and ionic strength 0.02, $w = 0.065$ in the native state and $w = 0.023$ in the expanded state. These values of w require that the configurational change, at this temperature and ionic strength, at pH 3.5, should be accompanied by the binding of 29 protons per 67,000 g. If allowance is made for the fact that the radius of the expanded form is probably larger at ionic strength 0.02 than at ionic strength 0.04 (*cf.* serum albumin[5]), then the calculated proton uptake is increased to about 34. It thus appears that most, and perhaps all of the observed uptake of 36 protons can be accounted for.

Ferrihemoglobin used in this study was prepared from crystalline horse oxyhemoglobin (kindly furnished by Dr. J. H. Wang) by the method of Steinhardt and Zaiser.[8] The author is indebted to Dr. E. P. Geiduschek and Mr. G. Saliba for invaluable assistance in performing these measurements.

(8) J. Steinhardt and E. M. Zaiser, THIS JOURNAL, **75**, 1599 (1953).

(9) Department of Chemistry, State University of Iowa, Iowa City, Iowa. John Simon Guggenheim Memorial Fellow, 1956–57.

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RECEIVED JUNE 3, 1957

S-(DICHLOROVINYL)-L-CYSTEINE: AN AGENT CAUSING FATAL APLASTIC ANEMIA IN CALVES[1]

Sir:

We are reporting the synthesis of the compound S-(dichlorovinyl)-L-cysteine which upon oral administration produces a fatal aplastic anemia in young calves similar to that which we have observed with toxic trichloroethylene-extracted soybean oil meal (TESOM).

S-(Dichlorovinyl)-L-cysteine was synthesized by treating equimolar quantities of trichloroethylene (TCE) with the disodium salt of L-cysteine in liquid ammonia by a modification of the method of du Vigneaud.[2] The product precipitated on adjusting the pH to 5.0 with acetic acid and was crystallized from water–ethanol, 60–70% yield; m.p. 155–156° (decomp.). *Anal.* Calcd. for $C_5H_7O_2NSCl_2$: C, 27.29; H, 3.27; N, 6.48; S, 14.84; Cl, 32.82. Found: C, 27.83; H, 3.29; N, 6.47; S, 14.9; Cl, 32.8. *Ultraviolet absorption* in water: λ max. 210 mμ, $\epsilon = 8600$; λ max. 258 mμ, $\epsilon = 3200$. *Titration.* Neut. equiv., calcd. 216; found, 216; pI′, 5.4. *Paper chromatography.* 70:30 1-propanol–H_2O on Whatman No. 1, R_f 0.72; positive ninhydrin, 4-(*p*-nitrobenzyl)-pyridine (4-NBP),[3] ultraviolet and iodoplatinate tests.

Preliminary data indicate that the compound is S-(1,2-*trans*-dichlorovinyl)-L-cysteine.

The biological response of young calves to oral administration of S-(dichlorovinyl)-L-cysteine was studied by the methods established for the toxicity assay of TESOM.[4] The assay results are shown in Table I. The 500 and 200 mg./100 lb./day dosage levels were not tolerated by the assay calves and had to be discontinued after 5 and 7 days, respectively; however, all dosage levels tested produced the complete clinical, hematologic and postmortem picture of severe aplastic anemia of the bovine.[4]

TABLE I

BIOLOGICAL RESPONSE OF CALVES TO ORAL ADMINISTRATION OF S-(DICHLOROVINYL)-L-CYSTEINE

Dosage mg./100 lb./day	Days to develop						
	Thrombocytopenia[a]	Leucopenia[b]	Lymphocytosis[c]	Elev. temp.	Fecal blood	Visible hemor.	Death[d]
500	10	9	13	13	13	11	14
200	12	10	10	11	11	13	14
50	14	19	21	21	17	19	23
20	18	22	22	25	25	22	27
10	20	27	31	51	27	26	60

[a] Platelet count below 200,000. [b] Leucocytes below 5000. [c] Over 85% lymphocytes. [d] Necropsied when moribund. Typical severe lesions of aplastic anemia.

We recently reported[5] on fractionation studies of toxic TESOM which indicated that the aplastic anemia causing entity is associated with the protein component of the meal. Analyses indicated that toxic TESOM contained 0.5 mole less sulfhydryl groups per 10^6 g. and about 25 p.p.m. more chlorine than did hexane-extracted meal from the same beans. In addition evidence that TCE would react with cysteine was obtained from sealed tube experiments. Of fourteen amino acids tested, only cysteine gave more than one ninhydrin spot by paper chromatography and one of these absorbed ultraviolet light and gave a positive 4-NBP test. Likewise reduced glutathione liberated chloride equivalent to its sulfhydryl content in sealed tube reactions and gave strong spots of R_f 0.50 and 0.70 which absorbed ultraviolet light and were 4-NBP positive. These facts and the knowledge that heat is required for the apparent interaction of TCE with soybean flakes to form toxic TESOM suggested the attempt to interact TCE with cysteine on a larger scale suitable for characterization and assay of the product.

Oral administration of S-(dichlorovinyl)-L-cysteine at the 20 and 10 mg./100 lb./day levels produces the typical syndrome of aplastic anemia in calves consuming levels of toxic TESOM'S ranging from $^3/_4$ to $^1/_5$ lb./100 lb./day and to samples of our isolated toxic protein at levels ranging from $^3/_8$ to $^1/_{10}$ lb./100 lb./day.

Paper chromatograms of fractions from a proved toxic pancreatic digest of TESOM's protein show ultraviolet absorbing 4-NBP and S positive spots with an R_f 0.45 and 0.70 with 70–30 1-propanol–H_2O on Whatman no. 1 paper. Similarities of the chemical and physical characteristics of these spots with those of S-(dichlorovinyl)-L-cysteine and its derivatives (TCE + glutathione) plus the observation that S-(dichlorovinyl)-L-cysteine pro-

(1) A report of work done, in part, under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act. Contract supervised by Northern Utilization Research and Development Division, Agricultural Research Service.

(2) V. du Vigneaud and W. F. Patterson, *J. Biol. Chem.*, **114**, 533 (1936).

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duces in calves the typical TESOM toxicity syndrome, suggest that S-(dichlorovinyl)-L-cysteine may be related in structure to a part of, or may possibly be, the toxic principle of TESOM. Attempts to isolate sufficient toxic material from TESOM for chemical characterization are under way at the present time.

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RECEIVED JUNE 10, 1957

THE MAGNETIC SUSCEPTIBILITY OF MOLTEN NICKEL(II) COMPLEXES

Sir:

The paramagnetism exhibited by the bis-N-methylsalicylaldiminenickel(II) complex, diamagnetic in the solid state, when dissolved in "non-coördinating" solvents such as benzene and chloroform, has been interpreted as being due to the conversion of a proportion of the molecules of the complex from a planar to a tetrahedral configuration.[1] A similar behavior is observed for complexes of the series from bis-N-ethyl- to bis-N-amylsalicylaldiminenickel(II).[2]

The electric dipole moment measurements made on such complexes dissolved in dioxane and benzene have afforded evidence against such a view.[2] On the other hand, the hypothesis that paramagnetic octahedral disolvated complexes are formed, although improbable in the light of the investigations by Basolo and Matoush[3] on the coördinating tendencies of the methylbenzenes, cannot be ruled out.[4] In fact the existence of a silver perchlorate–benzene complex[5] shows that benzene and metal atoms may bind together.

In order to determine whether or not benzene molecules do coördinate with these nickel(II) complexes to yield paramagnetic solutions, magnetic measurements have been made on bis-N-alkylsalicylaldiminenickel(II) complexes, from bis-N-ethyl- to bis-N-decyl-, in the molten state. The magnetic susceptibilities of the molten compounds were measured between 80 and 200° by the Gouy method. In order to have samples of a lower melting point, mixtures of two complexes in the molecular ratio of 1:1 also were used.

Complexes which are diamagnetic in the solid state are paramagnetic with moments ranging from 0.8 to 1.15 B.M. in the molten state. Graphs of the magnetic moment *vs.* temperature for all of the complexes examined have very similar shapes and sometimes coincide. In the case of complexes or mixture of complexes melting below 100°, the curves have a minimum near 120°. Since the paramagnetism of bis-N-methylsalicylaldiminenickel(II) in benzene and chloroform, as measured by Clark and Odell,[1b] decreases steadily with increasing temperature from −16 to 43°, magnetic measurements have been also made on solutions of the complexes in dibutylphthalate which permits measurements up to 200°.

Curves of the magnetic moments of these complexes dissolved in this solvent likewise show a minimum. For the bis-N-methyl- complex this minimum falls near 120°. Beyond this point the magnetic moment rises steadily with increasing temperature. This suggests that the mechanism of the transition from diamagnetism to paramagnetism is the same for solutions as it is for the molten systems. The results of this investigation also show that the presence of solvents is not necessary to give rise to paramagnetism in these complexes, their diamagnetism being a property peculiar to the solid state only.

The equilibrium constants K = [paramagnetic form]/[diamagnetic form] have been calculated from values of magnetic susceptibility. The plots of log K against $1/T$ give a minimum which demonstrates an inversion of sign in the enthalpy of values of the equilibrium.

The possibility that the paramagnetism of the molten compounds can result from dissociation of the complexes into free Ni^{++} ions and chelate molecules has been excluded by measurements of electrical conductivity made on these complexes in the molten state.

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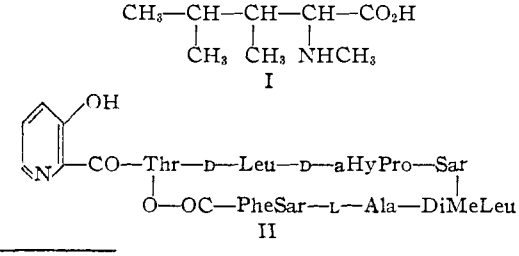
RECEIVED MAY 31, 1957

THE STRUCTURE OF ETAMYCIN

Sir:

This communication reports the complete structure of the antibiotic Etamycin[1] (Viridogrisein[2]), the isolation of which recently was described independently and simultaneously by two groups.[1,2] The antibiotic possesses interesting activity against Gram-positive organisms, and in addition causes a reversible leucopenia in dogs. Etamycin is a surprisingly lipophilic peptide (soluble in benzene and carbon tetrachloride) with a molecular weight in the range 800–900. The presence of 3-hydroxypicolinic acid, L-alanine, *allo*-hydroxy-D-proline, D-leucine and threonine was reported.[1,2] We have now shown Etamycin to be a macrocyclic lactone (22-membered ring) which contains in addition to the above-mentioned components sarcosine, α-



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