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A FACILE ONE-STEP SYNTHESIS OF ADENINE*

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Abstract—A facile one-step synthesis of adenine has been achieved by the reaction of formamide with $POCl_3$ in a sealed tube. A maximum yield reached 43·5% of theory calculated on formamide under optimal conditions. Hydrogen cyanide is not considered a possible intermediate for the formation of adenine because $H^{14}CN$ added to the reaction mixture is not incorporated. A speculative mechanism for the reaction is proposed.

PREBIOTIC syntheses of adenine from simple compounds have received considerable attention in recent years. These include (i) Oró's[1] synthesis of adenine from hydrogen cyanide and aqueous ammonia, (ii) a modified synthesis[2] from the same starting materials in non-aqueous media to facilitate an improved yield (23%) of the compound, (iii) a demonstration[3] of adenine formation from trimer or tetramer of hydrogen cyanide, and (iv) an electron irradiation[4] on a mixture of methane, ammonia and water.

The present paper deals with a facile one-step synthesis of adenine by heating a mixture of formamide (FA) and $POCl_3$ without solvent. Thus, a maximum yield of adenine (43·5%) was obtained when a mixture of FA and $POCl_3$ in a 1:2 mole ratio was heated at 120° in a sealed vessel for 15 hr. No UV-absorbing substances other than adenine were formed practically in the reaction mixture as demonstrated by paper chromatography and ion-exchange chromatography on Dowex 50 × 8 (H^+ form) equipped with a conventional UV-recording instrument.†

Initially, we had intended to seek a simple method for synthesizing aminomalononitrile, which we thought an important key intermediate for the synthesis of adenine. Shortly after we started our investigation, Ferris *et al.*[3] announced the first synthesis of the compound by the aluminium amalgam reduction of isonitrosomalononitrile, and the compound was utilized for the synthesis of adenine by the same authors.

Our attempt, at this stage, was to synthesize aminomalononitrile from FA and hydrogen cyanide with the aid of a Lewis acid catalyst.‡ Thus we heated a mixture of FA, $POCl_3$ and hydrogen cyanide in a 1:1:2 mole ratio in a sealed tube at 120° for 10 hr and found, somewhat surprisingly, that adenine was produced in the reaction mixture. Further scrutiny of the reaction led us to the finding that hydrogen cyanide was not necessary for the reaction. Despite our efforts to look for catalysts of the

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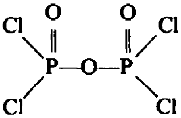
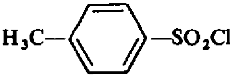
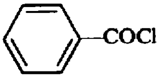
† Uvicord Type 4701A (LKB).

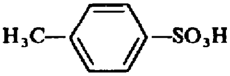
‡ The reaction between N,N-dimethylformamide and hydrogen cyanide in the presence of phosgene gives dimethylaminomalononitrile.[5]

TABLE 1. REACTION OF FORMAMIDE WITH ACID CHLORIDES

Acid chloride	Formamide:acid chloride (Mol.)	Yield of adenine (%)
$POCl_3$	1:1	36
	1:1	3
PCl_5	1:1	18
PCl_3	1:1	0
	1:1	9
$SOCl_2$	1:1	+
SO_2Cl	1:1	0
$H_3C—COCl$	1:1	0
	1:1	0
	1:1	0
$H_3C—SO_2Cl$	1:2	1·4

Reaction temp: 120°. Reaction time: 5 hr.

TABLE 2. REACTION OF FORMAMIDE WITH SOME REAGENTS

Reagent	Formamide: Reagent (Mol.)	Reaction condition Temp (°C)	Time (hr)	Yield of adenine (%)
liq. SO_2	1:10	120	10	0
liq. Cl_2	1:20	120	5	0
liq. $COCl_2$	1:50	150	10	0
$ClCOOC_2H_5$	1:1	120	5	0
$(CH_3)_2SO_4$	1:2	120	5	0
S_2Cl_2	2:5	120	5	0
$ClSO_3H$	1:3	120	5	0
	1:1	120	5	0
HCOOH	1:5	120	5	0
P_2O_5	10:3	120	5	+*
Al_2O_3	1:1	120	5	0
$AlCl_3$	1:1	200	5	0
$KHSO_4$	1:1	120	5	0
Molecular sieve 5A	/	120	5	0

* A trace of adenine was detected on PPC.

reaction, none of the compounds examined were superior to $POCl_3$; the results are summarized in Tables 1 and 2.

Our efforts were then directed towards the optimal conditions of the reaction between FA and $POCl_3$. First of all, we examined the yields of the reaction with varying mole ratios of the reactants. Thus it was demonstrated that a mole ratio of

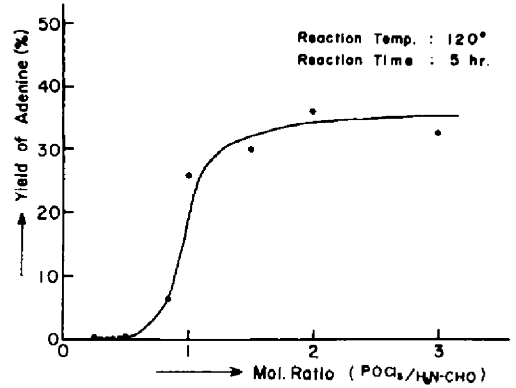


FIG. 1 Effect of mol. ratio on the formation of adenine.

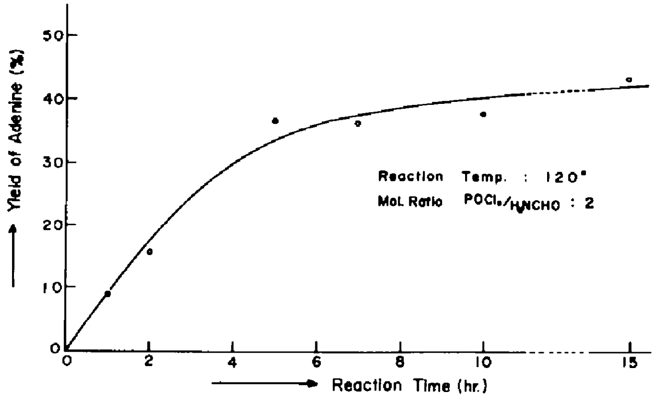


FIG. 2 Effect of reaction time on the formation of adenine.

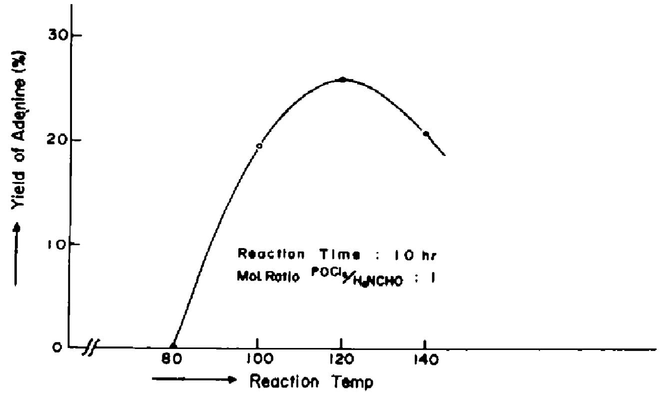


FIG. 3 Effect of reaction temperature on the formation of adenine.

$POCl_3$ to FA of less than unity led always to a very poor yield of adenine as shown in Fig. 1. On the contrary, in a mole ratio exceeding 1·5 a yield of more than 30% of theory calculated on FA was attained. Secondly, we carried out the experiments to find out the optimal temperature and the period of the reaction time. As shown in Fig. 2 and Fig. 3, it was made clear that the temperature was optimal at 120° and the yield reached a plateau after 7 hr and gradually increased up to 43·5% after 15 hr from the beginning of the reaction. With the aim of further improvement of the yield the reaction was carried out with an addition of other substances, however, none of them were effective to improve the yield of adenine as summarized in Table 3.

TABLE 3. EFFECT OF ADDITIVES ON THE FORMATION OF ADENINE

Additive	Formamide:additive (Mol.)	Yield of adenine (%)
KCN	1:2	21·5
Malononitrile	1:2	25
Dioxane	5:1	9
Dimethylformamide	1:1	0
P_2O_5	5:3	20
$AlCl_3$	1:1	3·7
HCl	/	26·5
Triethylamine	1:2	0
Pyridine	1:2	0
N,N-Dimethylaniline	1:2	0

Formamide: $POCl_3$ = 1:1. Reaction temp: 120°. Reaction time: 5 hr.

When the reaction was carried out in an open vessel a large quantity of ammonium chloride separated, which sublimed and condensed on the wall of the vessel, and practically no adenine was produced. Attempts to carry out the reaction in a vapor phase by the help of various dehydrating catalysts thus far met with failure.

For quantitative estimation of adenine in reaction mixtures, a 0·2–0·3 ml portion of the crude reaction mixture, which was diluted to a concentration of ca. 1 mg/ml of adenine, was applied on top of a column (Dowex 50 × 8, H^+ form), run with 2·2N HCl and finally measured by a UV-recorder at 253·7 mμ.* The UV-absorption thus recorded was referred to the calibration curve to calculate the quantity of adenine. The pure specimen of adenine was easily separated from the reaction mixture by adsorption on a column of active charcoal followed by elution with dilute aqueous methanolic ammonia solution, and identified by paper chromatography with two different solvent systems, UV, IR and mass spectra and elemental analysis.

A proposed mechanism[1,3] for the synthesis of adenine from hydrogen cyanide and ammonia involves that the reaction proceeds with intermediary formation of formamidine and 4-aminoimidazole-5-carboxamidine. This points to a possibility that hydrogen cyanide formed *in situ* might be playing a vital part in the present synthesis of adenine.

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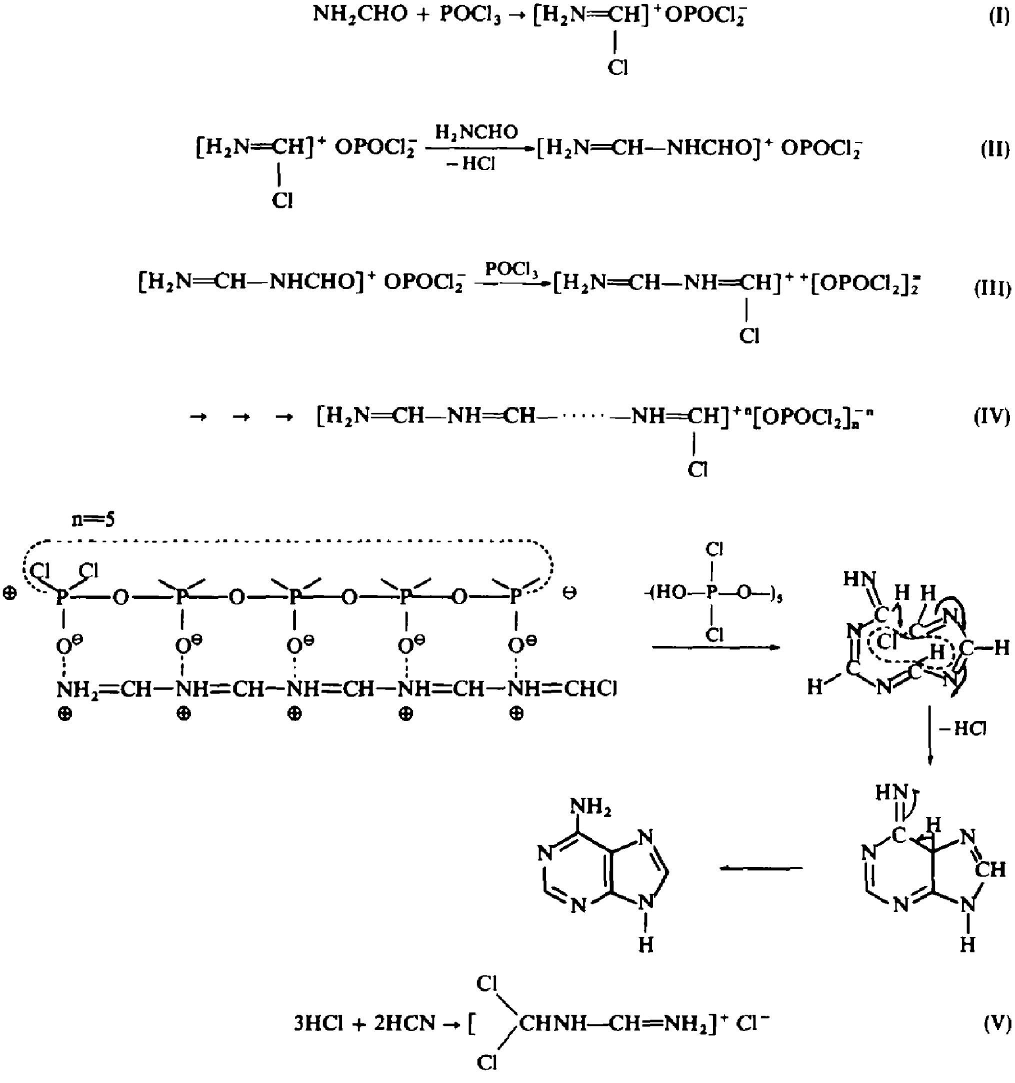
* See footnote † on page 5732.

In order to ascertain whether hydrogen cyanide is a genuine intermediate for the present reaction, a small quantity of $K^{14}CN$ was added to the reaction mixture and adenine isolated was assayed by a liquid scintillation counter. The result indicated that no hydrogen cyanide participation had taken place. Furthermore, gas chromatography of the vapor phase in the reaction vessel did not show the presence of hydrogen cyanide in any significant quantity. In addition, the reaction between hydrogen cyanide and $POCl_3$ afforded only a trace of adenine. These results suggest



$$NH_2CHO + POCl_3 \rightarrow [H_2N{=}\underset{\substack{|\\ Cl}}{C}H]^+OPOCl_2^- \qquad (I)$$

$$[H_2N{=}\underset{\substack{|\\ Cl}}{C}H]^+\ OPOCl_2^- \xrightarrow[-HCl]{H_2NCHO} [H_2N{=}CH{-}NHCHO]^+\ OPOCl_2^- \qquad (II)$$

$$[H_2N{=}CH{-}NHCHO]^+\ OPOCl_2^- \xrightarrow{POCl_3} [H_2N{=}CH{-}NH{=}\underset{\substack{|\\ Cl}}{C}H]^{++}[OPOCl_2]_2^- \qquad (III)$$

$$\rightarrow\ \rightarrow\ \rightarrow\ [H_2N{=}CH{-}NH{=}CH{-}\cdots\cdots{-}NH{=}\underset{\substack{|\\ Cl}}{C}H]^{+n}[OPOCl_2]_n^{-n} \qquad (IV)$$

$$3HCl + 2HCN \rightarrow [\overset{Cl}{\underset{Cl}{>}}CHNH{-}CH{=}NH_2]^+\ Cl^- \qquad (V)$$

Chart 1

that the reaction between FA and $POCl_3$ to yield adenine would have proceeded by a path in which hydrogen cyanide played no vital role in the mechanism. It is interesting to note that reactions of phosgene with FA under a variety of conditions always failed to give adenine. This appears somewhat peculiar because phosgene has been known equivalent to $POCl_3$ in regard to formation of the Vilsmeier reagent.[6]

At present we have no definite evidence to propose a mechanism for the present reaction, however, assuming that FA can possibly react with $POCl_3$*†[7] and that a chlorinated phosphoric acid is prone to polymerize under anhydrous conditions, it is tempting to speculate a possible mechanism for the reaction. In our view this might be illustrated as follows: First of all, FA reacts with $POCl_3$ to give a Vilsmeier compound (I), which may react further with FA‡ to give II, followed by the action of $POCl_3$ to give III. Thus polymerization of the Vilsmeier compound would proceed until it reaches a possible intermediate (IV: $n = 5$) which cyclizes to yield adenine as shown in Chart 1.

A fairly stable crystalline compound obtained from 2 moles of hydrogen cyanide and 3 moles of hydrogen chloride has been demonstrated[11] to be N-dichloromethylformamidinium chloride (V). When we heated this complex with $POCl_3$ in a sealed tube adenine was obtained in a 14% yield. Since this complex is considered a derivative of dimerized FA, the reaction may be a supporting evidence for the above described mechanism for the adenine synthesis.

EXPERIMENTAL

Adenine

(*a*) *From formamide and phosphorus oxychloride.* Formamide (0·01 M; 450 mg) was placed in a stainless steel vessel (30 ml), which was cooled with dry ice–acetone. To a solidified mass of formamide was added 3·1 g (0·02 M) $POCl_3$ and the vessel was sealed tightly. After being left standing for 30 min at room temp the vessel was heated at 120° for 10 hr in an oil bath. The reaction mixture was cooled, treated with water, and a slightly colored soln thus obtained was filtered to remove a very small quantity of resinous material. A portion of the filtrate was applied on the column (Dowex, 50 × 8, H^+ form, 6 × 80 mm) which was connected with Uvicord Type 4701A (LKB) and run with 2·2N HCl. From the calibration curve, the yield of adenine was calculated as being 103 mg (38%). A soln containing 703 mg of adenine (calculated as above) was run through a column packed with 17·5 g of active charcoal. After being washed with water, the column was eluted with aqueous methanolic ammonia (water–MeOH–28% NH_4OH = 6:6:1). The eluate (450 ml) was evaporated to yield a colorless powder (740 mg). The powder was identified by paper chromatography [ascending, Whatman No. 1, solvent systems; (i) NH_4OH (pH = 10), and (ii) n-butanol saturated with water–28% NH_4OH = 100:1] and by the comparison of the IR, UV and mass spectra with authentic adenine. The elemental analysis after being dried over P_2O_5 was consistent with the theoretical values. (Found: C, 44·23; H, 4·08; N, 51·61. Calc. for $C_5H_5N_5$: C, 44·44; H, 3·73; N, 51·83%). All other experiments were carried out in a similar fashion.

(*b*) *From* N-*dichloromethylformamidinium chloride and phosphorus oxychloride.* A mixture of 200 mg N-dichloromethylformamidinium chloride and 2 ml $POCl_3$ was heated in a sealed vessel at 180° for 5 hr in an oil bath. After cooling, the reaction mixture was treated as described to yield 9·3 mg adenine. (14%, assuming that 1 mole of adenine was formed from 2·5 moles of N-dichloromethylformamidinium chloride).

Incorporation test of $H^{14}CN$ *into adenine.* In a stainless steel vessel (30 ml) containing 450 mg formamide and 3·1 g $POCl_3$ as described above, a glass tube containing 1·3 g KCN ($K^{14}CN$: 84·5 mCi/Mol) was added so as to generate $H^{14}CN$ upon contact with HCl which arises from the reaction of formamide and $POCl_3$. The vessel was sealed and heated at 120° for 10 hr. After being worked up as described, formation of adenine was checked by ion-exchange chromatography (the yield was estimated as being 33% of theory)

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* It has been reported that FA and phosgene reacted to give a complex.[8]

† Although $POCl_3$ in a large excess of FA gives trisformylaminomethane,[9] we confirmed that adenine was not formed under these conditions.

‡ N-Monosubstituted formamide chloride is prone to dimerization.[10]

and paper chromatography. The spot of adenine on the paper chromatogram was eluted and its radio activity was measured by a liquid scintilation counter to show the radio activity was practically absent from the adenine formed.

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