Synthesis of Purine Derivatives from Single-carbon Compounds

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The reaction of chloroform with liq. ammonia in a sealed vessel at high temperatures furnished adenine, purine, and 8-aminopurine in 5, 7 and 1% yields, respectively. Similarly, the reaction between ethyl orthoformate and liq. ammonia yielded adenine (21%), purine (14%) and 8-aminopurine (3%). A possible mechanism has been proposed for the reaction, which involves a polymerization of formimidoyl derivative rather than an intermediary formation of aminomalononitrile.

Many reports have hitherto been published on the synthesis of purine derivatives, especially of adenine which is one of the most important components of nucleic acids. The conventional methods can be classified into two groups: The one is represented by Traube’s method which involves the cyclization of pyrimidine intermediates, and the other is Shaw’s method which involves the cyclization of appropriate imidazole derivatives. These syntheses, however, have some drawbacks from an industrial point of view, because some troublesome processes were inevitable. Orô, et al. were the first to show that adenine was produced under prebiotic conditions by warming a mixture of hydrogen cyanide and ammonia in water, although the yield was only 0.5%. A few years ago, Yamada, et al. succeeded in synthesizing adenine and 4,5-dicyanoimidazole both in about 20% yields by heating a mixture of hydrogen cyanide and liquid ammonia in non-aqueous media.

It has been reported that ammonium cyanide was produced by the reaction of trihalogenmethanes (e.g. chloroform or bromoform) and ammonia (or ethanolic ammonia) at high temperatures. The authors therefore assumed that adenine might be synthesized from a more readily available and less toxic trihalogenomethane.

Thus, chloroform was allowed to react with liquid ammonia (molar ratio 1:10) in a sealed vessel at 200° for 16 hours. The reaction mixture was subjected to the ion-exchange chromatography using Dowex-1 (acetate form) and 0.1M acetate buffer (pH 4.4) to separate three compounds A, B and C in the order of elution (Fig. 1). Each fraction was desalted with activated charcoal and the compound obtained was purified by

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1) Presented in part at the 21st Annual Meeting of Chemical Society of Japan, Osaka, April, 1968.
2) Location: Fuso-Nishino-cho, Higashiyodogawa-ku, Osaka.
3) W. Traube, Ann., 331, 64 (1904).
6) Y. Yamada, I. Kumashiro and T. Takenishi, Abstracts of Papers, the 18th Annual Meeting of Chemical Society of Japan, Osaka, April 1965, p. 243.
recrystallization or sublimation. Compound A was purified by sublimation to give colorless needles, mp 216°. Its ultraviolet absorption spectra (UV spectra, pH 1.0 and 13.0), infrared absorption spectrum (IR spectrum, KBr), nuclear magnetic resonance spectrum (NMR spectrum, D₂O), mass spectrum and Rf value of paper chromatography (PC, one solvent) were found all to be in accord with those of an authentic sample of purine. The structure was further substantiated by the elementary analysis (C₅H₄N₄). The yield was 7% based on chloroform. Compound B was recrystallized from hot water to give colorless needles, mp 360° (decomp.), which were identified as 8-aminopurine by the UV (pH 1.0, 7.0 and 13.0), NMR (NaOD) and mass spectra as well as the elementary analysis (C₅H₄N₄). The yield of 8-aminopurine was 1%. Compound C was recrystallized from hot water to afford colorless needles, mp 355—360° (decomp.), which were identified as adenine by the UV (pH 1.0, 7.0 and 13.0) and IR (KBr) spectra, the paper chromatography (five solvents) and the elementary analysis (C₅H₄N₄). The identification was also done by the deamination to hypoxanthine and by the acylation to N⁸-octanoyladenine (C₁₃H₂₆N₅O, mp 189—190°) and to N⁸, 9-dioctanoyladenine (C₉H₂₉O₂N₅, mp 157°). The yield of adenine was 5%.

Each yield of compounds A, B and C reached a maximum at a reaction temperature in the range of 180—200°. 8-Aminopurine was not formed at 160°. No detectable UV absorbing substances were given below 120°. Substitution of liquid ammonia with 30% methanolic ammonia gave a small quantity of a substance which was identical with adenine in the UV spectra (pH 1.0, 7.0 and 13.0), but different in the Rf values. The use of bromoform or iodoform in place of chloroform yielded no UV absorbing substances.

Reaction of ethyl orthoformate and liquid ammonia (molar ratio 1:12) at 200° for 16 hours, however, resulted in the synthesis of adenine, purine and 8-aminopurine in the yields of 21, 14 and 3%, respectively, based on ethyl orthoformate.

Yamada, et al. synthesized adenine and 4,5-dicyanoimidazole by heating a mixture of sodium cyanide, ammonium chloride and liquid ammonia (120°, 8 hr). The authors carried out the same experiment as Yamada, et al. to observe the formation of purine (1% yield) additionally, but not to detect any amount of 8-aminopurine. Both the reaction temperature and time were then modified to the authors' reaction conditions (200°, 8 hr or 16 hr), which, however, resulted in no change in product distribution. It was already mentioned that no UV absorbing substances were produced in the authors' reaction when the reaction was carried out at 120° for 16 hr, which was a preferred conditions in Yamada and coworkers' procedure. The authors' reaction was thus found to give a comparatively good yield of adenine, and have three significant differences as compared with Yamada and coworkers' (Table I): (1) 4,5-Dicyanoimidazole was not detected in any amounts. (2) Purine was synthesized in a much higher yield, which is comparable to that of adenine. (3) 8-Aminopurine was given as a reaction product.

### Table I. Yields of Reaction Products in Various Conditions

<table>
<thead>
<tr>
<th>Reaction system</th>
<th>Temp. (°C)</th>
<th>Time (hr)</th>
<th>Adenine (%)</th>
<th>Purine (%)</th>
<th>8-Aminopurine (%)</th>
<th>4,5-Dicyanoimidazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHCl₃-NH₃</td>
<td>200</td>
<td>16</td>
<td>5.0</td>
<td>7.0</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>16</td>
<td>3.5</td>
<td>3.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>16</td>
<td>— (a)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CH(OEt)₂-NH₃</td>
<td>200</td>
<td>16</td>
<td>21.0</td>
<td>14.0</td>
<td>3.0</td>
<td>—</td>
</tr>
<tr>
<td>NaCN-NH₄Cl-NH₃</td>
<td>200</td>
<td>8</td>
<td>4.0 (TOD₉₀⁻¹⁻¹ 11400)</td>
<td>0.5</td>
<td>—</td>
<td>TOD₁₉₀⁻¹⁻¹ 1139</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>16</td>
<td>7.0 (TOD₉₀⁻¹⁻¹ 19740)</td>
<td>0.5</td>
<td>—</td>
<td>TOD₁₉₀⁻¹⁻¹ 1480</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>8</td>
<td>20.0 (TOD₉₀⁻¹⁻¹ 57000)</td>
<td>1.0</td>
<td>—</td>
<td>TOD₁₉₀⁻¹⁻¹ 12000</td>
</tr>
</tbody>
</table>

(a)—- not detectable
Chart 1. Mechanism of Formation of Adenine from Hydrogen Cyanide

Chart 2. Mechanism of Formation of Adenine, 4,5-Dicyanoimidazole
and 4,8-Diaminopyrimido[5,4-d]pyrimidine

Chart 3. Mechanism of Formation of Purine Derivatives from Chloroform
or Ethyl Orthoformate
Oró, et al. and Yamada, et al. have proposed their reaction mechanisms as shown in Chart 1 and 2, where aminomalononitrile is assumed to be a key compound. If the authors' reaction is assumed to proceed via this compound, a similar result to Yamada and coworkers' should come about. The different result of the authors' reaction can be explained by another mechanism shown in Chart 3: Ethyl orthoformate (or chloroform) reacts with ammonia to form a formimidoyl derivative, $\text{HC}^\equiv\text{NH}$ (X is OCH$_3$, NH$_2$ or Cl), of which the polymerization yields adenine (I), purine (II) and 8-amino-purine (III). The mechanism receives support also by the fact that the formation of I, II and III is observed, but not of 4,5-dicyanoimidazole when formiminoethyl ether or formamidine hydrochloride is allowed to react with liquid ammonia at 200° for 16 hours.

**Experimental**

Paper Electrophoresis (PE) and Paper Chromatography (PC) — PE was carried out at 22 v/cm for 60 min using 0.05M borate buffer (pH 9.2). PC was performed by the ascending method using the following solvents: A, BuOH—AcOH—H$_2$O (4:1:1); B, BuOH saturated with H$_2$O; C, iso-PrOH—conc. NH$_4$OH—H$_2$O (60:35:1); D, dil. NH$_4$OH (pH 10); E, H$_2$O saturated with BuOH; F, BuOH—diethyleneeglycol—H$_2$O (4:1:1).

Reaction of CHCl$_3$ with liqu. NH$_4$ — CHCl$_3$ (35 ml, 435 mmoles) was allowed to react with liqu. NH$_4$ (105 ml, 4550 mmoles) in a stainless autoclave at 200° for 16 hr (pressure 95—100 kg/cm$^2$). An aliquot (0.5 ml) of the extract was subjected to Dowex—1(AcO$^-$; 200—400 mesh) column chromatography [column 0.9 cm x 158 cm, temp. 45°, solvent 0.1M acetate buffer (pH 4.4), flow rate 30 ml/hr] to separate three fractions (Fig. 1).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOD$_{380}$</td>
<td>37</td>
<td>10</td>
<td>(TOD$_{380}$)</td>
</tr>
</tbody>
</table>

A large scale experiment furnished a sufficient amount of the corresponding fractions, which were treated as follows: Fraction I was adjusted with HCl to pH 3, poured on a column of activated charcoal, the column washed with H$_2$O, eluted with conc. NH$_4$OH—BuOH—EtOH—H$_2$O (2:45:48) and the eluate evaporated to dryness to yield a light-brown powder. This compound was purified by sublimation (bath temp. 160—170°/7 mmHg) to yield colorless needles, mp 216°. UV $\mu$: $\lambda_{max}$ 259, $\lambda_{min}$ 275 (0.1M HCl); $\lambda_{max}$ 269, $\lambda_{min}$ 241 (0.1M NaOH). IR spectrum (KBr) and $Rf$ value (solvent A) were in accord with those of an authentic sample of purine.

NMR (D$_2$O): 8.6 (H$_2$), 8.9 (H$_2$), 9.1 (H$_5$). Mass spectrum $m/e$: 120 (M$^+$), 93 (M$^-$—CH$_3$), 66 (M$^-$—C$_3$H$_7$N$_2$). Anal. Calcd. for C$_5$H$_7$N$_2$: C, 50.00; H, 3.38; N, 46.85. Found: C, 50.03; H, 3.32; N, 46.78. Fraction II was worked up in a similar manner to yield colorless needles, mp 360° (decomp), after recrystallization from hot water. UV $\mu$: (e)$\lambda_{max}$ 283 (12650), 241 (shoulder) (4400), $\lambda_{min}$ 250 (2870) (H$_2$O); $\lambda_{max}$ 287 (15950), $\lambda_{min}$ 245 (1350) (2N HCl); $\lambda_{max}$ 292 (12350), $\lambda_{min}$ 254 (3300) (0.1N NaOH). PC (A): $Rf_{max}$ 0.95. NMR (NaOD): 8.23 (H$_2$), 8.35 (H$_2$). IR $\mu_{max}$ cm$^{-1}$: 1680 (NH$_2$), 1640 (NH). Mass Spectrum $m/e$: 135 (M$^+$), 81 (M$^-$—C$_3$H$_7$N$_2$), 67.5 (1/2 M$^-$).

Anal. Calcd. for C$_5$H$_7$N$_2$: C, 44.45; H, 3.73; N, 51.82. Found: C, 43.89; H, 3.46; N, 52.10. Similarly, fraction III was worked up to yield colorless needles, mp 355—360° (decomp) after recrystallization from hot water. UV $\mu$: $\lambda_{max}$ 258, $\lambda_{max}$ 225 (H$_2$O); $\lambda_{max}$ 260, $\lambda_{min}$ 229 (0.1N HCl); $\lambda_{min}$ 265, $\lambda_{max}$ 237 (0.1N NaOH). IR spectrum (KBr) and $Rf$ values of PC (A, B, D, E, F) were all in accord with those of authentic adenine.

Anal. Calcd. for C$_5$H$_7$N$_2$: C, 44.45; H, 3.73; N, 51.82. Found: C, 44.24; H, 4.00; N, 51.53. Deamination: A small sample of above described adenine was deaminated with AcOH and

9) A novel mechanism has been proposed by Ochiai, et al. in the synthesis of adenine from formamide and phosphorus oxychloride (M. Ochiai, R. Marumoto, S. Kobayashi, H. Shimazu and K. Morita, Tetrahedron, 24, 5731 (1968).
12) All melting points are uncorrected. NMR spectrum was measured using Me$_4$Si as an external reference and chemical shift was expressed in ppm.
13) TOD$_{380}$ $\mu$: optical density at 260 $\mu$m x ml.
14) $Rf_{max}$: $Rf$ ratio of a sample to adenine.
NaNO₃ by the conventional method, which showed the same mobility as that of authentic hypoxanthine in PE. UV mλ (nm): λ max 260, λ min 238 (0.1N NaOH); λ max 246, λ min 220 (0.1N HCl). N₄, 9-Dioctanoyladenine: Another sample (327 mg) was acetylated with octanoic anhydride (2 g) to yield colorless needles, mp 157°, after recrystallization from EtOH. Its IR spectrum (KBr) was superimposable on that of authentic N₈,9-dioctanoyladenine. Anal. Calcd. for C₈₃H₁₉₂O₅N₅: C, 65.10; H, 8.59; N, 18.05. Found: C, 65.06; H, 8.80; N, 18.09. N₄-Octanoyladenine: The diacylated compound described above was refluxed in EtOH for 3 hr and the solvent evaporated to give the residue, which was recrystallized from EtOH to yield colorless flakes, mp 189—190°. The IR spectrum was in accord with that of authentic N₄-octanoyladenine. Anal. Calcd. for C₈₃H₁₉₂O₅N₅: C, 59.77; H, 7.24; N, 26.71. Found: C, 59.02; H, 7.23; N, 26.80.

Reaction of CHCl₃ with 30% Methanolic NH₃—Chloroform (7 ml, 87 mmole) was allowed to react with 30% methanolic NH₃ (39 ml, NH₃ 589 mmole) at 200° for 16 hr. The reaction mixture was treated as described above and subjected to Dowex-50 (hydrogen form, 100—200 mesh) column chromatography using 2N HCl as an eluting solvent to yield a single fraction. The PC (A) of this fraction showed a single UV absorbing spot of R f=1.32. UV mλ: λ max 263.5, λ min 232 (H₂O); λ max 265.5, λ min 236 (pH 10<); λ max 260, λ min 233 (2N HCl).

Reaction of CH (OEt)₂ with liq NH₃—Ethyl orthoformate (4.15 ml, 28 mmole) was allowed to react with liq. NH₃ (8.5 ml, 350 mmole) at 200° for 16 hr. The reaction mixture was separated in the same way as described in the preceding section. Each component was identified from the retention time in the ion-exchange chromatography, R f value on PC (A) and UV spectrum (pH 4.4). The yields of the reaction products are shown in Table I.

**Reaction of the System NaCN-HCl-NH₃**

A mixture of NaCN (5.39 g, 0.11 mole), NH₃Cl (5.35 g, 0.10 mole) and liq. NH₃ (16 ml, 0.66 mole) was allowed to react at 200° or 180° for 16 hr or 8 hr. An aliquot (0.5 ml) of the hot water extract (100 ml) of the reaction mixture was subjected to Dowex-50 × 8 (hydrogen form, 200—400 mesh) column chromatography (column 1 cm x 138 cm, temp. 45°, gradient elution with Varigrad using a citrate buffer, flow rate 72 ml/hr) to yield 4,5-dicyanoimidazole, purine and adenine. These results are summarized in Fig. 2 and Table II.

**Table II. Yields of Reaction Products in NaCN-HCl-NH₃ System**

<table>
<thead>
<tr>
<th>Peak</th>
<th>λ max H₂O (nm)</th>
<th>λ min H₂O (nm)</th>
<th>200° 16 hr</th>
<th>200° 8 hr</th>
<th>120° 8 hr</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>248</td>
<td>260</td>
<td>7</td>
<td>6</td>
<td>60</td>
<td>4,5-dicyanoimidazole</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ghost peak by solvent</td>
</tr>
<tr>
<td>III</td>
<td>260</td>
<td>269</td>
<td>7 (0.5)</td>
<td>4 (0.5)</td>
<td>16 (1.1)</td>
<td>purine</td>
</tr>
<tr>
<td>IV</td>
<td>260</td>
<td>267</td>
<td>99 (7.0)</td>
<td>57 (4.0)</td>
<td>285 (20.0)</td>
<td>adenine</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8-aminopurine</td>
</tr>
</tbody>
</table>

**Reaction of CH (OEt)NH·HCl with liq NH₃**—A mixture of CH(OEt)NH·HCl (1.095 g, 10 mmoles), EtOH (1 ml, 20 mmole) and liq. NH₃ (15 ml) was allowed to react at 200° for 16 hr. An aliquot (5 ml) of the hot water extract (50 ml) of the reaction products was subjected to the ion-exchange chromatography described in the preceding section to separate purine (5.7%), adenine (1.2%) and 8-aminopurine (1.0%). 4,5-Dicyanoimidazole was not detected.

15) The reaction products in the system CHCl₃-NH₃ were also separated by the same chromatography to afford purine, adenine and 8-aminopurine, but 4,5-dicyanoimidazole was not detected.

16) Chamber | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td>Total 150 ml</td>
<td>NaOH</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.2</td>
<td>0.2</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

a) molar concentration of each chamber
Reaction of CH(NH)NH₂•HCl with liq. NH₃—A mixture of CH(NH)NH₂•HCl (805.2 mg, 10 mmoles), NH₄Cl (1.07 g, 20 mmoles) and liq. NH₃ (20 ml) was allowed to react at 200° for 16 hr. The reaction mixture was treated as described above to afford purine (2.3%), adenine (0.8%) and 8-aminopurine (0.7%). 4,5-Dicyanoimidazole was not detected.

Acknowledgement—The authors are grateful to Drs. Y. Abe and K. Tanaka for their interest and encouragement. Thanks are also due to Messrs. M. Hori and R. Marumoto for their useful suggestions, to Mr. M. Kan and his associates for elementary analyses, and to Dr. Y. Asahi and his associates for mass and NMR spectral measurements.