Table I

Compd	Dosage, mg/kg ip	Fraction showing prevention of tremors	Fraction showing lethality	Other effects
3	500	0/10	10/10	CNS depression
	1000	0/10	10/10	Writhing movements
7	500	9/10	3/10	
	1000	10/10	3/10	
6	500	0/10	3/10	
	1000	3/10	6/10	
dl-Dopa	500	0/10	5/10	
	1000	9/10	6/10	
l-Dopa	250	3/10	1/10	
	500	10/10	5/10	

Table II

	M	% control[a]	% inhibn[a]
l-Monoiodotyrosine	10^{-5}	42	58
6	10^{-5}	95	5
6	10^{-4}	97	3
6	10^{-3}	102	

[a]Means of triplicate.

silica gel plates from Quantum Industries were used for tlc development. Ir, nmr, uv, mass spectra, and tlc were all appropriate.

4-Hydroxy-3-hydroxymethylbenzaldehyde Diacetate (2). A mixture of 1[14] (1.7 g, 0.01 mol), KOAc (2 g, 0.2 mol), and AcOH (15 cc) was stirred at 60° for 5 hr and then at 25° for 16 hr. The solvent was evaporated to yield a solid, to which 100 g of ice-H_2O was added. The product was extracted into Et_2O, and the solvent was evaporated to yield crystals. Recrystallization from Et_2O provided 2 (1.1 g, 57% yield), mp 99–100°. Compound 2 gave a positive 2,4-DNP and negative $FeCl_3$ test. *Anal.* ($C_{12}H_{12}O_5$) C, H.

4-[4′-Hydroxy-3′-(hydroxymethyl)benzylidene]-2-methyl-2-oxazolin-5-one Diacetate (3). A mixture of 2 (23.6 g, 0.1 mol), acetylglycine (11.7 g, 0.1 mol), NaOAc (8.23 g, 0.1 mol), and Ac_2O (80 ml) was stirred at 110° for 1 hr and then poured into 500 g of ice-H_2O. The obtained solid was filtered and crystallized from EtOAc-Et_2O to give 3 (26 g, 81%), mp 116–118°. *Anal.* ($C_{16}H_{15}NO_6$) C, H, N.

4-[4′-Hydroxy-3′-(hydroxymethyl)benzylidene]-2-phenyl-2-oxazolin-5-one Diacetate (7). Compound 7 was prepared from 2 in the same way as described for the synthesis of compound 3, except the reaction time at 110° was extended for 6 hr. Obtained was 7 (28.1 g, 74%), mp 156–157°. *Anal.* ($C_{21}H_{17}NO_6$) C, H, N.

2-Acetamido(3-acetoxymethyl-4-acetoxy)cinnamic Acid (4). Compound 3 (12.2 g, 0.04 mol) in 75% aqueous Me_2CO (400 ml) was heated at reflux until a clear solution was obtained (approximately 6 hr). The solvent was evaporated to give a solid. Crystallization from THF-Et_2O gave 4 (10.4 g, 78%): mp 183–185°; tlc (Me_2CO-MeOH, 1:1) R_f 0.78. *Anal.* ($C_{16}H_{17}NO_7$) C, H, N.

***dl*-3-(Hydroxymethyl)tyrosine Triacetate (5).** A solution of 4 (3.4 g, 0.01 mol) in 75% aqueous MeOH (80 ml) was hydrogenated at 25° over 10% Pd/C (350 mg) at an initial H_2 pressure of 50 lb/in.^{2}. When hydrogenation was complete the catalyst was filtered, the solvent was evaporated, and the residue was crystallized from Me_2CO to give 5 (3.04 g, 91%): mp 157–158°; tlc (C_6H_6-EtOAc-MeOH, 7:3:3) R_f 0.50. *Anal.* ($C_{16}H_{19}NO_7$) C, H, N.

***dl*-3-(Hydroxymethyl)tyrosine (6). Procedure A.** A mixture of 5 (3.4 g, 0.01 mol) and 5 *N* HCl (100 ml) was stirred at reflux until a clear solution was obtained (approximately 5 hr). The solution was concentrated (to 20 ml) under reduced pressure, treated with activated charcoal, filtered, and kept at 0° for 2 days. The precipitated crystals were filtered and washed with EtOH and then with Me_2CO to yield the HCl salt of 6 (1.71 g, 71%), mp >300°. *Anal.* ($C_{10}H_{14}NO_4Cl$) C, H, N, Cl.

The HCl salt was dissolved in H_2O (30 ml), dilute NH_4OH was added to pH 5.5, and the solution was concentrated. The precipitated crystals were filtered and washed with H_2O to give 6 (1.34 g, 64%): mp 290–294° dec; tlc (96% EtOH-34% NH_4OH, 7:3) R_f 0.85; tlc (*n*-BuOH-H_2O-HOAc-pyridine, 15:5:3:2) R_f 0.62. Compound 6 gave a positive ninhydrin test: λ max (0.01 *N* HCl) 226 mμ (log ε 3.81) and 275 (3.58). Corresponding data for *dl*-Dopa: λ max (0.01 *N* HCl) 224 mμ (log ε 3.79) and 279 (3.40). *Anal.* ($C_{10}H_{13}NO_4$) C, H, N.

Procedure B. To a cooled mixture of 7 (7.6 g, 0.02 mol) and purified red P (4.65 g, 0.15 mol) in Ac_2O (60 ml) was added dropwise with stirring 55% HI (50 g, 0.2 mol). The mixture was refluxed for 1 hr and then cooled. The excess P was removed by filtration; the solvent was evaporated under reduced pressure to give a solid. The solid was partitioned between H_2O-Et_2O (400 ml, 1:1) and after separation the aqueous solution was adjusted to pH 6 with dilute NH_4OH. The solution was concentrated and the precipitated crystals were filtered and washed with H_2O, MeOH, and then Me_2CO to give 6 described in procedure A.

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Amino–Imino Tautomerism in the Antibiotic Formycin A as Studied by CNDO/2 Molecular Orbital Theory

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In a recent communication Krugh[1] reported that carbon-13 nmr studies of the nucleoside antibiotic formycin A (1) show that this molecule is involved in prototropic tautomeric equilibria. These results for formycin A, which is a cytotoxic adenosine analog, are particularly interesting since adenosine does not give any evidence of tautomerization in its ^{13}C spectra and is known to exist in the amine form.[2,3] In the present communication we show that CNDO/2 molecular orbital calculations indicate that, relative to adenine, there are significant changes in the stability of the amino and imino forms of formycin A and that this suggests a possible mechanism for the biological activity of this drug.

Using the Pople and Segal[4] version of CNDO/2, we have computed the total ground-state energy of formycin

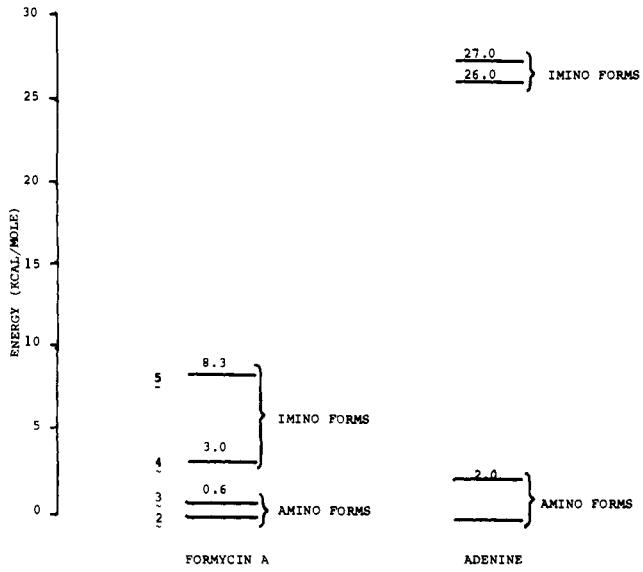
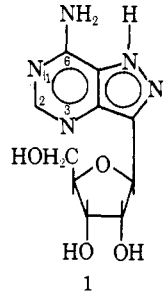
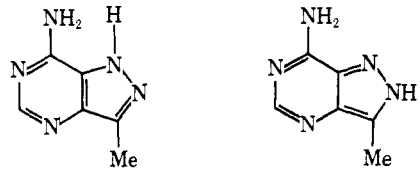


Figure 1. Relative stabilities of the amino and imino tautomers of formycin A and adenine. Total ground state for form **2** of formycin A was 66,681.7 kcal/mol. Adenine data were taken from ref 7.

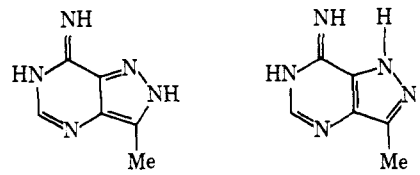


1

A in four of its possible tautomers (**2**-**5**). To reduce the complexity of the calculations, the sugar moiety was replaced by a methyl group. Geometries adopted for these calculations were based on X-ray diffraction measurements on formycin A and related pyrazolopyrimidines;[5,6] NH_2 and N=H distances were taken to be 1.39 and 1.28 Å, respectively. The results of these calculations are summarized in Figure 1 together with CNDO/2 data for adenine in similar tautomeric forms.[7]



2 **3**

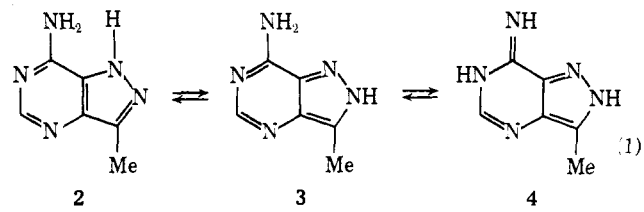


4 **5**

As in adenine, the amino forms of formycin A are predicted to be the most stable tautomers. More significantly, the imino forms of formycin A are found to be more stable than those of adenine. Assuming that these tautomers are in equilibria with one another and ignoring any entropy effects, we have calculated the relative populations of the four tautomers using a Boltzmann distribution. These numbers given in Table I show that in formycin A both amino forms (**2** and **3**) are heavily populated. This is in sharp contrast to adenine where only one of the amino forms is effectively present. While amino forms predominate in both formycin A and adenine, formycin A differs from adenine in that it has a small but significant concentration for one of its imino tautomers. These results, therefore, suggest that formycin A is involved in the kind of dynamic equilibria shown in eq 1. Such tautomeric equilibria would account for the line broadening of the base carbons that is observed in the ^{13}C nmr spectra.[1]

Table I. Relative Concentrations of Formycin A Tautomers at Equilibrium

	%
Amino form **2**	73
Amino form **3**	26
Imino form **4**	1
Imino form **5**	0



2 **3** **4** (1)

The biological consequences of the transitions in **1** may be important in explaining the antibiotic activity of formycin A. It is well known that this drug functions at the nucleotide level[8-13] and that the 6-amino group is necessary for its biological activity.[14,15] This drug with its pyrazolopyrimidine base in the amino form is analogous to adenosine and could be incorporated in its place. After incorporation, tautomerization to the imino form could occur as indicated by our calculations, and thus base pairing with a cytosine base results instead of the normal thymine pairing as in the case of adenosine. Miscoupling of the base pairs *via* such a mechanism may constitute the nature of the cytotoxic action of formycin A. It should be stressed that these calculations are preliminary and that a more complete theoretical treatment would include the sugar moiety and the possibility of syn-anti conformational changes.[16]

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Application of Regular Solution Theory to Biomembranes

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The solubility of small molecules in biological membranes is of importance in a number of areas including physiology, pharmacology, toxicology, and hyperbaric physiology. In spite of this widespread interest little direct attention has been paid to the problem. In this paper we present a semiempirical method based on the concepts of regular solution theory for the analysis of the solubility data of the simplest of solutes, namely inert gases. The treatment is tested against available data and is shown to provide a predictive framework for the gas solubility in a particular membrane. A more critical assessment of the treatment awaits accurate experimental work.

Recent studies of biological membranes have established the lipid bilayer as the basic structural component.[1] This bilayer has an interior hydrocarbon core some 25–35 Å thick and extensive in two dimensions.[2] Whereas the hydrocarbon chains of the phospholipids are (on time average) oriented across the plane of the bilayer, numerous studies attest to the fact that they are in an essentially fluid state, the freedom of motion increasing progressively toward the membrane interior.[3] It seems probable that for the solvation of small nonpolar molecules the lipid bilayer should be amenable to treatment as a three-dimensional liquid within certain limitations, and there are, in fact, good indications that such an approach might be successful for nonpolar solutes. For example, the strong correlation between oil solubility and the general anesthetic potency of gases[4] has long been held to imply a membranous site of action for these agents. Similarly, the permeability of membranes to nonionic solutes has been related to olive oil solubility.[5] Diamond and Wright[6] have discussed possible limitations of such a simplified approach.

Theoretical Background. Regular solution theory offers a framework for predicting solubility from a knowledge of certain parameters of the pure components.[7] Rigorous application of the theory to solutions of gases in nonpolar solvents is generally not possible, but used in a semiempirical mode, the theory has been successfully and extensively applied.

Regular solution theory characterizes solvents in terms of a solubility parameter, δ_1, defined as

$$\delta_1 = (\Delta E/V)^{1/2} \quad (1)$$

where ΔE is the molar heat of vaporization at constant volume and V is the molar volume. For dilute solutions plots of solubility *vs.* δ_1^2 for a gas in a number of solvents generally yield a regular relationship[7] (*e.g.*, Figure 1). The solubility of a gas in an additional solvent of known δ_1 can thus be predicted if solubility data for that gas in a number of other solvents are available. For solvents of limited stability and high boiling point, δ_1 cannot be evaluated from eq 1. In this case, δ_1 may be derived from solubility data using the reverse of the procedure outlined above and in Figure 1. Such solvents are often of high molecular weight and adopting this semiempirical approach is likely to be more successful in these cases because it is based directly on solubility properties and thus takes into account such factors as the large size difference between solute and solvent. The δ_1 so derived will be referred to herein as an "empirical solubility parameter" to distinguish it from the thermodynamic solubility parameter derived from the heat of vaporization of the pure solvent.

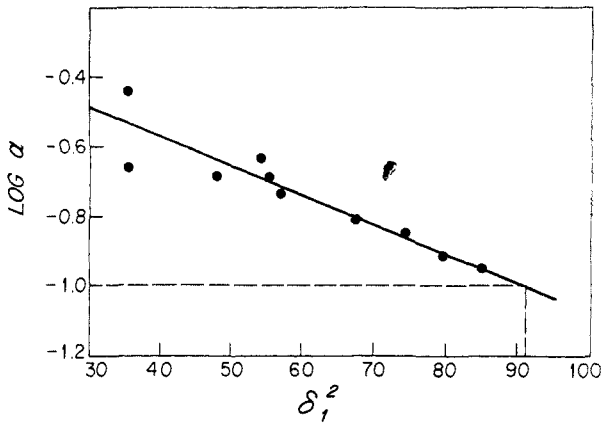

Figure 1. The relation between the Bunsen solubility coefficients (α) for nitrogen in a series of solvents and solvents' solubility parameters (δ_1 defined in eq 1). Data from ref 7. The dashed line demonstrates how the experimental solubility of nitrogen in erythrocyte ghosts can be used to define the solubility parameter.

A number of factors have to be taken into account when applying this approach to biological membranes. It is usual practice to express solubility as mole fraction, but this is not very meaningful for heterogeneous biological membranes. Instead, we have used the Bunsen coefficient (α), which is defined as the number of milliliters of gas (at STP) which dissolves in 1 ml of solvent or membrane at a partial pressure of 1 atm. Another factor is the failure of the geometric mean rule for forces between unlike molecules which causes deviations from the smooth curves usually obtained (*e.g.*, Figure 1). This is particularly true for perfluorinated compounds such as CF_4 and SF_6.[8] However, if only one class of solvent is chosen (for our purposes hydrocarbon solvents) the geometric mean rule breaks down in a systematic fashion so that smooth plots are still obtained and may be used predictively for similar solvents. The effects of size in solvents such as i-C_8H_{18} have been discussed by Hildebrand.[7] Selection of suitable solvents on this basis, however, enables reference plots of log α against δ_1^2 to be constructed for the gases of interest using data summarized in ref 7 (p 201) and 9.

Application of Regular Solution Theory to Biomembranes. In order to test whether regular solution theory may be applied to biomembranes, data for a series of gases in a membrane may be used with each of the appropriate reference plots to yield an estimate of the empirical solubility parameter as shown in Figure 1. If the theory is applicable the estimates of δ_1 given by each gas will be self-consistent.

The only suitable set of data available in the literature is that for six gases in the erythrocyte ghost membrane.[10] The experimental uncertainty in the Bunsen coefficients in that work is ±30% corresponding to an uncertainty in the estimated empirical solubility parameter of ±0.6