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# Structure–Activity Relationship for Antibacterial Action of Phenolic and Aromatic Nitro Compounds. An Attempt at Systematic Identification of New Antibacterial Agents

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A structure–activity relationship between antibacterial activity and the energy of the lowest unoccupied molecular orbital (LUMO) of phenolic and aromatic nitro compounds was derived based on results obtained with eight strains of Gram-positive and Gram-negative bacteria. The reliability of the correlation was confirmed by measuring the minimum inhibitory concentration at various pH values of the medium; it was found that the undissociated molecular species having a low lying LUMO is essential for strong antibacterial activity. Based on the above results, 2,4,6-trinitroanisole was examined, and it proved to have a remarkably strong antibacterial activity (200 times stronger than that of 2,4,6-trinitrophenol).

**Keywords**——structure–activity relationship; antibacterial activity; lowest unoccupied molecular orbital (LUMO); aromatic nitro compound; phenol; electronic structure; MINDO/3 method; pH ($pK_a$) effect

In the previous paper,[1] a relationship between antifungal activity and the energy of the lowest unoccupied molecular orbital (LUMO) of several phenolic compounds was found; all the highly active compounds possess a low-lying LUMO as compared with inactive compounds. The relationship may provide a useful basis for screening new antifungal drugs.

Because of the difficulty in the precise determination of antifungal activity, the research has been extended to antibacterial investigations on the same compounds, and similar correlations were derived with some Gram-positive as well as Gram-negative bacteria, as described in this paper. The extension of the examined species of phenolic and aromatic nitro compounds further established the structure–activity relationship between the energy of LUMO and the antibacterial action of these compounds. This structure–activity relationship was applied in an attempt to find highly active antibacterial agents.

For historical reasons, there are many papers on phenols, including extensive investigations by Fujita on the structure–activity relationship using $pK_a$ as a parameter[2] and by Hansch using $\log P$ ($P$ is the partition coefficient determined in the *n*-octanol–water system).[3] In this paper, the structure–activity relationship of phenols was generalized and extended to non-phenolic compounds by using the LUMO energy, *a priori* obtainable from the Schrödinger equation,[4] instead of the experimental parameters, $pK_a$ and $\log P$. Since the origin of biological activity remains obscure in many kinds of drugs, the generalization of a structure–activity relationship in terms of quantum-chemical concepts, overcoming limitations of available chemical species and experimental techniques, should be valuable for understanding drug mechanisms; experimental parameters are not always obtainable due to

the difficulty of measurement, and their physico-chemical meaning is sometimes obscure (*e.g.*, $pK_a$ for an undissociable species, $\log P$ for compounds insoluble in *n*-octanol, *etc.*).

## Materials and Methods

**Bacteria**——The Gram-positive bacteria *Staphylococcus aureus* (2 strains), and *Micrococcus luteus* and *Bacillus subtilis*, and the Gram-negative bacteria *Escherichia coli, Klebsiella pneumoniae, Salmonella typhimurium* and *Proteus vulgaris* were used for all studies. *S. aureus* U9NO was kindly provided by Dr. P. A. Pattee,[5] Dept. of Microbiology, Iowa State Univ., Ames, Iowa 50011, U.S.A.

**Chemicals**——*p*-Acetophenol was obtained from Tokyo Chemical Industry, Ltd., Tokyo; *o*-, *m*- and *p*-nitroanisoles from Wako Pure Chemical Industries, Ltd., Osaka; *p*-cyanophenol (purity: 95%) from Aldrich Chemical Co., Milwaukee, U.S.A.; and other chemicals from Nakarai Chemicals, Ltd., Kyoto. All chemicals used were of reagent grade. 2,4,6-Trinitroanisole was prepared by the condensation of picryl chloride and sodium methoxide.[6]

**Assay of Antibacterial Activity**——The antibacterial test was carried out by preparing serial 2-fold dilutions of each compound employed in agar media (Sensitivity Test Agar, Eiken Chemical, Ltd., Tokyo). The agar plate containing the compound (pH 7.4) was inoculated in a loop (1 mm) with an appropriately diluted suspension (inoculum size as indicated in Table I) of bacteria grown overnight in the broth (Sensitivity Test Broth, Eiken). The inoculated plate was incubated at 37 °C for 18 to 20 h. The antibacterial activity of each compound was expressed in terms of minimum inhibitory concentration (MIC).

**Adjustment of Medium pH**——The twice-concentrated agar media used were previously adjusted to pH 6.0 with 1 N HCl or to pH 8.0 with 1 N NaOH, and were sterilized. To correct the pH shift occurring during sterilization of the agar media, the media were poured into an equal volume of sterilized buffer. The buffer component was either sodium 2-(*N*-morpholino)ethanesulfonate for pH 6.0 or sodium *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate for pH 7.0 and 8.0 at a final concentration of 0.2 M. Neither of these buffer media inhibit bacterial growth. On the other hand, solutions of phenolic compounds were adjusted to the desired pH with NaOH unless otherwise stated. A suitable amount of 2,4-dinitroanisole, 2,4,6-trinitroanisole, or 2,4,6-trinitro-1-chlorobenzene was dissolved at 50 °C in a minimum quantity of ethanol. The final concentration of the alcohol in agar plates was less than 2.5%, which did not inhibit the growth of bacteria.

**Self-Consistent Field Molecular Orbital (SCF MO) Calculation**——Chemical structures of the compounds listed in Table I were fully optimized by the DFP (Davidon–Fletcher–Powell)[7] method based on MINDO/3[8] SCF MO calculations in order to normalize the theoretical values on the most stable conformations of the compounds. In the case of the nitro compounds, the geometry optimization was carried out under the constrained condition that the molecular structure is planar, as observed in X-ray diffraction analysis,[9] because full optimization based on the MINDO/3 calculation often gives an extraordinary rotation of the nitro group. Total energy and LUMO energy ($E_{LUMO}$) were obtained in these optimized molecular conformations. The results have been reported in detail elsewhere.[10]

**Correlation Analysis**——Correlation analysis was carried out by a standard least-squares method.[11] Regression lines were obtained with 95% confidence limits by means of the *t*-test. The standard deviation (*s*), the correlation coefficients (*r*), and the *F*-test values (*F*) are also shown with the correlation formulae.

## Results and Discussion

### Antibacterial Activity

Susceptibility (MIC) to the compounds shown in Table I was generally unaffected by a change in inoculum size. This suggests that these compounds probably exert bactericidal action. In a series of homologous nitrophenols, the position of the nitro group on the benzene ring did not have any great effect. Dinitration of phenol remarkably increased the antibacterial activity: 2,5-substitution was the most effective. Methyl ether formation of nitrophenols and nitroanisoles decreased the activity against all bacteria.

### Structure–Activity Relationship in Terms of the Energy of LUMO ($E_{LUMO}$)

Linear relationships between antibacterial activity towards various bacteria and the $E_{LUMO}$ were obtained. The correlative formulae are collected in Table II. The results are illustrated in Figs. 1 and 2 for a Gram-positive coccus and a Gram-negative bacillus, respectively, as examples. A characteristic feature is that the correlative expressions in Table

TABLE I. Antibacterial Activity and Electronic Parameters of Phenolic and Aromatic Compounds (Medium pH 7.4)

| | Minimum inhibitory concentration (mM) | | | | | | | | Electronic parameters | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G (+) | | | | G (−) | | | | | | | |
| | *S. aureus* 209P | *S. aureus* U9NO | *M. luteus* ATCC 9341 | *B. subtilis* PCI219 | *E. coli* NIHJ JC-2 | *K. pneumoniae* W52 | *S. typhimurium* LT-2 | *P. vulgaris* YO-1 | | | | |
| | Inoculum size (cells/ml) | | | | | | | | Calc. | | Exp. | |
| | $10^7$ | $10^7$ | $10^6$ | $10^5$ | $10^5$ | $10^5$ | $10^5$ | $10^5$ | $E_{LUMO}$ (eV) | $\Delta E$ (eV) | $pK_a$[a] | log $P$[b] |
| Phenol | 50 | 50 | 50 | 25 | 25 | 25 | 50 | 25 | 1.1459 | 14.763 | 9.998 | 1.46 |
| *p*-Cyanophenol | 10 | 10 | 10 | 10 | 8 | 8 | 8 | 8 | 0.8482 | 14.104 | 8.64 | |
| *p*-Hydroxymethyl benzoate | 25 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 0.5123 | 13.835 | 8.10 | 1.96 |
| *p*-Acetophenol | 50 | 25 | 25 | 25 | 10 | 25 | 25 | 10 | 0.4314 | 13.837 | 8.05. | |
| *o*-Nitrophenol | 8 | 2 | 4 | 8 | 4 | 8 | 4 | 4 | −0.4136 | 13.396 | 7.234 | 1.79 |
| *m*-Nitrophenol | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 2 | −0.5524 | 14.011 | 8.399 | 2.00 |
| *p*-Nitrophenol | 4 | 2 | 4 | 4 | 1 | 1 | 1 | 1 | −0.3188 | 13.338 | 7.149 | 1.91 |
| *o*-Nitroanisole | 10 | 8 | 8 | 10 | 8 | 8 | 8 | 8 | −0.4033 | | | 2.03 |
| *m*-Nitroanisole | 8 | 8 | 4 | 8 | 4 | 4 | 4 | 4 | −0.5425 | | | 2.16 |
| *p*-Nitroanisole | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | −0.3087 | | | 2.03 |
| 2,4-Dinitrophenol | 1 | 1 | 2 | 4 | 2 | 8 | 2 | 2 | −0.9624 | 12.569 | 4.110 | 1.51 |
| 2,5-Dinitrophenol | 0.31 | 0.31 | 0.625 | 0.625 | 0.31 | 1.25 | 0.31 | 0.31 | −1.5727 | 12.780 | 5.216 | 1.75 |
| 2,6-Dinitrophenol | 2 | 2 | 16 | 1 | 8 | 8 | 8 | 16 | −1.2308 | 12.558 | 3.706 | 1.25 |
| 2,4,6-Trinitrophenol | 50 | 50 | 50 | 50 | 6.25 | 12.5 | 12.5 | 50 | −1.6715 | 11.845 | 0.290 | 1.34 |
| Cinnamaldehyde | 0.8 | 0.4 | 0.8 | 0.4 | 0.8 | 1 | 0.8 | 0.4 | −0.1568 | | | |

*a*) Cited from ref. 12 except for *p*-cyanophenol and *p*-acetophenol. *b*) Ref. 11.

TABLE II. Regression Analysis Equations Based on $E_{LUMO}$ for the Compounds in Table I[a]

| | $\log(1/\text{MIC(mM)}) = a + bE_{LUMO}$ | | | $n=13$ | |
|---|---|---|---|---|---|
| G (+) | $a$ | $b$ | $s$ | $r$ | $F$[b] |
| *S. aureus* 209P | −0.979 (±0.198) | −0.696 (±0.245) | 0.308 | 0.884 | 39.2 (>99%) |
| *S. aureus* U9NO | −0.845 (±0.191) | −0.655 (±0.236) | 0.296 | 0.879 | 37.5 (>99%) |
| *M. luteus* ATCC 9341 | −0.934 (±0.216) | −0.463 (±0.267) | 0.335 | 0.756 | 14.6 (>99%) |
| *B. subtilis* PCI219 | −0.909 (±0.155) | −0.514 (±0.192) | 0.241 | 0.872 | 34.8 (>99%) |
| G (−) | | | | | |
| *E. coli* NIHJ JC-2 | −0.777 (±0.231) | −0.453 (±0.285) | 0.358 | 0.727 | 12.3 (>99%) |
| *K. pneumoniae* W52 | −0.891 (±0.219) | −0.324 (±0.270) | 0.339 | 0.623 | 7.0 (>95%) |
| *S. typhimurium* LT-2 | −0.835 (±0.253) | −0.556 (±0.312) | 0.392 | 0.764 | 15.4 (>99%) |
| *P. vulgaris* YO-1 | −0.770 (±0.271) | −0.427 (±0.335) | 0.421 | 0.646 | 7.9 (>95%) |

The numerals in parentheses are the ±95% confidence limits of $a$ and $b$. a) Not including trinitrophenol and cinnamaldehyde. b) $F_1^{11}$ (99%)=9.65 and $F_1^{11}$ (95%)=4.84.

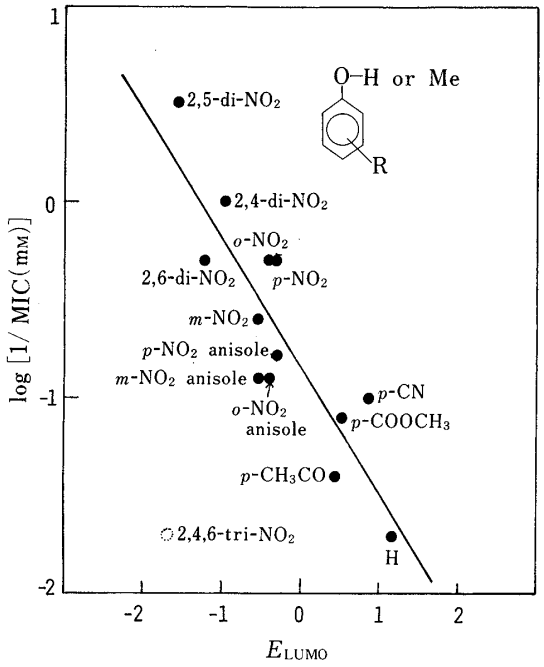


Fig. 1. The Structure–Activity Relationship with Gram-Positive *S. aureus* U9NO

——, $\log[1/\text{MIC(mM)}] = -0.845(\pm0.191) - 0.655(\pm0.236)E_{LUMO}$ ($n=13$, $s=0.296$, $r=0.879$, $F=37.5$ (>99%)).

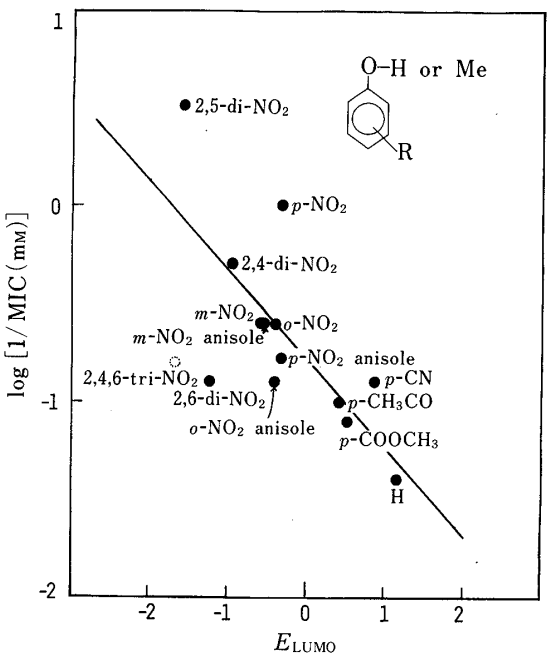


Fig. 2. The Structure–Activity Relationship with Gram-Negative *E. coli* NIHJ JC-2

——, $\log[1/\text{MIC(mM)}] = -0.778(\pm0.231) - 0.453(\pm0.285)E_{LUMO}$ ($n=13$, $s=0.358$, $r=0.727$, $F=12.3$ (>99%)).

II are very similar within each group (*i.e.*, Gram-positive and Gram-negative bacteria). Smaller values of the coefficients, $a$ and $b$, and poor correlations were characteristic in the latter group.

## Structure–Activity Relationship for the Undissociated Species

A failure of the structure–activity relationship based on $E_{LUMO}$ was observed in the case of such a very low $E_{LUMO}$ compound as 2,4,6-trinitrophenol, whose antibacterial activity is extraordinarily small. What is the reason for this discrepancy? The poly-substitution of the nitro group enhances the dissociation of the hydroxyl group of phenols, and the $pK_a$ value of 2,4,6-trinitrophenol is remarkably small, 0.290, compared with those of dinitrophenols (*e.g.* 4.110 for 2,4-dinitrophenol; see Table I). This fact suggests that the structure–activity

TABLE III. Regression Analysis Equations Based on $E_{LUMO}$ for the Undissociated Species of the Compounds in Table I[a)]

$\log(1/MIC_n(mM)) = a + bE_{LUMO}$

| [All compound] | | | | $n=14$ | |
|---|---|---|---|---|---|
| G (+) | $a$ | $b$ | $s$ | $r$ | $F^{b)}$ |
| *S. aureus* 209P | −0.425 (±0.840) | −2.23 (±0.934) | 1.32 | 0.832 | 27.0 (>99%) |
| *S. aureus* U9NO | −0.292 (±0.813) | −2.18 (±0.904) | 1.28 | 0.834 | 27.5 (>99%) |
| *M. luteus* ATCC 9341 | −0.372 (±0.766) | −2.04 (±0.852) | 1.21 | 0.833 | 27.2 (>99%) |
| *B. subtilis* PCI219 | −0.349 (±0.847) | −2.08 (±0.942) | 1.33 | 0.811 | 23.1 (>99%) |
| G (−) | | | | | |
| *E. coli* NIHJ JC-2 | −0.199 (±0.877) | −2.14 (±0.975) | 1.38 | 0.809 | 22.8 (>99%) |
| *K. pneumoniae* W52 | −0.312 (±0.845) | −2.01 (±0.940) | 1.33 | 0.802 | 21.7 (>99%) |
| *S. typhimurium* LT-2 | −0.266 (±0.812) | −2.18 (±0.903) | 1.28 | 0.835 | 27.7 (>99%) |
| *P. vulgaris* YO-1 | −0.211 (±0.759) | −1.99 (±0.844) | 1.19 | 0.829 | 26.3 (>99%) |
| [Nitro compounds] | | | | $n=10$ | |
| G (+) | $a$ | $b$ | $s$ | $r$ | $F$ |
| *S. aureus* 209P | −2.21 (±1.35) | −4.14 (±1.44) | 0.980 | 0.920 | 44.3 (>99%) |
| *S. aureus* U9NO | −1.98 (±1.41) | −3.97 (±1.49) | 1.020 | 0.908 | 37.5 (>99%) |
| *M. luteus* ATCC 9341 | −2.00 (±1.28) | −3.77 (±1.37) | 0.932 | 0.914 | 40.5 (>99%) |
| *B. subtilis* PCI219 | −2.21 (±1.35) | −4.05 (±1.43) | 0.976 | 0.918 | 42.6 (>99%) |
| G (−) | | | | | |
| *E. coli* NIHJ JC-2 | −2.07 (±1.49) | −4.12 (±1.58) | 1.079 | 0.905 | 36.2 (>99%) |
| *K. pneumoniae* W52 | −2.02 (±1.50) | −3.83 (±1.59) | 1.087 | 0.891 | 30.8 (>99%) |
| *S. typhimurium* LT-2 | −1.96 (±1.38) | −3.99 (±1.47) | 1.005 | 0.911 | 39.1 (>99%) |
| *P. vulgaris* YO-1 | −1.84 (±1.27) | −3.72 (±1.35) | 0.921 | 0.914 | 40.5 (>99%) |

The numerals in parentheses are the ±95% confidence limits of $a$ and $b$. *a*) Not including cinnamaldehyde. *b*) $F_1^{12}(99\%)=9.33$, $F_1^{12}(95\%)=4.75$ and $F_1^{8}(99\%)=11.26$.

relationship may be valid for the undissociated species and not for the total concentration of the examined compound, because the low concentration of undissociated 2,4,6-trinitrophenol could explain its low antibacterial activity (Figs. 1 and 2). The concentration of the undissociated species $C_n$ can be obtained from the total concentration of the examined compound $C_t$ by means of the following equation, $C_n = C_t[H^+]/(K_a + [H^+])$, using its $pK_a$ value.

The regression analysis between the values of $E_{LUMO}$ and the concentrations of undissociated species established a linear structure–activity relationship for all the examined compounds including phenols. The results are collected in Table III for all the examined bacteria. The formulae obtained are considered to have the same expression, irrespective of Gram-positive and Gram-negative bacteria, and are much improved compared with the formulae in Table II. The obtained regression lines are shown as dotted lines in Figs. 3 and 4, as examples of the results for Gram-positive and Gram-negative bacteria.

A better correlation is obtained when the range of analysis is restricted to the nitro compounds in Figs. 3 and 4. The regression line is shown as a bold line in each figure. Clearly non-nitro phenols form another group apart from the bold line. The formulae of the regression lines restricted to nitro compounds are also collected in Table III for all the examined bacteria. The significance level of all the formulae is over 99% by the $F$-test.

**Theoretical Estimation of $pK_a$**

There are a few compounds whose $pK_a$ values are not known. These unknown $pK_a$ values were estimated by the following procedure in this paper. From the thermodynamic relationship

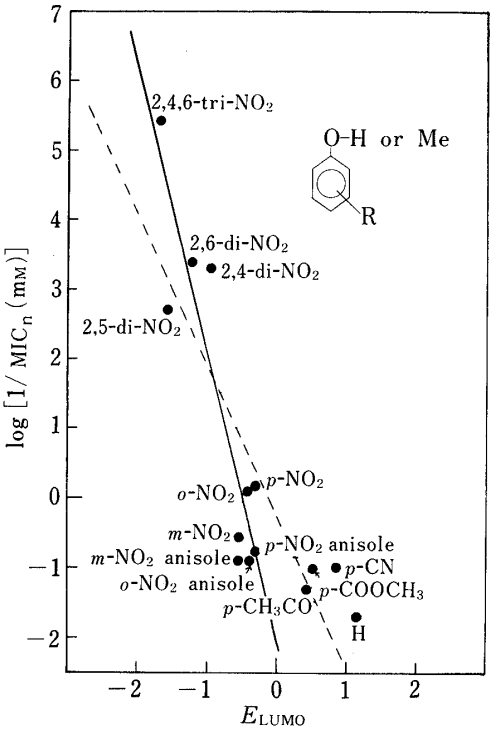


Fig. 3. The Structure–Activity Relationship in Terms of Undissociated Molecular Species with Gram-Positive *S. aureus* U9NO

---, log [1/MIC$_n$ (mM)] = $-0.292(\pm0.813)-2.18(\pm0.904)E_{LUMO}$ ($n=14$, $s=1.28$, $r=0.834$, $F=25.2$ (>99%)) [all compounds]. ——, log [1/MIC$_n$ (mM)] = $-1.98(\pm1.41)-3.97(\pm1.49)E_{LUMO}$ ($n=10$, $s=1.02$, $r=0.908$, $F=51.5$ (>99%)) [nitro compounds].

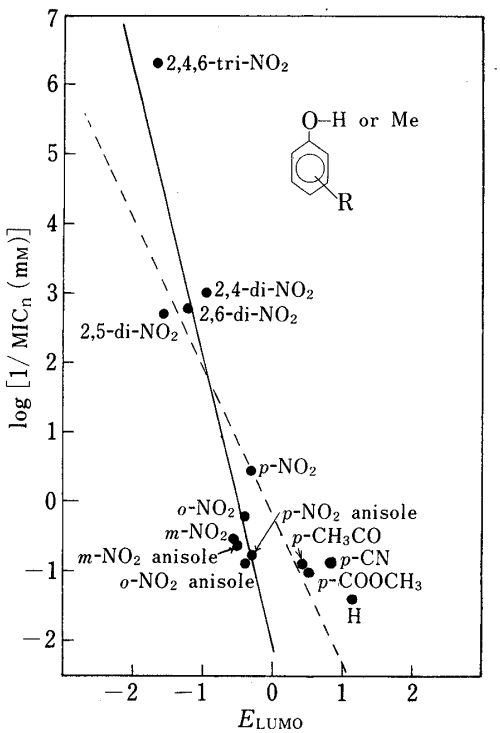


Fig. 4. The Structure–Activity Relationship in Terms of Undissociated Molecular Species with Gram-Negative *E. coli* NIHJ JC-2

---, log [1/MIC$_n$ (mM)] = $-0.199(\pm0.877)-2.14(\pm0.975)E_{LUMO}$ ($n=14$, $s=1.38$, $r=0.809$, $F=20.9$ (>99%)) [all compounds]. ——, log [1/MIC$_n$ (mM)] = $-2.07(\pm1.49)-4.12(\pm1.58)E_{LUMO}$ ($n=10$, $s=1.08$, $r=0.905$, $F=49.8$ (>99%)) [nitro compounds].

$$\Delta G^\circ = -\boldsymbol{R}T\ln K_a = 2.303\boldsymbol{R}T\mathrm{p}K_a \quad (1)$$

where $\Delta G^\circ$ is the standard molar Gibbs free energy change, and putting $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$ in Eq. 1, we obtain

$$-\ln K_a = \Delta H^\circ/\boldsymbol{R}T - \Delta S^\circ/\boldsymbol{R} \quad (2)$$

In solutions, the relation (3) will be valid.

$$\Delta H^\circ \simeq \Delta E^\circ = E_{dis} - E_{undis} + \Delta = \Delta E + \Delta \quad (3)$$

where $E_{dis}$, $E_{undis}$ and $\Delta$ are the total energies of the dissociated species, that of the undissociated species and the change of the solvation energy following the dissociation, respectively. The standard molar entropy change $\Delta S^\circ$ and the solvation energy change are assumed to be constant, because of the similar structures of the compounds examined in this paper. Therefore, p$K_a$ is proportional to the dissociation energy, $\Delta E = E_{dis} - E_{undis}$, which is obtained by quantum-chemical calculation of the total energy change of the following reaction (4).

$$HA \rightarrow A^- + H^+ \quad (4)$$

The values of $\Delta E$ are collected in Table I. The result of correlation analysis between $\Delta E$ and p$K_a$ supported our treatment, as shown in Fig. 5. The unknown p$K_a$ values of *p*-CN and *p*-COOCH$_3$ phenols were estimated from Fig. 5.

**Physico-Chemical Meaning of p$K_a$ and $E_{LUMO}$**

The physico-chemical meaning of p$K_a$ in relation to MO level has been clarified in the foregoing discussion: *i.e.*, p$K_a$ is directly obtainable from the total energy change following the proton dissociation of a proton-dissociable compound, and is straightforwardly connected by Eq. 1 to the concentration ratio of dissociated and undissociated species of the proton-dissociable compound. Therefore, we used the value of p$K_a$ for the calculation of the concentration of undissociated species in the structure–activity relationship correlation analyses in Figs. 3, 4, and Table III. It is well known that dissociation affects the absorption properties of a drug molecule. Absorption through a cell membrane is generally facilitated by conversion of a molecule to an undissociated species.[4]

On the other hand, $E_{LUMO}$ is a measure of the relative electron-accepter property of a molecule.[4] Many authors have discussed the charge-transfer interaction between a drug and its receptor using $E_{LUMO}$.[4] In this paper, we also used $E_{LUMO}$ as a measure of the interaction of a drug at the receptor site and established a linear structure–activity relationship for all the examined compounds including dissociable chemicals for which the active species is restricted to the undissociated molecule depending upon the p$K_a$ value.

**Parallelism between p$K_a$ and $E_{LUMO}$ in Dissociable Compounds**

The structure–activity relationship in phenols was studied by Fujita using p$K_a$ as a parameter and a reliable correlation between p$K_a$ and MIC for the undissociated species was obtained.[2] Is there any relationship between $E_{LUMO}$ and p$K_a$? Regression analysis was performed to investigate this problem (Fig. 6) and a fair correlation, which is similar to Fujita's result, was obtained. Detailed examination, however, revealed that there are contradictions: *e.g.*, among three examined dinitrophenols, the most active bactericidal 2,5-

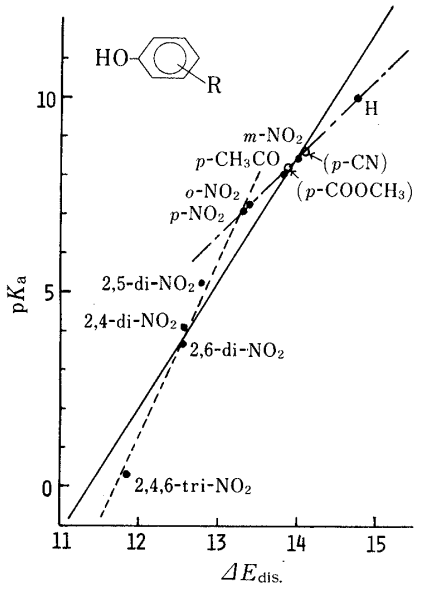


Fig. 5. The Relationship between p$K_a$ and the Dissociation Energy ($\Delta E$)

——, $pK_a = -36.8\,(\pm 9.52) - 3.24\,(\pm 0.72)\Delta E$ ($n=9$, $s=0.767$, $r=0.971$, $F=114$ (>99%)) [all compounds]. —·—, $pK_a = -19.6\,(\pm 2.15) + 2.00\,(\pm 0.16)\Delta E$ ($n=5$, $s=0.056$, $r=0.999$, $F=1497$ (>99%)) [mono-substituted compounds]. – – –, $pK_a = -53.1\,(\pm 14.1) + 4.59\,(\pm 1.12)\Delta E$ ($n=5$, $s=0.389$, $r=0.991$, $F=173$ (>99%) [*ortho*-substituted compounds].

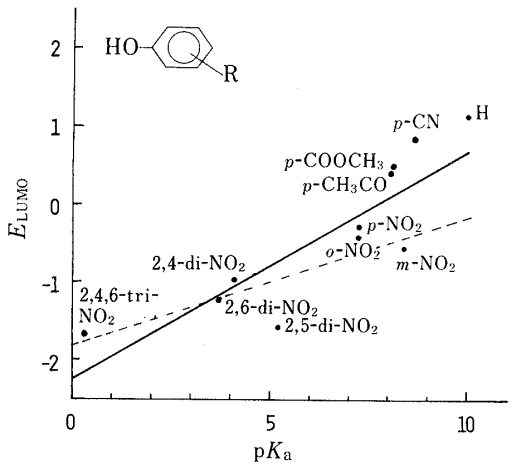


Fig. 6. The Relationship between LUMO Energy and p$K_a$

——, $E_{LUMO} = -2.25\,(\pm 0.923) + 0.296\,(\pm 0.132)pK_a$ ($n=11$, $s=0.523$, $r=0.860$, $F=25.6$ (>99%)) [all compounds]. – – –, $E_{LUMO} = -1.81\,(\pm 0.746) + 0.165\,(\pm 0.129)pK_a$ ($n=7$, $s=0.341$, $r=0.826$, $F=10.8$ (>95%)) [nitro compounds].

dinitrophenol has the lowest $E_{LUMO}$ in accordance with the obtained structure–activity relationship, but its $pK_a$ value is the highest among the three, in conflict with the relationship. Moreover, the correlation between $E_{LUMO}$ and $pK_a$ is less good when the examined compounds were restricted to nitro compounds. The correlation is generally improved when the examined species are restricted to similar compounds as shown in Fig. 5, for example, if the correlation reflects a particular physical meaning. Therefore, we can conclude that the relationship between $pK_a$ and $E_{LUMO}$ is not straightforward and is modified by other factors. In this paper, $pK_a$ is used for calculating the concentration of undissociated species and then $E_{LUMO}$ is used as a descriptor of the charge-transfer type drug–receptor interaction.

**Other Molecular Indices**

Besides $E_{LUMO}$, many other molecular orbital indices were calculated: *i.e.*, total electron density, frontier electron density (nucleophilic ($f_N$) and electrophilic ($f_E$)), superdelocalizability ($S_N$ and $S_E$), and highest occupied molecular orbital (HOMO) energy. The regression analyses with those parameters, however, did not give good correlations, even in the case of $f_N$, the electron density of LUMO. This means that there is no positional effect in the antibacterial action of phenolic and aromatic nitro compounds. Because of the small range of log $P$ of the compounds examined, as shown in Table I, no correlation with log $P$ was obtained even when the effective species was restricted to undissociated molecules.

**Effect of pH of the Agar Media on Antibacterial Testing**

Provided that only the undissociated species is effective in the antibacterial action of phenolic and aromatic nitro compounds, the pH of the agar media where the antibacterial test

TABLE IV. Antibacterial Activity of Aromatic Nitrophenols at Various pH Values of the Medium

| | | Minimum inhibitory concentration (mM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | G (+) | | | | G (−) | | | |
| | | *S. aureus* 209P | *S. aureus* U9NO | *M. luteus* ATCC 9341 | *B. subtilis* PCI219 | *E. coli* NIHJ JC-2 | *K. pneumoniae* W52 | *S. typhimurium* LT-2 | *P. vulgaris* YO-1 |
| | | Inoculum size (cells/ml) | | | | | | | |
| | | $10^8$ | $10^8$ | $10^8$ | $10^7$ | $10^5$ | $10^5$ | $10^5$ | $10^6$ |
| *m*-Nitrophenol | pH 6 | 4 | 4 | 2 | 4 | 4 | 4 | 2 | 2 |
| | pH 7 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 2 |
| | pH 8 | 8 | 8 | 4 | 8 | 4 | 4 | 2 | 2 |
| *p*-Nitrophenol | pH 6 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 |
| | pH 7 | 2 | 4 | 4 | 4 | 1 | 1 | 1 | 1 |
| | pH 8 | 8 | 8 | 8 | 8 | 4 | 4 | 4 | 8 |
| 2,4-Dinitrophenol | pH 6 | 0.063 | 0.063 | 0.25 | 0.25 | 0.25 | 4 | 0.25 | 0.25 |
| | pH 7 | 1 | 1 | 2 | 4 | 2 | 8 | 2 | 2 |
| | pH 8 | 2 | 4 | 4 | 16 | 4 | 8 | 4 | 8 |
| 2,5-Dinitrophenol | pH 6 | 0.039 | 0.078 | 0.155 | 0.039 | 0.039 | 0.625 | 0.078 | 0.155 |
| | pH 7 | 0.31 | 0.31 | 0.625 | 0.625 | 0.31 | 1.25 | 0.31 | 0.31 |
| | pH 8 | 1.25 | 1.25 | 1.25 | 2.5 | 1.25 | 2.5 | 0.625 | 0.625 |
| 2,6-Dinitrophenol | pH 6 | 0.25 | 0.25 | 2 | 0.5 | 0.5 | 4 | 0.5 | 0.5 |
| | pH 7 | 2 | 2 | 16 | 1 | 2 | 8 | 2 | 4 |
| | pH 8 | 8 | 32 | 32 | 2 | 8 | 8 | 8 | 16 |
| 2,4,6-Trinitrophenol | pH 6 | 12.5 | 12.5 | 12.5 | 12.5 | 3.2 | 6.25 | 6.25 | 3.2 |
| | pH 7 | 50 | 50 | 50 | 50 | 6.25 | 12.5 | 12.5 | 50 |
| | pH 8 | 50 | 50 | 50 | 50 | 6.25 | 12.5 | 12.5 | 50 |

TABLE V. Regression Analysis Equations Based on $E_{LUMO}$ for the Undissociated Species of the Compounds in Table IV at Various pH Values of the Medium

$\log(1/MIC_n(mM)) = a + bE_{LUMO}$ $n=6$

| | | $a$ | $b$ | $s$ | $r$ | $F^{a)}$ |
|---|---|---|---|---|---|---|
| G (+) | | | | | | |
| *S. aureus* 209P | pH 6 | −1.30 (±3.43) | −3.33 (±2.95) | 1.29 | 0.843 | 9.8 (>95%) |
| | pH 7 | −1.26 (±3.58) | −3.32 (±3.08) | 1.35 | 0.832 | 9.0 (>95%) |
| | pH 8 | −1.53 (±3.45) | −3.91 (±2.97) | 1.30 | 0.877 | 13.4 (>95%) |
| *S. aureus* U9NO | pH 6 | −1.31 (±3.11) | −3.29 (±2.67) | 1.17 | 0.863 | 11.6 (>95%) |
| | pH 7 | −1.54 (±3.28) | −3.46 (±2.82) | 1.24 | 0.862 | 11.6 (>95%) |
| | pH 8 | −1.76 (±3.43) | −3.99 (±2.95) | 1.29 | 0.883 | 14.1 (>95%) |
| *M. luteus* ATCC 9341 | pH 6 | −0.98 (±2.67) | −2.69 (±2.30) | 1.01 | 0.852 | 10.6 (>95%) |
| | pH 7 | −1.70 (±2.83) | −3.42 (±2.43) | 1.07 | 0.890 | 15.2 (>95%) |
| | pH 8 | −1.62 (±3.29) | −3.86 (±2.83) | 1.24 | 0.873 | 12.8 (>95%) |
| *B. subtilis* PCI219 | pH 6 | −1.53 (±2.67) | −3.26 (±2.29) | 1.01 | 0.892 | 15.6 (>95%) |
| | pH 7 | −1.67 (±2.97) | −3.53 (±2.55) | 1.12 | 0.887 | 14.7 (>95%) |
| | pH 8 | −1.89 (±3.75) | −4.16 (±3.23) | 1.42 | 0.873 | 12.8 (>95%) |
| G (−) | | | | | | |
| *E. coli* NIHJ JC-2 | pH 6 | −1.50 (±3.03) | −3.32 (±2.60) | 1.14 | 0.871 | 12.6 (>95%) |
| | pH 7 | −1.51 (±3.58) | −3.57 (±3.08) | 1.35 | 0.850 | 10.4 (>95%) |
| | pH 8 | −1.57 (±4.18) | −4.05 (±3.59) | 1.58 | 0.843 | 9.8 (>95%) |
| *K. pneumoniae* W52 | pH 6 | −1.48 (±3.58) | −2.74 (±3.08) | 1.35 | 0.777 | 6.1 |
| | pH 7 | −1.47 (±3.73) | −3.20 (±3.20) | 1.40 | 0.811 | 10.4 (>95%) |
| | pH 8 | −1.50 (±4.12) | −3.84 (±3.54) | 1.55 | 0.832 | 9.0 (>95%) |
| *S. typhimurium* LT-2 | pH 6 | −1.20 (±2.95) | −2.99 (±2.54) | 1.11 | 0.853 | 10.7 (>95%) |
| | pH 7 | −1.28 (±3.22) | −3.34 (±2.77) | 1.21 | 0.859 | 11.3 (>95%) |
| | pH 8 | −1.40 (±3.55) | −3.93 (±3.06) | 1.34 | 0.872 | 12.7 (>95%) |
| *P. vulgaris* YO-1 | pH 6 | −1.34 (±2.88) | −3.22 (±2.47) | 1.09 | 0.875 | 13.1 (>95%) |
| | pH 7 | −1.21 (±3.34) | −3.33 (±2.88) | 1.26 | 0.849 | 10.3 (>95%) |
| | pH 8 | −1.31 (±3.01) | −3.66 (±2.58) | 1.13 | 0.892 | 15.5 (>95%) |

The numerals in parentheses are ±95% confidence limits of $a$ and $b$. a) $F_1^4(95\%)=7.71$ and $F_1^4(99\%)=21.20$.

is carried out must affect the total dose of the antibacterial agent required for the minimum inhibitory effect: *i.e.*, lower pH increases the antibacterial activity of phenolic compounds based on the total dose and higher pH depresses it.

The experiments on the pH effect proved that this view is correct (Table IV). Formulae obtained by regression analysis based on the concentration of undissociated species determined from Table IV are collected in Table V with the correlation coefficients. The characteristic feature in these correlation formulae is that the coefficients $a$ and $b$ increase in absolute values with increasing pH.

**Requirements for Antibacterial Compounds**

Two requirements appear from the foregoing discussions for highly active antibacterial compound, *i.e.*, low $E_{LUMO}$ and undissociated molecular species. The low $E_{LUMO}$ is essential for both antifungal[1)] and antibacterial activities (Table I) regardless of the category of phenolic compounds: *e.g.* cinnamaldehyde has strong antifungal and strong antibacterial activities. This requirement may reflect an electron-donating character of the acceptor associated with the vital reaction site in the micro-organism. The requirement for undissociated species may be related to the cell membrane penetration process.

2,5-Dinitrophenol is the best fit for the two requirements among the compounds examined in this research, and has the most effective antibacterial action. Among the three dinitrophenols, the $E_{LUMO}$ of 2,5-dinitrophenol is extraordinarily low and is comparable with the value of 2,4,6-trinitrophenol. Moreover, the large $pK_a$ value shows that a high con-

TABLE VI. Antibacterial Activity of Undissociable Aromatic Nitro Compounds at Various pH Values of the Medium

| | | Minimum inhibitory concentration (mM) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | G (+) | | | | G (−) | | | | |
| | | *S. aureus* 209P | *S. aureus* U9NO | *M. luteus* ATCC 9341 | *B. subtilis* PCI219 | *E. coli* NIHJ JC-2 | *K. pneumoniae* W52 | *S. typhimurium* LT-2 | *P. vulgaris* YO-1 | |
| | | Inoculum size (cells/ml) | | | | | | | | $E_{LUMO}$ (eV) |
| | | $10^8$ | $10^8$ | $10^8$ | $10^7$ | $10^5$ | $10^5$ | $10^5$ | $10^6$ | |
| 2,4,6-Trinitro-anisole | pH 6 | 0.063 | 0.125 | 0.5 | 0.063 | 0.063 | 1 | 0.25 | 0.25 | −1.5064 |
| | pH 7 | 1 | 1 | 2 | 0.5 | 0.5 | 2 | 1 | 2 | |
| | pH 8 | 2 | 2 | 8 | 2 | 2 | 8 | 2 | 4 | |
| 2,4,6-Trinitro-1-chlorobenzene | pH 6 | 0.5 | 0.5 | 0.125 | 0.125 | 0.5 | 2 | 1 | 0.5 | —[a] |
| | pH 7 | 1 | 1 | 2 | 0.25 | 1 | 4 | 4 | 2 | |
| | pH 8 | 2 | 2 | 2 | 1 | 1 | 4 | 4 | 2 | |
| 3,4-Dinitro-benzamide | pH 7 | 0.8 | 0.8 | 0.8 | 0.2 | 0.4 | 0.8 | 0.8 | 0.8 | −1.3303 |
| 2,4-Dinitro-anisole | pH 6 | 16 | 16 | 16 | 16 | 4 | 16 | 4 | 8 | −0.8074 |
| | pH 7 | 16 | 16 | 16 | 16 | 4 | 8 | 4 | 16 | |
| | pH 8 | 16 | 16 | 16 | 16 | 2 | 4 | 2 | 8 | |

*a*) Preliminary calculation of $E_{LUMO}$ of 2,4,6-trinitro-1-chlorobenzene was very low, but the value is excluded because there is no concrete MINDO/3 parameter for the Cl atom.

centration of the undissociated species of 2,5-dinitrophenol can be expected in the culture media (Table I).

## Attempt to Prepare a Strongly Active Agent

These two requirements for strong antibacterial activity suggested to us that 2,4,6-trinitroanisole, 2,4,6-trinitro-1-chlorobenzene, and 3,4-dinitrobenzamide might be interesting candidates for research, for example. The results are shown in Table VI with the $E_{LUMO}$ values.

As expected, all the examined compounds have low $E_{LUMO}$ and show potent antibacterial activity, especially 2,4,6-trinitroanisole (3 to 100 times more active than 2,4,6-trinitrophenol at pH 7). Contrary to our expection, 2,4,6-trinitroanisole and 2,4,6-trinitro-1-chlorobenzene showed pH dependence of the activity, as shown in Table VI. Such a phenomenon was not observed with 2,4-dinitroanisole. The origin of this unexpected phenomenon is the formation of insoluble precipitates at pH 7.0 and 8.0, which may be complexes of these compounds with components of the culture medium (no precipitate formed in buffer solutions of pH 7 and 8). Therefore, the value of MIC at pH 6 should be the real value required for inhibition of bacterial growth because pH independence is reasonable in these cases as in the case of 2,4-dinitroanisole. At pH 6, 2,4,6-trinitroanisole shows a remarkably strong antibacterial activity (200 times stronger than that of 2,4,6-trinitrophenol).

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### References and Notes

1) S. Oikawa, M. Tsuda, S. Ohnogi, and N. Kurita, *Chem. Pharm. Bull.*, **28**, 1946 (1980).
2) T. Fujita, *J. Med. Chem.*, **9**, 797 (1966).

3) C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964).
4) L. B. Kier, "Molecular Orbital Theory in Drug Research," Academic Press, N. Y. 1971; Japanese edition, "Ryoshi Yakurigaku," Chijinshokan, Tokyo, 1978.
5) P. A. Pattee and J. N. Baldwin, *J. Bacteriol.*, **84**, 1049 (1962).
6) E. Chapman, A. G. Perkin, and R. Robinson, *J. Chem. Soc.*, **1927**, 3015.
7) W. C. Davidon, *Computer J.*, **10**, 406 (1968); R. Fletcher, *ibid.*, **8**, 33 (1965); R. Fletcher and M. J. D. Powell, *ibid.*, **6**, 163 (1963).
8) R. C. Bingham, M. J. S. Dewar, and D. H. Lo, *J. Am. Chem. Soc.*, **97**, 1285 (1975).
9) For example, 2,4,6-trinitrophenol: E. N. Duesler, J. H. Engelmann, D. Y. Curtin, and I. C. Paul, *Cryst. Struct. Commun.*, **7**, 449 (1978).
10) M. Tsuda and S. Oikawa, *Report of Environmental Research Organization of Chiba University* (in Japanese), **9**, 58 (1984).
11) T. Kubota, "Structure–Activity Relationships—Quantitative Approaches. The Significance in Drug Design and Mode-of-Action Studies" ("Yakubutsu no Kouzoukasseisoukan," in Japanese), T. Fujita ed., Nankoudo, Tokyo, 1979, p. 43.
12) "Handbook of Chemistry" ("Kagaku Benran," in Japanese), Vol. 2, Chemical Society of Japan ed., Maruzen, Tokyo, 1975, p. 996.