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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Attempted Cyclization of N-Chloroacetylthiazolidines

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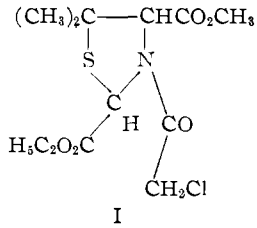
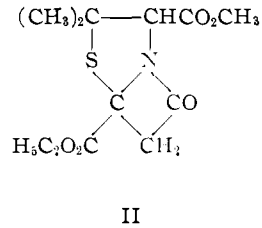
In an effort to extend our new β-lactam synthesis,[2] we have conducted a preliminary study of the possibility of cyclizing such N-chloroacetylated substituted thiazolidines as I by dehydrohalogenation to bicyclic β-lactams as for example, II.



I

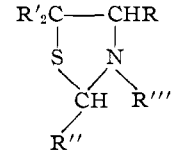


II

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.

(2) J. C. Sheehan and A. K. Bose, THIS JOURNAL, **72**, 5158 (1950).

(3) O. Süs, *Ann.*, **568**, 129 (1950).

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

which, after removal of the excess diazomethane and the solvent, was obtained as a light yellow liquid (5 g.). The crude ester was allowed to react overnight at room temperature with chloroacetic anhydride (4.5 g.) in benzene solution. The reaction mixture was warmed on a steam-cone for an hour and then the solvent was removed. On pouring the residue into water and stirring, crystals (5.95 g., 83.2%), m.p. 92–100°, separated. By recrystallization from a mixture of benzene and ligroin, the amide was obtained as colorless, stout prisms (5.6 g., 94%), m.p. 95–98°. After further recrystallization from ligroin, the m.p. was constant at 96–97°.

Anal. Calcd. for $C_{13}H_{14}O_5NClS$: C, 52.09; H, 4.71; N, 4.67. Found: C, 52.24; H, 4.84; N, 4.75.

2-Phenyl-3-chloroacetyl-4-carbomethoxythiazolidinesulfoxide. (a).—A solution of III (1.2 g.) in dioxane (15 ml.) was treated with potassium permanganate (0.17 g.) dissolved in glacial acetic acid (5 ml.). After one hour the reaction mixture was decolorized by the addition of a few drops of hydrogen peroxide. By pouring the colorless solution into water 0.54 g. of crystalline material was obtained. This was washed with ether to remove the starting material and then crystallized thrice from ethanol and twice from chloroform–ether. The m.p. was constant at 211–213° (dec.).

Anal. Calcd. for $C_{13}H_{14}O_4NClS$: C, 49.45; H, 4.47; N, 4.44. Found: C, 49.31; H, 4.44; N, 4.83.

(b).—To a solution of III (1.5 g.) in acetic acid was added 1.5 ml. of 35% hydrogen peroxide. After two weeks the solution was poured into water, 1.26 g. of crystalline material, yield 79.6%, m.p. 195–205°, was obtained. Recrystallization from ethanol raised the m.p. to 210–212°.

No reaction was observed when a solution of the sulfoxide in dry dioxane was treated with triethylamine.

2,4-Dicarbethoxythiazolidine (IV).—Cysteine ethyl ester hydrochloride (4.2 g.) and sodium acetate (2.7 g.) were dissolved in 20 ml. of water. Ethyl glyoxalate alcoholate (3.35 g.) in 80 ml. of alcohol was added and the reaction mixture was stored for two days. After removal of most of the alcohol by distillation, the oily layer which separated was collected in ether. The dried (sodium sulfate) ether extract was concentrated to a yellow liquid (4.5 g.). By evaporative distillation 2.8 g. (53%) of the thiazolidine was obtained. An analytical sample, $n^{25}D$ 1.4880, d^{25}_4 1.1991, was prepared by a second evaporative distillation.

Anal. Calcd. for $C_9H_{15}O_4NS$: C, 46.34; H, 6.48; N, 6.00; *MR*, 56.44. Found: C, 46.57; H, 6.50; N, 6.04; *MR*, 56.05.

2,4-Dicarbethoxy-3-chloroacetylthiazolidine (VI).—A mixture of IV (0.87 g.) and chloroacetic anhydride (0.8 g.) was allowed to react overnight at 60–70°. The reaction mixture was then poured into water and extracted with ether. The ether extract was washed successively with sodium bicarbonate solution and water. Removal of ether from the dried (sodium sulfate) solution left a slightly yellow, viscous oil (1.15 g., 81%). Evaporative distillation (120–130° (0.4 mm.)) gave a colorless, viscous oil (90% recovery). A second evaporative distillation afforded an analytical sample, $n^{25}D$ 1.5090.

Anal. Calcd. for $C_{11}H_{16}O_5ClNS$: C, 42.65; H, 5.21; N, 4.52. Found: C, 42.74; H, 5.18; N, 4.67.

2-Carbethoxy-4-carbomethoxy-5,5-dimethylthiazolidine (V).—To a solution of D-penicillamine methyl ester hydrochloride (3 g.) and sodium acetate (2 g.) in 20 ml. of water was added ethyl glyoxalate alcoholate (2.3 g.) in 50 ml. of alcohol. After two days the alcohol was removed, leaving oily droplets in an aqueous suspension. The oil was collected with ether, washed, and the dried ethereal solution was concentrated to a pale yellow liquid (3.71 g.). An evaporative distillation (60–70° (0.07 mm.)) of 1.85 g. of this liquid afforded 1.51 g. of colorless distillate—part of which crystallized. The crystalline material (0.8 g., 35%), m.p. 48–50°, is readily soluble in organic solvents but can be recrystallized from dilute alcohol.

Anal. Calcd. for $C_{10}H_{17}O_4NS$: C, 48.56; H, 6.93; N, 5.66. Found: C, 48.60; H, 7.03; N, 5.79.

The liquid fraction of the distillate (0.71 g., 31.3%) was thrice distilled for analysis.

Anal. Calcd. for $C_{10}H_{17}O_4NS$: C, 48.56; H, 6.93; N, 5.66. Found: C, 48.38; H, 6.77; N, 5.62.

Both the crystalline and liquid fractions were optically active, the specific rotations in alcohol, $[\alpha]^{25}D$ were −20.7° and +28.2°, respectively.

In another experiment using 5 g. of D-penicillamine methyl ester hydrochloride, the undistilled reaction mixture was seeded. After two days in a refrigerator, 3.3 g. (37.3%) of crystalline product, m.p. 43–45°, was obtained.

2-Carbethoxy-3-benzoyl-4-carbomethoxy-5,5-dimethylthiazolidine.—To a suspension of 2-carbethoxy-4-carbomethoxy-5,5-dimethylthiazolidine (0.5 g.) in 10% sodium hydroxide solution was added benzoyl chloride (0.25 ml.) with vigorous shaking. After one hour, the reaction mixture was warmed on a steam-cone to destroy excess benzoyl chloride and was then extracted with ether. The dried (sodium sulfate) ethereal solution was concentrated to an oil which was purified by an evaporative distillation (130–140° (0.5 mm.)); a colorless and very viscous oil (0.27 g., 38%) was obtained. The infrared spectrum has an amide band at 6.02 μ in addition to the carbonyl band (a double peak at 5.73 and 5.77 μ).

Anal. Calcd. for $C_{17}H_{21}O_5NS$: C, 58.10; H, 6.02; N, 3.99. Found: C, 57.95; H, 6.02; N, 3.86.

2-Carbethoxy-3-chloroacetyl-4-carbomethoxy-5,5-dimethylthiazolidine (I).—A mixture of 2-carbethoxy-4-carbomethoxy-5,5-dimethylthiazolidine (0.8 g.) and chloroacetic anhydride (1 g.) was kept overnight at 70–75°. The ethereal extract of the melt was washed successively with water, dilute hydrochloric acid, water, sodium bicarbonate solution and water. The dried ethereal solution gave a reddish brown oil (0.88 g.) after concentration. A faintly yellow and very viscous oil (0.77 g., 73.6%) was obtained by an evaporative distillation (130–140° (0.5 mm.)). A colorless analytical sample was prepared by two more evaporative distillations.

Anal. Calcd. for $C_{12}H_{18}O_5NClS$: C, 44.52; H, 5.64; N, 4.33. Found: C, 44.66; H, 5.68; N, 4.13.

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The Reaction of Azomethines with Methylmagnesium Iodide[1]

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The classical investigations of Busch[2] and his co-workers indicated that Schiff bases reacted with the Grignard reagent in a manner analogous to aldehydes, but only one example involving a keto anil was reported. Such reactions were used by subsequent workers, notably Moffett and Hoehn,[3] and Campbell and co-workers[4] to prepare substituted aromatic secondary amines, but the problem of isolating the intermediate Grignard addition compound and determining its composition was not studied.

Sachs and Sachs[5] attempted the isolation of the compounds resulting from the action of phenylmagnesium bromide and ethylmagnesium bromide on quinoline in ethyl ether. Their analyses indicated that one mole of the organomagnesium compound and one mole of quinoline had reacted, although the

(1) Taken from a thesis submitted by Thomas J. Gair to the Graduate School of Boston College in partial fulfillment of the requirements of the degree of Master of Science.

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