Studies in Purine Chemistry. XIII. Synthesis of the 5-Aza Analogs of Adenine and Hypoxanthine[1,2]

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5-Azaadenine (1) has been prepared from 3-amino-s-triazole (5) by initial conversion with triethyl orthoformate to N,N'-bis(3-s-triazolyl)formamidine (7), followed by reaction with calcium cyanamide. Although hydrolytic deamination of 1 was not successful, 5-azahypoxanthine (10) was prepared from 5 by reaction with potassium cyanate to give 3-amino-2-(aminocarbonyl)-s-triazole (12a), followed by cyclization with triethyl orthoformate. Chemical and spectroscopic evidence supporting these structural assignments are discussed.

Although many purine analogs must be converted to their ribosides in order to act as purine antagonists, numerous studies have shown that this is not a requisite for biological activity.[4a-e] It even appears probable that, in some cases, a potential purine antagonist may be rendered inactive by conversion *in vivo* to its riboside, and that retention of activity might result from blocking this conversion.[4a] In view of these considerations, derivatives of the s-triazolo[2,3-a]-s-triazine ring system[5] appear to be attractive candidates for purine

purine   s-triazolo[2,3-a]-s-triazine

antagonists. Since the purine ring N–H grouping is missing, conversion to a normal riboside is impossible and derivatives of this system might thus show antitumor activity because of their inability to become deactivated by ribosidation. Furthermore, the suggestion has recently been made[4a] that, in the purine series, positions 3 and/or 7 are the most likely sites for bonding to the receptor sites of enzyme systems utilizing the respective purines. In adenine and adenine analogs, position 1 may also be important as a bonding site. This bonding is presumably a consequence of the basicity of the 1,3- and/or 7-nitrogen atoms, *i.e.*, a sharing of the free electron pair on nitrogen with an

electron acceptor site on the enzyme surface. Consideration of the formal resonance hybrid structures for the adenine derivative 1 derived from the s-triazolo-[2,3-a]-s-triazine ring system indicates a negatively charged periphery, with part of the driving force for charge delocalization being the acquired (4n + 2) electronic distribution possible for the six-membered ring. The amino group in position 5 should assist in this charge separation through delocalization of the positive charge imposed upon the bridgehead nitrogen (structure 1f).[6]
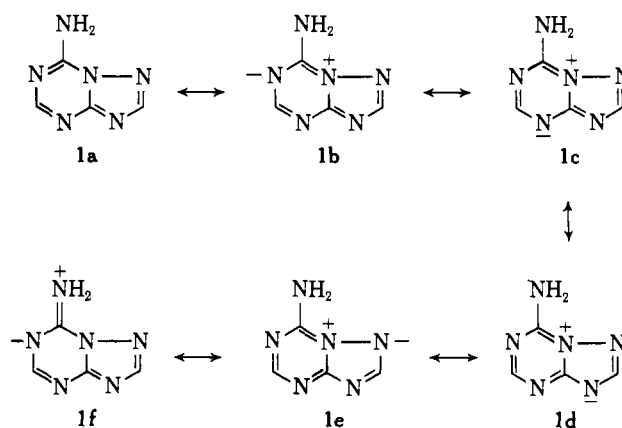
Extensive previous work in this laboratory[7] has shown that o-aminonitriles may be utilized as intermediates for the preparation of fused 4-aminopyrimidines by the sequence of reactions outlined below, in which the final cyclization step involves the nucleophilic
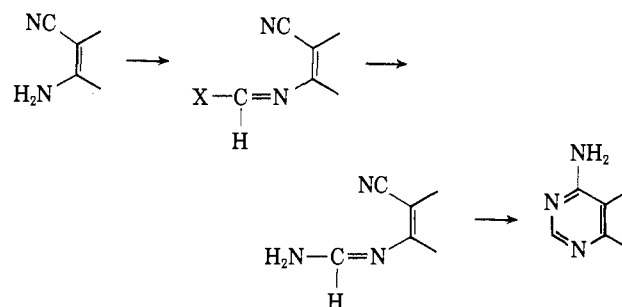
addition of an amino group (in this instance, incorporated as a part of the formamidino side chain) to the o-situated nitrile. A literally interpreted extrapolation of this reaction sequence to the preparation of the desired 5-azaadenine (1) would proceed *via* the inter-

(1) This work was supported in part by a research grant (CA-02551) to Princeton University from the National Cancer Institute, National Institutes of Health, Public Health Service.

(2) For Part XII see E. C. Taylor and E. E. Garcia, *J. Am. Chem. Soc.*, **86**, 4721 (1964).

(3) National Institutes of Health Predoctoral Fellow, 1961–1964.

(4) (a) R. K. Robins, *J. Med. Chem.*, **7**, 186 (1964); (b) G. A. LePage and M. Jones, *Cancer Res.*, **21**, 642 (1961); (c) A. P. Kimball and G. A. LePage, *ibid.*, **22**, 1301 (1962); (d) E. Y. Sutcliffe, K.-Y. Zee-Cheng, C. C. Cheng, and R. K. Robins, *J. Med. Pharm. Chem.*, **5**, 588 (1962); (e) J. Scholler and J. J. Bittner, *Cancer Res.*, **18**, 464 (1958).

(5) For a discussion of previous reports of derivatives of this and the isomeric s-triazolo[4,3-a]-s-triazine ring systems, see (a) W. L. Mosby in "The Chemistry of Heterocyclic Compounds," Vol. 15, A. Weissberger, Ed., Interscience Publishers, Inc., New York, N. Y., 1961, pp. 941–945; (b) V. A. Titkov and I. D. Pletnev, *Zh. Obshch. Khim.*, **33**, 1355 (1963); (c) V. A. Titkov and I. D. Pletnev, U.S.S.R. Patent 122,227; *Chem. Abstr.*, **54**, 8096 (1960).

(6) The importance of zwitterionic structures even for simple purine derivatives has recently been demonstrated on the basis of chemical reactivity studies [L. B. Townsend and R. K. Robins, *J. Am. Chem. Soc.*, **84**, 3008 (1962)]; analogous problems have been studied with spectroscopic techniques [C. W. Noell and R. K. Robins, *J. Heterocyclic Chem.*, **1**, 34 (1964)].

(7) See E. C. Taylor and R. W. Hendess (*J. Am. Chem. Soc.*, **87**, 1995 (1965)) for examples and leading references.

mediate **2**, which would require 2-cyano-3-amino-*s*-triazole (**3**) as the starting material; however, numerous difficulties can be foreseen in the preparation of this unknown precursor. On the other hand, a possible modification of the above synthetic principle which
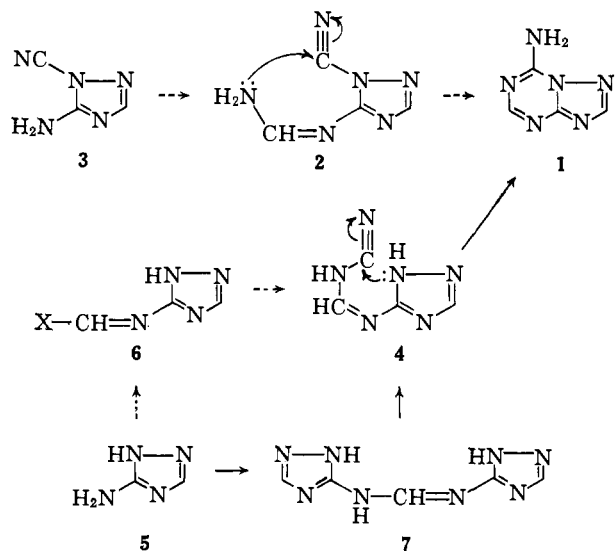
would lead to the same end product **1** would be the utilization of an isomer of **2** in which the nitrile group is incorporated into the formamidino side chain, and the amino group is incorporated into the *s*-triazole ring system. Thus, the intermediate **4** should likewise be capable of intramolecular cyclization to **1**. This intermediate in turn should be available from 3-amino-*s*-triazole (**5**) by initial conversion to the intermediate **6** in which the substituent grouping X is capable of elimination with its bonding electron pair. A search for readily accessible compounds of this type revealed that the ethoxymethylenamino derivative **6** (X = OC_2H_5) is not known, but that the N,N'-disubstituted formamidine **7** is known[8]; it is, in fact, the product formed from 3-amino-*s*-triazole (**5**) and triethyl orthoformate, a reaction which commonly leads to ethoxymethylenamino derivatives. We thereforet reated **7** with calcium cyanamide in the form of the commercially available "Temposil" ($^1/_3$ calcium cyanamide and $^2/_3$ citric acid by weight). The reaction proceeded smoothly to give 5-azaadenine (**1**) in good yield. The absence of a nitrile band in the infrared spectrum of the product indicates that cyclization of **4** had indeed taken place.

It should be pointed out at this time that a definite ambiguity exists as to the actual structure of the compound which we have designated as 5-azaadenine. First of all, it is possible that intramolecular cyclization of the intermediate **4** could have taken place by participation of N-4 rather than N-2, giving rise to the isomeric *s*-triazolo[4,3-*a*]-*s*-triazine derivative **8**.[5] We
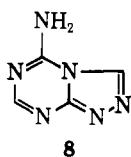
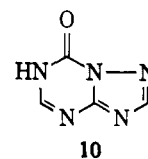feel that involvement of N-2 is more likely because of

(8) J. D. Kendall and H. G. Suggate, U. S. Patent 2,534,914 (Dec. 1950); *Chem. Abstr.*, **45**, 2350 (1951).

its greater basicity; it is adjacent to nitrogen rather than electron-deficient carbon (C-5). Secondly, the possibility exists that compound **7**, which has been assumed to have the structure assigned,[8] could alternately be represented by **7a**. Attempts to resolve this structural question by n.m.r. were inconclusive. If **7a** were correct, reaction with calcium cyanamide could take the course indicated below and lead to 2-amino-5-azapurine (7-amino-*s*-triazolo[2,3-*a*]-*s*-triazine (**9**)), a second structural isomer of **1**. Supporting, although not conclusive, evidence in favor of structure **1** as opposed to **8** or **9** is found in a comparison of the ultraviolet spectra of the reaction product ($\lambda_{max}^{0.1\ N\ HCl}$ 261 mμ; $\lambda_{max}^{H_2O}$ 258 mμ) with the spectra of adenine ($\lambda_{max}^{0.1\ N\ HCl}$ 262.5 mμ, $\lambda_{max}^{H_2O}$ 260.5 mμ)[9a] and 2-aminopurine ($\lambda_{max}^{pH\ 1.84}$ 237 and 314 mμ, $\lambda_{max}^{pH\ 7}$ 236 and 305 mμ).[9b] Thus, although we have been unable
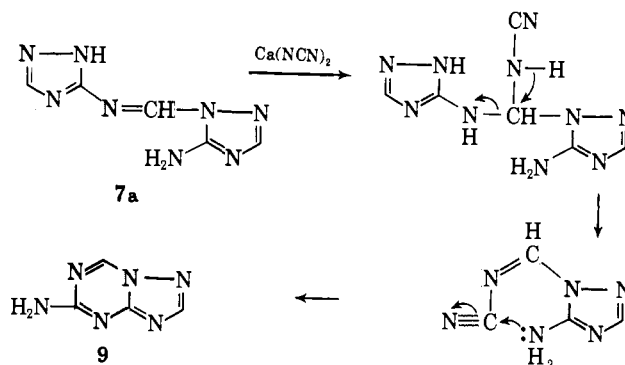
to design experiments which would distinguish unequivocally among these three isomeric structures, we feel that a reasonable choice can be made in favor of structure **1** for our azaadenine product.

Attempts to convert **1** to 5-azahypoxanthine (**10**) were unsuccessful. Compound **1** was stable to nitrous acid and to cold nitrosyl sulfuric acid and was de-

composed at higher temperatures by the latter reagent. The desired hypoxanthine analog **10** was, however, obtained by an independent synthetic sequence which is outlined below.

Reaction of 3-amino-*s*-triazole (**5**) with potassium cyanate in acid solution gave a monoureido derivative which could be considered to have any of the structures **11a–d**. In spite of the arguments advanced by Williams[10] that (in acid solution) the exocyclic nitrogen in 3-amino-*s*-triazole (**5**) is the most nucleophilic of the four nitrogen atoms, and contrary to the report of Kaiser and Peters[11] that 3,5-diamino-*s*-triazole gives the 3,5-diureido derivative, we were able to show conclusively that our monoureido derivative did *not* possess structure **11d**. This latter structure has two separate N–H groups and one NH_2 group, and its

(9) (a) "The Nucleic Acids," Vol. 1, E. Chargaff and J. N. Davidson, Ed., Academic Press Inc., New York, N. Y., 1955, p. 502; (b) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).
(10) L. A. Williams, *ibid.*, 3046 (1961).
(11) D. W. Kaiser and G. A. Peters, U. S. Patent 2,723,275 (Nov. 1955); *Chem. Abstr.*, **50**, 10135 (1956).

n.m.r. spectrum would therefore be expected to exhibit a single, sharp, CH peak and three broader NH peaks in the ratio 1:1:1:2. The monoureido derivative


11a

11b

11c

11d
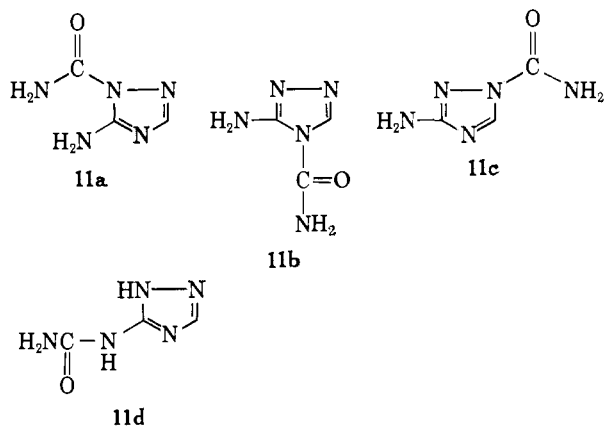
obtained in the above reaction, however, showed one CH peak and two NH peaks in the ratio 1:2:2. Structure 11d is thus excluded, although this evidence cannot be used to distinguish among the other three possibilities.

Structure 11c was eliminated as a result of the following experiment. Treatment of the monoureido derivative (11a, 11b, or 11c) with triethyl orthoformate gave a cyclized derivative which must be either 10 or 12. This result firmly excludes structure 11c which cannot

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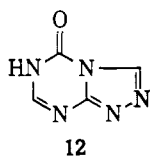
undergo cyclization with triethyl orthoformate. Of the two isomeric cylization products 10 and 12, we prefer the former for the same reasons which were previously advanced in favor of 1 as opposed to 8. This assignment receives support in the striking similarity of the ultraviolet spectra of 10 ($\lambda_{max}^{0.1\ N\ HCl}$ 247 mμ; $\lambda_{max}^{H_2O}$ 250 mμ (broad), and $\lambda_{max}^{0.1\ N\ NaOH}$ 244 (sh) and 257 mμ) with the spectra of hypoxanthine[9a] ($\lambda_{max}^{0.1\ N\ HCl}$ 248 mμ, $\lambda_{max}^{H_2O}$ 249.5 mμ, and $\lambda_{max}^{pH\ 9-12}$ 258.5 mμ).

Methylation of 10 by treatment of its silver salt with refluxing methyl iodide, or by reaction in ethereal slurry with diazomethane, or with dimethyl sulfate in ethanol, was shown to give the 6-methyl derivative 13
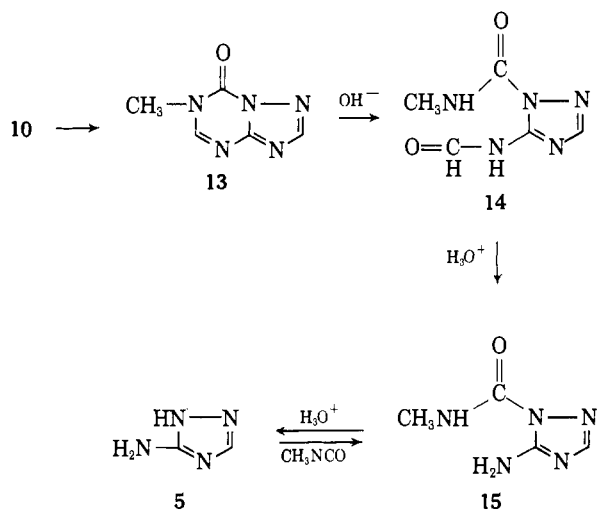
by means of the following sequence of reactions. Mild alkaline hydrolysis of the monomethyl derivative 13 gave a product whose microanalysis indicated the addition of one molecule of water. The same product was obtained by reaction of 10 with dimethyl sulfate in aqueous sodium hydroxide solution. This hydrolysis product was shown to have structure 14 by examination of its n.m.r. spectrum, which exhibited (in dimethyl sulfoxide) four peaks (exclusive of the methyl peak which was obscured by solvent) in the ratio 1:1:1:1, due to two single N–H protons, one formamide C–H proton, and one aromatic C–H proton. Dilute acid hydrolysis of this formylamino derivative 14 resulted in loss of the formyl group to give the methylamide 15 which on further acid hydrolysis gave 3-amino-s-triazole (5). Finally, treatment of the latter compound with methyl isocyanate regenerated the methylamide 15. This reaction sequence not only firmly establishes the position of methylation on the nitrogen atom adjacent to the oxygen in 10 (or 12), but suggests that structure 10 is probably correct, since the reaction of 3-amino-s-triazole with methyl isocyanate to give a methylamide should lead to 15 rather than the corresponding product derived from 12 because of the anticipated greater basicity of N-2 as contrasted with N-4.

Experimental[12]

5-Amino-s-triazolo[2,3-a]-s-triazine (5-Azaadenine) (1). A mixture of 9.0 g. of N,N'-bis(3-s-triazolyl)formamidine,[8] 56 g. of Lederle "Temposil" ($^1/_3$ calcium cyanamide and $^2/_3$ citric acid by weight), and 200 ml. of dimethylformamide was heated under reflux for 24 hr. with mechanical stirring. It was then filtered hot and the residue was extracted with two 50-ml. portions of hot dimethylformamide. The combined filtrate and extracts were evaporated to dryness under reduced pressure and the dry residue was sublimed at 240° (0.05 mm.) to give 4.0 g. (58%) of a white solid. Recrystallization from water then gave fine white needles: m.p. 320° dec., $\lambda_{max}^{0.1\ N\ NaOH}$ 249 and 274 (sh) mμ ($\epsilon \times$ 10^{-3} 11.5 and 6.0), $\lambda_{max}^{0.1\ N\ HCl}$ 261 mμ ($\epsilon \times$ 10^{-3} 7.8).

Anal. Calcd. for C$_4$H$_4$N$_6$: C, 35.29; H, 2.96; N, 61.75. Found: C, 35.43; H, 3.14; N, 61.80.

3-Amino-2-(aminocarbonyl)-s-triazole (11a). A solution of 8.4 g. of 3-amino-s-triazole and 8.1 g. of potassium cyanate in 150 ml. of water containing 8.5 ml. of concentrated hydrochloric acid was stirred at room temperature for 2 hr. During this period the product separated out as white crystals. The cooled mixture was filtered and the collected product was washed with cold water and dried to give 9.8 g. (77%). Recrystallization from water gave white crystals: m.p. 172–174° dec., $\lambda_{max}^{ethanol}$ 236 mμ ($\epsilon \times$ 10^{-3} 7.0), $\lambda_{max}^{0.1\ N\ HCl}$ 222 mμ ($\epsilon \times$ 10^{-3} 8.5).

Anal. Calcd. for C$_3$H$_5$N$_5$O: C, 28.35; H, 3.97; N, 55.10. Found: C, 28.35; H, 3.88; N, 54.92.

s-Triazolo[2,3-a]-s-triazin-5(6H)-one (5-Azahypoxanthine) (10). A slurry of 50.8 g. of 3-amino-2-(aminocarbonyl)-s-triazole in 450 ml. of triethyl orthoformate was heated on a steam bath for 18 hr. with mechanical stirring. The ethanol which was formed in the cycli-

zation reaction was removed by distillation under reduced pressure and the residual mixture was cooled in ice. The precipitated solid was collected by filtration and dried to give 47.6 g. which upon recrystallization from water gave white crystals of the hemihydrate, m.p. 244° dec.

Anal. Calcd. for $C_4H_3N_5O \cdot 0.5H_2O$: C, 32.85; H, 2.74. Found: C, 32.44; H, 3.03.

Drying the hemihydrate at 100° *in vacuo* for 6 hr. over phosphorus pentoxide gave 34 g. (62%) of the anhydrous product: m.p. 271° dec., $\lambda_{max}^{0.1\ N\ NaOH}$ 244 (sh) and 257 mμ ($\epsilon \times 10^{-3}$ 6.6 and 7.1), $\lambda_{max}^{0.1\ N\ HCl}$ 247 mμ ($\epsilon \times 10^{-3}$ 5.3).

Anal. Calcd. for $C_4H_3N_5O$: C, 35.04; H, 2.21; N, 51.09. Found: C, 35.04; H, 1.87; N, 51.40.

Hydrolysis of this material either with hot 6 N hydrochloric acid or with hot 1 N sodium hydroxide resulted in ring cleavage to give 3-amino-s-triazole in approximately 50% yield.

6-Methyl-s-triazolo[2,3-a]-s-triazin-5(6H)-one (1-Methyl-5-azahypoxanthine) (13). *Method A.* A solution of 10 g. of 5-azahypoxanthine in 1 l. of water at *ca.* 65° was treated with aqueous silver nitrate until no further precipitation occurred. The mixture was then cooled in ice, filtered, and the collected solid was dried to give 17.0 g. (98%) of the silver salt as a white powder. Ten grams of this material was then heated under reflux with 50 ml. of methyl iodide for 12 hr. with constant stirring. Excess methyl iodide was evaporated by means of a stream of air and the pale yellow, dry residue was extracted in a Soxhlet extractor with dioxane. The residual yellow residue was again treated with methyl iodide and the reaction product was treated again as described above. This process was repeated until no further product could be obtained by dioxane extraction. The combined dioxane extracts were then evaporated to dryness to give 2.85 g. (45%), m.p. 213–217° dec., which upon recrystallization from dioxane gave white crystals: m.p. 220–222° dec., $\lambda_{max}^{ethanol}$ 257 mμ ($\epsilon \times 10^{-3}$ 3.5), $\lambda_{max}^{0.1\ N\ HCl}$ 254 mμ ($\epsilon \times 10^{-3}$ 6.5).

Anal. Calcd. for $C_5H_5N_5O$: C, 39.73; H, 3.33; N, 46.34. Found: C, 39.71; H, 3.61; N, 45.77.

Method B. A solution of 1 g. of 5-azahypoxanthine in 20 ml. of ethanol containing 0.34 g. of sodium was treated dropwise over a period of 15 min. and with shaking with 1.0 ml. of dimethyl sulfate. The mixture was then heated under reflux for 5 min., cooled, and neutralized with dilute hydrochloric acid. The precipitated solid was collected by filtration, washed with water, dried, and sublimed at 160° (0.05 mm.) to give a white product, m.p. 185–195° dec. Recrystallization from dioxane then gave white crystals, m.p. 220–222° dec., identical with the material prepared by method A, as determined by a comparison of infrared spectra.

Method C. A slurry of 10.0 g. of 5-azahypoxanthine in 100 ml. of ether was treated with an ethereal solution of diazomethane (from 25 g. of Du Pont's EXR-101 precursor). The reaction mixture was stirred at 0° for 2 hr. and filtered to give 10.3 g. (93%) of a tan solid, m.p. 220–222° dec., identical with the material prepared by methods A and B, as described above, as determined by a comparison of infrared spectra.

3-Formylamino-2-(methylaminocarbonyl)-s-triazole (14). *Method A.* To a mixture of 12.2 ml. of 6 N sodium hydroxide (2 equiv.) and 73 ml. of water, maintained at 0°, was added 5.0 g. of 5-azahypoxanthine. During a 10-min. period, 4.6 g. of dimethyl sulfate was added while the solution was stirred at 0°. After an additional 10 min. of stirring, the mixture was neutralized with 6 N hydrochloric acid and the precipitated solid was collected by filtration to give 0.5 g. which upon recrystallization from acetonitrile gave white needles; m.p. 163–164° dec., $\lambda_{max}^{0.1\ N\ NaOH}$ 239 mμ ($\epsilon \times 10^{-3}$ 6.7), $\lambda_{max}^{0.1\ N\ HCl}$ 222 mμ ($\epsilon \times 10^{-3}$ 11.4).

Anal. Calcd. for $C_5H_7N_5O_2$: C, 35.50; H, 4.17; N, 41.41. Found: C, 35.35; H, 4.15; N, 41.50.

Acidification of the reaction filtrate to pH 4 resulted in the separation of 2.4 g. of unchanged starting material.

Method B. A solution of 3.6 g. of 6-methyl-s-triazolo[2,3-a]-s-triazin-5(6H)one in 40 ml. of 0.5 N sodium hydroxide was allowed to stand at room temperature for 20 min., cooled to 0°, and neutralized with hydrochloric acid. The product which separated was collected by filtration to give 2.7 g. of a white solid, m.p. 163–164°, identical with the product obtained by method A above.

3-Amino-2-(methylaminocarbonyl)-s-triazole (15). *Method A.* A mixture of 0.8 g. of 3-formylamino-2-(methylaminocarbonyl)-s-triazole in a solution of 4 ml. of 6 N hydrochloric acid and 40 ml. of water was heated under reflux for 30 min., cooled to room temperature, and neutralized with dilute sodium hydroxide. The precipitate which separated upon cooling was collected by filtration to give 0.4 g. of product. Evaporation of the filtrate to dryness and sublimation of the residue at 160° (0.5 mm.) gave an additional 0.14 g. of product; total yield, 0.54 g. (81%). Recrystallization from water followed by vacuum sublimation gave a white crystalline product: m.p. 191–193° dec., $\lambda_{max}^{ethanol}$ 235 mμ ($\epsilon \times 10^{-3}$ 7.9).

Anal. Calcd. for $C_4H_7N_5O$: C, 34.04; H, 5.00; N, 49.63; NCH_3, 10.63. Found: C, 34.31; H, 4.86; N, 49.65; NCH_3, 10.63.

Method B. To a mixture of 8.4 g. of 3-amino-s-triazole in 100 ml. of water containing 8.5 ml. of concentrated hydrochloric acid was added 5.7 g. of methyl isocyanate dissolved in 50 ml. of ethanol, and the solution was stirred for 48 hr. at room temperature. The precipitated solid was collected by filtration to give 2.1 g. (15%), m.p. 191–193° dec., identical with the product obtained by method A above.

Hydrolysis of 3-Amino-2-(methylaminocarbonyl)-s-triazole (15). A solution of 0.55 g. of 3-amino-2-(methylaminocarbonyl)-s-triazole in 8 ml. of concentrated hydrochloric acid was heated on a steam bath for 2 hr. and then evaporated to dryness. The residue was dissolved in water, neutralized with sodium hydroxide, and again evaporated to dryness. The residue was extracted with ethanol and the extract was concentrated to *ca.* 5 ml. and cooled to give a small amount of solid which was discarded. Evaporation of the filtrate to dryness and recrystallization of the residue from a small amount of ethanol then gave 0.30 g. (91%) of 3-amino-s-triazole, identical with an authentic sample.