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A Theoretical Approach to Structure–Activity Relationships of Chloramphenicol and Congeners

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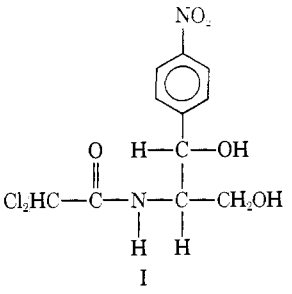
In this study the most stable conformation of the chloramphenicol molecule was calculated by means of extended Hückel theory and found to agree with nmr data. Based on those conformational results a three-dimensional picture of the possible binding sites of the molecule to a receptor is displayed. The influence of substitution at the phenyl ring and the acylamino side chain on the biological activity of the chloramphenicol molecule has been investigated by CNDO/2 and model interaction calculations. An explanation of the influence of the para-substituted phenyl ring of chloramphenicol is presented.

Many studies have been made on the structure–activity relationships of the chloramphenicol molecule, I, which are of interest due to its important antibacterial action.



I

The pharmacological evaluations of numerous congeners of the natural molecule have revealed that apparently three parts of the molecule are specifically involved in drug–receptor events. The propanediol moiety critically determines the activity,[1] since changes at this part of the molecule, like replacement[2] or acetylation[3,4] of the hydroxyl groups or extension of the 3-carbon chain,[5,6] result in a complete loss of antibiotic activity. The other two features, the para substituent at the benzene ring and the dichloromethyl group at the acylamino side chain, have been the subject of several chemical modifications.[7,8] Recently the first substance has been described, which exerts a higher antibacterial activity than the mother substance.[9] This modification possesses a trifluoromethyl group instead of the dichloromethyl group at the acylamino side chain. There are several other congeners in this series known, with a broad range of activity.[7,10] Besides the dichloromethyl group, the *p*-nitro substituent has been found to be replacable with a variety of groups or atoms,[7,11] but all of them are lower in activity. Efforts have been made to retain activity by replacing the phenyl ring with other aromatic systems, but there has been little success. Only the nitrothienyl congener retains some antibiotic activity.[12,13]

Despite the fact that much has been accomplished, there is still considerable uncertainty concerning the drug–receptor interactions. In this study we try to derive further information which might help to elucidate these events by means of theoretical methods.

Molecular Conformation. The confirmation of chloramphenicol has been previously studied by nmr and Raman spectroscopy[14] and more recently by ORD and

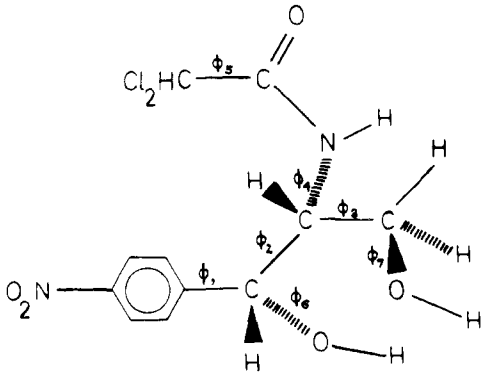


Figure 1. The seven rotational axes of chloramphenicol.

CD.[15] The crystal structure of bromamphenicol has been determined by X-ray analysis.[16] A recent study by Bustard and Egan[17] combines nmr and ir spectral analyses with theoretical potential energy calculations (PEF) in order to estimate quantitatively the rotamer populations. In this study we have undertaken the prediction of the conformation using molecular orbital theory.

The chloramphenicol molecule contains seven potential rotational axes, ϕ_1–ϕ_7, shown in Figure 1. The first four axes determine the preferred conformation of the molecule to a large extent, while the remaining three have only a modest effect.

Our preliminary approach was the calculation of the total energies for different values of ϕ_2 and ϕ_3. Using the calculated preferred conformation, we calculated the influence of changing angles ϕ_1 and ϕ_4. The three rotors ϕ_5, ϕ_6, and ϕ_7 were held fixed until the last step where they were then rotated independently to predict the global minimum. This is essentially the same approach taken by Bustard in his conformation calculations.[17] The amide moiety of the chloramphenicol molecule was maintained in the natural trans conformation in accordance with the current view.[18] The nitro group was assumed to lie in a plane with the phenyl ring. The calculations were made using extended Hückel theory[19] and the parameters and dimensions previously employed.[20]

We recognize that the commonly employed semiempirical MO methods have limitations. The entire approach is based upon an isolated molecule consideration. The results require interpretation in terms of complex biological systems. The conformational predictions have not been unanimously in agreement with experimental data. Enough encouragement has been gained by us and other investigators to proceed with this approach and to treat our results with these shortcomings in mind.

Table I. Values of the Rotational Axes ϕ_1–ϕ_7 of the Preferred Conformations 1 and 2

Rotational axes	Conformation 1	Conformation 2
ϕ_1	90	90
ϕ_2	300	300
ϕ_3	180	60
ϕ_4	180	180
ϕ_5	60	60
ϕ_6	300	180
ϕ_7	300	30

The calculations revealed two conformations with virtually equivalent energies, shown in Table I. The values of the angles relate to the structures in Figure 2. We have depicted these two structures in Figure 3, with distances separating key structural features.

Benzyl Radical Hypothesis. In a recently published in-

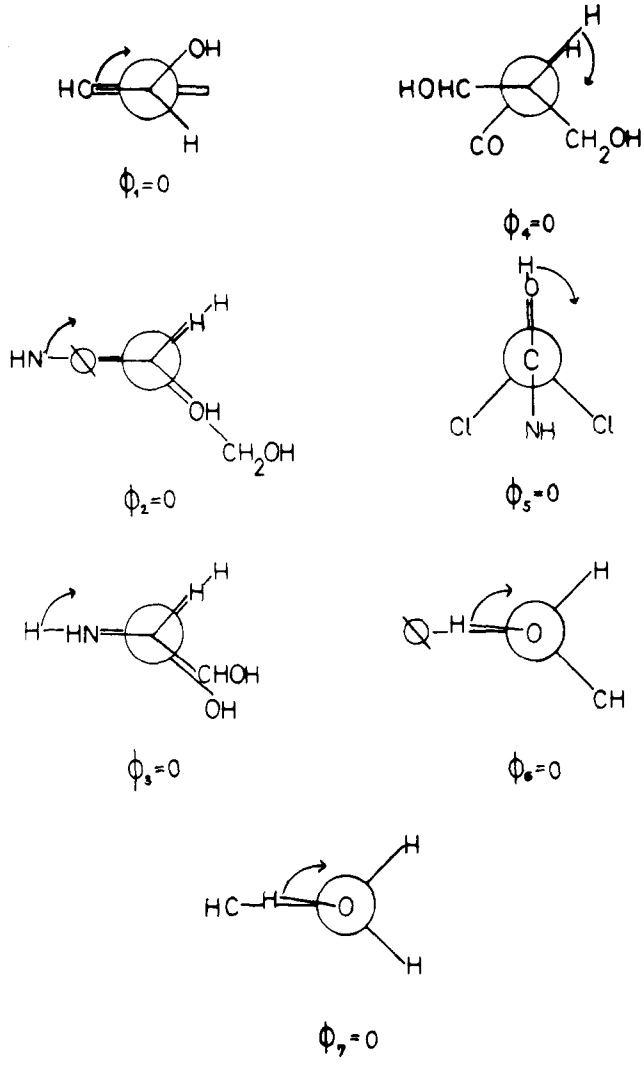


Figure 2. Newman projections of the rotational axes ϕ_1–ϕ_7 presenting the zero values and the directions of rotation.

vestigation concerning the influence of the substituents on the benzene ring on the antibiotic activity of the chloramphenicol molecule, Hansch and coworkers approached this problem by means of regression analysis.[9,21] Their results led them to offer an explanation of those effects, which we can test by MO calculations.

It has been suggested that the para substituents should be able to stabilize a free radical, formed at the benzyl carbon atom, which may act by inhibiting the electron transport system of the bacterial respiration chain.[9] Assuming that the differences in the ease of radical formation or radical stability in a series of congeners are related to the energy differences between the original molecule and the radical, we have calculated the total energies for a series of para-substituted acyloins, modeling the chloramphenicol congeners and the para-substituted planar acyloin radicals, as shown in Figure 4. Two methods have been used for these calculations, the CNDO/2 method[22] and the INDO method (intermediate neglect of differential overlap[23]).

In order to examine the influence of the para substituents at the possible radical formation site more clearly, we also have calculated the charges for the congener model compounds. The ΔE is constant in the series of free-radical models and the charges induced by the para substituents on the hydroxymethylene moiety are also virtually constant through the congener series.

Acylamino Side-Chain SAR. A suggestion of Hansch

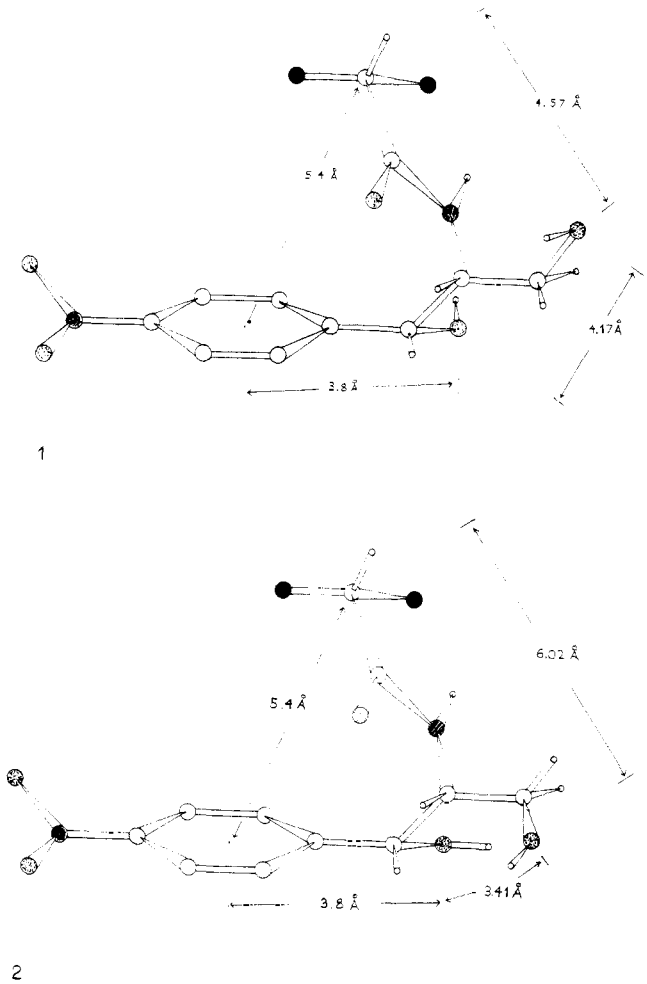


Figure 3. Illustration of the predicted preferred conformations of chloramphenicol showing distances between potential receptor-active moieties.

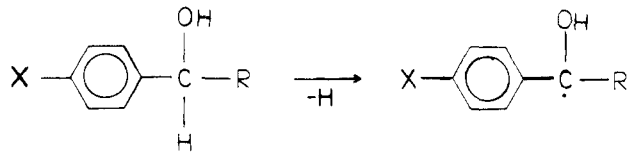


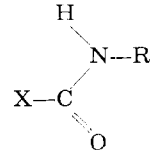
Figure 4. Proposed radical formation in chloramphenicol.

and coworkers[9] concerning the influence of the substituents at the acylamino side chain was that the electron-withdrawal effect of those substituents has a prominent influence on the activity of these derivatives.

In an effort to test this possibility we estimate the electron-charge influence of substituents by CNDO/2 calculations of the charges in the acylamide moieties. The model compound, surrogate for the chloramphenicol molecule in this approach, is the *N*-monomethylamide, shown in Table II. This table includes our results together with the pharmacological activities of the chloramphenicol congeners. From an inspection, there is no detectable relationship revealed between the charges and the activity, although a more detailed treatment of combinations of atom charges should perhaps be made.

Phenyl Substituent SAR. In some recent papers we have employed a method which enables us to study drug-receptor interactions by means of interaction energy calculations between a moiety on a drug molecule and a model compound simulating a possible receptor feature. We believe that the influence of the para substituent may be its direct participation in binding to a receptor feature;

Table II. Biological Activities and Calculated Charges for an Acylamino Side-Chain Model of a Series of Chloramphenicol Congeners



X	O_q	C_q	N_q	H_q	Biological act. log
CF_3	−0.281	+0.284	−0.191	+0.133	2.24
$CHCl_2$	−0.354	+0.377	−0.185	+0.126	2.0
CH_2Cl	−0.367	+0.371	−0.185	+0 125	1.7
CHClMe	−0.333	+0.346	−0.209	+0.116	1.47
CHF_2	−0.333	+0.317	−0.175	+0.125	1.3
CH_2F	−0.363	+0.340	−0.179	+0.122	1.16
$CHMe_2$	−0.364	+0.346	−0.201	+0.107	0.88
CCl_3	−0.368	+0.398	−0.169	+0.125	0.75
C_3H_7	−0.356	+0.342	−0.206	+0.133	0.71
CH_3	−0.373	+0.360	−0.187	+0.106	0.48
CH_2CN	−0.367	+0.367	−0.191	+0.120	0.14

therefore, this should be an opportunity to use this approach in constructing a heuristic model which may perform in an isomorphic way with the actual system.

In the model interaction system we simulate the receptor site with a single amino acid side chain. In reality, the drug moiety is probably in a more composite field of several side chains when in an active complex. We can reasonably deal with a single molecule simulating the receptor feature to avoid enormous complexity; hence, we assume single side-chain dominance at this position.

The two hydroxyl groups may likely be involved in bonding, anchoring the molecule at the receptor. The benzene part of the molecule will therefore be directed in space in a constant way, through the series of congeners. The substituent groups (R) extend from a fixed point, the last atom of the benzene ring, and assume their preferred conformations.

The calculations of interaction energies are performed between a series of chloramphenicol models and the receptor site models with the benzene part of the drug model always at the same position relative to the receptor. Comparisons of energies among the chloramphenicol congeners must then be made at constant distances separating the planes of drug and receptor models. This distance then defines the overall relationship of any para-substituted chloramphenicol congener to a particular receptor site model.

Admittedly our model is arbitrary since we assume a limited variability in the relationship between the phenyl ring and the receptor model. However, there must be some rigidity in the interaction of this structural feature with the receptor site since the molecule is concurrently interacting through several other moieties in a pharmacophore, with their corresponding receptor features.

The models chosen for study are designed not to depict a precise reality but are designed on the basis of a simplistic reasonableness. The principle intent is to find a system which is isomorphic with reality. The criteria for such a result is the ability of the model to parallel reality, expressed here in terms of the biological effect.

The calculations are those developed by Claverie and Rein[24] and are referred to as the monopole bond polarizabilities method. Rein and others[25-27] have used the method with success in studying biochemical molecular interactions. We have used the method to predict the side-chain interactions of prostaglandin E1, hence, its preferred conformation.[28] We have also used the method

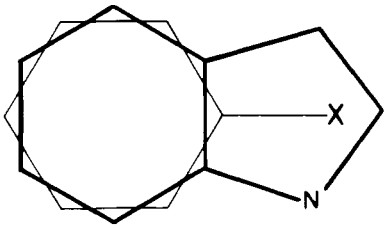


Figure 5. Geometry of interaction between the chloramphenicol phenyl ring and the receptor model indole, surrogate for tryptophan.

to study onium group interactions[29] and sweet taste receptor structure.[30] These are examples of what we refer to as receptor mapping using model interaction calculations.

The monopole bond polarizabilities method of Claverie and Rein[24] and elaborated by Huron and Claverie[31] was utilized for the actual numerical calculations. Within this approximation, the interaction energy can be written in terms of the charge distributions of the two molecules approximated by point charges centered at the nuclei and the polarizabilities of the bonds, together with an algorithm for computing the repulsive energy component as the charge distributions begin to overlap.

Standard bond lengths and angles are assumed. The ionization potentials are derived from the CNDO/2 calculations. The polarizabilities are taken from Denbigh[32] or Le Fevre.[33] The van der Waals radii are as follows: H = 1.2, C = 1.6, C(arom) = 1.8, N = 1.5, O = 1.4, F = 1.4, Cl = 1.8, Br = 2.0, I = 2.2, S = 1.9 Å.

Assuming that the functional features of the receptor are derived from amino acid side chains the calculations were performed with a variety of amino acid side-chain models, such as serine, isoleucine, tyrosine, phenylalanine, and tryptophan, and for various modes of approach.

The best correlation between the constant separation binding energies and biological activity was obtained using the tryptophan model. Indole has been used as surrogate for this amino acid side chain. Figure 5 shows the geometry of the approach. Table III includes the calculated interaction energies in comparison to the biological activity of the 14 congeners which were considered in our study.

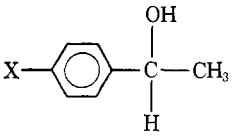
The binding energy *vs.* biological activity relationship for a drug-receptor distance of 4.5 Å reveals the best linear relation among most of the 14 congeners (Figure 6). The correlation coefficient considering 12 of them, excluding only the amino and the phenyl substituent, is r = 0.914.

Further support for this receptor model comes from a solution of the approximation used by Burgen[34] and Villa[35] relating the different binding energies of the two extremes in the series with their potency ratio ρ as $\Delta E = -RT \ln \rho$.

In the case under study the potency ratio between the *p*-nitro- and the sulfomethyl-substituted congeners is ρ = 17.8 and the expected binding energy difference is calculated to be 1.77 kcal/mol. Table III reveals a 1.79 kcal/mol difference in our model interaction energies at 4.5 Å for this pair of substituents.

The failure of the amino and the phenyl congeners to fit in the correlation has to be explained. In both cases our calculations predict higher activity than they show in the experimental data. In the amino case a possible explanation may be the alteration *in vivo* by an easily accomplished metabolic acylation. This would result in a substituent of such a dimension (over 5 Å out from the ring in the case of a simple acetylamino group) that it may exceed the limits of the receptor site. Putting it another way, it may encounter steric interference beyond the receptor site, which would tend to lower its interaction with that site. The same argument would hold for the phenyl derivative. It is noteworthy that most of the active derivatives[9] have a shorter linear dimension from the phenyl ring of chloramphenicol.

Table III. Biological Activities and Calculated Model Interaction Energies for a Series of Para-Substituted Chloramphenicols



X	Biological act. log	Interaction energies in kcal/mol 4.25 Å	4.5 Å	4.75 Å
p-NO_2	2.0	5.64	4.59	3.67
SCH_3	1.71	4.81	4.08	3.35
I	1.51	3.75	3.34	2.83
CN	1.29	4.26	3.56	2.9
m-NO_2	1.28	4.84	4.03	3.26
Br	1.28	3.54	3.12	2.62
OCH_3	1.21	3.87	3.26	2.67
Cl	1.05	3.53	3.06	2.55
$CH(CH_3)_2$	1.03	0.74	2.97	3.29
C_6H_5	1.01	6.06	5.17	4.23
H	0.8	3.16	2.73	2.26
$COCH_3$	0.76	0.61	2.91	3.26
SO_2CH_3	0.75	+	2.8	4.05
NH_2	0.5	4.15	3.45	2.79

Discussion

The prediction of two virtually equally preferred conformations is in agreement with a recent combined nmr and pef analysis.[17] The conformation 1 which was obtained as the most stable one by EHT calculations in this study is equivalent to the one predicted by Bustard and Egan,[17] with the slight difference that in their work, angle ϕ_5 was found to be 0 degrees according to our definition of this rotational axis (Figure 2). The preferred conformation 2 in our study is essentially the same as the one predicted by Bustard and Egan[17] to be the secondary minimum conformation. The differences are confined to the less important rotational axes ϕ_5, ϕ_6, and ϕ_7.

The two preferred conformations 1 and 2 differ only in the relationship of the hydroxyl groups to each other. In neither conformation is a hydrogen bond connecting those two groups predicted. This is in contrast to Jardetzky's findings[14] but consistent with Bustard's.[17]

The stereospecificity of the molecule requires the presence of at least three binding sites in the pharmacophore. Structure-activity studies to date implicate the substituted phenyl moiety, the acylamide region, and at least one and perhaps both hydroxyl groups. The three-dimensional relationships of these groups are displayed in Figure 3 for both preferred conformations.

An influence of the *p*-phenyl substituent upon the ease of benzyl radical formation has been suggested.[9] Our calculations on a series of para-substituted benzyl models reveal a constant energy difference between the ground state and the radical form. Furthermore, the influence of the *p*-phenyl group on the charge densities in the region of the carbinol moiety is also constant.

As an alternate explanation of the influence of the phenyl substituents we have hypothesized that the phenyl moiety is engaging a receptor feature in a composite of binding forces influenced by the para substituent. To test

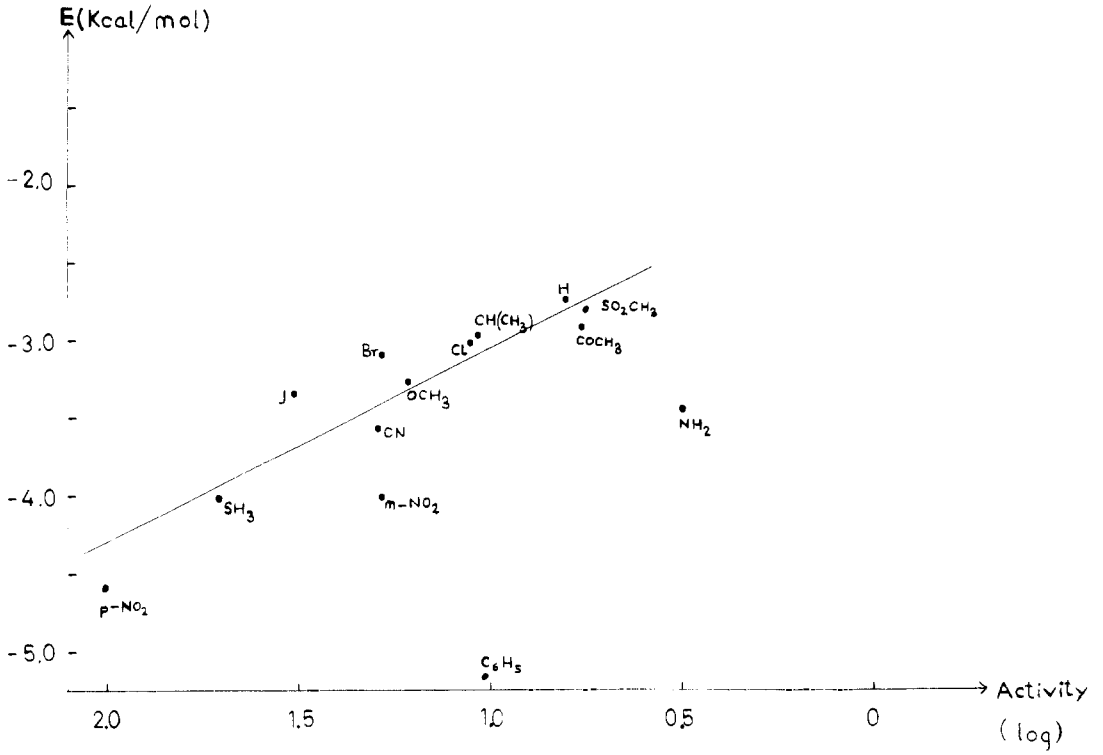


Figure 6. Calculated interaction energy *vs.* biological activity for a separation distance of 4.5 Å, Table III.

this hypothesis we have considered several models of molecules representing the chloramphenical analog and receptor features derived from amino acid side chains.

The best model in our study is derived from a receptor model simulating the tryptophan side chain. The calculated interaction energies for 12 of 14 analogs correlate fairly well with activity, considering that we are studying only a single parameter. In addition to the correlation, r = 0.914, the predicted interaction energy range, 1.79 kcal/mol (Table III), compares very well with the 1.77 kcal/mol expected for the range of potency from the thermodynamic expression.

In our view, our model is isomorphic with a phenomenon in which the phenyl moiety is interacting with a receptor site. The strength of this interaction may relate to the activity of the analog. Certainly, we cannot claim that tryptophan is the receptor site in reality. We do feel that our calculations show that this is a good model, isomorphic with reality.

It has been suggested that the variation in the electronic structure at the acylamino side chain may be responsible for changes in the activity of the peptide moiety.[9] Our calculations of charges on the C, O, N, and H atoms for 11 acyl variants revealed no correlation of charge with activity, based upon an inspection. A more detailed treatment of charges on combinations of atoms would be of further interest.

The possibility exists that the acyl moiety itself may be the active feature interacting with a receptor through dispersion forces. If interaction of these groups were the critical mechanism, the nitrile and chloro derivatives should be more active than the fluoro compounds, based on a comparison of atom polarizabilities.

Another explanation may be the influence of the acyl group on the phenyl moiety across space. Figure 3 shows that this distance is not large. The effect could be a modest electronic or steric perturbation of the phenyl moiety which alters its receptor binding ability.

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β-Amino Ketones. Synthesis and Some Biological Activities in Mice of 3,3-Dialkyl-1,2,3,4-tetrahydro-4-quinolinones and Related Mannich Bases†

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A series of Mannich bases, based on the α-alkyl-β-dimethylaminopropiophenone skeleton, and closely related cyclic analogs, *viz.* 3-alkyl-substituted 1-methyl-1,2,3,4-tetrahydro-4-quinolinones, was synthesized. They were screened in mice for analgetic and anticonvulsant activities. In addition, a number of these compounds were tested for sedative properties through the method involving sodium pentobarbital sleeping time potentiation.

A series of open-chain β-amino ketones and closely related 1,2,3,4-tetrahydro-4-quinolinones was previously reported to have analgetic properties[1] (Haffner tail-pinch method[2]). Sufficient biological activity was exhibited by several members in the series to warrant further investigation of a number of related compounds (Table I).

Mannich Bases. From prior studies which investigated β-dimethylaminopropiophenones, $ArCOCH_2CH_2NMe_2$, for analgetic activity, there emerged the lead compound **5** (Table I). In this particular structure, the active methylene protons were replaced by two methyl groups. Structural modification of this type of molecule was sought and a project initiated to synthesize a number of Mannich bases. The first phase of this study was to prepare compounds of type I (Table I). The aim was to detect changes in biological activity when only one alkyl group flanks the ketone in $C_6H_5COCHRCH_2NMe_2$ and assess the affect of lengthening the alkyl chain in R from CH_3 to n-C_3H_7.

These Mannich bases possess an enolizable system, -COCHR-. The possibility that the enol form may be the active species was tested by synthesizing type II Mannich bases in which enolization is impossible. These showed good analgetic activity and a series of para-substituted derivatives, type II in Table I, was planned. The para substituent in this series was designed to vary in lipophilic and electronic character (type II, Table I).

To ascertain if the ketone function was essential to these biological activities, several of these Mannich bases were reduced with $NaBH_4$ to the corresponding amino alcohol (type III, Table I). These alcohols were at least as effective as anticonvulsants, if not more so, than the corresponding ketones, type II.

Quinoline Derivatives. The analgetic effectiveness of $C_6H_5COC(CH_3)_2CH_2NMe_2$ (**5**) prompted us to synthesize several cyclic analogs, **30–32.** Rather than attempt to mono- and bisalkylate 1-methyl-1,2,3,4-tetrahydro-4-quinolinone at C-3, alternate routes to compounds of general type VI were sought.

1- and 3-substituted 4-hydroxycarbostyrils have been synthesized by reacting N-substituted anilines with alkyl- or arylmalonic esters.[3] For our needs, *N*-methylanilines were condensed with methylmalonic esters to produce **17–19.** Prior investigators had shown that 4-hydroxycarbostyrils were totally enolic, based on their uv and ir spectra, as well as chemical conversions.[3,4] We established that **17–19** existed in solution entirely as 4-hydroxycarbostyrils (instead of 4-keto lactams, type V, where R = H) since their proton magnetic resonance (pmr) spectra showed singlets between δ 2.05–2.15 and 10.15–10.30 (in DMSO-d_6) for the CH_3 and OH protons, respectively. Although meta-substituted anilines could have given mixtures of 5- and 7-substituted 4-hydroxycarbostyrils, our preparations afforded only the 7-chloro and methoxy analogs.‡

Alkylations of the potentially tautomeric compounds, 17–19, are expected to proceed *via* the corresponding ambident anions to *O*- and *C*-alkyl derivatives,[3,4,6] *viz.* **20–22** and **23–26,** respectively. Although there are claims in the literature that the ratio of isomers can be manipulated by the use of diverse solvent systems,[4,6] we found that the ratio varied little for reactions conducted in aqueous, alcoholic, or DMF media. C-Alkylation took place predominantly to furnish type V keto amides. Near the end of this investigation, we became aware of the relatively facile synthesis of dialkylmalonanilic acids, $ArNHCOCR_2CO_2H$, and their cyclization to keto amides of type V.[7] This route involved the condensation of the half acid chloride of a disubstituted malonic acid with an aniline and subsequent cyclization with polyphosphoric acid to produce V. We could not effect the direct synthesis of this quinoline system by heating a mixture of $C_6H_5NHCH_3$ with $(CH_3)_2C(CO_2CH_3)_2$.

To reach the target compounds, **30–32,** directly from

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‡The structures of these 7 isomers were established by examining the pattern of the aromatic proton signals in their pmr spectra in solvents which separated the signals sufficiently to permit analysis and comparison of their chemical shifts and spin-spin coupling constants with data reported for similar 5- and 7-substituted quinoline derivatives (for noted examples, see ref 5). Attempts were made to isolate the 5 isomers of 18 and 19 by closely examining the mother liquors, but these could not even be detected (tlc, pmr spectra).