Tetrahedron Letters Vol. 21, pp 251 - 254 0040-4039/80/0115-0251$02.00/0

THE CRYSTAL AND MOLECULAR STRUCTURE OF NONIONIZED 6-THIATETRACYCLINE FREE BASE

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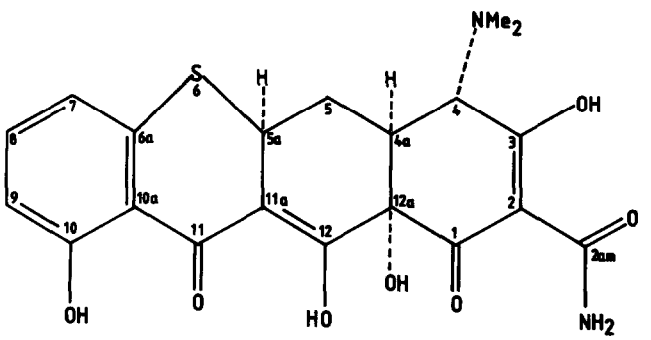
ABSTRACT: A high resolution crystal structure analysis of 6-thiatetracycline, a new broad spectrum tetracycline antibiotic, has substantiated the integrity of the chemical structure and conformation of the free base under hydrophobic conditions.

In an accompanying report, Kirchlechner and Rogalski (1) describe the total synthesis of 6-thiatetracycline, 6-STC, which has been found to be a potent broad spectrum antibiotic. The presence of the heteroatom, sulfur, in the C-ring of 6-STC gives it a chemical structure markedly different from that of other members of the tetracycline family. In view of its antibacterial activity, 6-STC has the potential to be the first of a class of new tetracyclines



6-STC

of medicinal importance. We therefore thought it particularly important that high precision structural data be obtained by low temperature crystallographic techniques in an effort to closely examine the effects the heteroatom has on the bonding, particularly in the BCD-chromophore, and on the conformation of the free base, both in the nonionized and zwitterionic forms. This report presents the principal findings from the crystal structure analysis of the nonionized free base.

High quality single crystals of racemic 6-STC, obtained from methylene chloride, were kindly provide by R. Kirchlechner. Their space-group symmetry is $P2_1/c$ with Z = 4 and lattice parameters: a = 5.536(1), b = 19.538(2), c = 17.540(2)Å, $\beta = 99.93(1)^{\circ}$ for a crystal maintained at *ca.* 120K. Intensity data were measured for the cooled crystal to a resolution of $(\sin\theta/\lambda)_{max} = 0.951 Å^{-1}$ by the ω-scan technique typically utilized in this laboratory (2). A total of 11243 reflections contributed to the refinement of 351 variables, including all atomic coordinates, anisotropic temperature factors for C, N, O, and S atoms and isotropic temperature factors for H atoms, to give a conventional residual R = 0.043.

The conformation for 6-STC in this crystalline modification is very similar to that of other nonionized tetracycline free bases as exemplified by that of oxytetracycline free base (2,3). As can be seen from the stereoscopic projection (4) presented in Figure 1, the chemical structure of 6-STC is that of the nonionized free base in which the A-ring chromophore is demonstrated to be in the enolic form and the dimethylamino group is not protonated. The crys-

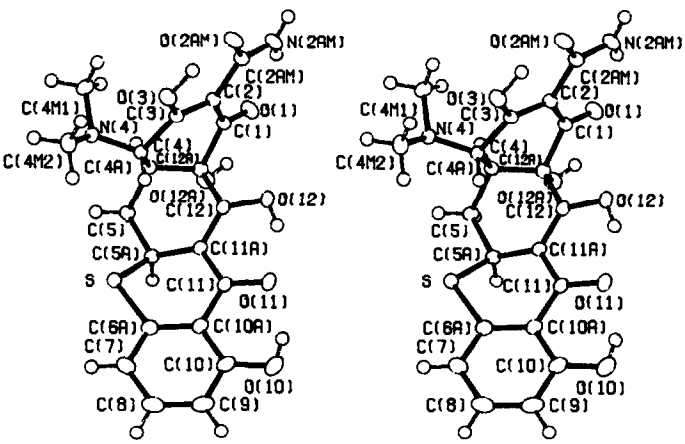


Figure 1. A stereoscopic projection of 6-STC nonionized free base. The atom labeling is depicted therein. The A-ring chromophore is at the upper portion of the figure; the BCD-chromophore includes the D-ring (bottom ring in the projection) and the atoms of the right hand portion of the two middle rings.

tal structure clearly demonstrates that 6-STC crystallizes from nonaquesous solution with the chemical structure and conformation expected (3) of tetracyclines capable of *in vivo* antibacterial activity.

Because of the position of substitution, the sulfur atom may be expected to influence the bonding geometry of the BCD-chromophore. Figure 2 presents bond lengths from this structure determination of 6-STC and the averaged values compiled from two examples of nonionized oxytetracycline free base (2,3), non-

ionized 5,12a-diacetyloxytetracycline free base (5), nonionized N-*t*-butyl-6-deoxy-6-demethy-8-methoxytetracycline free base (6), zwitterionic oxytetracycline and tetracycline free bases (3), and two examples of cationic α-6-deoxyoxytetracycline (7). All of these examples display very similar bond distances in the BCD chromophore. The near identity of the bond lengths in the

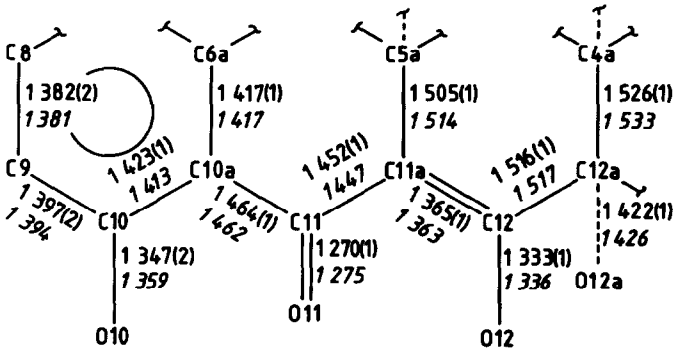


Figure 2. A comparison of bond distances in the BCD-chrompohore. The values with estimated standard deviations (in parentheses) are for 6-STC, those without are the averages of numerous tetracycline structures described above.

BCD-chromophore of 6-STC with the averaged values provides convincing evidence that the presence of the heteroatom has had little if any effect on the bonding in the chromophore.

The bonding geometry of the sulfur atom is as follows: C(5a)-S = 1.842(1) and C(6a)-S = 1.756(1)Å and C(5a)SC(6a) = 100.40(5)°. The nonbonded intramolecular separation between C(5a) and C(6a) in 6-STC, the distance most likely to be directly effected by the presence of the heteroatom, is 2.751Å; the average distance for the two examples of nonionized oxytetracycline free base and for N-*t*-butyl-6-deoxy-6-demethy-8-methoxytetracycline in which the conformations are the same as that of 6-STC observed here, is 2.504Å.

To date, we have not succeeded in getting adaquate crystals of 6-STC in the other important conformation displayed by the medicinally important tetracyclines (3,8) that associated with either the zwitterionic free base or the cation. Proton NMR spectra from the hydrochloride salt are, however, indicative that this conformation is accessible to 6-STC (1).

While quantitative data are not yet available, it appears that the free base of 6-STC displays a higher propensity to adopt the nonionized chemical structure and its associated conformation than does oxytetracycline. If so,

the solvent dependent equilibrium between the two forms of the free base (8) is most likely more favorable to the nonionized form of this tetracycline under physiological conditions than it is for the usual derivatives. Such a shift in the equilibrium may give rise to a natural "time release" mechanism whereby 6-STC is more slowly released from lipophilic regions of the body for delivery to the bacteria.

We attribute the apparent preference of 6-STC for the nonionized chemical structure to the reduced steric repulsion between components of the A- and C-rings. Specifically, the interaction between the β-substituent at C(6) and the hydrogen atom at C(4) tends to destabilize the conformation associated with the nonionized form of the tetracycline free bases (see the discussion of relative lipophilicities of several tetracyclines in (3)). Figure 1 presents visual evidence that these interactions are very minor in 6-STC.

ACKNOWLEDGEMENT: We thank the Institut für Organische Chemie, Biochemie und Isotopenforschung der Universität Stuttgart for making this research possible.

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(Received in Germany 2 November 1979)