in the same manner as that given for the *Helicostylum* fermentation above. The semicrystalline residue obtained upon evaporation of the extraction solvent showed by papergram[12] one major product which was more polar than I and differed in mobility from that produced by the *Helicostylum*. The residue was triturated with acetone and filtered to give 610 mg. of crystals. These were dissolved in 65 ml. of hot acetone, filtered through a bed of Magnesol,[14] and concentrated to crystallization; yield of V, 360 mg., m.p. 252–254°, $[\alpha]_D^{23}$ +107° (c 0.86, dioxane), $\lambda_{max}^{alc.}$ 233 mμ (13,850), λ_{max}^{Nujol} 3500, 3410, 1697, 1667, and 1617 cm.$^{-1}$

Anal. Calcd. for $C_{21}H_{28}O_4$ (344.43): C, 73.22; H, 8.19. Found: C, 73.53; H, 8.47.

The mother liquors were combined, concentrated to remove the acetone, and chromatographed on 300 g. of Florisil,[13] taking five 200-ml. fractions of each of the following solvents: methylene chloride, 12%, 20%, 30%, and 50% acetone in petroleum ether (b.p. 62–72°). Fractions 17–21 (1.21 g.) were combined and crystallized from acetone to give 500 mg. of V, melting at 243–247°. Recrystallization of this material from acetone gave 400 mg. with m.p. 246–250°.

6β,21-Dihydroxypregna-4,17(20)-diene-3,11-dione diacetate (VI). A solution of 400 mg. of V in 1 ml. of pyridine and 1 ml. of acetic anhydride was allowed to react overnight at room temperature. Addition of ice and water caused crystallization. The mixture was filtered and the solid dried, yield 440 mg., m.p. 129–135°. Recrystallization from ethyl acetate (1 ml.) and petroleum ether (b.p. 62–72°) (2 ml.) gave 270 mg., m.p. 136–138.5°, $[\alpha]_D$ +66° (c 0.72, dioxane), $\lambda_{max}^{alc.}$ 231 mμ (13,250), λ_{max}^{Nujol} 1732, 1700, 1676, 1617, 1250, and 1230 cm.$^{-1}$

Anal. Calcd. for $C_{25}H_{32}O_6$: C, 70.07; H, 7.53. Found: C, 69.70; H, 7.90.

6β-Hydroxycortisone 6,21-diacetate (VII). 6β,21-Dihydroxypregna-4,17(20)-diene-3,11-dione diacetate (580 mg.) was oxidized with phenyliodosoacetate in the presence of osmium tetroxide to yield 400 mg. of 6β-hydroxycortisone 6,21-diacetate by a procedure identical with that given above for the preparation of 9α-hydroxycortisone acetate. Recrystallization from acetone–petroleum ether (b.p. 62–72°) gave 260 mg., m.p. 236–238.5° (reported[12] 225–233°), $[\alpha]_D^{23}$ +124° (c 0.33, dioxane), λ_{max}^{Nujol} 3540, 1735, 1712, 1697, 1675, 1620, and 1240 cm.$^{-1}$ The ultraviolet spectrum in sulfuric acid agrees with that given by Burstein and Dorfman.[9] A sample obtained by chromic acid oxidation of 6β-hydroxyhydrocortisone 6,21-diacetate, prepared *via* the 5,6-oxide by G. B. Spero of these laboratories, melted at 231–232°, $[\alpha]_D^{23}$ +127° (dioxane) and was identical with our sample by infrared and paper chromatographic analysis.

Acknowledgment. The authors are indebted to A. Koning for technical assistance; to L. M. Reineke and group for papergram analysis; to Dr. J. L. Johnson, J. E. Stafford, and Mrs. G. S. Fonken for infrared and ultraviolet absorption studies; and to W. A. Struck and associates for analytical data.

KALAMAZOO, MICH.

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF HOWARD UNIVERSITY]

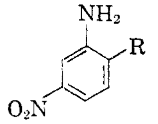
Ultraviolet Spectroscopic Studies of Some Sweet and Nonsweet Isomeric m-Nitroanilines[1]

LLOYD N. FERGUSON AND LILLIAN GREEN CHILDERS[2]

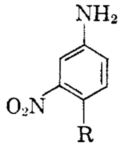
Received March 7, 1960

The ultraviolet spectra of some 2- and 4-substituted 5-nitroanilines and of their respective disubstituted constituent compounds have been measured in 95% alcohol. Their absorption bands have been classified and discussed in terms of electronic transitions within the molecules. Certain solvent and salt effects are also reported. Taste-structure relationships which possibly might be drawn from the spectra are pointed out.

It has been observed[3] that many 2-substituted 5-nitroanilines (I) are intensely sweet, whereas the isomeric 4-substituted-5-nitroanilines (II) are bitter or tasteless.



I



II

Relative sweetness[4]
(Sucrose = 1)

R	I	II
$-OCH_3$	167	Tasteless
$-CH_3$	298	Tasteless
$-Br$	715	Tasteless

This striking difference in taste of isomeric pairs has aroused our interest in their physicochemical properties and the present paper reports some of their spectroscopic properties. There is even a noticeable difference in the color of the isomers. All of the compounds are yellow to red but the sweet isomer of each couple is lighter than the nonsweet member.

Two types of spectroscopic studies were made in this investigation. The first was to measure the complete ultraviolet spectra down to 210 mμ. It was hoped, through an interpretation of the spectra, to learn if there is any significant difference in electronic interactions of the substituents in the

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(1) Number V in a program of physicochemical studies of the sense of taste; No. IV, *J. Org. Chem.*, **25**, 1220 (1960).

(2) Taken from the M.S. thesis of L.G.C., Howard University, 1959.

(3) J. J. Blanksma and P. W. M. van der Weyden, *Rec. trav. chim.*, **59**, 629 (1940); **65**, 329 (1946); *cf.* P. E. Verkade, *et al.*, *Rec. trav. chim.*, **68**, 639 (1949) and earlier papers in this series.

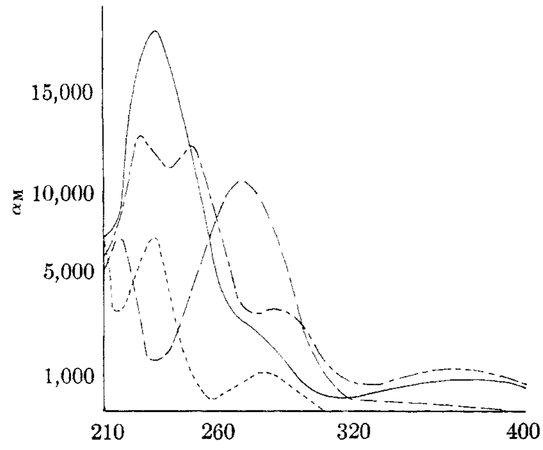

Fig. 1. Absorption spectra of 2-methyl-5-nitroaniline (— — —) and its disubstituted constituents all in alcohol: *o*-toluidine (-------), *p*-nitrotoluene (- - - -), and *m*-nitroaniline (———)

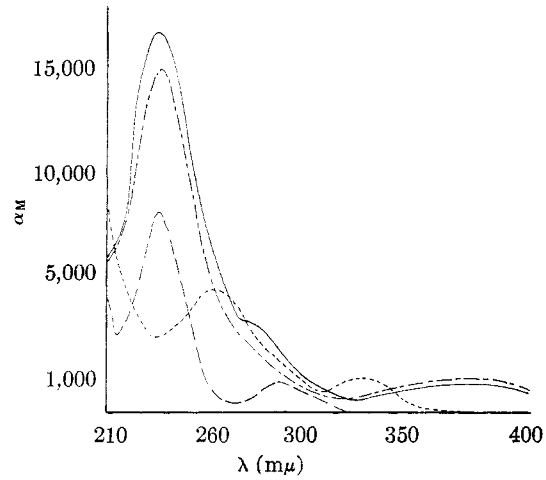

Fig. 2. Absorption spectra of 4-methyl-3-nitroaniline (— — —) and its disubstituted constituents all in alcohol: *p*-toluidine (- - - -), *o*-nitrotoluene (-------), and *m*-nitroaniline (———)

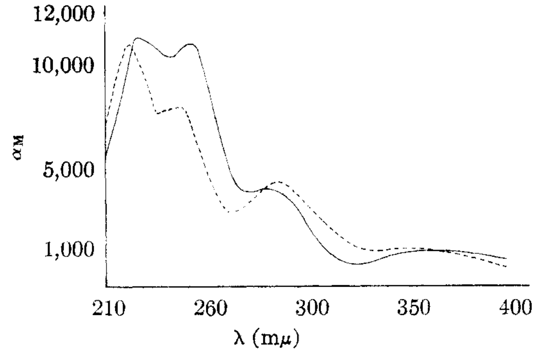

Fig. 3. Absorption spectrum of 2-methyl-5-nitroaniline in alcohol (———) and in water (-------)

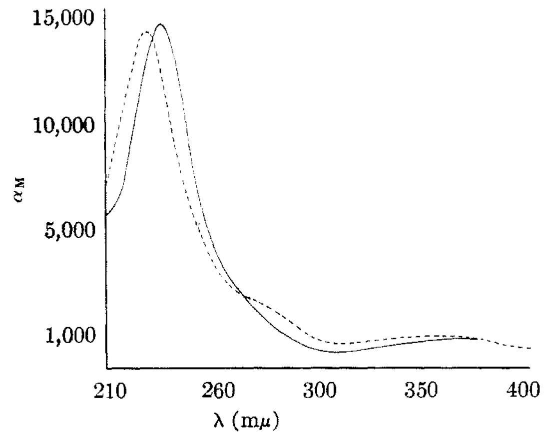

Fig. 4. Absorption spectrum of 4-methyl-3-nitroaniline in alcohol (———) and in water (······)

sweet and nonsweet isomers. The second type of spectroscopic study involved measuring the absorbancy of the compounds at a wave length of maximum absorption in the presence of various cations. The objective here was to detect any differential behavior of the sweet and tasteless isomers towards cations which are important in biological systems.

EXPERIMENTAL

Sources of compounds. With the exception of 4-carboxy-3-nitroaniline, all of the substituted *m*-nitroanilines were available from other phases of the major investigation.[4] The other organic compounds were obtained from commercial sources. The solid compounds were recrystallized to constant melting points which agreed with those reported in the literature. The liquid compounds were redistilled under reduced pressure and used soon thereafter. The inorganic salts were the purest commercial grades available and used as purchased.

4-Carboxy-3-nitroaniline.[5] This compound was prepared by nitrating *p*-toluidine in concd. sulfuric acid at −15° to −10° to yield 4-methyl-3-nitroaniline. The latter compound was acetylated with acetic anhydride in benzene, m.p. 142–143° (lit.,[6] m.p. 144°). The acetanilide was oxidized with aqueous potassium permanganate in the presence of magnesium sulfate and the product hydrolyzed in 6*N* hydrochloric acid on a steam bath. The reaction mixture was brought to *p*H 6 with ammonia and evaporated to dryness. The residue was extracted with hot alcohol and the alcohol solution evaporated to dryness. This residue was then washed with water and recrystallized twice from alcohol, m.p. 239–240° (lit.[1] m.p. 239.5° corr.).

Spectral measurements. All measurements were made on a Beckmann Model DU spectrophotometer in 1-cm. matched, fused-silica cells. The solute concentrations were adjusted to give absorbancy readings between 0.2 and 0.8. Measurements were made in distilled water, aqueous solutions of sodium chloride, potassium chloride, magnesium chloride, and manganous chloride, or in 95% ethanol, and in all cases the respective solvent was used as a blank.

DISCUSSION AND RESULTS

Doub and Vandenbelt[8] found for a particular class of trisubstituted compounds (III), in which only

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[4] *Cf.* A. R. Lawrence and L. N. Ferguson, *Nature*, **183**, 1469 (1959).

[5] Reported to have a very sweet taste by Bogert and Kropff.[7]

[6] K. Brand and H. Zöller, *Ber.*, **40**, 3324 (1907).

[7] M. T. Bogert and A. H. Kropff, *J. Am. Chem. Soc.*, **31**, 847 (1909).

TABLE I

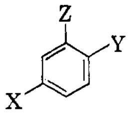
SPECTRAL CHARACTERISTICS OF THE SWEET *m*-NITROANILINES AND THEIR DISUBSTITUTED CONSTITUENTS[a]

(Wave Lengths in mμ)

	Secondary Band		First Primary Band		Second Primary Band		Third Primary Band	
Compound	λ_m	ϵ	λ_m	ϵ	λ_m	ϵ	λ_m	ϵ
2-CH_3-5-NO_2-Aniline[8]	370	1,800	288	4,660	250[b]	11,900	227	12,460
	(354)	(1,860)	(292)	(4,970)	(245)	(8,730)	(222)	(11,420)
m-Nitroaniline[11a]	370	1,358	275[b]	4,000	235	17,200)		
	(358)[9]	(1,450)[9]	(280)[9]	(4,800)[9]				
o-Toluidine	285	1,810			233	7,790		
	ca. (280)[16]	*ca.* (1,600)[16]			*ca.* (230)[16]	*ca.* (7,100)[16]		
p-Nitrotoluene			275	10,410			215	7,790
			(285)[10]	(9,250)[10]			(217)[10]	(6,700)[10]
2-OCH_3-5-NO_2-Aniline[8]	370	3,560	304	4,760	257[b]	13,240	226	10,560
	(367)	(4,330)	(309.5)	(5,170)	(256)[b]	(10,040)	(222)	(10,680)
m-Nitroaniline[11a]	370	1,358	275[b]	4,000	235	17,200		
	(358)[9]	(1,450)[9]	(280)[9]	(4,800)[9]				
o-Anidisine[11b]	284	3,110			236	8,100		
p-Nitroanisole[12]			306	10,780			224	7,690
			(317)[8]	(11,000)[8]			(227)[8]	(7,000)[8]
2-Br-5-NO_2-Aniline	370	2,308	285[b]	5,500	253.5	13,210	229	13,950
	(357)[b]	(1,980)	(290)	(5,200)	(250)	(8,420)	(224)	(11,100)
m-Nitroaniline[11a]	370	1,358	275[b]	4,000	235	17,200		
	(358)[9]	(1,450)[9]	(280)[9]	(4,800)[9]				
o-Bromoaniline	293	2,700			237	8,580		
	299[c]	3,110[c]						
p-Nitrobromobenzene[13,14]			276	11,380			215	7,760
			270[c]	11,500[c]				
4-COOH-3-NO_2-Aniline			282	13,050	253[b]	9,600		
m-Nitroaniline[11a]	370	1,358	275[b]	4,000	235	17,200		
	(358)[9]	(1,450)[9]	(280)[9]	(4,800)[9]				
o-Nitrobenzoic acid			*ca.* 250[12]	3,500[12]				
			(266)[12]	(5,300)[12]				
p-Aminobenzoic acid			285	17,500			219	9,260
			(288)[14]	(19,000)[14]			(219.5)[10]	(9,900)[10]

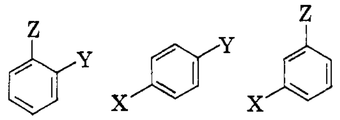
[a] All data are from this study unless indicated otherwise. The values in parentheses are for an aqueous solvent. [b] The mean value of a shoulder or broad band. [c] In hexane.[14] The shift is in the correct direction in going to the less polar solvent.

one substituent is a *meta*-orienting group, that there is a relationship of their ultraviolet spectra to the spectra of their disubstituted constituents.



III

A trisubstituted benzene



Its disubstituted constituents

As compounds I and II fall in the class reported by Doub and Vandenbelt, the spectra of I and II were compared with those of their disubstituted constituents. Typical spectra for compounds of type I and II and of their respective constituents are shown in Figs. 1 to 4. The spectral characteristics for all of the compounds studied are given in Tables I and II. The bands are assigned on the basis of three considerations[17]: (1) First use the criteria of Doub and Vandenbelt,[10] although these authors pointed out[18] that the intensity criteria do not always strictly apply, especially in the case of the secondary bands. In most instances, our assignments agree with those of Doub and Vandenbelt. (2) A more polar solvent will produce a bathochromic shift of the first primary band and a hypsochromic

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(8) L. Doub and J. M. Vandenbelt, *J. Am. Chem. Soc.*, **77**, 4535 (1955).

(9) L. Doub and J. M. Vandenbelt, *J. Am. Chem. Soc.*, **71**, 2414 (1949).

(10) L. Doub and J. M. Vandenbelt, *J. Am. Chem. Soc.*, **69**, 2714 (1947).

(11) (a) R. A. Morton and A. J. McGookin, *J. Chem. Soc.*, 901 (1934). (b) I. P. Grammaticakis, *Bull. soc. chim. France*, **18**, (5) 220 (1951).

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

(13) H. E. Ungnade, *J. Am. Chem. Soc.*, **76**, 1601 (1954).

(14) A. Burawoy and A. R. Thompson, *J. Chem. Soc.*, 4314 (1956); G. Förster and J. Wagner, *Z. für physik. Chem.*, **B35**, 343 (1937).

(15) W. F. Forbes, A. S. Ralph, and R. Gosine, *Can. J. Chem.*, **36**, 869 (1958).

(16) L. Dede and A. Rosenberg, *Ber.*, **67**, 147 (1934). These authors report a solvent effect in agreement with that noted in the present paper.

(17) Other descriptions have been used, such as X and X′ bands, R, B, and K bands, N → V, N → R, and others [*cf.* E. A. Braude, *Determination of Organic Structures by Physical Methods* edited by E. A. Braude and F. C. Nachod, Academic Press, N. Y., 1955, Chap. 4].

(18) Footnote 3 of ref. 9.

TABLE II

SPECTRAL CHARACTERISTICS OF THE NONSWEET *m*-NITROANILINES AND THEIR DISUBSTITUTED CONSTITUENTS[a]
(Wave Lengths in mμ)

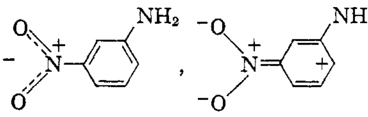
Compound	Secondary Band		First Primary Band		Second Primary Band		Third Primary Band	
	λ_m	ϵ	λ_m	ϵ	λ_m	ϵ	λ_m	ϵ
4-CH_3-3-NO_2-Aniline	366	1,395			234.5	15,500		
	(356)[b]	(1,462)			(228)	(15,200)		
m-Nitroaniline[11a]	370	1,358	275[b]	4,000	235	17,200		
	(358)[9]	(1,450)[9]	(280)[9]	(4,800)[9]				
o-Nitrotoluene	332.5	1,800	257	5,720				
	(325)[9]	(1,300)[9]	(266)[9]	(5,300)[9]			(202.5)[9]	(13,000)[9]
p-Toluidine[16]	289	1,515			234	9,120		
	(286)[10]	(1,600)[10]			(232)[10]	(8,900)[10]		
4-OCH_3-3-NO_2-Aniline	385	1,400			233	13,420		
	(390)	(1,923)			(228)	(15,910)		
m-Nitroaniline[11a]	370	1,358	275[b]	4,000	235	17,200		
	(358)[9]	(1,450)[9]	(280)[9]	(4,800)[9]				
o-Nitroanisole	324	2,370	259	3,220				
	(338)[8]	(2,800)[8]	(267)[8]	(4,000)[8]			(211)[8]	(15,000)[8]
p-Anisidine[11b]	300	2,138			235	9,250		
4-Br-3-NO_2-Aniline	365	1,199			241	18,250		
	(355)[b]	(1,107)			(236.5)	(15,450)		
m-Nitroaniline[11a]	370	1,358	275[b]	4,000	235	17,200		
	(358)[9]	(1,450)[9]	(280)[9]	(4,800)[9]				
o-Nitrobromobenzene[13]	295	1,380	253	2,980				
p-Bromoaniline	296	1,440			244	12,580		
	(290)[10]	(1,340)[10]			(239.5)[10]	(12,800)[10]		
2-COOH-5-NO_2-Aniline	400	2,830	265	11,000	242	19,690		
m-Nitroaniline[11a]	370	1,358	275[b]	4,000	235	17,200		
	(358)[9]	(1,450)[9]	(280)[9]	(4,800)[9]				
Anthranilic acid	333	4,400	247	6,880				
	(327)[9]	(1,940)[9]	(248)[9]	(3,900)[9]				
p-Nitrobenzoic acid			261	12,040				
			(271)[15]	(10,000)[15]				

[a] All data are from this study unless indicated otherwise. The values in parentheses are for an aqueous solvent. [b] The mean value of a shoulder or broad band.

shift of the second and third primary bands. This criterion is based on empirical observations; however, explanations of solvent effects on successive absorption bands have been offered by others.[19] (3) Bands below 250 mμ are arbitrarily designated third primary bands. In some cases, as with compounds of type I, such an assignment is natural because the first and second primary bands are clearly observable.

An accounting of the four bands of these compounds can be given in terms of electronic transitions within the molecules. It can be seen in Tables I and II that *m*-nitroaniline, a constituent of all of the trisubstituted benzenes, has a band at 370 mμ. A similar band appears in the spectra of all but one of the trisubstituted benzenes. This band can be designated an $n \rightarrow \pi^*$ band on the basis[20] of its low intensity, its broadness in alcohol solvent, its disappearance in acid solution, the effect of an electron-withdrawing substituent, and the *blue-shift* in going to more polar solvents.[21] It is observed from the data in Tables I and II that with few exceptions, the secondary band suffers a *blue-shift* in going from an alcohol to an aqueous solvent (latter values are in parentheses). This *blue-shift* has been explained[22] as originating from the effect of solvent molecules orienting themselves around the solute molecules in a favorable orientation to the ground state charge distribution of the solute molecules. Upon excitation, the charge distributions of the solute molecules are markedly changed and the solvent molecules are no longer oriented for strong binding to the solute molecules. This produces a greater solvation energy for the ground state of the solute than for the excited state.

The first primary band can be associated with the nitrobenzene $\pi \rightarrow \pi$ resonance of the type:



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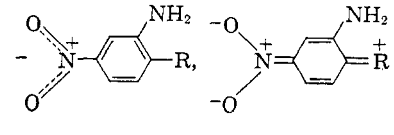

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(19) N. S. Bayliss and E. G. McRae, *J. Phys. Chem.*, **58**, 1002, 1006 (1954).

(20) J. W. Sidman, *Chem. Rev.*, **58**, 689 (1958).

(21) H. McConnell, *J. Chem. Phys.*, **20**, 700 (1952).

In *m*-nitroaniline this band is only a shoulder at about 275 mμ. The presence of the electron-donating substituent R produces a bathochromic and hyperchromic shift, and is greatest for the 2-OCH_3 derivative. Furthermore, in the case of substituted *m*-nitoanilines, the band persists in acid solution, although the $^+NH_3$ group produces a small shift to shorter wavelengths. In fact, the band resembles very much that for the correspondingly substituted nitrobenzene in which there is no $^+NH_3$ group.[23] This band in the 2-COOH derivative is very similar to that in *p*-nitrobenzoic acid.

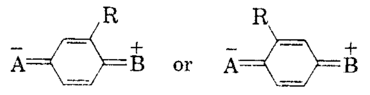
The *red-shift* of the primary band with change of solvent from ethanol to water has been explained in terms of increased solvation of the solute molecules in the excited state owing to their greater dipole moments.[19]

The second primary band apparently involves the lone-pair electrons on the amino nitrogen atom because, for all cases studied, it disappears in acid solution where the lone-pair electrons are not available for the transition. As this band too undergoes a *blue-shift* with a change to more polar solvents, it can be readily distinguished from the first primary band. On this basis, for example, the more intense bands of *o*-toluidine, *p*-toluidine, and *p*-bromoaniline are designated second rather than first primary bands, and that of anthranilic acid as a first rather than a second primary band.

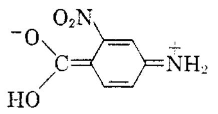
The third primary band is the least tenable assignment. It has been pointed out[24] that this band could possibly be the third primary band of benzene shifted to longer wave lengths. It is significant that a band in the 210–223 mμ region appears in the spectra of all of the substituted *m*-nitroanilines in acid solution.[23]

The first primary band is sensitive to the presence of a group *ortho* to the nitro group.[13,25] Thus, the band is either missing or is at shorter wavelengths and of much lower intensity for the *o*-substituted nitro compounds than for the *p*-substituted isomers. This is understandable in view of the coplanarity required for the nitro-phenyl group electronic interaction responsible for this band.

There is no readily apparent correlation between the spectra of the substituted *m*-nitroanilines and their tastes. It can be observed that only the sweet compounds have a first primary band above 280 mμ *and* a second primary band in the 230–260 mμ range. Considering the electronic transitions responsible for these two bands, one might deduce that intense sweetness is associated with a molecule having a flatness and a charge distribution as found in a *p*-disubstituted benzene with an electron-donor and an electron-withdrawing group plus a third group off center.



The sweetness of a molecule would then depend on the degree to which it approximates this structure. For example, *m*-nitroaniline, lacking a more electron-donating group than the hydrogen atom *para* to the nitro group, is only weakly sweet.[8] Furthermore, when R in I and II is an electron-donating group, I is the sweet isomer and II is nonsweet; but we have found in two cases that when R is an electron-withdrawing group (COOH or SO_2NH_2), II becomes the sweet isomer.



This possible taste-structure relationship is being explored further through a study of the molecular addition complex forming ability of these compounds and through an examination of the tastes of other structural systems approximating that of I and II.

The intensities of the second primary band (as the nonsweet isomers have no first primary band) of the 2- and 4-substituted 5-nitroanilines were measured in aqueous solutions with salt concentrations of 0.005M, 0.01M, 0.1M, and 1.0M. The molar extinction coefficients observed in water were unaffected by the presence of sodium chloride and magnesium chloride. However, potassium chloride produced a *decrease* in intensity of 5 to 10% for both isomers at each of the salt concentrations, and manganous chloride produced a variable 10% *increase* at the two higher salt concentrations. As these salt effects did not differ for the sweet and the nonsweet isomers, they were not investigated further at this time. These ions were chosen because of their key roles in nerve transmission and enzyme activations.

It is believed that the critical set of properties which determines the tastes of these compounds involves their loose complex-forming ability with some biological constituent in the tongue area. One does not expect a correlation with a single property of structure, basicity, or charge distribution, but with a combination of these and other factors. It is hoped that a factor analysis of the spectroscopic and other physicochemical properties of these compounds will provide a function which may be used for predicting the tastes of compounds, or at least give a clue as to why one isomer is several hundred times as sweet as sucrose while the other isomer is tasteless.

WASHINGTON 1, D. C.

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(22) G. J. Brealey and M. Kasha, *J. Am. Chem. Soc.*, **77**, 4462 (1955).

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(24) Page 2418 of ref. 9.

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