0.25 ml. (4 millimoles) of base methyl iodide for 4 hours. The product was precipitated with water, centrifuged and washed. Hydrolysis was carried out as previously described. The yield was 0.35 g. or 90% of theoretical. The melting point was 68–70°. One recrystallization would bring the melting point up to the desired 73–75°.

The specific activity as determined with an external counter (2.1 mg./cm.^{2}) on a Raychronometer was 2.54 microcuries/mg.; this was 99.4% of theoretical. The overall radioactive yield based on the number of milligrams of compound obtained was 89.5%.

CANCER RESEARCH LABORATORY
UNIVERSITY OF FLORIDA
GAINESVILLE, FLORIDA

Countercurrent Distribution of Sheep Adrenocorticotropic Protein Preparations

BY GEORGE P. HESS, FREDERICK H. CARPENTER AND CHOH HAO LI

RECEIVED MARCH 3, 1952

Although the adrenocorticotropic hormone (ACTH) preparations isolated from sheep and pig pituitary glands by the method of Li, *et al.*, and Sayers, *et al.*,[1] have been found to behave as homogeneous proteins in electrophoretic, ultracentrifugal and solubility studies, considerable evidence[2] has accumulated which indicates that the ACTH activity is not restricted to a protein with the properties described by Li, *et al.*, and Sayers, *et al.*[1] When submitted to partial pepsin and acid hydrolysis, the protein preparations retained biological activity.[2a-e] These results could be interpreted to mean that only a portion of the protein molecule was necessary for biological activity. However, material with an ascorbic acid depleting activity[3] up to 100 times greater than the activity of the protein preparations, has been prepared from the extracts of the pituitary gland.[2f,g,h] In addition, Dixon, *et al.*,[4] obtained a separation of the adrenal weight-increasing activity from the adrenal ascorbic acid-depleting activity in a pig ACTH protein preparation, using ion exchange chromatography. These observations may be explained in part by the assumption that the protein preparations were not homogeneous.

In an attempt to answer the question of homogeneity, we have subjected the protein preparations to other separation techniques. In a recent communication from this Laboratory[5] it was reported that the ACTH protein preparation could be separated into two fractions by electrodialysis. One fraction, the cathode material, possessed nearly all the biological activity of the original protein preparation as measured by the adrenal ascorbic acid depleting method.[3] Further evidence for the inhomogeneity of the ACTH protein preparations has now been obtained from investigations which have made use of the countercurrent distribution method of Craig.[6]

The ACTH protein preparations were submitted to countercurrent distribution between 2,4,6-collidine and water, a solvent system which has been described previously[7] for the investigations of partial peptic hydrolysates of the ACTH protein.[2e] The results of a typical experiment in which 47 mg. of ACTH protein preparation was subjected to a 25-transfer distribution are shown in Table I and Fig. 1. At least three components were revealed. The main component (Tubes 0-14) contained

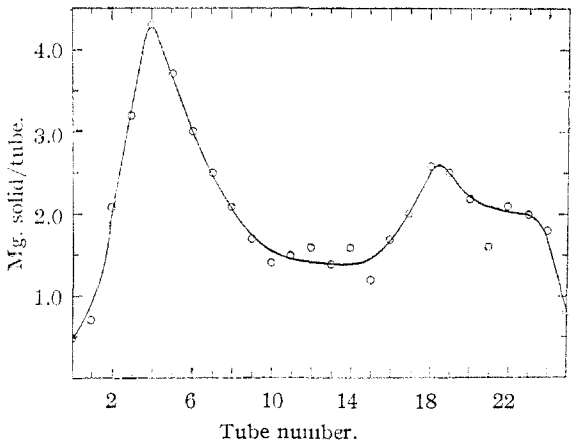

Fig. 1.—Twenty-four transfer distribution of sheep ACTH protein between 2,4,6-collidine and water.

TABLE I

DISTRIBUTION OF SOLIDS AND BIOLOGIC ACTIVITY IN VARIOUS FRACTIONS OBTAINED BY A 25-TRANSFER DISTRIBUTION OF A SHEEP ACTH PROTEIN PREPARATION BETWEEN COLLIDINE AND WATER

Fraction	Amount, mg.	Dose, μg.	Depletion of ascorbic acid, mg./100 g. adrenal	ACTH potency, I.U./mg.
Starting material	47	2	−140, −118, −143, −117, −78, −99, −112, −142, −101	2
Tubes[a] 0–14	31	5	−29, −62, −64, −57, +28, +32	0.04
Tubes[a] 15–20	12	1	−68, −75, −76, −49, −59, −52, −120, −91, −41, −76, −44, −48, −49, −44	1
Tubes[a] 21–25	8	0.2	−69, −78, −78, −107, −81, −142, −89, −74, −85, −63, −116	10

[a] See Fig. 1.

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(2) (a) C. H. Li, *Trans. Macy Conf. on Metabolic Aspects of Convalescence*, **17**, 114 (1948); (b) N. G. Brink, M. A. P. Meisinger and K. Folkers, THIS JOURNAL, **72**, 1040 (1950); (c) J. B. Lesh, J. D. Fisher, I. M. Bunding, J. J. Koesis, L. S. Walaszek, W. F. White and E. E. Hays, *Science*, **112**, 43 (1950); (d) C. H. Li, THIS JOURNAL, **73**, 4146 (1951); (e) N. G. Brink, F. A. Kuehl, Jr., M. A. P. Meisinger, M. N. Bishop and K. Folkers, *ibid.*, **74**, 480 (1952); (f) R. W. Payne, M. S. Raben and E. B. Astwood, *J. Biol. Chem.*, **187**, 719 (1950); (g) B. Cortis-Jones, A. C. Crooke, A. A. Henly, P. Morris and C. J. O. R. Morris, *Biochem. J.*, **46**, 173 (1950); (h) H. B. F. Dixon, S. Moore, M. P. Stack-Dunne, and F. G. Young, *Nature*, **168**, 1044 (1951).

(3) M. Sayers, G. Sayers and L. A. Woodbury, *Endocrinology*, **42**, 379 (1948).

(4) H. B. F. Dixon, M. P. Stack-Dunne, F. G. Young and D. B. Cater, *Nature*, **168**, 1084 (1951).

(5) G. P. Hess, J. I. Harris, F. H. Carpenter and C. H. Li, THIS JOURNAL, **73**, 5918 (1951).

(6) (a) L. C. Craig and D. Craig in A. Weissberger, "Techniques of Organic Chemistry," Interscience Publ., New York, N. Y., Vol. III, 1950, Chap. 4; (b) E. J. Harfenist and L. C. Craig, THIS JOURNAL, **73**, 877 (1951).

(7) F. H. Carpenter, "XIIth International Congr. of Pure and Applied Chem.," Abstracts of Papers, pp. 69 (1951).

61% of the recovered dry weight and was practically devoid of potency, while the center component (Tubes 15–20) contained 23% of the recovered dry weight, and possessed about 13% of the total activity. The material in Tubes 21–25 contained 16% of the dry weight, and most of the biological potency. Thus it is apparent that the adrenal ascorbic acid depleting activity of the hormone protein can be separated from the main components. Whether other adrenal-stimulating activities (adrenal weight-increasing, adrenal repair, eosinopenic, etc.) are associated with the main components remains to be investigated. At any event, it must be concluded that the protein with an apparent molecular weight of 20,000 which was isolated from sheep pituitaries[2a] is a mixture. Since electrophoretic, ultracentrifugal and solubility methods, as they were applied, did not reveal this inhomogeneity, the results reported here are a further demonstration[6b] of the utility of the countercurrent method in homogeneity studies on low molecular weight proteins.

Experimental

Because of the formation of emulsions, the distributions were performed in glass centrifuge tubes. Volumes of 5 ml. for the upper and 5 ml. for the lower layer were used. The solvent was obtained by equilibrating 7 parts of 2,4,6-collidine (Reilly Coal Tar and Chemical Company, b.p. 170°) with 11 parts of water. The experiment was performed with the lower layers as the moving phase, but the data are recorded as though the upper layers were the moving phase. The solid in each tube was determined by transferring the contents of the tube to a tared Florence flask weighing about 2.2 g.,[8] and removing the solvents by evaporation from the frozen state. The residues were finally kept for several hours over phosphoric anhydride *in vacuo* before being weighed. A total of 51 mg. of material was present in the tubes. This increase in weight over the starting material was probably due to the formation of a small amount (*ca.* 5 mg.) of non-volatile collidine hydrochloride. The residues were dissolved in water and the solutions were pooled as indicated in Table I. These pooled solutions were assayed by the adrenal ascorbic acid depleting method[3,9] in hydrophysectomized male rats. A preparation of the International Standard was employed for comparison to estimate the ACTH potency of the various fractions obtained.

Acknowledgment.—This work was aided in part by grants from the National Institutes of Health, the United States Public Health Service, the Armour Laboratories, Merck and Company, Inc., and the Eli Lilly Laboratories.

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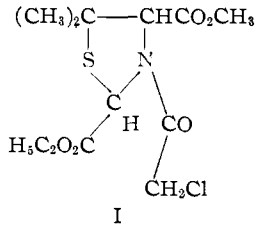
DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

Attempted Cyclization of N-Chloroacetylthiazolidines

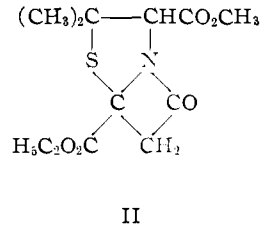
BY JOHN C. SHEEHAN AND AJAY KUMAR BOSE[1]

RECEIVED APRIL 18, 1952

In an effort to extend our new β-lactam synthesis,[2] we have conducted a preliminary study of the possibility of cyclizing such N-chloroacetylated substituted thiazolidines as I by dehydrohalogenation to bicyclic β-lactams as for example, II.



I

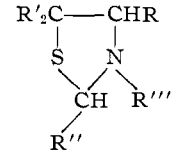


II

Recently Süs[3] has reported some experiments of a similar nature. The first compound we investigated was 2-phenyl-3-chloroacetyl-4-carbomethoxythiazolidine (III), obtained by the reaction of chloroacetic anhydride with 2-phenyl-4-carbomethoxythiazolidine.[4] A benzene solution of III was heated under reflux with triethylamine but it failed to afford a β-lactam. In the expectation that the sulfone of III might be more reactive, III was submitted to oxidation with potassium permanganate or hydrogen peroxide. The only oxidation product isolated was the sulfoxide, which was unchanged after heating in dioxane solution with triethylamine.

It seemed that a more favorable case for closing the fused β-lactam-thiazolidine ring would be that of a derivative of 2-carboxythiazolidine since the methenyl group would be activated by the carboxy function and perhaps also by the sulfur atom. Therefore, 2,4-dicarbethoxythiazolidine (IV) and 2-carbethoxy-4-carbomethoxy-5,5-dimethylthiazolidine (V) were prepared by condensing ethyl glyoxalate alcoholate with L-cysteine ethyl ester hydrochloride and D-penicillamine methyl ester hydrochloride, respectively. An evaporative distillation of a crude sample of V afforded a crystalline (Va) and a liquid (Vb) distillate. It was found that Va and Vb had identical elemental analysis and infrared spectra but different optical rotations ($[\alpha]^{25}D$ −20.7° and +28.2°, respectively). Evidently Va and Vb are diastereoisomers formed when the new center of asymmetry was created.

The N-chloroacetylated thiazolidines (VI, I) (corresponding to IV and V, respectively) were unaffected by treatment with triethylamine. Efforts to oxidize VI and I to sulfoxides or sulfones failed.



III, R = CO_2CH_3, R′ = H, R″ = C_6H_5, R‴ = $ClCH_2CO$
IV, R = $CO_2C_2H_5$, R′ = H, R″ = $CO_2C_2H_5$, R‴ = H
V, R = CO_2CH_3, R′ = CH_3, R″ = $CO_2C_2H_5$, R‴ = H
VI, R = $CO_2C_2H_5$, R′ = H, R″ = $CO_2C_2H_5$, R‴ = $ClCH_2CO$

Experimental[5]

2-Phenyl-3-chloroacetyl-4-carbomethoxythiazolidine (III). —Benzaldehyde and L-cysteine hydrochloride were condensed[4] and the crude thiazolidine (5 g.) suspended in ether was treated with diazomethane to give the methyl ester

(1) Overseas scholar of the Government of India.

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(5) All melting points are corrected. We are indebted to Dr. S. M. Nagy and his associates for the microanalyses.