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

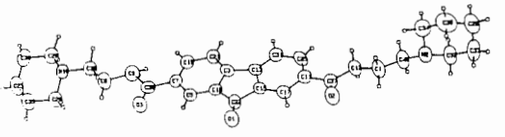
PS-04.01.19 STRUCTURAL AND FUNCTIONAL STUDIES OF TILORONE, AN INTERFERON INDUCER. CRYSTAL STRUCTURE AND CONFORMATION OF 2,7-BIS-[PIPERIDINOPROPOXY]-9H-FLUOREN-9-ONE. T. Srikrishnan+*, Kanthi, B. Dasari+ and Boris Albini++, Center for Crystallographic Research+, Roswell Park Cancer Institute, Buffalo, 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.

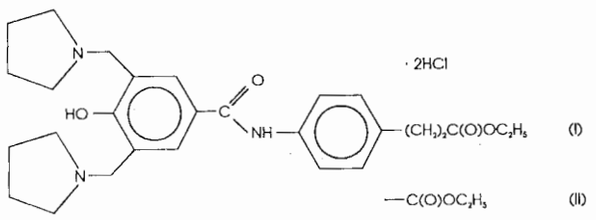


PS-04.01.20 CONFORMATION OF 4-SUBSTITUTED 2,6-BIS(1-PYRROLIDINYLMETHYL)PHENOL ANTIARRHYTHMICS IN DIFFERENT PROTONATION STATE. M.L.Główka , Technical University, Łódź, Poland; P.W. Codding, University of Calgary, Canada

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-

protonated compounds of that type (*J.Med.Chem.* **34**, 2678; *Acta Cryst.* C47, 1038) showed that protonation of the pyrrolidine ring N atom is a key determinant of conformation; hence, we have determined the structures of two diprotonated derivatives to complete the study.

Crystal data: (I) H_2O, 0.5 CH_3OH, C2/c, Z=8, a=20.301, b=11.296, c=24.901Å, β=93.19°, R=0.071. (II) $P2_1/c$, Z=4, a=12.903, b=16.430, c=13.731Å, β=103.64°, R=0.057.

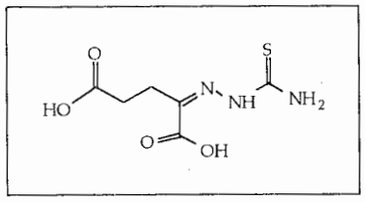


PS-04.01.21 **A STRUCTURAL INVESTIGATION ON THE α-KETOGLUTARIC ACID THIOSEMICARBAZONE IN METAL COMPLEXES** By M. Belicchi Ferrari, G. Gasparri Fava, C. Pelizzi, P. Tarasconi and G. Pelosi, Istituto di Chimica Generale ed Inorganica, Centro di Studio per la Strutturistica Diffrattometrica del CNR, Viale delle Scienze, 43100 Parma, Italy

Thiosemicarbazones present biological and chemical properties which cover a broad spectrum of potentially useful chemotherapeutic properties (antitumoral, antibacterial, as well as antiviral) (S.Padhyè & G.B.Kauffman, Coord.Chem.Rev. ,1985, 63, 127-160).

Our research aims at the understanding of the correlation between the structural features and the biological properties of this class of compounds under different experimental conditions. Many pyruvic acid and pyridoxal thiosemicarbazone complexes have already been synthesised and characterised. Some of them containing copper or cobalt, tested in vitro, have shown an inductive effect on FLC (Friend erythroleukemia cells) erythroid differentiation and a suppressive efficacy on their proliferation (M.Belicchi Ferrari, G.Gasparri Fava, P.Tarasconi, R.Albertini, S.Pinelli & R.Starcich, J.Inorg. Biochem., 1993, accepted for publication).

At present we are studying the behaviour of the α-ketoglutaric acid thiosemicarbazone, a ligand which presents many potential donor



atoms and a variety of potential conformations, in complexes with different co-ordinating ions such as zinc, copper and nickel. All of the cited compounds have been synthesised and characterised spectroscopically and their crystal structures have been determined by X-ray analysis.

PS-04.01.22 **CRYSTAL STRUCTURE OF A QUINOLONE DERIVATIVE WITH ANTIMALARIAL ACTIVITY**. By **Mario V. Capparelli*** (Fac. Ciencias, Univ. Central de Venezuela, Caracas 1050, Venezuela), **Jaime E. Charris** (Fac. Farmacia, Univ. Central de Venezuela, Caracas 1050, Venezuela) and **Duilio Cascio** (Molec. Biology. Inst., UCLA. Los Angeles, CA 90024, USA)

According to the World Health Organization malaria is still the chief cause of human death, aside from natural causes. The antimalarial drugs currently in use, mainly aminoquinolines, pyrimidines, sulfonamides and sulfones, are being rendered increasingly ineffective by the appearance of resistant strains of *Plasmodium*.
A number of new N-methylated quinolones were synthesized as a part of a program to develop more active and less toxic new antimalarial drugs. These compounds were tested *in vitro* for antimalarial activity and seven of them were found to be between 100% and 75% effective in inhibiting the growing of *P. falciparum*.
The crystal structure of one of the active compounds, *viz.* 2,4-diamino-10-methyl-9-methoxypyrimido[4,5-b]5-(10H)-quinolone (activity 89%), was determined. The tricyclic molecule is essentially planar, with both methyl groups out of the plane. Bond lengths and angles are within normal values; the C=O distance, 1.263(2) Å, indicates a weakened double bond. The molecules are stacked along [101], with N-H...O and N-H...N bonds connecting molecules in adjacent piles.

Crystal Data: $C_{13}H_{13}N_5O_2$, M_r = 271.28, monoclinic, $P2_1/n$, a = 10.664(2), b = 10.111(2), c = 11.318(2) Å, β = 99.351(4)°, D_x = 1.50 gcm^{-3}, Z = 4, F(000) = 568, μ(Mo-Kα) = 1.00 cm^{-1}. The structure was solved by direct methods and refined to R = 0.049 for 1941 unique reflections with I > 3 σ(I).

PS-04.01.23 X-RAY CRYSTAL STRUCTURAL ANALYSIS OF TWO NEW POTENTIAL ACTIVE ANTIMALARIAL DRUGS. D. Gómez de Andérez[1], R. Avila[1], J. Domínguez[2], and S. Khan[3]. [1]Universidad de Los Andes, Facultad de Ciencias, Mérida-Venezuela, [2]Universidad Central de Venezuela, Facultad de Farmacia, Caracas-Venezuela, [3]University of California, Chemistry Dept., Los Angeles, CA-USA.

Malaria is distributed widely throughout populated areas where the average temperature is 60°F or higher and some extends to more temperate climates. Because of the acute toxicity of some antimalarial drugs, there is interest in testing new compounds against this disease. Methylthio-4-ciano-5-pyrazolone ($C_5H_5N_3OS$) and 3-amino-5-(2-methoxyanilino)-4-metoxy-carbonylpyrazol ($C_{12}H_{14}N_4O_3$) are compounds with potential antimalarial activity.
X-Ray single crystal analysis shows: (a) C_5H_5OS is monoclinic, $P2_1/c$, a=4.514(1), b=13.536(1), c=11.455(1)A and B=92.82(1), Mo K=0, 7107A. F(000)=320, 2349 unique reflections measured, 1167 with I>3σ(I). The structure was solved by direct methods, the final anisotropic refinement using full matrix least-square gave R=0.047, Rw=, GOF=1.77. (b) $C_{12}H_{14}N_4O_5$ is monoclinic $P2_1/n$, a=13.108(3), b=7.739(2), c=13.224(3)A, B=110.65(1) MoKα=0.7107, F(000)=552. 4053 unique reflections measured, 1890 with I>3σ(I). The final anisotropic refinement gave R=0.049, Rw=0.060, GOF=1.683.

PS-04.01.24 **BETICOLINS, A NEW CLASS OF TOXINS PRODUCED BY CERCOSPORA BETICOLA, A PARASITIC STRAIN OF SUGAR BEETS.** By A. Neuman[1], T. Prangé[1], M.L. Millat[3], J. Einhorn[2], J.P. Blein[3]. . [1]Chimie Biomoléculaire (URA 1430 CNRS) UFR-Biomédicale, 93012-BOBIGNY CEDEX, France ; [2] Laboratoire de Phytopharmacie, I.N.R.A., F-78026 VERSAILLES CEDEX, France ; [3] Laboratoire de Phytopharmacie, I.N.R.A., BV 1540, 21034-DIJON CEDEX, France

Cercospora Beticola is a parasitic fungus responsible for leaf spot disease on sugar beet. The pathogen agent produces, in addition to the red cercosporin, several yellow toxic metabolites with a new