[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HOWARD UNIVERSITY]

Dissociation Constants of Some Sweet and Tasteless Isomeric *m*-Nitroanilines[1]

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The dissociation constants of some 2- and 4-substituted 5-nitroanilines were determined spectrophotometrically. Their relative basicities were also determined by potentiometric titration in glacial acetic acid. A good linear relationship was found between the potentials at half-neutralization in acetic acid and the dissociation constants in aqueous solutions.

There is a marked resemblance of the spectra of the substituted *m*-nitroanilines in acid to the spectra of the correspondingly substituted nitrobenzenes in ethanol. This relationship indicates that the effects of electronegativity, resonance, steric requirements, etc., which control the spectra of the substituted nitrobenzenes have the same total effects on the spectra of the *m*-nitroanilines in acid solution.

No simple correlation was found or anticipated between the tastes and base strengths of these substituted *m*-nitroanilines.

It is well known that 2-substituted 5-nitroanilines are intensely sweet while the isomeric 4-substituted 5-nitroanilines are bitter or tasteless (*cf.* Table IV). It is an intriguing challenge to be able to account for this sharp difference in taste in terms of the chemical or physical properties of the isomeric pairs. In spite of the attempts of many chemists, no reliable, widely applicable correlation has been found between the tastes and molecular properties of substances. It appears that chemoreception depends upon several properties. As a part of a program to find a set of molecular properties which may be used as a parameter for predicting the tastes of substances, attention has been given to the electron distributions in these *m*-nitroanilines. This paper reports their relative base strengths.

The pK_a values for the substituted *m*-nitroanilines were determined spectrophotometrically by the method of Flexser, Hammett, and Dingwall.[3] The order of relative base strengths was also established by potentiometric perchloric acid titration in glacial acetic acid.[4] Good agreement was found for the relative basicities of the *m*-nitroanilines by the two methods.

EXPERIMENTAL

Materials. With the exception of the 2-fluoro- and the 4-fluoro-5-nitroanilines, the preparation of all of the substituted *m*-nitroanilines has been described.[5]

Twenty-three grams of *o*-fluoroaniline, b.p. 82°/41 mm., was dissolved in 100 ml. of concd. sulfuric acid and nitrated with 9.7 ml. of fuming nitric acid dissolved in 100 ml. of concd. sulfuric acid at −10°.[6] The crude product was recrystallized from aqueous alcohol, m.p. 101–102° (lit.,[7] m.p. 101.5°); acetanilide, m.p. 179–180° (lit.,[8] m.p. 178.4°).

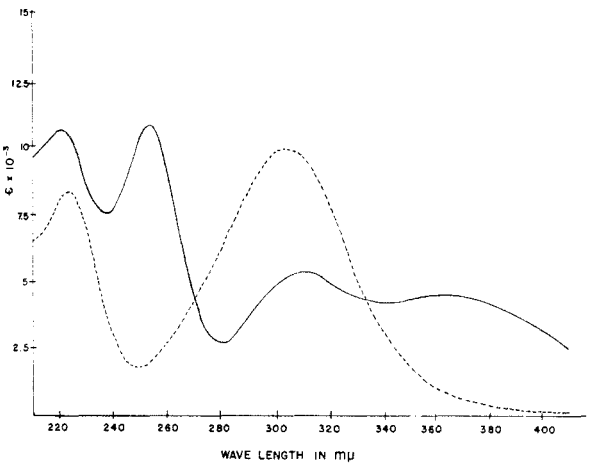


Fig. 1. Absorption spectrum of 2-methoxy-5-nitroaniline in 0.1*N* sodium hydroxide ———; and in 0.1*N* sulfuric acid .....

Spectroscopic measurements. Stock solutions for each base were prepared by dissolving a weighed amount of base (about 0.1 g. except in the case of the slightly soluble 2-bromo-5-nitroaniline, where 0.05 g. was used) in 1 l. of carbon dioxide-free distilled water. All of the other required solutions were prepared from distilled water and analytical grade reagents.

The spectra of the bases were determined with a Beckman DU line-powered spectrophotometer with the same pair of matched 1 cm. silica cells. The solutions in the cells were maintained at 25.0 ± 0.1° by means of thermospacers. Spectra of the bases were measured in approximately 0.1*N* sodium hydroxide, 0.1*N*, 1.0*N*, 2.5*N*, or 5.0*N* sulfuric acid, and buffer solutions containing various proportions of 0.02*N* sodium acetate and 0.02*N* hydrochloric acid. These solutions were prepared by diluting an aliquot volume of stock base solution with the appropriate volume of acid, base, or buffer solution. In each instance, blank cell solutions were prepared in the same manner with distilled water in place of the stock base solution. The spectrum of each base was measured twice in acid and in alkaline solution with fresh solutions each time. The averaged values of the extinction coefficients so obtained were used in the calculations. Beer's law was tested for two compounds of each

(1) Number IV in a program of physicochemical studies of the sense of taste. No. III, *Nature*, in press.

(2) Taken in part from the Ph.D. thesis of A.R.L., Howard University, 1959.

(3) L. A. Flexser, L. P. Hammett, and A. Dingwall, *J. Amer. Chem. Soc.*, **57**, 2103 (1932).

(4) J. S. Fritz, *Acid-Base Titrations in Non-aqueous Solvents*, The G. Frederick Smith Chemical Co., Columbus, Ohio, 1952, p. 9.

(5) A. R. Lawrence, M.S. Thesis, Howard University, 1957.

(6) F. D. Chattaway, K. J. P. Orton, and R. C. T. Evans, *Ber.*, **33**, 3062 (1900).

(7) A. F. Holleman and J. W. Beekman, *Rec. trav. chim.*, **23**, 237 (1904).

(8) F. Swarts, *Rec. trav. chim.*, **35**, 142 (1916).

type in acid, base, and buffer solution and was found to hold in each case.

Typical spectra are shown in Fig. 1 for 2-methoxy-5-nitroaniline in alkali and in acid.

pH Measurements. The *p*H measurements were made with a Beckman Model G *p*H meter standardized immediately before use at *p*H's of 4.01 and 1.08 by the use of 0.05*M* potassium acid phthalate[9] and 0.1*N* hydrochloric acid, respectively.

Potentiometric titrations in glacial acetic acid.[4] Reagent grade glacial acetic acid[10] was refluxed with 3–5% by weight of potassium permanganate for 2–6 hr., distilled from the reaction mixture, and the fraction boiling at 117–118° collected. Traces of water were removed from this fraction with two to three times the amount of triacetyl borate required for reaction of the estimated amount of water present. The material was then redistilled and the fraction boiling at 117–118° collected. This acetic acid was used throughout the study.

A 0.05*N* perchloric acid solution was prepared by dissolving 4.16 ml. of 72% perchloric acid in 200 to 300 ml. of acetic acid and adding 9.3 ml. of freshly distilled acetic anhydride. This mixture was then made up to 1 l. with acetic acid, allowed to stand overnight, and standardized against potassium acid phthalate dissolved in acetic acid. Solutions of the anilines in acetic acid were prepared by dissolving weighed samples of the bases (about 0.03 g.) in 50.0 ml. each of acetic acid. All of the bases used were readily soluble in this medium.

Titrations[4] were performed by adding perchloric acid solution to the solution of the base in increments of 0.2 ml. except near the equivalence point where 0.1 ml. increments were used. A period of 2 min. was allowed to elapse between each addition and subsequent reading to permit the solution to reach equilibrium. A 5-ml. microburet graduated in 0.02-ml. intervals was used. The cell was a 100-ml. Pyrex glass beaker and stirring was achieved by means of a stream of nitrogen gas bubbling through the solution.[11] Trace amounts of oxygen were removed from the nitrogen by passing it through an alkaline solution of pyrogallol. The cell was immersed in a thermostated water bath maintained at 25.10 ± 0.01°.

The millivolt scale of a Beckman Model G *p*H meter was used to measure the potentials during the titrations. A glass electrode, Beckman Model 40498, was used as the indicator electrode. The reference electrode was a silver–silver chloride electrode, Beckman Model 39261, which was prepared by the electrolysis of a saturated potassium chloride solution. An isolation cell[12] containing acetic acid saturated with potassium chloride connected the silver–silver chloride electrode with the main body of the solution. The equivalence points were determined from a large scale plot of $\Delta MV/\Delta V$ against ΔV, where ΔMV is the change in the millivolt reading and ΔV is the corresponding volume of acid added.

All apparatus was placed upon a large sheet of aluminum foil to provide adequate grounding.[13] Particular care was taken to ground the case of the *p*H meter and the thermostat bath.

A typical titration curve is shown in Fig. 2 for 2-methyl-5-nitroaniline, and the millivolt readings at the half-neutralization points for all compounds are given in Table I.

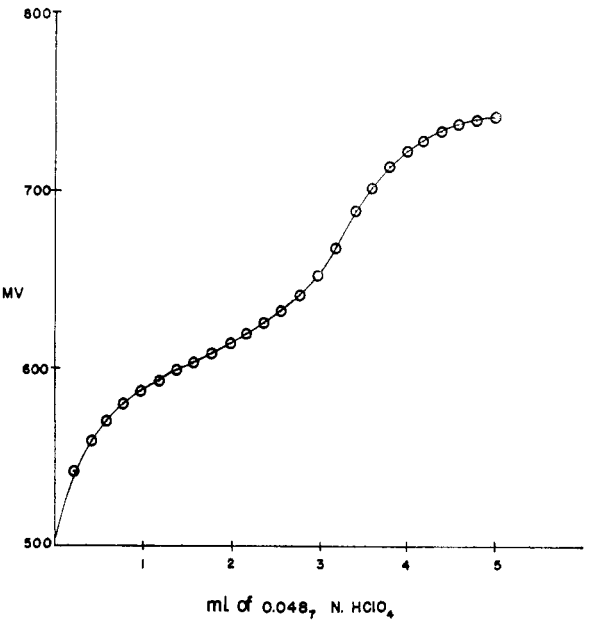


Fig. 2. Graph of the potentiometric titration of 2-methyl-5-nitroaniline in glacial acetic acid

TABLE I

MILLIVOLT READINGS AT HALF NEUTRALIZATION POINTS FOR SUBSTITUTED 5-NITROANILINES

Substituent	$MV_{1/2}$	Mean $MV_{1/2}$
2-CH_3O	601	601
	601	
2-CH_3	605	605
	605	
2-F	700	701
	702	
2-Cl	736	735
	734	
2-Br	739	739
	740	
4-CH_3O	524	523
	523	
4-CH_3	564	565
	565	
4-F	600	602
	603	
4-Cl	641	642
	643	
	642	
4-Br	641	642
	643	
p-Toluidine	502	503
	503	
p-Anisidine	495	500
	500	
1,3-Diphenylguanidine	456	456
	456	

Computation of dissociation constants. The *p*Ka's were calculated from Equation 1[3]

$$pK_a = pH + \log \frac{(e_2 - e_3)}{(e_1 - e_2)} + \log f_{BH^+} \qquad (1)$$

where e_1 and e_3 are the molar absorbancy indexes at a given wave length of the amine in acid and in base; e_2 is the apparent molar absorbancy index in the buffer; and f_{BH^+} is the activity coefficient of the protonated base. The activity

(9) V. E. Bower and R. G. Bates, *J. Research Nat. Bur. Standards*, 59, 261 (1957).

(10) L. F. Fieser, *Experiments in Organic Chemistry*, 3rd ed., D. C. Heath Co., Boston, 1957, p. 281.

(11) R. P. Linstead, J. A. Elvidge, M. Whalley, *A Course in Modern Techniques of Organic Chemistry*, Butterworths Scientific Publications, London, 1955, p. 153.

(12) R. A. Glenn, *Anal. Chem.*, 25, 1916 (1953).

(13) W. M. Clark, *The Determination of Hydrogen Ions*, 3rd ed., The Williams and Wilkins Co., 1928, p. 357.

coefficient of the nonionic free base is taken as unity.[14] The activity of the protonated base was calculated from the approximate Debye-Hückel relationship,

$$\log f_{BH^+} = -\frac{0.509z^2\sqrt{\mu}}{(1 + a\sqrt{\mu})}$$

where z is the valence of the protonated base, μ is the ionic strength of the medium, and the ionic radius a was given a value of 5.[14]

For the very weak bases 2-fluoro-, 2-chloro-, 2-bromo-, 4-chloro-, and 4-bromo-5-nitroanilines, Equation 2 was used,

$$pK_a = H_0 + \log\frac{(e_2 - e_3)}{e_1 - e_2} \qquad (2)$$

where H_0 is Hammett's acidity function,[15] and the e's have the same meaning as in equation (1). Values of H_0 have been determined by a number of investigators,[16] but for this study the data of Hammett and Deyrup were used. The H_0 values at the particular normalities of interest were determined from a large-scale plot of H_0 *vs.* normality. The normalities of the acid solutions were established by titration against standardized base.

The dissociation constants so determined are given in Table II. It is comforting to observe the good agreement of the pKa's for p-toluidine and p-anisidine determined here spectrophotometrically (5.05 and 5.26, respectively) with those determined by other methods (5.07 and 5.29, respectively).[17]

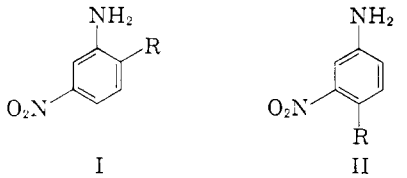
TABLE II

DISSOCIATION CONSTANTS OF 2- AND 4-SUBSTITUTED 5-NITROANILINES FROM SPECTROSCOPIC MEASUREMENTS

Substituent	pK_a	Substituent	pK_a
2-CH_3O	2.49 ± 0.01	4-CH_3O	3.36 ± 0.02
2-CH_3	2.30 ± 0.02	4-CH_3	2.86 ± 0.01
2-F	1.09 ± 0.02	4-F	2.36 ± 0.004
2-Cl	0.64 ± 0.02	4-Cl	1.93 ± 0.02
2-Br	0.52 ± 0.02	4-Br	1.80 ± 0.01
p-Toluidine	5.05 ± 0.01		
p-Anisidine	5.26 ± 0.04		

RESULTS AND DISCUSSION

If a nitro group with its strong electron-withdrawing effect is introduced into the ring of o- or p-substituted anilines to give I or II, one



I II

might expect 1) the base strengths to decrease, and 2) little or no change in the relative order of base strengths of the monosubstituted anilines.

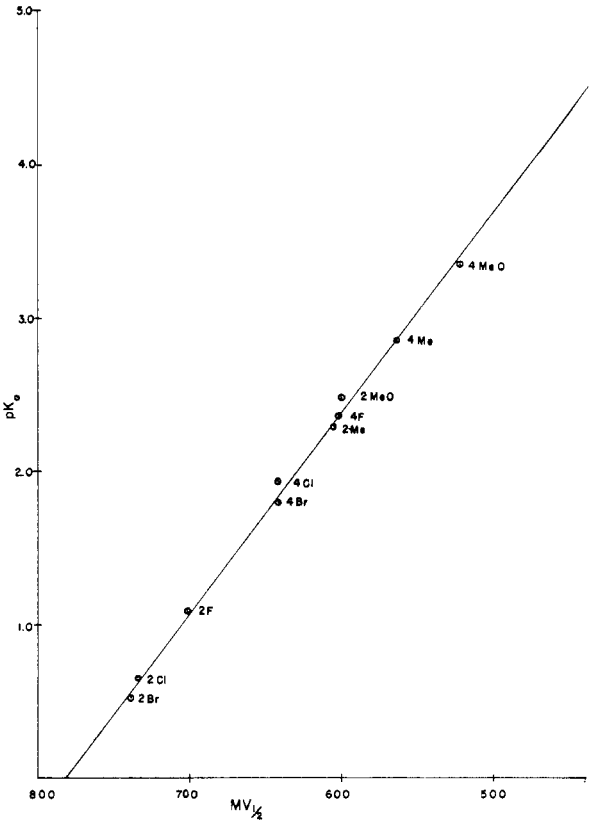


Fig. 3. Plot of pK_a values measured in water *vs.* potentials at half neutralization measured in glacial acetic acid for substituted 5-nitroanilines

Examination of the data of Table III reveals that these expectations are met. This approximate additivity of the effects of the nitro and other substituents indicates that the relative base strengths of the 2- and 4-substituted 5-nitroanilines can be explained on the same bases as are monosubstituted anilines in terms of resonance, steric requirements, inductive effects, and hydrogen bonding.[18]

TABLE III

pK_a VALUES FOR SOME SUBSTITUTED ANILINES

Substituent	pK_a of Aniline	pK_a of 5-Nitroaniline	ΔpK_a
H	4.58	2.54	2.04
2-CH_3O	4.49	2.49	2.00
2-CH_3	4.38	2.30	2.08
2-F	2.96	1.09	1.87
2-Cl	2.62	0.64	1.98
2-Br	2.60	0.52	2.08
4-CH_3O	5.26	3.36	1.90
4-CH_3	5.05	2.86	2.19
4-F	4.51	2.36	2.15
4-Cl	4.00	1.94	2.06
4-Br	3.91	1.80	2.11
		Mean ΔpK_a = 2.05	

(14) S. Glasstone, *The Elements of Physical Chemistry*, D. Van Nostrand Co., Inc., New York, N. Y., 1946, p. 486.

(15) L. P. Hammett, *Physical Organic Chemistry*, McGraw-Hill, New York, 1940, p. 267.

(16) L. P. Hammett and A. J. Deyrup, *J. Am. Chem. Soc.*, **57**, 2721 (1932); M. A. Paul and F. A. Long, *Chem. Rev.*, **57**, 1 (1957).

(17) *Cf.* H. C. Brown, D. H. McDaniel, and O. Hafliger, in *Determination of Organic Structures by Physical Methods*, edited by E. A. Braude and F. C. Nachod, Academic Press, N. Y., 1955, p. 590.

(18) H. C. Brown, D. H. McDaniel, and O. Hafliger, "Dissociation Constants" in *Determination of Org. Structures by Physical Methods*, edited by F. C. Nachod and E. A. Braude, Academic Press, Inc., N. Y., 1955, chap. 14; B. M. Wepster, *Rec. trav. chim.*, **71**, 1159 (1952).

Fig. 3 is a plot of the pK_a values measured in water for the substituted 5-nitroanilines against the potentials at half-neutralization measured in acetic acid. The equation for this line, computed by the method of least squares, is

$$pK_a = 10.336 - 0.0132\ MV_{1/2}.$$

Such a linear relationship is not unusual[19] and it provides strong support to the order found for the base strengths measured in acetic acid. Also, it indicates that there is not a significant difference in solvation effects for these two solvents for this series of compounds.

The pK_a values for the 4-substituted 5-nitroanilines are plotted against the respective Hammett *sigma* constants in Fig. 4. A reasonably linear relationship exists. The point for the 4-methyl derivative lies slightly above the line. This deviation could be attributed to an effect upon the resonance of the nitro group by the adjacent methyl group, resulting in a decrease of the base strength of the aniline.

In Table IV are listed the pK_a values and the relative sweetnesses of the compounds. Except for the 2-fluoro derivative, whose sweetness was measured by a different group of workers, the relative sweetnesses of the sweet nitroanilines parallel the order of their decreasing basicities. However, no rigid correlation can be made between taste and this single property. Nevertheless, it is hoped that the basicity may later be combined with other properties to give a function which may be used as a single parameter for interpreting or predicting the tastes of compounds.

TABLE IV

pK_a AND RELATIVE SWEETNESSES OF SUBSTITUTED 5-NITROANILINES

Substituent	pK_a	Relative sweetness (Sucrose = 1)
H	4.58	40[20]
2-CH_3O	2.49	167
2-CH_3	2.30	298
2-F	1.09	40[21]
2-Cl	0.64	375
2-Br	0.52	714
4-CH_3O	3.36	tasteless
4-CH_3	2.86	tasteless
4-F	2.36	tasteless
4-Cl	1.93	tasteless
4-Br	1.80	tasteless

It is noteworthy that the spectra of the substituted *m*-nitroanilines in acid solution show a great similarity to the spectra of the corresponding 2-

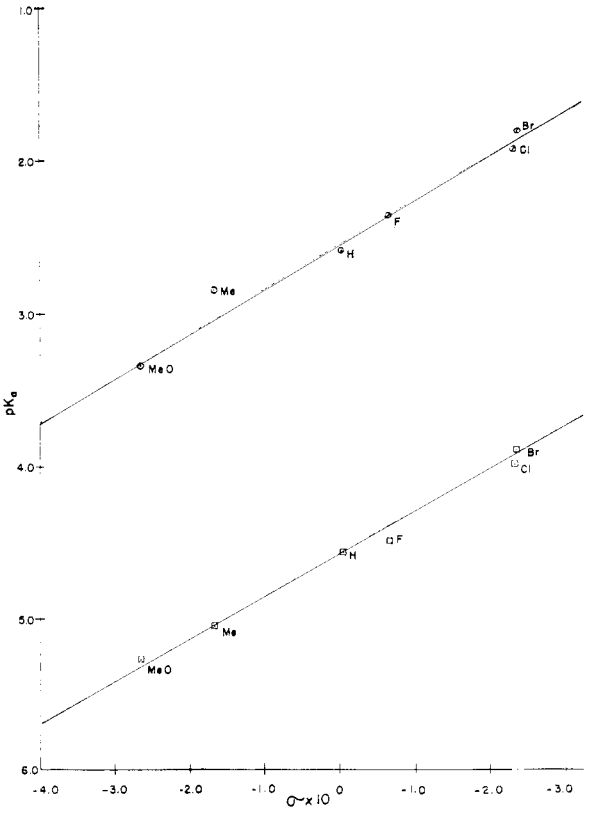


Fig. 4. Plot of pK_a values for 4-substituted 5-nitroanilines (⊙) and *p*-substituted anilines (⊡) against Hammett *sigma* constants

and 4-substituted nitrobenzenes measured in ethanol. This resemblance is not surprising, because in acid solution the lone pair electrons on the amino nitrogen is no longer available for electronic interaction with the nitrophenyl group and, accordingly,

TABLE V

ABSORBANCY INDEXES FOR SUBSTITUTED 5-NITROANILINES IN ACID AND SUBSTITUTED NITROBENZENES IN ETHANOL AT THEIR λ_{max}'S

Substituent	2-Substituted 5-Nitroaniline		*p*-Substituted Nitrobenzenes[22]	
	λ	a_M	λ	a_M
H	256 mμ	7500	257 mμ	8100
F	260	7200	266	7900
CH_3	272	8950	275	10400[23]
Cl	270	9650	272	10000
CH_3O	305	9950	306	10780
Br	275	10300	276	11100
	4-Substituted 5-Nitroaniline		*o*-Substituted Nitrobenzene[22]	
H	256	7500	257	8100
F	248	7000	250	6900
CH_3	255	5350	257	5400
Cl	250	4050	252	3500
CH_3O	259	3850	258.5	3450
Br	250*	3400	255	3000

* = shoulder

(19) N. F. Hall, *J. Am. Chem. Soc.*, **52**, 5115 (1930); N. K. Hall, *J. Phys. Chem.* **60**, 63 (1956); S. Viebel, B. J. Nielsen, and S. Refn, *Acta Chem. Scand.* **6**, 1066 (1952).

(20) J. J. Blanksma and P. W. M. van der Weyden, *Rec. trav. chim.* **59**, 629 (1940).

(21) J. J. Blanksma, W. J. van den Broek, and D. Hoegen, *Rec. trav. chim.* **65**, 329 (1946).

(22) W. F. Forbes, *Can. J. Chem.*, **36**, 1350 (1958).

(23) L. L. Green, M.S. Thesis, Howard University, 1959.

the spectra similate those for corresponding compounds not containing the $^{+}NH_3$ group. Furthermore, it is observed that the order of decreasing absorbancy indexes of the λ_{max}'s of the *p*-substituted nitrobenzenes is the same as that of the 2-substituted 5-nitroanilinium ions, where the substituents are *para* to the nitro group, and the order for the *o*-substituted nitrobenzenes is identical with that of the 4-substituted 5-nitroanilinium ions. The values are given in Table V.

WASHINGTON 1, D. C.