Purine Studies. Part XII.[1] Derivatives Resulting from Acylation and Subsequent Ethylation of 4,5-Diamino-6-methyl-2-methylthiopyrimidine

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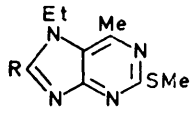
The structures of the various products arising from acetylation of 4,5-diamino-6-methyl-2-methylthiopyrimidine have been identified by means of ^{1}H n.m.r. and by their conversion into known compounds. Ethylation of the acetylated derivatives does not take place at the same exocyclic nitrogen atom in every case; the site of reaction is a reflection of the change in relative basicity of the 4- and 5-amino-groups which results from their acetylation.

One route to the less readily obtained 7-alkylpurines involves alkylation of 5-acylamino-4-aminopyrimidines in an aprotic solvent, in the presence of potassium carbonate, followed by ring closure of the resulting 5-(*N*-alkyl)acylamino-4-aminopyrimidines.[2] As our programme on the amplifying effect of purines on the biological activity of the antibiotic phleomycin [3,4] required derivatives of the type (1), the foregoing route was adopted for their preparation. Thus, in hot formic acid 4,5-diamino-6-methyl-2-methylthiopyrimidine (2; R = H) was converted into the 5-formamido-derivative (2; R = CHO),[5] which on treatment with iodoethane, in cold dimethylformamide containing potassium carbonate, afforded the *N*-ethyl homologue (3). Ring closure to the purine (1; R = H) was effected by heating under reflux in the same, but fresh, solvent–base mixture. From an attempt to carry out this cyclisation in hot formamide–hydrochloric acid was isolated not only (1; R = H) but also the novel dipurin-2-yl sulphide (4).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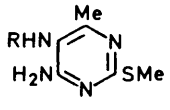
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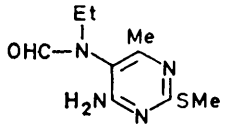
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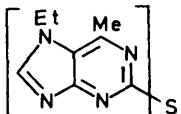
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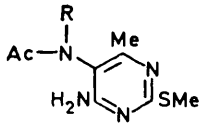
Hot acetic acid and the diaminopyrimidine (2; R = H) react similarly to give the 5-acetamido-derivative (5; R = H). The latter, which was also formed by the action of cold acetic anhydride on the diamine, gave, on ethylation and cyclisation as before, a purine having properties and analysis consistent with the structure (1; R = Me).

Under reflux conditions acetylation of (2; R = H) with the anhydride afforded a triacetylated pyrimidine. Although the major product of ethylation of the latter was an *N*-ethyl monoacetyl derivative this was not the same as the ethylation product from (2; R = Ac). In addition, from the reaction was isolated an *N*-ethyl diacetylated pyrimidine. As brief heating of the *N*-ethyl monoacetyl compound with acetic anhydride converted it into the diacetylated form, the ethylated nitrogen atom must be the same in both derivatives. Ring closure of the foregoing monoacetyl derivative gave the known [3] 9-ethyl-8-methyl-2-methylthiopurine (6). Ethylation in the case of the triacetyl derivative therefore involved the 4- rather than the 5-amino-group. Confirmation of this came from the preparation of the *N*-ethyl monoacetylated pyrimidine by the action of cold acetic anhydride on the 5-amino-4-ethylamino-pyrimidine (7; R = H).

From the mother liquors of the triacetylated pyrimidine preparation was isolated a diacetyl derivative, arising most likely through loss of one of the three acetyl groups during isolation. This lability of an acetyl group is demonstrated by the almost complete conversion of the tri- into the di-acetylated form when the former is run on a silica t.l.c. plate in ethyl acetate. Partial acetyl group loss also occurs on recrystallisation of the triacetyl derivative from aqueous solvents. Ethylation of the diacetyl derivative gives the same *N*-ethyl monoacetylated pyrimidine as was obtained from alkylation of the triacetyl compound.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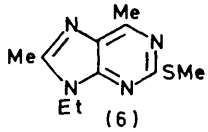
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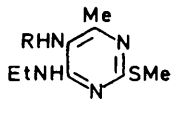

(7)

Although a number of acetylated derivatives of 5,6-diaminouracils have been reported [6-8] as arising from

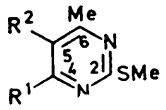
[1] Part XI, D. J. Brown and R. K. Lynn, *J.C.S. Perkin I*, 1974, 349.
[2] J. A. Montgomery and K. Hewson, *J. Org. Chem.*, 1961, **26**, 4469.
[3] R. J. Badger, D. J. Brown, and J. H. Lister, *J.C.S. Perkin I*, 1974, 152.
[4] D. J. Brown, R. L. Jones, A. M. Angyal, and G. W. Grigg, *J.C.S. Perkin I*, 1972, 1819.
[5] R. N. Prasad, C. W. Noell, and R. K. Robins, *J. Amer. Chem. Soc.*, 1959, **81**, 193.
[6] H. Bredereck, I. Hennig, and W. Pfleiderer, *Chem. Ber.*, 1953, **86**, 321.
[7] H. Bredereck, I. Hennig, W. Pfleiderer, and G. Weber, *Chem. Ber.*, 1953, **86**, 333.
[8] E. S. Golovchinskaya, *Zhur. obshchei Khim.*, 1954, **24**, 136.

acetylation of uric acid, little direct evidence to support the various structures proposed has been put forward. In view of the possible bearing of these studies on ours, a detailed ^1H n.m.r. examination of the compounds in the present work was undertaken in order to define the locations of the acetyl groups.

The 5-amino-group protons of the parent 4,5-diaminopyrimidine (2; R = H) show a resonance at δ 4·22 which is moved downfield to δ 9·0 when the group is acetylated to give (2; R = Ac). No direct comparison is possible with the displacement of the 4-amino-group resonance (δ 6·3) following acetylation, as the 4-acetamido-5-aminopyrimidines are unstable from the triacetyl derivative, on the other hand, shows two NH signals, one of which is downfield, denoting that ethyl and acetyl groups are sited on different nitrogen atoms, and that the structure is therefore (7; R = Ac). As the triacetyl derivative shows a single downfield resonance at δ 10·2, monoacetylation of the 4-amino-group is indicated and the structure is (9; R = H).

Acetylation of the diamine (2; R = H) to the monoacetyl and then stepwise to the triacetyl derivative can be achieved by controlling the reaction time and temperature and follows the sequence 5-NH_2 ⟶ 4-NH_2 ⟶ 5-NHAc. As acylation usually involves

^1H N.m.r. spectra [a] for



R^1	R^2	Solvent	SMe	6-Me	4-NH_2	5-NH_2	N(4)Ac	N(5)Ac	CH_3·CH_2	CH_3·CH_2
NH_2	NH_2 [b]	$(CD_3)_2SO$	2·35	2·15	6·30	4·22				
NH_2	NHAc [b]	$(CD_3)_2SO$	2·40	2·07	6·70	9·00		2·03		
NH_2	N(Et)Ac	$(CD_3)_2SO$	2·38	2·08	7·00			1·67	0·95 [c]	3·53 [d]
		$CDCl_3$	2·27	2·53	5·67			1·87	1·10	3·75
NHEt	NHAc	$(CD_3)_2SO$	2·40	2·50	6·90	8·95		2·00	1·10 [c]	3·50 [d]
		$CDCl_3$	2·40	2·17	6·65	7·20		1·82	1·20	3·60
NHEt	NH·CO·CD_3	$(CD_3)_2SO$	2·40	2·50	6·9	9·00			1·10 [c]	3·50 [d]
NHAc	NHAc	$(CD_3)_2SO$	2·45	2·20	10·10	9·10	2·05	2·25		
		$CDCl_3$	2·40	2·48	8·50	8·40	2·12	2·12		
N(Et)Ac	NHAc	$(CD_3)_2SO$	2·35	2·50		9·50	2·05	1·95	1·05 [c]	3·60 [d]
		$CDCl_3$	2·43	2·53		7·50	2·10	2·27	1·20	4·00
N(Et)·CO·CD_3	NHAc	$CDCl_3$	2·35	2·55		7·40		2·15	1·20 [c]	4·00 [d]
NHAc	NAc_2	$(CD_3)_2SO$	2·29	2·55	10·20		2·18	2·25, 2·25		
		$CDCl_3$	2·34	2·58	9·05		2·62	2·28, 2·34		
N(Et)Ac	NAc_2	$(CD_3)_2SO$	2·33	2·55			2·08	2·30, 2·30	1·13 [c]	3·60 [d]
		$CDCl_3$	2·37	2·57			2·15	2·37, 2·37	1·27	3·70
N(Et)·CO·CD_3	$N(COCD_3)_2$	$CDCl_3$	2·38	2·58					1·28 [c]	3·70 [d]
4,5-$(NH_2)_2$-2-SMe [b]		$(CD_3)_2SO$	2·35	7·55 [f]	6·45	4·50				
4,5$(NH_2)_2$-6-Me [b]		$(CD_3)_2SO$	7·82 [e]	2·17	6·20	4·45				

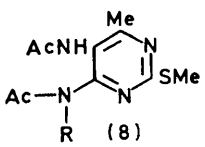
[a] Singlet resonances unless otherwise indicated. [b] Derivative not soluble in $CDCl_3$. [c] Triplet, *J* 7 Hz. [d] Quartet, *J* 7 Hz. [e] Singlet, 2-H. [f] Singlet, 6-H.

and their attempted isolation has led to ring closure to the corresponding 8-methylpurines.[9,10] The diacetylated derivative shows two amino-group resonances (at δ 9·1 and 10·1) indicating that this is the 4,5-diacetamidopyrimidine (8; R = H). If environmental and hydrogen bonding effects due to the 5-acetamido-group are not taken into account then the foregoing results suggest that with a 4-acetamido-5-aminopyrimidine a similar downfield shift would be observed for the 4-amino-proton signal. Recent results [11] for (*inter alia*) 4,5-diacylaminopyrimidines have confirmed our assignments for the amino-proton resonances of (8; R = H). Our data also allow structures to be assigned to the two isomeric *N*-ethyl monoacetyl derivatives. Thus, the product from ethylation of the monoacetylated pyrimidine is (4; R = Et) as only one NH resonance is obtained. The isomer, derived from the most basic nitrogen atom present the 5-amino-group is the one initially acylated. The 4-amino-group then becomes the more basic centre and the second acyl group is directed to this position. The nitrogen atom at the 5-position is then the centre of greatest basicity and secondary acetylation takes place here. This alternation in basicity explains the differences in sites of ethylation between the mono- and di-acetyl derivatives. Alkylation, under the conditions employed, would be expected to take place at the more anionic of the two exocyclic nitrogen atoms, *i.e.* that of the 5-acetamido-group in (5; R = H) and that of the 4-acetamido-group in (8; R = H).

Ethylation of (9; R = H) can only take place at the 4-acetamido-group but on account of degradation the product was not isolated. However, the *N*-ethyl derivative (9; R = Et) was obtained from the action of hot acetic anhydride on the diamine (7; R = H).

[9] J. H. Lister and G. M. Timmis, *J. Chem. Soc.*, 1960, 1113.
[10] F. E. Kempter, H. Rokos, and W. Pfleiderer, *Angew. Chem. Internat. Edn.*, 1967, **6**, 258.
[11] C. V. Z. Smith, R. K. Robins, and R. L. Tolman, *J.C.S. Perkin I*, 1973, 1855.

Because of the close groupings of the chemical shifts of the protons of the 6-methyl, 2-methylthio-, and acetyl groups, deuteriated acetylating reagents were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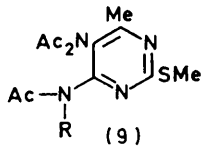
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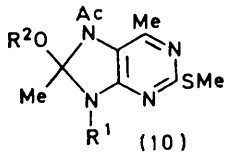
(10)

used to prepare the monoacetyl analogue (7; R = $CO{\cdot}CD_3$), the 4-trideuterioacetyl analogue of (8; R = Et), and the tristrideuterioacetyl form of (9; R = Et). From the spectra of the labelled derivatives the individual acetyl group resonances could be identified. Furthermore, the assignments given for the 6-methyl group and the *S*-methyl group, which had been derived from spectra of 4,5-diamino-6-methyl- and 4,5-diamino-2-methylthio-pyrimidine, respectively, were verified.

The correspondences shown by the chemical shifts in the two solvents used seem to indicate that hydrogen bonding, either inter- or intra-molecular, does not occur to any significant degree with these derivatives. Also, no evidence was found for the existence of any di- or tri-acetyl derivative in the form of a cyclic adduct of the type (10; R^1 = H or Et, R^1 = H) or (10; R^1 = H or Et, R^2 = Ac), respectively. Such structures have been proposed [7,12] for the acetylated forms of 5,6-diaminouracils.

EXPERIMENTAL

^{1}H N.m.r. spectra were recorded at 33° on a Perkin-Elmer R10 60 MHz instrument, with tetramethylsilane or sodium 3-trimethylsilylpropane-1-sulphonate as standard. In order to obtain good amino-group proton signals in dimethyl sulphoxide it was necessary to store the solvent over molecular sieves (type 4A) in a diaphragm-sealed container and withdraw it, as required, by hypodermic syringe.

4-Amino-5-(N-ethyl)formamido-6-methyl-2-methylthiopyrimidine (3).—To 4-amino-5-formamido-6-methyl-2-methylthiopyrimidine (1·9 g) [5] in dimethylformamide (12 ml) containing dried potassium carbonate (1·5 g) was added iodoethane (2 g), and the mixture was stirred for 46 h. Inorganic salts were removed and the filtrate was diluted with water (20 ml) and evaporated to dryness. The residue was taken up in hot water (carbon) then the solution was concentrated to 5 ml to give the *4-amino-5-(N-ethyl)formamidopyrimidine* (1·15 g), m.p. 137—138° (Found: C, 48·1; H, 6·2; N, 24·8. $C_9H_{14}N_4OS$ requires C, 47·8; H, 6·2; N, 24·8%).

5-Acetamido-4-ethylamino-6-methyl-2-methylthiopyrimidine (7; R = Ac).—The 5-amino-4-ethylaminopyrimidine [3] (0·5 g) in ethanol (8 ml) was treated with acetic anhydride (2·5 ml) and left for 2·5 h. Evaporation to dryness and crystallisation of the residue from ethyl acetate containing a small amount of ethanol gave the *5-acetamido-4-ethylaminopyrimidine* (0·3 g), m.p. 218—219° (Found: C, 49·7; H, 6·7; N, 23·1. $C_{10}H_{16}N_4OS$ requires C, 50·0; H, 6·7; N, 23·3%).

5-Diacetylamino-4-(N-ethyl)acetamido-6-methyl-2-methylthiopyrimidine (9; R = Et).—The foregoing 5-amino-4-ethylaminopyrimidine (0·3 g) and acetic anhydride (5 ml) were heated under reflux for 1·5 h. Evaporation left a brown oil which solidified on trituration with a little water. The *triacetylated derivative* was obtained crystalline (0·07 g) from hot water; m.p. 102—103° (Found: C, 51·9; H, 6·2; N, 17·5. $C_{14}H_{20}N_4O_3S$ requires C, 51·9; H, 6·2; N, 17·3%).

5-Acetamido-4-amino-6-methyl-2-methylthiopyrimidine (2; R = Ac).—A solution of 4,5-diamino-6-methyl-2-methylthiopyrimidine (1 g) in ethanol (15 ml) was treated with acetic anhydride (5 ml) and left for 2 h. Evaporation to dryness left a residue (1·14 g) which was recrystallised from ethanol giving the *5-acetamidopyrimidine*, m.p. 239—240° (Found: C, 45·6; H, 5·5; N, 26·2. $C_8H_{12}N_4OS$ requires C, 45·3; H, 5·7; N, 26·4%). The same product, but in lower yield, resulted from heating the diaminopyrimidine with acetic acid under reflux for 3 h.

4-Amino-5-(N-ethyl)acetamido-6-methyl-2-methylthiopyrimidine (5; R = Et).—This was obtained from the preceding 5-acetamido-4-aminopyrimidine (0·7 g) and iodoethane (0·75 g) under the same conditions as for the 5-(*N*-ethyl)formamido-analogue. The *5-(N-ethyl)acetamidopyrimidine* (0·2 g) had m.p. 174—175° (from water) (Found: C, 50·1; H, 6·2; N, 23·3. $C_{10}H_{16}N_4OS$ requires C, 50·0; H, 6·7; N, 23·3%).

4-Acetamido-5-diacetylamino-6-methyl-2-methylthiopyrimidine (9; R = H) *and 4,5-Diacetamido-6-methyl-2-methylthiopyrimidine* (8; R = H).—A solution of 4,5-diamino-6-methyl-2-methylthiopyrimidine (2·3 g) in acetic anhydride (20 ml) was heated under reflux for 1 h. The oil which remained after removal of the anhydride slowly solidified. This was taken up in hot water (80 ml), the pH was adjusted to 7 with ammonia solution, and on cooling a crystalline precipitate of the *4-acetamido-5-diacetylamino-compound* (2·3 g) was obtained; m.p. 147—148° (Found: C, 48·4; H, 5·4; N, 18·7. S, 10·7. $C_{12}H_{16}N_4O_3S$ requires C, 48·7; H, 5·4; N, 18·9; S, 10·8%). Concentration of the aqueous mother liquors gave the *4,5-diacetamido-compound* (0·4 g), m.p. 204—205° (from water) (Found: C, 46·9; H, 5·4; N, 22·1. $C_{10}H_{14}N_4O_2S$ requires C, 47·2; H, 5·6; N, 22·0%). On heating (1 h) the didiacetyl derivative with anhydride the triacetylated form was obtained.

Ethylation of 4,5-Diacetamido-6-methyl-2-methylthiopyrimidine.—The diacetyl derivative (0·15 g) in dimethylformamide (3 ml) containing anhydrous potassium carbonate (0·15 g) was treated with iodoethane (0·12 g) and then stirred for 30 h. Work-up as before gave *5-acetamido-4-ethylamino-6-methyl-2-methylthiopyrimidine* (0·03 g), identical (m.p., i.r., and n.m.r.) with the product obtained from monoacetylation of the 5-amino-4-ethylaminopyrimidine.

Ethylation of 4-Acetamido-5-diacetylamino-6-methyl-2-methylthiopyrimidine.—The triacetylated pyrimidine (1·5 g) and iodoethane (0·8 g) in dimethylformamide containing potassium carbonate (0·9 g) were stirred for 26 h. After removal of the inorganic solids the solution was diluted with water, brought to pH 7 with ammonia, and

[12] H. Biltz and W. Schmidt, *Annalen*, 1923, **431**, 70.

taken to dryness. Dissolution of the residue in ethanol followed by the addition of ether, in excess, caused further inorganic matter to precipitate. The filtrate was evaporated; crystallisation of the residue from water then gave 5-acetamido-4-ethylamino-6-methyl-2-methylthiopyrimidine, identical with samples prepared by the other routes. A second product obtained on taking the aqueous mother liquors to dryness was *5-acetamido-4-(N-ethyl)acetamido-6-methyl-2-methylthiopyrimidine* (0·1 g), m.p. 148—149° (from water) (Found: C, 51·3; H, 6·3; N, 20·0. $C_{12}H_{18}N_4O_2S$ requires C, 51·1; H, 6·4; N, 19·9%). The diacetyl derivative was also obtained when 5-acetamido-4-ethylamino-6-methyl-2-methylthiopyrimidine in acetic anhydride was heated under reflux for 35 min.

7-Ethyl-6-methyl-2-methylthiopurine (1; R = H).—A solution of 4-amino-5-(*N*-ethyl)acetamido-6-methyl-2-methylthiopyrimidine (0·46 g) and potassum carbonate (0·18 g) in dimethylformamide (5 ml) was heated under reflux for 2 h, diluted with water, and evaporated to dryness. The residue was extracted with chloroform. After removal of the solvent the product was crystallised from benzene–petroleum (b.p. 40—60°) to give the *purine* (0·2 g), m.p. 118—119° (Found: C, 52·2; H, 5·8; N, 26·9. $C_9H_{12}N_4S$ requires C, 51·9; H, 5·8; N, 26·9%).

7-Ethyl-6,8-dimethyl-2-methylthiopurine (1; R = Me).—Ring closure of 4-amino-5-(*N*-ethyl)acetamido-6-methyl-2-methylthiopyrimidine (0·1 g) as in the previous preparation gave the *7-ethylpurine*, m.p. 110—111° [from petroleum (b.p. 100—120°)] (Found: C, 53·7; H, 6·3; N, 25·4. $C_{10}H_{14}N_4S$ requires C, 54·0; H, 6·4; N, 25·2%).

9-Ethyl-6,8-dimethyl-2-methylthiopurine (6).—The foregoing procedure with the 5-acetamido-4-ethylaminopyrimidine gave the 9-ethylpurine, identical (m.p., i.r.) with the product previously [3] reported.

Bis-(7-ethyl-6-methylpurin-2-yl) Sulphide (4).—As the i.r. spectrum of the product from the attempted cyclisation of 4-amino-5-(*N*-ethyl)formamido-6-methyl-2-methylthiopyrimidine (0·7 g) (in a metal bath at 260° for 20 min) showed uncyclised material to be present, the mixture was taken up in formamide (7 ml) and hydrochloric acid (1 ml) and heated under reflux for 6 h. Water (20 ml) was added, the solution was taken to dryness, and the residual gum was dried on a porous tile. The solid was extracted with hot petroleum (b.p. 60—80°) to remove any 7-ethyl-6-methyl-2-methylpurine present, and the residue was crystallised from water to give the *dipurin-2-yl sulphide*, m.p. 213—214° (Found: C, 53·7; H, 4·6; N, 31·5. $C_{17}H_{21}N_8S$ requires C, 54·2; H, 5·1; N, 31·6%).

Preparations of Deuteriated Derivatives.—These employed the same procedures as for the corresponding unlabelled derivatives, but hexadeuterioacetic anhydride or tetradeuterioacetic acid was used as acylating agent. The residues remaining after removal of the reagent were washed with dry ether giving fairly pure products. These were characterised by comparisons of their n.m.r. and i.r. spectra with those of the unlabelled analogues.

We thank Drs. D. J. Brown and E. Spinner for discussions and Mr. S. E. Brown for ^1H n.m.r. spectra.

[4/096 *Received*, 21*st January*, 1974]