Synthesis and Reactions of 7,8-Dihydro-8-methylpterin and 9-Methylguanine 7-Oxide

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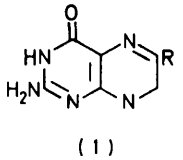
7,8-Dihydro-8-methylpterin (3) has been prepared by reduction of [2-amino-5-(*p*-chlorophenylazo)-6-hydroxypyrimidin-4-yl(methyl)amino]acetaldehyde (7; X = $N_2C_6H_4Cl$) and by reduction of the corresponding 5-nitropyrimidine. The addition of nucleophilic reagents to the dihydropterin is reported and further reactions with the products are described. In a new purine synthesis the 5-nitropyrimidine (7; X = NO_2) is cyclised to 9-methylguanine 7-oxide (26) with sodium hydroxide. The latter undergoes typical *N*-oxide reactions and has been used for the synthesis of other purine derivatives.

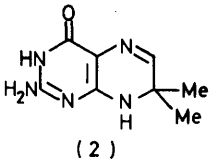
DURING the bacterial intracellular conversion of 7,8-dihydroneopterin [1; R = CH(OH)·CH(OH)·CH_2·OH] into folic acid the pteridine nucleus remains in the dihydro-form. It has been suggested that naturally occurring pteridines from other sources are produced by carbanion addition to 7,8-dihydropterin (1; R = H).[1] 'Blocked' 7,8-dihydropterins of closely related structures [(2) and (3)], in which aromatisation of the pyrazine ring is prevented, are therefore of interest.

Examples of type (2) were first reported by Pfleiderer and Zondler [2] and subsequently by Wood and his coworkers.[3] The synthesis of 7,8-dihydropterin (1; R = H) by reductive cyclisation of (2-amino-6-hydroxy-5-nitropyrimidin-4-ylamino)acetaldehyde was reported by both groups.[1,4] In an attempted extension of this synthesis to compound (3), Pfleiderer and Zondler [4] failed to obtain the required intermediate [2-amino-6-hydroxy-5-nitropyrimidin-4-yl(methyl)amino]acetaldehyde (7; X = NO_2) owing to ready pyrimidine ring scission to produce the imidazoline (8; X = NO_2) during the acidic hydrolysis of (5; X = NO_2).

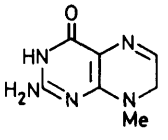
We have synthesised compound (3) by a similar route *via* the 5-arylazopyrimidine (5; X = $N_2C_6H_4Cl$) which is not so susceptible to ring scission during hydrolysis of the acetal. An 80% yield of (7) was obtained by hydrolysis of (5) with concentrated hydrochloric acid. Subsequently the corresponding 5-nitro-compound (7; X = NO_2) was also obtained, in 50% yield, together with (8). The starting material for this last synthesis,



(1)

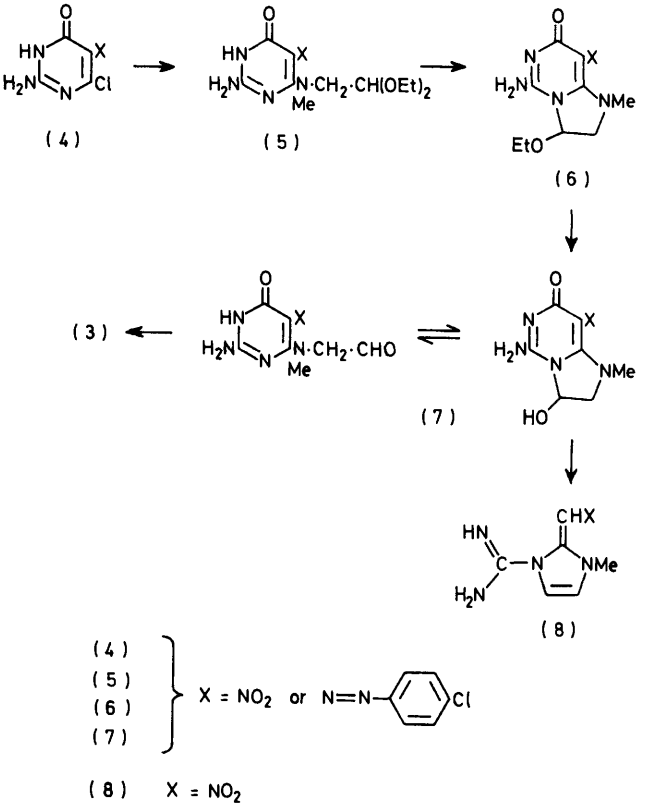


(2)



(3)

(4; X = NO_2), is unstable,[5] and cannot be recrystallised. Fortunately it could be stored and used as the triethylamine salt. The latter crystallises from boiling ethanol and may be stored unchanged indefinitely. The reductive cyclisation (7) ⟶ (3) was accomplished with sodium dithionite. Catalytic reduction of (7) gave the 5,6,7,8-tetrahydropterin.



(4) (5) (6) (7) X = NO_2 or N=N-C$_6$H$_4$Cl

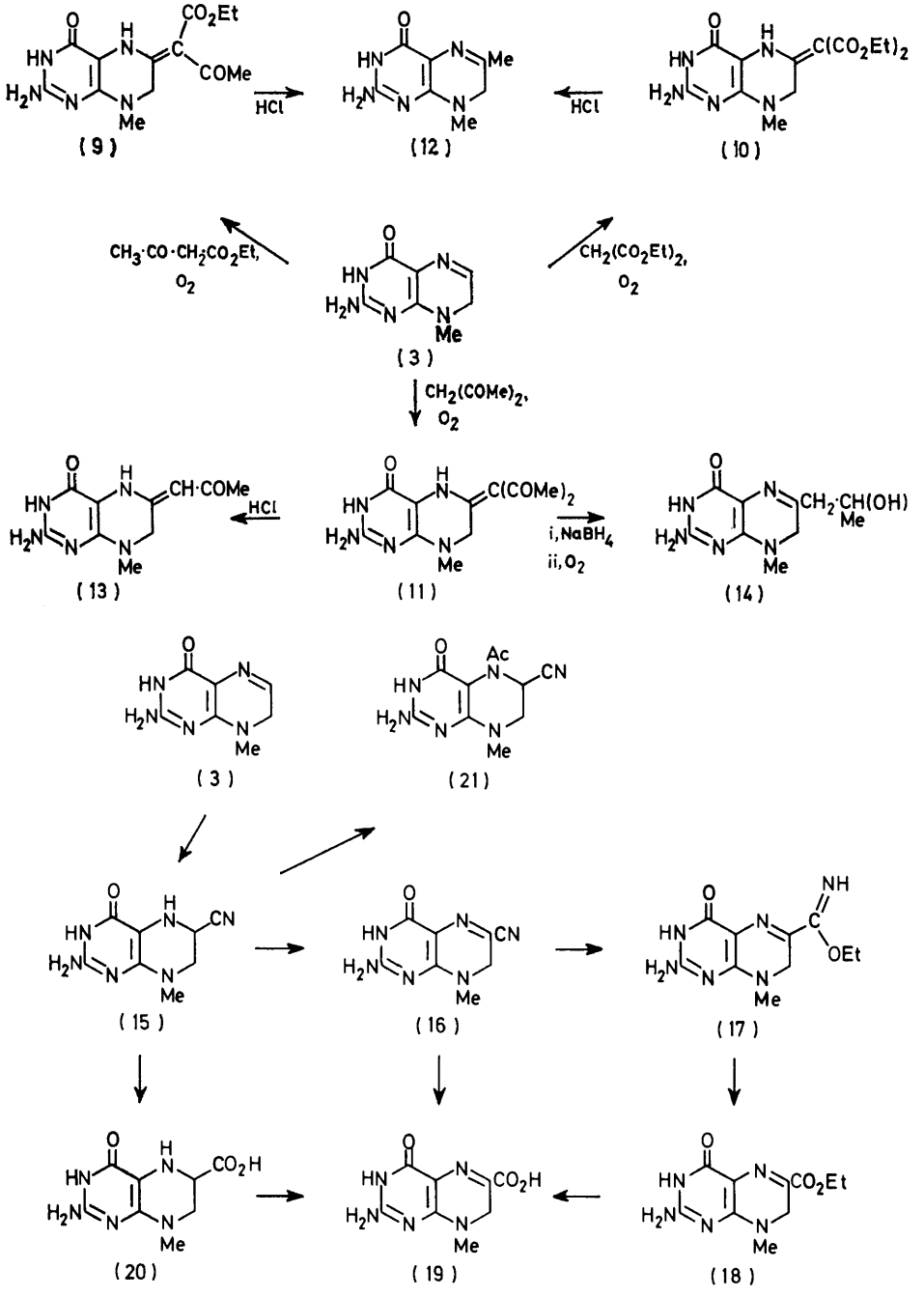
(8) X = NO_2

The two aldehydes (7; X = $N_2C_6H_4Cl$ or NO_2) crystallised from the hydrolysis solution as hydrochlorides and further purification was unnecessary. They can, however, be crystallised from boiling water without decomposition, the former crystallising as the mono- and the latter as the hemi-hydrochloride. They were obtained as the free bases and also as crystalline sodium salts. The hemi-hydrochloride of the nitro-derivative was the only modification of (7) (X = $N_2C_6H_4Cl$ or NO_2) which showed aldehydic absorption in the i.r. spectrum. Another example of the formation of a hemihydrochloride of the type BHB^+Cl^-, as a result of the strong hydrogen bonding in these heterocycles, is

[1] A. Stuart, H. C. S. Wood, and D. Duncan, *J. Chem. Soc.*, 1966, 285.
[2] W. Pfleiderer and H. Zondler, *Chem. Ber.*, 1966, **99**, 3008.
[3] B.P. 1,303,171.
[4] W. Pfleiderer and H. Zondler, *Chem. Ber.*, 1966, **99**, 2984.
[5] A. Stuart, D. W. West, and H. C. S. Wood, *J. Chem. Soc.*, 1964, 4769.

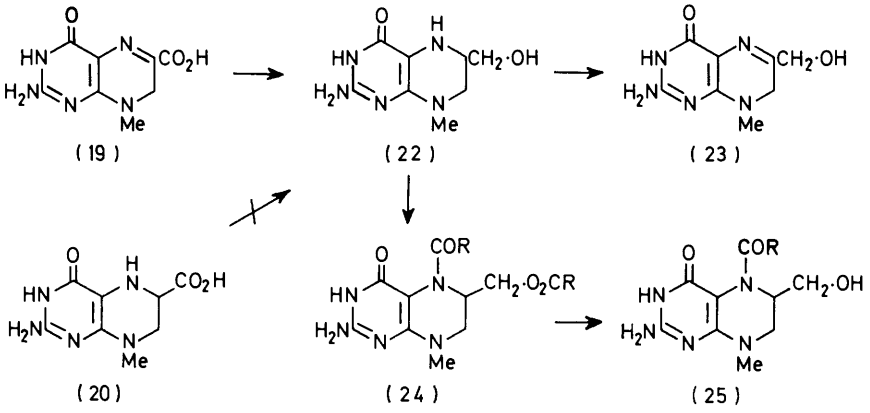
given in the Experimental section. This general phenomenon has been discussed by Katritzky and Taylor.[6]

Michael additions of acetylacetone, diethyl malonate, and ethyl acetoacetate to the dihydropterin, followed by aerial oxidation, gave the stable 6-methylene derivatives (9)—(11). These were used for the preparation of compounds (12)—(14). The intermediate tetrahydro-compounds were not isolated. The absence of protons at C-6 and at the exocyclic carbon atom in the oxidised adducts was indicated by n.m.r. spectra. From the failure of compound (9) to give the vinyl ketone (13) it appears that in going from (9) to (12) the first step is loss of acetyl. Removal of an acetyl group also occurred when the 6-(2-hydroxypropyl)pterin (14), was produced by reduction of (11) with sodium borohydride followed by aerial oxidation.

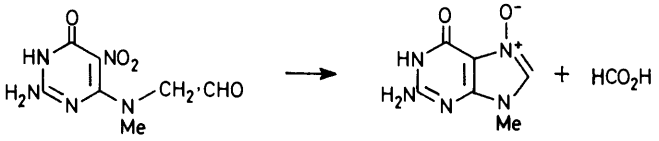


Addition of cold aqueous potassium cyanide to the hydrogen sulphite salt of (3) gave the potassium salt of 5,6,7,8-tetrahydro-8-methylpterin-6-carbonitrile. In the absence of air this was hydrolysed with sodium hydroxide to the tetrahydro-acid (20). The 7,8-dihydro-nitrile (16),

[6] A. R. Katritzky and P. J. Taylor, 'Physical Methods in Heterocyclic Chemistry,' ed. A. R. Katritzky, Academic Press, London and New York, 1971, vol. IV, p. 273

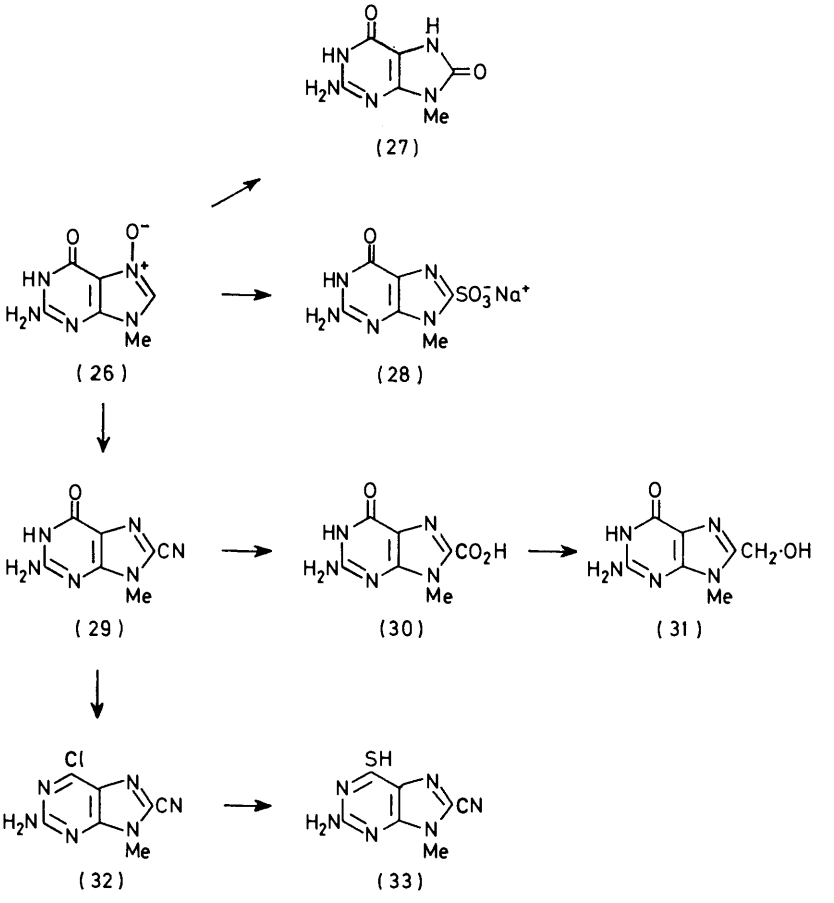


was conveniently crystallised from a solution of (15) in dimethylformamide by passing in oxygen. On dissolving the oxidised nitrile in cold dilute sodium hydroxide the



solution immediately deposits the sparingly soluble sodium salt of the amide. The dihydro-nitrile reacts equally rapidly with sodium ethoxide to give the sodium salt of the imino-ether (17). This can be dissolved in cold dilute hydrochloric acid and precipitated unchanged by addition of sodium hydrogen carbonate. Warming with hydrochloric acid converts the imino-ether into the ester (18), and the constitution of the latter was confirmed by alkaline hydrolysis, to the acid (19). The reactions, (15) ⟶ (16) ⟶ (17) ⟶ (18) proceed cleanly, in high yield, and provide an excellent route to the pure ester. This was fortunate since an attempt to esterify (19) under Fischer–Speier conditions gave an intractable mixture.

7,8-Dihydro-6-hydroxymethylpterin (1; R = CH_2·OH) is an intermediate in the biochemical conversion



of 7,8-dihydroneopterin [1; R = CH(OH)·CH(OH)·CH_2·OH] into folic acid, and it was of interest to prepare the corresponding 8-methyl compound (23). 5,6,7,8-Tetrahydro-6-hydroxymethyl-8-methylpterin (22) was obtained by reducing a suspension of the carboxylic acid (19) in tetrahydrofuran with diborane. Despite the much greater solubility of the corresponding tetrahydropterincarboxylic acid (20), because it exists as a zwitterion this was not reduced under these conditions. Partial hydrolysis of the tetrahydropterin diacyl derivatives (24; R = H or Me) with sodium hydroxide furnished the 5-formyl- and 5-acetyl-5,6,7,8-tetrahydro-6-hydroxymethyl-8-methylpterins (25; R = H or Me). Subsequently this selective hydrolysis was more cleanly accomplished by adding (24) to an aqueous solution of methylamine.

An interesting property of the pterin precursor, the 5-nitropyrimidinylaminoacetaldehyde (7), is that with cold dilute sodium hydroxide it cyclises to 9-methylguanine 7-oxide (24) in almost quantitative yield. Structural requirements for this reaction are specific. The formyl group may be replaced by benzoyl (benzoic acid is then eliminated) but not by methoxycarbonyl or cyano, and no reaction occurs with the unmethylated amino-aldehyde. The constitution of (26) was confirmed by rearrangement with acetic acid to the 8-oxopurine [7] (27). Other typical *N*-oxide reactions were used to prepare the new guanine derivatives (28)—(33).

EXPERIMENTAL

[2-*Amino*-5-(*p-chlorophenylazo*)-6-*hydroxypyrimidin*-4-*yl*(*methyl*)*amino*]*acetaldehyde Diethyl Acetal* (5; X = $N_2C_6H_4$Cl).—To an ice-cooled, stirred mixture of compound (4; X = $N_2C_6H_4$Cl) (43 g) [8] and dimethylformamide (80 ml) was added methylaminoacetaldehyde diethyl acetal (45 g) during 30 min, and stirring was continued at room temperature overnight. The mixture was filtered and the orange coloured solid was washed with a little dimethylformamide and then with ether to obtain the *hemihydrochloride* of (5) [Found: C, 48.5; H, 5.4; Cl, 12.9; N, 19.7. $(C_{17}H_{23}ClN_6O_3)_2HCl,H_2O$ requires C, 48.4; H, 5.8; Cl, 12.6; N, 19.9%].

2-*Amino*-4-*chloro*-6-*hydroxy*-5-*nitropyrimidine* (4; X = NO_2) *Triethylamine Salt*.—The nitropyrimidine (4; X = NO_2) (118 g) [5] was added to a solution of triethylamine (80 ml) in propan-2-ol (1 l) and the mixture was boiled. After filtering to remove impurity the solution was cooled to precipitate the *triethylamine salt* as thick yellow needles, m.p. 135° [Found: C, 41.2; H, 6.1; N, 24.0. $C_4H_3ClN_4O_3,N(C_2H_5)_3$ requires C, 41.2; H, 6.2; N, 24.0%]. The constitution was confirmed by dissolving in water and acidifying to give a quantitative recovery of (4).

[2-*Amino*-5-(p-*chlorophenylazo*)-6-*hydroxypyrimidin*-4-*yl*(*methyl*)*amino*]*acetaldehyde* (7; X = $N_2C_6H_4$Cl).—A mixture of compound (5; X = $N_2C_6H_4$Cl) (12 g) and concentrated hydrochloric acid (120 ml) was heated to 80 °C for 15 min. During the first 5 min (7) began to crystallise from solution. After cooling the orange solid was collected and stirred with water for 2 h. The resulting yellow solid was filtered off and washed with water to obtain the *hydrochloride* of (7; X = $N_2C_6H_4$Cl) (9.5 g), m.p. 240° (decomp.). The product is pure, and can be crystallised without decomposition from boiling water (Found: C, 42.7; H, 4.1; Cl, 19.2; N, 23.0. $C_{13}H_{12}ClN_6O_2,HCl,0.5H_2O$ requires C, 42.6; H, 4.1; Cl, 19.4; N, 23.0%).

[2-*Amino*-6-*hydroxy*-5-*nitropyrimidin*-4-*yl*(*methyl*)*amino*]*acetaldehyde* (7; X = NO_2).—The acetal (5; X = NO_2) [4] (270 g), dissolved in cold concentrated hydrochloric acid (1 l), was heated to 80 °C for 15 min. On cooling (7) crystallised as the dihydrochloride (148 g), m.p. 171° (decomp.) (Found: C, 29.7; H, 4.2; Cl, 12.6; N, 24.9. Calc. for $C_7H_9N_5O_4,2HCl,H_2O$: C, 29.8; H, 4.3; Cl, 12.6; N, 24.9%). This is hydrolysed with cold water and may be crystallised from boiling water to obtain pale yellow needles of the hemihydrochloride of (7; X = NO_2) [Found: C, 32.2; H, 4.5; Cl, 6.6; N, 26.6. Calc. for $(C_7H_9N_5O_4)_2HCl,2H_2O$: C, 32.0; H, 4.2; Cl, 6.8; N, 26.8%]. Aqueous sodium hydrogen carbonate converted it into the sodium salt (Found: C, 27.4; H, 4.1; N, 23.1. $C_7H_8N_5NaO_4,3H_2O$ requires C, 27.0; H, 4.6; N, 23.1%). The latter is hydrolysed on crystallising from hot water to give the *nitropyrimidine* (7; X = NO_2), m.p. 250° (decomp.) (Found: C, 37.3; H, 4.2; N, 30.8. $C_7H_9N_5O_4$ requires C, 37.0; H, 4.0; N, 30.8%).

The hydrochloric acid filtrate from the above experiment was heated at 80 °C for a further 1 h. It was concentrated *in vacuo* (to 100 ml) and to the resulting cold solution was added sufficient 5N-sodium hydroxide to adjust the pH to 8.0. The precipitate was collected and crystallised from hot water to obtain orange red crystals of the imidazoline (8). The m.p. (231°) and n.m.r. spectrum were the same as those recorded.[4]

7,8-*Dihydro*-8-*methylpterin* (3).—To a stirred suspension of the hydrochloride of (7; X = $N_2C_6H_4$Cl) (10 g) in water (250 ml) was added sodium dithionite (20 g) during 5 min. After stirring for a further 30 min the resulting cream coloured suspension was filtered and the solid was washed with water to obtain the hydrogen sulphite salt of (3) (7.0 g). Addition of 2N-sodium hydroxide (30 ml) to a suspension of the hydrogen sulphite salt (3.0 g) in water (6 ml) immediately produced a clear solution from which the sodium salt of (3) quickly crystallised (1.8 g). This was converted into the *free base* by dissolving in dilute hydrochloric acid and precipitating with sodium hydrogen carbonate (Found: C, 45.5; H, 5.3; N, 37.8. $C_7H_9N_5O,0.25H_2O$ requires C, 45.8; H, 5.3; N, 37.8%). The same conditions were used for the preparation of (3) from the nitro-compound (7; X = NO_2).

5,6,7,8-*Tetrahydro*-8-*methylpterin*.—A mixture of compound (7; X = $N_2C_6H_4$Cl) (10 g), water (200 ml), and platinum oxide (0.5 g) was stirred under hydrogen at 20 lb in^{-2} for 3 h. The mixture was filtered and concentrated *in vacuo* to 20 ml. A solution (50 ml) of sodium hydrogen carbonate (7.0 g) was added and the precipitated solid was collected and washed; m.p. >300°, yield 3.6 g (Found: C, 46.3; H, 5.8; N, 38.2. Calc. for $C_7H_{11}N_5O$: C, 46.4; H, 6.1; N, 38.7%). The *hydrochloride* crystallised on adding the base to a small volume of N-hydrochloric acid (Found: C, 38.6; H, 5.4; Cl, 16.3; N, 31.7. $C_7H_{11}N_5O,HCl$ requires C, 38.7; H, 5.5; Cl, 16.3; N, 32.2%).

6-(1-*Ethoxycarbonyl*-2-*oxopropylidene*)-5,6,7,8-*tetrahydro*-8-*methylpterin* (9).—Ethyl acetoacetate (65 ml) was added to a stirred suspension of the sodium salt of compound (3) (10 g) in water (2 l) and a slow stream of oxygen was passed into the mixture for 24 h. The *solid* was collected and washed with water and ethanol (12.8 g). A portion was crystallised from ethanol; m.p. >300° (Found: C, 50.1;

[7] F. Perini and H. Tieckelmann, *J. Org. Chem.*, 1970, **35**, 812.
[8] B.P. 677,342.

H, 5.6; N, 22.5. $C_{13}H_{17}N_5O_4,0.25H_2O$ requires C, 50.1; H, 5.6; N, 22.5%).

6-[*Bis*(*ethoxycarbonyl*)*methylene*]-5,6,7,8-*tetrahydro*-8-*methylpterin* (10).—From the sodium salt of compound (3) (5 g) and diethyl malonate (40 g), *compound* (10) was prepared (7.5 g) as described for the preparation of (9) (Found: C, 49.1; H, 5.7; N, 21.0. $C_{14}H_{19}N_5O_5,0.25H_2O$ requires C, 49.3; H, 5.7; N, 20.6%).

6-(1-*Acetyl*-2-*oxopropylidene*)-5,6,7,8-*tetrahydro*-8-*methylpterin* (11).—This was prepared from the sodium salt of compound (3) (22.5 g) and acetylacetone (110 ml) by the method described for (9) except that the reaction time was increased to 70 h and the pH was maintained at 8.0 by occasional addition of sodium hydroxide; yield 26.2 g. A *portion* (200 mg) recrystallised from ethanol (40 ml) for analysis (Found: C, 49.9; H, 5.8; N, 24.2. $C_{12}H_{15}N_5O_3,0.5H_2O$ requires C, 50.3; H, 5.6; N, 24.5%).

7,8-*Dihydro*-6,8-*dimethylpterin* (12).—A mixture of compound (9) (1 g) and N-hydrochloric acid (20 ml) was heated on a steam-bath. After 30 min undissolved material was removed and the filtrate was evaporated *in vacuo* almost to dryness. Ethanol (10 ml) was added and the mixture filtered to obtain 7,8-dihydro-6,8-dimethylpterin hydrochloride (12) (0.5 g). The material crystallised from methanol (20 ml) as pale yellow *needles* (Found: C, 41.0; H, 5.0; Cl, 15.1. $C_8H_{11}N_5O,HCl,0.25H_2O$ requires C, 41.2; H, 5.3; Cl, 15.2%). The same compound resulted when (10) was heated with hydrochloric acid.

6-*Acetonylidene*-5,6,7,8-*tetrahydro*-8-*methylpterin* (13).—A mixture of compound (11) (200 mg) and 0.1N-hydrochloric acid was boiled to obtain a clear solution. On cooling (13) separated as light brown *needles* (130 mg) (Found: C, 47.2; H, 5.8; N, 27.5. $C_{10}H_{13}N_5O_2,H_2O$ requires C, 47.4; H, 5.9; N, 27.6%).

7,8-*Dihydro*-6-(2-*hydroxypropyl*)-8-*methylpterin* (14).—Sodium borohydride (0.4 g) was added to a stirred mixture of compound (11) (1.2 g) and ethanol (50 ml) during 45 min. On passing a current of oxygen into the red solution the colour changed to yellow and a yellow solid was precipitated. After oxygenation for 1 h the solid was collected and washed with water; yield 0.5 g. The product was purified by dissolving in N-sodium hydroxide (10 ml) and passing carbon dioxide into the solution; fawn coloured *needles* were precipitated (0.3 g) (Found: C, 44.5; H, 6.9; N, 26.2. $C_{10}H_{15}N_5O_2,2H_2O$ requires C, 44.0; H, 6.9; N, 25.7%).

5,6,7,8-*Tetrahydro*-8-*methylpterin*-6-*carbonitrile* (15).—A saturated solution of potassium cyanide was quickly added to a mixture of the hydrogen sulphite salt of compound (3) (18 g) and water (30 ml) at 5 °C. A clear orange solution was obtained from which the potassium salt of (15) crystallised during a few minutes. The mixture was filtered and the potassium salt was washed with a little ice-cold saturated potassium cyanide solution. The salt was added to 5% sodium hydrogen carbonate (100 ml) which had previously been saturated with carbon dioxide. Carbon dioxide was bubbled into the mixture for 1 h. The solid was collected and washed with water and ethanol to obtain the product (15) (11.5 g). The material (1 g) dissolved in cold 2N-hydrochloric acid (10 ml) to give a clear solution from which the *hydrochloride* (0.5 g) crystallised over a few minutes (Found: C, 39.8; H, 4.9; N, 34.3. $C_8H_{10}N_6O,HCl$ requires C, 39.7; H, 4.5; N, 34.7%).

5-*Acetyl*-5,6,7,8-*tetrahydro*-8-*methylpterin*-6-*carbonitrile* (21).—The tetrahydropterin (15) (3.0 g) was dissolved in a cold mixture of dimethylformamide (10 ml) and acetic anhydride (10 ml). After 1 h water (100 ml) was added and (21) crystallised as *prisms* (2.2 g), m.p. 308° [unchanged on crystallising from water (80 ml)] (Found: C, 47.2; H, 5.1; N, 33.4. $C_{10}H_{12}N_6O_2,0.25H_2O$ requires C, 47.5; H, 5.0; N, 33.3%).

5,6,7,8-*Tetrahydro*-8-*methylpterin*-6-*carboxylic Acid* (20).—The potassium salt of the nitrile (21) [prepared as described above from the hydrogen sulphite salt of 7,8-dihydro-8-methylpterin (18 g)] was stirred with N-sodium hydroxide (200 ml) in nitrogen for 90 h. The pH of the resulting clear solution was adjusted to 4.0 with acetic acid and the precipitate of (20) was collected. This was purified by dissolving in cold 2% sodium hydrogen carbonate (800 ml), filtering, and reprecipitating by adjusting the pH to 4.0. Washing with ethanol left the product (20) (9.0 g). The pale yellow crystalline solid was readily soluble in cold sodium carbonate [the sodium salt of the dihydro-acid (19) is virtually insoluble in water].

7,8-*Dihydro*-8-*methylpterin*-6-*carboxylic Acid* (19).—A slow stream of air was passed into a solution of the tetrahydro-acid (20) (9.0 g) in 5% sodium hydrogen carbonate (600 ml) for 12 h; the sodium salt of (19) was precipitated as a gelatinous mass. Hydrochloric acid was added until the pH was 2.0 and after 1 h the mixture was filtered and the *solid* (19) was washed (7.0 g) (Found: C, 40.7; H, 4.2; N, 30.6. $C_8H_9N_5O_3,0.5H_2O$ requires C, 41.3; H, 4.3; N, 30.2%).

7,8-*Dihydro*-8-*methylpterin*-6-*carbonitrile* (16).—The tetrahydro-nitrile (15) (20 g) was dissolved in cold dimethylformamide and a slow stream of oxygen was passed into the solution for 12 h; yellow crystals of (16) were precipitated. The *solid* was washed with a little dimethylformamide and then ethanol (16.3 g) (Found: C, 45.5; H, 4.1; N, 39.3. $C_8H_8N_6O,0.5H_2O$ requires C, 45.1; H, 4.2; N, 39.4%).

Ethyl 7,8-*Dihydro*-8-*methylpterin*-6-*carboximidate* (17).—The nitrile (16) (4.0 g) was added to a solution of sodium ethoxide in ethanol (100 ml) [from sodium (3 g)]. It quickly dissolved and after a few minutes the sodium salt of (17) crystallised. The mixture was filtered and the solid was stirred with cold 0.1N-hydrochloric acid (400 ml) to obtain a clear orange solution. Addition of sodium hydrogen carbonate solution precipitated a yellow *solid* (17) which was collected and washed with water and then with ethanol; yield 2.7 g (Found: C, 44.4; H, 6.0; N, 31.3. $C_{10}H_{14}N_6O_2,H_2O$ requires C, 44.8; H, 6.0; N, 31.3%).

Ethyl 7,8-*Dihydro*-8-*methylpterin*-6-*carboxylate* (18).—The imino-ether (17) (0.1 g) was dissolved in 0.1N-hydrochloric acid and heated on a steam-bath. After a few minutes the yellow *ethyl ester* (18) crystallised (0.1 g) (Found: C, 47.4; H, 5.3; N, 28.0. $C_{10}H_{13}N_5O_3$ requires C, 47.8; H, 5.1; N, 27.9%).

5,6,7,8-*Tetrahydro*-8-*methylpterin*-6-*ylmethanol* (22).—To a stirred suspension of compound (19) (6 g) in dry tetrahydrofuran (300 ml) at 0—5 °C was added 1M-diborane (130 ml) in tetrahydrofuran during 30 min. After stirring in nitrogen for 24 h the mixture was allowed to settle and the clear supernatant tetrahydrofuran was decanted. Ice–water (200 ml) was added to the residue and the resulting solution was filtered. Hydrochloric acid (15 ml; 2N) was added and the solution was evaporated *in vacuo* below 40 °C. The solid was dissolved in methanol (150 ml) and the solution was again evaporated *in vacuo* to remove boric acid as methyl borate. The residue was dissolved in water (10 ml), butan-1-ol (100 ml) was added, and the mixture was evaporated *in vacuo* until crystallisation occurred. The product

was collected and washed with butanol and ether to obtain crystals (4.8 g) of the *hydrochloride* of (2). The compound is hygroscopic and must be stored in a desiccator (Found: C, 34.4; H, 5.8; N, 24.7. $C_8H_{13}N_5O_2,HCl,2H_2O$ requires C, 33.9; H, 6.0; N, 24.7%).

7,8-Dihydro-8-methylpterin-6-ylmethanol (23).—The tetrahydropterin (22) (1.0 g) was dissolved in a solution of sodium hydrogen carbonate (1 g) in water (15 ml) and a slow stream of oxygen was passed into the solution for 3 h. The yellow *precipitate* (0.7 g) was collected and recrystallised from hot water (10 ml) (Found: C, 41.2; H, 5.8; N, 29.7. $C_8H_{11}N_5O_2,1.25H_2O$ requires C, 41.5; H, 5.8; N, 30.2%).

5-Acetyl-5,6,7,8-tetrahydro-8-methylpterin-6-methyl Acetate (24; R = Me).—Acetic anhydride (1.0 ml) was added to a mixture of the hydrochloride of (22) (0.5 g), dimethylformamide (2.0 ml), and anhydrous sodium acetate (0.5 g). After 1 h, ice–water (5 ml) was added and the solution was evaporated *in vacuo* to a cream-coloured solid. Recrystallisation from boiling water (4 ml) gave *needles* (0.4 g), m.p. 182° (decomp.) (Found: C, 43.8; H, 6.0; N, 21.2. $C_{12}H_{17}N_5O_4,2H_2O$ requires C, 43.5; H, 6.0; N, 21.1%).

5-Acetyl-5,6,7,8-tetrahydro-8-methylpterin-6-ylmethanol (25; R = Me).—To an aqueous 40% solution of methylamine (12 ml) was added compound (24; R = Me) (1.0 g). After 5 min at room temperature a clear solution resulted and this was evaporated *in vacuo* to an oily solid. Methanol (5 ml) was added to obtain a clear solution from which (25; R = Me) soon crystallised as *needles* (0.5 g). The compound is very soluble in cold water. The i.r. spectrum no longer showed the ester carbonyl absorption of the starting material (Found: C, 47.1; H, 6.0; N, 27.3. $C_{10}H_{15}N_5O_3$ requires C, 47.4; H, 5.9; N, 27.6%).

5,6,7,8-Tetrahydro-6-hydroxymethyl-8-methylpterin-5-carbaldehyde (25; R = H).—The hydrochloride of (22) (0.4 g) was added to a mixture of formic acid (5 ml) and sodium formate (0.4 g). Acetic anhydride (2.0 ml) was added dropwise during 5 min. After 30 min at room temperature the solution was evaporated to dryness *in vacuo*. The solid was triturated with acetone, filtered off, and washed with more acetone. Recrystallisation from water (1.0 ml) gave the *ester* (24; R = H) (0.2 g), m.p. 265° (decomp.) (Found: C, 42.9; H, 4.9; N, 25.5. $C_{10}H_{13}N_5O_4,0.5H_2O$ requires C, 43.5; H, 5.1; N, 25.4%).

The ester (0.3 g) was dissolved in 0.5N-sodium hydroxide (4 ml). After 10 min N-hydrochloric acid (2 ml) was added to precipitate crystals of the product (25; R = H). Unlike the corresponding acetyl compound this was insoluble in water. It was purified by dissolving in 0.2N-sodium hydroxide and passing carbon dioxide into the solution to give pale yellow *crystals* (0.15 g). The i.r. spectrum no longer showed the ester carbonyl absorption (24) (Found: C, 44.5; H, 5.5; N, 28.9. $C_9H_{13}N_5O_3,0.25H_2O$ requires C, 44.5; H, 5.2; N, 28.8%).

9-Methylguanine 7-Oxide (26).—The hydrochloride of (7) (10 g) was made into a paste with water (20 ml), and 2N-sodium hydroxide (80 ml) was quickly added; a clear orange solution immediately resulted. After a few minutes the colourless sodium salt of (26) crystallised. This was filtered off and washed with 0.05N-sodium hydroxide and then with ethanol (7.3 g). A portion was recrystallised from hot 0.05N-sodium hydroxide (Found: C, 32.6; H, 4.3; N, 31.5. Calc. for $C_6H_7N_5NaO_2,H_2O$: C, 32.4; H, 4.0; N, 31.5%). Another portion was mixed with water, and dilute acetic acid was added until the aqueous pH was 6.0. The *product* (26) was filtered off and washed with ethanol. It was insoluble in water and organic solvents; m.p. >300° (Found: C, 38.2; H, 3.9; N, 37.0. $C_6H_7N_5O_2,0.5H_2O$ requires C, 37.9; H, 4.2; N, 36.8%).

9-Methylguanin-8(7H)-one (27).[8]—A mixture of compound (26) (1.5 g) and glacial acetic acid was heated on a steam-bath for 12 h. The solid was collected and dissolved in 0.2N-sodium hydroxide (20 ml). Carbon dioxide was passed into the solution to precipitate crystals of the product (27) (1.0 g) (Found: C, 35.9; H, 4.5; N, 35.1. Calc. for $C_6H_7N_5O_2,H_2O$: C, 36.2; H, 4.6; N, 35.2%).

The purine (27) was also prepared as follows. A mixture of 2,5-diamino-4-hydroxy-6-methylaminopyrimidine hydrochloride (1.0 g) and urea (2.0 g) was heated to 180 °C for 1 h. The mixture was cooled and ground to a powder, which was washed with water and then with ethanol. The product was dissolved in 0.2N-sodium hydroxide, the solution was treated with charcoal, and the purine was precipitated by passing in carbon dioxide. This purification treatment was repeated to obtain the product (27) (0.4 g). Perini and Tieckelmann [8] report the preparation of 9-methylguanin-8(7*H*)-one by a third method. The u.v. spectra of the compounds prepared by these three routes were identical.

Sodium 9-Methylguanine-8-sulphonate (28).—To a solution of the sodium salt of (26) (1 g) in water (70 ml), sodium dithionite (4 g) was added, and the mixture was heated on a steam-bath for 1 h. The mixture was filtered hot and on cooling deposited crystals of the sodium salt of (28). These were dissolved in 0.2N-sodium hydroxide and carbon dioxide was passed in to reprecipitate the *sodium salt* (0.6 g) (Found: C, 25.0; H, 2.2; N, 24.2; S, 11.3. $C_6H_6N_5NaO_4S,H_2O$ requires C, 25.2; H, 2.1; N, 24.5; S, 11.2%).

9-Methylguanine-8-carbonitrile (29).—Benzoyl chloride (1.5 g) and potassium cyanide (1.0 g) were added to a solution of the sodium salt (2 g) of (26) in water (120 ml). After stirring for 30 min the *precipitate* was collected and washed with water and then with ethanol (0.9 g). A portion was purified by dissolving in 0.1N-sodium hydroxide and precipitating with carbon dioxide (Found: C, 43.7; H, 3.0; N, 43.9. $C_7H_6N_6O$ requires C, 44.2; H, 3.2; N, 44.2%).

9-Methylguanine-8-carboxylic Acid (30).—A solution of compound (29) (1 g) in 2N-sodium hydroxide was heated on a steam-bath in an open flask until the volume was reduced to 5 ml (during 1 h); the disodium salt of (30) crystallised out. This was dissolved in water (100 ml) and 2N-hydrochloric acid (25 ml) was added to precipitate the acid (30) (0.9 g) (Found: C, 36.6; H, 3.9; N, 30.5. $C_7H_7N_5O_3,H_2O$ requires C, 37.0; H, 4.0; N, 30.8%).

9-Methylguanin-8-ylmethanol (31).—1M-Diborane in tetrahydrofuran (80 ml) was added to a stirred suspension of compound (30) (4 g) in tetrahydrofuran (400 ml) during 15 min. The mixture was stirred in nitrogen for 3 days, then cooled in an ice-bath, and methanol (100 ml) was added, followed by 2N-hydrochloric acid (10 ml). The mixture was evaporated *in vacuo* to a solid. More methanol (100 ml) was added and the mixture was again evaporated to remove boric acid as methyl borate. The residue was dissolved in water and the solution was treated with charcoal. Saturated aqueous sodium hydrogen carbonate was added until the pH was 3.0—4.0 to precipitate the *product* (31) (0.5 g). A portion (0.2 g) was recrystallised from boiling water (20 ml); yield 0.13 (Found: C, 41.1; H, 4.4; N, 34.7. $C_7H_9N_5O_2,0.5H_2O$ requires C, 41.2; H, 4.9; N, 34.3%).

2-Amino-6-mercapto-9-methylpurine-8-carbonitrile (33).—A mixture of compound (29) (10 g), phosphoryl chloride (450 ml), *NN*-diethylaniline (10 g), and water (1 ml) was

refluxed for 1 h. The solution was evaporated *in vacuo* to a red oil which was poured onto ice. The resulting solid (32) was washed with water and dried in a desiccator (yield 4.0 g).

A mixture of the crude chloropurine (32) (8.0 g), thiourea (8.0 g), and ethanol (500 ml) was refluxed for 2 h. The mixture was filtered and the solid was washed with water and then ethanol. The product was suspended in water (300 ml) and sufficient 2N-sodium hydroxide was added to effect dissolution. The solution was treated with charcoal and carbon dioxide was passed in to precipitate the *product* (33) (4.0 g). This purification procedure was repeated (Found: C, 38.0; H, 3.4; N, 38.1; S, 14.3. $C_7H_6N_6S,0.75H_2O$ requires C, 38.3; H, 3.4; N, 38.3; S, 14.6%).

[6/1869 *Received, 6th October*, 1976]