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# SULFUR BASE COMPLEXES OF 4,6-DINITRO-BENZOFUROXAN. ISOLATION OF THE CYSTEINE COMPLEX; SUPPORT FOR CELLULAR THIOL ADDITION AS A PRIMARY STEP IN THE IN VITRO INHIBITION OF NUCLEIC ACID SYNTHESIS BY NITROBENZOFUROXANS.

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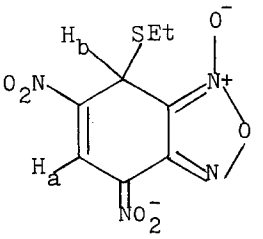
Nitrobenzofuroxans have been observed as powerful inhibitors of nucleic acid biosynthesis, with an especially toxic effect on leukocyte metabolism in vitro.[1,2,3] This activity is thought to result from a primary interaction of such heterocycles with intracellular thiol functionality, producing, initially, the corresponding Meisenheimer complex.[1,2] There is much indirect evidence to support these proposals, including characterization of several adducts of nitrobenzofurazans and nitrobenzofuroxans with oxygen bases.[4,5,6] We report here the first examples of thiol addition to 4,6-dinitrobenzofuroxan (DNBF), as well as characterization and isolation of the cysteine complex of DNBF which forms completely in water at physiological pH. Several other examples of amino acid and amino acid derivative complexes with DNBF are also reported.

Addition of one equivalent of ethane thiol to a solution of DNBF in DMSO results in a dark red solution which exhibits resonances characteristic of the complex 1. The two doublets for DNBF at δ9.19 and 8.92 are replaced by singlets at δ8.84 and 5.42 for the ring protons $H_a$ and $H_b$ in 1. The quartet and triplet of the ethyl group appear at δ2.75 and 1.16. The spectrum does not change at all, even after several weeks. Interestingly, $H_a$ in 2 appears at δ8.60[4], (close to the shift of $H_a$ in 1) whereas $H_b$ in 2 appears at δ6.0, about 0.6 ppm further downfield than that of $H_b$ in 1. In fact, chemical shifts of hydrogens on the tetrahedral ring carbon in such



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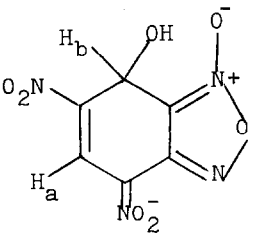

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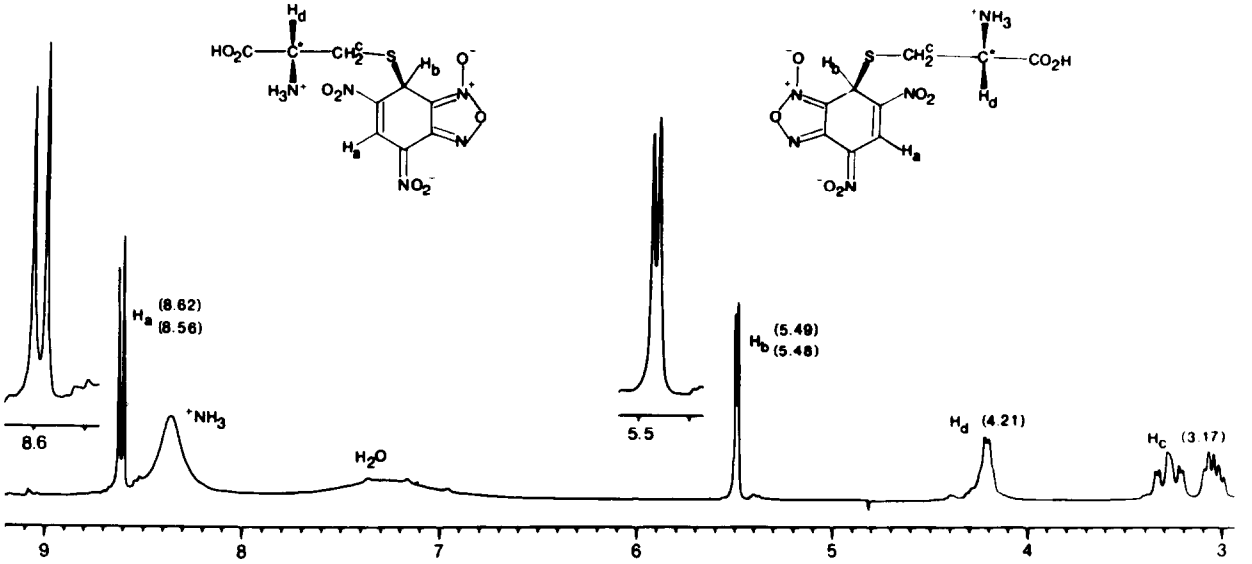
DNBF complexes are characteristic of the heteroatom bonded to this carbon (vide infra).

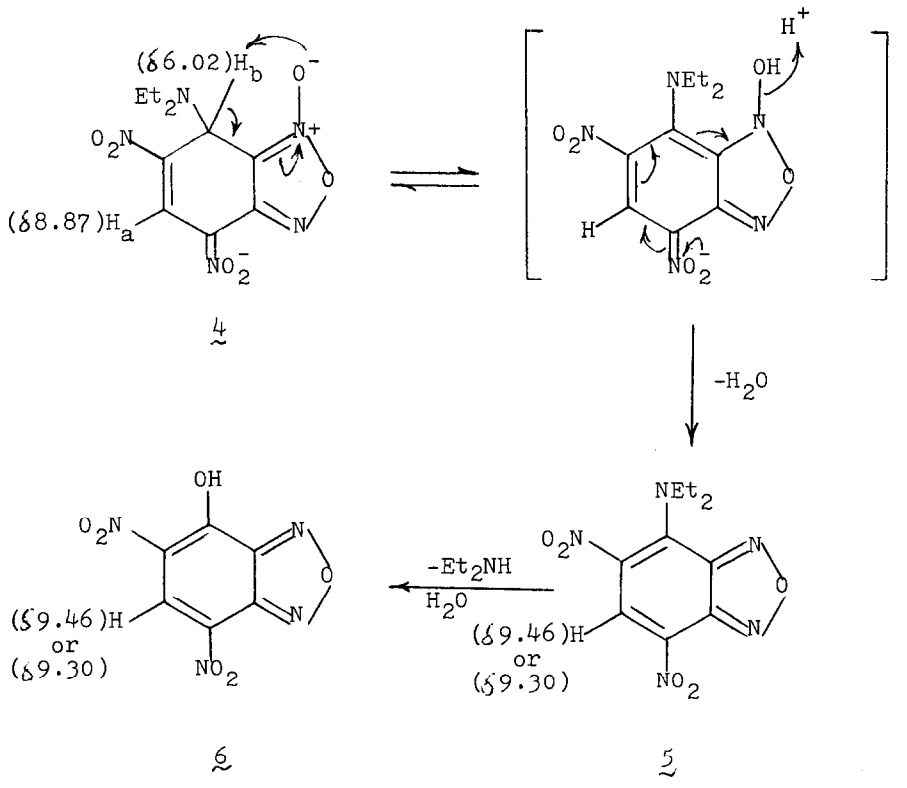
Addition of one equivalent of L-cysteine to a DMSO solution of DNBF yields a dark red solution. The pmr spectrum of this mixture is shown below, along with the structures of the diastereomeric complexes which form.




Because the asymmetric center in L-cysteine is relatively far from the nucleophilic sulfur, both diastereomers are formed in essentially equal amounts. It is interesting to note that the effect of this asymmetry (diastereomeric shift difference) is greater for $H_a$ than $H_b$, even though $H_b$ is only three bonds removed from the asymmetric center, whereas $H_a$ is six bonds removed. This suggests a conformation in which the cysteine moiety may be oriented towards the anionic ring, perhaps because of a favorable H-bonding interaction or other stabilizing electrostatic effect.

We were able to isolate the complex by adding cysteine to an aqueous suspension of DNBF in water, where the complex 2 is readily formed (at pH 7).[5] The hydroxy complex (as well as some uncomplexed DNBF) rapidly reacts to form the sulfur complex of cysteine, which after a few moments precipitates from solution. When thoroughly dried in a vacuum over $P_2O_5$ this material, while not particularly shock sensitive, exploded when heated. Attempts to take a melting point resulted in violent explosive decomposition at 128°. Attempts to obtain a mass spectrum resulted in explosive decomposition when the inlet temperature was raised above 100°. M-1 and M+1 peaks were particularly prominent at m/e 346 and 348. This explosive behavior is similar to that previously described for the potassium salt of 2, and extreme caution must be taken when such complexes are prepared in the dry state.

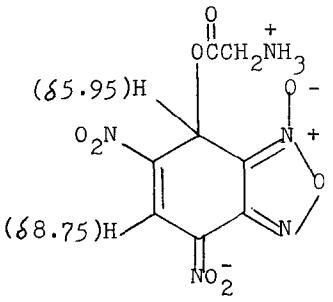
It is important to note that the complex of DNBF with cysteine is quite stable at room temperature, or in DMSO solution. This is in contrast to some oxygen[6b] and nitrogen base complexes (vide infra) which slowly rearrange as follows:




4 5 6

For example, adding equimolar amounts of diethylamine and DNBF to DMSO results in formation of 4, easily characterized by its pmr absorptions. After several days however, the resonances for 4 are replaced by those for 5 and 6. Evaporation of the solvent yields a solid mixture which exhibits strong parent peaks at m/e 281 and 226 for 5 and 6.

Interestingly, when glycine is added to a DMSO solution of DNBF the oxygen complex 7 is formed whereas with glycine ethyl ester the nitrogen complex 8 results. With potassium acetate the complex 9 is formed.

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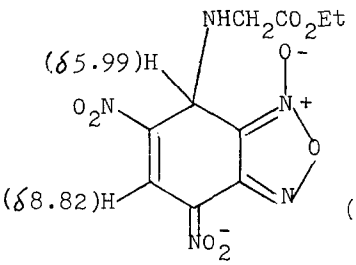


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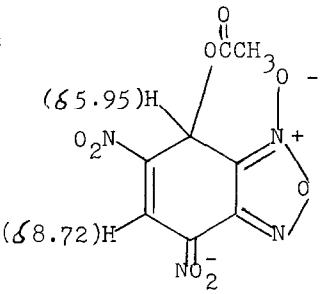


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These complexes are not as stable as the sulfur complexes formed from ethane thiol and cysteine. They all decompose over a period of days (in DMSO) to give a mixture of products, probably via rearrangement analogous to that which yields 5 and 6.

## References

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