$HFe(III)C_{18}H_{16}O_6N_2$,[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 $C_{18}H_{23}O_5N_3Cl_2$: 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 *p*H 4 to precipitate EDDHA. Purification of EDDHA was effected by dissolving the crude I in dilute ammonia and reprecipitating at *p*H 4. Calcd. for $C_{18}H_{20}O_6N_2$; 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 *p*H 8, while Mn(II) is chelated at *p*H 6 and above. Although Cu(II) forms a strong chelate, titration data indicate that chelate formation probably goes through the following stepwise process

$$Cu^{+2} + H_4C_{18}H_{16}O_6N_2 \longrightarrow Cu(II)H_2(C_{18}H_{16}O_6N_2) + 2H^+$$
$$\downarrow 2OH^-$$
$$[Cu(II)(C_{18}H_{16}O_6N_2)]^{-2}$$

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 mμ over the *p*H 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, $HFe(III)C_{18}H_{16}O_6N_2$, which separates as a purple solid from concentrated solutions.

$$Fe^{+3} + H_4C_{18}H_{16}O_6N_2 \longrightarrow HFe(III)C_{18}H_{16}O_6N_2 + 3H^+$$
$$\downarrow OH^-$$
$$[Fe(III)C_{18}H_{16}O_6N_2]^{-1}$$

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 trifoliate 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 *p*H 5.0 p.p.m. Fe	Florida *p*H 7.5 p.p.m. Fe	Utah *p*H 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.

CHEMICAL RESEARCH LABORATORIES
GEIGY CHEMICAL CORPORATION
ARDSLEY, NEW YORK
H. KROLL[6]
M. KNELL
J. POWERS[6]
J. SIMONIAN

RECEIVED JANUARY 21, 1957

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 S_2 = H; S_3 = SO_3H), a compound

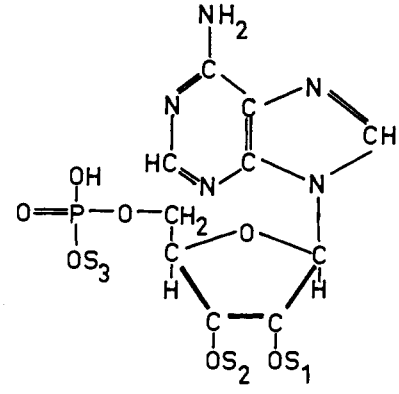

Fig. 1.

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.

reaction. Reasonable yields were obtained only if the reaction was carried out in a very small volume.

In a typical experiment 50 mg. of adenosine-5′-phosphoric acid, 0.02 ml. of concentrated H_2SO_4 and 240 mg. of dicyclohexylcarbodiimide were shaken vigorously at room temperature in 0.05 ml. of pyridine + 0.03 ml. of water. After 5 and 10 hours, 250 mg. of carbodiimide in 0.15 ml. of pyridine was added. After 15 hours, ice-cold water was added, the *p*H was adjusted to 3 and the solution was filtered and extracted several times with ether. The solution was then subjected to electrophoresis on a cellulose column[7] (length = 40 cm., diam. = 3 cm., 4/cm. for 18 hours; 0.1 *M* ammonium formate, *p*H 3). After elution the different compounds were localized by radioactivity and ultraviolet absorption (Fig. 2).

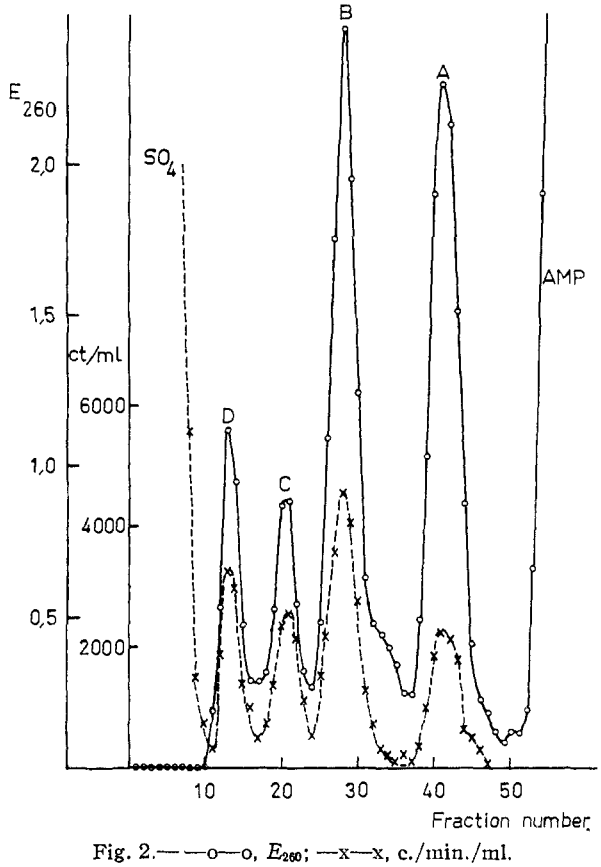

Fig. 2.— —o—o, E_{260}; —x—x, c./min./ml.

The compounds corresponding to peaks A-C all contained adenine, ribose and phosphate in the approximate ratio 1:1:1. For every molecule of adenine, A contained one molecule of sulfate, B contained two molecules of sulfate and C contained three molecules of sulfate.

Compound A could be separated into two components during electrophoresis at *p*H 8.7, both of which contained one sulfate group per molecule of AMP. The slower moving component (A_1) was identified as adenosine-5′-phosphosulfate and the faster moving component (A_2) as a mixture of adenosine-5′-phosphate-2′-sulfate and adenosine-5′-phosphate-3′-sulfate (Fig. 1; S_3 = H; S_1 or S_2 = SO_3H, S_2 or S_1 = H) by the following criteria.

(1) The sulfate group was removed completely from A_1 by treatment at 100° either with 0.5 *N* perchloric acid for 5 minutes or with calcium oxide[8] for 30 minutes, while by the same treatments only *ca.* 20 and 10% free sulfate was produced from A_2.

(2) Compound A gave rise on deamination with HNO_2 to a component which consisted of inosine-5′-phosphate and sulfate in stoichiometric amounts. This demonstrates that neither A_1 nor A_2 contains sulfuric acid bound to the amino group of adenine.

(3) One mole of A_1 consumed one mole of periodate[9] at room temperature during a period of three hours, while one mole of A consumed less than 0.1 mole of periodate. Very little free sulfate was liberated during this treatment. This demonstrates the presence of two hydroxyl groups attached to adjacent carbon atoms in A_1.

(4) The electrophoretic behavior at *p*H 8.7: adenosine-5′-phosphosulfate (A_1) contains at this *p*H two negative charges and moves slower toward the anode than adenosine-5′-phosphate-2′(or 3′)-sulfate (A_2), which contains three negative charges (Fig. 1: S_3 = H).

(5) A study of the time course of the synthesis revealed that A_1 was the dominating primary product. This is in accordance with what is known about the general mechanism of the carbodiimide method.

(6) On paper chromatography in Na_2HPO_4: isoamyl alcohol A_2 separated equally in amount into two components. This medium was originally used for the separation of adenosine-2′-phosphate and adenosine-3′-phosphate.[10]

Compound B did not consume periodate and was partially hydrolyzed by acid and alkali. We believe this disulfate to be a mixture of three compounds (Fig. 1; two of S_1, S_2 or S_3 = SO_3H, the third one = H).

The criteria for compound C indicate that it contains sulfate groups linked to both ribose hydroxyls and to the phosphate group (Fig. 1; S_1, S_2 and S_3 = SO_3H).

The identity of compound D is not yet clear.

The relative proportions of the different adenosine-5′-phosphosulfates could to some extent be modified at will by choosing the proper experimental conditions. It has thus been possible to obtain a 40–50% conversion of AMP to adenosine-5′-phosphosulfate on a one gram scale.

(7) J. Porath, *Biochem. Biophys. Acta*, **22**, 151 (1956).
(8) K. Lohmann, *Biochem. Z.*, **233**, 460 (1931).
(9) J. S. Dixon and D. Lipkin, *Anal. Chem.*, **26**, 1092 (1954).
(10) C. E. Carter, THIS JOURNAL, **72**, 1466 (1950).

DEPT. OF BIOCHEMISTRY
DEPT. OF CELL RESEARCH AND GENETICS P. REICHARD
KAROLINSKA INSTITUTET N. R. RINGERTZ
STOCKHOLM, SWEDEN

RECEIVED FEBRUARY 19, 1957

MOLECULAR WEIGHTS OF "LIVING" POLYMERS*

Sir:

The polymerization initiated by negative aromatic ions, like naphthalene$^-$, and carried out in non-proton donating solvents like tetrahydro-

(*) This research was supported by the generous grant of the National Science Foundation, NSF-G.2761.