194. *Chemistry of Micrococcin* P. *Part III.**

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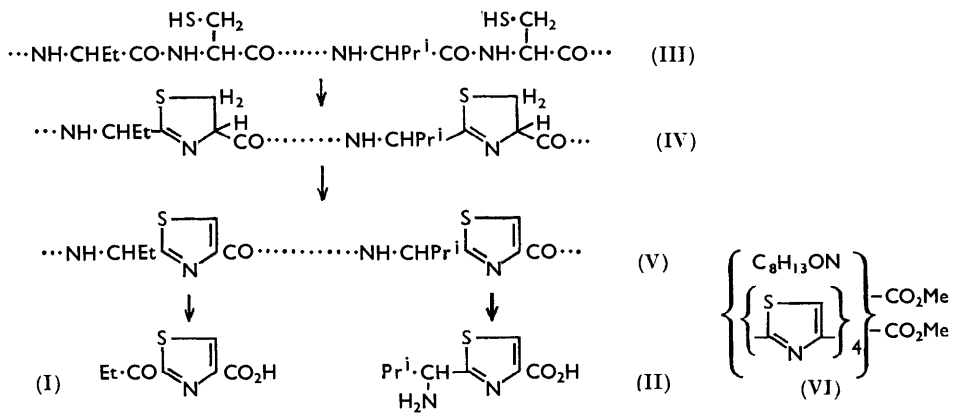
The substance $C_{24}H_{23}O_5N_5S_4$, obtained from the " acid-insoluble fraction " from the acid hydrolysis of micrococcin *P* by treatment with methanol and sulphuric acid, afforded methyl 2-carbamoylthiazole-4-carboxylate (VII) on oxidation with chromic acid in acetic acid, thus providing proof of its thiazole nature.

A series of methyl 2,4′-linked polythiazole-4-carboxylates has been synthesised and their behaviour on oxidation has been studied for comparison with that of the substance $C_{24}H_{23}O_5N_5S_4$. The ultraviolet absorption spectra of the synthetic polythiazole esters show that limited conjugation occurs between 2,4′-linked thiazole nuclei. The substance $C_{24}H_{23}O_5N_5S_4$ and micrococcin *P* must contain a more extended chromophoric system.

The effect of fortifying the medium in which micrococcin *P* is produced has been studied, and cysteine was markedly stimulatory, indicating that availability of this amino-acid is a limiting factor in the biosynthesis of the antibiotic.

In Part I[1] it was shown that treatment of the " acid-insoluble fraction " from the acid hydrolysis of micrococcin *P* with methanol and sulphuric acid gave a substance $C_{24}H_{23}O_5N_5S_4$ containing two methoxyl groups. As micrococcin *P* is methoxyl-free, the appearance of two methoxyl groups in the substance $C_{24}H_{23}O_5N_5S_4$ was attributed to the



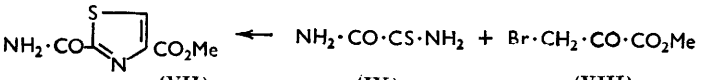
esterification of two carboxyl groups. Two other soluble hydrolytic fragments from micrococcin *P* proved to be thiazoles, namely, 2-propionylthiazole-4-carboxylic acid (I) and 2-(1-amino-2-methylpropyl)thiazole-4-carboxylic acid (II), and it was suggested that they could originate from the incorporation of appropriate amino-acids into a peptide

* Part II, preceding paper.

[1] Brookes, Fuller, and Walker, *J.*, 1957, 689.

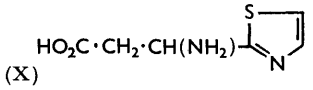
chain (III) followed by cyclisation at cysteine residues with formation of thiazoline rings (IV) and thence, by dehydrogenation, of thiazole rings (V); thus the precursors of 2-propionylthiazole-4-carboxylic acid (I) were considered to be α-aminobutyric acid and cysteine, and those of 2-(1-amino-2-methylpropyl)thiazole-4-carboxylic acid (II) to be valine and cysteine. With these facts in mind, it was suggested that the nitrogen : sulphur ratio, stability of the parent dibasic acid to further acid hydrolysis, and light absorption properties of the substance $C_{24}H_{23}O_5N_5S_4$ were compatible with a polythiazole structure (VI), which could arise by cyclisation and dehydrogenation of a polycysteine peptide in the manner outlined above.

Evidence for at least one thiazole ring in the compound $C_{24}H_{23}O_5N_5S_4$ was forthcoming when it was found that oxidation with chromic acid in acetic acid gave a substance $C_6H_6O_3N_2S$, which afforded on alkaline hydrolysis a mixture of thiazole-2,4-dicarboxylic acid and its partial decarboxylation product thiazole-4-carboxylic acid, together with ammonia. The substance $C_6H_6O_3N_2S$ could therefore only be one of the two possible methyl ester amides of thiazole-2,4-dicarboxylic acid. In view of the ease with which a carboxyl group is lost in a thiazole-2-carboxylic acid,[1] it was thought unlikely, apart from biogenetic considerations, that the parent acid of the dimethyl ester $C_{24}H_{23}O_5N_5S_4$ could have had a free carboxyl group in the 2-position of a thiazole ring, and the more likely structure for the substance $C_6H_6O_3N_2S$ was methyl 2-carbamoylthiazole-4-carboxylate (VII), since the alternative structure would have indicated a thiazole-2-carboxylic acid structure for the acidic precursor of the substance $C_{24}H_{23}O_5N_5S_4$. Proof of the structure (VII) followed by condensation of methyl bromopyruvate (VIII) with monothio-oxamide (IX), which afforded authentic methyl 2-carbamoylthiazole-4-carboxylate (VII), identical with the oxidation product $C_6H_6O_3N_2S$.

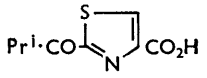


(VII) (IX) (VIII)

The formation of an amide group in an oxidation is unusual and it seemed likely that it could arise by destruction of another nitrogenous heterocyclic ring. On the other hand, it may be noted that β-(2-thiazolyl)-β-alanine (X), a degradation product of the antibiotic bottromycin, is stated [2] to give thiazole-2-carboxamide on oxidation with potassium permanganate, an observation that is at variance with our own experience [1] in which 2-(1-amino-2-methylpropyl)thiazole-4-carboxylic acid (II) afforded the keto-acid, 2-isobutyrylthiazole-4-carboxylic acid (XI), under these conditions. We are, however, of the opinion that the carbamoyl group in methyl 2-carbamoylthiazole-4-carboxylate (VII), as obtained by the oxidation of the substance $C_{24}H_{23}O_5N_5S_4$, arises by the oxidation of a second heterocyclic ring, probably of a thiazole ring. That oxidative destruction of a thiazole ring with chromic acid in acetic acid can generate a carbamoyl group was amply demonstrated by the study of a series of synthetic 2,4′-linked polythiazoles now to be described.



(X)



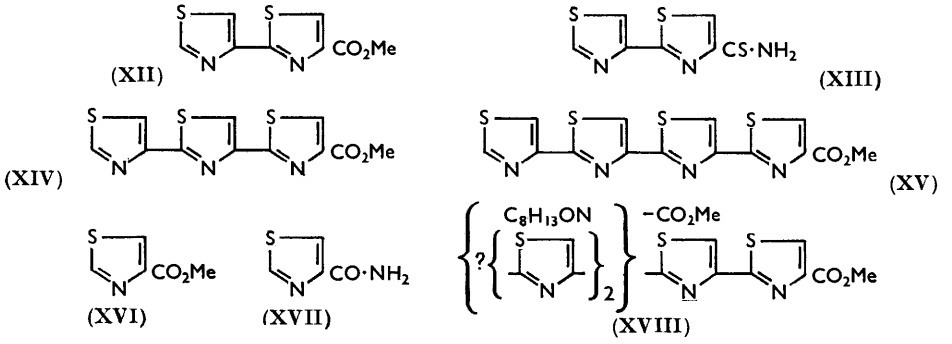
(XI)

Condensation of thiazole-4-carboxythioamide with methyl bromopyruvate afforded methyl 2,4′-bithiazolyl-4-carboxylate (XII), and the derived thioamide (XIII) gave access to the next two members of the series. Thus, condensation with methyl bromopyruvate gave methyl 2,4′:2′,4″-terthiazole-4-carboxylate (XIV), and condensation with methyl 2-bromoacetylthiazole-4-carboxylate, best obtained by treatment of methyl 2-1′-hydroxyethylthiazole-4-carboxylate with *N*-bromosuccinimide, afforded methyl 2,4′:2′,4″:2″,4‴-quaterthiazole-4-carboxylate (XV). Oxidations of these synthetic methyl polythiazole

[2] Waisvisz, van der Hoeven, and te Nijenhuis, *J. Amer. Chem. Soc.*, 1957, **79**, 4524.

carboxylates with chromic acid in acetic acid were carried out under the same conditions as were used for the substance $C_{24}H_{23}O_5N_5S_4$ and the products were carefully examined and identified. Methyl 2,4′-bithiazolyl-4-carboxylate (XII) afforded the ester-amide (VII), identical with the synthetic material and with the substance obtained by oxidation of the compound $C_{24}H_{23}O_5N_5S_4$, together with methyl thiazole-4-carboxylate (XVI), unchanged starting material (XII), and, in one experiment, thiazole-4-carboxamide (XVII). The terthiazole ester (XIV) afforded a mixture of methyl thiazole-4-carboxylate (XVI) and thiazole-4-carboxamide (XVII), but no trace of the ester-amide (VII) was detected. The quaterthiazole ester (XV) afforded the ester amide (VII), methyl thiazole-4-carboxylate (XVI), thiazole-4-carboxamide (XVII), and methyl 2,4′-bithiazolyl-4-carboxylate (XII). With the experience gained in examining the products of these oxidations, re-investigation of the oxidation of the substance $C_{24}H_{23}O_5N_5S_4$ showed that some methyl thiazole-4-carboxylate (XVI) was formed in addition to the ester-amide (VII).

These results prove that oxidative destruction of a thiazole ring can generate a carboxamide group in two ways, either the 2- or the 4-carbon atom of the ring furnishing the carbon atom of that group; thus, the carbon atom of the carboxamide group in the ester-amide (VII), when obtained by oxidation of methyl 2,4′-bithiazolyl-4-carboxylate (XII), came from the 4′-position, while that of the thiazole-4-carboxamide (XVII) must come from the 2-position of an adjacent thiazole ring. Methyl thiazole-4-carboxylate (XVI) and, of course, the ester-amide (VII) can only arise from the terminal ring bearing the methoxycarbonyl group, but thiazole-4-carboxamide could arise from any of the other thiazole rings in the synthetic polythiazoles and not solely from the terminal ring remote from that bearing the methoxycarbonyl group. Some indications of the formation of thiazole-4-carboxythioamide, rather than of thiazole-4-carboxamide (XVII), might have been expected, but it is known that thioamides are converted at moderate temperatures and under oxidising and hydrolytic conditions into carboxamides.[3] The oxidations of the



$$(XII) \xrightarrow{CrO_3} (VII) + (XVI) + (XVII) + (XII)\ (unchanged)$$

$$(XIV) \xrightarrow{CrO_3} (XVI) + (XVII)$$

$$(XV) \xrightarrow{CrO_3} (VII) + (XVI) + (XVII) + (XII)$$

$$C_{24}H_{23}O_5N_5S_4 \xrightarrow{CrO_3} (VII) + (XVI)$$

synthetic polythiazoles (XII), (XIV), (XV) showed a number of interesting features; thus, the bithiazolyl (XII) proved relatively resistant to oxidation, some remaining unchanged, and it was also formed as one of the oxidation products of the quaterthiazole (XV). This suggests that the non-terminal thiazole nuclei in the terthiazole (XIV) and the quaterthiazole (XV) may be the preferential points of attack in these compounds, and oxidation may proceed *via* an acylthioamide, R·CS·NH·CO·R′, or *via* the corresponding diacylimide,

[3] Cf. Boudet, *Bull. Soc. chim. France*, 1951, 846; *Compt. rend.*, 1951, **233**, 796.

R·CO·NH·CO·R′. The terthiazole (XIV), in contrast to the bithiazolyl (XII), the quaterthiazole (XV), and the substance $C_{24}H_{23}O_5N_5S_4$, gave no ester-amide (VII) that could be detected. On the basis of the foregoing observations the formula (VI) for the compound $C_{24}H_{23}O_5N_5S_4$ may be provisionally expanded to the expression (XVIII).

FIG. 1. FIG. 3.

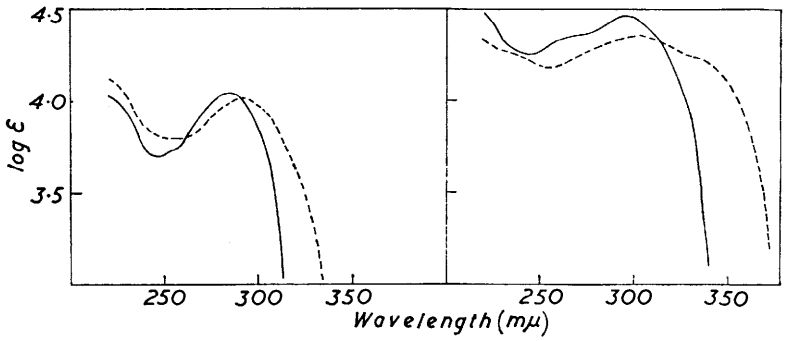


FIG. 1. *Ultraviolet absorption spectrum of methyl 2,4′-bithiazolyl-4-carboxylate* (XII); ——— *in* EtOH, – – – – – – *in* 10N-HCl.

FIG. 3. *Ultraviolet absorption spectrum of methyl 2,4′:2′,4″:2″,4‴-quaterthiazole-4-carboxylate* (XV); ——— *in* MeOH, – – – – – – *in* 10N-HCl.

FIG. 2. FIG. 4.

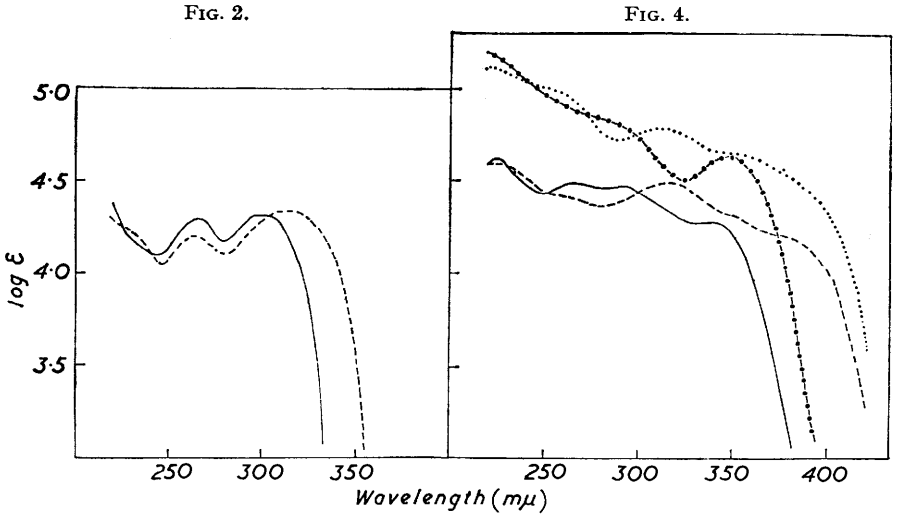


FIG. 2. *Ultraviolet absorption spectrum of methyl 2,4′:2′,4″-terthiazole-4-carboxylate* (XIV); ——— *in* MeOH, – – – – – – *in* 10N-HCl.

FIG. 4. *Ultraviolet absorption spectrum of the substance* $C_{24}H_{23}O_5N_5S_4$; ——— *in* EtOH, – – – – – – *in* 10N-HCl. *Ultraviolet absorption spectrum of micrococcin* P; —·—·— *in* EtOH, ... *in* 10N-HCl.

The ultraviolet absorption spectra of the synthetic polythiazoles seemed to indicate that limited conjugation exists between 2,4′-linked thiazole nuclei. Methyl 2,4′-bithiazolyl-4-carboxylate (XII) (Fig. 1) showed a single maximum in ethanol at 285 mμ, which agrees closely with observations on 2-acylthiazole-4-carboxylic acids,[1,4] and on methyl 2-propionylthiazole-4-carboxylate.[1] Methyl 2,4′:2′,4″-terthiazole-4-carboxylate (XIV) (Fig. 2), on the other hand, showed two maxima in methanol, one at longer and one at

[4] Hausmann, Weisiger, and Craig, *J. Amer. Chem. Soc.*, 1955, **77**, 730; Weisiger, Hausmann, and Craig, *ibid.*, p. 3123.

shorter wavelength than the single maximum shown by the bithiazolyl (XII). Methyl 2,4′:2′,4″:2″,4‴-quaterthiazole-4-carboxylate (XV) (Fig. 3) showed a similar but less well resolved spectrum, and, surprisingly, the long-wave maximum was observed at slightly shorter wavelength than that in the terthiazole (XIV). The substance $C_{24}H_{23}O_5N_5S_4$, which contains the essential chromophoric system of micrococcin *P*, showed absorption (Fig. 4) at significantly longer wavelength than any of the synthetic polythiazole esters, indicating a more extended chromophoric system in the substance $C_{24}H_{23}O_5N_5S_4$. All of these substances exhibited a shift of absorption to longer wavelength in acid solution. Infrared absorption spectra will be considered in a later communication.

Micrococcin *P*, with a molecular weight of about 2290,[5] contains approximately 16% of sulphur,[6] indicating no less than eleven atoms of sulphur in the molecule. Of these *at least* four have been shown to be present in thiazole rings, and the structures of two of the fully identified products of acid hydrolysis, 2-propionylthiazole-4-carboxylic acid (I) and 2-(1-amino-2-methylpropyl)thiazole-4-carboxylic acid (II) suggested their derivation from amino-acid and peptide precursors in the manner outlined above. We therefore explored fortification of the culture medium with the appropriate amino-acids, singly and in combination, to see whether their availability might be a limiting factor in the production of micrococcin *P*; in particular, we studied the addition of DL-α-aminobutyric acid, DL-valine, and L-cystine (*vice* L-cysteine) to the culture medium in which *Bacillus pumilus* was producing micrococcin *P*. The results shown in the Table indicate that the organism can supply its own requirements of α-aminobutyric acid and valine, but that those cultures which received supplements of cystine (*vice* cysteine) produced roughly twice as much micrococcin *P* as the others, and the same was found to hold for large-scale cultures (320 l.). This observation accords well with our view that micrococcin *P* is derived biogenetically from a cysteine-rich peptide by cyclisation at cysteine residues to form (ultimately) thiazole structures that are resistant to acid hydrolysis. The lack of dependence on α-aminobutyric acid is perhaps not surprising since it is derivable biosynthetically from threonine,[7] which has already been demonstrated [1] as a product of the acid hydrolysis of micrococcin *P* and considered in relation to the origin of the aminoacetone contained in the antibiotic.[5] Similarly, lack of dependence on valine may be attributed to its relatively ready availability biosynthetically from simple glycolytic precursors.[8]

Effect of supplements on yield of micrococcin P.

Medium	Antibiotic titre (in arbitrary units)
Basal	1500
Basal + cystine	3200
Basal + valine	1600
Basal + α-aminobutyric acid	1300
Basal + α-aminobutyric acid + valine	1200
Basal + α-aminobutyric acid + cystine	2900
Basal + valine + cystine	3200
Basal + α-aminobutyric acid + valine + cystine	3300
Basal + potassium sulphate (0·1%)	1300
Basal + potassium sulphate (0·35%)	1900
Basal + mercaptoacetic acid	1300

Experimental

Light petroleum refers to the fraction of boiling range 60—80°.

Further Purification of the Substance $C_{24}H_{23}O_5N_5S_4$.—The dimethyl ester (100 mg.) was chromatographed on a column (16 × 3 cm.) of " Solka-floc " (S.W.40B) (30 g.) with 1 : 1 v/v

[5] Mijović and Walker, preceding paper.
[6] Fuller, *Nature*, 1955, **175**, 722; Abraham, Heatley, Brookes, Fuller, and Walker, *ibid.*, 1956, **178**, 44; cf. Heatley and Doery, *Biochem. J.*, 1951, **50**, 247.
[7] Lien and Greenberg, *J. Biol. Chem.*, 1953, **200**, 367.
[8] Strassman, Thomas, and Weinhouse, *J. Amer. Chem. Soc.*, 1955, **77**, 1261; Wagner, Radhakrishnan, and Snell, *Proc. Nat. Acad. Sci. U.S.A.*, 1958, **44**, 1047.

chloroform–light petroleum saturated with water. When this system was used the diester travelled fairly rapidly down the column, leaving impurities adsorbed at the top. Recrystallisation from chloroform–ether gave white needles (69 mg.), m. p. 258° (Found: C, 49·1; H, 4·0; N, 11·7. Calc. for $C_{24}H_{23}O_5N_5S_4$: C, 48·9; H, 3·9; N, 11·9%), $\lambda_{max.}$ 225—226, 264, 290, and 328 (infl.) mμ (log ε 4·62, 4·48, 4·48, and 4·28) in EtOH, 316 mμ (log ε 4·50) in 10N-HCl (Fig. 4).

Oxidation of the Substance $C_{24}H_{23}O_5N_5S_4$ *with Chromium Trioxide.*—(*A*) The substance $C_{24}H_{23}O_5N_5S_4$ (200 mg.) was suspended in glacial acetic acid (16·7 c.c.) and added to a solution of chromium trioxide (830 mg.) in 90% acetic acid (3·3 c.c.). The mixture was kept at 100° for 3 hr., insoluble material (87 mg.) was then removed by centrifugation, excess of chromium trioxide was destroyed by addition of ethanol, and the clear solution was evaporated to dryness; the last traces of acetic acid were removed by repeated evaporation to dryness with toluene. The residue was taken up in water (4 c.c.) and continuously extracted with ether for 3 hr. Evaporation of the dried ether extract gave a white solid (42 mg.), which was best purified by sublimation at 140° (bath-temp.)/0·5 mm., followed by recrystallisation from chloroform–light petroleum, affording colourless prisms (21 mg.), m. p. 176—179° [Found: C, 38·8; H, 3·2; N, 15·1; S, 17·3; OMe, 15·4%; *M* (Rast), 195. $C_6H_6O_3N_2S$ requires C, 38·7; H, 3·3; N, 15·1; S, 17·2; OMe, 16·6%; *M*, 186], $\lambda_{max.}$ 235 and 265 mμ (log ε 4·00 and 3·83) in MeOH.

(*B*) In a similar manner, the substance $C_{24}H_{23}O_5N_5S_4$ (1·0 g.) was later oxidised with chromium trioxide (8·3 g.). The crude product (165 mg.), obtained by continuous ether-extraction for 16 hr., was crystallised from chloroform–light petroleum, affording colourless plates (81·3 mg.), m. p. 169—173°, not depressed on admixture with methyl 2-carbamoylthiazole-4-carboxylate; identity was confirmed by comparison of infrared absorption spectra and paper chromatography. The mother-liquors were evaporated to dryness and the residue was chromatographed on alumina (2·4 g.) in the manner described below. Benzene eluted a substance (36·7 mg.) shown by comparison of infrared absorption spectra and paper chromatography to be methyl thiazole-4-carboxylate.

(*C*) The same two substances were isolated on oxidation of the substance $C_{24}H_{23}O_5N_5S_4$ (750 mg.) with twice its weight of chromium trioxide.

Alkaline Hydrolysis of the Substance $C_6H_6O_3N_2S$.—The preceding product (20 mg.) was dissolved in water (2 c.c.), and 40% aqueous sodium hydroxide (0·2 c.c.) was added. The solution was boiled until no more ammonia was evolved (5 min.), and it was then cooled, acidified, and continuously extracted with ether for 1 hr. Evaporation of the dried extract gave a solid, which partly dissolved in cold chloroform. The insoluble portion was shown by m. p. behaviour and infrared absorption spectrum to be thiazole-2,4-dicarboxylic acid. Concentration of the chloroform solution and addition of light petroleum precipitated a solid shown to be thiazole-4-carboxylic acid by its m. p. and infrared absorption spectrum.

Monothio-oxamide (IX).—Attempts to follow the directions given by Weddige [9] for the preparation of monothio-oxamide from ethyl thio-oxamate were unsatisfactory but the following procedure was convenient. Ethyl thio-oxamate [10] (2·0 g.) was shaken in a separating funnel with 2N-ammonia (10 c.c.) until the change in crystalline form of the solid appeared to be complete (about 2 min.). The mixture was then extracted repeatedly with ether until the extracts ceased to be coloured. The combined dried extracts were evaporated below 50°, and crystallisation of the resulting solid from ethanol afforded monothio-oxamide (IX) as yellow needles (1·2 g., 77%), m. p. 180—183° (Found: C, 22·9; H, 3·7; N, 26·2. Calc. for $C_2H_4ON_2S$: C, 23·1; H, 3·8; N, 26·9%). Weddige [9] did not record an m. p.

Methyl 2-Carbamoylthiazole-4-carboxylate (VII).—Methyl bromopyruvate was obtained by the action of *N*-bromosuccinimide on methyl lactate by following the method described for the ethyl ester.[11] Methyl bromopyruvate (0·45 g.) was added to a solution of monothio-oxamide (0·25 g.) in methanol (20 c.c.) and the mixture was boiled under reflux for 3 hr. The solid left on evaporation of the solvent was extracted with chloroform, insoluble material (0·15 g.) being rejected. Concentration of the chloroform solution gave methyl 2-carbamoylthiazole-4-carboxylate (VII) (0·40 g.), and purification by sublimation and crystallisation from chloroform–light petroleum gave colourless needles, m. p. 175—178° (Found: C, 38·6; H, 3·3; N, 15·2. Calc. for $C_6H_6O_3N_2S$: C, 38·7; H, 3·3; N, 15·1%); the infrared absorption spectrum of this

[9] Weddige, *J. prakt. Chem.*, 1874, **9**, 137.
[10] Reissert, *Ber.*, 1904, **37**, 3721.
[11] Kruse, Geurkink, and Grist, *J. Amer. Chem. Soc.*, 1954, **76**, 5796.

compound was identical with that of the substance $C_6H_6O_3N_2S$, obtained by oxidation of the substance $C_{24}H_{23}O_5N_5S_4$.

Methyl 2,4′-Bithiazolyl-4-carboxylate (XII).—(i) *Thiazole-4-carboxamide.* A mixture of ethyl thiazole-4-carboxylate [12] (70 g.), dissolved in ethanol (50 c.c.), and ethanolic ammonia solution (saturated at 0°) (60 c.c.) was heated in a stainless-steel autoclave at 105° for 7 hr. Evaporation of the solution and crystallisation of the solid residue from water afforded the amide as colourless needles (46·2 g., 81%), m. p. 149—152° (lit.,[13] m. p. 150°).

(ii) *4-Cyanothiazole.* The preceding amide (70·0 g.) was suspended in dry pyridine (275 c.c.), and redistilled phosphoryl chloride (50·0 c.c.) was added slowly with cooling. An immediate exothermic reaction took place. After ½ hr. the mixture was added to water and extracted thrice with ether. The combined ether extracts were washed with dilute hydrochloric acid, dried, and evaporated, giving the nitrile as an oil, which crystallised in colourless needles (26·15 g., 44%), m. p. 57—59° (lit.,[14] m. p. 59·5—60·5°). Better conversion was obtained in small-scale experiments.

(iii) *Thiazole-4-carboxythioamide.* Hydrogen sulphide was passed for 8 hr. into a solution at 0° of the preceding nitrile (29·8 g.) in ethanol (200 c.c.) containing triethanolamine (4 c.c.). The mixture was kept at room temperature overnight and the crude product, which had separated, was collected. Recrystallisation from ethanol gave the thioamide as pale yellow flattened prisms (37·2 g., 98%), m. p. 194—198° (lit.,[14] m. p. 195—196°).

(iv) A mixture of the preceding thioamide (21·6 g.) and methyl bromopyruvate (27·2 g.) in dry methanol (200 c.c.) was boiled under reflux for 3 hr., the product separating. After cooling, the product (30·6 g., 90%; m. p. 178—181°) was collected. Recrystallisation from ethyl acetate afforded *methyl 2,4′-bithiazolyl-4-carboxylate* (XII) as off-white needles, m. p. 179—181° (Found: C, 42·4; H, 2·8; N, 12·4. $C_8H_6O_2N_2S_2$ requires C, 42·5; H, 2·7; N, 12·3%), $\lambda_{max.}$ 285 mμ (log ε 4·04) in EtOH, 290 mμ (log ε 4·02) in 10N-HCl (Fig. 1).

2,4′-Bithiazolyl-4-carboxamide.—A suspension of methyl 2,4′-bithiazolyl-4-carboxylate (24·1 g.) in methanol (250 c.c.) was heated with methanolic ammonia solution (saturated at 0°) (500 c.c.) in a stainless-steel autoclave at 100° for 8 hr. After cooling and evaporation of the solvent, crystallisation of the residue from water gave *2,4′-bithiazolyl-4-carboxamide* as colourless needles (19·1 g., 85%), m. p. 185—188° (Found: C, 39·9; H, 2·1; N, 19·8. $C_7H_5ON_3S_2$ requires C, 39·8; H, 2·4; N, 19·9%).

4-Cyano-2,4′-bithiazolyl.—Phosphoryl chloride (15·3 g.) was added slowly with ice-cooling to a suspension of the preceding amide (21·1 g.) in dry pyridine (25 c.c.). An immediate exothermic reaction took place. After ½ hr. at room temperature, water (250 c.c.) was added to the mixture and the product was collected. Recrystallisation from propan-1-ol afforded *4-cyano-2,4′-bithiazolyl* as colourless needles (11·1 g., 57%), m. p. 184—186° (Found: C, 43·3; H, 1·7; N, 21·4. $C_7H_3N_3S_2$ requires C, 43·5; H, 1·5; N, 21·8%).

2,4′-Bithiazolyl-4-carboxythioamide (XIII).—Hydrogen sulphide was bubbled gently for 4 hr. through a solution of the preceding nitrile (11 g.) in 2-ethoxyethanol (750 c.c.) containing triethanolamine (2 c.c.) cooled to 0°. The mixture was kept at room temperature overnight and then evaporated almost to dryness. The solid was collected, and recrystallisation from propan-1-ol afforded the *thioamide* as yellow needles (7·7 g., 60%), m. p. 195—198° (Found: C, 37·4; H, 2·1; N, 18·4. $C_7H_5N_3S_3$ requires C, 37·0; H, 2·2; N, 18·5%).

Methyl 2,4′:2′,4″-Terthiazole-4-carboxylate (XIV).—A solution of the preceding thioamide (1·13 g.) and methyl bromopyruvate (0·90 g.) in dimethylformamide (15 c.c.) was heated on the boiling-water bath for 2 hr. and then cooled. The product was collected and washed with methanol. Recrystallisation from dimethylformamide–ether afforded *methyl 2,4′:2′,4″-terthiazole-4-carboxylate* (XIV) as buff-coloured plates (1·03 g., 67%), m. p. 232—234° (Found: C, 42·6; H, 2·3; N, 13·7. $C_{11}H_7O_2N_3S_3$ requires C, 42·7; H, 2·3; N, 13·6%), $\lambda_{max.}$ 268 and 300 mμ (log ε 4·28 and 4·32) in MeOH, 264 and 313 mμ (log ε 4·20 and 4·34) in 10N-HCl (Fig. 2).

Ethyl 2-1′-Benzoyloxyethylthiazole-4-carboxylate.—A mixture of *O*-benzoyl-lact-thioamide [15] (16 g.), ethyl bromopyruvate (14·9 g.), and powdered calcium carbonate (16 g.) in ethanol (160 c.c.) was boiled under reflux on the water-bath for 15 hr. The solution, freed from inorganic salts by filtration, was concentrated to small bulk, and the residue was distributed

[12] Erne, Ramirez, and Burger, *Helv. Chim. Acta*, 1951, **34**, 143.
[13] Erlenmeyer and Morel, *ibid.*, 1945, **28**, 362.
[14] Menassé, Prijs, and Erlenmeyer, *ibid.*, 1957, **40**, 554.
[15] Olin and Johnson, *Rec. Trav. chim.*, 1931, **50**, 72.

between 2N-sodium carbonate and ether. Evaporation of the dried ethereal solution gave a brown oil, which was passed in benzene down a column of activated alumina (Peter Spence & Sons, Ltd., Type H; activity IV/V) (440 g.). The effluent, on evaporation, gave *ethyl 2-1'-benzoyloxyethylthiazole-4-carboxylate* as a low-melting solid (12·4 g., 53%), which was analysed directly (Found: C, 59·2; H, 5·0; N, 4·4. $C_{15}H_{15}O_4NS$ requires C, 59·0; H, 5·0; N, 4·6%).

2-1'-Hydroxyethylthiazole-4-carboxylic Acid.—The preceding benzoyl derivative (5·32 g.) was dissolved in ethanol (25 c.c.) and treated with a solution of potassium hydroxide (2·25 g.) in ethanol (25 c.c.). The mixture was boiled under reflux for 1 hr. and then taken to dryness under reduced pressure. The residue was taken up in water (75 c.c.) and washed twice with ether. The aqueous solution was acidified with concentrated hydrochloric acid and extracted with half its volume of ether to remove benzoic acid (Found: 2·25 g. Calc.: 2·18 g.). The aqueous phase was then continuously extracted with ether for 4½ hr., affording a yellow oil which crystallised in contact with ethyl acetate. Recrystallisation from ethyl acetate–light petroleum gave *2-1'-hydroxyethylthiazole-4-carboxylic acid* as colourless needles (2·17 g., 72%), m. p. 148—150° (Found: C, 41·3; H, 4·0; N, 7·8. $C_6H_7O_3NS$ requires C, 41·6; H, 4·1; N, 8·1%).

The methyl ester, prepared with the aid of ethereal diazomethane, crystallised from ethyl acetate–light petroleum in plates, m. p. 89—92°.

Methyl 2-Acetylthiazole-4-carboxylate.—Chromic acid solution [16] (8N; 0·5 c.c.) was added with cooling and stirring to a solution of the preceding methyl 2-1'-hydroxyethylthiazole-4-carboxylate (0·62 g.) in purified acetone (10 c.c.). After a few minutes of further stirring, saturated aqueous potassium carbonate solution was added together with ether. The organic phase was separated, washed with water, dried, and evaporated, affording an oil, which rapidly solidified. Recrystallisation from benzene–light petroleum gave *methyl 2-acetylthiazole-4-carboxylate* as colourless plates (0·38 g., 62%), m. p. 78—80° (Found: C, 45·3; H, 4·2; N, 7·6. $C_7H_7O_3NS$ requires C, 45·4; H, 4·0; N, 7·6%).

Methyl 2,4':2',4'':2'',4'''-Quaterthiazole-4-carboxylate (XV).—(*A*) Bromine (0·24 c.c.) in glacial acetic acid (2 c.c.) was added dropwise with stirring to a hot solution of methyl 2-acetylthiazole-4-carboxylate (0·40 g.) in glacial acetic acid (10 c.c.). The bromine colour was rapidly discharged and hydrogen bromide was evolved. After ½ hr. at the b. p. the solvent was removed under reduced pressure. To the residual oil were added 2,4'-bithiazolyl-4-carboxythioamide (482 mg.) and dimethylformamide (5 c.c.), and the mixture was heated on the water-bath for 3 hr.; a crystalline solid separated. Recrystallisation from dimethylformamide–ether afforded *methyl 2,4':2',4'':2'',4'''-quaterthiazole-4-carboxylate* (XV) as buff-coloured plates (0·42 g., 50%), m. p. 303—306° (Found: C, 42·6; H, 2·0; N, 14·3. $C_{14}H_8O_2N_4S_4$ requires C, 42·8; H, 2·0; N, 14·3%), $\lambda_{max.}$ 273 (infl.) and 296 mμ (log ε 4·37 and 4·47) in MeOH, 302 mμ (log ε 4·36) in 10N-HCl (Fig. 3).

(*B*) A mixture of methyl 2-1'-hydroxyethylthiazole-4-carboxylate (0·94 g.) and *N*-bromosuccinimide (1·78 g.) in dry carbon tetrachloride (30 c.c.) was boiled under reflux on a water-bath. After 2½ hr. the deep red colour had become much lighter and evolution of hydrogen bromide had slackened; heating was continued for a further 2½ hr. Evaporation of the colourless filtrate, after removal of succinimide, gave an oil (1·26 g., 95%), which crystallised on cooling. When a solution of this substance and 2,4'-bithiazolyl-4-carboxythioamide (1·09 g.) in dimethylformamide (7·5 c.c.) was heated on the steam-bath, a crystalline solid began to separate in about 3 min. After 2 hr. on the steam-bath the cooled product was collected and washed with methanol. Recrystallisation from dimethylformamide–ether afforded buff-coloured plates (1·45 g., 74%), m. p. 303—306°, identical (m. p., infrared absorption spectrum) with methyl 2,4':2',4'':2'',4'''-quaterthiazole-4-carboxylate obtained as in (*A*) (above).

Chromatography of the Products obtained by Oxidation of Synthetic Methyl Polythiazole-4-carboxylates with Chromium Trioxide.—In the following experiments alumina denotes Savory and Moore Ltd. aluminium oxide (" for chromatographic analysis "; Brockmann activity II). Paper chromatograms were run on Whatman No. 1 chromatographic paper, with butanol–acetic acid–water (63 : 10 : 27), and spots were detected by suspending dried papers in an atmosphere containing iodine vapour.

Oxidation of Methyl 2,4'-Bithiazolyl-4-carboxylate (XII).—The bithiazolyl ester (2·26 g., 0·01 mole) was suspended in glacial acetic acid (50 c.c.) and treated with a solution of chromium trioxide (4·52 g.) in 90% acetic acid (100 c.c.). The mixture was heated on the steam-bath for 3 hr. Ethanol was added to destroy any excess of chromium trioxide, and the solution was

[16] Bladon, Fabian, Henbest, Koch, and Wood, *J.*, 1951, 2407.

taken to dryness, the last traces of acetic acid being removed by repeated evaporation to dryness with toluene. The residue was treated with water (50 c.c.), then continuously extracted with ether for 16 hr. Evaporation of the dried ether extract gave a colourless solid (1·68 g.), and recrystallisation from chloroform–light petroleum afforded a mixture (870 mg.), which was chromatographed on alumina (26 g.). Benzene eluted a substance (323 mg.), m. p. 179—181°, shown by mixed m. p., comparison of infrared absorption spectra, and paper chromatography to be unchanged methyl 2,4′-bithiazolyl-4-carboxylate (XII). Benzene–ether eluted a colourless solid (263 mg.), affording on recrystallisation from chloroform–light petroleum, colourless plates, m. p. 151—154°, raised to 175—177° on further crystallisation from methanol; there was no depression of m. p. on admixture with methyl 2-carbamoylthiazole-4-carboxylate (VII), and identity was confirmed by comparison of infrared absorption spectra and paper chromatography (Found: C, 38·7; H, 3·4; N, 15·3. Calc. for $C_6H_6O_3N_2S$: C, 38·7; H, 3·3; N, 15·1%).

The mother-liquors were bulked and taken to dryness, yielding a colourless solid (660 mg.) which was chromatographed on alumina (19·8 g.). Benzene eluted a crystalline solid (489 mg.), which, on sublimation at 100—120°/15 mm., gave colourless flattened needles (255 mg.), m. p. 72—75°. The substance was shown by comparison of infrared spectra, paper chromatography, m. p. and mixed m. p. to be methyl thiazole-4-carboxylate (XVI). Further sublimation of the residue at 120—150°/15 mm. afforded colourless plates (208 mg.), m. p. 178—180°, not depressed on admixture with methyl 2,4′-bithiazolyl-4-carboxylate (XII); identity was confirmed by comparison of infrared absorption spectra and paper chromatography.

Oxidation of Methyl 2,4′:2′,4″-Terthiazole-4-carboxylate (XIV).—This substance (1·0 g.) was oxidised by the method described above for methyl 2,4′-bithiazolyl-4-carboxylate. The ether extract afforded an oil which rapidly solidified. Sublimation at 90°/0·005 mm. afforded a colourless microcrystalline solid, m. p. 70—127°. Crystallisation of the sublimate from methanol gave a mixture (470 mg.) of colourless needles and stout prisms, which was chromatographed on alumina (15 g.). Benzene eluted a substance which sublimed at 100—120°/15 mm., to give *methyl thiazole-4-carboxylate* (XVI) as colourless plates (45 mg.), m. p. 72—75°, not depressed on admixture with an authentic specimen obtained by esterification of the free acid with diazomethane (Found: C, 42·3; H, 3·8; N, 9·8. $C_5H_5O_2NS$ requires C, 42·0; H, 3·5; N, 9·8%). Identity was further confirmed by comparison of infrared absorption spectra and paper chromatography.

Elution with methanol–ether afforded a substance which crystallised from chloroform–light petroleum in colourless needles (164 mg.), m. p. 151—153°, not depressed on admixture with thiazole-4-carboxamide (XVII) (Found: C, 37·4; H, 3·2. Calc. for $C_4H_4ON_2S$: C, 37·5; H, 3·1%). Identity was confirmed by comparison of infrared absorption spectra and paper chromatography.

Oxidation of Methyl 2,4′:2′,4″:2″,4‴-Quaterthiazole-4-carboxylate (XV).—This substance (1·0 g.) was oxidised by the method described above. The ether extract afforded an oil which rapidly solidified and this was submitted to fractional sublimation. The material (270 mg.) subliming at 86—135°/16 mm. was chromatographed on alumina (8·3 g.). Benzene eluted a substance which sublimed at 100—120°/15 mm. as colourless plates (52 mg.), m. p. 72—75°, identified by m. p., mixed m. p., infrared absorption spectrum, and paper chromatography as methyl thiazole-4-carboxylate (XVI) (Found: C, 42·2; H, 3·4%). Methanol–ether then eluted a substance which crystallised from chloroform–light petroleum in colourless needles (44 mg.), m. p. 151—153°, identified by m. p., mixed m. p., infrared absorption spectrum, and paper chromatography as thiazole-4-carboxamide (XVII) (Found: C, 37·8; H, 3·5. Calc. for $C_4H_4ON_2S$: C, 37·5; H, 3·1%).

The material (146 mg.) subliming at 135—198°/16 mm. was similarly chromatographed on alumina (4·4 g.). Benzene eluted a substance (68 mg.) which sublimed at 100—140°/15 mm. as colourless plates, m. p. 165—168°, raised to 178—181° by crystallisation from ethyl acetate; the substance was identified as methyl 2,4′-bithiazolyl-4-carboxylate (XII) by m. p., mixed m. p., and infrared absorption spectrum. Benzene–ether then eluted a substance (33 mg.), which crystallised from chloroform–light petroleum in colourless plates, m. p. 164—170°, raised to 175—177° on recrystallisation from methanol; the substance was identified as methyl 2-carbamoylthiazole-4-carboxylate (VII) by m. p., mixed m. p., and infrared absorption spectrum.

Effect of Supplements on Yields of Micrococcin P produced by a B. pumilus *Species.*—A basal medium containing glucose (1%), ammonium citrate (1%), "Lab-lemco" (Oxoid) (0·5%),

dipotassium hydrogen phosphate (0·5%), and other inorganic salts (traces) was used and supplemented in the appropriate experiments with L-cystine (0·1%), DL-valine (0·1%), and DL-α-aminobutyric acid (0·1%), singly or in combination; potassium sulphate (0·1 and 0·35%) and mercaptoacetic acid (0·1%) were also used in separate experiments. The various media, basal or supplemented, were inoculated with an 18 hr. culture of the *B. pumilus* species and shaken for up to 60 hr. at 35°. The resulting antibiotic titres, observed in two series of experiments and expressed in arbitrary units, are shown in the Table.

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