Purine Studies. Part II. The Ultra-violet Absorption Spectra of Some Mono- and Poly-substituted Purines.*

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The ultra-violet absorption spectra of a number of mono- and poly-substituted purines have been measured in aqueous solution. The pH of each solution was so adjusted, by reference to the pK values of the compound studied, that simultaneous examination of more than one ionic species was avoided, as far as possible. Shifts in the wave-length and intensity consequent upon substitution are tabulated and discussed.

The ultra-violet absorption spectra of polysubstituted purines have been fairly extensively examined, but comparatively little work has been reported on monosubstituted derivatives. Much of this earlier work was carried out with solutions of arbitrary pH values, and the rest without control of pH, so that the results referred to mixtures of ions and neutral molecules uncertain in composition. In the present work the absorption spectra of each ionic and molecular species were measured, wherever possible, by using buffered solvents with pH values two units above (or below) the pK values of the compound studied. The pK_a values, the wave-lengths of maximum absorption, and the logarithms of the molecular extinction coefficients of the purines examined are recorded in Table 1, some typical absorption curves being illustrated by the Figures.

The absorption spectrum of purine resembles that of pyrimidine in aqueous solution (Boarland and McOmie, *J.*, 1952, 3716) more closely than that of glyoxaline which shows only end absorption below 260 mμ (McFarlane, *Biochem. J.*, 1936, **30**, 1199). A closer parallel exists between the spectra of the 2- or 6-monosubstituted purines and those of the corresponding 2- or 6-substituted 4 : 5-diaminopyrimidines, as has been noted (Cavalieri, Bendich, and Brown, *J. Amer. Chem. Soc.*, 1948, **70**, 3875) in the case of the 2 : 6-disubstituted purines and the corresponding 2 : 6-substituted 4 : 5-diaminopyrimidines. Thus 2-OH, and 2-NH_2 substituents are bathochromic and hypochromic in both the purine nucleus and 4 : 5-diaminopyrimidines, whilst 6-OH and 6-NH_2 substituents are hypsochromic and hyperchromic in those compounds. The absorption curves of the substituted purines and the correspondingly substituted 4 : 5-diaminopyrimidines resemble one another in general form and even in some detail, the curves for 2-aminopurine and 2 : 4 : 5-triaminopyrimidine both possessing shoulders at 240 mμ (Fig. 1). However, the neutral molecules of the 4 : 5-diaminopyrimidines substituted in the 6-position, and of

* Part I, preceding paper.

TABLE 1.

The values in italics refer to wave-lengths at which shoulders or inflexions occur in the absorption curves.

Substituents	Basic pK_a	Acidic pK_a	pH	Species charge	$\lambda_{max.}$ (mμ)	log ε
Purine derivatives.						
None	2·39	8·93	0·28	+	<220,[j] 260	>4·09, 3·79
			5·70	0	<220,[j] 263	>3·48, 3·90
			11·00	−	219, 271	3·92, 3·88
2-Hydroxy	1·69	8·43, 11·90	−0·75[e]	+	264, 322	3·67, 3·81
			6·05	0	238, 315	3·46, 3·69
			10·15	−	271, 313	3·68, 3·68
			13·0[f]	mainly =	219, 265, 312	4·29, 3·60, 3·83
6-Hydroxy	1·98	8·94, 12·10	−0·75[e]	+	248	4·02
			5·18	0	249	4·02
			10·35	−	258	4·05
			13·0[f]	mainly =	262	4·04
8-Hydroxy	2·58	8·24, >12	0	+	280	4·02
			5·40	0	235, 277	3·51, 4·05
			10·13	−	285	4·11
			13·0[f]	mixed − and =	284	4·10
2-Methoxy	2·44	9·2	0	+	284	3·83
			6·0	0	246, 283	3·41, 3·91
			11·4	−	283	3·88
6-Methoxy	2·21	9·16	0·20	+	254	4·01
			5·60	0	252—253	3·99
			11·3	−	261	3·99
2-Amino	3·80, −0·28	9·93	−3·5[g]	++	*235*, 325	3·81, 3·62
			1·84	+	*237*, 314	3·62, 3·60
			7·0	0	*236*, 305	3·70, 3·78
			12·0	−	*276*, 303	3·61, 3·76
6-Amino	4·22	9·8	2·10	+	262	4·12
			7·03	0	260	4·13
			12·01	−	267	4·08
8-Amino	4·68	9·36	2·40	+	288	4·20
			7·05	0	241, 283	3·51, 4·16
			12·01	−	*230*, 290	3·98, 4·07
6-Methylamino	4·18	9·99	2·02	+	267	4·18
			7·12	0	266	4·21
			12·00	−	273	4·20
8-Methylamino	4·78	9·56	2·70	+	*230*, 296	3·90, 4·24
			7·15	0	245, 290	3·50, 4·22
			12·00	−	*230*, 298	4·00, 4·16
2-Dimethylamino	4·02	10·22	1·70	+	228, *248*, 340	4·52, 3·97, 3·48
			6·98	0	223, 248, 332	4·41, 4·02, 3·71
			12·70	−	232, 327	4·39, 3·67
6-Dimethylamino	3·87	10·5	1·70	+	276	4·19
			6·98	0	275	4·25
			13·0	−	221, 281	4·21, 4·25
8-Dimethylamino	4·80	9·73	2·70	+	*230*, 305	4·04, 4·29
			7·25	0	250, 296	3·47, 4·27
			12·00	−	*230*, 306	3·91, 4·22
2-Mercapto	0·50	7·15, 10·40	−1·2[h]	+	227–232, 287, 382	3·87 4·27, 3·26
			4·98	0	241, 285—286, 345—348	4·10 4·25 3·18
			8·80	−	235, 263, 328	4·12, 4·19, 3·49
6-Mercapto	<2·5[b]	7·77, 10·84	−3·5[g]	? +	225, 324	3·82, 4·17
			5·09	0	225, 325	3·87, 4·27
			9·30	−	228, 312	3·98, 4·16

TABLE 1. (*Continued.*)

Substituents	Basic pK_a	Acidic pK_a	pH	Species charge	$\lambda_{max.}$ (mμ)	log ε
8-Mercapto	<2·5 [b]	6·64, 11·16	−3·5 [g]	? +	238, *280*, 331	4·17, 3·61, 4·26
			4·50	0	231, 310	4·01, 4·46
			8·90	−	228, 313	4·13, 4·37
			13·0	=	230, 315	4·18, 4·31
2-Methylthio	1·91	8·91	0	+	241—242, *250*, 314	4·13, 4·09, 3·64
			5·90	0	232, *250*, 305	4·22, 3·93, 3·78
			11·6	−	240, 300—302	4·28, 3·79
6-Methylthio	0	8·74	−3·5 [g]	+	222, 313	4·08, 4·41
			5·80	0	*255*, 290	3·66, 4·35
			11·15	−	222, 290	4·27, 4·31
8-Methylthio	2·95	7·67	0	+	232, 305	4·04, 4·32
			5·07	0	246, 290	3·59, 4·30
			9·90	−	220, 296	4·23, 4·27
6-Methyl	2·6	9·02	0	+	265	3·88
			5·85	0	261	3·92
			11·53	−	271	3·93
8-Methyl	2·85	9·37	0	+	264	3·92
			5·85	0	266	4·01
			12·0	−	274	3·92
6-Chloro [a]	—	7·82	5·50	0	265	3·96
			10·00	−	274	3·92
8-Phenyl	2·68	8·09	0	+	237, 304	4·13, 4·41
			5·40	0	231, 298	4·06, 4·42
			10·28	−	233, 304	4·24, 4·42
2 : 6-Dihydroxy	—	7·44, 11·12	5·05	0	267	3·90
			9·99	−	240, 277	3·91, 3·92
			13·0 [f]	mainly =	283	3·94
2 : 8-Dihydroxy	—	7·45, >12	5·08	0	*230*, 310	3·90, 3·70
			10·00	−	262, 306	3·98, 3·87
			13·0 [f]	mixed − and =	220, 310	4·23, 3·98
6 : 8-Dihydroxy	—	7·65, 9·87	5·08	0	257, *280*	4·08, 3·76
			8·67	mainly [i] −	265	4·04
			12·01	=	271	4·14
6-Amino-2-hydroxy	4·51	8·99	2·00	+	*230*, 284	3·69, 4·03
			6·98	0	240, 286	3·89, 3·90
			11·14	−	*235*, 284	3·72, 4·09
2-Amino-6-hydroxy	3·3	9·2, 12·3	1·00	+	248, 271	4·03, 3·85
			6·20	0	246, 275	4·01, 3·89
			10·71	mainly [i] −	245, 273	3·78, 3·87
			13·0 [f]	mainly =	221, 274	4·12, 3·94
2 : 6-Diamino	5·09, <1·0	10·77	−1·2 [h]	partly ++	247, 296	4·01, 3·88
			3·0	+	241, 282	3·96, 4·02
			7·48	0	246—247, 279—280	3·85, 3·95
			13·0	−	*243*, 284	3·67, 3·97
2 : 6 : 8-Triamino	2·41, 6·23	10·79	0·30	++	248, 305	4·12, 4·11
			4·32	+	221, *250*, 299	4·31, 3·69, 4·24
			8·50	0	249, 293	3·80, 4·08
			13·0	−	226, 261, 295	4·31, 3·69, 4·08
2-Amino-8-phenyl	3·98	9·20	1·0	+	257, 332	4·38, 4·08
			6·50	0	238, 329	4·22, 4·28
			11·40	−	239, 330	4·31, 4·28
Pyrimidine derivatives.						
4 : 5-Diamino	6·03	—	3·20	+	284	3·94
			8·05	0	246, 289	3·89, 3·86
2 : 4 : 5-Triamino	2·56, 7·63	—	0	++	268	3·61
			5·15	+	228, *240*, 295	4·20, 4·07, 3·62
			9·80	0	232—234, 303	3·92, 3·73

TABLE 1. (*Continued.*)

Substituents	Basic pK_a	Acidic pK_a	pH	Species charge	$\lambda_{max.}$ (mμ)	log ε
4 : 5 : 6-Triamino	1·47, 5·78	—	−0·75 [a]	++	265	4·01
			3·55	+	287	4·01
			7·98	0	277, 380	3·89, 2·37
4 : 5-Diamino-2-hydroxy ...	4·37	11·45	2·30	+	305	3·76
			6·98	0	292	3·58
			13·0	−	226, 303	3·91, 3·67
4 : 5-Diamino-6-hydroxy ...	1·34, 3·57	9·86	−0·75 [a]	++	257	3·85
			2·45	+	258	3·74
			6·70	0	278, 372	3·95, 2·44
			12·00	−	272, 370	3·87, 2·62
4 : 5-Diamino-2-mercapto ...	2·96	10·39	1·0	+	231, 293, *338*	3·96, 4·28, 3·68
			6·50	0	*250*, 271, *335*	4·19, 4·22, 3·26
			12·40	−	221, 269, 316	4·17, 4·17, 3·71
Various compounds.						
S-Methyl*iso*thiourea	9·83 [b]	—	7·05	+	220	4·58
			12·0	0	238	3·74
Thiourea	*ca.* −1 [d]	—	7·05	0	236	4·08
O-Methyl*iso*urea	9·80 [c]	—	7·50	+	<220	>0·48
			12·0	0	<220	>1·43
Benzamidine	11·6 [c]	—	7·00	+	268, 229	2·91, 3·96
			13·00	0	228	3·93
Acetamidine	12·52 [c]	—	7·00	+	<220	>1·58
			13·0 [f]	mainly 0	219	3·04
Acetylguanidine	8·33 [c]	—	6·05	+	220	2·76
			10·5	0	229	4·14

[a] The basic pK_a and the absorption spectrum of the cation were not measurable owing to hydrolysis in acid solution. [b] The basic pK_a was not determinable, either potentiometrically or spectroscopically. [c] Albert, Goldacre, and Phillips, *J.*, 1948, 2240. [d] Walker, *Z. physikal. Chem.*, 1889, **4**, 319. [e] 4N-Sulphuric acid. [f] The highest pH used with silica absorption cells. [g] 13·5N-Sulphuric acid. [h] 5N-Sulphuric acid. [i] The two acid dissociation constants are too close together to permit the examination of the spectrum of the mono-anion. [j] The wave-length of maximum absorption of the *y* band in the spectra of many purines lies below 220 mμ and so cannot be measured with accuracy. The logarithm of the molar extinction coefficient at 220 mμ is tabulated only in the cases of the purine cation and the purine neutral molecule.

4 : 5-diaminopyrimidine itself, give rise to a band at 370—380 and 246 mμ respectively which disappears on cation formation, a phenomenon which is not observed in the case of any of the purine derivatives examined. Such a band may be due to an $n \longrightarrow \pi$ forbidden transition in the case of the 6-substituted 4 : 5-diaminopyrimidines as it is only of moderate intensity (ε 300) in the spectra of these compounds (Platt, *J. Opt. Soc. Amer.*, 1953, **43**, 252), whilst in the case of 4 : 5-diaminopyrimidine itself, where the intensity of the band is higher (ε 7700), it may be due to the conjugation of an amino-group with the pyrimidine nucleus, a conjugation which is destroyed by cation formation.

The ultra-violet absorption spectra of the substituted purines consist in general of two broad bands. These lie at 260 and <220 mμ in the case of the purine molecule itself, and may be designated the *x*, and the *y* band respectively. The corresponding molecules with fewer, or no, nitrogen atoms in the nucleus give rise to three distinct bands in the same region, and so too does 9-methylpurine when examined in *cyclo*hexane, this compound being the only purine that could be studied in a non-polar solvent (Table 2). The introduction of a nitrogen atom into the *cyclo*pentadiene ring of indene brings about a marked increase in the intensity of the first (longest-wave-length) band, and a decrease in that of the second, no great intensity changes being brought about in these bands by the substitution of further nitrogen atoms in this ring (Table 2). The introduction of a nitrogen atom into the benzene ring of benziminazole causes a further increase in the intensity of the first band and a further decrease in that of the second, the substitution of another nitrogen atom into this ring again causing no great intensity changes in these

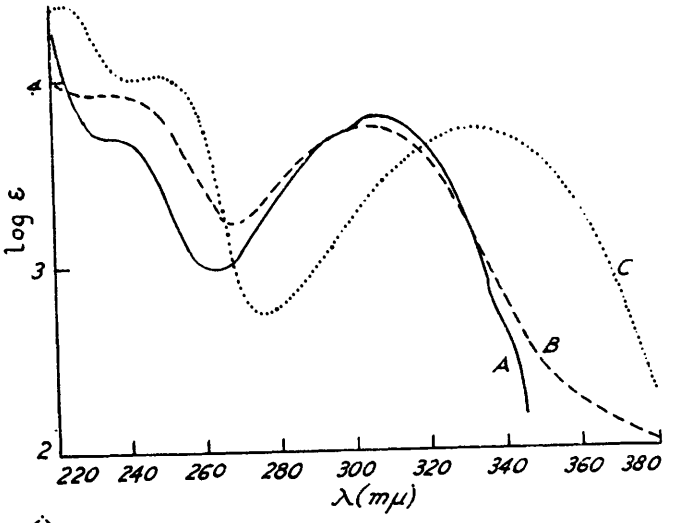


FIG. 1.

2-Aminopurine:
A, neutral molecule.
2 : 4 : 5-*Triaminopyrimidine*:
B, neutral molecule.
2-*Dimethylaminopurine*:
C, neutral molecule.

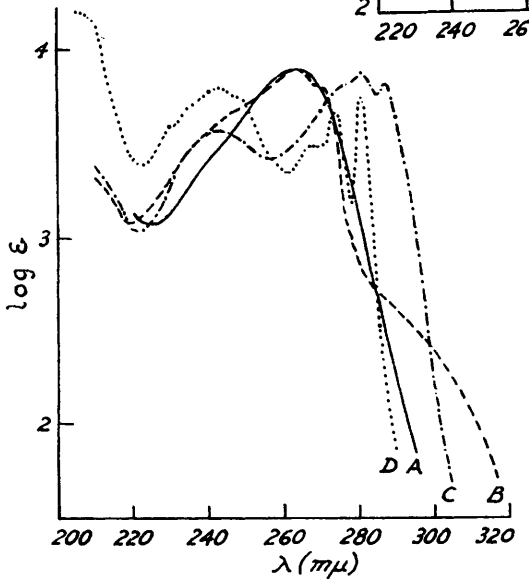


FIG. 2.

9-*Methylpurine*:
A, neutral molecule (pH 5·05).
B, in cyclo*hexane.*
1 : 3 : 4-*Triazaindene*:
C, in cyclo*hexane.*
Benziminazole:
D, in cyclo*hexane.*

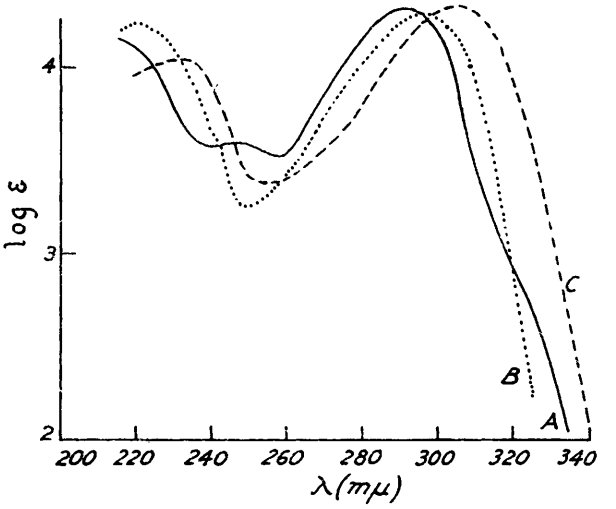


FIG. 3. 8-*Methylthiopurine*:
A, neutral molecule.
B, anion.
C, cation.

bands. However, in the spectrum of 9-methylpurine in *cyclo*hexane these two bands lie close together, so that the second appears only as a shoulder on the short-wave side of the first, and in the spectrum of this compound in aqueous solution, these two bands appear to be merged under a common envelope (Fig. 2).

Thus it seems, from a comparison with the spectra of the azaindenes, that the x band in the spectra of the purines is an envelope covering two transitions, the first more intense than the second. The spectra of indene and indole have been placed on a sequence linking the spectrum of styrene with that of naphthalene (Platt, *J. Chem. Phys.*, 1951, **19**, 101), the first two transitions of both styrene and naphthalene being longitudinal and transverse respectively (*idem*, *ibid.*, 1950, **18**, 1168). Hence it is probable that the x band in the spectra of the purines is due principally to a longitudinal polarization of the purine nucleus with a smaller contribution from a transverse polarization, making the band composite in nature. Such a view is supported by the effect of substituents upon the position of the x band in the purine spectra.

The changes in the wave-lengths of maximum absorption and in the molecular extinction coefficients induced by single substituents placed in different positions of the purine nucleus are given in Table 3. It may be seen that, for a given substituent, the changes vary according to the position of the substituent in the purine nucleus, the order of increasing bathochromic shift being, in general, $6 < 8 < 2$, and the order of increasing hyperchromic effect, $2 \ll 6 \ll 8$, for the x bands. Substituents in the 6-position are transversely disposed with respect to the longer axis of the purine molecule and lower the transition energy only of the weaker component of the x band, whilst substituents in the 8-position are longitudinally disposed with respect to that axis and lower the transition

TABLE 2.

The values of the wave-length of maximum absorption and the molecular extinction coefficient are taken from the smooth curve drawn through the vibrational fine structure in the cases where such fine structure was observed. Values in italics refer to shoulders or inflexions.

Compound	pK_a	Solvent	λ_1 (mμ)	ε_1	λ_2 (mμ)	ε_2	λ_3 (mμ)	ε_3
Indene [a]	—	*n*-Hexane	280	500	250	10,000	220	20,000
Indole [a]	—	*cyclo*Hexane	280	4,000	265	6,500	219	25,000
Benziminazole [b]	—	*cyclo*Hexane	275	3,800	243	6,200	<210	>12,000
	5·53 [d]	Aq. pH 2·0	268	5,500	*240*	3,800	<210	>8,300
	—	Aq. pH 9·1	271	4,250	244	5,100	<220	>2,000
	12·3 [d]	Aq. pH 13 [e]	274	5,400	250	3,600	217	6,900
Benzotriazole [c]	—	*iso*Octane	278	4,800	248	6,000	<220	>1,500
1 : 3 : 4-Triazaindene [b]	—	*cyclo*Hexane	282	6,900	243	3,600	<210	>2,400
	3·95	Aq. pH 1	282	8,300	231	1,800	<210	>2,700
	—	Aq. pH 7	282	9,500	244	3,380	<210	>2,800
	11·08	Aq. pH 13	289	10,000	*265*	3,600	218	3,300
9-Methylpurine [b]	—	*cyclo*Hexane	263	7,600	*240*	3,700	<210	>2,000
	2·36	Aq. pH 0	262	5,500	—	—	<220	>8,400
	—	Aq. pH 5	264	7,700	—	—	<220	>1,300

[a] Friedel and Orchin, "Ultra-violet Spectra of Aromatic Compounds," Wiley & Sons, New York, and Chapman & Hall, London, 1951, Nos. 189 and 192; Platt, *J. Chem. Phys.*, 1951, **19**, 101. [b] Present work. [c] Ewing, *J. Amer. Chem. Soc.*, 1951, **73**, 4360. [d] Albert, Goldacre, and Phillips, *J.*, 1948, 2240. [e] Highest pH used with silica cells.

energy of the stronger component of the x band. Substituents in the 2-position are orientated at an angle of 30° to the longer axis of the purine molecule and thus lower the transition energy of both of the components making up the x band. In benziminazole and indole, where the first two transitions give rise to distinct absorption bands, a methyl group has the greatest bathochromic effect on the first (longitudinal) transition when placed in the 6-position, corresponding to the 2-position in purine, whilst a methyl group in benziminazole exerts the greatest hyperchromic effect upon the second (transverse) transition when substituted in the 4-position, corresponding to the 6-position in purine (Beaven, Holiday, and Johnson, *Spectrochim. Acta*, 1951, **4**, 338).

Substituents in the 2- and the 8-position of the purine nucleus appear in some cases to exert a bathochromic effect on the first transition of the purine spectrum sufficient to separate the two transitions making up the x band of purine itself. The neutral molecules

of purines substituted in the 8-position by groups carrying unshared electrons show small peaks at 235—250 mμ, of molecular extinction coefficient 2900—3900, in all of the cases studied save 8-mercaptopurine, where there is but a slight shoulder at 240 mμ which is overshadowed by the *y*-band peak at 231 mμ. Similar peaks are found in the spectra of purines substituted in the 2-position by groups carrying unshared electrons, but here such peaks, whilst present predominantly in the spectra of the uncharged species, are found also in the spectra of some cationic, and some anionic forms, and they vary much more widely in intensity (ε 2500—10,500). These peaks are not due to the absorption of radiation by the substituted amidine chromophore (–N:CX–N=, where X = OH, OMe, SH, SMe, NH_2, NHMe, NMe_2), a grouping common to the 2- and the 8-substituted purines, for, of the substituted amidines studied (Table 1), only thiourea and *S*-methyl*iso*thiourea gave absorption bands in the required region.

TABLE 3.

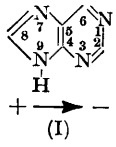
The shifts are measured relative to the corresponding ionic species of purine.

		Position of substituent					
		2	6	8	2	6	8
		Shift in wave-length of the *x*-band (mμ)			Shift in mol. extinction coefficient (× 10^{-3})		
Substituent	Species						
Amino	+	54	2	28	−2·18	7·11	10·02
	0	42	−3	20	−1·92	5·61	6·45
	−	32	−4	19	−1·83	4·53	4·17
Methylamino	+	—	7	36	—	8·98	11·22
	0	—	3	27	—	8·28	8·66
	−	—	2	27	—	8·27	6·87
Dimethylamino	+	80	16	45	−3·12	9·47	13·34
	0	69	12	33	−2·85	9·68	10·68
	−	56	10	35	−2·95	10·20	9·02
Hydroxy	+	62	−12	20	0·29	4·31	4·19
	0	52	−14	14	−3·04	2·53	3·28
	−	42	−13	14	−2·57	3·64	5·30
Methoxy	+	24	− 6	—	0·55	4·07	—
	0	20	−10	—	0·09	1·85	—
	−	14	−10	—	−0·07	2·33	—
Mercapto	+	122	66	70	−4·34	10·44	13·79
	0	84	62	47	−6·44	10·68	20·90
	−	57	41	42	−4·51	6·87	15·86
Methylthio	+	54	53	45	−1·80	19·54	14·73
	0	42	27	27	−2·06	14·45	14·45
	−	29	19	25	0	12·84	11·04
Methyl	+	—	5	4	—	1·42	2·15
	0	—	−2	3	—	0·37	2·29
	−	—	0	3	—	0·93	0·73
Phenyl	+	—	—	44	—	—	19·25
	0	—	—	35	—	—	18·42
	−	—	—	33	—	—	18·66
Chloro	0	—	2	—	—	1·85	—
	−	—	3	—	—	0·77	—

It is probable that these small peaks are the second transition contributing to the composite *x* band of the purine spectrum, as they approximate in position and intensity to the second band observed in the spectrum of 9-methylpurine in *cyclo*hexane solution (Table 2) in the case of the 8-substituted purines. Here the substituents are orientated along the longitudinal axis of the purine molecule, so that they do not greatly affect the position and intensity of the second transition, which is transverse, whilst they bring about a marked bathochromic shift of the first transition, which is longitudinal, separating it from the second. Similar considerations apply to substituents in the 2-position of purine, though these, being orientated at an angle of 30° to the longitudinal axis of the molecule, exert a greater influence on the second transition, as may be seen from the wider variation in the intensity of the bands between 235 and 250 mμ in the spectra of the 2-substituted purines.

It is noteworthy that the second transition of the purine spectrum appears as a distinct band predominantly in the spectra of the neutral molecules of 2- and 8-substituted purines, in fact, exclusively so in the case of the 8-substituted purines. The second transition of the spectral sequence connecting benzene with naphthalene, to which the spectrum of purine is linked through the azaindenes, has been analysed as a transition to a multipolar excited state with wave-function antinodes at the ring atoms (Klevens and Platt, *J. Chem. Phys.*, 1949, **17**, 470; Platt, *ibid.*, 1949, **17**, 484; 1951, **19**, 101). Accordingly this transition is particularly sensitive to perturbations, and it is likely that anion or cation formation in 2- and 8-substituted purines displaced this transition into the envelope of one or other of the more intense bands on either side. In the spectrum of 1 : 3 : 4-triazaindene the band of the second transition, which is distinct in the neutral molecule, is much reduced in intensity and is markedly displaced towards shorter wave-lengths in the cation, whilst in the anion it is greatly displaced towards longer wave-lengths and is almost completely merged into the band of the first transition. In the case of 9-methylpurine even the change from a non-polar to a polar solvent brings the first and the second transition of the spectrum of the neutral molecule under a common envelope.

The intensity changes caused by a substituent in the first band of the spectra of benzenoid ring systems have been shown to be proportional to the "spectroscopic moment" of the substituent, a parameter which may be loosely correlated with the mesomeric moment of the substituent and its directing power (Platt, *ibid.*, 1951, **19**, 263; and earlier references quoted by this author). The stronger transition of the purine *x* band corresponds to the first band of the spectra of benzenoid ring systems according to the sequence drawn up by Platt (see p. 2076), and some indication of the effect of substituents on the intensity of the *x* band of purine may be given in terms of their mesomeric effect on the separation of charge in the purine nucleus. Glyoxaline, benziminazole, and purine form a series of diminishing basic strength and increasing acid strength (Albert, Goldacre, and Phillips, *J.*, 1948, 2240; also Part I), indicating that the benzene, and more particularly the pyrimidine, nucleus exerts an electron-withdrawing effect on the glyoxaline ring, giving rise to longitudinal dipole (I). Electron-donating substituents, by virtue of their mesomeric effect, would enhance this dipole when placed in the 8-position and diminish it when placed in the 2-position, an observation which helps to explain the hyperchromic effect of substituents in the 8-position, and the hypochromic effect of substituents in the 2-position of the purine nucleus. Similarly, such substituents in the 6-position would enhance, and in the 2-position would diminish, a transverse dipole across the pyrimidine ring of the purine nucleus caused by the electron-attracting property of the aza-nitrogen atoms of that ring, substituents in the 6-position being hyperchromic, and in the 2-position being hypochromic, in conformity with this view.



(I)

Substituents in the 2-position of the purine nucleus exhibit the following order of increasing bathochromic shift, the same order being observed with the anions and cations as with the neutral molecules: MeO < MeS ≪ NH_2 < HO < NMe_2 < HS. With substituents in the 6- and the 8-position the orders of increasing bathochromic effect vary somewhat from one species to another. In the 6-position some substituents are bathochromic and others hypsochromic. For the neutral molecules the order of increasing bathochromic shift may be written: Cl < NHMe < NMe_2 < MeS < HS, and the order of increasing hypsochromic effect: Me < NH_2 < MeO < HO. The neutral molecules of the 8-substituted purines may be placed in the following order with regard to increasing bathochromic shift: Me < HO < NH_2 < MeHN = MeS < NMe_2 < Ph < HS.

The *y* (short-wave) bands of the absorption spectra of the substituted purines lie, in general, at too short a wave-length to be measured with accuracy, save in the cases of the purine anion and the anions of a number of monosubstituted purines. With the results from these cases the effect of substituents in the purine nucleus on the *y* band can be indicated to some degree. Substituents in the 2-position exert a bathochromic effect, increasing in the order: MeO < NMe_2 < MeS < HS < HO < NH_2. Substituents in the 6-position tend in general to bring about a hypsochromic shift in the *y* band; so do substituents in the 8-position, in contrast to their effect upon the *x* band.

The above series do not necessarily express the true order of the bathochromic effect of substituents on the purine spectrum, as it is possible that potentially tautomeric substituents exist in their corresponding oxo-, thiono-, or imino-forms, giving rise to different chromophore systems. It has been shown theoretically that such tautomers may give the same spectrum as the true hydroxy-, mercapto-, or amino-forms (Brown and Lahey, *Austral. J. Sci. Res.*, 1950, **3**, 615), but for comparison with other chromophore systems only the order of the bathochromic effect of non-potentially tautomeric substituents will be considered here, apart from the amino-group which appears to exist in the true amino-form (see below). The order of the bathochromic effects of substituents in the 2- and the 8-position on the *x* band of the purine spectrum resembles that of the effect of the same auxochromes on the 250-mμ band of benzene, a band related by a spectral sequence with the stronger transition of the purine *x* band (see above). The magnitude of the bathochromic shifts induced by the substituents in the 2-position are somewhat greater, and in the 8-position somewhat less, than the shifts induced by the same substituents in the benzene ring (Bowden and Braude, *J.*, 1952, 1068), an observation which may be related to the greater polarizability of the pyrimidine ring, and the smaller

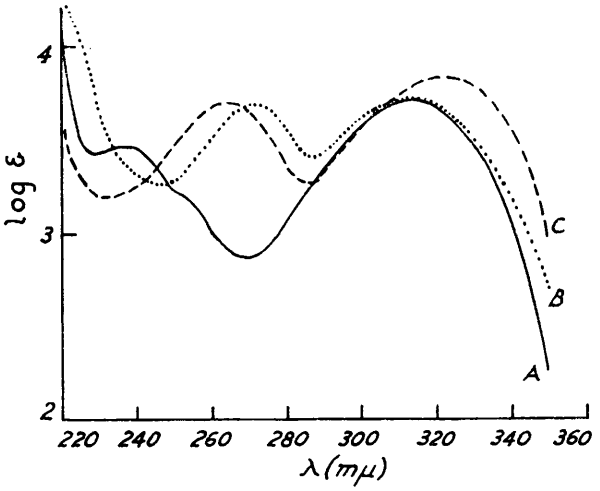


FIG. 4. *2-Hydroxypurine*:
A, neutral molecule.
B, anion.
C, cation.

polarizability of the glyoxaline ring in the purine nucleus, which is to be expected from the migration of π-electrons from the glyoxaline ring to the pyrimidine ring in purine.

Of the shifts induced in the *x* band of the purine spectrum by substituents in the 6-position those which are bathochromic follow the order observed with the ethylenic chromophore, though they are smaller, whilst those that are hypsochromic follow the order found in the C:C·C(X):O system (Bowden, Braude, and Jones, *J.*, 1946, 948). Substituents in the 6-position of purine form the C:C·C(X):N system with part of the pyrimidine ring, a system which might be expected to be intermediate in spectroscopic properties between the corresponding enone and diene chromophores.

In the di- and tri-substituted purines, the effect of the individual substituents on either the wave-length of maximum absorption or the molecular extinction coefficient is not additive even where there is not more than one potentially tautomeric substituent in the purine nucleus. Additivity has been observed for disubstituted pyrimidines containing not more than one potentially tautomeric group (Boarland and McOmie, *J.*, 1952, 3722), indicating a lack of interaction between the two substituents. Thus it seems that there is some conjugation between the substituents of a di- or tri-substituted purine. An amino- or hydroxy-group introduced into the 6-position of an 8- or 2-substituted purine brings about a greater hypsochromic shift than it does in the case of the purine molecule itself, whilst such a group introduced into the 2-position of a 6- or an 8-substituted purine gives rise to a smaller bathochromic shift than it does in the parent molecule. A hydroxyl group placed in the 8-position of a 2- or 6-substituted purine produces no very great changes

in wave-length of maximum absorption, but a phenyl group introduced into the 8-position of such a substituted purine gives rise to marked bathochromic shifts, though these shifts are smaller than those observed on substitution of the parent molecule with an 8-phenyl group.

The spectra of the amino- and hydroxy-purines might be expected to afford some indication of the structures of their ions as the NH_3^+ group should exert the same bathochromic effect as the H or CH_3 group, owing to the fixation of the unshared electrons of the nitrogen atom in the cationic form, whilst the O^- group should exert the same bathochromic effect as the NH_2 group, owing to the similar polarizabilities of those groups (Jones, *J. Amer. Chem. Soc.*, 1945, **67**, 2127).

The presence of an NH_3^+ group in *N*-heteroaromatic molecules has not yet been demonstrated, and in the one case where the spectrum of the cationic form of an aminopurine resembles that of the neutral molecules of the corresponding methyl derivative (6-aminopurine cation, $\lambda_{max.}$ 262 mμ, and 6-methylpurine neutral molecule, $\lambda_{max.}$ 261 mμ), it is known from crystallographic evidence that the cationic proton of the aminopurine is attached to the 1-nitrogen atom of the purine nucleus (Cochran, *Acta Cryst.*, 1951, **4**, 81). The spectrum of the anionic form of 6-hydroxypurine resembles that of the neutral

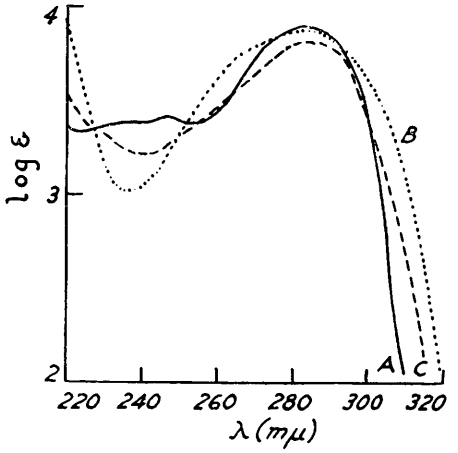
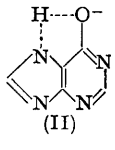


FIG. 5. *2-Methoxypurine:*
A, neutral molecule.
B, anion.
C, cation.

molecule of 6-aminopurine ($\lambda_{max.}$ 258 and 260 mμ respectively), but the spectra of the 2-hydroxy- and 8-hydroxy-purine anions differ from those of the neutral molecules of the corresponding aminopurines (Figs. 1 and 4). Thus it is probable that anion formation in the 2-hydroxy- and 8-hydroxy-purine involves the dissocation of a proton from a nitrogen atom of the glyoxaline ring, not from the hydroxyl group. In the case of 6-hydroxypurine there is some evidence that the first acidic dissociation constant belongs to the hydroxyl group (Albert, *Biochem. J.*, 1953, **54**, 646). Here the same anion could be formed whether the proton dissociated from the hydroxyl group or the imino-group of the glyoxaline ring, the oxygen atom substituted in the 6-position and the nitrogen atom in the 7-position being linked by a hydrogen bond in the anion (II). In 6-aminopurine also there is some evidence of hydrogen bonding between the substituent and the 7-nitrogen atom of the purine nucleus, at least in the cation (Cochran, *loc. cit.*), an observation indicating a similarity between the structures of the 6-hydroxypurine anion and 6-aminopurine which helps to explain the resemblance of their spectra.



(II)

The hydroxy-, mercapto-, and amino-purines may possibly exist, wholly or in part, in their corresponding tautomeric forms. Some indication of the nature of such potentially tautomeric groups substituted in the purine nucleus may be obtained from comparisons between the spectra of such purines and those of the corresponding methoxy-, methylthio-, and dimethylamino-purines. However, such indications can only be tentative in the present work as comparisons with the spectra of the corresponding *N*-methyl-oxo-, -thiono-,

and -imino-purines would be required for definitive assignments of structure. The spectra of all the monoaminopurines resemble those of the same ionic forms of the corresponding dimethylamino-derivatives (Fig. 1), suggesting that the amino-purines exist predominantly in the true amino-form. The spectra of 6-hydroxypurine, too, are similar to those of the corresponding ionic forms of 6-methoxypurine, but there are considerable differences between the spectral absorption curves given by 2-hydroxypurine and 2-methoxypurine (Figs. 4 and 5). These observations neither prove nor exclude the possibility that 6-hydroxypurine exists principally in the hydroxy-form, as cases are known in other *N*-heteroaromatic structures where the spectrum of a hydroxy-derivative resembles the spectra of both the corresponding methylated tautomers (Albert, Brown, and Cheeseman, *J.*, 1952, 4219). However, the differences between the spectra of 2-hydroxy- and 2-methoxy-purine strongly suggest that 2-hydroxypurine exists principally in the keto-form.

With regard to the sulphur-substituted purines, the spectra of all the mercaptopurines differ from those of the same ionic forms of the corresponding methylthiopurines, the differences being the most prominent with the 2-substituted derivatives and least marked with the 6-substituted derivatives. Thus it is possible that 2-mercaptopurine, at least, exists to some extent in the thiono-form, this being the more probable because all the ionic forms of 2-mercaptopurine exhibit absorption peaks at 330 mμ or longer wavelengths, a region in which the C:S group shows an absorption maximum (Braude, *Ann. Reports*, 1945, **42**, 112). However, the intensities of the peaks in the spectra of 2-mercaptopurine are rather high (ε 2—20 × 10^3) for a spin-forbidden transition of the type found in the 330-mμ band of the thione chromophore (ε *ca.* 5), though it is possible that intensity is " borrowed " from the neighbouring high-intensity *x* band of the purine absorption in the case of 2-mercaptopurine.

Experimental

Source of Materials.—The sources of the purine derivatives studied were as in Part I. 4 : 5-Diaminopyrimidine was prepared according to Brown (*J. Appl. Chem.*, 1952, **2**, 239), 4 : 5-diamino-2-mercaptopyrimidine according to Elion and Hitchings (*J. Amer. Chem. Soc.*, 1947, **69**, 2553), 4 : 5-diamino-2-hydroxypyrimidine according to Johns (*Amer. Chem. J.*, 1911, **45**, 79), and 2 : 4 : 5- and 4 : 5 : 6-triaminopyrimidine and 4 : 5-diamino-6-hydroxypyrimidine according to Albert, Brown, and Cheeseman (*J.*, 1951, 474).

Benziminazole and thiourea specimens from British Drug Houses Ltd. were recrystallized. 1 : 3 : 4-Triazaindene was prepared according to Kögl (*Rec. Trav. chim.*, 1948, **67**, 29).

O-Methylurea hydrochloride was kindly supplied by Dr. E. Hoerger (Talladega College, Talladega, Alabama).

Acetylguanidine was prepared according to Traube (*Ber.*, 1910, **43**, 3586), benzamidine according to Pinner (" Die Imidoäther und ihre Derivate," R. Oppenheim, Berlin, 1892, p. 152 ff.), *S*-methyl*iso*thiourea sulphate according to *Org. Synth.*, Coll. Vol. II, p. 411, and acetamidine hydrochloride according to *Org. Synth.*, 1928, **8**, 1.

Absorption Spectra.—These were measured with a Hilger Uvispek H700/301 Quartz Spectrophotometer, with buffer solutions with the pH values recorded in Table 1. The buffer solutions were 0·01M-glycine (for pH 1·5—3·5), 0·01M-acetate (for pH 3·8—5·7), 0·01M-phosphate (for pH 6·0—7·9, and pH 10·3—11·3), 0·01M-borate (for pH 8·2—10·0), together with N- (pH 0) and 0·1N-hydrochloric acid (pH 1·0), and 0·1N- (pH 13) and 0·01N-potassium hydroxide (pH 12).

pK_a *Determinations.*—The pK_a values of 1 : 3 : 4-triazaindene and of the 2- and the 6-substituted 4 : 5-diaminopyrimidines were determined by potentiometric titration of 0·01M-aqueous solutions with a glass electrode and a Cambridge pH meter. The pK_a values recorded in Table 1 are correct within ±0·08 pH unit.

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