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Development of Structure–Taste Relationships for Monosubstituted Phenylsulfamate Sweeteners Using Classification and Regression Tree (CART) Analysis

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Twenty monosubstituted phenylsulfamates (cyclamates) have been synthesized and have had their taste portfolios determined. These have been combined with 63 compounds already in the literature to give a database of 83 ortho, meta, and para compounds. A training set of 75 compounds was randomly selected leaving eight compounds as a test set. A series of nine predictors determined with Corey–Pauling–Koltun models, calculated from the PC SPARTAN PRO program and Hammett σ values taken mainly from the literature, have been used to establish structure–taste relationships for these types of sweeteners. The taste panel data for all compounds were categorized into three classes, namely, sweet (S), nonsweet (N), and sweet/nonsweet (N/S), and a novel "sweetness value" or weighting was also calculated for each compound. Linear and quadratic discriminant analysis were first used with the S, N, and N/S data, but the results were somewhat disappointing. Classification and regression tree analysis using the sweetness values for all 75 compounds was more successful, and only 14 were misclassified and six of the eight test set compounds were correctly classified. For the 29 meta compounds, one subset using just two parameters classified 83% of these compounds. Finally, using various methods, predictions were made on the likely tastes of a number of meta compounds and a striking agreement was found between the tree prediction and those given by earlier models. This appears to offer a strong vindication of the tree approach.

KEYWORDS: Sweetness; sulfamates; cyclamates; CART analysis; phenylsulfamates

INTRODUCTION

Cyclamates, the well-known alternative sweeteners, have been around now for over 50 years, and despite the arrival in the marketplace of many new nonnutritive sweeteners, they still enjoy widespread usage worldwide in both the European Union and over 50 other countries (*1*, *2*). In the United States, their reintroduction is under review by the Food and Drug Administration. There are probably many reasons for their popularity and continued usage not the least being that they are excellent in synergistic applications (*3*), for example, with saccharin as Sucaryl, have a relatively low production cost (*4*), and are now (like saccharin) accepted as being safe to use (*2*).

In the original seminal work of Audrieth and Sveda on the structure–taste relationships (SARs) of sulfamate (cyclamate) sweeteners, the strong sweetness of a number of phenylsulfamates was missed since only three such materials (with X = H, X = *p*-OEt, and X = *p*-Me) were synthesized in that study (*5*) (**Figure 1**). The compound with X = H was reported to have a sweet aftertaste, and the other two compounds were not S. However, some years ago, the marked sweetness of some of these compounds, especially the meta compounds, was discovered serendipitously (*6*). There has been some controversy regarding the phenylsulfamates, and a theory proposed to account for the sweetness/nonsweetness of various cyclamates (*7*) was extended in the seventies to explain the nonsweetness of phenylsulfamates (*8*), but this was later refuted using X-ray data and MINDO theoretical calculations (*6*).

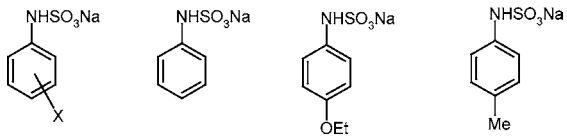

Figure 1. (Left to right) Molecular structures of general monosubstituted phenylsulfamate salt, sodium phenylsulfamate (X═H), sodium *p*-ethoxyphenylsulfamate (X═OEt), and sodium *p*-methylphenylsulfamate (X═Me), respectively.

A program involving the syntheses and taste assessment of a large number of monosubstituted phenylsulfamates was carried out (*9*, *10*). Through this work, taste data became available for 63 *ortho*-, *meta*-, and *para*-phenyl-substituted compounds, and by employing a combination of experimentally measured and theoretically calculated parameters, a series of appropriate descriptors were available for all 63 compounds. These were

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Table 1. Percentage of Assessors Giving the Tastes of Monosubstituted Phenylsulfamates[a,b]

			%						sweetness	predominant
entry	sulfamate	pH	S	sour	bitter	salty	tasteless	S aftertaste	value	taste: N, S, N/S
1	*o*-CO_2Et	6.13	40	20	0	20	40	20	60	N/S
2	*o*-CO_2-*n*-Bu	6.13	0	0	100	0	0	20	16.7	N
3	*m*-CO_2Me	5.62	40	20	60	0	0	20	42.9	N/S
4	*m*-CO_2Et	5.56	0	40	60	0	20	0	0	N
5	*m*-iso-Pr	9.32	80	0	20	0	20	20	83.3	S
6	*m*-CO_2–[c]	4.01	0	37.5	25	37.5	50	0	0	N
7	*m*-SO_3–[c]	3.79	0	62.5	0	25	50	0	0	N
8	*m*-CO_2-*t*-Bu	9.27	40	0	60	0	20	20	50	N/S
9	*p*-CO_2Me	6.06	0	40	80	20	0	0	0	N
10	*p*-CO_2Et	6.00	0	0	0	0	100	0	0	N
11	*p*-$(CH_2)_2$OH	9.86	20	20	20	0	40	0	33.3	N/S
12	*p*-CHMeOH	7.37	0	0	60	0	40	0	0	N
13	*p*-CO_2–[c]	4.27	0	50	25	12.5	75	0	0	N
14	*p*-CO_2-*n*-Pr	3.87	0	20	20	0	60	0	0	N
15	*p*-CO_2-*n*-Bu	9.27	60	0	40	0	0	20	66.7	S
16	*p*-CO_2-iso-Pr	7.60	20	20	40	0	20	80	62.5	S
17	*p*-CO_2-*t*-Bu	7.46	0	0	100	20	0	0	0	N
18	*p*-CO_2-iso-Bu	8.87	60	0	40	0	20	20	66.7	S
19	*p*-NEt$(CH_2)_2$OH	9.89	20	0	0	0	80	0	100	S

[a] All compounds were tasted as 0.01 M solutions made in distilled water of pH values varying from 5.7 to 5.9. The solutions were tasted by the assessors within 24 h of preparation. [b] Five assessors were used, and their responses are shown as percentages of 100/5, i.e., 20%. Thus, for example, for compound **1**, two assessors found immediate sweetness and this is shown as 40%, one assessor found sourness and this is given as 20%, none of the five found bitterness and this is shown as 0%, one assessor found saltiness given as 20%, two of the assessors found the compound to be tasteless shown as 40%, and finally one assessor found a S aftertaste displayed as 20%. [c] Eight assessors were used for these compounds (Coyle, C. M. Ph.D. Thesis, National University of Ireland, 2002), and thus, the taste response per assessor was 100/8, i.e., 12.5%.

then used to derive some SARs for this set of compounds. In the development of these SARs, linear (LDA) and quadratic (QDA) discriminant analyses were carried out using various subsets of the calculated and theoretical parameters, and generally, the overall % classification was reasonable at about 87%. However, despite this high rate of classification, the results seemed to be skewed in favor of the 28 sweet (S) compounds since in some cases up to 100% of these were correctly classified while as little as 60% of the 35 nonsweet (N) compounds were correctly classified. Because the database was not very large, all 63 compounds were used in the analysis, and although some predictions were made (*10*), a legitimate test set was not available.

A number of reasons would suggest that it seems timely to readdress the development of SARs for this class of cyclamate: (i) the limitation and possible inadequacy of the earlier attempts; (ii) there are now an additional 20 sulfamates available in our laboratory, and thus, the database of monosubstituted phenylsulfamates has increased in size to 83 compounds allowing some to be used as a test set; and (iii) the wide use of tree-based approaches in many areas (*11*) and the likely suitability of these for classifying the present taste data. Over 80% correct classification using a tree method with a set of very structurally diverse heterosulfamates has been obtained in this laboratory (*12*, *13*).

MATERIALS AND METHODS

Chemistry. The 20 aromatic sulfamates synthesized in this work were made by literature procedures (*5*, *14*). A commercially available pyridine sulfur trioxide complex was allowed to equilibrate in excess pyridine at room temperature. An equimolar quantity of amine was added under anhydrous conditions and allowed to stir overnight at room temperature. Sodium hydroxide (10%) was then added to the solution, which was worked up leading to the isolation of the sulfamates as their sodium salts. This procedure was used for synthesizing compound **5**. However, during the sulfamation of the 12 aniline esters, i.e., $ArCO_2R$ in **Table 1**, sodium carbonate was used instead of sodium hydroxide as it is a milder base and does not cleave the ester group as sodium hydroxide could. The addition of the hydroxide/carbonate continued with stirring until a pH of 10 was achieved. The solution was left to stir for an additional 20 min. The aqueous layer was washed several times with portions of diethyl ether to remove any unreacted amine that may be present, and it was then evaporated under reduced pressure. The resulting crude sodium salt product was extracted and recrystallized several times from aqueous ethanol.

Because three of the starting anilines are quite acidic and exist as zwitterions, i.e., *meta*- and *para*-$^+NH_3C_6H_4CO_2^-$ and *meta*-$^+NH_3C_6H_4SO_3^-$, the following procedure had to be employed to achieve sulfamation of these materials leading to compounds **6**, **13**, and **7**, respectively. 4-Pyrrolidinopyridine, which was equimolar with the aniline acid, was added. The pK_a of the 4-pyrrolidinopyridine is greater than that of the NH_3^+ group of the aniline acids, and this ensures that a free amino group, which is essential for sulfamation, is present. Thus, in this method, 4-pyrrolidinopyridine and available pyridine sulfur trioxide complex were allowed to equilibrate in excess pyridine at room temperature. An equimolar quantity of the aniline acid was added and allowed to react overnight at room temperature, and 10% sodium hydroxide was added to the solution until a pH of 10 was attained. The resulting solution was washed with diethyl ether, and it was concentrated to dryness. Once again, the crude product was purified by recrystallization with aqueous ethanol.

Sulfamation of the three aniline alcohols *para*-$(CH_2)_2$OH, *para*-CHMeOH, and *para*-NEt$(CH_2)_2$OH leading to compounds **11**, **12**, and **19**, respectively, was achieved using procedures based on those of Warner and Coleman (*15*) and Inoue and Nagasawa (*16*). The hydroxyl group of these compounds is attached to an aliphatic region of the target molecule, and under normal sulfamation conditions, the hydroxyl group is sulfated by the sulfur trioxide adduct. By using the procedure of the above authors, selective sulfamation of the amino group may be accomplished. The solid aniline was dissolved or suspended in distilled water in a round-bottomed flask. Sodium hydroxide (10%) was added until a pH within a range of 11.2−11.4 was achieved. An equimolar quantity of pyridine sulfur trioxide was added over a 1 h period during which enough 10% sodium hydroxide was added to keep the pH between 11.2 and 11.4; upon completion of this addition, the reaction was left to stir for 1 h. At the end of the reaction time, the crude mixture was reduced to 25 mL on a rotary evaporator. Ethanol was added, and

Table 2. Concentrations and pH Values of the Four Primary Standards

taste	standard	concentration (M)	pH
S	sucrose	4.4×10^{-2} (1.5%)	5.05
sour	citric acid	5.2×10^{-4} (0.01%)	3.69
bitter	quinine sulfate	6.4×10^{-6} (0.0005%)	5.03
salt	sodium chloride	3.4×10^{-2} (0.2%)	5.60

the precipitated inorganic impurities were removed by filtration. Acetone was then added to the resulting filtrate causing the precipitation of the sodium sulfamate product. The crude product was filtered and recrystallized from aqueous ethanol to yield the pure sulfamate product.

Characterization of Monosubstituted Phenylsulfamates. All 20 monosubstituted phenylsulfamates gave C, H, and N microanalyses within ±0.5% except the following four compounds. Compound **2**·$1H_2O$: Theory C, 42.17%; H, 5.15%; N, 4.47%. Found C, 42.11%; H, 5.14%; N, 3.95%. Compound **16**·$1H_2O$: Theory C, 40.13%; H, 4.72%; N, 4.68%. Found C, 40.45%; H, 4.37%; N, 3.16%. Compound **18**·$1H_2O$: Theory C, 42.17%; H, 5.15%; N, 4.47%. Found C, 42.19%; H, 4.71%; N, 3.43%. Compound **11**·$1H_2O$: Theory C, 37.35%; H, 4.70%; N, 5.44%. Found C, 37.42%; H, 4.38%; N, 4.44%. Each sulfamate was characterized by ^{1}H and ^{13}C NMR and IR spectroscopy. The expected peaks were observed in both the proton and the carbon-13 spectra. Each sulfamate gave the characteristic IR frequencies, νNH 3400−3190 cm^{-1}, νNS 730−660 cm^{-1}, νSO_3 (asymm) 1070−1040 cm^{-1}, νSO_3 (symm) 1203−1170 cm^{-1}, and νSO_3 (asymm) 1240−1210 cm^{-1}. C, H, and N microanalyses indicated that some of the products crystallized with varying quantities of water of hydration. Each product was tested for sulfate and sulfamate. Recrystallization continued until the sulfate test was negative and a clean sharp sulfamate resulted. The percentage yields of pure sulfamates varied from 11 to 64% with an average of 35%.

Instrumentation. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a JEOL 400 mHz spectrometer. IR analysis was carried out using a Perkin-Elmer FT-IR spectrum 1000 while a Perkin-Elmer 2400 series II analyzer was utilized for C, H, and N elemental microanalyses. pH determinations for all sodium sulfamate salts were made using a Jenway model 3310 pH meter buffered at pH 4.0, 7.0, and 9.2.

Sensory Analysis of Sulfamates (1). A panel of five subjects was used for the taste determinations for the 19 compounds reported in this work, and a sip and spit methodology was utilized (*9*). Taste assessments made on the sulfamates synthesized previously involved on average six assessors (*9, 10*). Taste evaluation was carried out at 18 ± 0.5 °C. Solutions for tasting were prepared in grade B volumetric flasks using distilled water and were tasted within 24 h of their preparation. All sodium sulfamates were tasted as 0.01 M solutions, a concentration that is found to give detectable tastes. Each sulfamate solution was presented to an experienced group of panelists in clean white plastic cups. The panel was given a maximum of five samples per tasting session, and fixed aliquots of 8 mL were used.

To enable the panelists to report accurately the taste of the sodium sulfamate solutions standards for the four primary tastes, sweet, sour, salty, and bitter, were utilized. The concentrations and pH values of the primary standards are outlined in **Table 2**. Each of the standards was tasted at or above their recognition threshold to enable the panelists to detect a definite taste (*17, 18*). All tasters were first given a sample of each of the primary standards and a pure distilled water sample until it was felt that they were competent in their ability to identify each solution correctly, followed by the sulfamate test solutions. Each of the tasters was then asked to identify one or more of the primary standards that best described the taste of the sulfamate solution under scrutiny. The panelists were also asked to record the presence of aftertastes. The aftertaste refers to the taste sensation that remains once the test solution has been removed from the mouth (*19*). Each of the 19 sulfamates synthesized in this work was tasted once. In runs to establish the reliability and to get a standard deviation of the tasting procedures employed, the same five assessors were each given one sweet (S), one nonsweet (N), and one sweet/nonsweet (N/S) compound and each was tasted five times by the assessors. The test solutions were given in a randomized manner interspersed with the four standards and distilled water. Seventy-five tastings on the three test solutions were carried out giving values of 80, 16, and 50, respectively, with an average ± standard deviation of 49 ± 9.

The results of the taste study on 19 sulfamates are reported in **Table 1**. The other "new" sulfamate included in this work is compound **61**—the parent phenylsulfamate. Compound **61** was tasted previously (*5, 10*) but was not included in our earlier analysis so we have now included it here. Percent tasteless was ignored in assessing whether the compound was predominantly S, N, or displayed substantial amounts of both in which case it was labeled N/S. Thus, for example, the first entry in **Table 1** is compound **1** and it was found to be 60% S and 40% N (combining sourness and saltiness) and is designated as being N/S; the second entry is compound **2** classified as being N since the panel found it to be 100% bitter and only 20% S; the third entry is compound **3** categorized as N/S since it shows 60% sweetness and 80% nonsweetness. Assignment to the N/S category was made if the difference between the % S and % N was 25 or less; if the difference was greater than this, then the compound was assigned to the N or S category depending on which taste predominated. Continuing in this way, all 19 compounds were assigned to one of the three categories. Finally, to prepare the data for further analysis, a "sweetness value" based on a scale running from 0 (totally N) to 100 (totally S) was devised. These weightings were calculated as follows:

$$\text{sweetness value} = (\%\ \text{S} + \%\ \text{S aftertaste}) \times 100 / \text{total taste (excluding tasteless) \%}$$

For the compounds **1**−**3** in **Table 1**, the calculations are as follows: **1**, (40 + 20) × 100/(40 + 20 + 0 + 20 + 20) = 60; **2**, (0 + 20) × 100/(0 + 0 + 100 + 0 + 20) = 16.7; **3**, (40 + 20) × 100/(40 + 20 + 60 + 0 + 20) = 42.9. Since the next compound **4** displays no sweetness, it is assigned a weighting of 0 and the last compound **19** shows only 20% sweetness and it is given a sweetness value of 100.

RESULTS AND DISCUSSION

In seeking SARs for the monosubstituted phenylsulfamates, we used a series of predictors from several sources. First, the spatial parameters *x*, *y*, and *z* measured using Corey−Pauling−Koltun (CPK) models were determined for the 19 sulfamates newly synthesized in this work. These values give the length, height, and width, respectively, of the XC_6H_4− part of $XC_6H_4NHSO_3Na$. The procedures for measuring these have been described previously (*10*), and the V_{CPK} is defined as *xyz*. These measurements were carried out on the whole sulfamate anion, $XC_6H_4NHSO_3^-$. Four additional parameters, the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies (eV), the aqueous solvation energy, E_{solv} (kcal mol^{-1}), and another measure of volume, $V_{Spartan}$, were obtained using the PC SPARTAN PRO '02 (Wave function Inc.) program (*10*). For the calculation of E_{solv}, the SM 5.4 procedure within the program was utilized. The equilibrium geometry for each molecule was obtained using the empirical AM1 module within the program. For the initial set of 63 compounds, eight of these parameters (*x*, *y*, *z*, V_{CPK}, HUMO, LUMO, E_{solv}, and $V_{Spartan}$) were generally available or were calculated.

The Hammett σ values (see **Table 3**), which measure the electronic effects of the substituents (X) in the various positions of the benzene ring, were available for most of the 83 molecules and were estimated for others by the methods now outlined. Some σ values were determined by plotting the HOMO energy and Hammett σ values of known compounds while the unknown Hammett σ value was determined by extrapolation/interpolation. In this way, σ values for *o*-CO_2Et (in compound **1**) and *o*-CO_2-*n*-Bu (in compound **2**) were obtained by interpolating their known HOMO energies against a standard curve constructed

Table 3. Parameters, Predominant Taste, and Sweetness Value for Monosubstituted Phenylsulfamates

entry	sulfamate	x^e	y^f	z^g	V_{CPK} [h]	$V_{Spartan}$ [i]	HOMO	LUMO	E_{solv}	σ^j	taste	sweetness value
20	*o*-Et[b]	7.08	4.56	8.18	264.1	212.25	–4.052	3.605	–212.85	–0.13	N	0
21	*o*-OEt[b]	7.54	4.92	7.32	271.7	224.97	–3.85	3.509	–250.214	0.02	S	71.4
22	*o*-iso-Pr[b]	7.61	6.02	7.46	341.6	229.15	–5.08	3.638	–227.865	–0.15	S	71.4
23	*o*-Me[b]	6.67	3.72	7.52	186.9	191.9	–3.951	3.656	–209.96	–0.15	N/S	50
24	*o*-NH_2 [b]	6.89	3.39	7.32	170.8	184.08	–5.067	3.609	–216.314	–0.27	N	0
25	*o*-CF_3 [b]	7.46	4.68	7.78	271.8	213.98	–4.16	3.085	–261.372	0.81	N	33.3
26	*o*-F[b]	6.17	3.37	6.94	144.5	177.24	–5.172	3.027	–258.397	0.54	N	0
27	*o*-Cl[b]	6.84	3.48	7.67	122.3	188.18	–5.278	3.302	–217.185	0.67	N	0
28	*o*-Br[b]	7.06	3.77	7.94	211.4	195.36	–6.278	3.043	–203.363	0.70	N	0
29	*o*-I[b]	7.35	4.13	8.31	252.2	197.08	–4.255	2.156	–123.532	0.63	N	0
30	*o*-OMe[b]	7.14	4.78	8.08	279.1	202.62	–3.822	3.54	–246.697	0.34	N/S	42.9
31	*o*-NO_2[b]	6.47	4.83	7.56	235.2	204.09	–4.525	2.397	–157.847	1.40	N	0
32	*o*-CN[b]	7.02	3.36	8.05	189.9	200.86	–3.9	3.4	–198.579	1.20	N	0
33	*o*-CH_2Ph[c]	6.9	7.5	10.8	559	277.15	–4.235	3.176	–175.395	0.02	N	0
34	*o*-sec-Bu[c]	5.6	9	7	353	249.53	–4.005	3.615	–209.189	–0.18	N	38.5
35	*o*-COMe[c]	7.5	5.4	8.2	332	214.31	–5.482	2.884	–250.934	0.04	N	33.3
36	*o*-OPh[c]	9.6	7.5	8.1	583	270.11	–5.3	2.55	–213.933	0.67	N/S	50
37	*o*-Ph[c]	11.2	3.4	6.3	240	256.92	–5.197	2.687	–187.877	0.74	N	0
38	*o*-CH_2OH[d]	6.8	3.8	8.8	227	200.32	–5.258	3.441	–271.099	0.04	N/S	48.8
1	*o*-CO_2Et[d]	7.33	4.59	10.18	342.5	245.66	–5.6248	2.88218	–302.583	0.80	N/S	60
2	*o*-CO_2-*n*-Bu[d]	7.57	4.46	12.42	419.33	286.53	–5.4755	2.77176	–319.524	0.74	N	16.7
39	*o*-C_4H_8NO[c]	7.7	6.5	9.2	460	264.55	–5.163	3.412	–248.052	–0.28	N	0
40	*m*-Et[b]	5.86	4.47	7.56	198.1	211.02	–5.129	3.611	–228.52	–0.05	S	80
41	*m*-OEt[b]	5.94	6.1	8.14	294.7	222.27	–5.219	3.664	–259.66	0.10	S	62.5
42	*m*-O^- [b]	5.62	3.37	6.86	130	178.93	–0.159	8.301	–340.16	–0.71	N	0
43	*m*-COMe[b]	6.33	3.74	8.57	202.7	214.9	–5.409	2.472	–255.46	0.35	N	0
44	*m*-NH_2 [b]	5.71	3.38	7.35	142.1	185.08	–5.097	3.686	–220.37	–0.07	N	0
45	*m*-I[b]	6.02	4.13	8.27	205.6	203.17	–5.42	3.042	–199.28	0.35	N	0
46	*m*-OMe[b]	5.86	4.78	7.98	223.5	201.63	–5.183	3.615	–254.6	0.12	N/S	57.1
47	*m*-NO_2 [b]	5.74	4.83	7.47	207.1	200.73	–5.868	1.799	–211.39	0.71	N	0
48	*m*-CF_3 [b]	6.24	4.88	7.97	242.9	210.63	–5.618	2.822	–369.45	0.44	N	0
49	*m*-F[b]	5.75	3.37	6.84	132.5	177.02	–5.379	3.359	–259.39	0.34	S	100
50	*m*-Cl[b]	5.7	3.48	7.65	151.7	188.28	–5.385	3.303	–221.89	0.37	S	100
51	*m*-Br[b]	5.74	3.77	7.92	171.6	194.6	–5.416	3.196	–210.46	0.39	S	100
52	*m*-CN[b]	5.79	3.36	8.11	157.8	194.16	–5.571	2.606	–183.41	0.62	S	100
53	*m*-Me[b]	5.76	3.71	7.39	157.8	190.48	–5.119	3.622	–223.23	–0.06	S	100
54	*m*-*t*-Bu[c]	6.6	6	8.5	337	249.64	–5.128	3.605	–232.77	–0.12	N	0
55	*m*-SMe[c]	5.9	4.9	7.9	229	212.55	–5.222	3.104	–217.99	0.14	N/S	60
56	*m*-NMe_2 [c]	6.6	3.8	8.6	216	225.94	–5.154	3.598	–206.84	–0.15	N	36.4
57	*m*-OPh[c]	10.6	9.4	5.6	558	269.14	–5.425	2.426	–214.62	0.25	N/S	55.6
58	*m*-OCF_3 [c]	6.8	4.7	8.7	278	221.09	–5.517	3.169	–414.19	0.36	S	75
59	*m*-CHMeOH[c]	5.9	8	6.1	288	221.35	–5.148	3.533	–273.75	0.08	S	63.6
60	*m*-CH_2OH[c]	5.8	4.3	8.1	202	201.31	–5.333	3.323	–272.98	0.10	N/S	57.1
61	H[a,b]	5.77	3.37	6.33	122.9	170.18	–5.13	3.701	–216	0.00	S	87.5
3	*m*-CO_2Me[d]	5.62	6.71	7.5	282.83	226.51	–5.6325	2.8623	–295.071	0.33	N/S	42.9
4	*m*-CO_2Et[d]	6.9	7.49	7.46	385.54	246.17	–5.6277	2.83418	–300.443	0.36	N	0
5	*m*-iso-Pr[d]	5.63	6.24	8.39	294.75	231.13	–5.1432	3.59997	–232.331	–0.07	S	83.3
6	*m*-CO_2^- [d]	6.3	3.2	7.8	157.24	203.15	–2.205	6.83	–390.12	0.01	N	0
7	*m*-SO_3^- [d]	6.6	5.1	8.4	282.74	218.92	–2.495	6.37	–440.03	0.30	N	0
8	*m*-CO_2-*t*-Bu[d]	8.5	5.86	9.42	469.21	285.68	–5.4406	2.5538	–308.105	0.35	N/S	50
62	*m*-OCH_2Ph[c]	12.1	4.4	7.6	405	290.17	–5.34	2.101	–223.92	0.04	N	0
63	*p*-Et[b]	7.5	4.54	6.34	216	211.16	–5.077	3.695	–228.593	–0.14	N/S	50
64	*p*-OEt[a,b]	8.62	3.98	6.34	217.3	222.6	–5.161	3.521	–258.944	–0.25	N/S	50
65	*p*-iso-Pr[b]	7.61	6.03	6.33	290.4	230.99	–5.092	3.686	–232.247	–0.10	N/S	42.9
66	*p*-COMe[b]	7.73	3.73	6.32	182.2	214.89	–5.512	2.625	–256.456	0.48	N	0
67	*p*-F[b]	6.36	3.37	6.29	134.7	176.88	–5.202	3.386	–258.936	0.15	N	33.3
68	*p*-Cl[b]	7.16	3.48	6.28	156.4	188.14	–5.292	3.404	–221.912	0.23	N	33.3
69	*p*-Br[b]	7.44	3.77	6.29	176.7	194.53	–5.373	3.336	–211.088	0.23	N/S	50
70	*p*-I[b]	7.94	4.13	6.4	209.7	202.93	–5.422	3.274	–199.88	0.24	N/S	50
71	*p*-OMe[b]	7.24	4.78	6.28	217.7	202.35	–5.159	3.518	–253.066	–0.21	N/S	50
72	*p*-NO_2 [b]	7.13	4.83	6.33	218.1	200.66	–6.005	2.015	–213.804	0.78	N	0
73	*p*-CN[b]	7.55	3.36	6.28	159.4	194.21	–5.581	2.78	–184.717	0.69	N/S	42.9
74	*p*-*t*-Bu[c]	7.8	6	6.3	295	249.52	–5.09	3.69	–232.912	–0.20	N	16.7
75	*p*-sec-Bu[c]	8.9	5.6	6.4	319	250.06	–5.098	3.66	–236.315	–0.12	N	12.5
76	*p*-SMe[c]	10.1	6.9	3.8	265	213.47	–5.352	3.024	–216.751	0.00	N	33.3
77	*p*-NMe_2 [c]	7.9	5.5	6.3	274	226.18	–4.969	3.625	–206.286	–0.72	S	66.7
78	*p*-C_4H_8NO[c,s]	11.2	6.3	7.6	536	264.43	–5.097	3.508	–250.252	–0.50	N/S	60
79	*p*-OCH_2Ph[c]	13.6	6.2	4.7	396	278.59	–5.198	2.442	–196.132	–0.41	N	0
80	*p*-$NO_2C_6H_4SO_2$[c]	15.8	14.2	8	1795	328.83	–6.362	0.387	–250.341	0.76	N	0
81	*p*-Me[a,c]	6.6	3.8	6.3	158	190.49	–5.046	3.705	–223.146	–0.14	N	37.5
82	*p*-*n*-Bu[b]	7.9	7.42	6.24	365.6	251.19	–5.074	3.674	–240.302	–0.19	N	0
9	*p*-CO_2Me[d]	7.17	6.4	6.15	282.21	225.71	–5.7132	2.7399	–295.924	0.43	N	0
10	*p*-CO_2Et[d]	8.54	5.86	6.24	312.28	246.1	–5.7003	2.77484	–300.979	0.41	N	0
11	*p*-$(CH_2)_2OH$[d]	8.45	4.76	6.18	248.57	222.25	–5.2104	3.54163	–280.967	–0.06	N	33.3

Table 3 (Continued)

entry	sulfamate	x^e	y^f	z^g	V_{CPK} [h]	$V_{Spartan}$ [i]	HOMO	LUMO	E_{solv}	σ^j	taste	sweetness value
12	*p*-CHMeOH[d]	7.76	5.53	6.12	264.05	221.41	–5.3009	3.47251	–275.371	–0.14	N	0
13	*p*-CO_2^- [d]	6.56	6.32	3.37	139.72	203.18	–2.1837	6.8484	–389.825	0.04	N	0
14	*p*-CO_2-*n*-Pr[d]	10.67	5.14	6.99	383.36	266.72	–5.7034	2.77489	–307.472	0.48	N	0
15	*p*-CO_2-*n*-Bu[d]	10.2	4.7	9.43	452.07	287.72	–5.5917	2.66869	–317.621	0.47	S	66.7
16	*p*-CO_2-iso-Pr[d]	7.75	5.91	6.31	289.01	265.94	–5.5812	2.70335	–308.111	0.46	S	62.5
17	*p*-CO_2-*t*-Bu[d]	9.57	6	7.42	426.06	285.78	–5.5715	2.71958	–309.356	0.46	N	0
18	*p*-CO_2-iso-Bu[d]	9.7	6.32	8.27	507.11	286.71	–5.6953	2.78331	–311.185	0.48	S	66.7
83	*p*-$SO_2NC_5H_{10}$ [c]	11.8	6.4	7.5	566	313.4	–6.021	2.198	–301.488	0.65	N	33.3
19	*p*-NEt $(CH_2)_2OH$[d]	7.83	5.35	8.13	340.57	276.27	–4.8777	3.50304	–266.076	–0.51	S	100

[a] See ref *5*. [b] See ref *9*. [c] See ref *10*. [d] Present work. [e] *x* (Å) is the length of the XC_6H_4– group in $XC_6H_4NSO_3Na$. [f] y (Å), the width of the XC_6H_4–, is offset at 90° to and shares the same plane as that of the *x*-axis. [g] *z* (Å), the height of the XC_6H_4–, is perpendicular to the plane of the *x*- and *y*-axes. [h] V_{CPK} (Å^3) is a product of *x.y.z* and represents the volume occupied by the XC_6H_4– group. [i] $V_{Spartan}$ is the volume of a sulfamate as calculated by the PC SPARTAN PRO program. [j] Hammett σ values were obtained from Hansch, C.; Leo, A.; Hoekman, D. *Exploring QSAR Hydrophobic, Electronic and Steric Constants*; American Chemical Society: Washington, DC, 1995.

from the known HOMO energies and Hammett σ values of *o*-CO_2^-, *o*-CO_2H, and *o*-CO_2Me. A number of σ values could not be readily calculated by this method. So, a Hammett σ value for a group similar in structure to the target group was used instead. The Hammett σ value for *o*-morpholino (in **39**) was not given in the literature so the corresponding Hammett σ value for an *o*-diethylamino group, $C_4H_{10}N$, was used instead. The Hammett σ value for *m*-CO_2-*t*-Bu (in **8**) was not given in the literature; however, its corresponding value was interpolated from a graph constructed by plotting the HOMO energies and the Hammett σ values of *m*-CO_2^-, *m*-CO_2H, *m*-CO_2Me, and *m*-CO_2Et. The Hammett σ value for the *m*-benzoxy group, *m*-OCH_2Ph (needed for **62**), was not available so a substitute Hammett σ value for *m*-CH_2OPh was used. The Hammett σ values for *p*-CO_2-*n*-Pr (in **14**), *p*-CO_2-*n*-Bu (in **15**), *p*-CO_2-iso-Pr (in **16**), *p*-CO_2-*t*-Bu (in **17**), and *p*-CO_2-iso-Bu (in **18**) were not available; however, they were interpolated from a graph constructed by plotting the HOMO energies and the Hammett σ values of *p*-CO_2^-, *p*-CO_2H, *p*-CO_2Me, and *p*-CO_2Et. The Hammett σ value for the *p*-piperidino-1-sulfonyl group, *p*-C_5H_{10}-NSO_2 (in **83**), was not available so the corresponding Hammett σ value for *p*-dimethylsulfonamido, *p*-$C_2H_6NSO_2$, was used. The Hammett σ value for the *p*-ethylethanolamino grouping, *p*-NEt-$(CH_2)_2OH$ (in **19**), was not available so instead the σ value for the *p*-diethylamino group, $C_4H_{10}N$, was used.

A full listing of values of the nine parameters is brought together for all available 83 phenylsulfamates in **Table 3**. In **Table 3**, compounds have been grouped in the order ortho, meta, and para, and thus, the entry numbers do not run in sequence.

This table also contains N, S, or N/S assignments and novel sweetness values for all of the compounds. These sweetness values or weightings place all of the tastants on a scale from 0 (N) to 100 (S) and are particularly useful where relative sweetness data are not available or cannot be easily measured as in the present case. The taste portfolios for the 63 compounds previously synthesized are available, and N, S, N/S, and sweetness values were determined for them by reference to the original taste panel data (*9*, *10*) using the procedures detailed and the equation given in the sensory analysis section above.

Statistical Methods. The main idea in this work is to try to classify present and future compounds in the database into three distinct classes, i.e., S, N, and predominant N/S using the nine variables listed above. A key objective is to find out which variables are best for discriminating between the categories and to use a rule based on these to classify future compounds for which the true taste is unknown. A test set of eight compounds was chosen using stratified random sampling in order to mirror the S:N:N/S ratio in the original data. Thus, the computer removed four N (**27**, **37**, **75**, and **82**), two N/S (**70** and **78**), and two S (**58** and **59**), and in the remaining 75 compounds, it left 41 N, 18 N/S, and 16 S. These 75 were used as a training set, and the eight removed compounds became a test set. The distribution of the taste categories in the test set thus reflects the distribution in the training set and also probably mirrors the situation in nature where usually about 10−20% of the compounds in a particular class of tastants tend to possess varying degrees of sweetness and the rest are principally nonsweet. Additionally, in any study of this type, one expects to see some gray areas where sweetness and nonsweetness are both to the fore. The Splus 6.1 (Insight) statistical package was used throughout for discriminant and tree analysis.

Table 4. QDA and LDA Classification of 75 Substituted Phenylsulfamates

subset	method	correctly classified % N	% N/S	% S	overall % correctly classified	cross-validation % correct
HOMO, LUMO, E_{solv}, $V_{Spartan,X,Y,Z,\sigma}$	LDA	51.2	61.1	56.3	54.7	41.3
HOMO, LUMO, E_{solv}, $V_{Spartan,X,Y,Z,\sigma}$	QDA	61.0	100.0	93.8	77.3	49.3

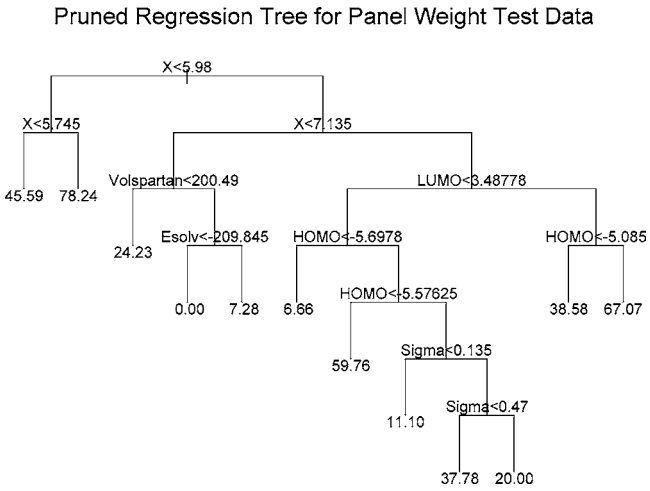

Figure 2. Pruned regression tree using a training set of 75 monosubstituted phenylsulfamates, 61 of which were correctly classified by the tree (81.3%).

Discriminant Analysis. Some success in the past has been achieved in developing SARs for heterosulfamates using dis-

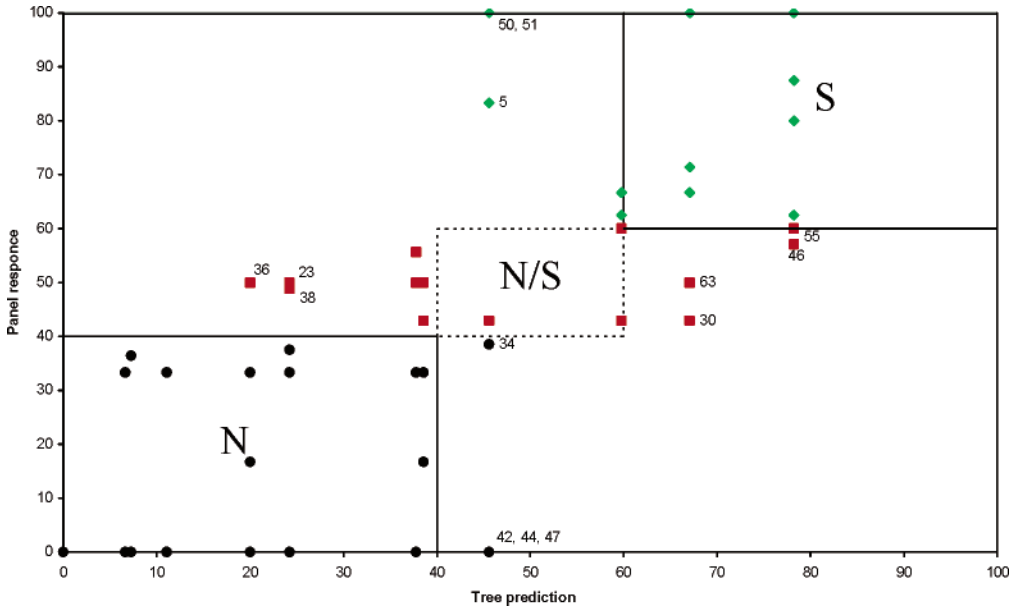

Figure 3. Scatterplot of tree predicted vs taste panel sweetness values using the training set of 75 monosubstituted phenylsulfamates—Some compounds are superimposed on others since they have the same coordinates and thus the scatterplot displays slightly less than 75 compounds: green diamond, S; red square, nonsweet/sweet (N/S); and black circle, nonsweet (N). The numbered compounds are the ones that are misclassified.

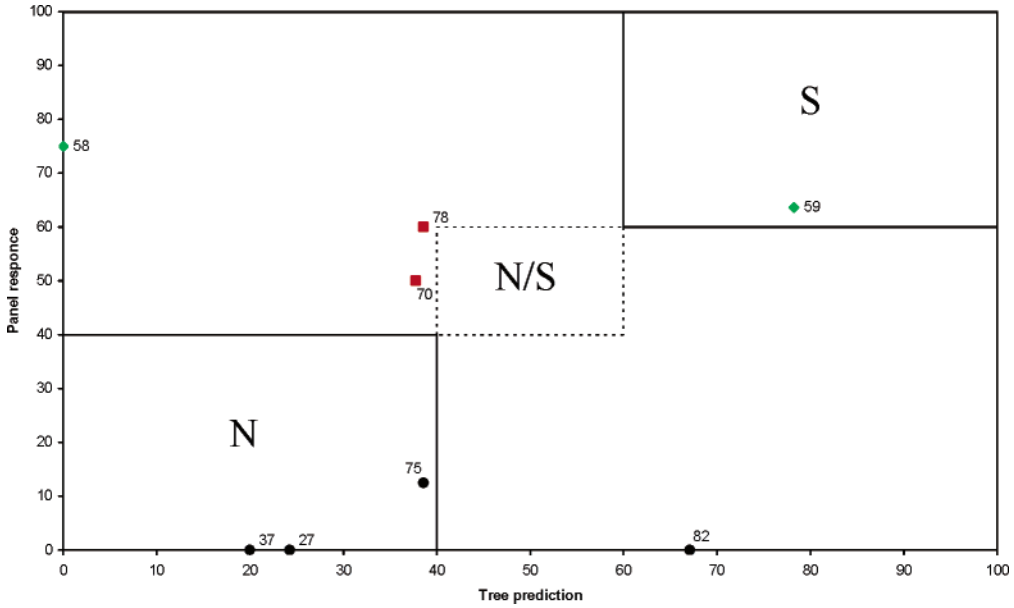

Figure 4. Scatterplot of tree predicted vs taste panel sweetness values using the test set of eight monosubstituted phenylsulfamates: green diamond, S; red square, nonsweet/sweet (N/S); and black circle, nonsweet (N).

criminant analysis (*20*) so both LDA and QDA analysis were tried in this work using the eight predictors *x*, *y*, *z*, HOMO, LUMO, E_{solv}, $V_{Spartan}$, and σ and the training set of 75 compounds. Because V_{CPK} is dependent on *x*, *y*, and *z*, it was dropped from this analysis. As seen in **Table 4**, the results are poor. In the LDA, only 54.7% of the compounds are correctly classified and cross-validation shows only 41.3% are being correctly classified. In QDA, the situation is improved with about 77% correctly classified and cross-validation gives 49.3% correctly classified. However, when the test set of eight compounds was examined, LDA could only predict four of the tastes correctly and QDA only two. One of the limitations of the LDA/QDA methods is that they do not allow us to incorporate the panel-derived sweetness values. These values can be used in the classification and regression trees (CART) analysis below.

CART. Tree-based approaches have become increasingly popular over the last 20 or so years since the publication of a useful guide on CART (*11*). These applications have arisen in many fields, but particularly, it appears in medicine, agriculture, and allied areas. This approach has been used successfully in this laboratory recently in a sensory analysis study (*12*, *13*). Furthermore, the frequent high levels of classification achieved (sometimes 90+%) with CART analysis (*21*, *22*) and reliable predictability (*22*) make it attractive to use. Tree-based approaches have many advantages over discriminant analysis as they may be easier to interpret, are nonparametric, and make no assumptions regarding the covariance structure in the two groups.

In essence, the CART procedure is used to gain a better understanding of the dependence of the response variables on the structure of the relationships of potential explanatory

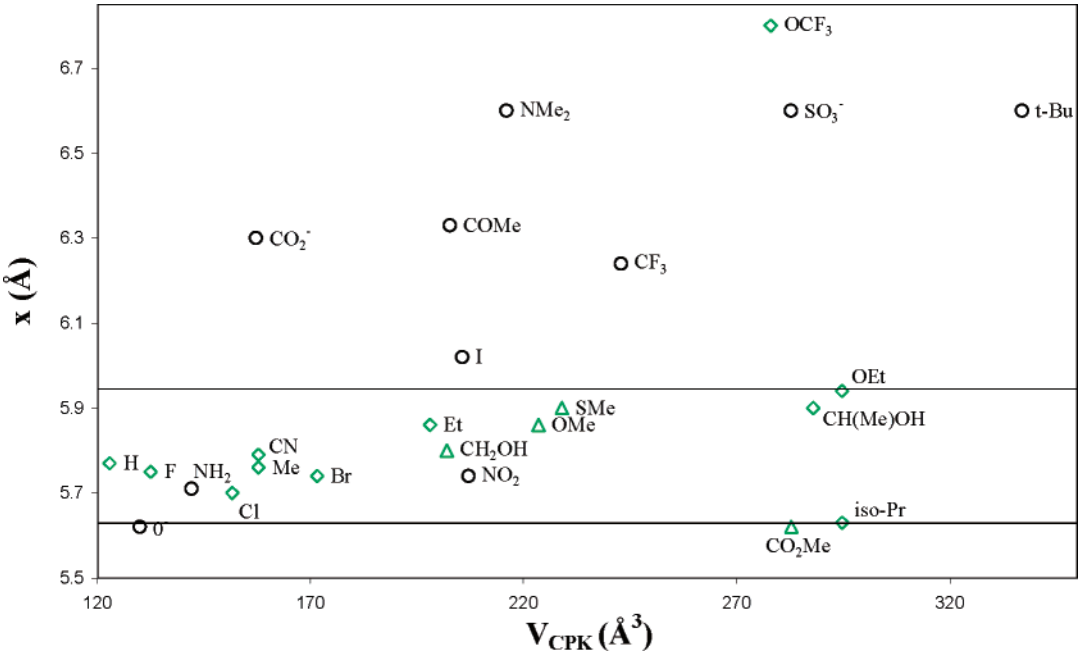

Figure 5. Plot of *x* (Å) vs V_{CPK} (Å^3) for *meta*-phenylsulfamates for which taste data are available as follows: Green diamond, exclusive or predominant sweetness; green triangle, reduced sweetness; and black circle, nonsweet. The following meta compounds, *x* and V_{CPK} and taste symbols in brackets, were omitted form this plot as they would have made it inordinately large: **57** (black circle, 10.6, 558), **62** (black circle, 12.1, 405), **4** (black circle, 6.9, 385.54), and **8** (green triangle, 8.5, 469.21).

Table 5. Taste Predictions on Unsynthesized *meta*-Substituted Phenylsulfamates

sulfamate (*m*-$XC_6H_4NHSO_3Na$) X =	*x*/V_{CPK} [a]	QDA[a]	LDA[a]	x[b]	$V_{Spartan}$ [b]	E_{solv} [b]	taste	sweetness value
n-Pr	N	N	N	6.3	231.59	−235.04	N	7.28
n-Bu	N	N	N	6.8	252.14	−240.79	N	7.28
sec-Bu	N	N	N	7.0	250.95	−237.67	N	7.28
$(CH_2)_2OH$	N	S	N	6.3	221.92	−280.42	N	7.28
CH_2F	S	S	S	5.8	197.18	−269.39	S	78.24
CH_2Cl	S	S	S	5.8	208.75	−230.72	S	78.24

[a] See ref *10*. [b] These values were calculated for each sulfamate using CPK models to obtain"*x*" and V_{CPK}, and the Spartan program was used to obtain $V_{Spartan}$ and E_{solv}.

variables (e.g., *x*, *z*, HOMO) and their combinations, together with their high-order interactions. If the response variable is binary, the procedure produces a classification tree while a regression tree is produced if the response variable is continuous. The first option therefore that we tried was to build a classification tree (using cross-validation) employing the compound designations S, N, and N/S. The tree had an overall misclassification rate of 23%, or 17 compounds of the 75 were misclassified, and we felt that it might be possible to do better than this. The second alternative method was to use the sweetness values for the 75 compounds calculated from the experimental panel tasting and six of the nine available predictors (omitting *y*, *z*, and V_{CPK}) because these six were found by the program to be the most significant to construct a regression tree (see data in **Table 3**). The best tree was then selected using 10-fold cross-validation, and its performance was assessed using the Pearson correlation coefficient, which was 0.792 with a *P* value of <0.0001, which suggests a real correlation in the population of these compounds and provides evidence of the predictive power of the tree. The pruned classification tree shown in **Figure 2** was found. The way this works is explained below in conjunction with **Figures 3** and **4**.

Tree results can also be presented in another way as a Scatterplot of taste panel vs tree predicted sweetness values in **Figure 3**. The program has decided that the total number of misclassified compounds is 14, and these are indicated on **Figure 3** (with their entry numbers from **Table 3**). In the Scatterplot, some compounds are superimposed on others because they have the same coordinates, and thus, the plot appears to display somewhat less than 75 compounds. The S (green diamond) and N (black circle) misclassified compounds are those that lie outside the areas designated S and N, respectively. Three S (**5**, **50**, and **51**) and four N (**34**, **42**, **44**, and **47**) compounds have been misclassified. Seven of the N/S (red square) compounds (**23**, **30**, **36**, **38**, **46**, **55**, and **63**) are deemed to be misclassified, and this is because they lie well outside the N/S designated area in **Figure 3**; four others (**57**, **64**, **65**, and **71**) are just very slightly to the left of the N/S area and cannot be regarded as being misclassified. Thus, this classification tree misclassifies 14 of the 75 compounds in the data set (18.6%) or it has assigned approximately four of out five compounds to the correct category, i.e., S, N, or N/S. Finally, for the test set of eight compounds, the Scatterplot shown in **Figure 4** was obtained. Two compounds, **58** (green diamond) and **82** (black circle), are obviously misclassified, and the other six have their tastes correctly predicted.

The significant correlation coefficient (r = 0.792) and reliability found with this tree, the low misclassification within

the training set, the lack of underlying parametric assumptions unlike LDA and QDA, and the good predictability of the test set provide strong evidence for the superiority of the tree-based method over discriminant analysis in the analysis of this present data.

The use of the tree in **Figure 2** can be illustrated as follows: in **Figure 4**, **59** is in the S area and if one looks at its parameters in **Table 3** the tree can be used to show that it is predicted to be S. Thus, its *x* value is 5.9, which is <5.98 as required by the tree so one follows to the left, at the next node *x* should be less than 5.745, which is not the case so one goes to the right giving a predicted sweetness value of 78.24 implying definite sweetness, which is what the panel found with their figure of 63.6. Conversely, for a misclassified compound in **Figure 4** such as the nonsweet compound **82**, the panel sweetness value is 0 and *x* for this compound is 7.9, which is not <5.98 as required by the tree so one follows to the right, at the next node $x < 7.135$ is required so one moves on to the right again, now a LUMO < 3.48778 is required but **82** has a LUMO value of 3.674 so one moves on to the right once again, at the next node the tree requires HOMO < −5.085 for a fit and the HOMO value for **82** is −5.074 so one again moves to the right indicating a sweetness value of 67.07 in clear disagreement with the panel finding of 0 so this compound is deemed to be misclassified.

Some years ago, we found that plots of the CPK *x* values vs V_{CPK} for the *meta*-phenylsulfamates tended to cluster those sulfamates that had a strong sweetness in a rather narrow area more or less defined by $\sim 5.65 \leq x \leq \sim 5.95$ (*2*−*4*). With the synthesis of several new meta compounds in this current work, it has been possible to confirm the lower limit of 5.65 for *x* in the x/V_{CPK} plot, and the latest version of this is shown in **Figure 5**. For this plot, those compounds with a sweetness value >60 are classed as being exclusively or predominantly sweet (green diamond), those with a value in the range of 40−60 are classed as having reduced sweetness (green triangle), and those with a value <40 are taken as being nonsweet (black circle). This simple plot using the subset *x* and V_{CPK} performs well and correctly classifies 88% (14/16) S and 77% (10/13) N meta compounds misclassifying only 17% (5/29) of the total set of 29 meta compounds. Furthermore, because it only uses two spatial predictors, it is easy to give it a physical interpretation and it suggests that the S meta compounds have to access at some point a receptor site one of whose dimensions is in the range of 5.65 to ∼5.95 Å.

Finally, measurements with CPK models show that the best chance of obtaining a meta compound with good sweetness is to choose small substituent X groups in the phenylsulfamate such as *n*-Pr−, *n*-Bu-, sec-Bu−, $HO(CH_2)_2-$, FCH_2-, and $ClCH_2-$, all of which will fall in the desired area most likely to give sweetness. So far, none of these can be readily synthesized since none of the required starting anilines are available, but one can use the various SARs developed to predict their tastes. In the second column of **Table 5**, the taste predictions from the x/V_{CPK} plot are given for these six unknown compounds, and the third and fourth columns contain predictions made using previous QDA and LDA SARs developed from the database of 63 compounds. The variables needed for this analysis are available in an earlier paper (*10*). Last, using the tree developed here (**Figure 2**) and the *x*, $V_{Spartan}$, and E_{solv} values given in columns five, six, and seven, sweetness values and hence taste predictions can be obtained for these six compounds, and they are shown in columns eight and nine. The results are quite striking in that the various SARs all more or less predict the same tastes for each of the compounds signaling that these predictions are probably valid. Furthermore, the tree analysis tells us more in that the four N compounds have predicted sweetness values (7.28), which are very close to the defined zero for nonsweetness, and the two S compounds have predicted values (78.24) that are high on the sweetness scale developed in this paper.

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