Structure of a Copper-Isoniazid Complex

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It is well-known that complex formation with copper ions increases the in vitro mycobactericidal action of the antituberculosis agent isoniazid. We report here the preparation and structure of a copper(II)-isoniazid complex. Unit cell parameters are $a = 9.575$, $b = 14.855$, and $c = 7.056$ Å and space group $P2_12_12_1$. Copper bonding geometry is square planar with the isoniazid carbonyl oxygen and hydrazide amino nitrogen atoms and two chlorines occupying coordination positions. Complexing with copper(II) does not significantly alter the isoniazid molecular conformation.

Isoniazid (isonicotinic acid hydrazide, INH) is an extremely important antituberculosis drug whose rediscovery in the early 1950's resulted in a virtual revolution in the treatment of that disease. The specific inhibitory action of INH on *Mycobacterium tuberculosis* has led to numerous studies of its mechanism of action; although these investigations have uncovered a host of biochemical effects on susceptible mycobacteria,[1] the primary mode of action of INH still remains unclear.

It has been known for many years that copper ions enhance the in vitro activity of INH against *M. tuberculosis*,[2] prompting speculation that a copper-INH complex may be an important entity in the mycobacterial action.[3] Chemical and spectroscopic studies have verified the existence of copper-INH complexes, and several different complexes have been proposed involving Cu(I) or Cu(II) in 1:1 or 1:2 association with neutral or anionic INH. Thus, Krivis and Rabb concluded[4] that Cu(II) is reduced by INH to Cu(I) and that the bactericidal complex is Cu(I)-neutral INH, whereas Rieber and Bemski found[5] no significant reduction of cupric ion and postulated a 1:1 or 1:2 Cu(II)-INH complex as the important species. The 1:1 Cu(II)-INH was further shown to cause nearly immediate cell lysis of a streptomycin-resistant strain of mycobacteria.[5]

Despite the widespread interest in the identity of the antibacterial copper-INH product, no stable complex has been chemically or structurally characterized. We have prepared mixtures of copper ions and isoniazid and have crystallized a Cu(II)-INH complex. We report here the results of the structure determination of this complex.

Crystallographic Data. A 1:1 aqueous solution of cuprous chloride and INH resulted in a brown precipitate [presumably Cu(I)-INH], which was dissolved in 1 N HCl.[4] Upon standing, the solution turned green, and green crystals, subsequently determined to be of Cu(II)-INH-Cl_2·HCl, formed upon solvent evaporation. The crystal unit cell is orthorhombic, with dimensions $a = 9.575$ (2), $b = 14.855$ (3), and $c = 7.056$ (1) Å and space group $P2_12_12_1$, and contains four formula units of the complex salt (one per asymmetric unit). X-ray diffraction intensity data were collected on an automated diffractometer (Nb-filtered Mo Kα radiation) from a crystal $0.1 \times 0.1 \times 0.26$ in size and corrected for coincidence loss and absorption, and structure factors were derived. A total of 1461 reflections were measured to a resolution of 0.77 Å, of which 1378 had intensity greater than twice their standard deviations and were classified as observed. The structure was obtained by interpretation of the Patterson function and successive difference electron-density maps. Refinement was by full-matrix least squares with the quantity minimized $\sum w(|F_o| - |F_c|)^2$, where $w = 1/\sigma_F^2$. Hydrogen atoms were located in difference electron-density maps and were refined with isotropic thermal parameters; final discrepancy index, $R = 0.033$. Atomic coordinates and thermal parameters are listed in Table I. A table of observed and calculated structure factors is available.[6]

Results

The results of the crystallographic structure determination are shown in Figures 1 and 2. The coordination of the copper ion is roughly square planar, as is usual for Cu(II) compounds; two coordination positions are occupied by the carbonyl oxygen and hydrazide amino nitrogen atoms of the INH molecule, and the other two by chlorines. The amino nitrogen atom is bonded to two hydrogens and is neutral rather than anionic. The four ligands lie alternately approximately 0.1 Å above and below the best plane through them, with the Cu(II) ion 0.2 Å from this plane (on the same side as N3 and Cl1). The INH molecule consists of two planar portions: the pyridine ring and the C-C(=O)-N-N portion. These two are almost coplanar: the angle between normals to them is 4.4°, and the C3-C4-C1-N2 torsion angle is 178°. The Cu(II) ion is situated 0.4 Å out of the latter plane.

Bond distances and angles in INH are normal for this type of compound and are remarkably similar to the values in neutral uncomplexed INH.[7] Protonation, in forming the hydrochloride, has taken place at N1; the increased value (+7°) of the angle at N1 compared to the neutral INH is similar to that observed in other pyridinium compounds. Nearly all bond lengths in the INH moiety of Cu(II)-INH-Cl_2·HCl are within 0.01 Å of corresponding distances in neutral INH.

(1) For a review of INH biochemistry, see Krishna Murti, C. R. *Antibiotics* **1975**, *3*, 623-651.
(2) Sorkin, E.; Roth, W.; Erlenmeyer, H. *Helv. Chim. Acta* **1952**, *35*, 1736-1741.
(3) Albert, A. *Experientia* **1953**, *9*, 370.
(4) Krivis, A. F.; Rabb, J. M. *Science* **1969**, *164*, 1064-1065.
(5) Rieber, M.; Bemski, G. *Arch. Biochem. Biophys.* **1969**, *131*, 655-658.
(6) See paragraph at end of paper regarding supplementary material.
(7) Jensen, L. H. *J. Am. Chem. Soc.* **1954**, *76*, 4663-4667.

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Table I. Fractional Atomic Coordinates and Anisotropic Thermal Parameters ($\times 10^2$)[a]

atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
Cu	0.18065 (5)	0.08309 (3)	0.03119 (7)	1.97 (2)	2.01 (2)	1.64 (2)	−0.31 (2)	−0.34 (2)	0.31 (2)
Cl(1)	0.34448 (11)	0.16322 (7)	0.18151 (14)	2.37 (5)	2.41 (5)	1.99 (5)	−0.58 (4)	−0.35 (4)	0.01 (4)
Cl(2)	0.14271 (11)	−0.01380 (7)	0.27233 (16)	2.38 (6)	2.80 (5)	2.63 (5)	−0.43 (4)	−0.39 (5)	1.17 (5)
Cl(3)	0.22001 (11)	0.44141 (7)	0.93833 (17)	2.25 (5)	3.00 (6)	2.84 (6)	0.14 (4)	−0.23 (5)	0.69 (5)
N(1)	0.0378 (4)	0.3040 (3)	−0.7971 (6)	3.5 (2)	2.3 (2)	1.6 (2)	0.7 (2)	0.4 (2)	0.6 (2)
N(2)	0.0020 (4)	0.0726 (3)	−0.2838 (5)	2.5 (2)	2.5 (2)	1.8 (2)	−0.7 (2)	−0.6 (2)	0.4 (2)
N(3)	0.0253 (4)	0.0234 (3)	−0.1150 (6)	2.2 (2)	2.1 (2)	1.9 (2)	−0.1 (2)	−0.1 (2)	0.3 (2)
O(1)	0.1426 (3)	0.1798 (2)	−0.1659 (4)	2.7 (2)	2.0 (1)	2.1 (2)	−0.3 (1)	−1.0 (1)	0.2 (1)
C(1)	0.0656 (4)	0.1520 (3)	−0.2964 (6)	1.7 (2)	1.8 (2)	1.9 (2)	0.2 (2)	0.4 (2)	−0.3 (2)
C(2)	0.1150 (5)	0.3348 (3)	−0.6521 (7)	2.9 (2)	2.0 (2)	3.7 (3)	0.2 (2)	0.3 (2)	0.2 (2)
C(3)	0.1189 (4)	0.2873 (3)	−0.4870 (6)	2.2 (2)	2.4 (2)	2.1 (2)	0.0 (2)	−0.1 (2)	0.4 (2)
C(4)	0.0478 (4)	0.2049 (3)	−0.4742 (6)	1.7 (2)	1.9 (2)	1.9 (2)	0.3 (2)	0.3 (2)	0.2 (2)
C(5)	−0.0320 (5)	0.1763 (3)	−0.6273 (7)	2.0 (2)	2.3 (2)	2.6 (2)	0.1 (2)	−0.3 (2)	0.0 (2)
C(6)	−0.0367 (5)	0.2280 (3)	−0.7887 (7)	3.0 (2)	2.9 (2)	2.0 (2)	0.3 (2)	−0.6 (2)	−0.1 (2)

atom	x	y	z	B	atom	x	y	z	B
H(1)	−0.058 (7)	0.023 (4)	−0.055 (9)	6.0 (2)	H(5)	0.163 (5)	0.308 (3)	−0.370 (6)	2.0 (1)
H(2)	0.063 (8)	−0.017 (5)	−0.156 (10)	3.0 (3)	H(6)	0.163 (5)	0.395 (3)	−0.683 (7)	3.0 (1)
H(3)	−0.051 (6)	0.045 (3)	−0.364 (8)	3.0 (2)	H(7)	0.918 (4)	0.122 (3)	−0.639 (6)	2.0 (1)
H(4)	0.056 (6)	0.333 (4)	−0.893 (8)	5.0 (2)	H(8)	0.908 (5)	0.209 (3)	−0.881 (7)	3.0 (1)

[a] Standard deviations are given in parentheses.

Table II. Short Intermolecular Contacts in Cu(II)–INH·HCl

atom A	atom B	distance, Å	Cl···H–N angle, deg	symmetry position B
Cl(1)	H(N3)	2.61	171.2	$^1/_2 - x, -y, ^1/_2 + z$
Cl(3)	H(N1)	2.54	148.1	$x, y, 2 + z$
Cl(3)	H(N2)	2.29	152.0	$-x, ^1/_2 + y, ^1/_2 - z$
Cl(3)	H(N3)	2.34	165.0	$^1/_2 + x, ^1/_2 - y, 1 - z$

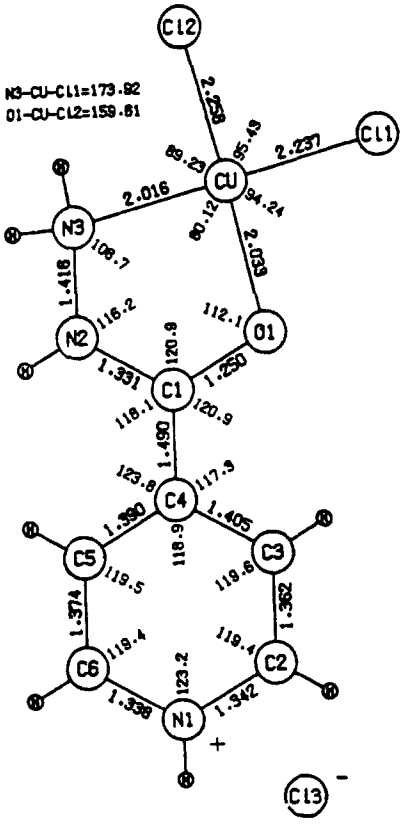

Figure 1. Atomic numbering and bond distances and angles in Cu(II)–INH-Cl_2·HCl.

The conformations of neutral INH and INH·HCl–copper(II) complex, as found in their respective crystal structures, are also virtually identical. Figure 2 shows a stereoscopic view of the two structures maximally superposed: the only difference is that the ring and the C–C(=O)–N–N planes are slightly more nearly coplanar in the copper complex (4.4° between plane normals) than in INH (16.1°). Formation of the Cu(II) complex has, thus, not resulted in any significant alteration of the native INH conformation.

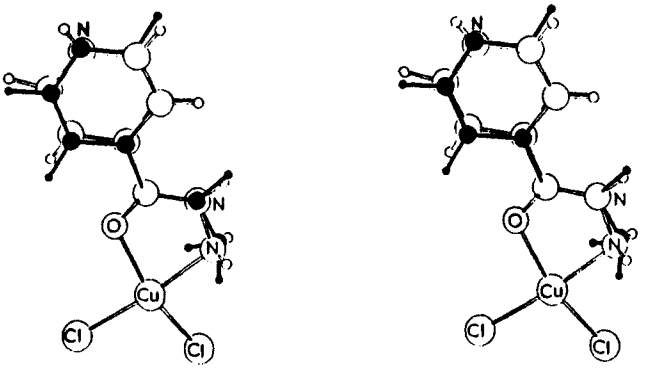

Figure 2. Stereoscopic diagram of superposed conformational structures of Cu(II)–INH-Cl_2·HCl (open circles and bonds) and neutral uncomplexed isoniazid[7] (blackened circles and bonds). Carbon and hydrogen atoms are unlettered.

All four hydrogens attached to the nitrogen atoms of INH form short intermolecular contacts with symmetry-related ions and atoms. Hydrogens on N1, N2, and N3 all interact with the chloride ion, Cl3, while the second hydrogen on N3 interacts with Cl1. Distances and angles describing intermolecular contacts shorter than 2.8 Å are listed in Table II.

The relationships between the diverse biochemical effects of isoniazid and its lethal action of *Mycobacterium tuberculosis* remain unclear. A satisfactory explanation of the enhanced activity of the copper–INH complex must await elucidation of the molecular basis of INH mycobactericidal action. The present structural investigation has established (1) the stability and ease of formation under aerobic conditions of the Cu(II)–INH complex, (2) that the complex consists of Cu(II) coordinated to neutral INH nitrogen and oxygen atoms, and (3) that whatever the

nature of the copper enhancement of INH action, it is not due to a conformational change induced in INH upon formation of the Cu(II) complex.

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Supplementary Material Available: A listing of observed and calculated structure factors (2 pages). Ordering information in given on any current masthead page.

Book Reviews

Progress in Pharmacology. Volume 3. Number 1. Structure–Activity Relationships in Clonidine-Like Imidazolidines and Related Compounds. By P. B. M. W. M. Timmermans, W. Hoefke, H. Stahle, and P. A. van Zwieten. Gustav Fisher Verlag, Stuttgart and New York. 1980. vi + 104 pp. 17 × 24 cm. $42.50.

The monograph "Structure–Activity Relationships in Clonidine-Like Imidazolidines and Related Compounds" is a logical continuation of the first book on this subject published by Van Zwieten in 1975. This new book, Volume 3, is considerably enlarged over the previous contribution, mainly due to the results taken form the Ph.D. thesis of Timmermans and those of other experts such as Hoefke, Stähle, and Van Zwieten. This volume is organized into six parts: a brief introduction and five major sections which are concerned with the chemical (Chapter 2) and physical (Chapter 3) properties of clonidine-like derivatives, SAR (Chapter 4), followed by a comparison between various pharmacological actions (Chapter 5), and finally by QSAR (Chapter 6) using the Hansch method. This volume contains 62 figures and 39 tables with numerous pharmacological and chemical data (e.g., pD_2, ED_{50}, α_1 and α_2 actions, log P, pK_a, etc.).

The over 200 literature references given in alphabetical order and followed by a subject index are also useful. Unfortunately, the references go only up to 1978 with the exception of a few author's references which are from 1979. It is regrettable that the paragraph devoted to the site of action of clonidine is omitted. Also, it has been recently shown that in the nucleus reticularis lateralis, clonidine inhibited excitatory neurons. The authors, on the contrary, state that the hypotensive action of clonidine is accounted for by an "increased activity of hypothetical inhibitory neurones" (pp 2, 1, 35). With regard to the paragraph devoted to the nature of α-adrenoceptor (p 85), I hope that the reader will understand that the work of Timmermans et al. in 1977 confirmed previous results from several researchers. Nevertheless, this volume can be considered essential for anyone working or undertaking research in adrenergic drugs. Researchers not directly involved in that field might find it interesting because this monograph gathers contributions from physical and medicinal chemists, biochemists, and pharmacologists. The price of $42.50 for this volume containing 104 pages might be discouraging.

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Specific GABA Receptor Agonists and Uptake Inhibitors: Design, Development and Structure–Activity Studies. By Povl Krogsgaard-Larsen. Login Brothers, Chicago. 1980. 198 pp. 15.5 × 22 cm. $12.50.

Professor Krogsgaard-Larsen is a major contributor to the medicinal chemistry of γ-aminobutyric acid (GABA), an inhibitory neurotransmitter. This monograph presents a clearly written account of his research in the period 1972–1979 which has resulted in the discovery of many selective GABA agonists and GABA uptake inhibitors and has advanced our knowledge of the structure–activity relationships of these agents. Many of the compounds reported are widely used by biologists to study GABA function, including the interaction of GABA with other neurotransmitters and with benzodiazepines. The GABA agonist, 4,5,6,7-tetrahydro[5,4-c]pyridin-3-ol (THIP), synthesized during the course of this research, is a potent analgesic now undergoing clinical trials. The monograph, Professor Krogsgaard-Larsen's thesis for the Doctor of Pharmacy from the Royal Danish School of Pharmacy, is recommended to workers in the field as offering a perspective not available in previous publications and to students of medicinal chemistry as an account of excellent and successful research in the emerging field of amino acid neurotransmitters. Its utility is hampered somewhat by the lack of an index, but the table of contents is quite detailed.

Chapter I, "Introduction", briefly reviews the evidence that GABA is an inhibitory neurotransmitter. Since decreased GABA function is implicated in the pathogenesis of certain diseases (Chapter II, "GABA Dysfunctions and Neurological and Psychiatric Disorders"), Chapter III, "Pharmacological Interventions in the GABA System", reviews the potential for increasing GABA neurotransmission with inhibitors of GABA metabolism, inhibitors of GABA uptake, and GABA agonists. Chapter IV, "Biochemical and Pharmacological Evaluation of Gabaergic Compounds", describes the methods used to assess GABAergic activity and selectivity. Chapter V, "Specific GABA Receptor Agonists: Design and Development", and Chapter VI, "Specific Inhibitors of GABA Uptake: Design and Development", present the rationale for selecting muscimol as the starting point for the research and detail the structural changes, results, and hypotheses that led to the discovery of the title compounds. Chapter VII, "Neuropharmacology of GABA Receptor Agonists", and Chapter VIII, "Neuropharmacology of GABA Uptake Inhibitors", review the metabolism, distribution, potential prodrugs, and selected pharmacology of the title compounds. Chapter IX, "Syntheses of Heterocyclic GABA Analogues", outlines the synthesis of compounds discussed in preceding chapters. Chapter X is a "Summary in Danish".

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Chemotherapy of Cancer. Second Edition. By Stephen K. Carter, Marie T. Bakowski, and Kurt Hellmann. Wiley, New York. 1981. 379 pp. 13.5 × 21 cm. $18.50.

The revised edition of *Chemotherapy of Cancer* appears 4 years after the first edition and reflects the considerable changes that have taken place in clinical cancer chemotherapy. This book has a strong clinical orientation and is notable for its lack of chemical structures. As with the first edition, the authors have divided the book into four sections, each containing two to four clearly written and compact chapters. The emphasis on conciseness, however, leads to an inability to discuss some topics in detail; for example, the mechanism of drug action, pharmacokinetics, and drug metabolism are poorly developed themes. Nevertheless, the unusually straightforward and uncomplicated style makes this book ideally suited for medical and graduate students, postdoctoral fellows, residents, and researchers and clinicians who have recently become involved in cancer research or therapy.

The introductory section presents the general stratagies and concepts of cancer chemotherapy and the current steps involved in clinical testing of new antineoplastic agents. Although less than