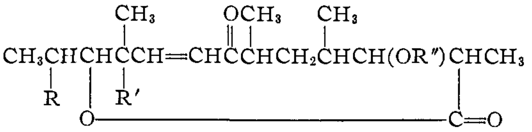
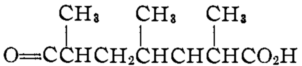
2.90, 5.73 and 5.83 μ, negative single Cotton effect in rotatory dispersion (typical of saturated ketone); found for $C_{17}H_{28}O_5$: C, 65.48; H, 9.00) is isomeric with neomethynolide (IIb) but lacks the unsaturated carbonyl chromophore. Since cycloneomethynolide (VIa) forms only a monoacetate (VIb) (m.p. 194° (subl.), $[\alpha]_D$ +1°, $\lambda_{\max}^{CHCl_3}$ 5.69, 5.83 and 8.05 μ (but *no* OH absorption); found for $C_{19}H_{30}O_6$: C, 64.64; H, 8.16; CH_3CO, 11.93), does not give a positive iodoform test and does not react with periodic acid after lithium aluminum hydride reaction, a new ether linkage must have been formed by addition of the C-12 hydroxyl group to the double bond of the α,β-unsaturated ketone. In conformity with this view, cycloneomethynolide (VIa) can be oxidized readily to the ketone VIc (m.p. 122–124°, $[\alpha]_D$ +60°; found for $C_{17}H_{26}O_5$; C, 65.94; H, 8.30) which furnished 63% of CO_2 after treatment with alkali followed by acidification. These results are best rationalized by structure VIa for cycloneomethynolide from which it follows that neomethynolide must be IIb and neomethymycin IIa.[9] This structure represents a novel substitution pattern of considerable biogenetic interest[10] among macrolide antibiotics and explains the absence of spiroketal formation, so facile in the methymycin series (I). The details of these and related experiments as well as the conclusions to be drawn from rotatory dispersion measurements of various macrolide transformation products will be reported in a complete paper.



Ia, R = H; R′ = OH; R″ = desosamyl[4a]
Ib, R = R″ = H; R′ = OH
IIa, R = OH; R′ = H; R″ = desosamyl[4a]
IIb, R = OH; R′ = R″ = H



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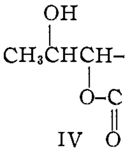

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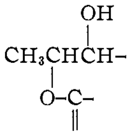
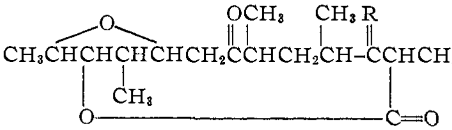


V



H
VIa, R = ᴸOH
b, R = H
c, R = O ᴸOAc

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RECEIVED MARCH 12, 1957

FORMATION OF A THREE-STRANDED POLYNUCLEOTIDE MOLECULE

Sir:

It has been shown that molecules of the synthetic polyribonucleotides, polyadenylic acid (poly-A) and polyuridylic acid (poly-U), associate in aqueous solution,[1] and further demonstrated[2,3] that fibers drawn from mixtures of the acids produce an X-ray diffraction pattern similar to that of desoxyribose nucleic acid (DNA). This complex is a two-stranded helical molecule in which the poly-A and poly-U are intertwined.

A continuous variation study has been made of the complexing reaction in which the total concentration of the bases in solution is maintained constant while varying their ratio. The complexes which form have a lower optical density at 259 mμ than their uncombined constituents, and the minimum of optical density occurs where the largest amount of complex is formed.

Experiments were carried out with poly-A and poly-U prepared in our laboratory with polyribonucleotide phosphorylase from *E. coli* obtained by the method of Littauer and Kornberg[4] as well as with polymers kindly supplied by S. Ochoa.[5] The solutions contain 0.1 *M* sodium chloride and 0.01 *M* glycylglycine at *p*H 7.4. The open circles of Fig. 1 show the variation with composition of the optical density at 259 mμ. The position and sharpness of the minimum clearly shows formation of a very strong 1:1 complex.

In some experiments, it was noted that the points on the right side of Fig. 1 (open circles) fall below the straight line, producing a bowed appearance. The shape of the curve was such as to suggest the formation of a new relatively weak complex involving two poly-U strands for each poly-A strand.

Ultracentrifuge studies at low concentrations using ultraviolet optics showed that the sedimentation coefficient of the new complex (s_{20} = 17.3) was greater than that of the 1:1 complex (s_{20} = 12.6). (The values of s_{20} for this poly-A and poly-U were 8.0 and 3.2, respectively.)

The addition of divalent cations has been found to drive this reaction to completion. In solutions which are 0.01 *M* in $MgCl_2$ the shape of the optical density curve changes to that seen in the solid curve of Fig. 1. This curve has a minimum at 67% U, 33% A, *i.e.*, 2:1.

We have interpreted these results to mean that the original two-stranded molecule has taken on a third strand of poly-U which fills the helical groove in the (A + U) complex. We have built models to show that such a three-stranded complex is possible with divalent cations neutralizing the charges on

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[9] The glycosidic linkage is placed at C-3 (rather than C-12) because warming of neomethymycin or dihydroneomethymycin with alkali (opening of lactone ring) followed by sodium metaperiodate cleavage yielded acetaldehyde under conditions where the glycosidic linkage would not be expected to be affected.

[10] *Cf.* R. B. Woodward, "Festschrift Arthur Stoll," Birkhäuser, Basel, 1957, pp. 524–544; *Angew. Chem.*, **69**, 50 (1957).

(1) R. C. Warner, *Federation Proc.*, **15**, 379 (1956).

(2) A. Rich and D. R. Davies, THIS JOURNAL, **78**, 3548 (1956).

(3) A. Rich, *Ann. N. Y. Acad. Sci.*, in press (1957).

(4) U. Z. Littauer and A. Kornberg, *J. Biol. Chem.*, in press.

(5) M. Grunberg-Manago, P. J. Ortiz and S. Ochoa, *Science*, **122**, 907 (1955).

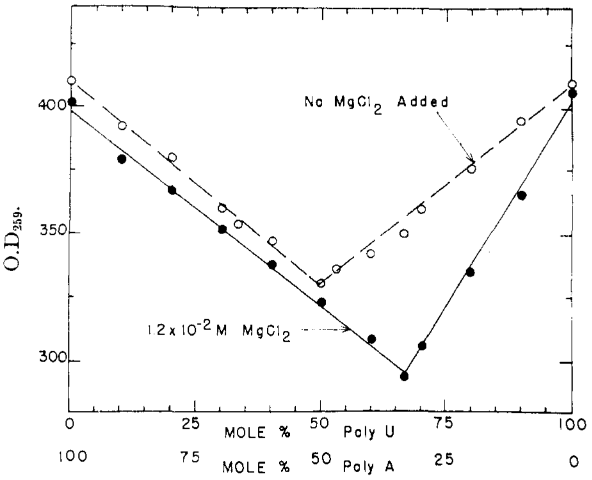

Fig. 1.—The optical density of various mixtures of polyadenylic acid and polyuridylic acid. Optical densities were measured two hours after mixing. All solutions are in 0.1 *M* NaCl, 0.01 *M* glycylglycine, *p*H 7.4, T = 25°.

the phosphate groups and the additional uracil hydrogen bonded to either one or both bases of the adenine–uracil pair. It should be noted that there is only one position whereby the new uracil residue can make two strong hydrogen bonds, namely, by bonding uracil O_6 and N_1 to adenine N_{10} and N_7. The addition of the third strand need not involve an increase in radius or helical pitch of the molecule, and could therefore account for the increase of approximately 50% in sedimentation coefficient.

The transition of the mixture of (A + U) and U molecules to (A + 2U) was examined in more detail. The optical density at 259 mμ was measured for 1:1 mixtures of (A + U) and U. Changes in this optical density with time are plotted in Fig. 2 for various concentrations of $MgCl_2$. The fact that the final optical density depends upon the concentration of Mg^{++} indicates that the latter must be reacting with the polynucleotides. At concentrations greater than 0.1 *M* $MgCl_2$, the optical density does not decrease further. If we assume that the drop in optical density is proportional to the number of U molecules which have reacted with (A + U), then these curves show that the reaction is second order in magnesium.

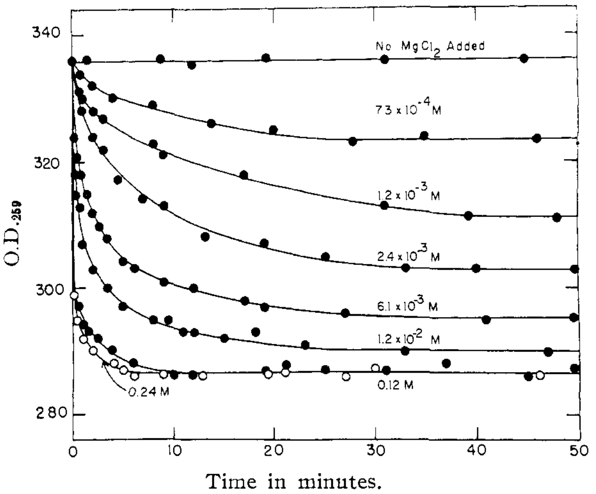

Fig. 2.—The time dependence of optical density of a mixture of two moles of polyuridylic acid to 1 mole of polyadenylic acid, for various concentrations of $MgCl_2$. All solutions are in 0.1 *M* NaCl, 0.01 *M* glycylglycine, *p*H 7.4, *T* = 25°.

It has been found that ethylenediaminetetraacetic acid can reverse the reaction shown in Fig. 2. It should be pointed out that other ions such as Ca^{++} and Zn^{++} can also promote the reaction.

This reaction has a fair amount of specificity. Thus, even in the presence of large concentrations of Mg^{++}, the (A + U) molecule will not react with polycytidylic acid, polyinosinic acid or polyadenylic acid, but only with polyuridylic acid.

Since the two-stranded (A + U) structure is similar to the Watson and Crick[6] structure for DNA, with uracil instead of thymine, this three-stranded structure may have significance as a prototype for a biologically important three-stranded complex, as, for example, a single ribonucleic acid chain wrapped around a two-stranded DNA.

(6) J. D. Watson and F. H. C. Crick, *Cold Spring Harbor Symposia*, XVIII (1953).

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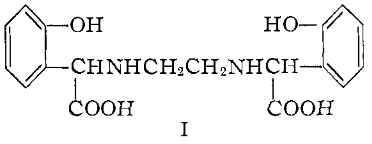
RECEIVED MARCH 21, 1957

A PHENOLIC ANALOG OF ETHYLENEDIAMINETETRAACETIC ACID

Sir:

The use of the ferric chelate of ethylenediaminetetraacetic acid (EDTA) for treating iron chlorosis in plants is restricted to crops grown on acid soils.[1] The Fe(III)EDTA is ineffective in correcting this mineral deficiency in plants grown on alkaline soils, and this can be attributed in part to the instability of the ferric chelate in soil media of this type.[2]

We wish to report the synthesis of a new chelating agent, ethylenediamine di-(*o*-hydroxyphenylacetic acid),[3] EDDHA (I), and its ferric chelate,



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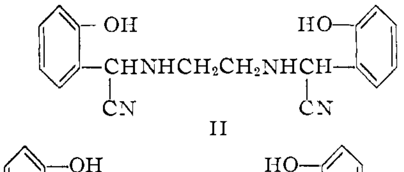

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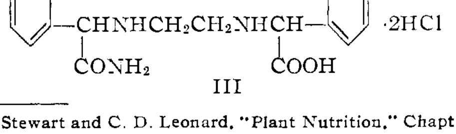

III

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(1) I. Stewart and C. D. Leonard, "Plant Nutrition," Chapter XVI, N. F. Childers, Editor, Rutgers University, New Brunswick, N. J., 1954, p. 775.

(2) A. Wallace, R. T. Mueller, O. R. Lunt, R. T. Ashcroft and L. M. Shannon, *Soil Science*, **80**, 101 (1955).

(3) Ethylenediamine di-(*o*-hydroxyphenylacetic acid) and its ferric chelate, $HFe(III)C_{18}H_{16}O_6N_2$, were distributed for agricultural testing under the code numbers Chel 138 and Chel 138 HFe.