Purine Studies. Part XV.[1] Addition of Hydrogen Sulphite Ion to Purines

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Several 6-unsubstituted purines underwent addition of potassium hydrogen sulphite or sulphurous acid across the 1,6-double bond. The effect of substituents in the 2- and 8-positions on the stability of the adducts is discussed with reference to their u.v. and ^{1}H n.m.r. spectra.

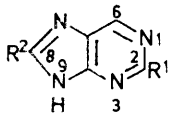
ADDITION of nucleophiles across a carbon–nitrogen double bond is of interest as a possible mechanism in the enzymic oxidation of purines to purinones.[2a] Addition of water has also been proposed as a first step in the enzymic deamination of adenosine and 4-aminopteri-

[1] Part XIV, M. D. Fenn and J. H. Lister, *J.C.S. Perkin I*, 1975, 485.

[2] (*a*) F. Bergmann and S. Dikstein, *J. Biol. Chem.*, 1956, **223**, 765; (*b*) B. E. Evans and R. V. Wolfenden, *Biochemistry*, 1973, **12**, 393; *J. Amer. Chem. Soc.*, 1972, **94**, 5902.

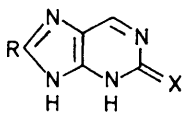
dine.[2b] In a recent study [3] of the addition reactions of 6-unsubstituted purines with nucleophiles it was reported that several purines showed a hypsochromic shift in their u.v. spectra when dissolved in aqueous potassium hydrogen sulphite. This effect has been further examined, and several unstable purine–hydrogen sulphite adducts have been isolated: the effect on their stability of substitution at positions remote from the addition site is described.

Purine (1a) and its 2-amino- (1b), 2-oxo- (2a), and 2-thioxo- (2b) derivatives with a two-fold excess of potassium hydrogen sulphite in water gave moderate yields of crystalline compounds in which the purine was bound in a 1 : 1 ratio with the elements of either sulphurous acid (purine and 2-aminopurine) or potassium hydrogen sulphite (purin-2-one and -thione). The upfield shift of a proton resonance in the ^1H n.m.r. spectra in D_2O of the adducts by *ca.* 3.5 p.p.m. with respect to the purines (Table 1) and the strong similarity between the



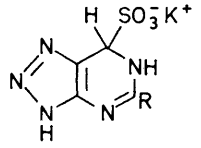
(1)

a; $R^1 = R^2 = H$
b; $R^1 = NH_2, R^2 = H$
c; $R^1 = H, R^2 = CF_3$
d; $R^1 = NH_2, R^2 = CF_3$
e; $R^1 = H, R^2 = SO_2Me$
f; $R^1 = H, R^2 = SO_3K$
g; $R^1 = R^2 = SO_2Me$

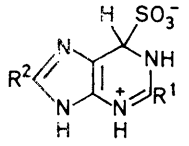


(2)

a; X = O, R = H
b; X = S, R = H
c; X = O, R = CF_3

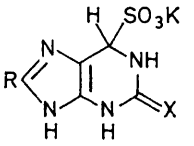


(3)



(4)

a; $R^1 = R^2 = H$
b; $R^1 = NH_2, R^2 = H$
c; $R^1 = NH_2, R^2 = CF_3$
d; $R^1 = H, R^2 = CF_3$
e; $R^1 = H, R^2 = SO_2Me$
f; $R^1 = H, R^2 = SO_3K$



(5)

a; X = O, R = H
b; X = S, R = H
c; X = O, R = CF_3

u.v. spectra of the purine adducts and the 1,6-adducts of the corresponding 8-azapurines (3) [4,5] indicated that addition had occurred across the 1,6-bond. This was confirmed by the n.m.r. spectrum of the adduct of purine partially (50%) deuteriated in the 6-position. Thus the hydrogen sulphite adducts of purine and 2-aminopurine were formulated as (4a and b) and those of purin-2-one and -2-thione as (5a and b), respectively.

Dilute aqueous solutions of these adducts were unstable, their u.v. spectra reverting to those of the starting purines with half-lives ranging from 3 to 11 min at 20 °C. Although pK_a values for these unstable substances could not be measured, it was estimated by analogy with the hydrates of the corresponding 8-azapurines [6] that the purine portion of the purine–hydrogen sulphite adduct and its 2-amino-derivative would exist at the pH of maximum stability (which varied between 0 and 3) as the monocation (4a or b) stabilised by amidinium- and guanidinium-type resonance; those of the purin-2-one and -2-thione derivatives would exist as the neutral species (5a or b) stabilised by urea-type resonance.[4a] This is supported by the effect of adjusting the pH of the solution to 8, where the adducts (4a and b) would exist as neutral (purine) species, and the adducts (5a and b) as anions. Under these conditions, where the special resonance stabilisations would be absent, the adducts dissociated so rapidly and completely that only the final purine spectrum was observed.

8-Trifluoromethylpurine (1c) and its 2-amino- (1d) and 2-oxo- (2c) derivatives gave similar hydrogen sulphite adducts (4d), (4c), and (5c) which were further stabilised by the presence of the electron-withdrawing group (*cf.* covalent hydration of 8-azapurines [6]). For example, a dilute solution of the adduct (4c) in N-hydrochloric acid is stable indefinitely (by u.v. spectroscopy), whereas the spectrum of the adduct (4b) of 2-aminopurine quickly reverted to that of the purine cation. Its n.m.r. spectrum [in $(CD_3)_2SO$] 1 min after dissolution showed the presence of a mixture of the adduct and the purine in a 3 : 1 ratio, unlike that of the adduct (4b) which immediately reverted to 2-aminopurine in $(CD_3)_2SO$. Similarly the n.m.r. spectrum of the adduct (4d) of 8-trifluoromethylpurine could be measured in $(CD_3)_2SO$ whereas the spectrum of the corresponding adduct (4a) of purine reverted immediately to that of purine (1a) in this solvent. Further, u.v. spectra reveal that the former adduct (4d) dissociates in water much less rapidly than the latter (4a) (Table 2). The u.v. spectrum of the adduct (5c) of 8-trifluoromethylpurin-2-one was measured at pH 5 without rapid decomposition, whereas the corresponding 8-unsubstituted compound (5a) immediately reverted to the purine at this pH. However, the adducts of these purines were less stable than those of the corresponding 8-azapurines,[5] indicating that the electron-withdrawing power of the 8-trifluoromethyl group only partly compensates for the loss of the doubly-bonded N atom of the latter.

Rather than stabilising the adducts, an 8-methylsulphonyl substituent tended to undergo nucleophilic displacement with hydrogen sulphite. Thus 8-methylsulphonylpurine (1e) gave not the expected adduct (4e)

[3] W. Pendergast, *J.C.S. Perkin I*, 1973, 1906.
[4] For reviews, see (*a*) A. Albert and W. L. F. Armarego, *Adv. Heterocyclic Chem.*, 1965, **4**, 1; (*b*) D. D. Perrin, *ibid.*, p. 43.
[5] A. Albert and W. Pendergast, *J.C.S. Perkin I*, 1972, 457.
[6] A. Albert, *J. Chem. Soc.* (*B*), 1966, 427.

TABLE 1

^{1}H N.m.r. data (33.3 °C)[a]

	H-2	H-6	H-8	Others	Solvent [c]
1,6-Dihydropurine-6-sulphonates[b]					
Unsubstituted	1.85	3.64	1.66		$KDSO_3$–D_2O [d]
2-Amino		4.80	2.70		DCl–D_2O
2,3-Dihydro-2-oxo-9-methyl [e]		4.52	2.31	6.34 (Me)	D_2O
2,3-Dihydro-2-thioxo		4.23	2.34		D_2O
8-Trifluoromethyl	1.51	3.71			
2-Amino-8-trifluoromethyl		4.61		0.86 (NH), 2.44 (NH_2)	$(CD_3)_2SO$
2,3-Dihydro-2-oxo-8-trifluoromethyl		5.02		3.14 (NH)	$(CD_3)_2SO$
Purines					
Unsubstituted	0.72	0.54	1.06		$KDSO_3$–D_2O [d]
2-Amino		1.50	1.79		DCl–D_2O
2,3-Dihydro-2-oxo-9-methyl [e]		1.66	2.45	7.07 (Me)	D_2O
2,3-Dihydro-2-thioxo					
8-Trifluoromethyl	0.40	0.12			2N-DCl–D_2O
2-Amino-8-trifluoromethyl		1.20		3.20 (NH_2)	$(CD_3)_2SO$
2,3-Dihydro-2-oxo-8-trifluoromethyl		0.67			$(CD_3)_2SO$
1,6-Dihydro-8-azapurine-6-sulphonate (for comparison) [f]		4.00			D_2O

[a] Those compounds reported in the Experimental section but not listed here were too insoluble for an n.m.r. spectrum to be measured; the instability of these compounds would not permit an accumulated spectrum to be obtained where the reaction proceeded to completion. [b] The values for the adduct and the purine were taken from the same solution after the adduct began to dissociate into the purine, except in the case of 1,2,3,6-tetrahydro-2-thioxopurine-6-sulphonate, which was stable in D_2O. [c] For spectra in $(CD_3)_2SO$, tetramethylsilane was the internal standard. For spectra in D_2O the internal standard was sodium 3-trimethylsilylpropane-1-sulphonate. [d] Accumulated spectrum of a saturated solution of purine in a saturated solution of potassium hydrogen sulphite in D_2O. Signal ratios indicate an adduct–purine ratio of 1 : 1. [e] Accumulated spectrum; adduct–purine ratio 1 : 3. [f] Value from ref. 5.

TABLE 2

U.v. spectroscopy in water (20°)

	Species [a]	Basic pK_a	$t_{\frac{1}{2}}$/min [b]	$\lambda_{max.}$/nm [c]	log ε	pH
Purine						
1,6-Dihydro-6-sulphonate	+		3	235	3.06	0
Unsubstituted [d]	+	2.39		260	3.79	0.28
2-Amino-1,6-dihydro-6-sulphonate	+		11	242	4.00	2
2-Amino [d]	+	3.80		314	3.60	1.84
1,2,3,6-Tetrahydro-2-oxo-6-sulphonate	0		8	243	3.76	3
2,3-Dihydro-2-oxo [d]	0	1.69		315	3.69	6.05
1,2,3,6-Tetrahydro-2-thioxo-6-sulphonate [e]	0			260, 278		−1
2,3-Dihydro-2-thioxo [d]	0	0.5		382	3.26	−1.2
1,6-Dihydro-8-trifluoromethyl-6-sulphonate	+		7	244	3.79	0
8-Trifluoromethyl [f]	+	1.0		267.5	3.85	(3N-HCl)
2-Amino-1,6-dihydro-8-trifluoromethyl-6-sulphonate	+		Stable	243		0
2-Amino-8-trifluoromethyl [g]	+	2.59		318	3.64	0
1,2,3,6-Tetrahydro-2-oxo-8-trifluoromethyl-6-sulphonate [e]	0			249		2
2,3-Dihydro-2-oxo-8-trifluoromethyl	0			316	3.75	4
1,6-Dihydro-6,8-disulphonate [h]	+ [i]			249		2.5
8-Sulphonate [j]	0	2.22		269		2.5

[a] Cation (+), neutral species (0); charge refers to purine portion of molecule. [b] Half-life estimated from repeat scans of spectrum. [c] Longest wavelength absorption only given for parent purines to illustrate hypsochromy. [d] Values from S. F. Mason, *J. Chem. Soc.*, 1954, 2071. [e] Log ε values and $t_{\frac{1}{2}}$ for dissociation not measured as change is complex; final spectrum not of the purine. [f] Values from A. Bendich and A. Giner-Sorolla, *J. Amer. Chem. Soc.*, 1958, **80**, 5744. [g] Values from ref. 7. [h] Prepared both from 8-methylsulphonylpurine and from potassium purine-8-sulphonate (see Experimental section). [i] Reaction followed on cation to minimise rate of decomposition. Parent purine unstable in acid, hence log ε values and $t_{\frac{1}{2}}$ for dissociation not determined. [j] Values from D. J. Brown and J. A. Hoskins, *Austral. J. Chem.*, 1972, **25**, 2641.

but the disulphonic acid (4f), confirmed by comparison with condensation of the product of hydrogen sulphite with potassium purine-8-sulphonate (1f). 2,8-Bismethylsulphonylpurine (1g) gave only 2-methylsulphonylpurin-2-one, which was also obtained as a by-product in the preparation of the starting material by oxidation of 2,8-bismethylthiopurine with permanganate.

EXPERIMENTAL

Samples for microanalysis were dried at room temperature and 0.1 mmHg. ^{1}H N.m.r. spectra were measured with a Perkin-Elmer R10 spectrometer at 33.3 °C and 60 MHz. U.v. spectra were determined with a Unicam SP 800 recording spectrophotometer. In order to obtain rapidly the spectra of unstable species the following procedure was adopted. An arbitrary quantity of the compound (*ca.* 1 mg) was shaken with the appropriate buffer solution for 10 s. The solution was rapidly filtered into a u.v. cell and the decomposition was followed to completion, repeat scans of the spectrum being made at 1—2 min intervals. The absorbance and wavelength of the absorption maxima were extrapolated to the moment of dissolution. The pH values of the solutions were adjusted to correspond with pure ionic species of the final purine to determine its concentration, and hence the initial concentration of adduct.

Condensation of Purines with Potassium Hydrogen Sulphite.—The finely ground purine (0.0005 mol) was heated at

100 °C in water (0.5 ml) with potassium hydrogen sulphite (0.0005 mol). The hot solution was clarified by centrifugation, and allowed to cool. The precipitated *addition compound* was filtered off and washed with ice-cold water. Yields and analyses are given in Supplementary Publication No. SUP 21478 (2 pp.)† The salt-like adducts did not have sharp m.p., but decomposed gradually without melting above 150°. They were analysed without further purification (after drying). Attempted crystallisation from water or other solvents resulted in decomposition to the purines, as did attempted removal of water of crystallisation by drying at elevated temperatures.

2,8-*Bismethylsulphonylpurine*.—A stirred suspension of 2,8-bismethylthiopurine [7] (0.1 g) in aqueous 1% acetic acid (5 ml) was cooled to 0 °C prior to addition of solid potassium permanganate (0.28 g) in small portions over 5 min. The ice-bath was removed and the mixture stirred for a further 10 min. Sulphur dioxide was passed into the suspension, with the temperature kept below 10 °C. Immediately after decolourisation the *crystals* (0.06 g) were filtered off, washed with ice-cold water and dried at 20° and 0.05 mmHg; m.p. 204° (efferv.) (Found: C, 30.5; H, 3.15; N, 20.2. $C_7H_8N_4O_4S_2$ requires C, 30.4; H, 2.9; N, 20.3%). When the reaction was carried out at 25 °C for 2 h, with no cooling in the decolourisation step, a mixture of the above compound and a carbonyl compound ($\nu_{max.}$ 1 740 cm^{-1}) was obtained. This was shown to be 7,9-*dihydro*-2-*methylsulphonylpurin*-8-*one* as follows. (*a*) 2,8-Bismethylsulphonylpurine [7] (0.05 g) was heated under reflux with water (5 ml) for 4 h and cooled to 0 °C to give the oxo-compound (0.031 g), decomp. >250° (Found: C, 31.6; H, 3.7; N, 24.9. $C_6H_6N_4O_3S$, 0.75 H_2O requires C, 31.6; H, 3.3; N, 24.6%). (*b*) 2-Methylthiopurin-8-one [8] (0.1 g) was oxidised with potassium permanganate (0.14 g) as described above for 2,8-bismethylthiopurine. The product (59%) was shown by i.r. spectroscopy to be identical with that obtained in (*a*). It was shown by comparative chromatography on Whatman no. 1 paper [with aqueous 3% NH_4Cl and butanol–5N-acetic acid (7 : 3)] to be identical with the carbonyl component of the above oxidation product.

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* For details of Supplementary Publications see Notice to Authors No. 7, *J.C.S. Perkin I*, 1974, Index issue.

[7] A. Albert, *J. Chem. Soc.* (*B*), 1966, 438.
[8] C. W. Noell and R. K. Robins, *J. Org. Chem.*, 1959, **24**, 320.