## 402. *Peptides. Part XIV.*[1] *Thiazoleamino-acids, Degradation Products of Thiostrepton.*

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Hydrolysis of the antibiotic thiostrepton has yielded alanine, threonine, isoleucine, cystine, and two new amino-acids, 2-aminomethylthiazole-4-carboxylic acid (Ia) and 2-1′-aminopropylthiazole-4-carboxylic acid (Ic). The relationship of these and other sulphur-containing degradation products is discussed in terms of possible structural features of the antibiotic. A general synthetical route to thiazole amino-acids of the general formula (I) has been applied to the synthesis of six members of the series.

The isolation, and chemical and biological properties of the antibiotic thiostrepton were described in reports from the Squibb Institute for Medical Research in 1956.[2-4] A subsequent study of the sensitivity of 289 pathogenic organisms to thiostrepton confirmed the earlier promising biological results, and suggested that thiostrepton had a useful spectrum of antibiotic activity which warranted much further consideration.[5] Through the kindness of the Squibb Institute, we have been able to examine further some of the chemical features of the antibiotic, and we report here some of our initial observations concerning the amino-acid content of thiostrepton, especially in relation to the relatively high sulphur content of the antibiotic.*

That thiostrepton was at least partially of a peptide nature was soon recognised by the Squibb group,[3] and a number of amino-acids liberated from the antibiotic by acidic hydrolysis were tentatively identified by paper chromatography. The identification of alanine, threonine, and isoleucine † was subsequently confirmed by ion-exchange chromatography, and a further peak on the chromatogram was tentatively regarded as phenylalanine.[8]

Studies on thiostrepton in this laboratory also began with an examination of the amino-acid content. Acidic hydrolysis resulted in the formation of alanine, threonine, isoleucine, and cystine, with lesser amounts of several other unidentified ninhydrin-reacting components. Quantitative analysis by the ion-exchange method of Moore and Stein [9] ‡ yielded peaks corresponding to alanine, threonine, and isoleucine (molecular ratio 2·0 : 0·95 : 0·89; minimum molecule weight 1600), together with cystine (not analysed quantitatively by the technique used); a further peak corresponded more closely

* A preliminary account of part of this work has been published.[6]

† These amino-acids together with cystine have recently been isolated from thiostrepton. The cystine is in the D-form.[7]

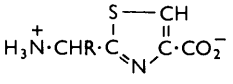
‡ We are grateful to Dr. Emil L. Smith for performing this analysis.

[1] Part XIII, *Tetrahedron*, 1963, **19**, 95.
[2] Pagano, Weinstein, Stout, and Donovick, "Antibiotics Annual 1955—56," Medical Encyclopædia, Inc., New York, p. 554.
[3] Vandeputte and Dutcher, *ibid.*, p. 560.
[4] Steinberg, Jambor, and Suydam, *ibid.*, p. 562.
[5] Kutscher, Seguin, Rankow, and Piro, *Antibiotics and Chemotherapy*, 1958, **8**, 576.
[6] Kenner, Sheppard, and Stehr, *Tetrahedron Letters*, 1960, No. 1, 23.
[7] Bodanszky, Sheehan, Fried, Williams, and Birkhimer, *J. Amer. Chem. Soc.*, 1960, **82**, 4747.
[8] Dutcher, personal communication.
[9] Moore, Spackman, and Stein, *Analyt. Chem.*, 1958, **30**, 1185.

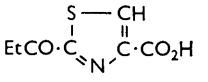
to the expected position for tyrosine rather than for the phenylalanine previously reported. Examination by paper chromatography in the benzyl alcohol–water system of Consden *et al.*[10] showed conclusively, however, that neither tyrosine nor phenylalanine was present in hydrolysates of the antibiotic, and prompted a careful search among the hydrolysis products for aromatic amino-acids which might appear on the Moore and Stein chromatogram close to the normal positions of tyrosine and phenylalanine.

Acidic and basic hydrolysates of thiostrepton were chromatographed on columns of activated charcoal. Elution with water yielded the expected mixture of aliphatic amino-acids. Elution with aqueous acetic acid containing phenol furnished the " aromatic fraction." In the case of thiostrepton hydrolysed with aqueous hydrochloric acid, this contained in low yield three ninhydrin reacting components with $R_F$ values of 0·05, 0·25, and 0·35.* A preliminary separation by paper chromatography showed that the components had similar ultraviolet absorption ($\lambda_{max.}$ *ca.* 230 mμ). A higher yield of the two substances with $R_F$ 0·05 and 0·25 was obtained when thiostrepton was hydrolysed first with alkali and then with acid; under these conditions the " aromatic fraction " contained in addition a third ninhydrin-reacting substance with $R_F$ 0·44. This too had the characteristic ultraviolet absorption maximum at *ca.* 230 mμ, and all four substances were clearly closely related.

Chromatography on ZeoKarb-225 cation-exchange resin effectively separated two components of the mixture, and yielded the substance of $R_F$ 0·25 as its crystalline hydrochloride, $C_5H_6N_2O_2S$,HCl, and the substance of $R_F$ 0·44 more easily crystallised as the free base, $C_7H_{10}N_2O_2S$. The water solubility, ninhydrin reaction, stability to further hydrolysis, and infrared spectra showed clearly that both compounds were amino-acid derivatives, although the atypical ninhydrin colours (yellow slowly turning purple) precluded their being of the normal α-amino-acid type. Since both were electrophoretically neutral at pH 6·5, the second nitrogen atom was present in a stable, effectively non-basic

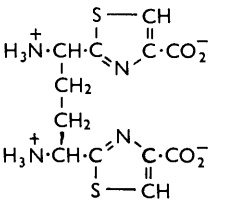


(Ia; R = H)
(Ib; R = Me)
(Ic; R = Et)
(Id; R = CHMe$_2$)
(Ie; R = CH$_2$·CHMe$_2$)



(II)

(If; R = CH$_2$Ph)
[Ig; R = CMe(OH)·CHMe·OH]



(III)

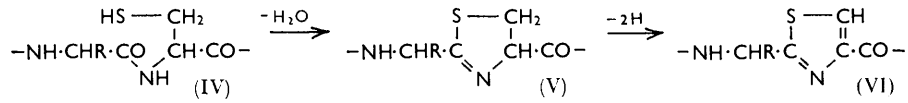
structure, probably in a heterocyclic ring. At this stage we were aided by the observation of Brookes *et al.*,[11] that acidic hydrolysis of micrococcin P., an antibiotic from a species of *Micrococcus*, yielded an amino-acid formulated by them as 2-1′-amino-2′-methylpropylthiazole-4-carboxylic acid (Id), and which had ultraviolet absorption ($\lambda_{max.}$ 234 mμ) identical with that of our pure products. We therefore considered that the aromatic amino-acids from thiostrepton were most likely members of the same homologous series, *viz.*, 2-aminomethylthiazole-4-carboxylic acid (Ia) hydrochloride and 2-1′-aminopropylthiazole-4-carboxylic acid (Ic), in agreement with the analytical results. Synthesis as described below, established the correctness of these two structures. The synthetic products (Ia) and racemic (Ic) were identical with the products derived from thiostrepton. Evidently complete racemisation of the aminopropylthiazole (Ic) occurs under the drastic conditions necessary for its liberation from the antibiotic.[12]

More recently, Bodanszky and his collaborators have isolated three further thiazole

* $R_F$ values in the text refer to solvent system A (Experimental section).

[10] Consden, Gordon, and Martin, *Biochem. J.*, 1944, **38**, 224.
[11] Brookes, Fuller, and Walker, *J.*, 1957, 689.
[12] Cf. Dean, Mijovic, and Walker, *J.*, 1961, 3394.

derivatives from thiostrepton.[7,13] These include 2-propionylthiazole-4-carboxylic acid (II), a substance previously encountered as a degradation product of micrococcin P.,[11] and which has also been isolated from thiostrepton in this laboratory, and two additional thiazole amino-acids corresponding to the substances mentioned above with $R_F$ 0·05 and 0·35. For the first of these (" thiostreptoic acid ") the structure (III) has been advanced.[7]



This follows from the oxidation of (III) to the corresponding diketone, identical with a synthetic sample. The second amino-acid (" thiostreptine ") has been formulated as the dihydroxythiazole amino-acid (Ig) on the basis of firm chemical and spectroscopic evidence.[13]

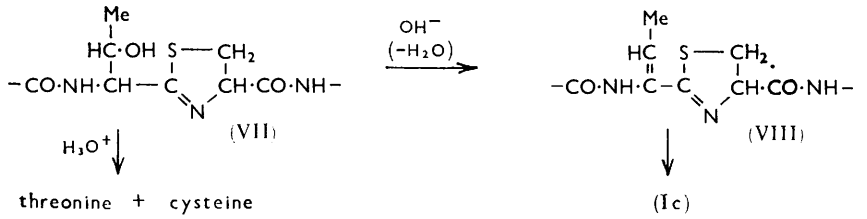
Thiostrepton has therefore yielded hydrolysis products containing six sulphur atoms in thiazole rings, in addition to the sulphur-containing amino-acid cystine (which is probably derived from cysteine initially liberated[7]). The origin of thiazole and thiazoline rings in the peptide antibiotics bacitracin,[14] micrococcin P,[11] and bottromycin [15] has been discussed in terms of biosynthetic condensation of cysteine-containing peptides (IV) to thiazolines (V) and thence, by oxidation, to thiazoles (VI).[16] Cleavage of the peptide bonds by hydrolysis then liberates the thiazoles (I) with side-chains characteristic of the amino-acids from which they were biogenetically derived. On this basis, the products from thiostrepton (Ia, Ic, Ig, and III) might simply be derived biogenetically from parts of a peptide chain (or chains) containing glycine, α-amino-n-butyric acid, βγ-dihydroxyisoleucine, and αα′-diaminoadipic acid residues respectively adjacent to cysteine residues. It has further been suggested that the ketothiazole (II) might arise as a secondary degradation product through oxidation of the corresponding thiazole amino-acid (Ic) or its precursor in the peptide chain.[7,11] Certainly in the case of thiostrepton which contains only five atoms of sulphur per molecule * considerable duplication of degradation products must have occurred, and the identical carbon skeletons of (Ic) and (II) suggest their similar genesis. However, as indicated briefly in our earlier communication,[6] we are also considering favourably alternative explanations based upon the expected modes of decomposition of α-amino-β-hydroxyalkylthiazole derivatives to account for the multiplicity of sulphur-containing degradation products from thiostrepton.

The activating effect of thiazole and thiazoline rings in their 2-alkyl derivatives is well known. This is illustrated by the condensation reactions with aldehydes and acid chlorides at the methyl groups of 2-methylthiazole [18] and 2-methylthiazoline,[19] as well as by deuterium-exchange studies.[20] Consequently, the common β-elimination and reverse aldol reactions associated with β-hydroxy-carbonyl compounds should take place also with 2-2′-hydroxyalkyl-thiazole and -thiazoline derivatives. That this is the case has been shown recently with the isolation and identification of thiostreptine (Ig) as a thiazole amino-acid of this type.[13] Thiostreptine is unstable to prolonged acidic hydrolysis, and

* Thiostrepton has a molecular weight of 1613 (±5%) as determined by a thermoelectric method,[17] in good agreement with the minimum molecular weight deduced from amino-acid analysis. The sulphur content is 9·5%.

[13] Bodanszky, Alicino, Birkhimer, and Williams, *J. Amer. Chem. Soc.*, 1962, **84**, 2003.
[14] Craig, Konigsberg, and Hill, " Amino-Acids and Peptides with Antimetabolic Activity," ed. Wolstenhome and O'Connor, Churchill, London, 1958, p. 226.
[15] Waisvisz, van der Hoeven, and te Nijenhuis, *J. Amer. Chem. Soc.*, 1957, **79**, 4524.
[16] For a review, see Arnstein, *Ann. Reports*, 1957, **54**, 339.
[17] Tomlinson, *Mikrochim. Acta*, 1961, **3**, 457.
[18] Erlenmeyer *et al.*, *Helv. Chim. Acta*, 1948, **31**, 1142.
[19] Kuhn and Drawert, *Annalen*, 1954, **590**, 55; Sheehan, Beck, Henery-Logan, and Ryan, *J. Amer. Chem. Soc.*, 1956, **78**, 4478.
[20] Erlenmeyer, Weber, and Wiessmer, *Helv. Chim. Acta*, 1938, **21**, 1017.

we have not detected its formation at all when thiostrepton is hydrolysed under basic conditions. The products of the acidic degradation have been identified as acetoin, acetaldehyde, and 2-aminomethylthiazole-4-carboxylic acid (Ia).[13] Clearly this decomposition is of the reverse aldol type and indicates that the thiazoles (Ia) and (Ig) stem from a common part of the thiostrepton molecule.




Certain features of the chemistry of thiostrepton suggest that the antibiotic contains also a 2-2′-hydroxyalkylthiazoline nucleus. The presence of a thiazoline ring in thiostrepton is in agreement with the negative disulphide and thiol tests, the positive thiol test after treatment with dilute acid under the same conditions as those in which the thiazoline ring of bacitracin is cleaved,[21] and the formation of cysteine on vigorous acidic hydrolysis. Thiazolines are more stable under basic conditions however, and it is significant that the 2-aminopropylthiazolecarboxylic acid (Ic) is obtained from thiostrepton only when the antibiotic is hydrolysed initially with alkali. We have not observed the formation of (Ic) when thiostrepton is hydrolysed with acid,* even with rigorous exclusion of oxygen to prevent possible oxidative deamination to the propionylthiazole (II). These results are readily accommodated by the presence in the antibiotic of a thiazoline system (VII) derived from adjacent threonine and cysteine residues. Under the normal conditions of acidic hydrolysis, such a system would regenerate threonine and cysteine, both of which are established hydrolysis products of thiostrepton. Under alkaline conditions, however, elimination of the β-hydroxyl group would be favoured, yielding the unsaturated thiazoline (VIII). This by subsequent prototropic shift could lead to the aminopropylthiazole derivative (Ic). It is possible that the system (VII) or the corresponding thiazole may also account for the formation of the propionylthiazole (II) from thiostrepton.

*Synthesis of α-Aminoalkylthiazole-carboxylic Acids* (I).—The Hantzsch synthesis of thiazoles from thioamides and α-halogenocarbonyl compounds affords a convenient general route to thiazole amino-acids of type (I). The method has been applied to the synthesis of six members of the series (Ia—f), corresponding in side-chain to glycine, alanine, α-amino-n-butyric acid, valine, leucine, and phenylalanine. After most of this work was complete, Brookes *et al.*[22] described the synthesis of (Ib) and the hydrochlorides of (Ia) and (Id) by similar methods. The synthesis of 2-(4-amino-4-carboxybutyl)thiazole-4-carboxylic acid, a degradation product of cephalosporin C, has also been recorded.[23]

The appropriate α-amino-nitriles were obtained by Strecker syntheses and converted into their benzoyl derivatives. (The *N*-acetyl derivatives were used in our early work,[6] but the benzoyl derivatives were easier to crystallise and purify throughout the series. The phthaloyl derivative was also prepared in one case.) These acylamino-nitriles are also accessible by dehydration of the appropriate acylamino-acid amides.[22] Hydrogen sulphide in aqueous ethanolic ammonia converted the acylamino-nitriles into the desired thioamides. The reaction failed with α-benzamido-β-phenylpropionitrile but conversion

* An early reported [7] isolation of (Ic) from acidic hydrolysates of the antibiotic has been corrected.[13]

[21] Lockhart, Abraham, and Newton, *Biochem. J.*, 1955, **61**, 534.
[22] Brookes, Clark, Majhofer, Mijovic, and Walker, *J.*, 1960, 925.
[23] Jeffery, Abraham, and Newton, *Biochem. J.*, 1960, **75**, 216.

into the thioamide was satisfactory with thioacetamide and anhydrous hydrogen chloride.[24] Condensation with ethyl bromopyruvate in boiling ethanol then afforded the fully protected thiazole amino-acids, from which the acyl and ester groups were removed by hydrolysis.

## Experimental

Ultraviolet spectra were measured for 95% ethanol solutions unless otherwise stated.

*Paper Chromatography.*—Solvent systems: A, butan-1-ol–ethanol–water–propionic acid (10 : 10 : 5 : 2); B, butan-1-ol–acetone–water–dicyclohexylamine (10 : 10 : 5 : 2).[25]

*Isolation of Thiazole Amino-acids from Thiostrepton.*—Thiostrepton (1 g.) was heated with 5N-sodium hydroxide (27 ml.) in a sealed tube at 100° for 4·5 hr. The hydrolysate was acidified, extracted with ethyl acetate, and evaporated *in vacuo*. The residue was dissolved in 5N-hydrochloric acid (25 ml.) and the solution heated in a sealed tube at 100° for 24 hr. The hydrolysate was evaporated and the residue dissolved in the minimum amount of water and applied to a column of activated charcoal (10 g.) mixed with Celite (10 g.). The column was washed with water until the eluate no longer gave a positive reaction with ninhydrin; it was then eluted with a solution of phenol (5%) and acetic acid (20%) in water. The eluate containing ninhydrin-positive material was collected, extracted several times with ether to remove phenol, and evaporated *in vacuo* to give a solid residue (158 mg.). This was combined with similar material (7 mg.) isolated from a previous acidic hydrolysis of thiostrepton, dissolved in N-hydrochloric acid and chromatographed on a column (10 cm. × 3 cm. diameter) of ZeoKarb-225 cation-exchange resin ($H^+$ form). The column was eluted with N-hydrochloric acid (flow rate 20—30 ml./hr., 10 ml. fractions collected). Ninhydrin-positive substances in the eluate were detected by spotting samples (0·01 ml.) on filter-paper strips which had been soaked in M-phosphate buffer (pH 6·8), and developing with ninhydrin. Fractions were also examined in the ultraviolet spectrophotometer at 235 mμ.

Fractions 68—83 were combined, and evaporated *in vacuo*. The residue (33 mg.), recrystallised twice from ether–ethanol, yielded 2-aminomethylthiazole-4-carboxylic acid hydrochloride, m. p. 263—265° (decomp.), $\lambda_{max.}$ 234 mμ (ε 5250 in $H_2O$) (Found: C, 31·9; H, 3·9; N, 14·7; inorganic residue, 2·4. Calc. for $C_5H_6N_2O_2S,HCl$: C, 30·85; H, 3·6; N, 14·4%). The $R_F$ values and infrared spectrum were identical with those of the synthetic substance, prepared as described below.

Fractions 86—110 were combined and evaporated. The hygroscopic residue (45 mg.) was dissolved in dilute acetic acid and poured through a column of Dowex-1 × 4 anion-exchange resin (acetate form). The eluate was evaporated and the residue (32·5 mg.) recrystallised twice from ethanol–ether to yield DL-2-1′-aminopropylthiazole-4-carboxylic acid, m. p. 255—257°, $\lambda_{max.}$ 234 mμ (ε 4970 in $H_2O$) (Found: C, 42·5; H, 5·9; N, 13·9; inorganic residue, 4·1. Calc. for $C_7H_{10}N_2O_2S$: C, 45·2; H, 5·4; N, 15·05%). The infrared spectrum and $R_F$ values were identical with those of the synthetic amino-acid.

*2-Amino-4-methylvaleronitrile Hydrochloride.*—A solution of potassium cyanide (52·1 g.) in water (130 ml.) was added dropwise with stirring to a cooled (5°) mixture of redistilled isovaleraldehyde (64 g.) in ether (410 ml.) and ammonium chloride (44·6 g.) in water (12 ml.). After the addition was complete, the mixture was stirred for a further 4 hr. at room temperature and the ether layer separated. The aqueous solution was washed twice with ether, the combined extracts were dried ($MgSO_4$) and dry hydrogen chloride was passed into the solution. The precipitated *nitrile* was recrystallised from ethanolic ether (30 g., m. p. 178° (Found: C, 48·9; H, 8·65; N, 19·15. $C_6H_{13}ClN_2$ requires C, 48·6; H, 8·8; N, 18·9%).

*2-Amino-3-phenylpropionitrile Hydrochloride.*—In an experiment similar to the above, phenylacetaldehyde (6 g.), ammonium chloride (3 g.), and potassium cyanide (3·5 g.) yielded the nitrile (2·15 g.), m. p. 168°, which was characterised as the benzoyl derivative.

*2-Benzamidobutyronitrile.*—2-Aminobutyronitrile hydrochloride[26] was benzoylated with benzoyl chloride and aqueous sodium hydroxide by the procedure of Klages and Haak.[27] The *product* (94%) had m. p. 105° (from aqueous ethanol) (Found: C, 70·2; H, 6·7; N, 15·0. $C_{11}H_{12}N_2O$ requires C, 70·2; H, 6·4; N, 14·9%).

*2-Benzamido-3-methylbutyronitrile.*—2-Amino-3-methylbutyronitrile hydrochloride,[28] m. p.

[24] Taylor and Zoltewicz, *J. Amer. Chem. Soc.*, 1960, **82**, 2656.
[25] Hardy, Holland, and Naylor, *Analyt. Chem.*, 1955, **27**, 971.
[26] Zelinsky and Stadnikoff, *Ber.*, 1908, **41**, 2061.
[27] Klages and Haak, *Ber.*, 1903, **36**, 1646.
[28] Lipp, *Annalen*, 1880, **205**, 9.

204° (sealed capillary), similarly yielded the benzoyl derivative (92%), m. p. 109—110° (from aqueous ethanol) (Found: C, 71·1; H, 6·9; N, 13·95. Calc. for $C_{12}H_{14}N_2O$: C, 71·3; H, 7·0; N, 13·85%). Brookes *et al.*[22] give m. p. 110° for material prepared from DL-valine.

*2-Benzamido-4-methylvaleronitrile.*—The *benzoyl derivative* (98%) had m. p. 111—112° (from aqueous ethanol) (Found: C, 72·0; H, 7·4; N, 12·9. $C_{13}H_{16}N_2O$ requires C, 72·2; H, 7·5; N, 12·95%).

*2-Benzamido-3-phenylpropionitrile.*—The *benzoyl derivative* (61%) had m. p. 151° (from ethanol) (Found: C, 76·9; H, 5·9; N, 11·3. $C_{16}H_{14}N_2O$ requires C, 76·8; H, 5·6; N, 11·2%).

*2-Benzamidobutyrothioamide.*—Hydrogen sulphide was passed for 16 hr. through a solution of 2-benzamidobutyronitrile (1·5 g.) in methanol (10 ml.) and ammonia solution (*d* 0·880; 2 ml.). The solution was filtered and evaporated, and the residue recrystallised from aqueous ethanol. The *thioamide* (1·25 g., 83%) had m. p. 182°, $\lambda_{max.}$ 226—228, 265—267 mμ (ε 12,100, 12,050) (Found: C, 59·2; H, 6·45; N, 12·9; S, 14·5. $C_{11}H_{14}N_2OS$ requires C, 59·45; H, 6·35; N, 12·6; S, 14·4%).

*2-Benzamido-3-methylbutyrothioamide.*—2-Benzamido-3-methylbutyronitrile (2·0 g.), ammonia solution (*d* 0·88; 2 ml.) and methanol (20 ml.), treated with hydrogen sulphide overnight, likewise yielded the thioamide, which (1·75 g., 74%) had m. p. 195—197° (from ethanol), $\lambda_{max.}$ 227, 268—272 mμ (ε 12,600, 11,850) (Found: C, 61·1; H, 6·8; N, 11·8; S, 13·75. Calc. for $C_{12}H_{16}N_2OS$: C, 61·0; H, 6·8; N, 11·9; S, 13·5%). Brookes *et al.*[22] give m. p. 202° (decomp.).

*2-Benzamido-4-methylvalerothioamide.*—A solution of 2-benzamido-4-methylvaleronitrile (15·0 g.) and ammonia solution (*d* 0·88; 20 ml.) in methanol (75 ml.) was treated with hydrogen sulphide during 16 hr. Most of the *product* crystallised, and more was recovered by vacuum concentration (total yield 14·9 g., 86%). After recrystallisation from aqueous ethanol, the m. p. was 187·5—188°, $\lambda_{max.}$ 226—230, 266—268 mμ (ε 11,350, 12,150) (Found: C, 62·6; H, 7·5; N, 11·2; S, 13·0. $C_{13}H_{18}N_2OS$ requires C, 62·4; H, 7·25; N, 11·2; S, 12·8%).

*2-Benzamido-3-phenylpropiothioamide.*—A mixture of 2-benzamido-3-phenylpropionitrile (1·25 g.) and thioacetamide (0·75 g.) in dry dimethylformamide (15 ml.) was saturated with hydrogen chloride and then heated for 30 min. on the steam-bath. The mixture was concentrated *in vacuo* to half volume and neutralised by the dropwise addition of aqueous sodium hydrogen carbonate solution. The crystalline *thioamide* (0·9 g.) was collected and recrystallised (from methanol); it had m. p. 190—192°, $\lambda_{max.}$ 224—225, 269—270 mμ (ε 8610, 7570) (Found: C, 67·7; H, 5·7; N, 9·8; S, 11·6. $C_{16}H_{16}N_2OS$ requires C, 67·6; H, 5·7; N, 9·85; S, 11·3%).

*Ethyl 2-Benzamidomethylthiazole-4-carboxylate.*—A solution of benzamidoacetothioamide [29] (15·5 g.) and ethyl bromopyruvate (15·5 g.) in ethanol (150 ml.) was heated under reflux for 2 hr. Most of the product (9·3 g.) crystallised when the solution was set aside overnight. The filtrate was evaporated, and the residue washed in benzene solution with aqueous sodium carbonate and water, dried ($Na_2SO_4$), and evaporated. The crystalline residue was combined with the previous product and recrystallised from ethanol (charcoal), and then from chloroform–light petroleum. The *thiazole ester* (14·8 g.) had m. p. 144·5—145·5°, $\lambda_{max.}$ 229—232 mμ (ε 21,800) (Found: C, 57·8; H, 4·8; N, 9·85. $C_{14}H_{14}N_2O_3S$ requires C, 57·9; H, 4·9; N, 9·65%).

*Ethyl 2-Phthalimidomethylthiazole-4-carboxylate.*—Phthalimidoacetothioamide [30] (15 g.) and ethyl bromopyruvate (13·4 g.) in ethanol (200 ml.) were heated under reflux for 1 hr. and the solution cooled. The precipitated *thiazole* was collected and recrystallised from ethanol (yield 15·6 g.); it had m. p. 129°, $\lambda_{max.}$ 219 mμ (ε 50,000) (Found: C, 56·9; H, 3·9; N, 8·7. $C_{15}H_{12}N_2O_4S$ requires C, 57·0; H, 3·8; N, 8·9%).

*Ethyl 2-1′-Benzamidoethylthiazole-4-carboxylate.*—A mixture of 2-benzamidopropiothioamide [29] (4·6 g.) and ethyl bromopyruvate (4·3 g.) in ethanol (100 ml.) was heated under reflux for 1·5 hr. and the solution then evaporated. The product was isolated with ethyl acetate which was washed with aqueous sodium hydrogen carbonate and water. The residue from evaporation was recrystallised from aqueous ethanol and yielded the *thiazole* (4·8 g.), m. p. 119°, $\lambda_{max.}$ 231—232 mμ (ε 19,600) (Found: C, 59·3; H, 5·1; N, 8·9; S, 10·25. $C_{15}H_{16}N_2O_3S$ requires C, 59·2; H, 5·3; N, 9·2; S, 10·5%).

*Ethyl 2-1′-Benzamidopropylthiazole-4-carboxylate.*—Prepared in a similar manner from 2-benzamidobutyrothioamide (2·4 g.) and ethyl bromopyruvate (2·1 g.), this thiazole was

[29] Johnson and Burnham, *Amer. Chem. J.*, 1912, **47**, 232.
[30] Chi and Tschin, *J. Amer. Chem. Soc.*, 1942, **64**, 90.

obtained as a viscous oil (3·3 g.) which did not crystallise. It was characterised as the derived carboxylic acid.

*Ethyl* 2-(1-*Benzamido-2-methylpropyl*)*thiazole-4-carboxylate*.—2-Benzamido-3-methylbutyrothioamide (2·9 g.) and ethyl bromopyruvate (2·4 g.) similarly yielded the crystalline *thiazole* (3·4 g.), m. p. 112° (from ethanol), $\lambda_{max.}$ 231 mμ (ε 18,800) (Found: C, 61·5; H, 6·1; N, 8·65; S, 9·8. $C_{17}H_{20}N_2O_3S$ requires C, 61·4; H, 6·1; N, 8·4; S, 9·6%).

*Methyl* 2-(1-*Benzamido-3-methylbutyl*)*thiazole-4-carboxylate*.—Condensation of 2-benzamido-4-methylvalerothioamide (5 g.) and ethyl bromopyruvate (3·9 g.) in ethanol in the usual manner yielded the oily thiazole ethyl ester (6·15 g.). However, when the reaction was carried out in methanolic solution, complete transesterification took place and yielded the crystalline *methyl ester*, m. p. 123—124° (from ethanol), $\lambda_{max.}$ 232—234 mμ (ε 20,400) (Found: C, 61·5; H, 6·2; N, 8·0; S, 9·9. $C_{17}H_{20}N_2O_3S$ requires C, 61·4; H, 6·1; N, 8·4; S, 9·6%). The structure of the methyl ester was confirmed by its proton magnetic resonance spectrum (60 Mc./sec.; $CDCl_3$ solution) which had a singlet of intensity 3 protons at 5·8 τ, characteristic of the methoxyl group.

*Ethyl* 2-(1-*Benzamido-2-phenylethyl*)*thiazole-4-carboxylate*.—Condensation of 2-benzamido-3-phenylpropiothioamide (1·4 g.) and ethyl bromopyruvate (1·0 g.) in ethanol in the usual manner yielded the oily thiazole derivative (1·7 g.) which was characterised as the free acid.

2-*Benzamidomethylthiazole-4-carboxylic Acid*.—A solution of ethyl 2-benzamidomethylthiazole-4-carboxylate (3·5 g.) in ethanol (10 ml.) was heated under reflux with 2N-sodium hydroxide (30 ml.) for 1·5 hr. The solution was concentrated, extracted with ethyl acetate and acidified. The precipitated *carboxylic acid* was collected and recrystallised from methanol (1·8 g., 57%); it had m. p. 220—221°, $\lambda_{max.}$ 231 mμ (ε 19,200) (Found: C, 54·8; H, 3·9; N, 10·5; S, 12·4. $C_{12}H_{10}N_2O_3S$ requires C, 55·0; H, 3·8; N, 10·7; S, 12·2%).

2-*Phthalimidomethylthiazole-4-carboxylic Acid*.—In a similar manner, ethyl 2-phthalimidomethylthiazole-4-carboxylate (3·1 g.) yielded the corresponding phthalamic acid which was recrystallised from aqueous methanol (2·3 g., 77%). On being dried at 100°, the phthalamic acid cyclised to the *phthalimido-derivative*, m. p. 234—235° (Found: C, 54·2; H, 3·0; N, 9·5; S, 11·3. $C_{13}H_8N_2O_4S$ requires C, 54·2; H, 2·8; N, 9·7; S, 11·1%).

2-1′-*Benzamidoethylthiazole-4-carboxylic Acid*.—The ethyl ester (1·5 g.), dissolved in methanol (5 ml.), was hydrolysed with 2N-sodium hydroxide (5·5 ml., 2 equiv.) for 17 hr. at room temperature. The mixture was worked up as described above for the benzamidomethyl compound, and the product recrystallised from aqueous ethanol. The *thiazolecarboxylic acid* (1·24 g., 81%) had m. p. 173°, $\lambda_{max.}$ 231 mμ (ε 19,600) (Found: C, 56·4; H, 4·2; N, 9·9; S, 11·85. $C_{13}H_{12}N_2O_3S$ requires C, 56·5; H, 4·4; N, 10·1; S, 11·6%).

2-1′-*Benzamidopropylthiazole-4-carboxylic Acid*.—The corresponding ester was hydrolysed as described above, and yielded the *carboxylic acid* (86%), m. p. 177°, $\lambda_{max.}$ 231—232 mμ (ε 18,250) (Found: C, 57·9; H, 4·7; N, 9·4; S, 11·3. $C_{14}H_{14}N_2O_3S$ requires C, 57·9; H, 4·9; N, 9·65; S, 10·95%).

2-(1-*Benzamido-2-methylpropyl*)*thiazole-4-carboxylic Acid*.—This *acid* (84%) had m. p. 233—234°, $\lambda_{max.}$ 231—232 mμ (ε 15,800) (Found: C, 59·1; H, 5·4; N, 9·1; S, 10·8. $C_{15}H_{16}N_2O_3S$ requires C, 59·2; H, 5·3; N, 9·2; S, 10·5%).

2-(1-*Benzamido-3-methylbutyl*)*thiazole-4-carboxylic Acid*.—The *acid* (83·5%) had m. p. 199—200°, $\lambda_{max.}$ 230—231 mμ (ε 18,700) (Found: C, 60·5; H, 5·55; N, 9·0; S, 10·4. $C_{16}H_{18}N_2O_3S$ requires C, 60·4; H, 5·7; N, 8·8; S, 10·1%).

2-(1-*Benzamido-2-phenylethyl*)*thiazole-4-carboxylic Acid*.—The *acid* (81%) had m. p. 202—203°, $\lambda_{max.}$ 230—231 mμ (ε 19,250) (Found: C, 64·5; H, 4·4; N, 7·7; S, 9·35. $C_{19}H_{16}N_2O_3S$ requires C, 64·8; H, 4·6; N, 7·95; S, 9·1%).

2-*Aminomethylthiazole-4-carboxylic Acid* (Ia).—2-Benzamidomethylthiazole-4-carboxylic acid (1·85 g.) in dioxan (5 ml.) was heated under reflux with 5N-hydrochloric acid (25 ml.) for 20 hr. The cooled mixture was extracted with ethyl acetate and evaporated. Recrystallisation of the solid residue from methanol–ether yielded the amino-acid hydrochloride (0·6 g., 44%), m. p. 269—270·5° (decomp.) (Found: C, 31·0; H, 3·7; N, 14·5. Calc. for $C_5H_6N_2O_2S$,HCl: C, 30·85; H, 3·6; N, 14·4%). Brookes *et al.*[22] give m. p. 280° (decomp.). A part of the hydrochloride was dissolved in dilute acetic acid and passed through a column of Dowex-1 × 4 anion exchange resin (acetate form). The column was washed with acetic acid until the ninhydrin reaction became negative, and the total eluate evaporated. Recrystallisation from ethanol–ether yielded the *amino-acid*, m. p. 277—280° (decomp.), $R_F$ (A) 0·22; (B) 0·58; $\lambda_{max.}$ 234 mμ (ε 5460

in $H_2O$), $\nu_{max.}$ (KBr disc) 3·22, 3·40, 3·60, 3·68, 4·24, 6·18, 6·42, 6·64, 6·73, 6·89, 7·25, 7·64, 7·73, 8·25, 8·48, 8·81, 9·12, 10·10, 10·50, 10·92, 11·52, 11·74, 12·43, 12·96 μ (Found: C, 38·1; H, 3·9; N, 17·8; S, 20·3. $C_5H_6N_2O_2S$ requires C, 38·0; H, 3·8; N, 17·7; S, 20·2%).

The thiazole amino-acid could also be obtained by similar acidic hydrolysis of the benzoyl and phthaloyl ethyl esters (yields 43% and 31%, respectively).

2-1′-*Aminoethylthiazole-4-carboxylic Acid* (Ib).—The benzoyl derivative was hydrolysed as in the foregoing experiment, and yielded the *amino-acid hydrochloride*, m. p. 233·5—234·5° (from ethanol–ether) (yield 68%) (Found: C, 34·8; H, 4·5; N, 13·1; S, 15·4. $C_6H_3N_2O_2S$,HCl requires C, 34·5; H, 4·3; N, 13·4; S, 15·3%). The free amino-acid, obtained as above, had m. p. 265—267° (decomp.) (from ethanol–ether) (lit.[22] m. p. 274—278°), $R_F$ (A) 0·35; (B) 0·62; $\lambda_{max.}$ 234 mμ (ε 5510 in $H_2O$), $\nu_{max.}$ (KBr disc) 2·90, 3·40, 3·70, 3·92, 4·80, 6·18, 6·37, 6·52, 6·68, 6·74, 6·84, 6·88, 7·28, 7·84, 8·34, 8·81, 9·16, 9·34, 9·78, 9·90, 10·12, 10·50, 10·60, 11·28, 11·45, 12·10, 12·84, 13·42 μ (Found: C, 41·5; H, 4·7; N, 16·4; S, 18·4. Calc. for $C_6H_8N_2O_2S$: C, 41·8; H, 4·7; N, 16·3; S, 18·6%).

2-1′-*Aminopropylthiazole-4-carboxylic Acid* (Ic).—Acidic hydrolysis of the benzoyl derivative in the usual manner afforded the hygroscopic hydrochloride, which was converted into the free *amino-acid*, m. p. 253—254° (decomp.) (from ethanol), $R_F$ (A) 0·44, (B) 0·74; $\lambda_{max.}$ 234 mμ (ε 5250 in $H_2O$), $\nu_{max.}$ (KBr disc) 3·22, 3·42, 3·65, 3·86, 4·56, 4·80, 6·18, 6·40, 6·80, 7·38, 7·88, 7·92, 8·24, 8·44, 8·91, 9·34, 9·52, 10·12, 10·42, 10·64, 11·56, 12·10, 12·40, 12·81, 12·96, 13·64 μ (Found: C, 45·2; H, 5·8; N, 14·95; S, 17·0. $C_7H_{10}N_2O_2S$ requires C, 45·2; H, 5·4; N, 15·05; S, 17·2%).

2-(1-*Amino-2-methylpropyl*)*thiazole-4-carboxylic Acid* (Id).—The hydrochloride, recrystallised from ethanol–ether (charcoal), had m. p. 265—267° (decomp.), $\lambda_{max.}$ 235 mμ (ε 5740 in $H_2O$, 6700 in EtOH) (Found: C, 40·3; H, 5·7; N, 11·8; S, 13·6. Calc. for $C_8H_{12}N_2O_2S$,HCl: C, 40·6; H, 5·5; N, 11·8; S, 13·55). Brookes *et al.*[22] record m. p. 269—272° (decomp.). The infrared spectrum was identical with that of the (+)-isomer.[11] The free *amino-acid*, liberated in the usual manner and recrystallised from ethanol–ether, had m. p. 252—253° (decomp.), $R_F$ (A) 0·53, (B) 0·80; $\nu_{max.}$ (KBr disc) 2·96, 3·40, 3·74, 3·82, 4·28, 4·78, 6·14, 6·40, 6·75, 7·25, 7·73, 7·85, 8·45, 8·94, 9·32, 9·96, 10·54, 11·48, 12·04, 12·12, 12·50, 12·92, 13·10 μ (Found: C, 47·9; H, 6·3; N, 13·6; S, 15·8. $C_8H_{12}N_2O_2S$ requires C, 48·0; H, 6·0; N, 14·0; S, 16·0%).

2-(1-*Amino-3-methylbutyl*)*thiazole-4-carboxylic Acid* (Ie).—The *hydrochloride*, recrystallised from methanol–ether, had m. p. 269—270° (decomp.) (Found: C, 42·9; H, 6·2; N, 10·9; S, 12·85. $C_9H_{14}N_2O_2S$,HCl requires C, 43·1; H, 6·0; N, 11·2; S, 12·8%). The free *amino-acid*, prepared by dissolving the hydrochloride in the minimum amount of 2N-hydrochloric acid and neutralising the filtered solution with concentrated aqueous ammonia, had m. p. 263—265° (decomp.), $R_F$ (A) 0·64 $\lambda_{max.}$ 236—238 mμ (ε 7200 in 2N-hydrochloric acid), $\nu_{max.}$ (Nujol) 3·21, 3·41, 3·50, 3·70—3·82, 4·25, 4·83, 6·15, 6·40, 6·51, 6·57, 6·80—6·83, 7·13, 7·32, 7·65, 8·05, 8·29, 8·44, 8·83, 8·95, 9·36, 9·73, 10·26, 10·58, 11·21, 11·46, 12·10, 12·86, 12·89, 13·38 μ (Found: C, 50·5; H, 6·6; N, 13·1; S, 14·9. $C_9H_{14}N_2O_2S$ requires C, 50·4; H, 6·7; N, 12·9; S, 14·9%).

2-(1-*Amino-2-phenylethyl*)*thiazole-4-carboxylic Acid* (If).—The *hydrochloride*, recrystallised from ethanol–ether (charcoal), had m. p. 258—259° (decomp.) (Found: C, 51·3; H, 4·7; N, 9·7. $C_{12}H_{12}N_2O_2S$,HCl requires C, 50·6; H, 4·6; N, 9·8%). The *amino-acid*, prepared as above, had m. p. 250—252° (decomp.), $R_F$ (A) 0·62, $\lambda_{max.}$ 235—237 mμ (ε 6430 in 2N-hydrochloric acid), $\nu_{max.}$ (Nujol) 3·20, 3·38, 3·70—3·80, 4·25, 4·83, 6·15, 6·40, 6·80, 7·33, 7·81, 8·04, 8·76, 9·30, 9·70, 10·11, 10·26, 11·15, 11·50, 12·65, 12·89, 13·22, 13·40, 13·78, 14·23 μ (Found: C, 57·8; H, 5·0; N, 11·0. $C_{12}H_{12}N_2O_2S$ requires C, 58·1; H, 4·9; N, 11·3%).

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