04-Crystallography of Biological Small Molecules

PS-04.01.18 STRUCTURE AND CONFORMATION STUDIES OF CRYSTALLINE ANTIBIOTICS: CRYSTAL STRUCTURE AND ABSOLUTE CONFIGURATION OF KANAMYCIN. A. Yoram, A. Puius*, Todd Stievater and T. Srikrishnan, Center for Crystallographic Research, Roswell Park Cancer Institute, Buffalo, NY 14263, USA.

Kanamycin, an antibiotic complex produced by Streptomyces Kanamyceticus from the Japanese soil, was isolated by Okami, Umezawa and their coworkers as early as 1957. The Kanamycin antibiotic family consists of three components, namely Kanamycin A (the major component), Kanamycin B and C. The disulfates of Kanamycin A [4-O-(6-amino-6-deoxy-α-D-glucopyranosyl)-6-O-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine inhibits the growth of many kinds of bacteria and is widely used in the treatment of gonorrhea, salmonella, tuberculosis and many other diseases. Kanamycin A consists of a central deoxystreptamine ring linked to two glucopyranosyl rings at the 4 and 6 positions.

The absolute configuration of Kanamycin A is very important in the understanding of its mode of action. An earlier study of the absolute configuration was carried out using chemical methods. As part of our ongoing project on accurate structural investigations of crystalline oligosaccharides of biological importance, we carried out a structural analysis of Kanamycin A monosulfate monohydrate using CAD-4 diffractometer data. These crystals obtained from water are triclinic, space group P1 with a = 7.2294 (1), b = 12.4922 (15), c = 7.1168 (9) Å, α = 94.74 (1), β = 89.16 (1), γ = 91.59 (1)°, V = 640.2 (2) Å^3, μ(cuk α) = 18.4 cm^{-1}, F.W. 600.6, D_{calc} = 1.558 g/cm^3. The structure was obtained using SHELXS-86 and refined by full matrix least squares to a final R value of 0.042. Both the D-glucose moieties are attached to the deoxystreptamine ring by α-linkages. This absolute configuration confirms the earlier determination using photographic data. The (Φ, ψ) values for the glycosidic linkages are +101.9, -121.2, -131.5 and -140.4 respectively.

Most aminoglycoside antibiotics including streptomycin and kanamycin, not only inhibit protein synthesis but also cause misreadings of the genetic code. It interacts with the ribosomal S12 protein to stabilize codon-anticodon binding between mRNA and aminoacyl tRNA and inhibits the elongation of the peptide chains through a series of reactions resulting in prevention of ribosomes from moving along mRNA. The specific details at to which step of ribosome cycle, Kanamycin blocks the protein synthesis are not yet fully understood. Our attempts to grow crystals of Kanamycin B and C are in progress.

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Kanamycin Antibiotics (sulfates)

Kanamycin A:
R = NH_2; R' = OH

Kanamycin B:
R = R' = NH_2

Kanamycin C:
R = OH; R' = NH_2

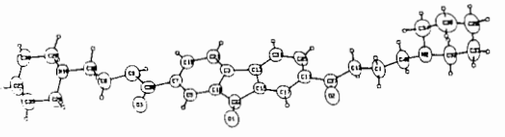
PS-04.01.19 STRUCTURAL AND FUNCTIONAL STUDIES OF TILORONE, AN INTERFERON INDUCER. CRYSTAL STRUCTURE AND CONFORMATION OF 2,7-BIS-[PIPERIDINOPROPOXY]-9H-FLUOREN-9-ONE. T. Srikrishnan+*, Kanthi, B. Dasari+ and Boris Albini++, Center for Crystallographic Research+, Roswell Park Cancer Institute, Buffalo, NY 14263 and Departments of Microbiology and Medicine++, State University of New York at Buffalo, School of Medicine, Buffalo, NY 14214, USA.

Tilorone is an orally active pharmacological agent that protects mice against infections with RNA and DNA viruses. It is also the only known small molecule that is an interferon inducer. It has anti-tumor and anti-inflammatory properties and was shown to interact and influence the immune mechanism in humans and animals. Tilorone has been postulated to act by intercalating preferentially between A-T base pairs (Chen, Gresh & Pullman, Nucl. Acid Res. 16, 3061, 1988). Twenty-eight different analogs of Tilorone have been synthesized by changing the substituents at the 2,7, positions of the central chromophore, fluoren-9-one and have been tested for their antiviral activity (Andrews et al J. Med. Chem. 17, 882, 1974).

Structural and functional studies of these compounds have been undertaken in our laboratories with an objective to correlate the structural features with the immunosuppressive activity. Crystals of the title compound are triclinic, space group P1, with a = 7.068 (2), b = 9.030 (3), c = 23.635 (3) Å, α = 103.91 (2), β = 108.49 (2), γ = 93.84 (4)°, V = 1371.9 (4) Å^3, D_o = 1.17 g/c.c.; D_c = 1.168 g/c.c. and Z = 2. The structure was solved with CAD-4 data (2450 reflections, 1873 ≥ 3σ), using SHELX-86 program and refined to a final R value of 0.063. The propoxy group is in an extended zig-zag conformation and is coplanar with the central fluorenone chromophore. The two terminal piperidine rings are in the preferred chair conformation. The crystal structure lends support to the postulated intercalative mechanism of action for Tilorone. Attempts are being made to crystallize the other Tilorone analogs as well as a complex of Tilorone with an oligonucleotide fragment, which could afford a detailed investigation of the intercalation mechanism.

We hypothesize that the stereochemistry and the molecular geometry of the drugs and/or their active metabolite affect both the receptor interaction and the mode of immunomodulation. Lymphocyte proliferation studies were conducted on mice (Cunningham et al, Immunobiol. 184, 53, 91). The drugs were studied in vitro to assess their effect on immunological function of murine lymphocytes, focusing on cell proliferation assays and on the response to IL-2 and expression of IL-2 receptors. Our preliminary studies showed that Tilorone does modulate the IL-2 response in high responder strains (e.g. AKM) we well as in low responder (e.g. C3HA); this effect seems to be dose dependent. Comparison of the biological effects of the various Tilorone analogs should allow us to obtain some insight into one effect of structure-function relationships.

Work supported by the New York State Department of Health.



PS-04.01.20 CONFORMATION OF 4-SUBSTITUTED 2,6-BIS(1-PYRROLIDINYLMETHYL)PHENOL. ANTIARRHYTHMICS IN DIFFERENT PROTONATION STATE. M.L. Główka, Technical University, Łódź, Poland; P.W. Codding, University of Calgary, Canada

Class I antiarrhthymics bind specifically to sodium channels and thus interfere with interconversions of channels to different activity states. Most of them are comprised of flexible chains connecting the key functional groups. The flexibility makes prediction of the specific molecular shape required by the receptor binding site difficult. We have studied a more rigid series of antiarrhythmics based on the 4-substituted 2,6-bis(1-pyrrolidinylmethyl)phenols developed in 1980s by Stout and coworkers (*J. Med. Chem.* 32, 1910 and references therein). Our studies on free bases and mono-

