Hückel Molecular Orbital Calculations for Some Antimalarial Drugs and Related Molecules

JUDITH A. SINGER AND WILLIAM P. PURCELL

Department of Medicinal Chemistry, College of Pharmacy, University of Tennessee, Memphis, Tennessee 38103

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Hückel molecular orbital (HMO) π electronic charge densities and energy levels of the highest occupied molecular orbital (HOMO) and lowest empty molecular orbital (LEMO) of several representative antimalarial compounds and their parent compounds have been calculated. Comparison of results on the antimalarial molecules quinine, chloroquine, primaquine, quinacrine, pyrimethamine, proguanil, cycloguanil, and 3-piperonylsydnone with those on their appropriate parent or analogous molecules have elucidated the contributions of the substituents to the π electronic properties of the antimalarials. The interaction between components of the antimalarial complex between 2-hydroxy-4,6-dimethylpyrimidine and 4,4′-dinitrocarbanilide was studied. The specificity of this interaction seems to result from the fact that 4,4′-dinitrocarbanilide has a low-lying LEMO. The electronic aspects of the interaction with DNA of the antimalarials, quinine, chloroquine, and quinacrine, were investigated, with inclusion of the effect of amine salt formation on electronic properties of antimalarials. The experimentally observed specific interaction of chloroquine with the guanine of DNA seems to be explained satisfactorily by electron-donating or -accepting characteristics of these molecules.

The rational approach to drug design is a field of current and growing interest. Many aspects of the relationship between chemical constitution and pharmacologic response have been discussed by Schueler.[1] Application of molecular orbital calculations to biochemical compounds and their interactions has been pioneered in particular by Pullman and Pullman.[2] The application of quantum chemistry specifically to drug design has been considered recently.[3] Correlations of cholinesterase inhibition with electron densities have been achieved.[4,5] A close correlation has been found[6] between hallucinogenic potency and the calculated energy of the highest filled molecular orbital of the hallucinogens.

Our interest in drug design has been currently focused on the design of new antimalarial compounds which would overcome the resistance which has developed toward previously effective synthetic antimalarial compounds.[7] We have therefore carried out Hückel molecular orbital (HMO) calculations for a number of antimalarial compounds, in neutral and salt form, from the major classes of these drugs, for related parent compounds, and for the bases of deoxyribonucleic acid (DNA), as well as for inosine, with which several of these antimalarial compounds have been shown to interact.

Our purpose has been: (1) to provide the general patterns of π-electron charge densities and electron-donor and -acceptor properties for the various classes of antimalarials; (2) to investigate the effect of those substituents considered essential for antimalarial activity on the π-electronic properties of the parent moiety; and (3) to study theoretically the interactions recently studied experimentally,[8] of chloroquine, quinacrine, and quinine with DNA bases.

Methods

Hückel molecular orbital (HMO) calculations were conducted with an IBM 1620 computer using a program kindly furnished by Mr. G. V. O'Bleness[9] and modified by Dr. L. J. Schaad.

Unless otherwise indicated, the semiempirical parameters,[10] h and k, defined in eq 1 and 2, were those recommended by Streitwieser.[10] The terms α_X and α_C refer to the coulomb integrals of a heteroatom and a carbon, respectively; β_0 is the carbon–carbon resonance integral

$$\alpha_X = \alpha_C + h\beta_0 \quad (1)$$

$$\beta_{XY} = k\beta_0 \quad (2)$$

and β_{XY} is the resonance integral for carbon–heteroatom or heteroatom–heteroatom. Methyl groups bonded to carbon were treated as hyperconjugated according to the heteroatom model. The methyl group of a methoxy group was given an h value of 3.0 and a methyl to oxygen k value of 0.3, parameters employed by Streitwieser.[12] Portions of the molecules which are not conjugated with the major π-electron system were not included in the calculations.

It should be emphasized that the HMO method is applicable only to planar molecules. Several of the molecules (pyrimethamine in Figure 3, compounds in Figure 4, 3-piperonylsydnone in Figure 6, and 4,4′-dinitrocarbanilide (DNC) and carbanilide in Figure 7) in this study may not be planar. As a first approximation, however, they were assumed to be planar in order to examine *relative* changes in the quantum mechanical indices.

Our program was verified by calculating pyridine with the same parameters used to give results listed in a table by Coulson and Streitwieser.[13]

(1) F. W. Schueler, "Chemobiodynamics and Drug Design," McGraw-Hill Book Co., Inc., New York, N. Y., 1960.

(2) B. Pullman and A. Pullman, "Quantum Biochemistry," Interscience Publishers, Inc., New York, N. Y., 1963.

(3) R. L. Schnaare and A. N. Martin, *J. Pharm. Sci.*, **54**, 1707 (1965).

(4) W. P. Purcell, *J. Med. Chem.*, **9**, 294 (1966).

(5) W. B. Neely, *Mol. Pharmacol.*, **1**, 137 (1965).

(6) S. H. Snyder and C. R. Merril, *Proc. Natl. Acad. Sci. U. S.*, **54**, 258 (1965).

(7) W. H. Tigerd, *Military Med., Suppl.*, **131**, 853 (1966).

(8) F. E. Hahn, R. L. O'Brien, J. Ciak, J. L. Allison, and J. G. Obenick, *ibid., Suppl.*, **131**, 1071 (1966).

(9) G. V. O'Bleness, Proceedings of the 1620 Users Group Joint Eastern-Midwestern Region, Pittsburgh, Pa., Oct 1963, p 226.

(10) W. P. Purcell and J. A. Singer, *J. Chem. Eng. Data*, **12**, 235 (1967).

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

(12) A. Streitwieser, Jr., *J. Am. Chem. Soc.*, **82**, 4123 (1960).

(13) C. A. Coulson and A. Streitwieser, Jr., "Dictionary of π-Electron Calculations," W. H. Freeman and Co., San Francisco, Calif., 1965, p xix.

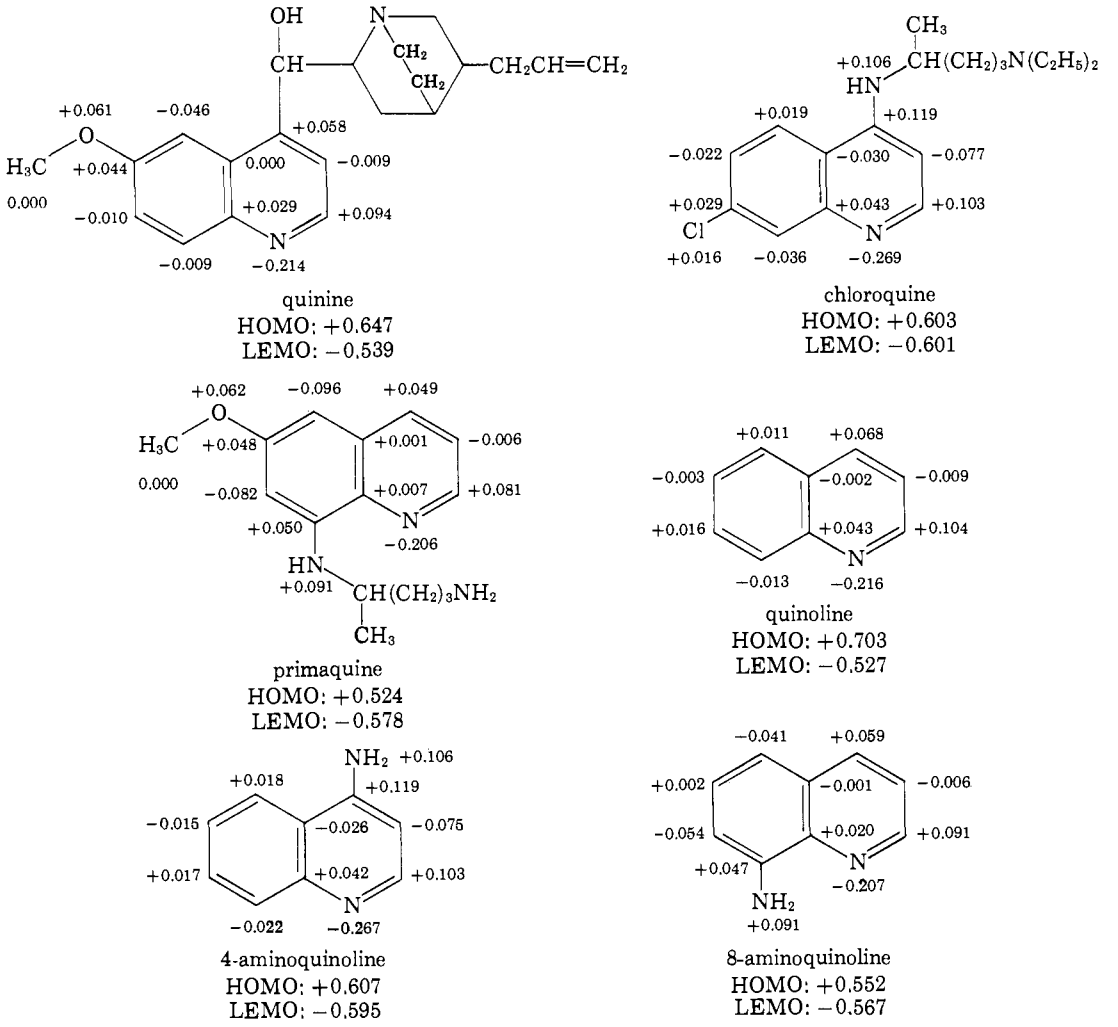


Figure 1.—HMO charge densities and energy levels of the HOMO and LEMO of the quinoline antimalarials, quinine, chloroquine, and primaquine, and their parent compounds, quinoline, 4-aminoquinoline, and 8-aminoquinoline.

Results and Discussion

Electronic Structures and Energy Levels of Antimalarial Molecules and Related Compounds.—Antimalarial drugs fall into several categories:[14] 4-quinolylcarbinols, 4-aminoquinolines, 8-aminoquinolines, 4-quinazolones, 9-aminoacridines, diaminopyrimidines, biguanides, 4,4′-diaminodiphenyl sulfones, and a new category, sydnones. We have selected representative antimalarial compounds from these classes for our calculations, with the exception of the sulfones. Sulfones were omitted because there is controversy over their molecular orbital description;[15] we are studying this problem in more detail. It should be noted that sydnones are mesoionic compounds[16] and also present difficulties in their molecular orbital treatment.

We recognize that the active antimalarial agent may for some drugs be a metabolite of the administered drug. This is known to be true for proguanil.[17] We have therefore included an active metabolite[17] of proguanil and an analog[17] of the metabolite.

Although these antimalarial drugs are often administered in salt form, we have initially compared the neutral forms. The effect of salt formation on some of these structures is considered in the latter part of this paper.

The indices of electronic structure considered are the net π-electronic charge densities[18] and energy levels in units of β_0 (eq 1) of the highest occupied molecular orbital (HOMO) and the lowest empty molecular orbital (LEMO).[19] We have assigned a plus sign to those charge densities representing a deficit of electrons. The energy level of the HOMO is taken as a measure of electron-donor ability[20] and that of the LEMO as a measure of electron-acceptor ability.[21] These properties are of primary importance in influencing charge-transfer complex formation,[22] an interaction which is proving of interest in biological systems.[23] The smaller the energy level of the HOMO, the lower the energy required to remove a π electron from the molecule, and therefore the greater are the electron-donor properties. The closer to zero the

(14) W. H. Nyberg and C. C. Cheng, *J. Med. Chem.*, **8**, 531 (1965).
(15) G. Cilento, *Chem. Rev.*, **60**, 147 (1960).
(16) W. Baker and W. D. Ollis, *Quart. Rev.* (London), **11**, 15 (1957).
(17) H. C. Carrington, A. F. Crowther, D. G. Davey, A. A. Levi, and F. L. Rose, *Nature*, **168**, 1080 (1951).
(18) Reference 2, p 116.
(19) It should be kept in mind that the value of β_0 is negative, as a result of which the energy values given by us have the opposite sign compared to the absolute values of these energies. Throughout this discussion the energy values are always in units of β_0.
(20) Reference 2, p 128.
(21) Reference 2, p 132.
(22) Reference 2, p 135.
(23) E. M. Kosower, *Progr. Phys. Org. Chem.*, **3**, 81 (1965).

energy level of the LEMO, the greater would be the electron affinity, and therefore the greater are the electron-acceptor properties. A positive energy level for the LEMO indicates a bonding unfilled orbital which would have electron-acceptor properties superior to the usual negative-valued LEMO.

It should be understood that the significance of the values calculated by the HMO method lies in the relative values and trends, not in absolute values. Comparisons may be made with more confidence among closely related series of molecules.

One may compare charge densities (Figure 1) of the quinoline-related antimalarial molecules, quinine, chloroquine, and primaquine, to each other and to those of their parent compounds, quinoline, 4-aminoquinoline, and 8-aminoquinoline. Of the three antimalarials, chloroquine stands out as having by far the most negative heterocyclic nitrogen, with a charge density of −0.269, compared with that for quinine, −0.214, and for primaquine, −0.206. By way of comparison to a more familiar compound, the heterocyclic nitrogen of pyridine, calculated similarly, has a charge density of −0.195. For mononitrogen heterocyclic compounds, the charge density on the nitrogen has been taken as a measure of basicity.[24] Differences in the ring carbon charge densities are not so great, although the ring carbon bonded to the amino nitrogen is significantly more positive in chloroquine than in primaquine. Chloroquine also has a charge of opposite sign on its 5-, 6-, and 7-carbons compared to the corresponding carbons of the other two compounds. The difference between the charge densities at the amino nitrogens of chloroquine and primaquine is much less than that of the corresponding ring nitrogens. The methyl of the methoxy group and the chloro substituent do not carry appreciable charge, while the methoxy oxygen has a charge density of about +0.06 for both quinine and primaquine.

Comparison of these antimalarials to quinoline, 4-aminoquinoline, and 8-aminoquinoline shows that the charge-density patterns of chloroquine and primaquine are close to those, respectively, of 4-aminoquinoline and 8-aminoquinoline. Of the substituents on the quinoline nucleus of these two antimalarials, it is apparent that the 4-amino and 8-amino groups effect the greatest perturbation in charge distribution. It is interesting that, although charge yielded to the ring system by the 4-amino group is only 0.015 greater than that contributed by the 8-amino group, when the 4-amino group is present there is greater electron density (0.06 more) at the quinoline nitrogen than when the 8-amino group is present. Even though the amino group is electron releasing in both instances, the effect of the 8-amino group on the quinoline nitrogen is to make it slightly less negative, while the 4-amino group increases the negativity of this nitrogen considerably. It may be concluded that the chloro substituent of chloroquine does not have a very pronounced effect on the π-electronic structure. The 6-methoxy group of quinine and primaquine has little effect on the heterocyclic ring of, respectively, quinoline and 8-aminoquinoline; it does have some influence on the ring to which it is bonded, increasing the negative charge of the 5-carbon by almost 0.06.

(24) Reference 2, p 125.

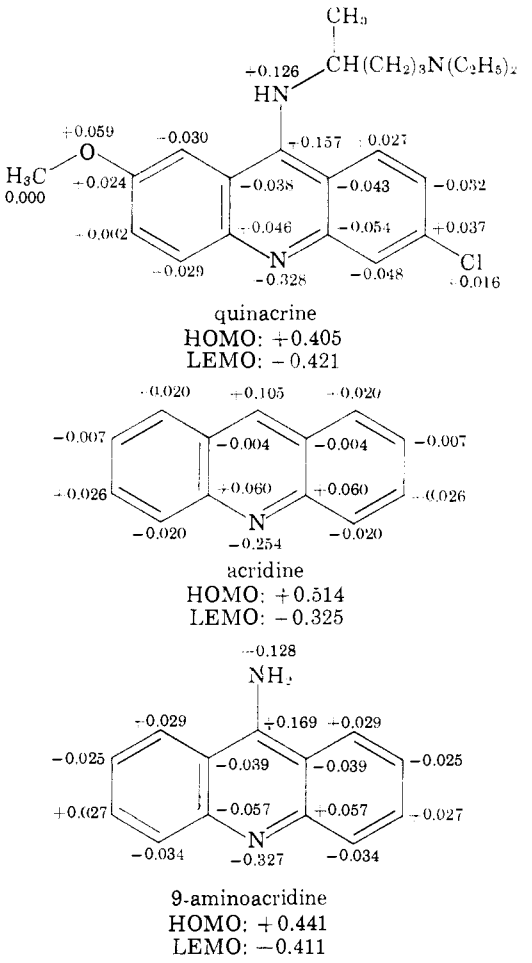
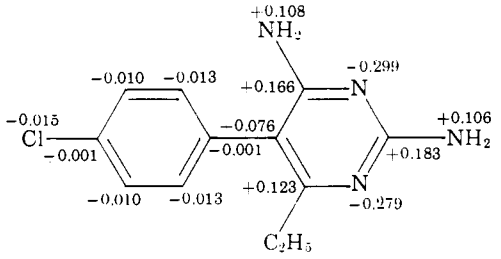


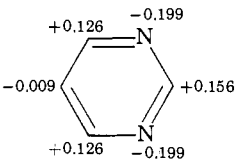
Figure 2.—HMO charge densities and energy levels of the HOMO and LEMO of the acridine antimalarial, quinacrine, and its parent compounds, acridine and 9-aminoacridine.

With respect to the energy levels, the amino groups again have the greatest influence on the quinoline nucleus. The energy of the HOMO, or electron-donor ability, seems somewhat more sensitive to the substituents than does the energy of the LEMO, or electron-acceptor ability. The amino substituents increase the electron-donor ability and decrease the electron-acceptor ability, relative to the quinoline nucleus. It is interesting that, although the 8-amino group does not influence the quinoline nitrogen charge density to nearly the extent that the 4-amino group does, it has an appreciably greater effect on the energy level of the HOMO than does the 4-amino group. The effects of the methoxy and chloro substituents follow these trends, although to a lesser extent. The HOMO energy level of pyridine by our calculation is, for comparison, +1.000, while the LEMO energy level is −0.841. Quinine should be the best electron acceptor and primaquine the best electron donor of these three antimalarials.

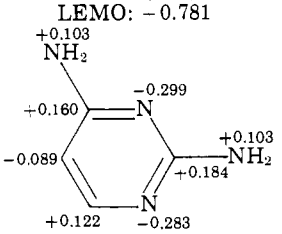
Quinacrine (Figure 2), an acridine, when compared to the preceding quinoline antimalarials (Figure 1), shows a very high charge density, −0.328, on the heterocyclic nitrogen and a comparatively high charge density, +0.126, on the amino nitrogen. The methyl group of the methoxy substituent shows neutrality, comparable to the neutrality shown by this group in the quinoline series. Energy levels show that quina-



pyrimethamine
HOMO: +0.570
LEMO: −0.754



pyrimidine
HOMO: +1.08
LEMO: −0.781



2,4-diaminopyrimidine
HOMO: +0.799
LEMO: −0.857

Figure 3.—HMO charge densities and energy levels of the HOMO and LEMO of the 2,4-diaminopyrimidine antimalarial, pyrimethamine, and its parent compounds, pyrimidine and 2,4-diaminopyrimidine.

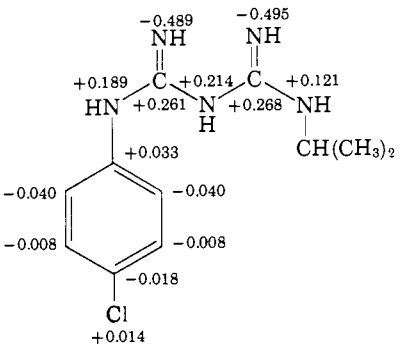
erine should be both a better electron donor and electron acceptor than the previously considered quinoline antimalarials.

Comparison of quinacrine to acridine and 9-aminoacridine (Figure 2) shows that most charge density differences between quinacrine and acridine are effected by the 9-amino group. The effect of the 9-amino group on the acridine ring nitrogen charge density is appreciably greater than the effect of the 4-amino group on the quinoline ring nitrogen. Comparison of energy levels shows that the 9-amino group of acridine does not increase the electron-donor ability as much as do the 8-amino and 4-amino groups of quinoline, but acridine itself is a better electron donor than either 4-amino- or 8-aminoquinoline. The good electron-acceptor ability of acridine is decreased by the 9-amino substituent, but the effect is not sufficient to make 9-aminoacridine (or quinacrine) a poorer electron acceptor than the quinoline antimalarials.

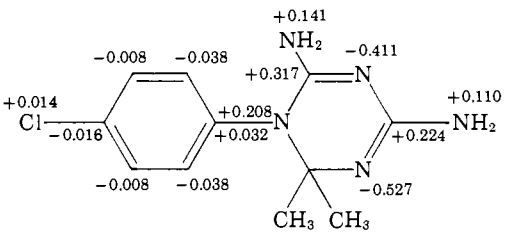
Comparing pyrimethamine (Figure 3) with the quinoline (Figure 1) and acridine (Figure 2) compounds, one sees that the negativity of the ring nitrogens of pyrimethamine is intermediate between that of the ring nitrogens of the quinoline compounds and quinacrine. The pyrimethamine amino group charge densities are comparable to the quinoline amino group charge densities. While the amino groups have by far the most influence on charge densities of pyrimethamine (Figure 3), the chlorophenyl group aids the amino groups in improving electron-donor ability (Figure 3) and counteracts their effect of decreasing electron-acceptor ability (Figure 3).

The biguanide representative, proguanil (Figure 4), was calculated using the parameters of Streitwieser[11] for an aromatic nitrogen for those nitrogens shown with a double bond and those for an amine nitrogen for those nitrogens shown with three single bonds. We do not feel that this is necessarily the best selection for guanide nitrogens, but, since proguanil is known to be metabolized to a more active compound,[17] we are emphasizing calculations on its active metabolite and its analog in the discussion below. It is nevertheless interesting to note the much higher positive charge on the amino nitrogen adjacent to the chlorophenyl group as compared with the terminal amino nitrogen.

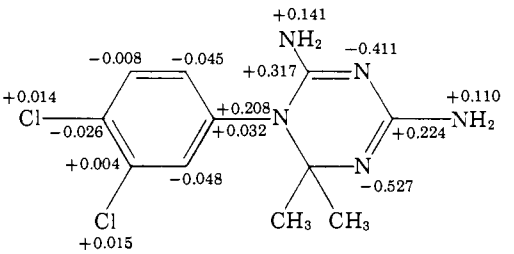
The metabolite of proguanil, 2,4-diamino-1-(*p*-chlorophenyl)-1,6-dihydro-6,6-dimethyl-1,3,5-triazine, or cycloguanil (Figure 4), active as an antimalarial, is notable for its relatively high and low π charge densities; even the carbons in the dihydrotriazine ring



proguanil
HOMO: +0.676
LEMO: −0.910



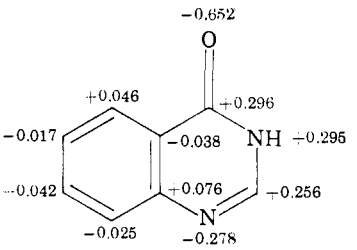
2,4-diamino-1-(*p*-chlorophenyl)-1,6-dihydro-6,6-dimethyl-1,3,5,-triazine(cycloguanil)
HOMO: +0.632
LEMO: −0.744



2,4-diamino-1-(3,4-dichlorophenyl)-1,6-dihydro-6,6-dimethyl-1,3,5-triazine

HOMO: +0.630
LEMO: −0.744

Figure 4.—HMO charge densities and energy levels of the HOMO and LEMO of the biguanide antimalarial, proguanil, its active metabolite, 2,4-diamino-1-(*p*-chlorophenyl)-1,6-dihydro-6,6-dimethyl-1,3,5-triazine (cycloguanil), and the active analog of cycloguanil, 2,4-diamino-1-(3,4-dichlorophenyl)-1,6-dihydro-6,6-dimethyl-1,3,5-triazine.



4-quinazolone
HOMO: +0.672
LEMO: −0.572

Figure 5.—HMO charge densities and energy levels of the HOMO and LEMO of 4-quinazolone.

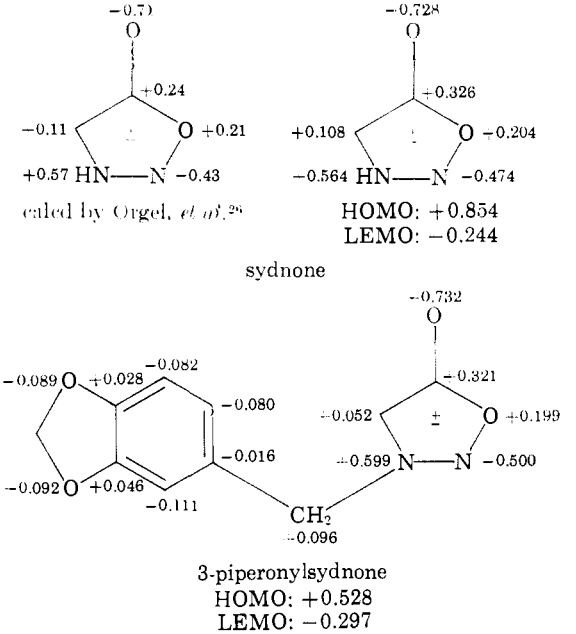
are quite positive. Cycloguanil, as the pamoate salt, has been recently shown to have repository antimalarial properties.[25]

The derivative of cycloguanil, 2,4-diamino-1-(3,4-dichlorophenyl)-1,6-dihydro-6,6-dimethyl-1,3,5-triazine (Figure 4), which is ten times more active against *Plasmodium gallinaceum*[17] than cycloguanil, differs very little in charge density or electron-donor or electron-acceptor properties from cycloguanil. The higher activity would logically be ascribed to some factor other than π-electron distribution or energy levels of the HOMO or LEMO.

Although the structure of 4-quinazolone was calculated (Figure 5), no specific antimalarial of this class was treated since the antimalarials we found in the literature had no substituents which entered into the π-electron system. The carbon between the two nitrogens is unusually positive.

Sydnones, as mentioned previously, present difficulties. We have calculated sydnone (Figure 6) using the parameters of Orgel, *et al.*[26] ($h_{-O-\text{(cyclic)}} = 3.2$, $h_{=O\text{(exocyclic)}} = 2.0$, $k_{CO} = 1.4$, $h_{N<} = 2.0$, $h_{-N=} = 1.0$, $k_{CN} = 1.2$, $k_{NO} = 0.6$, and $h_C = 0.2$), and selecting a value for k_{NN}, which was not cited by Orgel, *et al.*,[26] of 1.0. The charge densities calculated by Orgel, *et al.*,[26] are given for comparison (Figure 6). We have also calculated the antimalarial,[14] 3-piperonylsydnone (Figure 6), using the parameters employed for sydnone, and treating the methylene bridge between the two cyclic moieties according to the heteroatom model of hyperconjugation as given by Streitwieser.[11] We do not feel that a direct comparison between these calculations on the sydnones and the calculations on the more conventional π-electron systems using the established Streitwieser parameters[11] is justified. One might expect qualitatively, however, from the LEMO energy levels that the sydnones would be good electron acceptors and from the charge distributions, as well as from knowledge that sydnones are mesoionic, that they would be capable of pronounced electrostatic interactions.

We would like to point out the recent treatment of sydnones by the ω-HMO technique, which correlates well with dipole moments and ultraviolet maxima.[27] These authors feel that part of the failure of the simple



calcd by Orgel, *et al.*[26]

HOMO: +0.854
LEMO: −0.244

sydnone

3-piperonylsydnone
HOMO: +0.528
LEMO: −0.297

Figure 6.—HMO charge densities and energy levels of the HOMO and LEMO of sydnone (calculated according to the parameters of Orgel, *et al.*,[26] and $k_{NN} = 1.0$) and 3-piperonylsydnone (with the methylene group between the ring moieties treated as hyperconjugated according to the heteroatom model).

HMO method with the mesoionic sydnones may be attributed to lack of consideration of electron correlation, a feature taken into account by the ω-HMO method. It seems reasonable to us that the ω-HMO method would be more appropriate for treating the sydnones.

We have also studied a type of antimalarial agent which does not fall into the categories listed earlier. It was discovered that 4,4′-dinitrocarbanilide (DNC) formed a 1:1 molecular complex with 2-hydroxy-4,6-dimethylpyrimidine (HDP), as well as with other polar compounds.[28] The DNC–HDP complex had significant antimalarial and anticoccidial activity.[28] Neither DNC nor HDP separately, or as an uncomplexed mixture, showed significant anticoccidial activity. Further, the unsubstituted carbanilide did not complex with HDP. The authors suggested that hydrogen bonding between HDP and the urea portion of the substituted carbanilide was likely.[28] Such hydrogen bonding did not seem to us to be specific enough to explain the requirement of the *p*-nitro groups for complexing. We find for HDP (Figure 7) a fairly pronounced charge-density distribution and moderate electron-donor properties. The nitro groups as well as the urea portion of DNC would appear to be likely hydrogen bonding sites. The results for carbanilide and 4,4′-dinitrocarbanilide (Figure 7) are revealing. We find that the LEMO energy level for 4,4′-dinitrocarbanilide is *positive*, *i.e.*, the orbital is a bonding one. Bonding unoccupied orbitals, while not unheard of, are rare and signify exceptional electron-acceptor properties.[29] In this instance there are actually two bonding unoccupied orbitals. For carbanilide (Figure 7) one finds that there are no bonding unoccupied orbitals; indeed, the energy level of the LEMO.

(25) P. G. Contacos, G. R. Coatney, J. S. Linn, and J. W. Kilpatrick, *Am. J. Trop. Med. Hyg.*, **13**, 386 (1964).

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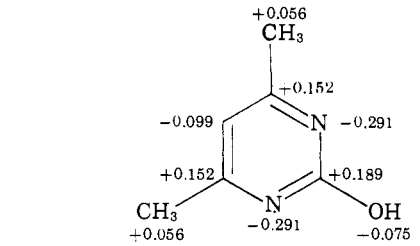
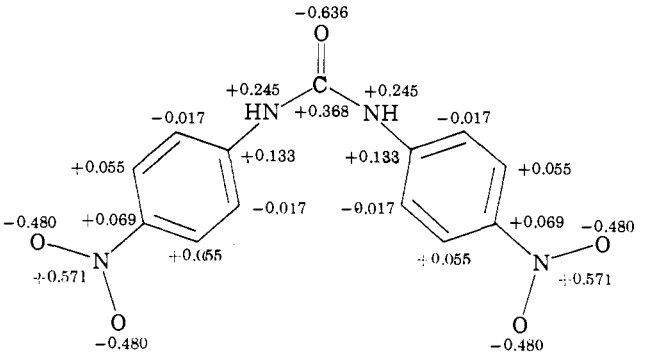
(27) L. B. Kier and E. B. Roche, *J. Pharm. Sci.*, **55**, 807 (1966).

(28) A. C. Cuckler, C. M. Malanga, A. J. Basso, and R. C. O'Neid, *Science*, **122**, 244 (1955).

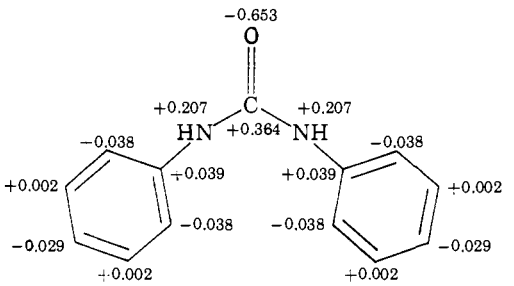
(29) A. Pullman and B. Pullman, *Biochim. Biophys. Acta*, **54**, 384 (1961).



2-hydroxy-4, 6-dimethylpyrimidine (HDP)
HOMO: +0.873
LEMO: −0.889



4, 4′-dinitrocarbanilide (DNC)
HOMO: +0.789
LEMO'S: +0.318, +0.305, −0.897



carbanilide
HOMO: +0.744
LEMO: −0.865

Figure 7.—HMO charge densities and energy levels of the HOMO and LEMO of 2-hydroxy-4,6-dimethylpyrimidine (HDP), 4,4′-dinitrocarbanilide (DNC), and carbanilide.

−0.865, does not indicate especially good electron-acceptor properties.

We believe that the HMO calculations indicate strongly that the attractive forces in the DNC–HDP complex arise in large part from a charge transfer and that the specificity of DNC as compared to carbanilide results from the extraordinary electron-acceptor properties of DNC.

Electronic Aspects of the Interaction between Some Antimalarials and Deoxyribonucleic Acid.—A most interesting and valuable study by Hahn and coworkers[8] has provided convincing evidence that chloroquine, quinacrine, and quinine complex with native DNA, blocking enzymatic synthesis of DNA and ribonucleic acid (RNA) *in vitro* and the biosynthesis of DNA and RNA in susceptible cells. The selectivity of these drugs for the malaria parasite is attributed to the unusually high accumulation of the drug by the parasitized erythrocytes. Resistance to the drugs is due to an impairment of the accumulation mechanism or permeability.

The ultraviolet spectra of chloroquine, quinacrine, and quinine were altered by DNA. The chloroquine interaction, the most intensively studied, was shown specific for native, double-stranded DNA. The spectra of chloroquine was markedly altered by DNA and the double-helical polymer of deoxyguanylic acid and deoxycytidylic acid (dGdC) but not appreciably by the polymer of deoxyadenylic acid and deoxythymidylic acid (dAdT) or of deoxyinosinic acid and deoxycytidylic acid (dIdC). Since dGdC is identical with dIdC, with the exception of the 2-amino group of guanine present in dGdC, the spectroscopic evidence was taken as indicating conclusively the 2-amino group of guanine is a specific attachment site for chloroquine. More particularly, it was suggested that the 7-chloro substituent of chloroquine interacted electrostatically with the 2-amino group.

Chloroquine and quinacrine stabilized double-helical DNA against thermal strand separation. Chloroquine also stabilized dAdT, even though dAdT did not influence the spectrum of chloroquine. It was therefore postulated that chloroquine interacts with DNA in two ways: (1) through interaction between the ring of chloroquine and guanine, with the previously discussed specific electrostatic interaction between the 7-chloro substituent of chloroquine and the 2-amino group of guanine, and (2) through interaction between chloroquine's positively charged amino groups (in the salt form) and the anionic phosphate groups of DNA.

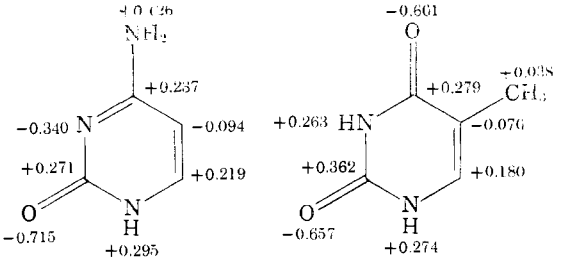
Goldberg[30] has questioned the requirement for the 2-amino group of guanine in DNA for complex formation and emphasized the difficulty of relating spectral changes quantitatively to complex formation. He suggests that the difference in the effect on the chloroquine spectrum by adenine and guanine may be due to either the better electron-donor ability of guanine or to the influence of guanine on the secondary structure of DNA.

We suggest that the interaction between a chloro substituent and an amino group, both of which carry a positive π charge, would probably not be strong enough to confer specificity on the guanine moiety for interaction with the chloroquine ring, even considering the negative σ charge which the chloro substituent should have. We were interested in (1) exploring further the role of the 2-amino group of guanine, (2) comparing, with a consistent set of HMO parameters, electronic properties of the DNA bases and of the antimalarials with which they interact, and (3) investigating the effect of salt formation at the amino group bonded to the ring on the electronic structure of the antimalarials.

We would like to point out that HMO calculations on the DNA bases adenine, guanine, cytosine, and thymine, as well as the hydrogen-bonded pairs adenine–thymine and guanine–cytosine, have been carried out by the Pullmans[2] using their set of parameters. For our calculations, we employed hydrogen bonding parameters suggested by the Pullmans.[31]

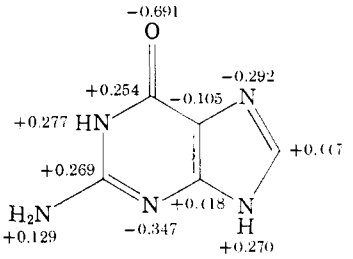
Comparing the two pyrimidine bases, cytosine with thymine, and the two purine bases, guanine with adenine (Figure 8), one notes pronounced charge-density differences. In part, these differences are due to the

(30) I. H. Goldberg, *Military Med., Suppl.*, **131**, 1092 (1966).
(31) Reference 2, p 110.

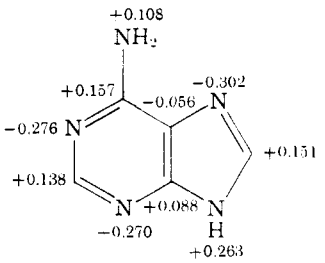


cytosine
HOMO: +0.768
LEMO: −0.602

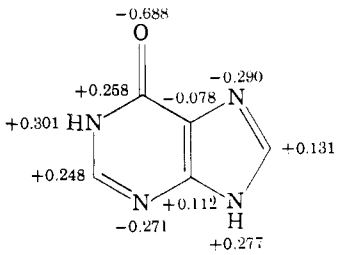
thymine
HOMO: +0.665
LEMO: −0.597



guanine
HOMO: +0.481
LEMO: −0.754



adenine
HOMO: +0.640
LEMO: −0.772

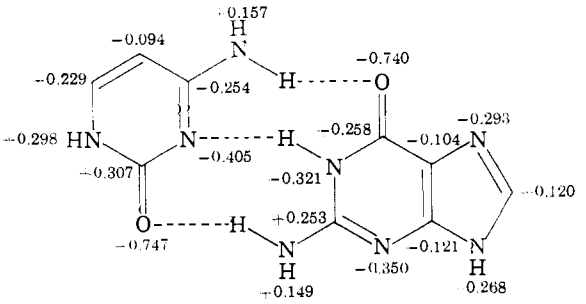


inosine
HOMO: +0.546
LEMO: −0.677

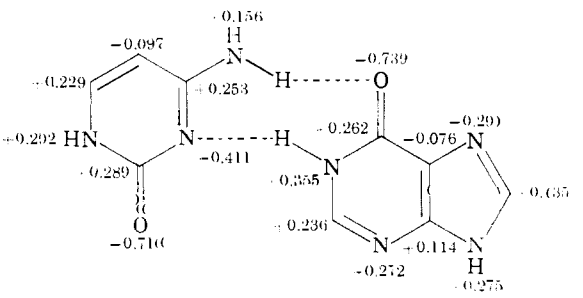
Figure 8.—HMO charge densities and energy levels of the HOMO and LEMO of cytosine, thymine, guanine, adenine, and inosine.

choice of the lactam and amino forms, which have been shown to preponderate[32] as opposed to the lactim and imino forms, of these molecules. Since the contributions, however, of the lactim and imino forms are relatively minor,[32] the charge distributions of the molecule as represented should be close to that of the molecule as it exists. The purines are better electron donors than the pyrimidines, and guanine is, as indicated previously,[2,30] far superior in this respect to adenine. We would expect that electrostatic interactions involving the five-membered purine ring would not be too different for adenine and guanine. An electrostatic attraction to a positive site by the 3-nitrogen would be significantly stronger for guanine as compared to adenine. An electrostatic interaction at the 1-nitrogen would be profoundly different for guanine and adenine, for this site in guanine carries considerable positive π charge, while in adenine it carries a π charge of the same magnitude and opposite sign. Guanine also furnishes a center of very high negative charge density, the carbonyl oxygen, which adenine lacks.

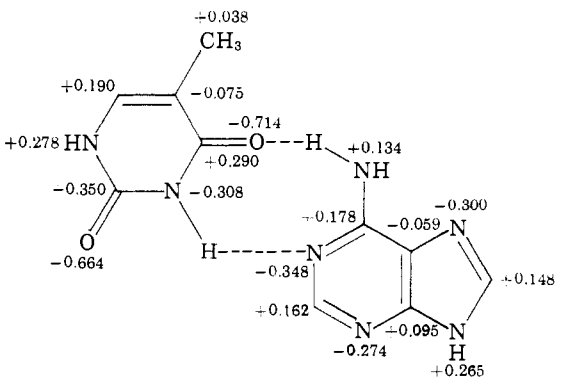
To investigate the role of the 2-amino group of guanine, we calculated the electronic properties of the guanine molecule with the 2-amino group removed, *i.e.*, inosine (Figure 8). The 2-amino group was found to be a major contributor to the relatively superior electron-donor properties of guanine, its removal reducing these properties considerably. This role



guanine-cytosine
HOMO: +0.487
LEMO: −0.592



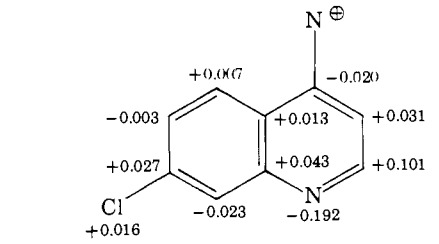
inosine-cytosine
HOMO: +0.564
LEMO: −0.590



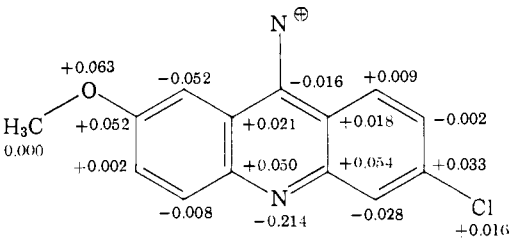
adenine-thymine
HOMO: +0.605
LEMO: −0.584

Figure 9.—HMO charge densities and energy levels of the HOMO and LEMO of the hydrogen-bonded pairs guanine–cytosine, adenine–thymine, and inosine–cytosine, with hydrogen bonding treated according to Pullman and Pullman.[31]

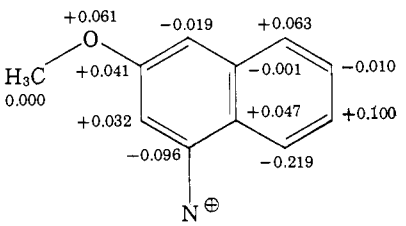
(32) Reference 2, p 207.



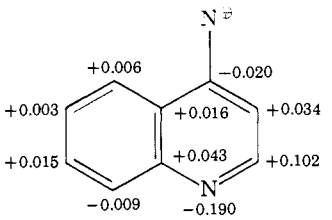
chloroquine (amine salt)
HOMO: +0.719
LEMO: −0.486



quinacrine (amine salt)
HOMO: +0.507
LEMO: −0.291



primaquine (amine salt)
HOMO: +0.682
LEMO: −0.511



4-aminoquinoline (amine salt)
HOMO: +0.729
LEMO: −0.481

Figure 10.—HMO charge densities and energy levels of the HOMO and LEMO of the amine salt forms of chloroquine, quinacrine, primaquine, and 4-aminoquinoline, with the positive amine nitrogen treated as purely inductive.

of the 2-amino group makes it important in influencing charge-transfer interactions, which may be observed spectroscopically.[33] We feel that this function of the 2-amino group of guanine may very well explain why the interaction of chloroquine and dGdC is observed spectroscopically, while that of chloroquine and dIdC is not, without invoking a specific electrostatic interaction involving the 2-amino group. Electrostatic interactions with the guanine ring should not be greatly different than those with the inosine ring, with the possible exception of those at the 3-nitrogen, the charge density of which is influenced by the 2-amino group.

Consideration of hydrogen bonding for the pairs, guanine–cytosine, inosine–cytosine, and adenine–thymine, does not change these general conclusions. It is found that charge densities vary somewhat for those atoms directly involved in the hydrogen bonding (Figure 9). The changes in HOMO energy levels on hydrogen bonding bring the inosine–cytosine pair closer in electron-donor properties to adenine–thymine than to guanine–cytosine (Figure 9), which is interestingly in accord with the respective influences on the chloroquine spectrum by dIdC, dAdT, and dGdC polymers.[8]

Since Hahn, *et al.*,[8] found the interactions of chloroquine took place only with double-stranded (*i.e.*, base-paired) DNA polymers, and since this base pairing does not cause great differences in electronic properties, the hypothesis that chloroquine must interact simultaneously with both members of a base pair is attractive.

To take account of the proposed participation of the amine salt form of chloroquine in the DNA complex,[8] we treated the protonated amino group according to Streitwieser,[34] who has indicated that an amine salt group may be considered purely inductive. We employed his auxiliary inductive parameter of $0.1h_X$[11] where h_X is taken as the h value of a positively charged nitrogen, 2.0,[11] giving us an h value for the carbon bonded to the amine salt group of 0.2.

Comparison of the calculation for the amine salt of chloroquine (Figure 10) with that for neutral chloroquine (Figure 1) indicates that the quinoline nitrogen is sensitive to this alteration; this charge for the salt is appreciably lower. Even more outstanding is the effect on the energy levels. There is a marked improvement in the electron-acceptor properties of the salt which should enhance interaction with the electron-donating purines. The effect of salt formation (Figure 10) on quinacrine (Figure 2) is in the same direction as for chloroquine and is even more pronounced. The amine salt of quinacrine should be by far the best electron acceptor of the antimalarials considered. Because the quinacrine salt is so much better an electron acceptor than the chloroquine salt, it is reasonable that its complexation is observable spectroscopically with dIdC and dAdT, poorer electron donors than dGdC and DNA, while the weaker electron acceptor, chloroquine salt, must interact with the better electron donors, dGdC and DNA, before the spectroscopic changes are observed. This explanation is an alternate to the conclusion that the compared effects of DNA, dGdC, dAdT, and dIdC on the spectra of chloroquine and quinacrine point to the general interactions of quinacrine and the guanine-specific interactions of chloroquine with DNA.

It is interesting that quinine, which does not form an amine salt and has poorer electron-acceptor properties than the salt forms of chloroquine and quinacrine, was thought to involve hydrogen bonding[8] in the DNA complex, in contrast to the "ionic" interactions[8] suggested for chloroquine and quinacrine.

We have also calculated the effect of salt formation (Figure 10) on primaquine (Figure 1). Surprisingly, the quinoline nitrogen's charge density for the salt is raised slightly. Electron-acceptor properties are im-

(33) L. J. Andrews and R. M. Keefer, "Molecular Complexes in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964.

(34) Reference 11, p 231.

proved, although not to such an extent as for quinacrine and chloroquine.

Finally, to estimate the effect of the 7-chloro substituent on the electronic properties of the amine salt of chloroquine, we calculated the amine salt of 4-aminoquinoline (Figure 10). Differences between the amine salts of 4-aminoquinoline and chloroquine are slight, indicating that the 7-chloro substituent does not have much influence on the ring structure. In conjunction with the fact that replacement of the 7-chloro substituent diminishes, but does not abolish binding to DNA,[35] this calculation suggests, in accord with Hahn, *et al.*,[5] that perhaps the 7-chloro substituent does enter into specific (though not essential) interaction in the DNA complex.

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(35) D. Sgoutas and L. Levine, *Arch. Biochem. Biophys.*, **101**, 335 (1963).

Nucleic Acids. I. The Synthesis of Nucleotides and Dinucleoside Phosphates Containing *ara*-Cytidine[1a]

WILLIAM J. WECHTER

Research Laboratories of The Upjohn Company, Kalamazoo, Michigan

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With the hope of influencing the mechanism of action, transport, or cross-resistance phenomena of the potent cytotoxic antiviral nucleoside 1-β-D-arabinofuranosylcytosine (*ara*-cytidine, CA), the nucleoside was incorporated into 25 dinucleoside phosphates of the type CApX and XpCA where X represents a second nucleoside. Where possible, all three internucleotide linkages, 2′→5′, 3′→5′, and 5′→5′, were prepared with each structural type, by the condensation of suitably protected nucleosides and nucleotides followed by deblocking. While ion-exchange chromatography was employed for small-scale isolation, continuous-flow film electrophoresis was utilized for larger scale preparations. For biological comparisons, the three mononucleotide derivatives of *ara*-cytidine, as well as two single esters of *ara*-cytidine 5′-phosphate, were prepared. The structures of all phosphorus-containing products were confirmed enzymatically. In the course of the enzymatic characterizations, a new specificity of venom diesterase toward unsymmetrical (with respect to the sugar) 5′→5′-dinucleoside phosphates was uncovered. The structures of all phosphorus-containing intermediates and products were confirmed by their nmr spectra. From these latter studies, we were able to confirm the nature and ratio of heterocyclic bases present, establish the position of nucleotide or internucleotide linkages, differentiate the sugar moieties, and confirm the gross structures of the synthetic products.

For about 14 years[1b,c] chemists, particularly in the United States, have been vigorously engaged in the synthesis of analogs of the naturally occurring nucleosides. The rationale for the most of this work was based on the supposition that one could produce unique selective antimetabolites which would be useful in the treatment of neoplastic and viral diseases. While a number of these compounds are presently being employed clinically, almost all of them are highly toxic to mammalian cells. Further, the infective agent or neoplasm develops resistance to these antimetabolites. In the hope of increasing the cellular selectivity of such antimetabolites, either by alternative mechanisms of action or by alternative transport mechanisms, we have begun a program to incorporate a variety of antimetabolites into oligonucleotides.[1c] By this means we hope to contribute to a partial understanding of the effects of charge, molecular weight, and molecular configuration on cellular penetration incorporation and transport of oligonucleotides into living systems. We thus desire knowledge of the cellular metabolism and possibly biologically unique properties of oligonucleotides. For this purpose, we synthesized a series of dinucleoside phosphates, nucleotides, and simple esters of these nucleotides derived from the cytotoxic,[2a,b] antiviral[2c-e] nucleoside 1-β-D-arabinofuranosylcytosine[2f] (*ara*-cytidine, *ara*-C, CA). Cytotoxicity studies with these compounds will be reported in the accompanying paper.[2g]

Employing the procedures pioneered principally by Khorana and his co-workers[3] all of the desired compounds were prepared, but on larger scales than those employed in the literature preparations. The products were isolated by ion-exchange chromatography. Large-

(1) (a) Presented at the 152nd National Meeting of the American Chemical Society, Division of Medicinal Chemistry, New York, N. Y., Sept 12, 1966. (b) Early workers in this field included B. R. Baker, G. B. Brown, J. J. Fox, C. A. Dekker, J. A. Montgomery, and their co-workers; leading references may be found in *Advan. Carbohydrate Res.*, **14**, 283 (1959); **17**, 301 (1962). (c) Fradulent 3′→5′- and 5′→5′-linked dinucleoside phosphates have been reported subsequent to the interception of this work: J. A. Montgomery, G. J. Dixon, E. A. Dulinage, H. J. Thomas, R. W. Brockman, and H. E. Skipper, *Nature*, **199**, 769 (1963); D. G. Parsons and C. Heidelberger, *J. Med. Chem.*, **9**, 159 (1966); J. Smrt and F. Šorm, *Collection Czech. Chem. Commun.*, **28**, 61, 887 (1963); R. H. Hall and R. Thedford, *J. Org. Chem.*, **26**, 1506 (1962); A. Block, M. H. Fleysher, R. Thedford, R. J. Mane, and R. H. Hall, *J. Med. Chem.*, **9**, 886 (1966).

(2) (a) M. Y. Chu and G. A. Fischer, *Biochem. Pharmacol.*, **11**, 423 (1962); (b) C. G. Smith, H. H. Buskirk, and W. L. Lummis, *Proc. Am. Assoc. Cancer Res.*, **6**, 60 (1965); (c) G. E. Underwood, *Proc. Soc. Exptl. Biol. Med.*, **111**, 660 (1962); (d) H. E. Renis and H. G. Johnson, *Bacteriol. Proc.*, **45**, 140 (1962); (e) D. A. Buchala, *Proc. Soc. Exptl. Biol. Med.*, **115**, 89 (1964); (f) J. H. Hunter, U. S. Patent 3,115,282 (1963); (g) Part II: G. G. Smith, H. H. Buskirk, and W. L. Lummis, *J. Med. Chem.*, **10**, 774 (1967).