and sulphur), (b) N-DNA ~ D-DNA (Acr and hemoglobin, pH 3.5) and (c) N-DNA < D-DNA (MB, TB). Different modes of binding of N- and D-DNA with the various cationic compounds can be inferred from this. Different binding patterns of DNA have also been reported by other investigators[10,11] using techniques quite distinct from the present one. As evident from the Table, the influence of D-DNA on the clot formation by N-DNA is conditioned both by the nature of the cationic compound and the proportion of the two forms of DNA in the system.

The ultrasonicated DNA (N_2 atmosphere, 12 Kcs, 2.5 A, 60 min)loses its clot-forming ability to different extents as tested with different basic compounds. The change is, however, drastic and fast in an atmosphere of air.

The unique behaviour of clot formation by N-DNA may be due to its high viscosity as well as large extension in space of its molecules as compared to other polymers[12]. Preliminary experiments with some of the systems indicate that DNA is recoverable from the clots. LERMAN[1] has reported the recovery of N-DNA from its complexes with acridines[14].

Zusammenfassung. Es wird ein Test angewendet, der native und denaturierte DNS unterscheiden lässt. Er besteht auf einer Verklumpung nativer DNS bei Zugabe von kationisierten Stoffen, während denaturierte DNS nur eine Trübung, respektive einen Niederschlag gibt.

CHANAN SINGH and J. MISRA

Central Drug Research Institute, Lucknow (India), November 30, 1965.

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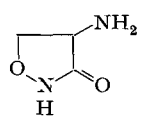
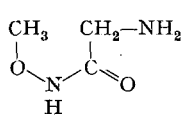
Glycine Methoxyamide, Acyclic Analog of Cycloserine

There has been considerable speculation on the molecular mechanism by which cycloserine (1) inhibits certain enzymes in microbiological systems[1]. The formation of a Schiff base with pyridoxal followed by opening of the isoxazolidone ring are the principal steps in the proposed mechanism. It seemed, therefore, of interest to synthesize the acyclic analog of cycloserine, glycine methoxyamide (GMA) (2), to determine whether the isoxazolidone ring was necessary to antibiotic activity. GMA does not appear in the chemical literature, but was readily synthesized by the procedure of KNOBLER[2] et al., using glycine N-carbonic anhydride and methoxyamine. The purified product of this reaction showed no activity against 29 representative bacteria, fungi and yeasts, demonstrating clearly that the ring is necessary for biological activity.



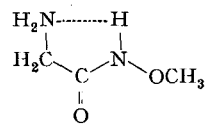
(1)



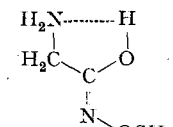
(2)

The structure of GMA was established by elemental analysis, IR-, UV- and NMR-spectroscopy. The solid-state IR-spectrum of GMA showed major bands at 6.1 (C=O), 6.22 (N–H), 6.52 (amide II) and 9.44 μ (C–O). Much hydrogen bonding of the α-amino group was indicated by broad absorption in the 3.0–4.0 μ region. These results are consistent with those of EXNER[3], but, in our opinion, do not support either the amide (3) or imide (4) structure.

UV data, λ^{MeOH} = 214 (log ε, 3.07), 222 nm (log ε, 3.38), was similar to that for cycloserine and NMR peaks in NaOD/D_2O appeared at δ 3.72 (–O–CH_3) and 3.35 (–CH_2–) which are consistent with the structure. The expected paramagnetic shifts to δ 3.78 and 3.55 occurred when the solution was acidified with trifluoroacetic acid.



(3)



(4)

It seemed pertinent to compare the ionization constants and metal binding affinities of GMA with those of cycloserine since large differences in these properties might account for the difference in biological activity between these two compounds. In our hands, cycloserine showed pKa values of 4.53 (–CONHO–) and 7.31 (–NH_2) in agreement with those reported by NEILANDS[4]. GMA, however, was much less acidic than cycloserine, having 7.20 (–CONHO–) and 9.51 (–NH_2) pKa values. This large difference of 1.8 pK units in the acidity of the cyclic and acyclic alkoxyamides is quite surprising. The flat cycloserine ring[5] undoubtedly stabilized the anion by facilitating overlap of the nitrogen *p*-electrons with the carbonyl group, thus increasing the acidity almost one

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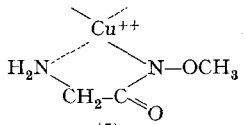
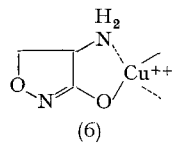
hundredfold[6]. The Table shows the metal-binding constants of GMA and those reported for cycloserine by NEILANDS[4]. Here again there is a very large difference in a biologically important property. GMA is a much stronger metal-binding agent than cycloserine and even binds Cu^{++} more tightly than EDTA (Ks = 18.8)[7]. This difference between cycloserine and GMA may reside in the fact that Cu^{++} can be bound between 2 *nitrogen* atoms (5) in GMA while only a less stable type[8] of binding between the nitrogen and oxygen atoms of cycloserine (6) is sterically possible. The stability order, $Cu^{++} > Co^{++} > Zn^{++}$, of these ions with GMA is consistent with N,N-binding in the complexes[8]. There was, however, very little difference in the visible spectra of the two copper chelates: GMA, λ^{H_2O} = 675 nm (log ε, 1.64); cycloserine, λ^{H_2O} = 700 nm (log ε, 1.45).



(5)



(6)

	Cu^{++}	Zn^{++}	Co^{++}
Cycloserine	9.7	6.0	5.7
GMA	22.2	9.8	16.6

We found also that GMA formed a crystalline Schiff base with 5-chlorosalicylaldehyde under the same mild conditions which gave a cycloserine Schiff base[1a]. We have not investigated the chemistry of this compound further, but its facile formation indicated that GMA could react in vivo with pyridoxal in the same manner that cycloserine most probably does.

In summary, we have found that when the functional groups of cycloserine are arranged in an acyclic structure, no antibiotic activity is observed. This remarkable total loss of activity may be due to a requirement for the ring in the reaction sequence leading to enzyme inhibition or it may be that the large differences in ionization and metal-binding propensities between the cyclic and acyclic compounds lead GMA into biological pathways far removed from the cycloserine site of action[9].

Zusammenfassung. Methoxy-glycinamid, ein offenkettiges Isomeres von Cycloserin, wurde hergestellt. Seine physikalischen Eigenschaften sind von denjenigen des Cycloserins stark verschieden. Es besitzt keine antibiotische Eigenschaft mehr.

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Department of Chemistry, University of Georgia, Athens (Georgia 30601, USA), April 22, 1966.

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[9] Acknowledgment: We are grateful for the support of NIH Grant No. AI-05539-03 and for a generous sample of D-cycloserine from Dr. W. F. RUNGE, Commercial Solvents Corporation. We thank also Dr. D. LEYDEN of this department for his helpful discussions in connection with the titration data reported herein, and Dr. G. R. GALE, Veterans Administration Hospital, Charleston, South Carolina, for the biological test results reported.

Presence of Sialopolysaccharidic Components in Egg Gelatinous Mantle of *Rana latastei* and *Bufo vulgaris*

CHIARUGI[1] first reported that frog spawn of *Rana esculenta* was able to give metachromasia with aniline basic dyes. RE[2] confirmed the metachromasia in egg gelatinous mantle of *R. esculenta* and *Bufo vulgaris*. A large review on the histochemistry and morphology of Amphibian spawn has recently been published by GHIARA[3]. GIACOSA[4] demonstrated the presence of reducing substances. WOLFENDEN[5] reported the presence of nitrogen-containing reducing substances in egg jelly mucins of *R. temporaria*. SCHULTZ et al.[6] found glucosamine together with other reducing substances. The presence of *d*-galactose[7–9] and fucose[10] has been confirmed by FOLKES et al.[11]. This group was also interested in separating the hexosamines (glucosamine and galactosamine). MINGANTI was interested in the chemical analysis of egg gelatinous mantle from *B. vulgaris, R. esculenta, Discoglossus pictus, Axolotl* and *Triton cristatus*[12–15]; recently MINGANTI[16] reported comparative data on the chemical composition of Amphibian egg mucins.

From the analysis reported on the chemical composition of egg casings we have not found data on the presence of sialic acids, so frequently described as constituents of glycoproteins.

In the present paper we report the data obtained during researches carried out in order to investigate the

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