Hydrazinolysis of Some Purines and Pyrimidines and their Related Nucleosides and Nucleotides

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Uracil and thymine and their related nucleosides and nucleotides are degraded quantitatively by treatment with hydrazine hydrate at 90°. The reaction products are, respectively, pyrazol-3-one, and 5-methylpyrazol-3-one together with, in the case of the nucleosides and nucleotides, approximately quantitative yields of urea and a sugar or sugar phosphate hydrazone. Cytosine and its derivative are similarly degraded but two heterocyclic products, 3-aminopyrazole and *NN'*-di(3-pyrazolyl)hydrazine are obtained. Adenine and guanine, their related nucleosides, and guanine nucleotides are not degraded by hydrazine hydrate at 90° but adenine nucleotides are slowly attacked. In this reaction the only degradation product which has been identified is inorganic phosphate.

THE hydrazinolysis of certain nucleic acid components and related compounds has been studied.[1-7] More recently, the reaction of anhydrous hydrazine with ribo- and deoxyribo-nucleic acids has been investigated [8-18] and has been used [19] in studies of the purine nucleotide distribution in the latter macromolecule. Preliminary examinations [20] of the reaction of ribo- and deoxyribonucleic acids with hydrazine hydrate made clear the need for a fuller understanding of the reaction of nucleic acids and their components with this reagent. The present work was therefore undertaken and has yielded results confirming and extending those of the studies mentioned above.

The rates of degradation of a number of pyrimidines, and purine and pyrimidine nucleosides and nucleotides in hydrazine hydrate at 90° and in 15% aqueous hydrazine hydrate at 65° are listed in Table 3. Previous reports have given data on the degradation of some of the compounds listed in Table 3 in anhydrous hydrazine at 37°,[15] and at 60°;[17,18] in hydrazine hydrate and 15% aqueous hydrazine hydrate at 70°;[1] and in 7M-hydrazine hydrate at pH 5·5, 7, and 9 at 0, 37, and 100°.[6] Similar relative rates of reaction for the various compounds tested have been obtained in all cases. The following generalisations can be made. Within a series of pyrimidine derivatives the order of reactivity is nucleotide $\geqslant$ nucleoside $\gg$ free base (deoxyribose derivatives react more slowly than the corresponding ribose derivatives). Within a group of pyrimidines or pyrimidine derivatives the order of reactivity is uracil $\gg$ cytosein $>$ thymine.

Levene and Bass [3] isolated pyrazol-3-one by treatment of uridine with hydrazine hydrate and suggested that the ribose moiety of uridine was converted into ribosylurea. The work of Chargaff [17] confirmed in the present report has shown that in fact the hydrazinolysis of pyrimidine nucleosides and nucleotides yields a pyrazole, free urea, and a sugar or sugar phosphate hydrazone (Table 4). Chargaff and his co-workers [17] did not isolate the carbohydrate derivative obtained by hydrazinolysis of thymidine but converted it into the known deoxyribose 2,4-dinitrophenylhydrazone. Recently simple aliphatic hydrazones [21] and carbohydrate hydrazones [22,23] have been isolated as stable crystalline solids. Identification of the product of reaction of ribose with hydrazine hydrate as ribosehydrazone and the data collected in Table 6 describing the properties of the sugar derivatives obtained by hydrazinolysis of ribose, deoxyribose, ribose-5-phosphate, and several ribo- and deoxyribonucleosides and -nucleotides suggest that in all cases a sugar or sugar phosphate hydrazone is produced.

Fosse, Hieulle, and Bass [2] described the preparation of pyrazol-3-one and 5-methylpyrazol-3-one by hydrazinolysis of uracil and thymine, respectively. Isolation of small yields of 3-aminopyrazole after hydrazinolysis of cytidine or cytidylic acid has also been reported.[1,17] Lingens and Schneider-Bernlöhr [7] showed that in hydrazine hydrate at 80° cytosine is converted into a mixture of 3-aminopyrazole and 4-hydrazinopyrimidin-2-one (I), and in 4M-hydrazine at pH 6 and 80° into a mixture of (I) and 4,4'-hydrazodipyrimidin-2-one (II). The present report shows that in hydrazine hydrate at 90° two pyrazole derivatives are formed in low yield from cytosine and in high yield from cytosine nucleosides and nucleotides (Table 4). These are 3-aminopyrazole and a related compound which we tentatively formulate as *NN'*-di-(3-pyrazolyl)hydrazine (III).

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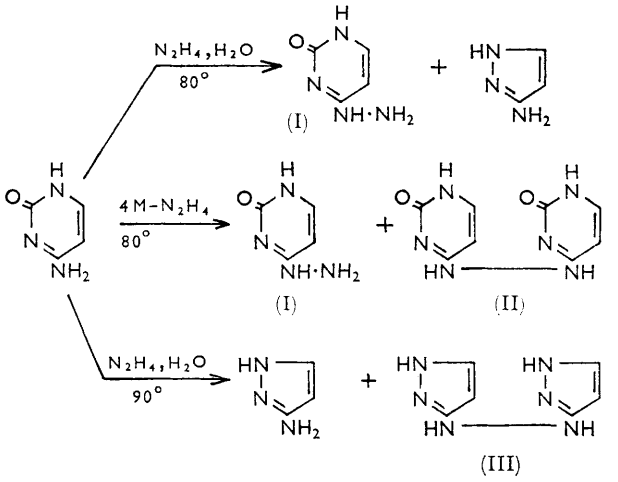
This structure accounts for the non-diazotisability of the compound and for its partial, presumably reductive, cleavage to 3-aminopyrazole during prolonged heating in hydrazine hydrate. Table 1 shows that the u.v. absorption spectra of compound (III) are very similar



to those of 3-aminopyrazole over a wide range of pH values but possess $\varepsilon_{max.}$ values several times higher. A similar quantitative relationship exists between the spectra [24] (ethanol solution) of aniline ($\varepsilon_{max.}$ *ca.* 7800) and hydrazobenzene ($\varepsilon_{max.}$ *ca.* 21,000) but their absorption maxima (235 and 248 mμ, respectively) differ considerably. Confirmation of structure (III) will require further work.

The following observations suggest that production of 3-aminopyrazole and *NN'*-di-(3-pyrazolyl)hydrazine (III) during hydrazinolysis of cytosine and its derivatives is concurrent rather than sequential. 3-Aminopyrazole is not converted into (III) during prolonged heating in hydrazine hydrate. It has been reported that 5-methylpyrazol-3-one is converted into a bis-compound under such conditions.[25] *NN'*-Di-(3-pyrazoly)hydrazine is cleaved slowly to 3-aminopyrazole (34% in 4 hr. at 90°) in hydrazine hydrate but is produced rapidly from cytidine-2',3' phosphate in the same yield as 3-aminopyrazole (quantitative destruction of CMP * and 35% and 32% yields of amino-pyrazole and dipyrazolyl-hydrazine, respectively, in 25 min. at 90°; Tables 3 and 4). Since both 3-aminopyrazole and *NN'*-di-(3-pyrazolyl)hydrazine are stable under conditions which cause complete decomposition of cytosine (Table 3) but give low yields of these compounds (Table 4) it is evident that hydrazinolysis of cytosine produces mainly fragments not detected by the analytical procedures used, *i.e.*, devoid of u.v. absorbing properties. This type of degradation is quantitatively very much less important in the case of cytosine nucleosides and nucleotides.

Hydrazinolysis has been regarded as a pyrimidine specific reaction [17,19] although it was noted [19] that treatment of $\phi X174$ deoxyribonucleic acid with anhydrous hydrazine gave results suggesting some differential loss of adenine residues. Ellery and Symons,[18] however, showed that anhydrous hydrazine at 60° causes relatively rapid destruction of adenine residues in DNA and in 5'-deoxyadenylic acid. Guanine residues are not attacked under such conditions. As shown here (Table 5) 2'(3')-adenylic acid and 5'-deoxyadenylic acid are attacked by hydrazine hydrate at 90° in a reaction which apparently causes destruction of the purine ring (loss of u.v. absorption) and of the sugar moiety of these compounds with liberation of inorganic phosphate. Guanosine, 2'(3')-guanylic acid, and 5'-deoxyguanylic acid are not attacked detectably under these conditions (Table 3). Hence neither anhydrous hydrazine at 60° nor hydrazine hydrate at 90° can be considered as a truly pyrimidine-specific reagent.

* AMP, CMP, GMP, TMP, and UMP refer to the monophosphates of adenosine, cytidine, guanosine, thymidine, and uridine.

EXPERIMENTAL

Preparation of Pyrazoles.—(a) *Pyrazol-3-one.* Uracil (4·48 g.) and hydrazine hydrate (10 c.c.) were heated at 100° for 60 min. The reaction mixture was evaporated to dryness *in vacuo* at 100° and the residue was re-evaporated to dryness twice with water (20 c.c.) and finally dissolved in water (50 c.c.). The solution was acidified (H_2SO_4) to pH 4 and extracted continuously with ether for 12 hr. Evaporation of the ether extract yielded pyrazol-3-one (3·06 g., 90%) as crystals, m. p. 156—160°, recrystallised from methanol, m. p. 163—164° [Found, in material dried (P_2O_5) at 100°/1 mm. for 12 hr.: C, 42·65; H, 4·8; N, 33·6. Calc. for $C_3H_4N_2O$: C, 42·85; H, 4·8; N, 33·3%]. Light absorption characteristics are in Table 1. Dixanthydryl pyrazol-3-one was obtained as prisms from acetone, m. p. 217—220° (decomp.) [lit., 215—217,[1] 210—213° (decomp.) [2,3]].

(b) *5-Methylpyrazol-3-one.* Thymine (2·0 g.) and hydrazine hydrate (10 c.c.) were heated at 100° for 4 hr. The reaction mixture was evaporated to dryness *in vacuo* at 100° and the crystalline residue re-evaporated twice to dryness with water (20 c.c.). Recrystallised from water, 5-methylpyrazol-3-one had m. p. 220—222° (sublimes below m. p.) [Found, in material dried (P_2O_5) at 100°/1 mm. for 12 hr.: C, 48·85; H, 6·4; N, 28·5. Calc. for $C_4H_6N_2O$: C, 48·95; H, 6·15; N, 28·55%]. Light absorption characteristics are in Table 1. Picrate, needles from water, m. p. 205° [Found, in material dried (P_2O_5) at 70°/1 mm. for 24 hr.: C, 36·9; H, 2·65; N, 21·8. Calc. for $C_{10}H_9N_5O_8$: C, 36·7; H, 2·75; N, 21·4%]. *Dixanthydryl 5-methylpyrazol-3-one*, plates from acetone, m. p. 204—205° [Found, in material dried (P_2O_5) at 70°/1 mm. for 24 hr.: C, 78·2; H, 4·35; N, 5·75. $C_{30}H_{22}N_2O_3$ requires C, 78·6; H, 4·8; N, 6·1%].

(c) *3-Aminopyrazole.* (i) Cytosine (2 g.) and hydrazine hydrate (15 c.c.) were heated at 90° for 3 hr. The reaction mixture was then subjected to fractional distillation. After removal of hydrazine hydrate at ordinary pressure distillation was continued *in vacuo* and crude 3-aminopyrazole was collected as a heavy yellow oil (400 mg.), b. p. 155—160°/10 mm. Picrate, needles from water, m. p. 219—221° [Found, in material dried (P_2O_5) at 100°/1 mm.

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for 12 hr.: C, 34·8; H, 2·35; N, 27·1. Calc. for $C_9H_8N_6O_7$: C, 34·6; H, 2·6; N, 26·7%]. *Trixanthydryl derivative*, needles from acetone, m. p. 185—187° [Found, in material dried (P_2O_5) at 100°/1 mm. for 12 hr.: C, 80·35; H, 4·55; N, 6·8. $C_{42}H_{29}N_3O_3$ requires C, 80·8; H, 4·7; N, 6·75%]. Light absorption characteristics of 3-aminopyrazole prepared in solution by regeneration from its analytically pure picrate (treatment with washed Dowex 2 × 10 ion-exchange resin, hydroxide form) are in Table 1.

(ii) Cytosine (4 g.) and hydrazine hydrate (40 c.c.) were heated at 90° for 3 hr. The reaction mixture was evaporated to dryness *in vacuo* at 100° and the residue dissolved in 0·01N-HCl (40 c.c.) and passed on to a column (20 × 2 cm.) of Dowex 50 × 8 ion-exchange resin (200—400 mesh; hydrogen form) previously washed with 0·01N-HCl (200 c.c). The column was eluted with 0·25N-HCl (6 l.), fractions of 25 c.c. being collected at a flow rate of 100 c.c. per hr. Two major u.v. (240 mμ) absorbing peaks (fractions 120—155, and 165—220, respectively) and several minor peaks were eluted. Fractions 120—155 were combined, evaporated *in vacuo* to dryness, and the residue, dissolved in water (3 c.c.), was passed through a column (3 × 0·5 cm.) of Dowex 2 × 10 ion-exchange resin (200—400 mesh, hydroxide form). The column was washed with water (5 c.c.) and the combined column eluate and washings were treated with a hot solution of picric acid (460 mg.) in water (10 c.c.). On cooling the mixed solutions deposited crude 3-aminopyrazole picrate as needles (300 mg.), m. p. 210—220°, which after recrystallisation from water had m. p. and mixed m. p. 217—219°. Fractions 165—220 were combined and evaporated to dryness *in vacuo*, leaving a white crystalline residue (500 mg.; softens at 205°, decomposes above 265°) which was recrystallised twice from ethanol. Purified NN′-*di*-[3-*pyrazolyl*]*hydrazine dihydrochloride* did not have a sharp m. p. but softened at 205° and decomposed with effervescence above 280° [Found, in material dried (P_2O_5) at 100°/1 mm. for 12 hr.: C, 30·2; H, 4·45; N, 34·25. $C_6H_8N_6$,2HCl requires C, 30·4; H, 4·2; N, 35·45%]. NN′-*Di*-(3-*pyrazolyl*)*hydrazine* was obtained as a hydrate by passing a solution of its hydrochloride (100 mg.) in water (10 c.c.) through a column (10 × 1 cm.) of Dowex 1 × 10 ion-exchange resin (200—400 mesh, hydroxide form). The column was washed with water (150 c.c.) until elution of u.v. (225 mμ) absorbing material was completed. Column eluate and washings were evaporated to dryness *in vacuo* and the crystalline residue (68 mg.), m. p. 112—118°, was recrystallised from water as prisms, m. p. 120—121° [Found, in material dried (silica gel) at 1 mm. for 12 hr.: C, 34·45; H, 5·8; N, 40·05. $C_6H_8N_6$,2½H_2O requires C, 34·45; H, 6·2; N, 40·2%]. Light absorption characteristics are in Table 1. Attempts to dehydrate this material by drying (P_2O_5) at 100°/0·1 mm. yielded a high-melting product (270—275°) which showed no u.v. absorbing properties (210—300 mμ). This product was not further investigated. NN′-*Di*-(3-*pyrazolyl*)*hydrazine picrate* was obtained as needles from water, softens at 188—190°, melts and decomposes 210—230° [Found, in material dried (P_2O_5) at 100°/1 mm. for 12 hr.: C, 34·15; H, 3·0; N, 27·25. $C_{18}H_{14}N_{12}O_{14}$ requires C, 34·7; H, 2·25; N, 27·0%], *M* (Rast) 220 (c 1·92), 320 (c 10·43), 406 (c 13·66), and 600 (c 18·01). These results suggest dissociation of a ternary salt having a formula weight 622.

TABLE 1

Light absorption of pyrazoles

Compound		Solvent: 6N-HCl	0·1N-HCl	pH 5·2	pH 7·2	pH 9·0	0·1N-KOH
Pyrazol-3-one	$\lambda_{max.}$	223	222	240	234	231	237
	$\varepsilon_{max.}$	7500	7000	4900	5700	6900	5300
	$\varepsilon_{230}/\varepsilon_{240}$	5·7	5·3	0·8	1·07	1·3	0·84
5-Methylpyrazol-3-one	$\lambda_{max.}$	232	231	248	247	242	242
	$\varepsilon_{max.}$	7250	7000	7100	7100	7100	6500
	$\varepsilon_{230}/\varepsilon_{240}$	1·39	1·53	0·60	0·62	0·69	0·51
3-Aminopyrazole	$\lambda_{max.}$	240	239	225	224	224	233
	$\varepsilon_{max.}$	6100	5200	3700	3900	3900	1650
	$\lambda_{min.}$		214				
	ε_{min}		2050				
	$\varepsilon_{230}/\varepsilon_{240}$	0·70	0·79	1·90	2·34	2·18	1·36
NN′-Di-(3-pyrazolyl)hydrazine	$\lambda_{max.}$	238	236	223	223		223
		(238)	(237)		(223)		(225)
	$\varepsilon_{max.}$	23,100	21,700	11,600	11,100		11,700
		(25,600)	(21,700)		(12,100)		(10,700)
	$\lambda_{min.}$	213	212				
		(218)	(213)		(215)		
	$\varepsilon_{min.}$	2700	3350				
		(3970)	(3800)		(11,900)		
	$\varepsilon_{230}/\varepsilon_{240}$	0·73	0·83	3·28	3·51		2·41
		(0·73)	(0·84)	(2·92)			(2·46)

Treatment of 3-*Aminopyrazole and* NN′-*Di*-(3-*pyrazolyl*)-*hydrazine Dihydrochloride with Hydrazine Hydrate.*—3-Aminopyrazole, regenerated from its picrate (3·1 mg., 10 μmoles) by treatment with washed Dowex 2 × 10 ion-exchange resin and evaporation of the resin supernatant and washings to dryness *in vacuo*, and NN′-di-(3-pyrazolyl)-hydrazine dihydrochloride (2·4 mg., 10 μmoles) were dissolved in hydrazine hydrate (1 ml.) and the solutions heated at 90°. No change in the u.v. absorption (236 mμ; 0·1N-HCl) of the reaction mixtures was observed during 4 hr. at 90°. The 4 hr. reaction mixtures were evaporated to dryness *in vacuo*, the residues re-evaporated with water (2 × 10 ml.) to dryness, and finally dissolved in water (1 ml.). Aliquots of these solutions were chromatographed, with appropriate marker substances, on Whatman No. 1 paper in solvent 2 (see below). Ultraviolet-absorbing

spots on the dried developed chromatograms were cut out together with blank areas of the same R_F, eluted with 0·1N-HCl (5 ml.), and the u.v. absorption spectra of the eluates measured. The reaction product from 3-aminopyrazole contained a single component, R_F 0·6, whose u.v. absorption identified it as unchanged starting material (96%). Three products were present in the reaction mixture from *NN'*-di-(3-pyrazolyl)hydrazine. These were unchanged starting material (R_F 0·52; 66%), 3-aminopyrazole (R_F 0·6; 20%), and an unidentified substance (R_F 0·24) whose u.v. spectrum was very similar to those of 3-aminopyrazole and *NN'*-di-(3-pyrazolyl)hydrazine.

Light Absorption Characteristics of Pyrazoles and Pyrazolones.—Compounds were dissolved in the solvents listed at concentrations of approximately 0·1mM. Solvents: pH 5·2, potassium acetate–acetic acid buffer 0·05M; pH 7, potassium phosphate buffer 0·02M; pH 9, Tris–hydrochloric acid buffer 0·05M. 3-Aminopyrazole was regenerated from a weighed quantity of its analytically pure picrate by treatment with washed Dowex 2 × 10 ion-exchange resin. Figures in parentheses for *NN'*-di-(3-pyrazolyl)-hydrazine were obtained using the analytically pure base dihydrochloride.

Paper Chromatography of Pyrazoles.—Ascending chromatograms on Whatman No. 1 paper were run in the following solvent systems: 1, butan-1-ol–water (86 : 14), 2, propan-2-ol–hydrochloric acid–water (170 : 41 : 39); 3, propan-2-ol–ammonium hydroxide (*d* 0·88)–water (7 : 1 : 2). Spots on the developed chromatograms were detected by (*a*) u.v. absorption, (*b*) aqueous ferric chloride (1%) spray:[26] 3-aminopyrazole, grey-brown; pyrazol-3-one, brown; 5-methylpyrazole-3-one, red-violet; *NN'*-di-(3-pyrazolyl)-hydrazine, brown; pyrimidines, no reaction, (*c*) diphenylcarbazone spray:[27] pyrazoles and pyrazolones, blue; pyrimidines, blue-violet, (*d*) phenol–sodium hypochlorite spray:[28] 3-aminopyrazole, pyrazol-3-one, 5-methylpyrazol-3-one, brown; *NN'*-di-(3-pyrazolyl)hydrazine, no reaction; pyrimidines, blue. (*e*) 3-Aminopyrazole alone of the compounds in Table 2 is diazotisable and can be selectively detected by spraying with an aqueous acetic acid solution of sodium nitrite, followed by an alkaline aquous solution of 1-naphthol (1%) (brown colour). The R_F values observed are in Table 2.

TABLE 2

R_F Values of pyrazoles and pyrimidines

Compound	Solvent, R_F 1	2	3
Pyrazol-3-one	0·60	0·80	0·40
5-Methylpyrazol-3-one	0·67	0·90	0·50
3-Aminopyrazole	0·45	0·61	0·67
NN'-Di-(3-pyrazolyl)hydrazine		0·55	
Uracil	0·33	0·70	0·60
Thymine	0·50	0·80	0·45
Cytosine	0·12	0·43	0·50

Kinetics of Reaction of Bases, Nucleosides, and Nucleotides in Hydrazine Hydrate at 90°, *and in* 15% *Aqueous Hydrazine Hydrate at* 65°.—The compounds in Table 3 were dissolved at a concentration of 30 μmoles per c.c. in hydrazine hydrate preheated to 90°, or in 15% aqueous hydrazine hydrate preheated to 65°. Zero time was taken to be the time at which complete solution was obtained. Solutions were incubated in stoppered tubes at 90 and 65°, respectively, and at zero time and suitable intervals thereafter aliquots of the reaction mixtures were diluted (100 or 200 fold) in 0·1N-sodium hydroxide and their u.v. absorption at 250 mμ measured. Table 3 summarises the results.

TABLE 3

Kinetics of hydrazinolysis reactions

Compound	Hydrazine hydrate, 90° Half life (min.)	Time for 100% decomp. (min.)	15% Aq. hydrazine hydrate, 65° Half life (min.)	% Decomp. after 60 min.	% Decomp. after 210 min.
Uracil	4	30	150	20	63
Uridine	<1	10	18	100	
Deoxyuridine	<1	20	30	100	
2′(3′)-UMP	*ca.* 1	10	18	100	
5′-UMP	<1	5			
Cytosine	20	180			7
Cytidine	4	25			
Deoxycytidine	8	50			10
2′(3′)-CMP	*ca.* 1	25		13	38
5′-DeoxyCMP	6	25			10
Thymine	55	250			10
Thymidine	12	80			11
5′-TMP	8	55			25
Adenosine	No detectable decomp. in 4 hr.				
2′(3′)-AMP	210				
5′-DeoxyAMP	360				
Guanosine	No detectable decomp. in 6 hr.				
2′(3′)-GMP					
5′-DeoxyGMP					

Stoicheiometry of the Reaction of Hydrazine Hydrate with Pyrimidine Derivatives at 90°.—Compounds to be examined (120 μmoles) were dissolved in hydrazine hydrate (2 c.c.) at 90° and the solutions heated at 90° for periods long enough to cause complete decomposition (see Table 3). Reaction mixtures were evaporated to dryness *in vacuo* on a water-bath, and the residues dissolved in water (2 c.c.) and passed through columns (3 × 1 cm.) of ion-exchange resin IRC 50 (hydrogen form) to remove residual hydrazine. The columns were washed with water until total eluate volumes of 10 c.c. were obtained. Pyrazoles were assayed in the eluates by their u.v. absorption. Colorimetric assays were used to measure the amounts of ribose [29] and deoxyribose [30] reacting material, urea,[31] and inorganic phosphate.[32] The results are summarised in Table 4.

Stoicheiometry of the Reaction of Hydrazine Hydrate at 90° *with* 2′(3′)-*AMP and* 5′-*DeoxyAMP.*—Solutions of 2′(3′)-AMP and 5′-deoxyAMP (60 μmoles) in hydrazine hydrate (2 c.c.) were heated at 90° and aliquots (0·1 c.c.) removed at intervals, evaporated to dryness *in vacuo* on a water-bath, and the residues in water (2 c.c.) passed through columns (2 × 1 cm.) of IRC 50 ion-exchange resin (hydrogen form) to remove residual hydrazine, and the columns washed with water until 20 c.c. of eluate had been collected. The eluates were concentrated *in vacuo* to 2 c.c. Ascending paper chromatography on Whatman No. 1 paper in the 5% aqueous disodium hydrogen phosphate–isopentyl

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TABLE 4

Stoicheiometry of hydrazinolysis of pyrimidine derivatives

Compound	Hydrazinolysis conditions	Reaction products μmoles per 100 μmoles of starting material: Ribose or deoxyribose reacting material [a]	Urea [b]	Pyrazoles [c]	Inorganic [d] phosphate
Uridine	N_2H_4,H_2O, 90°	50	90	93	
2′(3′)-UMP		65, 81	82, 100	75	5
5′-UMP		100			0
Cytosine				6, Amino-pyrazole 3, Dipyrazolyl-hydrazine	
Cytidine		75, 86	62	49, Amino-pyrazole 28, Dipyrazolyl-hydrazine	
Deoxycytidine		100	75	—	
2′(3′)-CMP		80, 90	68	35, Amino-pyrazole 32, Dipyrazolyl-hydrazine	
5′-CMP		100			0·3
5′-DeoxyCMP		100	52		1·3
Thymidine		100, 85	95	90	
5′-TMP		100	91	92	1·5
Uridine	15% aqueous N_2H_4,H_2O, 65°	79	70	83	
5′-DeoxyCMP					0·1
5′-TMP					1·0

[a] The hydrazinolysis products do not contain free ribose or deoxyribose but the sugar derivatives present react as free sugar, in the colorimetric assays used. [b] Free urea was found by paper chromatography of the hydrazinolysis products of uridines cytidine, and 2′(3′)-UMP. [c] Cytosine derivatives yield mixtures of 3-aminopyrazole and *NN′*-di-[3-pyrazolyl]hydrazine. The yield of each of these two products was measured in three cases by chromatography of the crude reaction mixtures on ion-exchange columns as described in procedure (ii) for the preparation of 3-aminopyrazole. [d] Chromatographic examination of hydrazinolysis reaction mixtures does not reveal the presence of inorganic phosphate (see below). The varying low amounts found by colorimetric analyses are probably produced during the manipulations subsequent to hydrazinolysis.

TABLE 5

Stoicheiometry of hydrazinolysis of adenine nucleotides

Reaction products μmoles per 100 μmoles of starting material

	2′(3′)-AMP			5′-d-AMP		
Time (hr.)	2′(3′)-AMP	Ribose reacting material	Inorganic phosphate	5′-d-AMP	Deoxyribose reacting material	Inorganic phosphate
0	100	100	0	100	100	0
1	82	84	5	88	85	7·5
2	64	69	16			10
3½	46	44	31	61	75	16
5	36	44	49	60		26
6½	26	41	60	52	55	29

alcohol (3 : 2) system showed that the only u.v. absorbing components present in the reaction products were unreacted nucleotides. Spots on the developed chromatograms were cut out, eluted with 0·05M-trishydroxymethylmethylamine–hydrochloric acid buffer (pH 7, 5 c.c.), and the eluates assayed spectrophotometrically. The amounts of inorganic phosphate, and ribose and deoxyribose reacting material in the IRC 50 eluates were assayed as previously described (Table 5).

Nature of the Sugar Derivatives obtained by Hydrazinolysis of Nucleosides.—(a) *Paper chromatography.* The sugars, sugar phosphates, nucleosides, and nucleotides in Table 6

TABLE 6

Sugar derivatives obtained by hydrazinolysis of nucleosides

Solvent system	Substance tested	Reference R_F	Observed R_F: Treatment of hydrazinolysis products. Method of detection. None A	None P	None C	IRC 50 A	IRC 50 P	IRC 50 C	Dowex 50 A	Dowex 50 P	Dowex 50 C	PhCHO A	PhCHO P	PhCHO C
4	Ribose	0·34	0·13			0·10 0·34			0·34			0·34		
	Uridine		0·14			0·06 0·34			0·34			0·34		
	Deoxyribose	0·43			0·20			0·20 0·43			0·43			0·43
	Deoxycytidine				0·20			0·20 0·43			0·43			0·43
5	Inorganic phosphate	0·79		0·79			0·79			0·79			0·79	
	Ribose-5-phosphate	0·62	0·44	0·44					0·60	0·60 0·79 [a]				
	5′-CMP		0·44	0·44		0·54	0·54 0·79 [a]		0·62	0·62 0·79 [a]		0·62	0·62 0·79 [a]	
	2′(3′)-CMP		0·44	0·44		0·55	0·55 0·79 [a]		0·61	0·61 0·79 [a]		0·61	0·61 0·79 [a]	
	Deoxyribose	0·73			0·70 0·74			0·74			0·74			0·74
	Deoxyribose-5-phosphate	0·65		0·47	0·47		0·57	0·57		0·65 0·80 [a]	0·65 0·73		0·65 0·80 [a]	0·65 0·73
	5′DeoxyCMP			0·47	0·47		0·57	0·57		0·65 0·80 [a]	0·65 0·73		0·65 0·80 [a]	0·65 0·73
	Glucose-3-phosphate	0·58	0·25	0·25					0·58	0·58			0·58	0·58

[a] Traces of inorganic phosphate were detected only after IRC 50, Dowex 50, or benzaldehyde treatment of the crude hydrazinolysis products.

were dissolved in hydrazine hydrate at 90° at a concentration of 30 μmoles per c.c. Heating was continued for 10 min. (sugars and sugar phosphates) and for times sufficient to bring about complete reaction in the case of nucleosides and nucleotides (see Table 1). The reaction solutions were evaporated to dryness *in vacuo* on a water-bath, and the residues dissolved in water (5 c.c.). Aliquots (1 c.c.) of these solutions were treated as follows, (i) passed through columns (2 × 1 cm.) of ion-exchange resin IRC 50 (hydrogen form) which were then washed with water (2 c.c.), (ii) passed through columns (2 × 1 cm.) of ion-exchange resin Dowex 50 (hydrogen form) which were then washed with water (2 c.c.), (iii) shaken with redistilled benzaldehyde (0·1 c.c.) for 5 min. at 100° and the benzaldehyde then removed by extraction with ether. The crude reaction products and those obtained by the above treatments were examined by ascending chromatography on Whatman No. 1 paper. Solvent systems used were: 4, ethyl acetate–pyridine–water (2 : 1 : 2); 5, methanol–88% formic acid–water (80 : 15 : 5). Sugars and sugar derivatives were detected on the developed chromatograms by spraying with the aniline phthalate [33] (A), cysteine–sulphuric acid [34] (C), and phosphate [35] (P) sprays. Table 6 summarises the results.

(b) *Reaction of ribose with hydrazine hydrate.* Ribose (6 g.) and hydrazine hydrate (10 c.c.) were heated at 100° for 10 min. and the resulting solution evaporated *in vacuo* on a water-bath. The gummy residue (7·1 g.) was rubbed with ethyl acetate, and the solid product thus obtained was crystallised from methanol (3·8 g.), m. p. 108—109°. Recrystallised as prisms from aqueous methanol (1 : 15 v/v) ribose hydrazone had m. p. 119—121 (lit.,[22] 127—129, 126—127° [23]) [Found, in material dried (P_2O_5) at 50°/1 mm. for 24 hr.: C, 36·9; H, 7·25; N, 16·3. Calc. for $C_5H_{12}N_2O_4$: C, 36·6; H, 7·3; N, 17·05%]. Light absorption: in water, $\lambda_{max.}$ 204 (ε 4070) and 221 mμ (ε 3430), $\lambda_{min.}$ 218 (ε 3410); in 95% ethanol, $\lambda_{max.}$ 204·5 (ε 3730) and 226 mμ (ε 3370), $\lambda_{min.}$ 216 (ε 3200).

(c) *Reaction of ribose hydrazone with benzaldehyde.* A solution of ribose hydrazone (660 mg., 4 mmoles) in water (20 c.c.) was shaken at 100° for 10 min. with redistilled benzaldehyde (850 mg., 8 mmoles), and the mixture then set aside at 4° overnight. Crystalline benzaldehyde azine was collected by filtration (630 mg., 3·0 mmoles), m. p. and mixed m. p. 91—92°.

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