# Prediction of *in Vitro* Activity of Sulfonamides, Using Hammett Constants or Spectrophotometric Data of the Basic Amines for Calculation

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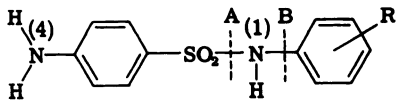
SUMMARY

A homologous series of substituted benzenesulfonamides has been synthesized, and the minimum inhibitory concentration (MIC) against two strains of bacteria has been determined. It is shown that there is an approximately linear relationship between the MIC of the substituted benzenesulfonamides and the $\sigma$-Hammett values as well as the $b$ values calculated from infrared-spectrophotometric data of the substituted anilines used for the synthesis of the benzenesulfonamides. Thereby it is possible to predict the *in vitro* activity of sulfonamides using either Hammett or $b$ values of the basic amines.

## INTRODUCTION

Previous studies on structure activity correlations of benzene- and pyridinecarbothionamides (1, 2) have shown that the stretching frequencies and the infrared (IR)-absorption intensities arising from vibrations of functional groups can be related to their reactivity. However, these studies could only be done using a KBr-disk technique because of the insolubility of the compounds in nonpolar solvents. As pointed out, no claim can be made for the accuracy of the frequency values because of the association effects in the solid state. Moreover, there is no possibility for the determination of absorption intensities in solid state measurements. These difficulties could be overcome by studying the benzonitriles, which are the precursors of benzenecarbothionamides in synthesis. These substances are highly soluble in nonpolar solvent systems and the vibration frequency of interest is very well isolated from other vibrations occurring in the IR spectra of the benzonitriles. The results obtained (3) have verified the results calculated from the C=S bond of the carbothionamides (2). This paper deals with structure-activity studies on substituted benzene derivatives of sulfanilamide, based on considerations very similar to those discussed above. Since sulfa drugs of this type are highly insoluble in nonpolar organic solvents we thought of studying the substituents rather than the complete sulfa drugs. For this purpose one may split the sulfonamide molecule according to the dotted line $B$, studying, for example, substituted chlorobenzenes, which may be used for the synthesis of the sulfa drugs.




Since it is known for sulfathiazole and sulfapyridines (4, 5) and for some 4-sulfapyrimidines and sulfaphenazole (6, 7) that the substituent may be bound to the $N^1$-nitrogen atom by a double bond, it seems to be more appropriate to split the sulfa drug molecule according to the dotted line $A$, thus studying the substituted anilines. In this paper correlations will be shown between physicochemical properties of these substituted anilines and the antibacterial activities of the corresponding sulfonamides.

Since the discovery of the antimicrobial action of sulfonamides, many studies have been done to get an insight into the mode of action of this type of chemotherapeutic agent and to find some structure-activity correlations which might be helpful for the synthesis of new sulfa drugs and for the prediction of their activity. The theory of Bell and Roblin (8), published in 1942, is the most important and stimulating one. Plotting the minimum inhibitory concentration (MIC) against the $pK_a$ value of several sulfonamides, Bell and Roblin found a bell-shaped curve with a minimum for the MIC between $pK_a$ 6 and 7. This relationship was used to predict the MIC of a sulfonamide by measuring its $pK_a$ value. The reason for this relationship is, according to Bell and Roblin: "the more negative the $SO_2$ group of a sulfonamide type compound, the greater the bacteriostatic activity of the compound. The correlation between acidic dissociation ($pK_a$) and the activity is shown to be directly associated with the negative character of the $SO_2$ group."

When we tried to reexamine the hypothesis of Bell and Roblin we could not find such a good relationship between the $pK_a$ and the MIC for many additional sulfonamides (9). There are sulfonamides with a $pK_a$ value higher or lower than 6 or 7 and still very active, and there are sulfonamides with nearly the same $pK_a$ and different MIC (10). Infrared studies to determine the polarity of the sulfone group in the sulfonamides (9, 12) disproved the hypothesis of Bell and Roblin that a sulfonamide with a more polar $SO_2$ group is more active. Additional studies on the primary amino group in a series of sulfonamides seemed to support our theory (9, 11) that the reactivity of the primary amino group is most important for sulfonamide action. However, further studies on a series of acyl-substituted sulfonamides (10) and studies by Wahl (12), using Raman- and IR-spectrometry, do not support our hypothesis. The difficulties are mostly due to the limitation of IR-spectrophotometry to solid state measurements for this type of compound. The association effects of solid-state measurements overlap the small spectral shifts that can be expected normally. Based on our results in benzenecarbothionamide studies where we have taken for calculation IR data from the benzonitriles used for the synthesis of the carbothionamides we tried to overcome the difficulties in the sulfonamide series by studying the various component amines.

## METHODS

*Synthesis of substituted benzene derivatives of sulfanilamide.* The sulfonamides were prepared according to (a) Crossley *et al.* (13) or (b) Knorr and Rössler (14). Most of the sulfonamides used are described in the literature (15–17).

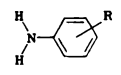
Method a: To a solution of 0.1 mole of the aniline in 25 ml of dry pyridine 0.1 mole of crystalline *p*-acetylaminobenzenesulfochloride is added under stirring. The temperature is kept below 60°. The stirring is maintained for about 2 hr. The solution is then poured into water and the precipitate is collected.

Method b: To a solution of 0.1 mole of the aniline and 0.1 mole of sodium carbonate in water, 0.1 mole *p*-acetylaminobenzenesulfochloride is added slowly under vigorous stirring. The stirring is maintained for about 1 hr, the solution is acidified, and the precipitate is collected. The *p*-acetylaminobenzenesulfonamide is hydrolyzed to the free benzenesulfonamide by short boiling in 50% sulfuric acid or 20% sodium hydroxide. The sulfonamides are recrystallized from ethanol–water for purification. Melting points are given in Table 1, column 7.

Infrared spectra were measured with a Leitz grating spectrophotometer and GeS filter, the apparent slit width being about 4 $cm^{-1}$. Standard absorption lines of polystyrol and a mixture of indene, camphor, and cyclohexanone according to Jones (18) were used as wavelength calibrants. A variable path length cell with sodium chloride windows was used. Measurements were made with different path lengths and several concentrations in the range of 0.01 M.

The anilines used were either commercial products or samples supplied by Hcechst AG, Germany. All were recrystallized or redistilled until physical constants indicated

TABLE 1
*Structure-activity correlations in the sulfonamide series*[a]

| 1 | 2 | 3 | | 4 | 5 | 6 | 7 Sulfonamides synthesized using the amines listed in column 1 | |
|---|---|---|---|---|---|---|---|---|
|  | $\sigma$ Hammett | $\nu_a$ ($cm^{-1}$) | $\nu_s$ ($cm^{-1}$) | $10^5 \times k$ (dyn $cm^{-1}$) | $\theta$ | $b$ | Melting point (°C) | MIC $\mu$moles/l E. coli |
| *p*-$N(CH_3)_2$ | −0.600 | 3453 | 3377 | 6.46 | 109.4 | 0.499 | 234–239 | 45.0 |
| **o-$OCH_3$** | −0.390[b] | 3487 | 3396 | 6.56 | 113.2 | **0.531** | 203–206 | 45.0 |
| **o-$OC_2H_5$** | −0.350[b] | 3486 | 3396 | 6.56 | 112.9 | **0.535** | 150–153 | 45.0 |
| *p*-$OCH_3$ | −0.268 | 3460 | 3382 | 6.48 | 109.9 | 0.504 | 198 | 34.5 |
| *p*-$OC_2H_5$ | −0.250 | 3459 | 3381 | 6.48 | 109.8 | 0.503 | 195 | 32.0 |
| *p*-$CH_3$ | −0.170 | 3470 | 3390 | 6.51 | 110.3 | 0.508 | 188–195 | 27.2 |
| **o-$CH_3$** | −0.170[b] | 3482 | 3398 | 6.55 | 113.2 | **0.520** | 157–158 | 32.0 |
| *m*-$CH_3$ | −0.069 | 3475 | 3393 | 6.53 | 110.8 | 0.512 | 135–137 | 22.5 |
| H | 0 | 3479 | 3396 | 6.54 | 111.1 | 0.514 | 156–157 | 16.0 |
| *m*-$OC_2H_5$ | +0.115 | 3482 | 3397 | 6.55 | 111.6 | 0.518 | 180 | 13.3 |
| *m*-$OCH_3$ | +0.115 | 3483 | 3398 | 6.55 | 111.6 | 0.518 | 164–166 | 11.2 |
| **o-Cl** | +0.200[b] | 3494 | 3401 | 6.58 | 113.7 | **0.535** | 174–175 | 2.8 |
| *p*-Cl | +0.227 | 3482 | 3398 | 6.55 | 111.3 | 0.516 | 202 | 16 |
| *p*-Br | +0.232 | 3485 | 3399 | 6.56 | 111.8 | 0.521 | 203–206 | 11.25 |
| *p*-I | +0.276 | 3486 | 3398 | 6.55 | 112.4 | 0.525 | 208–216 | 10.1 |
| *m*-I | 0.352 | 3487 | 3399 | 6.56 | 112.3 | 0.525 | 132–134 | 8 |
| *m*-Cl | 0.373 | 3490 | 3402 | 6.575 | 112.4 | 0.525 | 129–135 | 8 |
| *m*-Br | 0.391 | 3490 | 3401 | 6.57 | 112.6 | 0.527 | 138–141 | 11.25 |
| *m*-$NO_2$ | 0.710 | 3497 | 3407 | 6.60 | 112.9 | 0.529 | 180 | 2 |
| *p*-$COCH_3$ | 0.874[c] | 3502 | 3410 | 6.61 | 113.4 | 0.533 | 195–199 | 1.4 |
| *p*-$NO_2$ | 1.27[c] | 3509 | 3416 | 6.64 | 113.6 | 0.535 | 169–172 | 1.4 |
| **o-$NO_2$** | — | 3527 | 3407 | 6.66 | 121.1 | **0.583** | 179 | 1.4 |

[a] Structures of anilines used in this study (column 1); $\sigma$-Hammett constant for the substitutents of the anilines (column 2); the position of the symmetric $\nu_s$ and the antisymmetric $\nu_a$ stretching vibration of the amino group in anilines (columns 3); the N—H stretching vibration force constant $k$ calculated from $\nu_s$ and $\nu_a$ using Eq. (I) (column 4); the H—N—H bond angle $\theta$, calculated from $\nu_s$ and $\nu_a$ using Eq. (II) (column 5); the "s" character of the nitrogen hybrid orbitals of the N—H bonds expressed as the coefficient $b$ using Eq. (III) (column 6); and the melting point and minimum inhibitory concentration (MIC) (columns 7) of the sulfonamides synthesized by using the anilines listed in column 1. The ortho-substituted compounds are printed in boldface type.

[b] Using Taft's ortho-substituent parameter.

[c] Special $\sigma$-values for aniline derivatives (37).

purity. The carbon tetrachloride used as solvent was spectral grade (Merck AG., Darmstadt, Germany). The force constant $k$, the bond angle $\theta$, and the coefficient $b$ were calculated from the stretching frequencies of the primary amino group of the anilines using the following equations (see references 19, 20):

$$k = \frac{\pi^2 \times c^2 \times m'}{\frac{1}{M'} + \frac{1}{M''}} (\nu_s + \nu_a)^2 \text{ dyne cm}^{-1}$$

where: $c$ = velocity of light $2.99776 \times 10^{10}$ cm $sec^{-1}$;
$m$ = mass of a particle having molecular weight of 1;
$M'$ and $M''$, atomic weight of the atom participated, e.g., $NH_2$: $M_N = 14$. $M_H = 1$.

$$\cos\theta = \left(1 + \frac{M'}{M''}\right) \cdot \frac{\left(\frac{\nu_a}{\nu_s}\right)^2 - 1}{\left(\frac{\nu_a}{\nu_s}\right)^2 + 1}$$

$$b^2 = -\cos\theta/(1 - \cos\theta)$$

*Microbiological assay method.* The MIC of the $N^1$-benzenesulfonamides was determined according to Krüger-Thiemer *et al.* (21) in a special standardized bacteriological screening test. The strains of bacteria used were *Escherichia coli* (mutaflor) and *Mycobacterium smegmatis* 169. Readings were taken after 24 hr (*E. coli*) or 4 days (*M. smegmatis*) of growth on a synthetic Sauton medium. The MIC is given as the lowest concentration of the sulfa drug that showed inhibition of bacterial growth. The dilution steps used were $1:\sqrt{2}$. The MIC determined is given as $\mu$M.

## RESULTS AND DISCUSSION

To keep the model as simple as possible and to have amines which are easily soluble in nonpolar solvent systems for IR- and NMR-spectrometry we have chosen benzeneamines, substituted in ortho, meta, and para position. The differences in the *in vitro* activity of the synthesized benzenesulfonamides were large enough to look for some structure-activity relationships (Table 1, column 7). As shown by Fig. 1, there is an approximately linear relation between the $\sigma$-Hammett values (22) and the logarithm of the MIC. Similar approaches for other systems have been made by Hansch, Zahradnik, Boček, and their associates (23–27). From this plot it can be seen that the more positive the $\sigma$-Hammett constant of the amine, the more active the corresponding sulfonamide, with the exception of the orthohalogenated amine, which proved to give a more active sulfonamide than could be expected by its $\sigma$-Hammett value (Table 1). Thus, there is a simple Hammett or Taft relationship between the activity of a sulfonamide and its correlated amine. This can be useful for the synthesis of new sulfonamides and the prediction of their activity. Limiting is the fact that only a very small number of Hammett constants are available and only for very simple amine molecules. For this reason we looked for other molecular parameters of the amines which could be used for the prediction of sulfonamide activity instead of or in addition to $\sigma$-Hammett values.

Many authors have shown that the

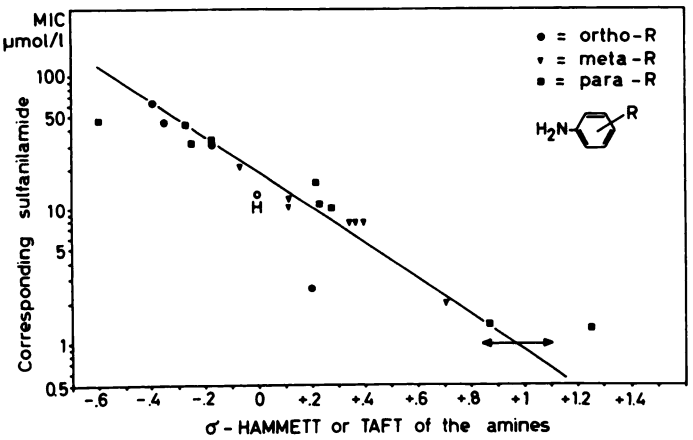



FIG. 1. *Plot of the $\sigma$-Hammett values (Table 1, column 2) of the amines (Table 1, column 1) versus the logarithm of the MIC (Table 1, columns 7) of the corresponding sulfonamides*

For *o*-nitroaniline no $\sigma$-Hammett is available, only the MIC of the corresponding sulfonamide is given (arrow).

stretching frequencies and the absorption intensities arising from vibrations localized in functional groups attached to an aromatic ring can be related to the electronic nature of the ring substituent constant (28, 29), based on reactivity studies. Correlations have been published, for instance, for the OH stretching vibrations in phenols (30), the C≡N stretching vibrations in benzonitriles (3, 30–35), the C=O stretching vibrations in aldehydes (36), and the $NH_2$ stretching vibration in anilines (19, 35, 37–40). Meanwhile these correlations, which had remained so long largely empirical are based on a more satisfactory theoretical treatment. Brown (41) has shown, on the basis of molecular orbital calculation, that such correlation of spectrophotometric parameters with reactivity constants are reasonable, since the changes in electron distribution which occur in the molecule during vibrational distortions closely parallel those which occur in the formation of the transition state during chemical reactions.

The N—H stretching vibrations in substituted anilines have already been studied in great detail by Krueger and co-workers (35, 37) and in heterocyclic amines by Mason (19). Infrared data for some of the anilines used in this study have not yet been published, as far as we know. Therefore we decided to measure all the amines again to have better comparable data. The data obtained by us from the stretching frequencies of the amino group are in excellent agreement with those obtained by Krueger (35, 37). We tried to find a correlation of either the stretching frequencies, the force constant ($k$), the band intensities, or the "s" character of the nitrogen hybrid orbitals of the N—H bonds expressed as the coefficient $b$ of the amines to the MIC of the corresponding sulfonamides, where the relation between $b$ and the nitrogen hybrid orbitals is given by

$$\psi_{\text{hybrid}} = b\psi_s + \sqrt{(1 + b^2)}\,\psi_b.$$

Each of these parameters gave acceptable correlations to the *in vitro* activity of the sulfonamide. We decided to use the "s" character, for it had the best correlation and it is possible to interpret the results in a more fundamental manner. The HNH bond angle calculated from the N—H stretching frequencies using the Eqs. I (20) and II (19, 40)

$$k = \frac{\pi^2 \times c^2 m}{\frac{1}{M'} + \frac{1}{M''}} (\nu_s + \nu_a)^2 \text{ dyne cm}^{-1} \quad \text{(I)}$$

$$\cos\theta = 1 + \frac{M'}{M''} \times \frac{\left(\frac{\nu_a}{\nu_s}\right)^2 - 1}{\left(\frac{\nu_a}{\nu_s}\right)^2 + 1} \quad \text{(II)}$$

increases smoothly from 109.4° in *p*-dimethylaminoaniline to 111.1° in aniline, to 113.6° in *p*-nitroaniline (19). The increase in the bond angle is synchronized with the change of the nitrogen atom from almost pure $sp^3$ hybridization toward a state with higher s:p ratio when the lone pair electrons become more and more delocalized over the aromatic ring. The increase in "s" character is accompanied by an increase in the $NH_2$ stretching frequencies and in the C—N stretching frequencies as these acquire more and more double-bond character.

The constant $b$ is the coefficient of the 2s orbital of nitrogen in the hybrid (19). It can be used as a measure of the "s" character of the hybrid nitrogen orbitals binding the hydrogen atoms and can be calculated by the following equation:

$$b^2 = -\cos\theta/(1 - \cos\theta) \quad \text{(III)}$$

For some *p*-substituted anilines the calculated $b$ values are already available in the literature (42). The results of our calculations are given in Table 1 together with data of the $NH_2$ stretching frequencies and the bond angles. The double bond character expressed by $b$ increases as the substituents become more and more electron-withdrawing. Plotting the $b$ values of the amines against the logarithm of the MIC of the sulfonamides, we get an approximately linear relationship (Fig. 2), showing that the sulfonamide becomes more active with increasing $b$ values, i.e., with increasing double-bond character of the C—N bond. The aniline derivatives with ortho-substituents deviate from the line in most cases. This behavior can be explained by intramolecular hydrogen bonding between the protons of the primary amino group and the

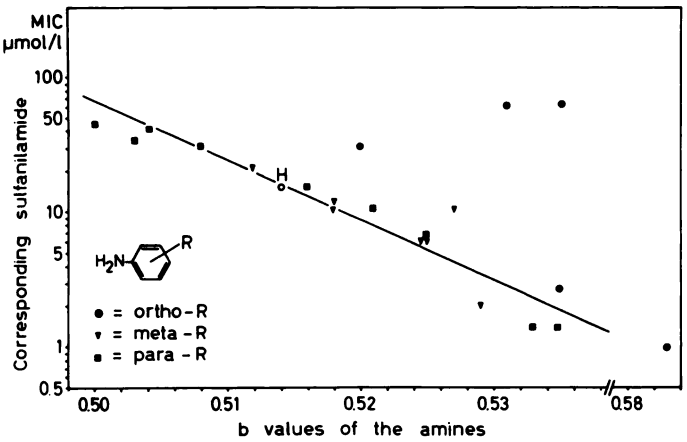



FIG. 2. *Plot of the b values (Table 1, column 6, calculated by Eq. III) of the amines versus the logarithm of the MIC of the corresponding sulfonamides (Table 1, column 7)*

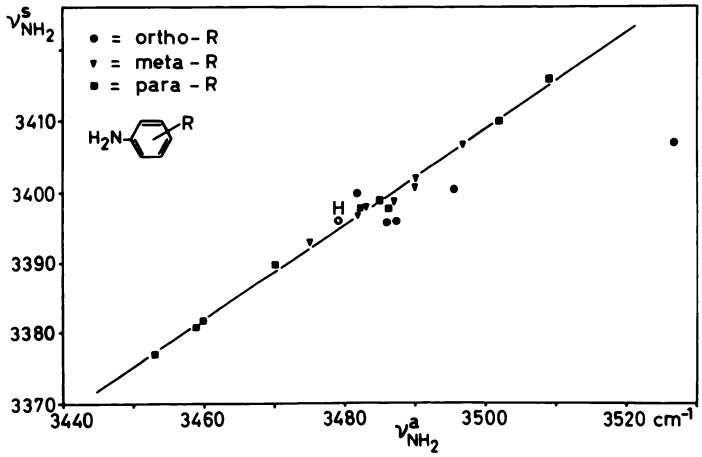



FIG. 3. *Plot of symmetric $\nu^s_{NH_2}$ versus the antisymmetric $\nu^a_{NH_2}$ stretching frequencies (Table 1, column 3) of the anilines used for the synthesis of sulfonamides*

substituents in the ortho position. The hydrogen bonding causes a larger bond angle, calculated from IR data, than can be attributed to delocalization of the lone electron pair only (Fig. 3). The preliminary studies on heterocyclic amines show that the relationship described above holds in general and is not limited to amines of the aniline type. Additional linear combination of atomic orbitals calculations and nuclear magnetic resonance data, which are useful for the correction of IR data of ortho-substituted sulfonamides will be published shortly.

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REFERENCES

1. S. Kakimoto, J. K. Seydel and E. Wempe, *Jahrb. Borstel* **5**, 240 (1961).
2. J. K. Seydel, E. Wempe and R. Fetting, *Arzneimittel-Forsch.* **13**, 200 (1963).

3. J. K. Seydel, *Z. Naturforsch.* **16b**, 419 (1962).
4. S. J. Angyal and W. K. Warburton, *Australian J. Sci. Res. Ser. A.* **4**, 93 (1951).
5. R. G. Shepherd, A. C. Bratton and K. C. Blanchard, *J. Am. Chem. Soc.* **64**, 2532 (1942).
6. J. K. Seydel, *Proc. 3rd Intern. Congr. Chemotherapy, Stuttgart, 1963*, Vol. 1, p. 617 (1964).
7. J. K. Seydel and E. Krüger-Thiemer, *Arzneimittel-Forsch.* **14**, 1294 (1964).
8. P. H. Bell and R. O. Roblin, *J. Am. Chem. Soc.* **64**, 2905 (1942).
9. J. K. Seydel, E. Krüger-Thiemer and E. Wempe, *Z. Naturforsch.* **15b**, 620 (1960).
10. J. K. Seydel and E. Wempe, *Arzneimittel-Forsch.* **14**, 705 (1964).
11. J. K. Seydel, E. Krüger-Thiemer and E. Wempe, *Jahrb. Borstel* **5**, 652 (1961).
12. M. Wahl, Spektroskopische Untersuchungen an einigen Sulfonamiden. Diplomarbeit a.d. I. Physik. Institut der Universität München, Abt. Prof. Brandmüller (May 1965).
13. M. L. Crossley, E. H. Northey and M. E. Hultquist, *J. Am. Chem. Soc.* **62**, 374 (1940).
14. L. Knorr and P. Rössler, *Ber. Deut. Chem. Ges.* **36**, 1279 (1903).
15. H. Erlenmeyer, M. Aeberli and E. Sorkin, *Helv. Chim. Acta* **30**, 2066 (1947).
16. R. Behnisch, *Chem. Ber.* **81**, 297 (1948).
17. C. Marchant, C. C. Lucas and L. McClelland (Appendix by P. H. Greey), *Can. J. Res.* **20**, 5 (1942).
18. R. N. Jones, P. K. Faure and W. Zaharias, *Rev. Universelle Mines* [9] **15**, 417 (1959).
19. S. F. Mason, *J. Chem. Soc.* **1958**, 3619 (1958).
20. D. Barnard, J. M. Fabian and P. H. Koch, *J. Chem. Soc.* **1949**, 2442 (1949).
21. E. Krüger-Thiemer, E. Wempe and M. Töpfer, *Arzneimittel-Forsch.* **15**, 1309 (1965).
22. L. P. Hammett, *J. Am. Chem. Soc.* **59**, 96 (1937).
23. C. Hansch and T. Fujita, *J. Am. Chem. Soc.* **86**, 1616 (1964).
24. C. Hansch, E. W. Deutsch and R. N. Smith, *J. Am. Chem. Soc.* **87**, 2738 (1965).
25. C. Hansch, K. Kiehs and G. L. Lawrence, *J. Am. Chem. Soc.* **87**, 5770 (1965).
26. R. Zahradnik, *Experientia* **18**, 534 (1962).
27. K. Boček, J. Kopecký, M. Krivucová and D. Vlachová, *Experientia* **20**, 667 (1964).
28. L. P. Hammett, "Physical Organic Chemistry," p. 186. McGraw-Hill, New York, 1940.
29. H. H. Jaffé, *Chem. Rev.* **53**, 191 (1953).
30. P. J. Stones and H. W. Thompson, *Spectrochim. Acta* **10**, 17 (1957).
31. H. W. Thompson and G. Steel, *Trans. Faraday Soc.* **52**, 1451 (1956).
32. M. W. Skinner and H. W. Thompson, *J. Chem. Soc.* **1955**, 487 (1955).
33. P. Sensi and G. G. Gallo, *Gazz. Chim. Ital.* **85**, 235 (1955).
34. E. Lippert, *Z. Elektrochem. Ber. Bunsenges. Physik. Chem.* **59**, 534 (1955).
35. P. J. Krueger and H. W. Thompson, *Proc. Roy. Soc.* **A250**, 22 (1959).
36. H. W. Thompson, R. W. Needham and D. Jameson, *Spectrochim. Acta* **9**, 208 (1957).
37. P. J. Krueger and H. W. Thompson, *Proc. Roy. Soc.* **A243**, 143 (1957).
38. M. S. C. Flett, *Trans. Faraday Soc.* **44**, 767 (1948).
39. S. Califano and R. Moccia, *Gazz. Chim. Ital.* **86**, 1014 (1956).
40. J. W. Linnett, *Trans. Faraday Soc.* **41**, 223 (1945).
41. T. L. Brown, *J. Phys. Chem.* **64**, 1798 (1960).
42. P. J. Krueger, *Z. Naturforsch.* **17a**, 692 (1962).