

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, pH 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 O₆ and N₁ to adenine N₁₀ and N₇. 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 MgCl₂. The fact that the final optical density depends upon the concentration of Mg⁺⁺ indicates that the latter

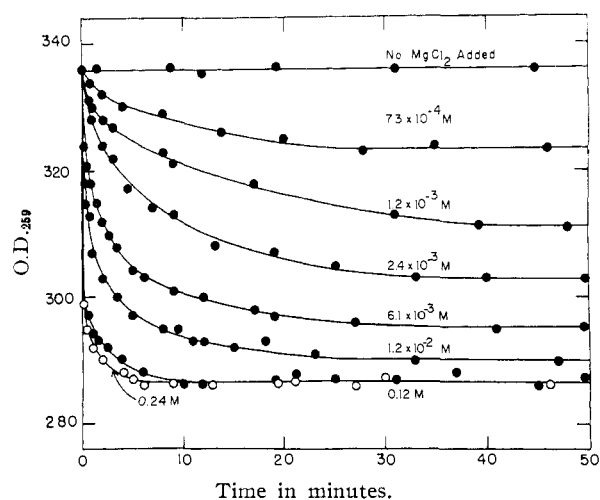


Fig. 2.—The time dependence of optical density of a mixture of two moles of polyuridylic acid to 1 mole of polyadenylic acid, for various concentrations of MgCl₂. All solutions are in 0.1 *M* NaCl, 0.01 *M* glycylglycine, pH 7.4, *T* = 25°.

must be reacting with the polynucleotides. At concentrations greater than 0.1 *M* MgCl₂, 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.

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 Crick⁶ 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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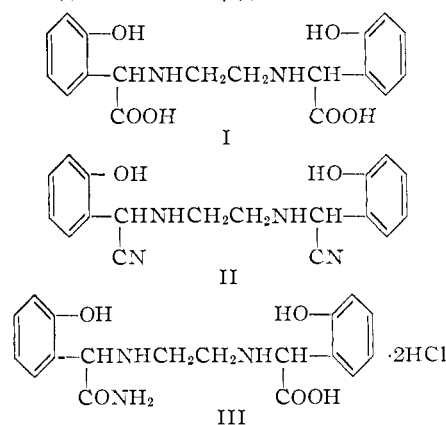
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A PHENOLIC ANALOG OF ETHYLENEDIAMINE-TETRAACETIC ACID

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,



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

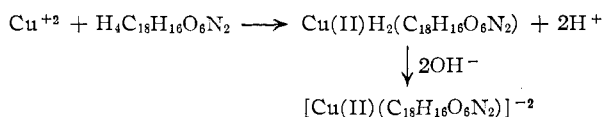
(2) A. Wallace, R. T. Mueller, O. R. Lunt, R. T. Ashcroft and L. M. Shannon, *Soil Science*, 80, 101 (1955).

(3) Ethylenediamine di-(*o*-hydroxyphenylacetic acid) and its ferric chelate, HFe(III)C₁₈H₁₆O₆N₂, were distributed for agricultural testing under the code numbers Chel 138 and Chel 138 HFe.

$\text{HFe(III)C}_{18}\text{H}_{16}\text{O}_6\text{N}_2$,³ and their use in correcting iron deficiencies in a variety of crops grown on the alkaline, clay soils of the western United States.

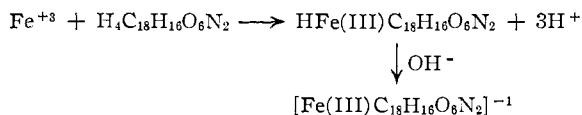
EDDHA was prepared by the addition of two moles of hydrogen cyanide to the Schiff base from salicylaldehyde and ethylenediamine to yield the dinitrile II. The dinitrile decomposed at 113–115°. All attempts to purify II by recrystallization were unsuccessful owing to its instability. Titration of II in glacial acetic acid with perchloric acid yielded a neutral equivalent of 163, calculated 161. Compound II was hydrolyzed in concentrated hydrochloric acid at 40° to the monoamide dihydrochloride III. Calcd. for $\text{C}_{18}\text{H}_{23}\text{O}_5\text{N}_3\text{Cl}_2$: N, 9.7; Cl, 16.4. Found: N, 9.6; Cl, 16.6. Neut. equiv. (in dimethylformamide using KOH as the titrant), calcd.: 108. Found: 108. EDDHA was obtained from III by refluxing in 6 *N* HCl and neutralizing the reaction mixture to pH 4 to precipitate EDDHA. Purification of EDDHA was effected by dissolving the crude I in dilute ammonia and reprecipitating at pH 4. Calcd. for $\text{C}_{18}\text{H}_{20}\text{O}_6\text{N}_2$; N, 7.8. Found: N, 7.6. Neut. equiv. (glacial acetic acid, perchloric acid titrant), calcd.: 180. Found: 179.

EDDHA is insoluble in water, but soluble in dilute mineral acids and alkali. The metal chelating properties of the new compound are significantly different than those of EDTA. Ca(II) is bound weakly at pH 8, while Mn(II) is chelated at pH 6 and above. Although Cu(II) forms a strong chelate, titration data indicate that chelate formation probably goes through the following stepwise process



Ferric iron combines with EDDHA in water to form a deep red solution which, in contrast to the ferric chelates of catechol disulfonate,⁴ maintains a relatively constant optical density at 480 m μ over the pH range from 4 to 9.

The titration data of EDDHA in the presence of Fe(III) indicate that chelate formation proceeds stepwise through the formation of the acid, $\text{HFe(III)C}_{18}\text{H}_{16}\text{O}_6\text{N}_2$, which separates as a purple solid from concentrated solutions.



The stability constant of the Fe(III)EDDHA chelate is estimated to be approximately 10^{30} .

EDDHA shows promise as a sensitive analytical reagent for ferric iron. The ferric chelate can be used to determine iron at levels of 1 to 3 gammas.

The uptake of chelated iron labeled with Fe^{59} by bean plants grown on three soil types, an acid New Jersey soil, an alkaline Florida soil, and a calcareous Utah soil, was determined by measuring the tagged iron in the first trifoliolate leaves of the assay plants. The data are shown in the Table.

(4) J. H. Yoe and A. L. Jones, *Anal. Chem.*, **16**, 111 (1944).

Sample	Soil types		
	New Jersey pH 5.0 p.p.m. Fe	Florida pH 7.5 p.p.m. Fe	Utah pH 7.3 p.p.m. Fe
FeCl_3 ^a	15	5	25
Fe(III)EDTA	210	5	50
Fe(III)HEEDTA ^b	60	50	40
Fe(III)EDDHA	140	200	200

^a Five mg. of Fe as FeCl_3 was used to prepare the ferric chelates. ^b Hydroxyethyl ethylenediaminetriacetic acid.

The effectiveness of Fe(III)EDDHA in translocating iron into plants grown on alkaline soils has been substantiated in field trials.⁵

(5) Unpublished Reports, J. R. Kuykendall, Geigy Agricultural Chemicals, Ardsley, New York.

(6) Olin Mathieson Chemical Corporation, New Haven, Connecticut.

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SYNTHESIS OF ADENOSINE-5'-PHOSPHOSULPHATES BY THE CARBODIIMIDE METHOD

Sir:

Chemical synthesis of biologically important compounds containing a pyrophosphate linkage *via* the carbodiimide route has been carried out recently (*e.g.* ref.^{1,2}). The same approach also has been used for the preparation of sulfonic acid anhydrides.³ It seemed reasonable to investigate the possibility of utilizing the carbodiimide method for the synthesis of adenosine-5'-phosphosulphate (Fig. 1; S_1 and $\text{S}_2 = \text{H}$; $\text{S}_3 = \text{SO}_3\text{H}$), a compound

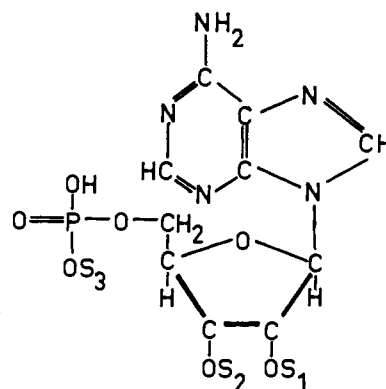


Fig. 1.

which contains a phosphate group and sulfuric acid in an anhydride linkage. This substance has recently been implicated as an intermediate in the enzymic activation of sulfate.^{4,5} Its chemical synthesis by a different route also independently has been carried out by Baddiley and co-workers.⁶

In our synthesis we used commercial crystalline adenosine-5'-phosphoric acid and S^{35} -labeled sulfuric acid. The presence of the radioactivity facilitated characterization of the products of the

(1) H. G. Khorana, *THIS JOURNAL*, **76**, 3517 (1954).

(2) G. W. Kenner, A. R. Todd and R. F. Webb, *J. Chem. Soc.*, 2843 (1954).

(3) H. G. Khorana, *Can. J. Chem.*, **31**, 585 (1953).

(4) R. S. Bandurski, L. G. Wilson and C. L. Squires, *THIS JOURNAL*, **78**, 6408 (1956).

(5) P. W. Robbins and F. Lipmann, *ibid.*, **78**, 6409 (1956).

(6) J. Baddiley, personal communication.