Acute Toxicity. Male ddN strain mice, weighing 18–21 g, were used. Groups of ten mice received food and water ad libitum. The test compounds were administered orally. The LD_{50} was calculated according to the method of Litchfield and Wilcoxon[10] on the 7th day after administration.

(10) J. T. Lichtfield, Jr., and F. W. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).

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Notes

A Consideration for Structure–Taste Correlations of Perillartines Using Pattern-Recognition Techniques

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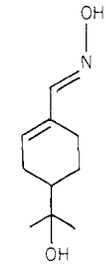
The relationships between molecular structure and taste quality (sweet or bitter) or several perillartine derivatives were investigated using pattern-recognition techniques. For the classification of these compounds into two classes (sweet or bitter), a significant discriminant function was developed by the use of linear learning machine. All the compounds were assigned correctly to their observed taste classes by the function involving three parameters (one hydrophobic and two steric). In addition, the K–L transformation technique was used for examination of classification results.

Recently, Iwamura[1] has performed a quantitative analysis of the structure–taste relationships of 49 perillartine derivatives reported by Acton and co-workers.[2] In his paper, Iwamura used the taste potencies of the compounds as dependent variables in the regression analysis with physicochemical parameters and STERIMOL parameters[3] and suggested the commonness between sweet and bitter receptor from the results. However, his analysis was based on only relative taste potencies of sweetness and bitterness, and he did not give precise consideration to the absolute taste potencies of them.

According to Acton's paper, only half of the 49 compounds give more than 50% bitter or sweet taste potencies for total taste potencies. Moreover, there are several compounds that give far less than 50% potencies even for the sum of bitterness and sweetness. Therefore, it does not seem appropriate to apply the result of the regression analysis based on the data set containing those compounds which give mainly other tastes to the discussion of structure–activity relationships in bitter and sweet tastes. In other words, for those compounds that give more than 50% of other taste potencies, the structure–activity relationships in bitter and sweet tastes could not be clearly determined.

Acton has also presented the quantitative structural features for bitter and sweet compounds. In this case, some compounds that do not give bitter or sweet taste as their main taste were studied. For example, compound I gives 4% sweetness and 18% bitterness for total taste potency; therefore, bitterness or sweetness could not be superior to other tastes. However, Acton categorized it to be a bitter compound based on a ratio of sweetness vs. bitterness of 0.222.



I

To avoid this ambiguity, we have selected those compounds that give more than 50% bitterness or sweetness for total taste potencies as typical examples, and we studied them from the standpoint of discrimination between sweetness and bitterness by using various pattern-recognition techniques.

Data Set and Preprocessing. Pattern-recognition methods are powerful techniques for the investigation of structure–activity relations (SAR).[4-7] In chemical applications, pattern-recognition techniques are generally implemented in three successive procedures: preprocessing; feature selection, by which significant parameters for classification are selected; and development of the clas-

(1) H. Iwamura, *J. Med. Chem.*, **23**, 308 (1980).
(2) E. M. Acton and H. Stone, *Science*, **193**, 584 (1976).
(3) A. Verloop, W. Hoogenstraaten, and J. Tipker, in "Drug Design", Vol. 7, E. J. Ariëns, Ed., Academic Press, New York, 1976, p 165.
(4) G. L. Kirschner and B. R. Kowalski, in "Drug Design" Vol. 8, E. J. Ariëns, Ed., Academic Press, New York, 1977, p 73.
(5) Y. Takahashi, Y. Miyashita, H. Abe, S. Sasaki, Y. Yotsui, and M. Sano, *Anal. Chim. Acta*, **122**, 241 (1980).
(6) Y. Miyashita, Y. Takahashi, Y. Yotsui, H. Abe, and S. Sasaki, Proceeding of 7th International CODATA Conference, No. 41, Pergamon Press, Oxford, 1981, p 37.
(7) Y. Miyashita, T. Seki, Y. Takahashi, S. Daiba, Y. Tanaka, Y. Yotsui, H. Abe, and S. Sasaki, *Anal. Chim. Acta*, **133**, 603 (1981).

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Chart I

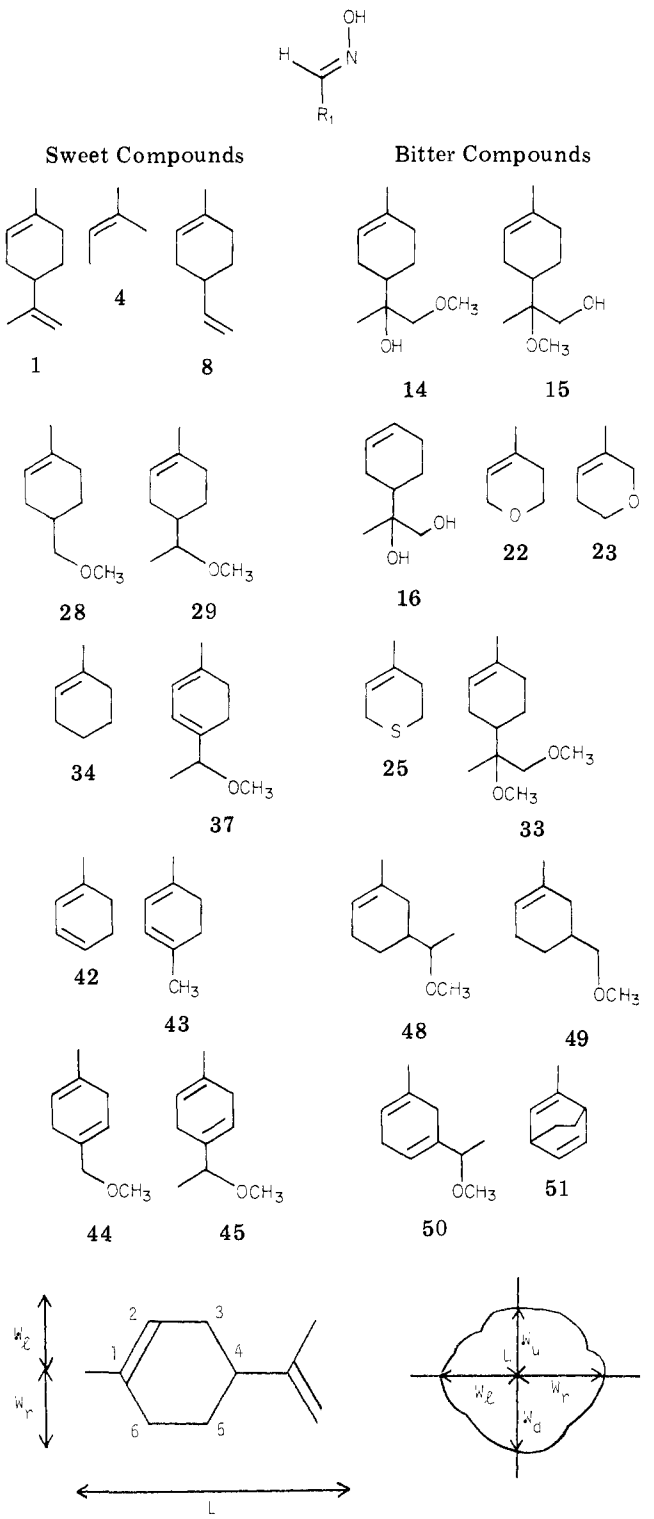

Figure 1. Schematic representation of the STERIMOL parameters (L, W_l, W_u, and W_d).

sifier, to decide the class of a given pattern vector. If a compound is represented by d parameters, a d-dimensional pattern vector $\boldsymbol{X}_i$ is denoted by eq 1. Therefore, patterns

$$\boldsymbol{X}_i = \boldsymbol{x}_{i1}, \boldsymbol{x}_{i2}, ..., \boldsymbol{x}_{id} \quad (1)$$

with d parameters can be represented as points in a d-dimensional space.

In the present study, we have defined either of two classes (sweet or bitter) for each of 22 compounds shown in Chart I. The structures of the molecules were described by hydrophobic (log P) and the STERIMOL (L, W_l, W_u, W_r and W_d) parameters as shown in Table I and Figure 1.

Table I. Taste Class and Parameters[a] of Perillartine Derivatives

no.[b]	taste[c]	log P	L	W_l	W_u	W_r	W_d
1	sweet	2.58	8.52	3.13	2.85	3.42	1.99
4	sweet	0.87	5.10	3.13	1.91	2.94	1.90
9	sweet	2.28	8.69	3.19	2.84	3.42	1.99
28	sweet	1.10	9.36	3.14	2.94	3.41	1.98
29	sweet	1.40	9.36	3.14	3.26	3.56	2.10
34	sweet	1.48	6.06	3.09	2.08	3.01	1.71
37	sweet	1.10	8.87	3.30	2.63	3.07	2.52
42	sweet	1.48	6.29	3.09	1.91	3.41	1.91
43	sweet	0.78	7.10	3.09	1.91	3.41	1.91
44	sweet	0.80	9.01	3.09	2.20	3.41	2.02
45	sweet	1.10	9.01	3.08	2.52	3.43	2.53
14	bitter	−0.10	10.67	3.33	4.11	3.56	2.10
15	bitter	−0.10	9.36	3.04	3.79	3.62	2.22
16	bitter	−0.92	9.37	3.14	3.56	3.56	2.10
22	bitter	−0.72	5.51	3.05	2.53	3.41	1.97
23	bitter	−0.72	6.15	3.16	2.67	3.01	1.72
25	bitter	0.34	6.05	3.25	2.62	3.43	2.03
33	bitter	0.72	10.67	3.51	4.08	3.63	2.22
48	bitter	1.40	7.98	3.12	3.42	5.96	2.00
49	bitter	0.80	7.68	3.09	2.32	5.84	1.96
50	bitter	1.10	7.68	3.09	2.43	5.89	2.57
51	bitter	1.90	5.88	2.72	2.95	3.92	3.85

[a] The values of parameters were taken from ref 2. [b] The compounds are numbered according to ref 1. [c] The compounds with sweet/total taste ≥ 0.5 are taken as sweet and those with bitter/total taste ≥ 0.5 are taken as bitter.

Table II. A Result of Feature Selection Using Fisher Ratio for Sweet/Bitter Classification

parameter	Fisher ratio	rank
log P	7.84×10^{-2}	1
L	1.74×10^{-5}	5
W_l	1.59×10^{-5}	6
W_u	6.01×10^{-2}	2
W_r	4.95×10^{-2}	3
W_d	9.14×10^{-3}	4

Table III. Classification Results by LLM and KNN Method

method	recognition, %	prediction, %	misclassified compds
1NN		90.9	25 and 51
3NN		90.9	25 and 51
5NN		90.9	25 and 51
LLM	100.0	100.0	

Thus, all compounds in the data set are represented as six-dimensional pattern vectors. Then, they are preprocessed in such a way that each component of a pattern vector has a zero mean value and unit standard deviation; in other words, every component of a pattern vector is weighted equally.

Feature Selection. Feature (parameter) selection was performed with the Fisher ratio.[8] The Fisher ratio is a quantitative estimate of the significance of a given parameter for separating two classes. It is the ratio between the square of the difference in interclass means and the sum of squared intraclass standard deviation.

Then each parameter for the classification was ranked in the order of significance according to the Fisher ratio (Table II). Three features, log P, W_u, and W_r, were selected as the most significant parameters because of the

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Table IV. Results of K-L Transformation

axis	eigenvalue	cumulative variance, %	eigenvector log P	eigenvector W_u	eigenvector W_r
z_1	1.24	41.5	0.725	−0.683	0.091
z_2	1.08	77.4	0.246	0.380	0.892
z_3	0.68	100.0	0.644	0.624	−0.443

rather large value of the Fisher ratio.

Classification. K-nearest neighbor[9] (KNN) and linear learning machine (LLM)[10,11] methods were applied for classifications of the data set.

The results are shown in Table III. The KNN method, which utilizes the distance between class-known and class-unknown pattern vectors for the decision criterion, is used to classify a pattern vector into the class to which the majority of the K-nearest known pattern vectors belong. The Euclidean distance between samples was used in this study. The distance was defined by the above three parameters.

By the use of this method for k = 1, 3, and 5, the prediction rates were 90.9% in every case. The compounds **25** and **51** were always misclassified. A discriminant function developed by LLM classifies the compounds into two classes and predicts the class to which each pattern vector belongs.

Equation 2 is the discriminant function obtained by

$$g(\boldsymbol{X}) = 0.33 \log P - 0.21 W_u - 0.89 W_r - 0.22 \quad (2)$$

LLM. If $g(\boldsymbol{x}) > 0$, then the compound is assigned to the sweet class; if $g(\boldsymbol{x}) < 0$, then the compound is assigned to the bitter class. As shown in Table III, the recognition rate with LLM was 100%, and the prediction rate by leave-one-out procedures was also 100%. In this case, it is suggested that the data set is linearly separable. These compounds could not be completely classified by the use of any combination of two parameters. Thus, it seems that log P, W_u, and W_r are indispensable for classification of taste qualities. Within this compound set, compounds having large values of log P and small values of W_u and W_r will be classified as sweet, because of the positive coefficient of the log P term and the negative coefficients of the W_u and W_r terms in eq 2. These results seem to support more quantitatively Iwamura's suggestion, which states that sweet compounds have relatively smaller substituents, in dimensions, than bitter compounds. However, it is shown that there is some substantial dependency on hydrophobicity for the taste qualities. This is a significant difference between Iwamura's study and the present study. Some considerations are given about this point as follows: we have used a more limited data set than was used in Iwamura's study because those compounds that show rather weak taste would make classification results ambiguous and would lead to ambiguous conclusions.

In the following studies, the above results will be more clarified by the mapping and cluster analysis methods in pattern recognition.

Consideration with Mapping Results and Cluster Analysis. In order to display three-dimensional significant parameter space (log P, W_u, and W_d), the Karhunen-Loēve (K–L) plot[12] is employed. This method is one of the linear mapping methods, which is useful for interpretation of classification results. Figure 2 illustrates the mapping results, and 77.4% of total variance has retained (Table IV).

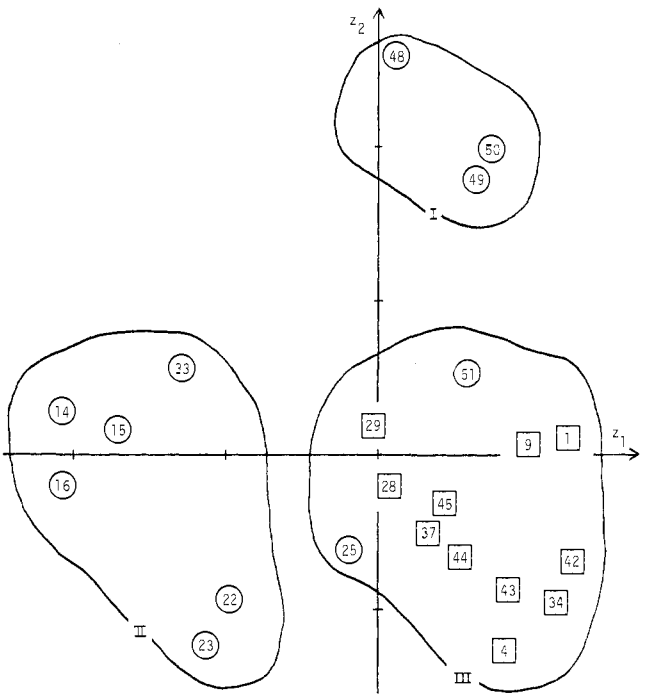

Figure 2. K–L plot of 22 perillartines and 3 clusters: (□) sweet; (○) bitter compounds.

Then, all the compounds are disparted into three clusters, which are shown by circles using cluster analysis[5] (Figure 2). Cluster analysis is a technique for finding homogeneous groups in a given data set. In Figure 2, compounds in the same cluster are similar to each other with respect to their defined properties and are different from those of compounds in other clusters.

Clusters I and II consist of only bitter compounds. On the other hand, cluster III contains sweet compounds, except for two bitter compounds, **25** and **51**, which are missclassified by the KNN method. From the results, it appears that compounds **25** and **51** are located in a certain similar area of the three-dimensional parameter space which was previously defined with log P, W_u, and W_d.

It is clear from Figure 2 that sweet compounds must have larger values of z_1 and simultaneously have smaller values of z_2. Table IV shows eigenvalues and eigenvectors obtained by the K–L transformation. The eigenvalues indicate contribution to the variance, and the eigenvectors account for contribution to the parameters.

This result shows that z_1 is subjected to log P and W_u; on the other hand, z_2 is subjected to W_r. Taking into account the sign of the eigenvector and sweet region, it is consistent with the results obtained from the LLM. As shown in Figure 2, the sweet compounds are located in a limited region of parameter space. The above results may suggest that the sweet taste receptor is more restricted, in the viewpoint of spatial shape, than the bitter receptor.

In conclusion, the STERIMOL parameters can be used to identify taste qualities of perillartine derivatives if they are accompanied by the hydrophobicic parameter, log P. Since it is well known that the hydrophobicity of molecules is related to various biological phenomena,[13] these results

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would be very interesting if it were important for transportation of taste compounds to a certain receptor and for exertion of taste. For applying pattern-recognition techniques to SAR studies, it is important to consider the data structure in parameter space of objective samples with a visible display method.

Acknowledgment. The authors thank the Computer Center, Institute for Molecular Science, for affording facilities for computation.

Probes of the Active Site of Norepinephrine *N*-Methyltransferase: Effect of Hydrophobic and Hydrophilic Interactions on Side-Chain Binding of Amphetamine and α-Methylbenzylamine[1a]

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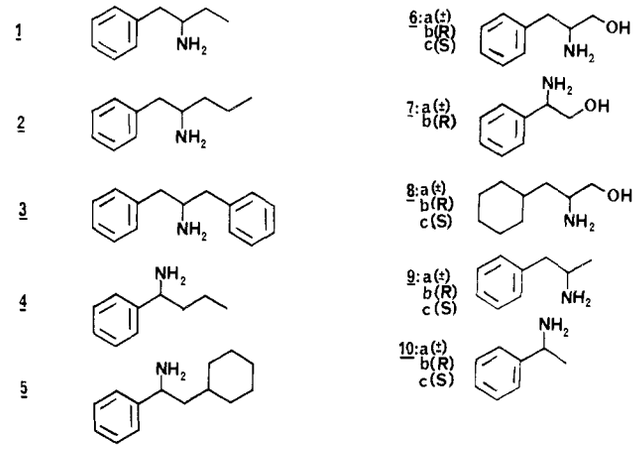
A series of ω-substituted analogues of amphetamine and α-methylbenzylamine were prepared and evaluated as inhibitors of norepinephrine *N*-methyltransferase (NMT). These included several alkyl side chain extended analogues (**1–5**), as well as the terminally hydroxylated derivatives phenylalanol (**6a**) and phenylglycinol (**7a**). None of the alkyl-substituted derivatives displayed appreciable activity as inhibitors; however, the hydroxylated analogues were up to twofold more potent than the parent compounds. The positive contribution of the side-chain hydroxy suggests that the terminal methyl group of the lead compounds is situated close to a hydrophilic area or hydrogen bonding functional group within the active site.

The enzyme norepinephrine *N*-methyltransferase (NMT, EC 2.1.1.28; also known as phenylethanolamine *N*-methyltransferase, PNMT) catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine (AdoMet) to the primary amino group of norepinephrine, yielding epinephrine. In pursuit of specific inhibitors of NMT which could prove to be useful pharmacological tools, we have been attempting to characterize select features of the active site through the use of analogues of norepinephrine and of the competitive NMT inhibitors amphetamine and α-methylbenzylamine. Our efforts to date have focused on the properties of the active-site region that accommodates the aromatic ring of bound substrates[2,3] and inhibitors,[4] and also on the side-chain conformation of bound phenylethylamines.[5] In this report, we describe initial results of an investigation that is aimed at characterizing a different region of the active site, the area which lies in the vicinity of the terminal methyl group of bound amphetamine (**9a**) and α-methylbenzylamine (**10a**).

The ability of phenylethylamines, such as amphetamine, to inhibit NMT was first noted by Axelrod[6] and later explored in depth by Fuller et al.[7] The potent inhibitory activity of NMT by benzylamines was first reported by



Fuller et al.[8] The α-methyl derivatives of phenylethylamine and benzylamine were of interest with respect to the development of some metabolically stable inhibitors suitable for in vivo studies, since the added methyl group renders these compounds resistant to oxidation by the amine metabolizing enzyme monoamine oxidase (MAO). To our knowledge, no further investigations have been conducted regarding the nature and tolerances of the region of the active site of NMT occupied by this additional methyl group. Since an understanding of the active site area that binds substrates and inhibitors is important for the rational design of feasible inhibitors to bind to this site, we have prepared some simple side chain extended analogues of **9a** and **10a** that were designed to probe the characteristics of the region of the enzyme in the vicinity of the methyl group of the ligands. Basically, the compounds were selected in order to detect either hydrophobic or hydrophilic character within this region by adding additional hydrocarbon bulk to the methyl group or by adding an hydroxy group to the side-chain terminus. The

(1) (a) Taken from the Ph.D. Dissertation submitted to the Graduate School of the University of Kansas by M.F.R., 1982. (b) University of Kansas undergraduate research participant (Grant KU-3944). (c) Supported by NIH Predoctoral Training Grant GM 07775.
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