0.2 mm. and was somewhat unstable.[7] The substance was identified as a decahydroheptalene dione (or a mixture of isomers) by formation of two derivatives.

The di-2,4-dinitrophenylhydrazone, for which no solvent for recrystallization was found, was purified by washing with hot methanol, hot water and hot methanol; m.p. 125–126°.

Anal. Calcd. for $C_{24}H_{24}N_8O_8$: C, 52.17; H, 4.35; N, 20.28. Found: C, 52.42; H, 4.84; N, 19.94.

The disemicarbazone, for which no satisfactory solvent could be found, was purified by successive washing with hot methanol, hot water and hot methanol. It had no discernible melting point but decomposed above 250°.

Anal. Calcd. for $C_{14}H_{24}N_6O_2$: C, 54.54; H, 7.79; N, 27.27. Found: C, 54.76; H, 7.79; N, 26.93.

(7) Other ketonic compounds in this series have been observed to be unstable. See G. Buchi and O. Jeger, *Helv. Chim. Acta*, **32**, 538 (1949).

DEPARTMENT OF CHEMISTRY
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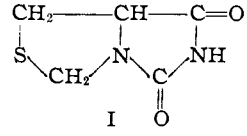
The Hydantoin Derivative of 4-Thiazolidinecarboxylic Acid

BY MARVIN D. ARMSTRONG[1]

RECEIVED JUNE 15, 1955

The hydantoin derivative of 4-thiazolidinecarboxylic acid (I) is of interest as a new derivative of cysteine and as an example of a fused ring system analogous to that of some hydantoins and thiohydantoins prepared during the studies of the chemistry of penicillin.[2] Both the D- and L-forms of the hydantoin have been prepared by causing the corresponding thiazolidinecarboxylic acids to react with potassium cyanate and then treating the reaction mixture with acid. Neither isomer showed penicillin activity when assayed with *B. subtilis*, nor biotin activity for *S. cerevisiae*.



I

Experimental

L-4-Thiazolidinecarboxylic acid was prepared according to the procedure of Schubert.[3] To a suspension of 0.50 g. of this compound in 25 ml. of water was added 0.61 g. of potassium cyanate; all of the thiazolidinecarboxylic acid went into solution. The solution was heated on a steam-bath for 30 minutes, then acidified by the addition of concd. HCl, an additional 0.5 ml. of concd. HCl was added, and the solution was evaporated to a low volume on a steam-bath. The solid that crystallized when the solution was cooled was collected and recrystallized from a small volume of water; yield 0.41 g. (69%), m.p. 167°. The compound is soluble in dil. alkali, slightly soluble in water and insoluble in acids, alcohol or acetone. It gives a negative test for sulfhydryl when treated with sodium nitroprusside, both before and after treatment with sodium cyanide.

When the rotation is measured immediately upon dissolving in 1 *N* NaOH, $[\alpha]^{20}D$ −115° (*c* 1); the rotation gradually decreases over a period of 12 hours, and then remains constant at $[\alpha]^{20}D$ −23°.

Anal. Calcd. for $C_5H_6O_2N_2S$: C, 37.98; H, 3.80; N, 17.72; S, 20.25. Found: C, 38.06; H, 4.21; N, 17.61; S, 20.53.

The corresponding derivative prepared from D-cysteine hydrochloride was identical with the above compound in all its chemical and physical properties with the exception of the optical rotation; for a 1% solution in 1 *N* NaOH, $[\alpha]^{20}D$ +115°, decreasing to $[\alpha]^{20}D$ +23°.

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(1) Univ. of Utah College of Medicine, Salt Lake City, Utah.

(2) "The Chemistry of Penicillin," Princeton University Press, 1949, pp. 302, 970, 971.

(3) M. P. Schubert, *J. Biol. Chem.*, **114**, 341 (1936).

Experimental Chemotherapy of Tuberculosis. IV. 2-Piperazinecarboxylic Acid and Related Compounds

BY F. L. BACH, JR., S. KUSHNER AND J. H. WILLIAMS

RECEIVED JULY 5, 1955

In view of the high antituberculous activity of Aldinamide,[1a] pyrazinamide,[1b] it was considered necessary to investigate the effect of various changes in the chemical constitution of this compound. One variation of interest was the reduced form of pyrazinamide, namely, 2-piperazinecarboxamide. Since pyrazine derivatives of this type are not easily reduced, 2-piperazinecarboxylic acid, a new acid, was synthesized as an intermediate.

The disodio derivative of N,N′-di-*p*-tosylethylenediamine was condensed with ethyl α,β-dibromopropionate in a refluxing ethanolic potassium hydroxide solution to yield the ethyl ester of 1,4-di-*p*-tosyl-2-piperazinecarboxylic acid. Hydrolysis of the ester and detosylation occurred when this derivative was refluxed in 48% hydrobromic acid. The free acid released by silver carbonate in an aqueous medium was very soluble in water, insoluble in the ordinary organic solvents and was characterized as a white, crystalline solid which melted with decomposition at 275–277°. The infrared spectrum of this substance shows a typical amino acid carboxylate ion absorption at 6.32 μ.[2] Attempts to esterify 2-piperazinecarboxylic acid by the usual methods such as refluxing with ethanol and hydrogen chloride or by treatment with diazomethane were unsuccessful. However, esterification can be accomplished by a prolonged refluxing of the acid in ethanol, benzene and concentrated sulfuric acid, followed by a periodic distillation from the reaction of an azeotropic mixture consisting of ethanol, benzene and water. The ester prepared in this manner was treated with hydrazine hydrate (100%) to yield 2-piperazinecarboxylic acid hydrazide and an ammonolysis of ethyl 2-piperazinecarboxylate afforded the desired product, 2-piperazinecarboxamide.

The 2-piperazinecarboxylic acid which was purified by sublimation *in vacuo* gave an elemental analysis and neutralization equivalent in accord with the calculated amounts and an electrometric titration of the dihydrochloride of this acid showed end-points at *p*H 3.7, 7.5 and 10.6. One molar equivalent of this cyclic amino acid when condensed with two molar equivalents of ninhydrin in a warm, neutral, aqueous solution gives a deep-red colored solution. A similar reaction between proline and

(1) (a) The trade-mark of American Cyanamid Company for pyrazinamide is Aldinamide; (b) S. Kushner, H. Dalalian, J. L. Sanjurjo, F. L. Bach, Jr., S. R. Safir, V. K. Smith, Jr., and J. H. Williams, THIS JOURNAL, **74**, 3617 (1952).

(2) H. M. Randall, R. G. Fowler, N. Fuson and J. R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand Co., Inc., New York, N. Y., 1949, p. 16.

ninhydrin has been reported.[3] The structure of the 2-piperazinecarboxylic acid was established by dehydrogenating 2-piperazinecarboxamide over palladized charcoal. The sublimate obtained from this aromatization was collected as a white, crystalline material, m.p. 173–183° (pure samples of pyrazinamide have been found to melt sharply at different temperatures ranging between 183 to 193°). The unsaturated compound gave a positive result when subjected to the color test[4] used to identify pyrazinamide as pyrazinoic acid and an infrared analysis of the dehydrogenated compound showed agreement with the infrared spectra of pyrazinamide (see Fig. 1).

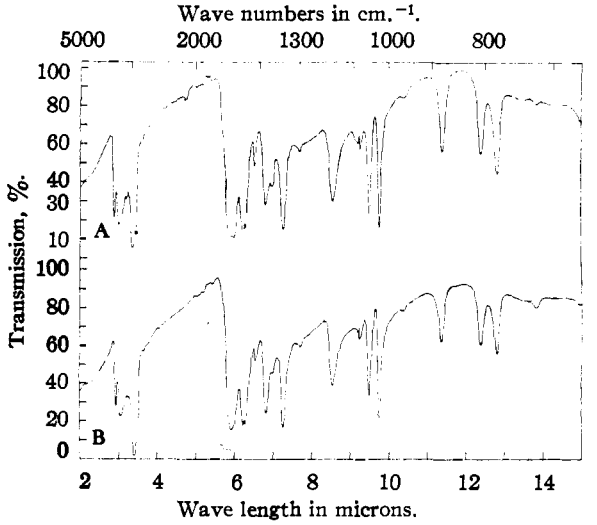

Fig. 1.—A, dehydrogenated 2-piperazinecarboxamide; B, pyrazinamide (2-pyrazincarboxamide). The infrared spectra were determined in Nujol mull (Nujol bands at 3.43, 3.50, 6.86, 7.26, 13.89 μ).

The reduced form of pyrazinamide and the other derivatives reported herein were found inactive when screened for antituberculous activity in the mouse test.[5]

Experimental[6]

Ethyl Ester of 1,4-Di-*p*-tosyl-2-piperazinecarboxylic Acid (I).—A mixture consisting of 40.1 g. (0.15 mole) of ethyl α,β-dibromopropionate, 50.0 g. (0.12 mole) of the disodio derivative of N,N'-di-*p*-tosylethylenediamine and 6.1 g. (0.11 mole) of potassium hydroxide in 300 ml. of ethanol was refluxed for three hours. The resulting dark-brown solution was filtered hot and, on standing, the filtrate deposited a tan, crystalline mass, 25 g. (45%). This crude material was washed with two 100-ml. portions of warm water, air-dried and recrystallized twice from warm ethyl acetate; m.p. 140–141°.

Anal. Calcd. for $C_{21}H_{26}N_2O_6S_2$: C, 54.1; H, 5.4; N, 6.0; S, 13.8. Found: C, 53.8; H, 5.7; N, 5.9; S, 13.9.

1,4-Di-*p*-tosyl-2-piperazinecarboxylic Acid Hydrazide (II).—A solution of 9.3 g. (0.020 mole) and 2.0 g. of hydrazine hydrate (100%) in 100 ml. of absolute ethanol was refluxed for 15 hours. The resulting clear reaction mixture was concentrated to a tan, crystalline residue which was recrystallized twice from ethanol; m.p. 175–177°.

Anal. Calcd. for $C_{18}H_{24}N_4O_5S_2$: C, 50.0; H, 5.3; N, 12.4; S, 14.2. Found: C, 50.5; H, 5.5; N, 12.2; S, 14.2.

2-Piperazinecarboxylic Acid Dihydrobromide (III).—Sixteen grams (0.034 mole) of ethyl 1,4-di-*p*-tosyl-2-piperazinecarboxylate was refluxed in 75 ml. of 47.5% hydrobromic acid for three hours. After approximately one hour the solid material completely dissolved and after three hours a crystalline material separated. The solution was allowed to cool and the dihydrobromide was collected, washed with 30 ml. of acetone and air-dried. This material (8.5 g.) was recrystallized from an ethanol–water solution; m.p. 282–284° dec.

Anal. Calcd. for $C_5H_{12}N_2O_2Br_2$: C, 20.7; H, 3.5; N, 9.7; Br, 55.1. Found: C, 21.3; H, 4.2; N, 9.5; Br, 54.6.

1,4-Di-*p*-nitrocarbobenzyloxy-2-piperazinecarboxylic Acid (IV).—To 7.3 g. (0.025 mole) of III dissolved in 19 ml. of 4 *N* sodium hydroxide and 10 ml. of water was added with stirring a solution consisting of 13.5 g. (0.063 mole) of *p*-nitrobenzyl chloroformate in 32 ml. of dioxane and 24 ml. of 4 *N* sodium hydroxide. These additions were made portionwise at approximately five-minute intervals. After the last addition, stirring was continued for one hour and the oily layer which separated from the reaction mixture was collected and dissolved in 1.5 liters of warm water. This solution was allowed to stand two days, then filtered and acidified with concentrated hydrochloric acid. The acidified solution on standing deposited 2.0 g. of a white, granular precipitate, m.p. 210–224°. A sample recrystallized from warm ethyl acetate melted at 222–224° (sintering at 218°).

Anal. Calcd. for $C_{21}H_{20}N_4O_{10}$: C, 51.6; H, 4.1; N, 11.5. Found: C, 51.9; H, 4.5; N, 12.0.

2-Piperazinecarboxylic Acid (V).—The dihydrobromide of 2-piperazinecarboxylic acid (7.0 g., 0.024 mole) was dissolved in 20 ml. of water and treated with 6.7 g. (0.024 mole) of silver carbonate added in three portions. After the silver bromide was removed, the clear solution was treated with hydrogen sulfide and then filtered. The filtrate was evaporated to dryness under reduced pressure at steam-bath temperature, leaving a white, granular residue. The yield of V was 2.9 g. (91.0%), m.p. 268–270° dec. (sintering at 265°). An analytical sample of this material was obtained by sublimation at 0.25 mm. with a heating block temperature of 170°; m.p. 275–276° dec.

Anal. Calcd. for $C_5H_{10}N_2O_2$: C, 46.1; H, 7.7; N, 21.5; neut. equiv., 203.0. Found: C, 45.6; H, 7.8; N, 21.5; neut. equiv., 195.5.

2-Piperazinecarboxylic Acid Dihydrochloride (VI).—The dihydrochloride of V was formed by adding two molar equivalents of 3.5 *N* hydrochloric acid to 0.5 g. of the acid in 5 ml. of water. When acetone was added to this solution a white, granular precipitate separated. This salt after drying over phosphoric anhydride at 110° (0.2 mm.) for 15 hours weighed 0.61 g. (90%), m.p. 261–262° dec.

Anal. Calcd. for $C_5H_{12}N_2O_2Cl_2$: C, 29.7; H, 5.9; N, 13.9; Cl, 34.7. Found: C, 30.0; H, 6.2; N, 13.9; Cl, 35.0.

Ethyl 2-Piperazinecarboxylate (VII).—Ethanol (70 ml.), benzene (10 ml.) and concentrated sulfuric acid (2.0 ml.) were refluxed with 11.7 g. (0.040 mole) of III for approximately 60 hours. After this period of time the reflux condenser was replaced by a heated, packed, distillation column and a ternary azeotropic mixture (benzene, ethanol and water) was collected at 64.9° (1 atm.). When the temperature at the still-head rose above 64.9°, the reaction was again refluxed until the boiling point of the azeotropic mixture was obtained. This procedure was repeated until a constant temperature of 78–79° (1 atm.) was observed. Distillation was then discontinued and the volatile materials were removed under reduced pressure at steam-cone temperature. The quasi-crystalline residue was made slightly alkaline with cold, concentrated potassium carbonate solution and extracted with three 50-ml. portions of dry benzene. The combined extracts were concentrated to a yellow, oily residue which solidified on cooling; 3.5 g. (55%). A pure sample of the ester was obtained after two recrystallizations from warm ether; m.p. 62–64°.

Anal. Calcd. for $C_7H_{14}N_2O_2$: C, 53.2; H, 8.9; N, 17.7. Found: C, 53.3; H, 9.0; N, 17.6.

2-Piperazinecarboxamide (VIII).—Two grams (0.013 mole) of VII was dissolved in 50 ml. of ethanol and to this

(3) W. Grassman and K. V. Arnim, *Ann.*, **509**, 297 (1934).

(4) W. S. Allen, S. M. Aronovic, L. M. Brancone and J. H. Williams, *Anal. Chem.*, **25**, 895 (1953).

(5) A standardized mouse test using survival as a criterion was used to evaluate activity. These compounds were tested at the arbitrary level of 0.2% of diet, fed *ad libitum* (8 mg. /day).

(6) Melting points uncorrected.

solution was added 25 ml. of concentrated ammonium hydroxide. The ammoniacal solution was allowed to stand seventy hours in a stoppered flask at room temperature and then concentrated to a gummy residue. The crude material was triturated with an ethanol–ether solution and the tan, granular solid which separated was collected and recrystallized from warm ethanol. The yield of the amide was 1.1 g. (65%), m.p. 144–145°.

Anal. Calcd. for $C_6H_{11}N_3O$: C, 46.5; H, 8.5; N, 32.6. Found: C, 46.2; H, 8.8; N, 32.6.

2-Piperazinecarboxylic Acid Hydrazide (IX).—A mixture consisting of 0.80 g. (0.0051 mole) of VII, 1.0 ml. of hydrazine hydrate (100%) and 10 ml. of dry ethanol was refluxed for 15 hours. The volatile material was then removed at reduced pressure and steam-cone temperature leaving a semi-crystalline residue which solidified when triturated with two 10-ml. portions of dry ether; 0.70 g. (95%). An analytical sample that was recrystallized twice from hot benzene had a m.p. 100–101°.

Anal. Calcd. for $C_6H_{12}N_4O$: C, 41.7; H, 8.3; N, 38.8. Found: C, 42.0; H, 8.5; N, 38.2.

Dehydrogenation of 2-Piperazinecarboxamide (X).—A sublimation tube equipped with inlet and outlet tubes was charged with a mixture of 0.040 g. (0.00031 mole) of 2-piperazinecarboxamide and 0.040 g. of palladium-on-charcoal (5%). The sublimation apparatus was set upright in a Wood metal-bath and the system was swept with nitrogen (an atmosphere of nitrogen was maintained by means of a slight positive pressure, *ca.* 40 mm., during the course of the reaction). When the temperature of the metal-bath reached 290°, gas was evolved and the temperature was regulated between 290–305° until the evolution of gas ceased. The crystalline sublimate which formed on the sides of the tube during the dehydrogenation was collected and triturated with small portions of ether and acetone, m.p. 173–183° (micro-stage melting block). The infrared absorption spectrum of this material is shown in Fig. 1.

Acknowledgment.—The authors wish to thank Mr. L. Brancone and staff for the analytical results and Mr. W. Fulmor for the infrared absorption measurements.

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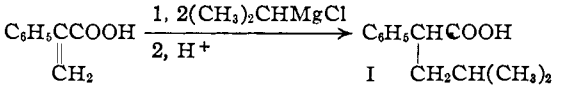
The Reaction of Isopropylmagnesium Chloride with Atropic, Tropic and α-Phenylisocaproic Acids

BY F. F. BLICKE AND HAROLD ZINNES

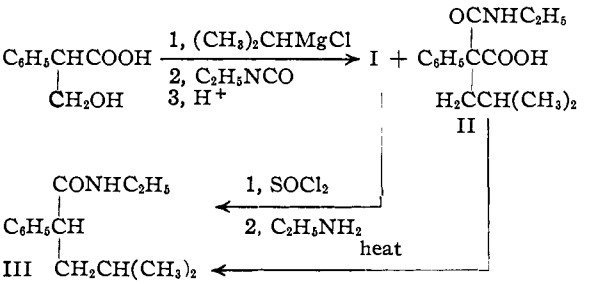
RECEIVED JULY 5, 1955

The interaction of isopropylmagnesium chloride with crotonic acid by 1,4-addition has been described.[1] It has now been found that this Grignard reagent reacts with atropic acid in a similar manner. When the α,β-unsaturated acid was refluxed with a solution of isopropylmagnesium chloride and the resulting mixture was hydrolyzed, α-phenylisocaproic acid (I) was obtained in 72% yield. This acid

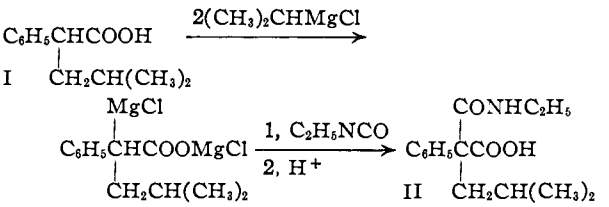


was found to be identical with the α-phenylisocaproic acid obtained by hydrolysis of α-phenylisocapronitrile according to the method of Bodroux and Taboury.[2]

Compound I was also produced in 51% yield when tropic acid was treated with isopropylmagnesium chloride and the mixture was hydrolyzed. When ethyl isocyanate was added to the reaction mixture before hydrolysis there was obtained, in addition to I (25% yield), a 40% yield of α-(ethylcarbamyl)-α-phenylisocaproic acid (II). The structure of II was established by its conversion, by decarboxylation, into the same α-phenyl-N-ethylisocaproamide (III) which was formed by successive treatment of I with thionyl chloride and ethylamine. These reactions indicate that tropic acid may have undergone initial dehydration to atropic acid under the influence of the Grignard reagent.[3]



Compound II was also prepared by an Ivanov reaction from compound I and ethyl isocyanate.



Experimental

α-Phenylisocaproic Acid (I) from Atropic Acid.—Isopropylmagnesium chloride was prepared from 2.0 g. of magnesium, 10 cc. of isopropyl chloride and 80 cc. of ether. After the addition of 3.7 g. of atropic acid,[4] dissolved in 150 cc. of benzene, the material was refluxed for 18 hours. The mixture was hydrolyzed with ammonium chloride solution, the aqueous layer was separated and the ice-cold solution was made acidic to congo red. The solid precipitate weighed 3.4 g. (71%), m.p. and mixed m.p. 77–78°[5] after recrystallization from petroleum ether (40–60°). Calcd. for $C_{12}H_{16}O_2$: neut. equiv., 192.3. Found: neut. equiv., 193.2.

α-Phenylisocaproic Acid (I) from Tropic Acid.—To the stirred isopropylmagnesium chloride solution obtained from 5.4 g. of magnesium, 25 cc. of isopropyl chloride and 100 cc. of ether, there was added 225 cc. of benzene and 8.3 g. of powdered tropic acid.[6] The mixture was refluxed for 18 hours. The product was isolated in the manner described above; yield 5.5 g. (57%), m.p. and mixed m.p. 77–78° after recrystallization from petroleum ether (40–60°).

α-Phenyl-α-(ethylcarbamyl)-isocaproic Acid (II). (A) From Tropic Acid.—Benzene (400 cc.) and then 16.6 g. of powdered tropic acid were added to the stirred solution obtained from 10.7 g. of magnesium, 50 cc. of isopropyl chloride and 200 cc. of ether. A solution of 25.9 g. of ethyl isocyanate in 100 cc. of benzene was added and the mixture was refluxed for 4 hours. After treatment of the material in the usual manner, the precipitated oily mixture of products was extracted with ether. The residue, obtained after removal of the ether, was triturated with cold

(1) F. F. Blicke and Harold Zinnes, THIS JOURNAL, **77**, 5399 (1955).

(2) F. Bodroux and F. Taboury, *Bull. soc. chim.*, [4] **7**, 666 (1910).

(3) In the study of a reaction between methyl tropate and phenylmagnesium bromide, A. McKenzie and E. R. Winton (*J. Chem. Soc.*, 840 (1940)) suggested that the initial reaction consisted of a dehydration of the tropate to methyl atropate.

(4) H. S. Raper, *J. Chem. Soc.*, 2558 (1923).

(5) Reference 2, m.p. 78–79°.

(6) F. F. Blicke, H. Raffelson and B. Barna, THIS JOURNAL, **74**, 253 (1952).