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One Step Syntheses of Adenine, Xanthine and Guanine from Phenylazomalonic Acid Derivatives

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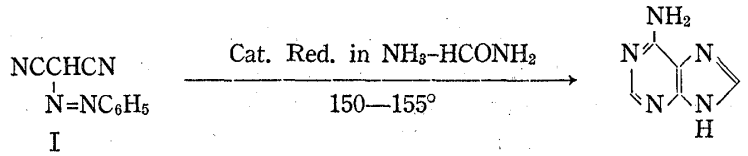
Facile one step purine syntheses from phenylazomalonic acid derivatives are described. Adenine, xanthine and guanine were synthesized directly from phenylazomalononitrile, N-(2-cyano-2-phenylazoacetyl)urea and 2-cyano-2-phenylazoacetylguanidine, respectively, by catalytic hydrogenation in formamide in the presence of ammonia (in the latter two the presence of ammonia was not indispensable) in excellent or good yields. Course of the reaction was revealed to involve the initial pyrimidine ring-closure followed by the imidazole ring-closure.

Search for a better synthetic method for the industrial production of the purines versatile as synthetic intermediates has been a problem of increasing importance. After our first communication[2] outlining the facile one step purine syntheses from phenylazomalonic acid derivatives the details of the hypoxanthine synthesis have been already reported.[3] In continuation we now wish to disclose the one step syntheses of adenine, xanthine and guanine in detail.

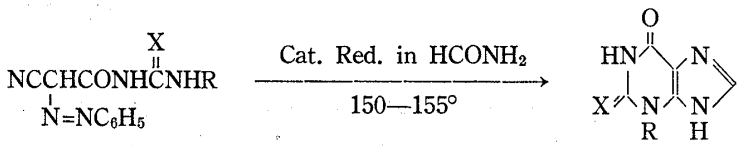
The means of the catalytic hydrogenation of 2-cyano-2-phenylazoacetamide in the ammonia-formamide system applied to the synthesis of hypoxanthines was extensively processed with some other phenylazomalonic acid derivatives. Wide scope of this type of the reaction has been recognized by the success in the syntheses of adenine, xanthine and guanine.

High pressure hydrogenation of phenylazomalononitrile (I) at 150—155° over Raney nickel or palladium-charcoal catalyst in 7% ammonia-formamide led to the production of adenine in 70—80% yield. Optimum concentration of ammonia was shown to be in a range of about 7—15%. At concentrations above and below the optimum range the yield of adenine was markedly decreased.



Extensions using other varied functional derivatives of phenylazomalonic acid as substrates needed preparations of N-(2-cyano-2-phenylazoacetyl)urea (II), N-(2-cyano-2-phenylazoacetyl)-N'-methylurea (III) and 2-cyano-2-phenylazoacetylguanidine (IV). Compound II had been known[4] and III and IV were newly synthesized. These three substrates, II, III, and IV, were successfully converted into xanthine, 3-methylxanthine and guanine by the catalytic hydrogenation not only in ammonia-containing formamide but also merely in formamide under similar conditions. In these cases supply of ammonia was not indispensable, but somewhat better yield, 87%, of xanthine was obtained in 7% ammonia-formamide than that in formamide itself, 79%. Similarly 3-methylxanthine and guanine were obtained in 80—92% yield in 7% ammonia-formamide and in formamide. Thus the catalytic hydrogenation method

1) Location: *2-2-1, Oshika, Shizuoka.*
2) M. Sekiya and J. Suzuki, *Chem. Pharm. Bull* (Tokyo), **20**, 209 (1972).
3) M. Sekiya and J. Suzuki, *Chem. Pharm. Bull* (Tokyo), **23**, 2401 (1975).
4) M. Ishidate, M. Sekiya, H. Ozaki, I. Kurita, and Y. Harada, *Chem. Pharm. Bull.* (Tokyo), **3**, 224 (1955).

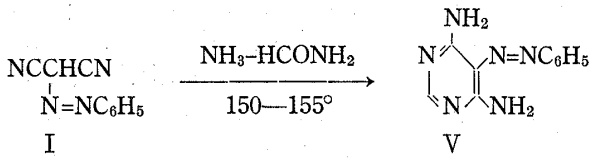


X=O or NH, R=H or CH_3

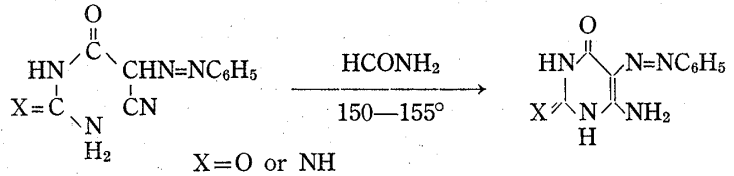
in the system of ammonia-formamide and of formamide has paved a generally applicable and practically useful way for the preparation of the purine derivatives.

Examinations were also made to see adaptabilities of some other chemical reduction methods using sodium dithionite, ammonium sulfite, zinc amalgam and aluminum amalgam in modified ways. Among these trials sodium dithionite, zinc amalgam and aluminum amalgam were somewhat efficient in affording xanthine, whereupon 43%, 76%, and 66% yields were obtained, respectively, by means of addition of II in formamide at the temperature of 120° which was raised to 150° after the addition. The same reduction procedures similarly gave guanine, but did not give adenine.

As for the previously reported[3] one step hypoxanthine synthesis from 2-cyano-2-phenylazoacetamide by the catalytic hydrogenation method the hypoxanthine-forming process has been stated to involve mechanistically the pyrimidine ring-closure at the initial stage followed by the imidazole ring-closure. On the analogy of these courses of the one step syntheses of adenine, xanthine and guanine were investigated as in the following. Conversion of I into 5-phenylazo-4,6-diaminopyrimidine (V) was effected to an appreciable extent (85—87%) by heating at 150—155° in ammonia-containing formamide, of which optimum ammonia concentration was in a range of 3—6%. Since no conversion occurred in the absence of ammonia,

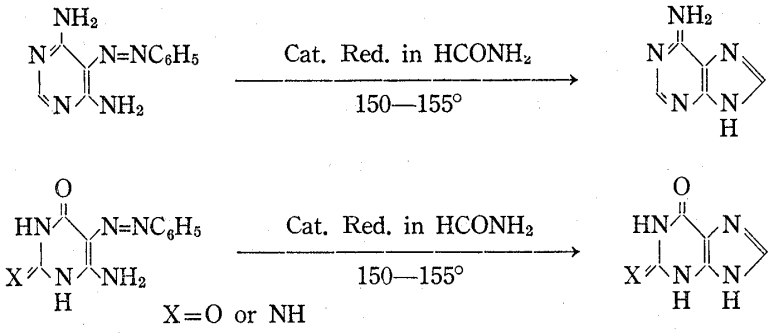


this pyrimidine formation is supposed to proceed by initial amidination of, at least, one of the nitrile groups of I by action of ammonia, followed by condensation with formamide. The resulting V was shown to be denatured gradually into an amorphous brown substance when heated in ammonia-formamide of higher ammonia concentration for longer period. This is because of existence of the optimum concentration of ammonia in the above reaction. The pyrimidine ring-closures of II and IV were similarly realized to give 5-phenylazo-substituted 4-aminouracil (VI) and 2,4-diamino-6-hydroxypyrimidine (VII), respectively, in about 90% yields, but in these cases the presence of ammonia was not indispensable.



X=O or NH

Catalytic hydrogenation in formamide at elevated temperature has been reported[4] to effect the conversion of VI into xanthine. On the analogy of this reaction the three 5-phenylazo-substituted pyrimidines, V, VI, and VII were converted into adenine, xanthine and guanine in above 90% yields under the conditions of their one step syntheses, *i.e.*, the catalytic hydrogenation in formamide at 150—155°. The above facts, the pyrimidine ring-closures and the purine skeleton formations in the settled conditions, insist on the courses of the



one step syntheses of adenine, xanthine and guanine that the pyrimidine ring-closure is induced initially and followed by the imidazole ring-closure.

It was also shown that adenine, xanthine and guanine were produced from V, VI, and VII in excellent yields using trimethylammonium formate (TMAF),[5] known as the distillable liquid formate composed of trimethylamine and formic acid, in place of formamide in the above catalytic hydrogenation method.

Experimental[6]

Phenylazomalononitrile (I)——To an aqueous solution of 66 g (1 mole) of malononitrile in the 700 ml of water aqueous benzenediazonium chloride, which was prepared from 102 g (1.1 mole) of aniline by the usual method, was added with stirring. This mixture was adjusted to about pH 4 by addition of aqueous sodium acetate and allowed to stand overnight. The resulting precipitates were collected by filtration, washed with water, then with ethanol and dried, 153 g (90%). Yellow prisms (benzene), mp 137—138°. IR ν_{max}^{KBr} cm^{-1}: 2249, 2230 (CN), UV λ_{max}^{EtOH} nm (ε): 244 (8200), 359 (20000). *Anal.* Calcd. for $C_9H_6N_4$: C, 63.52; H, 3.55; N, 32.93. Found: C, 63.44; H, 3.84; N, 32.91.

N-(2-Cyano-2-phenylazoacetyl)-urea (II) and -N'-methylurea (III)——Compound II has been known and III has not. These two compounds were prepared by azo-coupling of N-(2-cyanoacetyl)-urea and -N'-methylurea according to the method reported previously.[4] III (yield 72%): yellow needles (AcOH), mp 209—210° (decomp.). *Anal.* Calcd. for $C_{11}H_{11}O_2N_5$: C, 53.87; H, 4.52; N, 28.56. Found: C, 53.93; H, 4.59; N, 28.73.

2-Cyano-2-phenylazoacetylguanidine (IV)——To 193 g of 28% sodium methoxide–methanol solution 95.5 g (1 mole) of guanidine hydrochloride was added with stirring and then 99.1 g (1 mole) of methyl cyanoacetate was added. The mixture was stirred for further 30 min to give methanolic solution of cyanoacetylguanidine. To this solution an aqueous benzenediazonium chloride, prepared from 93 g (1 mole) of aniline by the usual method, was added. The mixture was diluted with 600 ml of water and adjusted to pH 6.5—7 with 15% aqueous NaOH and stirred at 10—20° for further 10 hr. Reddish brown precipitates were collected by filtration, washed with water and dried, 218 g (95%). IR ν_{max}^{KBr} cm^{-1}: 2180, 2205 (CN), 1620 (CO). UV λ_{max}^{EtOH} nm: 243.5, 368. *Anal.* Calcd. for $C_{10}H_{10}ON_6$: C, 52.17; H, 4.38; N, 36.51. Found: C, 52.01; H, 4.37; N, 36.46.

Adenine from Phenylazomalononitrile (I)——In an autoclave 6.8 g (0.04 mole) of I, 75 g of 7% ammonia–formamide and Raney nickel freshly prepared by usual means from 1 g of 50% alloy were placed. Under 80 kg/cm^2 of initial hydrogen pressure at room temperature the whole was heated at 150—155° and the constant shaking was started. Shaking and heating were continued for 4 hr, while uptake of hydrogen proceeded. After cooling the catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The residual solid was washed with 20 ml of ethanol and dissolved in 200 ml of hot water. After removal of an insoluble material the aqueous solution was decolorized with charcoal and concentrated under reduced pressure to give powder, which was recrystallized from water to give adenine, 3.9 g (72%). mp>300°. IR ν_{max}^{KBr} cm^{-1}: 3350—2050 (NH_2, NH), 1662 (C=N). UV $\lambda_{max}^{0.1N\ HCl}$ nm (ε): 263 (13000), $\lambda_{max}^{H_2O}$ nm (ε): 261 (13100), $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 269 (12200). (lit.,[7] UV $\lambda_{max}^{0.02N\ HCl}$ nm: 263, $\lambda_{max}^{H_2O}$ nm: 262, $\lambda_{max}^{0.1N\ NaOH}$ nm: 269). These spectral data were in agreement with those of an authentic sample. *Anal.* Calcd. for $C_5H_5N_5$: C, 44.44; H, 3.73; N, 51.83. Found: C, 44.20; H, 4.00; N, 51.58.

Adenine from 4,6-Diamino-5-phenylazopyrimidine (V)——A mixture of 10.7 g (0.05 mole) of V and 135g of formamide was hydrogenated over Raney nickel catalyst (from 2 g of 50% alloy) at 150—155° for 5 hr

5) M. Sekiya and K. Ito, *Chem. Pharm. Bull.* (Tokyo), **12**, 677 (1964).
6) All melting points are uncorrected. Infrared (IR) and ultraviolet (UV) spectra were recorded on a Hitachi EPI-G2 spectrophotometer and a Hitachi EPS-3T spectrophotometer, respectively.
7) Y. Fujimoto and N. Ono, *Yakugaku Zasshi*, **85**, 364 (1965).

under 80 kg/cm² of initial hydrogen pressure. After cooling the deposited precipitates were collected by filtration. Additional amount of the same was obtained by concentration of the filtrate and combined with the above precipitates. This material was added to 400 ml of water and the mixture was refluxed for 1 hr. An insoluble material was filtered off, then the filtrate was decolorized with charcoal and concentrated to give adenine, 6.1 g (90%).

In the above procedure, TMAF[5] and palladium-carbon in place of formamide and Raney nickel could be used with nearly the same yield of adenine.

Xanthine from N-(2-Cyano-2-phenylazoacetyl)urea (II)——1) Catalytic Reduction: A mixture of 6.9 g (0.03 mole) of II, 69 g of formamide and Raney nickel freshly prepared by the usual means from 1 g of 50% alloy was placed in an autoclave under 80 kg/cm² of initial hydrogen pressure and allowed to react at 150—155° with shaking for 5 hr. After cooling an insoluble material was collected by filtration and dissolved in 5% aqueous NaOH. The catalyst was filtered off and xanthine was precipitated by acidifying this alkali solution with acetic acid. The filtered formamide solution was concentrated under reduced pressure. A solution of the residual powder dissolved in 5% aqueous NaOH was decolorized with charcoal and acidified with acetic acid to give powder of xanthine. Total yield, 3.6 g (78%). mp—300°. IR ν_{max}^{KBr} cm^{-1}: 3200—2400 (OH, NH), 1705, 1660 (CO, C=N). UV $\lambda_{max}^{H_2O}$ nm (ε): 269 (9100) (lit.,[8] UV $\lambda_{max}^{pH\ 5.05}$ nm: 267). These spectral data were in agreement with those of an authentic sample.

When the hydrogenation reaction was carried out in the presence of ammonia, yield of xanthine was 87%.

2) Reduction with Aluminum Amalgam: To a mixture of 4.6 g (0.02 mole) of II and 69 g of formamide was added gradually aluminum amalgam prepared from 1.4 g of aluminum with stirring at 120°. After addition the mixture was stirred at 120° for 1 hr and then the reaction temperature was raised to 150—155° and stirring was continued for further 30 min. After cooling an insoluble material was filtered and washed with formamide. The filtrate combined with washings was treated in the same manner as described above 1) to give xanthine, 2.1 g (66%).

3) Reduction with Zinc Amalgam: By the use of zinc amalgam in place of aluminum amalgam the reaction was carried out by the same procedure as described in 2) to give 76% yield of xanthine.

4) Reduction with Sodium Dithionite: A mixture of 3.8 g of II, 8.7 g of sodium dithionite and 55 g of formamide was stirred at 120° for 2 hr and then at 150° for further 30 min. After cooling an insoluble material was filtered off and the filtrate was treated in the same manner as described above (1) to give xanthine, 0.8 g (30%).

When the reaction was carried out by using formamide containing water (3%), yield of xanthine was 43%.

3-Methylxanthine——A mixture of 9.8 g (0.05 mole) of III, 98 g of 7% ammonia–formamide and Raney nickel freshly prepared from 1 g of 50% alloy was allowed to be reduced under high hydrogen pressure by the same procedure as described above for xanthine. Yield, 3.6 g (85%). Needles (H_2O), mp>300°. *Anal.* Calcd. for $C_6H_6O_2N_4$: C, 43.37; H, 3.64; N, 33.73. Found: C, 43.64; H, 3.70; N, 34.17.

Guanine from 2-Cyano-2-phenylazoacetylguanidine (IV)——1) Catalytic Reduction: A mixture of 93.2 g (0.4 mole) of IV, 91 g of formamide and Raney nickel freshly prepared from 14 g of 50% alloy was placed in an autoclave and allowed to react with stirring under 30 kg/cm² of initial hydrogen pressure at 150—155°. During the reaction hydrogen pressure was maintained at 30 kg/cm² by charging hydrogen on requirement. After removal of the catalyst by filtration under cooling the filtrate was concentrated under reduced pressure. The residue was dissolved in 5% aqueous NaOH and an insoluble material was filtered off. This alkaline solution was acidified with hydrochloric acid to give powder of guanine, 52 g (86%). mp>300°. UV $\lambda_{max}^{0.1N\ HCl}$ nm: 248, 274 (lit.,[8] UV $\lambda_{max}^{pH\ 1}$ nm: 248, 271). The IR and UV spectra of the product were in agreement with those of an authentic sample.

2) Reduction with Sodium Dithionite: A mixture of 18.4 g (0.08 mole) of IV, 108 g of formamide and 13.9 g of sodium dithionite was stirred at 100—120° for 2 hr and then at 150—155° for further 5 hr. After cooling formamide was evaporated under reduced pressure. The residue was treated with 5% H_2SO_4 to give guanine sulfate, 5.6 g (35%).

Guanine from 2,4-Diamino-6-hydroxy-5-phenylazopyrimidine (VII)——A mixture of 6.9 g (0.03 mole) of VII and 120 g of formamide was hydrogenated over Raney nickel catalyst (2 g of 50% alloy) at 120—130° for 1 hr and then at 150—155° for 4 hr under 40 kg/cm² of initial hydrogen pressure. The deposited precipitates and the residual solid resultant from concentration of the filtrate were dissolved in 3% aqueous NaOH. An insoluble material was removed by filtration and the filtrate was acidified with acetic acid to give guanine, 4 g (89%).

4,6-Diamino-5-phenylazopyrimidine (V)——In an autoclave 13.6 g (0.05 mole) of I and 136 g of 6% ammonia-formamide were placed and the mixture was heated with shaking. The temperature was maintained at 150—155° for 5 hr. After cooling the deposited crystals were collected by filtration and washed with methanol, 15.0 g (87%). Prisms (DMF), mp 298—300° (lit.,[9] mp 302°). UV λ_{max}^{EtOH} nm: 246, 378. Their IR and UV spectra were in agreement with those of a specimen prepared according to the previously reported

8) S.F. Mason, *J. Chem. Soc.*, **1954**, 2071.
9) E. Richter and E.C. Taylor, *J. Am. Chem. Soc.*, **78**, 5848 (1956).

method.[10] *Anal.* Calcd. for $C_{10}H_{10}N_6$: C, 56.06; H, 4.71; N, 39.23. Found: C, 56.31; H, 4.62; N, 39.26.

4-Amino-5-phenylazouracil (VI)——A mixture of 11.6 g (0.05 mole) of II and 116 g of formamide was heated at 150—155° with stirring for 5 hr. After cooling the deposited crystals were collected by filtration and washed with methanol, 10.3 g (89%). Needles (AcOH), mp>300°. Their IR and UV spectra were in agreement with those of authentic 4-amino-5-phenylazouracil. *Anal.* Calcd. for $C_{10}H_9O_2N_5$: C, 51.91; H, 3.83; N, 30.29. Found: C, 51.83; H, 3.98; N, 30.04.

2,4-Diamino-6-hydroxy-5-phenylazopyrimidine (VII)——A mixture of 23 g (0.1 mole) of IV and 46 g of formamide was allowed to react with stirring at 150—155° for 2 hr. After cooling the deposited crystals were collected by filtration and the filtrate was concentrated under reduced pressure to give additional small amount of solid, which was washed with methanol. Total yield, 20.8 g (90%). mp>300°. Its IR and UV spectra were in agreement with those of a specimen prepared according to the previously reported method.[11]

Acknowledgement The authors are indebted to the members of Analysis Center of this college for microanalyses.

10) L.F. Cavalieri, J.F. Tinker, and A. Bandich, *J. Am. Chem. Soc.*, **71**, 533 (1949).
11) H.G. Garg and R.A. Sharma, *J. Pharm. Sci.*, **59**, 1961 (1970).