Anal. Calcd. for $C_7H_6N_3O_3Cl$: C, 39.0; H, 2.8; N, 19.5. Found: C, 38.8; H, 2.6; N, 19.5.

4-Chloro-2-nitrophenyl Isocyanate.—Seventy-one grams of 4-chloro-2-nitrophenylurea was suspended in 3600 ml. of a 10% solution of sodium hydroxide. The mixture was warmed on the steam-bath for 60 hours, at which time a clear red solution had formed. The solution was cooled, acidified with 500 ml. of concentrated hydrochloric acid and the resulting yellow flocculent precipitate collected and dried, weight 35 g., m.p. 85–86°, 53% yield. Recrystallization from ethanol yielded material of m.p. 87°.

Anal. Calcd. for $C_7H_3N_2O_3Cl$: N, 14.1. Found: N, 14.2.

7-Chloro-3-hydroxy-1,2,4-benzotriazine-1-oxide.—Three hundred and thirty grams of 4-chloro-2-nitrophenylurea was suspended in nine liters of 30% sodium hydroxide. The mixture was heated at 90–95° with good stirring for one-half hour and then made acid with glacial acetic acid. After cooling the crude 7-chloro-3-hydroxy-1,2,4-benzotriazine-1-oxide was collected. The hydroxy compound was further purified by dissolving in a 5% sodium hydroxide solution, filtering and reprecipitating with concentrated hydrochloric acid. The yellow precipitate was filtered off, washed with water and dried, weight 266 g., m.p. 225–226°, 88% yield. A sample recrystallized from Cellosolve had a melting point of 230–231° which is the same melting point as observed with a sample of 7-chloro-3-hydroxy-1,2,4-benzotriazine-1-oxide prepared by the diazotization of 7-chloro-3-amino-1,2,4-benzotriazine-1-oxide. A mixed melting point of the two samples showed no depression.

3,7-Dichloro-1,2,4-benzotriazine-1-oxide.—Twenty-five grams of 7-chloro-3-hydroxy-1,2,4-benzotriazine-1-oxide was dissolved in 50 ml. of dimethylaniline (free from mono-) and 100 ml. of phosphorus oxychloride and heated to reflux for three-quarters of an hour. The mixture was cooled and quenched in 1500 ml. of an ice–water mixture. The precipitate that formed was slurried with 300 ml. of hot 8 *N* hydrochloric acid to remove any methylanilino compound and then washed with water. The insoluble material consisting of crude 3,7-dichloro-1,2,4-benzotriazine-1-oxide weighed 20.7 g., m.p. 149–152°, 75.4% yield. Neutralizing the acid filtrate gave 4.0 g. of 7-chloro-3-(N-methylanilino)-1,2,4-benzotriazine-1-oxide, m.p. 155° (10% yield).

When dimethylaniline not especially purified was used, 25 g. of the hydroxy compound yielded 8.3 g. of the dichloro compound (32%) and 8.2 g. of methylanilino compound (30%). A sample of 3,7-dichloro-1,2,4-benzotriazine-1-oxide was recrystallized from alcohol, m.p. 153–154°.

Anal. Calcd. for $C_7H_3N_3Cl_2O$: C, 38.9; H, 1.4; N, 19.5. Found: C, 39.3; H, 1.5; N, 20.0.

7-Chloro-3-methoxy-1,2,4-benzotriazine-1-oxide.—To a solution consisting of 6.5 g. of 3,7-dichloro-1,2,4-benzotriazine-1-oxide in 50 ml. of absolute methanol was added 0.7 g. of sodium. The solution was then heated to reflux for 18 hours; on cooling, yellow-white crystals separated. The precipitate was recrystallized from methanol, weight 3.1 g., m.p. 155–156°, 50% yield. A sample was recrystallized from methanol, m.p. 157°.

Anal. Calcd. for $C_8H_6N_3O_3Cl$: C, 45.4; H, 2.9; N, 19.9. Found: C, 45.8; H, 3.2; N, 20.5.

7-Chloro-3-butylamino-1,2,4-benzotriazine-1-oxide.—Replacement of the 3-chloro group by a substituted amine is illustrated by this and the following example which are typical of the methods used for the preparation of compounds in Table I.

A mixture of 10 g. of 3,7-dichloro-1,2,4-benzotriazine-1-oxide and 6.8 g. of *n*-butylamine in 100 ml. of carbon tetrachloride was heated to reflux for 18 hours. At the end of this time the mixture was filtered to remove *n*-butylamine hydrochloride and the filtrate concentrated to dryness *in vacuo*. The residue was dissolved in the minimum quantity of hot absolute ethanol, treated with activated carbon and filtered. On cooling the filtrate, yellow needles, m.p. 170°, were formed, weight 3.7 g., 70% yield.

7-Chloro-3-(*p*-methoxyphenylamino)-1,2,4-benzotriazine 1-oxide.—A solution consisting of 6.6 g. of 3,7-dichloro-1,2,-4-benzotriazine-1-oxide and 7.2 g. of *p*-anisidine in 150 ml. of absolute alcohol was heated to reflux for 18 hours. Upon cooling the alcohol solution, a dark red precipitate was obtained. This material was filtered and recrystallized from Cellosolve. A yield of 6.0 g. (65%), m.p. 210–211°, was obtained.

7-Bromo-3-benzylamino-1,2,4-benzotriazine.—A mixture of 9.7 g. of 7-bromo-3-amino-1,2,4-benzotriazine-1-oxide and 75 ml. of benzylamine was heated to reflux for eight hours. The solution was cooled and poured into 500 ml. of methanol. The resulting precipitate was filtered off and washed with 100 ml. of methanol. The crude product was recrystallized from Cellosolve. A yield of 5.0 g. (40%), m.p. 172–173°, was obtained. Recrystallization from methanol yielded material, m.p. 173–174°.

Anal. Calcd. for $C_{14}H_{11}N_4Br$: C, 53.4; H, 3.5; N, 17.9. Found: C, 53.8; H, 3.7; N, 18.2.

7-Chloro-3-benzylamino-1,2,4-benzotriazine.—This compound was readily obtained by refluxing 7-chloro-3-amino-1,2,4-benzotriazine with benzylamine. The light yellow product was obtained in 78% yield, m.p. 175°.

Anal. Calcd. for $C_{14}H_{11}N_4Cl$: C, 62.2; H, 4.1; N, 20.7. Found: C, 61.9; H, 4.1; N, 21.3.

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NOTES

L-Pyrrolidonecarboxylic Acid

BY A. F. BEECHAM

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King and McMillan,[1] after thermal dehydration of glutamic acid, isolated two compounds, pyrrolidonecarboxylic acid, m.p. 180–183°, and "3,6-diketopiperazine-2,5-dipropionic acid," m.p. 160–161°. This work has been repeated and the two substances examined in more detail. The acid m.p. 180–183° is found to be optically inactive; the acid m.p. 160–161° has $[\alpha]_D$ −11.7° (in water) and is shown to be the optically active form of the first by the following considerations: (1) The infrared absorption of the tributylamine salts of the two compounds in chloroform solution is identical. Several well-defined absorption bands are assignable to the salts rather than to tributylamine or the solvent. (2) The ultraviolet absorption curves of the two acids in aqueous solution are featureless but identical. (3) The two acids have identical R_f values on paper chromatograms run with three different solvent systems. (4) The acid m.p. 160–161° can be converted in good yield to the acid m.p. 180–183° by heating at 190°.

The melting point and the specific rotation found for the acid m.p. 160–161° agree with the constants

(1) J. A. King and F. H. McMillan, THIS JOURNAL, **74**, 2859 (1952).

recorded for L-pyrrolidonecarboxylic acid.[2] As well as by thermal dehydration, L-glutamic acid may be converted to this compound by boiling an aqueous solution.[3] A sample of L-pyrrolidonecarboxylic acid prepared in this manner was shown by m.p. and mixed m.p. to be identical with the acid m.p. 160–161°. There seems little doubt, therefore, that the compounds isolated by King and McMillan were DL- and L-pyrrolidonecarboxylic acid.

However, pyrrolidonecarboxylic acid and 3,6-diketopiperazine-2,5-dipropionic acid would have molecular weights of 129 and 258, respectively. By cryoscopic determinations in glacial acetic acid King and McMillan found values of 128 for the acid m.p. 180–183° and 250 for the acid m.p. 160–161°. Using the same solvent, the ebullioscopic method has now given values of 142 for the DL-acid and 139 and 143 for the L-compound. In camphor solution both compounds are highly associated giving values of 220–360 over the concentration range 5–10%.

A second synthesis used by King and McMillan to prepare their "3,6-diketopiperazine-2,5-dipropionic acid" seems open to doubt. Le Quesne and Young[4] acted on carbobenzyloxy-L-glutamic acid anhydride with ethanol to obtain a mixture of the α- and γ-esters which were separated by fractional extraction with aqueous sodium carbonate. Hydrogenation of the resulting carbobenzyloxy-L-glutamic acid α-ethyl ester gave them α-ethyl L-glutamate which could not be obtained free from solvent. King and McMillan prepared α-ethyl L-glutamate by this method, heated the product at 100° for 24 hours and leached the resulting dark brown material with water, from which, after evaporation and standing, the acid m.p. 160–161° was isolated in 10% yield. It seems possible that the α-ethyl L-glutamate still contained a proportion of the γ-isomer, or it may be that α-ethyl L-glutamate is converted in part to L-pyrrolidonecarboxylic acid under the conditions used.

Experimental

Thermal Dehydration of L-Glutamic Acid.—L-Glutamic acid (73.5 g., (0.5 mole) was dehydrated exactly as described by King and McMillan[1] by heating at 175° until liquefaction was complete (1 hour) then continuing to heat at 150° and 40–50 mm. pressure until no further bubbles appeared in the melt (3 hours). The resulting material was taken up in boiling ethanol (600 cc.) filtered from insoluble material (glutamic acid, 5 g.) and the filtrate allowed to crystallize at 0°. Fractions 1 (3.5 g., m.p. 145–174°), 2 (1.3 g., m.p. 155–180°) and 3 (3.5 g., m.p. 155–175°) were collected, then the mother liquor concentrated to 300 cc. Immediately on cooling, fraction **4** (0.35 g., m.p. 315–320°) was collected. On further standing, fractions 5 (3.6 g., m.p. 160–181°), 6 (10.6 g., m.p. 145–159°) and 7 (4.8 g., m.p. 144–155°) were obtained. No further crystallization took place on standing or concentration.

DL-Pyrrolidonecarboxylic Acid.—The combined fractions 1, 2 and 3, after several recrystallizations from ethanol, yielded pure material, 4.8 g., m.p. 181.5–183°. The optical rotation of a 4% aqueous solution was zero.

A second pyrolysis of L-glutamic acid under the same conditions followed by crystallization of the melt from its own weight of water yielded the same material.

3,5,8,10-Tetraketoperhydrodipyrrolo[a,d]pyrazine.[1]—Fraction 4 on recrystallization from aqueous ethanol gave 0.18 g. of pure material, m.p. 335–337° dec., $[\alpha]_D$ zero.

L-Pyrrolidonecarboxylic Acid. A.—The combined fractions 6 and 7 were crystallized several times from solution in methyl ethyl ketone by the addition of carbon tetrachloride to give 10.9 g., m.p. 160–161°, $[\alpha]^{20}D$ −11.7° (*c* 4 in water).

B.—L-Glutamic acid, 100 g., was dissolved in 2500 cc. of boiling water, and the solution allowed to evaporate over 3 days in an air oven at 110°. The resulting clear sirup was taken up in 300 cc. of hot ethanol, and diluted with 2500 cc. of acetone to precipitate 7 g. of unreacted glutamic acid. Evaporation of the filtrate and recrystallization of the residue from methyl ethyl ketone gave 22 g. of material, m.p. 155–158°. Concentration of the mother liquor and addition of carbon tetrachloride gave a further 52 g., m.p. 145–155°. Recrystallization yielded a total of 48 g. of pure compound, m.p. 160–161°, undepressed on admixture with the material from A.

Racemization of L-Pyrrolidonecarboxylic Acid.—The compound from A above, 5 g., was heated at 190° (oil-bath) for 75 minutes. The melt was dissolved in 5 cc. of hot water, charcoal added, the solution filtered and the filter washed with 2 cc. of hot water. The combined filtrate and washings on standing overnight deposited DL-pyrrolidonecarboxylic acid, 2.0 g., m.p. 181–182.5°.

Infrared Absorption.—The acid m.p. 181.5–183° (0.13 g., 0.01 mole), suspended in 2 cc. of chloroform gave a clear solution upon addition of tributylamine (0.24 cc., 0.01 mole). An identical solution of the tributylamine salt of the acid m.p. 160–161° was prepared. The absorption of these solutions, measured over the range 800–1800 cm.$^{-1}$, was identical. Comparison with blanks run on chloroform alone and on 0.24 cc. of tributylamine in 2 cc. of chloroform showed that the following bands were due to absorption by the salts.

957 cm.$^{-1}$	1153 cm.$^{-1}$		1468 cm.$^{-1}$
1009 cm.$^{-1}$	1261 cm.$^{-1}$	doublet	1612 cm.$^{-1}$
1091 cm.$^{-1}$ doublet	1291		1696 cm.$^{-1}$
1107	1397 cm.$^{-1}$		

Ultraviolet Absorption.—Solutions of each acid, 0.10 g., in 25 cc. of water were examined over the range 3000–2300 Å. The curves were identical, although featureless.

Paper Chromatography.—The two acids had identical R_f values using the following solvent systems; phenol/water (R_f 0.71), ethanol/concd. aqueous ammonia (R_f 0.66), *n*-butyl alcohol/formic acid/water (R_f 0.78).

Molecular Weights.—Ebullioscopic determinations were carried out using glacial acetic acid, b.p. 118.10° (766 mm.), purified by refluxing for 24 hours with acetic anhydride and chromic oxide.

Anal. Calcd. for $C_5H_7NO_3$: mol. wt., 129. Found: acid m.p. 181.5–183°, 142 ± 4; acid m.p. 160–161°, 139 ± 4, 143 ± 4.[5]

The following values were obtained by cryoscopic determinations in camphor[6] (concentrations in parentheses): acid m.p. 181.5–183°: 240 (5.4%), 236 (5.5%), 331 (9.0%), 366 (9.8%). Acid m.p. 160–161°: 225 (4.9%), 311 (9.9%), 308 (10.4%).[7]

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(2) See, for instance, A. Menozzi and G. Appiani, *Gazz. chim. ital.*, **24**, I, 370 (1894); E. Abderhalden and K. Kautzsch, *Z. physiol. Chem.*, **68**, 493 (1910); M. Bergmann and L. Zervas, *ibid.*, **221**, 51 (1933); N. Lichtenstein, THIS JOURNAL, **64**, 1021 (1942).

(3) F. W. Foreman, *Biochem. J.*, **8**, 481 (1914); H. Wilson and R. K. Cannan, *J. Biol. Chem.*, **119**, 309 (1937).

(4) W. J. Le Quesne and G. T. Young, *J. Chem. Soc.*, 1954 (1950).

(5) The b.p. elevation constant used, 30.7°, was taken from the literature, E. Beckmann and O. Liesche, *Z. physik. Chem.*, **88**, 419 (1914).

(6) Carried out by the C.S.I.R.O. Microanalytical Laboratory under the supervision of Dr. K. W. Zimmerman.

(7) B. L. Hutchings, *et al.*, *Ann. N. Y. Acad. Sci.*, **48**, 273 (1946), found, in camphor, for a sample of pyrrolidonecarboxylic acid mol. wt. 284.