material and some yeast adenylic acid whereas the latter contained mostly yeast adenylic acid. In neither case was any 2′,3′-cyclic phosphate detected. The precipitate was collected, washed with water and dried; m.p. 226° undepressed on admixture with authentic N,N′-dicyclohexylurea.

(c) 0.1 cc. of the stock solution acidified with 0.1 cc. of 0.01 *N* hydrochloric acid was allowed to stand at room temperature. After 9 hr., spherical crystals were observed which were shown by paper chromatography to be pure starting material (as the free acid). The supernatant also contained the adenylylureas as well as a large amount of adenosine 2′,3′-cyclic phosphate and a small amount of yeast adenylic acid. In a similar experiment employing 0.1 *N* hydrochloric acid, complete degradation to N,N′-dicyclohexylurea (m.p. and mixed m.p. with authentic sample 226–227°) occurred within one hour at room temperature.

One hundred mg. of the pyridinium salts of III and IV, obtained by cellulose column chromatography (Method A above) and dried *in vacuo* over phosphorus pentoxide, was triturated thoroughly with 1 cc. of *N* hydrochloric acid and the insoluble dicyclohexylurea was collected after one hour, washed with water and dried over phosphorus pentoxide; wt. 37 mg.; theoretical yield for the pyridinium salts, 35.2 mg.[39]

**Action of Sodium Benzoxide on "x," "y" and Adenosine-2′,3′-cyclic Phosphate.**—Twenty-five mg. of the pyridinium salts of a mixture of III and IV obtained by cellulose column chromatography were dried *in vacuo* over phosphorus pentoxide and dissolved in 0.3 cc. of anhydrous benzyl alcohol; 0.1 cc. of a solution of sodium benzoxide in benzyl alcohol (from 100 mg. of sodium in 5 cc. of anhydrous benzyl alcohol) was added and the reaction vessel was sealed and allowed to stand at room temperature. Aliquots of 0.1 cc. were removed at intervals, diluted with 0.2 cc. of 2.5% acetic acid (to *p*H 3–4) and then extracted twice with ether. The residual aqueous solutions were examined by paper chromatography in solvent 1 and spectrophotometric estimation was made of the amounts of the reaction products and the unreacted adenylylureas. The results are summarized in Table II.

TABLE II

| | 1 hr. | 7 hr. | 17 hr.[40] |
|---|---|---|---|
| "x" and "y," % | 70.0 | 25.0 | 7.4 |
| Benzyl esters, % | 26.4 | 49.8 | 31.7 |
| Yeast adenylic acid, % | 3.6 | 25.2 | 60.9 |

Table II. The benzyl esters were separated by large scale paper chromatography in solvent 1 and after elution and concentration were rechromatographed in solvent 2 and in butanol–acetic acid–water (4:1:5). In solvent 2 the reported[24] $R_f$ values for the benzyl esters of *a* and *b* are, respectively, 0.67 and 0.56; found: 0.64 and 0.52. In butanol–acetic acid–water the reported[24] values are: 0.44 and 0.50; found: 0.49 and 0.57. Elution of the benzyl esters followed by spectrophotometric estimation revealed that the ester of adenylic acid *b* was present in much larger amount than that of adenylic acid *a* (4:1). Adenosine 2′,3′-cyclic phosphate and the individual isomeric adenylylureas were treated also with sodium benzoxide under similar conditions to those described above. The benzyl esters were formed in good yield (40–55%) in each instance. The determination of the relative amounts of the two benzyl esters revealed that in every case the *b* ester predominated (80–85%). Further identification of the mixed benzyl esters was made by acid (80% acetic acid at 100°) and alkaline (0.5 *N* sodium hydroxide at 30°) hydrolysis and chromatographic examination of the products of the reaction in solvent 2 and in butanol–acetic acid–water.[24] In both cases a mixture of adenylic acids *a* and *b* resulted.

**Acknowledgments.**—One of us (H.G.K.) wishes to thank the National Research Council of Canada, Ottawa, for financial support of this work. We are indebted to Dr. R. H. Wright for the determination of the infrared spectra.

(39) The slightly higher yield indicates some loss of pyridine from the pyridinium compounds.

(40) The absolute exclusion of moisture is difficult and will profoundly influence such a small scale reaction. This probably accounts for the increased formation of yeast adenylic acid with time.

VANCOUVER 8, B. C., AND
BERKELEY 4, CALIFORNIA

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[CONTRIBUTION FROM VENEREAL DISEASE EXPERIMENTAL LABORATORY, U. S. PUBLIC HEALTH SERVICE, SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF NORTH CAROLINA]

# The Electrical Effect of the N-Oxide Group in Pyridine 1-Oxide[1]

BY H. H. JAFFÉ

RECEIVED MARCH 5, 1954

Pyridine 1-oxide is shown to violate Brown's "chemical non-crossing rule."[3] The two major semi-empirical molecular orbital methods of calculating the reactivity of this compound are considered. The *static* method cannot correctly predict the reactivity in both electrophilic and nucleophilic substitution in this case. Hammett substituent constants for the replacement of a CH group in benzene by an $N^+ \rightarrow O^-$ group are derived from the *pK*'s of the N-oxides of nicotinic acid, isonicotinic acid, 3- and 4-hydroxy- and 4-aminopyridine, and are used in calculation, by the *localization* method, of the relative reactivity of the various positions in pyridine 1-oxide. The electronic structure and the dipole moment of this compound are also discussed.

Two main semi-empirical wave mechanical methods are available for the prediction of reactivity of conjugated organic compounds. These methods are referred to as the *localization* and the *static* methods[2]; both have been used extensively, and are generally found to lead to the same conclusions.[3] Unfortunately, neither method achieves a calculation of the true activation energy. In the *static* method the $\pi$-electron contribution to the total potential energy of the reacting system is calculated at some point along the reaction path before the transition state is reached. The *localization* method, on the other hand, is concerned with the same quantity at some point on the reaction path beyond the transition state.[3] The fact that predictions made by both methods usually agree with each other and with the experimental findings has led Brown to propose the "chemical non-crossing rule." This rule expresses the notion that curves representing the variation of the potential energy of the reacting system along the reaction path for similar compounds, or for different positions in a single compound, usually do not cross.[3] This rule is not postulated to be universally valid; it has received some theoretical support from the

(1) Paper V in the Series: Theoretical Considerations Concerning the Hammett Equation. For Paper IV see H. H. Jaffé, *J. Chem. Phys.*, **21**, 415 (1953).

(2) H. H. Greenwood, *Trans. Faraday Soc.*, **48**, 585 (1952).

(3) R. D. Brown, *Quart. Rev.*, **6**, 63 (1952).

work of Greenwood,[2] and has generally been found applicable in aromatic substitution reactions.[4]

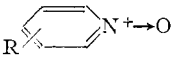
Pyridine 1-oxide undergoes electrophilic substitution primarily in the 4-position, and to a lesser degree in the 2-position.[5-7] Derivatives of this compound are most susceptible to nucleophilic substitution in these same two positions.[5,7,8] The *static* method cannot correctly predict the reactivity of these compounds, since it cannot predict maximum electrophilic *and* nucleophilic reactivity for the same position. Accordingly, pyridine 1-oxide is an example of a compound violating the non-crossing rule. A similar situation holds for azobenzene and possibly for nitrosobenzene.[7]

To gain better insight into the non-crossing rule we decided to determine what predictions for pyridine 1-oxide could be made by *localization* theory. The calculations require the knowledge of coulomb integrals for the two heteroatoms. Theoretical calculation of these parameters is not practical. Therefore, we resorted to the empirical procedure based on the theoretical treatment of Hammett's substituent constants.[9] This procedure has the advantage that the parameters obtained apply to the solvated group in solution, rather than to a gaseous state. There is no *a priori* reason to believe that the Hammett equation should be applicable to such heterocyclic compounds as pyridine 1-oxide, except for the moderate success encountered in the treatment of pyridine and quinoline.[10] However, use of the Hammett equation appears justified in the absence of a better empirical method.

**Substituent Constants.**—In order to be able to calculate the needed coulomb integrals, we proceeded to evaluate substituent constants for the replacement of a CH group in benzene by an $N^+\rightarrow O^-$ group. For this purpose we determined the $pK$'s of the N-oxides of nicotinic acid, isonicotinic acid, 4-amino- and 4-hydroxypyridine. Literature data for the N-oxides of 3- and 4-hydroxypyridine[11] and 4-aminopyridine[12] also have been used. The relevant $pK$'s are summarized in Table I. Calculation of the substituent constants from these $pK$'s was based on data recently reviewed by this author.[13] The values obtained are listed in Table I. The substituent constants obtained from the N-oxide of nicotinic acid in water and in 50% ethanol, and from 3-hydroxypyridine 1-oxide agree well with each other; it is worth noting that they are the largest $\sigma$-values so far encountered. The substituent constants obtained from the N-oxide of isonicotinic acid in the two solvents also agree well. The $pK$ of 4-hydroxypyridine 1-oxide leads to an appreciably larger $\sigma$-value than the $pK$ of the N-oxide of isonicotinic acid. Since resonance between structures I and II must contribute appreciably to the ground state of derivatives of pyridine 1-oxide[14] with electron repelling substituents in the 4-position, it is not surprising that special substituent constants ($\sigma^*$)[13] are required in reactions of these compounds.[13]

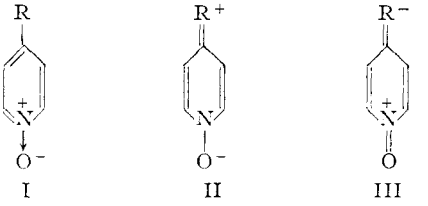
TABLE I

THE $pK$'S OF SOME DERIVATIVES OF PYRIDINE 1-OXIDE,



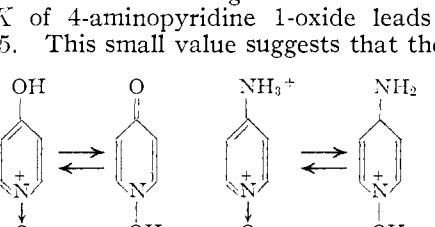
| R | Solvent | $pK$ | $\sigma$ | Reaction no.[a] |
|---|---|---|---|---|
| 4-COOH | $H_2O$ | 2.86 | 1.34 | 1a |
| | 50% $C_2H_5OH$ | 3.71 | 1.37 | 1e |
| 3-COOH | $H_2O$ | 2.73 | 1.47 | 1a |
| | 50% $C_2H_5OH$ | 3.54 | 1.50 | 1e |
| 4-$NH_2$ | $H_2O$ | 3.65[b] | (0.365)[c] | 26a |
| 4-OH | $H_2O$ | 5.76[d] | (1.88)[c] | 23a |
| 3-OH | $H_2O$ | 6.4[e] | 1.59 | 23a |

[a] The numbers of the reactions in Table I of ref. 13, from which the data for the calculation of substituent constants were taken. [b] Hirayama and Kubota give $pK$ = 3.54 (*cf.* ref. 12). [c] These values are minimum values; *cf.* the discussion of the tautomerism in these compounds in the text. [d] Shaw (ref. 11) gives $pK$ = 5.9. [e] Taken from ref. 11.



I II III

It appears likely that still other substituent constants (of smaller magnitude) will be needed with reactions of strongly electron attracting groups in the 4-position, since in this case resonance of structures I and III must be important.[14] 4-Hydroxypyridine 1-oxide (IV) is tautomeric with N-hydroxy-4-pyridone (V), and hence the measured $pK$ corresponds to the weaker of the two acids. Since it is not known whether IV or V is the weaker acid,[11] the value $\sigma^*$ = 1.82 is only a lower limit; nevertheless, this value is the largest $\sigma^*$-value on record. The $pK$ of 4-aminopyridine 1-oxide leads to $\sigma$ = 0.365. This small value suggests that the ionization process observed is the ionization of the tautomeric ion VII rather than that of VI. This conclusion is in agreement with the assignment made by Hirayama and Kubota.[12]



IV V VI VII

**Localization Energies.**—The substituent constants derived permit the evaluation of coulomb integrals $\alpha_N$ = 2.004 for the nitrogen atom and

(4) (a) *Cf.* G. W. Wheland, THIS JOURNAL, **64**, 900 (1942); (b) G. W. Wheland and L. Pauling, *ibid.*, **57**, 2086 (1935).

(5) E. Ochiai, *J. Org. Chem.*, **18**, 534 (1953).

(6) H. J. denHertog, *Rec. trav. chim.*, **69**, 468 (1950).

(7) M. Colonna and S. Patutta, *Gazz. chim. ital.*, **83**, 622 (1953).

(8) E. Shaw, J. Bernstein, K. Losee and W. A. Lott, THIS JOURNAL, **72**, 4362 (1950).

(9) H. H. Jaffé, *J. Chem. Phys.*, **20**, 279 (1952).

(10) (a) R. C. Elderfield and M. Siegel, THIS JOURNAL, **73**, 5622 (1951); (b) H. H. Jaffé, *J. Chem. Phys.*, **20**, 1554 (1952).

(11) E. Shaw, THIS JOURNAL, **71**, 67 (1949).

(12) H. Hirayama and T. Kubota, *J. Pharm. Soc. Japan*, **73**, 140 (1953).

(13) H. H. Jaffé, *Chem. Revs.*, **53**, 191 (1953); see the appendix of his reference for the method of calculation of new substituent contants.

(14) Evidence for this resonance is discussed below and in ref. 5.

$\alpha_O = 1.016$ for the oxygen atom.[15] These coulomb integrals were then used in the calculation of the localization energy in the 2-, 3- and 4-position of pyridine 1-oxide. The results are given in Table II.

TABLE II

LOCALIZATION ENERGIES[a]

| Compound | Position | $E^r$ | Electrophilic substitution $E$ | Electrophilic substitution $L$ | Nucleophilic substitution $E$ | Nucleophilic substitution $L$ |
|---|---|---|---|---|---|---|
| Benzene | | 8.000 | 5.464 | 2.536 | 5.464 | 2.536 |
| Pyridine | 2 | 8.651 | 5.939 | 2.712 | 6.339 | 2.312 |
| | 3 | 8.651 | 6.164 | 2.487 | 6.264 | 2.378 |
| | 4 | 8.651 | 6.014 | 2.637 | 6.414 | 2.228 |
| Pyridine 1-oxide | 2 | 12.894 | 10.260 | 2.634 | 10.205 | 2.689 |
| | 3 | 12.894 | 9.676 | 3.218 | 10.537 | 2.357 |
| | 4 | 12.894 | 10.420 | 2.474 | 11.180 | 1.714 |

[a] $E^r$ is the $\pi$-electron energy in the starting compound, $E$ that in the "transition" state, and $L$ the localization energy (*cf.* ref. 3). All quantities are given in units of $\beta$ (*cf.* footnote 15).

It is seen that the 4-position is most active (lowest activation energy) in both nucleophilic and electrophilic substitution, in agreement with experimental findings.[5-8] In electrophilic substitution the order of reactivity is correctly predicted 4 > 2 > 3,[5-7] but in nucleophilic substitution the predicted order is 4 > 3 > 2, although the scant experimental evidence seems to indicate the order 4, 2 > 3.[5,7,8] The disagreement between predicted and experimental reactivity in the 2-position is disappointing, and may to be due to several reasons. (a) The application of the localization method assumes constancy of entropies of activation for the reactions being compared.[3] Such constancy is usually encountered in reactions occurring at the *meta* and *para* position of benzene, but is rarely found for the *ortho* position.[16] Since the 2-position of pyridine 1-oxide is analogous to an *ortho* position in benzene, the discrepancy between theoretical and experimental reactivities may be due to an entropy effect. (b)The large amount of positive charge on the nitrogen atom adjacent to the reaction site of reactions at the 2-position may have an appreciable effect on the $\sigma$-bond strength in the transition state, and may thus invalidate the basic assumption of localization theory that the $\pi$-electron contribution to the activation energy is the only contribution which distinguishes the various positions in a given compound. (c) In the transition state the carbon atom at which localization occurs is in a different state of hybridization than the other carbon atoms, and carries a fractional charge. Neither of these facts can be adequately taken into account in the calculations. Their effect must have the greatest importance in dealing with the 2-position due to the proximity of the two perturbations (at the nitrogen atom and the reacting carbon atom), and hence may lead to the observed discrepancy. For these reasons it appears that too much significance should not be given to predictions made for the 2-position, and that the most significant comparisons are those between the 3- and 4-positions.[17]

Thus, the calculations by the localization method are in agreement with experiment, at least as far as the 3- and 4-positions are concerned, and the violation of the non-crossing rule encountered in pyridine 1-oxide is of the type illustrated in Brown's Fig. 4.[3]

Table II also contains localization energies for benzene and the various positions in pyridine and thus permits comparison of the reactivities of the three compounds. The data correctly predict the order of reactivity in nucleophilic substitution to be 4-pyridine 1-oxide > 4-pyridine > benzene,[18] and 4-pyridine, 2-pyridine > 3-pyridine > benzene.[19] The data also predict that the 4-position in pyridine is more activated than the 2-position, in agreement with some of the experimental evidence; however, other evidence suggests the opposite conclusion.[19a] By the *static* method one would predict 2-pyridine, 4-pyridine > 3-pyridine > benzene.[20] The relative positions of the 2- and 4-positions depend on the assumptions made about the inductive effect. The older assumption leads to 2-pyridine > 4-pyridine,[20] while the assumption we have employed leads to the opposite order.[21]

The predictions of the relative activity of different compounds in electrophilic substitution are much less satisfactory. This fact may be largely due to the basic nature of pyridine and its N-oxide, since both compounds are probably present as their respective conjugate acids in the strongly acid reaction media used in electrophilic substitution reactions. The coulomb integrals applicable to the free bases of course are not valid for their conjugate acids. Use of the correct coulomb integrals would not greatly affect the relative order of the three positions in each compound, but would completely change the comparison between the three compounds.

**The Charge Distribution in Pyridine 1-Oxide.**—The evaluation of the coulomb integrals also permits calculation of the electron distribution in pyridine 1-oxide. The electron densities found are indicated in Fig. 1. This electron distribution is

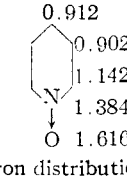



Fig. 1.—The electron distribution in pyridine 1-oxide.

not consistent with resonance between structures of types VIII–X only, as has been suggested by

(15) In accordance with customary practice in LCAO calculations in the coulomb integrals of the ring carbon atoms were equated to zero, thus defining the origin of the energy scale. The resonance integral of the carbon–carbon bond ($\beta$) was taken as the unit of energy, and all coulomb integrals and other energy quantities are expressed in this unit.

(16) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 121.

(17) R. D. Brown, THIS JOURNAL, **75**, 4077 (1953), has carefully examined MO predictions of reactivity in the *ortho* position of biphenyl, and has concluded that the failure of the predictions are due, not to a breakdown of the MO calculation, but to the intervention of steric effects.

(18) M. Katada, *J. Pharm. Soc. Japan*, **67**, 56 (1947); *C. A.*, **45**, 9537*e* (1951).

(19) (a) J. F. Bunnett and R. E. Zahler, *Chem. Revs.*, **49**, 273 (1951); (b) M. Katada, *J. Pharm. Soc., Japan*, **67**, 59 (1947); *C. A.*, **45**, 9537*j* (1951).

(20) H. C. Longuet-Higgins and C. A. Coulson, *J. Chem. Soc.*, **971** (1949).

(21) This conclusion follows from the data reported in ref. 7b.

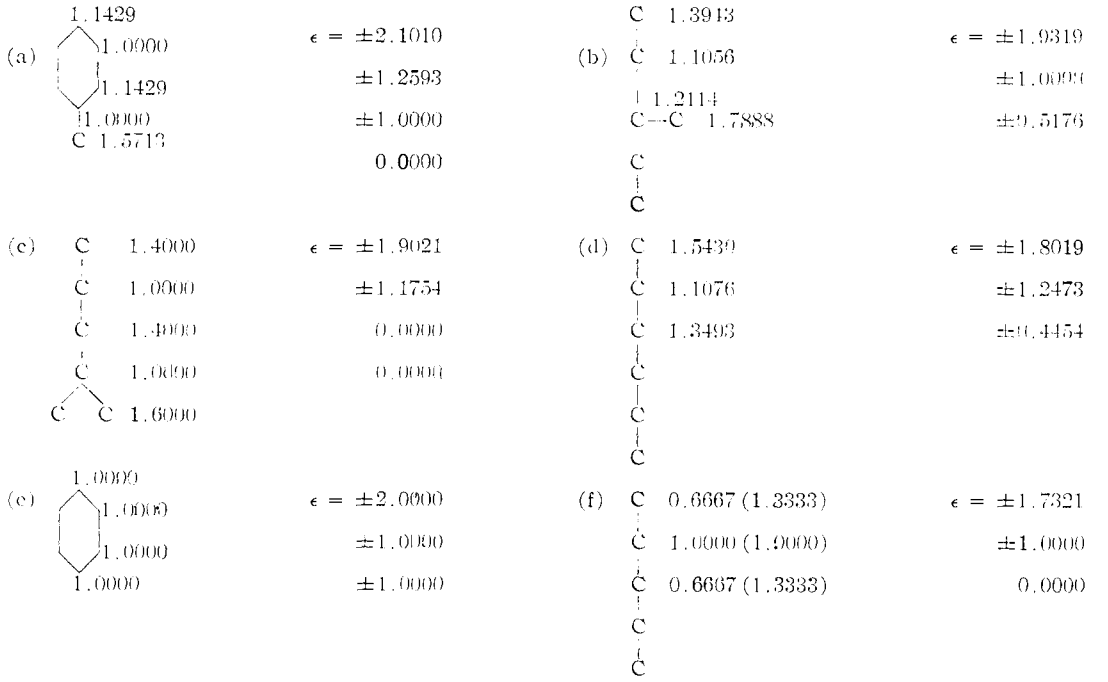



Fig. 2.—Molecular orbital energies ($\epsilon$) and electron densities for reference compounds for perturbation calculations. For (a)–(d) the figures given next to the atoms are electron densities for 8 electrons (nucleophilic substitution) in the molecules. For (b)–(d) the electron densities for 6 electrons in the molecules (electrophilic substitution are all 1.000. For (e) the electron densities given are for 6 electrons. For (f) the electron densities given are for 4 electrons (electrophilic substitution), and the values in parentheses are for 6 electrons (nucleophilic substitution). The compounds are the unperturbed reference compounds for pyridine 1-oxide (a), the transition state of substitution at its 4- (b), 3- (c) and 2- (d) positions, and for benzene and pyridine (e), and for the transition state for substitution in either of these compounds (f).

most authors, but also requires consideration of the structures of types XI and XII first proposed by Ochiai.[5] The electron distribution given in Fig.


1 permits calculation of the $\pi$-electron contributions to the dipole moment, $\mu_\pi = 0.32\ D$. This moment has the same direction and order of magnitude as the difference between the dipole moments of pyridine 1-oxide ($\mu = 4.24\ D$)[22] and of an "ordinary amine oxide,"[5] such as trimethylamine oxide ($\mu = 5.02\ D$).[22] The small magnitude of $\mu_\pi$ and the magnitude of the charges on the nitrogen and oxygen atoms indicate that the contribution to the ground state from structures of types IX and X is approximately balanced by a contribution of structures of types XI and XII.

**Acknowledgments.**—The author is indebted to Dr. J. F. Bunnett for several stimulating discussions; and to Dr. R. L. McKee for the N-oxides of nicotinic and isonicotinic acids.

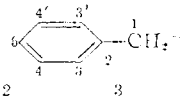
## Experimental

The N-oxides of nicotinic and isonicotinic acids were obtained through the kindness of Dr. R. L. McKee of the Department of Chemistry, University of North Carolina. 4-Amino and 4-hydroxypyridine 1-oxides were prepared in this Laboratory by known methods.[5,23] The $pK$'s were determined by standard methods of potentiometric titration in water and in 50% ethanol. Duplicate determinations agreed to within less than $\pm 0.03$ $p$H unit.

**Calculations.**—All theoretical calculations are based on molecular orbital theory in the LCAO approximation, neglecting overlap integrals. In spite of its poor theoretical foundation, this approximation frequently has been used successfully for the prediction of reactivities.[1-4,6,7b] The calculations were based on perturbation theory.[24] In the calculation of coulomb integrals the inductive effect of the nitrogen atom was treated as in earlier work,[6,7b] and the inductive effect of the oxygen atom was included in the coulomb integral of the nitrogen atom.[25] All resonance integrals were assumed equal. Although this assumption may not be completely justifiable, particularly for the nitrogen–oxygen bond, it is doubtful that the results would be appreciably affected by more realistic resonance integrals.

TABLE III

THE ATOM–ATOM POLARIZABILITIES ($\pi_{r,s}$) IN




| r s = | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 0.4116 | −0.1323 | −0.0716 | −0.0099 | −0.1162 |
| 2 | − .1323 | .3075 | − .0728 | .0050 | − .0397 |
| 3 | − .0716 | − .0728 | .4689 | − .1895 | − .0043 |
| 3' | − .0716 | − .0728 | − .0538 | − .0770 | − .0043 |
| 4 | − .0099 | .0050 | − .1895 | − .3932 | − .1323 |
| 4' | − .0099 | .0050 | − .0770 | .0105 | − .1323 |
| 5 | − .1163 | − .0397 | − .0043 | − .1323 | .4290 |

(22) E. P. Linton, THIS JOURNAL, **62**, 1945 (1940).

(23) The author is indebted to Dr. G. O. Doak and Mr. E. L. Petil for the preparation of these compounds.

(24) C. A. Coulson and H. C. Longuet-Higgins, *Proc. Roy. Soc.*, (*London*), **A191**, 39 (1947).

(25) *Cf.* the treatment of the inductive effect of the $H_3$ group in toluene, H. H. Jaffé, *J. Chem. Phys.*, **20**, 778 (1952).

The proportionality constant ($\kappa$) relating substituent constants ($\sigma$) and electron density changes was also taken from earlier work.[7b,25] The mutual atom polarizabilities ($\pi_{r,s}$)[24] for the compound $C_6H_5CH_2^-$, which were required for the evaluation of the coulomb integrals, are recorded in Table III.

The calculation of the localization energies was also based on standard perturbation methods.[3,24] The only comment required is the treatment of the inductive effect. As stated above, the inductive effect of the carbon atom at which localization occurs cannot be evaluated readily. Hence, the inductive effect of the nitrogen atom was treated by assigning to the $\alpha$-carbon atoms coulomb integrals $\alpha_C = \alpha_N/8$.[4b] The approximation used in our earlier papers[6,7b,25] would not improve the agreement between calculated and observed reactivities. The molecular orbital energies and the electron densities used in the calculations are shown in Fig. 2. The coulomb integral $\alpha_N = 0.60$ for the nitrogen atom in pyridine was taken from work on substituent constants[7b] and the dipole moment[26] of pyridine.

(26) P.-O. Löwdin, *J. Chem. Phys.*, **19**, 1323 (1951).

CHAPEL HILL, N. C.

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[CONTRIBUTION FROM THE BIOCHEMISTRY DEPARTMENT, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY AND THE FRANCIS DELAFIELD HOSPITAL]

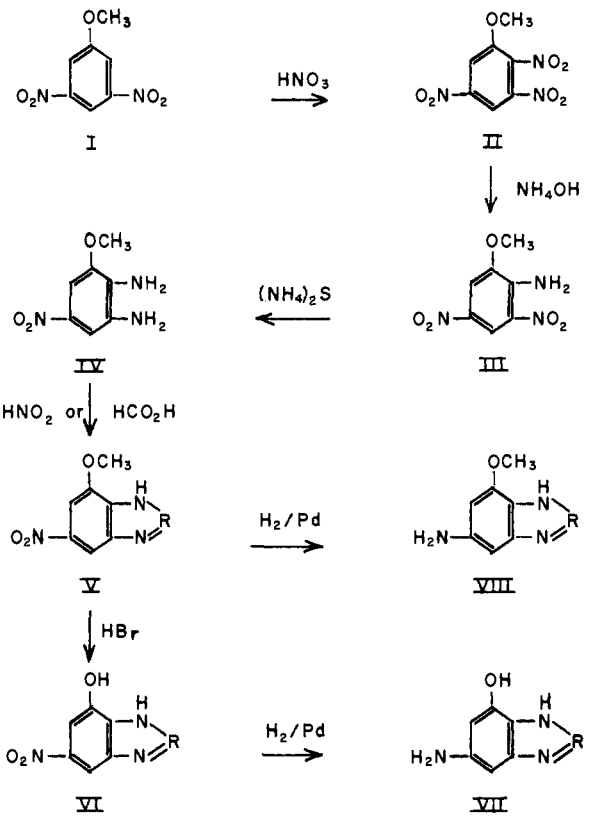
# Benzimidazoles and Benzotriazoles as Growth Antagonists

BY H. B. GILLESPIE, MORRIS ENGELMAN AND SAMUEL GRAFF

RECEIVED JANUARY 22, 1954

Analogs of the naturally existing purines are useful reagents for the study of metabolic processes. Several new benzimidazoles and benzotriazoles structurally related to guanine have been synthesized. Some of these compounds, among them 4-methoxy-6-nitrobenzimidazole and 4-methoxy-6-nitrobenzotriazole, were found to be effective growth inhibitors of *T. gelii*, a guanine requiring protozoan, and of developing embryos of *R. pipiens*. 6-Amino-4-hydroxybenzimidazole, a guanine analog, was a poor growth inhibitor, and 6-amino-4-hydroxybenzotriazole, an analog of 8-azaguanine, was not inhibitory in the systems tested. The growth of the transplanted mouse tumor, EO 755, was not affected by any of the compounds reported.

8-Azaguanine, an extremely effective antimetabolite to *T. gelii*[1] and also a carcinostatic agent for certain mouse tumors[2,3] differs from guanine only by N in place of CH in the 8-position. It seemed desirable, therefore, to simulate the guanine structure in another manner but without alteration of the ring substituents or the imidazole portion of the ring. 6-Amino-4-hydroxybenzimidazole therefore was prepared. This guanine analog, the synthetic intermediates, and the related benzotriazoles were all assayed biologically. The desired benzotriazoles and the benzimidazoles were prepared by ring closure with nitrous or formic acids from 2,3-diamino-5-nitroanisole which Borsche[4] reported merely as being formed "by reduction of 2-amino-3,5-dinitro anisole with ammonium sulfide." The product was described without other experimental details as dark red needles from water having m.p. 131–132°, which condensed with benzil in alcohol to give 2,3-diphenyl-7-nitro-5-methoxyquinoxaline (yellow needles from alcohol having m.p. 207–208°). Although the melting point of the quinoxaline derivative obtained in this Laboratory corresponded with the melting point reported by Borsche[4] the 2,3-diamino-5-nitroanisole obtained melted at 165–167° instead of 131–132°. The synthesis of 2-amino-3,5-dinitroanisole is so meagerly described by Blanksma[5] that a procedure for its preparation is included. The reactions utilized in the preparation of the compounds reported are indicated in the accompanying chart.




It has been reported that various benzimidazoles and benzotriazoles[6] are effective inhibitors of the growth of yeast,[7] lactobacilli,[8] vaccinia virus,[9] poliomyelitis virus[10] and influenza virus.[11] The

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