reaction is suppressed at the isoxazole stage due to the failure of I to absorb this light. If this latter alternative were correct then the oxazole III should be formed more rapidly by irradiation of the isoxazole I than by irradiation of the azirine II with 2537-A light. Since the contrary is true (the oxazole III is initially formed over eight times as efficiently from the azirine) III cannot be formed to a significant extent by direct rearrangement of the isoxazole I. Accordingly, *the azirine III is truly an intermediate in the phototransposition reaction, and its photochemical reactivity is dramatically sensitive to different wavelengths of light; the oxazole III is formed with 2537-A light while the isoxazole I is formed with >3000-A light.* Although it was not possible to determine accurately the degree of specificity of these reactions, the fact that perfect isosbestic points were not obtained suggests that the two reactions of II are not quite mutually exclusive. Thus, the spectra suggest that as much as 4% of the isoxazole may temporarily accumulate during 2537-A irradiation of II, while temporary buildup of about 2% of the oxazole III was observed during >3000-A irradiation of II.

We believe this wavelength dependence is probably caused by selective excitation of one of the two isolated chromophores in the azirine II followed by a molecular rearrangement which proceeds more rapidly than energy transfer between the two chromophores. Excitation of the benzoyl chromophore, which should contribute most heavily to the long wavelength tail due to its $n \rightarrow \pi^*$ transition, should occur quite selectively with >3000-A light. Thus, excitation of this grouping is probably responsible for the rearrangement to the isoxazole I. On the other hand, both chromophores should absorb the 2537-A light, and thus it is probably excitation of the ketimine chromophore which leads to the formation of the oxazole III.

A decision concerning the generality of this mechanism in phototransposition reactions of other five-membered ring heterocycles must await further study.

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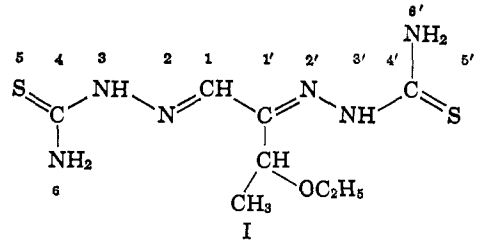
The Crystal Structures of Compounds with Antitumor Activity. 2-Keto-3-ethoxybutyraldehyde Bis(thiosemicarbazone) and Its Cupric Complex[1]

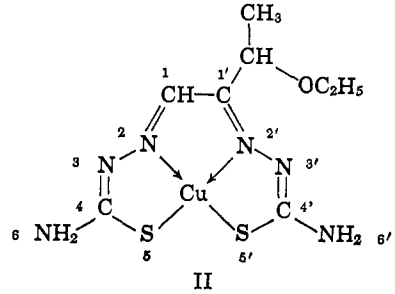
Sir:

2-Keto-3-ethoxybutyraldehyde bis(thiosemicarbazone) (I) has been shown to be effective as an antitumor agent in certain animals[2] and is currently being tested clinically. Its activity is dependent on the presence of certain metal ions in the diet,[3] and in *in vitro* test systems the cupric complex II has been found to be the most active form.[4]

The crystal structures of I and II have been determined by X-ray diffraction methods[5] in order to obtain information about any electronic and sterochemical differences between them that could account for the increased activity of II.



I



II

I crystallizes in the monoclinic space group $P2_1/c$ with $a = 20.846$, $b = 13.809$, $c = 9.557$ A, $\beta = 95° 30'$, and $Z = 8$. There are two molecules of $C_8H_{16}N_6OS_2$ per asymmetric unit. The intensities of 6018 unique reflections from a crystal ground as a sphere were measured on a G.E. XRD-5 diffractometer, using nickel-filtered Cu Kα radiation. Of these reflections 3831 were significantly above the background intensity. Absorption corrections were applied. The structure was solved by an application of the symbolic addition procedure.[6] Refinement by differential syntheses and least-squares techniques using the observed data has reduced R to 0.052. All 32 hydrogen atoms were located in a difference map at $R = 0.076$ and they were included in the calculations from that point, but their parameters have not yet been refined.

II may be prepared by dissolving freshly precipitated cupric hydroxide in an aqueous solution of I. It crystallizes from hot aqueous solution as dark red-brown, soft, triclinic plates[7] with $a = 9.306$, $b = 10.443$, $c = 7.479$ A, $\alpha = 90° 38'$, $\beta = 114° 15'$, $\gamma = 98° 25'$, and $Z = 2$. The space group is probably $P\bar{1}$. The intensities of 2874 unique reflections were measured as for I and 2454 were significantly above the background. Absorption corrections were applied.

(1) Research financed by Grants 1-SO1-FR-05539, AM 02884, and CA 06927 from the National Institutes of Health, U. S. Public Health Service.

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(5) We are indebted to Dr. H. G. Petering for a generous sample of the pure ligand I.

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(7) An orthorhombic modification of II crystallizes from the same solution at room temperature, $a = 10.45$, $b = 13.83$, $c = 9.32$ A, and $Z = 4$. The space group is probably $Pmn2_1$. These crystals could not be grown large enough for X-ray data collection purposes.

The structure was solved by Patterson and Fourier methods and refined by least squares to an R of 0.058. The ethoxy group is disordered, but as yet the nature of this disorder is not understood. The positions of the five hydrogen atoms of the bis(thiosemicarbazone) chains have been located in a difference map but have not yet been included in the calculations.

In the crystal structure of I the chain from S(5) to S(5′) is fully extended, and the conjugated system from N(3) to N(3′) (excluding the side-chain atoms) is approximately planar. The hydrogen atoms on N(3) and N(3′) of I are ionized on chelate formation to give II, where one molecule of I is coordinated to one cupric ion as a tetradentate chelate. The coordination is *via* the nitrogen atoms N(2) and N(2′) and the two sulfur atoms, which are at the corners of a very distorted square.

All the atoms of II [except for hydrogen atoms and those of the side chain on C(1′)] lie very close to the same plane, the maximum deviation from the least-squares best plane through Cu, N(6), C(4), N(3), N(2), C(1), C(1′), N(2′), N(3′), C(4′), N(6′) being about 0.06 A. The two sulfur atoms lie one above (+0.33 A) and one below (−0.11 A) this plane. These deviations are directed toward the copper atoms of adjacent complex molecules, which pack with their planes parallel to the reference molecule. Therefore there are sulfur atoms from neighboring molecules on both sides of the planar complex, completing a sixfold coordination for the copper atom. Although these Cu···S packing distances of 3.10 and 3.31 A are considerably longer than single bonds, there appears to be a real tendency toward bond formation since the Cu···S directions are very close to normal to the plane of the complex. Owing to repulsion between other parts of the molecules these Cu···S distances cannot be any shorter.

It is possible that in solution one or both of these octahedral positions are strong binding sites for a ligand that is not sterically hindered by the remainder of the atoms of II. This type of binding could bring I into favorable stereochemical relationships with other reacting species and thereby enhance its biological activity.

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Steroid Hydroxylations. III. The Role of Reduced Nicotinamide–Adenine Dinucleotide Phosphate (NADPH)

Sir:

Proof of the exact mechanism of steroid hydroxylations in biological systems has not been demonstrated. Hayano and co-workers showed that the oxygen atom incorporated during adrenal C-11 hydroxylation came from molecular oxygen rather than from water.[1] Subsequent experiments have shown that the same holds true for other positions on the steroid nucleus.[2] To date, reduced pyridine coenzymes and adenosine 5-triphosphate (ATP) have been shown to be necessary for hydroxylations.[3] Stoichiometric experiments by Cooper, *et al.*, performed during C-21 hydroxylation show that a 1:1 molar relationship exists between O_2 and NADPH.[4] Several different steroid hydroxylation mechanisms, all involving essentially the hydroxonium ion (OH^+), have been proposed by Hayano.[5] The hydroxylation reaction of progesterone to yield 17-hydroxyprogesterone was chosen to demonstrate the role of NADPH.

Glucose-1-^{3}H 6-phosphate was prepared by the phosphorylation of D-glucose-1-^{3}H (New England Nuclear Corp.).[6] Subsequent reaction of the glucose-1-^{3}H 6-phosphate with NADP and glucose 6-phosphate dehydrogenase gave the reduced product (340 mμ absorbancy), which was separated chromatographically according to the procedure of Pastore and Friedkin.[7] The microsomal fraction of sow ovaries was isolated[8] and the particles were checked for homogeneity by electron microscopy. Incubations, in duplicate, of 1 mg of progesterone (Calbiochem) with added NADPH (expt 1, 803,460 dpm, specific activity 6950 dpm/μg; expt 2, 1,606,920 dpm, specific activity 13,910 dpm/μg) were performed as previously described.[8] A Packard TriCarb liquid scintillation spectrometer, Model 314EX-2, was used for measurement of radioactivity. The steroids were dissolved in 10 ml of scintillation mixture prepared by dissolving 1 g of PPO + 0.06 g of POPOP in 200 ml of toluene. A Vanguard Model 880-D paper strip autoscanner was used for location of radioactive compounds on paper. Zaffaroni paper chromatographic systems with formamide as the stationary phase were utilized throughout this investigation.

After incubation for 1.5 hr at 37° the reaction was stopped with four volumes of acetone. Storage in a cold room overnight completed the precipitation of proteins, which were removed by filtration. The acetone was carefully removed *in vacuo*, and the resulting water layer was extracted three times with equal volumes of chloroform. The water layer was subjected to a distillation, and the distillate was found to contain a high amount of radioactivity (expt 1, 600,000 dpm, and expt 2, 1,050,000 dpm). The residue from the distillation, and the protein precipitates, were dissolved in 0.5 ml of solubilizing solution (Nuclear-Chicago Corp.) and measured for radioactivity. No significant counts over background could be detected.

The chloroform extract, after addition of authentic 17-hydroxyprogesterone (300 μg), was chromatographed in a hexane system for 20 hr and then further developed in a hexane–benzene system. After elution of this area with methanol and rechromatography in a benzene system, structure proof was undertaken to determine where the tritium label was located on the steroid nucleus. This was accomplished by using one portion from each experiment for C-20 ketone reduction with sodium borohydride followed by periodic acid cleavage

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