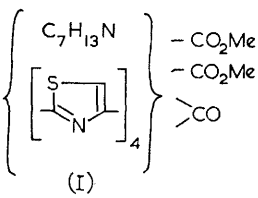
Chemistry of Micrococcin *P*. Part VII.[1] Dimethyl Micrococcinate and Some Synthetic Pyridine–polythiazole Carboxylic Esters

By R. J. Clark and James Walker

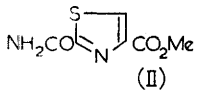
Dimethyl micrococcinate is shown to have the molecular formula $C_{21}H_{17}N_5O_5S_4$ and to consist probably of a pyridine ring and four thiazole rings with two ester functions, a carbonyl group, and an ethyl group. The ultraviolet absorption spectra of dimethyl micrococcinate and of 2,6-bis-(4′-methoxycarbonyl-2′,4-bithiazolyl-2-yl)pyridine show striking similarity in alcoholic and acid solutions.

Hydrolysis of the antibiotic micrococcin *P* with 20% hydrochloric acid afforded among other products an insoluble acidic substance, which was characterised as a dimethyl ester giving analytical figures suggesting a molecular formula $C_{24}H_{23}N_5O_5S_4$.[2] For convenience we now designate this acid micrococcinic acid and the ester dimethyl micrococcinate.

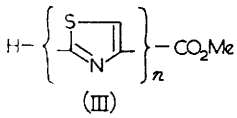
The infrared absorption spectrum of dimethyl micrococcinate in a potassium chloride disc showed three strong bands in the carbonyl-stretching region, and these were tentatively attributed to two ester carbonyl groups and (?) an aryl (heterocyclic?) ketone carbonyl group;[2] in chloroform solution the bands attributed to the ester carbonyl groups were superimposed and the ketonic carbonyl group band was shifted to slightly higher frequency.[3] The nitrogen : sulphur ratio, stability of the parent dibasic acid to further acid hydrolysis, light-absorption properties, and other evidence were deemed compatible with a polythiazole structure (I)[2] for dimethyl micrococcinate on the hypothesis that it was derived biogenetically from a cysteine-rich peptide.



(I)

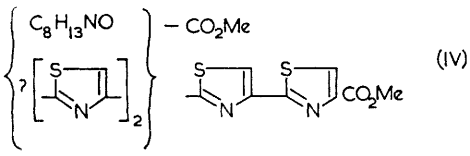


(II)

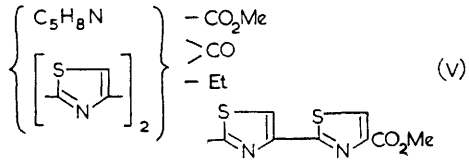


(III)

Further clarification of the structure of dimethyl micrococcinate came when methyl 2-carbamoylthiazole-4-carboxylate (II) was isolated as an oxidation product, and a study of the oxidation products of a series of synthetic 2,4′-linked polythiazole-4-carboxylic esters (III; $n = 2$—4) showed that the amide group in methyl 2-carbamoylthiazole-4-carboxylate (II) could have been generated by the oxidation of a second thiazole ring. Methyl thiazole-4-carboxylate (III; $n = 1$) was also detected as an oxidation product of dimethyl micrococcinate, and the modified expression (IV) was therefore advanced for the latter.[4] Nuclear magnetic resonance measurements, kindly made for us in November 1959, by Dr. N. Sheppard at the University Chemical Laboratory, Cambridge, demonstrated the presence of an ethyl group and of three or four thiazole-5 protons in dimethyl micrococcinate, and permitted revision of the expressions (I) and (IV) for dimethyl micrococcinate to the expression (V).



(IV)



(V)

We have already discussed the ultraviolet light-absorption characteristics of the synthetic polythiazole esters (III; $n = 2$—4), and, as it showed absorption at significantly longer wavelength than any of these, dimethyl micrococcinate, which contains the essential chromophore of micrococcin *P*, was shown to contain a more extended chromophoric system than the quaterthiazole ester (III; $n = 4$). Now the indeterminate portion, C_5H_8N, of the expression (V), suggestive of a reduced pyridine ring, was obviously too saturated to contribute effectively to an extension of the chromophoric system of a quaterthiazole ester, and we became aware that the early analyses of dimethyl micrococcinate had given higher hydrogen values than those obtained subsequently on specimens prepared at different times and purified in a variety of ways. The outcome of these determinations was to indicate $C_{24}H_{19}N_5O_5S_4$ or, more probably, $C_{24}H_{17}N_5O_5S_4$ as more appropriate molecular formulæ for dimethyl micrococcinate; the H_{17} formula was conclusively shown to be correct by Dr. G. E. Hall by nuclear magnetic resonance measurements (Part X) and follows from the *X*-ray crystallographic examination of the derived bis-4-bromoanilide by Mr. M. N. G. James and Mr. K. J. Watson (Part IX), The indeterminate portion, C_5H_8N, of the expression (V) could therefore be revised to C_5H_2N, indicative of a pyridine nucleus, which, in view of the occurrence of pyridine-ring precursors in bacterial metabolism and

[1] Part VI, B. M. Dean, M. P. V. Mijović, and J. Walker, *J. Chem. Soc.*, 1961, 3394.
[2] P. Brookes, A. T. Fuller, and J. Walker, *J. Chem. Soc.*, 1957, 689.
[3] M. P. V. Mijović and J. Walker, *J. Chem. Soc.*, 1961, 3381.
[4] P. Brookes, R. J. Clark, A. T. Fuller, M. P. V. Mijović, and J. Walker, *J. Chem. Soc.*, 1960, 916.

the formation of dipicolinic acid by spore-forming organisms, was perfectly acceptable biogenetically. Dimethyl micrococcinate therefore appeared likely to consist of an aggregate of four thiazole rings and one pyridine nucleus, linked but not condensed, with two ester functions, a carbonyl group, and an ethyl group.

We then decided to compare the ultraviolet light absorption properties of dimethyl micrococcinate with those of the accessible compound (VI) containing four thiazole nuclei, a pyridine ring, and two esterified

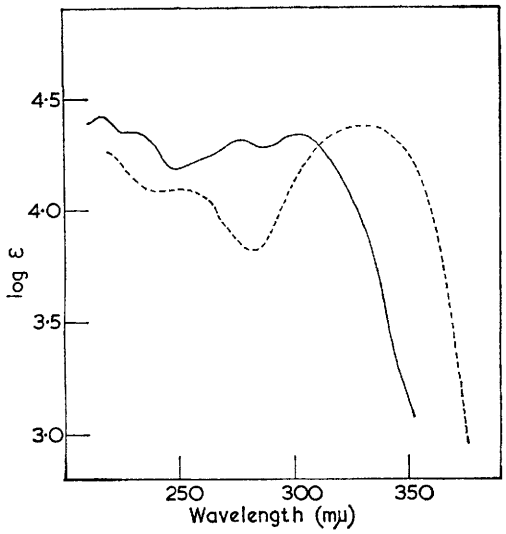
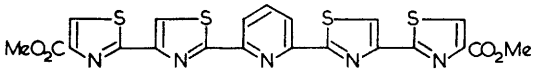
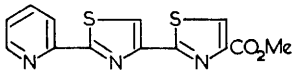


FIGURE 1 Ultraviolet absorption spectrum of methyl 2′-(pyrid-2-yl)-2,4′-bithiazolyl-4-carboxylate (VII); ——— in MeOH, – – – – in 10N-HCl

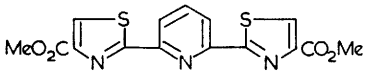
carboxyl substituents; this substance (VI) could plausibly be formed biogenetically from one molecule of dipicolinic acid, or diaminopimelic acid, and two



(VI)



(VII)

(VIII) 

molecules of cysteinylcysteine followed by appropriate thiazoline cyclisations and dehydrogenation in the manner already discussed.[2,4] In a preliminary experiment, thiopicolinamide, which has previously been employed in a thiazole synthesis,[5] was condensed with methyl 2-bromoacetylthiazole-4-carboxylate to give methyl 2′-(pyrid-2-yl)-2,4′-bithiazolyl-4-carboxylate (VII), whose ultraviolet absorption spectrum in methanol (Figure 1) closely resembled that of methyl 2,4′:2′,4″-terthiazole-4-carboxylate (III; $n = 3$) but it absorbed at rather longer wavelength than the latter in 10N-hydrochloric acid. Condensation of 2,6-dithiocarbamoylpyridine with methyl bromopyruvate afforded

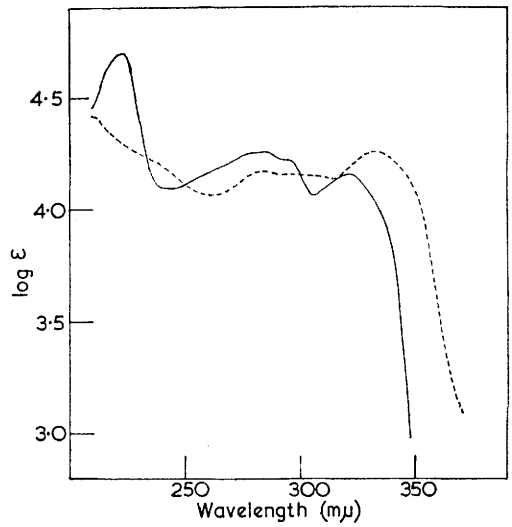


FIGURE 2 Ultraviolet absorption spectrum of 2,6-bis-(4-methoxycarbonylthiazol-2-yl)pyridine (VIII); ——— in MeOH, – – – – in 10N-HCl

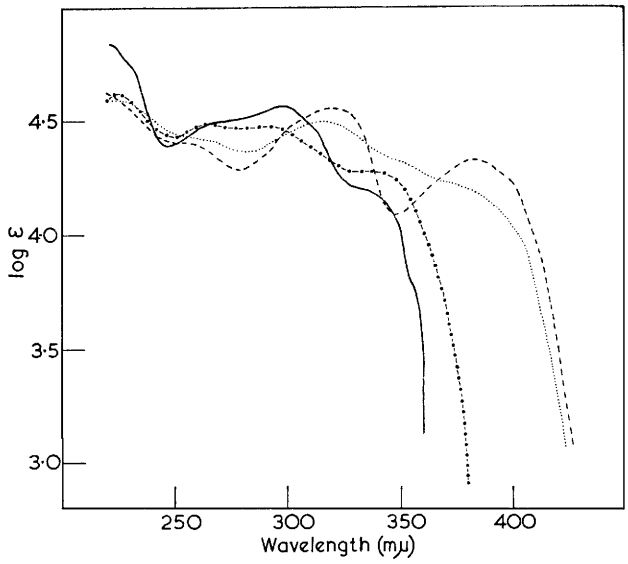


FIGURE 3 Ultraviolet absorption spectrum of 2,6-bis-(4′-methoxycarbonyl-2′,4-bithiazolyl-2-yl)pyridine (VI); ——— in ethanol, – – – – in 10N-HCl

Ultraviolet absorption spectrum of dimethyl micrococcinate; —·—·— in ethanol, · · · · in 10N-HCl

2,6-bis-(4-methoxycarbonylthiazol-2-yl)pyridine (VIII), and condensation with methyl 2-bromoacetylthiazole-4-carboxylate gave the required 2,6-bis-(4′-methoxycarbonyl-2′,4-bithiazolyl-2-yl)pyridine (VI), whose ultraviolet spectra in alcoholic and acid solution showed quite remarkable similarities with those of dimethyl micrococcinate (Figure 3), thus lending support to the general view of the structure of the latter outlined above.

[5] P. Karrer and J. Schukri, *Helv. Chim. Acta*, 1945, **28**, 820.

After examining the convenience of the procedure with a model substrate (methyl thiazole-4-carboxylate), the bis-4-bromoanilide was prepared from dimethyl micrococcinate by means of the Grignard reagent [6] for *X*-ray crystallographic investigation.[7] Although this had the disadvantage of introducing a considerable number of carbon atoms into an already large molecule it ensured that the bromine atoms were relatively inert chemically.

EXPERIMENTAL

Dimethyl Micrococcinate.—Cleaner preparations of crude micrococcinic acid (1·3 g.) were obtained by use of concentrated hydrochloric acid–98% formic acid (1 : 1) [8] (75 c.c.) under reflux for 5—6 hr. rather than 20% hydrochloric acid [2] as the hydrolysing agent for micrococcin *P* (5·4 g.). The dimethyl ester was prepared as described before [2] and crystallised well from dimethyl sulphoxide in colourless prisms, m. p. 250—251° (Found, on four different specimens purified in a variety of ways: C, 49·4, 49·0, 49·1, 49·1, 49·3, 49·3, 49·2; H, 2·9, 3·0, 3·0, 3·0, 3·0, 3·2, 3·1; N, 12·0, 11·75, 11·8; S, 21·7, 22·2, 22·0. Calc. for $C_{24}H_{23}N_5O_5S_4$: C, 48·9; H, 3·9; N, 11·9; S, 21·8. Calc. for $C_{24}H_{19}N_5O_5S_4$: C, 49·2; H, 3·3; N, 12·0; S, 21·9. Calc. for $C_{24}H_{17}N_5O_5S_4$: C, 49·4; H, 2·9; N, 12·0; S, 22·0%). A Kuhn–Roth oxidation afforded 0·88 molar proportion of volatile acid.

Methyl 2′-(Pyrid-2-yl)-2,4′-bithiazolyl-4-carboxylate (VII).—A mixture of methyl 2-bromoacetylthiazole-4 carboxylate [4] (1·17 g.) and thiopicolinamide (0·61 g.) in dry methanol (10 c.c.) was heated on the water-bath, and a crystalline solid soon began to separate. After 2 hr. under reflux the mixture was cooled and the product was collected. Recrystallisation from butan-1-ol afforded the *methyl pyridylbithiazolylcarboxylate* (VII) as fine needles (0·65 g., 45%), m. p. 226—228°; $\lambda_{max.}$ 216, 230, 278, and 301 mμ (log ε 4·41, 4·35, 4·31, and 4·33) in methanol, 250 and 332 mμ (log ε 4·09 and 4·37) in 10N-hydrochloric acid (Figure 1) (Found: C, 51·7; H, 3·3; N, 13·8; S, 20·7. $C_{13}H_9N_3O_2S_2$ requires C, 51·5; H, 3·0; N, 13·8; S, 21·1%).

2,6-*Bis*-(4-*methoxycarbonylthiazol*-2-*yl*)*pyridine* (VIII).—(i) 2,6-Dicyanopyridine [9] (4·25 g.) was added to liquid hydrogen sulphide (*ca.* 5 c.c.) followed by cold (−80°) ethanol (20 c.c.) containing triethanolamine (0·5 g.), and the mixture was heated at 55° for 8 hr. in the manner previously described.[10] Recrystallisation of the product from dimethylformamide–ether afforded 2,6-dithiocarbamoylpyridine as yellow needles (3·43 g., 53%), m. p. 250—252° (decomp.) (Found: C, 42·5; H, 3·75. Calc. for $C_7H_7N_3S_2$: C, 42·6; H, 3·5%).

(ii) A mixture of 2,6-dithiocarbamoylpyridine (0·49 g.) and methyl bromopyruvate (0·90 g.) in dimethylformamide (10 c.c.) was heated on a boiling-water bath for 3 hr. and then cooled. Recrystallisation of the product (0·75 g., 83%), m. p. 273°, from pyridine gave 2,6-*bis*-(4-*methoxycarbonylthiazol*-2-*yl*)*pyridine* (VIII) as colourless, fine, fluffy needles, m. p. 282°; $\lambda_{max.}$ 222, 278 (infl.), 285, 295 (infl.), and 321 mμ (log ε 4·70, 4·25, 4·25, 4·21, and 4·15) in methanol, 237 (infl.), 283, 294, and 334 mμ (log ε 4·22, 4·17, 4·15, and 4·26) in 10N-hydrochloric acid (Figure 2) (Found: C, 50·05; H, 3·2; N, 12·0; S, 17·5. $C_{15}H_{11}N_3O_4S_2$ requires C, 50·0; H, 3·1; N, 11·7; S, 17·8%).

2,6-*Bis*-(4′-*methoxycarbonyl*-2′,4-*bithiazolyl*-2-*yl*)*pyridine* (VI).—Methyl 2-1′-hydroxyethylthiazole-4-carboxylate (3·7 g.) was treated with *N*-bromosuccinimide (7·12 g.) in dry carbon tetrachloride (120 c.c.) as already described,[4] and the resulting crude methyl 2-bromoacetylthiazole-4-carboxylate (5·25 g., 99%) was heated with 2,6-dithiocarbamoylpyridine (1·96 g.) in dimethylformamide (35 c.c.) on the water-bath. A crystalline solid rapidly separated, and, after 2 hr. on the water-bath, the mixture was cooled, and the product was collected. Recrystallisation from dimethyl sulphoxide afforded 2,6-*bis*-(4′-*methoxycarbonyl*-2′,4-*bithiazolyl*-2-*yl*)*pyridine* (VI) as colourless, microscopic needles (4·33 g., 82%), m. p. 323—325°; $\lambda_{max.}$ 232 (infl.), 268 (infl.), 298, 314 (infl.), 335 (infl.), and 355 mμ (infl.) (log ε 4·73, 4·49, 4·57, 4·44, 4·20, and 3·78) in ethanol, 258 (infl.), 320, and 382 mμ (log ε 4·40, 4·55, and 4·32) in 10N-hydrochloric acid (Figure 3) (Found: C, 47·8; H, 2·8; N, 13·5; S, 24·2. $C_{21}H_{13}N_5O_4S_4$ requires C, 47·8; H, 2·5; N, 13·2; S, 24·3%).

Thiazole-4-carbon-4-bromoanilide.—A solution of 4-bromoaniline (0·59 g.) in dry ether (5 c.c.) was slowly added to a cold solution of methylmagnesium iodide, prepared from magnesium (80 mg.) and methyl iodide (0·65 g.) in dry ether (5 c.c.). When evolution of methane had ceased, methyl thiazole-4-carboxylate (0·23 g.) in dry chloroform (5 c.c.) was added, the mixture was warmed on the water-bath for 10 min., and then cooled. Excess of 2N-hydrochloric acid was added, and the mixture was warmed under reduced pressure to eliminate the organic solvent. The solid product was collected, washed with water, and dried (0·40 g., 84%). Crystallisation from aqueous ethanol afforded the 4-*bromoanilide* as colourless needles, m. p. 165—166° (Found: C, 42·5; H, 2·7; Br, 28·3; N, 9·4; S, 11·4. $C_{10}H_7BrN_2OS$ requires C, 42·5; H, 2·5; Br, 28·2; N, 9·9; S, 11·3%).

Micrococcinic Bis-4-bromoanilide.—A solution of 4-bromoaniline (1·11 g.) in dry anisole (5 c.c.) was slowly added to a cold solution of methylmagnesium iodide, prepared from magnesium (0·15 g.) and methyl iodide (0·96 g.), in dry anisole (5 c.c.). When evolution of methane had ceased, the solution was added to one of dimethyl micrococcinate (0·86 g.) in hot anisole (25 c.c.). A solid separated immediately and the mixture was stirred until it reached room temperature. Excess of 2N-hydrochloric acid was added, and the anisole layer was separated, dried, and evaporated to give a light brown solid (1·11 g., 84%), m. p. 273—276°. Crystallisation from pyridine and then from anisole afforded the *bis*-4-*bromoanilide* as light fawn needles, m. p. 275—277° (Found, on material dried at 140°/0·001 mm.: C, 47·3; H, 2·6; Br, 18·7; N, 10·9; S, 15·0. $C_{34}H_{21}Br_2N_7O_3S_4$ requires C, 47·3; H, 2·5; Br, 18·5; N, 11·35; S, 14·85%).

National Institute for Medical Research,
Mill Hill,
London N.W.7. [6/023 *Received, January 10th*, 1966]

[6] Cf. F. Bodroux, *Bull. Soc. chim. France*, 1905, **33**, 382; C. F. Koelsch and D. Tenenbaum, *J. Amer. Chem. Soc.*, 1933, **55**, 3049; D. V. N. Hardy, *J. Chem. Soc.*, 1936, 398.

[7] M. N. G. James and K. J. Watson, *J. Chem. Soc.(C)*, 1966, 1367.

[8] Cf. G. L. Miller and V. du Vigneaud, *J. Biol. Chem.*, 1937, **118**, 101; M. Bodanszky, J. T. Sheehan, J. Fried, N. J. Williams, and C. A. Birkhimer, *J. Amer. Chem. Soc.*, 1960, **82**, 4747.

[9] R. Lukeš and M. Pergàl, *Coll. Czech. Chem. Comm.*, 1959, **24**, 41.

[10] P. Brookes, R. J. Clark, B. Majhofer, M. P. V. Mijović, and J. Walker, *J. Chem. Soc.*, 1960, 925.