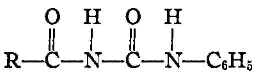
TABLE I



$$R-\overset{\overset{O}{\|}}{C}-\overset{\overset{H}{|}}{N}-\overset{\overset{O}{\|}}{C}-\overset{\overset{H}{|}}{N}-C_6H_5$$

R-	M. p., °C.	Solvent	Color	Yield, %	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
2-Furyl	186–188	EtOH	White	50	62.60	62.75	4.38	4.55	12.27	12.18
5-Bromo-2-furyl-	198–200	HOAc	White	98	46.59	46.91	2.93	2.95	9.06	9.35
5-Methyl-2-furyl-	162–163	EtOH	White	45	63.91	63.98	4.95	4.99	11.47	11.58
5-*t*-Butyl-2-furyl	186–188	EtOH	White	64	67.49	67.66	6.34	6.32	9.79	9.96
5-Nitro-2-furyl-	249–251	HOAc	Yellow	79	52.37	52.57	3.30	3.53	15.27	15.27
β-(2-Furyl)-vinyl-	198–201	EtOH	Tan	73	65.61	65.64	4.77	4.99	10.94	11.03

N-substituted furamides did not add normally to phenyl isocyanate and no characterizable product could be isolated from the reaction mixture.

Experimental

Starting Materials.—Details of the preparation of the substituted 2-furamides will appear in a later paper in this series.

Addition of Amides to Phenyl Isocyanate.—A mixture of the amide with a slight excess of phenyl isocyanate was heated under gentle reflux until entirely liquid (5–10 minutes). Ten milliliters of absolute ethanol was added cautiously to react with excess phenyl isocyanate and the solution was chilled. The solid which separated was isolated on a filter, washed with cold ethanol and recrystallized to constant melting point. The properties of the resulting 1-acyl-3-phenylureas are given in Table I.

Acylation of Phenylurea.—A solution of 6.75 g. (0.05 mole) of phenylurea in 20 ml. of benzene was heated to the reflux temperature and to the hot mixture was added slowly a solution of 6.5 g. (0.05 mole) of 2-furoyl chloride in 10 ml. of dry benzene. The mixture was heated under reflux while stirring vigorously for 6 hours.

The solution was cooled to room temperature and the solid which separated was isolated and recrystallized from hot ethanol to give 0.8 g. (7%) of 1-(2-furoyl)-3-phenylurea which melted at 187–188°. Mixing with the product from addition of 2-furamide to phenyl isocyanate did not depress the melting point of this product.

1-(5-Bromo-2-furoyl)-3-phenylurea was prepared similarly in 1% yield by acylation of a toluene solution of phenylurea with 5-bromo-2-furoyl chloride.

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COMMUNICATIONS TO THE EDITOR

ENZYMATIC PHOSPHORYLATION OF *l*-ASPARTATE

Sir:

The biological incorporation of amino acids into more complex compounds is known in certain instances[1] to utilize energy from ATP.[2] However, phosphorylated intermediates have not been demonstrated in these processes, and the mechanisms of energy transfer are obscure. The enzymatic formation of an "energy-rich" amino acid-phosphate compound, β-aspartyl phosphate, is described here. Suggestive evidence that this substance is a precursor of asparagine is the presence in the enzyme preparation of a very active asparagine "transferase" similar to the enzymes found by Waelsch and co-workers[3] in certain bacteria.

The enzyme was obtained from an extract of baker's yeast and purified about 10-fold with weak acid and ammonium sulfate. It appears to catalyze the reaction

$$\text{ATP} + l\text{-aspartate} \rightleftharpoons \text{ADP} + \beta\text{-aspartyl phosphate}$$

Though its equilibrium is unfavorable to accumulation of the new compound, the reaction may be followed readily if hydroxylamine is used to trap the aspartyl phosphate, forming a hydroxamic acid, as has been done with acetyl phosphate.[4] In Table I are shown the essential components of the system and the effect on hydroxamate formation of omitting each. Substitution of *d*-aspartate or *l*-glutamate reduced hydroxamic acid formation to 2 and 6%, respectively, of the value with *l*-aspartate.

TABLE I

All tubes contained 0.4 μM. hydroxylamine hydrochloride brought to *p*H 8.0 with tris. The incubation was at 30° for 30 minutes in a total volume of 1.0 ml.

Component omitted	Hydroxamic acid formed, μM.
None	3.2
25 μM. *l*-aspartate (potassium salt)	0.09
10 μM. ATP (sodium salt)	0.00
10 μM. $MgCl_2$	0.14
Enzyme, 0.1 ml.	0.00

High concentrations of aspartate and ATP favor synthesis of aspartyl phosphate, as shown in Table II. In this experiment hydroxylamine at *p*H 4.0 was added to stop the enzymatic activity after samples had been taken for phosphate analyses. Acyl phosphate was determined as hydroxamic acid,[5] and as the difference between inorganic

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[1] P. P. Cohen in W. D. McElroy and B. Glass, "Phosphorus Metabolism," The Johns Hopkins Press, Baltimore, Md., 1951, Vol. I, p. 630.

[2] Abbreviations used are ATP (adenosine triphosphate), ADP (adenosine diphosphate), AMP (adenosine-5-phosphate), and tris-[tris-(hydroxymethyl)-methylamine].

[3] H. Waelsch, *Advances in Enzymol.*, **13**, 237 (1952).

[4] F. Lipmann, *ibid.*, **6**, 231 (1946).

[5] F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945).

phosphate values obtained with the Fiske–SubbaRow[6] and Lowry–Lopez[7] methods.

TABLE II

The complete system contained in 1.0 ml. 10 μM. $MgCl_2$, 40 μM. ATP, 650 μM. *l*-aspartate, and 0.1 ml. enzyme. Both substrates were adjusted to *p*H 8.0 with tris. The incubation was at 30° for 45 minutes. Shown in parentheses are the inorganic phosphate values from which acyl phosphate was calculated.

	Acyl phosphate	
	Hydroxamic acid method, μM.	Fiske–SubbaRow P minus Lowry-Lopez P, μM.
Omit aspartate	0.02	0.1 (0.6–0.5)
Complete system	2.6	2.7 (5.6–2.9)

ADP appears to be the other reaction product, though side reactions prevent its stoichiometric demonstration. Using low initial substrate concentrations, and hydroxylamine as a trapping agent, disappearance of 1 μM. of ATP was accompanied by formation of 1.3 μM. of inorganic phosphate, 1.04 of hydroxamate, 0.64 of ADP, and 0.13 μM. of AMP. This result was not significantly changed by the presence of 0.05 molar potassium fluoride. ATP was determined by the method of Kornberg,[8] ADP and AMP according to Kalckar.[9]

Asparthydroxamic acid derived from the reaction product was characterized as the beta isomer by chromatographic comparison with beta and alpha asparthydroxamic acids. These were prepared by heating the corresponding amides, asparagine and isoasparagine, with hydroxylamine. When mixed with the substance obtained from the enzymatic reaction, only the mixture with alpha isomer could be separated into two hydroxamate fractions.

We wish to express our appreciation to Dr. J. P. Greenstein for a generous gift of isoasparagine.

(6) C. H. Fiske and Y. SubbaRow, *J. Biol. Chem.*, **66**, 375 (1925).

(7) O. H. Lowry and J. A. Lopez, *ibid.*, **162**, 421 (1946).

(8) A. Kornberg, *ibid.*, **182**, 779 (1950).

(9) H. M. Kalckar, *ibid.*, **167**, 445 (1947).

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RECEIVED MARCH 17, 1953

THE PREPARATION OF SAMARIUM AND YTTERBIUM METALS[1]

Sir:

After many attempts, we have succeeded in preparing massive samarium metal. Previous workers have reported the preparation of this metal by reducing samarium chloride with potassium[2] and by electrolyzing a molten salt-bath containing samarium chloride[3], but neither of these preparations gave a product which permitted characterization of this metal. Attempts to prepare samarium in our laboratory by these methods, while not exhaustive, were not fruitful, and indicated that a better method of preparing samarium was very desirable. Numerous attempts were made to reduce samarium halides by active metals including lithium, sodium, potassium, barium, calcium and magnesium,[4–7] but in each case the divalent halide of samarium was the product, indicating a high position in the electromotive series for the $Sm–Sm^{++}$ couple.

In preparing some of the heavy rare earth metals,[8] several of them were found to be distinctly more volatile than lanthanum. Vapor pressure measurements have been made on lanthanum[5] and dysprosium which indicate that dysprosium has a vapor pressure about 300 times that of lanthanum at the same temperature. Preliminary chemical and metallurgical studies on samarium indicated that it too might be more volatile than lanthanum. This low vapor pressure of lanthanum compared to other rare earth metals, its low melting point and the high heat of formation of its oxide, along with the possible high vapor pressure of samarium, suggested the preparation of samarium by distilling it from a heated mixture of samarium oxide and lanthanum metal.

Using a welded tantalum crucible[9] eight inches long and one inch in diameter with walls 2.5 mils thick, 20 g. of freshly ignited samarium oxide (98% pure, the balance consisting of other rare earths) and 20 g. of freshly prepared lanthanum turnings were heated under a vacuum of less than 1 micron to 1450° and held at this temperature for 30 minutes. The upper half of the crucible extended out of the furnace and had a perforated tantalum lid. On opening, a silvery crystalline metallic deposit was found on the upper walls of the crucible and on the bottom of the cap. Analysis of the deposit showed it to be samarium metal of greater than 98% purity with no lanthanum detectable. In a subsequent preparation, 25 g. of metal was obtained representing a yield of over 80% from the original oxide.

Thirty grams of metal prepared in this manner was melted in a tantalum crucible under a pressure of one atmosphere of purified argon. The melting point as determined with an optical pyrometer was between 1025 and 1050°, which is considerably below the figure of 1300 to 1350° given for previous preparations.[3,10] The bulk density of this fused specimen was found to be 7.53 g./cc., which would represent an atomic volume of 20 cc./mole. This would place samarium in line with the "regular" rare earths on this basis, instead of with europium and ytterbium with which it is chemically associated. Preliminary X-ray diffraction studies on single crystals separated from the condensate indicate that samarium is

(1) Contribution No. 220 from the Institute for Atomic Research and Department of Chemistry, Iowa State College. Work was performed in the Ames Laboratory of the Atomic Energy Commission.

(2) W. Klemm and H. Bommer, *Z. anorg. allgem. Chem.*, **231**, 138 (1937).

(3) W. Muthmann and L. Weiss, *Ann.*, **331**, 1–46 (1904).

(4) D. H. Ahmann, U. S. Atomic Energy Commission, AECD-3205 1950.

(5) A. H. Daane, U. S. Atomic Energy Commission, AECD-3209, 1950.

(6) F. H. Spedding, H. A. Wilhelm, W. H. Keller, D. H. Ahmann, A. H. Daane, C. C. Hach and R. P. Ericson, *Ind. Eng. Chem.*, **44**, 553 (1952).

(7) F. H. Spedding and A. H. Daane, THIS JOURNAL, **74**, 2783 (1952).

(8) A. H. Daane and F. H. Spedding, accepted for publication in *J. Electrochem. Soc.*

(9) A. H. Daane, *Rev. Sci. Instr.*, **23**, 245 (1952).

(10) W. Guertler and M. Pirani, *Z. Metallkunde*, **11**, 1 (1919).