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Concerning Amino-acids, Peptides, and Purines.

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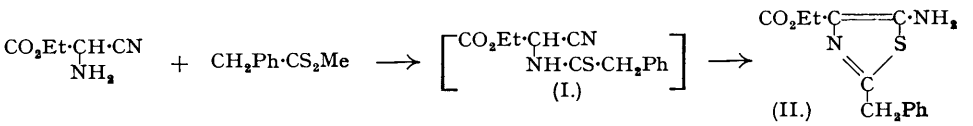
INTRODUCTION.

DURING an attack, with Dr. A. H. Cook and younger colleagues, upon the difficult problem of investigating possible routes for the synthesis of penicillin or biologically active analogues of it, a new reaction of α-amino-nitriles was discovered which provided an easy route to many hitherto inaccessible thiazole derivatives. At the same time some quite unexpected aspects of the chemistry of these compounds was revealed. Although the thiazole ring exhibits aromatic character, we have found in fact that suitably substituted thiazoles undergo a series of remarkable transformations, with the formation of further heterocyclic systems, hitherto accessible only with difficulty, whilst again other members undergo ready ring-fission leading to α-amino-acids or near derivatives thereof. This versatility has been turned to advantage in developing during the past three years new and general synthetic routes to amino-acids, peptides, and purines, the outstanding merit of which is that almost every step may be conducted at room temperature. The purpose of this Address then is to survey some of these new and flexible methods and to draw attention to the unity behind many diverse naturally occurring nitrogenous compounds, which has hitherto mostly escaped notice.

At the outset I would like to emphasize that the work I am about to describe is a team effort in which Dr. Cook has been responsible for the detailed development of the researches as a whole. I would like also to mention the name of Dr. A. L. Levy who has been concerned with the project since its initiation and has largely contributed to the work on peptides, and also that of Dr. J. D. Downer for his work in connection with the development of the purine syntheses. Finally I gratefully record the loyal co-operation of many others including Drs. J. D. Billimoria, C. W. Capp, J. R. Catch, R. Chatterjee, G. Harris, S. F. Macdonald, A. P. Mahadevan, G. Shaw, E. S. Stern and, among more recent collaborators, Messrs. A. C. Davis, G. D. Hunter, J. R. A. Pollock, E. Smith, and G. H. Thomas.

SOME 5-AMINOTHIAZOLES AND THEIR REACTIONS.

The origin of the researches under discussion was the observation that, when ethyl amino cyanoacetate was stood with methyl phenyldithioacetate, methanethiol was evolved. In place, however, of the expected thioamide (I), a basic product was obtained, in excellent yield,



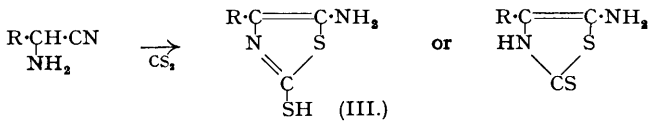
which proved to be ethyl 5-amino-2-benzylthiazole-4-carboxylate (II) (Bentley, Catch, Cook, Heilbron, and Shaw, CPS 267). This has now been found to be representative of a general reaction between α-amino-nitriles and dithio-acids or their esters (Studies in the Azole Series, Part I, Cook, Heilbron, and Levy, *J.*, 1947, 1594; Part XVII, Cook, Heilbron, and Smith, *J.*, this vol. p. 1440). Thioamides are undoubtedly intermediates in this synthesis and have been isolated in certain cases.

Whilst 2-aminothiazoles are among the best known of all thiazole compounds, 5-aminothiazoles have hitherto been almost unknown. It is only recently that Jensen and Hansen (*Dansk Tidskr. Farm.*, 1943, **17**, 189) and Ganapathi and Venkataraman (*Proc. Indian Acad. Sci.*, 1945, **22**, A, 343), stimulated by the valuable properties of sulphathiazole, a 2-aminothiazole derivative, prepared comparable 5-aminothiazole compounds by using orthodox reactions such as the Curtius degradation of ethyl thiazole-5-carboxylates; however, these reactions were found to be not generally applicable and the starting materials were often difficultly accessible. The only other previously known 5-aminothiazoles were " chrysean " and its derivatives. " Chrysean " was first obtained in 1874 by Wallach as a product of the interaction of hydrogen sulphide and aqueous sodium cyanide (Beilstein's " Handbuch," Vol. **27**, 334; Arnold and Scaife, *J.*, 1944,

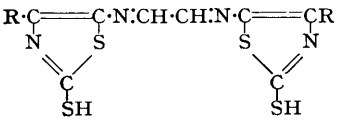
103), its formulation as 5-amino-2-thiocarbamylthiazole having only now received confirmation from the work of Erlenmeyer, Mengisen, and Prijs (*Helv. Chim. Acta*, 1947, **30**, 1865).

The 5-aminothiazoles are essentially aromatic in character, resembling the deactivated *o*- and *p*-nitroanilines. Thus they can be diazotised, acylated, condensed with aldehydes or ketones to give Schiff's bases, etc. Like the nitroanilines they lose the amino-group as ammonia by hydrolysis. This grouping exerts an activating influence on thiazoles containing an unsubstituted 4-position, where coupling with diazonium salts is readily effected.

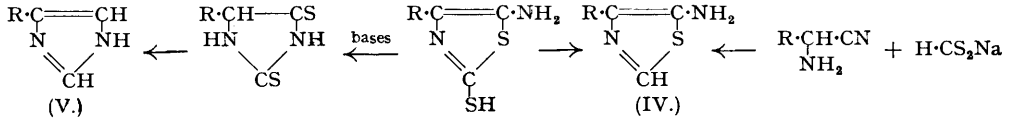
More arresting than the compounds so far described are the related compounds obtained by the use of carbon disulphide as a thioacylating agent in place of a dithio-acid or -ester. Interaction with α-amino-nitriles occurs at room temperature to afford the highly crystalline 5-amino-2-mercaptothiazoles (III) (Part II, Cook, Heilbron, and Levy, *J.*, 1947, 1598; Part X, Cook,



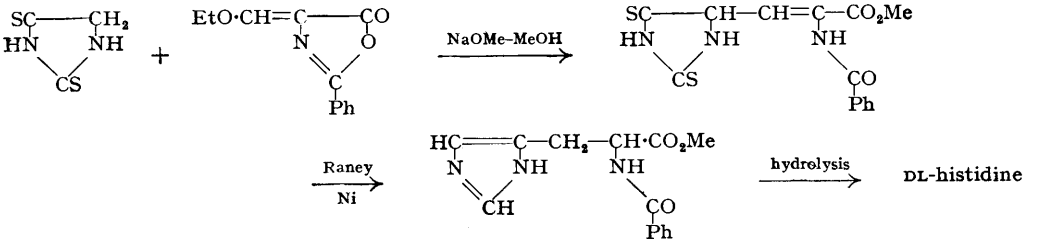
Heilbron, and Stern, *J.*, 1948, 2031). In many ways these bases afford functional derivatives which call for no particular mention, while in other ways their behaviour differs from that which might be expected. For instance, they condense with aldehydes and ketones with remarkable ease to give the highly crystalline Schiff's bases. Noteworthy is the reaction with glyoxal whereby deep red bis-compounds are obtained :



the appearance of which offers a valuable diagnostic test for 5-aminothiazoles. The most interesting reaction of the 5-amino-2-mercaptothiazoles is that which occurs when these compounds are allowed to react with aqueous alkalis or organic bases : molecular rearrangement ensues with formation of the isomeric 2 : 4-dithiohydantoins. The dithiohydantoins are strongly

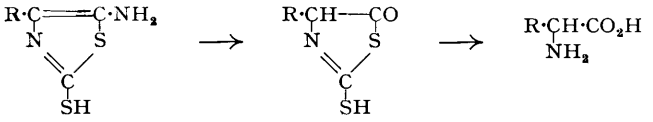


acidic substances from which the sulphur atoms can be readily removed with Raney nickel. It may be noted that when the precursor thiazoles are treated with Raney nickel only the extra-nuclear sulphur atom is removed, with the formation of 5-aminothiazoles (IV) identical with those obtained directly by the interaction of α-amino-nitriles with sodium dithioformate. With the rearranged dithiohydantoins, however, both sulphur atoms are removed to give the corresponding 5(4)-substituted glyoxalines (V). This provides a new general route to such monosubstituted glyoxalines, and a novel synthesis of DL-histidine has been effected along these lines (Davis and Levy, *J.*, in the press) :



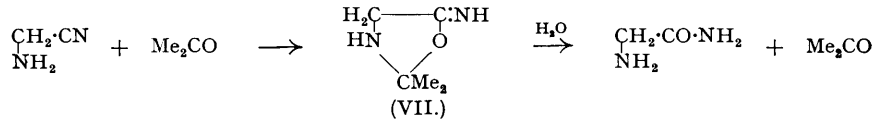
I revert now to the chemical transformation of the 5-amino-2-mercaptothiazoles : they are readily broken down by dilute mineral acids to give the corresponding α-amino-acids in good yield. This is frequently an improvement when preparing amino-acids by the Strecker route as the highly crystalline thiazoles can usually be isolated more conveniently and in better yield

than the free amino-nitriles (see, for example, Catch, Cook, Graham, and Heilbron, *J.*, 1947, 1609). The fact that α-amino-amides are not isolated from this hydrolysis suggests that the



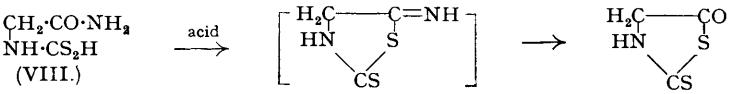
(VI.)

initial reaction is removal of the 5-amino-group to give a thiazolone (VI) which then loses carbon disulphide. Indeed the parent member of this aminothiazole series is converted into 2-mercaptothiazole (VI; R = H) with remarkable ease. The last compound, itself an isomeride of rhodanine, is the first representative of a new class of heterocyclic substances, and is more conveniently prepared by a series of novel reactions which were discovered accidentally during a study of the liberation of aminoacetonitrile from its sulphate (Part III, Cook, Heilbron, and Levy, *J.*, 1948, 201). It was found that suspending the sulphate in acetone and treating the suspension with sodium ethoxide, followed by carbon disulphide, unexpectedly gave a salt of the dithiocarbamic acid from aminoacetamide (VIII), which on acidification gave 2-mercaptothiazolone. It subsequently transpired that the mechanism of these surprising reactions involved condensation of the aminonitrile with the solvent, acetone, to give in quantitative yield a crystalline solid, probably 5-imino-2 : 2-dimethyloxazolidine (VII), which was readily split, again quantitatively, by cold water to aminoacetamide and acetone :



(VII.)

These reactions have since been found to be general with respect to both the amino-nitrile and the ketone components. Finally dithiocarbamates from α-amino-amides generally have been found to afford thiazolones on acidification, *e.g.*,

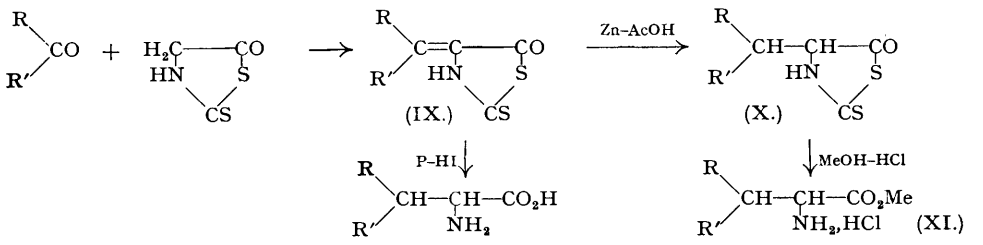


(VIII.)

AMINO-ACIDS.

A number of features of the parent thiazolone just described have been turned to advantage in devising new syntheses of α-amino-acids and their near derivatives.

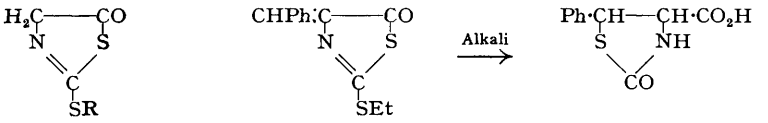
Like rhodanine, hydantoin, and 2-substituted oxazolones, 2-mercaptothiazolone exhibits a reactive methylene group which may be condensed with a wide variety of aldehydes and ketones. Though these condensations may in many instances be carried out in hot acid media, it is a special feature of the thiazolones that such condensations are very easily effected in presence of inorganic or organic weak bases, the alkylidene or arylidene products (IX) being obtained in excellent yield substantially at room temperature (Billimoria and Cook, Part XIX, in the press) :



The double bond in these compounds can be reduced by means of zinc and acid, thereby giving substituted derivatives (X) of the parent compound. Although the whole range of thiazolones has not yet been fully studied, enough has been established to show that their ring stability is generally similar to that of the unsubstituted thiazolone. For example, they are converted by

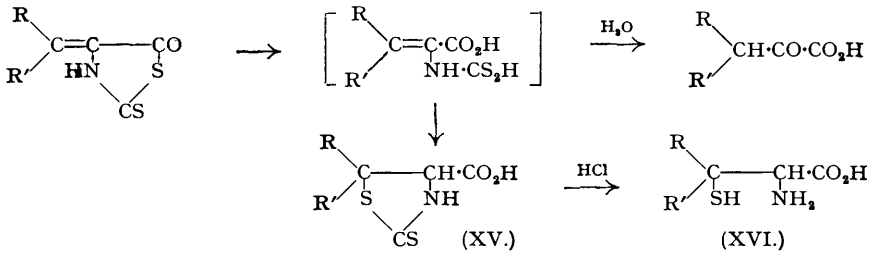
means of methanolic hydrogen chloride into α-amino-acid ester hydrochlorides (XI). Moreover, the unsaturated thiazolone derivatives may be reduced and the ring opened in a single stage. Thus with phosphorus and hydrogen iodide as the reducing agent the formation of α-amino-acids is readily achieved. In this manner β-phenylalanine, tyrosine, valine, leucine, norleucine, and other amino-acids have been obtained in good yield.

In addition to the free mercaptothiazolones a variety of 2-alkylthiothiazolone derivatives (XII) had earlier been studied (Cook, Harris, Heilbron, and Shaw, Part IV, *J.*, 1948, 1056) with generally similar results. It had, moreover, been observed that, when 2-ethylthio-4-benzylidene-5-



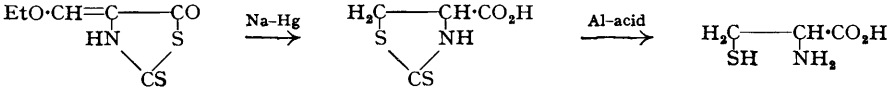
(XII.) (XIII.) (XIV.)

thiazolone (XIII) was treated with aqueous ethanolic alkali, 5-phenyl-2-thiazolidone-4-carboxylic acid (XIV) was obtained. On acid hydrolysis the latter product afforded β-phenylcysteine (Cook, Harris, and Heilbron, Part V, *J.*, 1948, 1060), and a precisely similar series of reactions was used to provide ββ-dimethylcysteine or penicillamine (Catch, Cook, Harris, and Heilbron, CPS 678). It has recently been observed that 2-mercapto-4-alkylidene- or -4-arylidene-thiazolones similarly rearrange in presence of sodium hydroxide, sodium alkoxides, or primary or secondary amines, giving respectively 5-substituted 2-thiothiazolidone-4-carboxylic acids (XV), esters, or *N*-substituted or unsubstituted amides. The mechanism of their origin was indicated by the formation in aqueous media of substituted pyruvic acids :



(XV.) (XVI.)

When the thiothiazolidones were heated with hydrochloric acid they were converted into substituted cysteines (XVI), many of which have so been synthesised. Cysteine itself was obtained by a modified procedure whereby 2-mercaptothiazolone was condensed with ethyl orthoformate to give an ethoxymethylene derivative (XVII), which was reduced and rearranged to the desired thiothiazolidone (XVIII) by sodium amalgam (Chatterjee, Cook, Heilbron, and Levy, Part VII, *J.*, 1948, 1337).



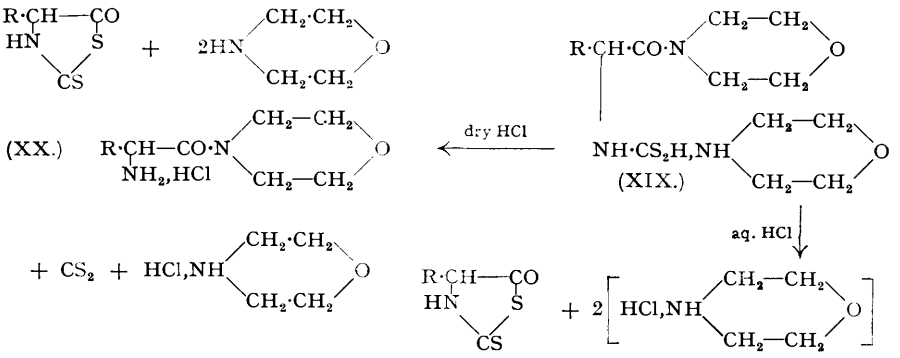
(XVII.) (XVIII.)

It is interesting to find that certain substituted cysteines, such as those derived by the above-mentioned routes from *cyclo*pentanone or *cyclo*hexanone, when condensed with 2-benzyl-4-ethoxymethyleneoxazolone under the conditions which synthesise benzylpenicillin in small yield, lead to the development of antibiotic activity which is presumably due to a small quantity of an analogue of benzylpenicillin. On the other hand, several β-arylcysteines have failed to afford antibiotic solutions under similar conditions and it may well be that the efficacy of substituted cysteines as precursors of penicillins is strictly limited by the substituents present.

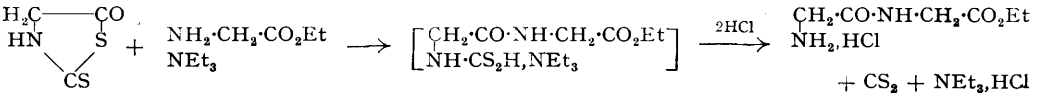
Peptides.

Whereas the reactions just outlined had their origin in the reactive methylene group of 2-mercapto-5-thiazolone or its near derivatives, those now to be reviewed depend more immediately on cleavage of the heterocyclic ring in these compounds. The earlier section of this work has been carried out entirely by Dr. A. L. Levy.

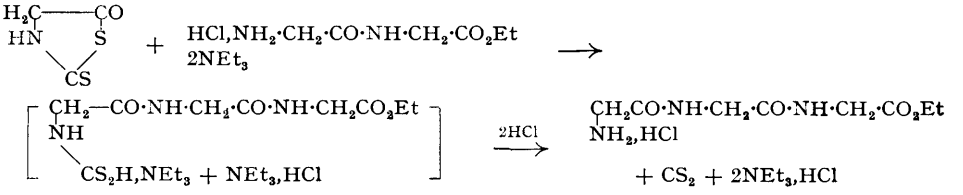
2-Mercapto-5-thiazolones are formally dithio-analogues of the carboxy-anhydrides of α-amino-acids, which have excited interest in many laboratories in recent years because of their ability to give rise to polypeptides. Such carboxy-anhydrides are mostly unstable and hardly permit of their ring-fission in a controlled manner except under acid conditions. By contrast the 2-mercaptothiazolones, while more stable, are still readily attacked by primary or secondary bases to give dithiocarbamate salts derived from the corresponding amide (*e.g.*, XIX) :



Whereas in presence of aqueous acids these revert to the original thiazolones, under anhydrous conditions carbon disulphide is liberated and the corresponding amides (XX) are obtained. By use of α-amino-esters this behaviour has, with modifications, been developed into a new method for the controlled synthesis of polypeptides. This may best be illustrated by reference to an example.

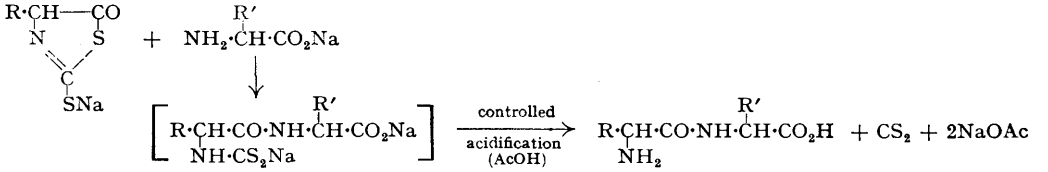


Treatment of 2-mercaptothiazolone with one equivalent each of glycine ethyl ester and triethylamine in ethanol causes rapid ring fission. Acidification of the solution with ethanolic hydrogen chloride without isolation of the intermediate dithiocarbamate salt leads to the separation of the hydrochloride of glycylglycine ethyl ester. This product is then used directly in presence of an additional molar quantity of triethylamine, liberating the dipeptide free base *in situ*, to attach another glycyl unit.



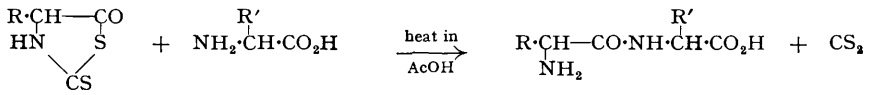
By further repetition of the operation the tetra- and penta-peptide esters have been prepared in crystalline form and in good yield.

With use of 2-mercaptothiazolone on the one hand, the method is applicable on the other to amino-esters generally, in both their racemic and their optically active forms. With 4-substituted 2-mercaptothiazolones the yields have so far been less satisfactory because acidification of the intermediate dithiocarbamate salt tends, even in non-aqueous media, to regenerate



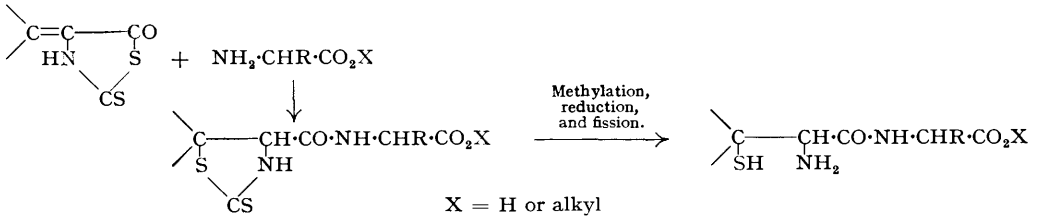
the original thiazolone. Incidentally, amino-acid and peptide ester hydrochlorides are very suitable for analysis by partition chromatography on paper, the above glycylpeptides, for example, forming a well-spaced series with butanol–acetic acid as the mobile phase, whereas under the same conditions the free peptides are closely crowded together.

The reaction of thiazolones with α-amino-acids in alkaline solution can conveniently be followed by measuring the marked decrease in pH which accompanies ring-fission. When acidification is then effected under carefully controlled conditions (*e.g.*, acetic acid at 70°) the desired peptide is produced, mixed invariably with its component amino-acids. Cation exchange resins have been found suitable for the separation of such mixtures, and for the isolation of the crystalline peptide.



A simpler procedure involves heating a thiazolone with an α-amino-acid in glacial acetic acid or water for a few moments; the peptide is produced, though again contaminated with some free amino-acid. Unlike the ester method, this is least satisfactory with the parent thiazolone (R = H), some of the lower polyglycyl peptides being simultaneously produced. Once again paper chromatography has proved of very great assistance in these studies for demonstrating the presence of the expected peptides. Though the tendency of the dithiocarbamic derivatives of peptides to lose the terminal amino-acid unit as a thiazolone may limit the synthetic usefulness of the series of reactions, it may in complementary fashion provide a simple means of recognising those terminal units in preformed peptides; this possibility is at present under investigation.

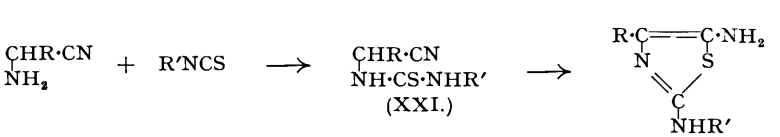
It will be recalled that 2-mercapto-4-alkylidene- or 2-mercapto-4-arylidene-thiazolones undergo rearrangement in presence of bases to give derivatives of 2-mercaptothiazolidone-4-carboxylic acids from which a variety of α-amino-β-mercapto-acids was prepared. This general reaction now appears to be of still more useful flexibility in that the rearrangement is brought about simply by salts or esters of α-amino-acids.



In this way peptides derived from substituted cysteines are obtained, and their interest as precursors of penicillins and glutathione analogues is at present being examined.

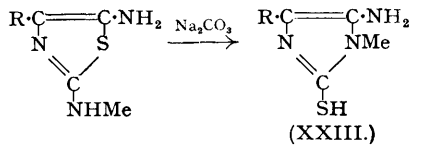
Purines.

The facility with which derivatives of glyoxalines can be obtained from α-amino-nitriles suggested that such glyoxalines might form the means of preparing purines in variety by closing the six-membered ring on to the 4 : 5-positions of the glyoxaline system. A few syntheses of this general kind are known (literature : Sarasin and Wegmann, *Helv. Chim. Acta*, 1924, **7**, 713; Montequi, *Anal. Soc. Fís. Quím.*, 1927, **25**, 182; Mitter and Chatterjee, *J. Ind. Chem. Soc.*, 1934, II, 867; Allsebrook, Gulland and Story, *J.*, 1942, 232; Mann and Porter, *J.*, 1945, 751; Baxter and Spring, *ibid.*, p. 232), but difficultly accessible intermediates were used. The work now to be reviewed has entirely overcome such disadvantages and provides a route for the synthesis of a great variety of purines, many of which were hitherto unknown.

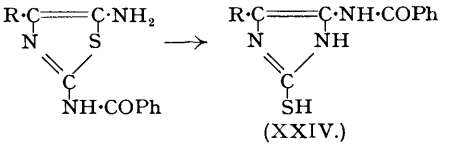


As a link between the 5-aminothiazoles of the earlier work and the desired 5-aminoglyoxalines, the action of *iso*thiocyanates on α-amino-nitriles has been studied. In all the cases examined (R = H, Me, Ph, CO_2Et, or $CO{\cdot}NH_2$; R′ = Me, Ph, COPh, or CO_2Et) the ultimate product is a substituted 2 : 5-diaminothiazole (XXII); the acyclic thioureas (XXI) are intermediates and have been isolated in certain instances. This new series of 5-aminothiazoles is in many respects similar to that already discussed.

When warmed with aqueous sodium carbonate, these 5-aminothiazoles undergo molecular rearrangement to give 5-amino-2-mercaptoglyoxalines, the R′ grouping being finally located at position 1 or 5 according to whether it is a hydrocarbon residue (XXIII) or an acyl group (XXIV), respectively. 1-Acyliminazoles may be first formed in the latter case, and in view of the known

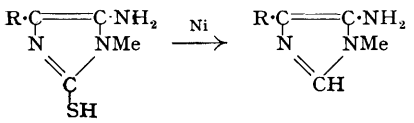


(XXIII.)



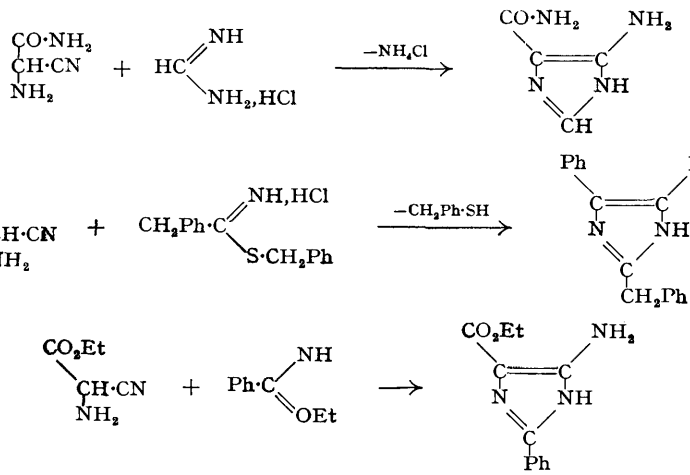
(XXIV.)

lability of such compounds it is probable that the acyl group then migrates to the more basic centre. Both types of rearrangement product are readily desulphurised by Raney nickel, and where R = H and R′ = CO_2Et (XXII) the nature of the rearrangement was confirmed by the emergence of the known 5-carbethoxyaminoglyoxaline (XXV) (Cook, Downer, and Heilbron, Parts VI and IX, *J.*, 1948, 1262, 2028; Capp, Cook, Downer, and Heilbron, Part VIII, *J.*, 1948, 1340; Cook, Heilbron, and Smith, Part XVII, p. 1440).



(XXV.)

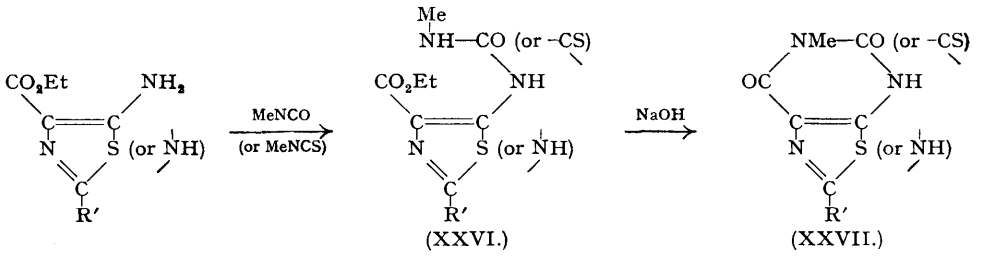
Still other and more direct syntheses of 5-aminoglyoxalines, which do not involve the intermediate formation of thiazoles, have been devised by condensing α-amino-nitriles with imino esters, thioimino-esters, or amidines. The following examples are representative of the compounds thus prepared (Cook, Davis, Heilbron, and Thomas, Part XIV, this vol., p. 1071) :



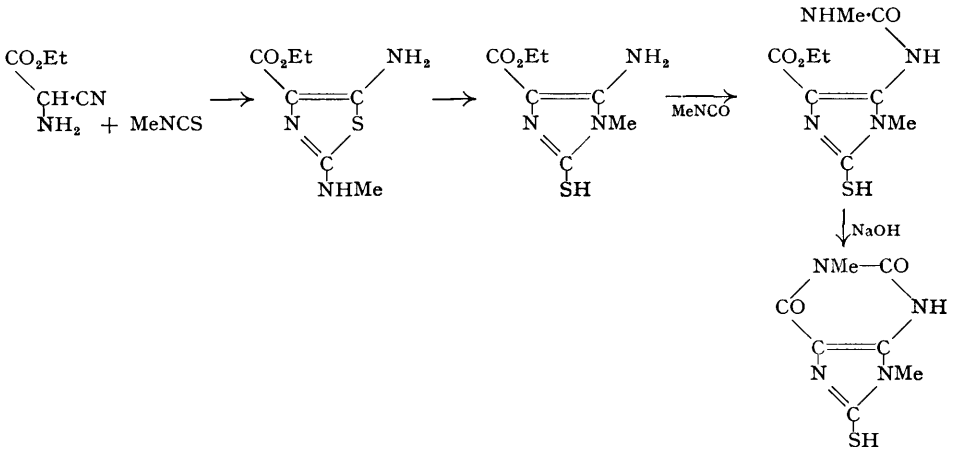
Like the thiazoles described earlier these glyoxalines reacted with *iso*thiocyanates or *iso*cyanates.

Somewhat surprisingly it was found that when ethyl 5-ureido-or 5-thioureidoazole-4-carboxylates (*i.e.*, glyoxalines or thiazoles) (XXVI) were allowed to stand in cold dilute aqueous

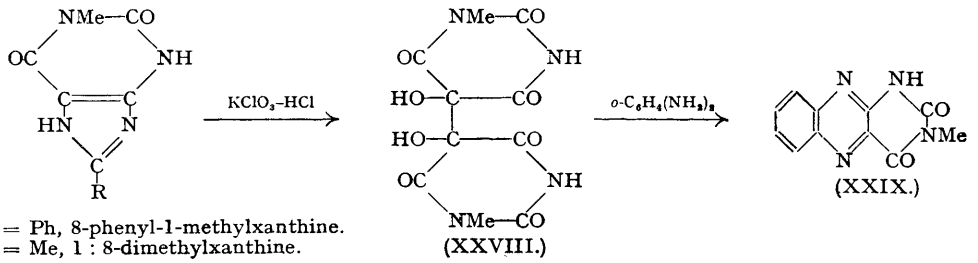
sodium hydroxide the elements of ethanol were eliminated, with simultaneous cyclisation to the corresponding purines or thiazolopyrimidines (XXVII), *e.g.*,



Though the physical and simple chemical properties of the products left little doubt of their bicyclic character, several independent proofs of the course of reaction have been revealed. Thus a direct comparison of authentic 1 : 9-dimethyl-8-thiouric acid with material prepared by the following stages shows their identity :

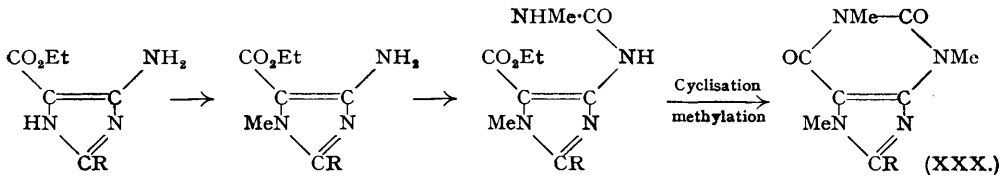


Again the presence of the pyrimidine ring has been proved in representative cases by oxidatively removing the glyoxaline moiety to give a known pyrimidine (XXVIII) and alloxazine (XXIX).



R = Ph, 8-phenyl-1-methylxanthine.
R = Me, 1 : 8-dimethylxanthine.

Finally by introducing methyl groups at appropriate points methylated purines arise, and in this way caffeine and several substituted caffeines (XXX) have been obtained :



(Cook, Davis, Downer, Heilbron, Macdonald, Mahadevan, and Thomas, Parts XII—XIV, this vol., pp. 1064—1074).

Recent developments in another field have lent an added significance to this work. 5-Amino-4-carbamylglyoxaline mentioned above appears to be an intermediate in the bacterial synthesis of purines and accumulates in cultures of *E. coli* undergoing sulphonamide bacteriostasis (Shrive, Ackermann, Gordon, Getzendaner, and Eakin, *J. Amer. Chem. Soc.*, 1947, **69**, 725). Thus the new synthetic route to purines just described appears to be, in outline at least, the method employed in Nature.

Many extensions of these reactions have been or are in the course of being worked out. Alternative syntheses of the pyrimidine ring have been devised by commencing with 5-amino-4 carbamylglyoxalines and inserting the last carbon atom by the agency of carbon disulphide, carbonyl chloride, ethyl orthoformate, and other compounds. Again progress has been made towards introducing sugar groupings, *e.g.*, by means of tetra-acetylglucose *iso*thiocyanate. Time does not permit of detailed discussion of these developments but it is hoped that sufficient has been unfolded to show that α-amino-acids and their near derivatives are, contrary to the view which chemists may sometimes have taken, far from being compounds of rather narrowly limited reactivity and synthetical scope.

Perhaps the simple connections between glycine and other α-amino-acids, glyoxalines, peptides, and purines here outlined may result in quickening interest in these compounds; maybe synthetic purines, which as a class have received such scant attention since the pioneering days of Emil Fischer, will come, with their obvious relations to biologically important compounds, to have an interest as drugs and in other ways comparable with that already attaching to many heterocyclic systems which have hitherto been more readily accessible.