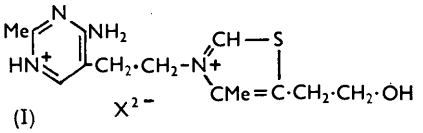
367. *Two Isomeric Homologues of Thiamine.*

By J. Biggs and P. Sykes.

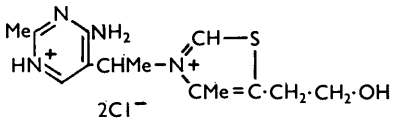
The synthesis of two isomeric homologues of thiamine is described, and their behaviour with alkali, potassium ferricyanide, and deuterium oxide and as catalysts for the conversion of pyruvate + acetaldehyde into acetoin + carbon dioxide is reported.

In a study of the behaviour of quaternary thiazolium compounds as catalysts for the conversion of pyruvate + acetaldehyde into acetoin + CO_2,[1] compounds having desirable features were the isomeric thiamine homologues (I; X = $2Cl^-$) and (II).

The essential intermediate for our synthesis of compound (I) was the amine (III; R = NH_2, R′ = $CH_2{\cdot}NH_2$). This is mentioned in a patent [2] but we were unable to repeat the reactions there outlined. We failed also in attempts to obtain the amine from readily available thiamine derivatives, *e.g.*, through the cyanide (III; R = NH_2, R′ = CN) [obtainable by dehydration of the amide (III; R = NH_2, R′ = $CO{\cdot}NH_2$) or from the bromide (III; R = NH_2, R′ = Br) and potassium cyanide], and by reduction of the amide (III; R = NH_2, R′ = $CO{\cdot}NH_2$).



(I)



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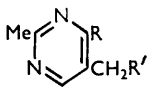

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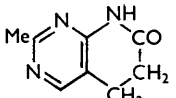

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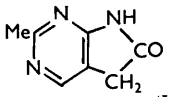


(V)

Condensation of acetamidine with dimethyl α-formylglutarate followed by cyclisation with acid yielded the hydroxy-ester (III; R = OH, R′ = $CH_2{\cdot}CO_2Me$), which with phosphorus oxychloride gave the chloro-ester (III; R = Cl, R′ = $CH_2{\cdot}CO_2Me$); but with methanolic ammonia at 110°, this chloro-ester was converted, not into the expected amino-amide (III; R = NH_2, R′ = $CH_2{\cdot}CO{\cdot}NH_2$), but into the cyclic lactam (IV). To ensure that this cyclisation was not due to over-vigorous reaction conditions, the chloro-ester was stirred with aqueous ammonia at room temperature, but only the amino-acid (III; R = NH_2, R′ = $CH_2{\cdot}CO_2H$) could be obtained. No attempt was made to prepare the desired amide by esterification and ammonolysis of this amino-acid, in the light of our previous experience of the spontaneous cyclisation of the esters of other pyrimidine amino-acids.[3]

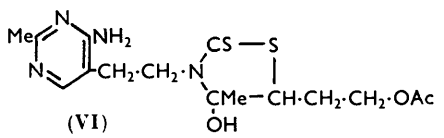
[1] Downes and Sykes, *Chem. and Ind.*, 1957, 1095.
[2] U.S.P. 2,377,395.
[3] Nesbitt and Sykes, *J.*, 1954, 3057.

Refluxing the lactam (IV) with 100% hydrazine hydrate, however, led to the hydrazide (III; R = NH_2, R′ = CH_2·CO·NH·NH_2). An acid solution of this compound was then treated with sodium nitrite, and the solution containing the azide (III; R = NH_2, R′ = CH_2·CO·N_3) heated until no more nitrogen was evolved, but basification followed by freeze-drying and vacuum-sublimation gave the lactam (IV) as the only product. This acylation of the 4-amino-group of the pyrimidine nucleus by the azide first formed was somewhat surprising in the face of the weakly basic nature of this amino-group. However, it appears to be a general reaction of suitable 4-aminopyrimidines, for on similar treatment of 4-amino-2-methyl-5-pyrimidylacethydrazide [3] (III; R = NH_2, R′ = CO·NH·NH_2), a lactam (V) with a 5-membered ring was obtained, though the corresponding 4-hydroxy-compound (III; R = OH, R′ = CO·NH·NH_2) yields the expected amine (III; R = OH, R′ = NH_2) in the normal way.[4]

If, however, the treatment with sodium nitrite was carried out in the presence of a large excess of acid, protonation of the amino-pyrimidine system protected the 4-amino-group, and the desired amine (III; R = NH_2, R′ = CH_2·NH_2) was then obtained. Treating [5] the amine with carbon disulphide, ammonia, and 5-acetoxy-3-chloropentan-2-one yielded the expected [6] 4-hydroxythiazolid-2-thione (VI) which underwent acid-catalysed dehydration to the thiazolin-2-thione (VII).

Hydrogen peroxide oxidised the thiazoline (VII) to the thiazolium sulphate (I; X = SO_4^{2-}), which with barium chloride yielded the desired chloride hydrochloride (I; X = $2Cl^-$).

In the synthesis of the second analogue an amine (VIII; R′ = NH_2) was needed. Condensing acetamidine with dimethyl α-formyl-β-methylsuccinate yielded the hydroxy-ester (VIII; R = OH, R′ = CO_2Me), a reaction extremely sensitive to the amount of sodium ethoxide used as catalyst and to the time of heating; diethyl α-formyl-β-methyl-succinate was rather more sensitive than the corresponding dimethyl ester which also



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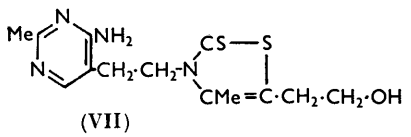

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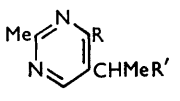

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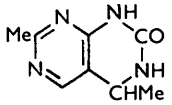

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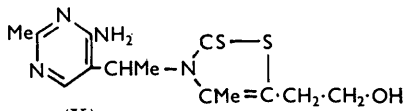

(X)

condensed in better yield. The hydrochloride of the 4-hydroxypyrimidine methyl ester was, however, extremely susceptible to ester exchange so it is readily converted into the corresponding ethyl ester if desired. The latter was then converted into the chloro-compound (VIII; R = Cl, R′ = CO_2Et), which with methanolic ammonia, in contrast to the previous series, yielded the expected amide (VIII; R = NH_2, R′ = CO·NH_2). But this, on reaction with sodium hypobromite by the method of Hoogewerff and van Dorp,[7] was converted, not into the expected amine, but into the ring compound (IX) by attack of the intermediate *iso*cyanate (VIII; R = NH_2, R′ = NCO) on the pyrimidine 4-amino-group, the yield being almost quantitative. This reaction was most unexpected as Gravin [8] was able to convert 4-amino-2-methyl-5-pyrimidylacetamide (III; R = NH_2, R′ = CO·NH_2) into the corresponding amine (III; R = R′ = NH_2) in reasonable yield. The cyclic urea (IX) could not be hydrolysed to the desired amine.

[4] Cerecedo and Pickel, *J. Amer. Chem. Soc.*, 1937, **59**, 1714.
[5] Sykes and Todd, *J.*, 1951, 534.
[6] Sykes, *J.*, 1955, 2390.
[7] Hoogewerff and van Dorp, *Rec. Trav. chim.*, 1887, **6**, 373.
[8] Gravin, *J. Appl. Chem.* (*U.S.S.R.*), 1943, **16**, 105.

The amide was, therefore, converted into the hydrazide (VIII; R = NH_2, R′ = $CO{\cdot}NH{\cdot}NH_2$) but on treatment with sodium nitrite in highly acid solution this yielded, not, as in the previous series, the desired amine, but the cyclic urea (IX). Reaction of the hydrazide with hydrogen chloride and pentyl nitrite in dry ethanol, however, yielded the ethylurethane (VIII; R = NH_2, R′ = $NH{\cdot}CO_2Et$). This has the nitrogen atom in the desired position but unfortunately proved extremely resistant to hydrolysis with acid or alkali and it was impossible to remove the ethoxycarbonyl group without further decomposition. The corresponding benzylurethane (VIII; R = NH_2, R′ = $NH{\cdot}CO_2{\cdot}CH_2Ph$) was therefore prepared; this was smoothly hydrogenolysed to the desired amine (VIII; R = R′ = NH_2). This most satisfactory overall reaction from hydrazide to amine was equally successful in the synthesis of the first homologue.

Treatment of the amine (VIII; R = R′ = NH_2) with carbon disulphide and 5-acetoxy-3-chloropentan-2-one, followed by acid-catalysed dehydration, yielded the thiazolin-2-thione (X) and thence as above the thiamine homologue (II).

Adding alkali to an aqueous solution of a salt (I) does not give a yellow colour, as thiamine does, nor does adding potassium ferricyanide to this solution produce thiochrome-like fluorescence. By contrast, the analogue (II) did give a yellow colour with alkali, and ferricyanide oxidation then produced a bright blue fluorescence. These observations are in accord with our views on the action of oxidising agents on thiamine itself.[5,9]

On treatment with one equivalent of alkali (to convert them into the quaternary chlorides) in deuterium oxide both homologues exchanged the hydrogen atom at $C_{(2)}$ of the thiazolium nucleus for deuterium virtually instantaneously; the exchange was followed and confirmed by nuclear magnetic resonance and infrared spectral measurements.[10] The speed of exchange was identical with that of thiamine under similar conditions.[11] Both the homologues acted as catalysts in the non-enzymic conversion, pyruvate + acetaldehyde ⟶ acetoin + CO_2,[12,13] compound (I) showing 67%, and compound (II) 5%, of the activity of thiamine under similar conditions. Neither homologue showed vitamin activity in a microbiological test with *Kloeckera brevis*.

Experimental

Dimethyl α-Formylglutarate.—Dimethyl glutarate (100·5 g.), ethyl formate (90 g., 1·92 mol.), sodium wire (24·8 g., 1·72 g.-atom), and dry ether (500 ml.) were heated under reflux for 4 hr., then kept overnight. All the sodium had then dissolved and a viscous red sodio-derivative separated. The ether layer was decanted, washed with water (50 ml.), dried (Na_2SO_4), and distilled under reduced pressure, yielding dimethyl glutarate (44·9 g.). The red sodio-derivative was dissolved in water, the aqueous washings of the ethereal layer were added, and the solution was acidified with dilute sulphuric acid and extracted repeatedly with ether. The ethereal extract was dried (Na_2SO_4) and distilled, yielding 46·4 g. (40%) of the formyl ester, b. p. 100—101°/0·1 mm. Formylation of diethyl glutarate yielded *diethyl α-formylglutarate* (36%), b. p. 129°/3 mm. (Found: C, 55·2; H, 7·1. $C_{10}H_{16}O_5$ requires C, 55·4; H, 7·4%).

Methyl β-(4-Hydroxy-2-methyl-5-pyrimidyl)propionate (III; R = OH, R′ = $CH_2{\cdot}CO_2Me$).—Acetamidine hydrochloride (36·6 g.) was added to a solution of sodium (8·8 g., 1 g.-atom) in ethanol (900 ml.), and the precipitated sodium chloride removed on a centrifuge. Dimethyl α-formylglutarate (72·2 g., 1 mol.) was added to the filtrate and the solution heated under reflux for 3 hr., cooled, treated with dry hydrogen chloride until no more sodium chloride separated, filtered (" Hyflo Supercel "), and further treated with hydrogen chloride until crystals began to separate (further material was obtained by saturating the original mother-liquors with hydrogen chloride and storing the solution). Recrystallising the *hydroxy-ester hydrochloride* from ethanol gave 41·7 g. (47%) of colourless needles, m. p. 223° (Found: C, 46·4; H, 5·6; N, 12·1. $C_9H_{13}O_3N_2Cl$ requires C, 46·5; H, 5·7; N, 12·1%).

[9] Nesbitt and Sykes, *J.*, 1954, 4585.
[10] Breslow, *J. Amer. Chem. Soc.*, 1957, **79**, 1762.
[11] Sykes and Downes, unpublished work.
[12] Mizuhara, Tamura, and Arata, *Proc. Japan Acad.*, 1951, **27**, 302.
[13] Mizuhara and Handler, *J. Amer. Chem. Soc.*, 1954, **76**, 571.

Treating an aqueous solution of the hydrochloride with sodium hydrogen carbonate, followed by freeze-drying and sublimation at 110°/10^{-4} mm., yields the free *base*, m. p. 109° (Found: C, 55·1; H, 6·4; N, 14·4. $C_9H_{12}O_3N_2$ requires C, 55·1; H, 6·2; N, 14·3%).

Similar treatment of diethyl α-formylglutarate yields *ethyl* β-(*4-hydroxy-2-methyl-5-pyrimidyl*)-*propionate hydrochloride*, m. p. 221—222° (Found: C, 48·7; H, 6·1; N, 11·6. $C_{10}H_{15}O_3N_2Cl$ requires C, 48·7; H, 6·1; N, 11·4%), which is converted by alkali into the *base*, m. p. 113° (Found: C, 56·9; H, 7·0; N, 13·6. $C_{10}H_{14}O_3N_2$ requires C, 57·2; H, 6·7; N, 13·3%).

Methyl β-(*4-Chloro-2-methyl-5-pyrimidyl*)*propionate* (III; R = Cl, R′ = $CH_2{\cdot}CO_2Me$).—Methyl 4-hydroxy-2-methyl-5-pyrimidylpropionate (12 g.) was powdered and added to redistilled phosphorus oxychloride (200 ml.), and the mixture was heated under reflux. All solid dissolved in 10 min. and heating was continued for a further 30 min. The excess of phosphorus oxychloride was then removed under reduced pressure, and the flask then rotated so that the product spread as a thin layer over its surface. The flask was then cooled in ice-water, and the viscous product dissolved in ice-water, brought to pH 8, and extracted repeatedly with ether. The extract was dried (Na_2SO_4), the solvent removed, and the residue distilled, yielding the *chloro-ester* (8·7 g., 78%), b. p. 134°/2·4 mm. (Found: C, 50·6; H, 5·3; N, 13·3. $C_9H_{11}O_2N_2Cl$ requires C, 50·4; H, 5·2; N, 13·1%). With phosphorus oxychloride the ethyl hydroxy-ester yielded the ethyl chloro-ester (68%), b. p. 105—108°/0·4 mm.

The oily methyl chloro-ester with hot 100% hydrazine hydrate yielded needles of the *hydrazide* (III; R = $NH{\cdot}NH_2$, R′ = $CH_2{\cdot}CO{\cdot}NH{\cdot}NH_2$), m. p. 182° (Found: C, 46·0; H, 6·9; N, 40·0. $C_8H_{14}ON_6$ requires C, 45·7; H, 6·7; N, 40·0%).

Action of Ammonia on Methyl β-(*4-Chloro-2-methyl-5-pyrimidyl*)*propionate*.—(*a*) The above chloro-ester (0·6 g.) was stirred with ammonia solution (*d* 0·880; 150 ml.) until dissolved (4 hr.). The solution was left overnight, then concentrated under reduced pressure to 5 ml. Ethanol (20 ml.) was added, and the solution cooled in ice; crystals separated which were collected and sublimed at 190°/10^{-4} mm., to yield β-(*4-amino-2-methyl-5-pyrimidyl*)*propionic acid* (III; R = NH_2, R′ = $CH_2{\cdot}CO_2H$), m. p. 233° (Found: C, 52·8; H, 6·1; N, 23·4. $C_8H_{11}O_2N_3$ requires C, 52·7; H, 6·1; N, 23·1%).

(*b*) The chloro-ester (4·2 g.) was heated in an autoclave with saturated methanolic ammonia (70 ml.) at 90—100° for 7 hr. The solution, from which some solid had separated, was evaporated to dryness under reduced pressure and the residue recrystallised (charcoal) from ethanol containing a little water, to yield 5 : 6 : 7 : 8-*tetrahydro-2-methyl-7-oxo*-1 : 3 : 8-*triazanaphthalene* (IV) (1·83 g., 57%) as colourless plates, m. p. 256° (Found: C, 58·9; H, 5·3; N, 26·0. $C_8H_9ON_3$ requires C, 58·9; H, 5·5; N, 25·8%).

4-*Amino-2-methyl-5-pyrimidylpropionhydrazide* (III; R = NH_2, R′ = $CH_2{\cdot}CO{\cdot}NH{\cdot}NH_2$).—The cyclic compound (IV) (0·88 g.) was heated under reflux in 100% hydrazine hydrate (20 ml.) for 45 min. On cooling, a mass of crystals separated. Recrystallisation from dry ethanol affords the *hydrazide* (0·84 g., 80%) as colourless needles, m. p. 201° (Found: C, 49·1; H, 6·5; N, 36·1. $C_8H_{13}ON_5$ requires C, 49·2; H, 6·7; N, 35·8%).

4-*Amino-5-2′-aminoethyl-2-methylpyrimidine* (III; R = NH_2, R′ = $CH_2{\cdot}NH_2$).—The preceding hydrazide (0·20 g.) was dissolved in 3N-hydrochloric acid (4·5 ml., 12 mol.), then cooled to −2°, and a solution of sodium nitrite (0·075 g., 1·06 mol.) slowly added with stirring. After a further 15 min. the solution was warmed to 60° (nitrogen was evolved), brought to pH 8·9 and freeze-dried. Three sublimations at 120°/10^{-4} mm. yielded the *amine* (0·064 g., 41%), m. p. 101° (Found: C, 55·0; H, 7·8; N, 36·5. $C_7H_{12}N_4$ requires C, 55·3; H, 8·0; N, 36·8%).

The action of nitrous acid on the hydrazide under a wide variety of other conditions yielded varying quantities of the cyclic lactam (IV), but no amine on sublimation of the freeze-dried reaction mixtures.

5-*2′-Acetoxyethyl*-3-*2′*-(*4-amino-2-methyl-5-pyrimidyl*)*ethyl*-4-*hydroxy*-4-*methylthiazolid*-2-*thione* (VI).—The hydrazide (1·20 g.) was treated with nitrous acid as above, and the solution made alkaline, evaporated to dryness under reduced pressure and extracted with ethanol (4 × 20 ml.). The ethanol extract was concentrated (3 ml.) and to it were added water (1·5 ml.), ammonia solution (*d* 0·880; 0·26 g.), and carbon disulphide (0·30 g.). The mixture was shaken until all the carbon disulphide had dissolved, 5-acetoxy-3-chloropentan-2-one (0·54 g.) was then added, and the whole shaken for 1 hr. and left overnight. Solid separated which on recrystallisation from aqueous ethanol yielded the thiazolid-2-thione (0·172 g., 7·5% calc. on hydrazide), m. p. 258°.

3-*2′*-(*4-Amino-2-methyl-5-pyrimidyl*)*ethyl*-5-*2′-hydroxyethyl*-4-*methylthiazolin*-2-*thione* (VII).—

The above thiazolid-2-thione (0·3 g.) was dissolved in 3N-hydrochloric acid (3 ml.) and heated just to the b. p., then cooled and made just alkaline. Recrystallisation of the separated material from methanol yielded the *thiazolin-2-thione* (0·1 g., 34%), m. p. 230° (Found: C, 50·7; H, 5·9; N, 18·3. $C_{13}H_{18}ON_4S_2$ requires C, 50·3; H, 5·8; N, 18·1%).

3-2′-(4-*Amino*-2-*methyl*-5-*pyrimidyl*)*ethyl*-5-2′-*hydroxyethyl*-4-*methylthiazolium Sulphate* (I; X = SO_4^{2-}).—The above thiazolin-2-thione (0·071 g.) was suspended in water (1·4 ml.), 30% hydrogen peroxide (0·081 g., 3 mol.) added, and the mixture left with occasional shaking until all the solid had dissolved (2 hr.). It was then concentrated (0·5 ml.); ethanol (5 ml.) and acetone (20 ml.) were added. Crystals separated at 0° which were collected and washed with acetone. Recrystallisation from water–ethanol–acetone yielded the *sulphate* (I; X = SO_4^{2-}) (0·075 g., 87%) as colourless needles, m. p. 221° (decomp.) (Found: C, 37·7; H, 6·3; N, 13·9. $C_{13}H_{20}O_5N_4S_2,2H_2O$ requires C, 37·8; H, 5·9; N, 13·6%). Treatment of the aqueous solution with aqueous picric acid, followed by recrystallisation of the product from ethanol, yielded the *picrate*, m. p. 161° (decomp.) (Found: C, 40·7; H, 3·3; N, 19·3. $C_{25}H_{24}O_{15}N_{10}S$ requires C, 40·7; H, 3·3; N, 19·0%). Treating the aqueous solution of the sulphate with barium chloride solution, removing the barium sulphate on a centrifuge, concentrating the solution, and treating it as above yielded the *chloride hydrochloride* (I; X = $2Cl^-$) as needles, m. p. 257° (decomp.) (Found: C, 42·1; H, 6·1; N, 15·0. $C_{13}H_{20}ON_4Cl_2S,H_2O$ requires C, 42·3; H, 6·3; N, 15·2%).

Dimethyl α-*Formyl*-β-*methylsuccinate*.—Dimethyl α-methylsuccinate (189·5 g.), ethyl formate (dried over K_2CO_3; 175 g., 2 mol.), sodium wire (27·2 g., 1 g.-atom), and dry ether (1420 ml.) were left at room temperature for 24 hr., then worked up as described for dimethyl α-formylglutarate. Dimethyl α-methylsuccinate (98·8 g.) was recovered, and the formyl ester (77·2 g., 34%) distilled (b. p. 100°/1 mm.). Similar formylation of diethyl α-methylsuccinate yielded the corresponding diethyl formyl ester [14] (53%), b. p. 114°/0·3 mm.

Methyl α-(4-*Hydroxy*-2-*methyl*-5-*pyrimidyl*)*propionate* (VIII; R = OH, R′ = CO_2Me).—Acetamidine hydrochloride (65 g., 1·3 mol.) was stirred with a solution of sodium (16·5 g., 1·35 g.-atom) in ethanol (400 ml.) for 15 min., and the separated sodium chloride removed on a centrifuge. Dimethyl α-formyl-β-methylsuccinate (99·2 g., 1 mol.) was added to the filtrate, and the deep red solution heated under reflux for 2 hr. The cooled solution was then treated with dry hydrogen chloride, the trace of separated sodium chloride removed, and the volume reduced to 200 ml. On cooling to 0°, crystals separated. More product was obtained by further concentration until acetamidine hydrochloride (m. p. 167°) began to be deposited. Recrystallisation from ethanol yielded the *hydroxy-ester hydrochloride* (61·7 g., 51%) as colourless needles, m. p. 205° (Found: C, 46·9; H, 5·7; N, 12·0. $C_9H_{13}O_3N_2Cl$ requires C, 46·5; H, 5·7; N, 12·1%). Similar treatment of diethyl α-formyl-β-methylsuccinate yields *ethyl* α-(4-*hydroxy*-2-*methyl*-5-*pyrimidyl*)*propionate hydrochloride* (37%), m. p. 211° (Found: C, 48·4; H, 5·9; N, 11·2. $C_{10}H_{15}O_3N_2Cl$ requires C, 48·7; H, 6·1; N, 11·4%), and the corresponding free *base*, m. p. 124° (Found: C, 57·0; H, 6·9; N, 13·4. $C_{10}H_{14}O_3N_2$ requires C, 57·1; H, 6·7; N, 13·3%).

Ethyl α-(4-*Chloro*-2-*methyl*-5-*pyrimidyl*)*propionate* (VIII; R = Cl, R′ = CO_2Et).—Powdered ethyl α-(4-hydroxy-2-methyl-5-pyrimidyl)propionate hydrochloride (27 g.) was heated with redistilled phosphorus oxychloride (250 ml.) for 20 min.; this yielded the *chloro-ester* (14·6 g., 59%), b. p. 99—100°/0·05 mm. (Found: C, 52·8; H, 6·0; N, 11·9. $C_{10}H_{13}O_2N_2Cl$ requires C, 52·6; H, 5·7; N, 12·2%).

α-(4-*Amino*-2-*methyl*-5-*pyrimidyl*)*propionamide* (VIII; R = NH_2, R′ = CO·NH_2).—The above chloro-ester (18 g.) was heated in an autoclave with saturated methanolic ammonia (140 ml.) at 110—120° for 10 hr. The mixture of solid and red solution was evaporated to dryness and the residue recrystallised from water (charcoal), to yield the *amino-amide* (9·2 g., 61%) as colourless needles, m. p. 231° (Found: C, 53·2; H, 6·2; N, 30·9. $C_8H_{12}ON_4$ requires C, 53·3; H, 6·6; N, 31·1%).

Action of Hypobromite on α-(4-*Amino*-2-*methyl*-5-*pyrimidyl*)*propionamide*.—The amide (0·592 g.) was dissolved in hot water (25 ml.), and the solution cooled to 0° and treated with sodium hypobromite (1·1 mol.) made from sodium hydroxide (0·78 g.) in water (4·5 ml.) to which bromine (0·19 ml.) was added. After 10 min. the temperature was raised to 80° and maintained for a further 20 min. Dilute sulphuric acid was then added to the cooled solution until the pH was 7. Crystals separated which, on recrystallisation from aqueous methanol, yielded 1 : 2 : 3 : 4-*tetrahydro*-4 : 7-*dimethyl*-2-*oxo*-1 : 3 : 6 : 8-*tetra-azanaphthalene* (IX) (0·58 g.,

[14] Fichter and Rudin, *Ber.*, 1904, **37**, 1611.

99%) as colourless prisms, m. p. 312° (decomp.) (Found: C, 53·9; H, 5·6; N, 31·4. $C_8H_{10}ON_4$ requires C, 53·8; H, 5·6; N, 31·4%).

α-(4-*Amino*-2-*methyl*-5-*pyrimidyl*)*propionhydrazide* (VIII; R = NH_2, R′ = $CO{\cdot}NH{\cdot}NH_2$).—The amide (1·03 g.) was heated in 100% hydrazine hydrate (7 ml.) under reflux for 1·5 hr.; on cooling, a mass of crystals separated. Recrystallisation from ethanol affords the *hydrazide* (1·01 g., 90%) as colourless needles, m. p. 263° (Found: C, 48·9; H, 7·0; N, 35·5. $C_8H_{13}ON_5$ requires C, 49·2; H, 6·7; N, 35·8%).

Action of Nitrous Acid on α-(4-*Amino*-2-*methyl*-5-*pyrimidyl*)*propionhydrazide*.—The hydrazide (0·216 g.) in 3N-hydrochloric acid (4·5 ml., 12 mol.) was treated at −2° with a solution of sodium nitrite (0·113 g., 1·06 mol. dropwise with continual shaking. After 15 min., the temperature was raised to 60° and maintained until no more nitrogen was evolved. Sodium hydrogen carbonate was added to give pH 8·3, causing the separation of solid, m. p. and mixed m. p. 312° (decomp.) with the cyclic urea (IX).

Action of Nitrous Acid on 4-*Amino*-2-*methyl*-5-*pyrimidylacethydrazide* [3] (III; R = NH_2, R′ = $CO{\cdot}NH{\cdot}NH_2$).—Similar treatment of this hydrazide, followed by freeze-drying of the basified solution and sublimation at 140°/10^{-3} mm. yielded 6-*methyl*-1 : 5 : 7-*triazaindan*-2-*one* (V), m. p. 220° (decomp.) (Found: C, 56·0; H, 5·2; N, 28·0. $C_7H_7ON_3$ requires C, 56·4; H, 4·8; N, 28·2%).

4-*Amino*-5-1′-*ethoxycarbonylaminoethyl*-2-*methylpyrimidine* (VIII; R = NH_2, R′ = $NH{\cdot}CO_2Et$).—A solution of the hydrazide (1·43 g.) in dry ethanol (190 ml.) containing dry hydrogen chloride (0·70 g., 2·2 mol.) and pentyl nitrite (1·24 g., 1·42 mol.; freshly distilled) was heated at 60° for 4 hr. The solvent was then removed, the residue dissolved in water (5 ml.), and the solution made just alkaline with sodium hydrogen carbonate. Solid separated which on recrystallisation from water (charcoal) yielded the *ethylurethane* (1·05 g., 64%) as colourless needles, m. p. 192° (Found: C, 53·7; H, 7·4; N, 24·9. $C_{10}H_{16}O_2N_4$ requires C, 53·6; H, 7·2; N, 25·0%).

4-*Amino*-5-1′-*benzyloxycarbonylaminoethyl*-2-*methylpyrimidine* (VIII; R = NH_2, R′ = $NH{\cdot}CO_2{\cdot}CH_2Ph$).—A solution of the hydrazide (0·60 g.) in benzyl alcohol (45 ml.; freshly distilled) containing dry hydrogen chloride (0·38 g., 2·2 mol.) and pentyl nitrite (0·43 g., 1·1 mol.; freshly distilled) was heated at 60° for 3 hr., the solution becoming yellow. The solvent was removed under reduced pressure and the oily residue dissolved in a mixture of ether and water. The aqueous layer was separated and extracted twice more with ether to remove benzyl alcohol. When the aqueous solution was made alkaline, the *benzylurethane* separated. Recrystallisation from water yielded colourless needles (0·45 g., 54%), m. p. 174° (Found: C, 62·8; H, 6·3; N, 19·6. $C_{15}H_{18}O_2N_4$ requires C, 62·9; H, 6·3; N, 19·6%).

4-*Amino*-5-2′-*benzyloxycarbonylaminoethyl*-2-*methylpyrimidine* (III; R = NH_2, R′ = $CH_2{\cdot}NH{\cdot}CO_2{\cdot}CH_2Ph$).—Similar treatment of 4-amino-2-methyl-5-pyrimidylpropionhydrazide (III; R = NH_2, R′ = $CH_2{\cdot}CO{\cdot}NH{\cdot}NH_2$) yielded the corresponding *benzylurethane* (65%) as needles, m. p. 142° (Found: C, 62·8; H, 6·1; N, 19·8. $C_{15}H_{18}O_2N_4$ requires C, 62·9; H, 6·3; N, 19·6%).

4-*Amino*-5-1′-*aminoethyl*-2-*methylpyrimidine* (VIII; R = R′ = NH_2).—A solution of the benzylurethane (0·225 g.) in ethanol (40 ml.) containing palladium black (0·07 g.) was stirred vigorously while hydrogen (carbon dioxide-free) was passed through until no more carbon dioxide was evolved (3 hr.). The solvent was removed under reduced pressure. The oily residue gradually crystallised. Sublimation (twice) at 100°/10^{-4} mm. yielded the *amine*, m. p. 103° (Found: C, 55·2; H, 8·1. $C_7H_{12}N_4$ requires C, 55·3; H, 8·0%).

The amine was characterised by reaction in aqueous solution with sodium dithioformate, the separated material being recrystallised from water to yield the *thioformyl derivative* (53%) as colourless prisms, m. p. 200° (decomp.) (Found: C, 49·3; H, 6·1; N, 28·5. $C_8H_{12}N_4S$ requires C, 49·0; H, 6·2; N, 28·6%).

4-*Amino*-5-2′-*thioformylaminoethyl*-2-*methylpyrimidine* (III; R = NH_2, R′ = $CH_2{\cdot}NH{\cdot}CHS$).—Similar hydrogenolysis of 4-amino-5-2′-benzyloxycarbonylaminoethyl-2-methylpyrimidine (III; R = NH_2, R′ = $CH_2{\cdot}NH{\cdot}CO_2{\cdot}CH_2Ph$) yielded the corresponding amine, characterised as its *thioformyl derivative* (56%), buff-coloured prisms, m. p. 197° (decomp.) (from water) (Found: C, 49·3; H, 6·3; N, 28·5. $C_8H_{12}N_4S$ requires C, 49·0; H, 6·2; N, 28·6%).

3-1′-(4-*Amino*-2-*methyl*-5-*pyrimidyl*)*ethyl*-5-2′-*hydroxy*)*ethyl*-4-*methylthiazolin*-2-*thione* (X).—The benzylurethane (4·35 g.) was hydrogenolysed as above, and the final aqueous solution evaporated to dryness. The residue was dissolved in water (4 ml.) and ethanol (10 ml.), ammonia

solution (*d* 0·880; 1·28 g.) and carbon disulphide (1·44 g.) then being added. The mixture was shaken until all the carbon disulphide had dissolved, 5-acetoxy-3-chloropentan-2-one (2·7 g.) was then added, and the mixture shaken vigorously for 3 hr. Next morning the mixture was evaporated to dryness under reduced pressure, and the residue taken up in 3N-hydrochloric acid (10 ml.), heated just to the b. p., allowed to cool slowly, and made just alkaline. Solid separated which on recrystallisation from aqueous methanol yielded the *thiazolin-2-thione* (2·23 g., 47%) as colourless needles, m. p. 230° (Found: C, 50·1; H, 6·0; N, 18·0. $C_{13}H_{18}ON_4S_2$ requires C, 50·3; H, 5·8; N, 18·1%).

3-1′-(4-*Amino*-2-*methyl*-5-*pyrimidyl*)*ethyl*-5-2′-*hydroxyethyl*-4-*methylthiazolium Chloride Hydrochloride* (II).—The thiazolin-2-thione (0·95 g.), water (30 ml.), and 24% hydrogen peroxide solution (1·26 g., 3 mol.) were stirred until the solid had dissolved (3 hr.), then treated with barium chloride solution until no more solid precipitated. The barium sulphate was removed on a centrifuge, and the solution evaporated to dryness. The residue was dissolved in methanol (20 ml.), and ether (30 ml.) added. The quaternary *chloride hydrochloride* (0·76 g., 70%) separated as needles, m. p. 177° (decomp.) (Found: C, 42·6; H, 6·2; N, 15·2. $C_{13}H_{20}ON_4Cl_2,H_2O$ requires C, 42·3; H, 6·3; N, 15·2%). Treatment of the aqueous solution with aqueous picric acid yields the *picrate* as yellow needles, m. p. 170° (decomp.) (Found: C, 40·7; H, 3·5; N, 18·9. $C_{25}H_{24}O_{15}N_{10}S$ requires C, 40·7; H, 3·3; N, 19·0%).

Exchange in Deuterium Oxide Solution.—Nuclear magnetic resonance spectra. In the hydrogen nuclear magnetic resonance spectra of a solution of thiamine monohydrate (0·05 g.) in water (0·5 ml.) a sharp peak was observed at 192 cycles/sec. to the lower field side of the resonance of the hydrogen nuclei in the solvent water. This peak was unequivocally assigned to the 2-hydrogen atom in the thiazole ring by comparison with spectra of a series of thiazolium salts, *e.g.*, 3-benzyl-4-methylthiazolium chloride [15] and oxythiamine [16] which give peaks at 203 and 197 cycles/sec. respectively. Thiamine monohydrate (0·25 g.) was dissolved in water (0·25 ml.) and treated with N-sodium hydroxide (0·70 ml., 1 mol.), and the solution was freeze-dried. The dried product was dissolved in deuterium oxide, and the hydrogen nuclear magnetic resonance spectrum examined immediately: the peak at 192 cycles/sec. was no longer detectable.

Similarly, solutions of the homologues (I) and (II) in water showed sharp peaks at 197 and 212 cycles/sec. respectively. After treatment with deuterium oxide, neither of these peaks was detectable.

Measurements were made with a Varian V-4300B high resolution spectrometer at 40 megacycles/sec. For comparison with Breslow's results [10] it should be noted that the resonance of benzene in an external capillary with respect to water occurs at 74 cycles/sec. to the lower field side of the water resonance. Further, because of the different frequency used his frequency separations have to be multiplied by a factor of $\frac{4}{3}$ for comparison with ours, *i.e.*, his quoted resonance frequency for the 2-hydrogen atom 3 : 4-dimethylthiazolium bromide of 108 cycles/sec. would occur at approximately 218 cycles/sec. on our scale.

Infrared spectra. 4-Methyl-3-phenethylthiazolium bromide,[11] when kept in deuterium oxide at room temperature for 3 days, then dried in a vacuum, shows a strong C–D stretching band [10] (in Nujol) at 2275 cm.$^{-1}$. This is supported by the disappearance of a band at 876 cm.$^{-1}$ which can be assigned to the out-of-plane bending of the 2-hydrogen atom.[10] Similarly thiamine shows the new band at 2280 cm.$^{-1}$ and the loss of a band at 897 cm.$^{-1}$.

The solution of the homologue (I) in deuterium oxide used for the nuclear magnetic resonance spectra was freeze-dried, and the infrared spectrum of the product examined. This showed the appearance of a band at 2300 cm.$^{-1}$ consistent with a C–D stretching frequency, and loss of a band at 913 cm.$^{-1}$ consistent with an out-of-plane bending of the 2-hydrogen atom. Comparable spectra for the homologue (II) are not available owing to difficulty in obtaining a satisfactory mull.

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[15] Karimullah, *J.*, 1937, 961.
[16] Rydon, *Biochem. J.*, 1951, **48**, 383.