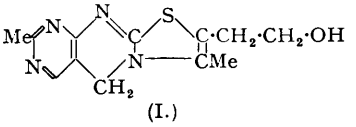
113. *Aneurin. Part X. The Mechanism of Thiochrome Formation from Aneurin and Aneurin Disulphide.*

By P. Sykes and A. R. Todd.

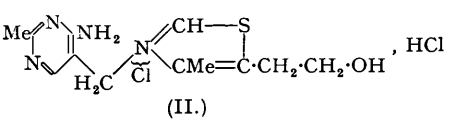
When aneurin disulphide is heated in various solvents it yields thiochrome and a colourless compound, m. p. 237° (cf. Zima and Williams, *Ber.*, 1940, **73**, 941). Evidence is presented for the view that this colourless substance is the thiazolone (IV), and an account is given of an extended series of experiments carried out in endeavours to synthesise (IV) by other routes. The thiazolone (IV) is not readily convertible into thiochrome. A mechanism is suggested to explain the production of thiochrome, together with (IV), from aneurin disulphide, and a new mechanism is advanced to account for the formation of thiochrome by oxidation of aneurin itself.

Although thiochrome (I) is readily formed by the oxidation of aneurin (vitamin B_1) (II) in alkaline solution with potassium ferricyanide (Barger, Bergel, and Todd, *Ber.*, 1935, **68**, 2257), its preparation in quantity by this route is inconvenient. The best preparative procedure rests on the observation of Zima and Williams (*Ber.*, 1940, **73**, 941) that aneurin disulphide (III) yields thiochrome (I), together with at least one colourless substance when heated in high-boiling solvents such as ethylene glycol. Some time ago we had occasion to prepare a substantial amount of thiochrome and we employed a method of this type in which a solution of aneurin disulphide in *iso*butanol is refluxed for two hours; we are indebted to Messrs. Hoffmann-La Roche & Co., Basle, for details of this method which had been used in their laboratories. Our results were similar to those of Zima and Williams (*loc. cit.*) in that

we obtained, in addition to thiochrome, substantial amounts of the colourless substance $C_{12}H_{16}O_2N_4S$, m. p. 237°, which these authors suggested might be the thiazolone derivative (IV) corresponding to aneurin, although they advanced no evidence to support their suggestion. The production of thiochrome from aneurin disulphide in this way is itself interesting, and if the colourless by-product is indeed (IV) then earlier views on the mechanism of thiochrome formation from aneurin (Barger, Bergel, and Todd, *loc. cit.*; Windaus, Tschesche, and Grewe, *Z. physiol. Chem.*, 1935, **237**, 98), in which (IV) is a postulated intermediate, would require revision. The present paper records some experiments designed to establish the validity of structure (IV) for the colourless product and to throw light on the mechanism by which thiochrome is produced from aneurin and aneurin disulphide.



(I.)



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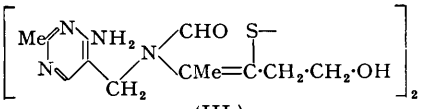

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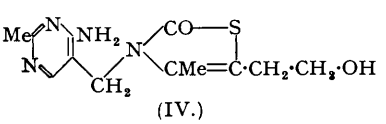
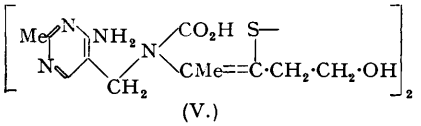


(IV.)

The decomposition of aneurin disulphide was examined in several solvents, but the yield of colourless substance, m. p. 237°—henceforth called, for convenience, " aneurin thiazolone "—was highest in *iso*butanol; thiochrome was formed simultaneously in every case. " Aneurin thiazolone," which is only very slightly soluble even in hot water, is a colourless, non-fluorescent, monoacid base which dissolves in dilute acid and is reprecipitated unchanged by addition of alkali in the cold. It is unaffected by treatment with sulphite under conditions which cause cleavage of aneurin, or by heating with aniline, benzylamine, or ammonia in alcoholic solution, and does not absorb hydrogen at room temperature in presence of a platinum catalyst; with acetic anhydride, it yields a monoacetyl derivative. When it is heated with dilute sodium hydroxide solution decomposition occurs with formation of sulphide, and similar treatment with hydrochloric acid yields 4-amino-5-aminomethyl-2-methylpyrimidine. After oxidation of a solution of " aneurin thiazolone " in one equivalent of dilute hydrochloric acid with hydrogen peroxide, two equivalents of alkali were required for neutralisation, and the oxidation product was isolated as its sodium salt. Acidification of a solution of this salt and then warming afforded carbon dioxide, and when the salt was heated with anhydrous formic acid decarboxylation occurred followed by formylation of the decarboxylation product, to give aneurin disulphide (III). On this evidence, the oxidation product is formulated as (V) and the " aneurin thiazolone " itself has almost certainly the originally postulated structure (IV). It is worthy of mention that, if the thiazolone is heated above its melting point for considerable periods, only negligible amounts of thiochrome (detected by its fluorescence) are produced.



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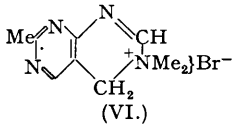

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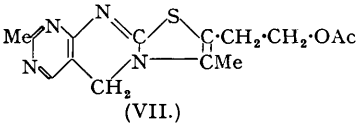

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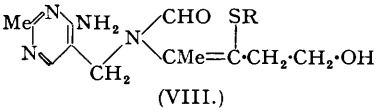
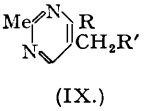
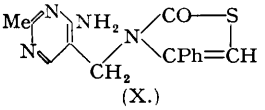


(VIII.)



(IX.)

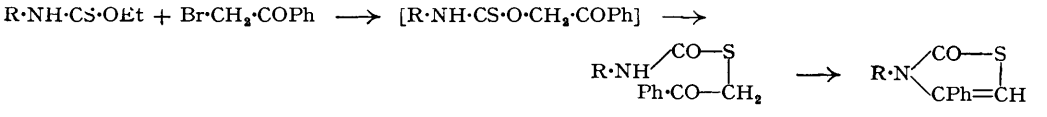


(X.)

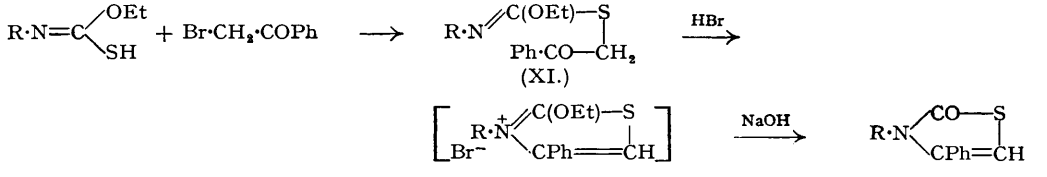
Although we were reasonably certain that the " aneurin thiazolone " had structure (IV), it was decided to attempt the synthesis of (IV) so as to set the matter beyond doubt. Many

possible methods were investigated, but none was successful. A number of features of chemical interest arose during these investigations, however. Initial attempts to condense 4-amino-5-bromomethyl-2-methylpyrimidine with 5-2′-acetoxyethyl-4-methylthiazol-2-one, either as such or as a metal derivative, were quite unsuccessful. When the two compounds were heated in butanol solution only 4-amino-5-butoxymethyl-2-methylpyrimidine (IX; R = NH_2, R′ = OBu^n) was obtained, while with dimethylformamide as a solvent a product was isolated which, on the evidence of analysis and general behaviour, is formulated as (VI). A more likely approach involved initial formation of a quaternary salt from 4-amino-5-bromomethyl-2-methylpyrimidine and 5-2′-acetoxyethyl-2-chloro-4-methylthiazole, but under the conditions employed quaternisation was followed by a spontaneous cyclisation yielding *O*-acetylthiochrome (VII). The compound (VIII; R = Na) which is the immediate precursor of aneurin disulphide in its production from aneurin, might form a suitable starting material for (IV) if the formyl group could be removed and the product caused to react with carbonyl chloride. Hydrolytic removal of this group in (VIII; R = Na) is impossible, since acid re-forms aneurin, and alkali causes elimination of sulphur as sulphide, and it was found, not unexpectedly, that the *S*-alkyl and *S*-aralkyl ethers (VIII; R = Me, $PhCH_2$, and p-$NO_2{\cdot}C_6H_4{\cdot}CH_2$) were hydrolysed at the sulphur linkage before any removal of the formyl group could be effected.

Attention was now turned to the method of Walther and Greifenhagen (*J. pr. Chem.*, 1907, [ii], **75**, 201), who synthesised various thiazol-2-ones by condensing arylthiourethanes with ω-bromoacetophenone. 4-Amino-2-methyl-5-thioncarbethoxyaminomethylpyrimidine (IX; R = NH_2, R′ = NH·CS·OEt) was prepared by reaction of *OS*-diethyl xanthate (Debus, *Annalen*, 1850, **75**, 121) with the corresponding 5-aminomethylpyrimidine. Heated in the form of its hydrochloride with ω-bromoacetophenone in dioxan, it gave a small yield of 3-(4-amino-2-methyl-5-pyrimidyl)methyl-4-phenylthiazol-2-one (X). This thiazolone, on prolonged heating above its melting point, gave a small amount of fluorescent material, just as did the "aneurin thiazolone." No trace of a thiazolone was obtained, however, in experiments in which the thiourethane (IX; R = NH_2, R′ = NH·CS·OEt) or its salts were treated under a varietv of conditions with 3-acetoxy-1-bromopropyl methyl ketone, or the corresponding chloro- and iodo-ketones, α-aceto-α-bromobutyrolactone, or bromoacetone. In each case, decomposition of the halogen compound occurred before condensation could take place. In the same way, the corresponding benzylthiourethane (IX; R = NH_2, R′ = NH·CS·O·CH_2Ph) failed to react with these aliphatic halogen compounds, although with ω-bromoacetophenone it yielded (X) more readily than did the ethylthiourethane. The mechanism proposed by Walther and Greifenhagen (*loc. cit.*) for the production of thiazolones by their method involved an initial elimination of alkyl halide and a subsequent migration of a group from oxygen to sulphur before cyclisation:



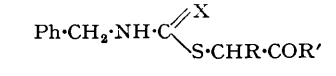
Migration of alkyl groups from oxygen to sulphur in simple thiocarbamates has been observed by Wheeler and Barnes (*Amer. Chem. J.*, 1899, **22**, 141), but we believe that, at least in the reactions we have studied, the following alternative mechanism plays a major rôle:

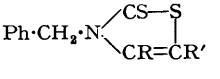


This belief rests on the fact that we were unable to detect any alkyl bromide during our reactions and bromide ion was always present in the resulting solutions. It is also significant that condensation of the thiourethane (IX; R = NH_2, R′ = NH·CS·OEt) with ω-bromoacetophenone occurs to a very small extent only unless the thiourethane is first converted into a salt or, better, the ethanol used as a solvent contains dissolved hydrogen chloride. Presumably, in the absence of additional acid, the hydrogen bromide liberated in the first stage of the reaction is used up in forming a salt of the intermediate pyrimidine derivative (XI) and

additional acid facilitates cyclisation. Intermediates of type (XI) in which the ethoxy-group is replaced by hydrogen or alkyl were isolated by Todd, Bergel, and Karimullah (*Ber.*, 1936, **69**, 217) as products of the reaction between *N*-substituted thioamides and α-halogeno-ketones and it was shown by these authors that their cyclisation to quaternary thiazolium salts occurred on heating their hydrochlorides, or, more rapidly by heating them in ethanol containing excess of anhydrous hydrogen chloride. When the pyrimidine thiourethane (IX; R = NH_2, R′ = NH·CS·OEt) was treated in ethanol with one equivalent of sodium ethoxide and then allowed to react with ω-bromoacetophenone, the intermediate (XI; R = 4-amino-2-methyl-5-pyrimidylmethyl) was obtained. This product was unaffected by refluxing ethanol, but heating it in ethanol containing dissolved hydrogen chloride converted it smoothly into the thiazolone (X); the same intermediate was also found in small amount in the mother-liquors from experiments in which the free thiourethane had been condensed with ω-bromoacetophenone in the absence of acid.

Although we considered that the thiazolone synthesis followed the route above indicated, it seemed possible that a compound of type (XII) postulated as an intermediate by Walther and Greifenhagen (*loc. cit.*) might be cyclised to give a thiazolone, and some model experiments were carried out to test this. Phenacyl *N*-benzylthiolcarbamate (XII; X = O, R = H, R′ = Ph), however, could not be dehydrated to yield a thiazolone. More success attended the use of dithiocarbamates. Phenacyl (XII; X = S, R = H, R′ = Ph) and 3-acetoxy-1-acetylpropyl *N*-benzyldithiocarbamate (XII; X = S, R = CH_2·CH_2·OAc, R′ = Me) were prepared; when these were heated above their melting points, gas was evolved and they were converted smoothly into 3-benzyl-4-phenylthiazol-2-thione (XIII; R = Ph, R′ = H) and 5-2′-acetoxyethyl-3-benzyl-4-methylthiazol-2-thione (XIII; R = Me, R′ = CH_2·CH_2·OAc) respectively. When an attempt was made to prepare a thiazol-2-thione in similar fashion from 3-acetoxy-1-acetylpropyl *N*-(4-amino-2-methyl-5-pyrimidyl)methyldithiocarbamate (XIV; R = NH_2, X = S), however, a cyclisation, involving the 4-amino-group on the pyrimidine nucleus, occurred, yielding 1 : 2 : 3 : 4-tetrahydro-7-methyl-2-thio-1 : 3 : 6 : 8-tetra-azanaphthalene (XV; X = S), a compound already described by Bergel and Todd (*J.*, 1938, 26; there called 2-thio-7-methyl-1 : 2 : 3 : 4-tetrahydro-1 : 3 : 6 : 8-benztetrazine). The corresponding monothiocarbamate (XIV; R = NH_2, X = O) was also prepared, but it cyclised in an analogous manner when heated to give (XV; X = O), which was identified by comparison with a specimen synthesised by heating the reaction product of 4-amino-5-aminomethyl-2-methylpyrimidine hydrochloride with potassium cyanate to 280° until ammonia evolution ceased. The ease with which these cyclisations occur is surprising in view of the difficulty encountered by Bergel and Todd (*loc. cit.*) in preparing (XV; X = S) by a not dissimilar method. One possible method of avoiding this unwelcome type of ring-closure would be to utilise 5-aminomethyl-4-chloro-2-methylpyrimidine as starting material for the thiocarbamate intermediate

(XII.) 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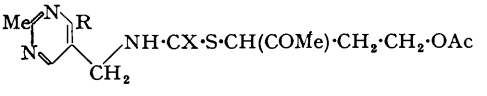
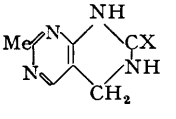
(XIV.) 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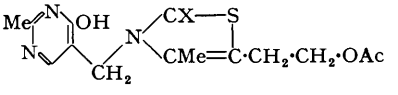
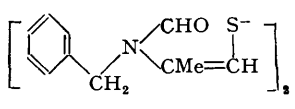
(XVI.) 

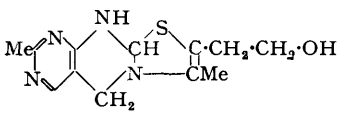
and to replace the chlorine atom in the final product by an amino-group. Unfortunately, however, we were unable to prepare this chloro-compound although various methods were tried. A second less attractive possibility was to start from 5-aminomethyl-4-hydroxy-2-methylpyrimidine and replace the 4-hydroxy-group in the final product by an amino-group. From this amine, the appropriate di- and mono-thiocarbamates (XIV; R = OH, X = S and O) were prepared and these, when heated, yielded 5-2′-acetoxyethyl-3-(4-hydroxy-2-methyl-5-pyrimidyl)methyl-4-methylthiazol-2-thione (XVI; X = S) and 5-2′-acetoxyethyl-3-(4-hydroxy-2-methyl-5-pyrimidyl)methyl-4-methylthiazol-2-one (XVI; X = O) respectively. Attempts to replace the hydroxy-group in either of these compound by chlorine caused gross decomposition.

The thiazolone (XVI; X = O) differs from the acetyl derivative of the " aneurin thiazolone " (IV) only in that the pyrimidine ring bears a hydroxy- rather than an amino-group in position 4, and it seemed possible that it might be obtainable from (IV). Cerecedo and Soodak (*J. Amer. Chem. Soc.*, 1944, **66**, 1988) describe a deamination procedure for the conversion of aneurin into its 4-hydroxy-analogue; we were unable to confirm the observations of these workers on aneurin itself and the method effected no deamination when applied to the " aneurin thiazolone," although the thiazolone ring was opened.

Assuming that structure (IV) correctly represents the " aneurin thiazolone," it is of interest to consider the manner in which it is produced together with thiochrome from aneurin disulphide. The reaction is carried out by simple heating in a variety of solvents and, although *iso*butanol appears to give the best results, *n*-butanol, ethylene glycol, glycerol, pyridine, and even water are all satisfactory. Although, as already mentioned, prolonged heating above its melting point converts a small amount of the thiazolone into thiochrome, there is no evidence that the two are interconvertible under the conditions used in their formation from aneurin disulphide; each of them is unaffected by prolonged refluxing in *iso*butanol. It seems very likely that thiochrome and the thiazolone are produced more or less simultaneously from one aneurin disulphide molecule, and the fact that they are not isolated in equimolecular proportions after the reaction is doubless due to its being much more difficult to isolate thiochrome than thiazolone. Some support for this view is found in the fact that the simple model disulphide (XVII) can be recovered unchanged after prolonged refluxing in various solvents. Although it could not yield a thiochrome analogue it could, in theory, yield a thiazolone analogous to (IV). It may well be that, before fission of a disulphide of this type will proceed, the possibility of forming a thiochrome-like compound must exist, *i.e.*, that the amino-group(s) in aneurin disulphide play an essential part in the fission.

Evidence regarding the mechanism by which thiochrome is produced from aneurin disulphide was obtained by refluxing the latter in *iso*butanol in a nitrogen atmosphere, the solvent having been previously freed from air by boiling it for some time while a stream of air-free nitrogen was passed through it. When the solution was allowed to cool in the absence of air, the thiazolone (IV) crystallised, but at no stage was there any trace of the brilliant blue fluorescence of thiochrome. When, however, air was admitted to the apparatus at the end of the experiment, with the solution either cold or hot, an intense blue fluorescence appeared almost immediately and thiochrome could subsequently be isolated. From these results it appears highly likely that dihydrothiochrome (XVIII) is the immediate precursor of thiochrome in the normal aneurin disulphide fission. Dihydrothiochrome, which is formed by sodium dithionite reduction of thiochrome (Kuhn, Wagner-Jauregg, van Klaveren, and Vetter, *Z. physiol. Chem.*, 1935, **234**, 196), is known to be oxidised spontaneously in air.

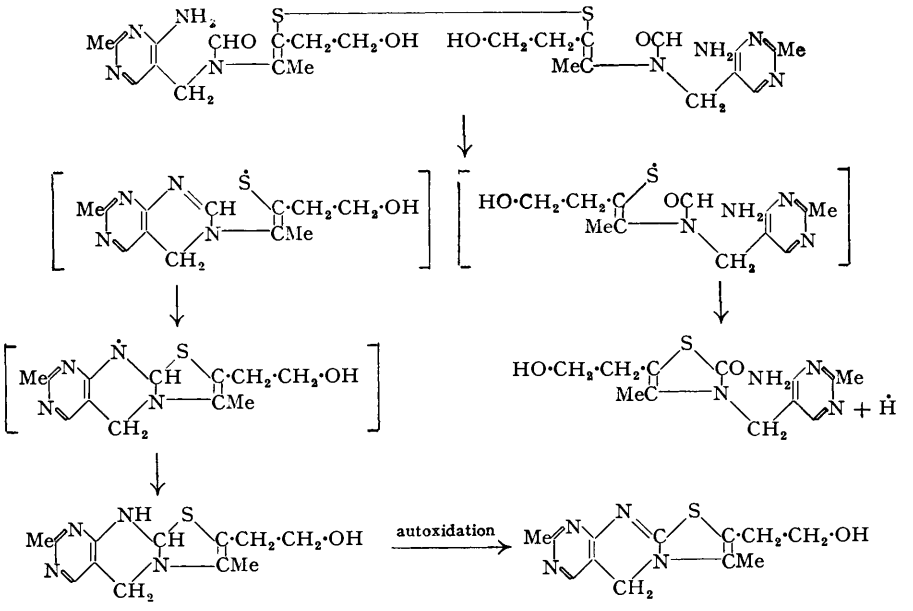
(XVII.) 



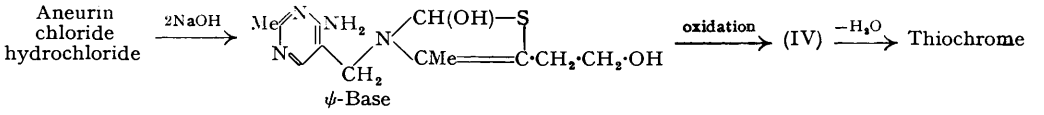
(XVIII.)

On the available evidence, it is tentatively suggested that when aneurin disulphide is heated in solvents there is initially a cyclisation by loss of water between one of the formyl groups and the adjacent 4-amino-group on a pyrimidine nucleus, followed by a fission of the molecule at the disulphide linkage to yield two thiol radicals. In the radical containing the cyclised fragment, intramolecular addition across the C=N system occurs, yielding the dihydrothiochrome radical which is converted into dihydrothiochrome by exchange with solvent, and then undergoes spontaneous oxidation to thiochrome itself in presence of air. The other radical produced in the initial fission yields the " aneurin thiazolone " (IV) by reaction of the thio-radical with the formyl group and elimination of a hydrogen radical. The proposed scheme is indicated on p. 539.

The initial dehydration postulated in the scheme presents no obstacle, since it seems likely that those derivatives of aneurin supposedly containing a free formyl group exist partly in such a cyclic form (Zima and Williams, *loc. cit.*). The addition of free radicals to unsaturated systems is well known, although those hitherto described involve the C=C rather than the C=N system (cf. Waters, " Chemistry of Free Radicals," Oxford, 1946, p. 188). When pyridine is used as a solvent, the radical-terminating action could be exerted by the water produced in the initial dehydration, if not by the pyridine itself; it is perhaps worthy of mention in this connection that the reaction is slower in pyridine than in other solvents, as judged by the development of fluorescence and by the amounts of the thiazolone isolated after varying periods of reaction.



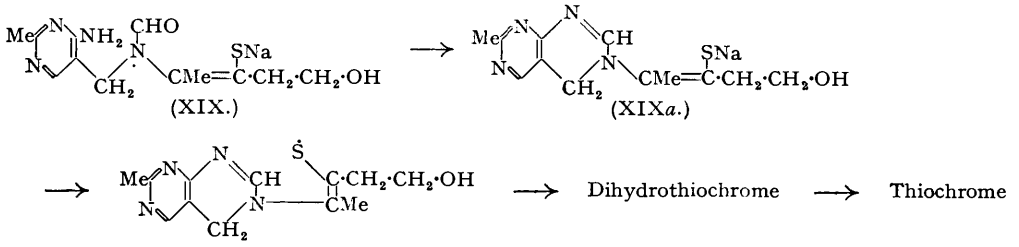
The mechanism originally postulated for the conversion of aneurin into thiochrome was oxidation of the aneurin ψ-base to the thiazolone (IV), followed by cyclisation (Barger, Bergel, and Todd, *loc. cit.*) :



This mechanism seemed satisfactory at the time, the more so as much the best reagent for bringing about the conversion was alkaline potassium ferricyanide, which is known to effect the change ψ-base ⟶ pyridone in the analogous pyridinium compounds. Porphyrexide (Kuhn and Vetter, *Ber.*, 1935, **68**, 2375) can also be used to prepare thiochrome but most other oxidising agents, *e.g.*, iodine, hydrogen peroxide, produce mainly aneurin disulphide from aneurin. On the evidence presented in this paper, in particular the observation that conversion of the thiazolone (IV) into thiochrome is very small even above 250°, it is clear that the mechanism of Barger, Bergel, and Todd (*loc. cit.*) cannot be correct. A modified mechanism which was favoured by Kuhn and Vetter (*loc. cit.*) postulated cyclisation at the ψ-base stage, followed by oxidation of the dihydrothiochrome (XVIII). A strong objection to this view is found in the fact that the oxidation of dihydrothiochrome to thiochrome proceeds spontaneously in air, so that no ferricyanide or other oxidising agent would be required. All attempts to demonstrate the existence of the aneurin ψ-base by oxidising aneurin in the presence of two equivalents of alkali under conditions most favourable to thiazolone formation failed, no trace of (IV) being obtained. It may well be that the ψ-base does not exist as such in alkaline solution and that aneurin exhibits an extreme case of the phenomenon observed in benzthiazole derivatives by Mills, Clark, and Aeschlimann (*J.*, 1923, **123**, 2353), where addition of one equivalent of alkali to the quaternary chloride converted half of it directly into the sodium salt of the thiol produced by opening the thiazole ring, while the other half of the chloride remained unchanged; in these experiments no ψ-base could be isolated.

We consider that the peculiar virtue of ferricyanide as an oxidising agent in converting aneurin into thiochrome lies in its being a one-electron oxidising agent which oxidises the sodium salt of the cyclised thiol-form of aneurin to the free radical; ferric salts are particularly suitable for this type of oxidation (cf. Waters, *op. cit.*, p. 283). Some of the cyclised form (XIX*a*) will always be present in alkaline solutions of the simple thiol salt (XIX) (Zima and Williams, *loc. cit.*). Formation of thiochrome from the thiol radical is believed to proceed in the manner postulated in our discussion of its formation from aneurin disulphide.

Doubtless, some of the ferricyanide takes part in oxidising the dihydrothiochrome intermediate, but this is not an essential function if air is present. It is noteworthy that mercuric oxide, which has been shown to be an effective substitute for potassium ferricyanide in the preparation



of thiochrome (Holman, *Biochem. J.*, 1944, **38**, 388), is also a one-electron oxidising agent. Two-electron oxidising agents such as hydrogen peroxide and iodine would be expected to convert the sodium salt of the thiol form of aneurin directly into aneurin disulphide without production of thiochrome; this is in fact what is observed. Some support for this view of the mechanism of thiochrome formation (which is indicated schematically above) is provided by the effect of varying the amount of alkali used in the ferricyanide oxidation of aneurin. If to a sample of aneurin three equivalents of alkali are added, followed immediately by potassium ferricyanide, the amount of thiochrome (estimated fluorimetrically) produced is greater than in a corresponding experiment using two equivalents of alkali. If the oxidation mechanism did not involve an initial opening of the thiazole ring system, there would have been little or no difference between the two.

Experimental.

Conversion of Aneurin Disulphide into Thiochrome (I) *and "Aneurin Thiazolone"* (IV).—Aneurin disulphide (III) (2 g.) was refluxed for 2 hours in *iso*butanol (40 c.c.), and the highly fluorescent solution then allowed to cool. Colourless needles of aneurin thiazolone (IV) [3-(4-amino-2-methyl-5-pyrimidyl)-methyl-5-2′-hydroxyethyl-4-methylthiazol-2-one] (0·47 g.), m. p. 237°, separated. From the mother-liquors, thiochrome (I) (0·14 g.) was obtained. (This follows the method used by Messrs. Hoffmann-La Roche & Co., Ltd., Basle.) Rather less conversion took place when ethylene glycol (Zima and Williams, *Ber.*, 1940, **73**, 941), glycerol, pyridine, or water was used in place of *iso*butanol.

When the experiment was repeated in an atmosphere of oxygen-free nitrogen (the solvent having previously been boiled out in a nitrogen stream), no fluorescence was observed until air was admitted to the apparatus, whereupon the characteristic colour due to thiochrome was seen at once. The yields of thiochrome and thiazolone obtained were almost exactly the same as when the conversion was carried out in the presence of air.

The thiazolone gave a negative result in the formaldehyde–azo-test (Kinnersley and Peters, *Biochem. J.*, 1934, **28**, 667). Heated to 280° for some time, the substance darkened and charred and then showed faintly the yellow-green fluorescence in the solid and blue fluorescence in butanol solution, characteristic of thiochrome.

Acetylation of "Aneurin Thiazolone."—A solution of the thiazolone (IV) (0·94 g.) in acetic anhydride (18 c.c.) was treated with pyridine (0·5 c.c.) and kept at room temperature for 48 hours, diluted with ethanol (50 c.c.), and then evaporated under reduced pressure. The residual gum was dissolved in a little water, made neutral with N-sodium hydroxide, and evaporated to dryness under reduced pressure, and the residue extracted with hot chloroform. The gum obtained by evaporating the chloroform extract crystallised from acetone, giving short colourles needles of the *acetyl* derivative (0·63 g., 58%), m. p. 174° (Found: C, 52·5; H, 5·6; N, 16·9%; *M*, 346. $C_{14}H_{18}O_3N$ requires C, 52·2; H, 5·6; N, 17·4%; *M*, 322).

Hydrogen Peroxide Oxidation of "Aneurin Thiazolone."—The thiazolone (IV) (0·45 g.) was dissolved in one equivalent of N-hydrochloric acid, excess of hydrogen peroxide (100-vol.; 5 c.c.) added, and the solution warmed initially to 30° and then kept at room temperature for 18 hours. Neutralisation with N-sodium hydroxide (2 equivs.) gave the oxidation *product* (V) (0·1 g., 20%) as platelets; recrystallised from methanol it had m. p. 176° (Found: C, 45·2; H, 4·9; N, 17·6. $C_{24}H_{32}O_6N_8S_2Na_2$ requires C, 45·2; H, 5·0; N, 17·6%).

On gentle warming of the oxidation product with N-hydrochloric acid, carbon dioxide was evolved. Neither the oxidation product itself nor the decarboxylated material gave a colour with sodium nitroprusside solution.

Conversion of the Above Oxidation Product into Aneurin Disulphide.—The product (V) (0·09 g.) was dissolved in anhydrous formic acid (1 c.c.), and the solution gently warmed till evolution of carbon dioxide ceased; it was then kept at room temperature for 10 days and evaporated to dryness in a vacuum from a bath at 30°. The residue was neutralised with N-sodium hydroxide and again evaporated to dryness in a vacuum. A solution of the residue, in a minimum of water, was diluted with ethanol, filtered to remove inorganic material, diluted with a large quantity of ether, and allowed to crystallise. The

product (0·02 g.) had m. p. 177°, undepressed on admixture with a sample of aneurin disulphide (III) (Found: N, 19·6. Calc. for $C_{24}H_{34}O_4N_8S_2$: N, 19·9%).

Attempted Reaction of 5-2′-Acetoxyethyl-4-methylthiazol-2-one with 4-Amino-5-bromomethyl-2-methylpyrimidine Hydrobromide.—(a) *In butanol.* 5-2′-Acetoxyethyl-4-methylthiazol-2-one (Andersag and Westphal, *Ber.*, 1937, **70**, 2035) (1·0 g.) and 4-amino-5-bromomethyl-2-methylpyrimidine hydrobromide (*idem, ibid.*) (1·42 g.) were refluxed in dry *n*-butanol for 1 hour. On concentration to half bulk, crystallisation took place; recrystallised from butanol, the *4-amino-5-butoxymethyl-2-methylpyrimidine hydrobromide* (IX; R = NH_2, R′ = OBu^n) formed extremely hygroscopic prisms (0·95 g., 69%), m. p. 138° (Found: C, 42·8; H, 6·2; N, 14·9; Br, 28·6. $C_{10}H_{18}ON_3Br$ requires C, 43·4; H, 6·5; N, 15·2; Br, 29·0%). The free base, obtained on treatment of the hydrobromide with alkali, was an oil which could not be distilled. The formation of this ether may account for the low yields of aneurin observed by Williams and Cline (*J. Amer. Chem. Soc.*, 1937, **59**, 1052) in condensation experiments with 4-amino-5-bromomethyl-2-methylpyrimidine in *n*-butanol.

(b) *In dimethylformamide.* Repetition of the above experiment in dry dimethylformamide led, on concentration *in vacuo* and cooling, to the formation of hygroscopic crystals. Very hygroscopic needles of the hydrobromide of the quaternary *salt* (VI) (0·59 g., 35%), m. p. 300° (decomp.), separated with difficulty from dry acetonitrile (Found: C, 32·5; H, 4·6; N, 17·3; Br, 46·9. $C_9H_{14}N_4Br_2$ requires C, 32·0; H, 4·1; N, 16·6; Br, 47·3%).

Reaction of 5-2′-Acetoxyethyl-2-chloro-4-methylthiazole with 4-Amino-5-bromomethyl-2-methylpyrimidine Hydrobromide.—5-2′-Acetoxyethyl-2-chloro-4-methylthiazole (Andersag and Westphal, *loc. cit.*) (1·1 g.) and 4-amino-5-bromomethyl-2-methylpyrimidine hydrobromide (1·42 g.) were refluxed in dry dioxan for 1 hour; all the pyrimidine had then dissolved and the solution showed a powerful greenish fluorescence. The solution was neutralised with dilute aqueous alkali and then evaporated to dryness under reduced pressure. The yellowish gum was extracted with dry chloroform, and the fluorescent extract evaporated till crystallisation occurred. Recrystallisation from acetone yielded *O*-acetylthiochrome (VII) (0·79 g., 52%), m. p. and mixed m. p. 118°.

O-*Acetylthiochrome* (VII).—Thiochrome (0·93 g.) was dissolved in warm acetic anhydride (20 c.c.); pyridine (0·5 c.c.) was added and the solution brought just to boiling, then kept overnight at room temperature, and excess of anhydride removed by evaporation in a vacuum. The gummy residue was dissolved in ethanol, made slightly alkaline with aqueous sodium hydroxide, and evaporated to dryness. The yellow gum crystallised from dry *n*-butanol, giving O-*acetylthiochrome* (0·61 g., 56%), which was obtained from acetone as pale yellow prisms, m. p. 118° (Found: C, 55·4; H, 5·2; N, 18·9. $C_{14}H_{16}O_2N_4S$ requires C, 55·2; H, 5·3; N, 18·5%).

Disulphide (XVII) *from 3-Benzyl-4-methylthiazolium Chloride.*—3-Benzyl-4-methylthiazolium chloride (Karimullah, *J.*, 1937, 961) (6·8 g.) (this compound forms a hydrate, colourless prisms, m. p. 136°, which on intense drying yields the anhydrous compound, m. p. 188°) was dissolved in N-sodium hydroxide solution (60 c.c., 2 equivs.). Iodine (3·8 g.), dissolved in aqueous potassium iodide solution, was added slowly to the stirred solution. The sticky resin which separated was extracted with chloroform, and the extract decolourised with thiosulphate solution. After the solution had been dried (Na_2CO_3), the solvent was removed in a vacuum, yielding a pale yellow syrup which crystallised from acetone, giving colourless leaflets of *di-*[2-(*N-benzylformamido*)*prop*-1-*enyl*] *disulphide* (5·5 g., 87%), m. p. 98° (Found: C, 63·6; H, 5·9; N, 6·8. $C_{22}H_{24}O_2N_2S_2$ requires C, 64·0; H, 5·8; N, 6·8%). This substance was recovered unchanged after being heated under reflux in solvents or heated above its m. p. for long periods.

S-p-*Nitrobenzyl Ether of "Open-chain" 3-Benzyl-4-methylthiazolium Chloride.*—3-Benzyl-4-methylthiazolium chloride (5 g.) was dissolved in aqueous sodium hydroxide solution (1·77 g. in 30 c.c.; 2 equivs.). The solution was evaporated to dryness in a vacuum in a nitrogen atmosphere, and the residue re-evaporated twice with dry toluene. The residue was then dissolved in liquid ammonia (100 c.c.) and treated with *p*-nitrobenzyl bromide (4·8 g.) suspended in liquid ammonia (150 c.c.). The dark red solution was stirred for 2 hours with exclusion of carbon dioxide and moisture, liquid ammonia being added as required to maintain the volume at 250 c.c. After being kept overnight the ammonia had evaporated and the resulting resin was warmed under reduced pressure to remove traces of ammonia, extracted with dry acetone, and filtered from the residual sodium halides. The extract was evaporated to dryness and the residue crystallised from ether. The S-p-*nitrobenzyl ether* separated from aqueous acetone as pale yellow prisms (5 g., 70%), m. p. 104° (Found: C, 62·9; H, 5·4; N, 8·1. $C_{18}H_{18}O_3N_2S$ requires C, 63·1; H, 5·3; N, 8·2%).

S-p-*Nitrobenzyl Ether* (VIII; R = p-$NO_2{\cdot}C_6H_4{\cdot}CH_2$) *of "Open-chain" Aneurin.*—Aneurin (2·5 g.) was dissolved in a little water, and sodium hydroxide solution (0·85 g. in 10 c.c.) added. This solution was evaporated to dryness in a vacuum, evaporated twice with dry toluene, and treated as before with *p*-nitrobenzyl bromide in liquid ammonia. The S-p-*nitrobenzyl ether* separated from acetone as pale yellow prisms (2 g., 66%), m. p. 203° (Found: C, 54·2; N, 5·6; N, 17·3. $C_{19}H_{23}O_3N_5S$ requires C, 54·6; H, 5·5; N, 16·8%). On treatment with sodium hydroxide, the substance rapidly blackened, sodium sulphide being formed.

The *p*-nitrobenzyl ether (0·5 g.) was refluxed for 1 hour in 10% hydrochloric acid, and the solution then evaporated in a vacuum. Extraction of the resin with hot ether and evaporation of the filtrate yielded di-*p*-nitrobenzyl disulphide (Price and Twiss, *J.*, 1909, **95**, 1728) (0·14 g., 69%), m. p. and mixed m. p. 126° (Found: N, 8·1. Calc. for $C_{14}H_{12}O_4N_2S_2$: N, 8·3%).

S-*Methyl Ether* (VIII; R = Me) *of "Open-chain" Aneurin.*—This material was obtained from aneurin (2·5 g.) as described above, the *p*-nitrobenzyl bromide being replaced by methyl iodide. It proved extremely difficult to free the product from sodium iodide; this was eventually done by precipitation as silver iodide from aqueous solution, followed by removal of the small excess of silver with

hydrogen sulphide. Care had to be taken to avoid a large excess of silver ion during the precipitation since the *S*-methylaneurin forms a sparingly soluble silver derivative which tends to separate from aqueous solution. The S-*methyl ether* separated from acetone in colourless plates (0·75 g., 34%), m. p. 133° (Found: C, 52·7; H, 6·4; N, 19·3. $C_{13}H_{20}O_2N_4S$ requires C, 52·7; H, 6·7; N, 19·0%). This compound underwent extensive decomposition when warmed with either acid or alkali.

4-*Amino*-2-*methyl*-5-*thioncarbethoxyaminomethylpyrimidine* (IX; R = NH_2, R′ = NH·CS·OEt).—4-Amino-5-aminomethyl-2-methylpyrimidine hydrochloride (2·1 g.) was dissolved in hot 95% ethanol, and sodium hydroxide (0·8 g.) in a little water was added, followed by enough water to dissolve the separated sodium chloride. Diethyl xanthate (Debus, *Annalen*, 1850, **75**, 121) (1·5 g.) was added and the whole gently refluxed for 1 hour; evolution of ethanethiol had then ceased and the product had begun to crystallise. The *thiourethane*, collected and recrystallised from methanol, formed colourless needles (1·8 g., 80%), m. p. 223° (decomp.) (Found: C, 48·1; H, 6·4; N, 25·1. $C_9H_{14}ON_4S$ requires C, 47·8; H, 6·2; N, 24·8%).

A small portion was dissolved in hot ethanol, a drop of constant-boiling hydrobromic acid added, and the solution diluted with ether till just cloudy. Colourless prisms of the *hydrobromide* separated, m. p. 179° (Found: N, 17·9. $C_9H_{14}ON_4S$,HBr requires N, 18·1%).

3-(4-*Amino*-2-*methyl*-5-*pyrimidyl*)*methyl*-4-*phenylthiazol*-2-*one* (X).—The above thiourethane hydrochloride (1·3 g.) was suspended in dry dioxan (40 c.c.), and ω-bromoacetophenone (3 g., excess) added. The solution was gently refluxed for 7 hours, then evaporated in a vacuum to yield a red gum. This was extracted with warm acetone to remove excess of ω-bromoacetophenone, and the deliquescent residue dissolved in aqueous ethanol. The solution was made just alkaline with N-sodium hydroxide and set aside in the ice-chest. Pale yellow prisms of the *thiazolone* (0·075 g., 5%), m. p. 230°, separated from methanol (Found: C, 60·1; H, 4·6; N, 18·8. $C_{15}H_{14}ON_4S$ requires C, 60·3; H, 4·7; N, 18·8%). Heated above its m. p. for long periods this compound yielded a small quantity of weakly fluorescent thiochrome-like material.

O-*Benzyl* S-*Ethyl Xanthate*.—Freshly prepared sodium benzylxanthate (Bulmer and Mann, *J.*, 1945, 671) (62 g.) was suspended in a little ethanol, and ethyl iodide (56 g., 1·2 mols.) added. After a few minutes' warming on the steam-bath, a vigorous reaction commenced and was complete after 15 minutes. Further warming produced a marked odour of ethanethiol. The mixture was cooled, diluted with water, and extracted with ether. The pale yellow ethereal extract was well washed with water and dried (Na_2SO_4). The solvent was removed under reduced pressure and gave the product as a pale yellow oil; attempts to distil it under reduced pressure resulted in considerable decomposition, ethanethiol being produced. The yield of crude material was 45 g. (70%).

4-*Amino*-2-*methyl*-5-*thioncarbobenzyloxyaminomethylpyrimidine* (IX; R = NH_2, R′ = NH·CS·O·CH_2Ph), prepared similarly to the corresponding ethyl compound, formed colourless needles (yield, 72%), m. p. 201°, from ethanol (Found: C, 58·3; H, 6·0; N, 19·6. $C_{14}H_{16}ON_4S$ requires C, 58·3; H, 5·6; N, 19·5%).

Ethyl N-(4-*Amino*-2-*methyl*-5-*pyrimidyl*)*methyl*-α-S-*phenacylthioformimidate* (as XI).—The ethyl thiourethane (1·13 g.) was suspended in ethanol (50 c.c.), and sodium (0·115 g., 1 equiv.), dissolved in ethanol (10 c.c.), was added; the solid dissolved on gentle warming. ω-Bromoacetophenone (1 g., 1 equiv.), dissolved in a little ethanol, was then added and the solution gently refluxed while a stream of nitrogen was passed through it. The solution rapidly became dark green and the colour remained after 30 minutes' refluxing but faded when the solution was allowed to cool. The mixture was then evaporated to dryness in a vacuum and the residue continuously extracted with ether for 3 hours. The extract was cooled, filtered from a little unchanged thiourethane, and extracted with N-hydrochloric acid, and the acid extract neutralised with N-sodium hydroxide. An oil separated which rapidly solidified. Recrystallised from acetone the *product* separated as colourless prisms (0·7 g., 41%), m. p. 131° (decomp.) (Found: C, 59·2; H, 5·4; N, 16·5. $C_{17}H_{20}O_2N_4S$ requires C, 59·4; H, 5·8; N, 16·3%).

A small quantity of the above compound could be obtained from reaction of the ethyl thiourethane and ω-bromoacetophenone by applying the above isolation procedure and seeding the product with authentic material.

The compound (as XI) (0·4 g.) was dissolved in dry ethanol (10 c.c.) and saturated with dry hydrogen chloride. The solution was brought to boiling for 2 minutes, kept at room temperature for one hour, then diluted with water and made alkaline with 30% sodium hydroxide solution. Pale yellow prisms of 3-(4-amino-2-methyl-5-pyrimidyl)methyl-4-phenylthiazol-2-one separated; recrystallised from methanol, the product (0·2 g., 52%) had m. p. and mixed m. p. 230° (Found: N, 18·6. Calc. for $C_{15}H_{14}ON_4S$ N, 18·8%).

The benzylthiourethane reacted with ω-bromoacetophenone in the absence of acid to yield the thiazolone (X) in 17% yield, whereas, in the presence of ethanolic hydrogen chloride, the benzyl- and the ethyl-thiourethane both gave yields of up to 80% of thiazolone. 3-Acetoxy-1-chloro-, -bromo-, and -iodo-propyl methyl ketones, α-acetyl-α-bromobutyrolactone, and bromoacetone were all too unreactive to combine with the thiourethanes even in the presence of acid catalysts, decomposition taking place at temperatures below that required for reaction. Attempts to make the thiocarbamate intermediate by treating sodium or silver salts of the thiourethane with the halogenated ketones were also unsuccessful.

S-*Phenacyl* N-*Benzylthiolcarbamate* (XII; X = O, R = H, R′ = Ph).—Benzylamine (5 g.) in ethanol (100 c.c.) was treated with a stream of carbon oxysulphide till no more solid separated. The solution was then warmed until all the solid had dissolved and ω-bromoacetophenone (5 g.) was added. The mixture was then set aside overnight at room temperature and evaporated to small bulk in a vacuum; on dilution with water, the *thiolcarbamate* crystallised. Recrystallised from aqueous ethanol (charcoal), it (3·1 g., 49%) had m. p. 132° (Found: C, 67·3; H, 5·0; N, 4·7. $C_{16}H_{15}O_2NS$ requires C, 67·3; H,

5·3; N, 4·9%). The substance was recovered unchanged on being heated to 180° for 15 minutes or refluxed in acetic anhydride for 2 hours.

A similar experiment using 3-acetoxy-1-chloropropyl methyl ketone in place of ω-bromoacetophenone led only to the production of dibenzylurea.

S-*Phenacyl* N-*Benzyldithiocarbamate* (XII; X = S, R = H, R′ = Ph).—Benzylamine (5 g.) was dissolved in ethanol (100 c.c.), and carbon disulphide (1·9 g.) added. The solution was heated to dissolve the benzylamine salt of the dithiocarbamic acid, and ω-bromoacetophenone (5 g.) added. The *dithiocarbamate* slowly separated. It formed colourless prisms (5·2 g., 74%) from ethanol and had m. p. 140° (Found: C, 63·9; H, 5·1; N, 4·7. $C_{16}H_{15}ONS_2$ requires C, 63·8; H, 5·0; N, 4·7%).

3-*Benzyl*-4-*phenylthiazol*-2-*thione* (XIII; R = Ph, R′ = H).—*S*-Phenacyl *N*-benzyldithiocarbamate (1 g.) was heated at 150° till gas evolution had ceased (*ca.* 30 minutes). Recrystallised from ethanol the thiothiazoline had m. p. 101° (yield, quantitative) (Found: C, 67·7; H, 4·8; N, 5·1. Calc. for $C_{16}H_{13}NS_2$: C, 67·9; H, 4·6; N, 5·0%). The m. p. was undepressed on admixture with an authentic specimen (von Walther and Roch, *J. pr. Chem.*, 1913, [ii], **87**, 45).

S-(3-*Acetoxy*-1-*acetylpropyl*) N-*Benzyldithiocarbamate* (XII; X = S, R = CH_2·CH_2·OAc, R′ = Me).—A mixture of benzylamine (5 g.), water (50 c.c.), and aqueous sodium hydroxide solution (2 g. in 10 c.c.) was shaken with carbon disulphide (3·8 g.) till all had dissolved. The orange solution was filtered and vigorously shaken with 3-acetoxy-1-chloropropyl methyl ketone (9 g.), a solid gradually being deposited. The *dithiocarbamate* (9 g., 60%), recrystallised from ether, had m. p. 94° (Found: C, 55·2; H, 5·3; N, 4·6. $C_{15}H_{19}O_3NS_2$ requires C, 55·3; H, 5·8; N, 4·3%).

5-2′-*Acetoxyethyl*-3-*benzyl*-4-*methylthiazol*-2-*thione* (XIII; R = Me, R′ = CH_2·CH_2·OAc).—The foregoing dithiocarbamate (1 g.) was heated at 140° till gas evolution ceased (*ca.* 5 minutes). Recrystallised from ether, the *thiazolthione* had m. p. 88° (yield, quantitative) (Found: C, 59·0; H, 5·6; N, 4·9. $C_{15}H_{17}O_2NS_2$ requires C, 58·6; H, 5·5; N, 4·6%).

3-*Acetoxy*-1-*acetylpropyl* N-(4-*Amino*-2-*methyl*-5-*pyrimidylmethyl*)*dithiocarbamate* (XIV; R = NH_2, X = S).—4-Amino-5-aminomethyl-2-methylpyrimidine hydrochloride (5·3 g.) was dissolved in water (50 c.c.), and potassium hydroxide (4·2 g.) in a little water was added, followed by carbon disulphide (1·9 g.), and the mixture was vigorously shaken until homogeneous. The orange solution was filtered to remove a little amorphous solid that had separated, 3-acetoxy-1-chloropropyl methyl ketone (4·5 g.) was added, and the mixture was shaken vigorously. Solid began to separate after 30 minutes; it was collected and crystallised from aqueous methanol, giving the *dithiocarbamate* as colourless plates (5·3 g., 60%), m. p. 250° (decomp.) (Found: C, 47·2; H, 5·3; N, 16·1. $C_{14}H_{20}O_3N_4S_2$ requires C, 47·2; H, 5·6; N, 15·8%). Treatment of the dithiocarbamate with mercuric oxide or hydrogen peroxide failed to convert it into the monothiocarbamate.

When the dithiocarbamate (1 g.) was refluxed in dry ethanol (50 c.c.) for 4 hours and the solution concentrated in a vacuum and set aside in an ice-chest, the separated solid, after recrystallisation from aqueous ethanol, was identical [m. p. and mixed m. p. 276° (decomp.)] with 1 : 2 : 3 : 4-tetrahydro-7-methyl-2-thio-1 : 3 : 6 : 8-tetra-azanaphthalene (XV; X = S) (Bergel and Todd, *J.*, 1938, 26).

S-(3-*Acetoxy*-1-*acetylpropyl*) N-(4-*Amino*-2-*methyl*-5-*pyrimidylmethyl*)*thiocarbamate* (XIV; R = NH_2, X = O).—4-Amino-5-aminomethyl-2-methylpyrimidine hydrochloride (5·3 g.) was dissolved in water (50 c.c.), potassium hydroxide (4·2 g.) in a little water was added, and carbon oxysulphide passed in from a generator for 45 minutes, the solution being continuously shaken. The solution was filtered to remove a little precipitated solid, and 3-acetoxy-1-chloropropyl methyl ketone (4·5 g.) added. The mixture was then vigorously shaken until a homogeneous solution resulted (2 hours), neutralised with acetic acid, and set aside in the ice-chest till solid separated. The *thiocarbamate* crystallised from methanol in colourless prisms (4·9 g., 58%), m. p. 240° (softening and gas evolution at 165°) (Found: C, 49·9; H, 5·9; N, 16·6. $C_{14}H_{20}O_4N_4S$ requires C, 49·5; H, 5·9; N, 16·5%).

The thiocarbamate (1 g.) was dissolved in dry ethanol (40 c.c.), and the solution gently refluxed for 4 hours, concentrated in a vacuum, and set aside in the ice-chest. The separated solid, after recrystallisation from water, was found to be sulphur-free and identical (m. p. and mixed m. p. 290°) with 1 : 2 : 3 : 4-tetrahydro-7-methyl-2-keto-1 : 3 : 6 : 8-tetra-azanaphthalene (XV; X = O). Similar results were obtained on varying the solvent employed.

1 : 2 : 3 : 4-*Tetrahydro*-7-*methyl*-2-*keto*-1 : 3 : 6 : 8-*tetra-azanaphthalene* (XV; X = O).—4-Amino-5-aminomethyl-2-methylpyrimidine hydrochloride (3 g.) was dissolved in water (20 c.c.), and potassium cyanate (3 g.) added. After 30 minutes, the solution was evaporated to dryness in a vacuum and the resulting solid heated to 280° for 15 minutes, no further ammonia being evolved. Recrystallised from water (charcoal), the *compound* formed colourless prisms, m. p. 290° (Found: C, 51·1; H, 4·8; N, 34·2. $C_7H_8ON_4$ requires C, 51·2; H, 4·9; N, 34·2%).

S-(3-*Acetoxy*-1-*acetylpropyl*) N-(4-*Hydroxy*-2-*methyl*-5-*pyrimidyl*)*methyldithiocarbamate* (XIV; R = OH, X = S).—5-Aminomethyl-4-hydroxy-2-methylpyrimidine hydrochloride (7 g.) was dissolved in water (50 c.c.), and sodium hydroxide (3·2 g.) in a little water was added. After addition of carbon disulphide (3·1 g.), the solution was shaken vigorously for 2 hours; 3-acetoxy-1-chloropropyl methyl ketone (7·3 g.) was then added and the shaking continued. The solution was extracted with ether to remove a little unchanged chloro-ketone and then brought to pH 7 by adding dilute acetic acid. After being kept overnight at 0°, the *dithiocarbamate* (8 g., 57%) was filtered off and recrystallised (charcoal) from aqueous ethanol; it had m. p. 174° (decomp.) (Found: C, 47·3; H, 5·3; N, 11·9. $C_{14}H_{19}O_4N_3S_2$ requires C, 47·1; H, 5·3; N, 11·8%).

5-2′-*Acetoxyethyl*-3-(4-*hydroxy*-2-*methyl*-5-*pyrimidyl*)*methyl*-4-*methylthiazol*-2-*thione* (XVI; X = S).—The above dithiocarbamate (0·5 g.) was heated at 185° till gas evolution ceased (*ca.* 15 minutes). The

pale yellow liquid set to a mass of crystals on cooling. Recrystallised from aqueous ethanol, the *thiazolthione* (0·41 g., 85%) had m. p. 185—186° (Found: C, 50·0; H, 5·1; N, 12·4. $C_{14}H_{17}O_3N_3S_2$ requires C, 49·6; H, 5·0; N, 12·4%).

S-(3-*Acetoxy*-1-*acetylpropyl*) N-(4-*Hydroxy*-2-*methyl*-5-*pyrimidyl*)*methylthiocarbamate* (XIV; R = OH, X = O).—5-Aminomethyl-4-hydroxy-2-methylpyrimidine hydrochloride (7 g.) was dissolved in water (50 c.c.), and sodium hydroxide (3·2 g. dissolved in a little water) was added. A stream of carbon oxysulphide was then passed through the solution for 40 minutes, by which time one equivalent had been absorbed. 3-Acetoxy-1-chloropropyl methyl ketone (7·3 g.) was added and the mixture shaken vigorously. The solution, which was now at pH 7, was filtered to remove some insoluble material, concentrated under reduced pressure to less than half its original bulk, and set aside. The yellow solid which separated was collected, dissolved in hot *n*-butanol, and re-precipitated by addition of ether. This process was repeated several times but the crude *thiocarbamate* (5 g.) could not be further purified. It had m. p. 130° (decomp.), followed by solidification and re-melting at *ca.* 175° (Found: N, 12·6. $C_{14}H_{19}O_5N_3S$ requires N, 12·3%).

5-2′-*Acetoxyethyl*-3-(4-*hydroxy*-2-*methyl*-5-*pyrimidyl*)*methyl*-4-*methylthiazol*-2-*one* (XVI; X = O).—The above crude thiocarbamate (0·3 g.) was heated to 150° till gas evolution ceased (15 minutes). The pale brown oil solidified when allowed to cool. Recrystallised from 95% ethanol (charcoal), the *thiazolone* formed colourless prisms, m. p. 180° (Found: C, 52·4; H, 5·5; N. 12·7. $C_{14}H_{17}O_4N_3S$ requires C, 52·1; H, 5·3; N, 13·0%).

Oxidation of the Aneurin ψ-Base.—Attempts were made to obtain the "aneurin thiazolone" (IV) by carrying out experiments in which any transiently formed aneurin ψ-base might be expected to undergo oxidation. Dropwise addition of a mixture of oxidising agent and one equivalent of alkali to a solution of aneurin in one equivalent of alkali, or of one equivalent of alkali to a mixture of aneurin and oxidising agent dissolved in one equivalent of alkali, failed to yield any trace of thiazolone. The product was always thiochrome or aneurin disulphide, depending on the oxidising agent used.

Oxidation of Aneurin (II).—Two portions of aneurin (0·005 g.) were treated with 2 and 3 equivalents of sodium hydroxide respectively, followed by excess of potassium ferricyanide. The thiochrome produced was extracted with *iso*butanol, and the solution was diluted, and the products were estimated in the usual way. The more alkaline solution showed approximately twice as much thiochrome as that to which only 2 equivalents of alkali had been added.

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