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CONFORMATIONAL STUDIES ON CHLORAMPHENICOL AND RELATED MOLECULES

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Abstract—A conformational analysis has been performed on chloramphenicol and related molecules by potential energy functions, NMR and IR spectroscopy. Specific solvent effects have been considered *via* calculation in the case of chloramphenicol. The results establish that essentially only one conformer of chloramphenicol exists in solution with respect to the C_α—C_β bond, but that this stabilization is not due to hydrogen bonding as proposed in a previous study. The importance of stabilization of polar solute conformations in polar media is also discussed.

INTRODUCTION

The solution conformation of chloramphenicol (D(-)-*threo*-2,2-dichloro-N-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]-acetamide) (1) has been previously studied by NMR and Raman spectroscopy,[1] and more recently by ORD and CD.[2] Chloramphenicol's specific inhibition of bacterial protein synthesis[3,4] has been initimately linked with the steric configuration and conformation of its propanediol moiety.[5] The present conformational analysis on 1 was undertaken in an attempt to establish quantitative estimates of rotamer populations, made possible by newer interpretive methods and theoretical techniques. The conclusions are experimentally based upon NMR and IR spectral analysis and theoretically upon potential energy functions (PEF).

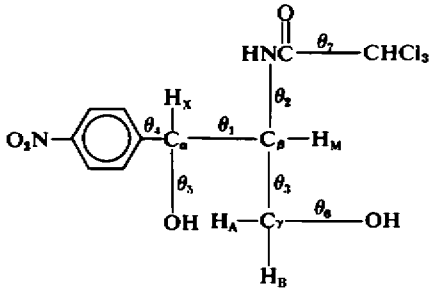
Moreover, we have extended our studies to chloramphenicol phenylboronate (6) and desacylchloramphenicol (free base 7a and hydrochloride 7b) to further validate our conclusions obtained from the chloramphenicol analysis.

Chloramphenicol

Theoretical analysis. The conformational energies of chloramphenicol were calculated using a computational technique based upon empirical potential energy functions. As applied to molecules, the method expresses the potential energy as a function of rotational angles about one or more bonds. The total energy is partitioned into a sum of pairwise van der Waals interactions and a sum of ethane-like torsional potentials over all rotation axes considered.[6–8] For chloramphenicol, the total conformational energy expression is given by Eq (1).

$$E(\theta_1, \theta_2, \ldots \theta_7) = \sum_i (a_i \exp(-b_i r_i) - c_i/r_i^6) + \sum_{K=1}^{7} U_k^\circ (1 + \cos n_k \theta_k)/2. \quad (1)$$

The summation with respect to i is only over all pairs of non-bonded atoms whose interatomic distance r_i is dependent upon one or more of the θ. The U_k° represent barrier heights for the inherent torsional potential about the k'th bond. The angle assignments used for both the theoretical and NMR work as well as the C atom and proton labels discussed later, are defined by the structure (1).



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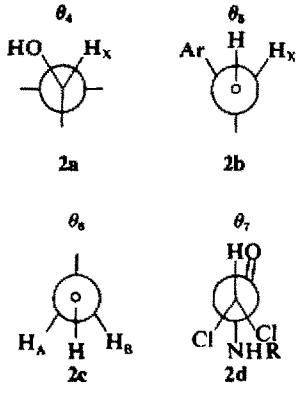
The values of a, b and c in Eq (1) were taken from Liquori *et al.*[9] for pairwise interactions among H, C, N and O atoms. These parameters for the interaction between two Cl atoms were taken from Mason and Kreevoy,[9] while parameters between Cl and the other atoms were obtained after Mason and Rice.[10] The torsional barrier heights and the axis order, n_k, were taken largely from Scheraga[11] and summarized in Table 1.

Table 1. Torsional potential parameters

Parameter	Axis number (k) 1	2	3	4	5	6	7
U_k°(Kcal/mole)	2·7	0·6	2·7	0·5	0·9	0·9	1·0
n_k	3	3	3	6	3	3	3

The bond lengths used in the calculations were taken from the *Table of Selected Bond Lengths* and X-ray data on nitrobenzene in Sutton's compilation.[12] All angles were taken to be tetrahedral except for those of *p*-nitrophenyl, which were taken from nitrobenzene, and the amide angles, which were taken from Scherega.[11]

The first three rotational degrees of freedom indicated in 1 determine the preferred conformations of chloramphenicol to a large extent, while the remaining four have little effect on former ones. The relative orientations of the latter four end groups were determined by systematically varying the angles to establish their effect on the first three optimized angles. If these four degrees of freedom are maintained at their minimized values, their effect can be factored out of Eq (1) to yield a problem of lower dimensionality. These relative orientations are given by structures **2a–2d**. The amide moiety of **1** was maintained in the *trans* conformation, since the available evidence clearly indicates that this conformer is largely predominant in solution in amides which are structurally similar to chloramphenicol.[13, 14]



Of the three remaining rotational degrees of freedom, θ_1 and θ_2 are expected to be strongly interrelated, since these bear a formal analogy to aromatic amino acids in which the corresponding angles are known to be interdependent. If θ_1 and θ_2 are systematically varied, the energy contour map shown in Fig. 1 can be drawn to illustrate the functional behavior. The low-lying minima are indicated by capital letters in Fig 1; the energies, optimized angles corresponding to these minima and mole fractions (n) of the respective conformers are summarized in Table 2. The values of n were calculated from the values of ΔE *via* Boltzmann factors.

The angles θ_1 and θ_2 are measured as indicated in **3a–3c** and **4**, respectively, in a clockwise sense. During the simultaneous variation of θ_1 and θ_2 by five degree increments, θ_3 was held constant such that the hydroxymethyl group was oriented as in

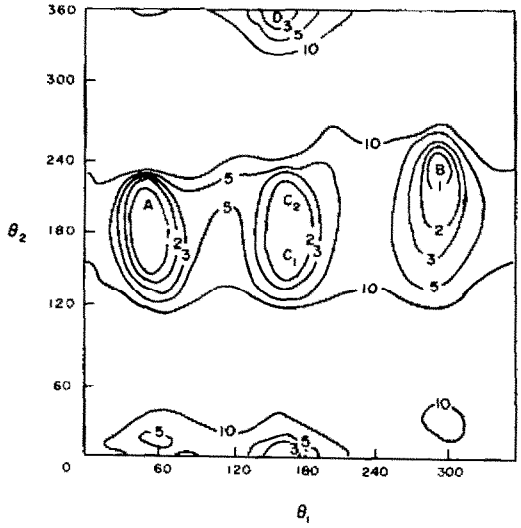


Fig 1. Potential energy map for chloramphenicol as a function of θ_1 and θ_2. Contour lines connect isoergic points with the energies (Kcal/mole) indicated. Minima in Table 2 are indicated by capital letters.

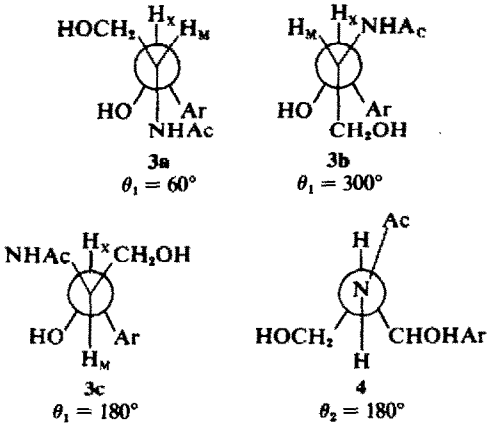
Table 2. Calculated minima and populations with respect to θ_1 and θ_2 at $\theta_3 = 180°$ for chloramphenicol

Conformation	θ_1 (deg)	θ_2 (deg)	E[a] (Kcal/mole)	n
A	65	205	0·000	0·71
B	305	225	0·824	0·18
C_1	175	150	1·617	0·045
C_2	175	205	1·617	0·045
D	170	355	2·241	0·02

[a]Energy with respect to global minimum at A

5b. In structures **3–5**, the proton subscripts label these atoms for the subsequent NMR analysis.

The effect of rotation of the hydroxymethyl group on the molecular potential energy was taken



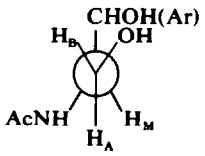
Ac = dichloroacetyl
Ar = *p*-nitrophenyl

into account by varying θ_3 in ten degree increments through a 360-degree cycle at each of the five minima reported in Table 2. The minimized values of θ_3 at each of the minima in Table 2 have been summarized in Table 3.

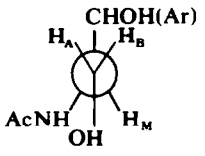
Table 3. Calculated minima with respect to θ_3 at each of the (θ_1, θ_2) minima for chloramphenicol

Minima from Table 2	Minima with respect to θ_3[a] θ_3 5a	ΔE	θ_3 5b	ΔE	θ_3 5c	ΔE
A	64	0·173	180	0·000	297	1·009
B	70	2·148	180	0·734	280	3·327
C_1	80	2·468	190	1·521	300	1·931
C_2	80	2·441	190	1·613	300	1·648
D	80	2·859	190	2·076	300	4·680

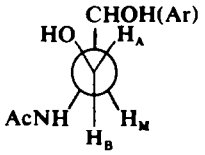
[a]Angles in degrees; energies in Kcal/mole.



5a
$\theta_3 = 60°$



5b
$\theta_3 = 180°$



5c
$\theta_3 = 300°$

Conformer populations with respect to θ_3 can be estimated from the data in Table 3 by calculating the partition functions (p.f.) for the three rotamers (**5a–5c**), summed over the minima A through D. The ratios of the p.f.'s yield relative conformer populations,[15] which can then be used to calculate mole fractions. The theoretical population analysis based on p.f.'s appears in Table 4 along with population estimates based only on the energies at the global minimum A.

Table 4. Calculated conformer populations of the chloramphenicol hydroxymethyl group at 30° and 150°

Conformer	T = 30° n(p.f.)	n(at A)	T = 150° n(p.f.)	n(at A)
5a	0·32	0·39	0·30	0·39
5b	0·57	0·52	0·53	0·47
5c	0·11	0·10	0·17	0·14

NMR analysis. The NMR spectra of chloramphenicol were obtained in four different solvents and over the temperature range 30°–150°. The spectra were analyzed by simple first order rules since deviations caused by higher order effects were minimal due to favorable chemical shifts dispersion at 100 MHz. The data obtained, summarized in Table 5, agree with the published values of Jardetzky.[1]

The small magnitude of J_{MX} clearly demonstrates that the most populated conformers with respect to θ_1 are those which have *gauche* protons (dihedral angle ~ 60°), *i.e.* **3a** and **3b**. NMR cannot readily differentiate between **3a** and **3b**. If the *trans* and *gauche* vicinal J_{MX} couplings are known, an estimate of the combined mole fraction of **3a** and **3b**, represented by n_g, can be obtained from Eq (2).

$$J_{MX} = n_g + (1 - n_g)J_t. \quad (2)$$

Table 5. Chemical shifts and coupling constants of chloramphenicol (1)

	Chemical shifts[a] Acetone-d_6 amb.	DMSO-d_6 amb.	90°	150°	Methanol-d_4 amb.	Pyridine-d_5 amb.	70°	110°
H–A	3·64	3·30	3·34	3·42	3·59	4·13	4·07	4·00
H–B	3·80	3·55	3·57	3·61	3·72	4·39	4·28	4·17
H–M	4·17	3·90	3·93	3·96	4·15	4·84	4·72	4·59
H–X	5·30	5·00	5·00	5·01	5·16	5·80	5·70	5·58
	Coupling constants[b]							
$J_{A,B}$	10·5	10·5	10·5	10·5	10·7	10·8	11·0	11·0
$J_{A,M}$	6·5	6·2	6·1	6·1	6·2	6·0	5·8	5·8
$J_{B,M}$	7·0	7·4	6·9	6·3	6·9	7·5	7·4	6·4
$J_{M,X}$	2·5	2·2	3·0	3·4	2·6	2·5	2·8	3·0

[a]measured from internal TMS in ppm (δ).

[b]determined from simple first order analysis, no attempt was made to determine the relative signs although the geminal coupling is presumed to be negative.

When the couplings for the individual rotamers are assumed to be $J_g = 2{\cdot}0$ Hz and $J_t = 12{\cdot}0$ Hz, the measured coupling in DMSO-d_6 solution predicts a combined mole fraction of 0·98 for **3a** and **3b**. The theoretical data in Table 2 supports a combined mole fraction of 0·89, which is in reasonable agreement considering the assumptions inherent in the NMR calculations which do not consider deviations from 60° staggering or tetrahedral symmetry.

In DMSO-d_6 solution, before the addition of D_2O, the amide NH proton appears as a doublet, $J_{NH,M} = 9{\cdot}5$ Hz. Narayanan and Sawant[16] have shown that NH–CH couplings of this magnitude arise in cases where the dihedral angle between the interacting protons is 180° as would be expected from the Karplus relationship. Thus NMR supports conformer **4** when $\theta_2 = 180°$ in agreement with the theoretical analysis.

Conformer populations with respect to θ_3 can be determined from the J_{AM} and J_{BM} couplings using the method of Bailey *et al*[17,18] who have shown that the populations of rotamers **5a–5c** can be calculated using Eqs (3)–(5).

$$n(\mathbf{5a}) = \frac{J_{AM} - J_g}{J_t - J_g} \tag{3}$$

$$n(\mathbf{5b}) = \frac{J_{BM} - J_g}{J_t - J_g} \tag{4}$$

$$n(\mathbf{5c}) = \frac{J_t + J_g - (J_{AM} + J_{BM})}{J_t - J_g}. \tag{5}$$

The calculated populations, using the vicinal coupling constants determined in DMSO-d_6 and with $J_t = 12{\cdot}0$ Hz and $J_g = 2{\cdot}0$ Hz, are collected in Table 6. The agreement of these populations with those in Table 4 which arose from the theoretical analysis is excellent.

In summary, the vicinal coupling constants are useful in defining the conformation of chloramphenicol with respect to θ_1, θ_2 and θ_3. Furthermore, the conclusions reached from the NMR analysis are in complete agreement with and offer support for the theoretical analysis in every detail.

Infrared analysis. Using NMR and Raman spectral data, Jardetzky[1] concluded that chloramphenicol (**1**) contained an intramolecular H-bond between the primary and secondary OH groups. This assumed H-bond was felt to play an important role in the stabilization of chloramphenicol's preferred conformation, *i.e.* conformer A, Table 2.

Since the theoretical analysis indicated that H-bonding energy was not necessary for the stabilization of chloramphenicol in its preferred conformation, we looked for additional data to support the claim that a H-bond was indeed present. Previously cited evidence based on Raman spectra[1] is questionable since it is known that Raman spectroscopy is particularly insensitive to OH groups and frequently the band due to H-bonded hydroxyls and free hydroxyls does not even appear.[19,20] It is thus probable that the 3400 cm^{-1} band reported for chloramphenicol is due to the —NH— group.

High-dilution infrared absorption studies have been used to determine the extent of intramolecular H-bonding in other compounds.[21] Although chloramphenicol is not soluble in CCl_4, it is sufficiently soluble in $CHCl_3$ to obtain high-dilution spectra. The spectra obtained at 0·001 M and 0·003 M in $CDCl_3$ were identical and showed two sharp bands at 3415 cm^{-1} and 3620 cm^{-1} due to —NH— and —OH absorption. A much less intense, broad band at 3510 cm^{-1} was also present. The intense band at 3620 cm^{-1} due to free OH absorption was particularly significant, since it was not detected in a previous study.[1] These results indicate that the hydroxyls in chloramphenicol exist primarily in a non H-bonded environment.

Chloramphenicol phenylboronate (NMR analysis)

The $CDCl_3$ solution NMR parameters for chloramphenicol phenylboronate (**6**) are collected in Table 7.

The NMR of the phenylboronate was examined in order to characterize a compound of rigid and

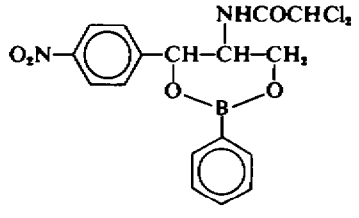


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Table 6. Calculated conformer populations of the chloramphenicol hydroxymethyl group from NMR data

Conformer	T = 30°	T = 150°
5a	0·42	0·41
5b	0·54	0·43
5c	0·04	0·16

Table 7. Chemical shifts and coupling constants of chloramphenicol phenylboronate (**6**)

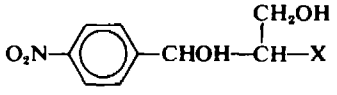
Chemical shifts ppm (δ)		Coupling constants Hz	
H–A	4·25	$J_{A,B}$	12·2
H–B	4·56	$J_{A,M}$	2·1
H–M	4·76	$J_{B,M}$	2·8
H–X	5·65	$J_{M,X}$	3·0

known conformation in the chloramphenicol series. Of necessity, the phenylboronate either incorporates conformer **3a** + **5c** or **3b** + **5a**. The three small vicinal coupling constants, which arise from all *gauche* relationships, indicate that the former circumstance is present. The contrast with chloramphenicol is especially apparent at θ_3 where a previously minor conformer is now completely populated.

The phenylboronate, by construction, incorporates a conformation very similar to that which has been ascribed to chloramphenicol.[1] However the very marked differences in coupling constants indicates that **1** and **6** do not have similar conformations and further stipulates that a conformation of the type that would result from intramolecular H-bonding between the two OH groups of **1** is not a major contributor to the solution conformation.

Dedichloroacetylchloramphenicol: Hydrochloride and free amine

Theoretical analysis. The conformational calculations on desacylchloramphenicol hydrochloride (**7a**) and the free amine (**7b**) were a simplification of those for chloramphenicol itself.

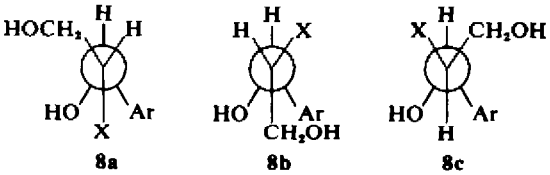


7a: X = NH_3^+
7b: X = NH_2

The main differences are a tetrahedral hydridization of the amine nitrogen instead of the trigonal one for the amide, restriction of the amine (or ammonium) group hydrogens to be staggered with respect to the hydrogen on the adjacent β-carbon (defines θ_2) and the assumption that all of the remaining angles (except θ_1) remain at their globally minimized values obtained for chloramphenicol. The angle assignments correspond to those denoted in structure **1**. The energy calculations yield minima with respect to θ_1 corresponding to conformers (**8a–8c**) for both the hydrochloride and the free amine.

The energies above the lowest one and calculated populations of each conformer appear in Table 8.

NMR analysis. The DMSO-d_6 NMR parameters for both the free amine and hydrochloride of desacylchloramphenicol are collected in Table 9.



8a **8b** **8c**

X = NH_3^+ or NH_2

Table 8. Calculated population analysis about C_α—C_β bond at 30°C. for **7a** and **7b**

Conformer	X = NH_3^+ ΔE (Kcal/mole)	n	X = NH_2 ΔE (Kcal/mole)	n
8a	0·000	0·43	0·000	0·37
8b	0·537	0·17	0·035	0·35
8c	0·043	0·40	0·150	0·28

Table 9. Chemical shifts and coupling constants of desacylchloramphenicol hydrochloride (**7a**) or free amine (**7b**)

	Chemical shifts ppm (δ)			Coupling constants Hz	
	7a	**7b**		**7a**	**7b**
H–A	~ 3·4	3·17	$J_{A,B}$	—	10·5
H–B	~ 3·4	3·37	$J_{A,M}$	—	5·8
H–M	~ 3·4	2·71	$J_{B,M}$	—	5·8
H–X	~ 4·46	4·67	$J_{M,X}$	7·5	4·5
			n_g	0·45	0·75

The increasing magnitude of $J_{M,X}$ supports the theoretical analysis that the population of conformers like **8a** increases in going from **1** to **7b** to **7a**. The calculated total mole fractions of **8a** + **8b** (Table 9) using equation **2** reflect the same trend as those from the theoretical analysis.

DISCUSSION

While our analysis indicates that conformer **3a** is the largely predominant one with respect to θ_1 in agreement with Jardetzky,[1] his analysis was predicated upon the existence of a H-bond between the primary and secondary OH groups, in order to eliminate three of the four possible conformations consistent with his data. We have demonstrated that such a H-bond does not exist to any appreciable extent and that conformer **3a** must be stabilized by other factors.

Other studies dealing with amides in solvents of varying dielectric constant indicate that inter-solute H-bonds of the type N—H . . . O=C do not form in media of high dielectric constant.[22] It is likely that the same factors operate in the case of chloramphenicol and that intramolecular H-bonds would form only in very low dielectric media or perhaps in a particular region of very low dielectric constant created by an aggregation of non-polar moieties.

The theoretical and experimental analyses of chloramphenicol have shown that the most populated conformation is that shown in Fig 2, which corresponds to global minimum A (Table 2). Clear preferences have been established for rotamers with respect to angles θ_1, θ_2 and θ_4 through θ_7 which have been incorporated into Fig 2; however, it should be stressed that a second rotamer with

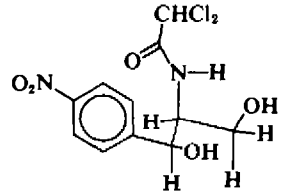


Fig 2. The lowest energy conformation of chloramphenicol, which corresponds to rotamers 3a + 5b. A second conformation of slightly higher energy corresponds to rotamers 3a + 5a (*vide supra*).

respect to θ_3 is also significantly populated (*i.e.* hydroxymethyl positioned as in 5a).

Since the polarity of the solute and solvent play an important role in the determination of conformer populations,[23] we have further extended our theoretical treatment to include such factors. It has long been known that the more polar conformers are stabilized to a greater extent in polar media than non-polar conformers.[23] Many years ago, Mizushima and Okazaki[24] proposed that this stabilization energy (ΔE_s) is related to the dipole moment (μ) of the conformer by Eq (6), in which ϵ is the solvent dielectric constant and a is the radius in Å of the solute molecule (assumed to be spherical).

$$\Delta E_s \text{ (Kcal/mole)} = \frac{-14{\cdot}40\,(\epsilon - 1)}{a^3\,(2\epsilon + 1)}\,\mu^2. \qquad (6)$$

We have applied this method to the minima indicated in Table 2 using $\epsilon_{DMSO} = 45$, $a^3 = 85{\cdot}1$ Å^3 (calculated from a density of 1·49 g/cc)[25] and the bond dipole moments summarized in Table 10.

The results of these calculations are summarized in Table 11 and are in excellent agreement with our NMR results. The postulation of a hydrogen bond to stabilize conformer 3a is thus completely unnecessary, and reflects rather a stabilization of that conformer by a combination of minimum non-bonded interactions and a marked polar stabilization effect.

At the global minimum A defined in Table 3, the calculated populations of rotamers 5a–5c also change when solvation energy from Eq (6) is included. These results, listed in Table 12, are still in reasonable agreement with the NMR results in Table 6. The lack of agreement of these results is likely due to inherent inaccuracies in the determination of dipole moments from bond dipole tables and the rather simple nature of the stabilization energy, as well as the oversimplification inherent in the NMR determinations. A more sophisticated treatment[26,27] could possibly improve these results. Nevertheless, the results are complimentary and very clearly indicate the trend of rotamer stabilities.

Table 11. Calculated mole fractions of the potential energy minima in Table 2 including solvent stabilization

Minima from Table 2	Potential[a] energy E	Stabilization energy $-\Delta E_s$	Total energy $E + \Delta E_s$	Mole fraction[b] n
A	192·74	9·23	183·51	1·00
B	193·90	1·20	192·70	—
C_1	194·92	4·93	189·99	—
C_2	194·68	5·77	188·91	—
D	195·13	1·00	194·13	—

[a]Includes all non-bonded interactions in Eq (1). The differences between the relative potential energies in this table and those in Table 2 arise from the minimization of angles θ_4–θ_7 in the stabilization energy calculations.

[b]Conformation A is the only significantly populated one.

Table 12. Calculated mole fractions of conformers with respect to θ_3 at the global minimum A of Table 11 including solvent stabilization.

Conformer	Potential energy E	Stabilization energy $-\Delta E_s$	Total energy $E + \Delta E_s$	Mole fraction n
5a	192·90	8·92	183·98	0·30
5b	192·74	9·23	183·51	0·66
5c	194·09	8·85	185·24	0·04

The conformational analysis of dedichloroacetyl chloramphenicol (7a and 7b) was undertaken to determine whether the rotamer populations of this molecule were similar to those of other phenethylamines. In the case of the hydrochloride (7a), the *trans* conformer 8c appears to be the most highly populated rotamer, which is usually the case in phenethylamines.[8,17,18] However, the free amine (7b) and chloramphenicol itself exhibit clear conformational preferences for a *gauche* form, a rather unexpected observation for compounds of this structure.

In summary, we have shown that chloramphenicol

Table 10. Bond dipole moments

Bond	H—N	H—O	C—N	C—O	C=O	C—Cl	C_{ar}—NO_2
μ(D)[a]	1·3	1·5	1·15	1·5	2·7[b]	2·3	4·2[b]

[a]G. W. Wheland, *Resonance in Organic Chemistry* p. 209. Wiley, New York, (1955)

[b]E. S. Gould, *Mechanism and Structure in Organic Chemistry* p. 62. Holt, Rinehart and Winston, New York (1959)

col has a definitely preferred conformation (3a) with respect to θ_1 in solution, but that minimal nonbonded interactions and solvent stabilization are responsible, rather than intramolecular hydrogen bonding as originally postulated.[1] Consistent with this conclusion is the observation of substantial populations of conformers 5a and 5b, which cannot accomodate an intramolecular H-bond. The lowest energy conformation is that shown in Fig 2. It has further been demonstrated that theoretical methods of conformational analysis can be useful and reliable for complex polar molecules in polar solvents.

EXPERIMENTAL

The chloramphenicol used in this study was purchased from Parke-Davis and Co. and used without further purification. The NMR spectra were obtained on a Varian Associates HA-100 spectrometer and the IR spectra were obtained on a Perkin-Elmer Model 521 spectrophotometer. Chloramphenicol phenylboronate[28] was prepared by refluxing an equimolar amount of chloramphenicol and phenylboronic acid in acetone for 2 hr, and then evaporating to dryness, m.p. 176–177°.

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