THE CHEMISTRY OF THE GLYCOCYAMIDINES

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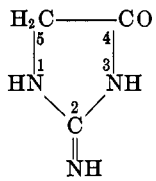
I. INTRODUCTION AND SCOPE

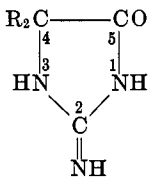
A. Nomenclature

Cyamidines are the anhydrides of α-guanidino acids, derived by *intramolecular* acylation of the ω-amino or imino group by the carboxy group. The simplest

member (I), the anhydride of glycocyamine (III) and the α-guanidino acid related to glycine, is called glycocyamidine; from this the whole group of compounds is designated as glycocyamidines.

The systematic name used for glycocyamidine in the indexes of *Chemical Abstracts* is 2-imino-4-imidazolidinone, formerly 2-imino-4-imidazolidone and 2,3-dihydro-2-imino-4(5*H*)-imidazolone.


I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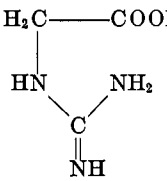
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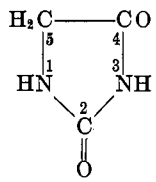
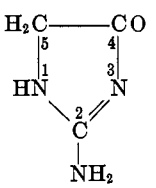

IV

Besides these, other names are also encountered in the literature, e.g., 4-oxo-2-iminoimidazolidine (Beilstein), 2-imino-4-ketotetrahydroimidazole, hydantoin-imide-(2) (Beilstein), expressing the close relationship to hydantoin (IV), and the incorrect name 2-iminohydantoin.

Many derivatives of glycocyamidine can be derived from tautomeric forms of I, e.g., V and VI. For these the names used in *Chemical Abstracts* are 2-amino-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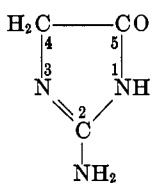
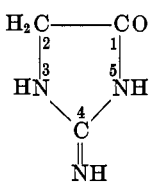

VI


VII

2-imidazolin-4(or 5)-one, formerly 2-amino-4(5*H*)- or -5(4*H*)-imidazolone. Other names encountered in the literature are 2-amino-4-glyoxalone and 2-amino-4-keto-4,5-dihydroglyoxaline.

Numbering

The tautomer I is numbered by analogy to the hydantoin ring as designated. The same numbering applies to all derivatives if their designation is based on the name "glycocyamidine" (in this case, derivatives substituted on the acyclic nitrogen are designated as N^2- or incorrectly as 2-substituted glycocyamidines), and also to all derivatives with a methylene group *not* substituted by alkyl or aryl groups if they are named as 2-imino-4-imidazolidinones. If, however, the methylene group is substituted by such groups, the numbering has to be altered (as in formula II), and the correct designation is as a 4-substituted-2-imino-5-imidazolidinone. The other two tautomers, V and VI, and their derivatives are always numbered as designated.

The rules for the numbering of the glycocyamidines outlined above have not always been strictly applied. Especially in the older literature, glycocyamidine itself was numbered according to formula II; also, the numbering designated in formula VII is sometimes encountered (129).

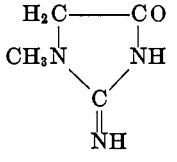
In this review, covering the literature up to the end of 1956 and papers published in the leading organic chemical journals up to September 1958, nomenclature based on the name "glycocyamidine" will be used and the rules of numbering outlined above will be applied.

Trivial names

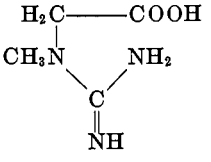
Besides the more or less systematic names specified, there exist also many trivial names for the derivatives of glycocyamidine: e.g., creatinine for 1-methylglycocyamidine, alacreatinine for 5-methylglycocyamidine, homocreatinine for 1,5-dimethylglycocyamidine, butyrocreatinine for 1-methyl-5-*n*-propylcreatinine, isovalerocreatinine for 1-methyl-5-isobutylcreatinine, and cystinecyamidine (XVII) for the compound related to cystine and containing two iminoimidazolidinone rings. The first two of these names are generally accepted and therefore they will be used also in this review.

B. Scope of the review

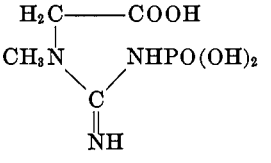
Creatinine (VIII), creatine (IX) (the guanidino acid related to it), and creatinephosphoric acid (X) (the *N*-phosphorylated derivative of the latter) are widely distributed in animals and plants and are of great biological importance. As this review confines itself to the *chemistry* of the glycocyamidines, these



VIII
Creatinine



IX
Creatine



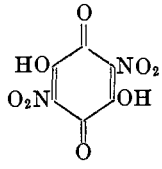
X

topics will not be treated here; however, for sake of completeness mention will be made of some important references, especially reviews and books dealing with the natural occurrence, biosynthesis, metabolism, and physiological rôle of these compounds (1, 19, 20, 49, 116–119, 140, 147a, 161, 165, 180, 181, 194, 209, 215, 234, 237, 245a, 248, 249, 269, 273, 274, 292, 295, 304).

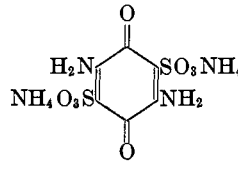
Some important reviews and books on the analytical chemistry of creatinine are given in references 22, 41, 48, 115, 147b, 156, 162, 245b, and 310. The creatinine occurring especially in urine or in other biological materials (287) can be precipitated in the form of its double salt with zinc chloride (25, 109–111, 207, 309) with the aid of potassium (122) or ammonium picrate (133; *cf.* 136); in the form of the reineckate (13, 294) with the aid of nitranilic acid (XI) (219), 2-nitro-1,3-indanedione (220), and the ammonium salt of diaminoquinonedisulfonic acid (XII) (126); in the form of its sozoiodolate (XIII) (6) with 1,24-phosphotungstic acid (14, 122) and 2-quinizarinsulfonic acid (325). A yellowish "isocreatinine" isolated from fishes (296) was shown later to be impure creatinine (188, 238).

In the literature there is described a substance designated as benzoglycocyamidine (XIV) with chemical properties resembling those of glycocyamidine. This

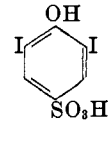
substance, 2-imino-1,2-dihydro-4(3*H*)-quinoxalone is in reality not a condensed derivative of glycocyamidine, but instead, one of 2-imino-1,2-dihydro-4(3*H*)-pyrimidone (XV), a ring homolog of it, and therefore is outside the scope of this review.



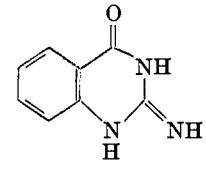
XI
Nitranilic acid



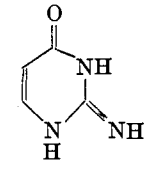
XII



XIII



XIV
Benzoglycocyamidine



XV

However, considering the close relationship of the glycocyamidines to the α-guanidino acids, the chemistry of the latter must occasionally be considered.

II. SYNTHESIS OF GLYCOCYAMIDINES

The relationship of the glycocyamidines and the α-guanidino acids is analogous to that of the hydantoins[1] and the hydantoic acids; by treatment with acids the α-guanidino acids can be cyclized to glycocyamidines and the reverse reaction can be brought about by careful treatment with bases. For other reactions occurring on treatment with bases, see Section IV,A. Therefore every synthesis of α-guanidino acids may be considered as a synthesis of glycocyamidines, and this makes it reasonable to consider the ring closure of α-guanidino acids in a separate section.

A. Ring closure of α-guanidino acids

The ring closure that has been most closely examined is that of creatine to creatinine, both in the living organism and outside it and by chemical and enzymatic means (273). The transformation

$$\text{Creatine} \rightleftharpoons \text{creatinine} + H_2O$$

is a reversible reaction of first order with a well-defined equilibrium (55, 141) which may be shifted by different factors such as temperature and pH (97). The elevation of the temperature, as well as the lowering of the pH, favors the formation of creatinine and these relations may be expressed in mathematical terms; for example, the equilibrium constant is related to the absolute temperature according to the equation (97):

$$\log K = -\frac{1084}{T} + 3.3652$$

Ring closure occurs readily if a sample of creatine containing water of crystallization or an aqueous solution of creatine is subjected to prolonged heating (112, 225, 226); moreover its velocity is already measurable at 36°C. (221; *cf.* 148–150), although the velocity of the reverse reaction in the pH range 6.7–7.6 and at a temperature of 37°C. proved to be approximately four times

[1] The chemistry of hydantoins has been reviewed by E. Ware (314).

as great (45). This reaction, occurring also in the organism, was shown by means of experiments in the presence of brain or muscle homogenates (221) to be non-enzymatic (144, 148–150, 205, 221). However, it should be mentioned that ih the organism creatine is transformed to creatinine not directly (27) but through creatinephosphoric acid (43, 157). In this connection the observation was made that the formation of creatinine in buffer solutions from creatinephosphoric acid is twice as great as from creatine (9).

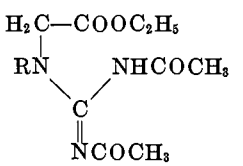
The creatine–creatinine equilibrium can be shifted by acids to the side of the cyclic product (141). The nature of the acid may have an influence on the initial rate of the ring closure (7, 8), but the position of the equilibrium itself is independent of the kind of acid or buffer solution used and is determined only by the pH of the medium (97). However, in other experiments the kind and the concentration of the buffer used proved to be a more important factor than the pH (9). The rate of the ring closure in hydrochloric acid of various concentrations (0.19–0.76 *N*) and at various temperatures (25–100°C.) was examined by Edgar and Wakefield (98); the influence of variation of the pH on the equilibrium and on the rate of attaining equilibrium is of greatest significance in the pH range 1.5–3.0 (97). In 2.667 *N* hydrochloric acid at 100°C. the rate constant of the cyclization was found to be 0.1685; that of the reverse reaction 0.0047 (123). Between 37.5° and 50°C. the rate of cyclization as a function of pH has a minimum in approximately 0.1 *N* and a maximum in 0.01 *N* hydrochloric acid. This can be explained by assuming that the measured rate of cyclization is the sum of the rates of two different reactions—namely, the ring closure of creatine and that of its hydrochloride—influenced to a different extent by the pH (54). In urine acidified with hydrochloric acid to normality the transformation to creatinine is practically complete at 60–65°C. (142). By heating with a solution of picric acid creatine can be cyclized more easily than hydantoic acid, but less easily than α-(*N*-methyl)hydantoic acid (121).

For preparative purposes the following methods of cyclization proved to be satisfactory: prolonged heating with hydrochloric acid (24, 83, 96, 152, 173, 202), treating of pulverized anhydrous creatine with gaseous hydrogen chloride (95, 202), evaporation of a solution in dilute sulfuric acid on the steam bath (202), heating with 90 per cent acetic acid (18) or with a solution of zinc chloride (76), and fusing with anhydrous zinc chloride at 120–130°C. (94, 96). In the latter case there is obtained a double salt of creatinine and zinc chloride; in the case of cyclizations with hydrogen chloride, the product is the hydrochloride of creatinine, which may be separated as such or may be transformed to the free base by treating its aqueous solution with aqueous ammonia with cooling (95, 96).

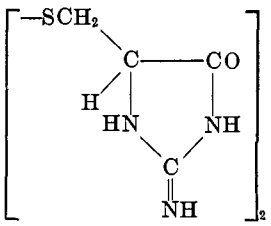
The ring closure of glycocyamine can be attained similarly, although the rate of formation of glycocyamidine is approximately only one-third of that of creatinine (10). The methods used for the cyclization are the following: careful heating of the hydrochloride of glycocyamine to 160–170°C. (189, 290), heating with concentrated hydrochloric acid to 130–140°C. (189), prolonged heating with dilute hydrochloric acid (28, 173) or with dilute sulfuric acid (10), and treating with concentrated sulfuric acid on the steam bath (264, 265). The glyco-

cyamidine may be liberated from its salts obtained by these methods by treating with lead hydroxide (290).

By treating with concentrated hydrochloric acid at 100°C. the ethyl esters of ω,ω'-diacetylglycocyamine (XVIa) and ω,ω'-diacetylcreatine (XVIb) may be cyclized to glycocyamidine and creatinine (29, 30), respectively. By treating with Amberlite IRA-400 the hydrochloride of the ethyl ester of glycocyamine is transformed partially to glycocyamidine, but hydrolysis to glycocyamine (211) also occurs.



XVIa: R = H
XVIb: R = CH_3



XVII

Other derivatives of glycocyamidine prepared by ring closure of α-guanidino acids are shown in table 1.

The case of 3-methylglycocyamidine is interesting, for theoretically the formation of the N^2-methyl isomer would also be possible. Apparently the reactivity ratio of the two nitrogen atoms in the ω-position is altered by methylation of one of them in such a way that the methylated one becomes more susceptible to the acylating action of the carboxyl (*cf.* the analogous situation in the nitrosation of *N*-methylurea to *N*-methyl-*N*-nitrosourea), and therefore this is exclusively acylated. The contrary observation of Johnson and Nicolet, according

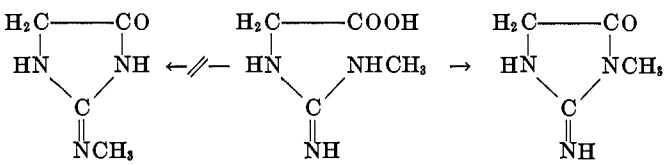


TABLE 1

Preparation of glycocyamidines by ring closure of α-guanidino acids

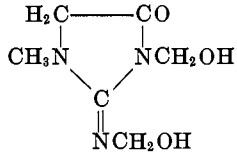
Substituent of the Nucleus	Method	References
5-(δ-Aminobutyl)	Heating with hydrochloric acid	(106, 284)
5-Benzyl	Heating with hydrochloric acid	(300)
5-Isobutyl	Heating with dilute sulfuric acid	(92)
1-Ethyl	Evaporation to dryness with concentrated hydrochloric acid	(11a)
5-Ethyl	Heating with dilute sulfuric acid	(86)
3-Methyl	Heating with hydrochloric acid	(189)
5-Methyl	Heating to 180°C. or heating with dilute sulfuric acid	(15, 16)
	Prolonged heating with hydrochloric acid	(28)
5-(1'-Methylpropyl)	Heating with hydrochloric acid	(300)
5-(β-Methylthioethyl)	Heating with hydrochloric acid	(300)
5-Isopropyl	Heating with dilute sulfuric acid	(86)
"Cystine-cyamidine" (XVII)	Heating with concentrated hydrochloric acid	(127)

to which the successive treatment of N^2-methylglycocyamidine first with dilute potassium hydroxide and then with hot hydrochloric acid furnishes a mixture of the isomeric N^2- and 3-methylglycocyamidines (177), may be perhaps explained by assuming that the hydrolysis of the N^2-methylglycocyamidine to ω-(*N*-methyl)-glycocyamine under the rather mild conditions applied was not complete.

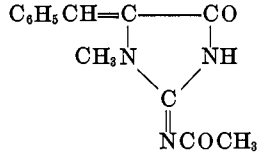
There existed some uncertainty in the literature regarding the nature of the products obtained by treating alcoholic solutions of creatine at ordinary temperature with gaseous hydrogen chloride (84; *cf.* 211). These products were assumed at first correctly to be the hydrochlorides of the corresponding esters of creatine, and it was shown that they could be transformed by heating above their melting points to the hydrochloride of creatinine (84), but later data appeared according to which the primary products should be derivatives of creatinine (179). Finally it was shown by examination of the pH dependence of the ultraviolet and infared spectra that the alcohol is present in a typical ester bond and that therefore no cyclization could have occurred (216). It is interesting to note that the methyl esters of both glycocyamine and creatine are cyclized irreversibly to glycocyamidine and creatinine, respectively, in alkaline solutions (104, 216).

The ring closure of creatinephosphoric acid to creatininephosphoric acid can not be accomplished; in treatment with acids or other reagents (thionyl chloride, sodium hydroxide, heating of the calcium salt) the cyclization is always accompanied by hydrolysis. For example, on treatment with dry hydrogen chloride creatinine and α-(*N*-methyl)hydantoic acid-ω-*N*-phosphoric acid are formed (323).

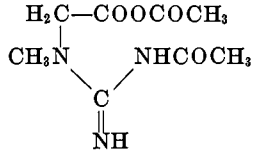
Besides the cyclization methods under acidic conditions, other reagents are able to effect closure of the cyamidine ring. For example, prolonged heating of creatine with aqueous formaldehyde solutions, even after preliminary neutralization of the latter, yields a bis(hydroxymethyl)creatinine (XVIII), the structure of which is proved by the fact that it can be prepared under similar conditions from creatinine and that it furnishes a dibenzoyl derivative (172). On the other hand, treatment of creatine with acetic anhydride and benzaldehyde gives N^2-acetyl-5-benzylidenecreatinine (XIX), which was first formulated as the 3-acetyl derivative (100).



XVIII



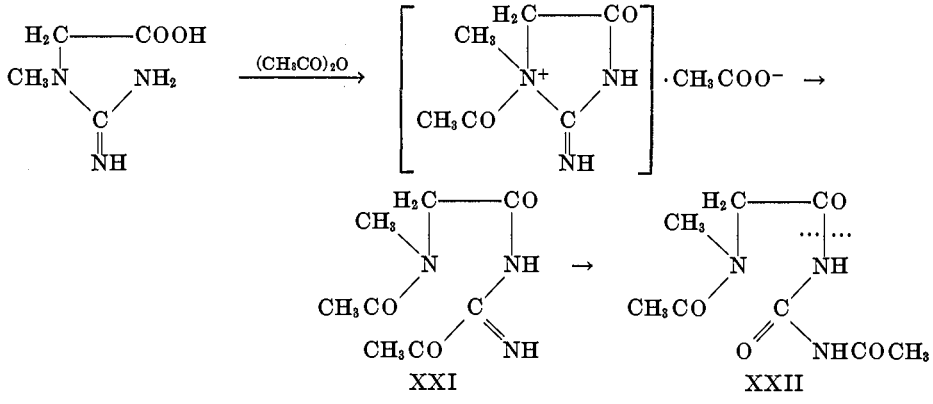
XIX



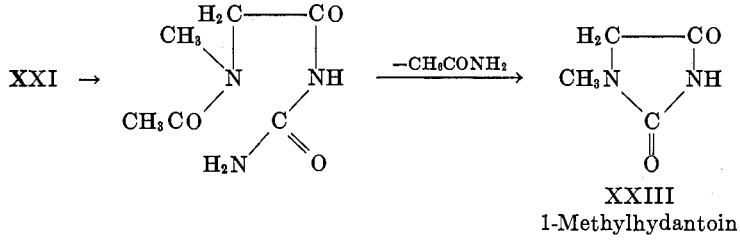
XX

In the absence of benzaldehyde no cyclization occurs; instead "diacetylcreatine" is formed (100), which was identified later as *sym*-(*N*-acetylsarcosyl)acetylurea (XXII) (167). The formula (XX) originally proposed (100) could not be correct, as the anhydride structure is disproven by the facts that the compound exhibits, in contrast with *N*-acetylguanidine (187), no basic properties, that it is

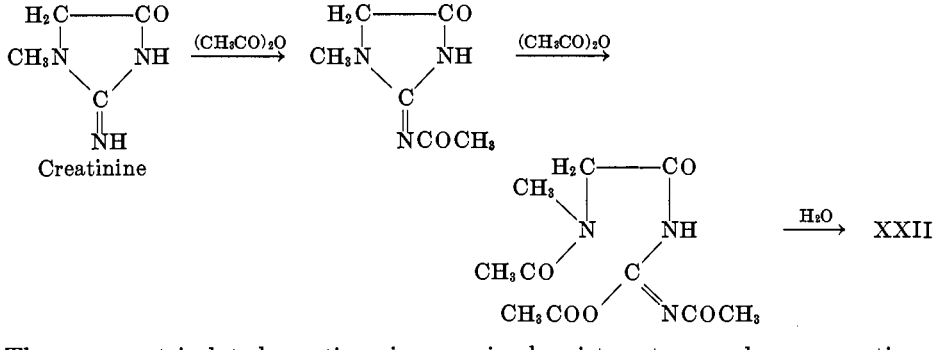
not hydrolyzed by water, and that it can be recrystallized without any change from alcohol. On the other hand, in agreement with structure XXII the compound furnishes on treatment with ammonia or methylamine (by splitting along the dotted line) acetylurea and sarcosineamide or *N*-methylsarcosineamide; this structure was proved also by synthesis (167). The mechanism of formation of XXII may be assumed as follows (167):



This mechanism accounts also for the formation of 1-methylhydantoin (XXIII) at any time by dilution of the mother liquors from XXII with water, a reaction which can be explained by the obviously ready hydrolysis of XXI and the splitting off of acetamide from the hydrolysis product (167).



XXII is formed also as a byproduct in the acetylation of creatinine (167, 168) and its formation can be explained on similar grounds.



These are not isolated reactions in organic chemistry; two analogous reactions from the older literature are the following:

$$HN{=}C(NHC_6H_5)_2 \xrightarrow{(CH_3CO)_2O} CH_3CONHC_6H_5 + CH_3CONHCONHC_6H_5 \quad (50)$$

$$R_2NCONH_2 \xrightarrow{(CH_3CO)_2O} [CH_3C\overset{+}{O}NR_2CONH_2] \xrightarrow{-H^+}$$

$$CH_3CONR_2 + HNCO \quad (305)$$

$$\downarrow$$

Cyanuric acid

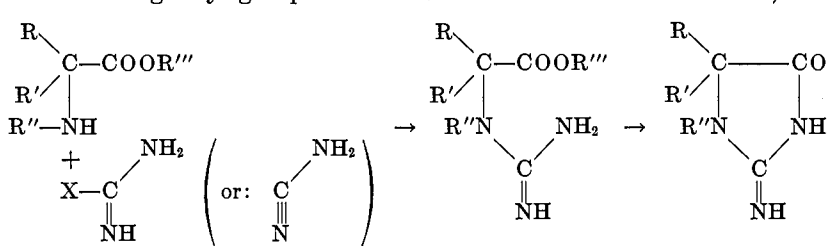
On treatment of creatine with phthalic anhydride cyclization occurs and a neutral salt, dicreatinine phthalate, is formed (167), which was formerly thought to be phthaloyldicreatine (303). Cyclization occurs also on treatment of creatine with benzoic anhydride and, as a result, N^2-benzoylcreatinine is formed (168, 303).

The closure of the cyamidine ring can be effected also by phosphorus oxychloride; when creatine is heated with this reagent simultaneous phosphorylation and ring closure to creatininephosphoric acid occur (320, 323).

B. Synthesis from α-amino acids

The reaction of α-amino acids and their esters with guanylating agents may lead, depending on the kind of the reagents, the conditions of the reaction, and the stability relationship of the expected reaction products, either to an α-guanidino acid or to the corresponding glycocyamidine. The former may be cyclized to the latter, as discussed in the preceding section.

From the general equation of the reaction, in which $XC(=NH)NH_2$ represents the guanylating agent (e.g., X = alkoxy, alkylthio, amino etc.), it is evident that this reaction may lead to glycocyamidines substituted in positions 1 and/or 5. If the guanyl group introduced itself contains a substituent, this will



appear in position 3 and/or N^2 of the cyamidine ring. Since only guanyl groups substituted symmetrically with identical substituents have been used, orientation phenomena have not yet been studied.

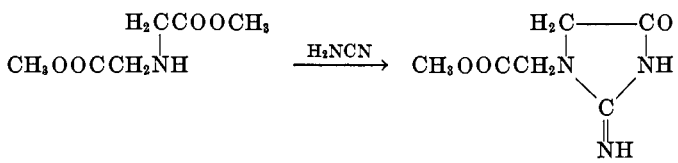
One of the best known guanylating agents is cyanamide. This gives with α-amino acids in concentrated aqueous solutions at room temperature in the presence of small quantities of ammonia on prolonged standing either α-guanidino acids or glycocyamidines.[2] Direct formation of glycocyamidines was observed from the following α-amino acids: *N*-ethyl-*dl*-alanine (90), α-ethylaminobutyric

[2] The cyanamide reacts not only with α-amino acids, but also with other amino acids with formation of guanidino acids, e.g., with α-(*N*-tosyl)-α-(*N*-methyl)- (283) and α-(*N*-benzoyl)ornithine (279a) to yield the respective arginine derivatives, and with α-(*N*-tosyl)lysine to yield α-(*N*-tosyl)-α-amino-ε-guanidinocaproic acid (284). Moreover, peptides, e.g., glycyl-*d*-alanine may also be guanylated by this reagent (179a).

acid (89), α-ethylamino-*n*-caproic acid (88), *N*-ethylglycine (91; see however 11a), *N*-methyl-*dl*-alanine (124) (*cf.* 204), α-methylaminobutyric acid (87; *cf.* 124), α-methylamino-*n*-caproic acid (88), and α-methylaminoisovaleric acid (87). Under similar conditions the following furnish only α-guanidino acids: α-aminobutyric acid (86), δ-(*N*-benzoyl)ornithine (279a), *dl*-hydroxyproline (300), leucine (92), and norvaline (86).

From this enumeration it becomes evident that substitution in position 1 by alkyl substituents confers upon the cyamidine ring greater stability with respect to the corresponding α-guanidino acids; in agreement with this generalization is the already mentioned fact that creatine is dehydrated under similar conditions faster than glycocyamidine. Sarcosine furnishes an apparent exception to the above generalization for, under the conditions specified, it gives not creatinine but creatine (33a, 33b); however, this is only a result of the special solubility relationships (11a). The latter reaction was also used for the synthesis of creatines labeled with isotopic nitrogen (33a, 33b).

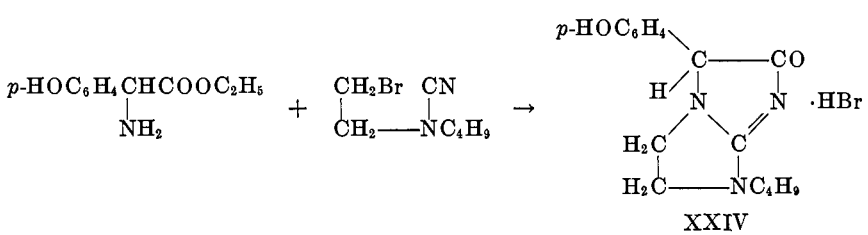
Glycocyamidines are obtained also on heating *N*-phenylglycine with cyanamide in alcoholic solution (in addition to *N*,*N*′-diphenyldiketopiperazine) (99) and from the dimethyl ester of iminodiacetic acid on standing in dry ether solution for some days (270):



However, ε-(*N*-benzoyl)lysine (284) and various other α-amino acids (306) at 60–100°C. and pH 9–10 yield only α-guanidino acids. Preliminary to the reaction with cyanamide the α-amino acids may be prepared *in situ* from α-halogeno acids by action with ammonia or ammonium carbonate (307).

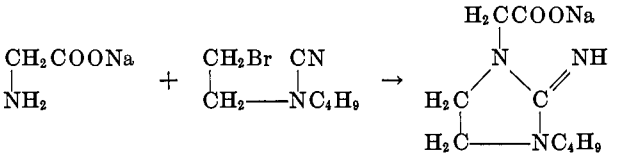
The addition of cyanamide to glycine and sarcosine is catalyzed by an enzyme occurring in muscle (but not in blood and in the liver); in the presence of this the cyanamide may be replaced by urea. Moreover, under more energetic conditions the reaction occurs in the absence of it (21).

Substituted cyanamides may also react with α-amino acids or their derivatives: e.g., *N*-(β-bromoethyl)-*N*-butylcyanamide yields with the ethyl ester of tyrosine the hydrobromide of 1,N^2-ethylene-N^2-butyl-5-(*p*-hydroxyphenyl)glycocyamidine (XXIV):

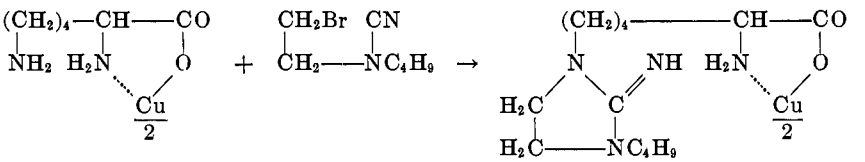


XXIV

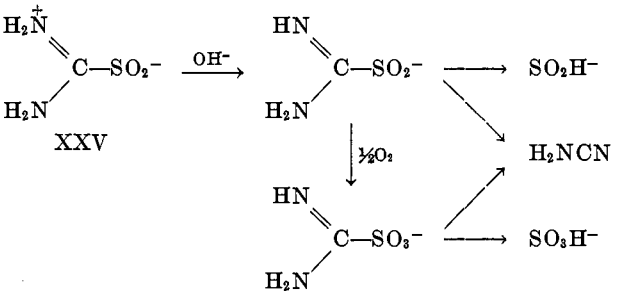
with sodium glycinate it gives only the analogous glycocyamine derivative (98a):



The same reagent is capable of guanylating selectively the ϵ-amino group of the copper complex of lysine (98a).



A recently discovered guanylating agent is formamidinesulfinic acid (XXV) (313). This otherwise powerful reducing agent does not reduce amino acids but guanylates them. The mechanism of its action seems at first sight obvious, namely, that the anion of formamidinesulfinic acid or its oxidation product, the anion of formamidinesulfonic acid, splits off cyanamide in alkaline solutions, which should be the true guanylating agent:



However, this mechanism is surely not correct, because formamidinesulfinic acid in ammoniacal solutions at room temperature forms glycocyamine much faster than cyanamide and, in the second place, at 70°C. formamidinesulfinic acid is capable of this reaction, but not the cyanamide (313).

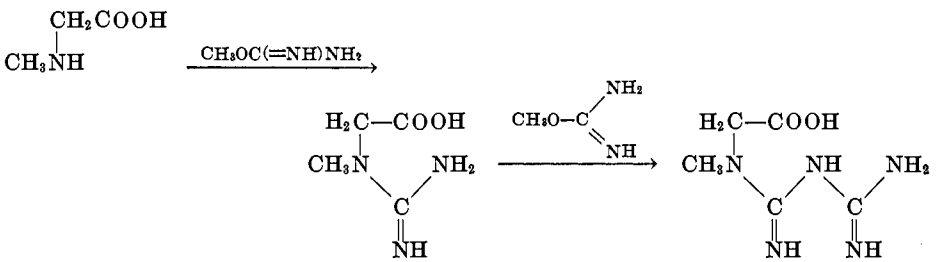
O-Alkylisoureas (see, e.g., 23a) are also capable of guanylating α-amino acids[3]:

[3] *O*-Methylisourea is capable of guanylating β-amino acids (246b) as well as the ϵ-amino group of α-(*N*-benzoyl)-*dl*-lysine (127a) and various dipeptides, e.g., *dl*-alanylglycine, glycyl-*dl*-phenylalanine, and glycylleucine (179a), in good yields.

The amino group in the α-position of diamino carboxylic acids may be protected against the guanylating action of *O*-methylisourea not only by benzoylation but also by preliminary preparation of the copper complexes; e.g., the copper complex of ornithine may be guanylated to that of arginine (300a).

Various other peptides (23a) and proteins (23a, 246a), e.g., human serum albumin (164a) and gelatin (93a), also react with *O*-methylisourea; in the latter two cases the amino groups in the ϵ-position of the lysine residues are guanylated with the formation of homoarginine residues.

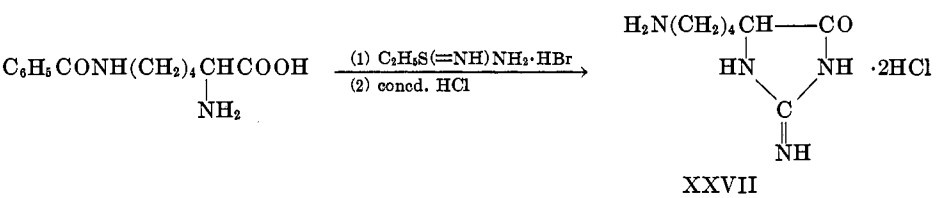
e.g., cystine, *d*-glutamic acid, *l*-hydroxyproline, *dl*-phenylalanine, *l*-proline, *dl*-serine, and *l*-tryptophan are guanylated in yields ranging from 66 to 94 per cent (179a). Sarcosine gives, with 2 moles of *O*-methylisourea in methanol, creatine in a yield of approximately 20 per cent (270); this yield is very low, for in ammoniacal solution with *S*-methylisothiourea the yield is 83 per cent (270). The yield cannot be improved by heating, because at 40–50°C. instead of creatine only small quantities of 1-methyl-1-biguanideacetic acid (XXVI) are obtained (270):



XXVI

Iminodiacetic acid is guanylated neither by *O*-methylisourea in methanol nor by *S*-methylisothiourea in ammoniacal solutions (270).

Like the *O*-alkylisoureas, their *S*-analogs are well-known guanylating agents, which were used first by Steib for the synthesis of glycocyamidines (284).[4] ε-(*N*-Benzoyl)lysine furnishes with *S*-ethylthiuronium bromide the corresponding α-guanidino derivative, which can be cyclized by hydrochloric acid to the dihydrochloride of 5-(δ-aminobutyl)glycocyamidine (XXVII) (284).



XXVII

In a similar manner glycocyamidine is obtained in 74 per cent yield from glycine (28, 44, 184), creatinine in 40 per cent yield from sarcosine (184), and alacreatinine in 65 per cent yield from alanine (28, 184). This method is applicable also to the preparation of 5-(γ-guanidinopropyl)glycocyamidine (324).

[4] Like the guanylating agents already mentioned, *S*-alkylisothioureas are capable of guanylating amino groups other than those in the α-position to a carboxyl group; β-alanine (217, 218), isoserine (218), β- and γ-aminobutyric acids (217b), α-(*N*-tosyl)-α-(*N*-methyl)-ornithine (283), α-(*N*-tosyl)lysine (284), and the α-(*N*-methyl) derivative of the latter (169a) may be guanylated by these reagents in the β-, γ-, δ-, or ε-position.

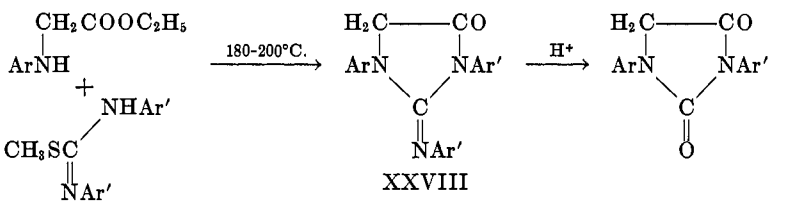
In the case of proteins, the ε-amino groups of the lysine residues are exclusively guanylated (217a, 246a); on this is based a method for the determination of the lysine content of α- and β-casein (217a).

The ω-amino group of α,ω-diamino carboxylic acids may be brought selectively to reaction with *S*-alkylisothiuronium salts by protecting the α-amino group through the formation of the copper complexes (275a).

The reaction is best performed in concentrated aqueous ammonia solution (108, 217, 270); this seems strange at first sight, for a great part of the *S*-alkylisothiourea must react obviously with the ammonia present in great excess to yield guanidine. But, keeping in mind that amino acids are easily capable of forming guanidino acids also with guanidines (see below), it becomes intelligible that many α-amino acids, e.g., alanine (217, 218, 270, 300), α-aminobutyric acid (108, 217b), aspartic acid (217, 300), citrulline (217b), cystine (127, 179a, 217, 300), glutamic acid (217b), glycine (217, 270, 300), histidine (217b), leucine (217b, 218, 300), isoleucine (217b, 218, 300), norleucine (217b, 218), lysine (217, 217b), methionine (168a, 217, 218, 300), phenylalanine (217, 300), proline (300), sarcosine (108, 270, 300), serine (217, 270, 300), threonine (217, 300), tryptamine (300), tyrosine (217), and valine (217b, 218, 300), as well as peptides, e.g., glycylglycine (217, 270) and glycyl-*l*-tyrosine (270), and proteins (217a, 246a, 271) may be guanylated in good yields by this method. An exception is furnished by *N*-ethylglycine, which yields with *S*-ethylisothiuronium bromide under these conditions no α-(*N*-ethyl)glycocyamine, but instead a stable complex of *N*-ethylglycine and guanidinium bromide (11a).

Instead of aqueous ammonia, solutions of the alkali hydroxides may be used (145, 146, 169a, 240a). *N*-Substituted *S*-alkylisothiuronium salts, e.g., the *N*-allyl or *N*-buten-(2)-yl derivative, may also react (145, 146).

Esters of amino acids react similarly to the parent compounds. For example, a number of *N*,*N*′,*N*″-triarylglycocyamidines (XXVIII) were prepared by heating *N*-arylglycine esters with *S*-methyl-*N*,*N*′-diarylisothioureas to 180–200°C.; the structures of the products are proved, apart from their formation, by their hydrolysis to 1,3-diarylhydantoins (72).

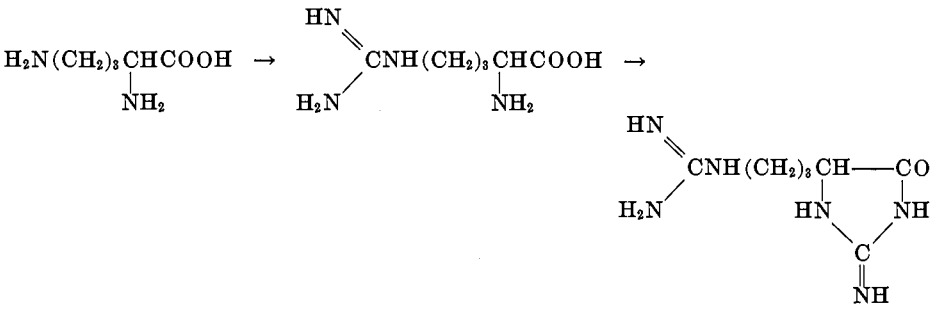


XXVIII

As the two aryl groups designated by Ar′ in each case were identical, no orientation phenomena could have occurred and no isomers could have been formed.

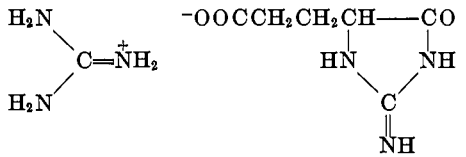
From the two amino groups of ornithine the one in the δ-position reacts exclusively with *S*-methylisothiuronium chloride in aqueous solution to give arginine in 68 per cent yield; upon heating the hydrochloride of the latter with 1.2 equivalents of cyanamide at 135°C. the α-amino group reacts, and the glycocyamidine (XXIX) is formed in 63 per cent yield (229). The same glycocyamidine may be prepared also from arginine with *S*-ethylisothiuronium bromide (324).

Guanidine and certain guanidinium salts are also capable of guanylating amino acids. When sarcosine is melted with guanidine carbonate at 140–160°C. creatinine is formed by guanylation and cyclization (163); similarly, α-phenylglycine yields at 180°C. 5-phenylglycocyamidine (99); however, with glycine no cyclization occurs at 140°C. and glycocyamine is obtained (211, 222).



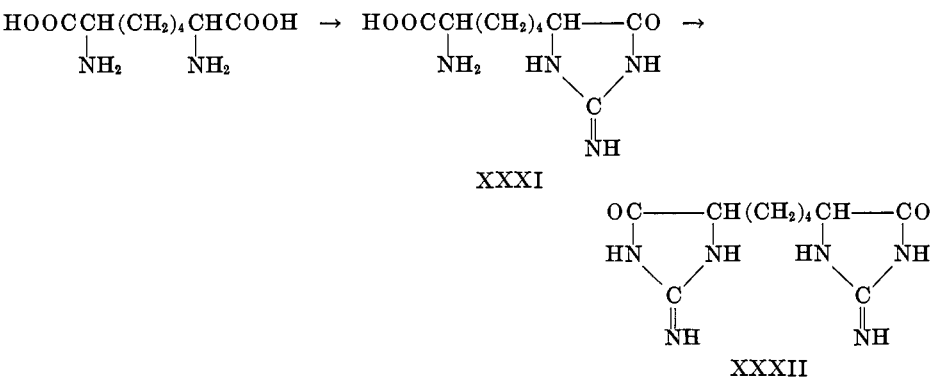
XXIX

With guanidine the ester of glycine furnishes glycocyamidine (299). This reaction was investigated extensively by Abderhalden (2–4), according to whom the free guanidine base reacts with esters of amino acids with evolution of ammonia and heat quite generally to yield glycocyamidines. The reaction proceeds even at 0°C.; e.g., with the ester of glycine the evolution of ammonia is quantitative, but with that of cystine only 55 per cent (2). In this manner the ester of glycine may be transformed to glycocyamidine (*cf.* 211), that of *dl*-leucine to *dl*-5-isobutylglycocyamidine, ethyl *d*-glutamate to the guanidinium salt of *d*-5-(β-carboxyethyl)glycocyamidine (XXX) (2), the ester of sarcosine at −15°C. to creatinine in 65 per cent yield, that of *dl*-tyrosine in 27 per cent yield to *dl*-5-



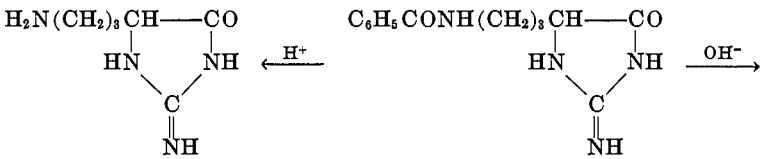
XXX

(*p*-hydroxybenzyl)glycocyamidine (3; *cf.* 300), and finally that of *N*-ethylglycine to *l*-ethylglycocyamidine (300). The reaction proceeds also with the esters of diamino carboxylic acids (4): e.g., the ester of α,α′-diaminosuberic acid reacts with guanidine in ethereal solution partly with only one, partly with both amino groups, to yield the amphoteric 5-(ε-carboxy-ε-aminopentyl)glycocyamidine (XXXI) along with the 5,5′-tetramethylenebisglycocyamidine (XXXII) of basic character; the two products combine with each other to yield a salt.

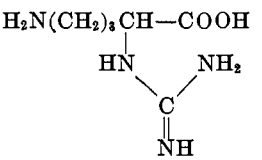


XXXII

Similarly, but at 15°C., the methyl ester of δ-(*N*-benzoyl)ornithine reacts with guanidine to form 5-(γ-benzoylaminopropyl)glycocyamidine (XXXIII), which may be debenzoylated by heating with concentrated hydrochloric acid without cleavage of the cyamidine ring; however, alkaline hydrolysis also cleaves the ring and isoarginine is formed (XXXIV) (4).



XXXIII



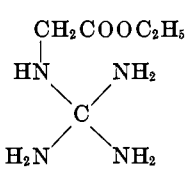
XXXIV

The reaction may not be extended, at least under the conditions studied, either to the free amino acids or to diketopiperazines and polypeptide esters (2). Moreover, ethyl hippurate and aceturate do not react in the manner mentioned with guanidine; the former gives hippurylguanidine, the latter guanidinium aceturate (4).

From the substituted guanidines thus far investigated monomethyl- and *asym*-dimethylguanidine react in this order with decreasing intensity with evolution of heat and ammonia and with formation of Jaffé-positive products (*cf.* Section VI,A), but the isolation of glycocyamidine derivatives was unsuccessful (4). On the other hand, with isoamyl-, α-naphthyl- and acetylguanidines even no Jaffé-positive products were formed (4).

Concerning the mechanism of the reaction, it is evident that the ammonia split off has its origin from the guanidine and not from the amino acid; otherwise, in the reaction of the ethyl ester of sarcosine with guanidine no creatinine and ammonia could be obtained.

Both mechanisms suggested by Abderhalden are in harmony with this fact (3). According to the first mechanism the guanidine should add the amino ester with formation of a derivative of tetraaminomethane (XXXV), which then should split off ammonia and cyclize to the glycocyamidine. According to the second mechanism the guanidine should suffer thermolysis to cyanamide



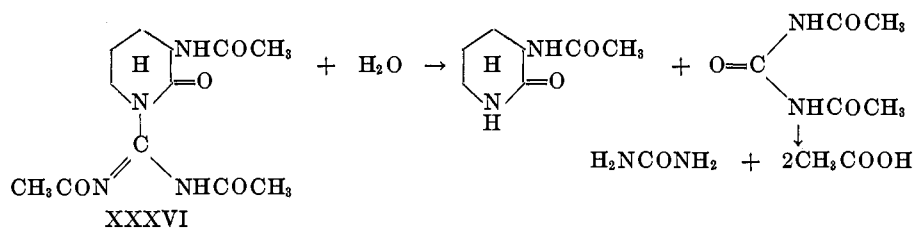
XXXV

and ammonia and the former should then add to the amino ester with simultaneous or subsequent ring closure. It could of course be supposed that in both

cases the guanidine is first acylated by the amino ester and that the transformations mentioned above occur subsequently.

Thus in each case the exchange of one of the amino groups of the guanidine by the carboxylalkylamino group is supposed not to proceed directly but through either an addition-elimination or an elimination-addition mechanism. However, this assumption is not justified by any experimental fact. On the contrary, there exist some experimental facts that cannot be reconciled with the second mechanism suggested by Abderhalden. Thus, although some glycocyamidine is obtained by the reaction of the ethyl ester of glycine with cyanamide in the presence of small quantities of guanidine, this reaction cannot be accomplished in dry liquid ammonia in the absence of guanidine (2). Moreover, the ethyl ester of sarcosine gives with cyanamide only a 29 per cent yield of creatinine, while the yield with guanidine attains 65 per cent, and the yield in the former reaction is even diminished if small quantities of guanidine are added (3). Finally, it is difficult to imagine that the guanidine should decompose under the mild conditions applied.[5]

An interesting guanylating agent is triacetylanhydroarginine (XXXVI), the hydrolysis of which leads to *dl*-β-acetylamino-α-piperidone and to urea (29).

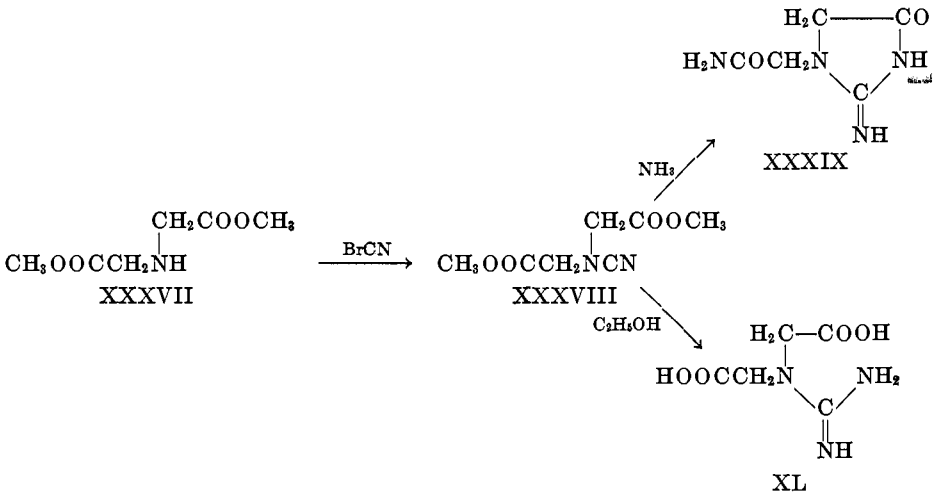


XXXVI

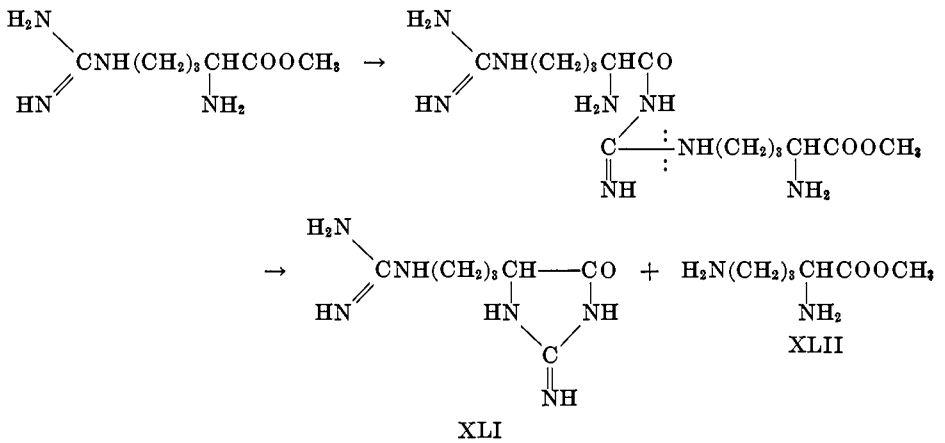
With amines, an analogous reaction takes place, as the result of which the diacetylguanyl group becomes transferred from the piperidone ring to the nitrogen of the amine. Thus with anhydrous methylamine in dry ether at 20°C. by transguanylation N,N'-diacetyl-N''-methylguanidine (30) is formed; with the ester of glycine under similar conditions 50 per cent of the ethyl ester of diacetylglycocyamine (XVIa) and some 3- or N^2-acetylglycocyamidine (which can be hydrolyzed by acids to glycocyamidine) along with 96 per cent of the piperidone; finally, with the ester of sarcosine the ethyl ester of diacetylcreatine (XVIb) is formed (29, 30).

The guanylation of the amino group of amino esters may be accomplished also in two steps. Thus the dimethyl ester of iminodiacetic acid (XXXVII) gives with cyanogen bromide in dry ether the dimethyl ester of N-cyanoiminodiacetic acid (XXXVIII) which, on prolonged heating with alcoholic ammonia at 85°C., is transformed to glycocyamidine-1-acetamide (XXXIX), hydrolyzable with hydrochloric acid to glycocyamidine-1-acetic acid; under somewhat more energetic conditions XXXVIII yields with alcoholic ammonia N-guanyliminodiacetic acid (XL) (270).

[5] With the ester of sarcosine the reaction sets in even at −15°C. and during the vigorous reaction the temperature rises no higher than 20°C. (3).



The glycocyamidine derivative XLI is the product of an interesting guanylation reaction. As it can be obtained by autocondensation of 2 molecules of the methyl ester of *d*-arginine, it was at first thought erroneously to be arginylarginine (105), but later the true structure was proved by synthesis (324). The mechanism of its formation, shown below, is proved by the fact that ornithine (XLII) may be isolated as a byproduct (324).



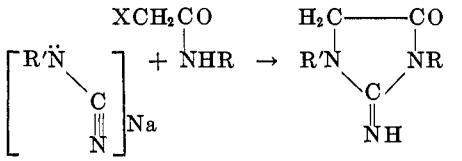
In aqueous ammoniacal solution glycine and β-alanine are guanylated at body temperature by the action of charcoal; however, α-alanine does not react (272a).

C. Synthesis from α-halogeno acids, α,β-unsaturated acids, and their derivatives

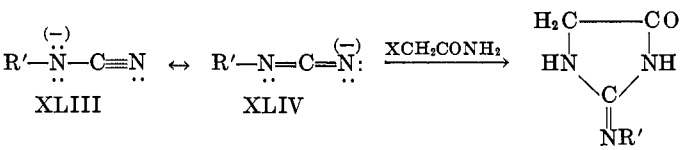
α-Halogeno acids are converted by mild heating with guanidine in aqueous solution to glycocyamidines substituted in position 5; thus α-bromoisovaleric acid furnishes at 60°C. 5-isopropylglycocyamidine (239), and α-(bromophenyl) acetic acid at 80°C. yields 5-phenylglycocyamidine (99, 239). However, ε-benzoyl-

amino-α-bromocaproic acid yields on treatment with a concentrated aqueous solution of guanidine the corresponding α-guanidino acid, which is converted to 5-(δ-aminobutyl)glycocyamidine only by heating with concentrated hydrochloric acid (106).

Similarly, α-halogenoacetamides and their *N*-monosubstituted derivatives yield with the alkali salts of monosubstituted cyanamides substituted glycocyamidines (158, 159). The substituent of the cyanamide is supposed to appear in position 1 of the glycocyamidine ring, but this is proved rigorously only in

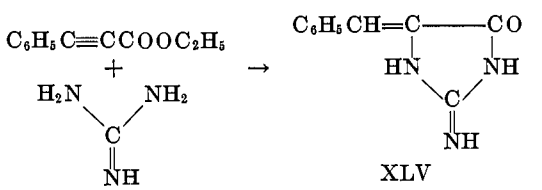


the case of the reaction of the sodium salt of benzylcyanamide with chloroacetamide (196)[5a]. Moreover, it can be imagined that the anion of cyanamide (XLIII) may react, depending on the electronic properties of the substituent R, according to the mesomeric carbodiimide form (XLIV), yielding thus N^2-substituted derivatives of glycocyamidine.



Glycocyamidine cannot be obtained from chloroacetylguanidine; on treatment with alcoholic ammonia no cyclization occurs, but instead, a transacylation, the result of which is the formation of chloroacetamide and guanidine (187, 189).

From α,β-unsaturated acids or their derivatives only in one case was the formation of a glycocyamidine observed: namely, from ethyl phenylpropiolate which, on treatment with guanidine in alcoholic solution in the presence of sodium ethoxide, yields 5-benzalglycocyamidine (XLV) (250).



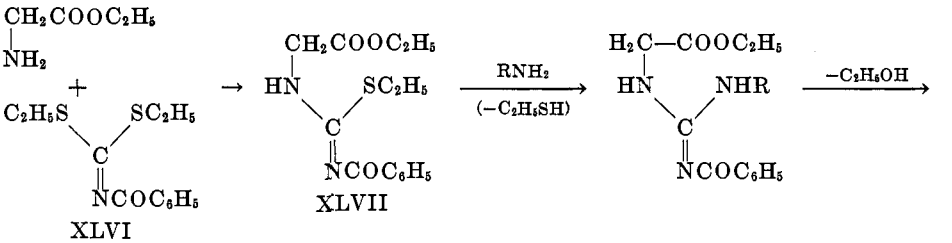
5-Benzalglycocyamidine

D. Synthesis from ethyl S-alkylpseudothiohydantoates

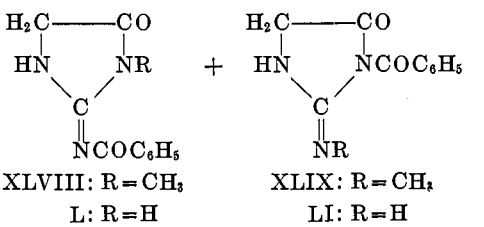
Ethyl *S*-ethyl-ω-(*N*-benzoyl)pseudothiohydantoate (XLVII), obtained by the reaction of ethyl glycinate and the benzimide of ethyl dithiolcarbonate

[5a] *Added in proof:* The same is true for the reaction of the sodium salts of phenyl- and *p*-ethoxyphenylcyanamide with chloroacetamide (196).

(XLVI) (317), yields with ammonia and amines in alcoholic solutions with evolution of ethyl mercaptan *N*-benzoylated α-guanidino esters which are able to split off ethanol and cyclize to benzoylated glycocyamidines (177).



XLVI XLVII

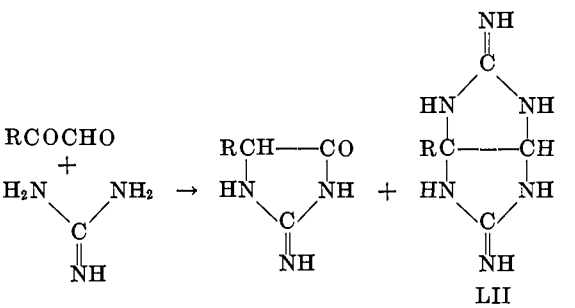


XLVIII: R = CH_3 XLIX: R = CH_3
L: R = H LI: R = H

Although the *N*-benzoylglycocyamidines formed could not be separated into isomers, they were nevertheless mixtures of isomers, for, in the case of the methyl derivative, successive debenzoylation with alcoholic hydrochloric acid and condensation with benzaldehyde led to two different *N*-methyl-5-benzylidene-glycocyamidines, in one of which the methyl is attached to position 3 and in the other to position N^2 (*cf.* Section III,A). The main product of the reactions is the N^2-benzoyl-3-methyl isomer (XLVIII); correspondingly, the product of debenzoylation contains mainly 3-methylglycocyamidine (177), identical with the substance obtained by the methylation of glycocyamidine (189). In the case of the reaction of XLVII with ammonia, the product leads on debenzoylation of course uniformly to glycocyamidine.

E. Condensation of glyoxals and benzils with guanidines

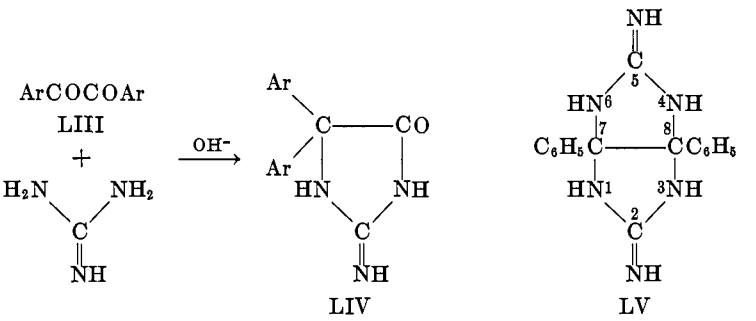
The well-known synthesis of hydantoins or thiohydantoins from the reaction of glyoxal or monoalkyl- and monoarylglyoxals with urea (107, 275) or thiourea (276, 277), respectively, may be extended to the synthesis of glycocyamidines, if guanidine is used instead of the urea or thiourea.



LII

Glyoxal and the hydrochloride of guanidine yield glycocyamidine on prolonged heating in aqueous solution; methylglyoxal leads similarly to 5-methylglycocyamidine (28).

As in the hydantoin or thiohydantoin series, disubstituted glyoxals may lead to analogous products only in those cases where the glyoxal itself or the intermediate formed by its reaction with urea, thiourea, or guanidine, respectively, is capable of undergoing a benzilic acid rearrangement; that is, only the benzils and other diarylglyoxals. Since the requirement for the benzilic acid rearrangement is alkalinity of the medium, the reaction must be performed in the presence of alkali.[5b] Thus the dimethyl ether of 5,5-dichlorosalicil (LIII) is transformed by heating with guanidine and alkali in alcohol solution to 5,5-bis(2-methoxy-5-chlorophenyl)glycocyamidine (LIV) (73); benzil leads similarly to 5,5-diphenylglycocyamidine (73, 160).



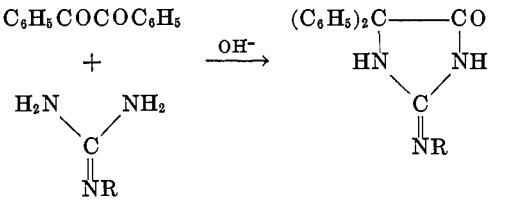
Ar = 2-CH_3O-5-ClC_6H_3—.

As in the condensations with urea or thiourea, which yield, in addition to (231) or instead of (31, 272) hydantoins or thiohydantoins, "acetylendiureins" (glycolurils) with guanidines, glycouril-2,5-diimide may be formed in addition to the glycocyamidine; e.g., in the reaction with benzil 7,8-diphenylglycoluril-2,5-diimide (LV) is formed as well as 5,5-diphenylglycocyamidine. These two products may be separated by means of their different basicity and solubility in water (56).

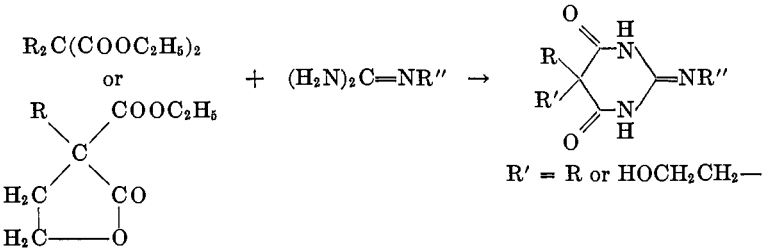
Monosubstituted guanidines may be expected theoretically to lead to one or several of the three isomeric glycocyamidines, substituted in position 1, N^2, or 3. While the condensation of butylguanidine and glyoxal in aqueous solution under different conditions was tried unsuccessfully (28), some monosubstituted guanidines have been successfully condensed in the presence of sodium hydroxide in alcoholic solution with benzil, leading in good yields to glycocyamidines substituted in position N^2 (195).[5c]

[5b] *Added in proof:* However, *N*-benzylguanidine can be condensed with benzil even in the absence of alkali to give a 60 per cent yield of 3-benzyl-5,5-diphenylglycocyamidine (195).

[5c] *Added in proof:* This is, however, true only if relatively large amounts of alkali are used. If the amount of alkali is lowered or the condensation is performed in the absence of alkali, the isomeric glycocyamidine substituted in position 3 becomes the chief product (195).



This reaction may be compared with the condensation of monosubstituted guanidines with malonic esters or α-carbethoxybutyrolactones, leading to barbituric acids in which likewise the unsubstituted nitrogen atoms of the guanidine have become members of the ring (278).

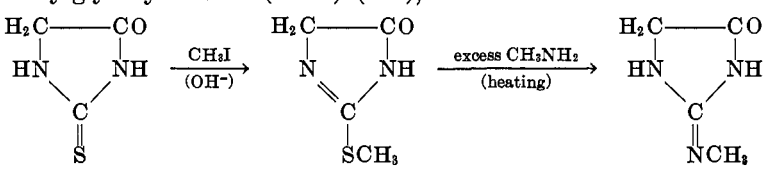


R′ = R or $HOCH_2CH_2$—

F. Synthesis from 2-thiohydantoins

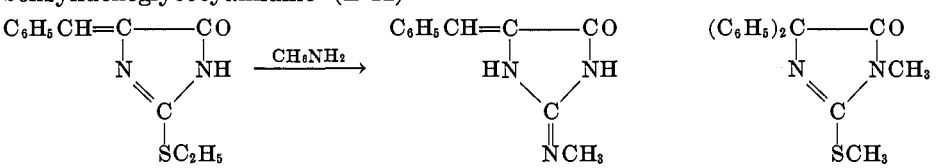
2-Thiohydantoins, being cyclic derivatives of thioureas (just as glycocyamidines are the corresponding derivatives of guanidines), several well-known reactions for the transformation of thioureas into guanidines (232) may be applied to the transformation of 2-thiohydantoins into glycocyamidines. The latter transformation may be achieved either directly by desulfurizing the 2-thiohydantoin in the presence of amines or indirectly by the ammonolysis or aminolysis of the *S*-alkyl derivatives of the 2-thiohydantoins. The second procedure is in general more convenient.

This type of reaction was first applied by Johnson and Nicolet for the synthesis of N^2-methylglycocyamidine (LVII) (177),



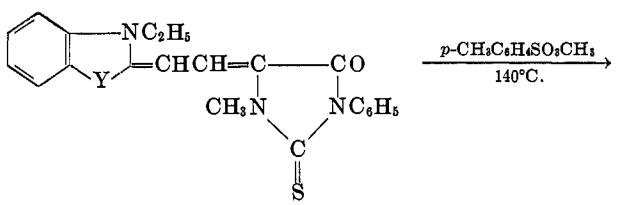
LVI LVII

the isolation of the intermediate 2-methylmercapto-2-imidazolin-4(or 5)-one (LVI) not being necessary. Similarly, 2-ethylmercapto-4-benzal-2-imidazolin-5-one (LVIII) yields with an alcoholic solution of methylamine N^2-methyl-5-benzylideneglycocyamidine (LIX):

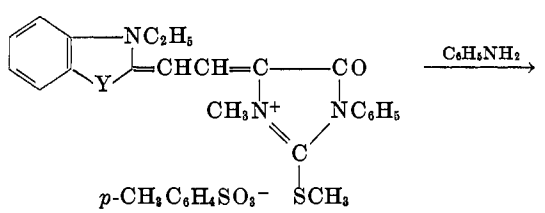


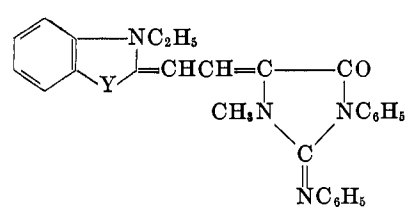
LVIII LIX LX

However, the yield in the latter case is low (177). Analogous reactions take place between 2-methylmercapto-4,4-(or 5,5)-diphenyl-2-imidazolin-5(or 4)-one and various primary amines, e.g., 2-aminoethanol (59), benzylamine (197), and secondary amines (52, 53), monoprimary monotertiary diamines (199), and hydrazine (311). The latter has also been subjected to the reaction with 1-methyl-2-methylmercapto-4,4-diphenyl-2-imidazolin-5-one (LX), the dimethyl derivative of 5,5-diphenyl-2-thiohydantoin (311), and the diamines mentioned above with the *S*-methyl derivatives of various other 2-thiohydantoins (200). The reaction is best performed by heating the alkylmercaptoimidazolinone and the amine in alcoholic solution, the necessary temperature depending on the nature of the amine used: primary amines react smoothly at temperatures about 100°C., but the reaction with secondary amines needs higher temperatures. 2-Methylmercapto-4,4(or 5,5)-diphenyl-2-imidazolin-5(or 4)-one may be transformed into 5,5-diphenylglycocyamidine by heating in acetic acid solution with ammonium acetate (195).



LXIa: Y = O
LXIb: Y = S



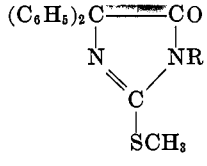


LXIIa: Y = O
LXIIb: Y = S

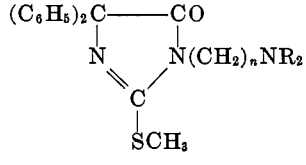
The reaction proceeds also with molecules which are more complicated than those mentioned: e.g., 1-methyl-3-phenyl-5-(3-ethylbenzoxazolin-2-ylidene-ethylidene)-2-thiohydantoin (LXIa) and the corresponding derivative of benz-

thiazoline (LXIb) yield, by successive treatment with methyl *p*-toluenesulfonate and aniline in the presence of an alcoholic solution of trimethylamine, N^2,3-diphenyl-5-(3-ethylbenzoxazolin-2-ylideneëthylidene)creatinine (LXIIa) and the corresponding derivative of benzthiazoline (LXIIb) (174).

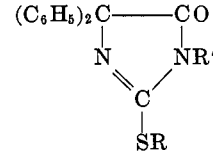
Except for this latter reaction and that with hydrazine (311) mentioned above, this reaction has never been applied to the synthesis of glycocyamidines substituted in positions 1 and/or 3. The cause of this is the sluggish reaction of *S*-alkyl derivatives of thiohydantoins substituted in position 1 and/or 3 with ammonia and amines. Thus, 1-methyl-2-methylmercapto-4,4-diphenyl-2-imidazolin-5-one (LXIIIa) could not be brought to react with diethylamine in spite of applying much more energetic conditions than those needed for the smooth occurrence of the analogous reaction with 2-methylmercapto-4,4(or 5,5)-diphenyl-2-imidazolin-5(or 4)-one (LXIIIb) (53). Similarly, the *S*-alkylated thiohydantoins of the types LXIV and LXV could not be transformed into the corresponding glycocyamidines, or at best with very low yields, by heating them



LXIIIa: R = CH_3
LXIIIb: R = H

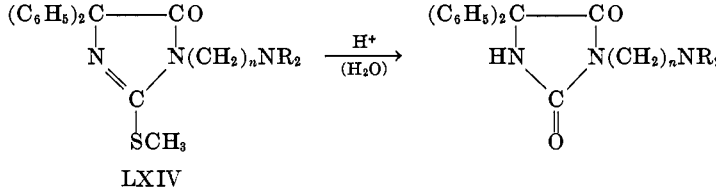


LXIV



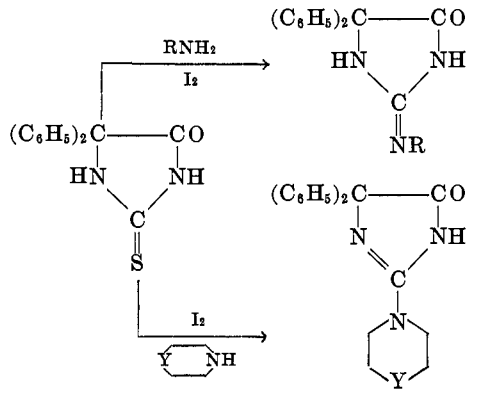
LXVa: R = R′ = CH_3
LXVb: R = R′ = $C_6H_5CH_2$
LXVc: R = CH_3, R′ = $C_6H_5CH_2$

with alcoholic ammonia (197, 198). However, by performing the latter reactions in the presence of ammonium salts, e.g., ammonium chloride, iodide, or acetate, the transformations were achieved smoothly and under much less vigorous conditions (197, 198). The catalytic activity of the ammonium salts on the ammonolysis of LXIV and LXV, which is a case of acid catalysis, may be considered as a parallel to the behavior of the same substances toward acids and bases; while being stable toward bases, they are transformed by acids even at room temperature slowly into the corresponding hydantoins.



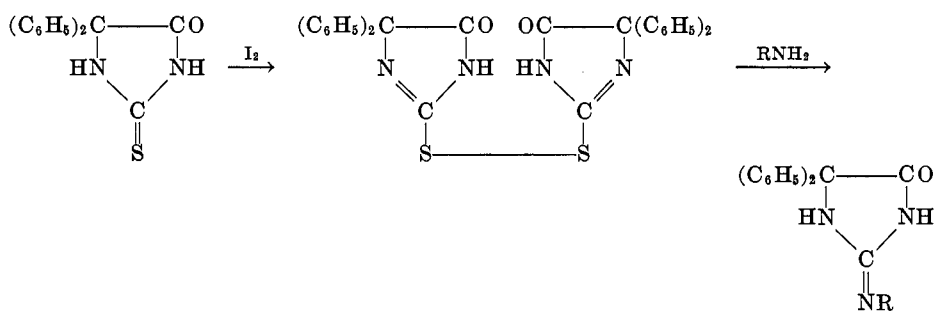
LXIV

As already mentioned, 2-thiohydantoins may be transformed also directly into glycocyamidines (103). This may be achieved by heating their alcoholic solutions in the presence of iodine and advantageously with pyridine (197) and ammonia or amines (62).

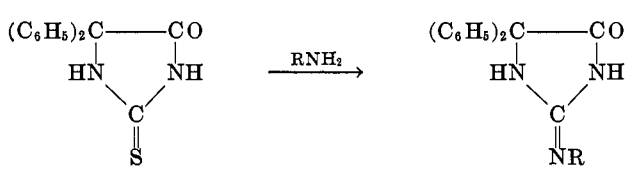


R = H, $C_6H_5CH_2$; Y = CH_2, O.

However, the yields are not good. The oxidation and the aminolysis may be performed also in separate steps; e.g., Huppert has obtained derivatives of hydantoinimide from mixtures of amino acids (protein hydrolysates) by treating them in mildly alkaline solution with carbon disulfide, then oxidizing the sulfurized products to disulfides, and finally treating the latter with ammonia, ammonium salts, or amines (166). Consequently, the mechanism of the direct transformation of 2-thiohydantoins into glycocyamidines may perhaps be formulated according to the following scheme:

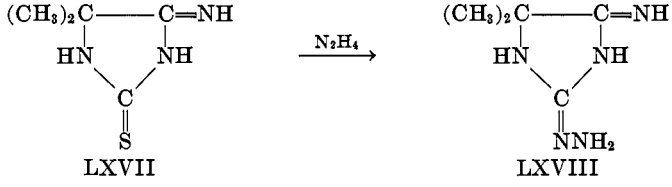


Sometimes the 2-thiohydantoins may react with amines even in the absence of oxidizing agents; e.g. 5,5-diphenyl-2-thiohydantoin yields with aniline (311), 2-aminoethanol (59; *cf.* 58), and phenylhydrazine (311) N^2,5,5-triphenyl-, N^2-(β-hydroxyethyl)-5,5-diphenyl-, and N^2-(phenylamino)-5,5-diphenylglycocyamidine (LXVI), respectively.



LXVIa: R = C_6H_5
LXVIb: R = $HOCH_2CH_2$
LXVIc: R = C_6H_5NH

Similarly, the reaction of 5,5-dimethyl-2-thiohydantoin-4-imide (LXVII) with hydrazine furnishes the 4-imide of N^2-amino-5,5-dimethylglycocyamidine (LXVIII) (311).



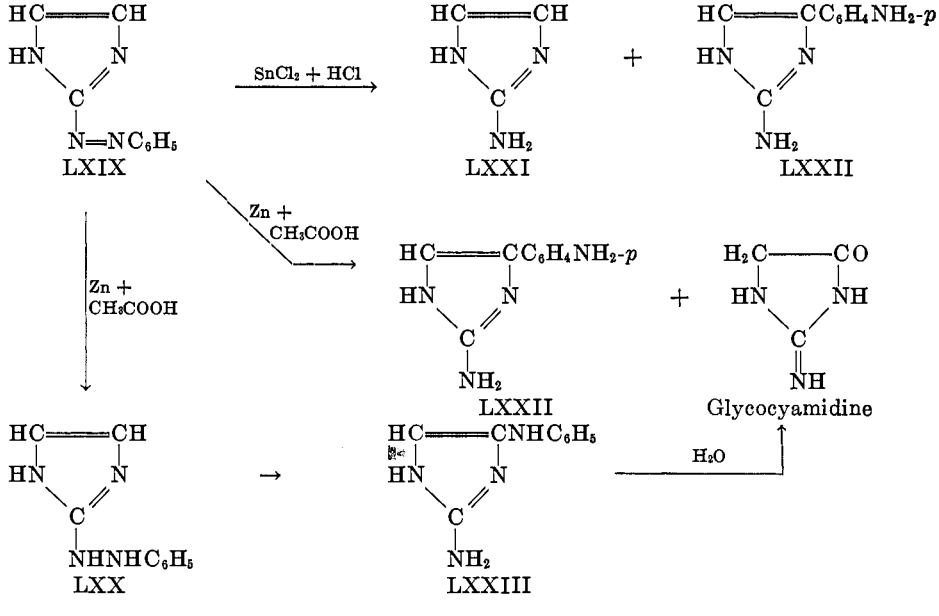
LXVII LXVIII

However, the aminolysis always proceeds much more smoothly after preliminary *S*-methylation (59, 311).

In the 2,4-dithiohydantoins the reactivity of the thiocarbonyl group in position 2 is less than the reactivity of the one in position 4. Therefore 5,5-diphenyl-2,4-dithiohydantoin furnishes with 2-aminoethanol the 4-(β-hydroxyethylimide) of 5,5-diphenyl-2-thiohydantoin; 1,3-dimethyl-5,5-diphenyl-2,4-thiohydantoin reacts similarly (59; *cf.* 151).

G. Rearrangement of 2- and 4(or 5)-arylhydrazoimidazoles

It was observed by Fargher and Pyman that 2-benzolazoimidazole (LXIX) may react with certain reducing agents to yield glycocyamidine among other products. Thus, stannous chloride reduces it partly to 2-aminoimidazole (LXXI), the normal product, and partly to 2-amino-4(or 5)-(*p*-aminophenyl)imidazole (LXXII), which is the product of a benzidine rearrangement. The reduction with zinc dust and acetic acid yields LXXII and glycocyamidine. The formation of the latter may be explained by assuming a semidine rearrangement of the intermediate 2-phenylhydrazoimidazole (LXX) leading to 2-amino-4(or 5)-phenylaminoimidazole (LXXIII) which, under the conditions applied, suffers hydrolysis to glycocyamidine:

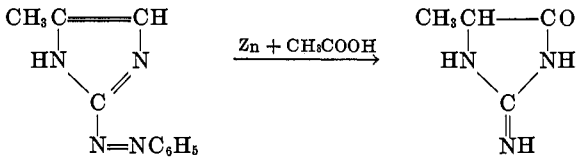


LXIX LXXI LXXII

LXXII

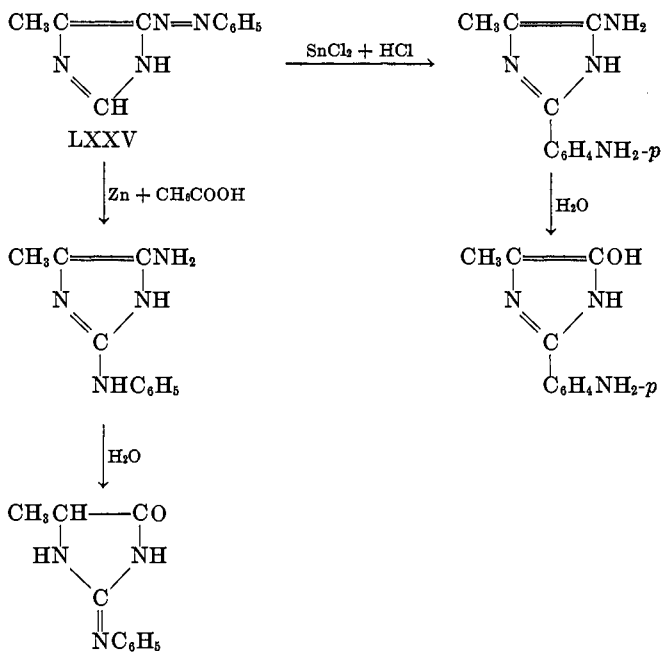
LXX LXXIII

Similarly, 2-benzolazo-4(or 5)-methylimidazole (LXXIV) furnishes on reduction with zinc dust and acetic acid small quantities of alacreatinine (103; *cf.* 47).



LXXIV

The behavior of 4(or 5)-benzolazo-5-(or 4)-methylimidazole (LXXV) on reduction is wholly analogous (102, 103).

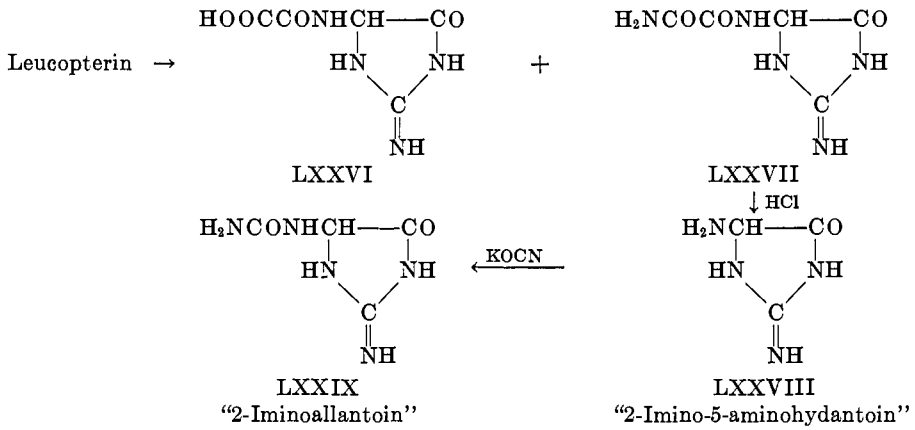


LXXV

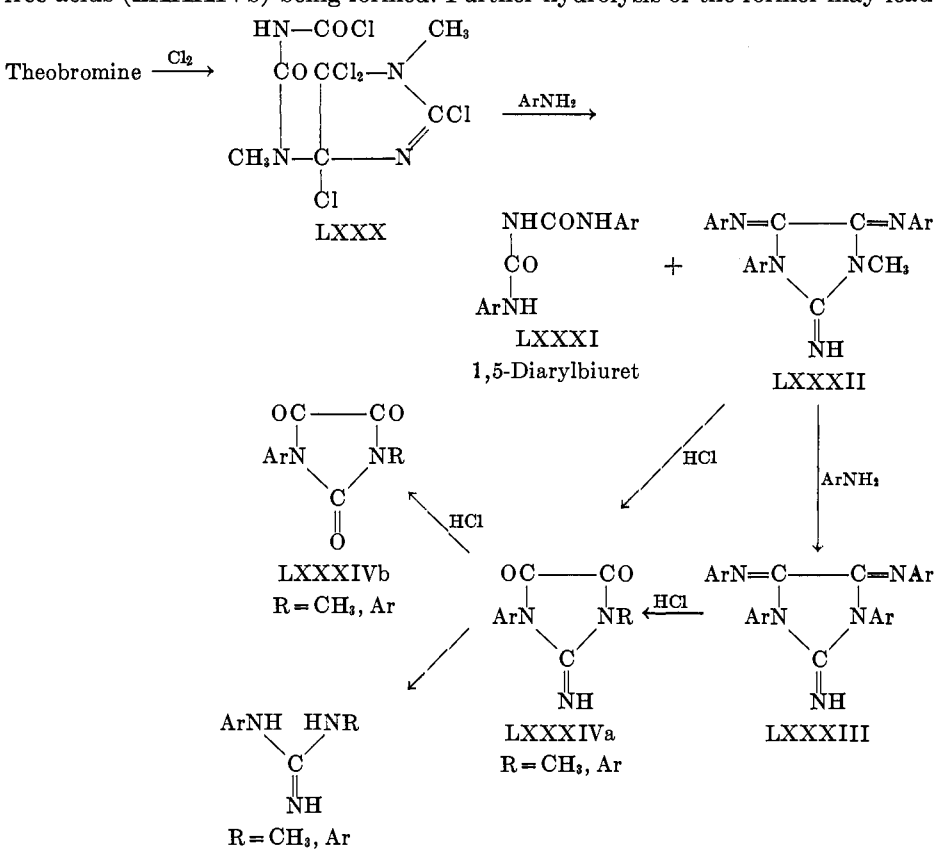
H. Other syntheses

During their well-known investigations on the wing pigments of butterflies Wieland and Purrmann degraded leucopterin *inter alia* to "iminohydantoinoxamic acid" (LXXVI) and "iminohydantoinoxamide" (LXXVII). The latter could have been transformed by successive treatment with hydrochloric acid and potassium cyanate to "2-imino-5-aminohydantoin" (LXXVIII) and "2-iminoallantoin" (LXXIX) (318). All these compounds are derivatives of 5-aminoglycocyamidine.

By exhaustive chlorination of theobromine, γ-methyl-γ-(2,4,5,5-tetrachloro-1-methyl-2-imidazolin-4-yl)allophanyl chloride (LXXX) was obtained by Todd and Whittaker, which, on treatment with arylamines, may be transformed



to 1,5-diarylbiuret (LXXXI) and to derivatives of the triimide of parabanic acid of either type LXXXII or type LXXXIII. By hydrolysis with hydrochloric acid the arylimino and imino groups of both types of compounds may be eliminated successively, the 2-imides of parabanic acids (LXXXIVa) and the free acids (LXXXIVb) being formed. Further hydrolysis of the former may lead

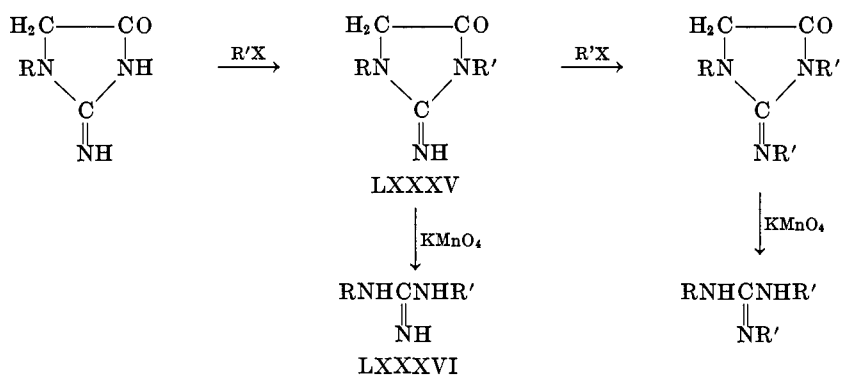


also to guanidines (298). Some of the substances mentioned in this paragraph are related to glycocyamidine; however, since they cannot be considered rigorously as derivatives of glycocyamidine, they will not be treated further here.

III. SUBSTITUTION REACTIONS OF GLYCOCYAMIDINES

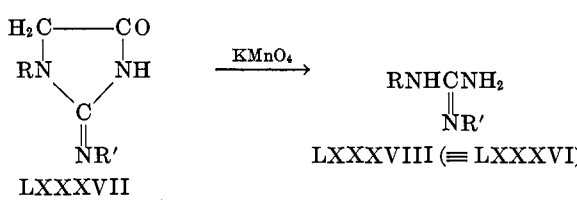
A. Alkylation

On treatment with alkyl halides or sulfates derivatives of glycocyamidine yield *N*-alkyl derivatives. Depending on the conditions applied, one or two alkyl groups may be introduced. That the alkyl groups are attached to nitrogen and not to carbon (C^5) or oxygen (enol ethers) is proved by oxidative degradation; oxidation of glycocyamidines alkylated by alkyl halides or sulfates leads always to oxalic acid and substituted guanidines (*cf.* Section IV,C).



R = H, CH_3.

However, the result of the oxidative degradation is not sufficient for complete proof of the structure of the monoalkylated compounds; for example, LXXXVII furnishes on oxidation the same guanidine (LXXXVIII) as the isomeric LXXXV.

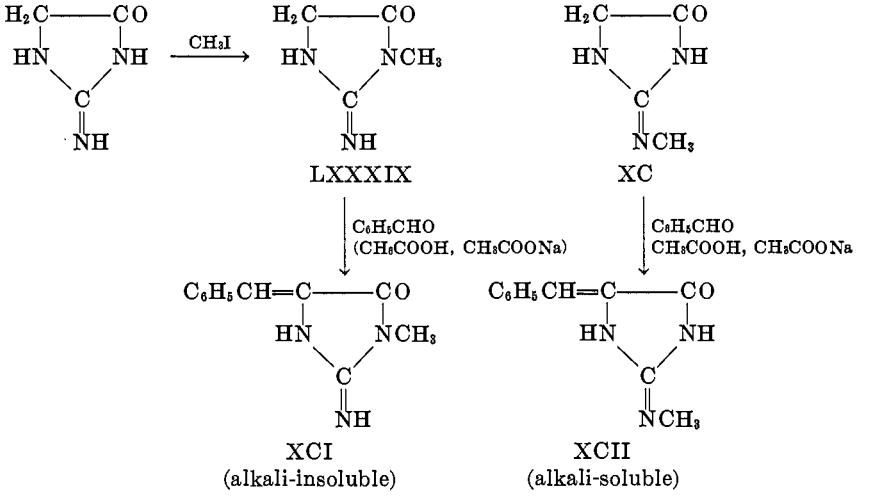


R = H, CH_3.

Before the theory of the tautomerism of substituted guanidines was correctly elaborated, the guanidines representated by formulas LXXXVI and LXXXVIII were considered by some authors as different substances. Therefore from the experimental fact that creatinine and the so-called α-methylglycocyamidine, obtained by the methylation of glycocyamidine with methyl iodide and not identical with creatinine (189), yield on oxidation the same methylguanidine (LXXXVI), it was concluded that in α-methylglycocyamidine the methyl group

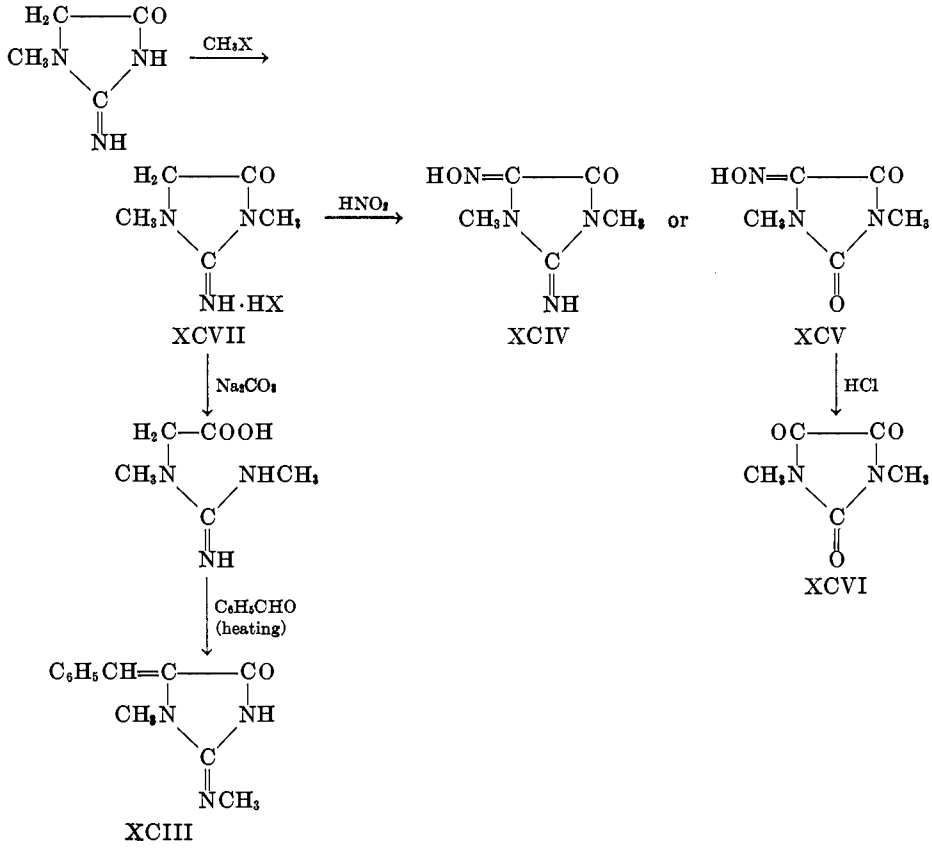
is attached to position 3 and not to N^2 (155, 193, 260, 263). This evidence, of course, cannot be accepted (177, 261, 262).

That α-methylglycocyamidine is really 3-methylglycocyamidine (LXXXIX) was proved by its condensation with benzaldehyde, which leads to an *N*-methyl-5-benzylidene glycocyamidine (XCI) insoluble in aqueous sodium hydroxide, while a third *N*-methylglycocyamidine (XC), likewise not identical with creatinine, yielded on similar treatment an *N*-methyl-5-benzylideneglycocyamidine (XCII), which was soluble in aqueous sodium hydroxide. The solubility in aqueous alkali pointed to the presence of an imide hydrogen in XCII and consequently in XC; insolubility in alkali indicated the absence of it in XCI and consequently LXXXIX (177).

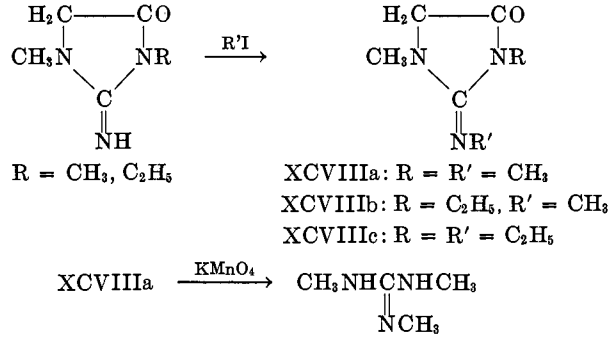


Under similar conditions creatinine may be converted to a monomethyl derivative (190). This was considered by Cornthwaite to be the N^2-methyl derivative, because it could have been transformed by condensation with benzaldehyde to N^2-methyl-5-benzylidinecreatinine (XCIII) (67), the structure of which was already known (228) (see Section III,C). However, as was pointed out later by Zeile and Meyer, the evidence furnished by Cornthwaite may not be accepted, because, under the conditions applied, a transitory opening of the ring of the methylcreatinine could have occurred (322). On the contrary, Zeile and Meyer proved the methylcreatinine obtained by the methylation of creatinine to be 3-methylcreatinine (XCVII), because on treatment with nitrous acid it furnishes, depending on the conditions applied, either "methylcreatinine oxime" (XCIV) or "dimethylhydantoin oxime" (XCV); the latter could be transformed by hydrolysis with hydrochloric acid to *N*,*N*′-dimethylparabanic acid (XCVI) (322). The reactions mentioned are pictured in the scheme on page 696.

By analogy to this the ethylcreatinine (223, 224) and benzylcreatinine (153) obtained from creatinine by ethylation with ethyl iodide, or by benzylation with benzyl chloride, may likewise be considered as derivatives substituted in position 3.

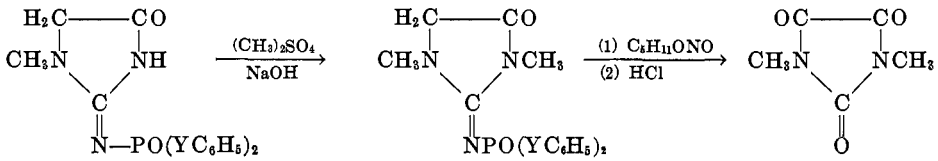


On treatment with further quantities of alkylating agents the monoalkylcreatinines may be transformed into N^2,3-dialkylcreatinines, e.g., the dimethyl- (XCVIIIa) (190), the N^2-methyl-3-ethylcreatinines (XCVIIIb) (155), as well as diethylcreatinine (XCVIIIc) (155). The structure of the latter substances is proved by their oxidation with potassium permanganate, which leads to N,N',N''-trialkylguanidines (193).



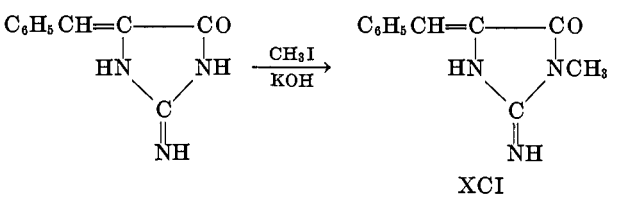
The dianilide (XCIXa) as well as the diphenyl ester (XCIXb) of creatinine-phosphoric acid yield on treatment with methyl sulfate the corresponding 3-

methyl derivatives, the structure of which is proved by their degradation to *N*,*N'*-dimethylparabanic acid (C) (322).

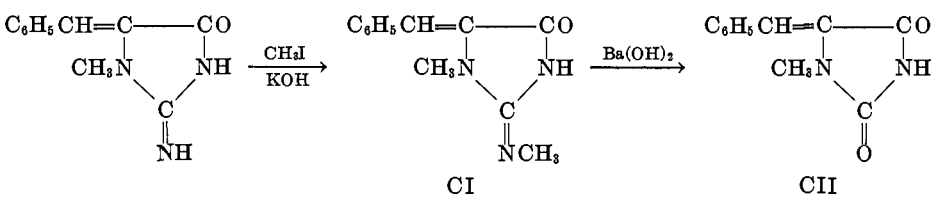


XCIXa: Y = NH
XCIXb: Y = O

Among the glycocyamidines substituted in the 5-position, 5-benzylideneglycocyamidine furnishes on treatment with methyl iodide a 3-methyl derivative (XCI), insoluble in aqueous alkali (177); however, 5-benzylidenecreatinine yields on similar treatment the N^2-methyl derivative (CI) (228). The structure of the latter is proved by the fact that, on hydrolysis by aqueous barium hydroxide, it yields, in addition to methylamine, 1-methyl-5-benzylidenehydantoin



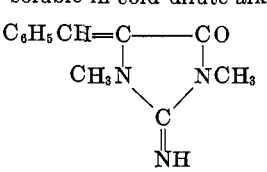
XCI



CI CII

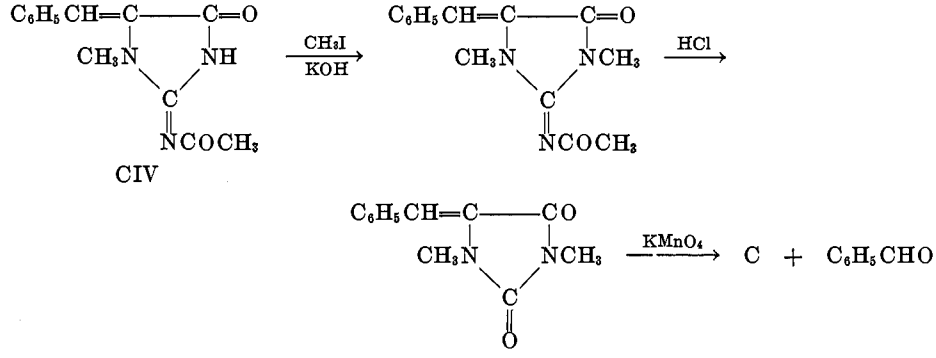
(CII) in nearly theoretical amounts and without formation even of traces of ammonia (228).[6] By analogy with this the product formed by the methylation of 5-furfuralcreatinine (67) may also be considered as an N^2-methyl derivative.

[6] Since the ring of glycocyamidines is in general unstable toward alkalis, the products formed on alkaline hydrolysis are in general not considered as proofs of structure. However (see Section IV,A), as is well known, the ring opening of aldehyde condensation products of glycocyamidines (as well as of hydantoins) by alkaline hydrolysis presents difficulties; moreover, the recyclization of the ring requires energetic treatment with *acids*. Therefore the proof of structure presented may be accepted (228). Against the structure CI one sole objection might be raised: namely, that the methyl-5-benzylidenecreatinine is not readily soluble in cold dilute aqueous alkalis, and this behavior would be more consistent with structure CIII. However, this objection is invalidated by the fact that 5-benzylidenecreatinine itself is only difficultly soluble in cold dilute alkalis (228).



CIII

N^2-Acetyl-5-benzylidenecreatinine (CIV) may be methylated in the form of its potassium salt by methyl iodide to the 3-methyl derivative, which undergoes degradation by successive treatment with hydrochloric acid and potassium permanganate to N,N'-dimethylparabanic acid (168).



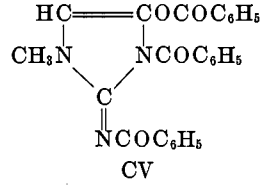
CIV

5,5-Diphenylglycocyamidine is methylated by methyl iodide to the 3-methyl derivative (196a) and benzylated by benzyl chloride to the 3-benzyl derivative (197).

By chemical means the methylation of glycocyamidine or its derivatives in position 1, that is, to creatinine or derivatives, has not yet been effected. However, the animal body is capable of effecting this reaction. In the body glycocyamidine may be transformed by successive hydrolysis and methylation to creatine (21), and the latter cyclizes to creatinine, as already mentioned (see Section II,A). The methylation of creatine is catalyzed by thyroxine; the methyl group comes from methionine (42), which in turn obtains it from choline (308).

B. Acylation

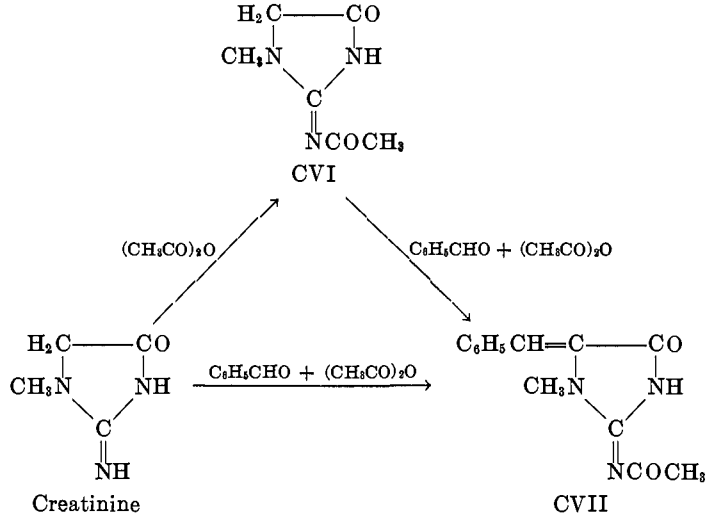
The behavior of creatinine toward acylating agents was first examined by Urano, according to whom creatinine is acylated by benzoic anhydride to benzoylcreatinine (303). That the benzoyl group is attached to one of the nitrogen atoms is proved by the fact that benzoylcreatinine is oxidized by potassium permanganate to *N*-benzoyl-*N'*-methylguanidine (129). The same benzoylcreatinine may be obtained by similar treatment from creatine (303). Later a product obtained from creatinine by treatment with benzoyl chloride was thought erroneously to be an isomer of the benzoylcreatinine already known (129), but finally it proved to be either a mixture of mono- and tribenzoylcreatinines (168) or another crystalline modification of benzoylcreatinine (137). By using benzoyl chloride in great excess, tribenzoylcreatinine (CV) could be obtained in pure form (129); it is considered to be a derivative of the enolic form of creatinine (129).



CV

With phthalic anhydride creatinine yields not an acyl derivative, as formerly thought (303), but simply a salt, dicreatinine phthalate (167). The same product is obtained from creatine (303).

With acetic anhydride at 60–65°C. creatinine furnishes a monoacetyl derivative (CVI) in 70 per cent yield; by elevation of the temperature the yield is considerably lowered and at 140°C. no monoacetylcreatinine is formed. From the mother liquors another product was obtained, which is hydrolyzed by water to "diacetylcreatine" (XXII) (168) (*cf.* Section II,A). Since, as will be shown in Section III,C, in 5-benzylideneacetylcreatinine (CVII), obtained by the treatment of creatinine with benzaldehyde in the presence of acetic anhydride (100), the acetyl group is attached to the acyclic nitrogen (168) and the acetylcreatinine mentioned above may be transformed by similar treatment to the same product (168), the structure CVI follows for acetylcreatinine (168).

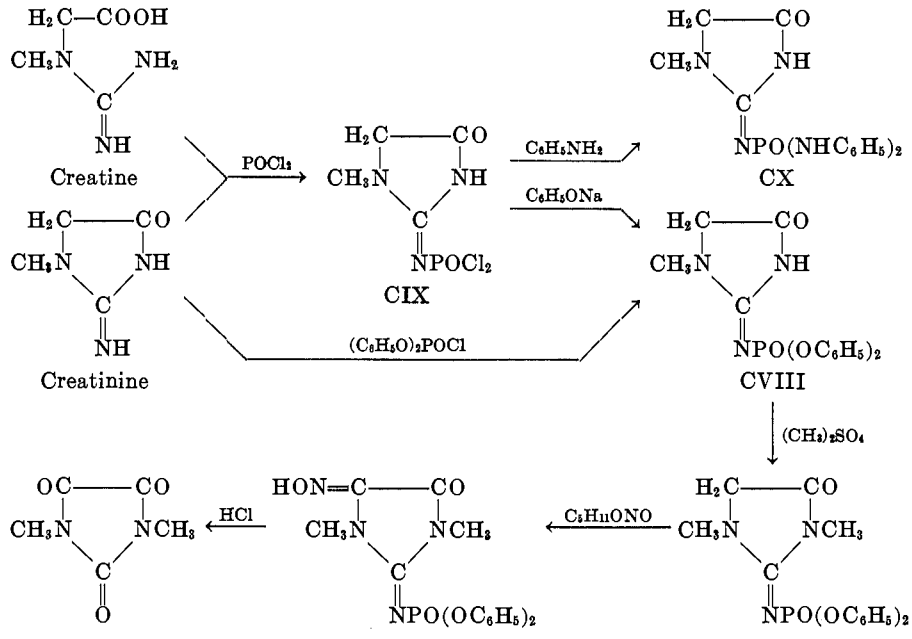


With diphenyl phosphorchloridate in boiling acetone creatinine yields the diphenoxyphosphoryl derivative (CVIII) (168). The same product may be obtained by heating creatinine or creatine with phosphorus oxychloride and then treating the creatininephosphoric acid dichloride (CIX) formed (320, 322), which was thought for some time to be isocreatinephosphoric acid dichloride (323),[7] with sodium phenoxide (322). Similarly, with aniline CIX yields the dianilide (CX) of creatininephosphoric acid (322). In all these compounds the phosphoric acid residue is attached to N^2, which was proved by methylating CVIII with methyl sulfate to the 3-methyl derivative, which latter, by successive treatment with amyl nitrite and hydrolysis with hydrochloric acid, was transformed to *N*,*N'*-dimethylparabanic acid (322).

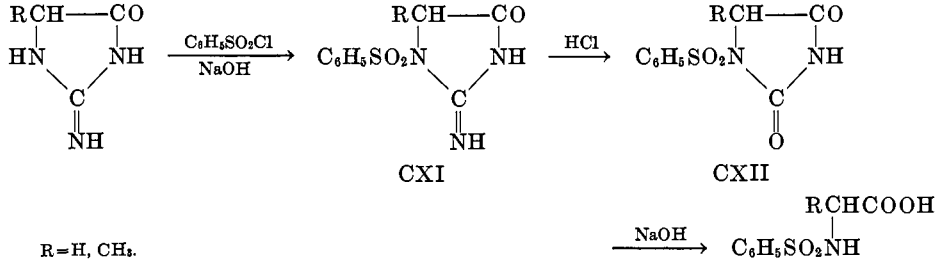
By analogy with these, all known monoacyl derivatives of creatinine—viz., benzoylcreatinine (168, 303), salicyloylcreatinine (256, 288), sulfanilylcreatinine,

[7] In aqueous sodium hydroxide solution with cooling creatine and phosphorus oxychloride yield *creatine*phosphoric acid, identical with the substance from natural sources (321).

and acetylsulfanilylcreatinine (65, 251)—are likewise considered as N^2-acyl derivatives.



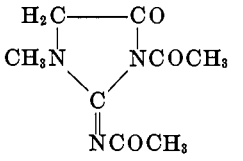
Both from glycocyamidine and from alacreatinine only one acyl derivative, the corresponding benzenesulfonyl derivative (CXI) obtained by treatment with benzenesulfonyl chloride in alkaline solution, is known (28). In these, the benzenesulfonyl group is attached to position 1, because hydrolysis under acidic conditions leads to the corresponding 1-benzenesulfonylhydantoins (CXII) and further hydrolysis under alkaline conditions to N-benzenesulfonylglycine or N-benzenesulfonylalanine (28):

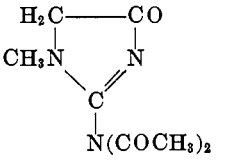


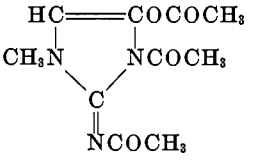
The di- and triacetyl derivatives of creatinine are likewise known (168). Their mixture is formed by treating creatinine with an excess of acetic anhydride at 100°C.; the main product of the reaction under these conditions is, however, an amorphous substance soluble in water and yielding by hydrolysis the already mentioned "diacetylcreatine" (XXII).

Since diacetylcreatinine gives with ferric chloride a color reaction character-

istic of enolic substances, it cannot be an *O*-acetyl derivative; hence its structure is either CXIII or CXIV. For some reasons, which today can not be accepted, the former was thought by Ing to be more probable (168) (*cf.* Section V,C). On the other hand, triacetylcreatinine is considered by analogy with the tribenzoyl derivative to be an enol acetate (CXV) (168).


CXIII


CXIV


CXV

C. Condensation with aldehydes

Like the hydantoins the glycocyamidines may be condensed with *aromatic* aldehydes to yield 5-arylidene derivatives. However, in contrast to hydantoins substituted in position 1, which may be condensed with aldehydes only with difficulty or not at all, creatinine, as well as other glycocyamidines substituted in position 1, is capable of being smoothly condensed. The condensation of glycocyamidines with *aliphatic* aldehydes has not yet been tried, perhaps because the condensation of these with hydantoins presented some difficulties. However, recently the condensation of 2-thiohydantoin with isobutyraldehyde has been performed in good yields, according to a new method using aqueous ammonia as medium (23), and this method can perhaps be successfully adapted to glycocyamidines.

The condensation of glycocyamidines with aromatic aldehydes may be effected: (*a*) by heating of the components at 140–180°C. (this may be performed also in solution in acetamide (69)[8]); (*b*) by heating the components in acetic acid solution in the presence of condensation agents of either basic (sodium acetate) or acidic (sulfuric acid) (71) character; (*c*) by heating the components in acetic anhydride and eventually in the presence of sodium acetate; (*d*) by heating the components in pyridine and eventually in the presence of small amounts of piperidine. (For reviews on the condensation of creatinine with aromatic aldehydes, see references 61 and 78.)

If the condensation is effected by method (*c*), it is accompanied by acetylation both in position N^2 of the glycocyamidine and, if present, on the phenolic hydroxyl of the aldehyde component. N^2-Butyryl derivatives may be prepared similarly (247). The acetyl derivatives may also be prepared by subsequent acetylation of the aldehyde condensation products obtained by another method (60, 79, 139).

The condensation product of creatinine first investigated was N^2-acetyl-5-benzylidenecreatinine (CXVIIa), prepared by heating *creatine* with benzaldehyde and acetic anhydride and originally considered to be the 3-acetyl derivative (CXVI) (100). Since, however, the compound is of acidic character—viz., it is soluble in alkalis and furnishes a potassium salt which may be methylated by

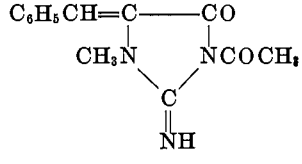
[8] In this condensation benzaldehyde may be replaced by benzylideneaniline (243).

TABLE 2

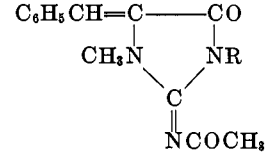
Aldehydes condensed with creatinine

Aldehyde	Method	References	Aldehycde	Method	References
Benzaldehyde	a	(243)	2,4-Dimethoxybenzaldehyde	a	(60)
	c	(81, 228)		d	(60)
o-Chlorobenzaldehyde	a	(69)	Vanillin	a	(60, 80, 139, 243)
m-Chlorobenzaldehyde	c	(60)		c	(60, 81, 139)
p-Chlorobenzaldehyde	a	(79)		d	(60, 139)
p-Bromobenzaldehyde	a	(79)	Veratraldehyde	a	(60)
p-Iodobenzaldehyde	a	(79)		b	(71)
m-Nitrobenzaldehyde	a	(79, 243)		c	(81)
p-Nitrobenzaldehyde	c	(79)		d	(60)
Salicylaldehyde	a	(68)	Piperonal	a	(69)
	c	(81)		c	(81)
m-Hydroxybenzaldehyde	c	(81)	3,4,5-Trimethoxybenzaldehyde	a	(60)
p-Hydroxybenzaldehyde	a	(60, 69)		d	(60)
	c	(60, 81)	*p*-Tolualdehyde	a	(69)
	d	(60)	Cinnamaldehyde	a	(68)
o-Methoxybenzaldehyde	a	(69)	*p*-Hydroxycinnamaldehyde	a	(69)
	c	(81)	Furfural	a	(60, 68)
m-Methoxybenzaldehyde	c	(81)	2-Thiophenaldehyde	c	(247)
Anisaldehyde	a	(69)	3-Thiophenaldehyde	c	(247)
	c	(81)	Imidazole-4(or 5)-aldehyde	c	(82)
o-Ethoxybenzaldehyde	a	(69)			

methyl iodide to the 3-methyl derivative (CXVIIb)— the acetyl group must be attached to N^2 (168).



CXVI



CXVIIa: R=H

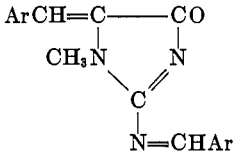
CXVIIb: R=CH_3

Other 5-arylidenecreatinines obtained from a derivative of *creatine* are N^2-methyl-5-benzylidene- and N^2-methyl-5-furfuralcreatinine (67; *cf.* 322), which were prepared from ω-(N-methyl)creatine by method (*a*). Some aromatic and heterocyclic aldehydes condensed with creatinine are shown in table 2.

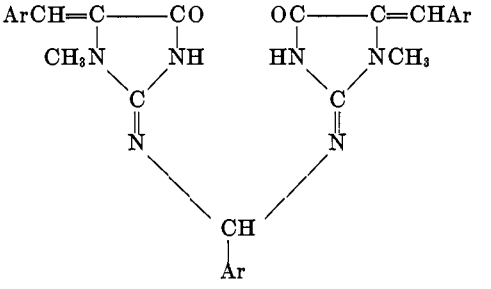
Besides creatinine, some other derivatives of glycocyamidine have been condensed with aldehydes: glycocyamidine and N^2-methylglycocyamidine with benzaldehyde according to method (*b*) (177); 3-methylcreatinine with furfural according to method (*a*) (68)[9]; some 1,N^2,3-triarylglycocyamidines with *m*-nitrobenzaldehyde (72); and finally the dianilide of creatininephosphoric acid (CX) with *p*-hydroxybenzaldehyde and anisaldehyde according to method (*d*) (322).

[9] In this case, 3-methylcreatinine was condensed with furfural under conditions under which a transitory opening of the cyamidine ring could have occurred; the situation is analogous to that in the condensation with benzaldehyde (67, 322; see above) and therefore the N^2-methyl derivative might have been formed.

Method (*a*) yields, in addition to the condensation products of composition 1:1, products of other composition (69); e.g., in the condensation of creatinine with cinnamaldehyde, furfural, furfurylideneacetaldehyde (68), and *p*-hydroxybenzaldehyde (81) and of 3-methylcreatinine with furfural[9] (68; see, however, 60). These products were considered originally as dialdehyde–monocreatinine condensation products and their structures (CXVIII) believed to be derivable from the amino tautomer (see Section V,C) of creatinine (81); later it was shown that they were rather trialdehyde–dicreatinine condensation products and their structures were supposed to be as shown in formula CXIX (69).



CXVIII



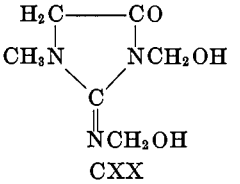
CXIX

However, the point of attachment of the arylidene bridge is not yet proved rigorously; considering that N^2 is more basic than N^3, the attachment designated in CXIX seems more probable than the alternative one, viz., to positions 3 and 3′.

The nature of the condensation products depends on the reactivity of the aldehyde used as well as on the temperature; at 140–150°C. the aldehydes examined furnished with only two exceptions (*m*-chloro- and *p*-nitrobenzaldehydes) 1:1 products, but gave 2:3 products at 180°C. *m*-Chlorobenzaldehyde furnishes the 2:3 product at 140–150°C. (60). Therefore 5-(*m*-chlorobenzylidene)creatinine may be prepared only by an indirect route, viz., by desacetylation of the N^2-acetyl derivative. *p*-Nitrobenzaldehyde reacts with creatinine at 140°C. to give a mixture of the 1:1 and 2:3 products (79).

Trialdehyde–dicreatinine condensation products may be obtained also by heating the arylidenecreatinine (1:1 product) with a further quantity of the aldehyde to 175°C. (69).

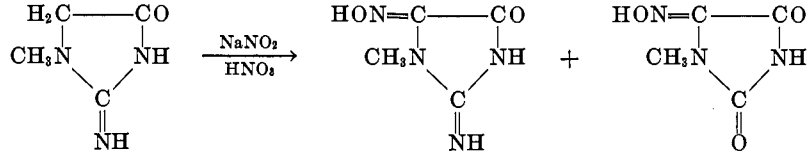
The reaction with formaldehyde is different from that with aromatic aldehydes; by prolonged heating formaldehyde furnishes with creatinine a bis(hydroxymethyl) derivative of the structure CXX (172).



CXX

D. Other substitution reactions

By treating creatinine, dissolved in cold dilute nitric acid, with sodium nitrite the 5-oxime of 1-methylparabanic acid (CXXII) is formed as the main product and some 5-isonitrosocreatinine (CXXI) is also formed (268). The former is apparently a product of hydrolysis or deamination of the latter. 3-Methylcreatinine reacts similarly, as does the diphenyl ester (CVIII) of 3-methylcreatinine-

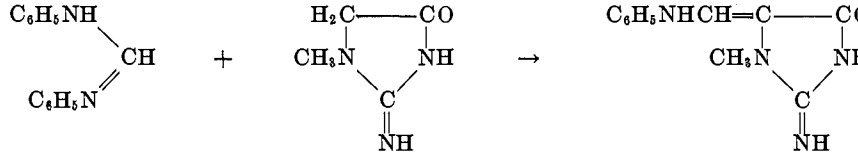


Creatinine CXXI CXXII

phosphoric acid (322).

Isonitrosocreatinine is likewise formed by treating creatinine in alkaline solution with sodium nitroprusside (Weyl color reaction for creatinine (316)) and subsequently acidifying with acetic acid (192, 258, 268, 316). Originally this substance was thought to be nitrosocreatinine (192).

Creatinine also condenses with diphenylformamidine, but the nature of the product is yet unknown (243). Perhaps, by a reaction analogous to that with 1,3-diphenyl-2-thiohydantoin, aniline is split off and 5-(anilinomethylene)-creatinine (CXXIII) is formed.



CXXIII

Creatinine does not couple with diazobenzenesulfonic acid (241).

IV. OTHER REACTIONS OF GLYCOCYAMIDINES

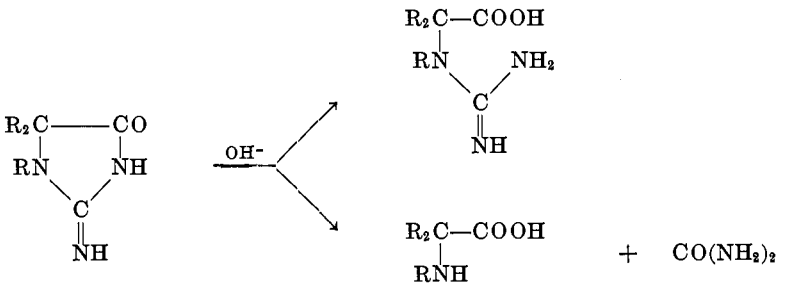
A. Hydrolysis by acids and bases

As already mentioned, glycocyamidines and α-guanidino acids are related genetically. The transformation of the latter into the former has been discussed; the reverse transformation will be discussed in the first part of this section.

The transformation of glycocyamidines into α-guanidino acids is a somewhat delicate task. The bond between C^4 and N^3 is, no doubt, disrupted by several basic reagents; however, the bond between N^1 and C^2 is scarcely more stable toward the same reagents. Therefore glycocyamidines are easily transformed by bases to urea and α-amino acids instead of α-guanidino acids (see page 705).

As already mentioned, the transformation creatine ⇄ creatinine is reversible; therefore creatinine may be hydrolyzed in aqueous solution partly to creatine (76). This reaction takes place also in urine,—rather rapidly at room temperature and yet at 4°C. with measurable velocity (164). The same reaction takes place

also in the organism and its velocity is the same as in aqueous solution; consequently this is a nonenzymatic reaction. Glycocyamidine is similarly hydrolyzed in the organism (21).



The hydroiodide of 3-methylglycocyamidine is transformed into ω-(*N*-methyl)-glycocyamine by treatment of its aqueous solution with silver oxide and subsequent evaporation to dryness (189). Creatinine is hydrolyzed to creatine by treating it with calcium hydroxide (203) or dilute ammonia (76), by heating with lead oxide (152), or by evaporating to dryness with ammonia (152). According to other statements however, mild heating with concentrated ammonia does not cause hydrolysis (25).

The glycocyamidines corresponding to the α-amino acids occurring in nature, e.g., 5-(*p*-hydroxyphenyl)glycocyamidine, are hydrolyzed by 0.5 per cent aqueous ammonia at 105°C. to α-guanidino acids (300). By brief heating with 5 per cent aqueous potassium hydroxide N^2-methylglycocyamidine is partly hydrolyzed to ω-(*N*-methyl) glycocyamine (177). Similarly, the ring of 5-(γ-benzamidopropyl)-glycocyamidine (XXXIII) is opened by basic reagents to form isoarginine (XXXIV) (4). By heating with 0.1 *N* sodium hydroxide or disodium hydrogen phosphate, creatininephosphoric acid is hydrolyzed to creatinephosphoric acid, while under these conditions creatinine itself is at best hydrolyzed only until reaching the equilibrium value (323).

However, the ring of 5-furfurylidenecreatinine may not be opened by 10 per cent sodium hydroxide (60); a contrary statement (68) is apparently erroneous. Similarly the N^2- and 3-methyl-5-benzylideneglycocyamidines are stable toward dilute alkalis (177). Thus a substituent in position 5, attached to the ring by a double bond, confers great stability upon the cyamidine (as well as upon the hydantoin) ring toward the action of basic reagents (228).

The reagent universally used in the hydantoin series for ring opening, viz., barium hydroxide, is not suitable for this purpose in the glycocyamidine series, because, instead of ring opening, it causes more profound changes. Thus, in accordance with the behavior of guanidine, which is split by this reagent to urea and ammonia, creatinine yields 1-methylhydantoin at 100°C. (225, 238); under other conditions the decomposition is still more extensive and leads to methylhydantoic acid or to sarcosine and urea (121, 225). The behavior of several glycocyamidines examined toward barium hydroxide is compiled in table 3.

TABLE 3

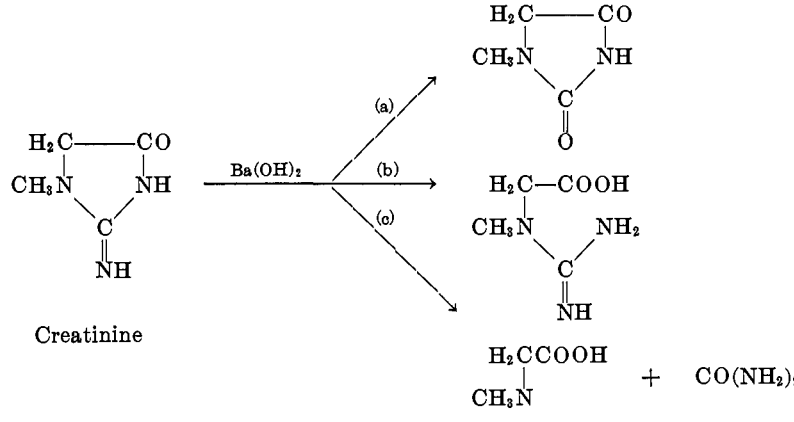
Behavior of glycocyamidines toward hydrolysis with barium hydroxide

Compound	Direction of the Reaction*	Products	References
N^2-Acetyl-5-(1'-acetyl-4'(or 5')-imidazolylmethylene)creatinine	c	*dl*-Acetyl-*N*-methylhistidine	(82)
N^2-Acetyl-5-benzylidenecreatinine	a	1-Methyl-5-benzylidenehydantoin	(228)
5-Benzylcreatinine	a	1-Methyl-5-benzylhydantoin†	(228)
	c	*N*-Methyl-*dl*-α-phenylalanine	(81, 228)
5-Benzylidenecreatinine	a	1-Methyl-5-benzylidenehydantoin‡	(60)
Creatinine	a	1-Methylhydantoin	(225, 238)
	b, c	α-(*N*-Methyl)hydantoic acid or sarcosine and urea	(121, 225)
3-Ethylcreatinine	c	Sarcosine, diethylamine, ammonia, and carbon dioxide	(155)
Glycocyamidine	b, c	Hydantoic acid or glycine	(189)
5-(*p*-Hydroxybenzyl)creatinine	c	*N*-Methyl-*p*-hydroxyphenylalanine	(81)
N^2-Methylbenzylidenecreatinine	a	1-Methyl-5-benzylidenehydantoin	(228)
5-(3'-Methoxy-4'-hydroxybenzyl)creatinine	c	*N*-Methyl-(3-methoxy-4-hydroxyphenyl)-alanine	(80, 139)
1-Phenylglycocyamidine	a	1-Phenylhydantoin	(99)

* The reaction leading to hydantoins is designated by a, that to hydantoic acids by b, and that to amino acids and urea by c.

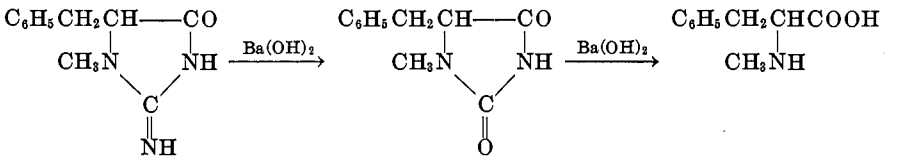
† This is formed probably directly and not through the corresponding hydantoic acid, because no stronger acid than carbon dioxide was used throughout the reaction (228).

‡ Some benzaldehyde may also be isolated from the reaction mixture (60).

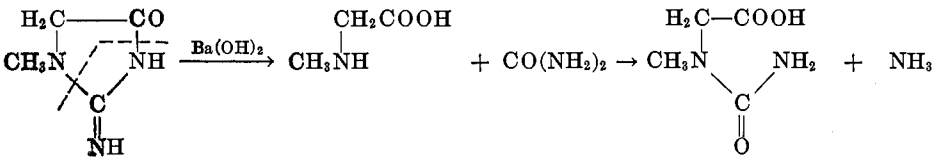


Creatinine is transformed by a 0.7 per cent aqueous solution of sodium carbonate to 1-methylhydantoin (99). Highly unstable towards alkalis is N^2-acetyl-3-methyl-5-benzylidenecreatinine, which is hydrolyzed by alkalis to 1,3-dimethyl-5-benzylidenehydantoin, this decomposition taking place partially even during the methylation of N^2-acetyl-5-benzylidenecreatinine (168).

Formerly it was thought that hydantoins are the products of partial hydrolysis of the glycocyamidines, being transformed by further hydrolysis to α-amino acids (228).

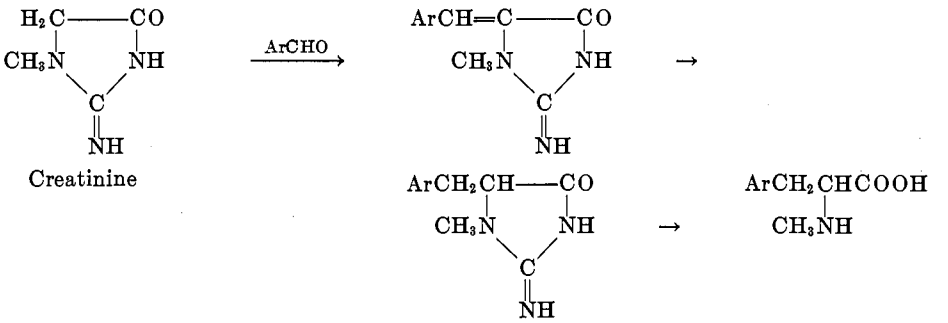


However, later it was shown that, to a certain extent, just the amino acid and urea are the primary products of hydrolysis, these products being able to condense under the conditions applied to hydantoic acids and, after acidifying, to hydantoins (121; *cf.* 54).



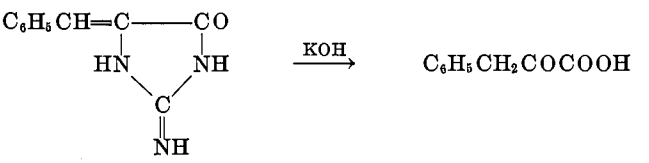
In accordance with this, the hydrolysis of creatinine may be controlled so as to lead to sarcosine, to methylhydantoic acid, or to 1-methylhydantoin (121).

The hydrolysis of derivatives of creatinine by barium hydroxide furnishes a method for the synthesis of *N*-methylamino acids which is suitable for preparative purposes. The method consists in principle in the condensation of an aromatic aldehyde with creatinine, followed by the reduction and subsequent hydrolysis of the product (78, 81, 139), and is as useful as the direct method,

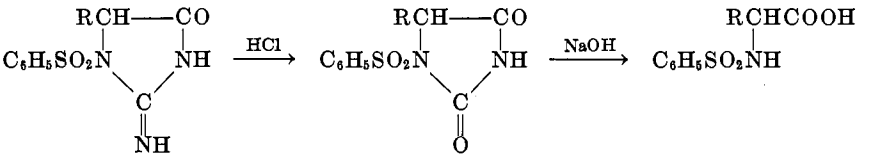


based on the condensation of aromatic aldehydes with *N*-benzoylsarcosine (77).

By treatment with alkalis glycocyamidines may be hydrolyzed even beyond the amino acid stage; e.g., 5-benzylideneglycocyamidine yields phenylpyruvic acid (250) on heating with potassium hydroxide; some benzaldehyde is also formed (250), but this is probably simply a product of oxidation.



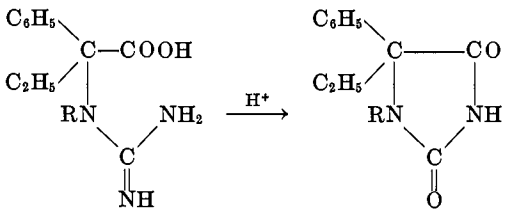
In some cases the cyamidine ring may be transformed into the hydantoin ring by the action of acids. 1-Benzenesulfonylglycocyamidine (CXXIVa) and the corresponding derivative of alacreatinine (CXXIVb) may be transformed by brief heating with hydrochloric acid to 1-benzenesulfonylhydantoin (CXXVa) or its 5-methyl derivative (CXXVb); further hydrolysis by alkali leads then to the corresponding *N*-benzenesulfonyl-α-amino acids (28).



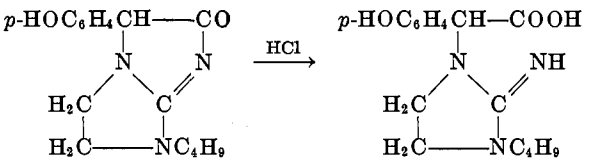
CXXIVa: R = H
CXXIVb: R = CH_3

CXXVa: R = H
CXXVb: R = CH_3

N^2-Acetyl-3-methyl-5-benzylidenecreatinine (CXXVIb) (168) (*cf.* below) and the N,N',N''-triarylglycocyamidines behave similarly (72). In this connection one may mention the anomalous behavior of some α-guanidino acids which, on heating with acids, yield the corresponding hydantoins instead of glycocyamidines (255).

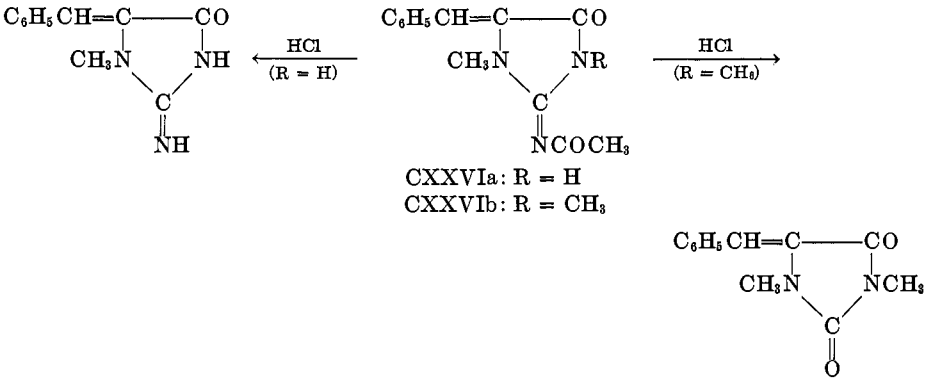


An anomalous reaction takes place with $1,N^2$-ethylene-N^2-butyl-5-(*p*-hydroxyphenyl)glycocyamidine, which is hydrolyzed by hydrochloric acid to the corresponding guanidino acid (98a).



Otherwise the cyamidine cycle is stable towards acids, and therefore treatment with acids is suitable for removing *N*-acyl groups. The following glycocyamidines were deacetylated or debenzoylated by heating with aqueous or alcoholic hydrochloric acid of various concentrations: N^2-benzoyl-3-methyl- and N^2-methyl-3-benzoylglycocyamidines (177), N^2-acetyl-5-benzylidenecreatinine (CXXVIa) (168, 228), N^2-acetyl-5-benzylcreatinine (81), N^2-acetyl-5-(3′-methoxy-4′-hydroxybenzyl)creatinine (139), and 5-(3-benzamidopropyl)-glycocyamidine (XXXIII) (4).

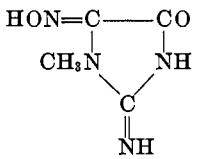
The behavior of N^2-acetyl-5-benzylidenecreatinine (CXXVIa) is influenced remarkably by introduction of a methyl group into position 3; while CXXVIa is simply deacetylated by hydrochloric acid, the 3-methyl derivative (CXXVIb) is transformed into the corresponding hydantoin derivative (168).



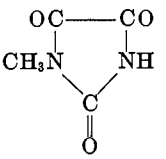
CXXVIa: R = H
CXXVIb: R = CH_3

Particularly stable towards the action of acids and bases are the trialdehyde–dicreatinine condensation products (CXIX) prepared from creatinine and aromatic aldehydes, which may be hydrolyzed only by heating with aqueous 40 per cent potassium hydroxide, the products of hydrolysis being small quantities of the 1:1 condensation product (69) or of 1-methyl-5-arylidenehydantoins (60), the corresponding aldehyde, and ammonia.

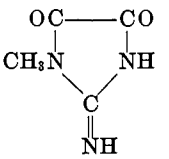
Isonitrosocreatinine (CXXVII) furnishes with hydrochloric acid, depending on the conditions, either simply its hydrochloride or methylparabanic acid (CXXVIII), hydroxylamine, methylamine, and ammonium oxalate (268). By performing the reaction in the presence of tin, the 2-imide of methylparabanic acid (CXXIX), the primary product, is hydrolyzed to methylguanidine (268).



CXXVII



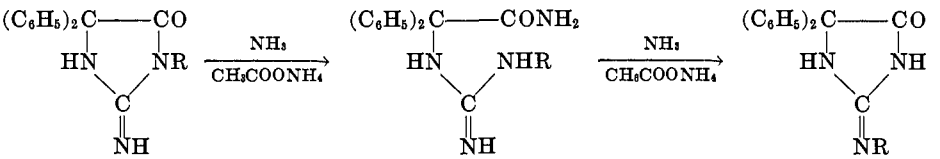
CXXVIII



CXXIX

B. Rearrangement

By prolonged heating with alcoholic ammonia in the presence of ammonium acetate the 3-methyl- and 3-benzyl-5,5-diphenylglycocyamidines are transformed partially to the N^2-methyl- and the N^2-benzyl derivatives (46), respectively. It seems probable that this is not a true rearrangement and that the reac-



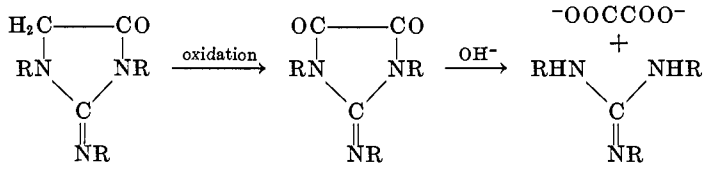
R = CH_3, $C_6H_5CH_2$.

tion may be explained by supposing temporary opening and subsequent closure of the cyamidine ring in the other direction. This seems probable, because alcoholic ammonia has proved to be a mild reagent suitable for both the separate opening and closure of the *hydantoin* ring. This reaction mitigates against the application

of alkaline reagents in structure determinations of derivatives of glycocyamidine. Since the reverse transformation has not yet been observed, apparently in the presence of ammonia the glycocyamidines substituted in position N^2 are more stable than their isomers substituted in position 3.

C. Oxidation and reduction

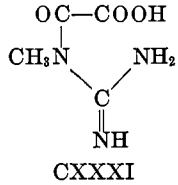
Glycocyamidines not substituted in position 5 are sensitive toward oxidizing agents. The oxidizing agent attacks the methylene group, leading to derivatives of the 2-imide of parabanic acid (CXXX). Since the oxidation is generally performed in an alkaline medium, the parabanic acids are hydrolyzed to oxalate and derivatives of guanidine.



CXXX

The most generally used oxidizing agent is alkaline permanganate, by which creatinine is oxidized at 50–60°C. to oxalate and methylguanidine (188, 223, 238); 3-methylglycocyamidine is oxidized likewise to methylguanidine (259), 3-methyl- (193), 3-ethyl- (155), and 3-benzylcreatinines (153) to the corresponding *N*,*N'*-disubstituted guanidines, and finally N^2,3-dimethylcreatinine is oxidized to *N*,*N'*,*N''*-trimethylguanidine (193). Exactly similar is the behavior of acylated glycocyamidines: N^2-benzoylcreatinine is oxidized to *N*-methyl-*N'*-benzoylguanidine and N^2-acetyl-5-benzylidenecreatinine to *N*-methyl-*N'*-acetylguanidine (129). An equally widely used oxidizing agent is mercuric oxide which, in aqueous or rather in ammoniacal solution, oxidizes glycocyamidine to oxalic acid and oxalate, respectively, and guanidine (266, 267), and creatinine to the former and *N*-methylguanidine (74, 75, 266, 267).

Other oxidizing agents used for the oxidation of creatinine to the same products are silver nitrate in the presence of barium hydroxide (101) or mercuric acetate (17), with which in aqueous solution at room temperature the primary product, *N*-methyl-*N*-guanyloxamic acid (CXXXI), may be isolated. Its hydrolysis leads



CXXXI

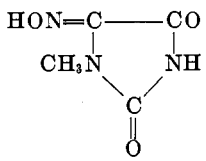
to the end products already mentioned. Creatinine is also capable of reducing Fehling's solution, from which the white cuprous salt of creatinine is precipitated (210); it also reduces alkaline solutions of potassium mercuric iodide and mercuric cyanide to metallic mercury (12).

Glycocyamidines substituted in position 5 may, of course, not be oxidized to derivatives of the 2-imide of parabanic acid. An exception is furnished by the 5-arylidene derivatives, in which the semicyclic double bond offers a point of attack for the oxidant. Thus, as already mentioned, N^2-acetyl-5-benzylidenecreatinine is oxidized by alkaline permanganate.

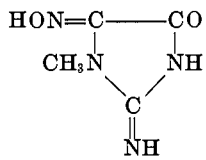
Towards reducing agents the cyamidine ring is stable. Therefore reducible groups in side chains may be reduced without affecting the ring. Thus 5-benzylidenecreatinine, as well as its N^2-acetyl derivative, may be reduced by zinc and acetic acid (129) or by red phosphorus and hydriodic acid or by tin and hydrochloric acid (228) to 5-benzylcreatinine, the N-acetyl group being split off at the same time as a result of the rather strong acidic reagents applied. The same substances may be reduced by sodium amalgam, this method being milder and proceeding without the splitting off of N-acetyl groups (60, 80–82, 139).

D. Deamination

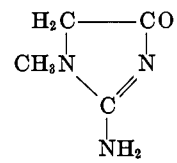
On treating creatinine in acetic acid solution with nitrous acid, one atom of nitrogen is split off; this reaction is hindered by mineral acids (137, 236, 319). Therefore, by performing the reaction in 23 per cent nitric acid both "1-methylhydantoin oxime" (CXXXII) and some "creatinine oxime" (CXXXIII) are obtained from creatinine (268). In the latter product all the nitrogens of creatinine



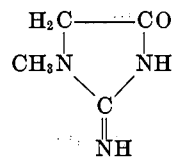
CXXXII



CXXXIII



CXXXIV

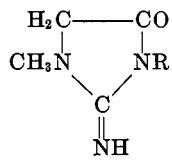


CXXXV

are still present. It is interesting that in the case of *creatine* the situation is reversed. This reacts with nitrous acid sluggishly; the reaction is, however, catalyzed by hydrochloric acid (236).

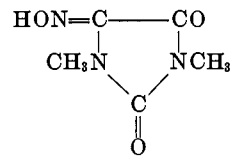
The peculiar behavior of creatinine was formerly explained by suggesting that in neutral solutions it is present as the amino tautomer (CXXXIV) but in the presence of hydrochloric acid as the imino tautomer (CXXXV), the nitrous acid being capable of deaminating only the former (236). This explanation is however wrong, because 3-methyl- and 3-benzylcreatinines (CXXXVI), in which the formation of amino tautomers is impossible, nevertheless do react quite rapidly with nitrous acid, the speed of the reaction in this case not being influenced by hydrochloric acid (137). From 3-methylcreatinine in the presence of an excess of nitrous acid "dimethylhydantoin oxime" (CXXXVII) (137) is formed. However, in the presence of an excess of hydrochloric acid "3-methylcreatinine oxime" (CXXXVIII) in the form of its hydrochloride is obtained (322) in addition to CXXXVII.

N^2-Acetyl- and N^2-benzoylcreatinines, containing neither a free amino nor a free imino group, evolve nitrogen with nitrous acid only very slowly; this can be explained by assuming slow hydrolysis of the acyl group (137). With nitrous acid N^2-benzoylcreatinine furnishes an oxime (CXXXIX) (137).

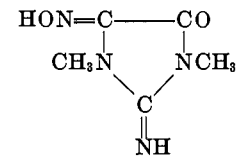


CXXXVI

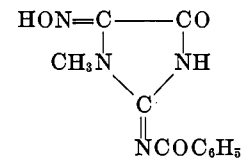
R = CH_3, $C_6H_5CH_2$.



CXXXVII



CXXXVIII



CXXXIX

The behavior of glycocyamidine is analogous to that of creatinine (137). The behavior of N^2,3-dimethylcreatinine is interesting; although all its nitrogen atoms are blocked by methylation, it nevertheless evolves nitrogen with nitrous acid quite rapidly. However, the products have not yet been identified. In some cases, two products with a composition corresponding to that of "dimethylhydantoin oxime" (CXXXVII), however not identical with this, were obtained. They could not be hydrolyzed to N,N'-dimethylparabanic acid (137).

By treatment with hypobromite one atom of nitrogen is split off from creatinine just as with nitrous acid (66).

E. Other reactions

By treatment with methylglyoxal creatinine (as well as creatine) is quickly decomposed in neutral solution with formation of ammonia, carbon dioxide, and oxo compounds (227).

V. PHYSICOCHEMICAL PROPERTIES OF GLYCOCYAMIDINES

The physicochemical properties of creatinine, the most important member of the derivatives of glycocyamidine, have been studied most thoroughly.

A. Crystal structure; ultraviolet and infrared spectra

Creatinine, as well as the monohydrate of creatine (213), crystallizes in the monoclinic system: space group $P2_1/c$. The elementary cell consists of four molecules: $a = 8.06$, $b = 5.97$, and $c = 13.34$ A., $\beta = 121°$. Along the c axis the molecules are held together by strong hydrogen bonds (85), as well as in the case of the monohydrate of creatine.

The ultraviolet absorption spectrum of creatinine is influenced by the pH (123, 138, 312). The position of the maximum in different solutions is shown in table 4. As may be seen, the position of the maximum is shifted by hydrogen ions. On the other hand, under the influence of hydroxyl ions the absorption band disappears completely (138). (For additional data on the absorption spectrum of creatinine in the range of 210–290 mμ at different pH values, see reference 312.)

Since in the vicinity of the absorption maximum of creatinine the absorption of *creatine* is continuous (138, 170) and weak (123), this region is suitable for the spectrophotometric determination of creatinine in the presence of creatine (123, 170). The absorption spectrum of creatine is likewise influenced by the pH; hydrogen ions shift the limit of absorption to shorter wavelengths and hydroxide ions shift it to longer wavelengths (138).

TABLE 4

Absorption maximum of creatinine in different solvents

Solvent	Wavelength	Extinction	Reference
Water	2345 A.	$\log \epsilon = 3.56$	(138)
Phosphate buffer (pH = 7.38)	234 mμ	$\epsilon = 0.742 \times 10^{-4}$	(123)
	216 mμ*	$\epsilon = 0.43 \times 10^{-4}$	(123)
Aqueous methyl, ethyl, and butyl alcohols	235–236 mμ		(170)
Aqueous acids	2360 A.	$\log \epsilon = 3.81$	(138)

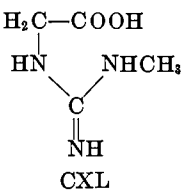
* Minimum.

In the infrared spectrum the maximum, corresponding to the C=N double bond, is at 6.29 μ, in harmony with the fact that the absorption maximum of this bond is situated in general in the region between 5.89 and 6.50 μ, and particularly in the case of derivatives of guanidine between 5.95 and 6.29 μ (233, 240). However, according to other data the absorption maximum of the C=N bond of various guanidines is in the region between 5.8 and 6.15 μ (201). (For the absorption spectrum of N^2-acetyl-5-benzylidenecreatinine, see reference 291.)

B. Acidity and basicity

The ionization constant of creatinine has been determined by several authors and by various methods (55, 93, 104, 212). The data published in the older literature, as well as the corresponding data for creatine, have been reviewed by Shiver (273). Some newer data, including those for some related compounds, are the following:

	pK_a	References
Creatinine	4.84	(104)
Glycocyamidine	4.80	(104)
Creatine	2.67	(104, 323)
"Isocreatine" (CXL)	2.88	(104)
Glycocyamine	2.86	(104)

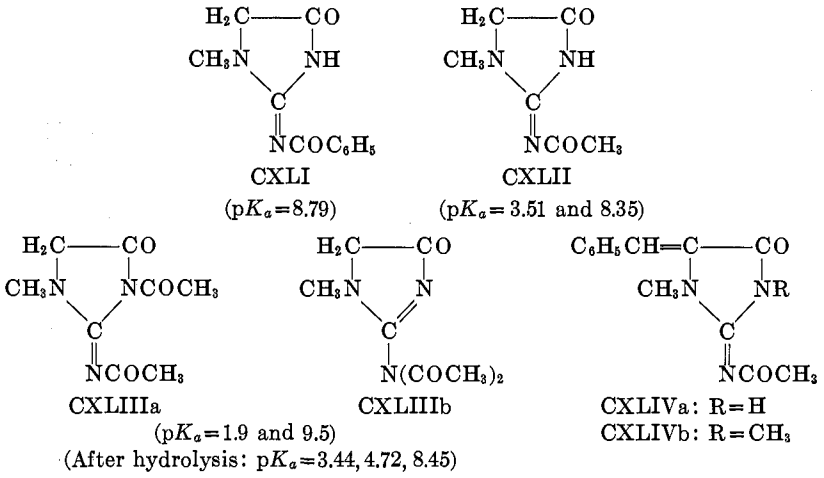


CXL

From these data it is seen that glycocyamines are weaker bases than glycocyamidines, although acylation of the guanidine group should have the opposite effect. This apparent contradiction is, of course, explained by the fact that glycocyamine exists as the dipolar ion (*cf.* 33, 128). As a result thereof, the ionization constant of the acidic group of creatine is likewise very low: K_a =

5.2×10^{-15} (143). The sodium salt of creatine is completely hydrolyzed at a dilution of 0.05–0.25 *N* (143).

The ionization constants of some derivatives of creatinine—benzoylcreatinine (CXLI), acetylcreatinine (CXLII) and diacetylcreatinine (CXLIIIa or CXLIIIb)—were determined by Ing, Kerwick, and Richardson (168); all these compounds contain acidic groups. The acidic character of both monoacylcreat-



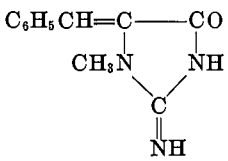
inines is a property of the intact molecules, not the result of a preliminary hydrolysis to the corresponding monoacyl*creatine*. This follows first from the composition of the anhydrous alkali salts, and secondly from the value of the ionization constant being very near to that of phenols, and may be caused by the presence of hydrogen atoms in either position 3 or 5, being rendered acidic by the vicinal carbonyl group. This is proved by the gradual disappearance of the acidic character when these hydrogen atoms are removed by substitution. For example, benzylideneacetylcreatinine (CXLIVa) is already a very weak acid; it reacts with bases only at about pH = 11, and its 3-methyl derivative (CXLIVb) is even completely free from acidic properties (168). Since the ionization constants of the acidic group of the monoacetyl- and diacetylcreatinines (8.35 or 9.5) are not very far apart, and in the latter no hydrogen is present in position 3, it may be supposed that the acidity of CXLI and CXLII is caused mainly by the hydrogens in position 5 (168). Diacetylcreatinine is hydrolyzed rapidly in both acidic and alkaline solutions; therefore its ionization constants must be determined by extrapolation (168).

The acidic character of derivatives of creatinine may not be explained only by the presence of hydrogen atoms in position 5 (or 3). Creatinine, for example, which contains such hydrogen atoms, is nevertheless totally free of acidic properties. It was once suggested that this might be explained by taking into account the possibilities for tautomerism (168); however, these considerations cannot be accepted.

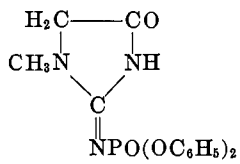
The ionization constants of creatininephosphoric acid and creatinephosphoric

acid were found to be pK_a = 3.41 and 7.38, and 2.44 and 2.81, respectively (323); the former contains no basic function, and the buffer region of its two acidic groups corresponds to the dissociation of the phosphoric acid residue. In the case of creatinephosphoric acid one buffer range, both on the acidic and on the alkaline branch of the titration curve, is situated beyond the limits of titratability. In harmony with this the substance forms at most secondary salts (323).

In complete agreement with the pK_a referred to above is the chemical behavior of the compounds mentioned. Thus, benzoylcreatinine is readily soluble in cold alkalis and precipitated from its solutions by carbon dioxide; its potassium salt can be prepared. On the other hand, acetylcreatinine is amphoteric; it yields stable salts both with acids (e.g., with hydrochloric and picric acids) and with potassium hydroxide. Benzylideneacetylcreatinine is soluble in alkalis and in 20 per cent potassium hydroxide solution, and a potassium salt is formed. However, the compound is precipitated from the alkaline solution by carbon dioxide, and from its aqueous solutions, as a result of hydrolysis, benzylideneacetylcreatinine is slowly, but completely deposited. Deacetylation of this compound results in further decrease in the acidic character; benzylidenecreatinine (CXLV) is insoluble in cold alkalis, but it may be crystallized from hot 2 *N* potassium hydroxide (168). At the same time the basic properties are enhanced by de-



CXLV

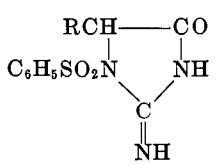


CXLVI

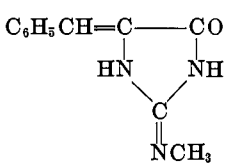
acetylation; thus benzylideneglycocyamidine is soluble in hot hydrochloric acid (250) and 5-benzylidenecreatinine forms a picrate which, however, is hydrolyzed during crystallization from water, alcohol, or acetic acid (129). Diphenoxyphosphorylcreatinine (CXLVI) is similar to the simple monoacylcreatinines mentioned, in that it is capable of forming a stable sodium salt (168).

The *N*,*N*′,*N*″-triphenyl derivative of glycocyamidine is soluble in acids and may be precipitated from its solutions by alkali (72); thus introduction of even three phenyl groups does not abolish the basicity of glycocyamidine.

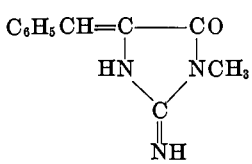
Both 1-benzenesulfonylglycocyamidine (CXLVIIa) and 1-benzenesulfonylalacreatinine (CXLVIIb) are amphoteric substances readily soluble in acids and bases (28); acylation of N^1 is accompanied by enhancement of the acidic character.



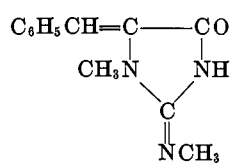
CXLVIIa: R=H
CXLVIIb: R=CH_3



CXLVIII



CXLIX

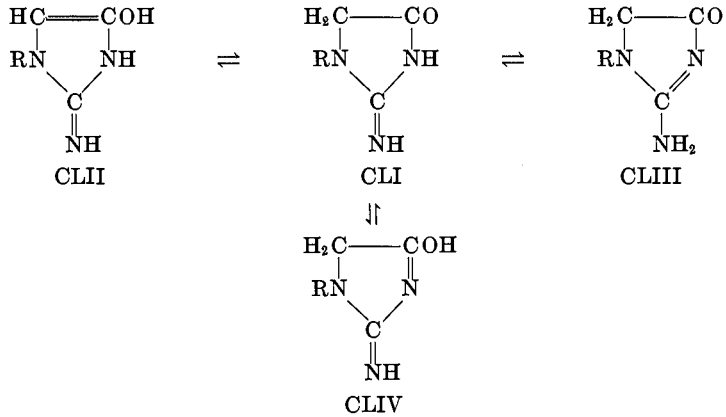


CL

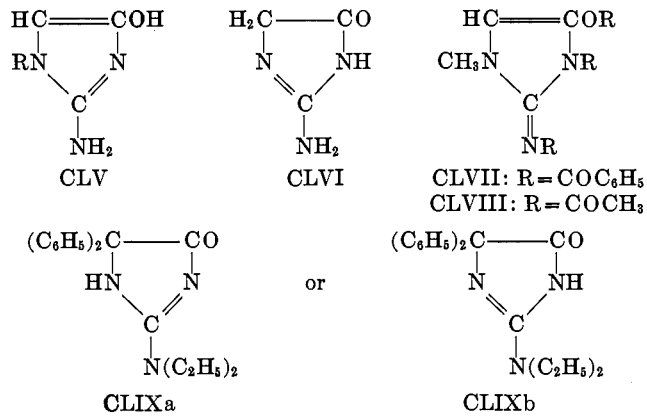
Among the isomeric *N*-methyl-5-benzylidenecreatinines the N^2-methyl derivative (CXLVIII) is readily soluble in cold dilute sodium hydroxide solution and precipitated from its alkaline solutions by acid; however, neither stereoisomer of the 3-methyl derivative (CXLIX) is soluble in 5 per cent alkali (177). Keeping in mind that benzylidenecreatinine (CXLV) is weakly acidic too, it may be concluded that introduction of a benzylidene group confers some acidic character on the glycocyamidine ring, but only if N^3 is not substituted. It is surprising however, that N^2-methyl-5-benzylidenecreatinine is insoluble and benzylidenecreatinine itself only weakly soluble in cold alkalis (228).

C. Tautomerism

It has already been mentioned that glycocyamidine (CLI: R = H) may be considered as a substance capable of existing in several tautomeric forms (CLII, CLIII, CLIV, CLV). The relation of CLI and CLII, CLIII, and CLIV is that of keto-enol, imino-amino, and lactam-lactim tautomers.



By simultaneous enolization and lactimization a further tautomeride (CLV) may be deduced, which could be stabilized by the presence of the aromatic system (168). All these considerations apply equally to the case of creatinine



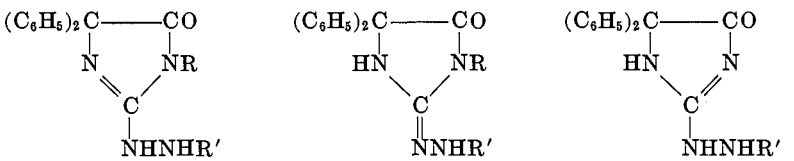
(CLI: R = CH_3); however, in the case of glycocyamidine one extra kind of tautomerism, that corresponding to the tautomerism occurring in imidazoles not substituted in position 1, may be expected; this is represented by the pair of tautomers CLVI and CLIII.

Derivatives of the enolic form are tribenzoylcreatinine (CLVII) (129) and triacetylcreatinine (CLVIII) (168). The presence of the enolic form explains the color reaction of creatinine with ferric chloride (168); formerly it was used to explain the Jaffé color reaction (see Section VI,A) on the basis of keto-enol tautomerism (11, 129).

Derivatives of the amino form (CLIII) or (CLVI) of glycocyamidine (e.g., CLIX) were obtained by the reaction of 2-methylmercapto-4,4-diphenyl-2-imidazolin-5-one with secondary amines (52, 53). Of the two possible structures, CLIXa and CLIXb, the former seems more probable because of the greater acidity of the amide group in comparison with the amino group. The fact that one atom of nitrogen may be split off from creatinine by nitrous acid (236, 319) or hypobromite (66) was formerly thought evidence in favor of the existence of the amino form of creatinine itself (CLIII: R = CH_3) (168); however this is wrong, because 3-methylcreatinine, in which no analogous amino form is possible, reacts similarly.

The silver salt of creatinine was formerly considered to be a derivative of the lactim form (CLIV: R = CH_3) (268); however, it may also be derived from the enol form (CLII: R = CH_3) (168). The aromatic tautomer CLV (R = CH_3) was advanced to explain the fact that while its N^2-acyl derivatives are acidic, creatinine itself is devoid of any acidic properties (168). However, these considerations seem to be out of date, and there is no evidence for the aromatic tautomer. The same argument applies to considerations concerning the two alternative structures for diacetylcreatinine (CXLIII a or b), according to which the former should be correct (168).

By examination of the ultraviolet spectra it was demonstrated that several derivatives of N^2-aminoglycocyamidines, for which the alternative structures CLX, CLXI, and CLXII are possible, possess the hydrazone structure (CLXI)

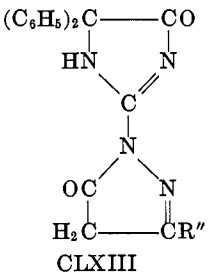


CLX CLXI CLXII

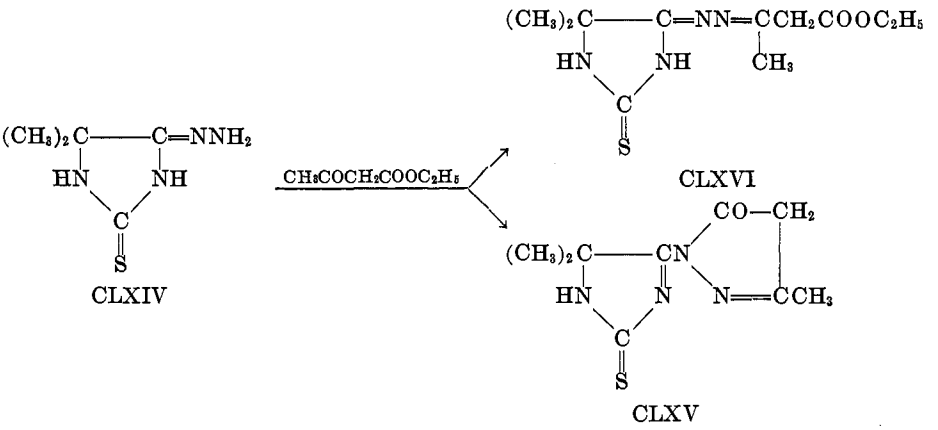
a: R=R'=H b: R=CH_3,R'=H[10] c: R=H,R'=C_6H_5

(311). In agreement with this, neither compound can be transformed into 1-(2-hydantoinyl)-5-pyrazolones (CLXIII). Similarly, CLXIV also possesses the hydrazone structure, and with ethyl acetoacetate it yields the azine CLXVI, not a pyrazolone (CLXV) (311).

[10] In this case the existence of the tautomer corresponding to formula CLXII is of course impossible.



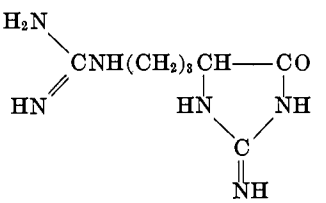
CLXIII



CLXIV CLXV CLXVI

D. Stereoisomerism

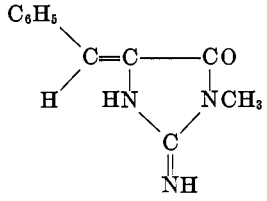
In the case of glycocyamidines monosubstituted in position 5 the appearance of optical isomerism may be expected. However, only one optically active glycocyamidine, 5-(γ-guanidinopropyl)glycocyamidine (CLXVII), obtainable from *d*-arginine, is known (324). This is optically stable in the form of the dinitrate;



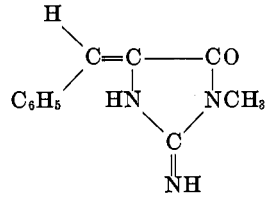
CLXVII

however, the free base is quickly racemized (324) in accordance with the well-known base-catalyzed racemization of derivatives of carboxylic acids containing in the α-position an asymmetric carbon atom to which a hydrogen atom is attached.

In the case of glycocyamidines containing a suitable substituent in position 5, attached to the ring by a double bond, cis-trans isomers may be expected. This isomerism is realized in the case of 3-methyl-5-benzylideneglycocyamidine (CLXVIIIa and b) (177); *cf.* the analogous situation in the case of the 5-benzylidenehydantoins (175, 176).



CLXVIIIa

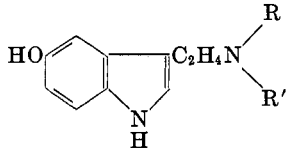


CLXVIIIb

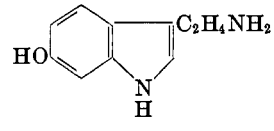
E. Molecular compounds

Creatinine forms molecular compounds. Thus, in alcoholic solution with sodium, potassium, and rubidium hydroxides 1:1 molecular compounds are formed (*cf.* 136). By treatment with ether these may be precipitated in crystalline form; the crystals contain one, or in the case of the compound with rubidium hydroxide, two molecules of water of crystallization (38). These may be intermediates in the hydrolytic reaction furnishing creatine, and may play a part in the formation of the red product of the Jaffé color reaction (38). The double salt of creatinine with zinc chloride has already been mentioned (see Section II,A).

A well-known and physiologically active double sulfate is formed by creatinine with serotonine (CLXIXa) (32, 70, 178, 206, 280, 281, 301, 302). Analogous double salts are formed with several other indole derivatives, such as 6-hydroxytryptamine (CLXX) (120, 289), 5-hydroxy-ω-(*N*-methyl)tryptamine (CLXIXb) (280, 302), and bufotenine (CLXIXc) (280, 289, 302).



CLXIXa: R=R′=H
CLXIXb: R=CH_3; R′=H
CLXIXc: R=R′=CH_3



CLXX

VI. COLOR AND OTHER REACTIONS OF GLYCOCYAMIDINE FOR ANALYTICAL PURPOSES

A. The Jaffé–Folin reaction

The best-known color reaction of creatinine is the Jaffé reaction (171), which was adopted by Folin for the quantitative determination of creatinine (109, 110, 114) and which is applicable also for the development of paper chromatograms (300). The reaction consists in the development of a red color when solutions containing creatinine are treated with aqueous picric acid and a few drops of alkali at room temperature. The reaction is applicable also to the determination of creatine if the solution is boiled for a few minutes after the treatment with picric acid but before making it alkaline; under these conditions, the creatine is cyclized to creatinine (113).

The color reaction is not specific for creatinine, as may be seen from table 5; many other compounds, some of quite different character, are capable of

TABLE 5

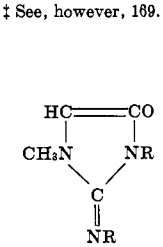
*Jaffé reaction of some organic compounds**

Positive Reaction	References	Negative Reaction	References
Creatinine			
Glycocyamidine	(26, 129, 173, 257)	N^2,3-Bis(hydroxymethyl)creatinine	(129)
1-Ethylglycocyamidine	(11a)	Tribenzoylcreatinine (CLXXIb)	(26, 129)
3-Methylglycocyamidine	(129)	5-Benzylidenecreatinine	(26, 129, 168)
3-Methylcreatinine	(26, 129)	N^2-Acetyl-5-benzylidenecreatinine	(26, 129)
3-Ethylcreatinine	(26)	Isonitrosocreatinine	(26, 129)
3-Benzylcreatinine	(26, 129)		
N^2,3-Dimethylcreatinine	(26, 129)	5-Phenyl-5-ethylhydantoin	(315)
N^2-Benzoylcreatinine	(129)	Parabanic acid	(315)
Diacetylcreatinine	(168)		
Triacetylcreatinine (CLXXIa)	(168)	Glycocyamine	(26)
5-Benzylcreatinine	(26, 129)†	Isoarginine (CLXXII)	(4)
5,5-Tetramethylenebiscreatinine	(4)	Benzaldehyde	(315)
5-(ε-Carboxy-ε-aminopentyl)creatinine	(4)	Benzophenone	(315)
5-(γ-Benzamidopropyl)creatinine	(4)	*d*-Camphor	(315)
		Benzoin	(315)‡
Hydantoin	(26, 315)	Ethyl acetate	(315)
1-Methylhydantoin	(121)	Acetamide	(315)
α-(*N*-Methyl)hydantoic acid†	(121)		
		Acetonitrile	(315)
Acetaldehyde	(315)	Ethyl oxalate	(315)
Acetone	(169, 315)	Oxamide	(315)
Methyl ethyl ketone	(169)	Styrene	(315)
Acetophenone	(169)	Diphenylmethane	(315)
Biacetyl	(315)	Dimedone (CLXXIV)	(169)
Cyclohexanone	(169, 315)		
Resorcin (cyclohexenedione)†	(169)		
Benzoin	(169)		
Pyruvic acid	(191)		
Ethyl phenylacetate	(315)		
Phenylacetamide	(315)		
Phenylacetonitrile	(315)		
Ethyl acetoacetate†	(169)		
Diethyl malonate	(169, 315)		
Malonamide	(315)		
Barbituric acid	(315)		
Estragol (CLXXIII)	(315)		
Indene	(315)		
Indole	(169)		
Nitromethane	(315)		
Diazomethane	(315)		

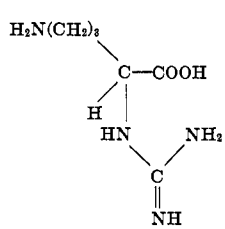
* *Cf.* 182.

† Slight coloration.

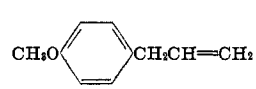
‡ See, however, 169.



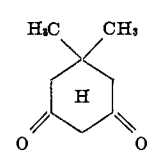
CLXXIa: R = $COCH_3$
CLXXIb: R = COC_6H_5



CLXXII



CLXXIII



CLXXIV

TABLE 6

Color reaction of aromatic nitro compounds with creatinine and other compounds containing active methylene groups

Positive Reaction	References	Negative Reaction	References
m-Dinitrobenzene	(169)	*o*-Nitrophenol	(135)
sym-Trinitrobenzene	(36, 135, * 169)	*p*-Nitrophenol	(135)
2,4,6-Trinitrotoluene	(36, † 135, * 182)	2,4-Dinitrophenol	(129, 135; see, however, footnote ‡)
2,4-Dinitrochlorobenzene	(169)	2,6-Dinitrophenol	(130; see, however, footnote §)
2,4,6-Trinitrochlorobenzene	(169, 182)	3,5-Dinitrophenol	(135)
2,4-Dinitrophenol	(11, ‡ 169)	3,5-Dinitro-*p*-cresol	(135)
2,6-Dinitrophenol	(11)§	2,4,6-Trinitro-*m*-cresol	(129, 135; see, however, footnote ‡)
Picric acid		2,4-Dinitrobenzoic acid	(135)
Methyl, ethyl, and phenyl picrates	(182)	3,5-Dinitrosalicylic acid	(135)
2,4,6-Trinitrophenyl benzoate	(182)	Picramic acid	(135)
2,4,6-Trinitro-*m*-cresol (3-methylpicric acid)	(11)‡		
2,4-Dinitro-1-naphtholsulfonic acid	(11)§		
Picramide	(182)		
N,*N*-Diethylpicramide	(182)		
N-Phenylpicramide	(182)		
β-(2,4,6-Trinitrophenyl)ethanol	(182)		
Methyl β-(2,4,6-trinitrophenyl)ethyl ketone	(182)		
3,5-Dinitrobenzoic acid ¶	(26, 34, 35)		
2,4,6-Trinitrobenzoic acid	(36, 135, * 182)		

* Slight coloration.
† Coloration only on heating.
‡ Coloration only on prolonged standing.
§ With this compound a transient coloration appears only on acidification of the solution containing creatinine.
¶ A contrary statement (135) is apparently erroneous.

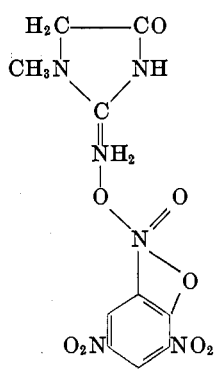
giving this reaction. Formerly it was thought that these included all derivatives of creatinine in which keto-enol tautomerism is possible (129); however, the group of substances yielding a positive color reaction is much wider, the Jaffé reaction for creatinine being only a special case of the color reaction with picric acid and alkali of substances containing an active methylene or methine group (182, 315). The activating groups in order of decreasing activating effect with respect to the color reaction are the following (315): diazo > nitro > carbonyl > cyanide > vinyl > carbamoyl > carbethoxy. However, as may be seen from table 5, the presence of active methylene groups is only a necessary, but not a sufficient condition for the positive color reaction. The only apparent exception is furnished by triacetylcreatinine (CLXXIa), which, although containing no active methylene group, nevertheless gives a positive reaction; this may be explained however, by the facile hydrolysis of the enolacetate group (168).

In addition to picric acid many other aromatic nitro compounds develop colors with creatinine and other substances containing active methylene groups (see table 6). One of these, 3,5-dinitrobenzoic acid, which gives a violet color, is also widely used for the determination of creatinine (26, 34, 35, 57, 186), as well as for the development of paper chromatograms (300). As might be expected, the reaction with 3,5-dinitrobenzoic acid is also not a specific one for creatinine

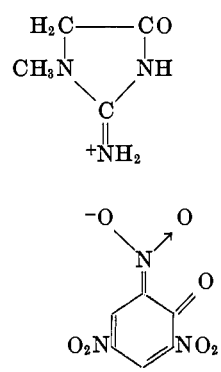
but is given by all glycocyamidine derivatives which give a positive reaction with picric acid (26).

Several red compounds of different compositions have been isolated from the red solution obtained from creatinine, picric acid, and alkali. First, by acidification of a red Jaffé solution with hydrochloric acid a red powder was isolated (135) which, on boiling with water (11) or when heated to 139°C., was transformed into the usual yellow creatinine picrate and therefore considered to be a tautomer thereof (135). The red tautomer is obtained in best yields by precipitating it immediately after the appearance of the red color (11). It consists of one molecule each of creatinine, picric acid, and water of crystallization. Its solubility in water is slight; however, in sodium bicarbonate solution it dissolves readily with red color as the sodium salt. By analysis of its red barium salt it proved to be a dibasic acid (11). Similar red isomers have been obtained from the picrates of glycocyamidine, 3-methylglycocyamidine, and 3-methylcreatinine (129).

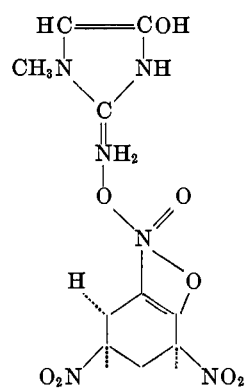
Later, by treating aqueous alkaline solutions of creatinine picrate of different compositions with alcohol, several crystalline substances of other composition were obtained (see table 7). Compounds 2 (37, 132) and 7, or more probably 10, (39) are considered as the ones responsible for the color produced in the Jaffé reaction. In agreement with the existence of many compounds of creatinine with picric acid, the absorption maximum of the creatinine–picric acid complex is dependent upon the concentration of the creatinine (125).



CLXXVa



CLXXVb



CLXXVI

As to the nature of the red tautomer of creatinine picrate, a structure (CLXXVI) was first proposed in 1925 (129). Since at that time the picric acid in the picrates was believed generally to be of quinoidal structure (*cf.* the structure (CLXXVa) proposed for the "normal" yellow creatinine picrate, which can be rewritten as in formula CLXXVb), this was supposed also to be the red tautomer. Moreover, as it was thought that only creatinine derivatives capable of enolization give the color reaction, the creatinine was supposed to be present in its enolic form. The dotted lines in formula CLXXVI should account for the fact that the hydrogen in the meta position must be somehow concerned during the formation of the red tautomer, and that consequently the distribution of the bonds in the benzene ring is uncertain. The important role of the

TABLE 7

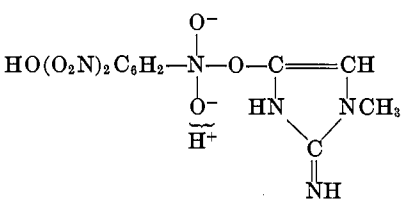
Red compounds isolated from solutions of creatinine and picric acid

No.	Composition*		Notes	References
1.....	$2C_4H_7N_3O \cdot C_6H_3(NO_2)_3OH \cdot 3NaOH \cdot 3H_2O$*		The color is less deep than that of the red substance obtained in the Jaffé reaction. The creatinine contained in the compound gives Jaffé's reaction only after standing in acetic acid (134). With hydrochloric acid dicreatinine picrate is formed; the creatinine contained therein gives Jaffé's reaction only after standing with acetic acid (134). Identical with compound 6?	(131)
2.....	$C_4H_7N_3O \cdot C_6H_3(NO_2)_3OH \cdot 2NaOH$		Readily soluble in water. On acidification of concentrated aqueous solutions the red creatinine picrate is precipitated. It gives Jaffé's reaction immediately, the intensity corresponding to the total creatinine present (134). Identical with compound 7?	(37, 132, 135)
3.....	$2C_4H_7N_3O \cdot C_6H_3(NO_2)_3OH$		These compounds are not red but become red after being dissolved in acetic acid-acetate buffer solution. The sodium derivatives, but not the free acid and the hydrobromide, split off nitrogen on being dissolved in sulfuric acid.	(134)
4.....	$2C_4H_7N_3O \cdot C_6H_3(NO_2)_3OH \cdot 1HBr \cdot 5\text{-}6H_2O$			
5.....	$2C_4H_7N_3O \cdot C_6H_3(NO_2)_3OH \cdot 2.5NaOH$			
6.....	$2C_4H_7N_3O \cdot C_6H_3(NO_2)_3OH \cdot 3NaOH$			
7.....	$C_4H_7N_3O \cdot C_6H_3(NO_2)_3OH \cdot 2NaOH \cdot 2H_2O$	(red)	Readily soluble in water. Their solutions show a decreasing tendency to turn yellow in the order given. On acidification the red creatinine picrate tautomer is precipitated.	(39)
8.....	$C_4H_7N_3O \cdot C_6H_3(NO_2)_3OH \cdot 2.5NaOH \cdot 2H_2O$	(red)		
9.....	$C_4H_7N_3O \cdot C_6H_3(NO_2)_3OH \cdot 3NaOH \cdot 2H_2O$	(red)		
10....	$C_4H_7N_3O \cdot C_6H_3(NO_2)_3OH \cdot 3.5NaOH \cdot 2H_2O$	(orange)		

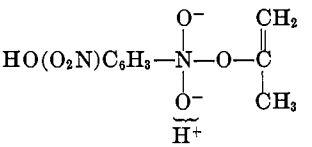
* $C_4H_7N_3O$ = creatinine.

meta hydrogen atom was concluded from the inability of 3-methylpicric acid to substitute for picric acid in the Jaffé test (129); however, this conclusion turned out later to be erroneous (11).

Formula CLXXVI was soon criticized (11), the main weakness being that it ascribes a basic function to creatinine, although the color is developed only in the presence of alkali and although nonbasic compounds may also give the same reaction. Therefore it was later suggested that the enolate anion of creatinine forms a coördinative bond with the positively charged nitrogen atom of one of the nitro groups; the structure of the red picrate being consequently CLXXVII (11). Then the completely analogous structure CLXXVIII would correspond



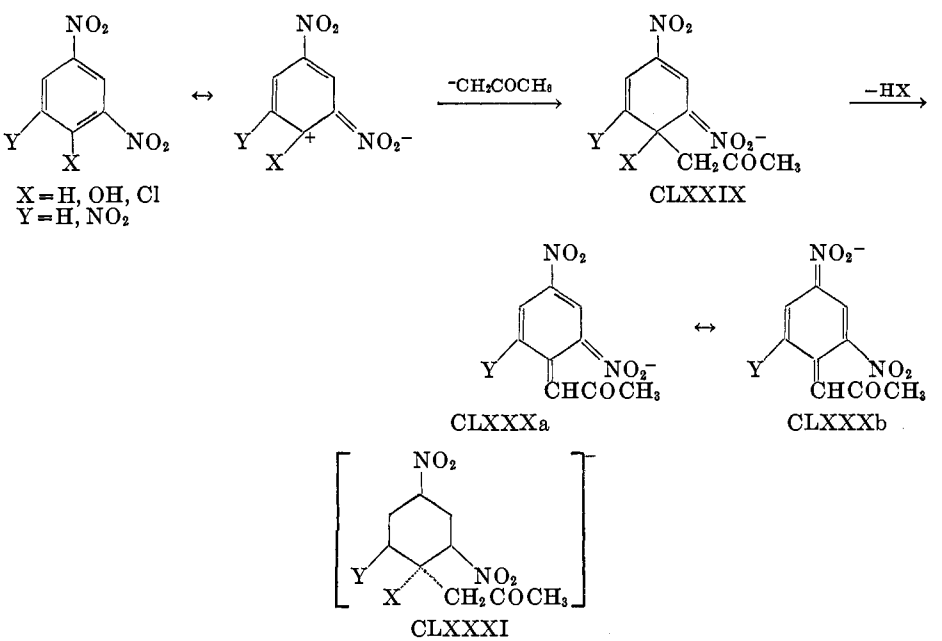
CLXXVII



CLXXVIII

to the colored product obtained from acetone and 2,4-dinitrophenol (242). In both cases it is uncertain whether the picric acid or the dinitrophenol reacts in the quinoid form. Moreover, it is not known to which nitro group the anion of creatinine or of acetone is attached. Formula CLXXVII represents, as may be seen, a dibasic acid, this being in agreement with the properties of the red picrate. By supposing that a second anion of creatinine could furnish a coordinative bond with another nitro group of the picric acid moiety, leading to the formation of a tribasic acid, a structure is suggested at the same time for compound 1 in table 7 (11). However, the structures of disodium creatinine picrate and trisodium dicreatinine picrate cannot be analogous, because, on treatment with concentrated sulfuric acid one or two atoms of nitrogen are split off only from the latter. On the other hand, the latter does not immediately give Jaffé's reaction (134). Another weakness of structure CLXXVII is the unusual distribution of the valences of the nitrogen.

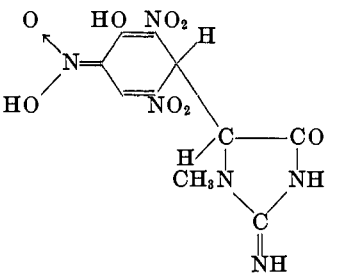
According to a recent suggestion the carbeniate anions of active methylene compounds should condense with *m*-dinitrobenzene and *sym*-trinitrobenzene, as well as with their hydroxy and chloro derivatives, in the position para to one of the nitro groups, leading to a primary condensation product (CLXXIX) which should be stabilized by splitting off one molecule of hydrogen, water, or hydrochloric acid and formation of a compound (CLXXX) of quinoid nature (169). In the case of acetone the transformations could be depicted as follows:



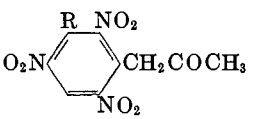
As may be seen, CLXXIX is identical with one of the limiting structures of the transition state (CLXXXI) of an aromatic nucleophilic substitution; therefore the Jaffé reaction becomes at once related to the common nucleophilic substitu-

tion reactions of aromatic polynitro compounds—e.g., hydroxylation by alkaline ferricyanide or introduction of a nitrile group by means of potassium cyanide. Since quinones likewise are capable of being substituted by nucleophilic reagents, the striking parallelism between the color reactions of active methylene compounds with polynitro compounds and 1,2-naphthoquinone-4-sulfonic acid (169) becomes intelligible.

According to the most recent suggestion the reaction of creatinine and other active methylene compounds with picric acid and related substances should be not a substitution, but instead an addition reaction (183). The structure of the red creatinine picrate, as deduced from the absorption spectrum, should be as shown in formula CLXXXII, the hydroxyl group of the picric acid moiety not being eliminated from the product (184). That the color reaction consists in addition of the active methylene compound and not in substitution by it is proved, in addition to the evidence gained from absorption spectra, by the fact that on treatment of the colored products from acetone and 2,4,6-trinitrochlorobenzene or phenyl picrate with hydrochloric acid, eventually in the presence of hydrogen peroxide, compounds of the structures CLXXXIII or CLXXXIV are obtained (183).



CLXXXII



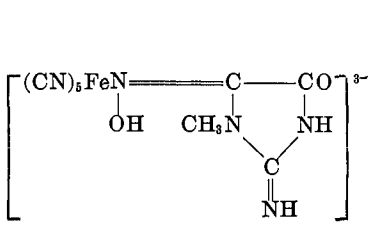
CLXXXIII: R = Cl
CLXXXIV: R = C_6H_5O

On treatment of aqueous alkaline solutions of creatinine and 3,5-dinitrobenzoic acid with alcohol, colored crystalline compounds are precipitated which contain one molecule of creatinine, two molecules of 3,5-dinitrobenzoic acid, and various amounts of sodium hydroxide. The more stable compound is brown, the less stable one violet. The latter, which contains three molecules of sodium hydroxide, decomposes in methanol solution into a compound consisting of one molecule each of creatinine and 3,5-dinitrobenzoic acid and four molecules of sodium hydroxide and in addition into creatinine and sodium 3,5-dinitrobenzoate (40). The corresponding silver salts have been likewise prepared (40). These compounds were considered, by analogy with the substances obtained with polynitro compounds, as molecular complexes (36).

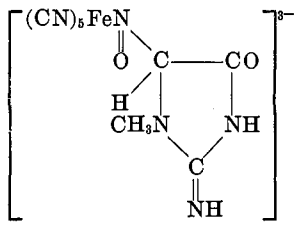
B. The Weyl reaction

Aqueous alkaline solutions of creatinine produce on treatment with sodium nitroprusside a transient red color, later turning yellow (235, 316), which, on acidifying with acetic acid and subsequent heating turns green and then blue

(254). It was suggested that the red coloration depends on the formation of an unstable iron complex, the structure of which is represented by either CLXXYV or CLXXXVI, and which is decomposed with formation of isonitrosocreatinine (258).



CLXXXV



CLXXXVI

Similarly, glycocyamidine furnishes with sodium nitroprusside a faint yellowish red (173, 189) or bluish red (29) color which, on acidification with acetic acid, turns deep red (173, 189). The color produced with 3-methylglycocyamidine is a transient red, turning crimson on acidification (189). A color is produced also with 5-(γ-benzamidopropyl)creatinine (4) and 1-methylhydantoin (121); however, α-(*N*-methyl)hydantoic acid does not react (121).

Creatine does not react with sodium nitroprusside. However, if the reagent is exposed to sunlight, it furnishes red colors with creatine, guanidine, and the methyl derivatives of the latter (297; *cf.* 208). Creatine may be caused to react with sodium nitroprusside by adding an oxidant such as sodium persulfate; the colored product obtained is stable towards oxidants and alkalis but unstable towards reducing agents and acids (235).

On paper chromatograms α-guanidinosuccinic acid and arginine give red spots with alkaline nitroprusside-ferricyanide reagent. After preliminary cyclization, the former gives blue and the latter orange spots (311a).

C. Other color reactions

With ferric chloride glycocyamidine (189) and diacetylcreatinine (168) furnish the red colors characteristic of enolic substances.

The Sakaguchi reaction for guanidines, which consists in the production of a red color by alkaline solutions of guanidine containing α-naphthol or oxine (253) on treatment with sodium hypochlorite or hypobromite (252), is also applicable to the determination of glycocyamine (253) as well as of other α-guanidino acids (217, 246), the intensity of the color produced by the different guanidino acids, calculated per guanidino group, however, not being equal (217, 246). The reaction may be applied also to the determination of creatinine if it is previously oxidized by alkaline mercuric oxide to oxalate and methylguanidine (244).

After suitable modification, viz., application of an alkaline α-naphthol–biacetyl reagent, the Sakaguchi reaction may be applied to the development of the spots of α-guanidino acids on paper chromatograms; however, glycocyamidines do not react (300).

Nitrosamine Red gives with creatinine in acetic acid–acetate buffer solutions a diazo reaction, the yellow dye produced being soluble in concentrated sulfuric acid with a red color. The sensitivity of the reaction is $1:10^{-5}$. Creatine does not give the reaction (185).

D. Other reactions for analytical purposes

Creatinine may be detected also with the aid of Ehrlich's reagent (*p*-dimethylaminobenzaldehyde) (214). It may be identified in the form of its compounds with aromatic isocyanates (154), its reineckate (13), or its salt with 2-nitroindane-1,3-dione (64).

For its determination the potassium mercuric rhodanide–dithizone method is very suitable. This consists in the reaction of creatinine with an excess of the potassium mercuric rhodanide and determination of the excess of the latter colorimetrically with dithizone. The determination is not influenced by the presence of guanidine, methylguanidine, ammonia, uric acid, urine, or even of 30 parts of creatine (285, 286). For the determination of creatinine, as well as of creatine, potentiometric titration with potassium mercuric iodide is likewise suitable (279).

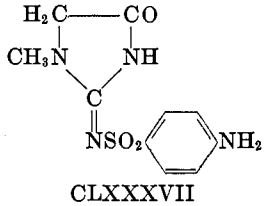
Creatinine may be separated from creatine by treating the mixture in saturated sodium bicarbonate solution with mercuric nitrate, by which only the former is precipitated (115). Similarly, in the presence of sodium acetate, creatinine (but not creatine) is precipitated by mercuric chloride in the form of a double salt (189). The separation may be effected also by means of paper chromatography (5, 211).

VII. PHARMACOLOGICAL PROPERTIES OF GLYCOCYAMIDINES

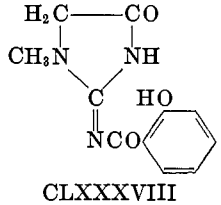
The pharmacology of creatinine has been investigated in great detail, the data prior to 1939 having been reviewed by Staub (282). According to the data, creatinine produces different effects on the brain and motor stimulation of mice and frogs, influences the electric excitability of muscle-nerve preparations, increases the action of adrenaline on the blood pressure and on the isolated frog heart, produces vasodilatation in the frog leg, and, after intravenous application, hypoglycemia in the sheep; finally, it inhibits the growth of *Bacillus probatus*. Higher doses are toxic for the smaller mammals.

According to more recent investigations creatinine has some protective action on certain experimental convulsions (51, 63, 230).

Among its derivatives sulfacreatinine (CLXXXVII), as well as the related sulfacreatine, does not damage the kidneys, in contrast to other sulfonamides (251). The salicyloyl derivative (CLXXXVIII) is a preservative (256).

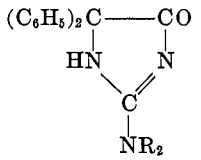


CLXXXVII



CLXXXVIII

Among the derivatives of glycocyamidine the pharmacology of different 2-(disubstituted amino)-5,5-diphenyl-2-imidazolin-4-ones (CLXXXIX) was studied; however, these compounds showed neither hypnotic and sedative,



CLXXXIX

nor lethal effects on mice in doses of 1 g./kg. (53). Some basic substituted derivatives of glycocyamidine have been found to produce various effects on different centers of the central nervous system (198, 199). N^2-Sulfanilylglycocyamidine has antibacterial properties (65).

Phthaloylbis(methylglycocyamidine), which is probably bis(methylglycocyamidine) phthalate (*cf.* page 675), lowers the blood sugar level in man and enhances the action of simultaneously given insulin; however, it does not influence the body weight (293). N^2-Sulfanilylglycocyamidine possesses antibacterial activity (65).

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