STRUCTURAL RELATIONSHIPS IN TETRACYCLINES. THE CRYSTAL STRUCTURE OF ANHYDROTETRACYCLINE HYDROBROMIDE

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SUMMARY

The crystal and molecular structure of anhydrotetracycline hydrobromide has been determined by single crystal X-ray diffraction methods. Although the molecule is similar to the tetracycline antibiotics, the biological activity is different. A comparison of the structures of anhydrotetracycline and the active tetracycline has revealed significant structural changes that may account for the differences in activity.

RESULTS

We wish to report the crystal structure of anhydrotetracycline hydrobromide, the first structure determination of a tetracycline-type compound with completely different biological activity. Anhydrotetracycline, formed by the removal of a water molecule from the C ring[1] of a tetracycline, has only a fraction of the activity of aureomycin against staphylococcus aureus. However, anhydrotetracycline has a much greater activity against actinomycetes compared to aureomycin.[2] Our results indicate definite molecular changes which may explain the fascinating differences in the activity of these drugs.

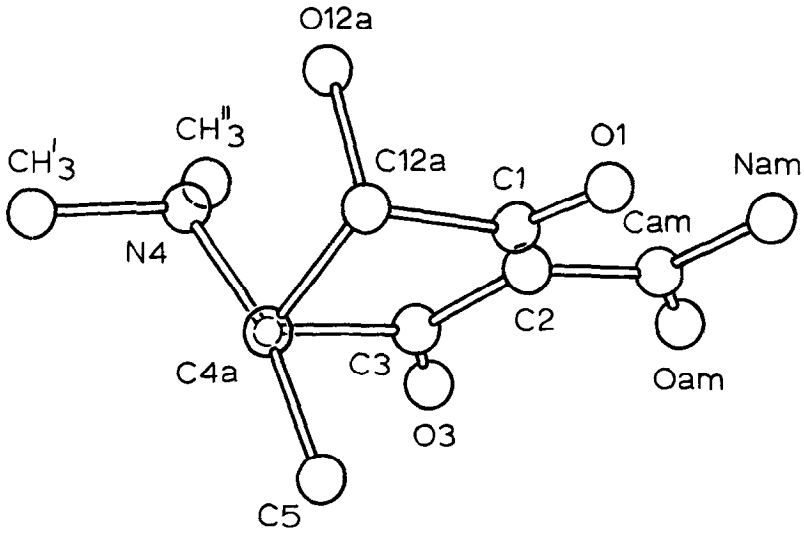
Anhydrotetracycline hydrobromide can be obtained as yellow, acicular crystals from methanol. The crystals are orthorhombic with the space group $P2_12_12_1$. There are four molecules in the unit cell of dimensions a = 19.562 $\pm$ 0.004, b = 16.586 $\pm$ 0.002 and c = 6.796 $\pm$ 0.002 Å. A crystal 0.08 x 0.07 x 0.05 mm^3 was used to measure 6613

reflections [with $2\theta < 135°$ (CuKα)] which were reduced to a unique set of 2296 reflections of which 2165 were considered to be observed.

The bromide ion was located in the Patterson Function and the light atoms were found in successive Fourier syntheses. The structure was refined by full-matrix least-squares methods with isotropic thermal parameters and then with the block approximation using anisotropic thermal parameters. The present residual (R) is 6.9% and the location of the hydrogen atoms is being carried out.

The atom numbering follows that proposed by Hochstein et al.[1] and also followed by Donohue et al.[3] and Cid-Dresdner[4]. Chemically, the derivatives differ by a water molecule which is removed from the III or C ring, making it aromatic. The overall result is a flattening of the anhydrotetracycline cation relative to the aureomycinium[3] or terramycinium cations.[4] Except for the C ring and the adjacent bonds, the bond lengths in the three molecules are in excellent agreement. The observed increased activity of anhydrotetracycline against actinomycetes appears to be related to either the changes in the C ring or to geometrical changes in the molecule. A comparison of anhydrotetracycline with a number of phenolic compounds[2] suggests that the activity is not related to the more phenolic nature of the C and D rings.

Since epimerization at the dimethylamino group on the I or A ring renders the antibiotics ineffective (less than 5% of the activity of the parent tetracycline[5]), we have examined this ring in more detail. A view down the C(4a)-C(4) bond in Figure 1, similar to Figure 5 of Donohue,[3] illustrates the configuration found in anhydrotetracycline hydrobromide. Several points of interest can be seen in Figure 1. We should point out that the relationship of the dimethylamino group indicates that we do not have epi-anhydrotetracycline. The most startling observation is the nearly staggered configuration about the



Caption for Figure 1.

A view down the C(4) to C(4a) bond in the A ring of anhydrotetracycline hydrobromide.

C(4a)-C(4) bond. The questions are whether the staggered configuration is retained in solution and how does the dimethylamino group influence the biological activity. Recent proton magnetic resonance studies[6] of tetracyclines were interpreted in terms of the eclipsed configuration found in the crystal structure studies on aureomycin and terramycin. In the case of anhydrotetracycline, the proton resonances are shifted in agreement with a different conformation of the dimethylamino group. Therefore, the assumption that the staggered configuration found in our study also exists in solution appears to be reasonable.

A comparison of the distances of N(4)-O(12a) of 2.77 Å and N(4)-O(3) of 3.13 Å in anhydrotetracycline with the distances of N(4)-O(12a) of 3.50 Å and N(4)-O(3) of 2.69 Å found in terramycin offers an interesting explanation for the differences in biological activity. If the tetracycline hydrogen bonds to a receptor site via N(4)-O(3) in

terramycin but with N(4)-O12a) in anhydrotetracycline, then the orientation of the remainder of the molecule relative to the receptor site is drastically altered. Other crystal structure investigations are in progress to provide additional structural evidence concerning the structural requirements for tetracycline activity. A detailed report of the structural data on anhydrotetracycline hydrobromide will be forthcoming.

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