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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## 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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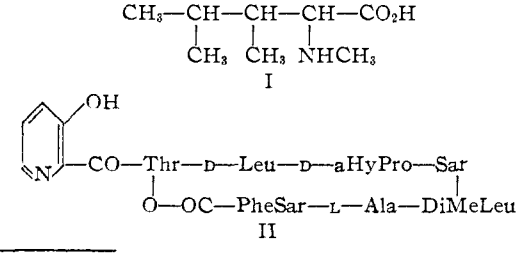
## THE STRUCTURE OF ETAMYCIN

*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-




(1) (a) J. B. Willis and D. P. Mellor, THIS JOURNAL, **69**, 1237 (1947); (b) H. C. Clark and A. L. Odell, *J. Chem. Soc.*, 3431 (1955).

(2) L. Sacconi, P. Paoletti and G. Del Re, THIS JOURNAL, in the press.

(3) F. Basolo and W. R. Matoush, *ibid.*, **75**, 5663 (1953).

(4) *Cf.* H. C. Clark and A. L. Odell, *J. Chem. Soc.*, 520 (1956).

(5) R. E. Rundle and J. H. Goring, THIS JOURNAL, **72**, 5337 (1950).

(1) B. Heinemann, *et al.*, *Antibiotics Annual*, **2**, 728 (1954–1955).

(2) Q. R. Bartz, *et al.*, *ibid.*, **2**, 777, 784 (1954–1955); the identity of Viridogrisein with Etamycin was established in the laboratories of the authors of refs. 1 and 2 and at M.I.T.

phenylsarcosine (PheSar) and β,N-dimethylleucine (DiMeLeu, I), the latter two of which have not been encountered previously in a natural product.

The presence of N-methylamino acids was indicated by color tests[3] on hydrolysates, and confirmed by an N-methyl determination, which demonstrated the presence of three N-methyl groups per molecule of Etamycin. For the isolation of the three N-methylamino acids, an Etamycin total hydrolysate was deaminated to destroy threonine, alanine and leucine and subjected to preparative paper chromatography.

One of the amino acids was identified as sarcosine by conversion to the dinitrophenyl derivative, m.p. 185–186°, undepressed upon admixture with an authentic sample.

The second amino acid, $C_9H_{11}NO_2$, m.p. 245–246° (sublimes) (Found: C, 65.30; H, 6.66; N, 8.57) had infrared and ultraviolet spectra indicating the presence of a benzene ring. It was proved to be identical with synthetic D,L-α-phenylsarcosine, m.p. 246–247° (sublimes), by infrared and ultraviolet spectra and chromatographic behavior. The optical inactivity is expected since optically active α-phenylglycine is known[4] to racemize rapidly with hot 10% hydrochloric acid.

The third amino acid, $C_8H_{17}NO_2$, m.p. 315–316° dec., (Found: C, 60.12; H, 10.47; N, 8.77) was optically active, $[\alpha]^{28}D$ +36.2° (*c*, 2.4 in 5*N* HCl). Hypochlorite degradation[5] gave methylamine, identified as the dinitrophenyl derivative, and the isopropylmethylacetaldehyde, isolated as the 2,4-dinitrophenylhydrazone, m.p. 125.2–126.0°, $[\alpha]^{28}D$ −38.7° (*c*, 1.5 in $CHCl_3$), which was identical (m.p., mixed m.p., infrared spectra) with a sample of the DNPH of (−)-isopropylmethylacetaldehyde obtained by ozonolysis[6] of ergosterol.[7] Thus, the third amino acid is β,N-dimethylleucine (I). It may be significant that the new amino acid and ergosterol have a common structural unit.

Etamycin contains only one each of the eight components according to quantitative paper chromatography and analyses of Etamycin, $C_{44}H_{62}N_8O_{10}$ (Found: C, 59.95; H, 7.40; N, 13.07; dried at 135° (0.01 mm.) and Etamycin hydrate, $C_{44}H_{62}N_8O_{10}\cdot H_2O$ (Found: C, 58.96; H, 7.17; N, 12.54; N-$CH_3$, 4.63; eq. wt., 890; dried at 110° (0.01 mm.).

(3) Paper chromatograms showed three weakly ninhydrin-positive spots, two of which gave red color tests with *p*-nitrobenzoyl chloride–pyridine according to P. A. Plattner and U. Nager, *Helv. Chim. Acta*, **31**, 220 (1948).

(4) F. Ehrlich, *Biochem. Z.*, **8**, 446 (1908).

(5) K. Langheld, *Ber.*, **42**, 2360 (1909); P. A. Plattner and U. Nager, *Helv. Chim. Acta*, **31**, 2192 (1948).

(6) G. Slomp, *et al.*, THIS JOURNAL, **77**, 1216 (1955).

(7) W. Bergmann and H. A. Stansbury, *J. Org. Chem.*, **9**, 281 (1944); the DNPH prepared by these authors had $[\alpha]^{26}D$ −37.7 and m.p. 124–124.5°.

Evidence that Etamycin contains a lactone structure was obtained as follows. On treatment with 0.1 *N* sodium hydroxide at room temperature it is converted into an antibiotically inactive acid, which contains the hydroxypicolinic acid and all amino acids. In Etamycin the threonine resists oxidation with chromic acid, whereas under the same conditions, in Etamycin acid the threonine is destroyed to the extent of 80%. Consequently the lactone link must involve the C-terminal carboxyl group and the hydroxyl group of threonine.

The absence of a terminal free amino group and the presence of unusual amino acids presented difficulties in the application of the conventional chemical and enzymatic methods of peptide sequence determination. However, catalytic hydrogenation of the obviously N-terminal hydroxypicolinic acid provided a secondary amino function, and alkali treatment gave a terminal carboxyl group, thus making possible the application of the Edman method.[8] Seven successive degradations revealed the sequence as hydroxypicolinic acid—Thr–D-Leu–D-aHyPro–Sar–DiMeLeu–L-Ala(?)–(PheSar). The course of the degradation was followed by paper chromatography of the phenylthiohydantoins,[9] dinitrophenylation and hydrolysis of each successive peptide, followed by paper chromatography of the resulting DNP-amino acids,[10] and by quantitative estimation of the amino acid content of each successive peptide after total hydrolysis and two dimensional paper chromatography. The sequence was completed by degradation from the carboxyl end with hydrazine,[11] which established phenylsarcosine as C-terminal. The structure of Etamycin is represented by II.

NOTE ADDED IN PROOF.—Comparison of the rotation of the DiMeLeu in neutral and acid solution has shown it to be in the L-series. Another isolation after mild hydrolysis gave PheSar having $[\alpha]^{31}D$ + 118° (*c*, 4.8 in *N* HCl), from which it can be deduced that it probably is in the L-series.

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(12) (a) Supported by a grant from the National Institutes of Health; (b) National Institutes of Health Postdoctoral Fellow, 1956-1957.