MOLECULAR STRUCTURE OF HELIOMYCIN, AN INHIBITOR OF RNA SYNTHESIS

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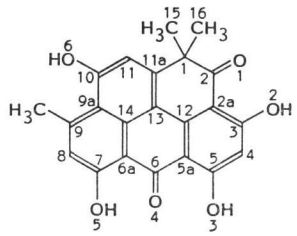
Heliomycin is produced by *Streptomyces variabilis* and was isolated by the extraction of the mycelium with acetone[1]. It possesses bactericidal and vasoconstrictive activity. The antibiotic has proved useful in the treatment of burns or polyderma and also for incorporation into cosmetics. It inhibits RNA and protein synthesis but has no effect on DNA synthesis[2].

There has been confusion regarding the structure and identity of this antibiotic. The handbook of antibiotic compounds[3] gives empirical formula as $C_{22}H_{18}O_6$ and states that it is identical with resistomycin ($C_{22}H_{16}O_6$), a difference of two protons. Another aspect which adds to this confusion is that the resistomycin also goes under the trade name of kanamycin sulfate (a glycosidic antibiotic).

NMR and X-ray studies were initiated to determine the structure and conformation of heliomycin (obtained from Cal Biochem, USA) and to see if it was similar to resistomycin, whose structure was deduced from chemical and spectral data[4~6] and also by X-ray analysis of dibromoresistomycin tetramethyl ester[5]. 200 MHz spectra of heliomycin in *d*-acetone was recorded at room temperature. The chemical shifts for the heliomycin are ^{1}H NMR (200 MHz, acetone-d_6), δ 1.65 (s, 6H, $(CH_3)_2$-C<), 3.05 (s, 3H, CH_3-aromatic), 6.36 (s, 1H, H-4), 7.12 (s, 1H, H-8), 7.41 (s, 1H, H-11). The corresponding chemical shifts for resistomycin[6] (100 MHz, acetone-d_6) are δ 1.66, 2.96, 6.37, 7.13, 7.42. These chemical shifts agree well except for methyl group at C-9, thus indicating similarity of structure between heliomycin and resistomycin (Fig. 1).

Single crystals of the antibiotic were obtained from dioxane in the form of needles. The crystals belong to the monoclinic space group $P2_1/C$ with cell dimensions of a=8.513(2), b=19.047(4), c=11.065(2) Å, β=105.6°(2), Z=4, d_c=1.506 g/cm^3, V=1,728.2 Å^3. A crystal of dimension 0.4×0.2×0.2 mm was used for data collection. The data was collected at −11°C because of the instability of crystals at room temperature. Intensities of 3,569 reflections, $4.0 \geq 2\theta \geq 53.0°$, were measured using MoKα radiation (λ=0.71069 Å) on a Syntax P2 diffractometer equipped with graphite monochromator and a Syntex LT-1 inert-gas (N_2) low temperature delivery system, using the omega scan technique, a variable scan rate (2.0−6.0°), a scan range of 2.0° with a scan to background ratio of 1.0. 1,566 reflections with $I \geq 3\sigma(I)$ were considered observed. The intensities were corrected for Lorentz and polarization effects but no absorption correction was applied (μ=1.15 cm^{-1}).

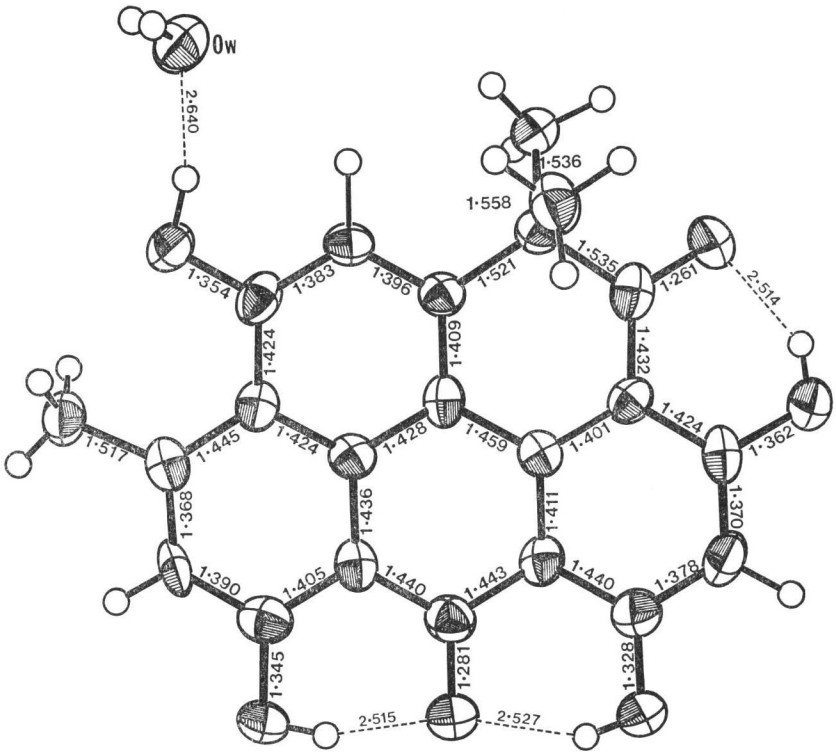
Fig. 1. Chemical formula and numbering system.



The structure was solved by the direct methods program MULTAN[7] with E's≥1.5. The first E map revealed 20 of the 28 non-hydrogen atoms in the asymmetric unit. The rest of the non-hydrogen atoms and water oxygen were located by difference Fourier methods. The structure was refined isotropically to an R factor of 0.117. Further least-squares refinement using anisotropic temperature factors reduced R to 0.098. At this stage all the hydrogens were located from a difference Fourier map and the structure was further refined using anisotropic temperature factors for non-hydrogen atoms and isotropic temperature factors for hydrogen atoms, which reduced R to the final value of 0.0495. The function minimized was $\sum w(Fo-Fc)^2$ and the scattering factors used were those of CROMER *et al*[8].

The fractional coordinates and other data have been sent to the Cambridge Crystallographic Data Centre. Fig. 2 shows the stereochemistry of the molecule and also the bond lengths and hydrogen bond distances. The standard deviations in the bond lengths are of the order of 0.006 Å. As expected, the molecule is planar except the ring with the *gem* dimethyl group. The torsion angles around this ring starting with

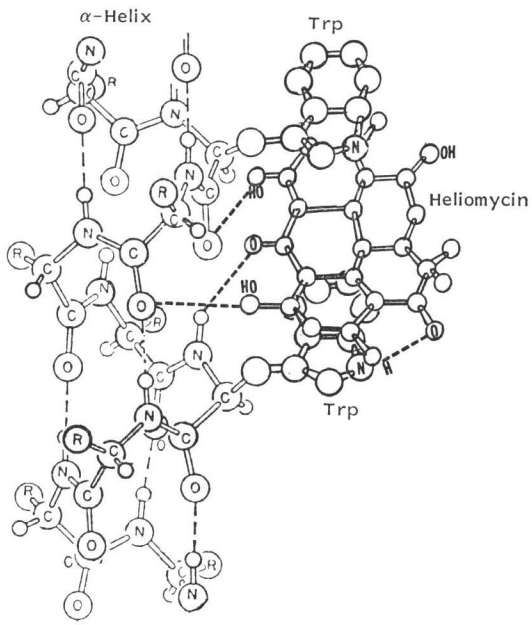
Fig. 2. Thermal ellipsoid plot of the molecule with bond lengths and intramolecular hydrogen bonding.



bond C(1)–C(2) and going clockwise are −22.3, 12.1, 2.7, −5.6, −6.8 and 19.8° respectively, thus indicating that conformation of this ring is half chair[9]. All the oxygen atoms in the molecule are involved in hydrogen bonding. The intramolecular hydrogen bonding (as shown in Fig. 2) involves O(1) and O(4) as proton acceptors while O(2), O(3) and O(5) are donors. The O(6) donates its proton to the water oxygen, which in turn is involved in intermolecular hydrogen bonding with O(1) (2.847 Å; 1+x, 1/2−y, 1/2+z). The molecules in the unit cell are packed parallel to the *ac* diagonal with partial stacking.

The mechanism of the action of heliomycin (resistomycin) is hardly understood. The only information available shows that it inhibits RNA and protein synthesis but not DNA synthesis. Studies by KOROLEV *et al.*[10,11] have shown that heliomycin inhibits the activity of the enzyme DNA-dependent RNA polymerase, thus inhibiting the synthesis of RNA. The forces most probably involved in the possible formation of the antibiotic-enzyme complex are (a) stacking of antibiotic between aromatic amino acids of α-helix and (b) hydrogen bonding between hy-

Fig. 3. Possible mode of interaction of heliomycin with the α-helix of enzyme.



droxyl and carbonyl groups on the antibiotic and various proton donor and acceptor atoms available on the α-helix of the enzyme. Fig. 3 depicts this possible model.

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