195. *Chemistry of Micrococcin* P. *Part IV.*[1] *A Method for the Structural Study of Thiazoles.*

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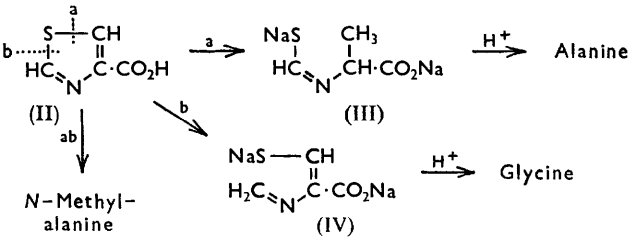
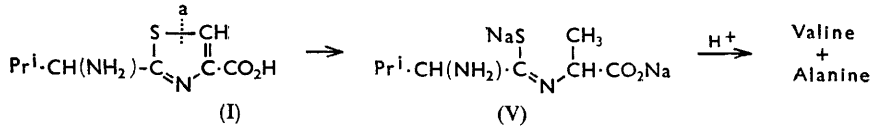
A method of fairly general applicability for the structural study of thiazoles consists of preliminary reduction with sodium in liquid ammonia and subsequent acid hydrolysis of the resulting intermediates: the products are indicative of the structures of the parent thiazoles. The method may be carried out on a micro-scale by using paper chromatography for identification purposes. It has been applied to a series of mono- and poly-nuclear thiazoles, to micrococcin *P*, and to the substance $C_{24}H_{23}O_5N_5S_4$ and the related diol with consistent results.

General methods for the structural study of thiazoles are unknown, and it appeared to us probable that if partial reduction of a thiazole nucleus could be effected under controlled conditions the resulting product might be capable of acid hydrolysis to recognisable fragments indicative of the structure of the parent thiazole. For example, it was hoped that a suitable process applied, say, to 2-(1-amino-2-methylpropyl)thiazole-4-carboxylic acid (I), isolated [2] from the acid hydrolysis of micrococcin *P* might yield evidence relating to its structure and possibly also to its hypothetical biological precursors.[1,2]



Thiazole-4-carboxylic acid (II) was used as a model. Reduction of a few milligrams with sodium in liquid ammonia followed by acid hydrolysis of the intermediate(s) gave a mixture that was shown by paper chromatography to contain alanine, *N*-methylalanine,



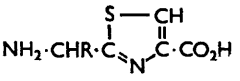
and glycine as the main products along with several other unidentified substances detectable with ninhydrin. The formation of alanine may be explained in terms of fission of the thiazole ring at " a " in thiazole-4-carboxylic acid (II), reduction of the double bond in the

[1] Part III, Brookes, Clark, Fuller, Mijović, and Walker, preceding paper.
[2] Brookes, Fuller, and Walker, *J.*, 1957, 689.

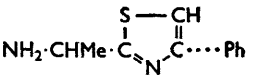
αβ-position to the carboxyl group, and acid hydrolysis of the derivative (III). *N*-Methylalanine would result directly from reductive desulphurisation at " ab " in thiazole-4-carboxylic acid (II) and reduction of the two double bonds, while fission of the thiazole ring at " b " in thiazole-4-carboxylic acid (II) and acid hydrolysis of the resulting derivative (IV) would afford glycine. The formation of *N*-methylalanine from thiazole-4-carboxylic acid (II) is analogous to the reduction of 4-methyl-5-phenylthiazole with sodium and alcohol to 2-methylamino-1-phenylpropane,[3] and of 2,4-dimethylthiazole to ethylisopropylamine,[4] although a previous report [5] had claimed ethylamine and propane-1-thiol to be products in the latter case.

When the process was applied to 2-(1-amino-2-methylpropyl)thiazole-4-carboxylic acid (I) reductive fission of the thiazole ring took place at " a " since acid hydrolysis of the presumed intermediate (V) afforded valine and alanine, but once again some glycine was detectable. Similar treatments of 2-aminomethyl- (VI) and of 2-1′-aminoethyl-thiazole-4-carboxylic acid (VII) both afforded alanine and glycine, and with the appearance of glycine in the latter case the regularity with which glycine was to accompany alanine in the degradation of a thiazole-4-carboxylic acid became more fully apparent, as it had been expected that the former compound (VI) would have given glycine and alanine and the latter (VII) alanine but no glycine.

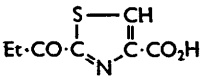
Reduction of 2-1′-hydroxyethylthiazole-4-carboxylic acid with sodium in liquid ammonia and acid hydrolysis of the intermediate product(s) afforded alanine and, again,



(VI) R = H
(VII) R = Me
(IX) R = Et

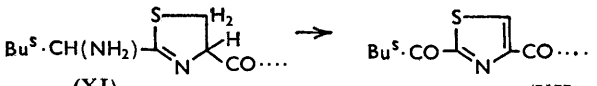


(VIII)



(X)

glycine, while similar treatment of 2-phenylthiazole-4-carboxylic acid afforded alanine, *N*-benzylalanine, and glycine as the main products, the formation of *N*-benzylalanine in this instance being analogous with that of *N*-methylalanine from thiazole-4-carboxylic acid. 2-1′-Aminoethyl-4-phenylthiazole (VIII) afforded alanine without, of course, any trace of glycine. When the process was applied to micrococcin *P* the amino-acids detected after acid hydrolysis were alanine, glycine, valine, threonine, and α-aminobutyric acid. Of these, only threonine is found on acid hydrolysis of micrococcin *P* itself,[2,6] so that the appearance of the others is indicative of their formation by the degradation of thiazole components in the antibiotic; thus alanine, valine, and glycine would arise from the 2-(1-amino-2-methylpropyl)thiazole-4-carboxylic acid (I) incorporated in the antibiotic.



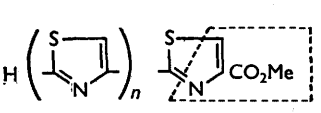
(XI) (XII)

The appearance of α-aminobutyric acid from micrococcin *P* was of interest, and raised the possibility of the presence in the native molecule of micrococcin *P* of a 2-1′-aminopropylthiazole-4-carboxylic acid (IX) unit, as outlined in our scheme for the biosynthesis of the thiazole components of the antibiotic;[1,2] the appearance of 2-propionylthiazole-4-carboxylic acid (X) on hydrolysis of micrococcin *P* would then be an artefact of the acid hydrolysis of the antibiotic, recalling formally the conversion by alkaline aeration of the

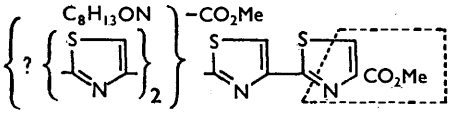
[3] Erlenmeyer and Simon, *Helv. Chim. Acta*, 1942, **25**, 528.
[4] Schuftan, *Ber.*, 1894, **27**, 1009.
[5] Schatzmann, *Annalen*, 1891, **261**, 1.
[6] Mijović and Walker, *J.*, 1960, 909.

N-terminal sequence [7] (XI) of bacitracin A into the terminal group [8] (XII) of bacitracin F. This question must, however, remain unanswered for the present, since it was found that application of the present process to 2-propionylthiazole-4-carboxylic acid (X) also afforded α-aminobutyric acid by way, obviously, of an intermediate ketimine formed by prior reaction of the carbonyl group with ammonia.

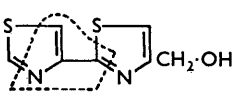
Application of the present process to the synthetic polythiazoles described in the preceding paper, *viz.*, methyl 2,4′-bithiazolyl-4-carboxylate (XIII; $n = 1$), 2,4′:2′,4″-terthiazole-4-carboxylate (XIII; $n = 2$), and 2,4′:2′,4″:2″,4‴-quaterthiazole-4-carboxylate (XIII; $n = 3$), and to the substance $C_{24}H_{23}O_5N_5S_4$ [1,2] gave almost identical results; in addition to alanine and glycine they showed the same pattern of unidentified ninhydrin-positive spots. In the preceding communication the expression (XIV) was suggested for the substance $C_{24}H_{23}O_5N_5S_4$ and it is obvious that in the above degradations of the polythiazoles (XIII; $n = 1$—3), and of the substance $C_{24}H_{23}O_5N_5S_4$, the alanine and glycine could come from those parts of these structures enclosed by the dotted lines. This contribution was therefore eliminated by prior reduction of the ester groups with lithium borohydride. As a model, methyl 2,4′-bithiazolyl-4-carboxylate (XIII; $n = 1$) was reduced to the alcohol (XV), and application of the present process then afforded alanine and glycine, which in this instance (XV) must have come from that part of the structure enclosed by the dotted line and could be taken as evidence of contiguous 2,4′-linked thiazole nuclei. Similarly, reduction of the substance $C_{24}H_{23}O_5N_5S_4$ (XIV) to the dihydric alcohol (XVI), and application of the present process to the latter compound again furnished alanine and glycine, thus providing further evidence for the presence of contiguous 2,4′-linked thiazole nuclei in the substance $C_{24}H_{23}O_5N_5S_4$, as suggested in the expression (XIV).[1] It is therefore now certain that *at least* five of the eleven sulphur atoms in the micrococcin *P* molecule are present in thiazole rings in line with the views that have been outlined relating to the biosynthesis of the antibiotic.[1,2]



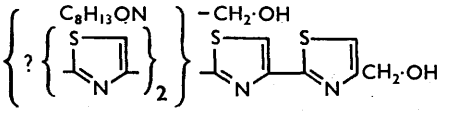
(XIII)



(XIV)



(XV)



(XVI)

The work described in the present communication gives some indication of the scope of our process for investigating the structures of thiazoles. With suitable modification of methods for the detection and identification of degradation products there is good reason to believe that it could be applied generally to this class of compound.

2-Aminomethylthiazole-4-carboxylic acid (VI) and 2-1′-aminoethylthiazole-4-carboxylic acid (VII) were synthesised by a general method which was also applied to the synthesis of 2-(1-amino-2-methylpropyl)thiazole-4-carboxylic acid (I). The synthetic 2-(1-amino-2-methylpropyl)thiazole-4-carboxylic acid (I) and the substance isolated from micrococcin *P* after acid hydrolysis [2] showed identical behaviour on paper chromatography and identical infrared absorption spectra. The colours given by these three substances with ninhydrin on paper chromatograms were of interest, since 2-aminomethyl- (VI) and

[7] Lockhart and Abraham, *Biochem. J.*, 1954, **58**, 633; Lockhart, Abraham, and Newton, *ibid.*, 1955, **61**, 534; Weisiger, Hausmann, and Craig, *J. Amer. Chem. Soc.*, 1955, **77**, 731.

[8] Hausmann, Weisiger, and Craig, *ibid.*, 1955, **77**, 730; Weisiger, Hausmann, and Craig, *ibid.*, p. 3123.

2-1′-aminoethyl-thiazole-4-carboxylic acid (VII) both gave yellow spots that slowly became purple, whereas 2-(1-amino-2-methylpropyl)thiazole-4-carboxylic acid (I) gave a purple spot directly.

Experimental

2-Aminomethylthiazole-4-carboxylic Acid (VI) *Hydrochloride.*—A mixture of benzamidoacetothioamide [9] (700 mg.) and ethyl bromopyruvate (700 mg.) in ethanol (15 c.c.) was heated under reflux for 2 hr., and then the solvent was removed under reduced pressure. The gummy residue was distributed between water and benzene, and the organic phase was washed with aqueous sodium carbonate and water, dried, and evaporated, giving a crystalline product (850 mg.). This (500 mg.) was heated under reflux with 20% hydrochloric acid (20 c.c.) for 4 hr.; benzoic acid was extracted with ether, and the aqueous phase was evaporated to dryness, giving a crystalline residue (320 mg.). Recrystallisation from methanol–ethyl acetate (charcoal) and then from methanol gave colourless prisms (230 mg.), m. p. 280° (decomp.) (Found: C, 30·8; H, 3·9; N, 14·1. $C_5H_6O_2N_2S$,HCl requires C, 30·9; H, 3·6; N, 14·4%). With the ninydrin spray on paper the *acid* gave a yellow spot that slowly became purple.

α-Benzyloxycarbonylaminopropionamide.—A solution of methyl α-benzyloxycarbonylaminopropionate (5·64 g.), prepared from DL-alanine, in methanol (30 c.c.) was treated with methanolic ammonia solution (50 c.c., saturated at 0°), and the mixture was kept at 0° for 5 days. Evaporation to dryness and crystallisation from chloroform–ether afforded the *amide* as colourless plates (5·0 g.), m. p. 127—128° (Found: C, 59·5; H, 6·4; N, 12·7. $C_{11}H_{14}O_3N_2$ requires C, 59·5; H, 6·3; N, 12·6%).

α-Benzyloxycarbonylaminopropionitrile.—Benzenesulphonyl chloride (4·5 g.) was added slowly with shaking to a slurry of the preceding amide (5·0 g.) in dry pyridine (10 c.c.). The resulting clear solution was heated at 100° for 10 min., then cooled and diluted with ether (100 c.c.). The ethereal solution was washed several times with 2N-hydrochloric acid and with water, dried, and evaporated, to give an oil which rapidly crystallised. The *nitrile* separated from ether–light petroleum in colourless needles (3·4 g.), m. p. 57—58° (Found: C, 64·6; H, 6·1; N, 13·6. $C_{11}H_{12}O_2N_2$ requires C, 64·7; H, 5·9; N, 13·7%).

α-Benzyloxycarbonylaminopropionothioamide.—Hydrogen sulphide was bubbled gently for 12 hr. through a solution of the preceding nitrile (1·7 g.) in dry ethanol (3 c.c.) containing triethanolamine (0·1 g.), and the crude product was obtained by dilution with water. Recrystallisation from aqueous ethanol afforded the *thioamide* as plates (1·0 g.), m. p. 101—103° (Found: C, 55·6; H, 5·7; N, 11·6. $C_{11}H_{14}O_2N_2S$ requires C, 55·5; H, 5·9; N, 11·8%).

Ethyl 2-(1-Benzyloxycarbonylaminoethyl)thiazole-4-carboxylate.—A solution of the preceding thioamide (0·8 g.) and ethyl bromopyruvate (0·66 g.) in ethanol (10 c.c.) was heated under reflux for 1½ hr., and then taken to dryness. The residual yellow gum was repeatedly extracted with boiling ether (10 × 20 c.c.); concentration of the combined extracts gave a solid 0·57 g.). Recrystallisation from aqueous ethanol afforded the *thiazole* as colourless plates, m. p. 90—91° (Found: C, 57·2; H, 5·3; N, 8·1. $C_{16}H_{18}O_4N_2S$ requires C, 57·5; H, 5·4; N, 8·4%).

DL-*2-1′-Aminoethylthiazole-4-carboxylic Acid* (VII).—The above ester (900 mg.) was heated under reflux with concentrated hydrochloric acid (30 c.c.) for 4 hr., and the mixture was then evaporated to a crystalline residue. Recrystallisation from methanol–ethyl acetate (charcoal) afforded needles (370 mg.), m. p. 223—228° (decomp.). The free *amino-acid* was liberated as a white precipitate by addition of pyridine to an ethanolic solution of the hydrochloride, and separated from aqueous ethanol in colourless plates, m. p. 274—278° (decomp.) (Found: C, 41·8; H, 4·7. $C_6H_8O_2N_2S$ requires C, 41·8; H, 4·7%). With the ninhydrin spray on paper the substance gave a yellow spot that slowly became purple.

α-Benzamidoisovaleronitrile.—(i) DL-Valine was benzoylated and esterified with diazomethane, and the resulting methyl ester was treated with methanolic ammonia at 110° for 8 hr. to give the amide (62%), m. p. 219—220° (lit.,[10] m. p. 220—221°).

(ii) Benzenesulphonyl chloride (3·5 g.) was added slowly and with shaking to a suspension of the amide (4·0 g.) in dry pyridine. The solid disappeared and the yellow solution was poured into ether (200 c.c.). The ethereal solution was washed in the manner described above, dried, and evaporated. The *nitrile* separated from benzene in prisms (2·6 g.), m. p. 110° (Found: C, 71·6; H, 7·2; N, 14·0. $C_{12}H_{14}ON_2$ requires C, 71·3; H, 6·9; N, 13·9%).

α-Benzamidoisovalerothioamide.—The preceding nitrile (1·1 g.) and triethanolamine (0·2 g.)

[9] Johnson and Burnham, *Amer. Chem. J.*, 1912, **47**, 232.
[10] Fox, Pettinga, Halverson, and Wax, *Arch. Biochem.*, 1950, **25**, 21.

in cold (−80°) ethanol (4 c.c.) were added to liquid hydrogen sulphide (3 c.c.) in a small bomb. The bomb was kept at 60° for 12 hr., then cooled, and opened. The crystalline residue was taken up in hot ethanol, filtered, and cooled, affording the *thioamide* as prismatic needles (1·06 g.), m. p. 202° (decomp.) (Found: C, 61·0; H, 6·6; N, 11·7. $C_{12}H_{16}ON_2S$ requires C, 61·0; H, 6·8; N, 11·9%).

DL-2-(1-*Amino*-2-*methylpropyl*)*thiazole*-4-*carboxylic Acid* (I) *Hydrochloride*.—The preceding thioamide (200 mg.) and ethyl bromopyruvate (170 mg.) were heated in ethanol (3 c.c.) under reflux for 2 hr. The ethanol was removed by distillation and the residue was taken up in benzene. The benzene solution was washed with aqueous sodium carbonate, and water, and evaporated to dryness. The residual yellow oil was heated under reflux for 4 hr. with 20% hydrochloric acid (10 c.c.). The cooled solution was extracted with ether to remove benzoic acid, then evaporated to dryness, affording a brownish-white crystalline residue (150 mg.). Recrystallisation from methanol–ethyl acetate (charcoal) gave the *amino-acid hydrochloride* as colourless prisms (100 mg.), m. p. 269—272° (Found: C, 40·5; H, 5·5; N, 11·9. $C_8H_{12}O_2N_2S,HCl$ requires C, 40·4; H, 5·5; N, 11·8%).

The behaviour of this substance on paper chromatography and the infrared absorption spectrum were identical with those of the compound obtained by acid hydrolysis of micrococcin *P*.[2]

2-1′-*Aminoethyl*-4-*phenylthiazole* (VIII) *Hydrochloride*.—(i) In a similar manner DL-alanine was benzoylated and esterified with diazomethane, and the resulting methyl ester was treated with methanolic ammonia at 100—110° for 7 hr., to give the amide (75%), m. p. 228° (lit.,[11] m. p. 227°).

(ii) Dehydration of the amide (12·0 g.) was effected with benzenesulphonyl chloride (10·8 g.) and pyridine (20 c.c.) at 90° for 1 hr. The nitrile, isolated in the usual way, crystallised from water in white needles (6·0 g.), m. p. 108° (lit.,[12] m. p. 108°).

(iii) A mixture of the preceding nitrile (5·6 g.) and liquid hydrogen sulphide (*ca.* 7 c.c.) in methanol (40 c.c.) containing triethanolamine (1·5 g.) was heated in a steel bomb at 50—60° for 12 hr. The thioamide, isolated in the usual way, crystallised from aqueous methanol in needles (4·2 g.), m. p. 145° (lit.,[13] m. p. 146°).

(iv) A mixture of the preceding α-benzamidopropionothioamide (2·0 g.), ω-bromoacetophenone (2 g.), and pyridine (1·5 g.) in methanol (15 c.c.) was heated under reflux for 2 hr. The methanol was removed by distillation and the residue was hydrolysed with boiling 20% hydrochloric acid in the usual way. Benzoic acid was extracted with ether and the aqueous phase was basified with 5N-sodium hydroxide and extracted with ether. Evaporation of the dried extract gave an oil (1·5 g.) which was treated with dry hydrogen chloride in ether. The *hydrochloride* separated from methanol–ethyl acetate in colourless needles (1·0 g.), m. p. 214—217° (Found: C, 54·7; H, 5·6; N, 11·3. $C_{11}H_{12}N_2S,HCl$ requires C, 54·9; H, 5·4; N, 11·6%).

4-*Hydroxymethyl*-2,4′-*bithiazolyl* (XV).—Methyl 2,4′-bithiazolyl-4-carboxylate (450 mg.) was dissolved in dry tetrahydrofuran (50 c.c.) and treated with lithium borohydride (100 mg. of solid containing 68% of borohydride). The mixture was kept at room temperature for 3 days and then treated with 3N-hydrochloric acid to destroy any excess of lithium borohydride. Tetrahydrofuran was removed under reduced pressure and the residue, after dilution with water, was extracted with butan-1-ol. Evaporation of the butanol gave a crystalline residue, and recrystallisation from ethanol (charcoal) afforded 4-*hydroxymethyl*-2,4′-*bithiazolyl* (XV) as colourless plates (335 mg.), m. p. 155—158° (Found: C, 42·5; H, 3·1; N, 14·2. $C_7H_6ON_2S_2$ requires C, 42·4; H, 3·0; N, 14·1%).

Reduction of the Substance $C_{24}H_{23}O_5N_5S_4$ *with Lithium Borohydride*: *the Diol* $C_{22}H_{23}O_3N_5S_4$.—The substance $C_{24}H_{23}O_5N_5S_4$ [1,2] (200 mg.) was dissolved in pure tetrahydrofuran (40 c.c.), and lithium borohydride (200 mg. of solid containing 68% of borohydride) was added. After 2 days at room temperature the mixture was worked up as described above. The residue, after dilution with water, was extracted with chloroform and then with butan-1-ol. The chloroform extract gave a yellow gum (10 mg.) and the butanol extract pale yellow crystals (153 mg.). Recrystallisation from propan-1-ol (50 c.c.) afforded the *diol* as pale yellow needles (68 mg.), m. p. 215—218°, raised to 220—222° by a further recrystallisation (Found: C, 49·6, 50·3, 49·8; H, 3·5, 4·0, 4·0; N, 12·6, 12·7; S, 23·6. $C_{22}H_{23}O_3N_5S_4$ requires C, 49·6; H, 4·3; N, 13·5; S, 24·1%).

[11] Mohr and Stroschein, *Ber.*, 1909, **42**, 2521.
[12] Delépine, *Bull. Soc. chim. France*, 1903, **29**, 1193.
[13] Goldberg and Kelly, *J.*, 1947, 1372.

Reductive Hydrolysis of Thiazoles.—General method. The thiazole (5—10 mg.) was suspended in liquid ammonia (15—20 c.c.) with stirring and cooling to −80°. Sodium (20 mg., or up to 100 mg for polythiazoles) was added and the mixture was stirred for 2 hr., or longer if necessary, until the colour of the sodium was discharged. The ammonia, after addition of ethanol (5 c.c.), was allowed to evaporate. 20% Hydrochloric acid (25 c.c.) was added to the residue and the mixture was heated under reflux for 16 hr. After evaporation to dryness, the residue was extracted with ethanol; the ethanolic extract was evaporated to dryness and the residue was again extracted with ethanol. This extract was passed down a column (8 × 1·2 cm.) of " Amberlite IRA-400(OH) " or " Deacidite FF(OH) " and the column was washed with ethanol until the effluent became neutral. Elution was continued with 2N-hydrochloric acid in 80% ethanol (10 c.c.) to elute amino-acids. The acid eluate was evaporated to dryness, and the residue was taken up in a little ethanol and spotted for paper chromatography. In the case of 4-hydroxymethyl-2,4′-bithiazolyl (XV) and the diol $C_{22}H_{23}O_3N_5S_4$ the initial ethanol washings of the column were evaporated to dryness, and the residues were spotted for chromatography; 2-aminopropan-1-ol (alaninol) was detected in each case.

All chromatograms were run on Whatman No. 3 paper; the solvent systems used were (a) butanol–acetic acid–water (63 : 10 : 27), (b) 2,6-lutidine–ethanol–water–diethylamine (55 : 25 : 20 : 2), and (c) phenol–water (5 : 2, with added concentrated aqueous ammonia and sodium cyanide), and spots were detected by spraying the paper with a 0·1% solution of ninhydrin in 98% butanol. The presence of *N*-methylalanine and of *N*-benzylalanine was confirmed by dipping the chromatograms in a 0·2% solution of *p*-nitrobenzoyl chloride in benzene, and, after drying, into pyridine–light petroleum (1 : 10),[14] before routine spraying with ninhydrin. Chromatograms were also sprayed with the Folin–Marenzi reagent [15] to test for cysteine. When α-aminobutyric acid was detected chromatograms were also run in ethanol–water (9 : 1).[16]

For the reductive hydrolysis of the following compounds the identified main spots are named; subsidiary and weaker unidentified spots were either slow-running or fast-running and are designated, *e.g.*, " slow 2a, 3c; fast 1abc," denoting that two slow-running spots were observed with solvent system (a) and three with solvent system (c), and one fast-running spot was observed with each of the solvent systems (a), (b), and (c).

Thiazole-4-carboxylic acid [17] (II): alanine, glycine, *N*-methylalanine; slow 2b, 3c; fast 1abc.

2-*Aminomethylthiazole-4-carboxylic acid* (VI): alanine, glycine; slow 2a, 3c; fast 2a, 1b.

2-1′-*Aminoethylthiazole-4-carboxylic acid* (VII): alanine, glycine; slow lab, 2c; fast 2a, 1bc.

2-(1-*Amino-2-methylpropyl*)*thiazole-4-carboxylic acid* (I): alanine, glycine, valine; slow 1abc; fast 2ab, 1c.

2-1′-*Hydroxyethylthiazole-4-carboxylic acid*: alanine, glycine; slow 1a, 2b, 4c; fast 3ab.

2-*Phenylthiazole-4-carboxylic acid*: [18] alanine, glycine, *N*-benzylalanine; slow 2c.

2-1′-*Aminoethyl-4-phenylthiazole* (VIII): alanine; slow 1a, 2c; fast 1a.

Micrococcin P: alanine, glycine, valine, threonine, α-aminobutyric acid; slow 1a, 2bc; fast 1ab.

2-*Propionylthiazole-4-carboxylic acid* (X): alanine, glycine, α-aminobutyric acid; slow 1abc; fast 2ab.

Methyl 2,4′-*bithiazolyl-4-carboxylate* (XIII; $n = 1$): alanine, glycine; slow 2c; fast 3abc.

Methyl 2,4′:2′4″-*terthiazole-4-carboxylate* (XIII; $n = 2$): alanine, glycine; slow 2c; fast 3ab, 2c.

Methyl 2,4′:2′,4″:2″,4‴-*quaterthiazole-4-carboxylate* (XIII; $n = 3$): alanine, glycine; slow 2c; fast 2ac, 3b.

Substance $C_{24}H_{23}O_5N_5S_4$: alanine, glycine; slow 2c; fast 1a, 3b, 4c.

4-*Hydroxymethyl*-2,4′-*bithiazolyl* (XV): alanine, glycine, 2-aminopropan-1-ol (alaninol); slow 1ab; fast 2abc.

[14] Sheehan, Zachau, and Lawson, *J. Amer. Chem. Soc.*, 1958, **80**, 3349.
[15] Folin and Marenzi, *J. Biol. Chem.*, 1929, **83**, 109.
[16] Cf. Block, Durrum, and Zweig, " Manual of Paper Chromatography and Paper Electrophoresis," 2nd edn., Academic Press, New York, 1958, p. 162.
[17] Erne, Ramirez, and Burger, *Helv. Chim. Acta*, 1951, **34**, 143.
[18] Erlenmeyer, Buchmann, and Schenkel, *ibid.*, 1944, **27**, 1432.

Diol $C_{22}H_{23}O_3N_5S_4$ *from the substance* $C_{24}H_{23}O_5N_5S_4$: alanine, glycine, 2-aminopropan-1-ol (alaninol); slow 1abc; fast 1a.

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