174~176° (IR ν_{max}^{Nujol} cm^{-1} : 1714 (C=O), 1656 (C=O), 1613, 1592, 1560 (arom.)). The methyl ester (XIb) was also obtained by methylation of XIa with diazomethane in ether-tetrahydrofuran. Oxidation of XIb employing a large excess of chromic trioxide in glacial acetic acid at room temperature gave methyl 2-ethyl-6,11-dioxo-5,7,10-trimethoxy-6,11-dihydro-1-naphthacenecarboxylate (Ib) as yellow needles, m.p. 235~237° (*Anal.* Calcd. for $C_{25}H_{22}O_7$: C, 69.11; H, 5.10. Found : C, 68.72; H, 5.06. IR ν_{max}^{KBr} cm^{-1} : 1721 (C=O), 1663 (C=O), 1608, 1586, 1569 (arom.)). Demethylation of Ib with boron tribromide in methylene chloride gave dark red needles as an acidic fraction, m.p. 260~262° (decomp.) (IR ν_{max}^{KBr} cm^{-1} : 1704 (C=O), 1648, 1600, 1587) (lit.,[4] dark red needles, m.p. 263° (decomp.), IR : ν_{max}^{KBr} 1704 cm^{-1} for η-pyrromycinonic acid).

All substances mentioned in this paper gave satisfactory elemental analyses.

Faculty of Pharmaceutical Sciences,
Osaka University,
Toneyama, Toyonaka, Osaka

Zen-ichi Horii (堀井善一)
Takefumi Momose (百瀬雄章)
Yasumitsu Tamura (田村恭光)

Received August 3, 1964

[Chem. Pharm. Bull. 12(10)1264~1268(1964)]

UDC 615.778 [547.722.5] : 541.69

Electronic Structure and Antibacterial Activity of Nitrofuran Derivatives

Stillman and Dodd[1] reported in 1944 that 5-nitrofurfural semicarbazone (nitrofurazone) and some related compounds possessed notable bacteriostatic and bactericidal activities. On account of their wide antibacterial spectrum and low development of bacterial resistance, attention has been attracted to those compounds from every quarter.

The relationship between the structure of nitrofuran derivatives and the antibacterial activity had been already discussed from the various point of view and the following results had been obtained. The nitro group is indispensable and in its absence there is very little or no activity. The introduction of a conjugated double bond between the nitrofuryl group and the end group of the side chain might result to enhance the activity to some extent.[2] The polarographic reduction half-wave potential of the nitro group is related partially to the activity.[3]

Moreover, many enzymological and bacteriological investigations[4~8] have been made to elucidate the mechanism of the antibacterial action of these compounds.*1 The above-stated empirical rules hold good partially, but they would be of little use for systematic elucidation of the structure-activity relationships.

*1 It may be worthy to note that those compounds inhibit some enzyme system (for example SH-enzyme system) involved in the carbohydrate metabolism of the microörganisms.

1) M.C. Dodd, W.B. Stillman : J. Pharmacol. Exptl. Therap., **82**, 11 (1944).
2) H. Saikachi, *et al.* : Yakugaku Zasshi, **69**, 285 (1949).
3) T. Sasaki : Pharm. Bull. (Tokyo), **2**, 104 (1951).
4) D.L. Cramer, M.C. Dodd : J. Bacteriol., **51**, 119, 293 (1947).
5) R.C. Bender, H.E, Paul : J. Biol. Chem., **191**, 217 (1951).
6) R.E. Asnis, J.S. Gots : Arch. Biochem., **30**, 25 (1951).
7) M.F. Paul, *et al.* : J. Biol. Chem., **206**, 491 (1954).
8) M.N. Green, E.C. Heath, Irving Yall : Proc. Soc. Exptl. Biol. Med., **76**, 152 (1951).

One of the characteristics of the nitrofuran derivatives is the fact that there is antibacterial action more or less only in the presence of 5-nitrofuryl radical. This is different from other chemotherapeutic agents, in which minor changes in the chemical structure influence greatly and activity and often result in decreased or disappeared activity. Consequently, in the nitrofuran derivatives it seemed reasonable to assume that the -CH=CH- or -CH=N- group standing between 5-nitrofuryl and the end group in the side chain is, as Saikachi reported,[9] no more than the supplementary group which enhances the antibacterial action to some extent. Therefore, the authors paid special attention to the nitrofuryl group and investigated the relation between its electronic structure and its antibacterial activity.

Fukui, *et al.* have already pointed out in quantum-chemical studies of carcinogenic compounds,[10] plant growth hormon compounds[11] and fungicides,[12] that the electronic structure of these compounds are closely related with their biological actions.

In the present paper the π-electron-energy and -distribution using a molecular orbital method, and in addition the superdelocalizability (Sr)[13] which has been defined in frontier electron method were calculated in order to find a clue of the reaction mechanism in the early stage of antibacterial action of nitrofuran derivatives.

Parameters used in Calculation

The calculation was carried out by using simple LCAO-MO (linear-combination-of-atomic-orbitals molecular orbital) treatment, solving the secular equation. The coulomb integral of the substituent X, that of the carbon atom attached to X, and the resonance integral between that carbon atom and X, are designated as $\alpha+a_X\beta$, $\alpha+a_r\beta$, and $l\beta$, respectively. Here α and β are coulomb integral of a carbon atom and the resonance integral of a carbon-carbon π-bond. The numerical values of a_X a_r and l adopted by us are shown in Table I.

Table I. Parameters used in the Calculation[a]

Substituent X	a_X	a_r	l
-O-	2	0.2	0.6
=N-	0.6	0.1	1
-N<	1	0.1	1
=O	2	0.2	1.4
$-NH_2$	0.4	0	0.6
-OH	0.6	0	0.7
$-NO_2$	$\begin{cases}\alpha_N=1\\ \alpha_O=1\end{cases}$	0.1	1

a) T. Yonezawa, *et al.*: "Introduction to Quantum Chemistry, Vol. 1," Kagaku-Dojin, Kyoto, 55 (1963).

The $-CH_2-$ group of compound (Ⅷ) in Table Ⅱ is treated as hyperconjugation and the parameters such as $\begin{matrix}N & \overset{0.7\beta}{\diagdown} & & \overset{2.5\beta}{} \\ & & C & = \\ C & \underset{0.7\beta}{\diagup} & & \underset{\alpha-0.1\beta}{} \end{matrix}\!\!\begin{matrix} & \\ H_2 & \\ \alpha-0.5\beta & \end{matrix}$ were adopted. Resonance integral between the nitrogen and nitrogen atoms was taken as β.

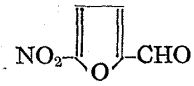
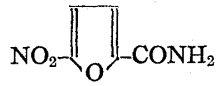
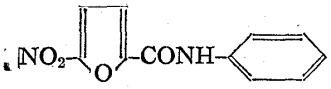
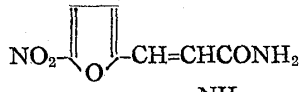
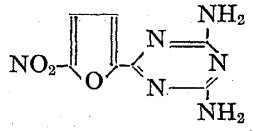
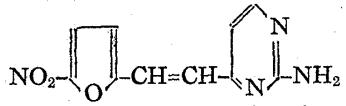
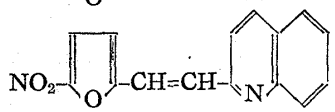
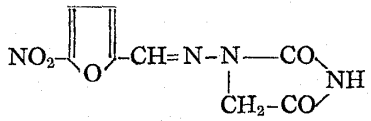
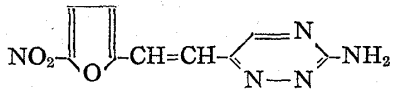
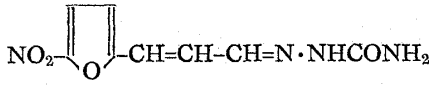
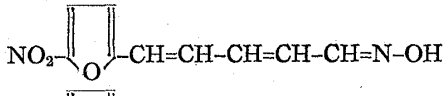
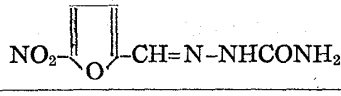
Results and Discussion

In Table Ⅱ, 12 typical compounds were selected from nitrofuran derivatives and those

9) H. Saikachi, K. Suzuki: This Bulletin, **6**, 693 (1958).
10) C. Nagata, K. Fukui, T. Yonezawa, T. Tagashira: Cancer Research., **15**, 233 (1955).
11) K. Fukui, C. Nagata, T. Yonezawa: J. Am. Chem. Soc., **89**, 2267 (1958).
12) K. Fukui, A. Imamura, C. Nagata: Bull. Chem. Soc. Japan, **33**, 122 (1960).
13) K. Fukui, T. Yonezawa, C. Nagata: *Ibid.*, **27**, 423 (1954).

lowest vacant energy level λ_{lv}, $Sr^{(N)}$, and net charges at the oxygen and nitrogen atoms in nitro group were calculated. $Sr^{(N)}$ represents the superdelocalizability for an nucleophilic reaction. These results will be mentioned conveniently in three sections as follow:

Table II. Relation between Reactivity Indics and Antibacterial Activity of Nitrofuran Derivatives

No.	Chemical structure	Lowest vacant energy level λ_{lv}	$Sr^{(N)}$ in the 2-position of furan nucleus	Net charge at the oxygen atom in nitro group, Q_O	Net charge at the nitrogen atom in nitro group, Q_N	Activity[b]
I		+0.006	[a]	0.981	−0.556	+
II		−0.078	4.098	0.973	−0.577	+
III		−0.079	4.030	0.975	−0.575	++
IV		−0.064	3.960	0.966	−0.590	+++
V		−0.098	3.463	0.974	−0.581	+++
VI		−0.102	2.973	0.962	−0.599	++++
VII		−0.106	2.866	0.961	−0.601	+++++
VIII		−0.138	2.532	0.957	−0.607	+++
IX		−0.140	2.458	0.954	−0.611	+++++
X		−0.135	2.355	0.948	−0.619	+++
XI		−0.139	2.127	0.937	−0.630	+++
XII		−0.157	2.117	0.954	−0.612	++

a) Since I has an bonding lowest vacant orbital in its normal state, its $Sr^{(N)}$ can not be treated in the same way as the others.
b) The antibacterial activity was tested for *Staph. aureus* and *E. coli*, and the larger the number of + sign is, the greater the activity is.

a) $Sr^{(N)}$: From these results of calculations it was found that the 2-position of furan nucleus is the most reactive to the nucleophilic radical, and the values of $Sr^{(N)}$ at this position show to be correlated closely with the antibacterial activity. As is clearly seen in Table II, the activity enhances proportionally with the increase of $Sr^{(N)}$, until it reaches maximum value, and then, decreases proportionally with the increase of $Sr^{(N)}$.

Since $Sr^{(N)}$ appears to be a suitable index for a discussion of nucleophilic reactivity, it may be concluded that an important factor determining its antibacterial activity is the chemical reactivity of nitrofuran derivatives at the 2-position with a nucleophilic group (for example SH-group) of a component in bacteria.

In studies by Fukui, *et al.*[10~12] it was show that for occurence of activity the upper and lower threshold values of reactivity might exist. In the case of nitrofuran derivatives, as described before, the existence of nitrofuryl radical means the occurrence of antibacterial activity and thus the upper and lower thresholds were not recognized. The relation between $Sr^{(N)}$ and antibacterial activity is therefore expressed with a curved line as is seen in Fig. 1. It is of much interest that in all recently discovered substances having strong activities $Sr^{(N)}$ values exist between 2.4 and 3.0.

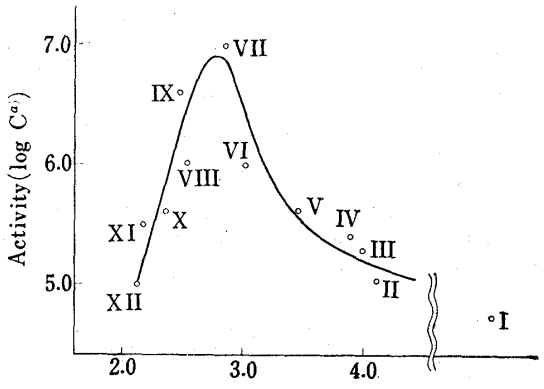


Fig. 1. The Relation between $Sr^{(N)}$ and Antibacterial Activity

a) C is maximum bacteriostatic dilution for *Staph. aureus*.

From the above facts the following consideration may be provided. The effective compounds are desired to have the suitable reactivities because they might reach effectively the reaction center concerned with the antibacterial action in the body of bacteria.*2 Of course it is unreasonable to say that antibacterial activity will be determined only by the values of $Sr^{(N)}$. In addition to the chemical reactivity, there are other factors such as permeability, diffusibility and solubility of the substance into the tissues and cells, and results of antibacterial test may be influenced by the procedures as well as by the kind of bacteria used in the test.

b) Net charge : In consideration of the importance of the nitro radical in nitrofuran derivatives, its reduction *in vivo* and its polarographic reduction were already studied. The relation between the reductivity of nitro group and the antibacterial activity, however, remains to be solved. It is considered that the electrostatic interaction of the nitro group with a site of the nitro reductive enzyme*3 may contribute to the *in vivo* reduction of nitro radical at least in the earliest stage. Hence the magnitude of the net charge at N or O atom of nitro radical comes into question. However, the result of calculation as shown in the Table II seems to indicate that the net charge at N or O atom of nitro radical has no evident correlation with the antibacterial activity.

c) λ_{iv} : Sasaki[3] attempted to compare the polarographic reduction potential of nitrofuran derivatives with the antibacterial action, and found that if the -CH=CH- group is

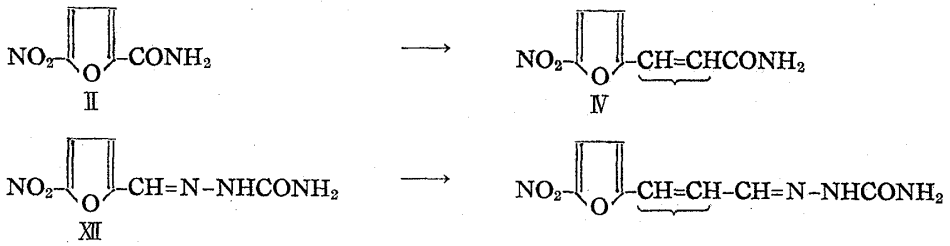


Chart 1.

*2 If these compounds are too reactive, they would react on the various groups in the body of bacteria before reaching the reaction center and consequently their antibacterial activities would decrease.

*3 Nitro-reductase has low affinities for his substrates and is flavoprotein having FAD as his prosthetic group. Cf. H. L. Richardson, A. R. Stier, E. Borsos-Nachtnebel : Cancer Research, **12**, 356 (1952); R. R. Brown, J. A. Miller, E. C. Miller : J. Biol. Chem., **209**, 211 (1954).

introduced between the nitrofuryl and the end group of the side chain, as shown in Chart 1, the half-wave reduction potential drops and at the same time the antibacterial activity increases.

On the other hand it is known that the potentials of a series of the compounds are linearly related to the heights of the lowest vacant π-electronic energy levels of these cmpounds calculated by the LCAO-MO method.[14] From the above fact it seems that the coefficient λ_{lv} of the lowest vacant π-electronic energy level indicated in Table II lends some support to the experimental results obtained by Sasaki. Namely IV or X is lower in the lowest vacant energy level and higher in antibacterial activity than in case of II or XII. However, as it is readily seen in Table II, the compound which is low in the lowest vacant π-electronic energy level, namely may be reduced more easily, is not always presented to be strong in an antibacterial activity. From the above stated consideration, it seems reasonably to be concluded that there is no striking relation between the nature of nitro radical and the antibacterial activity.

The authore express their deep gratitude to Dr. C. Nagata, Chief, Section on Biological Physics, National Cancer Center Research Institute, for his kind guidance and also to Dr. T. Akiba, Director of this laboratory for his encouragement throughout the course of this work.

Research Laboratories,
Chugai Pharmaceutical Co., Ltd.,
Takataminami-cho,
Toshima-ku, Tokyo

Fumio Yoneda (米田文郎)
Yoshihira Nitta (新田義博)

Received May 12, 1964

14) Fueno, *et al.*: Nippon Kagaku Zasshi, **79**, 116 (1958); Bull. Chem. Soc. Japan, **32**, 1003 (1959).

[Chem. Pharm. Bull. 12(10)1268~1269(1964)]

UDC 547.785.1 : 543.41-4

Imidazolylazo Compounds as Metallochromic Indicators

Although imidazole derivatives are expected to form complexes with various metal ions, the investigations of their complexing properties are mostly confined to imidazole itself, histidine and histamine, and no analytical applications of imidazole derivatives in general have been reported.

In the course of the systematic studies on chelating abilities and analytical applications of imidazole derivatives, it was found that some azo derivatives react with various metal ions to give intense colors and that they can be applied to analytical determinations. On the other hand, a number of *ortho*-hydroxyazo compounds, of which 1-(2-pyridylazo)-2-naphthol (PAN) and Eriochrome Black T are typical, are frequently used as indicators in complexometric titrations because of their marked color changes resulting from the reactions with metal ions. Since the successful introduction of PAN into complexometric analysis, many studies have been made on the properties of analogous compounds, such as those with thiazole nucleus in place of pyridine nucleus[1~4] and those with modified phenol components.[5,6]

1) G. Nakagawa, H. Wada : Nippon Kagaku Zasshi, 83, 1185 (1962).
2) A. Kawase : Bunseki Kagaku, **12**, 709 (1963).
3) *Idem* : *Ibid.*, **12**, 810 (1963).
4) *Idem* : *Ibid.*, **12**, 817 (1963).
5) A. Corsini, I. Mai-Ling Yih, Q. Fernando, H. Freiser : Anal. Chem., **34**, 1090 (1962).
6) G. Nakagawa, H. Wada : Nippon Kagaku Zasshi, **83**, 1190 (1962).