Tautomeric Preferences among Glycocyamidines

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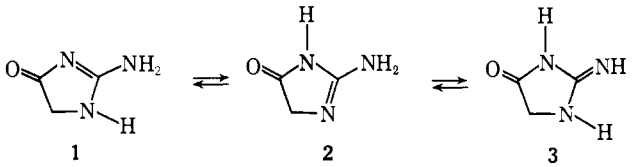
Abstract: The pK_a values, hydrolytic stabilities, deuterium exchange rates, and spectral properties of 12 glycocyamidines, seven of which have been isolated for the first time, have been investigated. The glycocyamidines fell into two distinct groups, those of the acylamino type (–N=C(N)—N(R)C(=O)R′, where R,R′ = alkyl) and those of the acylimino type (–NC(NR)=NC(=O)R′, where R,R′ = alkyl). Characteristically, the glycocyamidines of the acylimino type had pK_a values in the 4.48–4.84 range whereas those of the acylamino type had pK_a values in the 7.57–9.01 range. Glycocyamidines of the acylimino type were much more stable toward hydrolysis in strong base, had slower base-catalyzed deuterium exchange rates for their ring methylene hydrogens, and absorbed in the ultraviolet region at longer wavelengths than their acylamino counterparts. Within the acylamino group, three of the glycocyamidines investigated can each potentially exist in a pair of tautomeric forms in which the C=N bond is either endocyclic or exocyclic to the five-membered ring. Comparison of properties of these three compounds with glycocyamidines of the acylamino type locked into the endocyclic or exocyclic C=N bond forms by appropriate methyl substitution and other considerations have led to the tentative conclusion that the endocyclic C=N bond tautomers are preferred over the exocyclic C=N bond tautomers. A number of chemical transformations and interlocking interconversions were performed to substantiate further the structural assignments given, including the unusual reaction of 1-methyl-2-methylamino-2-imidazolidin-4-one with aqueous nitrous acid to give *N*-(*N*-nitrosomethylcarbamoyl)-2-(*N*-nitrosomethylamino)acetamide, a secondary *N*-nitrosoamine which prefers to be the *E* isomer in the solid state.

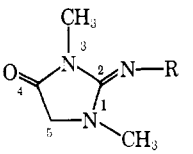
Creatinine (**7b**) is an important end product of nitrogen metabolism in vertebrates, about 1.1–1.7 g/24 hr appearing, for example, in the urine of normal adult humans.[2] Its relative abundance in urine is widely recognized as an indicator of certain disease states.[3] Creatinine belongs to a class of compounds called glycocyamidines, which are cyclic acylguanidines contained in five-membered rings. Only scattered reports concerning the structures of glycocyamidines have appeared,[4–13] and most of this work was performed prior to 1950. A review of this early work was published in 1959.[14] Although some of these early research groups recognized that the parent member of this series, glycocyamidine, could exist in three tautomeric forms [2-amino-2-imidazolin-4-one (**1**), 2-amino-2-imidazolin-5-one (**2**), and 2-iminoimidazolidin-4-one



1 **2** **3**

(**3**)], only recently has clear evidence been presented by Matsumoto and Rapoport[13] to show that tautomeric form **1** is preferred. In fact the structures of glycocyamidine and creatinine are almost universally written as tautomeric form **3**, which is probably the least preferred.

As part of our efforts to investigate the specificity of creatine kinase from rabbit muscle as reported in an accompanying paper,[15] we have synthesized and characterized numerous analogs of creatine. In some cases, particularly for the creatine analogs *N*-methylamidinoglycine (isocreatine, **19**) and *N*-methylamidino-*N*-methylglycine (**9**), important aspects of the characterization of these creatine analogs were the isolation and characterization of their ring closure products, the corresponding glycocyamidines. Thus, *N*-methylamidinoglycine (**19**) can cyclize to form either 1-methyl-2-amino-2-imidazolin-5-one (**5a**) or 2-methylamino-2-imidazolin-4-one (**7c**), depending on which group, the primary amino group or the secondary amino group, preferentially attacks the carbonyl (Scheme II). Similarly, *N*-methylamidino-*N*-methylglycine (**9**) can cyclize to form either 1,3-dimethyl-2-iminoimidazolidin-4-one (**4a**) or 1-methyl-2-methylamino-2-imidazolin-4-one (**7d**, Scheme I). A search of the literature revealed that of these four glycocyamidines only **4a** had been correctly characterized,[11] and its identity has been controversial.[7,9–12] We report here structural assignments, including, in some cases, tautomeric preferences, for 12 glycocyamidines, **4a–8**, seven of which have been isolated for the first time.



4a, R = H (1,3-dimethyl-2-iminoimidazolidin-4-one)
4b, R = CH_3 (1,3-dimethyl-2-methyliminoimidazolidin-4-one)

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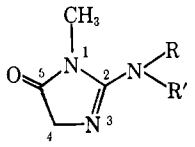
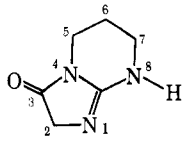
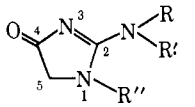


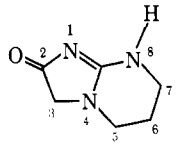
5a, R,R′ = H (1-methyl-2-amino-2-imidazolin-5-one)
5b, R = H; R′ = CH_3
(1-methyl-2-methylamino-2-imidazolin-5-one)
5c, R,R′ = CH_3 (1-methyl-2-dimethylamino-2-imidazolin-5-one)



6, 3-oxo-2,3,5,6,7,8-hexahydroimidazo[1,2-*a*]pyrimidine



7a, R,R′,R″ = H (2-amino-2-imidazolin-4-one (glycocyamidine))
7b, R,R′ = H; R″ = CH_3
(1-methyl-2-amino-2-imidazolin-4-one (creatinine))
7c, R = CH_3; R′,R″ = H (2-methylamino-2-imidazolin-4-one)
7d, R,R″ = CH_3; R′ = H
(1-methyl-2-methylamino-2-imidazolin-4-one)
7e, R,R′ = CH_3; R″ = H (2-dimethylamino-2-imidazolin-4-one)



8, 2-oxo-2,3,5,6,7,8-hexahydroimidazo[1,2-*a*]pyrimidine

Experimental Section

Methods. General analytical methods were described in the preceding paper.[15] A Consolidated Electrodynamics Corp. Model 21-110B double-focusing high-resolution mass spectrometer was used in performing accurate mass measurements. Ultraviolet spectra and rates of base-catalyzed hydrolysis were made using a Cary Model 14 recording spectrophotometer. All of the hydrolysis rate measurements were taken at 230 nm or longer wavelengths because of significant product absorption below this wavelength. Measurements of pK_a values were performed as described by Matsumoto and Rapoport[13] using a Sargent Recording pH Stat, Model S-30240, in conjunction with a Corning Model 12 Research pH meter. A slow stream of nitrogen was passed over the samples throughout these measurements. Rates of deuterium exchange for the ring methylene hydrogens were followed by nmr spectroscopy at 40° using 0.07 *M* glycocyamidine in 0.15 *M* sodium borate buffer, pD 9 in D_2O.

Materials. 2-Amino-2-imidazolin-4-one (Glycocyamidine, 7a). Glycocyamidine hydrochloride was prepared as described previously,[16] mp 206–208° dec (lit.[13] mp 211–212° dec), and was converted to the free base by crystallization from cold aqueous NH_3 as described by King.[17] The nmr spectrum of the glycocyamidine (D_2O) showed a singlet at δ 3.91 (see Table I).

1-Methyl-2-methylamino-2-imidazolin-5-one (5b) was prepared by Dr. K. Matsumoto[13] and was the gift of Professor Henry Rapoport (see Table I).

3-Oxo-2,3,5,6,7,8-hexahydroimidazo[1,2-*a*]pyrimidine (6) was prepared as described by McKay and Kreling,[18] mp 175–177° (lit.[18] mp 179.5–180°). The nmr spectrum showed peaks at δ 1.92 (2 H, multiplet), 3.21 (2 H, triplet, *J* = 6 Hz), 3.48 (2 H, triplet, *J* = 6 Hz), 3.86 (2 H, singlet). The uv spectrum is described in Table I.

1-Methyl-2-methylamino-2-imidazolin-4-one (7d). A. Cyclization of *N*-Methylamidino-*N*-methylglycine (9). *N*-Methylamidino-*N*-methylglycine (**9**, 145 mg, 1 mmol) was heated at reflux ($CaCl_2$ drying tube) for a total of 19.5 hr in 2.9 ml of anhydrous methanol. The course of the reaction was followed by periodically removing samples and analyzing by nmr spectroscopy (Figure 1). Peaks corresponding to the starting material decreased as peaks corresponding to 1,3-dimethyl-2-iminoimidazolidin-4-one (**4a**, see below) appeared; then gradually, the peaks corresponding to **4a** decreased and new peaks, corresponding to the product **7d**, appeared. After removal of the methanol *in vacuo*, the product was recrystallized from acetonitrile to give 65 mg (51% yield) of pale yellow plates, mp 196.5–198.5°.

Anal. Calcd for $C_5H_9N_3O$: C, 47.23; H, 7.14; N, 33.05. Found: C, 47.50; H, 7.38; N, 33.09.

The uv and nmr spectra are shown in Table I. The ir spectrum (Nujol) showed major bands at 3.07, 5.82, 6.12, 6.72, 8.22, 9.00, 10.13, 11.20, and 13.99 μ.

B. Isomerization of 1,3-Dimethyl-2-iminoimidazolidin-4-one (4a). Purified 1,3-dimethyl-2-iminoimidazolidin-4-one (**4a**) monohydrate (4.35 g, 30 mmol, see below) was heated in 90 ml of absolute methanol at reflux for 21.5 hr. A $CaCl_2$ drying tube was used to exclude moisture during the reaction. After removal of the methanol and recrystallization from acetonitrile, 3.46 g (91% yield) of large, pale yellow prisms, mp 197–199°, was obtained. This product had identical spectral properties with the product isolated above.

C. Methylamination of 1-Methyl-2-methylthio-2-imidazolin-4-one (10). 1-Methyl-2-methylthio-2-imidazolin-4-one hydriodide[15] (**10**, 27.2 mg, 0.1 mmol) was dissolved in 1 ml of 40% aqueous methylamine, and the solution was allowed to stand at room temperature for 20 min. The water and excess methylamine were then removed in a high vacuum to leave a pale yellow syrup. Sodium hydroxide (0.25 ml, 0.4 *N*) was added, and the solution was again evaporated to dryness to yield a pale yellow mixture of NaI and 1-methyl-2-methylamino-2-imidazolin-4-one, which was identified by its nmr and ir spectra.

1-Methyl-2-methylamino-2-imidazolin-4-one (7d) Hydriodide. 1-Methyl-2-methylamino-2-imidazolin-4-one (**7d**, 0.635 g, 5 mmol) was neutralized at 0° with HI solution. After removal of the water *in vacuo*, a quantitative yield (1.28 g) of tan solid remained. The product was washed with about 5 ml of 95% EtOH and recrystallized from the same solvent to give 0.70 g of colorless prisms, mp 266–268° dec.

Anal. Calcd for $C_5H_{10}IN_3O$: C, 23.54; H, 3.95; I, 49.76; N, 16.48. Found: C, 23.72; H, 3.99; I, 49.88; N, 16.29.

The nmr spectrum (D_2O) showed peaks at δ 3.05 (3 H, singlet), 3.11 (3 H, singlet), 4.32 (2 H, singlet). The ir spectrum had major bands at 3.28, 5.58, 5.67, 5.84, 5.89, 6.40, 6.75, 6.96, 7.50, 7.65, 7.85, 8.15, 8.80, and 9.96 μ.

1,3-Dimethyl-2-methyliminoimidazolidin-4-one (4b). 1-Methyl-2-methylamino-2-imidazolin-4-one (**7d**) (300 mg, 2.36 mmol) was heated at reflux for 13 hr in 20 ml of ethanol to which 10.0 g of iodomethane had been added. The ethanol and excess iodomethane were removed at reduced pressure, and the resulting crude hydriodide salt of **4b** was dissolved in 20 ml of acetonitrile. A small portion of the material was insoluble in the acetonitrile and was discarded. The acetonitrile was removed, and the product was dissolved in 5 ml of anhydrous methanol. A BioRad AG 3-X4 column (amino form, 1.5 × 45 cm) was prepared and washed with 400 ml of anhydrous methanol prior to use; the methanolic solution of the hydriodide salt of **4b** was then passed through this methanolic weakly basic ion-exchange resin column. After 100 ml of eluent had been collected, the methanol was removed *in vacuo* at 25° to leave 250 mg of solid material. The product **4b** was isolated by vacuum sublimation at 45–50° (0.4 mm) to give 124 mg (37% yield) of colorless crystals, mp 71–73°.

Anal. Calcd for $C_6H_{11}N_3O$: C, 51.04; H, 7.85; N, 29.77. Found: C, 50.82; H, 7.62; N, 29.58.

The ir spectrum (Nujol) showed major bands at 5.70–6.52 (broad), 7.69, 8.08, 8.93, 9.98, 11.08, 13.60, and 14.34 μ. The pK_a and uv and nmr spectral data are given in Table I.

1,3-Dimethyl-2-iminoimidazolidin-4-one (4a). A. Cyclization of *N*-Methylamidino-*N*-methylglycine (9). *N*-Methylamidino-*N*-methylglycine (**9**, 145 mg, 1 mmol) was heated at reflux in 2.9 ml of absolute methanol for 1.75 hr. A $CaCl_2$ drying tube was in the top of the condenser throughout the reaction. After removal of the methanol (25°, high vacuum), the syrupy product was repeatedly extracted with warm benzene until the weight of the material remaining in the flask became constant (53 mg). The benzene extracts were combined, and the benzene was removed *in vacuo* to leave 79 mg (62%) of crude pale yellow, solid product. A portion (43 mg) of this solid was sublimed at 42° (1.5 mm) for 2.5 hr. A small amount (2 mg) of yellow solid did not sublime. The white sublimate, which analyzed for a partial hydrate, had a mp 56–70°.

(16) I. S. Bengelsdorf, *J. Amer. Chem. Soc.*, 75, 3138 (1953).
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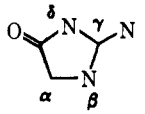
(18) A. F. McKay and M. E. Kreling, *Can. J. Chem.*, 40, 205 (1962).

Table I. Comparison of Properties of Glycocyamidines of the 2-Iminoimidazolidin-4-one (Acylamino Type I), 2-Amino-2-imidazolin-5-one (Acylamino Type II), and 2-Amino-2-imidazolin-4-one (Acylimino Type) Groups

Compd	λ_{max}, nm (ϵ)	Hydrolysis rate, min ($t_{1/2}$ at pH 12 in 0.01N NaOH)	pK_a	Deuterium exchange rate for CH_2 group, min ($t_{1/2}$ at pD 9)	Nmr chemical shift, δ[a] Ring CH_2 group	NCH_3 group β position	NCH_3 group γ-Amino or γ-imino position	NCH_3 group δ position
				Acylamino Type I				
4a	205 (13,600)[b]	16	8.07	3	3.95[c]	2.88		2.96
4b	208 (15,800)[b]	15	9.01	<1	3.90[c]	2.86[d]	3.10	2.89[d]
				Acylamino Type II				
5a	208 (5800)[b]	28	7.91	7	3.90			2.98
5b	205 (5700)[b]	61	7.96[e]	7	3.91		2.81	2.95
5c	208 (11,200)[b]	51	7.57	5	3.89		2.85	3.11
6	206 (9300)[b]	24	8.15[f]	7	3.86			
				Acylimino Type				
7a	225 (8125)[g]	>15 hr[g]	4.80[g]	23 hr	3.91			
7b	235 (7060)[g]	>15 hr[g]	4.83[h]	7 hr	3.94	2.98		
7c	213 (16,200)	>15 hr	4.84	49 hr	3.91		2.85	
7d	212 (18,400) (shoulder ~235)	>15 hr	4.55	15 hr	3.93	2.91[d]	2.88[d]	
7e	223 (21,900)	>15 hr	4.48	52 hr	3.92		2.94	
8	212 (18,600) (shoulder ~235)	>15 hr	4.75	23 hr	3.91			

[a] Chemical shifts are relative to external TMS. Measurements were made in D_2O (~5 % solutions) with the methyl group of creatinine used as internal standard. For purposes of simplifying this table, the following assignments have been made for a generalized formula for the glycocyamidines



[b] Since these compounds were hydrolyzing during measurement, the molar absorptivities are extrapolated values. [c] Deuterium exchange is so rapid in D_2O that the chemical shift could not be determined in this solvent; measurement was made in dimethyl d_6-sulfoxide. [d] The assignments for these pairs of methyl groups are uncertain and may be reversed. [e] K. Matsumoto and H. Rapoport, *J. Org. Chem.*, **33**, 552 (1968). [f] A. F. McKay and M.-E. Kreling, *Can. J. Chem.*, **40**, 1160 (1962). [g] C. F. Failey and E. Brand, *J. Biol. Chem.*, **102**, 767 (1933). [h] A. K. Grzybowski and S. P. Datta, *J. Chem. Soc.*, 187 (1964).

An analytical sample was prepared by allowing the crystals to stand overnight in a water-saturated atmosphere. The monohydrate obtained had mp 75–78° (softening at 72°).

Anal. Calcd for $C_5H_9N_3O \cdot H_2O$: C, 41.37; H, 7.64; N, 28.95. Found: C, 41.57; H, 7.53; N, 28.96.

The nmr and uv spectra are shown in Table I. The ir spectrum (Nujol) showed major bands at 3.19 (broad), 5.95, 6.23, 6.60, 6.75, 7.17, 7.70, 8.17, 8.79, and 9.75 μ. In contrast to the isomeric 1-methyl-2-methylamino-2-imidazolin-4-one (**7d**), product **4a** characteristically gives basic aqueous solutions (*cf.* pK_a values, Table I).

B. **Methylation of Creatinine (7b).** Creatinine (**7b**, 16.97 g, 150 mmol, Calbiochem Corp.) and iodomethane (25.60 g, 180 mmol) were dissolved in 150 ml of 95% EtOH, and the solution was heated at reflux for 3.5 hr. After removal of the solvent *in vacuo*, the crude product weighed 37.1 g (94% yield). A 1.0-g portion of this material was recrystallized twice from acetonitrile to give an analytical sample of **1,3-dimethyl-2-iminoimidazolidin-4-one (4a) hydriodide**, mp 213–215.5° dec (lit.[5] mp 212°).

Anal. Calcd for $C_5H_{10}IN_3O$: C, 23.54; H, 3.95; I, 49.76; N, 16.48. Found: C, 23.44; H, 3.97; I, 49.72; N, 16.52.

The nmr spectrum (D_2O) showed peaks at δ 3.19 (6 H, two overlapping singlets), 4.28 (2 H, singlet). The ir spectrum had major bands at 3.12, 3.23, 5.69, 5.95, 5.99, 6.20, 6.25, 6.49, 6.85, 7.14, 7.29, 7.37, 7.87, 9.04, 10.14, and 14.09 μ. A portion of the crude hydriodide of **4a** was converted to the free **4a** in the following manner. The crude hydriodide of **4a** (12.75 g) was dissolved in anhydrous methanol and passed through the methanolic weakly basic ion-exchange resin column (see above). After 200 ml of eluent had been collected, the methanol was removed *in vacuo* at 25° to give 6.5 g of pale yellow solid. This solid was extracted with three 30-ml portions of warm benzene, the extracts were combined, and the benzene was removed *in vacuo* at 25° to give 5.40 g (75% yield) of the pale yellow monohydrate of **4a**. The purity estimated by inspection of an nmr spectrum of the product was greater than 90%.

In a control experiment 1-methyl-2-methylamino-2-imidazolin-4-one (**7d**) hydriodide (0.255 g) was heated at reflux for 3.5 hr in 3.5 ml of 95% EtOH. By nmr spectroscopy the yield of 1,3-dimethyl-2-iminoimidazolidin-4-one (**4a**) hydriodide was approximately 3%. Since these reaction conditions are the same as those used in the methylation of creatinine (see above), the possibility that the hydriodide of **4a** could have been formed to any great extent from the isomerization of the hydriodide of **7d** is excluded.

Conversion of 1,3-Dimethyl-2-iminoimidazolidin-4-one (4a) to *N*-Methylamidino-*N*-methylglycine in H_2O. 1,3-Dimethyl-2-iminoimidazolidin-4-one monohydrate (43.5 mg, 0.300 mmol) was dissolved in 0.4 ml of D_2O and heated for 2.5 hr at 40° in a standard nmr tube. The conversion to *N*-methylamidino-*N*-methylglycine (**9**) was followed by nmr spectroscopy, and the $t_{1/2}$ for conversion was found to be 140 min. After standing at room temperature for an additional 18 hr, the sample was taken to dryness to leave a quantitative yield (45 mg) of *N*-methylamidino-*N*-methylglycine (**9**), which was identified by nmr and ir spectroscopy.

Reaction of 1-Methyl-2-methylamino-2-imidazolin-4-one (7d) with Nitrous Acid. 1-Methyl-2-methylamino-2-imidazolin-4-one (**7d**, 1.27 g, 10 mmol) was dissolved in 2 ml of H_2O, 4.15 ml of concentrated HCl (50 mmol) was added, and the resulting solution was added dropwise with vigorous stirring over 20 min to a solution of 6.21 g (90 mmol) of $NaNO_2$ in 12 ml of H_2O at −10 to −5°. After 5 min a white solid began to separate, and the solution became green. After the addition was complete, the mixture was stirred for 1 hr at −5°. The pale yellow product was filtered, washed with water and hexane, and dried in a stream of dry N_2 to give 0.97 g (48% yield) of very unstable product, mp 102–103° (decomposition with rapid gas evolution). From its analysis, spectral properties, and identification of its solvolysis products in ethanol and water, the product has been tentatively identified as (*E*)-*N*-(*N*-nitrosomethylcarbamoyl)-2-(*N*-nitrosomethylamino)acetamide (**11**). It was necessary to store this product at −10°, and it could not be recrystallized because of its instability. Therefore the analysis was performed on a crude sample.

Anal. Calcd for $C_5H_9N_5O_4$: C, 29.56; H, 4.47; N, 34.47. Found: C, 30.07; H, 4.82; N, 33.78.

The nmr spectrum (DMSO-d_6) showed peaks at δ 3.15 (3 H, singlet), 3.22 (3 H, singlet), 5.22 (2 H, singlet). The ir spectrum (Nujol) showed major peaks at 3.03, 5.73, 5.83, 6.19, 6.55, 6.65, 6.88, 6.98, 7.25, 7.55, 8.16, 9.23, 9.60, 10.26, 11.26, 11.94, 12.63, and 13.90 μ (broad). In the presence of small amounts of H_2O this unstable product gradually became converted (as observed by nmr spectroscopy) to (*E*)-2-(*N*-nitrosomethylamino)acetamide (**12**, see below), which in turn gradually isomerized to an equilibrium mixture of the *E* and *Z* isomers of 2-(*N*-nitrosomethylamino)acetamide (**12** and **13**).

Solvolysis of *N*-(*N*-Nitrosomethylcarbamoyl)-2-(*N*-nitrosomethylamino)acetamide (11) in Water. *N*-(*N*-Nitrosomethylcarbamoyl)-2-(*N*-nitrosoamino)acetamide (**11**, 0.954 g, 4.70 mmol) was dissolved in 20 ml of acetonitrile, 6 ml of H_2O was added, and the solution was heated on a steam bath for 15 min. Gas was evolved during the reaction. After removal of the solvents *in vacuo*, a quantitative yield (0.58 g) of pale yellow oil was obtained. The nmr spectrum indicated that the crude product was a 55:45 mixture of the *E* and *Z* isomers of 2-(*N*-nitrosomethylamino)acetamide (**12** and **13**). After recrystallization from 1,2-dichloroethane, 0.239 g (41% yield) of product, mp 62–72°, was obtained.

Anal. Calcd for $C_3H_7N_3O_2$: C, 30.77; H, 6.03; N, 35.88. Found: C, 31.08; H, 6.06; N, 35.58.

Two crystal forms, pale yellow needles and pale yellow plates, were found in the recrystallized product. For purposes of comparison, 2-(*N*-nitrosomethylamino)acetamide was independently synthesized by nitrosation of an authentic sample of sarcosinamide (**16**) hydrochloride. Thus sarcosinamide hydrochloride[19] (1.25 g, 10 mmol) was dissolved in 2.0 ml of H_2O and 0.57 ml (10 mmol) of glacial acetic acid was added with stirring at 0–5°. Sodium nitrite (0.69 g, 10 mmol) dissolved in 2.0 ml of H_2O was added dropwise with stirring over a period of 1.5 hr. The solution was stirred at 0° for an additional 1 hr and then at room temperature for another 1 hr. The solution was then neutralized with 1.0 ml of 10 *N* NaOH and extracted with six 20-ml portions of ethyl acetate. After removal of the ethyl acetate at reduced pressure, the crude product weighed 0.97 g (83% yield). The nmr spectrum showed peaks at δ 3.11, 3.83 (3 H, two singlets), 4.38, and 5.00 (2 H, two singlets), and was identical with that for the 55:45 mixture of (*E*)- and (*Z*)-2-(*N*-nitrosomethylamino)acetamide isolated by hydrolysis of *N*-(*N*-nitrosomethylcarbamoyl)-2-(*N*-nitrosomethylamino)acetamide (**11**). Upon recrystallization from 1,2-dichloroethane the same mixture of pale yellow needles and plates, mp 61–72°, was also obtained. By mechanical separation pure samples of needles, mp 62–63.5°, and plates, mp 81–82.5°, were obtained. Each form gave identical high-resolution mass spectra, showing major peaks at 117, 88, 87, 74, 73, 59, 45, 44, 43, 42, 41, 30, 28, 27, 18, 15. The parent ion of the needles was accurately mass measured.

Anal. Calcd for $C_3H_7N_3O_2$: 117.0538. Found: 117.0544.

The nmr spectrum of the plates in D_2O showed that they were a single isomer, presumably the *E* isomer (**12**): δ 3.11 (3 H, singlet), 5.00 (2 H, singlet). Gradually, the spectrum in D_2O changed to that of an equilibrium mixture of the *E* and *Z* isomers (Figure 2). In contrast, the nmr spectrum of the needles in D_2O showed an equilibrium mixture of the isomers even when the crystals were dissolved immediately before measurement of the spectrum (Figure 2). The ir spectra of the needles and plates in Nujol were also different: needles, major bands at 2.93, 5.97, 6.19, 6.86, 6.97, 7.18, 7.52, 7.78, 8.47, and 9.40; plates, major bands at 2.97, 3.15, 6.00, 6.95, 7.20, 7.60, 8.55, 9.56, 10.38, and 13.11 μ.

Solvolysis of *N*-(*N*-Nitrosomethylcarbamoyl)-2-(*N*-nitrosomethylamino)acetamide (11) in Absolute Ethanol. *N*-(*N*-Nitrosomethylcarbamoyl)-2-(*N*-nitrosomethylamino)acetamide (**11**, 203 mg, 1.0 mmol) dissolved in 4 ml of absolute ethanol [freshly distilled from $Mg(OEt)_2$] was heated at reflux for 10 min under rigorous exclusion of moisture. The reaction was accompanied by copious gas evolution. After removal of the solvent at reduced pressure, 185 mg of crude product was obtained. A portion of the material (110 mg) was subjected to fractional sublimation: first fraction, 18 mg (55°, 0.2–0.3 mm); second fraction, 82 mg (72°, 0.05–0.10 mm). The second fraction, mp 87.5–89.5° (75% yield), was the desired product. In order to obtain an analytical sample this fraction was resublimed under the above conditions and a central fraction (43 mg) was collected.

(19) C. S. Marvel, J. R. Elliott, F. E. Boettner, and H. Yuska, *J. Amer. Chem. Soc.*, **68**, 1681 (1946).

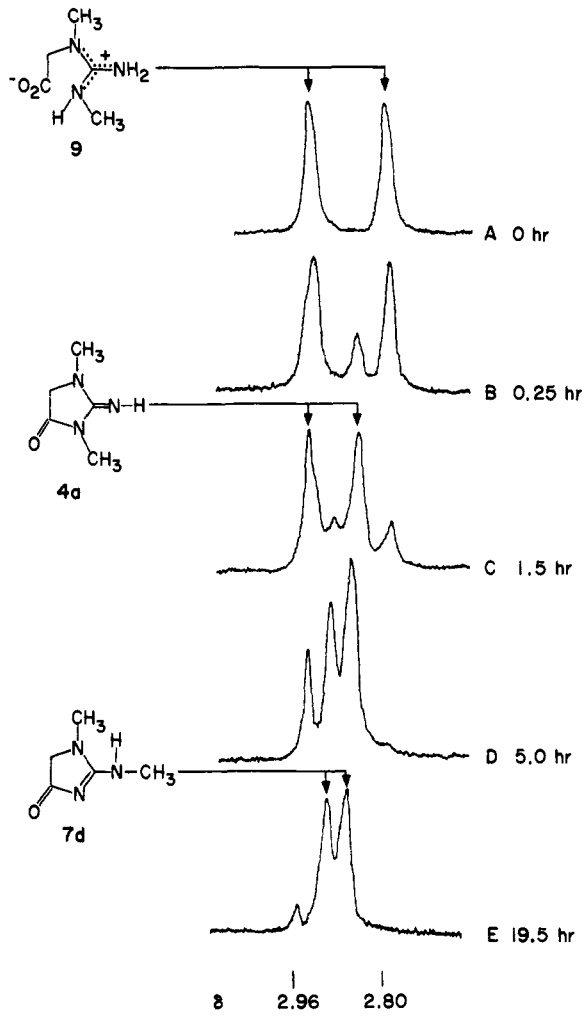


Figure 1. Proton nmr spectra taken at 60 MHz (D_2O) showing the *N*-methyl groups of (A) *N*-methylamidino-*N*-methylglycine and (B–E) showing the *N*-methyl groups of the products formed upon heating at reflux in methanol as a function of time. The product of kinetic control, 1,3-dimethyl-2-iminoimidazolidin-4-one (**4a**), formed first, which in turn isomerized upon further heating the methanol to the product of thermodynamic control, 1-methyl-2-methylamino-2-imidazolin-4-one (**7d**).

Anal. Calcd for $C_6H_{11}N_3O_4$: C, 38.09; H, 5.86; N, 22.21. Found: C, 38.26; H, 5.78; N, 22.66.

The nmr spectrum (D_2O) showed peaks at δ 1.27 (3 H, triplet, J = 7 Hz), 3.09 (3 H, singlet), 4.20 (2 H, quadruplet, J = 7 Hz), 5.23 (2 H, singlet), consistent with the structure (*E*)-*N*-(ethoxycarbonyl)-2-(*N*-nitrosomethylamino)acetamide (**14**). The high-resolution mass spectrum was also consistent with this structure. An accurate mass measurement was made on the parent ion.

Anal. Calcd for $C_6H_{11}N_3O_4$: 189.0749. Found: 189.0729.

A very large peak at 159, corresponding to the loss of NO, was also accurately mass measured.

Anal. Calcd for $C_6H_{11}N_2O_3$: 159.0770. Found: 159.0761.

Another large peak corresponded to the parent ion –NO and $-CH_3CH_2OH$ was likewise accurately mass measured.

Anal. Calcd for $C_4H_5N_2O_2$: 113.0351. Found: 113.0351.

The ir spectrum showed major peaks at 3.12, 5.69, 5.93, 6.71, 6.87, 6.96, 7.21, 7.59, 7.83, 7.99, 8.16, 8.54, 9.35, 9.64, 10.31, 10.42, and 13.01 μ (broad). After 20 hr at room temperature in D_2O this compound was transformed to a 49:51 mixture of *E* (**14**) and *Z* (**15**) isomers. Thus, the nmr spectrum showed peaks at δ 1.26 (3 H, two overlapping triplets, J = 7 Hz), 3.09, 3.93 (3 H, two singlets), 4.22 (2 H, two overlapping quadruplets, J = 7 Hz), 4.57, 5.23 (2 H, two singlets).

1-Methyl-2-amino-2-imidazolin-5-one (5a). A. Cyclization of *N*-Methylamidinoglycine Ethyl Ester (20). *N*-Methylamidinoglycine (**19**, 105 mg, 80 mmol) was heated at reflux for several hours in a concentrated solution of HCl in absolute ethanol. After

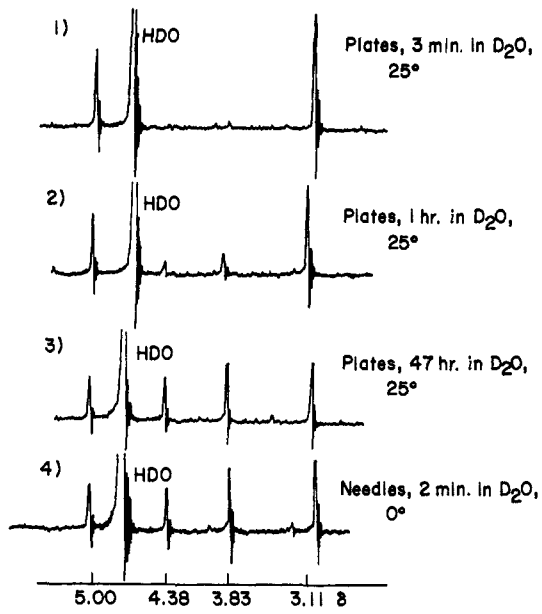


Figure 2. Parts 1–3: Proton nmr spectra taken at 60 MHz (D_2O) showing the conversion of (*E*)-2-(*N*-nitrosomethylamino)acetamide (**12**) (plates) to an equilibrium mixture of the *E* and *Z* isomers as a function of time. Part 4: proton nmr spectrum taken at 60 MHz (D_2O) of the equilibrium mixture of the *E* and *Z* isomers of 2-(*N*-nitrosomethylamino)acetamide (needles). This latter spectrum was measured as quickly as possible (~2 min) after dissolution in the D_2O at 0°.

cooling, the ethanol was removed *in vacuo* to leave an essentially quantitative yield of *N*-methylamidinoglycine ethyl ester hydrochloride (**20**). The nmr spectrum (D_2O) showed peaks at δ 1.37 (3 H, triplet, J = 7 Hz), 2.98 (3 H, singlet), 4.26 (2 H, singlet), 4.37 (2 H, quartet, J = 7 Hz). This product was not further purified but was used directly in the synthesis of **5a**. Thus the *N*-methylamidinoglycine ethyl ester hydrochloride (**20**, 1.57 g, 80 mmol) was dissolved in 1 ml of absolute methanol and passed through the BioRad AG 3-X4 methanolic weakly basic ion exchange column described above, using 200 ml of methanol as eluent. The crude product (1.2 g) was isolated by removal of the methanol in a high vacuum at 25°. Upon sublimation at 105° (0.1 mm), 36.4 mg (40% yield based on isocreatine) of 1-methyl-2-amino-2-imidazolin-5-one was obtained. An analytical sample was obtained by resublimation of 30 mg and collection of the fraction (11 mg) subliming between 95 and 97° (0.1 mm). This material melted at 233–235° dec.

Anal. Calcd for $C_4H_7N_3O$: C, 42.47; H, 6.24; N, 37.15. Found: C, 42.72; H, 6.44; N, 37.02.

The nmr spectrum (D_2O) showed peaks at δ 3.00 (3 H, singlet), 3.98 (2 H, singlet). Gradually the peak at δ 3.98, corresponding to the methylene hydrogens in the ring, exchanged with the D_2O solvent. Addition of both isocreatine and the isomeric 2-methylamino-2-imidazolin-4-one (**7c**) to the solution revealed, as expected, new sets of nmr peaks. The ir spectrum of **5a** (Nujol) showed major bands at 2.95–3.05 (broad), 5.91 (strong), 6.15, 8.60, 9.65, 12.70–14.00 (broad). The uv spectrum is shown in Table I. As in the case of other glycocyamidines of the acylamino type, **5a** characteristically gives basic aqueous solutions (*cf.* pK_a values, Table I).

B. Methylation of Glycocyamidine (7a). 1-Methyl-2-amino-2-imidazolin-5-one was also prepared by methylation of glycocyamidine with iodomethane followed by neutralization of the resulting hydriodide salt. Thus glycocyamidine (**7a**, 175 mg, 1.77 mmol) was heated at reflux for several hours in 50 ml of 95% ethanol which contained 1.26 g (8.85 mmol) of iodomethane. After removal of the solvent and excess iodomethane at reduced pressure, the crude 1-methyl-2-amino-2-imidazolin-5-one hydriodide was dissolved in 5 ml of anhydrous methanol and passed through the methanolic BioRad AG 3-X4 column. After removal of the methanol at 25° in a high vacuum, a nearly quantitative yield of product was obtained. The product was estimated to be at least 90% pure 1-methyl-2-amino-2-imidazolin-5-one as determined by ir and nmr spectroscopy.

2-Methylamino-2-imidazolin-4-one (7c). 1-Methyl-2-amino-2-imidazolin-5-one (**5a**, 50 mg, 44 mmol) was dissolved in 50 ml of absolute methanol and heated at reflux ($CaCl_2$ drying tube). The course of the reaction was followed by nmr spectroscopy. After 20 hr the peaks corresponding to **5a** had disappeared and new peaks corresponding to the expected product **7c** were the only detectable ones. The methanol was removed *in vacuo*, and the crude product was recrystallized from EtOH–CH_3CN to give 31 mg (62% yield), mp 256–256.5° dec.

Anal. Calcd for $C_4H_7N_3O$: C, 42.47; H, 6.24; N, 37.15. Found: C, 42.67; H, 6.41; N, 37.31.

The nmr spectrum (D_2O) showed peaks at δ 2.85 (3 H, singlet), 3.91 (2 H, singlet). Unlike its isomer **5a**, the hydrogens on the ring methylene group at δ 3.91 did not appear to exchange readily with the D_2O solvent. Similarly, aqueous solutions of product **7c** were neutral to pH paper. The ir spectrum of **7c** (Nujol) showed major bands at 5.91, 6.01, 6.48, 7.15, 7.28, 7.72, 8.16, 9.47, 13.20–14.15 (broad). The uv spectrum and pK_a value are shown in Table I.

2-Oxo-2,3,5,6,7,8-hexahydroimidazo[1,2-*a*]pyrimidine (8). 1-Carboxymethyl-2-iminohexahydropyrimidine[15] (200 mg, 114 mmol) was heated for 6 hr at reflux in 50 ml of concentrated ethanolic HCl. After removal of the ethanol at reduced pressure, the crude 1-carboxymethyl-2-iminohexahydropyrimidine ethyl ester hydrochloride was dissolved in 1 ml of MeOH and passed through the methanolic BioRad AG 3-X4 ion exchange column. After removal of the methanol *in vacuo* at 25°, the crude product was recrystallized from acetonitrile to give 107 mg (60% yield) of needles, mp 236–239° dec (sintering due to loss of water of hydration at *ca.* 100°).

Anal. Calcd for $C_6H_9N_3O \cdot H_2O$: C, 45.85; H, 7.05; N, 26.74. Found: C, 45.90; H, 7.02; N, 26.98.

The nmr spectrum (D_2O) showed peaks at δ 2.05 (2 H, multiplet), 3.34 (4 H, two overlapping triplets, J = 8 Hz), 3.91 (2 H, singlet). The ir spectrum (Nujol) showed major bands at 2.88, 2.93, 5.96, 6.09, 6.21, 6.82, 6.90, 6.98, 7.18, 7.28, 7.52, 7.58, 7.77, 8.03, 9.23, 10.31, 13.50, and 13.66 μ. The uv spectral data are shown in Table I. As in the case of the structurally analogous 1-methyl-2-methylamino-2-imidazolin-4-one (**7d**), this product gives neutral aqueous solutions (*cf.* pK_a data, Table I).

2-Dimethylamino-2-imidazolin-4-one (7e). 2-Thiohydantoin[20] (**21**, 4.2 g, 36 mmol) was heated at reflux for 3 hr in 50 ml of absolute ethanol to which 6.1 g (43 mmol) of iodomethane had been added. The solvent was removed at reduced pressure to leave 2-methylthio-2-imidazolin-4-one hydriodide (**22**), which was treated for 12 hr at room temperature with 7 g (156 mmol) of dry dimethylamine in 50 ml of absolute ethanol. After removal of the solvent *in vacuo*, the solid residue was washed onto a filter with three 30-ml portions of $CHCl_3$. Hydantoin, formed as a side product, was left on the filter (1.6 g). The $CHCl_3$ was removed from the filtrate, and the residual solids were dissolved in 3 ml of H_2O. This solution was passed through a Dowex-1 (^-OH form) column (1.2 × 30 cm), and the column was washed with a total of 100 ml of H_2O. After removal of the water *in vacuo*, there remained 2.0 g of crude product which was recrystallized from acetonitrile. The yield of purified product was 0.91 g (20%), mp 230–232° dec.

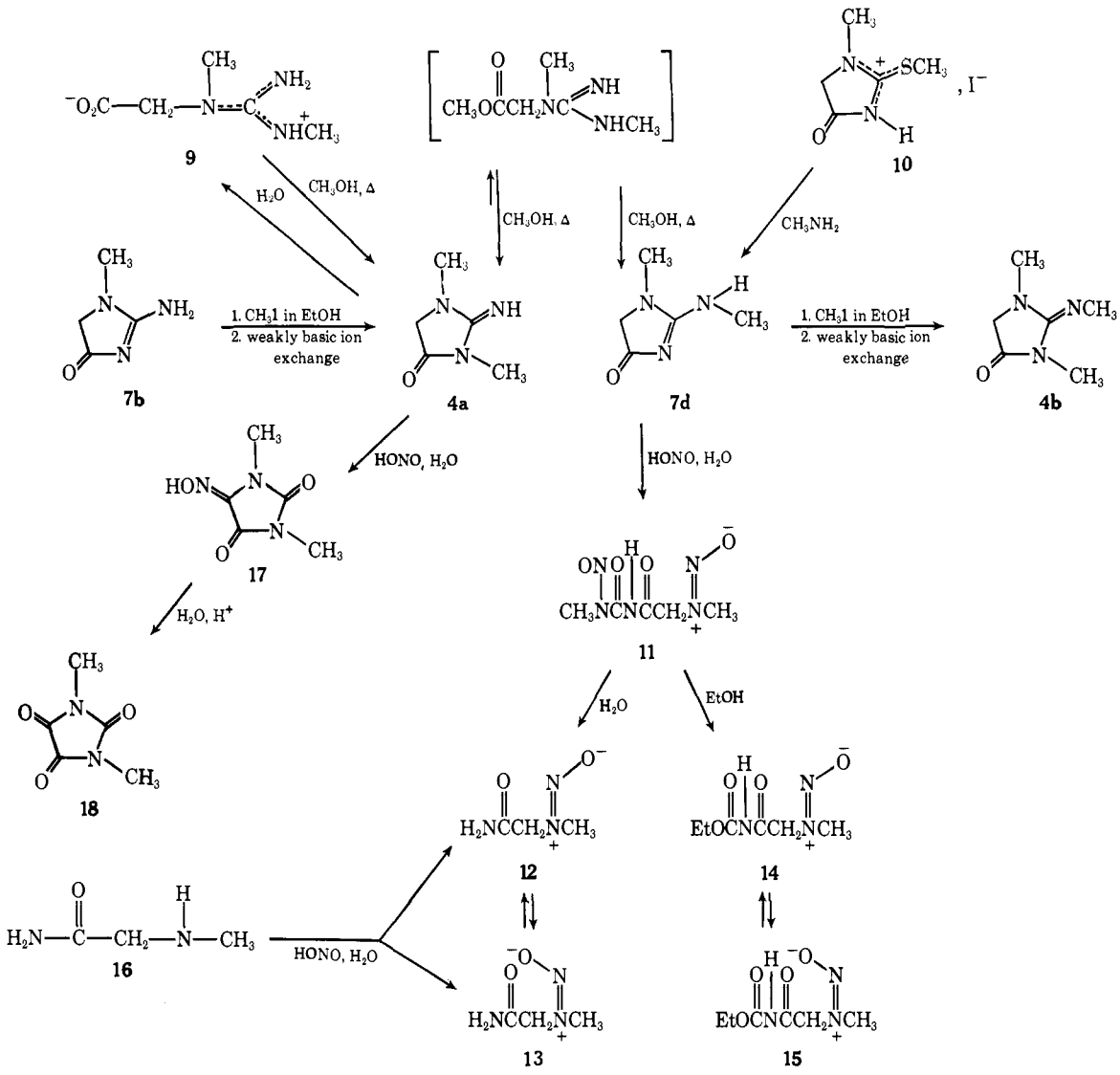
Anal. Calcd for $C_5H_9N_3O$: C, 47.23; H, 7.14; N, 33.05. Found: C, 47.06; H, 7.05; N, 33.14.

The ir spectrum (Nujol) showed major bands at 3.0–3.6 (broad), 5.90, 6.00–6.55 (broad), 6.75, 7.05, 7.29, 7.64, 8.11, 9.20, 9.70, and 10.74 μ. The pK_a, uv, and nmr spectral data are given in Table I.

1-Methyl-2-dimethylamino-2-imidazolin-5-one (5c). 2-Dimethylamino-2-imidazolin-4-one (**7e**, 365 mg, 2.87 mmol) was heated at reflux for 12 hr in 15 ml of *n*-propyl alcohol to which 0.60 g (4.8 mmol) of dimethyl sulfate had been added. After removal of the solvent and excess dimethyl sulfate at reduced pressure, the residue was dissolved in 5 ml of dry methanol and passed through the methanolic BioRad AG 3-X4 column. After removal of the methanol, the expected product was separated from the starting material by dissolution in 20 ml of CCl_4, a solvent in which the 2-dimethylamino-2-imidazolin-5-one is insoluble. After filtration, the CCl_4 solution was taken to dryness to leave 58 mg of crude product. The amount of recovered 2-dimethylamino-2-imidazolin-4-one was 227 mg. The 1-methyl-2-dimethylamino-2-imidazolin-5-one was purified by twice distilling in a small sublimation apparatus. A fraction collected at 38–40° (0.5 mm) gave a sample with no detectable impurities in its nmr spectrum. The yield was 20 mg

(20) T. B. Johnson and B. H. Nicolet, *J. Amer. Chem. Soc.*, 33, 1973 (1911).

Scheme I



(13%, based on unrecovered **7e**). The hydroscopic oil was analyzed by accurate mass measurement of its parent ion.

Anal. Calcd for $C_6H_{11}N_3O$: 141.0902. Found: 141.0906.

The infrared spectrum (neat) showed major bands at 3.42, 5.80 (broad), 6.18, 6.84 (broad), 7.24, 7.52, 8.70, 9.50 (broad), and 11.00 μ. The pK_a, uv, and nmr spectral data are given in Table I.

Results and Discussion

Isolation and Characterization of 1,3-Dimethyl-2-iminoimidazolidin-4-one (4a) and 1-Methyl-2-methylamino-2-imidazolin-4-one (7d). In refluxing methanol, *N*-methylamidino-*N*-methylglycine (**9**) cyclized unusually readily[21] compared to other glycocyamines to give the product of kinetic control, **4a**, which on further heating in methanol was converted to the product of thermodynamic control, **7d**, probably *via* *N*-methylamidino-*N*-methylglycine methyl ester (Scheme I and Figure 1). Product **4a** was also formed when creatinine **7b** was methylated with iodomethane followed by neutralization of the resulting hydriodide salt. Closely analogous reactions to the methylation of creatinine have been reported for the methylation of cytidine,[22,23] cytosine,[23] and guanosine.[22] In all cases the preferentially methylated nitrogen is the one which is structurally equivalent to the one methylated in creatinine, *i.e.*, the nitrogen atom adjacent to the carbonyl of the amide group.

The methylation of creatinine has been investigated by others.[5,7,9-12] Zeile and Meyer[11] succeeded in characterizing **4a** by conversion with nitrous acid into 1,3-dimethylhydantoin(5)oxime (**17**), which in turn was hydrolyzed in acid to the known compound, *N,N'*-dimethylparabanic acid (**18**) (Scheme I). We have found that product **4a** hydrolyzes spontaneously in water to generate the acyclic creatine analog **9**. This instability in water undoubtedly explains many of the difficulties which earlier research groups experienced in attempting to isolate and characterize **4a**.[9,10,12]

Isomer **7d** was also prepared by treatment of 1-methyl-2-methylthio-2-imidazolin-4-one hydriodide (**10**) with excess methylamine (Scheme I).

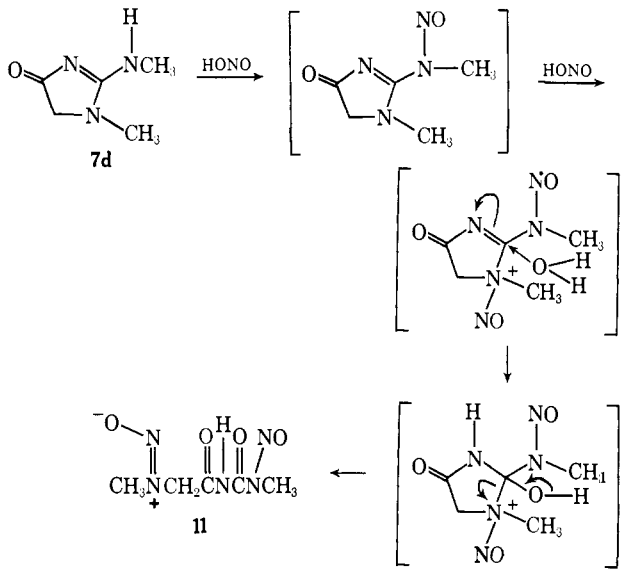
In order to substantiate further the structural assignment for isomer **7d** and also to test for the possibility that **7d** might have reverted to **4a** under the conditions used by Zeile and Meyer[11] for the characterization of **4a**, which would negate their proof of structure, we

(21) The unusual ease of cyclization of this compound is probably due to relief of steric repulsions between the two *N*-methyl substituents in going from the acyclic glycocyamine to the cyclic glycocyamidine. This is similar to the steric situation for DL-*N*-methylamidinoalanine, a glycocyamine which also cyclizes very readily.[15]

(22) H. Bredereck, H. Haas, and A. Martini, *Chem. Ber.*, **81**, 307 (1948).

(23) P. Brooks and P. D. Lawley, *J. Chem. Soc.*, 1348 (1962).

treated **7d** with cold, aqueous nitrous acid and examined the product formed. A very unusual and unexpected product, identified as (*E*)-*N*-(*N*-nitrosomethylcarbamoyl)-2-(*N*-nitrosomethylamino)acetamide (**11**), was isolated, which could have formed by the following mechanism



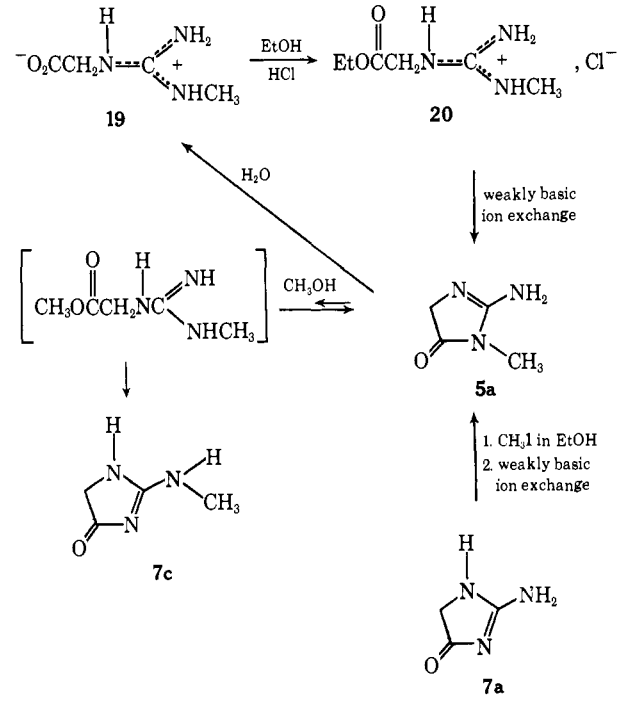
Product **11**, an unstable solid, was characterized by its elemental analysis, infrared spectrum, and nmr spectrum, and by its stable solvolysis products in both ethanol and water (Scheme I). The infrared spectrum, for example, had a pair of bands at 5.73 and 5.83 μ, similar to those found in succinimide[24] (5.65 and 5.88 μ).

The initial product from reaction of **11** with water was identified as (*E*)-2-(*N*-nitrosomethylamino)acetamide (**12**, Scheme I), which gradually isomerized to an equilibrium mixture of the *E* (**12**) and *Z* (**13**) isomers. We therefore conclude that product **11** preferentially has the *E* configuration about the N=N bond in the solid state. 2-(*N*-Nitrosomethylamino)acetamide was synthesized independently by the reaction of sarcosinamide (**16**) with nitrous acid in 83% yield. Two crystal forms of 2-(*N*-nitrosomethylamino)acetamide, needles and plates, were found when the compound was recrystallized from 1,2-dichloroethane. They were separated mechanically.[25,25a] The plates, mp 81–82.5°, were only the *E* isomer since the nmr spectrum in D_2O solution initially revealed only a single set of peaks, but gradually, over a period of many hours at 25°, changed to that of the equilibrium mixture of the two isomers (Figure 2). The needles, mp 62–63.5°, were presumably a 50:50 mixture of the *E* and *Z* isomers since the nmr spectrum in D_2O solution was consistent with the presence of the equilibrium mixture of the isomeric forms even when the spectrum was taken as rapidly as possible (~2 min) at 0° (Figure 2).[25a] Both needles and plates gave identical fragmentation patterns in their mass spectra, but, as expected, had different infrared spectra in the solid state. Interestingly, in the infrared spectrum of the plates there were bands present which were absent in the spectrum of the needles.

The product from reaction of **11** with anhydrous ethanol has been identified as *N*-(ethoxycarbonyl)-2-(*N*-nitrosomethylamino)acetamide (Scheme I). Upon isolation and purification by vacuum sublimation the nmr spectrum in D_2O showed that the product was predominantly the *E* isomer **14**.[26] Gradually, over a period of many hours at room temperature, the compound isomerized to give a spectrum consistent with the presence of an equilibrium mixture (~50:50) of the *E* (**14**) and *Z* (**15**) isomers.

Isolation and Characterization of 1-Methyl-2-amino-2-imidazolin-5-one (5a) and 2-Methylamino-2-imidazolin-4-one (7c). In an analogous set of reactions to some of those shown in Scheme I, isocreatine ethyl ester (**20**) was cyclized to form product **5a**, which in turn was converted on heating in methanol to the product of thermodynamic control (**7c**, see Scheme II). Also, in

Scheme II

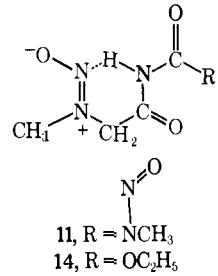


(24) J. R. Dyer, "Applications of Absorption Spectroscopy of Organic Compounds," Prentice Hall, Englewood Cliffs, N. J., 1965, p 36.

(25) A. Mannschreck, H. Münsh, and A. Mattheus (*Angew. Chem., Int. Ed. Engl.*, 5, 728 (1966)) report the first separation of a pair of *E* and *Z* isomers for an *N*-nitroso secondary amine, *N*-nitroso-*N*-benzyl-*N*-(2,6-dimethylphenyl)amine, using preparative thin-layer chromatography.

(25a) NOTE ADDED IN PROOF. The identity of the needles as a 50:50 mixture of the *E* and *Z* isomers of 2-(*N*-nitrosomethylamino)-acetamide has been confirmed by a single-crystal X-ray crystallographic study (L. Templeton and D. H. Templeton, University of California, Berkeley, unpublished results).

(26) The stereochemical assignments for this type of *E* and *Z* isomers are based on the work of Karabatsos and Taller[27] and Brown and Hollis.[28] The added stability of **11** and **14** in the solid state may be due to internal hydrogen bonding, *i.e.*



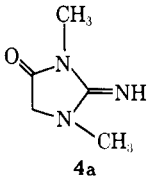
11, R = NCH_3
14, R = OC_2H_5

(27) G. J. Karabatsos and R. A. Taller, *J. Amer. Chem. Soc.*, **86**, 4373 (1964).

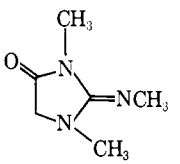
(28) H. W. Brown and D. P. Hollis, *J. Mol. Spectrosc.*, **13**, 305 (1964).

analogy to the methylation of creatinine, glycocyamidine (**7a**) was shown to react with iodomethane to give product **5a**. Korndörfer[4] has previously isolated the hydriodide of **5a** by this same method, and by treatment with Ag_2O in water generated a basic solution of what was undoubtedly the free base. Upon evaporation of the water, however, he noted that the basic solution became neutral, and only isocreatine itself could be isolated. We have confirmed the facility of the hydrolysis of **5a** back to isocreatine (Scheme II).

Comparison of Some Properties of Glycocyamidines. Seven new glycocyamidines were isolated for this study. Two glycocyamidines were prepared, which, because of appropriate methyl substitution, unequivocally have their C=N bonds exocyclic to the five-membered ring (Scheme I), *i.e.*

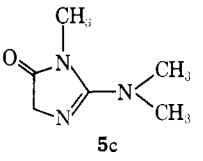


4a



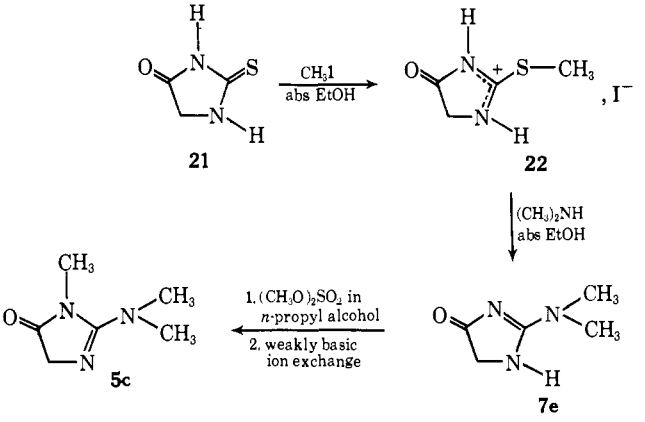
4b

and a third glycocyamidine was prepared which unequivocally contains the C=N bond endocyclic to the five-membered ring, but not conjugated with the carbonyl group (Scheme III), *i.e.*



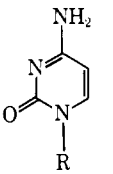
5c

Scheme III

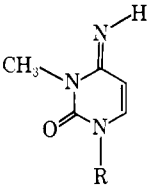


21 **22** **5c** **7e**

The pK_a values, relative stabilities toward alkaline hydrolysis, relative rates of base-catalyzed deuterium exchange for the methylene protons in the five-membered ring, uv and nmr spectral properties of these compounds, and the other glycocyamidines available for this study are given in Table I. There is clearly a sharp distinction between those glycocyamidines which can have the C=N bond conjugated with the carbonyl group (acylimino type) and those which are prevented by appropriate substitution from having such conjugation (acylamino types I and II). This distinction was first clearly recognized by Matsumoto and Rapoport,[13] who investigated a similar series of properties for a wider range of cyclic and acyclic acylimino- and acylaminoguanidines. Only three glycocyamidines were included in their study, however. Within our more homogeneous series of compounds the distinctions between the acylamino and acylimino types are more definitive than found by Matsumoto and Rapoport. For example, although all of their acylaminoguanidines, cyclic or acylic, had pK_a values (7.96–9.48) comparable to those which we have found for this structural type (7.57–9.01), we found a much narrower range for the pK_a values for the acyliminoglycocyamidines (4.48–4.84) than Matsumoto and Rapoport found for their more heterogeneous group of compounds (4.80–8.33). Presumably, this is due to the rigid, nearly completely coplanar geometries of the five-membered ring glycocyamidines which maximize overlap of the guanido nitrogen lone pairs with the extended π orbitals in the α,β-unsaturated carbonyl system. In closely analogous, nearly coplanar cytosine derivatives, the pK_a values of the parent compounds[29] and the products of monomethylation[23] are almost identical with those of the corresponding glycocyamidines and their monomethylated products, *i.e.*



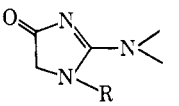
23, R = ribosyl; $pK_a = 4.1$
R = methyl; $pK_a = 4.55$



24, $pK_a = 8.7$
$pK_a = 9.4$

We have also consistently found a greater stability toward hydrolysis at pH 12 for the glycocyamidines of the acylimino type compared to those of the acylamino type (Table I), again a distinction which was more difficult to make with the more heterogeneous group of acylguanidines of Matsumoto and Rapoport. Similarly, the rates of base-catalyzed deuterium exchange for the methylene hydrogens in the five-membered ring are much slower for those glycocyamidines of the acylimino type compared to those of the acylamino type.

The ultraviolet spectra of the acyliminoglycocyamidines always showed absorption at longer wavelengths than the acylaminoglycocyamidines (Table I). None of the glycocyamidines of the acylamino type showed absorption maxima above 208 nm. Within the group of glycocyamidines of the acylimino type, two distinct ranges of longer wavelength maxima (sometimes appearing as shoulders on maxima at shorter wavelengths) were found, which correlate with the presence or absence of an alkyl substituent on the 1 position in the ring, *e.g.*, types A and B shown below. Structures of type A each



A, R = H
B, R = alkyl

showed a maximum between 223 and 225 nm whereas those of type B each showed a maximum at 235 nm. In compound **7c** (Table I) the expected shoulder at 223–225 nm is presumably masked by the broad chromophore at 213 nm.

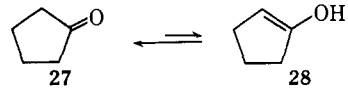
(29) J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, **9**, 369 (1952).

The distinctions between acylaminoglycocyamidines of type I (exocyclic C=N bond) and type II (endocyclic C=N bond) are much less clear cut. The similarities of both the ultraviolet absorption maxima and pK_a values for the two types do not offer any source of differentiation. It should be noted, however, that the molar extinction coefficients for the two type I compounds were observed to be greater than those for the rest of the acylaminoglycocyamidines. Similarly, the two type I compounds were less stable toward hydrolysis at pH 12 and showed more rapid deuterium exchange rates for their ring methylene hydrogens than the other acylaminoglycocyamidines. Finally, type I compound **4b** has an *N*-methyl group (presumably the C=N—CH_3) which showed an unusually low-field chemical shift (δ 3.10) in its nmr spectrum, whereas type II compound **5b** has no *N*-methyl peak below 2.95 (Table I), which is consistent with its assigned tautomeric preference.

Our assigned preference of endocyclic C=N bonds over exocyclic C=N bonds for the glycocyamidines is in agreement with the known preference of endocyclic C=C bonds over exocyclic C=C bonds in five-membered ring systems. For example, the equilibrium between methylenecyclopentane (**25**) and 1-methyl-1-cyclopentene (**26**) has been shown by Cope, *et al.*,[30] to lie far to the side of the endocyclic C=C bond system, *i.e.*

25 (0.09%) **26** (99.91%)

The preponderance of **26** over **25** had earlier been asserted by Brown.[31] On the other hand, as Brown pointed out,[31] there is a large preference for an exocyclic C=O bond (keto) over an endocyclic C=C bond



27 **28**

(enol) in cyclopentanone. We believe, however, that the equilibrium between the tautomers of the acylaminoglycocyamidines is more closely analogous to the equilibrium of **25** and **26** than to the equilibrium of **27** and **28** because the double bond is of the same type, C=N, for both the endocyclic and exocyclic isomers, whereas this is not the case for the equilibrium of **27** and **28**.

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(30) A. C. Cope, D. Ambros, C. Ciganek, C. F. Howell, and Z. Jacura, *J. Amer. Chem. Soc.*, **82**, 1750 (1960).

(31) H. C. Brown, *J. Org. Chem.*, **22**, 439 (1957).

Substrate-Induced p*K* Perturbations with Chymotrypsin[1]

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Abstract: Proflavin binding to chymotrypsin is pH dependent in the near-neutral pH range and the dissociation constants for the dye with the enzyme conjugate acid and base are 12.19×10^{-5} and 3.25×10^{-5} *M*, respectively. The p*K* of the proflavin–enzyme complex is 5.60 and the free enzyme has a p*K* of 6.18. Dye displacement has been used to study complexation of substituted benzamides and furoylamide with the enzyme to determine whether equivalent p*K* perturbations are produced on noncovalent binding of acyl groups as is seen with the corresponding groups covalently linked to the enzyme in isolable acyl enzymes. There is no p*K* perturbation produced by binding of benzamide and furoylamide; *p*-nitrobenzamide induces a small p*K* perturbation. Covalent binding of these acyl functions to the enzyme is required for p*K* perturbation. The dissociation constants for benzamide, *p*-nitrobenzamide, and furoylamide are 3.06 m*M*, 1.34 m*M*, and 13.86 m*M*, respectively, at pH 7.7, 25°. The dissociation constant for *p*-methoxybenzamide is approximately 1.22 m*M* under these conditions. It is proposed that acylation of the enzyme increases the p*K* of the active-site histidine residue by disruption of the hydrogen bond between His-57 and Ser-195. Hydrogen bonding between the histidine residue and the acyl linkage of the acyl enzyme results in further increases in the p*K*. Hydrogen bonding with the acyl linkage requires covalent bonding of the acyl group to the enzyme.

Studies of the acylation of chymotrypsin with anilides of a specific substrate—acetyltryptophan derivatives—and deacylation of isolable acyl enzymes formed from nonspecific substrates have shown that the pH dependence for activity depends on the electronic properties of the acyl function; electron withdrawal by either the acyl or aniline moiety results in a lowered apparent p*K* for activity.[2] Proton dissociation from the critical histidine residue required for activity is, therefore, coupled with some other equilibrium process. The reversible formation of a tetrahedral intermediate has been suggested to be responsible for p*K* perturbation with anilides;[2a] evidence against this process underlying the p*K* perturbation with acyl enzymes has been

(1) Supported by grants from the National Institutes of Health (DE 02761 and DE 03246).

(2) (a) M. Caplow, *J. Amer. Chem. Soc.*, **91**, 3639 (1969); (b) Vishnu and M. Caplow, *ibid.*, **91**, 6754 (1969); (c) S. A. Bernhard, E. Hershberger, and J. Keizer, *Biochemistry*, **5**, 4120 (1966).