754. *Coenzyme A. Part IV.* Derivatives of 2-Acetylthioethylamine as Acetylating Agents.*

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2-Acetylthioethylamine hydrochloride, *N*-2′-acetylthioethylacetamide (2-acetamidoethyl thiolacetate) (I), and the acetylated dipeptide (II) have been synthesised and their capacity to acetylate hydroxylamine in aqueous solution at room temperature has been investigated. All three substances reacted almost instantaneously under these conditions giving *N*-acetylhydroxylamine and the corresponding thiols. These experiments, which demonstrate the very reactive nature of the thiolacetate group in such substances, support the suggestion by Lynen and Reichert (*Angew. Chem.*, 1951, **63**, 47) that coenzyme A functions as an acetylating coenzyme in Nature by acetyl transfer at its terminal thiol group.

It was shown by Lynen and Reichert (*Angew. Chem.*, 1951, **63**, 47) that during enzymic acetylations involving coenzyme A an intermediate is formed in which the thiol group in the coenzyme is substituted, probably being present as a thiolacetate. The thiolacetate then transfers its acetyl group to the substrate. As a basis for the further study of these reactions a preliminary investigation of certain properties of related thiols seemed desirable. The terminal thiol group in coenzyme A is part of a 2-mercaptoethylamine residue in which the amino-group is involved in amide linkage with a substituted pantothenic acid (Snell, Brown, Peters, Craig, Wittle, Moore, McGlohon, and Bird, *J. Amer. Chem. Soc.*, 1950, **72**, 5349; Baddiley and Thain, *J.*, 1951, 2253). The chemistry of thiolacetates, particularly those derived from 2-mercaptoethylamine derivatives, has received little attention hitherto and in this paper we describe the preparation of some compounds of this type and their behaviour towards hydroxylamine.

2-Acetylthioethylamine hydrochloride was prepared readily by treatment of 2-mercaptoethylamine hydrochloride with an excess of acetyl chloride The amino-group was not acetylated under these conditions. A closer analogy to coenzyme A, however, is to be found in 2′-acetylthioacetamide (2′-acetamidoethyl thiolacetate) (I) where the basicity of the amino-group has been reduced by acetylation. This was formed by reaction of 2-mercaptoethylamine with acetic anhydride in pyridine. The acetylated dipeptide (II) possesses several features in common with coenzyme A. Besides the thiolacetate residue it contains the β-alanine amide structure and differs from the coenzyme only in that in place of the phosphorylated pantoic moiety an acetyl group has been substituted. It was prepared by treating acetyl-β-alanine methyl ester with 2-mercaptoethylamine and acetylating the resulting thiol with acetic anhydride. The free thiol was unstable in air, readily giving rise to the disulphide (III).

(I) $Me{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}S{\cdot}COMe$ $(Me{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}S)_2$ (III)

(II) $Me{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}S{\cdot}COMe$

2-Acetylthioethylamine hydrochloride was very unstable in aqueous solution. A fresh solution gave a negative reaction for thiol as determined by its stability towards an iodine solution. After the solution had been kept at room temperature for a few minutes, however, a strong positive thiol test was obtained, indicating fission of the thiolacetate bond. In neutral solution decomposition was also very rapid. The nature of the reaction products was not determined but it seems probable that two competing reactions were involved, namely, (i) hydrolysis to acetic acid and 2-mercaptoethylamine and (ii) transfer of the acetyl group from sulphur to nitrogen. The second reaction would tend to predominate in neutral or alkaline solution while the simple hydrolysis would occur mainly in acids. The amide (I) and the peptide (II), in both of which the basicity of the amino-group had been reduced by acetylation, were quite stable in neutral solution.

The three substances were examined by Dr. Lipmann as acetylating agents. He reports that all three reacted almost instantaneously with hydroxylamine in aqueous solution at room temperature, effecting *N*-acetylation. A little difficulty was experienced with 2-acetylthioethylamine hydrochloride in view of its instability in solution and for this reason acetylation by this substance was never quantitative. We have shown that (I) and (II) react readily with ammonia in aqueous solution at room temperature, liberating thiols.

* Part III, preceding paper.

These results indicate that certain thiolacetates, in particular those derived from 2-mercaptoethylamides, are effective acetylating agents and in this respect support the suggestion by Lynen and Reichert (*loc. cit.*) that coenzyme A brings about acetylation by acetyl transfer through its thiol group. Whether the final step in the biological acetylation process, namely, the transfer of acetyl from acetyl-coenzyme A to the substrate, is a non-enzymic (*i.e.*, spontaneous) reaction is not known.

The origin of the 2-mercaptoethylamine residue in the coenzyme is uncertain, but it would seem likely that it is derived from cysteine through decarboxylation. It is of interest that the neighbouring residue in coenzyme A, β-alanine, may have originated in a similar manner by decarboxylation of aspartic acid. While no evidence is available in connection with the first suggestion it has been shown that aspartic acid can be decarboxylated to β-alanine by certain bacteria (Ackermann, *Z. physiol. Chem.* 1907, **54**, 1; 1909, **60**, 482; Virtanen and Laine, *Enzymologia*, 1937, **3**, 266; Virtanen, Rintala, and Laine, *Nature*, 1938, **142**, 674). It appears that β-alanine is incorporated *per se* into pantothenic acid, and hence into coenzyme A, since pantoylaspartate is without activity as a substitute for pantothenic acid (Weinstock, May, Arnold, and Price, *J. Biol. Chem.*, 1940, **135**, 343). Whether 2-mercaptoethylamine is also incorporated *per se* into the coenzyme, or whether pantothenylcysteine could be utilised, is of considerable interest. In this connection and in other studies on biological precursors of coenzyme A the peptide (II) may provide a useful model.

Experimental.

2-Acetylthioethylamine Hydrochloride (*2-Aminoethyl Thiolacetate Hydrochloride*).—Finely powdered anhydrous 2-mercaptoethylamine hydrochloride (10 g.) was suspended in acetyl chloride (50 c.c.), and the mixture warmed gently. When evolution of hydrogen chloride had ceased the crystalline solid was removed by filtration, washed with ether, and recrystallised from alcohol (yield 7 g.). *2-Acetylthioethylamine hydrochloride* formed prisms, m. p. 146—147° (Found: C, 30·9; H, 6·2; N, 8·8; S, 20·6; Cl, 22·8. $C_4H_{10}ONSCl$ requires C, 30·9; H, 6·4; N, 9·0; S, 20·5; Cl, 22·8%).

2′-Acetylthioethylacetamide (*2-Acetamidoethyl Thiolacetate*).—Acetic anhydride (10·5 c.c.) was added to a solution of 2-mercaptoethylamine (3 g.) in anhydrous pyridine (40 c.c.). Heat was evolved during the initial stages of the reaction. Next morning methanol (10 c.c.) was added and the solution kept at room temperature for 30 minutes to permit decomposition of the excess of acetic anhydride. Solvent was removed by evaporation under reduced pressure and pyridine acetate removed by heating at 100°/*ca.* 0·1 mm. The syrupy residue was distilled under high vacuum. The main fraction (4 g.) was collected at 100°/10^{-5} mm. and crystallised in the receiver. *2′-Acetylthioethylacetamide* crystallised as long needles, m. p. 26—28° (Found: C, 44·2; H, 7·0; N, 8·6. $C_6H_{11}O_2NS$ requires C, 44·5; H, 6·8; N, 8·7%).

N-Acetyl-β-alanine Methyl Ester.—Dry, finely powdered β-alanine (8 g.) was added to acetic anhydride (25 c.c.), and the mixture heated at 100° for about 10 minutes, during which all solid had dissolved. Most of the acetic anhydride and acetic acid was removed by distillation under reduced pressure on a steam-bath and last traces of anhydride were decomposed by addition of water, then evaporation under reduced pressure. Sodium hydroxide (60 ml.; 2N) was added and after 30 minutes at room temperature sulphuric acid (2N) was added exactly equivalent to the alkali used. The solution was evaporated to dryness in a vacuum and the acetyl compound extracted from the residue with warm alcohol (100 c.c.). The filtered alcoholic solution was evaporated to dryness, leaving a clear syrup which did not crystallise. This was dissolved in methyl alcohol, then cooled to 5°, and an excess of ethereal diazomethane added. Solvents were removed by evaporation under reduced pressure and the remaining syrup distilled. After discarding of a small fraction boiling below 100°/0·6 mm., *acetyl-β-alanine methyl ester* was collected at 110—112°/0·6 mm., crystallising in the receiver, m. p. 20—25° (4·5 g.) (Found: C, 49·2; H, 7·7. $C_6H_{11}O_3N$ requires C, 49·6; H, 7·6%).

SN-Diacetyl-β-alanyl-2-mercaptoethylamine [*β-Acetamido-N-(2-acetylthioethyl)propionamide*] (II).—A mixture of 2-mercaptoethylamine (2 g.) and acetyl-β-alanine methyl ester (1·1 g.) was heated for 8 hours in an atmosphere of coal gas at 100°. The reaction mixture solidified on cooling. The product, which consisted largely of the monoacetyl dipeptide, melted at about 110° but was not characterised further since it oxidised readily in air to the disulphide (III). After being washed with ether it was heated for 1 hour on a steam-bath with acetic anhydride (about 20 c.c.). The reaction mixture was evaporated to a syrup under reduced pressure, the residue kept in methyl alcohol for 30 minutes, evaporated to dryness, and extracted with hot ethyl acetate. The insoluble part consisted of *di-*[*2-(β-acetamidopropionamido)ethyl*] *disulphide* (III), m. p. 190° (Found: C, 44·7; H, 7·1; N, 14·9; S, 17·3. $C_{14}H_{26}O_4N_4S_2$ requires C, 44·4; H, 6·9; N, 14·9; S, 16·9%). The ethyl acetate solution was evaporated to small volume and cooled. The *acetyl dipeptide* (II) crystallised out as rosettes, m. p. 131—132°, after recrystallisation from ethyl acetate (Found: C, 46·6; H, 7·2; N, 11·7; S, 13·7. $C_9H_{16}O_3N_2S$ requires C, 46·6; H, 6·9; N, 12·0; S, 13·8%).

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