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ELECTRONIC ASPECTS OF ENZYMIC ACETYL-TRANSFER REACTIONS

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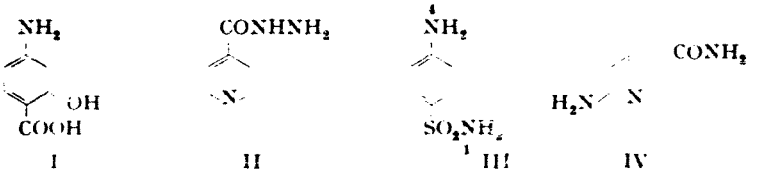
SUMMARY

The rate of enzymic acetylation of amines runs parallel to the electronic charge of the amino nitrogen, provided that the compound is not protonated under the experimental conditions. The ability of conjugated *N*-acetylamines to give up the acetyl group runs parallel to the dipositivity of the dissociable bond. The electronic factors governing the mechanism of transfer of the two-carbon unit are thus identical to those which have been shown previously to be responsible for the mechanism of transfer of one-carbon units by folic acid coenzymes.

INTRODUCTION

Among the important reactions catalyzed by the enzyme acetyl-transferase, with CoA as cofactor, is the acetylation of aliphatic and aromatic amines. This last type of compound involves the therapeutically active *p*-aminosalicylic acid, I (see ref. 1), isoniazid, II (see refs. 2, 3), a series of sulfonamides[4] (*e.g.* sulfanilamide, III) and some carcinostatic antimetabolites like 6-aminonicotinamide, IV (see ref. 4). The acetylation of these amines is due to CoASAc representing the "active acetate".



Recently it was discovered that the same enzymic system can also acetylate a large number of other arylamines such as aniline and its substituted derivatives[5]. Moreover, it has been shown that the acetyl-transferase active in these systems (and in a series of sulfonamides) does not need CoASAc as cofactor but can use instead different acetylated arylamines[6]. The enzyme catalyzes thus the reversible transfer of an acetyl group between different aromatic amines. The available data allow the clas-

Abbreviation: Acetyl-AABS, 4-acetaminoazobenzene-4'-sulfonic acid.

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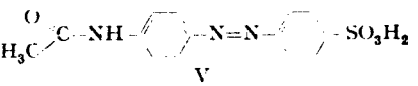
sification of the arylamines from the viewpoint of their relative acetyl-acceptor properties and the classification of the acetyl-arylamines from the viewpoint of their relative acetyl-donor properties.

The present paper is concerned essentially with the interpretation of these properties in terms of the electronic structure of the compounds involved.

I. ACETYL-ACCEPTOR PROPERTIES

The available data have been obtained essentially either with CoASAc[7,8] or with 4-acetaminoazobenzene-4'-sulfonic acid[5,6], V, as the acetyl donors.



V

The results indicate that:

1. When the arylamines are substituted with acidic groups *ortho* to the amino group no acetylation occurs[7]. When they are substituted with acidic groups *para* to the amino groups, acetylation occurs but at a very low rate[5,9]. These results are attributed to the occurrence of strong repulsions between the enzyme and the substrates, due to the presence of the negative charges[5], and to the great tendency of strongly acidic compounds for rapid excretion[9].

2. When non-acidic substituents are present the reaction occurs at a rate depending on the nature of the substituents and is to some extent proportional, at first sight, to their electron releasing capacity[5]. The quantitative data are reproduced in Table I which also contains information about the indices of the electronic structure of the amino groups which may be correlated with the observed reactivity. These indices have

TABLE I

ARYLAMINES AS ACETYL-ACCEPTORS

Amine	*Relative rate of acetylation*				*Electronic charge of the N atom of NH_2*	*Free valence of the N atom of NH_2*	$K_b \cdot 10^{10}$
	*	**	***	§			
p-Bromoaniline	1.12			Acetylated	1.849	1.021	
p-Chloroaniline	1.09				1.849	1.020	1.2§§
p-Methylaniline	1.00				1.853	1.028	12§§
Aniline	0.70	0.79			1.851	1.023	4.2§§
Benzidine		0.74			1.846	1.020	
p-Nitroaniline	0.34			Acetylated	1.827	0.996	0.001§§
Sulfanilamide	0.18	0.17	1.0		1.841	1.010	0.023§§§
Sulfathiazole			0.30		1.841	1.011	0.023§§§
Sulfadiazine			0.20		1.836	1.012	0.010§§§
p-Anisidine				Acetylated	1.861	1.042	15§§
o-Phenylenediamine				Acetylated	1.862	1.048	3.2§§
o-Anisidine				Acetylated	1.859	1.041	3.0§§
o-Toluidine				Acetylated	1.852	1.027	2.5§§
m-Nitroaniline				Acetylated	1.851	1.024	0.032§§
o-Nitroaniline				Not acetylated	1.824	0.991	0.00035§§

* Data from JACOBSON[5].
** Data from BESSMAN AND LIPMANN[6].
*** Data from LIPMANN[8].
§ Data from TABOR, MEHLER AND STADTMAN[7].
§§ Data from MORRISON AND BOYD[11].
§§§ Data from BELL AND ROBLIN[12].

been obtained by quantum-mechanical calculations based on the molecular orbital method[10].

It is observed that, on the whole, the rate of acetylation of related compounds is parallel to the value of the electronic charge and of the free valence of the amino nitrogen. The value of the electronic charge on the amino nitrogen represents the fraction of the "lone-pair" electrons of this atom which remains on it as a result of the participation of these electrons in the over-all π conjugation. The existence of the double parallelism does not allow to decide whether the reaction involves a free radical or an electrophilic mechanism. (For a discussion of a similar situation see ref. 13.) We may note that the parallelism seems, however, to be more complete with the electronic charge of the amino nitrogen than with its free valence.

A few complementary remarks and observations seem interesting:

1. The halogenated derivatives do not obey the correlation very well. The situation may perhaps be due to the approximations involved in calculations concerning these compounds because the parameters associated with the halogens are known with less certainty than those associated with nitrogen or oxygen. The situation may, however, reflect also a genuine exception, related perhaps to the specificity of the enzymic reaction (*vide infra*).

2. In the series of the sulfonamides only the 4-NH_2 group (see III) has been taken into consideration. This is in agreement with experiment which indicates that acetylation occurs much more readily (if not exclusively) on this amino group than on the 1-NH_2 group. It is also consistent with theoretical data: the electronic charge of the 1-NH_2 group of sulfanilamide is 1.730e and its free valence 0.835. Both indices are thus much smaller than the corresponding indices for the 4-NH_2 group.

3. The existence of a parallelism between the rate of acetylation of the arylamines and the electronic charge of their amino nitrogen suggests the possibility of a parallelism between the rate of this reaction and the basicity of the amines, inasmuch as this basicity is determined, too, by the value of the electronic charge on the amino nitrogen. Table II contains the appropriate data. Its examination indicates that if the *ortho*- and *para*-substituted compounds are considered separately (and such a separation is quite legitimate because of the possible *ortho* effects not taken into consideration in the calculations) an excellent correlation is observed between the basicity and the charge of the amino nitrogen. The correlation is even better than that between this charge and the rate of acetylation of the compounds: for instance, the halogenated derivatives satisfy the correlation with basicity, and the sulfonamides may be put in the same group as other *para*-substituted anilines.

4. A similar type of correlation exists between the relative rate of chemical acetylation of mercaptans by the acetate of pyridine-4-aldoxime methiodide and the acid dissociation constant of these mercaptans[14]. Although this reaction is a model for the acetylation of CoA rather than for the acetylation of amines, it is nevertheless significant that the rate of acetylation of thiophenol and of a series of its *para*-substituted derivatives runs parallel to the value of the electronic charge on the S atom and at the same time to the value of its pK_a (Table III). It may be noted that *p*-chlorothiophenol obeys this parallelism. The result suggests that the slightly abnormal behaviour of the halogenated derivatives of aniline during enzymic acetylation may be due to specificity factors.

5. Finally, it may be interesting to compare the results obtained for the acetyla-

tion of arylamines with those found for the acetylation of aliphatic amines. Although the two groups of amines are generally substrates to different enzymes, certain conclusions may be drawn if only from model reactions. The following points must be kept in mind as a basis for the understanding of the experimental data: (a) while the arylamines have a basic $pK_a < 7$ and thus exist at neutral pH largely in the neutral form, the aliphatic amines have a basic pK_a of the order of 9 and exist thus at neutral pH mainly in the protonated form. (b) The lone pair of the amino nitrogen is partially

TABLE II

BASICITY OF ARYLAMINES SUSCEPTIBLE TO ACETYLATION

Compound	$K_b \cdot 10^{10}$	*Charge of the amino nitrogen*	*Free valence of the amino nitrogen*
p-Aminoaniline	110*	1.866	1.051
p-Methoxyaniline	15*	1.861	1.042
p-Methylaniline	12*	1.853	1.028
Aniline	4.2*	1.851	1.023
p-Chloraniline	1.2*	1.849	1.020
Sulfanilamide	0.023**	1.841	1.010
Sulfathiazole	0.023**	1.841	1.011
Sulfadiazine	0.01**	1.836	1.012
p-Nitroaniline	0.001*	1.827	0.996
o-Aminoaniline	3.2*	1.862	1.048
o-Methoxyaniline	3*	1.859	1.041
o-Methylaniline	2.5*	1.852	1.027
o-Chloroaniline	0.05*	1.848	1.019
o-Nitroaniline	0.00035*	1.824	0.991

* Data from MORRISON AND BOYD[11].
** Data from BELL AND ROBLIN[12].

TABLE III

ACETYLATION OF THIOPHENOLS

Compound	*Relative rate of acetylation**	pK_a *(see ref. 14)*
p-Chlorothiophenol	0.0329	5.94
Thiophenol	0.252	6.82
p-Methylthiophenol	0.377	6.86

* Data evaluated from the molecular constant, k_2, at pH 5.0, given by O'NEILL, KOHL AND EPSTEIN[14].

TABLE IV

RATE OF ACETYLATION OF AMINO ACIDS BY ACETYLPHOSPHATE

Compound	*Relative rate of acetylation**	pK_a
Proline	0.9	10.6
Alanine	1.3	9.9
Glycine	10.7	9.8
Benzylamine	17	9.3
Phenylalanine	0.5	9.1
Glycylglycine	36	8.2
Glycine ester	55	7.7

* Data evaluated from the bimolecular coustants at pH 7.3, given by KOSHLAND[16].

delocalized in the arylamines, but, at first approximation, remains localized on that nitrogen in the aliphatic amines. In this last type of amines, inductive effects only influence the electronic charge of the amino nitrogen. The experimental results obtained for enzymic acetylation of histamine[7] and in model acetylation of amines with esters of thiocarboxylic acids[15] or with acetylphosphate[16] indicate, in the first place, that acetylation occurs only on the free, unprotonated compound. In the experiment with acetyl phosphate it is even observed that to a large extent the most reactive amines (in fact, amino acids) are the less basic ones (Table IV). At first sight, this correlation seems to be the reverse of the one observed for the enzymic acetylation of arylamines. In fact, the disagreement is only superficial: the correlation observed in the case of the aliphatic amines simply means that the most reactive amino acids are those which exist in the neutral form in higher percentage. On the other hand aniline, which is a much weaker base than the aliphatic amines, is acetylated only at a very low rate[16] (relative rate at pH 7.3: 2.9). This last result indicates that the factors governing the acetylation of the aliphatic and the aromatic amines are quite similar.

Thus, generally speaking, the ease of acetylation of amines depends essentially on the value of the electronic charge of the amino nitrogen: it is the greater the greater this charge (which frequently means the stronger the basicity), provided that the compound is not protonated under the experimental conditions.

II. ACETYL-DONOR PROPERTIES

The second column of Table V contains a series of data about the ability of different acetylated compounds to give up their acetyl group and the remaining columns of this table indicate the indices of the electronic structure of these molecules which may be related to this ability. It may be observed that as a result of electronic delocalization both the N and C atoms of the dissociable bond carry net positive charges (are electron deficient). The dissociable bond is therefore a dipositive bond in the sense defined by PULLMAN AND PULLMAN in the case, for instance, of energy-rich phosphates[17] and the substrates for enzymic hydrolysis[18]. Now, it can be seen that the ability of conjugated *N*-acetyl compounds to give up the acetyl group is greater the greater the dipositivity of the dissociable bond. To a small extent there is also a certain antiparallelism between the ability of the acetylated compound to give up its acetyl group and (a) the bond order of the dissociable bond or (b) the loss of resonance energy, ΔR, upon deacetylation. It may be interesting to add that the variations in the dipositivity of the dissociable bonds are due essentially to the variations of the net charge of the nitrogen atom, the net charge of the carbonyl carbon of the acetyl group remaining practically stationary in all the compounds studied.

This situation may be stated also in a different way. As we have seen previously, the rate of acetylation of an arylamine is parallel to the electronic charge of its amino nitrogen and, generally, to its basic strength. As the fixation of an acetyl group on a series of related amines, say anilines, brings about comparable perturbations in all these substrates it is obvious that, in general, the better acetyl-acceptor an aniline derivative is, the worse acetyl-donor will the corresponding acetanilide derivative be. It may also be said that in the aniline-acetanilide series, the best acetyl-acceptors are the most basic anilines, and the best acetyl-donors the less basic acetanilides.

A related observation of the same type may be found in the field of the acetyl-

TABLE V

COMPARISON OF CONJUGATED *N*-ACETYL COMPOUNDS AS ACETYL-DONORS

Compound	*Relative rate*	*The net charge on the N atom carrying the acetyl group*	*The net charge of the C atom of the carbonyl carbon*	*The dipositivity of the dissociable bond*	*The bond order of the dissociable bond*	ΔR^* *(in β unit)*
p-Nitroacetanilide	1.00**	+0.265	+0.268	+0.533	0.382	0.359
p-Chloroacetanilide	0.38**	+0.250	+0.267	+0.517	0.386	0.363
p-Methylacetanilide	0.18**	+0.247	+0.267	+0.514	0.387	0.364
Acetanilide	0.08**	+0.248	+0.267	+0.515	0.386	0.363
Acetyl-AABS	High***	+0.268	+0.268	+0.536	0.382	0.358
Acetylsulfanilamide	High***	+0.255	+0.268	+0.523	0.385	0.361
Acetylimidazole	Very high§	+0.488	+0.280	+0.768	0.310	0.287

* ΔR = Decrease of resonance energy upon the departure of the acetyl group.

** Values from JACOBSON[5]. Because of the variation in apparent K_s and V_{max} of these *para*-substituted acetanilides, and although *p*-nitroacetanilide may definitely be considered as the best acetyl-donor, the relative rate of deacetylation of the other compounds must be interpreted with caution.

*** BESSMAN AND LIPMANN[6] observed a reversible transfer of the acetyl group between *N*-acetylsulfanilamide and Acetyl-AABS, the equilibrium point appearing to be near to 1.

§ STADTMAN AND WHITE[20] observed a reversible enzymic transfer of acetyl group from CoASAc to imidazole; observing that, in JACOBSON's system[5], *p*-nitroacetaniiide is only $^1/_{200}$ as effective as CoASAc it can be said that acetyl-imidazole is a much better acetyl donor than the other compounds in Table V.

TABLE VI

DEACETYLATION OF THIOESTERS

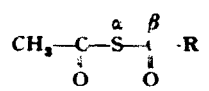
Compound	*Relative rate of deacetylation**	*pK_a of the corresponding mercaptans (see ref. 14)*
Acetylglutathione	0.19	9.20
2-Isopropylaminoethanethio-acetate	0.255	7.70
2-Diethylaminoethanethio-acetate	0.33	7.85
Acetylthiocholine	0.425	< 7.85
Acetylthiophenol	0.50	6.20

* Data evaluated from the molecular constants, at pH 5.0, given by O'NEILL, KOHL AND EPSTEIN[14].

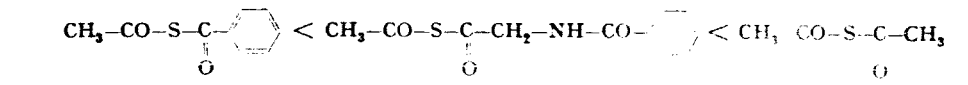
donor properties of a series of thioesters. As can be seen from Table VI the rate of acetyl donation by different thioesters (the acetyl-acceptor being in each case pyridine-4-aldoxime methiodide) runs parallel to the acidity of the corresponding mercaptans.

The *S*-acetyl linkage of all these thioesters is a dipositive bond[19] and this dipositivity is, of course, greater in thiophenol than in the aliphatic thioesters.

Finally, a similar observation may be made about SCHWYZER's results[15] concerning the yield of the acylation of amines RNH_2 by esters of thiocarboxylic acids



as a function of the nature of R. The order of increasing reactivity in three representative compounds, is



It runs parallel to the dipositivity of the dissociable bond (α-β) which amounts to 0.495, 0.501 and 0.517 respectively in the three compounds.

CONCLUSION

The electronic factors involved in the enzymic transfer of the acetyl groups seem thus to be essentially: (a) the value of the electronic charge of the amino nitrogen for the acceptance of the acetyl group and (b) the dipositivity of the dissociable bond for the donation of the acetyl group. It is most striking to observe that these two factors are the same as those which have been shown to be responsible, on the electronic level, for the mechanism of transfer of one-carbon units by folic acid coenzymes[13]. A very far-reaching analogy seems therefore to exist between the electronic factors responsible for the transfer of the metabolically important one- and two-carbon units. Anticipating on studies presently carried out in our laboratory we may add that the same factors seem also to be involved in other metabolic group transfer reactions (*e.g.* *O*- or *N*-methylation and demethylation). It appears thus probable that a large number of metabolic-group transfer reactions are governed by similar electronic factors.

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