ino acids remain stable for a long period, but N-carboxylic acid anhydrides are unstable in the presence of moisture and cannot survive storage, so that it is easier to copolymerize several sorts of amino acids. Some protein analogs were prepared by this method (Table I).

A detailed account of this work will be published in *J. Chem. Soc. of Japan*.

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ISOLATION, STRUCTURE AND SYNTHESIS OF A LATHYRUS FACTOR FROM *L. ODORATUS*[1]

Sir:

The isolation from *Lathyrus odoratus* seeds of a crystalline substance capable of producing the skeletal abnormalities characteristic of lathyrism has recently been accomplished.[2-4] The substance (I) obtained in this Laboratory, m.p. 193–194° dec.,[5] was water-soluble, ninhydrin-positive, and gave analytical values agreeing with the formula $C_8H_{13}O_3N_3$.[3] It showed only one ninhydrin spot when subjected to paper chromatography in three different solvent systems; however, after hydrolysis in 11.7 *N* hydrochloric acid for 8 hours at 120° this spot disappeared and was replaced by two others. On concentrating and cooling the hydrolysis mixture, L-glutamic acid hydrochloride precipitated. This fragment was identified by m.p., ultimate analysis, infrared spectrum, optical rotation and microbiological assay. When the filtrate was made alkaline and distilled, a volatile base was evolved which was identified as ammonia by conversion to ammonium chloride and demonstrating the absence of carbon by the method of Pepkowitz.[6] This result together with a sharp band at 4.45 μ in the infrared spectrum pointed to the presence of a nitrile function in I. On this basis the remaining hydrolysis product could be only sarcosine, α-alanine or β-alanine. Comparative paper chromatograms clearly pointed to β-alanine as the actual degradation product, and its presence in the hydrolysate was verified by isolation of the β-naphthalene sulfonate of β-alanine,[7] m.p. 134–136°, both alone and mixed with an authentic sample. It was concluded that I is β-(γ-L-glutamyl)-aminopropiononitrile or the α-glutamyl isomer. The γ-glutamyl structure was favored because I showed pK values[3] of 2.2 ± 0.2 and 9.1 ± 0.1. Accordingly a substance of this structure was synthesized by the method of King and Kidd[8] by condensation of β-aminopropiononitrile with N-phthaloyl-L-glutamic anhydride and subsequent removal of the phthaloyl substituent with hydrazine. The synthetic product melted with decomposition at 193.5–194°[5] and showed no depression on admixture with isolated I. The identity of the two products was confirmed by a comparison of their infrared spectra which were alike in all respects. The lathyrus activity of the synthetic compound in rats is being investigated.

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PHENYLACETYLGLUTAMINE AS A CONSTITUENT OF NORMAL HUMAN URINE

Sir:

Phenylacetylglutamine (PAG) was first described by Thierfelder and Sherwin[1] who isolated the compound from the urine of individuals fed phenylacetic acid. The conjugate is frequently referred to as a "detoxication" product. In investigating the source of the amino acids liberated by acid hydrolysis of human urine,[2] it has been found that PAG is excreted under normal conditions by the adult male to the extent of 250 to 500 mg. per day, and accounts for about 50% of the conjugated glutamic acid in urine.

Identification of PAG has been effected by chromatographic analyses of urine employing acidic and basic ion-exchange resins (Dowex 50 and Dowex 2). PAG and other conjugates were detected in the effluent by hydrolysis of 1-ml. effluent fractions with 6 *N* HCl or 2 *N* NaOH prior to the application of the photometric ninhydrin method.[3] Because of the affinity of the resin for aromatic compounds, PAG, despite its acidic nature, is retarded on columns of Dowex 50X8 and emerges at a position between urea and aspartic acid.[2] The conjugate was more readily determined by chromatography of 4-ml. samples of urine on 0.9 × 30 cm. columns of Dowex 2X4 (200–400 mesh) in the acetate form. Elution was begun with 0.2 *M* sodium acetate buffer at *p*H 5.3.[4] After 100 ml., the *p*H and ionic strength were gradually changed by allowing a 2 *M* sodium acetate buffer of *p*H 4.6[5] to flow into 100 ml. of the initial 0.2 *M* buffer stirred magnetically. The compound giving rise to a major peak at about 195 ml. was identified as PAG by the demonstration that the unknown and a sample of synthetic PAG[6] exhibited identical chromatographic behavior on columns of both Dowex 2 and Dowex 50, together with the finding of equimolar amounts of glutamic acid and ammonia in an acid hydrolysate of the unknown.

The same analytical procedure afforded a chro-

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(4) 27.2 g. of $NaOAc \cdot 3H_2O$ + 5.0 ml. of glacial HOAc + 2.5 ml. of 50% BRIJ-35 solution diluted to 1 l. with water. BRIJ-35 is a detergent manufactured by Atlas Powder Co., Wilmington, Delaware.

(5) 54.4 g. of $NaOAc \cdot 3\ H_2O$ and 10.0 ml. of glacial HOAc are made to a volume of 100 ml. with water. To this solution 30 ml. of glacial HOAc, 70 ml. of water and 0.2 ml. of BRIJ-35 solution are added.

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