[CONTRIBUTION NO. 1740 FROM THE GATES AND CRELLIN LABORATORIES OF THE CALIFORNIA INSTITUTE OF TECHNOLOGY]

A Reaction of Guanidine with Glyoxals in Aqueous Solution. The Preparation of Glycocyamidines

BY IRVING S. BENGELSDORF[1]

RECEIVED NOVEMBER 28, 1952

In the course of experiments to elucidate the chemistry of the preparation of a blood volume expander, "Oxypolygelatin," a new synthesis of 2-imino-4-imidazolidones (glycocyamidines) has been accomplished by the interaction of guanidine with glyoxals. The glycocyamidines, isolated as the 1-benzenesulfonyl derivatives, are converted by acidic hydrolysis to the corresponding 1-benzenesulfonylhydantoins. The latter are readily hydrolyzed with base to the N-benzenesulfonyl derivatives of α-amino acids. Six new compounds, 1-benzenesulfonylglycocyamidine and its picrate, 1-benzenesulfonylhydantoin, 1-benzenesulfonylalacreatinine and its picrate, and 1-benzenesulfonyl-5-D,L-methylhydantoin, have been prepared and their structures established by analyses and syntheses.

Glyoxal has been proposed and employed as a cross-linking reagent to change the shape and to increase the molecular weight of individual protein molecules in solution. The first step in the preparation of the blood volume expander, "Oxypolygelatin" (OPG) involves the reaction of gelatin molecules with glyoxal.[2] The chemistry of the cross-linking reaction of gelatin with glyoxal is incompletely understood at present. Some aspects of this reaction could perhaps be clarified by observing the reactions of glyoxal with model compounds.

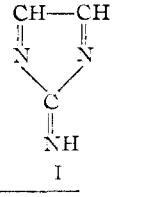
The coupling reaction of glyoxal with amino groups in the gelatin molecule, *e.g.*, the ϵ-amino group in the lysine side chain, can proceed in either of two directions; both reaction paths have been observed in non-aqueous media.

$$RNH_2 + OHC{-}CHO + H_2NR \longrightarrow RNHCH{-}CHNHR \quad (OH\ OH)$$

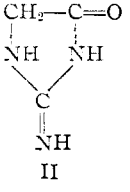
$$\rightarrow (a)\ RN{=}CH{-}CH{=}NR + 2H_2O^3$$

$$\rightarrow (b)\ RNHCCH_2NHR + H_2O^4 \quad (O)$$

The reaction of glyoxal with guanidino groups, *e.g.*, the δ-guanidino group of arginine, however, is more complex than the amine–glyoxal interaction, for the polyfunctionality of the guanidino group can lead to either an *inter*molecular condensation polymer (cross-linking) or to an *intra*molecular cyclization (no cross-linking). The analogous cyclic products of the reaction of glyoxal with guanidine should be either 2-imino-2-isoimidazole (I) or 2-imino-4-imidazolidone (II, glycocyamidine). Products corresponding to I have thus far not been isolated although the reaction of glyoxal sodium bisulfite with guanidine carbonate yields the sodium salt of 2-amino-4-imidazolesulfonic acid.[5] The isolation of glycocyamidine (II) from the interaction of glyoxal with guanidine is reported in this paper. This particular reaction apparently has not been previously described, although some similar general reactions have been observed. Thus glyoxal reacts with urea to give hydantoin[6,7] and acetylene diurein,[7,8] whereas methylglyoxal (pyruvic aldehyde) reacts with thiourea to give 5-methyl-2-thiohydantoin[9] and with urea to give methylacetylene diurein.[10] Phenylglyoxal has been reported to react with urea to yield 5-phenylhydantoin.[11]



I



II

There is no evidence to indicate that the cyclization reaction between glyoxal and the *substituted* guanidino group of the arginine side chains in gelatin does occur in the preparation of OPG. All reactions attempted between glyoxal and *substituted* guanidines led to viscous residues from which no definite product could be isolated (see Miscellaneous reactions).

The reaction of an equimolar mixture of aqueous technical glyoxal and guanidine hydrochloride at 95° followed by treatment with benzenesulfonyl chloride yields N-benzenesulfonylguanidine and the amphoteric 1-benzenesulfonyl-2-imino-4-imidazolidone (IIIa, 1-benzenesulfonylglycocyamidine). The latter reacts with picric acid to yield a picrate (IVa); it is also readily hydrolyzed with acid to 1-benzenesulfonyl-2,4-imidazoledione (Va, 1-benzenesulfonylhydantoin). The hydrolysis of Va with base results in the formation of N-benzenesulfonylglycine (VIa).

The reaction described above is probably general for glyoxals, for methylglyoxal (pyruvic aldehyde) reacts with guanidine hydrochloride (and subsequently with benzenesulfonyl chloride) to give 1-benzenesulfonyl-2-imino-5-D,L-methyl-4-imidazolidone (IIIb, 1-benzenesulfonylalacreatinine). The latter yields the corresponding picrate (IVb). Acidic hydrolysis of IIIb leads to 1-ben-

(1) Department of Chemistry, University of California, Los Angeles 24, California.

(2) D. H. Campbell, J. B. Koepfli, L. Pauling, N. Abrahamsen, W. Dandliker, G. A. Feigen, F. Lanni and A. Le Rosen, *Texas Repts. Biol. Med.*, **9**, 235 (1951).

(3) A. Chwala and W. Bartek, *Monatsh.*, **82**, 652 (1951).

(4) K. Maurer and E. H. Woltersdorf, *Z. physiol. Chem.*, **254**, 18 (1938).

(5) J. B. Ekeley and J. M. Fulmer, THIS JOURNAL, **52**, 2026 (1930).

(6) L. Siemonsen, *Ann.*, **333**, 101 (1904).

(7) H. Pauly and H. Sauter, *Ber.*, **63**, 2063 (1930).

(8) H. Biltz, *ibid.*, **40**, 4806 (1907).

(9) B. J. Sjollema and A. J. H. Kam, *Rec. trav. chim.*, **36**, 180 (1917); (b) B. J. Sjollema and L. Seekles, *ibid.*, **44**, 827 (1925).

(10) L. Seekles, *ibid.*, **46**, 77 (1927).

(11) H. J. Fisher, J. B. Ekeley and A. R. Ronzio, THIS JOURNAL, **64**, 1434 (1942).

zenesulfonyl-5-D,L-methyl-2,4-imidazoledione (Vb, 1-benzenesulfonyl-5-D,L-methylhydantoin). Basic hydrolysis of this hydantoin yields N-benzenesulfonyl-D,L-alanine (VIb).

Evidence for the position of the benzenesulfonyl group in the heterocyclic ring was obtained from the various hydrolytic degradations. The hydrolysis of glycocyamidine, substituted with a benzenesulfonyl group (IIIa), to the correspondingly substituted hydantoin (Va)[12] eliminates the possibility of the attachment of the benzenesulfonyl group to the imino group in the 2-position. As hydantoins with a substituent in the 1-position are hydrolyzed to amino acids in which the substituent is linked to the α-amino nitrogen atom,[13] the appearance of N-benzenesulfonylglycine (VIa) as the hydrolysis product of Va, indicates that the benzenesulfonyl group is attached to the 1-position of the glycocyamidine and not to the 3-position.

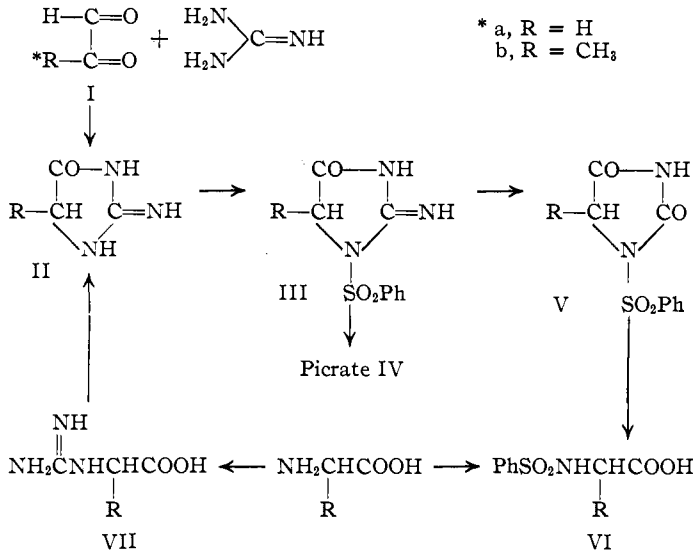


Fig. 1.

The identification of IIIa and IIIb as 1-benzenesulfonylglycocyamidine and 1-benzenesulfonylalacreatinine, respectively, was accomplished by their preparation *via* another synthetic route. Glycocyamine and alacreatine[14] were cyclized with hydrochloric acid to glycocyamidine and to alacreatinine. Treatment of the latter two compounds with benzenesulfonyl chloride gave compounds which were identical with IIIa and IIIb, respectively (Fig. 1).

The second step in the preparation of OPG involves the treatment of the cross-linked gelatin molecules with hydrogen peroxide.[2] The reaction of the glyoxal–guanidine hydrochloride reaction mixture with hydrogen peroxide was, therefore, investigated. Almost all of the glycocyamidine present was recovered (as the 1-benzenesulfonyl derivative, IIIa) after the reaction mixture was treated with hydrogen peroxide at a concentration greater than that encountered in the OPG preparation.

Experimental

General.—The reaction of both glyoxal and methylglyoxal with guanidine hydrochloride was conducted under conditions similar to those encountered in the preparation of "Oxypolygelatin" (aqueous solution, steam-bath temperature, slightly acidic *p*H) and no attention was paid to obtaining a maximum yield of product. Samples of technical and purified glyoxal, as well as technical methylglyoxal (pyruvic aldehyde) were provided by the Carbide and Carbon Chemicals Corp. The reaction of guanidine hydrochloride with technical glyoxal gives the same product as the reaction with purified glyoxal. The use of the latter material leads to less resinous reaction products with a resultant increase in the yield of glycocyamidine. All melting points are corrected.

Reaction of Glyoxal with Guanidine Hydrochloride.—Ten ml. of a purified glyoxal solution (30% w./v., 0.05 mole) was added to a solution of guanidine hydrochloride (9.55 g., 0.1 mole) in 50 ml. of water and the resultant solution was heated at 95° for 8 hours. After concentrating the dark reaction mixture under reduced pressure to a volume of 12 ml., the viscous residue was made alkaline with 10% sodium hydroxide and treated with benzenesulfonyl chloride. The alkali-insoluble N-benzenesulfonylguanidine separated (5.5 g., 27.6% yield, m.p. 215.5–216.5°, lit. m.p. 212°,[15a,b] 215–216°[15c]). It did not depress the melting point of an authentic sample (m.p. 217.5–219°).

1-Benzenesulfonyl-2-imino-4-imidazolidone (IIIa) was precipitated as an amphoteric solid on acidification of the filtrate. It was isolated by neutralization of its solution in excess acid with sodium bicarbonate. It was recrystallized from ethanol (charcoal) to give small colorless crystals (5.4 g., 45.2% yield, m.p. 223–224.5° with decomposition to a black tar).

Anal. Calcd. for $C_9H_9N_3O_3S$: C, 45.18; H, 3.79. Found: C, 45.44; H, 4.02.

1-Benzenesulfonyl-2-imino-4-imidazolidone picrate (IVa) was prepared and recrystallized from ethanol (needles, m.p. 185–186.5° with decomposition to a black tar).

Anal. Calcd. for $C_{15}H_{12}N_6O_{10}S$: C, 38.47; H, 2.58. Found: C, 38.79; H, 2.68.

Acidic Hydrolysis of 1-Benzenesulfonyl-2-imino-4-imidazolidone (IIIa).—A 25% hydrochloric acid solution of IIIa was refluxed for 15 minutes. A voluminous precipitate of 1-benzenesulfonyl-2,4-imidazoledione (Va) suddenly appeared; it was collected and recrystallized from ethanol (colorless needles, m.p. 241.5–242.5° without decomposition).

Anal. Calcd. for $C_9H_8N_2O_4S$: C, 45.00; H, 3.35. Found: C, 45.26; H, 3.26.

Basic Hydrolysis of 1-Benzenesulfonyl-2,4-imidazoledione (Va).—Six-tenths of a gram of Va in a solution of 0.3 g. sodium hydroxide in 100 ml. of water was refluxed for two hours; ammonia was rapidly evolved (odor, indicator paper). The hydrolysate was acidified and concentrated to a volume of 15 ml. The N-benzenesulfonylglycine which was deposited was recrystallized from water–ethanol (charcoal), m.p. 170–171.5°; lit. m.p. 165–166°.[16a,b] It did not depress the melting point of an authentic sample (m.p. 171°).

Reaction of the Glyoxal–Guanidine Hydrochloride Reaction Mixture with Hydrogen Peroxide.—The black viscous liquid (6.3 g.) from the interaction of guanidine hydrochloride (19.1 g., 0.2 mole) with 40 ml. of the 30% w./v. technical glyoxal (0.2 mole) was treated with 7 ml. of a 30% hydrogen peroxide solution for three hours at 95°. The reaction mixture, a pale orange-yellow viscous residue, was treated with benzenesulfonyl chloride to give IIIa (m.p. 224–225°) with decomposition, 3.5 g., 24% yield.

(12) (a) C. Neubauer, *Ann.*, **137**, 288 (1866); (b) B. H. Nicolet and E. D. Campbell, THIS JOURNAL, **50**, 1155 (1928); (c) J. F. Deck and F. B. Dains, *ibid.*, **55**, 4986 (1933).

(13) (a) T. B. Johnson and B. H. Nicolet, *Am. Chem. J.*, **47**, 459 (1912); (b) D. A. Hahn and A. G. Renfrew, THIS JOURNAL, **47**, 147 (1925).

(14) E. Brand and F. C. Brand, *Org. Syntheses*, **22**, 59 (1942).

(15) (a) D. Ackermann, *Z. physiol. Chem.*, **47**, 366 (1906); (b) H. T. Clarke and H. B. Gillespie, THIS JOURNAL, **54**, 1964 (1932); (c) R. Perrot, *Bull. soc. chim.*, 554 (1946).

(16) (a) H. Ihrfelt, *Ber.*, **22**, ref. 692 (1889); (b) E. Knoevenagel and H. Lebach, *Ber.*, **37**, 4094 (1904).

The Identification of IIIa as 1-Benzenesulfonyl-2-imino-4-imidazolidone. (A) Preparation of Guanidinoacetic Acid (Glycocyamine).—Guanidinoacetic acid (VIIa) was prepared by the interaction of glycine with S-ethylthiourea hydrobromide.[14] The prepared glycocyamine was surprisingly insoluble in water and did not melt at the reported temperature of 280–284° with decomposition; it darkened at 240° and charred up to 300° without melting. This behavior has been previously reported.[17,18] Glycocyanine, however, also has been reported to decompose at 250–260° without melting,[19] to melt at 280–284° with decomposition[14] and to decompose at about 300°.[20] Glycocyamine picrate, prepared as a derivative for analysis, was recrystallized from water (m.p. 204–205.5°; lit. m.p. 199–200° with decomposition,[21] 201°,[22] 202° with decomposition,[19,20] and 210° with decomposition[18]).

Anal. Calcd. for $C_9H_{10}N_6O_9$: C, 31.22; H, 2.91. Found: C, 31.59; H, 3.01.

(B) Cyclization of Glycocyamine to Glycocyamidine.—Two grams of glycocyamine was refluxed with 50 ml. of 18% hydrochloric acid for 18 hours. The solution was evaporated to dryness and three individual portions of the residue were treated in the following manners.

(1) The residue was recrystallized from ethanol (charcoal) to yield glycocyamidine hydrochloride (m.p. 213–214.5° with decomposition, lit. m.p. 208–210° with decomposition,[23] 210°,[24] 211–213°,[25] and 213°[18]).

(2) Glycocyamidine picrate was prepared and recrystallized from water (m.p. 212.5–213° with decomposition; lit. m.p. 195° uncor.,[26] 210°,[21] 214–215° with decomposition,[18] 215° decomposition,[24] 215–216°[25]).

(3) The residue was treated with aqueous sodium hydroxide and benzenesulfonyl chloride. The isolated 1-benzenesulfonyl-2-imino-4-imidazolidone, recrystallized from ethanol (charcoal), has the same melting point (224–225° with decomposition) as does IIIa.

Reaction of Methylglyoxal (Pyruvic Aldehyde) with Guanidine Hydrochloride.—To a solution of guanidine hydrochloride (19.1 g., 0.2 mole) in 300 ml. of water was added 43 ml. of a 33.4% technical methylglyoxal solution (0.2 mole). The resulting solution was heated to 95° for 1.5 hours. The reaction mixture was concentrated to a reddish-brown sirup.

Treatment of the sirup (10 g.) with benzenesulfonyl chloride gave some alkali-insoluble N-benzenesulfonylguanidine (m.p. 212–214°); the filtrate was acidified and neutralized with sodium bicarbonate. The collected amphoteric solid was recrystallized from ethanol (charcoal) to give 1-benzenesulfonyl-2-imino-5-D,L-methyl-4-imidazolidone (1-benzenesulfonylalacreatinine, IIIb, colorless prisms, m.p. 212.5–214° with decomposition to an orange liquid, 3.04 g., 25.2% yield).

Anal. Calcd. for $C_{10}H_{11}N_3O_3S$: C, 47.42; H, 4.38. Found: C, 47.67; H, 4.53.

1-Benzenesulfonyl-2-imino-5-D,L-methyl-4-imidazolidone picrate (IVb) was recrystallized from ethanol (needles, m.p. 184.5° with decomposition to a red liquid).

Anal. Calcd. for $C_{16}H_{14}N_6O_{10}S$: C, 39.83; H, 2.93. Found: C, 39.91; H, 3.00.

Acidic Hydrolysis of 1-Benzenesulfonylalacreatinine (IIIb).—A 36% hydrochloric acid solution of IIIb was refluxed for three hours. The 1-benzenesulfonyl-5-D,L-methyl-2,4-imidazoledione (Vb), which separated upon cooling, was recrystallized from water (m.p. 181°).

Anal. Calcd. for $C_{10}H_{10}N_2O_4S$: C, 47.24; H, 3.96. Found: C, 47.49; H, 3.85.

Basic Hydrolysis of 1-Benzenesulfonyl-5-D,L-methyl-2,4-imidazoledione (Vb).—A solution of 0.4 g. of Vb and 0.27 g. of potassium hydroxide in 50 ml. of water was refluxed for three hours. The acidified hydrolysate was concentrated to dryness. Extraction of the residue with boiling ethanol gave an impure material, m.p. 79–92°. Recrystallization from an absolute ethanol–benzene mixture (charcoal) gave N-benzenesulfonyl-D,L-alanine (needles, m.p. 124.5–126°; lit. m.p. 124–125°,[27] 126°[28a,b]). It did not depress the melting point (124.5–125.5°) of an authentic sample.

The Identification of IIIb as 1-Benzenesulfonyl-2-imino-5-D,L-methyl-4-imidazolidone. Preparation of D,L-α-Guanidinopropionic Acid (D,L-Alacreatine).—D,L-α-Guanidinopropionic acid (VIIb) was prepared by the interaction of D,L-alanine with S-ethylthiourea hydrobromide.[14] Portions of the reaction mixture were treated in the following manners.

(1) **Isolation of Alacreatine.**—The solution was concentrated, treated with charcoal and filtered. The crystals which appeared upon standing overnight (ice-box) were recrystallized from an ethanol–water mixture to give D,L-alacreatine (m.p. 228–230° with effervescence; lit. m.p. 226° (efferv.),[22] 228°,[29] 246–247° (efferv.)[18]).

Alacreatine picrate was recrystallized from water (m.p. 187–188.5° with decomposition; lit. m.p. 187° with decomposition[18]).

(2) **Cyclization of Alacreatine to Alacreatinine.** (a).—The reaction mixture was refluxed with an equal volume of 36% hydrochloric acid for 11 hours. Part of the hydrolysate was concentrated to dryness. The residue of alacreatinine hydrochloride was recrystallized from absolute ethanol (m.p. 200–202°; lit. m.p. 202–203°,[25] and 203–204°[18]).

(b).—Alacreatinine picrate, prepared from another portion of the hydrolysate, was recrystallized from water (prisms, m.p. 206–207° dec.; lit. m.p. 212° (dec.),[25] 214–215° dec.[18]).

(c).—A third portion of the hydrolysate was treated with benzenesulfonyl chloride to give 1-benzenesulfonyl-2-imino-5-D,L-methyl-4-imidazolidone (IIIb, m.p. and m.p. of a mixture with IIIb, 208–208.5° with decomposition.)

The 1-benzenesulfonylalacreatinine IIIb, prepared above, gave a picrate which was recrystallized from ethanol to give material identical with 1-benzenesulfonyl alacreatinine picrate (IVb, m.p. and m.p. of mixture with IVb, 183.5° with decomposition).

Miscellaneous Reactions.—The reaction of aqueous glyoxal with N-butylguanidine bicarbonate (in alkaline, neutral and sodium acetate solutions), N-benzylguanidine nitrate, glycine, glycine ethyl ester hydrochloride, ammonium chloride, dimethylamine hydrochloride, acetophenone and dimethylamine hydrochloride (extension of the Mannich reaction), *n*-butylamine (in alkaline, neutral and acidic solutions), ethylenediamine, aniline and D-glutamic acid under experimental conditions similar to those in the reaction with guanidine leads to either the recovery of the original reactants or to decomposition and resinification reactions. Some of this work is still in progress.

Acknowledgment.—The author wishes to thank Mr. Glenn A. Swinehart of the Kerckhoff Microanalytical Laboratory of this Institute for performing the reported microanalyses. This work was supported by grants from the National Institutes of Health, U. S. Public Health Service, and the Surgeon General's Office, Department of the Army, Contract Number DA-49-007-MD-254.

PASADENA, CALIF.

(17) F. Nicola, *Giorn. Farm. Chim.*, **51**, 241 (1902); *Chem. Zentr.*, **73**, II, 296 (1902).

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

(19) H. L. Wheeler and H. F. Merriam, *Am. Chem. J.*, **29**, 478 (1903).

(20) J. Söll and A. Stutzer, *Ber.*, **42**, 4532 (1910).

(21) M. Jaffe, *Z. physiol. Chem.*, **48**, 430 (1906).

(22) H. Ramsay, *Ber.*, **41**, 4385 (1908).

(23) E. Schmidt, *Arch. Pharm.*, **251**, 557 (1913).

(24) M. Bergmann and L. Zervas, *Z. physiol. Chem.*, **172**, 277 (1927).

(25) R. G. Fargher and F. L. Pyman, *J. Chem. Soc.*, **115**, 217 (1919).

(26) E. Abderhalden and H. Sickel, *Z. physiol. Chem.*, **173**, 51 (1928).

(27) C. S. Gibson and B. Levin, *J. Chem. Soc.*, 2754 (1929).

(28) (a) S. G. Hedin, *Ber.*, **23**, 3197 (1890); (b) W. Cocker and J. O. Harris, *J. Chem. Soc.*, 1290 (1940).

(29) K. Poller, *Ber.*, **59**, 1927 (1926).