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Synthesis of Possible Antimetabolites in the Purine Series[1]

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The elaborate research on both endocyclic and exocyclic changes in the natural purine bases has resulted in various analogues which are potent antagonists of many biological systems. However, the possibilities inherent in the replacement of the glycosyl group at position 9 of the natural nucleosides by more simple substituents were until recently[3,4] almost completely neglected. This paper deals with the synthesis of adenosine analogues of the general formula (VIII) in which the sugar moiety is replaced by a simple aliphatic chain of three to six carbon atoms, with a quaternary nitrogen at the end-position. The main homologues with $n = 3$ and 5 conform with Todd's postulation that the internucleotidic linkages are formed through phospho-di-esteric groups, between the 3′ and the 5′ positions of the nucleosides.[5] The significant anti-tumour activity of the nucleoside antibiotic puromycin[6] and its fragmental derivative[7] (IX), having an amino group in the 3′ position, lent support to the hope that the compounds (VII) or (VIII) might form suitable antimetabolites of natural purines.

9-Substituted purines have been prepared by three different routes. The procedure which involves cyclization of suitably substituted imidazoles[8] suffers from the disadvantages that it is of limited structural variation and that the intermediates are not easily accessible. Direct alkylation by the method of Davoll[9] is known to yield a mixture of the 9- and the 7-isomers, or sometimes only the 7-isomer,[10] and seems to be limited to the reactive glycosyl halides. In fact, our numerous attempts to alkylate several chloromercuripurines with alkyl chlorides, or even an

α-chloroketone, were not successful. More promising seemed to be the Traube synthesis which was first applied by Todd[11] to $N_{(4)}$-substituted-4,5,6-triaminopyrimidines.

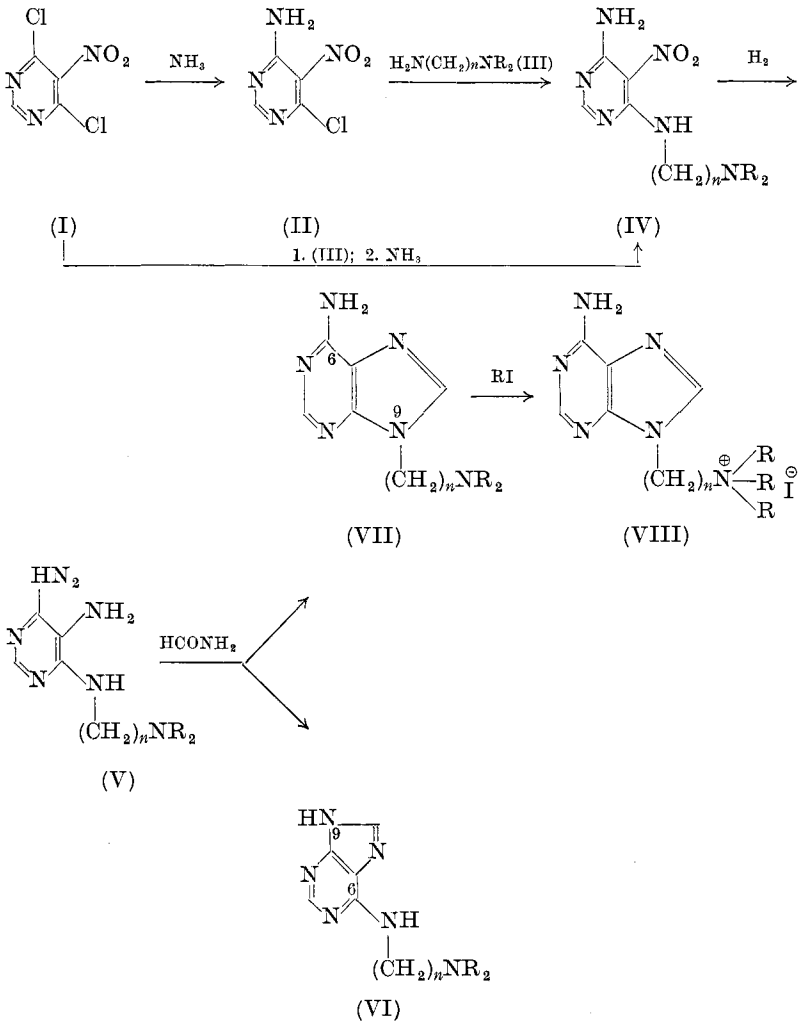
For the preparation of 4-(ω-dialkylaminoalkylamino)-5,6-diaminopyrimidines (V) we employed 4,6-dichloro-5-nitropyrimidine (I) as starting material. It was found that bisammonolysis could be reduced to a minimum when the reaction was carried out in tetrahydrofuran in the presence of sodium bicarbonate. 4-Chloro-5-nitro-6-aminopyrimidine[12]* (II) has thus been obtained in an 82 per cent yield. Condensation with the respective aliphatic diamines (III), followed by catalytic reduction of the nitro group, led to the desired $N_{(4)}$-substituted triaminopyrimidines (V). Alternatively, (IV) ($n = 3$, R = Me) was prepared by first condensing (I) with the diamine (III) ($n = 3$, R = Me) and treating the reaction product directly with excess of ammonia. This sequence, although more convenient, gave a somewhat lower yield. The triamines (V) are remarkably unstable in air and were isolated as their sulphates. Treatment of the latter with formamide following the procedure of Robins and Christensen[13] gave rise to a mixture of the isomers (VI) and (VII). Cyclizations in both directions have been previously observed by Todd and co-workers,[14] by Christensen,[15] and very recently by Hull[4e] and Timmis.[4f]

Initially we experienced considerable difficulty in separating the isomers, both being extremely soluble in water, thus excluding *a priori* their separation by the different solubilities in alkali. Separation was eventually achieved by fractional extraction with boiling *n*-heptane from which the 9-substituted isomer (VII) crystallized on cooling. Subsequent quaternization with the corresponding alkyl iodides was effected in boiling dry benzene. The structure of the synthesized adenines (VII) and (VIII) was proved by ultraviolet absorption spectroscopy.†

The aliphatic chain in (VII) or (VIII) is probably similar to a methyl group in its influence on the ultraviolet spectrum, and the absorption of these compounds should therefore resemble closely

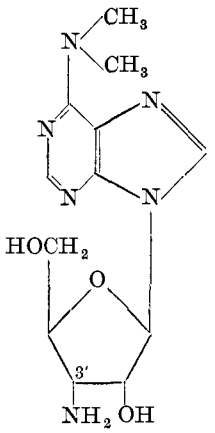
* This chloropyrimidine is, unless kept very tightly closed, unstable, especially in direct sunlight, evolving hydrogen chloride and apparently hydrolysing to 4-hydroxy-5-nitro-6-amino-pyrimidine on standing.

† In collaboration with Dr. S. Pinchas of the Weizmann Institute of Science.



Reaction Scheme. I.

that of 9-methyl-6-aminopurine. On the other hand, (VI)—or its quaternary derivative—is a 6-alkylaminopurine and its spectrum should therefore be virtually identical with that of 6-methylaminopurine.



(IX)

The ultraviolet absorption maxima of 9-methyl-6-aminopurine in neutral, acidic and basic aqueous solutions were found at 262 mμ (log ε 4·09), 260 mμ (log ε 4·08) and 262 mμ (log ε 4·11), respectively. Christensen[15] gives the value of 261 mμ (log ε 4·11) for the absorption maximum at pH 6, while Robins[4b] reports 261 mμ (log ε 4·16) for pH 1, and 262 mμ (log ε 4·08) for pH 11. 6-Methylaminopurine, on the other hand, shows its absorption maximum[16] in neutral, acidic and basic aqueous solutions respectively at 266 mμ (log ε 4·21), 267 mμ (log ε 4·18) and 273 mμ (log ε 4·20). That a dialkylaminoalkyl group has indeed the same effect as a methyl group is shown by the ultraviolet spectrum of 6-(γ-dimethylamino-*n*-propylamino)purine (VI, $n = 3$, R = Me) which possesses[17] a λ_{max} at 267–271 mμ in 95 per cent ethanol.

The isolated purines all absorb (see Table I) in neutral, acidic and basic aqueous solutions at 261·5 ± 0·5 mμ (log ε 4·04–4·24), 260 ± 1 mμ (log ε 4·10–4·23), and 260·5 ± 1·5 mμ (log ε 3·98–4·2) respectively, values which are practically identical with those of 9-methyl-6-aminopurine and completely at variance with those of 6-methylaminopurine. On the basis of these results it can be safely concluded that the structure of the isolated adenines is indeed (VII) and (VIII).

The extension of this work to the synthesis of ω-(adenyl)-alkylphosphonates will be reported later.

Table I. Ultraviolet spectra of certain purines.*

Purine derivative			Molecular species								
			cation			neutral			anion		
formula	n	R	pH	maxima λ(Å)	maxima log ε	pH	maxima λ(Å)	maxima log ε	pH	maxima λ(Å)	maxima log ε
(VII)	3	Me	1	2,590	4·14	7	2,620	4·04	12	2,600	3·98
(VII)	4	Et	1	2,590	4·22	7	2,610	4·24	12	2,590	4·17
(VII)	5	Et	1	2,590	4·12	7	2,610	4·15	12	2,610	4·15
(VII)	6	Et	1	2,600	4·20	7	2,620	4·15	12	2,620	4·18
(VIII)	3	Me	1	2,590	4·10	7	2,615	4·10	12	2,615	4·10
(VIII)	4	Et	1	2,600	4·17	7	2,620	4·17	12	2,620	4·17
(VIII)	5	Et	1	2,610	4·18	7	2,620	4·17	12	2,620	4·20
(VIII)	6	Et	1	2,600	4·19	7	2,630	4·14	12	2,640	4·18

* The spectra were measured with a calibrated Beckmann D.U. spectrophotometer, the probable experimental errors being $< \pm 0{\cdot}5$ mμ.

Pharmacological Results

Compounds (VIII) were screened by the Upjohn Company on a broad spectrum basis but have shown no outstanding biological activity.

Experimental*

4,6-Dihydroxy-5-nitropyrimidine. Prepared by the method of Boon[12] with the modification that 4,6-dihydroxypyrimidine[18] was added to the nitrating mixture at 85–90°.[15] To complete the reaction, stirring was continued for an additional 30 min at this temperature. The product, obtained in a 92 per cent yield, melted above 330°.

4-Chloro-5-nitro-6-aminopyrimidine (II). To a solution of 36·9 g of 4,6-dichloro-5-nitropyrimidine[12] (I) in 150 ml tetrahydrofuran was added 15·9 g sodium bicarbonate and the suspension warmed to 50–55°; then 17 ml of cold 12 N methanolic ammonia was added during 1 h through a dropping funnel with

* All melting points are uncorrected. Analyses were carried out in our micro-analytical laboratory under the direction of Mr. Erich Meier.

the stem dipping into the solvent. Stirring and warming were continued for a further 30 min. The filtrate was evaporated *in vacuo* at 30° to dryness, and the remainder extracted twice with boiling petroleum ether (60–80°) to remove unchanged starting material. Re-crystallization of the residue from benzene yielded 82 per cent of (II), m.p. 156°.

4-(ω - Dialkylamino - alkylamino) - 5 - nitro-6 - aminopyrimidines (Table II). To a stirred solution of 4-chloro-5-nitro-6-amino-

Table II. 5-Nitropyrimidines (IV).

n	R	M.p.	Formula	C		H		N	
				calcd.	found	calcd.	found	calcd.	found
3	Me	152–154*	$C_9H_{16}N_6O_2$	44·99	45·16	6·71	6·6	34·98	35·01
4	Et	82– 83†	$C_{12}H_{22}N_6O_2$	51·04	50·8	7·85	7·6	29·77	29·8
5	Et	83– 85*	$C_{13}H_{24}N_6O_2$	52·68	52·86	8·16	8·1	28·36	28·30
6	Et	78– 80‡	$C_{14}H_{26}N_6O_2$	54·17	54·49	8·44	8·2	27·08	26·96

Re-crystallized from: * ethanol; † methyl cellosolve-H_2O; ‡ dilute ethanol.

pyrimidine (II) in 20 vol dry dioxan was added, at 30–35°, a solution of 2 equiv of the diamine (III)* in 5 parts dry dioxane. Stirring was continued for 2 h. The filtered products were dissolved in water, the solutions decolourized with charcoal and precipitated with excess of concentrated ammonia. The pure products were obtained in yields of 60–70 per cent.

4-(ω-Dialkylamino-alkylamino)-5,6-diaminopyrimidine sulphates (Table III). The nitropyrimidines (IV) were dissolved in hot ethanol and hydrogenated with Raney nickel at a pressure of 45–60 lb./in². The theoretical amount of hydrogen was absorbed in 10–15 min. The catalyst was removed and the filtrates decolourized with charcoal. These operations were carried out under a nitrogen atmosphere to prevent darkening of the solution, the free triamines being unstable in air. An equivalent amount of 2 N ethanolic sulphuric acid was then added and the mixtures

* The dialkylaminoalkylamines were prepared by catalytic hydrogenation of the corresponding nitriles in the presence of Raney nickel and liquid ammonia at 105–110° and at a pressure of 1,500 lb./in².

Table III. 5-Aminopyrimidine sulphates (V).

n	R	M.p.	Formula	C		H		N		S	
				calcd.	found	calcd.	found	calcd.	found	calcd.	found
3	Me	268° (dec.)	$C_9H_{18}N_6.H_2SO_4$	35·06	35·08	6·54	6·70	27·26	26·94	10·4	10·41
4	Et	237–238°	$C_{12}H_{24}N_6.H_2SO_4.H_2O$	39·12	39·3	7·66	7·6	22·82	22·8	9·15	9·13
5	Et	261–262°	$C_{13}H_{26}N_6.H_2SO_4$	42·84	42·45	7·74	7·69	23·07	22·61	8·78	8·78
6	Et	above 300°	$C_{14}H_{28}N_6.H_2SO_4$	44·43	44·49	7·99	7·74	22·21	22·5	8·46	8·43

Table V. Quaternary compounds (VIII).

n	R	M.p.	Formula	C		H		N		I	
				calcd.	found	calcd.	found	calcd.	found	calcd.	found
3	Me	251* (dec.)	$C_{11}H_{19}N_6I$	36·48	36·5	5·30	5·30	23·20	23·4	35·02	34·92
4	Et	185–187†	$C_{15}H_{27}N_6I$			6·51	6·6	20·1	19·8	30·32	30·06
5	Et	208–210‡	$C_{16}H_{29}N_6I$	44·42	44·5	6·76	6·7	19·44	19·4	29·38	29·23
6	Et	198–200§	$C_{17}H_{31}N_6I$			7·0	7·12	18·83	18·6	28·43	28·46

Re-crystallized from: * methanol; † *n*-butanol; ‡ abs. ethanol-acetone; § 1-abs. ethanol: 5-*iso*propanol.

were allowed to stand in the refrigerator overnight. The precipitated sulphates were collected and re-crystallized from 70 per cent ethanol. Yields of 75–85 per cent were obtained.

9-(ω-Dialkylamino-n-*alkyl)-adenines (Table IV).* Two g of the triamine sulphates (Va) was added to 10 ml of C.P. formamide,

Table IV. 9-Substituted adenines (VII).

n	R	M.p.	Formula	C		H		N	
				calcd.	found	calcd.	found	calcd.	found
3	Me	103–105*†	$C_{10}H_{16}N_6$	54·5	54·3	7·32	7·8	38·16	38
4	Et	115–116†	$C_{13}H_{22}N_6$	59·5	59·1	8·45	8·5	32·04	31·9
5	Et	indefinite†	$C_{14}H_{24}N_6$	60·84	60·8	8·75	8·7	30·41	30·0
6	Et	68– 72†	$C_{15}H_{26}N_6.H_2O$	58·43	58·33	9·16	8·94	27·25	27·05

Re-crystallized from: * methanol; † benzene-*n*-heptane.

and the mixtures gently refluxed for 20 to 30 min. The cooled solutions were diluted with 150 ml of ethanol, concentrated ammonia added to complete the separation of ammonium sulphate and the filtrates treated with charcoal. The ethanol was distilled at reduced pressure (20 mm), and the excess of formamide removed under a nitrogen atmosphere at 110–120°/2 mm. The dark oily residues were extracted with several portions of boiling *n*-heptane, the combined extracts were cooled in ice-water and the solids collected. The white products were re-crystallized as shown in Table IV. Yields of 70–75 per cent were obtained.

9-(3-Dimethylamino-n-*propyl)-adenine (VII;* n = *3*, *R* = *Me*). Isolated in a somewhat different way, as follows. The reaction mixture was poured into 200 ml of cold ethanol, ammonium sulphate was filtered off, and the filtrate treated with ethanolic sulphuric acid. After leaving overnight in the cold, the sulphate was collected and dissolved in a minimum amount of water. The solution was made alkaline with ammonia whereupon 200 ml of methanol were added. The ammonium sulphate was separated after cooling, and the filtrate evaporated to dryness under reduced pressure and low temperature. Traces of water were removed azeotropically with dry benzene, and the dry residue was refluxed

for 30 min with 50 ml benzene, with addition of charcoal. The hot benzene extract was quickly filtered, and white flaky crystals of (VII) ($n = 3$, R = Me) separated rapidly.

ω-(9-Adenyl)-n-alkyl-trialkylammonium iodides (Table V). A solution of 1 g of the purine (VII) in 90 ml of dry benzene was refluxed for 4 h with the respective alkyl iodide. To the benzene solution, decanted from the precipitated quaternary iodide, more iodide was added, and refluxing was continued for another 2 h to complete the reaction. Yields of 80–90 per cent were obtained.

Summary. The synthesis of several substituted adenines ((VII) and (VIII)) is reported. 4-Chloro-5-nitro-6-aminopyrimidine (II) was condensed with various ω-dialkylaminoalkylamines (III). Catalytic reduction of the nitro group, followed by cyclization with formamide, yielded (VII) as well as the isomeric (VI). Treatment of (VII) with alkyl iodides led to the quaternary compounds (VIII). The structure of the synthesized 9-substituted adenines was proved by ultraviolet absorption spectroscopy.

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References

[1] Presented in a lecture delivered at the 21st Meeting of the Israel Chemical Society, Jerusalem, April 18, 1957. *Bull. Res. Counc. Israel*, **6A**, 297 (1957)

[2] From a thesis submitted to the Hebrew University, Jerusalem, Israel, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[3] The correlation of activity with 9-substitution was first demonstrated by Brown, G. B. and Weliky, V. S. *J. biol. Chem.*, **204**, 1019 (1953)

[4] [a] Timmis, G. M., Cooke, I. and Spickett, R. G. W. In *The Chemistry and Biology of Purines*, a Ciba Foundation Symposium held May 8–10, 1956 (eds. Wolstenholme and O'Connor), pp. 134–142 (1957). London; Churchill.

[b] Robins, R. K. and Hsi Hu Lin. *J. Amer. chem. Soc.*, **79**, 490 (1957)

[c] Parikh, J. R., Wolff, M. E. and Burger, A. *J. Amer. chem. Soc.*, **79**, 2778 (1957)

[d] Montgomery, J. A. and Temple, C. *J. Amer. chem. Soc.*, **79**, 5238 (1957); **80**, 409 (1958)

[e] Hull, R. *J. chem. Soc.*, 2746 (1958)

[4] [f] Leese, C. L. and Timmis, G. M. *J. chem. Soc.*, 4107 (1958).
[5] Brown, D. M. and Todd, A. R. *J. chem. Soc.*, 52 (1952)
[6] Baker, B. R., Schaub, R. E., Joseph J. P. and Williams, J. H. *J. Amer. chem. Soc.*, **77**, 12 (1955)
[7] Hutchings, B. L., see ref. 4a, p. 177
[8] Baxter, R. A. and Spring, F. S. *J. chem. Soc.*, 232 (1945)
[9] Davoll, J. and Lowy, B. A. *J. Amer. chem. Soc.*, **73**, 1650 (1951)
[10] Baker, B. R., Joseph, J. P., Schaub, R. E. and Williams, J. H. *J. org. Chem.*, **19**, 1780 (1954)
[11] Baddiley, J., Lythgoe, B., McNeil, D. and Todd, A. R. *J. chem. Soc.*, 383 (1943)
[12] Boon, W. R., Jones, W. G. M. and Ramage, G. R. *J. chem. Soc.*, 96 (1951)
[13] Robins, R. K., Dille, K. J., Willits, C. H. and Christensen, B. E. *J. Amer. chem. Soc.*, **75**, 263 (1953)
[14] [a] Baddiley, J., Lythgoe, B. and Todd, A. R. *J. chem. Soc.*, 318 (1944)
[b] Kenner, G. W., Lythgoe, B. and Todd, A. R. *J. chem. Soc.*, 652 (1944)
[c] Kenner, G. W. and Todd, A. R. *J. chem. Soc.*, 852 (1946)
[15] Daly, J. W. and Christensen, B. E. *J. org. Chem.*, **21**, 177 (1956)
[16] Mason, S. F. *J. chem. Soc.*, 2071 (1954)
[17] Skinner, C. G., Shive, W., Ham, R. G., Fitzgerald, D. C. and Eakin, R. E. *J. Amer. chem. Soc.*, **78**, 5097 (1956)
[18] Hull, R. *J. chem. Soc.*, 2214 (1951)