

through an 18-inch Vigreux until the pot temperature rose to 170°, then at reduced pressure to give a fraction, b.p. 93–95° (22.1 mm.). A second fractionation gave 54.0 g. (42%) of amide, b.p. 93–93.8° (23.5 mm.), m.p. 11.6°, n_D^{20} 1.4371.

Anal. Calcd. for $C_7H_{15}NO$: C, 65.07; H, 11.70; N, 10.84. Found: C, 64.9; H, 11.7; N, 10.8.

Di-*n*-propylformamide was prepared as previously described¹¹; b.p. 206–207° (715 mm.), n_D^{20} 1.4384. This amide could not be crystallized at –70°.

Anal. Calcd. for $C_7H_{15}NO$: C, 65.07; H, 11.70; N, 10.84. Found: C, 65.33; H, 11.80; N, 10.57.

(11) J. H. Robson, submitted to THIS JOURNAL for publication.

Di-*n*-butylformamide was prepared by the method of Massie,¹² b.p. 101° (7.8 mm.), n_D^{20} 1.4400.

Acknowledgment.—The authors wish to express appreciation to Dr. Walter Edgell of Purdue University for suggestions in preparation of this manuscript.

(12) S. P. Massie, *Iowa State Coll. J. Sci.*, **21**, 41 (1946).

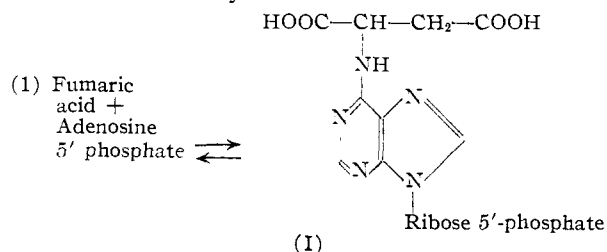
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COMMUNICATIONS TO THE EDITOR

ENZYMATIC SYNTHESIS OF ADENYLOSUCCINIC ACID¹

Sir:

From yeast autolysates a protein fraction has been prepared by ammonium sulfate precipitation and differential heat inactivation which is free of fumarase and catalyzes the reaction



The product of this reaction, tentatively assigned the structure I, [6-(succinylamino)-9-(ribofuranosyl 5'-phosphate)-purine] and the trivial name adenylosuccinic acid (AMP-S), has been isolated by ion exchange chromatography employing the Dowex-1, 2% cross linked resin in the chloride form and elution with a solution 0.02 *N* with respect to HCl and NH_4Cl . A solid amorphous preparation of the compound has been obtained by alcohol precipitation which is 92% pure on spectrophotometric analysis (E_M 267 $m\mu$ in 0.1 *N* HCl = 16.9×10^3 based on 1 mole of phosphorus), shows only one component on ion exchange and paper chromatography and whose elementary composition is in agreement with a mono-ammonium salt.

The following evidence supports the structure proposed: the organic phosphorus of the compound is quantitatively hydrolyzed to inorganic phosphate by bull semen 5'-nucleotidase² which is free of diesterase. Ribose and phosphate occur in the compound in equimolar proportion. The ultraviolet absorption spectrum of I exhibits a maximum at 267 $m\mu$ in acid (Fig. 1), a finding which also has been reported by Mason for 6-(methylamino)purine.³ Fumaric acid labeled with C^{14} in the carboxyl groups is incorporated enzymatically into I without dilution of relative molar specific activity.

(1) This work was supported by grants from the U. S. Public Health Service and the Atomic Energy Commission.

(2) L. A. Heppel and R. J. Hilmoe, *J. Biol. Chem.*, **188**, 665 (1951).

(3) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).

Carboxyl C^{14} labeled I is degraded by yeast enzyme free of fumarase at pH 7.0 to yield fumaric acid (90% radioactivity recovered) and AMP (equimolar with starting AMP-S), the products being determined by ion exchange and paper chromatography. Although titration data for I do not clearly demonstrate the carboxyl groups in the presence of the nucleotide phosphoryl group, ion exchange analysis indicates that I is more acidic than ADP, a finding in agreement with the structure proposed. That the amino group of adenine is the point of union with the succinyl residue is indicated by the spectral evidence cited and by the finding that muscle adenylic deaminase⁴ does not attack I until yeast enzyme has cleaved the compound to yield the AMP moiety. The foregoing evidence supports a reaction mechanism for the enzymatic synthesis of I analogous to the synthesis of argininosuccinic acid by "splitting" enzyme.⁵ An average equilibrium constant for reaction (1) calculated for

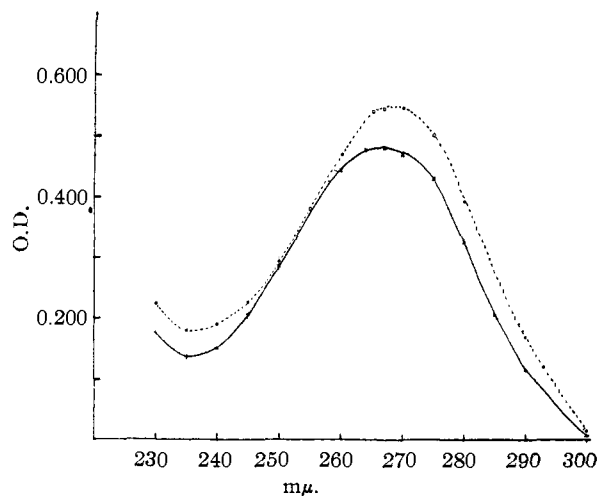


Fig. 1.—The ultraviolet absorption spectra of I at a concentration of 0.0285 μM . per ml. based on phosphorus determination: Solid line, 0.1 *N* HCl; broken line, 0.1 *N* KOH; E_M 267 $m\mu$, pH 1.0 = 16.9×10^3 ; E_M 270 $m\mu$, pH 12.0 = 19.2×10^3 .

(4) H. L. Kalckar, *J. Biol. Chem.*, **167**, 445 (1947).

(5) S. Ratner, "Advances in Enzymology," Vol. 15, p. 319, Interscience Publishers, Inc., New York, N. Y., 1954.

several concentrations of reactants and approaching equilibrium from synthesis and degradation is 12×10^{-3} (moles per liter) which corresponds to the constant 11×10^{-3} for the argininosuccinic acid reaction.⁵

Because of its relatively high absorption in the 280 m μ region, I may be readily determined in the presence of AMP and fumaric acid. Enzymatic synthesis and degradation of I is followed spectrophotometrically at this wave length and reversibility of the reaction is easily demonstrated.

Several metabolic consequences of the reaction described¹ are apparent. AMP-S may represent an intermediate in the mechanism for incorporating the amino group in purine nucleotides. Preliminary experiments which we have conducted with inosinic acid in pigeon liver preparations lend some support to this proposal. Should the analogy of reaction¹ with argininosuccinic acid metabolism be capable of extension, a role for carbamyl amino acid precursor or co-factor of both the amino group and the C-6 carbon of purines may be implicated.

The specificity of reaction (1) is limited at present to fumaric acid, adenosine 5'-phosphate, and adenine desoxyribosyl 5'-phosphate. The latter behaves in all respects like AMP. Similar reactions are being sought for other amino purine and pyrimidine nucleotides.

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APPLICATIONS OF FIELD IONIZATION TO MASS SPECTROMETRY

Sir:

The desorption of positive ions from surfaces by high electrostatic fields was first noted by E. W. Müller, who utilized the phenomenon in the field ion microscope.¹ In a recent publication from this laboratory preliminary mass spectrometric analyses of field produced ions from a few gases were reported,² and the mechanism of their formation discussed.³ This work has now been extended, and, as a consideration of Table I will indicate, promises to have interesting applications:

(1) Analysis of gas mixtures. Table I shows that parent peaks predominate in almost all cases investigated. For example, no peaks over 0.1%, except the parent ion, were observed with acetone. By contrast electron impact ionization of this gas produces 19 peaks of comparable intensity. The extreme simplicity of the spectra often permits the use of isotopic peaks for the direct determination of chemical formulas.

(2) Determination of transient intermediates in photochemical, thermal or radiation processes. For example, we are currently looking for the

(1) E. W. Müller, *Feldemission*, *Ergeb. d. Exakt. Naturwiss.*, **27**, 290 (1953).

(2) M. G. Inghram and R. Gomer, *J. Chem. Phys.*, **22**, 1279 (1954).

(3) It has recently come to our notice that similar conclusions regarding mechanism were reached by: F. Kirchner, *Naturwiss.*, **6**, 136 (1954), on the basis of Müller's results¹ and field induced changes in field emission patterns.

TABLE I
ION YIELDS FROM VARIOUS GASES WITH A TUNGSTEN FIELD IONIZATION SOURCE

Parent gas	Ions observed ^a (primary)	Ions observed ^b (secondary)
H ₂	H ⁺ (0.5), H ₂ ⁺ (0.5)	
D ₂	D ⁺ (0.5), D ₂ ⁺ (0.5)	
O ₂	O ₂ ⁺ (1.000)	
N ₂	N ₂ ⁺ (1.000)	
C ₂ H ₆	C ₂ H ₆ ⁺ (0.8), CH ₃ (or C ₂ H ₈ ⁺⁺)(0.2)	C ₂ H ₅ ⁺ , C ₂ H ₄ ⁺ , C ₂ H ₃ ⁺
C ₂ H ₄	C ₂ H ₄ ⁺ (1.000)	C ₂ H ₃ ⁺ , C ₂ H ₂ ⁺ , C ₂ H ⁺
CH ₄	CH ₄ ⁺ (1.000)	CH ₃ ⁺ , CH ₂ ⁺ , CH ⁺ , C ⁺
CH ₃ COCH ₃	CH ₃ COCH ₃ ⁺ (1.000)	
CH ₃ OH	CH ₃ OH ⁺ (0.7), CH ₃ O ⁺ (0.3)	

^a For the sake of clarity this table does not list isotopic peaks. Their magnitudes are in accord with accepted natural abundances. ^b Ions listed as secondary result from collisional or vibrational breakup of primary ions as shown by pressure dependence, peak shape and apparent fractional mass.

presence of CH₃ radicals in the photodecomposition of acetone. This application is feasible through the unique property of the field ionization source mentioned above.

(3) Information about fragmentation occurring on metal catalyst surfaces. We believe that the CH₃O⁺ peak observed in the case of methanol represents a substrate produced fragment. This is borne out by the observation that the CH₃OH⁺ peak broadens with increasing field while the CH₃O⁺ peak does not. This indicates that CH₃O⁺ ions can be formed only on the surface, since broadening corresponds to ion formation farther from the tip.

While substrate induced fragmentation interferes with the analytical applications indicated under (1) and (2), it is possible to suppress this phenomenon by suitable poisoning of the tip.

The use of pulsed fields should permit identification of ions produced in the chemisorbed layer, since the diffusion of gas to the vicinity of the tip can be made negligible in the time of a pulse. Variation of pulse repetition rates should permit the determination of sticking coefficients on individual crystal faces of the tip.

Our present experimental arrangement consists of a 12" radius of curvature direction focussing mass spectrometer equipped with an electron multiplier type of ion detector, capable of measuring currents of 10⁻¹⁹ ampere. A 30-mil hole in the screen of a field emission microscope permits a portion of the ion beam to pass into this instrument, so that the detector "sees" an area of about 900 Å.² of the tip surface. A focussing field ionization source for this mass spectrometer has been designed which should greatly increase the sensitivity of the method for analytical applications.

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