[Chem. Pharm. Bull. 18(8)1553—1557(1970)] UDC 545.822 : 547.551.04 : 577.15.02

Spectroscopic Studies on Molecular Interactions. V.[1] Charge-Transfer Property and Enzymatic Acetylation of Aniline Derivatives

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(Received January 30, 1970)

The first ionization potential, I_P, of 10 aniline derivatives was estimated from the charge–transfer absorption spectra arizing in the 510—560 mμ region due to their interactions with *p*-chloranil. The relation of I_P to the rate of enzymatic acetylation, V_{Ac}, indicated that V_{Ac}, and possibly affinity to the enzyme, increased with an increase in charge–transfer ability of the anilines. But it was also found that V_{Ac} became greater with an increase in basicity of the anilines; this was compatible with the theory for the usual organic acylation. Accordingly, whether or not the charge–transfer was actually concerned with the enzymatic reaction could not be determined from this study, except from the standpoint that organic ionic reactions were generally involved in the transfer of charge. The relation of I_P to pK_a was theoretically discussed.

There may be several properties determining the therapeutic activity of drugs, including affinity to enzymes. Recently, on the basis of a quantum–chemical calculation, Yomosa[3] has suggested that enzyme–substrate complexes may generally be charge–transfer complexes formed with the aid of a local field in living systems. In the previous paper,[1] it has been demostrated that the affinity of sulfonamides to acetylation enzymes increases with an increase in electron–donating ability of the compounds. It was expected that the rate of enzymatic acetylation was related to the enzyme–substrate affinity, and consequently to the charge–transfer ability of the substrates. Accordingly, in this paper, electron–donating ability of 10 aniline derivatives was spectroscopically determined by utilizing their interactions with *p*-chloranil, and the relations of the charge–transfer ability to the rate of enzymatic acetylation and other properties of the anilines were investigated.

Experimental

Materials——Aniline, *p*-benzoquinone, and their derivatives were of JIS special grade or the like, and purified by redistillation or recrystallization. Their boiling points or melting points were found to be similar to those listed in chemical handbooks and literatures. Purified methanol was used as the solvent throughout the investigation as in the previous study[1] in view of the solubility of the materials. The electron–donating effect of methanol on the spectra of *p*-benzoquinones was negligible under the conditions employed.

Measurements of Difference Spectra——The difference spectra were measured of donor–acceptor systems *vs.* acceptor in 1 cm cells at room temperature (17—20°) with a Hitachi model EPS-2 recording spectrophotometer about one week after preparing the test solutions. Standing for a week was necessary for the development of a new band in the case of *p*-nitroaniline, the weakest donor.

Determination of pK_a——The pK_a's of *p*-aminohippuric acid and *p*-aminosalicylic acid were evaluated to be 2.90 and 2.18, respectively, in water at 25° by the spectral method.[4] The UV spectra at several pH's were measured with a Shimazu model MPS-50L multipurpose spectrophotometer. The pK_a values for other compounds were refered to literatures.[5]

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[1] Part IV: I. Moriguchi and N. Kaneniwa, *Chem. Pharm. Bull.* (Tokyo), **17**, 2554 (1969).

[2] Location: *Hatanodai, Shinagawa-ku, Tokyo.*

[3] S. Yomosa, *Prog. Theor. Phys.*, **40**, Suppl. 249 (1967); "Seitai-Kōbunshi to Ion," ed. by N. Imai, T. Ooi, U. Kishimoto, M. Nagasawa, and S. Yomosa, Kagakudojin, Kyoto, 1968, p. 45.

[4] A. Albert and E.P. Serjeant, "Ionization Constants of Acids and Bases," Methuen & Co., London, 1962.

[5] N.F. Hall, *J. Am. Chem. Soc.*, **52**, 5115 (1930); "Kagaku Benran," ed. by Chemical Society of Japan, Maruzen Co., Tokyo, 1966, p. 1053.

Result and Discussion

p-Benzoquinone derivatives, known as good electron-acceptors,[6] have absorption maxima usually in the region of below 400 mμ in methanol, but in the presence of aniline new absorption bands arise in the 490—550 mμ region (Fig. 1). Between the transition energy for the new absorption bands and the electron affinity[7] of *p*-benzoquinones a good linear-relationship is recognized (Fig. 2). Furthermore, the observed peak position (545 mμ) for aniline–*p*-chloranil system is coinsident with that of the charge-transfer absorption band predicted from the observed ionization potential (7.69—8.23)[8] of aniline. Therefore, the new bands are thought to be charge-transfer ones indicating the formation of charge-transfer complexes between aniline and *p*-benzoquinones.[9] The similar spectral changes of *p*-benzoquinones were observed also in cases of several aniline derivatives, of which the rate of enzymatic acetylation was known.[10]

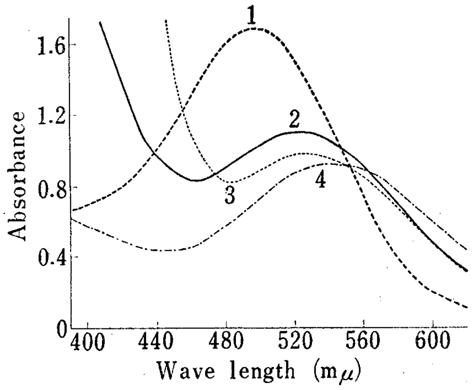

Fig. 1. Difference Spectra of Mixed Solutions of Aniline with *p*-Benzoquinone Derivatives in Methanol

1.25×10^{-3}M aniline with 2.50×10^{-3}M benzoquinones: 1. *p*-benzoquinone, 2. 2,5-dichloro-*p*-benzoquinone, 3. 2,6-dichloro-*p*-benzoquinone, and 4. *p*-chloranil (*vs.* 2.50×10^{-3}M benzoquinones)

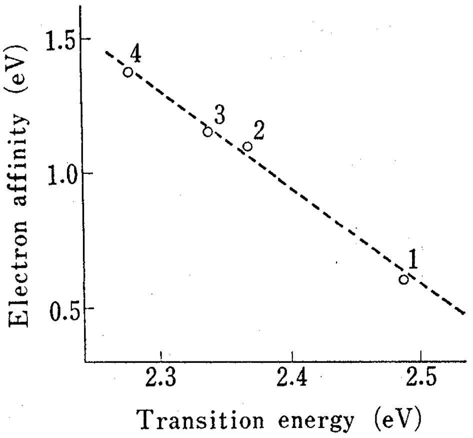

Fig. 2. Relation of Electron Affinity of *p*-Benzoquinones to Transition Energy for the New Absorption Bands of Aniline–Benzoquinone Systems

benzoquinones: 1. *p*-benzoquinone, 2. 2,5-dichloro-*p*-benzoquinone, 3. 2,6-dichloro-*p*-benzoquinone, and 4. *p*-chloranil

Consequently, the first ionization potential, I_P, of the anilines was estimated by utilizing the charge-transfer bands with *p*-chloranil. The following equation[11] was used for the calculation of I_P.

$$h\nu_{CT} = I_P - C_1 + C_2/(I_P - C_1) \qquad (1)$$

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[6] L.J. Andrews and R.M. Keefer, "Molecular Complexes in Organic Chemistry," Holden-Day, Inc., San Francisco, 1964.

[7] G. Briegleb, *Angew. Chem.*, **76**, 326 (1964).

[8] L.M. Branscomb, "Advances in Electronics and Electron Physics," Vol. 9, Academic Press, New York, 1957; K. Watanabe and J.R. Mottl, *J. Chem. Phys.*, **26**, 1773 (1957); F.I. Vilesov, *Soviet Phys. Usp.*, **6**, 888 (1964).

[9] In cases of dichloro-*p*-benzoquinone complexes, their spectra in Fig. 1 suggest that a considerable amount of inner complexes or reaction products may be mingled.

[10] K.B. Jacobson, *J. Biol. Chem.*, **236**, 343 (1961).

[11] S.H. Hastings, J.L. Franklin, J.C. Schiller, and F.A. Matsen, *J. Am. Chem. Soc.*, **75**, 2900 (1953).

Here $h\nu_{CT}$ is the charge–transfer transition energy which can be obtained from the wave length of the charge–transfer band, λ_{CT}, and the constants C_1 and C_2 are 5.70 eV and 0.44 (eV)^2, respectively, for *p*-chloranil complexes.[12] The values of λ_{CT}, $h\nu_{CT}$, and I_P are listed in Table I.

Table I. Charge–Transfer Properties of Aniline Derivatives with *p*-Chloranil

Run. No. anilines	λ_{CT} (mμ)	$h\nu_{CT}$(eV)	I_P (eV)
1 *p*-toluidine	554	2.238	7.720
2 aniline	545	2.275	7.762
3 *p*-bromoaniline	541	2.292	7.781
4 *p*-chloroaniline	540	2.296	7.785
5 *p*-aminohippuric acid	535	2.318	7.809
6 *p*-aminosalicylic acid	535	2.318	7.809
7 sulfanilic acid	535	2.318	7.809
8 *p*-aminobenzoic acid	532.5	2.329	7.822
9 sulfanilamide	528.5	2.346	7.840
10 *p*-nitroaniline	516	2.403	7.903

The relative rate for acetylation, V_{Ac}, of 10 aniline derivatives catalyzed by an acetyl transferase purified from pigeon liver was measured by Jacobson.[10] Fig. 3 is the plot of the values of V_{Ac} against I_P, showing a linear-relationship. For *p*-nitroaniline, the deviation from the line in Fig. 3 is considerably large, but the reason is unclear. The correlation coefficient (except *p*-nitroaniline) is -0.75 (significant at <0.02 level), indicating that the rate of the acetylation increases with an increase in electron–donating ability of the anilines.

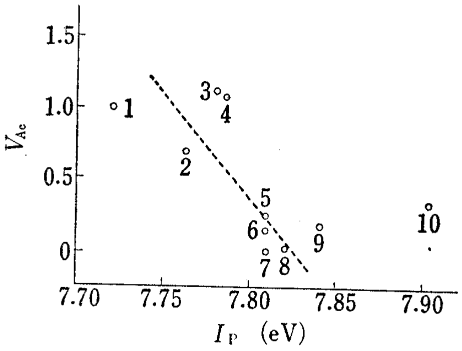

Fig. 3. Correlation of Relative Rate for Enzymatic Acetylation, V_{Ac}, with I_P

For numbering, see Table I.
correlation coefficient: -0.75 (except 10)

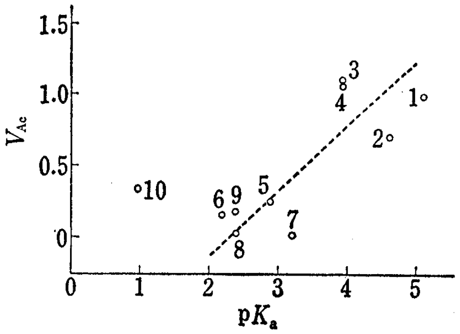

Fig. 4. Relationship between V_{Ac} and pK_a

For numbering, see Table I.
correlation coefficient: 0.80 (except 10)

It is expected that the rate of enzymatic reaction is related to the enzyme–substrate affinity, which is approximately represented by the reciprocal of K_m, the Michaelis constant, in the Michaelis–Menten enzyme kinetics.[13] At relatively low concentration of substrate, the rate constant of enzymatic reaction is approximately given as V_m/K_m,[14] where V_m denotes the maximal velocity of the reaction. In the enzymatic acetylation of the amino group of 16 sulfonamides with pigeon liver extract, the correlation coefficient of log K_m with log (V_m/

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[12] G. Briegleb and J. Czekalla, *Z. Elektrochem.*, 63, 6 (1959).

[13] E.E. Conn and P.K. Stumpf, "Outlines of Biochemistry," John Wiley & Sons, Inc., New York, 1963, Chapter 7.

[14] F.M. Snell, S. Schulman, R.P. Spencer, and C. Moos, "Biophysical Principles of Structure and Function," Addison-Wesley Publishing Co., Inc., Reading, Massachusetts, 1965, p. 349.

K_m)[15] was calculated to be -0.69 (significant at <0.01 level). This may indicate that the rate of enzymatic acetylation with pigeon liver enzymes increases with an increase in enzyme–substrate affinity. Therefore, the relationship between V_{Ac} and I_P (Fig. 3) may be in accord with the supposition that charge–transfer is concerned with enzyme–substrate complexations.

This relationship, however, may not be a positive evidence for the transfer of electronic charge between enzyme and substrate, because it is observed that V_{Ac} is also correlated with pK_a of the anilines (Fig. 4). The correlation coefficient (except *p*-nitroaniline, which exhibits large deviation as well as in the case of V_{Ac} with I_P) is 0.80 (significant at <0.01 level), indicating that the reaction rate becomes greater with an increase in basicity of the aniline derivatives. This is compatible with the theory[16] for the usual organic acylation where no enzyme is involved, and Jacobson[10] also has explained the data of V_{Ac} in terms of nucleophilic character of the amino group.

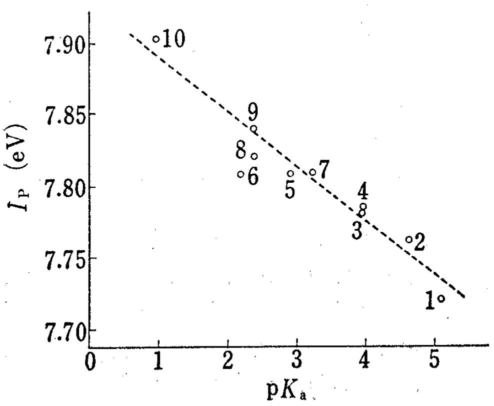

Fig. 5. Correlation of pK_a with I_P

correlation coefficient: -0.95
For numbering, see Table I.

From these relations shown in Fig. 3 and 4, it is predicted that pK_a is directly related to I_P. Fig. 5 is the plot of pK_a *vs.* I_P, exhibiting a good linear-relationship. It is known[17] that the energy of the highest occupied molecular-orbital, ε_{ho}, is a measure of electron-donating ability or I_P of molecules. And it is generally recognized[18] that pK_a or the Hammett constant is approximately correlated with the electron density on the atom (or atoms) concerned with ionization or reaction of molecules. Let $\Delta\alpha_X$ be the difference in Coulomb integral between the atoms X and X′ of aniline derivatives *p*-NH_2–C_6H_4–X and *p*-NH_2–C_6H_4–X′, respectively, for example. Then, according to the perturbation treatment of conjugated molecules in the simple LCAO MO method,[19] the difference in ε_{ho} between the two derivatives is expressed as

$$\Delta\varepsilon_{ho}=(C_X^{ho})^2\Delta\alpha_X+\sum_{k(\neq ho)}^{all}\frac{(C_{ho}^{X}C_X^{k})^2}{\varepsilon_{ho}-\varepsilon_k}(\Delta\alpha_X)^2+\cdots\cdots \tag{2}$$

where C_X^k is the atomic–orbital coefficient of the atom X in the *k*th molecular–orbital of *p*-NH_2–C_6H_4–X, C_X^{ho} is that in the highest occupied orbital, and ε_k refers to the energy of the *k*th molecular–orbital. Similarly, the difference in π-electron density on the amino-nitrogen atom between the two derivatives, Δq_N, is given as

$$\Delta q_N=\pi_{NX}\Delta\alpha_X+(\cdots\cdots)(\Delta\alpha_X)^2+\cdots\cdots\cdots \tag{3}$$

where π_{NX} is the atom–atom polarizability between the amino-nitrogen and X. In the first approximation, therefore, both $\Delta\varepsilon_{ho}$ and Δq_N are proportional to $\Delta\alpha_X$, and consequently $\Delta\varepsilon_{ho}$ is proportional to Δq_N. This may be valid with *meta*- or *ortho*-substituted anilines, and moreover, approximately applicable even in the case where the substituent is not an atom but a group.

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[15] The values of K_m and V_m were measured by Kakemi, *et al.* (K. Kakemi, T. Arita, and T. Koizumi, *Yakuzaigaku*, **25**, 22 (1965)).
[16] Y. Ogata, "Yūki-Hannōron," Maruzen Co., Tokyo, 1955.
[17] T. Koopmans, *Physica*, **1**, 104 (1933).
[18] H.C. Longuet-Higgins, *J. Chem. Phys.*, **18**, 275 (1950); H.H. Jaffé, *ibid.*, **20**, 279, 778, 1554 (1952).
[19] K. Fukui, K. Morokuma, T. Yonezawa, and C. Nagata, *Bull. Chem. Soc. Japan*, **33**, 963 (1960).

Thus, it can be said that charge–transfer ability is approximately correlated with pK_a or the Hammett constant. Accordingly, whether or not the charge–transfer is actually concerned with the enzymatic reaction may not be determined from such a way of approach as this work, except from the standpoint[20] that organic ionic reactions are generally involved in the transfer of change.

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[20] S. Nagakura, *Yuki Gosei Kagaku Kyokai Shi*, 27, 1033 (1969).