Molecular Structure Influencing Either a Sweet or Bitter Taste among Aldoximes

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Abstract □ A series of cyclohexylaldoximes was examined for their sweet or bitter taste using discriminant analysis. The structures of the molecules were described using molecular connectivity. A two-variable linear discriminant function and critical value were computed that correctly assigned 17 of the 20 molecules to their observed sweet or bitter taste categories. The same discriminant function can predict correctly the taste categories of seven of eight additional molecules.

Keyphrases □ Aldoximes—influence of molecular structure on sweet or bitter taste, discriminant analysis, molecular connectivity, taste category prediction □ Molecular connectivity indexes—aldoximes, influences of molecular structure on sweet or bitter taste □ Sweeteners, noncaloric—analysis of aldoximes for potential use as sweeteners, molecular connectivity indexes, discriminant analysis

A safe, stable, noncaloric sweetener continues to be an active research objective. The ban on cyclamate and the suspicion concerning the safe use of saccharin necessitate research for sweet-tasting molecules.

BACKGROUND

The prevailing hypothesis about the chemical structure governing sweet taste was proposed by Shallenberger and Acree (1) and extended by Kier (2). In essence, the hypothesis describes three structural features necessary, but not sufficient, for sweet taste. The first and second features are the presence of a potential hydrogen bond donor, designated AH, and the presence of a hydrogen bond acceptor, B (1). The optimum separation between A and B is about 3 Å. The third feature is a nonpolar region, X, capable of dispersion interaction at a receptor (2). It is a region of atoms located ~3.5 Å from A and ~5.5 Å from B.

One problem in the search for noncaloric sweeteners is the common occurrence of a bitter taste among a series of molecules under investigation. The bitter taste also can coexist with a sweet taste in the same molecule. A classic example is the perception of a bitter aftertaste with saccharin.

Some insight into structural influence on this taste dichotomy was revealed by Solms (3), who recorded that five D-amino acids are sweet while their L-forms are bitter. This finding implicates a stereochemical factor in the sweet and bitter response. Receptors for bitter and sweet taste have been shown to be activated by similar structural features so that closely related molecules impart either or both sensations (4).

The design of noncaloric sweeteners could be improved significantly if the structural characteristics influencing bitter and sweet taste could be discriminated. This knowledge would permit the design of molecules with an unambiguous taste.

A recent report (5) on a series of oximes presented an opportunity for such a discriminative structural analysis. In that study, 51 molecules were analyzed for taste potency and relative sweet–bitter contribution. The results from this oxime study (5) were used in these laboratories to analyze the structures by molecular connectivity, and a discrimination analysis was performed to find the best structural representation discriminating between these two tastes.

EXPERIMENTAL

Selection of Sweet- and Bitter-Tasting Molecules—Two taste characteristics that were defined previously (5) were considered in the present study to differentiate between sweet and bitter molecules. The first characteristic, taste potency compared to a 0.25 *M* sucrose solution, is a measure of taste intensity, regardless of whether the molecule is sweet or bitter. The other characteristic is the percentage of the taste identified as sweet and that identified as bitter.

For the present study, 10 molecules were selected from each taste category based on the largest percentage of sweet (or bitter) taste and the most potent taste response. All 20 molecules selected were aldoximes (Tables I and II).

Structural Analyses and Discrimination Analysis—The structural descriptions of the molecules in Tables I and II were made using molecular connectivity (6). Numerous studies on the structure–activity relationships of drug molecules have employed molecular connectivity to quantitate molecular structure effectively (6–9).

Discriminant analysis is an approach to differentiate between two or more classes of molecules. The procedure is a search for a linear combination of structural variables that describes a line, plane, or general surface that separates the molecule classes in the optimum manner. The value of y is related to the linear discriminant function by:

$$y = c_1x_1 + c_2x_2 + \ldots + c_nx_n \qquad \text{(Eq. 1)}$$

The function separates the categories with as little overlap as possible, thus providing discrimination between the categories. The coefficients, c_i, are determined so that the distance between the categories is maximized whereas the distance between members of the same category is minimized.

The analysis also produces the critical value, y^*, so that molecules with $y > y^*$ are assigned to one category and those with $y < y^*$ are assigned to the other category. The ratio of the between-category variance to the within-category variance is proportional to other statistical quantities such as D^2 (the Mahalanobis generalized distance), T^2 (defined by Hotelling), and the more standard F value (10).

The quality of the discriminant function may be assessed in three ways: comparison of the F value to tabulated values, determination of the

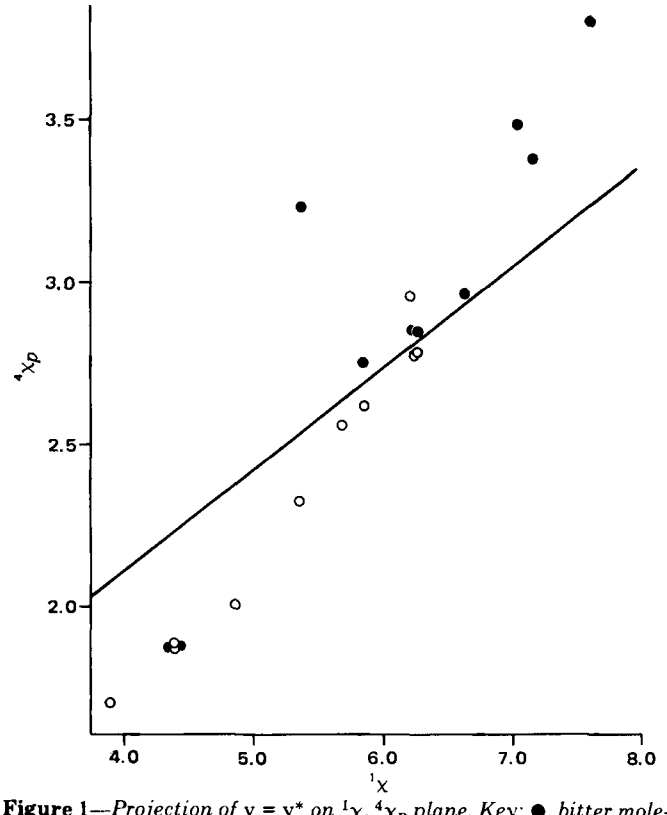

Figure 1—*Projection of* $y = y^*$ *on* $^1\chi$, $^4\chi_p$ *plane. Key:* ●, *bitter molecules; and* ○, *sweet molecules.*

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Table I—Discriminant Analysis of Sweet-Tasting Aldoximes R—CH=N—OH

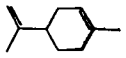
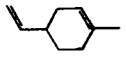
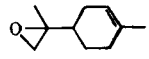
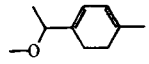
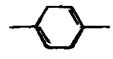
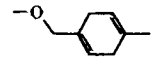
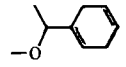
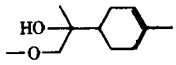
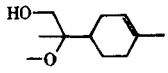
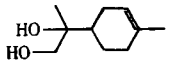
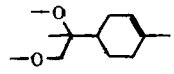
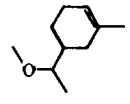
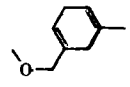
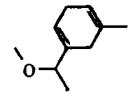
Compound	R	Taste Potency Relative to Sucrose	Percent of Taste, Sweet/Bitter	$^1\chi$	$^4\chi_p$	$y - y^*$
I		370	60/25	5.736	2.552	0.32
II		1150	50/10	5.364	2.337	0.70
III		50	65/10	6.244	2.965	−0.67
IV		55	48/7	3.932	1.707	1.41
V		55	40/16	4.432	1.884	1.33
VI		135	65/7	6.274	2.788	0.05
VII		200	70/3	4.432	1.884	1.33
VIII		500	78/3	4.826	2.026	1.25
IX		225	90/2	5.864	2.625	0.18
X		300	92/1	6.274	2.788	0.05

Table II—Discriminant Analysis of Bitter-Tasting Aldoximes R—CH=N—OH

Compound	R	Taste Potency Relative to Sucrose	Percent of Taste, Sweet/Bitter	$^1\chi$	$^4\chi_p$	$y - y^*$
I		4	6/70	7.098	3.489	−1.67
II		11	4/93	7.158	3.384	−1.19
III		30	5/73	6.598	2.930	−0.11
IV		1.5	0/50	4.432	1.884	1.33
V		2	2/65	4.432	1.884	1.33
VI		50	0/70	7.658	3.797	−2.19
VII		28	2/52	6.274	2.853	−0.20
VIII		140	0/75	5.864	2.743	−0.27
IX		92	0/67	6.274	2.853	−0.20
X		320	0/50	5.398	3.240	−2.77

percentage of molecules correctly classified, and prediction of the classification of molecules not included in the original study.

RESULTS

The best linear discriminant function, using two molecular connectivity index variables, is:

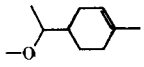
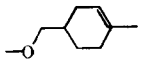
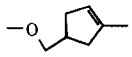
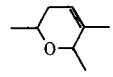
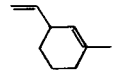
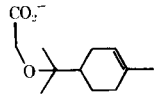
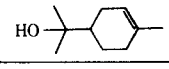
$$y = 1.21^1\chi - 3.88^4\chi_p \quad \text{(Eq. 2)}$$

and the critical value is:

$$y^* = -3.27 \quad \text{(Eq. 3)}$$

When each y value is solved for the sweet (Table I) and bitter (Table II) molecules, values of $y > -3.27$ are assigned to the sweet category while

Table III—Taste Prediction from Discriminant Analysis R—CH=N—OH

Compound	R	Taste Potency Relative to Sucrose	Percent of Taste, Sweet/Bitter	$^1\chi$	$^4\chi_p$	$y - y^*$
I		90	50/15	4.432	1.884	1.32
II		80	50/20	6.274	2.788	0.05
III		52	53/22	5.864	2.625	0.18
IV		24	39/9	5.364	2.519	0.00
V		40	55/5	3.308	0.697	4.568
VI		10	0/36	5.236	2.528	−0.20
VII		175	14/34	5.364	2.444	0.28
VIII		10	0/38	7.958	3.593	−1.04
IX		8	4/18	6.037	2.725	−0.01

values of $y < -3.27$ are assigned to the bitter category. Therefore, when $y - y^*$ is negative, the molecule is classified as bitter. The discriminant function is projected onto the $^1\chi$, $^4\chi_p$ plane for $y = y^*$ (Fig. 1) to illustrate the assignments to the two taste categories.

The discriminant function correctly assigns nine of the 10 sweet-tasting molecules (Table I) and eight of the 10 bitter-tasting molecules (Table II). The average percentage of correct assignment is 85%. The statistical significance can be stated as the T^2 value, which in this case is 5.9.

DISCUSSION

The linear discriminant function is an equation featuring two molecular connectivity indexes, $^1\chi$ and $^4\chi_p$. These indexes, weighted by the coefficients in the equation, describe two structural characteristics that influence whether a molecule is sweet or bitter.

The indexes convey structural information that is familiar to the medicinal chemist. The $^1\chi$ index describes the size of the molecule in terms of the number of bonds. Within the equation, the $^1\chi$ index indicates that the more bonds there are, the larger the y value will be. When $^1\chi$ is large enough, $y > y^*$ and the molecule is classified as sweet. The influence of size as expressed by $^1\chi$ is countered by the information conveyed by $^4\chi_p$. This index, as a negative term in the linear discriminant function, leads to lower y values when $^4\chi_p$ increases. The value of $^4\chi_p$ is influenced by the size of the molecule as well as by the substituents.

The indexes in the linear discriminant function indicate that the value of y is greater than that of y^* when the number of first-row atoms in the substituent of cyclohexylaldoxime is four or less. Thus, a *tert*-butyl group (*i.e.*, Compound IX, Table III) has a $y - y^*$ of virtually zero. Substituents with more than four first-row atoms, particularly if they are branched, give a y value less than y^* (Table II). The structural conclusions are that larger, more branched substituents on cyclohexylaldoximes increase the tendency for the molecule to be bitter. In contrast, smaller substituents, with four or fewer first-row atoms, influence sweet taste (Table I).

Another observation that can be made about the influence of structure on taste, based on the linear discriminant function, is that the two chi indexes are not of the valence class. This finding means that unsaturation or heteroatoms in the 20 molecules are not critical to the structures that influence the sweet or bitter taste. This conclusion is correct to the extent that 85% of the molecules are assigned correctly.

For the three incorrectly assigned molecules (III in Table I and IV and V in Table II), a molecular connectivity index of the valence type may be necessary for the correct assignment. However, the addition of a third molecular connectivity index in the discrimination analysis failed to improve the number of correctly assigned molecules in Tables I and II. The three incorrectly assigned molecules in Tables I and II have the lowest taste potencies among their respective taste classes. This observation suggests that high taste potency is important in the categorization of molecules as bitter or sweet tasting.

A test of the linear discriminant function and the molecular connectivity indexes that describe structures is the ability of the function and the associated critical value, y^*, to predict the taste categories of molecules not in the original study. For this test, nine additional molecules were selected from the study of Acton and Stone (5), based on their taste potencies and sweet–bitter taste ratios that favored one or the other category (Table III).

Calculation of the $^1\chi$ and $^4\chi_p$ indexes for each molecule and computation of $y - y^*$ for the linear discriminant function formed the basis of the taste category predictions shown in Table III. The first five molecules are reported to be sweet. The predictions for I–III and V are correct. The prediction for IV is ambiguous since $y = y^*$.

Compounds VI–IX are classified as bitter tasting. The solution of $y - y^*$ predicts VI, VIII, and IX to be bitter. Compound VII is predicted incorrectly.

Thus, seven of the predictions were correct, one was incorrect, and one was ambiguous. Without IV, the correct prediction for seven of eight compounds gave an accuracy of 87%, the same quality of classification as obtained with the assignments in Tables I and II combined.

The results of the study are encouraging in that molecular connectivity indexes appear to encode sufficient information about the structure influencing the taste category of cyclohexylaldoximes so that the discriminant analysis can assign correctly 17 of 20 molecules to the sweet or bitter taste category. Furthermore, the analysis based on these indexes can predict the taste category of additional molecules with the same accuracy. The study shows one approach to the possible separation of bitter taste from candidate molecules of interest as noncaloric sweeteners.

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Biological Disposition of Sodium Dichloroacetate in Animals and Humans after Intravenous Administration

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Received April 27, 1979, from the *Research Department, Pharmaceuticals Division, Ciba-Geigy Corporation, *Ardsley, NY 10502, and ‡Summit, NJ 07901.* Accepted for publication October 5, 1979.

Abstract □ Sodium dichloroacetate, a potential antidote for lactic acidosis, was administered intravenously to rats, dogs, and four humans. In three rats, maximum plasma sodium dichloroacetate concentrations were 120–164 μg/ml after a 100-mg/kg dose and declined with half-lives of 2.1–4.4 hr. In two dogs, maximal concentrations of 447 and 508 μg/ml were attained after a 100-mg/kg dose. The subsequent decline was relatively slow with approximate half-lives of 17.1 and 24.6 hr. An intravenous infusion of 10 mg/kg was administered over 20 min to two human subjects. Two other subjects received 20 mg/kg. After the infusion, maximum plasma concentrations of 19.9 and 24.7 μg/ml were seen with the lower dose and 57.3 and 74.9 μg/ml were achieved with the higher dose. Thereafter, concentrations declined rapidly with half-lives of 20–36 min. The observed large interspecies differences in half-lives could be explained in terms of differences in the apparent volume of distribution and/or clearance.

Keyphrases □ Pharmacokinetics—sodium dichloroacetate, plasma concentration level-time curve, rats, dogs, humans □ Sodium dichloroacetate—pharmacokinetics, plasma concentration levels, rats, dogs, humans □ Clearance, intrinsic—plasma sodium dichloroacetate concentration, pharmacokinetics, rats, dogs, humans

Sodium dichloroacetate (I) has been reported to reduce normal or elevated blood lactate and pyruvate levels in animals by activating the pyruvate dehydrogenase enzyme complex (1–3). Oral administration of I to patients having nonketotic diabetes mellitus also led to an appreciable lowering of plasma lactate concentrations (4). The compound has been of possible interest as an intravenous antidote in patients suffering from lactic acidosis. This study was undertaken to provide information on the biological disposition of I in animals and humans since no data are available.

EXPERIMENTAL

Materials—Labeled[1] and unlabeled[2] sodium dichloroacetate were used. The material labeled in both carbon atom positions had a specific activity of 23.4 μCi/mg. Radiochemical purity, determined by TLC on silica gel plates in ethanol–chloroform–concentrated ammonium hydroxide–water (53:30:15:1.5), was >98%.

Animal Experiments—Each of three male Sprague–Dawley rats, 160–170 g, was given an intravenous injection of ^{14}C-I, diluted 1:20 with nonlabeled I, in a tail vein. The compound (100 mg/kg) was administered as a 10% aqueous solution. At specified times, animals were subjected to light ether anesthesia, and a 0.2–0.3-ml blood sample was collected in a heparinized centrifuge tube through a glass capillary by puncture of the suborbital sinus. Animals then were permitted to recover until the next sampling time.

Each of two male beagles, 9.0 and 10.5 kg, was given 100 mg of I/kg as a 20% aqueous solution intravenously in the cephalic vein. Animals were placed in individual metabolism cages. At specified times, blood was collected from a jugular vein without anesthesia.

Human Study—Four normal subjects, having given informed consent, participated after an overnight fast. Subjects 1 and 2, ages 42 and 38 years, respectively, each weighed ~70 kg. Subjects 3 and 4, ages 52 and 26 years, respectively, weighed 80 and 83 kg. In each case, the history, physical examination, ECG, and all laboratory tests were within normal limits.

Following the collection of the control urine sample and the drawing of a control blood specimen from Subjects 1 and 2, a 10-mg/kg dose of I dissolved in 100 ml of saline was infused over 20 min. Blood specimens were obtained from the contralateral antecubital vein at the time the infusion was completed. Specimens were drawn subsequently at 1 (40 min after termination of the infusion), 2, 3, 4, 6, 8, 10, 12, 24, 32, and 48 hr. All urine was collected for 96 hr with collection periods ending at 1, 2, 4, 8, 12, 24, 48, 72, and 96 hr.

Several weeks later, the procedure was repeated on Subjects 3 and 4, the sole change being that each received 20 mg/kg. All subjects were given a standard breakfast after the 4th hr. They remained under clinical observation for 8 hr.

Blood specimens were centrifuged as soon as they were collected. Plasma was separated and stored frozen until analysis. The urinary volume was measured and recorded. Aliquots of 20 ml from each urine sample were labeled and frozen for analysis.

Analysis of Unchanged I—Plasma and urine I concentrations were analyzed by a reported GLC method (5) as modified (6). Briefly, the method involved addition of trichloroacetic acid as an internal standard and 14% boron trifluoride in methanol as the derivatizing reagent. The resulting methyl esters of I and the internal standard were extracted with benzene and analyzed on a Chromosorb 101 column using an electron-capture detector.

Preliminary analyses of plasma samples obtained from human subjects indicated that many specimens contained very low I concentrations. Therefore, method sensitivity was improved from 300 ng/ml as reported previously (6) to 40–50 ng/ml by three minor modifications: (*a*) the volume of solvent used in the extraction was reduced from 2 to 0.5 ml; (*b*) between the extraction and centrifugation steps, the entire contents of the vial were transferred to a 5-ml centrifuge tube to facilitate aliquot removal; and (*c*) the volume of extract analyzed was changed from 2 to 3 μl. The linearity of standard curves obtained during analysis demonstrated that these changes had no effect on the accuracy and precision of the results.

Analysis of Total Radioactivity—A 10-μl aliquot of plasma or urine was dissolved in 15 ml of scintillation cocktail and counted[3]. External standardization was used to correct for quenching. The cocktail had the following composition: naphthalene, 400 g; 2,5-diphenyloxazole, 20 g; 2,2′-*p*-phenylenebis(4-methyl-5-phenyloxazole), 1.2 g; dioxane, 2920 ml; toluene, 540 ml; and methanol, 140 ml.

[1] New England Nuclear Corp., Boston, Mass.
[2] Synthesized in these laboratories.
[3] Intertechnique model SL-40 liquid scintillation spectrometer.

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