[CONTRIBUTION FROM THE OHIO STATE UNIVERSITY RESEARCH FOUNDATION]

Triazines. XIX. Some Reactions of s-Triazine with Hydrazine and its Organic Derivatives

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s-Triazine (I) is cleaved by anhydrous hydrazine to 1,2-diformylhydrazine dihydrazone (IV). The reactions of IV with carbonyl compounds and acetic anhydride, and its thermal decomposition to 4-amino-1,2,4-triazole and 1,2,4-triazole are described. I and aromatic hydrazines yield N,N'-diaryl-formazans. Semicarbazide, thio-semicarbazide and aminoguanidine lead to the corresponding 1,2,4-triazole derivatives.

We recently reported that *s*-triazine (I) reacts easily with primary amines with complete cleavage of the ring forming ammonia and N,N'-disubstituted formamidines.[3a] The reaction of *s*-triazine with the hydrohalides of hydrazine and monoalkyl- or monoaryl hydrazines leads to 1,2,4-triazoles.[3b] It was anticipated that free hydrazine would react like primary amines. In this case, the reaction product should be the as yet unknown formhydrazide hydrazone (II) or formamide hydrazone (III). In fact, a mixture of *s*-triazine and anhydrous hydrazine reacts even at room temperature. The reaction becomes very vigorous on gentle heating and consequently is better performed in an inert diluent. The product, m.p. 122–123° dec., is formed in good yield and has been shown to be identical with a compound obtained long ago from hydrocyanic acid and hydrazine,[4] and not the expected II or III. The earlier procedure gave only very small yields, our reaction makes this interesting compound readily accessible. On the basis of their degradation experiments, Müller and Herrdegen assigned to this product the formula 1,2-diformylhydrazine dihydrazone (IV). Their findings, however, would also be compatible with the structures II and III. Since IV is, by elementary analysis, almost indistinguishable from III and since molecular weight determinations gave somewhat erratic results because of the instability of this compound, much effort was spent on proving its constitution. With picric acid under various conditions, only hydrazine picrate was isolated. Ethereal hydrogen chloride formed a dihydrochloride which was stable on storage in contrast to the parent compound IV. In water, however, the dihydrochloride was easily hydrolyzed and underwent extensive decomposition. Benzoylation of IV yielded only N,N'-dibenzoylhydrazine. The compound IV condensed readily with carbonyl compounds. The dibenzal derivative V already has been described,[4] but is not very suitable for the characterization of IV because of its instability. The product from acetone appears to be the best. The 1,2-diformylhydrazine bis-(isopropylidenehydrazone) (VI) is a fairly stable and recrystallizable compound which, however, is difficult to obtain analytically pure as is the dibenzal derivative. Contrary to V, VI was obtained from IV in an appreciable yield. Based on the formation of VI, then, it is reasonable to conclude that IV must contain at least two $-N-NH_2$ groups. This excludes IVa, a hydrazinium salt of 1,2-dihydro-1,2,-4,5-tetrazine,[5] which otherwise would be compatible with the experimental findings.[6] Final proof of the constitution of IV was found in the complete hydrolysis with aqueous hydrochloric acid which yielded, besides formic acid, only hydrazine and no ammonia. This result definitely excludes formula III for the primary reaction product from hydrazine and *s*-triazine.

The formation of 1,2-diformylhydrazine dihydrazone (IV) from *s*-triazine and hydrazine could be explained by the assumption that, at first, in a normal cleavage of the *s*-triazine ring, three moles of formhydrazide hydrazone (II) are formed

$$C_3H_3N_3\,(I) + 6N_2H_4 = 3C_6H_2H_4\,(II) + 3NH_3$$
$$2CH_6H_4\,(II) = C_2H_8N_6\,(IV) + N_2H_4$$

and then two moles of the unstable intermediate II condense with loss of hydrazine to IV.

The 1,2-diformylhydrazine dihydrazone (IV) is light sensitive , turning pink, especially in the presence of air. This color is due apparently to the formation of 1,2,4,5-tetrazine (VIII), since the coloring matter is readily soluble in ether and volatile with the vapors of this solvent.[5,7] The first stage of this light-induced decomposition of IV is presumably the formation of 1,2-dihydro-1,2,4,5-tetrazine (VII)—or an isomer thereof—which then suffers dehydrogenation either spontaneously or by air oxidation. That, in fact, VII can easily be formed from IV is demonstrated by the reaction of IV with acetic anhydride. This leads to a product to which we have assigned the structure 1,2-diacetyl-1,2-dihydro-1,2,4,5-tetrazine (IX). The other possible constitution, 4-diacetylamino-1,2,4-triazole (X), is excluded by the synthesis of X from 4-amino-1,2,4-triazole (XII) and acetic anhydride, which yields a compound quite different from IX. Furthermore, IX is hydrolyzed readily by methanolic potassium hydroxide and the resulting intense red-colored solution presents, by its reactions, strong evidence for the presence of 1,2,4,5-tetrazine. 1,2,4,5-Tetrazine has not yet been isolated from the light-induced degradation products of IV, however. This failure stems partly from the in-

(1) This article is based on work performed under project 116-B of The Ohio State University Research Foundation sponsored by the Olin Mathieson Chemical Corporation, New York, N. Y.

(2) Preceding communication: Ch. Grundmann and E. Kober, THIS JOURNAL, **79**, 944 (1957).

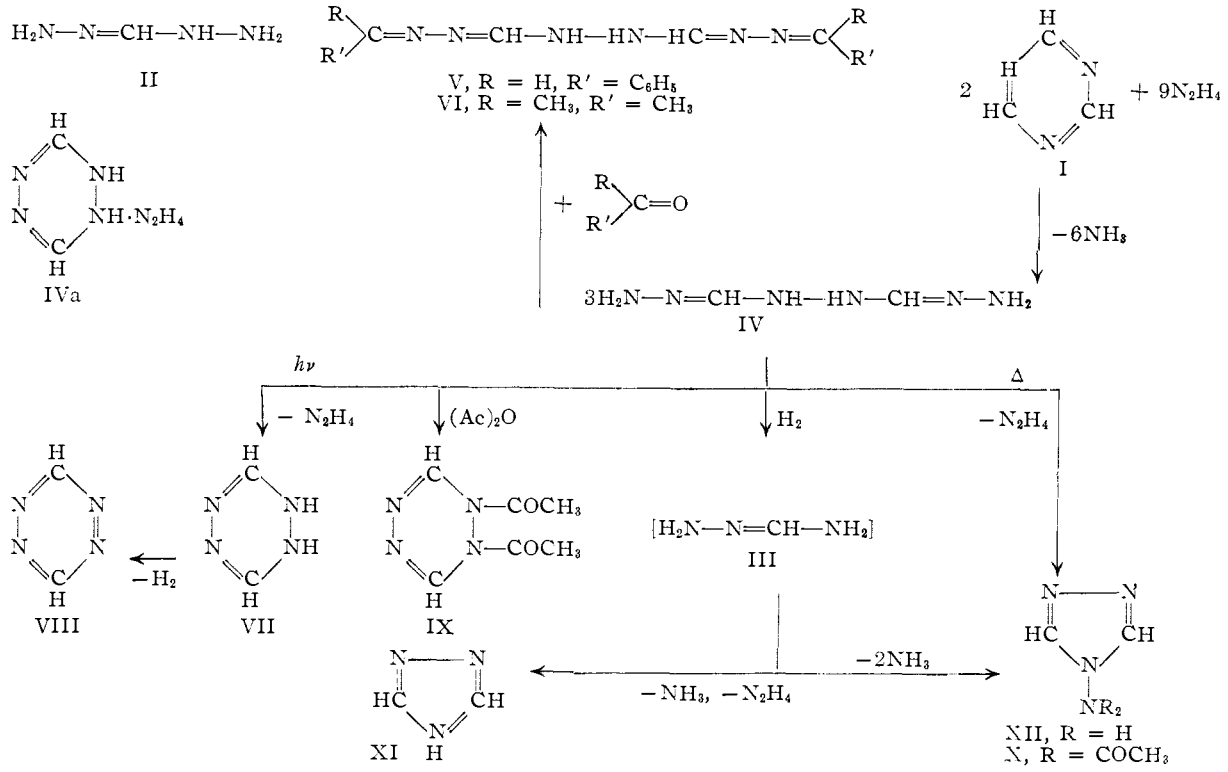
(3) (a) Ch. Grundmann and A. Kreutzberger, *ibid.*, **77**, 6559 (1955); (b) Ch. Grundmann and R. Rätz, *J. Org. Chem.*, **21**, 1037 (1956).

(4) E. Müller and L. Herrdegen, *J. prakt. Chem.*, [2] **102**, 113 (1921).

(5) 1,2-Dihydro-1,2,4,5-tetrazine is weakly acidic, Th. Curtius, A. Darapsky and E. Müller, *Ber.*, **40**, 837 (1907).

(6) Müller and Herrdegen, ref. 4, have already discussed this possibility.

(7) Th. Curtius, A. Darapsky and E. Müller, *Ber.*, **40**, 84 (1907); D. Wood, Jr., and F. W. Bergstrom, THIS JOURNAL, **55**, 3648 (1933).



herent difficulty in isolating small quantities of such a volatile and sensitive substance as VIII, and also from the presence of another route of spontaneous decomposition of IV.

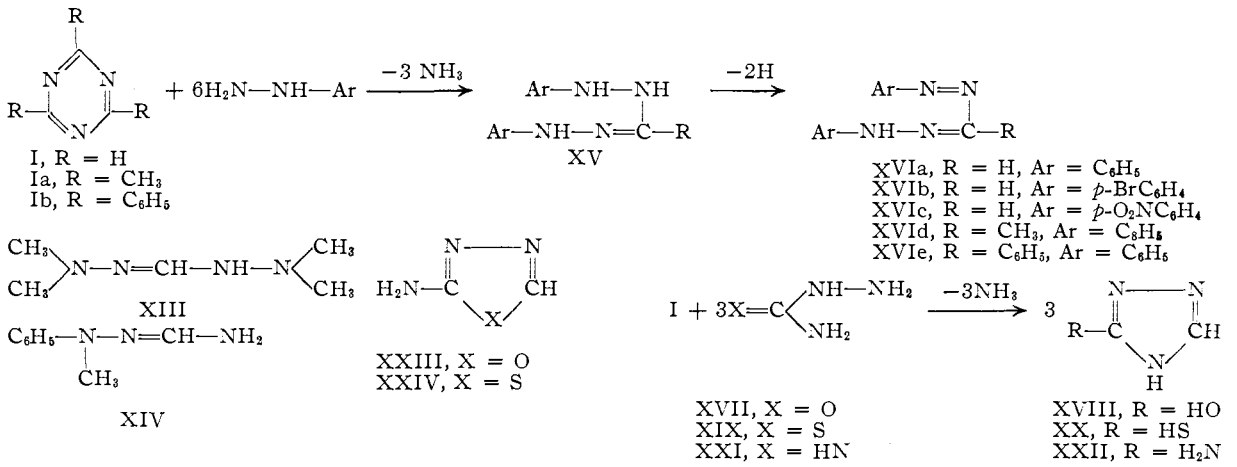
This second route of decomposition proceeds even in the dark and in a vacuum. It converts the hydrazoformaldehyde dihydrazone within several days at room temperature into a brownish, viscous oil. By fractional vacuum sublimation, we were able to isolate hydrazine, 1,2,4-triazole (XI, 38%), and 4-amino-1,2,4-triazole (XII, 15%) from this oil. The latter compound already has been noticed by Müller and Herrdegen among the thermal degradation products of IV.

While the formation of XII is easily understandable as the result of a ring closure of IV with the loss of one molecule of hydrazine, an explanation of the formation of XI seems much more difficult because it demands a reductive step in one or another phase of the self-decomposition of IV. At first, we made certain that 1,2,4-triazole is not an artificial product resulting from our mode of isolating the reaction product. 4-Amino-1,2,4-triazole is not converted into 1,2,4-triazole under the conditions of our experiments, either thermally or by the reductive action of hydrazine. The assumption that IV itself is reduced to formamide hydrazone (III), either by hydrazine, always present in the decomposing mixture, or by an internal oxidation–reduction process,[8] seems to be the only way to explain our results. That such a process may occur during the spontaneous decomposition of IV is indicated by a constant evolution of ammonia from the decomposing mixture. This ammonia can only stem from the reaction $2N_2H_4 = 2NH_3 + N_2 + H_2$, which yields the necessary reductive equivalent for the conversion of IV into III. Two molecules of this hypothetical intermediate III could then decompose to 1,2,4-triazole (XI) by loss of one molecule of ammonia and one molecule of hydrazine. By loss of two molecules of ammonia III could also yield 4-amino-1,2,4-triazole (XII).

Substituted hydrazines, as the free bases, react quite differently from the parent compound. In the first step aromatic hydrazines, like phenylhydrazine, apparently form diaryl-formhydrazide hydrazones (XV), which are not isolable but undergo spontaneous oxidation, either by air or by excess arylhydrazine, to the stable, intensively colored N,N′-diphenylformazans (phenylazo-formaldehyde phenylhydrazones). This reaction was successfully carried out with phenylhydrazine, *p*-bromophenylhydrazine and *p*-nitrophenylhydrazine, to yield the formazans XVIa, XVIb and XVIc. While the cleavage of *s*-triazines by primary amines is restricted to the parent compound, aromatic hydrazines cleave besides the parent compound I also trialkyl- and triaryl-*s*-triazines in the same way. From 2,4,6-trimethyl-*s*-triazine (Ia) we obtained the N,N′-diphenyl-C-methyl-formazan (XVId) and from 2,4,6-triphenyl-*s*-triazine (Ib) the N,N′-diphenyl-C-phenylformazan (XVIe).

With disubstituted hydrazines, less clear results were obtained. *asym*-Dimethylhydrazine reacts with *s*-triazine in a manner strictly analogous to a primary amine, yielding in this case the 1-formyl-2,2-dimethylhydrazine 2,2-dimethylhydrazone (XIII), the parent compound of which II is not known. With 1-methyl-1-phenylhydrazine, how-

(8) Reduction of hydrazo compounds by hydrazine derivatives to the corresponding amino compounds has been observed on different occasions, *cf.* R. Walther, *J. prakt. Chem.*, [2] **52**, 141 (1895); H. Beyer and G. Wolter, *Chem. Ber.*, **85**, 1079 (1952).



ever, the reaction proceeds only half-way. Without elimination of ammonia, but with breakdown of the *s*-triazine ring the N^3-methylphenylformamidrazone (XIV) is formed as the sole product. Even with a large excess of 1-methyl-1-phenylhydrazine and elevated temperatures, it was impossible to force the reaction to the tetra-substituted hydrazine derivative analogous to XIII. No explanation can be given at present for this striking difference in reactivity between the *asym*-dimethyl- and the 1-methyl-1-phenylhydrazine. As expected, symmetrically disubstituted hydrazines which do not contain an NH_2 group do not react with *s*-triazine under these conditions.

Among other hydrazine derivatives we have investigated the reaction of semicarbazide (XVII), thiosemicarbazide (XIX) and aminoguanidine (XXI) with *s*-triazine. In all cases these derivatives react as described previously for *o*-substituted diamines,[3a] forming, with cleavage of the triazine ring and liberation of ammonia, the corresponding 3-substituted-1,2,4-triazoles, 3-hydroxy-1,2,4-triazole (XVIII), 3-mercapto-1,2,4-triazole (XX) and 3-amino-1,2,4-triazole (XXII). It is interesting to note that in the case of XVII and XIX no formation of other possible products of ring closure, namely, 2-amino-1,3,4-oxadiazole (XXIII) and 2-amino-1,3,4-thiadiazole (XXIV) could be detected.[9]

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Experimental[10]

1,2-Diformylhydrazine Dihydrazone (IV).—With exclusion of direct light 8 g. of anhydrous hydrazine was added dropwise to a solution of 4.5 g. of *s*-triazine in 50 ml. of anhydrous ether. After standing overnight at room temperature, the almost colorless solid was collected under nitrogen and washed with small amounts of ice-cold anhydrous ether and then dried in a vacuum desiccator in the dark for two hours over phosphorus pentoxide; yield 80%. This crude product was best purified by digesting the finely powdered material with 5 ml. of cold absolute alcohol for 30 minutes followed by rapid filtration under nitrogen. A repetition of this procedure yielded 4.8 g. of pure IV as a micro-crystalline yellowish powder melting at 122–123° dec. No solvent could be found for IV in which this compound is soluble without decomposition.

Anal. Calcd. for $C_2H_8N_6$: C, 20.68; H, 6.95; N, 72.37. Found: C, 21.00; H, 7.09; N, 72.20.

Within a few seconds of exposure to the atmosphere IV turned pink, later red, but such samples kept *in vacuo* over concentrated sulfuric acid soon regained their original color (evaporation of the formed 1,2,4,5-tetrazine). The coloring matter could also be extracted with ether. After evaporation of such extracts, practically nothing remained, the red substance being entrained with the ether vapors.

The dihydrochloride of 1,2-diformylhydrazine dihydrazone was formed from a suspension of 5.8 g. of IV in 100 ml. of dry carbon tetrachloride by passing through a stream of dry hydrogen chloride for 24 hours, followed by vacuum filtration of the suspension. The filter cake was washed with dry carbon tetrachloride and dried on a porous plate in a desiccator over calcium chloride and paraffin chips for three days; m.p. 74–76° dec. All the above operations should be carried out with exclusion of moisture. The hydrochloride is extremely hygroscopic and is easily decomposed by water, the only isolable product in such cases being hydrazine hydrochloride.

Anal. Calcd. for $C_2H_8N_8 \cdot 2HCl$: C, 12.71; H, 5.33; Cl, 37.51. Found: C, 12.72; H, 6.12; Cl, 37.00.

The dihydrochloride apparently is stable for an unlimited time sealed in a glass tube.

With picric acid in aqueous solution or in alcoholic suspension, IV yielded a yellow crystalline picrate which was identified by analysis and mixed melting point (187–188°) with **hydrazine picrate.**[11]

Anal. Calcd. for $C_6H_7N_5O_7$: N, 26.82. Found: N, 26.62, 26.40.

Benzoylation of IV was carried out with benzoyl chloride in anhydrous quinoline and also by Pinner's procedure for the benzoylation of amidrazones,[12] but the only isolable product proved to be **N,N'-dibenzoylhydrazine**, according to analysis and a mixed melting point (245–246°) with an authentic sample.

Anal. Calcd. for $C_{14}H_{12}N_2O_2$: C, 69.98; H, 5.04; N, 11.66. Found: C, 70.01; H, 5.07; N, 11.71.

Acid Hydrolysis of IV.—Upon addition of 15 ml. of concentrated hydrochloric acid to 1.5 g. of freshly prepared pure IV the mixture became hot and foamed. When the initial reaction had subsided, the solution was evaporated on the steam-bath to dryness. The residue was dissolved in 30 ml. of water and again evaporated to dryness. This procedure was repeated twice more in order to remove all formic acid. The remaining white crystalline salt was dried in a desiccator over calcium chloride and analyzed for hy-

(9) Compare in this respect the formation of benzoxazole and benzthiazole from *s*-triazine and *o*-aminophenol, resp., *o*-aminothiophenol, Ch. Grundmann and A. Kreutzberger.[3]

(10) All melting points are corrected, microanalyses are from Galbraith Laboratories, Knoxville, Tenn., and Spang Microanalytical Laboratory, Ann Arbor, Michigan.

(11) R. von Rothenburg, *Ber.*, **27**, 690 (1894).

(12) A. Pinner, *ibid.*, **27**, 992 (1894).

drazine and ammonia by the method of the Mathieson Chemical Corporation.[13]

Anal. Calcd. for $N_2H_4 \cdot 2HCl$: N_2H_4, 30.53. Found: N_2H_4, 32.34, 32.69; NH_3, 0.00, 0.00.

Synthetic mixtures of ammonium formate and hydrazine dihydrochloride, treated as described above, yielded the calculated values for hydrazine and ammonia.

1,2-Diformylhydrazine Bis-(isopropylidenehydrazone) (VI).—A suspension of 3.2 g. of IV in 70 ml. of dry acetone gave a clear solution after refluxing for three hours. From the reddish solution 2.4 g. of large white crystals of VI separated on cooling. These crystals were rapidly filtered off and washed with cold dry acetone. Upon exposure to air, the crystals tend to turn slightly pink, a color which is afterwards difficult to remove. For purification they were recrystallized from acetone, under exclusion of air and moisture, yielding white prisms which, after drying over phosphorus pentoxide *in vacuo*, melted at 113°.

Anal. Calcd. for $C_8H_{16}N_6$: C, 48.95; H, 8.22; N, 42.83. Found: C, 47.78; H, 8.07; N, 42.32.

Repeated recrystallization of VI from acetone as well as other attempts at further purification did not yield better analytical values.

Condensation of IV was also attempted with benzaldehyde,[14] anisaldehyde, *o*-nitrobenzaldehyde, methyl ethyl ketone and acetophenone, but this resulted only in the isolation of the corresponding azines, while diethyl ketone and phenylacetone yielded only resins, and benzophenone and phenanthrenequinone did not react at all.

1,2-Diacetyl-1,2-dihydro-1,2,4,5-tetrazine (IX).—An amount of 10.5 g. of IV was added portionwise to 40 ml. of acetic anhydride. Dissolution occurred immediately with evolution of heat, while the color of the solution changed to orange. The reaction mixture was heated for two hours on the steam-bath and then the excess acetic anhydride distilled off *in vacuo*. The distillate was colored red by a volatile by-product, probably 1,2,4,5-tetrazine. In the distillation flask there remained a yellowish transparent sirup which slowly crystallized upon standing. The crude product was freed from adhering resinous material by dissolving in 17 ml. of hot acetone and decolorizing with charcoal. After cooling and filtration, 3.9 g. of a yellowish micro-crystalline substance was obtained. Upon repeated vacuum sublimation of this crude material, pure IX was obtained as faintly yellowish needles melting at 149–150°.

Anal. Calcd. for $C_6H_8N_4O_2$: C, 42.86; H, 4.80; N, 33.32; mol. wt., 168. Found: C, 42.57; H, 4.84; N, 33.07; mol. wt. (Rast), 168, 174.

IX has neither basic nor acidic properties. When a small sample was heated with an excess of 10% methanolic potassium hydroxide for a half-minute on the water-bath, the solution became intensively red. The colored matter could be extracted with ether and was volatile with the vapors of that solvent.

4-Diacetylamino-1,2,4-triazole (X).—When 10 g. of 4-amino-1,2,4-triazole (XII) was added portionwise to 100 ml. of acetic anhydride, a colorless solution resulted with evolution of heat. After heating for two hours on the steam-bath, the excess of acetic anhydride was removed by vacuum distillation. The remaining faintly brownish sirup crystallized slowly upon standing in a refrigerator for two weeks. The crude material was recrystallized with addition of charcoal from 30 ml. of boiling acetone, yielding 9.2 g. of white sturdy prisms, m.p. 129–131°.

Anal. Calcd. for $C_8H_8N_4O_2$: C, 42.86; H, 4.80; N, 33.32. Found: C, 42.75; H, 5.13; N, 33.22.

1,2,4-Triazole (XI) and 4-Amino-1,2,4-triazole (XII) from 1,2-Diformylhydrazine Dihydrazone (IV).—After 10.5 g. of IV had undergone spontaneous decomposition by storage in a vacuum desiccator in the dark for five days at room temperature, the resulting transparent brownish viscous oil was subjected to a slow fractional vacuum sublimation in a cold finger apparatus. After 12 hours at 140° bath temperature and 1 mm. 2.4 g. (38.4%) of white sturdy needles of 1,2,4-triazole (XI) was obtained. After one recrystallization from ethanol, the product was analytically pure, m.p. 120–121°. A mixed melting point with an authentic sample of XI[15] was without depression.

Anal. Calcd. for $C_2H_3N_3$: C, 34.78; H, 4.37; N, 60.85. Found: C, 35.11; H, 4.75; N, 60.80.

The resinous remainder in the distillation apparatus largely crystallized on standing. The crystals were freed from the main amount of adhering resins on a porous plate and then purified by dissolving in 30 ml. of ethanol and boiling with charcoal. After filtration and removal of the solvent from the filtrate, a slightly colored oil remained. This oil solidified on cooling to fine needles (1.1 g., 14.5%) of 4-aminotriazole (XII), m.p. 82–83°, which showed no depression in a mixed melting point with an authentic sample of XII.[16] The substance was further identified by its picrate,[17] m.p. 197–198°.

Anal. Calcd. for $C_8H_7N_7O_7$: N, 31.31. Found: N, 31.60.

From a mixture of 4-amino-1,2,4-triazole and anhydrous hydrazine heated for 12 hours at 140° and 1 mm., XII could be recovered unchanged. No 1,2,4-triazole was found in this experiment. Likewise, XII, heated alone to 180°, yielded no triazole.

1-Formyl-2,2-dimethylhydrazine 2′,2′-Dimethylhydrazone (XIII).—A mixture of *s*-triazine and *asym*-dimethylhydrazine (mole ratio 1:6) reacted slowly at room temperature under evolution of ammonia. The reaction was completed by heating for one hour at 100–115°. Subsequent vacuum distillation gave an 85% yield of XIII as a colorless mobile oil with an unpleasant odor, b.p. 73° (9 mm.); $n^{21}D$ 1.4664.

Anal. Calcd. for $C_5H_{14}N_4$: C, 46.13; H, 10.83; N, 43.04. Found: C, 45.92; H, 11.02; N, 43.02.

In an analogous manner, from 1-methyl-1-phenylhydrazine and *s*-triazine there was obtained the **N²-methylphenylformamidrazone (XIV).** In this case, the remaining solid reaction product (72%) was purified by repeated recrystallization from ethyl acetate, yielding beige-colored needles, m.p. 103–104°.

Anal. Calcd. for $C_8H_{11}N_3$: C, 64.40; H, 7.43; N, 28.17. Found: C, 64.55; H, 7.31; N, 28.12.

No definite product other than XIV could be obtained from a mixture of an excess of *asym*-methylphenylhydrazine and *s*-triazine at temperatures up to 190°.

Phenylazoformaldehyde Phenylhydrazone (XVIa).—Phenylhydrazine, either in alcoholic solution or undiluted, reacted slowly with *s*-triazine with evolution of ammonia at room temperature. The initial reaction product, presumably XV, was a viscous oil which could not be obtained crystalline by any means, and which very soon turned red, even in an atmosphere of nitrogen. Heating the mixture decomposed the primary product into unidentifiable resinous matters, but, when the primary product was poured in thin layers on flat disks and kept there for about 14 days in contact with the air, the color increased to deep red and gradually the mass crystallized to red needles of XVIa, yield 83.6%. XVIa was purified by several recrystallizations, first from aqueous and then from absolute methanol, m.p. 119–120°.

Anal. Calcd. for $C_{13}H_{12}N_4$: C, 69.63; H, 5.39; N, 24.98. Found: C, 69.60; H, 5.33; N, 24.69.

XVIa was identified by a mixed melting point with an authentic sample[18] and by conversion into the **acetyl phenylazo-formaldehyde phenylhydrazone**, m.p. 189°.[19]

Anal. Calcd. for $C_{15}H_{14}N_4O$: C, 67.64; H, 5.30; N, 21.04. Found: C, 67.84; H, 5.39; N, 20.85.

***p*-Bromophenylazo-formaldehyde *p*-bromophenylhydrazone (XIVb)** was obtained from *s*-triazine and freshly prepared free *p*-bromophenylhydrazine in 81% yield. In this case oxidation of the intermediate hydrazidine XV occurred spontaneously during the reaction. The remaining dark red microcrystalline mass yielded XIVb after recrystallization from glacial acetic acid as small reddish brown needles, m.p. 114–115°. These were identified by mixed melting

(13) Ch. C. Clark, "Hydrazine," Mathieson Chemical Corporation, Baltimore, Md., 1953.

(14) Contrary to Müller and Herrdegen, ref. 4, we were not able to isolate their dibenzal derivative V, but found only benzalazine.

(15) G. Pellizari, *Gazz. chim. ital.*, **24**, [II] 222 (1894).

(16) C. F. H. Allen and A. Bell, *Org. Syntheses*, **24**, 12 (1944).

(17) S. Ruhemann and H. E. Stapleton, *J. Chem. Soc.*, **75**, 1133 (1899).

(18) H. v. Pechmann, *Ber.*, **25**, 3186 (1892).

(19) E. Bamberger and E. Wheelwright, *ibid.*, **25**, 3204 (1892).

point with an authentic specimen.[20] In an analogous manner, the reaction of *s*-triazine with *p*-nitrophenylhydrazine resulted in ***p*-nitrophenylazo-formaldehyde *p*-nitrophenylhydrazone** (**XIVc**); yield 67%. After one recrystallization from benzene, reddish brown crystals, sintering at 94–95° and melting then slowly up to 104–105°, were obtained. They were identical with a sample prepared according to literature.[20]

A mixture of 2,4,6-trimethyl-*s*-triazine (Ia, 1.0 g.) and phenylhydrazine (5.3 g.) after heating for 30 minutes at 150–190° evolved ammonia and then turned deep red. From this crude product isolation of the **N,N'-diphenyl-C-methylformazan**, XVId (phenylazo-acetaldehyde phenylhydrazone) proceeded as described for XVIa. After recrystallization from ethanol the red, glittering needles of XVId (2.3 g.) melted at 122–123°. This melting point was not depressed upon mixing with an authentic sample.[21] With 2,4,6-triphenyl-*s*-triazine (Ib, 5.0 g.) and phenylhydrazine (10.5 g.) the reaction did not proceed to completion, even after two hours at 190°. After cooling the deep red solid obtained could be separated by extraction with boiling ethanol. The insoluble part (4.0 g.) was unreacted Ib, while the desired **N,N'-diphenyl-C-methylformazan**, XVIe, (phenylazo-benzaldehyde phenylhydrazone) crystallized from the concentrated alcoholic extracts (1.4 g.), m.p. 174–175°. The identity of XVIe was established by a mixed melting point with an authentic sample.[22]

(20) M. Busch and W. Wolbring, *J. prakt. Chem.*, [2] **71**, 374 (1905).
(21) E. Bamberger and W. Pemsel, *Ber.*, **36**, 87 (1903).
(22) H. v. Pechmann, *ibid.*, **27**, 1690 (1894).

3-Hydroxy-1,2,4-triazole (**XVIII**).—A vigorous reaction with evolution of ammonia occurred when a mixture of *s*-triazine (2.9 g.) and semicarbazide (XVII) (8 g.) in a flask was immersed into an oil-bath preheated to 100°. The mixture thereafter was heated for five minutes more at 120° and then allowed to cool. The resulting yellowish resin was extracted with hot ethanol and decolorized with charcoal. After evaporation to dryness the remaining crystalline XVIII (1.7 g., 18.7%) was recrystallized from alcohol, m.p. 234–235°.

3-Mercapto-1,2,4-triazole (**XX**) was obtained analogously from thio-semicarbazide (XIX) and *s*-triazine at a reaction temperature of 190°. The crude material (63.4%) was recrystallized from water, m.p. 215–216°.

3-Amino-1,2,4-triazole (**XXII**).—Free aminoguanidine (XXI) was prepared from the sulfate with an equivalent amount of barium hydroxide in aqueous solution, filtering off the precipitated barium sulfate and evaporating the filtrate to dryness *in vacuo*. The remaining crystalline free base is not stable. It soon turned red and must be used immediately. Its reaction with *s*-triazine at 210° as described above yielded 3-amino-1,2,4-triazole (47.4%), m.p. 158–159°, after recrystallization from ethyl acetate.

The compounds XVIII, XX and XXII were identified by mixed melting points with authentic samples prepared according to the literature.[23]

(23) O. Widmann and A. Cleve, *ibid.*, **31**, 379 (1898); M. Freund and C. Meinecke, *ibid.*, **29**, 2484 (1896); J. Thiele and W. Manchot, *Ann.*, **303**, 45 (1898).

COLUMBUS 10, OHIO

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Synthesis and Biological Activity of Some 6-(Substituted)-aminopurines

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Thirteen new 6-(substituted)-aminopurines have been synthesized and tested for biological activity in two assay systems. Some compounds which possessed the greatest activity in inhibition of hydra regeneration (*e.g.*, 6-(4-cyclohexylbutylaminopurine)) had relatively little effect on the rate of germination of lettuce seed (Early Curled Simpson); and those compounds (*e.g.*, 6-(α-naphthylmethyl)-aminopurine)) which possessed the greatest seed germination effect had relatively little activity in the hydra regeneration study.

The synthesis, and some biological activities, of several 6-(substituted)-purines has recently been reported from these laboratories.[1–4] In an effort to examine the relationship between biological activity and structure of the substituent group in the 6-positions of the purine nucleus, additional compounds have been prepared and are reported in this paper in conjunction with their biological activity in two assay systems.

6-(2-Furfuryl)-aminopurine (kinetin) has received considerable attention since it was reported by Miller, *et al.*,[5,6] to be a cell division factor in tobacco "wound" callus tissue. More recently, Miller has reported a stimulation of lettuce seed germination with kinetin, 6-benzylaminopurine and 6-hexylaminopurine,[7] and we have also observed such effects with various 6-(substituted)-thiopurine derivatives.[8] Liverman has observed an effect with both kinetin and several other adenine analogs in a leaf disk expansion type assay,[9] and the effects of some fifty 6-(substituted)-purines have been studied by Eakin, *et al.*, in these laboratories for their effect upon moss budding[10] and hydra tentacle regeneration.[2–4] More recently, a bacteriological assay for these purine analogs has been developed.[11] While it appears that no overall statement may be made with respect to most active compound(s) in the separate assay systems, a particular homolog is frequently the most active in more than one of the biological assays.

In the present study, differences in activity of some new 6-(substituted)aminopurines (Table I) in two different assay systems have been observed.

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