ISOLATION OF THREE NEW ANTIBIOTICS, THIOCILLINS I, II AND III, RELATED TO MICROCOCCIN P

(STUDIES ON ANTIBIOTICS FROM THE GENUS *BACILLUS*. VIII[1])

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Thiocillins I and II were isolated from the culture broth of *Bacillus cereus* G-15, and thiocillins II and III from that of *Bacillus badius* AR-91. Also, the former two were probably produced by *Bacillus megatherium* I-13. These antibiotics active against Gram-positive bacteria are soluble in a mixture of chloroform and methanol, show characteristic ultraviolet absorptions (maxima at *ca.* 275 nm and *ca.* 348 nm), and contain a high content of sulfur, as much as approximately 15 %. They are related to each other and also to micrococcin P, but differentiated by chromatographic behaviours.

In the course of our screening program for new antibiotics from the genus *Bacillus*,[1] a group of antibiotics characterized by UV absorptions and high sulfur contents were found to be produced by a variety of *Bacillus* species; *i.e.*, *B. cereus*, *B. badius* and possibly *B. pumilus* and *B. megatherium*. As representatives, three antibiotic components were isolated from the culture broths of strain G-15 identified with *B. cereus* and strain AR-91 identified with *B. badius*. Some of these antibiotics were found to be probably produced by strain I-13 identified with *B. megatherium* and also strain AR-140 which was preliminarily identified with *B. pumilus*, though the products were examined only by TLC. These antibiotics are closely related to micrococcin P, but were differentiated by direct comparison on TLC.

The term "micrococcin" originated for the antibiotic isolated from the culture broth of an unidentified species of *Micrococcus*, whose taxonomical data has been briefly described.[4] Later, a related antibiotic was isolated from the culture broth of a spore-bearing bacillus of *B. pumilus*.[5] The probable identity of both antibiotics and the naming of the latter micrococcin P have been reported.[6] Laborious work on the chemistry of micrococcin P by WALKER and his group[7] clarified most of the constituents of micrococcin P. Recently, WALKER* informed us that both specimens of micrococcin P and SU's original micrococcin are identical on TLC, and contain two components (P_1 and P_2).

The three antibiotics we isolated seem to belong to a group of antibiotics chemically related to micrococcin P. However, as already mentioned by WALKER,[6,7a] the term micrococcin is too narrowly suggestive of source to be satisfactory as a name for the antibiotic produced by a *Bacillus* species. Moreover, unlike in the case of naming micrococcin P which is now known to be identical with the SU's original micrococcin, the above antibiotics are produced not only by one species but a variety of species of the genus *Bacillus*. Therefore, we propose

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the new name "thiocillin" with approval of Dr. WALKER, which could apply to a substance produced by a bacillus, a coccus, or a bacterium without specific commitment to any particular family of microorganisms.

This paper deals with the taxonomic characteristics of the producing organisms as well as the isolation and characterization of thiocillins I, II and III.

Taxonomic Characters of the Producing Microorganisms

The taxonomic characteristics of the strain G-15 identified as *B. cereus* are as follows.

Morphology: The rods, on IM-agar, were 0.9~1.2 μ by 3.0~6.0 μ with slightly squared ends. They were Gram-positive, motile and occur in short to medium chain or singly (28°C, 1~2 days).

Spores, on IM-agar, were 0.9 μ by 1.6 μ elliptical, central to subterminal and were hardly stained by diluted methylene blue. The sporangia are not definitely swollen.

Colony on agar plate: Circular (1 day) to slightly filamentous (2 days or later) in form, raised in elevation, erose margin, not-shining grayish surface, soft to butyrous structure and opaque density. The colonies expand with aging.

Growth on agar stroke: Moderate to abundant, echinulate to arborescent, non-adherent grayish white growth was observed (Nutrient agar, 28°C, 1~5 days). The surface had a dull, mud-like appearance and was sometimes wrinkled. Diffusible pigment was not detected.

Nutrient broth: Moderate turbidity at 1 day but flocculent growth with sediment at 3 days or over. No pellicle and ring formation was observed (Nutrient broth containing 1 % glucose, 28°C, 1~5 days).

Conditions for growth: a. OF-Test; Weakly facultative anaerobe. Produces acid but no gas from glucose (28°C, 1~4 days). b. Temperature; Grew at 46°C to 20°C or below. The optimum was near 37°C (IM-agar, 1~2 days). c. pH; Grew at pH 5.75 to pH 8.50 or over. Did not grow at pH 4.9 (glucose nutrient broth, 28°C, 18 hours). The optimum was about pH 7 to 8. d. Resistance to NaCl; Grew weakly in 5 % NaCl broth but not in 7.5 % (glucose nutrient broth, 28°C, 1~4 days).

Physiological characters: The bacilli liquefied gelatin rapidly (yeast extract-supplied gelatin stab, 25°C, 1~15 days) and peptonized milk with no acid formation (28°C, 2~8 days). Coagulation of milk was not observed. Nitrite formation from nitrate, VOGES-PROSKAUER reaction, hydrolysis of starch, oxidase and catalase activities were all positive. Indole formation and H_2S production were both negative. These bacilli did not grow on KOSER's citrate medium but grew weakly on CHRISTENSEN's citrate medium. Acid formation was observed from glucose, fructose, sucrose, maltose, trehalose, starch, glycogen, glycerol and salicin but not from L-arabinose, D-xylose, mannose, galactose, lactose, inulin, inositol, sorbitol, arabitol and α-methyl-glucoside (28°C, 1~7 days).

Specification: The above descriptions suggest without any doubt that this organism is a strain of the species *cereus*.[2,3] The morphology and growth characteristics is also similar to that of *B. cereus* IFO 3131. Therefore, we named the bacillus as *Bacillus cereus* strain G-15.

A strain numbered I-13, whose products were identical with those of the strain G-15 on TLC, is characterized as follows.

Morphology: The rods, on IM-agar, are 1.1~1.5 μ by 2.5~5.0 μ with rounded ends. They occur in short chains or singly. On glucose-nutrient agar, many unstained granules were observed in lightly stained cells. Non-motile. Gram positive.

Spores, on IM-agar, are 1.0 μ by 1.5 μ, not easily stained ovals. The sporangia are not appreciably swollen.

Colony on agar plate: Colonies on nutrient agar were circular, convex (1 day) to raised (2 days or later), cream colored rough (1 day) to wrinkled (2 days or later) surface, butyrous (1 day) to gummy (2 days or later) consistency and opaque density (28°C, 1~7 days).

Growth on agar stroke: Moderate to abundant, echinulate, rough surface, butyrous to gummy growth of opaque density was observed on nutrient agar (28°C, 1~7 days). The cell mass is colored a pale yellowish brown and the tone becomes deeper on aging. Pale brown diffusible pigment was observed at 2 days or later.

Nutrient broth: Flocculent upper growth with sediment was observed on nutrient broth containing 1% glucose (28°C, 1~6 days). Pellicle or ring formation was not detected.

Conditions for growth: a. OF-Test; Oxidative. Did not produce significant amounts of acid or gas from glucose on deep culture (28°C, 1~4 days). b. Temperature; Grew at 20°C or below and 46°C or over (IM-agar, 1~2 days). The optimum is about 37°C. c. pH; Grew at pH 5.75 or below to 8.5 or over (IM-broth, 28°C, 1~4 days). Did not grow at pH 4.90. The optimum pH lies between pH 6.3 to 8.5. d. Resistance to NaCl; Grew weakly in 5% NaCl but not in 7.5% and 10% (glucose nutrient broth, 28°C, 1~4 days).

Physiological characters: Liquefaction of gelatin (yeast extract-supplied gelatin stab, 25°C, 1~7 days) was strongly positive but hydrolysis of starch was weakly positive (28°C, 1~7 days). On litmus milk, peptonization of milk was clearly observed but acid formation was not. Nitrite formation from nitrate, VOGES-PROSKAUER reaction, indole formation, and H_2S production were all negative (28°C, 1~7 days). Oxidase and catalase tests were both positive. The bacterium grew on CHRISTENSEN's citrate medium, but not on KOSER's citrate medium. Acid formation was observed from glucose, fructose, sucrose, maltose, and trehalose (weak), but not from L-arabinose (neglisibly positive), D-xylose, mannose, galactose, lactose, starch, glycogen, inulin, glycerol, inositol, adonitol, sorbitol, salicin and α-methyl glucoside.

Specification: The morphology, appearance and growth characteristics and many physiological characters of this bacterium were very similar to the description of *B. megatherium* in BERGEY's manual even though there exist minor differences, namely, i) motility and ii) acid formation from mannitol, L-arabinose and D-xylose.[2] The appearance and morphology of this bacterium also resembled that of *B. megatherium* IFO 1035. Therefore, we decided this bacterium was a strain of *B. megatherium* and named it as *B. megatherium* I-13.

The characteristics of the strain AR-91 identified with *B. badius* is described below.

Morphology: The rods, on Gly-IM-agar, were 0.7~0.8 μ by 3.0~5.0 μ, with rounded ends. A few cells were about 8 μ in length (28°C, 18 hours). Serially linked forms with two cells were observed commonly.

Spores, on Gly-IM-agar, were, 0.7 μ by 1.0~12 μ, elliptical to oval and located at central positition. The sporangia were not appreciably swollen. The free spores showed little surface stainability.

Colony on agar plate: Circular, convex, entire, smooth surface, semitranslucent (18 hours) to opaque density (48 hours or later) and slightly viscid to brittle-butyrous structure (Gly-IM-agar, 28°C, 1~7 days).

Growth on agar stroke: Filiform, moderate-abundant growth of creamy gray color and shining surface was observed on Gly-IM-agar (28°C, 1 day). The cell mass was slimy to viscid at 18 hours stage but becomes brittle-butyrous on aging. No diffusible pigment was detected.

Nutrient broth: Uniform, scanty growth at 18 hours and moderate, flocculent growth in upper layer at 3 days was observed, respectively (28°C, nutrient broth). Sometimes, brittle ring formation was observed.

Conditions for growth: a. OF-Test; Aerobic. No acid and gas was formed from glucose (28°C, 1~4 days). b. Temperature; Grew at 25°C to 37°C (Gly-IM-agar, 18 hours). Did not grow at 41°C. The optimum lies between 28°~32°C. c. Resistance to NaCl; Did not grow in 5% and 10% NaCl broth (28°C, 1~6 days).

Physiological characters: Liquefaction of gelatin was strongly positive but starch hydrolysis was very weak (28°C, 1~7 days). Nitrite formation from nitrate, urease-test were both positive but VOGES-PROSKAUER reaction, H_2S formation and growth on KOSER's citrate medium were all negative. Oxidase and catalase test are both positive. No acid or gas was formed from any

carbohydrates tested, *i.e.*, L-arabinose, xylose, L-rhamnose, ribose, glucose, mannose, galactose, fructose, sucrose, maltose, lactose, trehalose, raffinose, dextrin, starch, glycogen, inulin, glycerol, mannitol, sorbitol, salicin and α-methylglucoside.

Specification: The above observations clearly suggest that the bacterium should be attributed to the species "*badius*".[2,3] Namely, 0.7~0.8 μ by 3.0 μ~5.0 μ rods, elliptical to oval spores of not easily stainable, sporangia not definitely swollen, typical acerobic growth, no acid formation from glucose and other carbohydrates, negativity to VOGES-PROSKAUER and starch hydrolysis tests are the key characters for "*badius*". *B. brevis* differs from this on its definitely swollen sporangia and size of rods.[2] Of course, this culture differs from *B. cereus* largely on the points of its cell size, oxygen requirement, acid formation from carbohydrates *etc.*[2]

The only difference between *B. badius* ATCC 14574 and this strain is that the former grows on 5 % NaCl broth but the latter does not. Therefore, we decided this bacillus to be a strain of *badius* and named it *Bacillus badius* AR-91.

Production and Isolation of Thiocillins I and II

Spores of strain G-15 were inoculated into 600 ml of a medium consisting of 0.5 % glucose, 0.5 % meat extract, 1.0 % peptone and 0.3 % NaCl (pH 7.0) in a 2-liter Erlenmeyer flask, and cultured at 28°C for 24 hours on a rotary shaking machine. The culture was then transferred to a 30-liter jar fementer containing 15 liters of a medium consisting of 1.0 % glucose, 0.25 % glycerine, 0.25 % peptone, 1.0 % soy bean meal and 0.3 % NaCl (pH 7.0). Fermentation was carried out at 28°C for 3 days under aeration of 15 liters per minute and agitation of 200 r.p.m.

About 60 liters of the culture broth was adjusted to pH 2.0 with dil. HCl, to which 25 liters each of *n*-butanol and methanol and 1.5 kg of Hyflo Super-Cel were added. After being stirred for half an hour, it was filtered through a filter press. The filtrate (*ca.* 100 liters) was adjusted to pH 8.0 and concentrated under reduced pressure to *ca.* 70 liters, which was then extracted with 8 liters of *n*-butanol three times. The *n*-butanol extracts were combined and concentrated under reduced pressure to *ca.* 16 liters. This was washed with dil. HCl, dist. water, dil. $NaHCO_3$ and dist. water successively, and then further concentrated to a syrupy solution, which was extracted with 500 ml of a mixture of chloroform and methanol (1:1 by volume). The extract was again concentrated to a syrup, from which a crude powder (*ca.* 13 g) was precipitated by addition of ethyl ether.

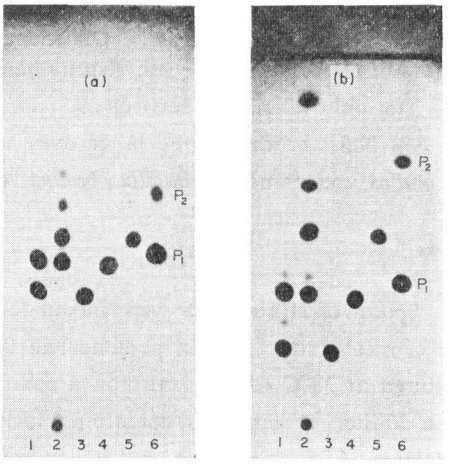
The crude powder (6.5 g) obtained as above was chromatographed on a silica gel column (Merck Silica Gel 0.05~0.2 mm, 300 g, 5×31 cm) with chloroform-methanol (9:1, by volume). The active eluate against *Staphylococcus aereus* assay plate was concentrated to dryness, giving a crude mixture of thiocillins I, II and a trace amount of two minor components (see Fig. 1) as a pale yellowish amorphous powder (0.9 g).

The crude mixture (170 mg) was applied on a silica gel GF plate (20×100 cm, 750 μ), which was developed by continus flow method[8] with chloroform-methanol (19:1) for 5 hours. Separated zones of thiocillins I and II were detected with a UV lamp, cut out and extracted with a mixture of chloroform-methanol (1:1). This chromatographic procedure was repeated with both extracts. Preparations of thiocillins I (50 mg) and II (60 mg) as colorless amorphous powders were obtained.

Production and Isolation of Thiocillin III

Strain AR-91 was inoculated into 100 ml of a medium consisting of 1.0% glucose, 0.5% peptone, 0.5% meat extract, 0.1% NaCl (pH 7.0) in a 500-ml shake flask (SAKAGUCHI flask) and cultured for a day at 28°C on a reciprocal shaker. About 3 ml of the culture was then seeded in 100 ml of a medium containing 0.25% starch, 1.0% glucose, 0.25% glycerin, 1.0% soy bean meal, 0.5% corn steep liquor, 0.1% yeast extract, 0.1% NaCl, 0.1% $CaCO_3$, 0.05% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $MnSO_4$ and 0.001% $Fe_2(SO_4)_3$ (pH 7.0) in a 500-ml shake flask. Fermentation was carried out for 3 days at 28°C on a reciprocal shaker.

Fig. 1. Photograph of thin-layer chromatograms. (a) Chloroform-methanol (9:1). (b) Chloroform-methanol (19:1) by continuous flow development. (1) Crude mixture from the strain G-15. (2) Crude mixture from the strain AR-91. (3) Thiocillin I. (4) Thiocillin II. (5) Thiocillin III. (6) Micrococcin P.



About 5 liters of the culture broth obtained as above was adjusted to pH 2.0 and mixed with 3 liters of a mixture of *n*-butanol and methanol (1:1, by volume). After being stirred for a while, it was filtered. The filtrate was evaporated under reduced pressure and extracted with 300 ml of *n*-butanol three times. The *n*-butanol extract was washed with dil. HCl, dist. water, dil. $NaHCO_3$ and dist. water successively, and concentrated to a syrup. The syrup was extracted with a mixture of chloroform and methanol (1:1), and the extract was again concentrated. Addition of ethyl ether to the concentrate gave a crude powder (890 mg).

The crude powder was chromatographed on a silica gel column (Merck, 0.05~0.2 mm, 35 g, 2.8×10 cm) with chloroform-methanol (9:1). The active eluate was concentrated to dryness giving a crude mixture of thiocillins II and III which contained two other minor component (Fig. 1) as a pale yellowish powder (140 mg).

The crude mixture was chromatographed on a silica gel GF plate in the manner described in the former section. From the separated zones, thiocillins II (30 mg) and III (70 mg) were obtained as colorless amorphous powders.

Thin-layer Chromatography of Thiocillins and Micrococcin P

Pre-coated silica gel plates (Merck Silica Gel 60 F-254) were used in this experiment with chloroform-methanol (9:1) in the usual developing manner (system a), and with chloroform-methanol (19:1) in continuous flow development[8] for 4 hours (system b).

Fig. 1 shows photographs of the thin-layer chromatograms of the crude mixtures of antibiotic components derived from strains G-15 and AR-91, purified samples of thiocillins I, II and III, and micrococcin P* kindly supplied by Dr. WALKER, which were run by systems a

* Dr. JAMES WALKER sent us specimens of micrococcin P and SU's original micrococcin, and informed us with TLC photographs that both specimens contain two components (P_1 and P_2) each of which is identical to its counterpart in the other specimen. We confirmed this information by TLC experiments.

and b. The crude mixture derived from strain G-15 showed two main components, corresponding to thiocillins I and II, and trace amounts of two minor antibiotic components, while that derived from strain AR-91 showed two main components, corresponding to thiocillins II and III, and two minor antibiotic components. The identity of both components corresponding to thiocillin II derived from strains G-15 and AR-91 was confirmed by modified circular development method[9] with a mixture of both specimens.

Differentiation of thiocillins I, II and III from micrococcin P_1 and P_2 was clear in system b, although distinguishing between thiocillin II and micrococcin P_1 was somewhat difficult in system a. A minor compnent, present in trace amount, of the crude mixtures derived from both strains could not be distinguished from micrococcin P_1 by these systems.

Physico-chemical Properties of Thiocillins I, II, and III

Thiocillins I, II and III are colorless crystalline powders, which gradually decompose above *ca.* 220°C. They are soluble in dimethylsulfoxide, dimethylformamide, tetrahydrofuran and a mixture of chloroform and methanol, slightly soluble in methanol, ethanol, acetone and chloroform, but insoluble in ethyl acetate, benzene, ethyl ether and water. They give positive reactions to DRAGENDORFF's and PAULY's reagents, and decolorize potassium permanganate, but are negative to ninhydrin reaction.

Elemental analysis, $[\alpha]_D$ values and UV maxima of these three antibiotics are listed in Table 1. Only slight differences are seen in their $[\alpha]_D$ values. From the elemental analysis and molecular weight determination measured by osmometry in tetrahydrofuran, an approximate empirical formula $C_{49}H_{57}N_{13}O_{12}S_6$ or $C_{56}H_{65}N_{15}O_{14}S_7$ is suggested for thiocillin I. $C_{49}H_{57}N_{13}O_{12}S_6$ requires: C, 48.54; H, 4.74; N, 15.02; O, 15.84; S, 15.87; M.W., 1212.45 and $C_{56}H_{65}N_{15}O_{14}S_7$ requires: C, 48.15; H, 4.69; N, 15.04; O. 16.04; S, 16.07; M.W., 1396.66.

Table 1. Physico-chemical properties of thiocillins I, II and III, and micrococcin P

	Thiocillin I	Thiocillin II	Thiocillin III	Micrococcin P[4b,d]
Anal. Found: C	48.33	48.62	49.71	48.9
H	4.68	4.66	4.80	4.7
N	14.83	14.68	15.30	13.7
O	14.34	14.25	12.62	
S	15.90	15.57	15.50	16.0
MW (osmometry in tetrahydrofuran)	1,292	1,392	—	
$[\alpha]_D^{24.5°}$ in 90% ethanol	+97.7±0.8° (*c*, 2.028)	+93.4±2.3° (*c*, 0.588)	+88.0±1.5° (*c*, 0.850)	+63.7° (*c*, 1.19)
λ_{max}^{MeOH}	217 nm ($E_{1cm}^{1\%}$ 734)	216 nm ($E_{1cm}^{1\%}$ 705)	217 nm ($E_{1cm}^{1\%}$ 729)	
	275 nm ($E_{1cm}^{1\%}$ 326)	278 nm ($E_{1cm}^{1\%}$ 291)	275 nm ($E_{1cm}^{1\%}$ 302)	
	348 nm ($E_{1cm}^{1\%}$ 199)	348 nm ($E_{1cm}^{1\%}$ 202)	347 nm ($E_{1cm}^{1\%}$ 208)	345 nm ($E_{1cm}^{1\%}$ 210)*

* In the uv spectrum of micrococcin,[4b] an additional absorption is seen as a shoulder at near 280 nm.

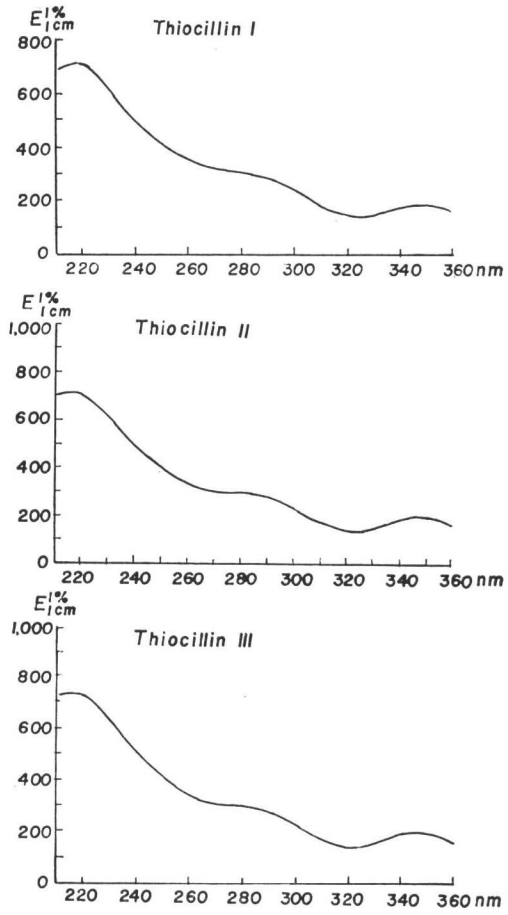
The UV and IR spectra of thiocillins I, II and III are shown in Figs. 2 and 3.

On comparison with known antibiotics isolated from the genus *Bacillus*, thiocillins I, II and III are similar to only micrococcin P. Similarities in UV absorptions and elemental analysis,

but an apparent difference in $[\alpha]_D$ value are shown in Table 1.

Thiocillins I, II and III were hydrolyzed in the following manner, as they are insoluble in hydrochloric acid. The samples were partially hydrolyzed with a mixture of formic acid and conc. hydrochloric acid (1:1, by volume) at 38°C for a day. Following this, the partial hydrolyzates were dried up and then completely hydrolyzed with constant boiling hydrochloric acid at 110°C for 2 days. On hydrolysis, thiocillins I, II and III produced a colorless precipitate. The acid-insoluble component was soluble in alkaline water and showed identical mobility with micrococcinic acid* on TLC [Rf *ca.* 0.35 on silica gel GF plate with chloroform-ethanol-14% ammoniacal water (4:7:2)]. When they were extracted from the plate and compared by UV measurement, identical maxima were shown (λ_{max}^{MeOH}: 296 nm, 345 nm). By amino acid analysis with an automatic amino acid analyzer Hitachi Model KLA-5, the acid-soluble parts of the hydrolyzates of thiocillins I, II and III gave *ca.* 0.8 μ moles of threonine per mg of antibiotic. These substances have been reported to be a part of the hydrolysis products of micrococcin P,[7a] showing the similarity of thiocillins I, II and III to micrococcin P in chemical structure. Differences in their constituents are not clear at present.

Fig. 2. Ultraviolet absorption spectra of thiocillins I, II and III in methanol.



Biological Properties

Thiocillins I, II and III show antimicrobial activity against Gram-positive bacteria *in vitro*, but not against Gram-negative bacteria. The minimal inhibitory concentrations obtained by the usual agar dilution method are shown in Table 2.

When thiocillin I was administered to the mice infected with *Streptococcus pyogenes*, *Staphylococcus aureus* or *Streptococcus pneumoniae* in two subcutaneous doses, given in 1 and 5 hours after infection, a therapeutic effect was observed only against *S. pyogenes* (ED_{50}: 2.91 mg/kg×2), but not against *S. aureus* and *S. pneumoniae*. The plasma level of thiocillin I was not detectable (<0.1 μg/ml) by bioassay in mice given 50 mg/kg subcutaneously. No toxic sign

* It was prepared from dimethyl micrococcinate,[7a,7f] kindly supplied by Dr. WALKER, by alkaline hydrolysis.

Fig. 3. Infrared absorption spectra of thiocillins, I, II and III (KBr).

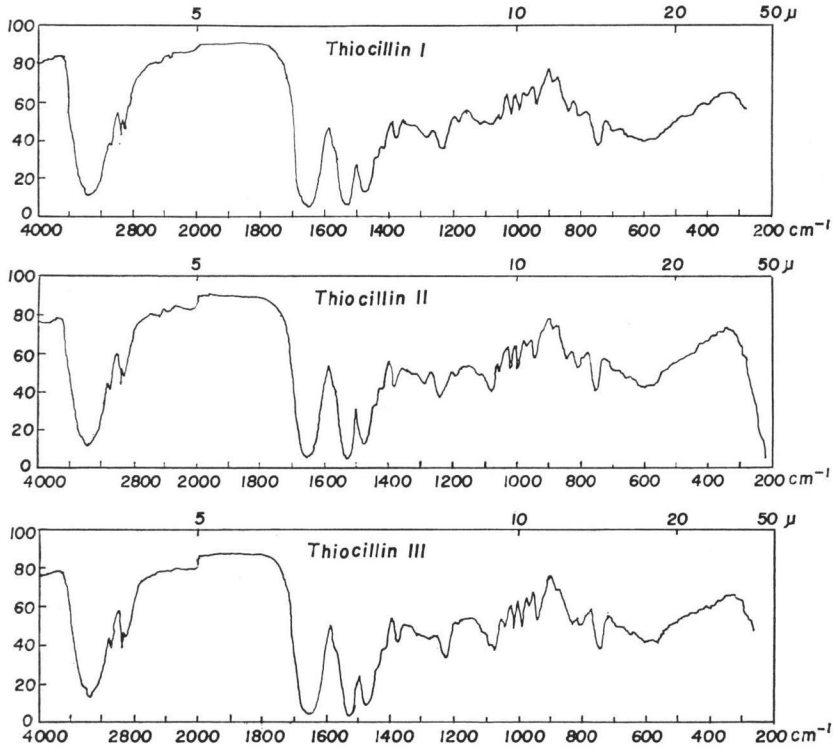


Table 2. Antimicrobial spectra of thiocillins I, II and III

Test organism	MIC (mcg/ml)		
	Thiocillin I	Thiocillin II	Thiocillin III
Bacillus subtilis PCI 219	1.56	0.39	—
Bacillus anthracis	0.2	0.1	—
Staphylococcus aureus FDA 209P	0.2	0.1	0.2
Staphylococcus aureus Smith	0.2	0.1	0.39
Staphylococcus aureus 80257	0.78	0.1	0.39
Streptococcus pyogenes C-203	3.13	1.56	1.56
Streptococcus pneumoniae type D	1.56	0.2	0.78
Escherichia coli NIHJ JC-2	>50	>50	>50
Escherichia coli 80750	>50	>50	>50
Klebsiella pneumoniae	>50	>50	>50
Salmonella typhimurium	>50	>50	>50
Pseudomonas aeruginosa PS-24	>50	>50	>50
Proteus vulgaris	>50	>50	>50

Obtained by the usual agar dilution method.

was observed in mice at a dose of 500 mg/kg by administration of this drug by intraperitoneal, subcutaneous or oral route.

The *in vivo* properties of thiocillins II and III were similar to that of thiocillin I described above.

Addendum

After the submission of the present paper, we noticed a paper which has reported the isolation of micrococcins M_1 and M_3 from several strains of *Staphylococcus* and *Micrococcus*. Close similarities of these substances to micrococcin and micrococcin P in physical properties and in degradation products but some differences in their molecular weights and in hydrolysis products are pointed out by comparison with literature data. However, the differences between micrococcins M_1 and M_3, and micrococcins P_1 and P_2 seem to be uncertain. By a private communication, Dr.

WALKER is now thinking that the molecular weight of the main component of micrococcin P may be around 1119. A difference between micrococcins M_1 and M_3, and thiocillins I, II and III can be seen in optical rotational data [Micrococcins M_1 and M_3: $[\alpha]_D^{20}$ +66.6 (*c* 1, ethanol)].[10]

Acknowledgement

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