

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF PURDUE UNIVERSITY]

The Conjugative Ability of the Trimethylsilyl Group

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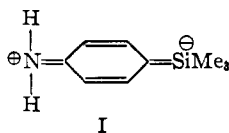
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The effect of the *m* and *p*-Me₃Si group upon the ionization constants of anilines, dimethylanilines, phenols and benzoic acids has been studied and the Hammett σ -values determined. These data indicate that the *m*-Me₃Si group exerts mainly an electron-releasing inductive effect in all these reactions. The same is true of the *p*-Me₃Si group in benzoic acids. The latter group, however, is capable of limited conjugation involving an expansion of the silicon valence shell in the reaction of phenols, anilines and dimethylanilines.

The study of the electronic influence of the trimethylsilyl group on an aromatic nucleus was undertaken to determine whether the trimethylsilyl group is capable of any appreciable resonance interaction or conjugation with other groups present on the aromatic nucleus.

It had previously been assumed that the trimethylsilyl group, although a rather indifferent group with respect to its directive abilities,^{1,2} still was able to exert its influence on an aromatic nucleus primarily by supplying electrons rather than withdrawing them. These conclusions had been reached from the following considerations: (a) the difference in electronegativity between silicon and carbon of +0.7 unit on the Pauling scale³ suggested an electron-releasing tendency for silicon, (b) the electron-releasing inductive effect of silicon-containing substituents upon the ionization constants of acetic acids,⁴ and aliphatic amines^{5,6} (c) isomer distribution in the nitration of phenyltrimethylsilane^{1,2} and (d) dipole moment studies of *p*-fluorophenyl- and *p*-chlorophenyltrimethylsilanes and Hammett σ -constants of *m*- and *p*-trimethylsilyl groups in benzoic acids obtained from ionization constants and rates of reaction with diphenyldiazomethane.⁷

From these considerations one is led to believe that the trimethylsilyl group is slightly electron releasing no matter to what moiety it happens to be attached. The recent dipole moment data of DeVries and Soffer,⁸ however, have indicated that the trimethylsilyl group may also act as an electron-accepting group if some additional group is present which has strong electron-releasing properties.



(1) R. A. Benkeser and P. E. Brumfield, *THIS JOURNAL*, **73**, 4770 (1951).

(2) Unpublished results of the nitration of *i*-butylbenzene with copper nitrate and acetic anhydride carried out in this Laboratory by R. A. Benkeser and D. S. Holton under the same conditions as described in ref. (1), indicate an *o*-*m*-*p* ratio of 10.0, 8.0, 82%, respectively, which is comparable to that obtained by K. L. Nelson and H. C. Brown, *ibid.*, **73**, 5805 (1951), using mixed acid, whereas nitration of phenyltrimethylsilanes results in an almost statistical distribution of nitro isomers.

(3) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1940, pp. 58-70.

(4) L. H. Sommer, J. R. Gold, G. M. Goldberg and N. S. Marans, *THIS JOURNAL*, **71**, 1509 (1949).

(5) J. E. Noll, B. F. Daubert and J. L. Speier, *ibid.*, **73**, 3871 (1951).

(6) L. H. Sommer and J. Rockett, *ibid.*, **73**, 5130 (1951).

(7) J. D. Roberts, E. A. McElhill and R. Armstrong, *ibid.*, **71**, 2923 (1949).

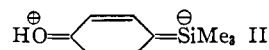
(8) H. Soffer with T. DeVries, *ibid.*, **73**, 5817 (1951).

These authors suggest that resonance structures such as I are possible, in which silicon expands its valence shell to ten electrons.

Additional evidence for the importance of such resonance forms can be obtained by comparing the base strengths of *m*- and *p*-trimethylsilylanilines and -dimethylanilines, and the acid strength of phenols.

If resonance is of any consequence in the trimethylsilylanilines and -dimethylanilines, then the base strength of the *p*-amine should be less than that of the unsubstituted amine itself because of contributions of resonance structures such as I, and will be reflected in a positive σ_p -value⁹; however, if an electron-releasing inductive effect is of prime importance then the *p*-amine should be a slightly *stronger* base than the unsubstituted amine itself with a consequent negative σ_p -value for the trimethylsilyl group.¹⁰

A similar argument holds for the trimethylsilylphenols, wherein possible resonance contributions such as II would tend to make the *p*-phenol a stronger acid than phenol itself.



Since the trimethylsilyl group is considered to exert its influence in the benzoic acids principally by an electron-releasing inductive effect,⁷ a comparison of the σ_p -values in benzoic acids *vs.* phenols and anilines should give an indication of the conjugative ability of the trimethylsilyl group. Thus two σ -values are needed to describe the effect of a *p*-nitro group⁹; one value (+1.27), is a measure of the effect of this group in anilines and phenols in which there is appreciable resonance interaction; the other value (+0.778) describes the effect of the *p*-nitro group in reactions of benzoic acids, where conjugative ability is of little importance. The difference (0.49) is thus a measure of the relative contributions of resonance structures in anilines and phenols.

Experimental

Preparation of Compounds.—All compounds except those containing silicon were prepared or treated as follows: The dimethylanilines were obtained either by redistillation or recrystallization of commercial products or by methylation with methyl iodide or dimethyl sulfate of the corresponding anilines by methods given in the literature.

The phenols consisted mostly of Eastman Kodak Co. White Label products further rectified or recrystallized.

(9) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 184.

(10) The correctness of this conclusion is of course contingent on the assumption that the inductive effect, even in an aromatic system, falls off smoothly with distance. See J. D. Roberts, *et al.*, *THIS JOURNAL*, **73**, 2181 (1951), for experimental evidence supporting this view.

Trimethyl-*p*-dimethylaminophenylsilane.—This compound was prepared as described by Gilman and Marshall.¹¹ Fifty-four grams (76%) of a product boiling at 136° (20 mm.), n_{D}^{20} 1.5344, was obtained.

Trimethyl-*m*-dimethylaminophenylsilane.¹²—To 5.6 g. (0.81 g. atom) of finely cut lithium suspended in 100 ml. of ether was added with stirring, a solution of 70 g. (0.35 mole) of *m*-bromodimethylaniline over a two-hour period. The reaction mixture was stirred and refluxed for an additional half-hour, after which time a solution of 37 g. (0.34 mole) of trimethylchlorosilane in 75 ml. of ether was added over a one-hour period. Upon completion of this addition, stirring and refluxing were continued for two hours; Color Test I¹³ was negative at this point.

The reaction mixture was hydrolyzed, and the ether layer was separated and dried over Drierite. This mixture was filtered, and the ether was stripped off. Distillation yielded 56.0 g. (84%) of a colorless product which boiled at 109–110° (8 mm.), n_{D}^{20} 1.5265.

***p*-Trimethylsilylphenol.**—*p*-Trimethylsilylphenol m.p. 76° (uncor.) was prepared by the method of Speier.¹⁴

***m*-Chlorophenol.**—*m*-Chlorophenol was prepared by an adaptation of the method of Koelsch.¹⁵ From one mole of *m*-chloroaniline was obtained 106.2 g. (88%) of *m*-chlorophenol boiling at 55° (3 mm.).

***m*-Chlorophenyltrimethylsiloxane.**—*m*-Chlorophenyltrimethylsiloxane was prepared by the general method of Speier.¹⁴ To 25.8 g. (0.2 mole) of *m*-chlorophenol was added an excess of trimethylchlorosilane (36 g., 0.33 mole). The mixture was heated to reflux while being protected from moisture. Evolution of hydrogen chloride steadily diminished over several hours. Refluxing was continued for a total of nine hours after which time the resulting solution was distilled under atmospheric pressure to remove unreacted trimethylchlorosilane. The remainder was distilled under vacuum to yield 36.20 g. (88%) of product boiling at 45° (2 mm.), n_{D}^{20} 1.4950, d_{4}^{20} 1.049, MR (calcd.) 55.64, MR (found) 55.78.

Anal. Calcd. for $C_9H_{13}ClOSi$: Si, 13.98. Found: Si, 13.33.

***m*-Trimethylsilylphenol.**—*m*-Trimethylsilylphenol was prepared by an adaptation of the method of Speier.¹⁴ To 6 g. (0.26 g.-atom) of molten sodium in 30 ml. of refluxing toluene in a 500-ml. 3-neck flask equipped with a high speed stirrer, reflux condenser and addition funnel, and under a nitrogen atmosphere was added about 2 g. of trimethylchlorosilane from a total of 14.3 g. (0.132 mole). This mixture was maintained at reflux temperature while a mixture of the remainder of the trimethylchlorosilane and 24.1 g. (0.12 mole) of *m*-chlorophenyltrimethylsiloxane was added cautiously at such a rate as to maintain a vigorous reaction and rapid refluxing. A Glascol heating mantle was used so that addition could be interrupted and still prevent the solidification of the sodium. It was found necessary to employ a large flask in relation to the volume of reagents used because of the tendency of the reaction mixture to froth badly. Fifteen minutes was required for the addition. The dark blue reaction mixture was stirred and refluxed for an additional hour, whereupon it was allowed to cool to room temperature and was then filtered. The filtrate was distilled at atmospheric pressure to remove the solvent, and then distilled under vacuum. This latter distillation yielded 20.9 g. (70%) of *m*-trimethylsilylphenyltrimethylsiloxane boiling at 60° (2 mm.), n_{D}^{20} 1.4770. There was no detectable yield of phenoxytrimethylsilane. Some higher boiling material (ca. 10 g.) boiling at 85° (2 mm.) was also obtained.

To 15 g. (0.063 mole) of *m*-trimethylsilylphenyltrimethylsiloxane was added 10 ml. of 95% ethanol and one drop of concd. hydrochloric acid. Two ml. of water was added to this solution during which time the mixture grew warm. It was then cooled in an ice-bath and diluted to turbidity with about 20 ml. of water. Upon standing a few minutes two layers formed. The upper layer consisting of the organic portion was separated and the ethanol and hexamethyldisiloxane were stripped off. Distillation of the residue yielded 8.67 g. of a clear colorless liquid which

boiled at 70° (1 mm.), n_{D}^{20} 1.5190, d_{4}^{20} 0.977, MR (calcd.) 51.63, MR (found) 51.66.

Anal. Calcd. for $C_9H_{11}OSi$: Si, 16.90. Found: Si, 17.10.

***p*-Aminophenyltrimethylsilane.**—*p*-Aminophenyltrimethylsilane had been prepared pure by catalytic hydrogenation of *p*-nitrophenyltrimethylsilane^{1,10a,b}; it was redistilled immediately before use.

***m*-Trimethylsilylbenzoic Acid.**—*m*-Trimethylsilylbenzoic acid was prepared by the carbonation of *m*-trimethylsilylphenyllithium using the procedure of Roberts, *et al.*⁷ The organometallic was prepared from the *m*-chlorophenyltrimethylsilane previously obtained.¹⁷ The yield of acid when recrystallized from 90–100° petroleum ether was 48%, m.p. 113–114°.

***m*-Trimethylsilylbenzamide.**—*m*-Trimethylsilylbenzoic acid (25 g., 0.13 mole) was refluxed with 100 ml. of thionyl chloride. Evolution of sulfur dioxide and hydrogen chloride ceased after about an hour. Refluxing was continued for an additional 15 minutes, whereupon the excess thionyl chloride was distilled off (ca. 30–40 ml.). The acid chloride was then added dropwise with vigorous stirring to 350 ml. of concd. ammonium hydroxide externally cooled by an ice-bath. When all the acid chloride had been added, stirring was continued for a few more minutes, and the precipitated amide was filtered off and washed well with cold water. The crude air-dried product melted at 117–120°. Crystallization from 90–100° petroleum ether yielded 21 g. (92%) of fine white crystals melting at 129.5–130°.

Anal. Calcd. for $C_{10}H_{15}NOSi$: N, 7.25, Si, 14.52. Found: N, 7.38; Si, 14.38.

***m*-Aminophenyltrimethylsilane. First Run.**—To 110 ml. of 0.5 *N* sodium hypochlorite solution¹⁸ maintained at 0 to 5° was added with stirring 5.0 g. (0.026 mole) of *m*-trimethylsilylbenzamide. The stirred reaction mixture was maintained at this temperature for 20 minutes after which time it was warmed to 65–70° over a half-hour period. The reaction mixture gradually became a dark orange solution, finally turning to a reddish-brown with a separation into two layers. The temperature was maintained at 65–70° for an additional 15 minutes, and the mixture was then steam distilled. A light yellow oil was collected with the steam distillate. Some sodium chloride was added to this, and the oil was extracted with ether and separated. The ethereal solution was dried over Drierite, filtered, and the ether was stripped off. Distillation yielded 2.9 g. (68%) of a colorless product boiling at 94° (5 mm.), n_{D}^{20} 1.5362. It is significant that no aniline resulting from cleavage of the silicon-carbon bond was detected as a forerun in this reaction.

Second Run.—The same procedure was used with an 11-g. (0.057 mole) sample of *m*-trimethylsilylbenzamide and 260 ml. of 0.5 *N* sodium hypochlorite solution; yield 5.8 g. (62%), boiling at 62° (1 mm.).

The acetyl derivative of the amine was prepared using acetic anhydride.^{16b} Crystallization from 90–100° petroleum ether yielded crystals melting at 114–115°. A mixed m.p. with an authentic sample^{16b,19} showed no depression; a mixed m.p. of the acetyl derivative and acetanilide was depressed.

***p*-Trimethylsilylbenzoic Acid.**—*p*-Trimethylsilylbenzoic acid was kindly supplied by Dr. P. E. Brumfield.¹⁹ This acid was recrystallized and dried before use.

Acidity Constants of Dimethylanilinium Ions.—Solutions of the respective dimethylanilines were prepared by dissolving accurately weighed samples ranging from 0.08 to 0.2 g. in 50% (1:1 by volume) aqueous ethanol and diluting to 100 ml. in volumetric flasks at 20°. All pH measurements including those on the phenols and anilines were recorded on a Beckman Model H-2 pH meter equipped with a glass electrode and saturated calomel half-cell. The meter was standardized with an aqueous buffer. The solutions of the dimethylanilines were thermostated at 20° while 5- or

(11) H. Gilman and F. J. Marshall, *THIS JOURNAL*, **71**, 2066 (1949).

(12) R. O. Ranck, M.S. Thesis, Purdue University, 1951.

(13) H. Gilman and F. Schultz, *THIS JOURNAL*, **47**, 2002 (1925).

(14) J. L. Speier, *ibid.*, **74**, 1003 (1952).

(15) C. F. Koelsch, *ibid.*, **61**, 969 (1939).

(16) (a) The sample of *p*-aminophenyltrimethylsilane was prepared by Dr. P. E. Brumfield; (b) R. A. Benkeser and P. E. Brumfield, *THIS JOURNAL*, **74**, 253 (1952).

(17) H. R. Krysiak, M.S. Thesis, Purdue University, 1952.

(18) E. S. Wallis and J. E. Lane, "Organic Reactions," Vol. III, R. Adams, Ed., John Wiley and Sons, Inc., New York, N. Y., 1946, p. 267.

(19) P. E. Brumfield, Ph.D. Thesis, Purdue University, 1951.

10-ml. increments of standard hydrochloric acid in 50% ethanol were added. Determinations were carried out at approximately 20, 30, 40 and 60% neutralization under a nitrogen atmosphere. Three or four determinations were carried out on individual amines. The apparent acidity constants were calculated in the same manner as described by Davies and Addis.²⁰ The pK_a values for a particular amine varied from 0.02 to 0.04 pK_a unit. The data are summarized in Table I. In Fig. 1 are plotted the logarithms of the apparent acidity constants against values of σ for eight dimethylanilines. A least squares treatment of the data gives the values presented in Table V.²¹

TABLE I
APPARENT ACIDITY CONSTANTS OF DIMETHYLANILINIUM IONS IN 50% ETHANOL AT 20°

Substituent	pK_a	Substituent	pK_a
H	4.35	<i>m</i> -Me	4.66
<i>p</i> -Br	3.52	<i>p</i> -I	3.43
<i>m</i> -Br	3.08	<i>m</i> -Cl	3.09
<i>p</i> -MeO	5.14	<i>p</i> -Me ₃ Si	3.98
<i>p</i> -Me	4.94	<i>m</i> -Me ₃ Si	4.41

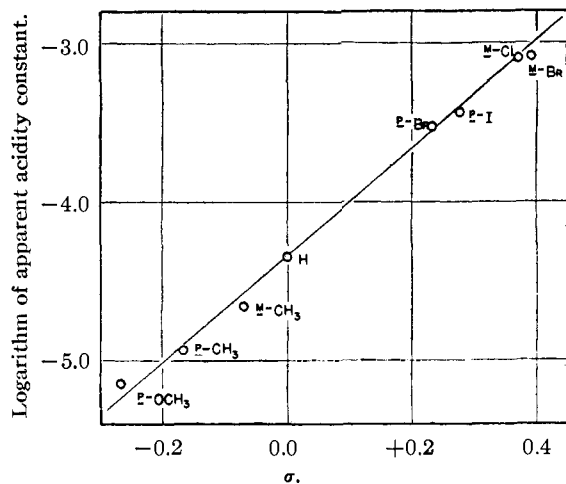


Fig. 1.—Relationship between apparent acidity constants of dimethylanilinium ions in 50% (1:1 by volume) ethanol-water at 20° and Hammett sigma constants.

Apparent Ionization Constants of Phenols.—Because of the limited solubility of *m*- and *p*-trimethylsilylphenol in water, it was necessary to carry out the potentiometric determination of these phenols in 30% ethanol. The pH meter was standardized with an aqueous buffer, and the solvent used for the phenols consisted of a solution of 30 volumes of absolute ethanol plus 70 volumes of carbon dioxide-free water. The standard carbonate-free sodium

hydroxide solution was prepared in the same solvent. All pH determinations were carried out at 25° and the procedure and calculations of the pK_a values were the same as those described by Bordwell and Cooper.²² For those phenols with pH values of 10 or greater, the correction for hydrolysis was applied, using pK_w equal to 14.38 as the value for water in 30% ethanol, obtained by graphical interpolation of the estimations of Branch, *et al.*²³ The pK_a values for a particular determination ranged from 0.01 to 0.04 pK_a unit. The results are given in Table II. Figure 2 illustrates a plot of logarithms of apparent ionization constants against values of σ for nine phenols. Treatment of the data by the method of least squares yields the results given in Table V.²⁴

TABLE II
APPARENT IONIZATION CONSTANTS OF PHENOLS IN 30% ETHANOL^a AT 25°

Substituent	pK_a	Substituent	pK_a
H	10.85	<i>p</i> -Br	10.15
<i>p</i> -NO ₂	7.69	<i>p</i> -Cl	10.20
<i>m</i> -NO ₂	9.02	<i>m</i> -Cl	9.89
<i>m</i> -MeO	10.67	<i>p</i> -Me ₃ Si	10.64
<i>p</i> -Me	11.14	<i>m</i> -Me ₃ Si	11.01
<i>m</i> -Me	10.98		

^a 30 vol. absolute ethanol plus 70 vol. carbon dioxide-free distilled water.

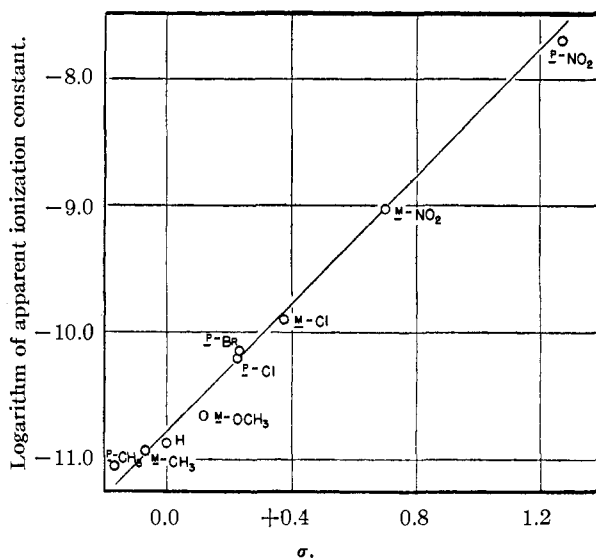


Fig. 2.—Relationship between apparent ionizations of phenols in 30% ethanol (30 vol. ethanol plus 70 vol. water) at 25° and Hammett sigma constants.

Acidity Constants of Anilinium Ions.—The acidity constants for the anilinium ions at 25° were determined by titration of the hydrochlorides in aqueous solution with standard carbonate-free sodium hydroxide in a manner similar to that of the phenols. Table III lists the results.

TABLE III
ACIDITY CONSTANTS OF ANILINIUM IONS IN WATER AT 25°

Substituent	H	<i>m</i> -Me ₃ Si	<i>p</i> -Me ₃ Si
pK_a	4.62	4.64	4.36

(22) F. G. Bordwell and G. D. Cooper, *THIS JOURNAL*, **74**, 1058 (1952).

(23) G. E. K. Branch, D. L. Yabroff and B. Bettman, *ibid.*, **56**, 937 (1934).

(24) G. M. Bennett, G. L. Brooks and S. Glasstone, *J. Chem. Soc.*, 1821 (1935), employing the same solvent and temperature, obtained pK values for halogenated phenols and phenol slightly lower than ours. Their use of an efficient salt bridge in conjunction with a calomel half-cell and glass electrode, however, may have minimized liquid junction potentials to a greater extent than in our determinations.

(20) W. C. Davies and H. W. Addis, *J. Chem. Soc.*, 1622 (1937).

(21) Hammett⁹ lists values of ρ and $\log K^\circ$ for this reaction as +3.99 and -4.05, respectively, for 12 dimethylanilines with a median deviation of 0.12. Davies and Addis,²⁰ and W. C. Davies, *J. Chem. Soc.*, 1865 (1938), from whose data this was calculated, obtained pK values 0.1 to 0.7 pK unit lower than ours. G. Thomson, *ibid.*, 1113 (1946), obtained a value of pK for dimethylaniline of 4.26 at 25° and also noted that Davies and Addis' value for dimethylaniline of 4.21 at 20° appeared to be slightly low since according to N. F. Hall and M. R. Sprinkle, *THIS JOURNAL*, **54**, 3469 (1932), the value of pK of organic bases is diminished with increase in temperature, *i.e.*, the temperature coefficient of pK is negative. Hall and Sprinkle noted an increase of 0.09 pK unit for dimethylanilinium ion which is the same difference observed in comparing our value with that of Thomson in going from 25 to 20°. Although Hall and Sprinkle's data for the change in pK with change in temperature is for water, it seems reasonable to assume that a change of similar magnitude over this limited temperature range should be expected for 50% ethanol, since the change in dielectric constant over a five degree temperature range is roughly the same magnitude (ΔD equal to 1.9 and 1.4 for water and 50% ethanol, respectively, as calculated from the tables of G. Åkerlöf, *ibid.*, **54**, 4125 (1932)).

Ionization Constants of Benzoic Acids.—Ionization constants of benzoic acids were determined in aqueous solution at $25 \pm 2^\circ$ at concentrations *ca.* 10^{-6} *M* in 0.1 *N* sulfuric acid, 0.1 *N* sodium hydroxide, and in a buffer solution 0.35 *M* in acetic acid and 0.10 *M* in sodium acetate, by the method of Flexser, *et al.*²⁵ Because of the limited solubility of the *m*- and *p*-trimethylsilylbenzoic acids, saturated solutions were prepared by suspending the acids in carbon dioxide-free distilled water and vigorously shaking these suspensions at intervals over a 12-hour period. These mixtures were filtered and the filtrates were diluted with half their volume of freshly boiled distilled water. These dilutions were necessary in order to prevent salting out of the acids in the buffer and sulfuric acid solutions. One hundred-ml. aliquots of these stock solutions were then pipetted into flasks into which were introduced 25-ml. aliquots of stock solutions of acid, base and buffer solutions. A Cary Recording Spectrophotometer model 10-11M was employed as the ultraviolet colorimeter using a one-cm. cell. The data are presented in Table IV.

TABLE IV

IONIZATION CONSTANTS OF BENZOIC ACIDS IN WATER AT $25 \pm 2^\circ$

Substituent	Approx. concn., <i>M</i> $\times 10^3$	λ_{max} ^a	D_{B} ^b	D_{BH} ^c	D^d	pK	Av. pK
H	5.08	245	0.075	0.150	0.115	4.28	4.20 \pm 0.04
		240	.140	.325	.230	4.20	
		235	.245	.490	.365	4.20	
		230	.360	.560	.450	4.13	
<i>m</i> -Me ₃ Si	3	245	0.115	0.250	0.180	4.19	4.24 \pm 0.03
		240	.210	.395	.305	4.24	
		235	.300	.465	.390	4.30	
		230	.370	.450	.410	4.22	
<i>p</i> -Me ₃ Si	3	255	0.170	0.435	0.305	4.24	4.27 \pm 0.02
		250	.370	0.815	.605	4.27	
		245	.705	1.115	.925	4.28	
		240	.940	1.210	1.090	4.30	

^a Optical densities were measured at or near maximum absorption. ^b Solvent was 0.1 *N* sodium hydroxide solution. ^c Solvent was 0.1 *N* sulfuric acid solution. ^d Solvent was buffer solution 0.10 *M* in sodium acetate and 0.35 *M* in acetic acid.

best straight line for this equation is 0.037. The data are included in Table V.

Discussion of Results.—Tables I and III clearly indicate that both *p*-trimethylsilylaniline and -dimethylaniline are weaker bases than aniline and dimethylaniline, respectively. Likewise, Table II indicates that *p*-trimethylsilylphenol is a stronger acid than phenol itself. While admittedly these trends are not large they are distinctly in accord with the results predicted by the resonance forms shown in I and II.

In Table V are presented along with other data, the σ -values of the trimethylsilyl group calculated from the Hammett equation.⁹ The average σ -values obtained from the six reactions listed are -0.07 for σ -meta, -0.05 for σ -para in the three benzoic acid reactions, and $+0.09$ for σ -para in the amines and phenols.

From these data it would appear that there are small but significant interactions in the *p*-amines and *p*-phenols in which the trimethylsilyl group is capable of conjugation to a limited extent possibly involving contributions of structures such as I and II. These conclusions are further substantiated by the $\Delta\sigma_p$ -values listed in Table V.

It is of interest to note the rather wide divergence of the σ -meta value of reaction 1 in Table V from the other σ -meta values in which the agreement is quite good.²⁷

Because of the uncertainty in the value -0.208 , the ionization constants of the benzoic acids in water were determined. These results listed in Table IV and reaction 3, Table V, clearly indicate the -0.208 to be an anomalous value. It is possible that the "bulk effect" suggested by Price and Lincoln²⁸ for the *t*-butyl group is perhaps in operation in this instance.

TABLE V
CONSTANTS FOR THE TRIMETHYLSILYL GROUP

Reaction	ρ	$\log k^0$	Median deviation, r	σ_m	σ_p	$\sigma_p - \sigma_m$	$\Delta\sigma_p^a$
1. Apparent ionization constants benzoic acids, 50% EtOH, 25 ^o	+1.464	-5.71	0.025	-0.208	-0.060	+0.148	
2. Reaction rates diphenyldiazomethane with benzoic acids, in abs. EtOH, 30 ^o	+0.937	+0.041	0.010	-0.018	-0.022	-0.004	
3. Ionization constants benzoic acids, water, 25 ^o	+1.000 ^c	-4.203 ^c	...	-0.04	-0.07	-0.03	
4. Apparent acidity constants dimethylanilinium ions, 50% EtOH, 20 ^o	+3.369	-4.35	0.042	-0.02	+0.11	+0.13	0.16
5. Acidity constants anilinium ions, water, 25 ^o	+2.730 ^c	-4.569 ^c	0.060 ^c	-0.03	+0.08	+0.11	0.13
6. Apparent ionization constants phenols, 30% EtOH, 25 ^o	+2.509	-10.82	0.058	-0.08	+0.07	+0.15	0.12
7. Ultraviolet absorption characteristics ^d						+0.03 ^e	-0.11 ^f

^a Calculated using an average σ_p -value of -0.05 for the benzoic acids. ^b Ref. 7. ^c Ref. 9. ^d Ref. 26. ^e Calculated from λ_{max} (241 $m\mu$) of trimethyl-*p*-aminophenylsilane. ^f Calculated from λ_{max} (236 $m\mu$) of *p*-triethylsilylbenzoic acid.

Ultraviolet Absorption Characteristics.—Ultraviolet absorption spectra were determined with a Cary recording spectrophotometer. Values of $\Delta\sigma$ (*i.e.*, $\sigma_p - \sigma_m$) were determined from λ_{max} using the equation

$$\Delta\sigma = 1.74 \times 10^{-2} (\Delta\lambda_{\text{obsd}}) - 6.14 \times 10^{-2}$$

by a least squares treatment of the data of Doub and Vandenbelt.²⁶ The median deviation of the points from the

(25) L. A. Flexser, L. P. Hammett and A. Dingwall, *THIS JOURNAL*, **57**, 2103 (1935).

(26) L. Doub and J. M. Vandenbelt, *ibid.*, **69**, 2714 (1947).

The data thus indicate that an electron-releasing inductive effect is of principal importance in the case of both the *m*-trimethylsilyl group, and the *p*-trimethylsilyl group in benzoic acids in agreement with the views of Roberts, *et al.*,⁷ but that the *p*-trimethylsilyl group in anilines, dimethylanilines

(27) The median deviation of σ -values as cited by Hammett for 52 reactions is 0.060.⁹

(28) C. C. Price and D. C. Lincoln, *THIS JOURNAL*, **73**, 5836 (1951).

and *phenols* apparently is capable of conjugation to a limited extent.

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A Fractionation of D-Amino Acid Oxidase by Electrophoresis-Convection¹

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Extracts of pig kidney cortex have been fractionated by electrophoresis-convection. Fractionations were carried out at pH 5.3, 7.0 and 8.1. Assay of the fractions for D-amino acid oxidase showed that considerable enrichment of the top fraction with respect to enzymatic activity was achieved in a single stage of fractionation at pH 7 after transport of 97% of the protein out of the top and into the bottom reservoir of the electrophoresis-convection cell. The specific activity of the top fraction, corrected for the effect of dilution upon enzymatic activity, was 3 to 5 times as great as that of the unfractionated extract. At pH 5.3 the specific activities of the top fractions were about 1.5 times that of the starting material, while at pH 8.1 very little if any fractionation was achieved.

Electrophoresis-convection is gaining extensive use in the fractionation of a variety of proteins.²⁻⁵ This method of fractionation, first proposed and tested by Kirkwood and Nielsen,^{6,7} is based upon the same principle as that of the Clusius column, except that electrophoretic transport, instead of transport by thermal diffusion, is superimposed on convective transport in a vertical convection channel. The separation of a heterogeneous protein into its constituents is accomplished by making use of small differences in their isoelectric points and mobilities. The details of construction and operation of the electrophoresis-convection apparatus have been described in detail by Cann and co-workers.^{8,9a,b} Incidental to other studies in the purification of D-amino acid oxidase¹⁰ we have found this method of fractionation to be useful in processing very crude extracts of the enzyme and believe that the study reported below should furnish a helpful direction for the application of electrophoresis-convection to enzyme purifications.

Experimental

Preparation of the Enzyme Extract.—2250 g. of fresh pig kidney cortex was minced batchwise in a Waring blender for one minute at room temperature and then stirred with 10.1 l. of *M*/60 sodium pyrophosphate at 12–13° for 40 minutes. The pH was maintained at 8.0 by the occasional addition of 6 *N* NaOH. This suspension was centrifuged cold and the insolubles discarded. To the supernatant,

8700 ml., was added 1827 g. of (NH₄)₂SO₄. After 24 hours at 5°, the suspension of the crude enzyme was centrifuged at 5° and the supernatant discarded. The precipitate was frozen immediately.

Fractionation.—For each fractionation an appropriate amount of the frozen precipitate was mixed with distilled water and dialyzed against cold tap water for 5 hours to remove the (NH₄)₂SO₄ and then against 2 or 3 changes of the desired buffer for 15 hours at 5°. This solution was centrifuged at 5° for about 3 hours and the supernatant used directly in the electrophoresis-convection cell.¹¹ Fractionations were carried out in phosphate buffer, ionic strength 0.1. Runs were made at pH 8.1 and 7.0 using a field strength of 2.0 volts/cm. and at pH 5.3 using 1.7 volts/cm. for times up to 48 hours. The progress of the fractionation was followed by withdrawing small samples from the top reservoir during the runs and analyzing them for protein content and enzymatic activity. These samples are designated according to the time of withdrawal from the apparatus, e.g., fraction top 24 hr. is the sample withdrawn from the top reservoir 24 hr. after the start of the fractionation. The various fractions were stored frozen. For electrophoretic analysis, fractions were concentrated by precipitating the protein with (NH₄)₂SO₄ and redissolving the precipitate in buffer.

Enzymatic Activity and Protein Assays.—Enzymatic activities, expressed as μ l. O₂/30 min./mg. protein, were determined manometrically with excess D,L-alanine as the substrate and in the presence of added excess flavin adenine dinucleotide. Protein concentrations were determined gravimetrically by precipitation of the protein from solution with an equal volume of 20% trichloroacetic acid.

Electrophoretic Analysis.—Electrophoretic analyses were carried out in a Perkin-Elmer Tiselius apparatus using the current regulating power supply described by Swingle.¹² The current was measured with a potentiometer circuit. Electrophoretic experiments were carried out in phosphate buffer pH 7.0 and ionic strength 0.1.

Results and Discussion

The results obtained in some representative fractionations of crude enzyme extracts are presented in Table I, where *V* is the volume of the fraction in ml., *c* is the concentration of the fraction in mg. protein/ml., *A* is the specific activity, μ l. O₂/30 min./mg. protein, at concentration *c*, and *f* is the ratio of specific activity of the fraction to the specific activity of the starting material adjusted to

(11) Fractionation of cloudy extracts resulted in the coating of membranes of the convection channel with colloidal material. These runs were accompanied by considerable osmotic transport of solvent into the electrophoresis-convection cell.

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