through Nihon-Kohden differentiator for recording left ventricular dP/dt max. All parameters were recorded on a strip chart recorder. The lead(II) electrocardiogram was recorded on a BPL monitor.

Compounds were dissolved in distilled water (concentration 1–10 mg/mL). Each dose of the test compound was administered in a volume of 0.1 mL/kg. According to the in vitro positive inotropic activity, doses were selected between 0.01 and 3 mg/kg. Four to six doses were selected in order to establish a dose–response relationship. A 10–30-min interval was given in between the doses. In each experiment the activity of one compound was assessed. The inotropic activity of the compound was determined by measuring the percent change in dP/dt max over the initial value. ED_{50} was calculated from the dose–response curve. Forskolin was used as a standard drug. Its ED_{50} value was 0.007 mg/kg iv.

Acute Toxicity Studies. Acute toxicity (LD_{50}) was evaluated in mice (18–20 g) of either sex. Test compounds were dissolved in distilled water or suspended in 0.5% (carboxymethyl)cellulose and injected intraperitoneally at dose levels of 10, 30, 100, 300, and 1000 mg/kg in a volume of 1 mL/100 g of body weight. The animals were observed for 48 h, and the approximate LD_{50} values were calculated according to the methods described by Campbell and Richter.[7]

Acknowledgment. We thank Dr. P. K. Inamdar and his group for providing analytical and spectral data. The skillful technical assistance provided by Greta Moraes, P. Subbarayan, A. V. Ghate, C. Gangadharan, and Dr. B. Jotwani is greatfully acknowledged.

Registry No. 1, 66575-29-9; 2, 64657-20-1; 3, 111124-74-4; 4, 105559-76-0; 5, 105535-46-4; 6, 115076-57-8; 6A, 115077-01-5; 7, 115076-58-9; 7A, 115076-98-7; 8, 105559-75-9; 9, 115076-59-0; 10·HCl, 115076-60-3; 11·HCl, 105535-50-0; 12, 115116-35-3; 13·HCl, 111124-59-5; 14, 105535-79-3; 14·HCl, 105617-19-4; 15·HCl, 115076-61-4; 16·HCl, 115076-62-5; 17·HCl, 115076-63-6; 18·HCl, 115076-64-7; 19, 115076-65-8; 20·HCl, 115076-66-9; 21·HCl, 105535-53-3; 22·2HCl, 111124-58-4; 23·HCl, 115076-67-0; 24, 115076-68-1; 25, 115076-69-2; 26, 115092-13-2; 27, 115076-70-5; 28, 115092-14-3; 29, 115076-71-6; 29 (R^6 = H, R^7 = $COCH(Cl)CH_3$), 115077-04-8; 30, 111124-61-9; 31·7·25HCl, 115076-72-7; 32·2HCl, 111124-63-1; 33, 115076-73-8; 34·HCl, 115076-74-9; 34(R^6 = H, R^7 = $COCCCH_3)_2Cl$, 115077-05-9; 35·HCl, 115076-75-0; 36·7·25HCl, 115076-76-1; 37·2HCl, 115076-77-2; 38, 115076-78-3; 39·HCl, 111124-60-8; 39 (R^6 = H, R^7 = $Co(CH_2)_3Cl$), 113462-51-4; 40·HCl, 115116-36-4; 41·2HCl, 115116-37-5; 42, 115076-79-4; 43·HCl, 115116-38-6; 43 (R^6 = H, R^7 = $CO(CH_2)_4Cl$), 115077-02-6; 44·HCl, 115076-80-7; 44(R^6 = H, R^7 = $CO(CH_2)_{11}Cl$), 115077-03-7; 45·HCl, 115076-81-8; 46·HCl, 115116-39-7; 47, 111149-17-8; 48·HCl, 115116-40-0; 49·HCl, 114376-11-3; 50·HCl, 115076-82-9; 51·1.25HCl, 115076-83-0; 52·HCl, 115076-84-1; 53·HCl, 115076-85-2; 54, 115076-86-3; 55, 115076-87-4; 56·1.25HCl, 115076-88-5; 57·HCl, 111188-69-3; 58·HCl, 115076-89-6; 59·2HCl, 111187-90-7; 60, 115076-90-9; 61, 115076-91-0; 62, 115076-92-1; 63·HCl, 111124-66-4; 64·HCl, 111124-65-3; 65·2HCl, 111124-67-5; 66·HCl, 115116-41-1; 67·HCl, 115076-93-2; 68·2.5HCl, 115076-94-3; 69·1.5HCl, 115116-42-2; 70·HCl, 115116-43-3; 71·2HCl, 115076-95-4; 72·HCl, 115092-15-4; 73, 115076-96-5; acrylic acid anhydride with *N,N'*-dicyclohexylcarbamimidic acid, 115076-97-6; *N*-methylcyclohexylamine, 100-60-7; *N*-4-piperidylbenzamide, 33953-37-6; 4-phenyl-4-piperidinol, 40807-61-2; thiomorpholine, 123-90-0; 1*H*-benzotriazole, 95-14-7; ethyl 4-oxo-1,2,3,4-tetrahydro-7-(trifluoromethyl)-3-quinolinecarboxylate, 115076-99-8; theophylline, 58-55-9; 7-[(2-methoxyethoxy)methyl]-3-methylxanthine, 115077-00-4; phthalimide, 85-41-6.

Ab Initio Molecular Electrostatic Potentials of Perillartine Analogues: Implications for Sweet-Taste Receptor Recognition

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A model for the recognition of the perillartine analogues has been determined from a consideration of the molecular electrostatic potentials calculated at the ab initio 3-21G level for a select set of biologically active analogues. The model stresses the importance of two regions of negative electrostatic potential. One region, near the oxime moiety, does not vary in shape or value with substitution in the hydrocarbon domain. A second region in the hydrocarbon domain varies in depth, extension, orientation, and shape, depending on the nature of the substituent. The depth, relative position, and orientation of this latter region in the most potent systems (the 1,4-cyclohexadiene analogue and its *p*-methyl derivative) serve as the basis for the optimum recognition pattern of these analogues. The rank order of taste potencies is in general agreement with predictions based on this model. In addition, some conclusions are drawn concerning the receptor–analogue interaction as well as the electrostatic features of the receptor.

I. Introduction

The perillartine analogues are a group of tastants that initiate both sweet and bitter taste response in humans. Many of these compounds display high taste potency with a predominance of either sweet or bitter taste. The perillartine analogues are oximes in the *E* conformation with a CC double bond in conjugation with the CN double bond. A select group of analogues are presented in Chart I (compounds **3**–**10**), along with their taste potency and the ratio of sweet to bitter taste.[1] The mechanism of action of these tastants is not clearly understood, since the receptor for such interactions has never been isolated. However, electronic and topological features as well as hydrophobic contributions[2,3] must play a role in the tastant–receptor interactions.

In previous quantitative structure–activity (QSAR) studies of these analogues, topological information was obtained concerning the important structural parameters (molecular width, length, and thickness) required for taste potency.[4,5] In particular, the appropriate dimensions for

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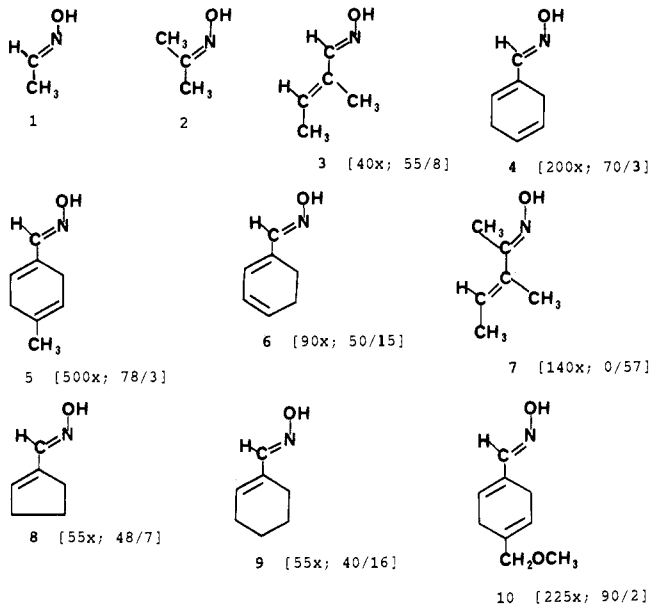
Chart I. Compounds of Interest in This Study[a]



[a] Compounds 3–10 are perillartine analogues. Their taste potencies are given by x (times sucrose). Also given are the ratios of the percentage of sweetness to that of bitterness estimated from the taste qualities. All the data is taken from ref 1.

an effective sweet tastant were described. The primary focus of these studies has been on the *variable* hydrocarbon domain of the analogues rather than on the *common* oxime group. In one of the studies,[4] in which the potency data of all the analogues studied by Acton and Stone were used, the length of the hydrocarbon section was directly correlated with taste potency. In both studies[4,5] the width and thickness of the hydrocarbon domain were inversely correlated with the taste potency. Also, certain features of the receptor site were deduced on the basis of the topology of the potent sweet-tasting perillartine analogues.[4] These results are in agreement with some of the features of a receptor-site model proposed by Temussi et al.[6] In the QSAR analysis, the role of hydrophobicity in the interaction involving the perillartine analogues indicates a positive correlation between the log of the 1-octanol/water partition coefficient and the taste potency of the analogues.[4,5]

In the above studies, the electronic features believed to be responsible for sweet taste have been derived from a simple model suggested by a consideration of a number of tastants as structurally diverse as saccharin, sucrose, and others.[7,8] This model suggests that the interaction takes place between an electronegative atom (B) on the tastant and an electropositive hydrogen on the receptor, as well as between a polarized system (A–H) on the tastant and an electronegative atom on the receptor. A distance of 2.5–4.0 Å between A and B has been postulated as a structural requirement to initiate the sweet-taste response.[7] A further electronic feature, in the form of an interactive site on the tastant at a distance of 5.0–6.0 Å from B, was assumed in this model in order to explain the relationship between the taste potency and the structure of the tastant.[8] However, it has been pointed out[3] that many organic compounds possess such electronic features and are clearly not intensely sweet. In addition, for the perillartine analogues, it is not possible to determine a polarized A–H system that would satisfy the above structural requirement and also form strong hydrogen bonds with the receptor.[3]

In this paper, the preliminary or recognition step in the interaction of a perillartine analogue with its receptor will be examined by means of the method of molecular electrostatic potentials (MEP). The MEP method not only focuses on the molecule as a whole rather than on specific functionalities within the molecule, but also does not make any a priori assumptions about the functional groups needed for biological activity. For example, in the case of the interaction of a drug with its receptor, the recognition step[9–11] has been characterized by requiring that the electrostatic potential for the drug and the receptor be complementary in regions of positive and negative potential.[9] Furthermore, the most potent drug is considered to generate the electrostatic potential that best complements, in the manner described above, the potential of the binding site of the receptor. In addition, at long range the electrostatic energy dominates the interaction between the drug and its receptor. Thus, as Weinstein et al. have demonstrated,[9–11] the electrostatic potential pattern has been shown to serve as a criterion for molecular reactivity at the drug receptor.

In our work, we use the molecular electrostatic potential to analyze the basis for tastant recognition at the receptor. The potential maps that are generated for the perillartine analogues are used to determine their recognition patterns, with the maps of the most potent sweet-tasting analogues serving as a model for optimum recognition. Although the electrostatic patterns that emerge can be compared to the electronic features postulated in "binding" models,[7,8] it must be stressed that our study deals only with recognition, a step *preliminary* to the actual tastant–receptor binding. In general, the conformational and electronic requirements for recognition may be somewhat different from those required for binding or activation of biological response. However, in many systems, the recognition pattern of the most potent compounds can be used as an indicator of the biological activity.[9–11]

We investigated the molecular electrostatic potential of a set of molecules (1–7 in Chart I), paying careful attention to the oxime moiety as well as (in the case of the perillartine analogues) to the π-electron region around the CC double bond(s) in the hydrocarbon domain of the analogue. We evaluated electrostatic potentials using ab initio methods for (a) two nonpotent oximes, (*E*)-acetaldoxime and acetoxime (**1** and **2** in Chart I), (b) four potent predominantly sweet-tasting perillartine analogues (**3–6** in Chart I), and (c) a potent but bitter-tasting perillartine analogue (**7** in Chart I). The analogues studied are representative of the systems analyzed by Acton and Stone.[1] In addition, the analogues chosen represent systems with relatively rigid hydrocarbon domains. The taste potencies of these compounds range over a modest spectrum of values. The least potent ((*E*,*E*)-tiglaldoxime, **3**, in Chart I) differs only in a single order of magnitude from the most

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potent (the *p*-methyl derivative of the 1,4-cyclohexadiene analogue, **6**, in Chart I). We determined the electrostatic potential in two planes at distances beyond covalent bond formation: (1) at 1.6 Å (in the plane near the maximum π-electron density, a recognition pattern at short range) and (2) at 2.2 Å (a more distant recognition pattern).[12]

In the next section we describe the analogues we studied and the methods we used. In the following section we present our results including the electrostatic potential contour maps of these tastants. Finally, in the last section we present our conclusions concerning receptor recognition and its implications for the taste potency of the sweet-tasting perillartine analogues.

II. Methods

The compounds analyzed in this study are shown in Chart I. The bond lengths and angles for (*E*)-acetaldoxime (**1**), acetoxime (**2**), and (*E,E*)-tiglaldoxime (**3**) were taken from our previous ab initio 3-21G basis set study of these compounds.[13] For (*E*)-acetaldoxime and acetoxime we found that the global energy minimum corresponded to a CNOH torsional angle of 180°. For (*E,E*)-tiglaldoxime the global minimum corresponded to a CNOH and CCCN torsional angle of 180°. However, for all three systems, the CNOH torsional angle can deviate from the optimum value by 60° and yield a structure in the 3-21G basis set that is no more than 3 kcal/mol higher in energy than the global minimum structure. Likewise, in the case of **3**, the CCCN torsional angle can deviate from the optimum value in the 3-21G basis set by 45° and yield a structure 3 kcal/mol higher in energy than the global minimum structure. Similar results were obtained with the STO-3G basis set.[13] Thus, although the optimum geometry corresponds to a planar structure, accessible conformations with respect to rotation around the CNOH and CCCN torsional angles lie close by.[13] In this study we have reported the electrostatic potential for these minimum energy planar conformations. Potential maps (not shown) for nonplanar conformations of (*E,E*)-tiglaldoxime at a CCCN torsional angle equal to 20° indicate contours similar in shape, extension, and orientation to the contours determined for the planar conformer. Therefore, for a CCCN torsional angle in the range of 0–45°, we might not expect any major differences in the contour maps of the planar and nonplanar conformers. The flexibility of the CNOH and CCCN angles may play a more important role in the binding of the analogues to the receptor.[14]

For the 1,4-cyclohexadiene analogues (**4**, **5**), the 1,3-cyclohexadiene analogue (**6**), and the ketoxime analogue (**7**) of (*E,E*)-tiglaldoxime, the oxime parameters (including the values for the CCCN and CNOH torsional angles) were taken from the optimized geometry of the (*E,E*)-tiglaldoxime, since in our previous work[13] we demonstrated the transferability of the oxime parameters. The parameters for the hexadiene substituents in **4**–**6** were taken from experimental bond lengths and angles.[15] The experimental geometry of the cyclohexadiene ring in **4** and **5** is planar. For the 1,3-cyclohexadiene analogue, the hexadiene ring is nonplanar with a C=C—C=C torsional angle equal to 18°.[15] STO-3G calculations on this ring[16] have yielded a value of 13.9° for this angle, with a flat energy surface in the vicinity of the minimum energy conformation. For **5** and **7**, the parameters for the methyl substituents were taken from standard bond lengths and angles.[17]

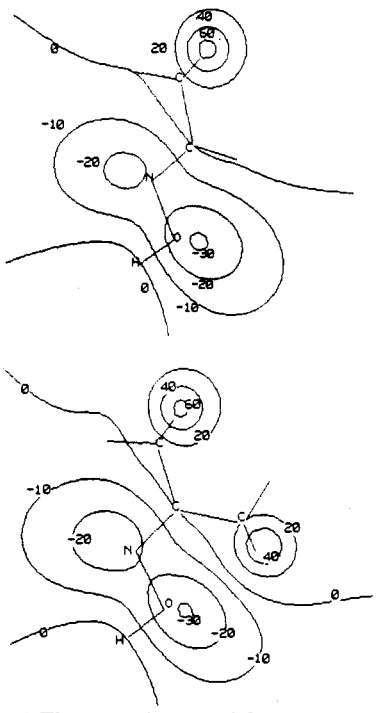

Figure 1. (a, top) Electrostatic potential contour map (calculated in the 3-21G basis set) of (*E*)-acetaldoxime in a plane 1.6 Å away from the CNOH plane. Contour levels were chosen at 60, 40, 20, 0, −10, −20, and −30 kcal/mol. All atoms except aliphatic hydrogens are labeled. (b, bottom) Electrostatic potential contour map of acetoxime in a plane 1.6 Å away from the CNOH plane. The basis set, contour levels, and labeling are the same as in Figure 1a.

The GAUSSIAN 82 package[18] was used for the calculation of the molecular electrostatic potentials (MEP). For compounds **1**–**7**, the potentials were calculated by using the extended 3-21G basis set. The 3-21G basis set was chosen since it was the smallest basis set that reproduced the general features of the contour map generated for the analogues **3** and **4** in the more extended 4-31G basis set.[19] On the other hand, the STO-3G basis set generated potential regions that were somewhat different in shape and depth from those generated in the 4-31G basis set.[19]

The potential was determined in two planes parallel to the plane containing the oxime group. The plane at 1.6 Å (which is at a distance just beyond the formation of a covalent bond) corresponds to points of maximum π-electron density for the conjugated systems.[9] The plane at 2.2 Å corresponds to the plane where the two minimum potential regions of the analogues have equipotential contours. The results of the potential calculations were displayed as contour maps by using the "READ MAP" facility of Chem-X.[20] All the contour levels are given in units of kilocalories/mole.

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(19) Venanzi, T. J.; Venanzi, C. A., unpublished results.
(20) Chem-X, developed and distributed by Chemical Design, Ltd., Oxford, England.

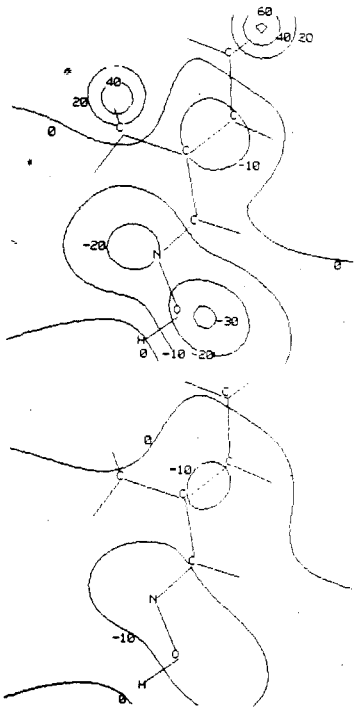

Figure 2. (a, top) Electrostatic potential contour map of (*E*,*E*)-tiglaldoxime in a plane 1.6 Å away from the CNOH plane. The basis set, contour levels, and labeling are the same as in Figure 1a. (b, bottom) Electrostatic potential contour map of (*E*,*E*)-tiglaldoxime in a plane 2.2 Å away from the CNOH plane. The basis set, contour levels, and labeling are the same as in Figure 1a.

III. Results

A. Contour Maps of (*E*)-Acetaldoxime and Acetoxime. The potentials of (*E*)-acetaldoxime and acetoxime were computed to determine the incremental effect of aliphatic substituents on the electrostatic potentials of the oxime moiety. The potential, in a plane 1.6 Å above the oxime moiety, is shown in Figure 1a,b for (*E*)-acetaldoxime and acetoxime, respectively.

The plots of both (*E*)-acetaldoxime and acetoxime show that the CNO group generates a very similar pattern of negative potential, with a deep region of −30 kcal/mol off the oxygen atom and a region of −20 kcal/mol off the nitrogen atom. As can be seen from Figure 1b, the addition of the methyl group to the acetaldoxime skeleton does not change the basic electrostatic potential pattern around the oxime group.

B. Contour Maps of (*E*,*E*)-Tiglaldoxime. The plots of the (*E*,*E*)-tiglaldoxime (**3** in Chart I) are shown in Figure 2 for planes at a distance of 1.6 and 2.2 Å from the oxime moiety.

As with the nonpotent oximes (Figure 1), a negative potential is found in the plane 1.6 Å above the CNO moiety of (*E*,*E*)-tiglaldoxime (Figure 2a). Furthermore, the features of this region in Figure 2a are exactly the same as in the nonpotent systems. At 2.2 Å (Figure 2b), the negative potential drops off, leaving only a single negative contour of −10 kcal/mol with a shape essentially the same as in Figure 2a.

On the other hand, the presence of the conjugated CC double bond lends a new dimension to the potential map. The hydrocarbon domain, which in the (*E*)-acetaldoxime and acetoxime molecules yields positive potential regions, here displays a negative contour at −10 kcal/mol due to the presence of the conjugated double bond. At 1.6 Å this region is not as negative as the region surrounding the oxime group (Figure 2a). On the other hand, at a further distance (2.2 Å), both the conjugated bond and the oxime group display contours of equal magnitude, −10 kcal/mol. This negative potential region in the hydrocarbon domain is a significant feature of the electrostatic potential of all the potent tastants, as will be seen below.

C. Contour Maps of the 1,4-Cyclohexadiene Analogue ((*E*)-1,4-Cyclohexadiene-1-carboxaldehyde Oxime) and Its *p*-Methyl Derivative ((*E*)-4-Methyl-1,4-cyclohexadiene-1-carboxaldehyde Oxime). The contour maps of the 1,4-cyclohexadiene analogue (**4**) and its *p*-methyl derivative (**5**) are shown in Figure 3a–d in planes at 1.6 and 2.2 Å away from the CNOH group.

As in the previous cases, a negative electrostatic potential region is found around the CNO moiety; the closer to the molecule, the more negative this region. At both 1.6 Å (Figure 3a,c) and at 2.2 Å (Figure 3b,d), the electrostatic potential of the region near the oxime group of both **4** and **5** is exactly the same as the region around the oxime moiety in (*E*,*E*)-tiglaldoxime (Figure 2a,b). In addition, the *p*-methyl derivative contour maps (Figure 3, parts c and d) are similar to the map of the unsubstituted 1,4-cyclohexadiene analogue (Figure 3a,b). The only difference is a truncation of the negative potential region around the ring due to the presence of the *p*-methyl group.

In both analogues, the presence of two conjugated bonds broadens and deepends the negative region associated with the conjugated double bond in (*E*,*E*)-tiglaldoxime. The minimum of this negative potential now appears around the CC double bond at the 4-position. On the other hand, in comparison to (*E*,E)-tiglaldoxime (Figure 2a,b), there is just a very slight increase in the ratio of the spatial extent of the negative to the positive potential regions.

At 1.6 Å, the depth of the potential well in the hydrocarbon domain in **4** and **5** is greater than in **3** (−20 kcal/mol as compared to −10 kcal/mol). At 2.2 Å the depth of the negative electrostatic potential regions over the cyclohexadiene ring (Figure 3, parts b and d) is comparable to that over the oxime group. It should be noted that the 1,4-cyclohexadiene analogue and its *p*-methyl derivative have two of the highest taste potencies among the analogues.[1]

D. Contour Maps of the 1,3-Cyclohexadiene Analogue ((*E*)-1,3-Cyclohexadiene-1-carboxaldehyde Oxime). The potential map of the 1,3-cyclohexadiene analogue (**6** in Chart I) is shown in Figure 4, parts a and b in planes 1.6 and 2.2 Å away from the planar CNOH group, respectively.

As in all the other analogues, the global minimum electrostatic potential is found around the CNO moiety. Again, this potential region is exactly the same as the previous systems with respect to shape, values of the potential, and the orientation of this region with respect to the molecule.

On the other hand, the presence of the conjugated double bond at the 3-position of the ring yields a negative potential region, which has a different orientation with respect to the oxime group than that of the corresponding 1,4-cyclohexadiene analogues **4** and **5** (Figure 3a–d). Whereas in both the 1,4-cyclohexadiene analogue **4** and its *p*-methyl derivative **5** the negative potential region over the hydrocarbon domain is parallel to the negative potential region over the oxime group, in the 1,3-cyclohexadiene analogue **6**, the comparable region is at an angle of about 45° to the negative potential region over the oxime moiety. This difference is obviously related to the position of the CC double bond at the 3-position on the ring. It is interesting to note that the taste potency of this molecule is not as large as that of the two 1,4-cyclohexadiene ana-

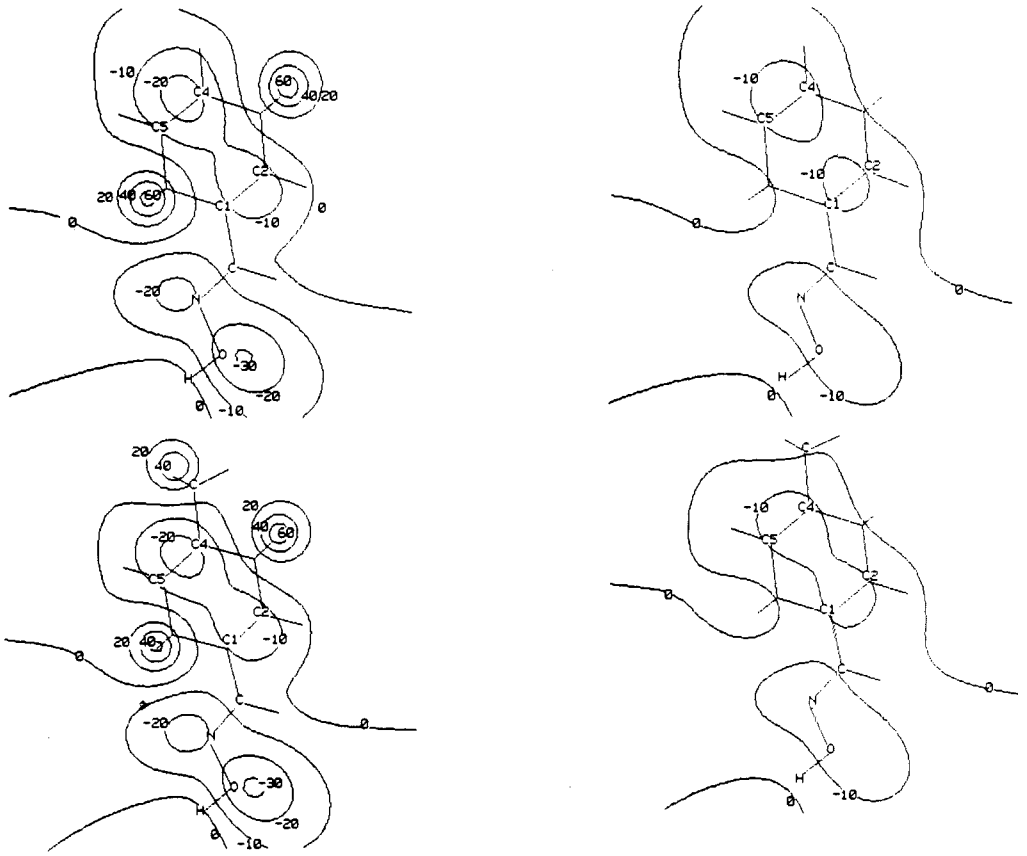

Figure 3. (a, top left) Electrostatic potential contour map of (*E*)-1,4-cyclohexadiene-1-carboxaldehyde oxime in a plane 1.6 Å away from the CNOH plane. The basis set, contour levels, and labeling are the same as in Figure 1a. (b, top right) Electrostatic potential contour map of (*E*)-1,4-cyclohexadiene-1-carboxaldehyde oxime in a plane 2.2 Å away from the CNOH plane. The basis set, contour levels, and labeling are the same as in Figure 1a. (c, bottom left) Electrostatic potential contour map of (*E*)-4-methyl-1,4-cyclohexadiene-1-carboxaldehyde oxime in a plane 1.6 Å a vay from the CNOH plane. The basis set, contour levels, and labeling are the same as in Figure 1a. (d, bottom right) Electrostatic potential contour map of (*E*)-4-methyl-1,4-cyclohexadiene-1-carboxaldehyde oxime in a plane 2.2 Å away from the CNOH plane. The basis set, contour levels, and labeling are the same as in Figure 1a.

logues **4** and **5**. Hence, while the degree of unsaturation may not be directly correlated with receptor recognition,[21] the placement of the double bond(s) in these analogues may be crucial for optimum recognition of the analogues by the receptor.

E. Contour Maps of the Ketoxime Analogue of (*E*,*E*)-Tiglaldoxime ((2*E*,3*E*)-3-Methyl-3-penten-2-one Oxime). The contour maps of the predominantly bitter-tasting ketoxime analogue (**7** in Chart I) are shown in Figure 5, parts a and b for planes 1.6 and 2.2 Å away from the CNOH moiety, respectively. The electrostatic potential maps share the same basic features of shape, placement, and number of minima as the maps of (*E*,*E*)-tiglaldoxime (see Figure 2). The only difference is a slightly less negative potential around the oxygen: a fact that is certainly due to the presence of a nearby methyl hydrogen in this system. Overall, the methyl substitution on (*E*,-*E*)-tiglaldoxime, like the methyl substitution on (*E*)-acetaldoxime, does not greatly affect the fundamental electrostatic potential associated with the oxime group.

IV. Discussion

The electrostatic potential maps for the perillartine analogues, especially the most potent ones, yield information on the electronic features that determine the recognition pattern of these molecules. This recognition pattern can be used for understanding not only the activity of the analogues in this study but also the activity of other structurally similar analogues. In addition, from an analysis of all the maps at the two different distances, some conclusions can be drawn about the preliminary stages of the receptor–analogue interaction.

From an analysis of the electrostatic potential contour maps of the perillartine analogues, the importance of two negative electrostatic potential regions emerges: (1) a broad region near the nitrogen and oxygen atoms of the oxime group, which reflects the effect of the lone pairs on each of these atoms and *does not generally vary in its position or depth from analogue to analogue*, and (2) a second region in the hydrocarbon section of the analogue; a region that *not only varies in its depth, shape, and extension but also in its orientation with respect to the region identified with the oxime moiety*. The depth and extent of this negative electrostatic region as well as its orientation with respect to the potential region around the oxime moiety appear to be crucial for receptor recognition and, thus, are significant indicators of taste potency. This result emphasizes the importance of the hydrocarbon domain: a point recently underscored by the structure–activity data[4,5] and first made, in a different context, by Kier.[8] In fact, for the systems considered in this study, the relationship between the taste potency and the electrostatic features required for receptor recognition is compatible with the results of the QSAR studies.[4,5]

Furthermore, since the 1,4-cyclohexadiene analogue and its *p*-methyl derivative are two of the most potent and purest sweet-tasting analogues, the depth and relative position and orientation of the negative electrostatic po-

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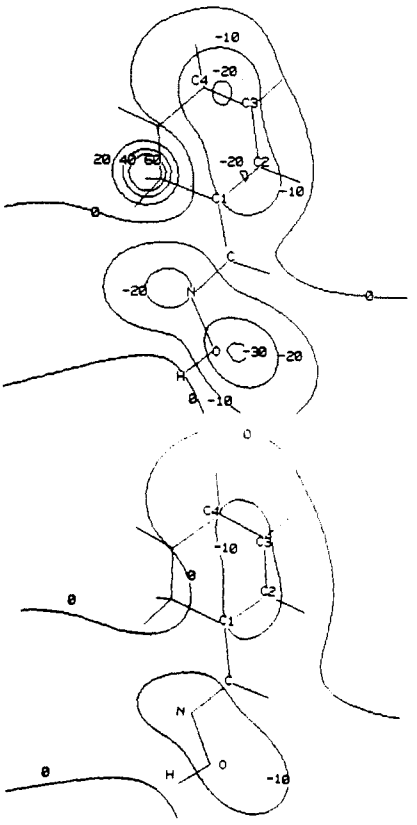

Figure 4. (a, top) Electrostatic potential contour map of (*E*)-1,3-cyclohexadiene-1-carboxaldehyde oxime in a plane 1.6 Å away from the CNOH plane. The basis set, contour levels, and labeling are the same as in Figure 1a. (b, bottom) Electrostatic potential contour map of (*E*)-1,3-cyclohexadiene-1-carboxaldehyde oxime in a plane 2.2 Å away from the CNOH plane. The basis set, contour levels, and labeling are the same as in Figure 1a.

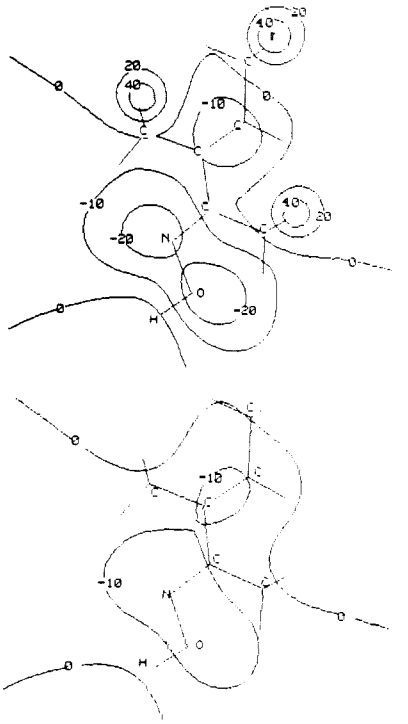

Figure 5. (a, top) Electrostatic potential contour map of the ketoxime analogue of (*E*,*E*)-tiglaldoxime ((2*E*,3*E*)-3-methyl-3-penten-2-one oxime) in a plane 1.6 Å away from the CNOH plane. The basis set, contour levels, and labeling are the same as in Figure 1a. (b, bottom) Electrostatic potential contour map of the ketoxime analogue of (*E*,*E*)-tiglaldoxime ((2*E*,3*E*)-3-methyl-3-penten-2-one oxime) in a plane 2.2 Å away from the CNOH plane. The basis set, contour levels, and labeling are the same as in Figure 1a.

tential regions in the hydrocarbon domain with respect to the potential region around the oxime group can be used as a *model for an optimum electrostatic recognition pattern at both near and far distances*. In comparison to this model, the electrostatic potential region in the hydrocarbon domain in the 1,3-cyclohexadiene analogue and (*E*,*E*)-tiglaldoxime is either oriented differently (1,3-analogue) or is shallower and less extensive ((*E*,*E*)-tiglaldoxime). As demonstrated in the results section, a drop in taste potency occurs as the negative electrostatic potential region in the hydrocarbon domain becomes either shallower, less extensive, or oriented in a manner different to that of the corresponding region in the 1,4-cyclohexadiene systems. In general, a drop in taste potency would be expected as the electrostatic potential pattern of other analogues deviates from the pattern of the 1,4-cyclohexadiene systems.

Given this model, predictions can be made concerning the activity of other analogues that are structurally similar to the small set of compounds considered in this study. For example, both the cyclopentene and cyclohexene derivatives (**8** and **9** in Chart I) would obviously have negative electrostatic potential regions very similar to the regions found in (*E*,*E*)-tiglaldoxime. Hence, from the electrostatic potential pattern and aside from considerations of hydrophobic and steric factors, one would predict a similar taste potency and sweet/bitter ratio for these compounds: a fact verified by the experimental results (see Chart I).[1]

In the case of the less rigid aldoxime molecule (**10** in Chart I), the presence of lone pair electrons on the oxygen atom of the methoxy group at the 4-position of the cyclohexadiene ring would increase the extension of the negative electrostatic potential region located in the hydrocarbon domain. The STO-3G contour maps of aldoxime,[18] using the geometry shown in **10**, indicate that, although the negative potential region in the hydrocarbon domain does indeed extend beyond the ring toward the methoxy oxygen, in other respects (position and orientation) the region in the hydrocarbon domain is similar to the one found in STO-3G contour maps of the 1,4-cyclohexadiene analogue. In the case, other factors notwithstanding, the potency would be predicted to be similar to the 1,4-cyclohexadiene analogues. In fact, Acton and Stone[1] found the taste potency of aldoxime to be 225 times that of sucrose with a sweet/bitter ratio of 90/2 (see Chart I).

The recognition model presented in this study is based on the electrostatic potential pattern generated by the minimum planar conformers of the analogues, i.e. CNOH and CCCN torsional angles = 180°. We would expect that the geometry of the conformers at recognition would be close to this minimum conformation and, thus, would generate similar electrostatic potentials (see Methods Section). Furthermore, since the potential region surrounding the oxime moiety is unaffected by substitution in the hydrocarbon domain, we would expect that this region would not be affected at all by changes in the CCCN torsional angle.

Although the recognition model is used here as an indicator of biological activity, a complete description of the taste potency must involve other factors among which are conformational dynamics, polarization, steric, and hydrophobic effects.[4,5] For example, a molecule that generates an electrostatic potential similar to the 1,4-cyclohexadiene analogues but is so bulky that it can not fit into the re-

ceptor site will not be active biologically and will, therefore, lack taste potency. In addition, the bitter-tasting ketoxime analogue of (*E,E*)-tiglaldoxime gives rise to the same electrostatic potential pattern as the parent compound. This suggests similar recognition patterns for the sweet and bitter taste receptors, a suggestion that is in agreement with the work of Temussi et al.[6] Whether the lack of a sweet-taste response with this analogue is due to the methyl group invading a steric barrier on the receptor is a question that cannot be answered by our model.

Finally, by analyzing the maps of the analogues at the two distances, conclusions can be drawn concerning the interaction between the analogues and the receptor. At a distance of 2.2 Å, where all the anaogues have two potential minima equal in value (contour levels of −10 kcal/mol), a long-distance electrostatic recognition pattern suggests that the perillartine tastant is directed into the receptor binding site such that the electropositive residues of the receptor align themselves to the negative electrostatic regions of the molecule.[22] Such electrostatic complementarity has been shown to exist between the molecular electrostatic potential patterns of enzymes, such as carboxypeptidase A, and their inhibitors,[23] as well as between cyclic urea "artificial enzymes" and their substrates.[24] At a distance of 1.6 Å, a distance at which the binding interaction might begin, the potential region around the oxime moiety becomes deeper than the one in the hydrocarbon domain. Also, the potential is more negative around the oxygen atom than the imino nitrogen, indicating that the site of the oxygen is the favorable site for oxime protonation: a result that agrees with most mechanisms for protonation.[25] At this short distance, the electrostatic potential pattern indicates that the electropositive residues on the receptor interact more strongly with the oxime moiety than with the conjugated sytem in the hydrocarbon domain. At either distance, the recognition pattern of the analogue implies a complementary pattern for the receptor, indicating the presence of electropositive residues on the receptor. In addition, since the 1,4-cyclohexadiene analogue and its *p*-methyl derivative are two of the most potent analogues, the electropositive residues on the receptor should be structurally positioned to best complement the electrostatic negative potential minima of these analogues. This model of the long-range electrostatic interaction between the analogue and the receptor serves as a starting point for the model of the binding of the analogue to the receptor: a model that must consider steric effects and dispersion forces (which may account for differences in potency between the 1,4-cyclohexadiene analogue and its *p*-methyl derivative) as well as charge-transfer and polarization effects.[11,26]

Given the electrostatic potential pattern generated by one class of tastants, it is now important to focus on other classes of structurally diverse tastants, such as rigid sweet-tasting molecules, where the question of conformation is less crucial. Our preliminary analysis of the acesulfame and saccharin systems indicate similar negative potential regions.[27] However, these regions are broader and deeper and positioned somewhat differently than the comparable regions generated by the planar conformations of the perillartine analogues. Ultimately, the generation of recognition patterns for various types of potent molecules can only give preliminary answers to a problem as complicated as the cause of sweet-taste response: a response that probably involves multiple receptors.[3] In any case, these patterns not only may be able to identify crucial factors in the biological response of sweet-taste perception but also may be used, along with a model of the geometrical features of the receptor site,[6] to provide information for the design of sweet-tasting molecules.

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