The Conversion of Isotopically Labeled Glycine to 1-Methyl-2-amino-2-imidazolin-4-one (Creatinine) (1)

G. L. Rowley (2) and G. L. Kenyon

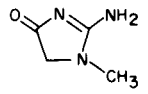
Department of Chemistry, University of California, Berkeley, California 94720

Received September 16, 1971

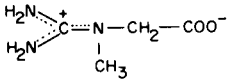
A procedure is presented which allows the conversion of glycine to creatinine (1-methyl-2-amino-2-imidazolin-4-one). The scheme was devised to optimize the yield based on the amount of glycine used so that the relatively readily available isotopically labeled glycines could be efficiently converted to the correspondingly labeled creatinines. In this case 1-(^{13}C)-glycine was converted to 4-(^{13}C)-1-methyl-2-amino-2-imidazolin-4-one.

Introduction.

The levels of creatinine, an important end product of nitrogen metabolism in vertebrates, are routinely determined clinically in human urine as an indicator of certain disease states (3). The favored tautomeric structure of creatinine (**1**) has only recently been established (4,5). As part of our research program to investigate synthetic approaches to creatine (**2**) and creatinine (**1**) we have



1

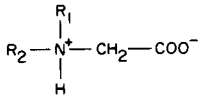


2

investigated several points of departure (5,6). The ready availability of isotopically labeled glycine makes it an attractive starting material for the synthesis of the corresponding labeled creatine, creatinine and closely related analogs. Despite numerous reports of procedures for the synthesis of creatine (7,8,9,10) no method has been reported for the chemical conversion of glycine to creatinine in good yield. The low yields of monosubstitution products obtained from direct alkylation of amino acids (11) were considered to be too wasteful. We have therefore devised a scheme which circumvents these problems and allows the synthesis of labeled creatine and creatinine from labeled glycine in good overall yield. The yield in none of the individual steps was below 75%.

Discussion.

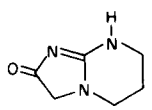
A survey of the literature revealed that there are at least four known procedures for the preparation of *N*-benzylglycine (**4**) from glycine (12,13,14,15). Our method, which was adapted from that of Velluz *et al.*, (16) involved forming the very water-insoluble *N,N*-dibenzylglycine (**3**) followed by catalytic hydrogenation to remove



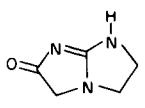
3 R_1, R_2 = $-CH_2C_6H_5$
4 R_1 = -H, R_2 = $-CH_2C_6H_5$
5 R_1 = $-CH_3$, R_2 = $-CH_2C_6H_5$
6 R_1 = $-CH_3$, R_2 = -H

selectively one of the benzyl groups. The high yield process which we employed for conversion of *N*-benzylglycine (**4**) to sarcosine (**6**) was patterned after similar conversions of other *N*-benzylamino acids to *N*-methylamino acids (17). The conversion of sarcosine (**6**) to creatine (**2**) was carried out by reaction of the sodium salt of sarcosine with *S*-methylthiuronium iodide so that unreacted sarcosine could be readily recovered and recycled by extracting the concomitantly formed sodium iodide with acetone.

The conversion of 1-(^{13}C)-creatine to 4-[^{13}C]-1-methyl-2-amino-2-imidazolin-4-one in 91% yield *via* cyclization of the 1-(^{13}C)-creatine ethyl ester hydrochloride by passage through a methanolic weakly basic ion-exchange resin column was patterned after the successful use of this cyclization method for the preparation of 2-oxo-2,3,5,6,7,8-hexahydroimidazo[1,2-*a*]pyrimidine (**7**) from 1-carboxymethyl-2-iminohexahydropyrimidine (5). This cyclization procedure had earlier been used in similar reactions by



7



8

McKay and Kreling (18). When the analogous synthesis of 6-oxo-2,3,5,6-tetrahydro-1*H*-imidazo[1,2-*a*]imidazole (**8**) was attempted from the recently prepared creatine analog, 1-carboxymethyl-2-iminoimidazolidine (6), however, the ethyl ester hydrochloride was converted rapidly on the methanolic column to the corresponding methyl ester hydrochloride, identified by its elemental analysis and its spectral properties. It is possible that the bicyclic compound (**8**) did form, but because of excessive strain, rapidly reacted with the methanol solvent.

The scheme presented here has been used for the synthesis of 1-[^{13}C]-creatine and 4-[^{13}C]-1-methyl-2-amino-2-imidazolin-4-one from 1-[^{13}C]-glycine, but in principle could be used for the preparation of other *N*-alkylamino acids and *N*-amidino-*N*-alkylamino acids from their appropriate isotopically labeled amino acid precursors.

EXPERIMENTAL

N,N-Dibenzylglycine (**3**).

To 1.20 g. (0.016 mole) of glycine in 9 ml. of water was added 3.2 g. of 85% potassium hydroxide (0.048 mole) and 9 ml. of ethanol. The solution was heated to reflux, and 4.0 ml. (0.035 mole) of benzyl chloride was added dropwise over 10 minutes. The resulting solution was heated at reflux for 1 hour, cooled and evaporated to one half the original volume. An equivalent volume of methylene chloride and about 20 ml. of water were added to give two liquid phases. The layers were separated, and the aqueous layer was extracted twice more with 10 ml. portions of methylene chloride. The combined methylene chloride layers were back extracted with a 10 ml. portion of water. The aqueous layers were combined, boiled briefly to drive off the dissolved methylene chloride, cooled, and acidified with acetic acid to *p*H 6. *N,N*-Dibenzylglycine precipitated from the solution immediately. The crystals were allowed to accumulate at 4° for 12 hours and then were removed by filtration. A small amount of additional product was obtained by concentrating the mother liquors and refiltering. The total yield of dried product, which required no further purification, was 3.46 g. (85%), m.p. 193-195° (lit. (16) m.p. 200°).

N-Benzylglycine (**4**).

The *N,N*-dibenzylglycine (3.32 g., 13.0 mmoles) was dissolved in 39 ml. of methanol, 1.63 ml. of concentrated hydrochloric acid (19.5 mmoles), 2.00 ml. of water and 1.30 g. of 10% palladium on charcoal (prereduced) were added. Hydrogenation was performed at 22° at atmospheric pressure. After 2 hours the quantitative amount of hydrogen had been taken up. After removal of the catalyst by centrifugation followed by filtration, the solvents were removed *in vacuo* to leave 2.45 g. of shiny plates of *N*-benzylglycine hydrochloride, m.p. 215.5-217.5° dec. (lit. (19), m.p. 216-218° dec.) (93% yield). This solid was dissolved in 25 ml. of water and passed through a column of Bio-Rad AG3-X4 resin in the amino form. A total of 500 ml. of wash water was used. After evaporation of the water *in vacuo* there remained 1.94 g. of white solid (97% recovery), m.p. 195-197.5° dec. (lit. (19), m.p. 198-199°).

N-Benzyl-*N*-methylglycine (**5**).

The *N*-benzylglycine (1.94 g., 11.8 mmoles) was mixed with 2.8 ml. (71 mmoles) of formic acid and 2.1 ml. of 36% aqueous formaldehyde (18 mmoles). After 15 minutes heating on a steam bath, carbon dioxide evolution had ceased. The product was cooled and the solvents were removed *in vacuo* at 55° to give a yellow syrup. On further drying 2.22 g. of white, slightly moist solid was obtained (theoretical yield, 2.11 g.). The crude product was dissolved in 18 ml. of boiling absolute ethanol and 108 ml. of absolute ether was added dropwise with vigorous stirring. The mixture was stirred an additional ½ hour, and the product was removed by filtration. The product was washed with three 3 ml. portions of ether, two 5 ml. portions of dry hexane and dried to give 1.87 g. (89%) of product, m.p. 188.5-191.5° dec. (lit. (20) m.p. 190-191°).

Sarcosine (*N*-methylglycine) (**6**).

The *N*-methyl-*N*-benzylglycine (1.87 g., 10.5 mmoles) was dissolved in 50 ml. of 90% aqueous acetic acid to which had been added 1.05 g. of 10% palladium on charcoal (prereduced). The mixture was hydrogenated for 2 hours as described in the synthesis of the *N*-benzylglycine. The catalyst was removed by centrifugation, and the solvents were removed at 60° *in vacuo*. There remained 0.98 g. of pale yellow sarcosine, which was used without further purification in the synthesis of the creatine.

Creatine (*N*-amidino-*N*-methylglycine) (**2**).

The sarcosine (10.5 mmoles) obtained above was dissolved in 1.5 ml. of water and neutralized with 1.05 ml. of 10 *N* sodium hydroxide to give a yellow solution. *S*-Methyl thiuronium iodide (6) (2.29 g., 10.5 mmoles), dissolved in 2.0 ml. of water, was added dropwise at 30° with vigorous stirring. Addition was complete in 2.5 hours, and the mixture was stirred for an additional 12 hours. The precipitate which formed was collected by filtration and dried to give 0.92 g. of pale yellow solid. An additional 0.10 g. was obtained by reducing the volume of the filtrate to about 3 ml. and slowly adding 12 ml. of absolute ethanol. The total crude yield was 1.02 g. The product was dissolved in 75 ml. of warm water and filtered through celite to remove a trace of insoluble yellow material. The volume was reduced to 40 ml. The resulting mixture was warmed on a steam bath to dissolve all the solid, and 160 ml. of warm absolute ethanol was added. After cooling overnight at 4°, the lustrous plates which had formed were removed by filtration and dried to yield 0.90 g. (6.9 mmoles) of analytically pure, anhydrous creatine. Examination of the mother liquors by nmr spectroscopy revealed that a reasonable amount of unreacted sarcosine was present. Accordingly, the solvents were removed *in vacuo*, and the crude sarcosine was triturated with 35 ml. boiling acetone. The resulting acetone-insoluble material was dissolved in 0.6 ml. of water, neutralized with 0.41 ml. of 10 *N* sodium hydroxide and reacted as described above with 0.89 g. (4.1 mmoles) *S*-methylthiuronium iodide. A total of 0.165 g. (1.1 mmoles) of recrystallized creatine hydrate was obtained as a second crop. Thus, the total yield of creatine based on *N*-benzyl-*N*-methylglycine was 8.0 mmoles (76%).

When the above sequence of reactions was performed with 1-[^{13}C]-glycine, the product obtained was 1-[^{13}C]-creatine (50 atom % ^{13}C-enriched). The anhydrous product was submitted for combustion analysis.

Anal. (21) Calcd. for $C_4H_7N_3O$: C, 36.88; H, 6.90; N, 31.96. Found: C, 37.28; H, 7.07; N, 31.95. Nmr[21] δ 2.97 (3H, s), δ 3.53 (2H, s and d, J ^{13}CC^{1}H = 5 Hz).

Creatinine (1-methyl-2-amino-2-imidazolin-4-one) (**1**).

A 200 mg. (1.52 mmoles) portion of anhydrous creatine was dissolved in 50 ml. of anhydrous ethanol, dry hydrogen chloride

was briefly bubbled into the solution, and the solution was heated at reflux for 20 hours. After removal of the solvent, the ethyl ester hydrochloride was obtained along with creatinine hydrochoride; nmr[21] δ 1.35 (3H, t, J = 7 Hz), 3.17 (3H, s), 4.34 (2H, s), 4.35 (2H, 8, J = 7 Hz). This product was not purified but was cyclized directly to creatinine. Thus, the sample was dissolved in the minimal amount of dry methanol and passed through a methanolic BioRad AG 3 x 4 column (amino form, 1.5 x 45 cm.). A total of 500 ml. of methanolic eluent was collected. After removal of the solvent the creatinine remained.

When this reaction sequence was performed using 1-[^{13}C]-creatine (200 mg.) the isolated yield of 4-[^{13}C]-creatinine was 156 mg. (91%). The ir spectrum (Nujol) was identical to that of authentic creatinine; nmr δ 2.98 (3H, s), 3.94 (2H, s and d, $J_{^{13}CC^1H}$ = 5 Hz).

Attempt to Prepare 6-Oxo-2,3,5,6-tetrahydro-1*H*-imidazo[1,2-*a*]-imidazole (**8**) by Cyclization of 1-Carboxymethyl-2-iminoimidazolidine Ethyl Ester Hydrochloride.

A 200 mg. (1.40 mmoles) portion of 1-carboxymethyl-2-iminoimidazolidine (5) was dissolved in 50 ml. of anhydrous ethanol, dry hydrogen chloride gas was bubbled into the solution for a brief period, and the solution was heated at reflux for 12 hours. After removal of the solvent, the 1-carboxymethyl-2-iminoimidazolidine ethyl ester hydrochloride remained as a solid; nmr δ 1.48 (3H, t, J = 7 Hz), 3.95 (4H, m), 4.43 (2H, q, J = 7 Hz), 4.53 (2H, s). This product was not purified further and was dissolved in the minimal amount of dry methanol and passed through the methanolic weakly basic ion-exchange resin column described above. A total of 500 ml. of methanol was used as eluent. After removal of the solvent, a white crystalline solid remained. After recrystallization from ethanol, large prisms (125 mg., 46% yield) of purified product were obtained, m.p. 188-190° dec. An aqueous solution of the sample gave a copious precipitate of silver chloride when dilute silver nitrate solution was added. The nmr spectrum was consistent with the structure of 1-carboxymethyl-2-iminoimidazolidine methyl ester hydrochloride, showing peaks at δ 3.97 (4H, m), 4.03 (3H, s), 4.53 (2H, s); ir (Nujol) : 5.78 and 6.00 μ.

Anal. (21) Calcd. for $C_6H_{12}ClN_3O_2$: C, 37.19; H, 6.25; N, 21.71. Found: C, 37.48; H, 6.34; N, 21.87.

Acknowledgment.

We would like to thank Mr. James Sinclair for supplying the 1-[^{13}C]-glycine used in these syntheses.

REFERENCES

(1) This work was supported by U. S. Public Health Service Grant AM13529, National Institute of Arthritis and Metabolic Diseases.

(2) U. S. Public Health Service Special Fellow, National Institute of General Medical Sciences, 1968-1971.

(3) H. Brainard, S. Margen and M. Shatton, "Current Diagnosis and Treatment," Lang Medical Publications, Los Altos, California, 1968, p. 502.

(4) K. Matsumoto and H. Rapoport, *J. Org. Chem.*, 33, 552 (1968).

(5) G. L. Kenyon and G. L. Rowley, *J. Am. Chem. Soc.*, 93, 5552 (1971).

(6) G. L. Rowley, A. L. Greenleaf and G. L. Kenyon, *ibid.*, 93, 5542 (1971).

(7) A. Strecker, *Jahresber. Fortschr. Chem.*, 686 (1868).

(8) H. King, *J. Chem. Soc. (London)*, 2374 (1930).

(9) E. Schütte, *Z. Physiol. Chem.*, 279, 52 (1943).

(10) K. Odo and E. Ishikawa, *Nippon Kaguku Zasshi*, 77, 1413 (1956).

(11) J. Novak, *Ber.*, 45, 834 (1912).

(12) E. Fischer and L. V. Mechel, *ibid.*, 49, 1355 (1916).

(13) H. Scheibler and P. Baumgarten, *ibid.*, 55, 1358 (1922).

(14) N. Gavrilov, A. W. Koperina and M. Klutcharova, *Bull. Soc. Chim. France*, 12, 773 (1954).

(15) H. Borsook, C. L. Deasy, A. J. Haagen-smit, G. Keighely, and P. H. Lowry, *J. Biol. Chem.*, 196, 669 (1952).

(16) L. Velluz, G. Amiard, and R. Heymes, *Bull. Soc. Chim. France*, 21, 1012 (1954).

(17) P. Quitt, J. Hellerbach and K. Vogler, *Helv. Chem. Acta*, 46, 327 (1963).

(18) A. F. McKay and M.-E. Kreling, *Can. J. Chem.*, 40, 205 (1962).

(19) C. K. Bradsher, F. C. Brown, and E. F. Sinclair, *J. Am. Chem. Soc.*, 78, 6189 (1956).

(20) D. E. Ames, R. E. Bowman, D. D. Evans and W. A. Jones, *J. Chem. Soc. (London)*, 1984 (1956).

(21) Melting points were taken using a Büchi apparatus and are uncorrected. Analyses were performed by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley. Nmr spectra were measured in deuterium oxide solution on a Varian T-60 spectrometer using sodium 2,2-dimethyl-2-silapentane-5-sulfonate as standard.