BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 40 2229—2233 (1967)

Electronic Aspect of the Antibacterial Activity of Nitrofuran Derivatives[1)]

Koichi HIRANO, Shigetaka YOSHINA,*1 Kimiko OKAMURA and Isamu SUZUKA*2

Department of Natural Sciences, Faculty of Education, Tohoku University, Kawa-Uchi, Sendai

(Received February 24, 1967)

In order to interpret the antibacterial activity of nitrofuran derivatives (N. F.) in connection with their electronic properties, an approximate superdelocalizability, $S_r(N)'$, the frontier electron density, $f_r(N)$, and the energy of the lowest vacant level, k_{lv}, were calculated. The simple Hückel MO method was adopted in this calculation. The activity for typical compounds of N. F. correlated closely with the values of k_{lv}. On the contrary, no significant correlation was found between the reactivity index, $S_r(N)'$, and this activity. These suggest that the bactericidal action of N. F. can be interpreted in terms not of the reactivity of N. F., but of the electron transfer from a biomolecule such as NADH to the N. F. molecule. On the other hand, between NADH and N. F. a molecular complex was found. This enables one to conclude that, by the use of their electron affinity, N. F. molecules inhibit the electron transfer coupled with the NADH of the respiratory chain in the body of bacteria.

Many studies[2-6)] have been made of the bactericidal action of nitrofuran derivatives in connection with their chemical constitution.

It was then found that the nitro group at the 5-position of the furan nucleus plays the most important role in the bactericidal action. On the other hand, an attempt[7)] was made to compare the antibacterial activity of nitrofuran derivatives with their polarographic reduction potential; it was thus found that the activity became stronger as the potential became more positive. Recently several authors have undertaken the study of the correlation between the reactivity indices calculated by the use of the LCAO MO method and the activity of the antibacterial substances. Fukui *et al.*[8)] pointed out that the antibacterial activity of 4-nitroquinoline-*N*-oxides was closely correlated with the approximate nucleophilic superdelocalizability, $S_r(N)'$, at the C atom to which nitro groups are attached.

From this fact the conclusion has been drawn that the antibacterial action of the compounds can be interpreted in terms of nucleophilic displacement between the $\rangle C\text{–}NO_2$ group and the –SH group in the enzymes. This concept was extended by Yoneda and Nitta[9)] to the bactericidal action of 5-nitrofuran derivatives; however, no significant correlation was found between the activity and reactivity index, $S_r(N)'$, at the 5-position of the furan ring, at which the nitro group was attached. These findings indicate that the reactivity index, $S_r(N)'$, is insufficient for interpreting the mechanism of the bactericidal action of nitrofuran compounds. In the present paper the authors will try to elucidate the bactericidal action of nitrofuran derivatives in the light of their electron affinity. We will deal here with 9 furan derivatives: furan, nitrofuran, furfural, nitrofurfural, miranon-M, furazolidon, nitrofurylacrylamide, furacine, and AF-2.

Parameters Used in the Calculation

In the calculation the simple Hückel[10)] molecular orbital method is used, and the secular equation is calculated numerically according to the usual variational method. The Coulomb integral, α_x, of the hetero atom, X, and the resonance integral, β_{rs}, between the r and s atoms, and the Coulomb integral, α_c', of the carbon atom adjacent to the hetero atom, X, are expressed as:

$$\alpha_x = \alpha + h\beta \quad (1)$$

$$\beta_{rs} = k\beta \quad (2)$$

$$\alpha_c' = \alpha + 0.1h\beta \quad (3)$$

The numerical values of parameters used are

1) Presented at 5th Annual Meeting of the Biophysical Society of Japan, Dec., 15, 1966.

*1 Department of Pharmacy, Meijo University, Yagoto, Showa-ku, Nagoya.

*2 Present address: Dapartment of Chemistry, Faculty of Science, Tohoku University, Katahira-cho, Sendai.

2) M. C. Dodd and W. S. Stillman, *J. Pharmacol. Exptl. Therap.*, **82**, 11 (1944).

3) J. Kranz and W. Evans, *J. Pharmacol.*, **85**, 324 (1945).

4) R. F. Raffauf, *J. Am. Chem. Soc.*, **72**, 753 (1950).

5) M. Green, E. Heath and I. Yall, *Proc. Exptl. Biol. Med.*, **76**, 152 (1951).

6) I. Yall and M. Green, *Proc. Exptl. Biol. Med.*, **79**, 306 (1952).

7) T. Sasaki, *Chem. Pharm. Bull.*, **2**, 104 (1954).

8) K. Fukui, A. Imamura and C. Nagata, This Bulletin, **33**, 122 (1960).

9) F. Yoneda and Y. Nitta, *Chem. Pharm. Bull.*, **12**, 1264 (1964).

10) E. Hückel, *Z. Physik*, **70**, 204 (1931).

listed in Table 1. In this case the hyperconjugation of the methyl group is neglected.

As may be seen from Table 2 the dipole moment of furan as calculated by this method is in good

TABLE 1. PARAMETERS[11]

Bond	h	k
=C-C=		0.9
-C=C-		1.1
$-\ddot{N}-$	1.5	0.8
=N-	0.5	1.0
$-N\langle^{O}_{O}$ (h_N)	1.0	(k_N) 1.0*1
$-N\langle^{O}_{O}$ (h_O)	1.0	(k_O) 1.0
$-\ddot{O}-$	2.0	0.8
=O	1.0	1.0
$-\ddot{N}-N=$		0.8*2

TABLE 2. DIPOLE MOMENT (DEBYE UNIT) OF FURAN

Calculated:	0.90*3
Experimental:	0.67—0.71[12,13]

TABLE 3. BOND-DELOCALIZATION ENERGIES (kcal/mol)*4

Compound	Furan	Furfural	Nitrofuran
Bond-delocalization energy	20	32	82
	21—23 (Exptl.)[14]		
Aromaticity of the furan ring	Furan < Furfural < Nitrofuran		

agreement with the experimental value. Table 3 lists the calculated values of the bond-delocalization energy for furan, furfural, and nitrofuran. These quantities are consistent with the chemical stability of the compounds.

Figure 1 illustrates the linear correlation curve of the half-wave reduction potential, $E_{1/2}$,*5 *vs.*

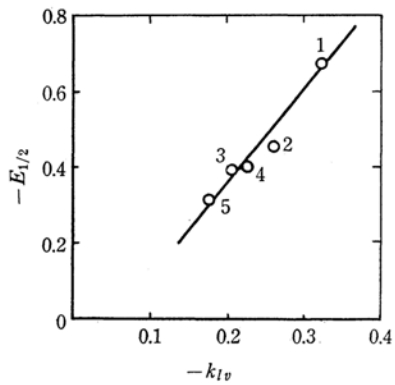


Fig. 1. The linear correlation curve of $-E_{1/2}$ *vs.* $-k_{lv}$.
$E_{1/2} = -bk_{lv} + c \quad b = -2.5$ eV

1 NO_2-furan-O

2 NO_2-furan-O-$CH{:}N{\cdot}NCONH_2$

3 NO_2-furan-O-$CH{:}CHCONH_2$

4 NO_2-furan-O-$CH{:}CH{\cdot}C{\cdot}CH{:}CH$-furan-O-$NO_2$
$H{\cdot}\overset{\overset{N}{\|}}{N}{\cdot}C(=NH)(NHCH_3)$

5 NO_2-furan-O-CHO

$-E_{1/2}$ *vs.* NHE

the lowest energy of the vacant level, $-k_{lv}$. From the slope of the curve, β value of -2.5 eV is determined.

This β value agrees closely with the corresponding values of similar compounds.[15] Therefore, the parameters used are adequate for the qualitative calculation of the electronic structures of the nitrofuran derivatives.

Results and Discussion

It was supposed hitherto that nitrofuran derivatives reacted *in vivo* with the SH-enzymes[16] or the nitro reductive enzymes[17] in the earliest stage of bactericidal action. Regarding the SH-enzymes, the nucleophilic displacement at the 5-position in the furan ring was assumed. Hence, we have also taken the quantities of $S_r(N)'$, $E_{1/2}$ and $-k_{lv}$ into account. Table 4 summarized the approximate nucleophilic superdelocalizability, $S_5(N)'$,[18] at the 5-position of the furan ring, at which the nitro group is attached; the polarographic half-wave reduction potential, $E_{1/2}$, and the

11) A. Streitwieser, Jr., "Molecular Orbital Theory for Organic Chemist," John Wiley and Sons, Inc., New York (1961), p. 135.

*1 J. I. F. Alonso, *Compt. rend.*, **233**, 403 (1951).

*2 The k value for $-\ddot{N}=N-$ was estimated from bond dissociation energy and bond length of -N=N- linkage. G. E. Coates and L. E. Sutton, *J. Chem. Soc.*, **1948**, 1187. W. J. Orville-Thomas, *Chem. Revs.*, **57**, 1179 (1957).

*3 The σ momment was estimated by the vector addition of the bond moment.

12) H. de V. Robles, *Rec. trav. Chim.*, **58**, 111 (1939).

13) A. A. Morton, "The Chemistry of Heterocyclic Compounds," McGraw-Hill Book Company, Inc., New York (1946), p. 52. (Japanese edition).

*4 β=20 kcal/mol is adopted for the evaluation of the bond-delovalization energy.

14) L. Pauling and J. Sherman, *J. Chem. Phys.*, **1**, 606 (1933).

*5 N. H. E. was adopted as a reference electrode.

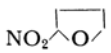
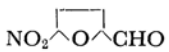
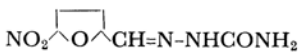
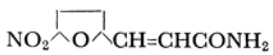
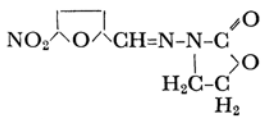
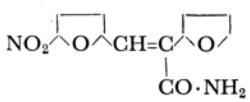
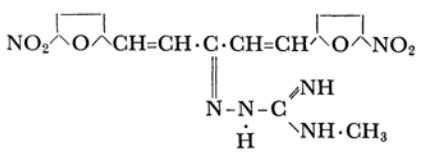
15) G. J. Hoijtink, *Rec. trav. Chim.*, **71**, 1089 (1952).

16) H. C. Lichstein and R. B. Boyd, *Proc. Soc. Exptl. Biol. Med.*, **57**, (2) 306 (1952).

17) J. O. Taylor and H. E. Paul, *J. Biol. Chem.*, **191**, 217 (1951).

18) K. Fukui, C. Nagata and T. Yonezawa, *J. Am. Chem. Soc.*, **80** 2267 (1958).

Table 4. Relation between reactivity indices and antibacterial activity

Compound	$S_5(N)'$	k_{lv}	$E_{1/2}$ volt	Activity (γ/ml)		
				E. Coli	*S. Aureus*	*B. Subitilis*
Furan 	0.723	−0.909	No reduction wave observed	1000	1000	1000
Furfural 	1.143	−0.356	−1.09	1000	1000	1000
Nitrofuran 	0.118	−0.322	−0.43	25	100	125
Nitrofrufural 	1.905	−0.179	−0.08	25	25	6.3
Fracine 	1.874	−0.270	−0.179	7	7	5
Nitrofruylacrylamide 	1.605	−0.213	−0.154	3.1	6.2	0.8
Furazolidon 	1.866	−0.269	−0.114	2	5	—
AF-2 	1.543	−0.204	−0.159	0.7	1.5	2.5
Miranon-M 	0.779	−0.232	−0.141	1	1	0.8

energy of the lowest vacant level, $-k_{ev}$ in comparison with the antibacterial activity of the compounds. The maximum bacteriostatic dilution is adopted as a measure of the activity.

From Table 4 it can be seen that no significant correlation is found between the reactivity index, $S_5(N)'$, and the antibacterial activity. This is in accord with the conclusion for a similar problem studied by Yoneda and Nitta.[9] On the contrary, the activity correlates closely with the values of the energy of the lowest vacant level. A similar relationship is found between the activity and the half-wave reduction potential. Accordingly, if other factors, such as the permeability, diffusibility, and solubility of the compounds into the tissues and cells, are the same, the activity of nitrofuran derivatives will not depend upon the nucleophilic displacement at the 5-position in the furan ring, but upon the quantities of $-k_{lv}$ and $E_{1/2}$, which are a measures of the electron affinity of the π conjugated molecule. Accordingly, the assumption is made that the reduction of the nitrofuran derivatives is the most important process in the bactericidal reaction of these bactericides.

It is worthwhile to give some information about the biomolecule acting as an electron donor to the bactericides. The FAD coenzyme was once pointed out as an electron donor[19,20]; in the present paper, however, the NADH coenzyme is assumed to be the electron donor, because NADH is capable of reducing the nitrofuran bactericides because of the difference in the redox potential. A redox reaction of this type may occur in the respiratory chain in the body of the bacteria.

For example, we take up nitrofurylacrylamide (N. F. A. A.) as a typical bactericide and assume that the redox reaction is initiated by the electron transfer from NADH to N. F. A. A. through the formation of an intermolecular complex such as is

$$\text{NADH} + \text{N.F.A.A.} \rightleftharpoons [\text{NADH}\cdot\text{N.F.A.A.}] \rightarrow \text{NAD}^+ + \text{N.F.A.A.H} \qquad (4)$$

shown in Scheme (4). To confirm this assumption, some spectral measurement is made. The ultraviolet absorption maximum of an aqueous solution of NADH in the presence of N. F. A. A. lies at a different wavelength than the corresponding maximum of an aqueous solution of NADH alone. These findings are illustrated in Fig. 2. If it is assumed that this shift reflects the formation of complex, the following relationship may be obtained at a fixed wavelength under the present experimental conditions[21]:

$$\frac{1}{[\text{NADH}]} \simeq \frac{\varepsilon K[\text{N.F.A.A.}]}{\Delta A} - K \qquad (5)$$

where K represents the equilibrium constant of a 1 : 1 complex of NADA and N. F. A. A.; ε, the molar absorbancy of the complex; [NADH], the total molar concentration of NADA, and [N. F. A. A.], the total molar concentration of N. F. A. A., and whose ΔA=(absorbancy for a NADH solution at the [NADH] concentration)+(absorbancy for a solution of N. F. A. A. at the [N. F. A. A.] concentration)−(absorbancy for a solution of NADH, N. F. A. A., and a complex in equilibrium at a total NADH concentration

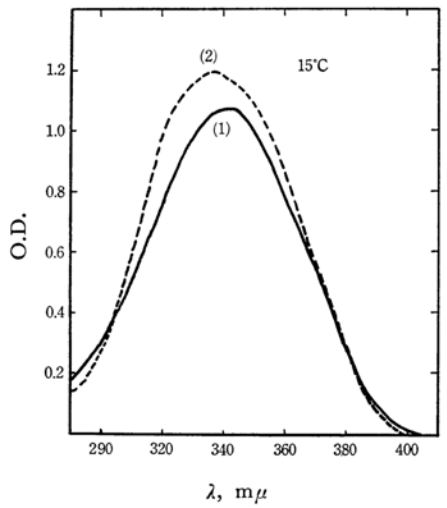


Fig. 2. The UV spectra of NADH solution in the presence of nitrofurylacrylamide (N.F.A.A.).
(1) Spectrum of 2.54×10^{-4} mol/l NADH waterly solution.
(2) Spectrum of NADH in the presence of N.F.A.A. (Reference: 5.28×10^{-5} mol/l N.F.A.A. waterly solution)

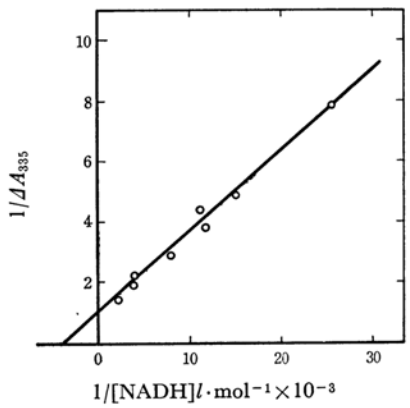


Fig. 3. A plot showing the Benesi-Hildebrand treatment of the data obtained in spectral studies of the interaction between NADH and nitrofurylacrylamide (N.F.A.A.).
Concentration of N.F.A.A.=5.28×10^{-5} mol/l.

19) H. L. Richardson, A. R. Stier and E. Borsos-Nachtnebel, *Caneer Research*, **12**, 356 (1952).
20) R. R. Brown, J. A. Miller and E. C. Miller, *J. Biol. Chem.*, **209**, 211 (1954).
21) S. D. Ross, M. Bassin, M. Finkelstein and W. A. Leach, *J. Am. Chem. Soc.*, **76**, 69 (1954).

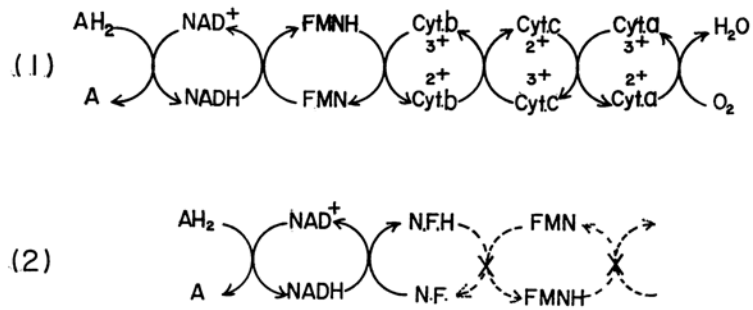


Fig. 4. The scheme of the electron transport in the respiratory chain.
(1) Normal electron transport.
(2) Electron transport inhibited by nitrofuran derivatives.

[NADH] and a total N. F. A. A. concentration [N. F. A. A.]). In Fig. 3, a linear plot of $1/\Delta A_{335}$ *vs.* 1/[NADH] is shown. The values of $\varepsilon_{335}=1.32\times10^4$ *l*/mol cm and $K=4\times10^3$ *l*/mol are found.

Within the range of concentration employed, the formation of a 1 :1 complex is consistent with the data obtained. This fact is very likely to support the assumption that the key reaction in the first stage of the bactericidal action is the formation of the intermediating molecular complex already mentioned above. If it is assumed that this complex is of a charge-transfer type, then the electron transfer from NADH to N. F. A. A. may proceed by way of Scheme (4). On the basis of the above assumptions, a model of the bactericidal action in the earliest stage was proposed by us in terms of the inhibition of the respiratory chain in the body of bacteria to the nitrofuran bactericides. Figure 4 shows the scheme of the respiratory chain in which the electron-transport cycle coupled with NADH is inhibited by the irreversible reduction of nitrofuran compounds in comparison with a normal respiratory chain.

It is interesting to evaluate the electron-accepting site in the nitrofuran bactericides, since such a locus may play an important role in the stage of complex formation in the model of bactericidal action presented here. It seems possible to consider that a transferred electron localizes on the *r*th atom having the largest $(C_r^{lv})^2$ value in the π conjugated molecule, where C_r^{lv} is the coefficient of atomic orbitals at the *r*th atom in the lowest vacant molecular orbital, and that the nucleophilic frontier electron density, $f_r(\mathrm{N})=2(C_r^{lv})^2$,[22] can be taken as the measure of the strength of an electron-attractive force at the *r*th atom in the molecule.

In Fig. 5, the molecular diagrams of $f_r(\mathrm{N})$ for several effective nitrofuran derivatives are illustrated. From Fig. 5 it may be seen that electron-accepting sites are located on the N and O atoms in the nitro group; in contrast to the case with

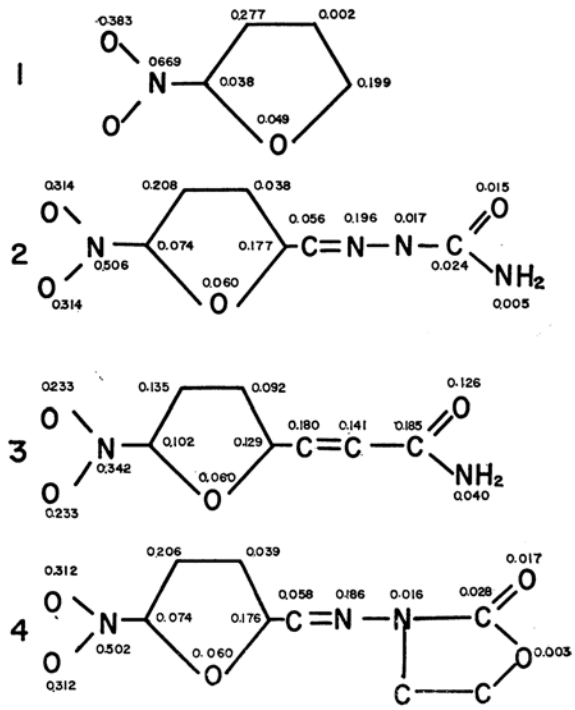


Fig. 5. Nucleophilic frontier electron density $f_r(\mathrm{N})$ for nitrofuran derivatives.
1 Nitrofuran
2 2-(5-Nitro)-furfuralsemicarbazone
3 2-(5-Nitro)-furylacrylamide
4 *N*-(5-Nitro-2-furfurylidene)-3-amino-2-oxazolidone

other atoms in the molecule. This fact suggests that the nitro group in nitrofuran bactericides is capable of playing the role of an electron-accepting locus; thus, the loss of the bactericidal action in the absence of a nitro group can be interpreted in terms of the interruption of the electron transport from biomolecules, such as NADH, to the bactericides.

The authors wish to express their appreciation to the Computing Center of Tohoku University for its generous provision of computer time.

22) K. Fukui, T. Yonezawa and H. Shingu, *J. Chem. Phys.*, **20**, 722 (1952).