musky odor is tightly linked to the molecular structure—more so than to the physical–chemical parameters. Indeed, changes in structure too minute to cause considerable shifts, if any, in the molecular weight (and therefore volatility) or lipophilicity (and therefore passive biotransport) can still produce dramatic impact upon the musky odor strength.

Just to mention a few examples, isomers P7-V and P9-VIII (Figure 2) have identical Log P values of 4.46 as calculated according to the computer automated structure evaluation (CASE) approach (Klopman and Wang, 1991). Yet P7-V is a strong musk, whereas P9-VIII is odorless. Very close structural analogs P25-F9-I and P36-XIII have similar Log P values in the range of 0.31 of each other, but, again, the former is a strong musk and the latter has no odor. Such examples abound in the database and bring out the importance of structural properties.

A total of 55 308 fragments were generated by breaking up each of the 152 molecules into linear chains of 2–10 heavy atoms with attached hydrogens. Fragments that passed the binomial probability test ($p < 15\%$) were considered as potential QSAR parameters. The 23 fragments (Figures 3 and 4) used in the final QSAR equation (Figure 5) were selected through a forward stepwise regression analysis. These 23 variables included 8 deactivating and 16 activating fragments. Combined with (Log P)^2 and a regression constant, these structural parameters

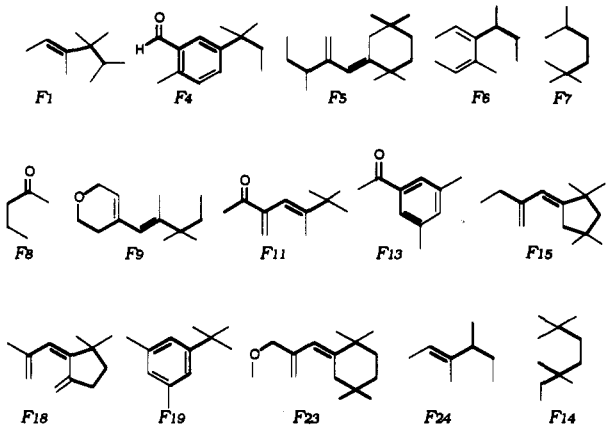

Figure 3. Activating structural descriptors used in the CASE QSAR eq 2. Fine lines represent bonds to non-hydrogen substituents.

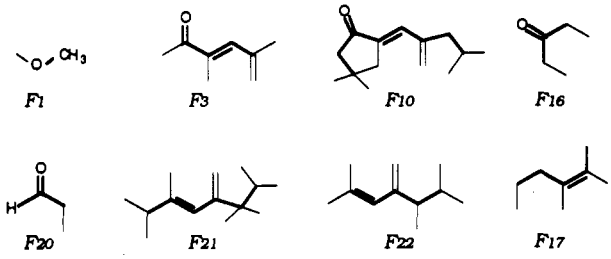

Figure 4. Deactivating structural descriptors used in the CASE QSAR eq 2. Fine lines represent bonds to non-hydrogen substituents.

$$\text{MUSK ODOR INTENSITY (MOI)} = 0.20n_1F_1 - 2.17n_2F_2 - 0.12n_3F_3 + 2.89n_4F_4 + 0.86n_5F_5 + 1.90n_6F_6 + 0.54n_7F_7 + 2.07n_8F_8 + 2.49n_9F_9 - 1.51n_{10}F_{10} + 1.14n_{11}F_{11} + 0.41n_{13}F_{13} + 1.44n_{14}F_{14} + 0.72n_{15}F_{15} - 1.34n_{16}F_{16} - 2.93n_{17}F_{17} + 1.75n_{18}F_{18} + 0.55n_{19}F_{19} - 0.97n_{20}F_{20} - 0.20n_{21}F_{21} - 0.26n_{22}F_{22} + 0.79n_{23}F_{23} + 0.25n_{24}F_{24} + 0.13(LogP)^2 + 1.61 \quad (2)$$

(n=152, F=26.11, r =0.83, s=7.90)

Figure 5. CASE QSAR equation relating the musky odor intensity (MOI) of aromatic nitro-free musks to their structural parameters (F_i) and hydrophobicity (Log P): F_i, ith fragment; n_i, number of times a fragment F_i occurs in a molecule.

provided the best retrofit of the experimental data. At each step of adding a parameter to eq 2, the partial F-test was evaluated to ensure that each descriptor was significant at least at the 95% confidence level.

Equation 2 in Figure 5 relates the musky odor intensity (MOI) of the chemicals in the database to the presence of 23 structural parameters and the square of the octanol/water partition coefficient. A simple set of rules based on the CASE activity scale can be used to translate the MOI values into the actual odor descriptions:

$$1 < \text{MOI} < 2 = \text{odorless/nonmusk (–)}$$

$$2 < \text{MOI} < 3 = \text{weak/faint musk (+)}$$

$$3 < \text{MOI} < 4 = \text{moderate musk (++)}$$

$$4 < \text{MOI} = \text{strong musk (+++)}$$

Interestingly, besides the structural parameters, eq 2 contained the squared value of the Log P with a positive coefficient, indicating that strong musky odor is associated with high lipophilicity. The index of determination R^2 was calculated to be 0.83 and the standard deviation of residuals 7.90. The F-test value of 26.11 was sufficiently high to exclude the possibility that the correlation was merely due to chance (Klopman and Kalos, 1985). A low incidence of false negatives and the absence of false positives contrasted with a rather "fuzzy" retrofit of weak/faint musks (Table II). Only 8 of 35 weak/faint musks were described as such in the retrofit, 21 having been predicted to be odorless/nonmusky and 5 to be marginal/strong musks. This may be a consequence of subjectiveness in the experimental odor intensity evaluation. Indeed, it is reasonable to expect that the degree of "doubt" during classification is highest when the odor is weak. This "boundary doubt" (boundary being the one between odoriferous and odorless) may have been carried over into the model.

The Multi-CASE analysis (Klopman, 1992) identified a number of structural determinants responsible for the musky smell with probability of relevance above 95% (Figure 6). Odorphobic fragments are presented in Figure 7.

Some of the conclusions that can be made upon examination of the list of odorphores in Figure 6 are the following:

First, a hydrophilic substituent on the central ring is required, its most electronegative atom being β to the central ring. This conclusion is exemplified by the fact that P34-VIII is a strong musk, whereas its isomer P36-XVIII is practically odorless (Figure 8). Nevertheless, a musk odorant can have an oxygen atom directly connected to the benzene ring (Figure 9). Assuming the same positioning of the aromatic ring inside the receptor cavity, in P18-XXI the oxygen on the aromatic ring is more distal from the hydrophilic spot on the receptor than the oxygen in P34-VIII. Notice, however, that the less favorable distance factor in P18-XXI is made up for by the higher hydrophilicity of the oxygen function in this case: in P18-XXI the hydrophobic constant π(OH) = –0.67, whereas in P34-VIII $\pi(CH_2O)$ = –0.47. Therefore, even though an oxygen can be further from the hydrophilic spot and more hindered, it can still maintain favorable ligand–receptor interaction due to its enhanced hydrophilicity. At the same time, placing an oxygen atom α to the ring in P36-XVIII (Figure 8) not only makes it more removed from the hypothesized hydrophilic interaction spot but also practically deprives the oxygen function of its hydrophilicity [$\pi(OCH_2)$ = –0.02]. Also, as will be explained later, an unfavorable hydrophobic interaction comes into play in the latter case.

Second, a visual analysis of odorphores occurring in rigid structures suggests that the electronegative atom of the hydrophilic function can be positioned both *cisoidal* and *transoidal* to the central ring. This leads to two possible assumptions.

(i) The hydrophilic spot in the receptor cavity is small, but the hydrophobic region is extensive and flexible enough to provide considerable freedom for the hydrophobic bulk such that it will still not disrupt the hydrophilic interaction of the electronegative function.

(ii) The hydrophobic cavity is spatially restrictive, but the effective span of the hydrophilic site is extensive enough to accommodate both transoidal and cisoidal location of the hydrophilic function.

Even at first sight, assumption (i) looked more attractive to us. Indeed, assumption (ii) implies that the hydrophobic part of the molecule would have to "sway" over quite a wide distance range. Not only does it appear unfeasible in terms of the receptor structure, but also the

Table II. Experimental Musk Odor Intensity vs That Calculated with CASE QSAR Equation 2: (-) Odorless/Nonmusk; (+) Weak/Faint Musk; (++) Moderate Musk; (+++) Strong Musk

		musky odor intensity					musky odor intensity		
	compound	actual	vs	calcd		compound	actual	vs	calcd
1	*m*-xylene	-		-	77	P21-F9-XI	-		-
2	*p*-cymene	-		-	78	P21-F9-XII	-		-
3	P7-F1-I	-		-	79	P21-F9-XIII	-		-
4	celestolide	+++		++	80	P21-F9-XIV	-		-
5	P7-IV	+++		+++	81	P21-F9-XV	-		-
6	P7-VI	+++		+++	82	P21-F9-XVI	-		-
7	P7-VII	+++		+	83	P21-F9-XVII	-		-
8	P9-IX	-		-	84	P21-F9-XIX	-		-
9	P9-VIII	-		-	85	P21-XIII	-		-
10	P9-F1-I	+		+	86	P21-XIII-i	-		-
11	P9-F2-II	+		+	87	P22-XIV	+		-
12	P9-F3-II	-		-	88	P22-XV	+		-
13	*p*-di-*tert*-butylbenzene	-		-	89	P22-XVI	-		-
14	P9-X	+		-	90	P23-XVII	+++		+++
15	P9-XI	-		-	91	P23-XVIII	+++		+++
16	phantolid	+++		+++	92	P23-XIX	+++		+++
17	versalid	+++		-	93	P23-XX	+++		+++
18	P12-V	+		-	94	P23-XXII	-		-
19	P12-F5-II	+		-	95	P23-XXII	-		-
20	P12-F6-I	-		-	96	P23-XXIII	-		-
21	P12-F6-II	+		+	97	P23-XXIV	-		-
22	P13-F7-I	-		-	98	P28-F7-I	+++		+++
23	P13-F7-II	+++		+++	99	P28-F7-II	+		-
24	P13-F7-III	-		-	100	P29-II	-		-
25	P13-F7-IV	+++		+++	101	P29-I	+		-
26	P13-F8-I	+		-	102	P30-VI	+++		+++
27	P13-F8-II	+		+	103	P31-F8-I	+++		++
28	P13-F8-III	+		-	104	P31-F8-II	+++		+
29	P13-F9-I	+++		+++	105	P31-F8-III	+++		+++
30	P13-F9-II	+++		+++	106	P31-F8-V	+++		++
31	P13-F9-III	+++		+++	107	P31-F8-VI	+++		+++
32	P13-VI	+++		+++	108	P31-F8-VII	+++		+++
33	α-methylstyrene	-		-	109	P31-F8-VIII	+++		+++
34	P14-F13-I	+++		+++	110	P31-F8-X	+		++
35	P14-F13-II	+		-	111	P31-F9-I	-		-
36	P16-F1-II	+		++	112	P31-F9-II	-		-
37	P16-F1-III	+++		++	113	P31-F9-III	-		-
38	P16-F1-IV	+		++	114	P31-F9-IV	-		+
39	P16-F1-V	+++		++	115	P31-F9-V	-		+
40	P16-F1-VI	+++		++	116	P31-F9-VI	-		-
41	P16-F1-VII	+		+	117	P33-I	+++		+++
42	P16-F1-VIII	+		+	118	P31-II	+++		+++
43	P16-F1-IX	+		+	119	P31-III	+		++
44	P16-F1-X	+		+	120	P33-F2-II	+++		+++
45	P16-F1-XI	+		-	121	P33-F2-III	+++		++
46	P16-F1-XII	-		-	122	P33-F2-IV	+++		+++
47	P17-F2-I	+++		++	123	P33-F2-VI	+++		++
48	P17-F2-II	+++		++	124	P33-F2-VI	+++		++
49	P17-F2-III	+		++	125	P33-V	-		-
50	P20-I	+++		+++	126	P33-F3-II	-		-
51	P20-III	+		-	127	P33-F3-IV	-		-
52	P20-IV	-		-	128	P34-F5-I	-		-
53	P20-V	-		-	129	P34-F5-II	-		-
54	P20-VII	-		-	130	P34-VII	+++		+++
55	P20-F4-I	+		-	131	P34-VIII	+++		+++
56	P20-F4-II	+		-	132	P25-F9-II	+++		+++
57	P20-F4-IV	-		-	133	P25-F9-III	+++		+++
58	P20-VIII	+++		+++	134	P25-F9-IV	+++		+++
59	P20-IX	+++		+++	135	P25-F9-VI	+++		+++
60	P20-X	+++		+++	136	P25-F9-VIII	+++		+++
61	P21-XIA	-		-	137	P25-XII	+		-
62	P21-XIC	-		-	138	P36-XIII	-		-
63	P20-I-BR	-		-	139	P36-XV	-		+
64	P20-I-CL	-		-	140	P36-XVI	+++		+++
65	P20-I-F	-		-	141	P36-XI	+++		++
66	P20-I-CN	-		-	142	P36-XVII	-		-
67	P20-I-CH_3OCH_2	-		-	143	P36-XVIII	-		-
68	P21-F8-VIII	-		-	144	P36-F12-IV	-		-
69	P21-F9-I	-		-	145	P37-F14-I	+		-
70	P21-F9-II	-		-	146	P37-F14	+		-
71	P21-F9-III	-		-	147	P37-F14-III	+++		++
72	P21-F9-III	-		-	148	P37-F15-I	+		-
73	P21-F9-V	-		-	149	P37-F15-II	+		-
74	P21-F9-VI	-		-	150	P37-F15-III	+		-
75	P21-F9-VII	-		-	151	P37-F15-IV	+		-
76	P21-F9-VIII	-			152	P30-IX	+++		+++
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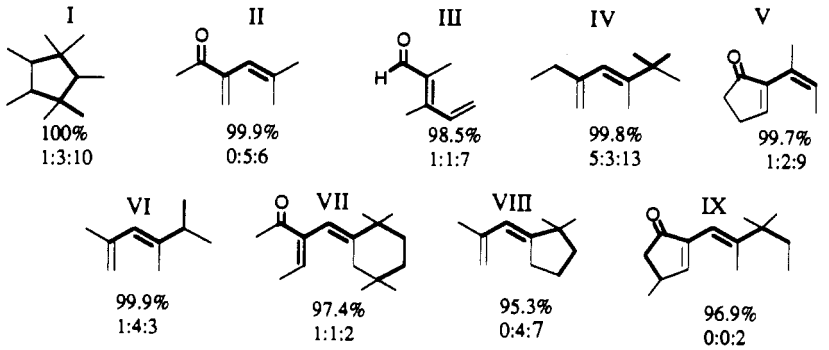

Figure 6. Structural features (odorphores) identified by Multi-CASE as responsible for the odor of aromatic musks. Fine lines represent bonds to non-hydrogen substituents. *X*:*Y*:*Z* is a distribution of an odorphore in nonmusks: weak musks:moderate/strong musks. Probability of relevance (*A*%) gauges the significance of each fragment.

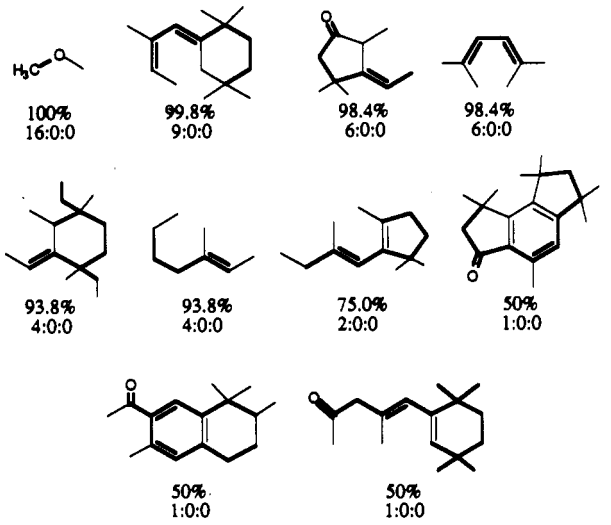

Figure 7. Structural features encountered exclusively in nonmusky congeners. These odorphobes were selected by Multi-CASE as responsible for the lack of musky odor.

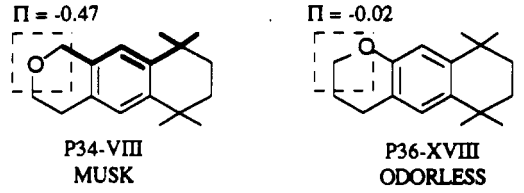

Figure 8. Odorphore IV (Figure 10) brings out the importance of the hydrophilicity of the electronegative substituent on the central ring.

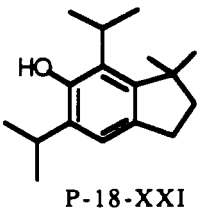

Figure 9. Enhanced hydrophilicity of the electrophilic substituent on the central ring preserves the odor-invoking receptor interaction through a longer distance.

distance-sensitive noncovalent interactions will hardly be preserved in this case. Moreover, it is (i) but not (ii) which allows us to explain the trend presented in Figure 10.

We used the ChemX molecular modeling software package (Chemical Design Ltd., Oxford, England) to fit P34-VIII, P33-F2-VI, and P33-F3-III rigidly in 3-D and compare the spatial requirements of the oxygen-bearing ring. Assumption (ii) permits us to choose the starred

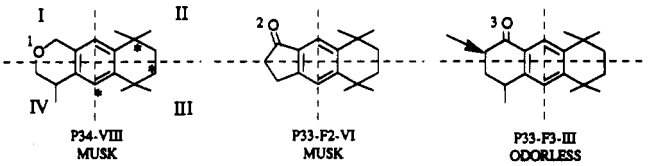

Figure 10. Exposure of the hydrophobic CH_2 group in quadrant I (pointed at with an arrow) may contribute to the odorlessness of P33-F3-III. Asterisks indicate atoms defined for rigid fitting.

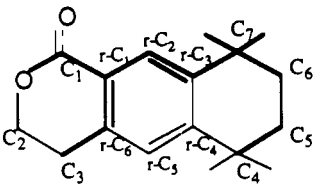

Figure 11. Structural backbone obtained by superimposing odorphores IV and VII. The *t*-Bu group is defined as a substituent of choice at r-C3. Substitution at r-C4 and r-C6 is mandated but is not defined.

points as the fitting atoms. The hydrophobic parts of the molecules fit together perfectly, whereas the oxygen functions are spread over a range of 2.341 Å. The latter number is a distance between O1 in odoriperous P34-VIII and O3 in odorless P33-Fe-III. The following observations can easily be made.

(1) The separation between O1 and O2 is 1.736 Å. Both chemicals, however, retain the musky odor.

(2) On the other hand, the distance between O2 and O3 is only 0.685 Å. Yet the disparity in odoriperous properties is fundamental—the former is a strong musk, whereas the latter is odorless.

Observation 1 suggests that the favorable interaction at the hydrophilic site is preserved within a span of at least 1.736 Å. Even though O3 of the odorless P33-F3-III could still be within the right distance margins, a hydrophobic methylene unit in quadrant II would be exposed to the hydrophilic site—a feature lacking in both musky P34-VIII and P33-F3-VI.

We then proceeded to superimpose the odorphores IV and VII to obtain a general musky structural backbone (Figure 11).

In the profile shown in Figure 11, the dimethyl substitution at C7 is mandated. This, in fact, defines a *t*-Bu group as a substituent of choice at r-C3. The substitution at C4 is required, but its pattern is not specified. Therefore, we looked at a series of structural analogs with different odor qualities to determine the optimum substitution pattern and generalize it in terms of substitution requirements at the r-C4 rather than at C4.

As is evident from Figure 12, an r-C4 ethyl-substituted compound is a nonmusk. However, transition from an

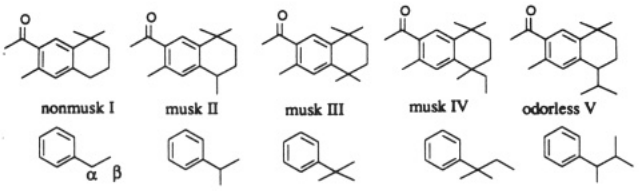

Figure 12. r-C4 requires a hydrophobic alkyl substituent of three or four carbons or five atoms maximally provided that the α, but not the β, position is branched.

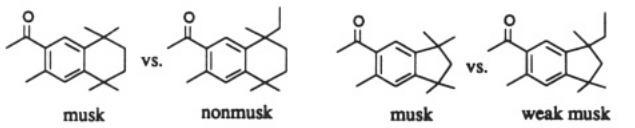

Figure 13. Maximally, four carbons can be accommodated at r-C3. Reducing the size of the saturated ring from six to five carbons somewhat loosens the spatial restrictions at r-C3.

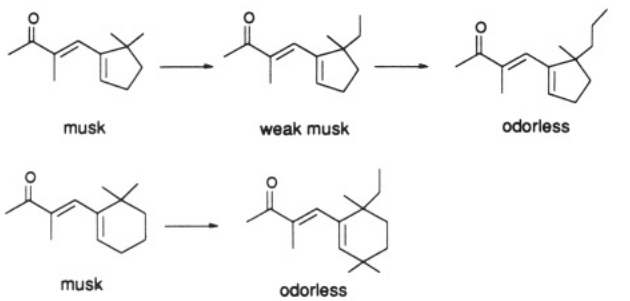

Figure 14. 1-Ethyl-1-methyl substitution makes tetralin derivatives odorless but still allows for the partial retention of odor in indan musks. Indan musks lose the odor if 1-ethyl is expanded by one methylene unit into 1-propyl. This means that the contraction of the saturated ring by one methylene brings about a corresponding increase in the allowable bulk at the 1 position.

ethyl to an isopropyl substituent brings about a musky odor, which again disappears as the size of the alkyl group goes up to five carbons and the β position becomes branched. So, the substitution at r-C4 allows limited variations. In the meantime, a *t*-Bu group at the r-C3 appears to represent an upper limit of the hydrophobic bulk allowed at that position and permits little tampering. Identification of the odorphobe V attests to the fact that exceeding the *t*-Bu bulk at this position quenches the odor.

Interestingly, shrinkage of the saturated ring from a six- to a five-membered ring somewhat offsets the odor-diminishing effect of the increased volume at r-C3 (Figure 13). This can be rationalized by assuming that such shrinkage "pulls back" the group attached to r-C3 and, by doing so, allows more space to accommodate an additional methyl group. Any further expansion of the r-C3 substituent, however, even on a five-membered ring, removes the odor, an event consistent with the one-methylene-long pull-back assumption (Figure 14).

Comparison of biophores V and IX with biophobe III makes it clear that the spatial requirements at the C3 position are also stringent. Indeed, a monomethyl substitution at C3 in alkylhydrindacenone musks preserves the odor, but the dimethylated derivative is odorless (Figure 15).

The structural rigidity of the alkylhydrindacenone musks allows a simple pictorial illustration of the possible cause for the odoripherous disparity between close structural analogs (Figure 16).

As Figure 15 shows, dimethylation, but not monomethylation, of the C3 in alkylhydrindacenone musks creates a steric hindrance to the odorant–receptor interaction and renders a compound odorless. At the same time, it is

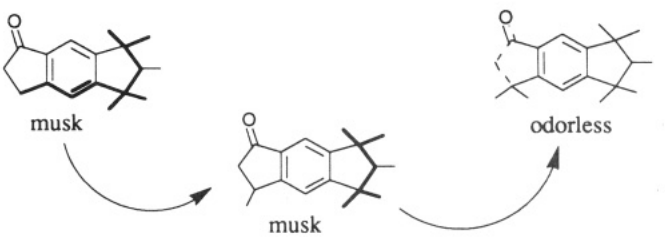

Figure 15. C3-monomethylated derivatives of alkylhydrindacenone musks are still musky. Dimethylation at C3 eliminates the odor. Highlighted are the fragments identified by the MultiCASE as (—) odorphoric and (- - -) odorphobic.

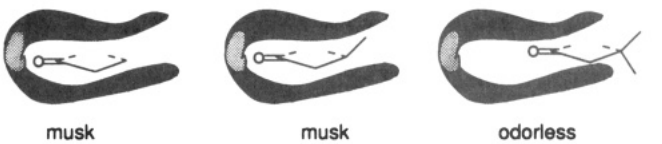

Figure 16. Narrow slot by the hydrophilic site of the receptor can accommodate no more than one methyl group at the C3 position of the alkylhydrindacenone musks (lighter region represents a hypothetical hydrophilic spot inside the musky odor receptor cavity).

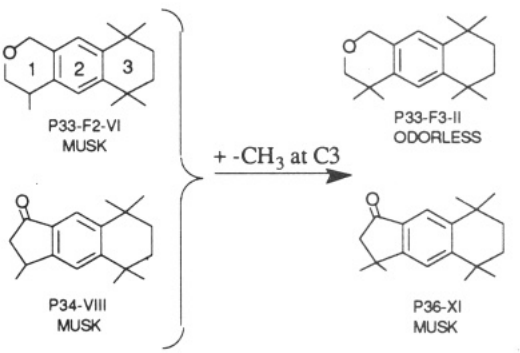

Figure 17. In isochromane musks, dimethylation at the C3 does not affect the odor, whereas in alkylhydrindacenone musks only one methyl substituent is allowed.

Figure 18. Dimethylated ring 1 in isochroman musks is less spatially demanding than that in alkylhydrindacenone musks.

noteworthy that the C3 dimethylated derivatives of the strong musks of the isochroman type are still musky. We compared two molecules—an odorless P33-F3-II and a musky P36-XI (Figure 17).

Both are derived from odoripherous molecules by adding a methyl group at C3. The result of such structural modification is, however, different in two cases—P33-F3-II is odorless, while P36-XI retains the odor, although of somewhat decreased intensity as compared to that of the parent odorant. Since rings 2 and 3 are identical in both odorants, we searched for an answer as to why such a difference occurs in the structure of ring 1. We again used the ChemX modeling package to optimize the molecular mechanics (MM2) energy of the two molecules and calculate the geometrical parameters of ring 1. For each molecule, the thickness of ring 1 was determined by summing up the distances between the C3 methyl groups and the plane of the aromatic ring. In a rigid five-membered ring of P33-F3-II, the methyls at C3 protrude from the plane of the aromatic ring far enough to require a span of 2.52 Å for their accommodation inside a receptor cavity (Figure 18). As follows from the above discussion of the C3 methylated derivatives of the alkylhydrin-

Table III. Comparison of the Experimental Musky Odor Strength with That Predicted by Multi-CASE for 20 Aromatic Compounds

		musk odor intensity	
no.	molecule	expt	Multi-CASE prediction
1	*p*-*t*-Bu-toluene (nonmusk)	–	–
2	P7-V (musk)	+++	+++
3	P9-F3-III (odorless)	–	– W
4	tonalid (musk)	+++	–
5	P13-F7-V (musk)	+++	+++
6	P20-VI (odorless)	–	+
7	P21-XIB (nonmusk)	–	–
8	P21-F9-IX (odorless)	–	–
9	P21-F9-X (odorless)	–	–
10	P21-F9-XVIII (odorless)	–	–
11	P30-VIA (musk)	+++	++++
12	P31-F8-IV (musk)	+++	+++
13	P33-IV (musk)	+++	+++
14	P33-F2-V (musk)	+++	+++
15	P33-F3-V (odorless)	–	–
16	P25-F9-I (musk)	++	++
17	P25-F9-VII (musk)	++	++
18	P36-XIV (odorless)	–	++ W
19	P33-F3-III (odorless)	–	–
20	P31-F8-IX (weak)	+	+

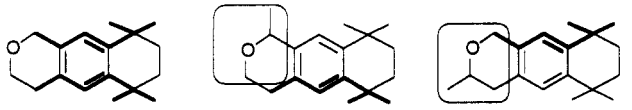

Figure 19. Unknown functionality $OCHCH_2$ is in fact a deactivating modulator in isochromane musks.

dacenone musks, this distance apparently exceeds the width of the receptor slot and prevents the perception of odor. On the other hand, in the musky P36-X, the more flexible six-membered ring allows such a positioning of the C3 methyls that their spatial demands become more modest than in the odorless P33-F3-II; the smallest width required to accommodate ring 1 in this case is only 2.27 Å. Therefore, we suggest that in musky P36-XI, in contrast to the odorless P33-F3-II, ring 1 can still fit into the receptor cavity after the C3 dimethylation and thus provide for the retention of musky odor.

Prior to submitting the database to the analysis, we had randomly removed a test set of 20 chemicals from it. When the test set was selected, the only consideration was to ensure an equal representation of both odoripherous and odorless chemicals. Finally, the set contained 10 odorless/nonmusk chemicals, 1 weak and 9 strong/moderate musks. These 20 chemicals were used later on to challenge the predictive ability of the model, and the results obtained were encouraging (Table III).

Of the 20 test chemicals, only 2 wrong predictions were made. One of those mishits fell on the odorless P36-XIV, for which the system issued a warning because P36-XIV contained a structural functionality never encountered by the program in the learning set. When we looked at the P36-XIV and the "unknown" functionality $OCHCH_2$ and compared it to the musky structural analogs, we found again that the spatial constraints around the hydrophilic interaction site of the receptor were relevant. Indeed, the unknown functionality identifies an alkyl substitution at either C1 or C2 positions in the ether ring of the isochromane musks (Figure 19).

As mentioned before, we found that an additional hydrophobic bulky group in α position to the oxygen of the ether ring wipes out the musky odor. Therefore, the unknown functionality encountered here is, in fact, a strongly deactivating modulator.

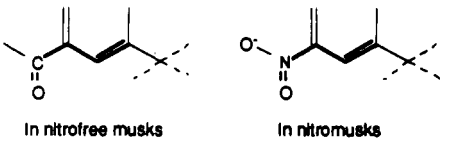

Figure 20. Similar odorphores found in nitro-free and nitro musks.

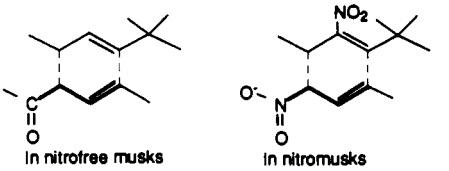

Figure 21. Two activating modulators of similar odorphores are related.

Comparison with Aromatic Nitro Musks. As mentioned, aromatic nitrated derivatives were the first synthetic chemicals discovered to have musky odor. Since the discovery of nitro-free aromatic musks, it has been a subject of interest to find the overlap, if any, between the nitrated and nitro-free musks in terms of structural features necessary for musky smell. This problem has not been a trivial one, since many of the nitro musks have a rather complex pattern of substitution which successfully conceals their possible structural resemblence to the nitro-free musks. In fact, some of the major contributors to the SAR research of musky odorants speculated that nitro musks might form a separate group having a mechanism of odorant–receptor interaction altogether different from that of the nitro-free odorants (Theimer and Davis, 1967).

However, we found that the odorphore II (Figure 6) present in 11 nitro-free musky odorants closely resembles an odorphoric fragment found during the analysis of nitro musks (Figure 20). Each odorphore identifies an electronegative functionality (a carbonyl or a nitro group) in a very similar environment. In both cases a meta position carries a *tert*-butyl or *tert*-butyl-like group. Moreover, at least two of the modulators of these odorphores are also closely related to each other (Figure 19). A modulator, in contrast to an odorphore, does not cause activity by itself (e.g., odor) but can modulate, negatively or positively, the activity caused by an odorphore.

The only difference between the modulators (Figure 21) is the presence of a nitro group at r-C2 in the case of the nitro musks. This group is conjugated with the same aromatic ring as the nitro group of the odorphore. It may well be that in the nitro musks with two nitro groups only one of these groups (the primary nitro group) participates directly in the receptor–odorant interaction and is equivalent, in this respect, to the carbonyl group of a nitro-free musk. The second nitro group (auxiliary group) could be a modulator, and we can only speculate about what role it may play. Since the auxiliary nitro group is invariably meta to the primary nitro group, it is unlikely that it participates in any resonance interaction with the latter. However, it can modulate the olfactory quality through its inductive influence on the aromatic ring.

Conclusions. We have shown that there exists a potential for the successful application of the (Multi-)CASE methodology for structure–activity relationship studies in olfaction. Both the lipophilicity and the molecular structure were shown to affect the muskiness of an aromatic chemical. Nitro-free and nitro musks share some common structural features. This implies that at least partial overlap may exist in the mechanism of odor perception in these two groups of odorants.

The 2-D structural determinants resulting from the (Multi-)CASE analysis can form a basis for the 3-D studies of the receptor structure odorant–receptor interaction.

The predictive ability of the (Multi-)CASE model can be utilized for the rational development and prioritization of synthetic efforts to discover novel odorants.

LITERATURE CITED

Baur, A. *Ber. Dtsch. Chem. Ges.* **1891**, *24*, 2836; Ger. Pat. 47,599, Appl. July 03, 1888; 62,362, Appl. Aug 14, 1891.

Boelens, H.; Haring, H. G.; Takken, H. J. The influence of molecular structure on olfactive quality (a quantitative approach). *Chem. Ind.* **1983**, *1*, 26–30.

Buck, L.; Axel, R. A. Novel Multigene Family May Encode Odorant Receptors: A Molecular Basis for Odor Recognition. *Cell* **1991**, *65*, 175–187.

Greenberg, M. J. Dependence of odor intensity on the hydrophobic properties of molecules. A quantitative structure odor intensity relationship. *J. Agric. Food Chem.* **1979**, *27*, 347–352.

Jennings-White, C. Human Primary Odors. *Perfum. Flavor.* **1985**, *9*, 46–58.

Jurs, P. C.; Narvaez, J. N.; Lavine, B. K. Structure Activity Studies of Olfactory Stimulants. *Chem. Senses.* **1986**, *11* (1), 145–151.

Kier, L. B.; Di Paolo, T.; Hall, L. H. Structure activity studies on odor molecules using molecular connectivity. *J. Theor. Biol.* **1977**, *67*, 585–596.

Klopman, G. Artificial Intelligence Approach to Structure-Activity Studies. Computer Automated Structure Evaluation of Biological Activity of Organic Molecules. *J. Am. Chem. Soc.* **1984**, *106* (24), 7315–7321.

Klopman, G. MULTICASE. 1. A Hierarchical Computer Automated Structure Evaluation Program. *Quant. Struct.-Act. Relat.* **1992**, *11*, 176–184.

Klopman, G.; Kalos, A. N. Causality in Structure-Activity Studies. *J. Comput. Chem.* **1985**, *6*, 28–38.

Klopman, G.; McGonigal, M. Computer Simulation of Physical-Chemical Properties of Organic Molecules. 1. Molecular System Identification. *J. Chem. Inf. Comput. Sci.* **1981**, *21*, 48–52.

Klopman, G.; Srivastava, S. Computer-Automated Structure Evaluation of Gastric Antiulcer Compounds. *Mol. Pharmacol.* **1989**, *37*, 958–965.

Klopman, G.; Wang, S. A Computer Automated Structure Evaluation (CASE) approach to Calculation of Partition Coefficient. *J. Comput. Chem.* **1991**, *12*, 1025–1032.

Nachbar, R. B.; Morton, T. H. A gas chromatographic (GLPC) model for the sense of smell. Variation of olfactory sensitivity with conditions of stimulation. *J. Theor. Biol.* **1981**, *89*, 387–407.

Theimer, E. T.; Davis, J. T. Olfaction, Musk Odor, and Molecular Properties. *J. Agric. Food Chem.* **1967**, *15*, 6–14.

Tute, M. QSAR and Drug Design. *Chem. Ind.* **1983**, *1*, 10–13.

Wolkowski, Z. W.; Moccatti, D.; Heymans, F.; Godfroid, J. J. A quantitative structure-activity approach to chemoreception, importance of lipophilic properties. *J. Theor. Biol.* **1977**, *66*, 181–193.

Wood, T. F. *Chemistry of aromatic musks*; Givaudan: Clifton, NJ, 1970; pp 1–37.

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Registry No. P9-VIII, 1144-40-7; P33-F3-II, 1226-16-0; P36-XIII, 1922-66-3; P36-XI, 102325-37-1; P36-XVIII, 135546-33-7; P7-V, 1756-31-6; P25-F9-I, 1217-06-7; P36-XIV, 1222-04-4; P33-F3-III, 102296-77-5; *m*-xylene, 108-38-3; *p*-cymene, 99-87-6; celestolide, 13171-00-1; *p*-di-*tert*-butylbenzene, 1012-72-2; phantolid, 15323-35-0; α-methylstyrene, 98-83-9; *p*-*t*-Bu-toluene, 98-51-1; tonalid, 21145-77-7; benzene, 71-43-2; indan, 496-11-7; tetralin, 119-64-2; coumarin, 91-64-5; versalid, 88-29-9; acenaphthene, 83-32-9; isochroman, 493-05-0; acetyltetramethyl-*s*-hydrindacene, 143791-22-4; acetyltetramethyl-*as*-hydrindacene, 143816-36-8.