in glacial acetic acid (15 ml.), treated with charcoal and filtered. Upon cooling, crystals of D-glucurono-6,3-lactone separated. These were filtered, washed successively with cold glacial acetic acid, absolute ethanol and anhydrous ether; yield 82 mg., $[\alpha]^{23}D$ +19 (*c* 3.3 in water), m.p. 171–172° and mixed m.p. with an authentic specimen 172–174°.

Anal. Calcd. for $C_6H_8O_6$: C, 40.92; H, 4.6. Found[30]: C, 40.9; H, 4.9.

C. D-Glucurono-6,3-lactone-*p*-nitroanilide.—Recrystallized-D-glucurono-6,3-lactone (20 mg.) obtained from enzymatic hydrolysis of urochloralic acid and recrystallized *p*-nitroaniline (22 mg.) were heated at 78° for 20 minutes with methanol (0.6 ml.) containing 0.07 ml. of concd. hydrochloric acid per 100 ml. as suggested by Spriestersbach.[29] After cooling, the *p*-nitroanilide separated as fine needles; it was collected by centrifugation, washed with cold methanol and recrystallized from methanol. The product (9 mg.), dried over P_2O_5, had m.p. and mixed m.p. with an authentic specimen of 129°.

Anal. Calcd. for $C_{12}H_{13}O_7N_2$: N, 9.46. Found[30]: N, 9.1.

Descending chromatography of *p*-nitroanilides on Whatman No. 1 paper was carried out with a solvent system of hexanol-1–concd. ammonium hydroxide–water (10:0.5:50, v./v.), the aqueous layer being the moving, the organic layer the stationary phase. R_f values of the yellow spots of different compounds were: *p*-nitroaniline, 0.48; D-glucose-*p*-nitroanilide,[31] 0.75; D-glucurono-6,3-lactone-*p*-nitroanilide (authentic specimen), 0.86; D-glucurono-6,3-lactone-*p*-nitroanilide from urochloralic acid, 0.85.

Isolation and Identification of Aglycone.—Application of differential analyses for trichloroacetic acid and trichloroethanol by the modified Fujiwara reaction[12b] showed that the biosynthetic urochloralic acid contained a component which reacted like trichloroethanol and not like trichloroacetic acid or trichloroacetaldehyde.

A deproteinized enzymatic hydrolyzate (30 ml.) prepared as described above containing an estimated amount of 50 mg. of trichloroethanol was saturated with sodium chloride and extracted three times with a total of 27 ml. of ethyl ether and the extract dried over anhydrous magnesium sulfate. Analyses of the ether extract by the oxidative Fujiwara procedure indicated that it contained only 23 mg. of trichloroethanol. After removal of the ether with a stream of dry air under slightly reduced pressure the residue was heated with *p*-nitrobenzoyl chloride (35 mg.) and dry pyridine (1 ml.) on a steam-bath for 40 minutes. The mixture was cooled to room temperature and diluted dropwise with water (1 ml.). After 30 minutes the solution was added dropwise to a mixture (10 ml.) of sodium bicarbonate (10%) and ice chips. The crystals which separated were centrifuged, washed with water and dried in a desiccator over P_2O_5. Recrystallization from petroleum ether (b.p. 70–80°) yielded a product (10 mg.), m.p. 69–70°, and mixed m.p. 69–70° with an authentic specimen of 2,2,2-trichloroethyl *p*-nitrobenzoate.

Acknowledgment.—Part of this work was supported by a grant from E. I. du Pont de Nemours and Co., Inc. We are indebted to Dr. A. L. Elder, Corn Products Refining Co., Argo, Ill., for a specimen of D-glucurono-6,3-lactone; to Dr. F. Smith of this Department for reference compounds and advice, and to Dr. V. Perman, School of Veterinary Medicine, University of Minnesota, for the specimens of calves' urine.

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(30) Huffman Microanalytical Laboratories, Wheatridge, Colo.

(31) F. Weygand, W. Perkow and P. Kuhner, *Ber.*, **84**, 594 (1951).

[CONTRIBUTION FROM DEFENCE RESEARCH CHEMICAL LABORATORIES]

Amino Acids. II. Synthesis of Cyclic Guanidino Acids[1]

BY A. F. McKAY[2] AND W. G. HATTON[2]

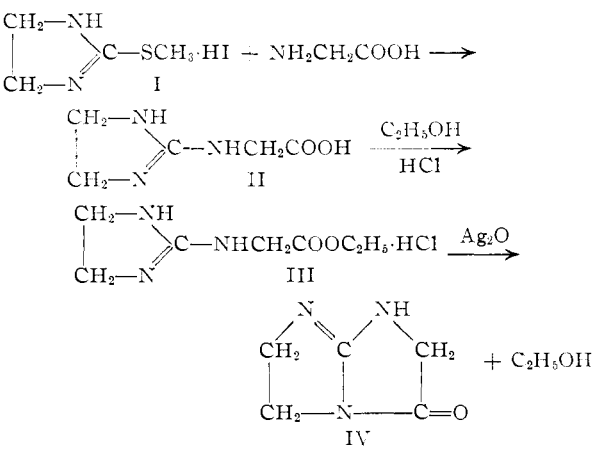
RECEIVED SEPTEMBER 23, 1955

A new series of N-substituted amino acids has been synthesized by the reaction of amino acids with S-methyl cyclic isothioureas. Some derivatives of these acids are described together with the bicyclic compounds 3-keto-Δ^8-hexahydro-1,4,8-pyrimidazole and 3-keto-2,3,5,6-tetrahydro-1-imidaz[1.2-a]imidazole.

A new series of cyclic guanidino acids analogous to those described by Elderfield and Green[3] has been synthesized. These acids (Table I) were prepared by allowing a sodium or potassium salt of an amino acid to combine with a S-methyl cyclic isothiourea at room temperature. Further characterization was effected by converting them to the picrates of their ethyl esters.

Although the acids could be purified by crystallization, the yields (25–50%) were very poor, owing to the similarity in solubility of the guanidino acids and the iodide salts. However, the acids could be recovered readily in good yield by passing the total aqueous reaction mixture through a mixed-bed of IRA-400 and IRC-50 resins which removed the potassium or sodium iodide salts completely, leaving the cyclic guanidino acids in solution.

2-Methylmercapto-2-imidazolinium iodide (I) and glycine in sodium hydroxide solution gave 2-(carboxymethylamino)-Δ^2-1,3-diazacyclopentene (II). This acid was then esterified to 2-(carbethoxymethylamino)-Δ^2-1,3-diazacyclopentene hydrochloride (III) and identified as its picrate. When the ester hydrochloride was shaken with silver oxide in water, a mixture of the bicyclic compound 3-keto-2,3,5,6-tetrahydro-1-imidaz[1,2-a]imidazole (IV) and the free acid 2-(carboxymethylamino)-Δ^2-1,3-diazacyclopentene (II) was obtained. The bicyclic compound IV was only isolated in the pure



(1) Issued as D. R. C. L. Report No. 170.

(2) Monsanto Canada Limited, Ville LaSalle, Quebec.

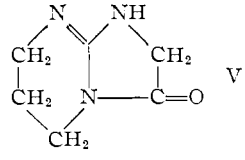
(3) R. C. Elderfield and M. Green, *J. Org. Chem.*, **17**, 442 (1952).

TABLE I

CYCLIC GUANIDINO ACIDS

Acids	M.p., °C. dec.	Yield, %	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
2-(Carboxymethylamino)-Δ^2-1,3-diazacyclopentene)	293	80.5	$C_5H_9N_3O_2$	41.96	41.92	6.29	6.42	29.37	29.00
DL-2-(α-Carboxyethylamino)-Δ^2-1,3-diazacyclopentene	251.5–252	86.5	$C_6H_{11}N_3O_2$	45.85	45.75	7.06	7.00	26.75	26.50
DL-2-(α-Carboxypropylamino)-Δ^2-1,3-diazacyclopentene	236.5–238	69.2	$C_7H_{13}N_3O_2$	49.11	49.05	7.66	7.54	24.55	24.23
2-(Carboxymethylamino)-4 (or 5)-methyl-Δ^2-1,3-diazacyclopentene	132–133.5	85.0	$C_6H_{11}N_3O_2$	45.85	46.08	7.06	7.28	26.75	26.56
1-(β-Hydroxyethyl)-2-(carboxymethylamino)-Δ^2-1,3-diazacyclopentene	187–189.5	76	$C_7H_{13}N_3O_3$	44.91	45.00	7.00	7.00	22.45	21.93
2-(ϵ-Carboxypentylamino)-Δ^2-1,3-diazacyclopentene	230.5–231	73	$C_9H_{17}N_3O_2$	53.18	52.87	8.43	8.75	20.67	20.15
2-(Carboxymethylamino)-Δ^2-1,3-diazacyclohexene	211.5–212	67.5	$C_6H_{11}N_3O_2$	45.85	45.91	7.06	7.05	26.75	26.35

form as its picrate (m.p. 235–236°). The free base was hygroscopic. When attempts were made to purify the bicyclic compound by crystallization, the corresponding acid II was obtained. By carrying through a similar series of reactions with 2-methylmercapto-Δ^2-tetrahydropyrimidinium iodide and glycine, 3-keto-Δ^8-hexahydro-1,4,8-pyrimidazole (V) was obtained. Here again attempts to purify the bicyclic compound V by crystallization gave the corresponding free acid, 2-(carboxymethylamino)-Δ^2-1,3-diazacyclohexene (m.p. 211.5–212° with dec.).



V

Some new cyclic thioureas and their S-methyl derivatives were prepared during this investigation. These are described in the experimental section. This work is being continued.

Acknowledgment.—The authors are grateful to American Cyanamid Company for the generous samples of 1,3-diaminopropane, 3-dimethylaminopropylamine and 3-isopropylaminopropylamine.

Experimental[4]

2-Methylmercapto-2-imidazolinium Iodide.—2-Methylmercapto-2-imidazolinium iodide (m.p. 142–143.5°) was prepared in 90% yield by the procedure of Aspinall and Bianco.[5]

1-(β-Hydroxyethyl)-2-methylmercapto-2-imidazolinium Iodide.—1-(β-Hydroxyethyl)-2-methylmercapto-2-imidazolinium iodide (m.p. 120.5–121.5°) was prepared in 95% yield as previously[6] described.

Hexahydropyrimidine-2-thione.—A solution of 37 g. (0.5 mole) of 1,3-diaminopropane in 125 cc. of ethanol was added dropwise to a stirred solution of 125 cc. of carbon disulfide in 125 cc. of ethanol. During the addition period, the temperature was held below 40°. The separated oil changed to a solid before the end of the addition period. This solid was removed by filtration and washed with ethanol; yield 75.9 g. (100%). It decomposed at 125–160° with the evolution of hydrogen sulfide. A sample of this γ-aminopropyldithiocarbamic acid inner salt was prepared for analysis by dissolving 1 g. in dilute ammonia solution. On evaporation of the ammonia, crystals of the inner salt separated.

Anal. Calcd. for $C_4H_{10}N_2S_2$: C, 31.97; H, 6.71; N, 18.65; S, 42.67. Found: C, 32.07; H, 6.78; N, 18.61; S, 42.51.

The remainder of the inner salt was placed in a 500-cc. erlenmeyer flask and heated at 150° in an oil-bath until the evolution of hydrogen sulfide ceased. The cream colored solid was separated into product and free sulfur by crystallizing from ethanol; yield 39.93 g. (69.0%). One more crystallization from ethanol raised the melting point from 209–211° to 211–211.5°. Schact[7] reported a melting point of 207°.

2-Methylmercapto-Δ^2-tetrahydropyrimidinium Iodide.—Hexahydropyrimidine-2-thione (25 g., 0.216 mole) was refluxed with a solution of 31 g. (0.216 mole) of methyl iodide in 100 cc. of absolute methanol for 30 minutes. After the solution cooled to room temperature, 100 cc. of peroxide free absolute ether was added. The white crystals (m.p. 146–146.5°) were filtered off and washed with ether; yield (99.5%). One crystallization from ethanol (30 cc.) using Norite raised the melting point to a constant value of 149–149.5°.

Anal. Calcd. for $C_6H_{11}IN_2S$: C, 23.26; H, 4.30; N, 10.85; I, 49.17. Found: C, 23.26; H, 4.39; N, 10.77; I, 48.82.

2-Methylmercapto-4(or 6)-methyl-Δ^2-tetrahydropyrimidinium Iodide.—4-Methylhexahydropyrimidine-2-thione[6] (25 g., 0.192 mole) was converted to 2-methylmercapto-4 (or 6)-methyl-Δ^2-tetrahydropyrimidinium iodide (m.p. 117–117.5°) in 94.5% yield by the method described above for 2-methylmercapto-Δ^2-tetrahydropyrimidinium iodide.

Anal. Calcd. for $C_6H_{13}IN_2S$: C, 26.47; H, 4.82; N, 10.29; S, 11.78; I, 46.64. Found: C, 26.41; H, 4.84; N, 10.06; S, 12.00; I, 47.00.

1-Isopropylhexahydropyrimidine-2-thione.—3-Isopropylaminopropylamine (23.24 g., 0.2 mole) in 100 cc. of 95% ethanol was added to a solution of 50 cc. of carbon disulfide in 100 cc. of ethanol under the conditions described above for the preparation of hexahydropyrimidine-2-thione. At the end of the addition period, the solvent was evaporated in the fume-hood under a stream of air. The oil which first separated soon changed into a white crystalline solid (m.p. *ca.* 116° with dec.); yield 37.07 g. (97%). A sample of this dithiocarbamic acid inner salt was prepared as described above for analysis.

Anal. Calcd. for $C_7H_{16}N_2S_2$: C, 43.71; H, 8.38; N, 14.57; S, 33.34. Found: C, 44.03; H, 8.32; N, 14.20; S, 33.50.

The inner salt (35.50 g., 0.184 mole) was placed in a 250-cc. erlenmeyer flask and then heated in an oil-bath at 140° with stirring. After the evolution of hydrogen sulfide had ceased, which required approximately 30 minutes, the cooled solid was crystallized from 95% ethanol in the presence of Norite; yield 21.75 g. (74.1%). The melting point was raised from 116–119.5° to 119.5–120° by crystallizing from ethanol.

(4) All melting points were determined on a Kofler block. Microanalyses were performed by Micro-Tech Laboratories, Skokie, Illinois.

(5) S. R. Aspinall and E. J. Bianco, THIS JOURNAL, **73**, 602 (1951).

(6) A. F. McKay and G. R. Vavasour, *Can. J. Chem.*, **32**, 59 (1954).

(7) Schact, *Arch. Exptl. Path.*, **235**, 461 (1897).

Anal. Calcd. for $C_7H_{14}N_2S$: C, 53.12; H, 8.91; N, 17.71; S, 20.26. Found: C, 53.31; H, 8.44; N, 17.51; S, 20.50.

4-Methylimidazolidine-2-thione.—1,2-Diaminopropane (15 g., 0.20 mole) in 100 cc. of ethanol was added to 50 cc. of carbon disulfide in 100 cc. of ethanol. This reaction and the isolation of the product were carried out under conditions similar to those described for 1-isopropylhexahydropyrimidine-2-thione. The dithiocarbamic acid inner salt (m.p. 102–104°) was isolated in 94.5% (28.44 g.) yield. It was converted to the cyclic thiourea by heating in an oil-bath at 140° in the above described manner. The cyclic thiourea (m.p. 101.5–103.5°) was obtained in 73.6% yield. One crystallization from ethanol raised the melting point to 103–103.5°.

Anal. Calcd. for $C_4H_8N_2S$: C, 41.35; H, 6.94; N, 24.12; S, 27.59. Found: C, 41.17; H, 6.35; N, 24.15; S, 27.85.

1-Isopropyl-2-methylmercapto-Δ^2-tetrahydropyrimidinium Iodide.—1-Isopropyl-2-methylmercapto-Δ^2-tetrahydropyrimidinium iodide (m.p. 191–192°) was prepared in 93% yield by the method described above for 2-methylmercapto-Δ^2-tetrahydropyridinium iodide.

Anal. Calcd. for $C_8H_{17}IN_2S$: C, 32.00; H, 5.71; N, 9.33; S, 10.68; I, 42.28. Found: C, 32.39; H, 5.78; N, 8.97; S, 11.07; I, 42.57.

The 4(or 5)-Methyl-2-methylmercapto-Δ^2-imidazolinium Iodide.—Ten grams (0.086 mole) of 4-methylimidazolidine-2-thione was refluxed in a solution of 12.80 g. (0.09 mole) of methyl iodide in 50 cc. of absolute methanol for one hour. In order to obtain the product, the methanol was removed *in vacuo* and the residue washed with peroxide-free ether, yield 21.49 g. (93.5%). The product was taken up in 80 cc. of hot acetone and then treated with Norite. The Norite was removed by filtration and the filtrate cooled to room temperature. This treatment raised the melting point from 87–92° to 91–92°.

Anal. Calcd. for $C_5H_{11}IN_2S$: C, 23.26; H, 4.30; N, 10.85; S, 12.42; I, 49.17. Found: C, 23.38; H, 4.31; N, 10.53; S, 12.85; I, 48.95.

Synthesis of the Cyclic Guanidino Acids.—The preparations of the acids described in Table I are similar; an example is given below in detail.

Thirty grams (0.123 mole) of 2-methylmercapto-2-imidazolinium iodide was dissolved in a solution of 5.2 g. (0.123 mole) of sodium hydroxide in 125 cc. of water. To this solution was added 10.95 g. (0.123 mole) of β-alanine in 50 cc. of hot water. This reaction mixture was allowed to stand in the fume-hood at room temperature for several days until the evolution of methyl mercaptan ceased. The solution was diluted with water to give about a 5% solution and then it was run through a mixed-bed resin column (150 cc. of activated IRC-50 and 150 cc. of activated IRA-400 resins well mixed) at the rate of 7–9 cc. per minute. The column had a diameter of $1^3/_8$". After the solution had passed through the column, the resin was washed with 1500 cc. of water. The combined eluate and water washings were evaporated *in vacuo*. This gave a solid residue of 15.69 g. (81.0%) of 2-(β-carboxyethylamino)-Δ^2-1,3-diazacyclopentene (m.p. 203–205°). One crystallization from aqueous ethanol raised the melting point to 211–212°.

Anal. Calcd. for $C_6H_{11}N_3O_2$: C, 45.85; H, 7.06; N, 26.75. Found: C, 45.59; H, 7.09; N, 26.82.

2-(Carbethoxymethylamino)-Δ^2-1,3-diazacyclopentene.—2-Carboxymethylamino)-Δ^2-1,3-diazacyclopentene (3.0 g., 0.02 mole) was refluxed for two hours in a solution of 2.18 g. (0.06 mole) of hydrogen chloride in 50 cc. absolute ethanol. At this point 10 cc. of benzene was added and the refluxing continued another hour, after which the benzene–water–ethanol azeotrope was removed; this procedure of treating with benzene was repeated three times and then the solvent was removed *in vacuo*, yield 4.23 g. (81.1%).

A sample was converted into the picrate salt in the usual manner. After crystallizing from water it melted at 193–195°.

Anal. Calcd. for $C_{13}H_{16}N_6O_9$: C, 39.00; H, 4.03; N, 21.00. Found: C, 39.33; H, 3.90; N, 20.92.

The picrates of the following ethyl esters of cyclic guanidine acids were prepared in a similar manner.

2-(β-Carbethoxyethylamino)-Δ^2-1,3-diazacyclopentene picrate (m.p. 137.5–138°). *Anal.* Calcd. for $C_{14}H_{18}N_6O_9$: C, 40.58; H, 4.37; N, 20.29. Found: C, 40.77; H, 4.23; N, 20.48.

2-(Carbethoxymethylamino)-Δ^2-1,3-diazacyclohexene picrate (m.p. 179.5–180°). *Anal.* Calcd. for $C_{14}H_{18}N_6O_9$: C, 40.58; H, 4.37; N, 20.29. Found: C, 40.39; H, 4.07; N, 20.42.

2-(ϵ-Carbethoxypentylamino)-Δ^2-1,3-diazacyclopentene picrate (m.p. 127–127.5°). *Anal.* Calcd. for $C_{17}H_{24}N_6O_9$: C, 44.70; H, 5.31; N, 18.42. Found: C, 44.83; H, 5.51; N, 18.39.

DL-2-(α-Carbethoxyethylamino)-Δ^2-1,3-diazacyclopentene picrate (m.p. 156–157°). *Anal.* Calcd. for $C_{14}H_{18}N_6O_9$: C, 40.58; H, 4.38; N, 20.29. Found: C, 40.65; H, 4.40; N, 20.38.

2-(Carbethoxymethylamino)-4 (or 5)-methyl-Δ^2-1,3-diazacyclopentene Picrate (m.p. 195–197°). *Anal.* Calcd. for $C_{14}H_{18}N_6O_9$: C, 40.58; H, 4.38; N, 20.29. Found: C, 40.60; H, 4.47; N, 20.20.

3-Keto-Δ^3-hexahydro-1,4,8-pyrimidazole.—2-(Carbethoxymethylamino)-Δ^2-1,3-diazacyclohexene hydrochloride (2.33 g., 0.01 mole) was dissolved in 30 cc. of water and 1.62 g. of silver oxide was added. This mixture was shaken mechanically for two hours, after which the silver chloride and unchanged silver oxide were removed by filtration. The filtrate was diluted with 400 cc. of acetone and the solid recovered; yield 0.9 g. (58.9%). The solid melted with decomposition at 195–203°. A sample (400 mg.) of this crude material was treated with a saturated aqueous picric acid solution. The picrate (310 mg.) was purified to a constant melting point of 155–155.4°.

Anal. Calcd. for $C_{12}H_{12}N_6O_8$: C, 39.13; H, 3.28; N, 22.83. Found: C, 39.39; H, 3.67; N, 22.76.

On attempting to purify the crude bicyclic compound itself by crystallization from ethanol only 2-(carboxymethylamino)-Δ^2-1,3-diazacyclohexene was obtained. It melted at 211.5–212° with decomposition alone and on admixture with an authentic sample.

3-Keto-2,3,5,6-tetrahydro-1-imidaz[1,2-a]imidazole.—Ten grams (0.048 mole) of 2-(carbethoxymethylamino)-Δ^2-1,3-diazacyclopentene hydrochloride was dissolved in 125 cc. of water and silver oxide (6.5 g.) was added. This mixture was shaken for 3.5 hours on a mechanical shaker. After the unchanged silver oxide and silver chloride were removed by filtration, the filtrate was taken to dryness. The yield of crystalline solid was 4.5 g. This solid was dissolved in 20 cc. of water and acetone was added to the point of turbidity. On standing white crystals separated; yield 1.08 g. (15.7%). These crystals melted at 290° with decomposition alone and on admixture with an authentic sample of 2-(carboxymethylamino)-Δ^2-1,3-diazocyclopentene. The filtrate from this acid gave a second crop of solid which did not melt below 300°; yield 2.47 g. This material was crystallized from aqueous acetone. It was hygroscopic and a sample which was allowed to come to constant weight in the atmosphere, was found to have taken up one and a half mole equivalents of water.

Anal. Calcd. for $C_5H_7N_3O \cdot 1^1/_2H_2O$: C, 39.45; H, 6.62; N, 27.61. Found: C, 39.22; H, 6.71; N, 27.42.

Its picrate, formed in the usual manner, melted at 235–236° with decomposition after purification. The decomposition point varied with the rate of heating.

Anal. Calcd. for $C_{11}H_{10}N_6O_8$: C, 37.29; H, 2.85; N, 23.73. Found: C, 37.39; H, 3.30[8]; N, 24.06.

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(8) Several analyses of this picrate gave high hydrogen values. The reason for this discrepancy is not known.