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CHLORAMPHENICOL: HIGH DILUTION FT-IR EVIDENCE FOR AN INTRAMOLECULAR HYDROGEN BOND[1]

ANTHONY L. FITZHUGH

PRI/DynCorp
NCI-Frederick Cancer Research and Development Center,
Medicinal Chemistry Section, Chemical Synthesis and
Analysis Laboratory, P.O. Box B, Frederick, MD 21702-1201

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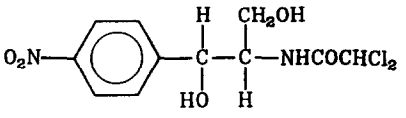
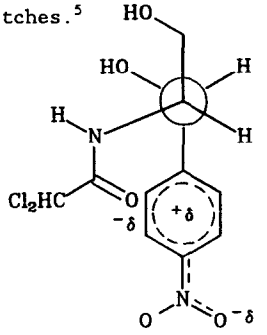
Abstract: FT-IR spectral evidence is presented which indicates that intramolecular hydrogen-bonding occurs in chloramphenicol.

Chloramphenicol is a broad-spectrum antibiotic which inhibits ($K_D \approx 1\text{-}2 \times 10\ M^{-6}$) protein biosynthesis at the level of the peptidyl transferase center of the 50S ribosomal subunit.[2] The naturally occurring configuration of chloramphenicol is D-threo (**Ia**).[3] In 1952, Dunitz published the results of an x-ray diffraction study of the dibromoacetyl analog of chloramphenicol which determined that the antibiotic exists in a preferred conformation (**Ib**) where the two hydroxyl groups are within 2.74 angstroms of one another.[4] Because of the close proximity of these two groups, Dunitz proposed that an intramolecular hydrogen bond was the force responsible for stabilizing the **Ib**-conformer. Other investigations of chloramphenicol using such spectroscopic techniques as IR, Raman and ^{1}H NMR supported Dunitz's hypothesis.[5,6] In particular, an early IR investigation showed two broad bands at 3630 and 3500 cm^{-1} which are characteristic of both free, and intramolecular hydrogen-bonded, O-H stretches.[5]



Ia



Ib

The existence of an intramolecular hydrogen-bond in chloramphenicol was questioned by Bustard et al. in their study involving high-dilution IR, ^{1}H NMR and potential energy calculations.[7] They postulated that the **Ib**-conformer might

be stabilized through minimal polar and/or solvent interactions. The existence of intramolecular hydrogen-bonding was further discounted by a study which posited that the **Ib**-conformer is stabilized **solely** by dipolar attractive forces between the carbonyl oxygen and the p-nitroaromatic ring.[8] This concept has precedent in the conformational stabilization of o-nitrophenyl esters of Boc amino acids.[9]

The opposing hypotheses of Dunitz and Bustard have heretofore remained unresolved. We decided to reinvestigate chloramphenicol with the aid of the powerful technique of FT-IR. Our findings are reported herein.

A review of the high-dilution (0.001 and 0.003 M in $CDCl_3$) IR data reported by Bustard discloses two sharp, intense bands at 3415 and 3620, and one broad, less intense band at 3510 cm^{-1}. The bands at 3415 and 3620 cm^{-1} were assigned as expected to an N-H stretch, and a free O-H stretch, respectively. Bustard, however, made no assignment of the 3510 cm^{-1} band despite its prior observation and assignment to an intramolecular hydrogen-bonded O-H stretch.[5]

When we obtained a high dilution FT-IR spectrum of chloramphenicol (0.001 M in $CDCl_3$), four prominent asymmetrical bands at 3691.2, 3616.1, 3516.0 and

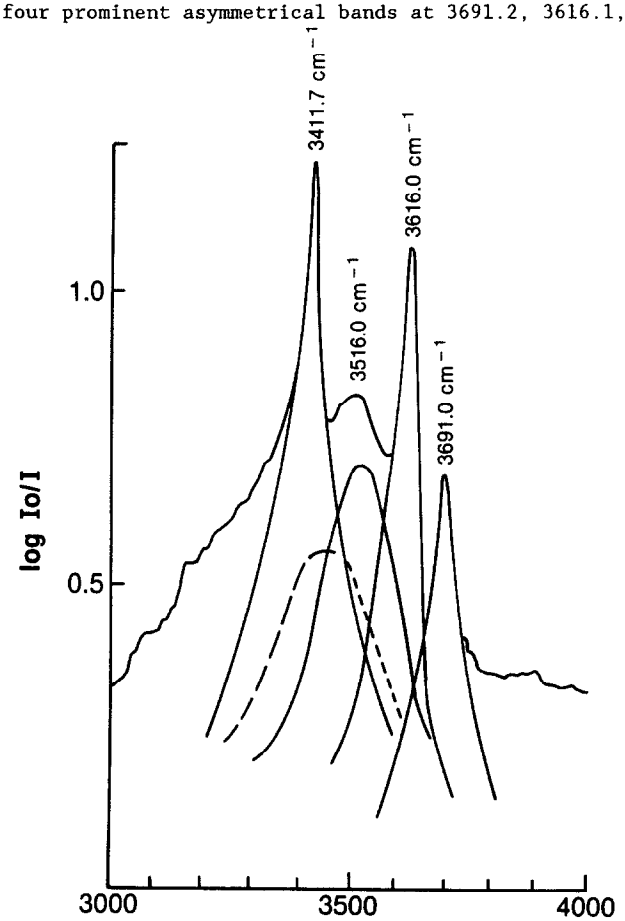
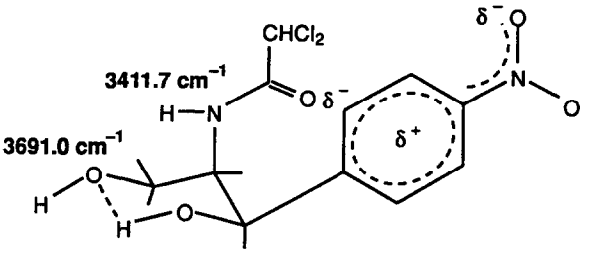


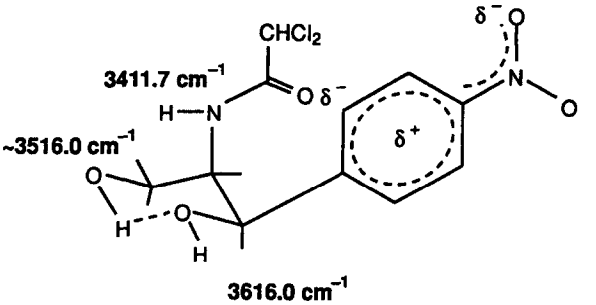
Fig. I High-Dilution FT-IR Spectrum of Chloramphenicol in the 3000 to 4000 cm^{-1} Region

3411.7 cm^{-1} were found. The 3616.1 and 3411.7 cm^{-1} bands were sharp and intense. The 3691.2 cm^{-1} band, which others had not previously detected, was sharp but of moderate intensity. The 3516.0 cm^{-1} band was quite broad and of intermediate intensity. The 3411.7 cm^{-1} band was assigned to the N-H stretch. The bands at 3691.2 and 3616.1 cm^{-1} were assigned to the stretches of two free O-H groups. The broad band at 3516 cm^{-1} was assigned to one of two intramolecular hydrogen-bonded O-H stretches.

The assignment of the 3516 cm^{-1} band to one of two intramolecular hydrogen-bonded O-H groups is reasonable based on the IR spectrum of 1-phenyl-1,3-propane diol, a close structural analog. A study[10] using high-dilution IR techniques (0.004 M $CHCl_3$) revealed that this compound displays a pattern in the 3000 to 4000 cm^{-1} region which, upon review, is nearly identical to that of chloramphenicol. Specifically, its spectrum disclosed two broad bands that when graphically factored, gave four asymmetrical bands at 3638, 3615, 3547 and 3531 cm^{-1}. This permitted the assignment of these bands to two pairs of intramolecular hydrogen-bonded O-H stretches. Theoretically then, the IR spectrum of intramolecular hydrogen-bonded chloramphenicol should have five bands (the additional band coming from its N-H group), rather than the four we observed. We attribute the failure to observe a fifth band experimentally is to the obscuration of the shoulder of one of the intramolecular hydrogen-bonded O-H stretches by the strongly overlapping N-H band. Except for this anomaly in the



IIa



IIb

Fig. II Preferred Conformer of Chloramphenicol showing two pairs of intramolecularly hydrogen-bonded O-H groups

high-dilution IR spectrum of chloramphenicol, the pattern of bands displayed in its spectrum and that of 1-phenyl 1,3-propane diol are virtually superimposable. The aforementioned correlation provides compelling evidence that intramolecular hydrogen-bonding occurs in chloramphenicol.

Consequently, it is proposed that the preferred conformation of chloramphenicol (Ib) is stabilized through the complementary action of both dipolar attractive and intramolecular hydrogen-bonding forces (IIa,b). Chloramphenicol therefore is an important example of a drug whose preferred conformation is stabilized entirely by non-covalent means. This conclusion provides support for the thesis that chloramphenicol maintains the Ib-conformation both in solution and at the active-site.[11] Hopefully, future studies of this fascinating antibiotic will provide insight into how the Ib-conformer of chloramphenicol achieves inhibition of the peptidyl transferase center.

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