about 40 positive charges. The effect of salt would be to decrease the repulsive forces due to these charges and allow a greater proportion of the molecular collisions to be effective in bonding.

At the regenerating *p*H of about 12.3 one would expect the amino groups to lose their charge while the carboxyl groups and some hydroxyl groups would now ionize and become charged. Thus the net charge would change from an average of roughly +40 to over −80. It seems clear that the great increase in net charge in going from acid to alkali would set up stronger repulsive forces which would aid in disrupting the inter-insulin bond. Evidence in support of this mechanism as being part of the process comes from the action of salt, which decreases reversion as shown in Fig. 3, and those experiments which indicate that reversion proceeds at the ends of the fibrils where the inter-insulin bonds would be weakest since fewer insulin units would be engaged in their stabilization. Reversion and the inter-insulin bond are receiving further attention.

Acknowledgment.—The author wishes to acknowledge the generous support which Armour and Company has given to this and other research.

Summary

Insulin fibrils may be reverted by treatment with alkali to give a crystalline product similar to native insulin: the alkali has therefore regenerated an insulin termed r-insulin. Limiting conditions for regeneration procedure were determined by studying the effect of alkali on native insulin. Using 0.5 ml. of 2% insulin (10 mg.) and 5.0 ml. of sodium hydroxide experiments indicated 0.03 *N* alkali, 0°, and forty-five minute treatment time would be optimal. This was found to be the case. In addition reversion of fibrils is greatly accelerated by increasing the number of available fibril ends suggesting that disaggregation occurs mainly at these positions. Sodium chloride in the alkali inhibits reversion by 90% in 1.0 *N* concentration. Thus, the repulsive forces between similarly charged groups may play a part in the mechanism of disaggregation.

The crystalline product from reverted fibrils is not significantly different from native insulin in certain intrinsic properties such as: crystallization, in which r-insulin will not crystallize in the absence of zinc, and recrystallization recovery; biological activity (20 I.U. per mg.); ultracentrifuge pattern (sedimentation constant 3.3–3.6); and fibril formation (at 20 and 100°). Tests for changes in labile groups, such as amino and disulfide, have been negative.

The absence of changes in labile groups, the retention by r-insulin of the characteristic properties of insulin, the known sensitivity of these characteristic properties to structural changes, and the fact that fibril elongation may take place at low temperatures in the *p*H region of maximum stability, are interpreted as showing that only small structural changes take place during fibril formation and that the process is therefore one in which globular or corpuscular units are linked endwise.

CAMBRIDGE 39, MASSACHUSETTS
RECEIVED NOVEMBER 18, 1947

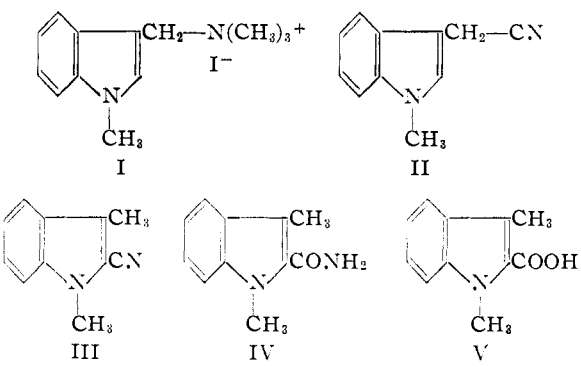
[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

An Allylic Rearrangement in an Alkylation by a Quaternary Ammonium Salt

BY H. R. SNYDER AND ERNEST L. ELIEL

In a previous communication[1] it was stated that the reaction of 1-methylgramine methiodide (I) with aqueous sodium cyanide produced not only 1-methyl-3-indoleacetonitrile (II) but also an isomer of this nitrile. This isomer has now been identified as 1,3-dimethyl-2-cyanoindole (III). The pure isomer (III) was isolated from the reaction mixture in 4.3% yield, but in view of the difficulties encountered in the purification it is believed to have been formed in an appreciably larger amount, perhaps to the extent of 10–15%.

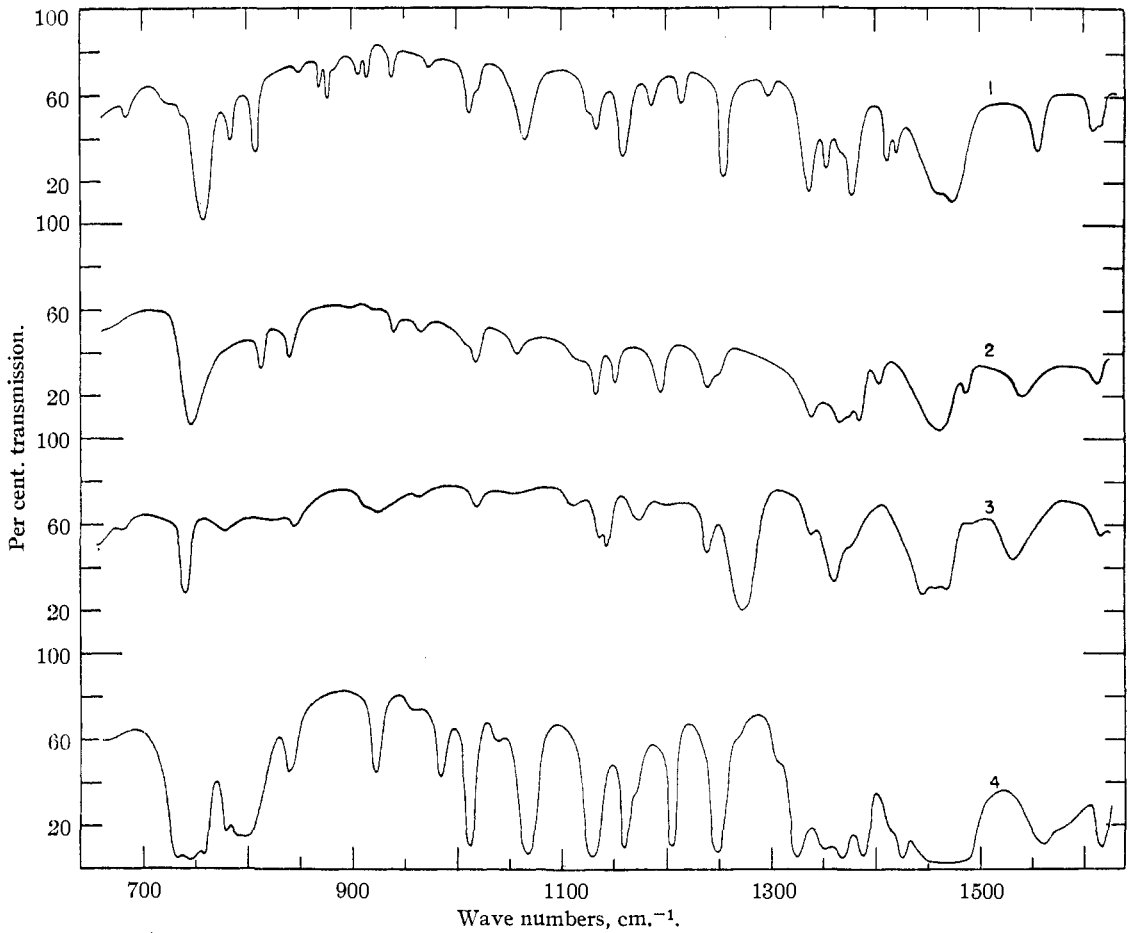
Comparison of the infrared absorption spectra of (II) and its isomer revealed a shift of the CN-absorption band of 36 cm.$^{-1}$ toward smaller wave numbers in the case of the isomer (III) (see the figures), indicating conjugation of the cyano group with one of the double bonds of the rings. Alkaline hydrolysis of the isomeric nitrile (III) yielded mainly the corresponding amide and only very



small amounts of the acid, probably because of steric hindrance of the nitrile function. The acid was finally obtained in poor yield by increasing the concentration of alkali and extending the reaction time in the hydrolysis.

The acid (V) was synthesized by a known

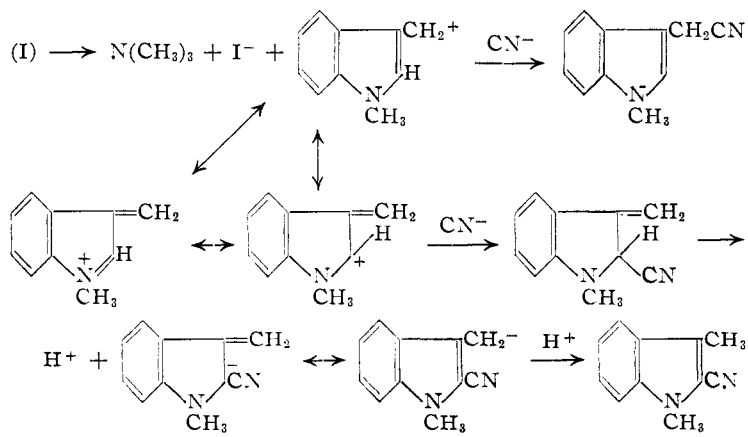
(1) Snyder and Eliel, THIS JOURNAL, **70**, 1703 (1948).



Figs. 1a, b.—Infrared absorption spectra: 1, 1-methyl-3-indoleacetonitrile; 2, 1,3-dimethyl-2-cyanoindole; 3, 1,3-dimethyl-2-indolecarboxylic acid (two identical spectra); 4, 1,3-dimethylindole (two identical spectra). Curves 1, 2 and 3 were obtained from Nujol suspensions. The absorption bands due to Nujol C–H frequencies occur

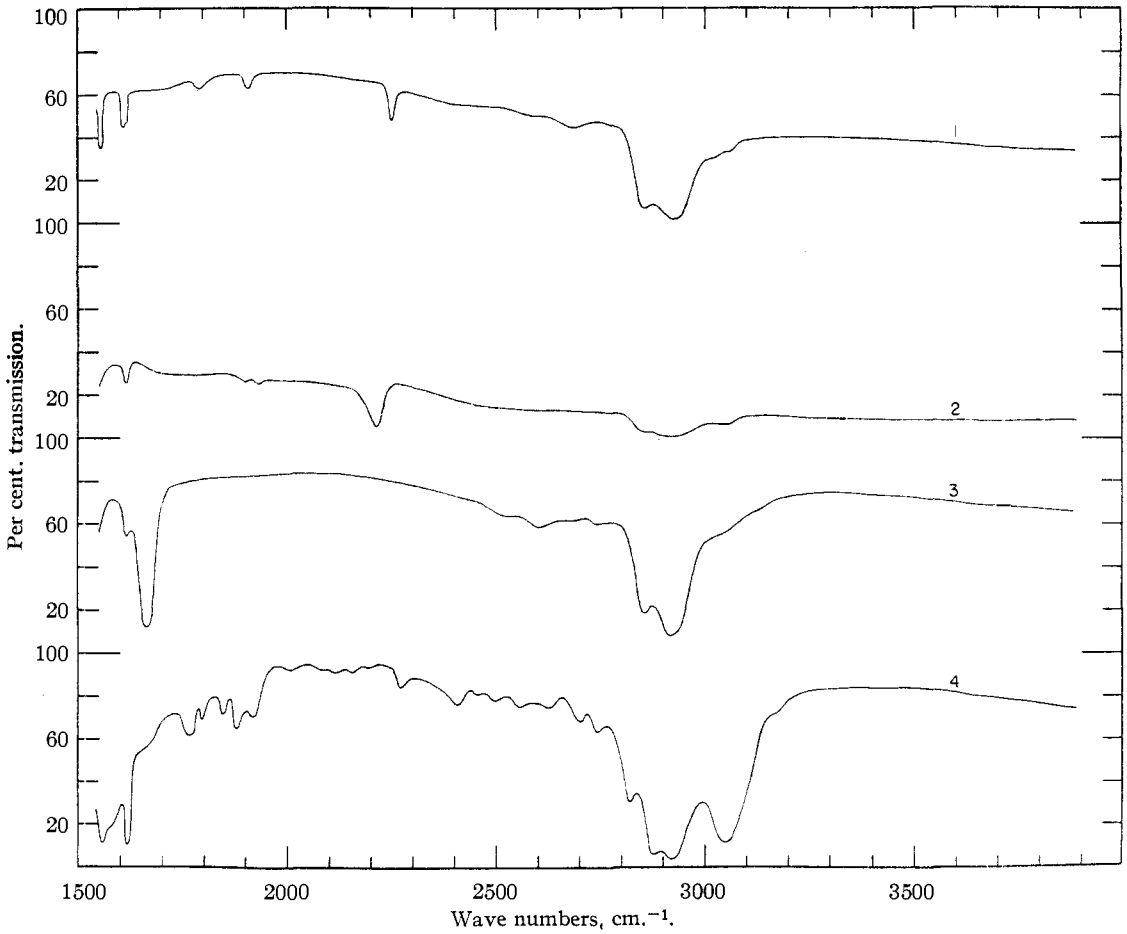
method[2] from *as*-methylphenylhydrazine and α-ketobutyric acid obtained by hydrolysis of α-benzoylaminocrotonic azlactone.[3,4] 1,3 - Dimethyl - 2 - indolecarboxylic acid (V) thus obtained proved to be identical with the hydrolysis product of the nitrile (III). The acid (V) prepared by nuclear synthesis was converted into its amide by treatment with phosphorus pentachloride in acetyl chloride solution followed by reaction with concentrated aqueous ammonia. This amide was identical with the one from the partial hydrolysis of the nitrile (III). Formulas III and IV must therefore be assigned to this nitrile and the corresponding amide.

It has thus been proved that in the reaction of 1-methylgramine methiodide (I) with sodium cyanide rearrangement occurs along with the normal reaction. The migration may be explained by the provisional assumption that the



quaternary base (I) dissociates into trimethylamine and a carbonium ion which may rearrange, as shown in the accompanying diagram. An analogy exists in the reaction of furfuryl chloride with sodium cyanide to give 5-methyl-2-furo-

(2) Kermack, Perkin and Robinson, *J. Chem. Soc.*, **119**, 1602 (1921).
(3) Carter, Handler and Melville, *J. Biol. Chem.*, **129**, 359 (1939).
(4) Carter and Stevens, *ibid.*, **133**, 117 (1940).



at 2920, 2855, 1460 and 1375 cm.$^{-1}$. Sample no. 4 was seen in a liquid cell of 0.05 mm. thickness. The low transmission of the two nitriles in the high frequency regions is due to the light scattering of the crystals in the Nujol suspension.

nitrile.[5,6] Simple α,β-unsaturated halides, on the other hand, do not seem to undergo rearrangement in reactions with sodium cyanide,[5] but do so in reactions with sodium carbonate in water and with various metallic acetates in acetic acid solution.[7–10]

Experimental[11,12]

2-Cyano-1,3-dimethylindole (III).[1]—The product of the reaction of 18.1 g. of 1-methylgramine methiodide with sodium cyanide[1] was fractionated *in vacuo* through a short Vigreux column. The fraction collected at 96–109° (0.15 mm.) and twice recrystallized from petroleum ether (b. p. 30–60°) formed large white prisms of m. p. 69.5–70.5°, weight 0.32 g. The next fraction, b. p. 109–120° (0.15 mm.), was twice extracted with hot petroleum ether (b. p. 30–60°). After the waxy residue of this extraction had been freed of insoluble oils by pressing on a porous plate, it dissolved in the hot petroleum ether extract. The crystals which separated from the cooled solution were recrystallized five times from petroleum ether to yield 0.08 g. of material of m. p. 69–70.5° [total yield, 0.40 g. (4.3%)].

1,3-Dimethyl-2-indolecarboxamide (IV). From III.—A solution of 0.39 g. of the above nitrile and 1 g. of potassium hydroxide in 1 ml. of water and 9 ml. of ethanol was refluxed for twenty-two hours. After dilution of the mixture with 4 ml. of water most of the ethanol was distilled. Crystals of the amide (IV) which separated from the cooled solution were collected, washed and dried; yield, 0.30 g. (69.5%). The amide crystallized from benzene in fine white needles, m. p. 213–214°.

Anal. Calcd. for $C_{11}H_{12}N_2O$: C, 70.18; H, 6.43; N, 14.89. Found: C, 70.18; H, 6.48; N, 14.70.

From the alkaline mother liquors of the hydrolysis of the nitrile, only 0.01 g. of acid could be isolated.

1,3-Dimethyl-2-indolecarboxylic Acid. From the Amide.—A solution of 0.19 g. of the above amide and 1 g. of potassium hydroxide in 0.5 ml. of water and 4.5 ml. of ethanol was refluxed for fifty-six hours. After dilution of the mixture with 4.5 ml. of water the alcohol was distilled. The cooled solution was filtered to remove unchanged amide. The filtrate was extracted with ether, boiled with charcoal, filtered, and acidified. The acid was collected, washed with water, and dried; yield, 0.04 g. (21%). After recrystallization from benzene–petroleum ether (b. p. 30–60°), from benzene, and again from benzene–petroleum ether, the acid melted at 215–216° (dec.).

(5) Reichstein, *Ber.*, **63**, 749 (1930).
(6) Runde, Scott and Johnson, THIS JOURNAL, **52**, 1284 (1930).
(7) Young and Andrews, *ibid.*, **66**, 421 (1944).
(8) Claisen, *J. prakt. Chem.*, [2] **105**, 65 (1922–1923).
(9) Meisenheimer and Beutter, *Ann.*, **508**, 58 (1933).
(10) Roberts, Young and Winstein, THIS JOURNAL, **64**, 2157 (1942).
(11) All melting points are corrected.
(12) Microanalyses by Miss Theta Spoor and Miss Jane Wood.

Anal. Calcd. for $C_{11}H_{11}NO_2$: C, 69.81; H, 5.86. Found: C, 69.97; H, 5.79.

From **α-Benzoylaminocrotonic Azlactone.**—Five grams of a mixture of the *cis* and *trans* forms of α-benzoylaminocrotonic azlactone[13] of m. p. 118–122° was hydrolyzed by refluxing for three hours with 200 ml. of 1 *N* hydrochloric acid.[3] After the solution had cooled, 3 g. of what was presumably a mixture of α-benzoylaminocrotonic and benzoic acids was removed by filtration. The filtrate was neutralized with 2 *N* sodium hydroxide, and a solution of 3.4 g. of *as*-methylphenylhydrazine in 10 ml. of water and 5 ml. of glacial acetic acid was added to it. After standing overnight the solution was chilled and the solid methylphenylhydrazone that had separated was collected and washed. It was suspended in a solution of 15 ml. of concentrated hydrochloric acid in 30 ml. of water, and the mixture was heated on the steam-bath with swirling for thirty minutes. The suspension was cooled and filtered, and the solid was dissolved in aqueous sodium hydroxide. After removal of oily impurities by ether extraction, the aqueous solution was boiled with charcoal, filtered and acidified. The acid that separated was collected, washed with water and dried at 60°; yield, 1.3 g. (29.4% from the azlactone). After three recrystallizations from benzene, the melting point of the acid (and of mixtures with the acid described in the preceding paragraph) was 215–216° (dec., lit.,[2] 213°). The infrared absorption spectra[14] of the samples obtained by the two different methods were identical (see the figures). Both samples separated from benzene solution as needles which in contact with the mother liquor soon changed into crystals of granular texture.

Decarboxylation of the above acid at 225° followed by distillation at 15 mm. (bath temperature 130–160°) yielded 1,3-dimethylindole, identified by its refractive index ($n^{20}D$ 1.5929), infrared absorption spectrum (see the figure), and the melting point and mixed melting point (142.5–143°) of the picrate. The sample used for comparison had been obtained by hydrolysis and decarboxylation of 1-methyl-3-indoleacetonitrile (II).[1]

1,3-Dimethyl-2-indolecarboxamide. From the Acid.—A suspension of 0.32 g. of the above acid (V) in 3.2 ml. of redistilled acetyl chloride was cooled in an ice-bath and 0.42 g. of phosphorus pentachloride was added. The mixture was swirled until homogeneous and then allowed to stand at room temperature for two and three-fourths hours. The solvent was removed *in vacuo* with the bath temperature not exceeding 45°. The solid residue was chilled and 10 ml. of ice-cold concentrated aqueous ammonia solution was added to it. The temperature was slowly raised to 72° over a period of thirty minutes with constant stirring. The suspension of the amide was then cooled and the solid was collected, washed with concentrated aqueous ammonia followed by water, and dried at 60°; yield, 0.28 g. (87.5%). After two recrystallizations from benzene–absolute alcohol the product melted at 213.5–214°. The mixed melting point with the amide obtained by hydrolysis of 1,3-dimethyl-2-cyanoindole (III) was 213–214°.

(13) The azlactone was kindly put at the authors' disposal by Dr. H. E. Carter.

(14) The authors are indebted to Mrs. Agatha Roberts Johnson for the absorption studies.

Summary

The reaction of the methiodide of 1-methyl-3-dimethylaminomethylindole with aqueous sodium cyanide affords, in addition to the normal alkylation product, a small amount of 1,3-dimethyl-2-cyanoindole. The structure of this product has been proved by conversion to the corresponding amide and acid which were identical with compounds obtained by independent syntheses.

URBANA, ILLINOIS RECEIVED JANUARY 2, 1948

[CONTRIBUTION FROM DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

Studies on Double Refraction of Flow. IV. Human Serum γ-Globulin and Crystallized Bovine Serum Albumin[1]

BY JOHN T. EDSALL AND JOSEPH F. FOSTER[2]

In previous papers of this series, the molecular dimensions of zein[3] and of fibrinogen[4] have been studied by the method of double refraction of flow, the results being interpreted in the light of viscosity, sedimentation and other measurements. In the present study, we report results of similar investigations on human serum γ-globulin[5] and crystallized bovine albumin.[6] The orientation of these molecules, by means of a velocity gradient, to an extent sufficient for accurate double refraction measurements, required the use of solvents of high viscosity, in order to diminish the rotary Brownian movement. Whereas fibrinogen, with a molecular length near 700 Å., could be readily studied in 40% glycerol, γ-globulin required 60–76% glycerol, and serum albumin approximately 90% glycerol.

(1) This paper is Number 68 in the series "Studies on the Plasma Proteins" from Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry, and Number XVII in the series "Preparation and Properties of Serum and Plasma Proteins" from the same laboratory.

The preparations of serum globulin employed were prepared from blood collected by the American Red Cross, under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

(2) Present address, Department of Chemistry, Iowa State College, Ames, Iowa.

(3) J. F. Foster and J. T. Edsall, THIS JOURNAL, **67**, 617 (1945).

(4) J. T. Edsall, J. F. Foster and H. Scheinberg, *ibid.*, **69**, 2731 (1947).

(5) J. L. Oncley, M. Melin, D. A. Richert, J. W. Cameron and P. M. Gross, Jr., in preparation.

(6) E. J. Cohn, W. L. Hughes, Jr., and J. H. Weare, *ibid.*, **69**, 1753 (1947).

Experimental Methods

The apparatus used[7] and the methods of measurement[3,4] have already been described in detail.

The great majority of the measurements on γ-globulin were made on a single preparation (IIGl-L371) prepared from Fraction II + III of human plasma[8] by method 3c as described by Oncley, *et al.*[5] Electrophoretically, this preparation contained 97% γ-globulin, 2% albumin, and 1% β-globulin. Such preparations, however, have been

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(8) E. J. Cohn, L. E. Strong, W. L. Hughes, Jr., D. J. Mulford, J. N. Ashworth, M. Melin and H. L. Taylor, THIS JOURNAL, **68**, 459 (1946).