Biochem. J. (1970) **119**, 861–869
Printed in Great Britain

Effect of Streptomycin on Lipid Composition with Particular Reference to Cyclic Depsipeptide Biosynthesis in *Serratia marcescens* and other Micro-organisms

By MARGARET A. C. BERMINGHAM, B. S. DEOL and J. L. STILL
Department of Biochemistry, University of Sydney, Sydney, N.S.W. 2006, *Australia*

(*Received* 18 *May* 1970)

The addition of low concentrations of streptomycin (5–10 μg/ml of medium) to *Serratia marcescens* caused significant alterations in the lipid composition of this organism, but neither growth nor pigmentation was affected. The acetone-soluble cyclic depsipeptides, which comprise on average 15% of the total lipid, were decreased almost to zero and the total lipid phosphorus was more than doubled in the presence of streptomycin. Most of the phospholipid increase was due to an increase in phosphatidylethanolamine. Cyclic depsipeptides were not leached from the cell in the presence of streptomycin, indicating a definite inhibition of the biosynthetic pathway. The effect of streptomycin on the reported peptidolipids of *Rhodopseudomonas spheroides*, *Halobacterium halobium*, *Nocardia asteroides* and *Pseudomonas tabaci* was investigated. In the case of the only strictly comparable cellular cyclic depsipeptide (that of *N. asteroides*) the biosynthesis was strongly inhibited by streptomycin, but cell weight was maintained or even slightly increased. A possible mode and site of action of low concentrations of streptomycin on bacterial lipids is discussed.

The existence of a family of 14-membered cyclic depsipeptides containing serine esterified to C_{10} and C_{12} hydroxy fatty acids has been demonstrated in *Serratia marcescens* (Bermingham, Deol & Still, 1970*a*). Preliminary work has shown that the biosynthesis of these compounds in *S. marcescens* is decreased sevenfold by the addition of very low concentrations of streptomycin, which affect neither growth nor pigmentation of the organism (Bermingham, Deol & Still, 1970*b*).

In the present paper we report the results of a detailed investigation of the effects of streptomycin on cyclic depsipeptides and other lipids of *S. marcescens*. In addition, the effect of streptomycin on known cellular peptidolipids in other species of bacteria, namely *Rhodopseudomonas spheroides*, *Halobacterium halobium* and *Nocardia asteroides*, exocellular peptidolipids such as the wildfire toxin of *Pseudomonas tabaci*, and the exocellular rhamnolipid of *Pseudomonas aeroginosa* (which does not contain an amino acid but has a 3-hydroxy-$C_{10:0}$ acid as its fatty acid moiety) was examined and compared with the effect on *S. marcescens* cyclic depsipeptides.

MATERIALS AND METHODS

Materials. Reagents used were commerical A. R. grade preparations. Radiochemicals were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. All solvents were purified, dried when necessary and redistilled before use.

Growth of cultures. S. marcescens, red-pigmented strains A.T.C.C. 8195, N.T.C.C. 1377 and white mutant N.T.C.C. 4612 were grown on (*a*) a medium containing inorganic N with glycerol as the sole carbon source (Bunting, 1946) and (*b*) a similar medium, but enriched with Difco Bacto-peptone in 1-litre conical flasks on a gyratory shaker (except for large-scale experiments, where growth was carried out in 12-litre carboys with continuous aeration at a rate of 4 litres/min) at 30°C. *H. halobium* (kindly supplied by Dr A. D. Brown, University of New South Wales) was grown on the medium described by Sehgal & Gibbons (1960) at 37°C. *Ps. aeruginosa*, *Ps. tabaci* and *N. asteroides* were grown on the Bacto-peptone-enriched medium under the same conditions as used for *S. marcescens*, at 30°C. *Rps. spheroides* was grown in the light at 34°C under semi-aerobic conditions as described by Gorchein (1968) for the maximum production of ornithine-containing lipid.

The growth of *S. marcescens* was followed by extinction measurements at 2h intervals and cells were harvested at late exponential or stationary growth phase. *H. halobium* was harvested after 3 days of growth, *Ps. tabaci* after 4 days, *Ps. aeruginosa* after 5 days and *N. asteroides* after 14 days. *Rps. spheroides* was harvested after 65h of growth. All cells used for extraction of lipid were harvested by centrifugation at 20000*g*, washed once with water and freeze-dried.

Extraction of cells. Dried cells of *S. marcescens* were extracted successively with (*a*) acetone and (*b*) chloroform–methanol (2:1, v/v) as described by Bermingham *et al.* (1970*a*). *N. asteroides* was extracted with pure

chloroform (which solubilizes the lipopeptide; Guinand, Michel & Lederer, 1958), and *Rps. spheroides* and *H. halobium* with chloroform–methanol (2:1, v/v) (Marshall & Brown, 1968). Cells of *Ps. tabaci* and *Ps. aeroginosa* were not extracted, as the relevant lipids are mainly excreted and may be measured as mg/ml of medium.

As a routine, lipid extracts were freed from non-lipid contaminants by passage through Sephadex G-25 (Wuthier, 1966).

Analytical methods. For *S. marcescens*, *N. asteroides* and *H. halobium*, the cyclic depsipeptide content was determined by measuring the release of amino N on hydrolysis of the lipid extracts with 10M-HCl for 1h. The wildfire-toxin content of the medium of *Ps. tabaci* could also be determined by this method since half the total N is released as amino N on strong-acid hydrolysis (Woolley, Schaffner & Braun, 1955).

The rhamnolipid content of the medium of *Ps. aeruginosa* was determined as described by Hauser & Karnovsky (1954), and the ornithine-containing lipid of *Rps. spheroides* by the method of Gorchein (1968) after removal of poly-β-hydroxybutyrate. T.l.c. was carried out on silica gel G and silica gel H.R. (E. Merck A.-G., Darmstadt, Germany) in chloroform–methanol–7M-ammonia (65:25:4, by vol.) and chloroform–methanol–water (65:25:4, by vol.) on 20cm × 20cm plates (analytical) and 20cm × 40cm plates (preparative). The detection and identification of individual lipids on chromatograms was as described by Bermingham *et al.* (1970*a*). Total lipid P was measured by the method of Chen, Toribara & Warner (1956). Radioactive compounds were detected by radioautography after exposure to Ilford X-ray film for 1–3 weeks. ^{14}C-labelled cyclic depsipeptides were prepared by growing *S. marcescens* up to stationary phase in the presence of [^{14}C]serine, acetone extraction of the labelled cyclic depsipeptides and purification by preparative t.l.c. in the chloroform–methanol–7M-ammonia system. ^{14}C-labelled mixed 'serratamic' acids were obtained by mild alkaline hydrolysis of a purified radioactive cyclic depsipeptide fraction followed by preparative t.l.c. in the chloroform–methanol–water system.

RESULTS

Initially the effects of four antibiotics on growth, prodigiosin (the red pigment characteristic of the genus *Serratia*) content and cyclic depsipeptide content in *S. marcescens* strain 1377 were investigated (Table 1). A large decrease in cyclic depsipeptide content is caused by very low concentrations of streptomycin, which affect neither growth nor pigmentation of the organism.

A further series of experiments showed that the decrease in cyclic depsipeptide content was on average about 700% (Table 2) with 10μg of streptomycin/ml of medium. Concentrations of streptomycin of more than 10μg/ml of medium, although still effecting a large decrease in cyclic depsipeptide

Table 1. *Effect of various antibiotics on growth, prodigiosin content and cyclic depsipeptide biosynthesis in* S. marcescens *N.T.C.C.* 1377

Each antibiotic (5μg/ml of medium) was added at the time of inoculation to 600ml of Bacto-peptone-enriched medium in 1-litre conical flasks and cells were harvested at stationary growth phase. Prodigiosin was measured by the method of Williams & Gott (1964).

Antibiotic	Growth of bacteria (mg/100ml of medium)	Prodigiosin content (E_{537}/5ml of cell suspension)	Acetone-extractable material (mg/g of dry cells)	Cyclic depsipeptide content (μg/mg of acetone extract)
Neomycin	20	—	35.0	22
Achromycin	88	0.19	25	172
Chloramphenicol	89	0.21	32	228
Streptomycin	81	0.17	35	54
Control	85	0.18	30	248

Table 2. *Effect of streptomycin on prodigiosin and cyclic depsipeptide synthesis in* S. marcescens *N.C.T.C.* 1377

Values given are the averages of seven separate observations carried out at the same time under identical conditions. Experimental details are the same as for Table 1.

	Cell yield (mg/100ml of medium)	Acetone extract (mg/g of dry cells)	Prodigiosin (E_{537}/5ml of cell suspension)	Cyclic depsipeptide (μg/mg of acetone extract)
Control	88	58.2	0.15	398
Streptomycin (10μg/ml)	84	45.8	0.14	55
Control/streptomycin ratio	1.05	1.2	1.0	7.2

content, caused a 75% decrease in the growth of the bacteria (Fig. 1). In the entirely unpigmented white strain N.T.C.C. 4612 the small amount of cyclic depsipeptide formed in control cells (0.5mg/g dry wt.) is completely absent in the presence of streptomycin. When acetone extracts of control and streptomycin-grown cells were prepared and subjected to t.l.c., the results confirmed that the cyclic-depsipeptide area is drastically decreased by streptomycin at both exponential and stationary growth phases.

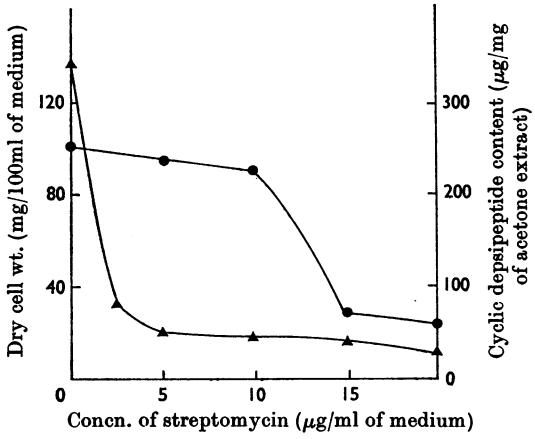


Fig. 1. Effect of streptomycin concentration on growth and cyclic depsipeptide content in *S. marcescens* N.C.T.C. 1377. Each observation was made in duplicate on 600ml cultures grown from a common inoculum under identical conditions (see Table 1 for details). Cells were harvested at the onset of stationary growth phase. ●, Dry cell wt.; ▲, cyclic depsipeptide content.

The above results were obtained with bacteria grown on Bacto-peptone medium where N is supplied in an organic form. When the experiments were repeated with cells grown on Bunting's (1946) medium (which supplies N as ammonium chloride), the cyclic depsipeptide content was found to be unaltered by the presence of streptomycin. An inhibition of the growth of pea seedlings by streptomycin may be reversed by divalent cations (Venis, 1969). In addition, the action of streptomycin on the chloroplast ribosomal system of *Euglena* may be partially reversed by divalent cations (Drown & Galloway, 1969). Cations such as Mg^{2+} are present in both media used for the growth of *S. marcescens*; however, it is possible that these cations could have been neutralized by anions contained in the Bacto-peptone medium and not present in Bunting's medium. In fact, when low concentrations of EDTA were added with streptomycin to Bunting's medium it had the same inhibitory effect on cyclic depsipeptide content as streptomycin alone in

Table 3. *Fractionation of lipid from control and streptomycin-grown cells of* S. marcescens *N.C.T.C.* 1377

Growth conditions are those described for the large-scale process in the Materials and Methods section. Cells were freeze-dried and portions taken for extraction with each solvent mixture.

	Control	Streptomycin (10μg/ml of medium)	Control/streptomycin ratio
Total dry cell weight (mg/100ml of medium)	92	77	1.2
Total lipid (chloroform–methanol-soluble) (mg/g of dry cells)	138.8	131.6	1.0
Acetone-soluble lipid (mg/g of dry cells)	62.2	37.8	1.6
Polar lipid (chloroform–methanol-soluble–acetone insoluble (mg/g of dry cells)	76.6	93.8	0.6
Phosphorus content (% of total lipid extract)	1.58	3.76	0.4

Table 4. *Determination of the major individual phospholipids in total lipid extracts of control and streptomycin-grown cells (large-scale growth)*

'Total lipid' indicates material extracted from freeze-dried cells with chloroform–methanol (2:1, v/v) without prior acetone treatment. Portions (300μg) were chromatographed on t.l.c. plates in chloroform–methanol–water and the major phospholipids were determined as described by Kahavcova & Odavic (1969) with phosphatidylethanolamine as standard. Results are expressed as % of total lipid weight.

	Phosphatidyl-ethanolamine	Phosphatidyl-glycerol	Lysophosphatidyl-ethanolamine	Unidentified ninhydrin-positive phospholipid (R_{PE} 2.4)	Unidentified ninhydrin-negative phospholipid (R_{PE} 0.43)
Control	25	4	0.7	—	2
Streptomycin-grown cells	52	1	3	2	3

Bacto-peptone medium. When acetone extracts of cells from the above experiment were subjected to t.l.c. in the chloroform–methanol–ammonia system it was noticed that a diffuse spot appeared ahead of phosphatidylglycerol in cases where streptomycin was effective in inhibiting cyclic depsipeptide biosynthesis (Bacto-peptone medium–streptomycin; Bunting's medium–EDTA–streptomycin). This material was purified by successive preparative t.l.c. experiments in chloroform–methanol–water, where its rate of flow in comparison with phosphatidylethanolamine (R_{PE}) was 2.3, and in chloroform–methanol–ammonia (R_{PE} 1.3) and found to consist of a mixture of free 3-hydroxy-$C_{10:0}$ and -$C_{12:0}$ acids.

A fractionation of lipid from control and streptomycin-treated cells grown on a large scale indicates that although there is little difference in the total lipid weight (chloroform–methanol-soluble material), the polar lipid is increased and the acetone-soluble lipid (cyclic depsipeptide-containing) is decreased (Table 3). In addition, the phosphorus content of the total lipid is more than doubled in the streptomycin-grown cells. When the major phospholipids were determined quantitatively by t.l.c., almost all were found to be increased in the presence of streptomycin, particularly the phosphatidylethanolamine fraction (Table 4). An unidentified ninhydrin-positive phospholipid with a high R_F value appears only in streptomycin-treated cells. This lipid becomes labelled when [^{14}C]serine is added to the culture medium (see below). On purification and hydrolysis of this lipid, ninhydrin-positive compounds that co-chromatographed with ornithine and ethanolamine were obtained but sufficient material was not available for a complete analysis.

When [^{14}C]serine was fed to *S. marcescens* it was rapidly incorporated into the cyclic depsipeptide fraction (Bermingham *et al.* 1970*a*). When this experiment was repeated in the presence of streptomycin (Table 5) incorporation of [^{14}C]serine into the cyclic depsipeptide fraction was inhibited to the same extent as the cyclic depsipeptide content when measured as μg of 'amino-bound-serine/mg of acetone extract'. Since we found no excess of radioactivity leached into the medium in the presence of streptomycin, there appears to be definite inhibition of the biosynthesis of the cyclic depsipeptides.

Radioactive cyclic depsipeptide and a serratamic acid fraction derived from it were added back to *S. marcescens* in the presence and absence of streptomycin (Table 6). The extreme stability of the cyclic depsipeptide molecule, once formed, is demonstrated in that it is neither metabolized nor broken down, but is recovered unchanged in the acetone extract (whether streptomycin is present or not). Serratamic acid, however, is rapidly utilized

Table 5. *Incorporation of* [U-^{14}C]*serine into lipids of* S. marcescens *N.C.T.C.* 1377

L-[U-^{14}C]Serine (sp. radioactivity 1.39 mCi/mg) was added at the time of inoculation to 600 ml of medium in conical flasks (10 μCi each). Cells were harvested at exponential and stationary growth phases and processed as described in the Materials and Methods section. Samples of lipid extracts were evaporated directly in Packard vials and the radioactivity was counted [after addition of scintillant: 0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis-(5-phenyloxazol-2-yl)benzene in toluene] in a Packard Tri-Carb liquid-scintillation counter (efficiency 82%). For determination of the radioactivity of the cyclic depsipeptide fraction, the acetone extracts were chromatographed quantitatively on t.l.c. and the cyclic depsipeptides located by radioautography; the relevant areas of silica gel were scraped off directly into Packard vials, mixed with thixotropic gel and their radioactivities counted.

	Growth of bacteria (mg dry wt./100 ml of media)		Acetone-soluble fraction		Chloroform–methanol-soluble–acetone-insoluble fraction		Label in cyclic depsipeptides	Cyclic depsipeptide content (μg/mg of acetone extract)
			wt. (mg/100 ml of medium)	(c.p.m./mg)	wt. (mg/100 ml of medium)	(c.p.m./mg)	(c.p.m./mg of acetone extract)	
Control	Exponential phase	106	7.3	5589	5.6	7685	5185	218
	Stationary phase	99	5.5	5298	5.6	6743	6745	304
Streptomycin-grown	Exponential phase	99	5.1	4377	7.5	7069	1615	86
	Stationary phase	103	4.1	4056	8.0	6523	2075	90

Table 6. *Incorporation of* ^{14}C-*labelled cyclic depsipeptide* (*sp. radioactivity* 952 *c.p.m./*μ*g*) *and* [^{14}C]*serratamic acid* (*sp. radioactivity* 279 *c.p.m./*μ*g*) *fractions into* S. marcescens *in the presence and absence of streptomycin* (10 μ*g/ml*)

The preparation of the radioactive compounds is described in the Materials and Methods section and the experimental details are as given for Table 5.

		Acetone-soluble lipid		Polar lipid	
		(c.p.m./mg)	t.l.c. analysis	(c.p.m./mg)	t.l.c. analysis
^{14}C-labelled cyclic depsipeptide	Control	4844	Cyclic depsipeptide	480	Cyclic depsipeptide (trace)
	Streptomycin	6098	Cyclic depsipeptide	1058	Cyclic depsipeptide (trace)
[^{14}C]Serratamic acid	Control	404	Acetone complex of phosphatidylethanolamine Cyclic depsipeptide	598	Phosphatidylethanolamine and phosphatidylglycerol
	Streptomycin	90	Cyclic depsipeptide (trace)	84	Phosphatidylethanolamine and phosphatidylglycerol

Table 7. *Effect of streptomycin on the peptidolipids of* N. asteroides, H. halobium, Ps. tabaci, Rps. spheroides *and the rhamnolipid of* Ps. aeruginosa

	Streptomycin (μg/ml of medium) ...	0	5	10	15	20	50	80
N. asteroides	Dry cell wt. (mg/100ml of medium)	166	183	236	282			
	Amino N released (μmol/mg of lipid hydrolysed)	0.24	0.06	0.03	0.05			
H. halobium	Dry cell wt. (mg/100ml of medium)	97	72	70	92	78	90	70
	Amino N released (μmol/mg of lipid hydrolysed)	0.06	0.07	0.11	0.13	0.11	0.12	0.13
Ps. tabaci	Dry cell wt. (mg/100ml of medium)	83	102	100	83			
	Amino N released (μmol/ml of medium)	18	17	16	16			
Ps. aeruginosa	Dry cell wt. (mg/100ml of medium)	112	110	110	112			
	Rhamnolipid concn. (mg/ml of medium)	3.2	3.2	2.4	2.4			
Rps. spheroides	Dry cell wt. (mg/100ml of medium)	88	18*		6†			
	Ornithine-containing lipid content (μmol/mg of lipid hydrolysed)	0.02	0.04		0.05			

* 0.25 μg of streptomycin/ml.
† 0.5 μg of streptomycin/ml.

and radioactivity is recovered in both lipid and non-lipid fractions. In the lipid fraction the radioactivity is largely in the form of phosphatidylethanolamine and phosphatidylglycerol, and this incorporation is not qualitatively affected by streptomycin.

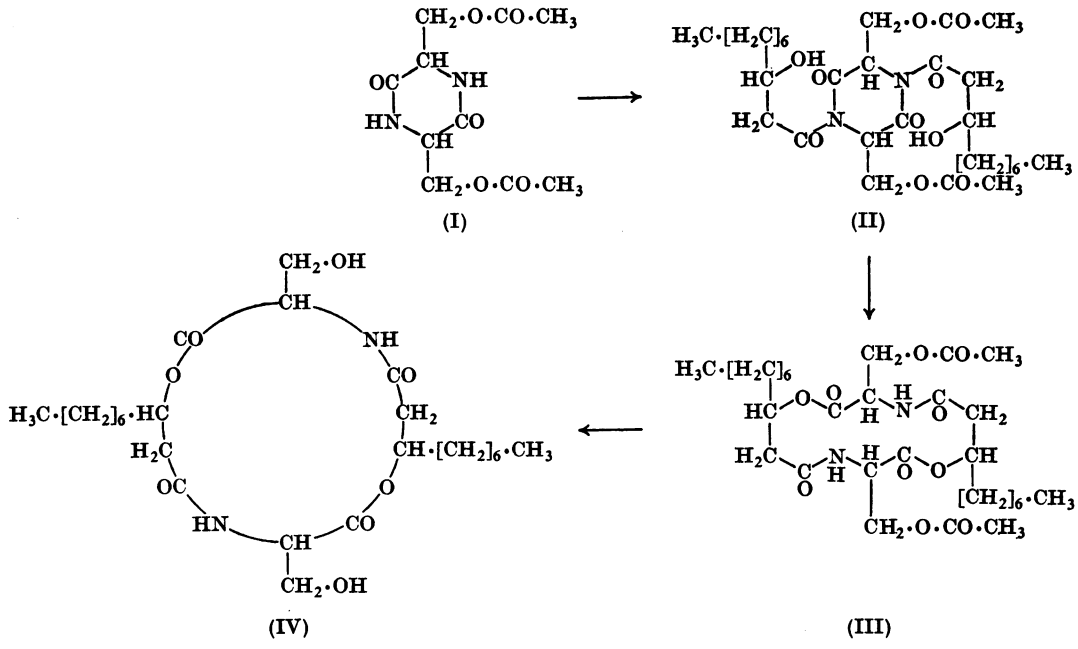
The effect of streptomycin on certain lipids in bacteria other than *S. marcescens* is shown in Table 7. The glycolipid concentration of the medium of *Ps. aeruginosa* is only slightly decreased (25%) by concentrations of streptomycin of 10 μg or more/ml of medium and the wildfire toxin of *Ps. tabaci* is unaffected by streptomycin treatment. Neither compound is strictly comparable with the cyclic depsipeptide of *S. marcescens*, since both are excreted into the medium. The lipopeptide of *H. halobium* is comparable insofar as it is retained within the cell, and although its structure is completely unknown (Marshall & Brown, 1968), there is evidence in favour of a cyclic molecule. The results show that the concentration of *H. halobium* peptidolipid is unaffected by streptomycin treatment. However, even very high concentrations of streptomycin (80 μg/ml of medium) failed to affect the growth of this bacterium and it is possible that the antibiotic action was affected by the high salt concentration necessary for the growth of halophils in a situation analogous to that obtaining during growth of *S. marcescens* on Bunting's medium in the absence of EDTA. Attempts to grow *H. halobium* on alternative media, or in the presence of EDTA, failed. The peptidolipid of *N. asteroides* is directly comparable with that of *S. marcescens*; it may be isolated from the cellular lipid fraction and has been shown to have a cyclic structure (Barber, Wolstenholme, Guinand, Michel & Lederer, 1965). A significant decrease (400%) is observed in the content of this lipid with concentrations of streptomycin as low as 5 μg/ml of medium. At the same time the total dry cell weight is maintained or even slightly increased. T.l.c. analysis of the lipids of *N. asteroides* confirmed that the peptidolipid fraction (R_F 0.75 in chloroform–methanol–ammonia) is significantly inhibited in the presence of streptomycin.

DISCUSSION

The biosynthetic pathway of the cyclic depsipeptides of *S. marcescens* is unknown, as is that of all similar compounds isolated from other sources (Russell, 1966). Serratamolide (IV, Scheme 1), which is a component of the cyclic depsipeptide fraction of *S. marcescens*, has been synthesized by a fairly complex chemical procedure involving the acylation of *OO'*-diacetylserylseryl-lactam (I) with D-β-benzyloxydecanoyl chloride to give the diacyl derivative (II), which on hydroxylamide interaction and rearrangement gave *OO'*-diacetylserratamolide (III) (Shemyakin *et al.* 1964). However, certain related 14-membered ring compounds, such as (V), have been synthesized by a simple condensation of their linear amides (Hassall, Martin, Schofield & Thomas, 1967); these authors suggested that the biosynthesis of serratamolide might also occur by twinning since the linear amide serratamic acid had been found in abundance in *S. marcescens* (Cartwright, 1955). Recent evidence, however, suggests that the occurrence of free serratamic acid is an artifact of alkaline extraction procedures (Bermingham *et al.* 1970*a*). The addition of [^{14}C]serratamic acid to *S. marcescens* gives rise to small amounts of labelled cyclic depsipeptide, but the radioactivity is largely found in phospholipid and non-lipid fractions. The pattern of incorporation seems to indicate a breakdown to [^{14}C]serine and incorporation of this amino acid into lipid.

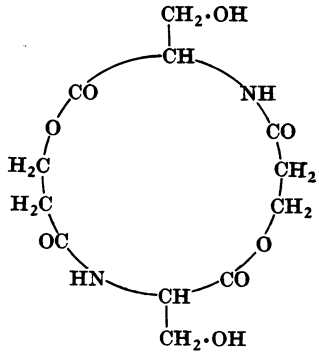
Serratamic acid is stable chemically, but could be susceptible to enzymic degradation immediately on entry into the cell. There is no evidence that a pool of free serratamic acid exists at all in the cell, which would argue against a synthesis by twinning. In addition, free β-hydroxy-$C_{10:0}$ and -$C_{12:0}$ acids, but not free serratamic acid, accumulate in the presence of streptomycin. In fact, there is no reason why the ester linkage between the serine carboxyl group and the β-hydroxyl group of the fatty acid could not be formed before the amide linkage, which would then effect ring closure, and free serratamic acid would exist only as a breakdown product. The biosynthesis of the cyclic depsipeptides of *N. asteroides* is also inhibited by streptomycin, which strongly suggests that certain features of the synthesis of these lipids are common to different bacteria, and no linear amides have been reported from the *Nocardia* species. Although classified as a cyclic depsipeptide by Russell (1966) the proposed structure of the wildfire toxin of *Ps. tabaci* (VI) is that of a small six-membered ring, the major part of the molecule being linear (Woolley *et al.* 1955). The lack of effect of streptomycin on structure (VI) might indicate a streptomycin inhibition of the cyclization to form larger rings, but the actual site remains to be elucidated.

The mode of action of streptomycin, although studied extensively, is not as yet completely resolved. Two early effects of streptomycin addition have been noted: (*a*) on ion efflux from the bacterial cell, and (*b*) on protein synthesis (Brock, 1966). The mechanism of the latter has been widely investigated both *in vivo* and *in vitro* and current theory suggests that protein synthesis is inhibited *in vivo* because of an accumulation of false initiation-complexes or 'streptomycin–70S-monosomes' (Luzzatto, Apirion & Schlessinger, 1968, 1969; Schlessinger, 1969; Modolell & Davis, 1969) due to binding of the streptomycin molecule to a

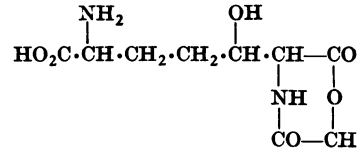


(I) (II) (III) (IV)

Scheme 1.



(V)



(VI)

specific protein in the 30S subunit of the ribosome (Weisblum & Davies, 1968). Translation errors may also be induced by streptomycin under certain abnormal conditions (Davies & Davis, 1968) but the fact that extensive misreading can occur in certain bacterial mutants without causing cell death (Gorini & Kataja, 1964) indicates that the lethal action of the drug *in vivo* is due to an inhibition of protein synthesis.

The effect of streptomycin concentration on growth and protein synthesis in *S. marcescens* has been studied by Siddiqui (1967). The pattern of streptomycin inhibition of total protein synthesis *in vivo* (as measured by incorporation of [^{14}C]-proline) found in strain Nima is very similar to the inhibition of growth of strain N.T.C.C. 1377 observed by us (Fig. 1), being unaffected by a streptomycin concentration of 5μg/ml of growth medium and decreasing rapidly after concentrations of 10μg/ml of medium have been reached.

The concentration of streptomycin needed to inhibit growth in *S. marcescens* is considerably in

excess of that needed to decrease cyclic depsipeptide biosynthesis drastically. Even if the serine involved in this synthesis were activated as aminoacyl-tRNA, the form in which amino acids react during the biosynthesis of certain *O'*-amino acid esters of phospholipids (Lennarz, Nesbitt & Reiss, 1966) there is no evidence that streptomycin has any effect on the formation of aminoacyl-tRNA complexes.

Since streptomycin inhibits the peptide-bond-forming site on the ribosome (Modolell & Davis, 1969), it could be argued that it binds in an analogous fashion to a similar peptide-bond-synthesizing enzyme elsewhere in the cell. However, the biosynthesis of homoeomeric cyclic peptides such as gramicidin S is completely different from the ribosomal system, involving as it does successive peptidyl-transfer reactions between thioester-bound intermediates on a multienzyme complex (Gevers, Kleinhauf & Lipmann, 1969); the biosynthesis of cyclic depsipeptides may be different yet again. Our results show that streptomycin does not inhibit the formation of the linear ornithine-containing lipoamino acid of *Rps. spheroides*, which contains a peptide bond (Gorchein, 1968), and it appears therefore that the streptomycin-mediated inhibition of cyclic depsipeptide biosynthesis is unrelated to its effect on protein synthesis.

The well-documented early effect of streptomycin on K^+ efflux from the cell has received little attention compared with its effect on protein synthesis. It has been speculated, however, that this ion efflux may in fact be the primary event, from which all other effects of streptomycin action arise (Brock, 1966).

Cyclic depsipeptides may be demonstrated to act as ion carriers through artificial barriers (Dobler, Dunitz & Krajewski, 1969). It is therefore notable that if these compounds were to form part of the cell membrane of *S. marcescens* [as other lipoamino acids such as the ornithine-containing lipid of *Rps. spheroides* are thought to (Gorchein, 1968)], it could be envisaged that addition of low concentrations of streptomycin would cause considerable alterations in the permeability of this membrane, especially in view of the phospholipid changes that accompany cyclic depsipeptide depletion. This phospholipid increase and cyclic depsipeptide decrease appear to be interdependent but could in fact be parallel events. Although results are extremely scarce, certain published results (see below) indicate that streptomycin may have a similar effect on phospholipids in other bacteria in which cyclic depsipeptides have not been reported: lipid content is doubled in a streptomycin-resistant human strain of *Mycobacterium tuberculosis* ($H_{37}R_v$) compared with a sensitive strain (Chandraseckhar, de Moute & Subramanian, 1958), and in a sensitive avian strain the streptomycin-treated bacteria had a higher phospholipid and glycolipid content than the control (Motomiya, 1960; Yamaguchi, Fukushi, Motomiya, Munakata & Shinohara, 1960); also, development of polyantibiotic resistance in several strains of *Salmonella* sp., *Escherichia coli* and *Staphylococcus aureus* appears to be associated with an increased lipid content (Vaczi & Farkas, 1961; Asselineau, 1966). If low concentrations of streptomycin can cause large alterations in bacterial lipid composition this could lead to changes in the membrane and the observed increased permeability to ions. Investigation of actual membrane lipid changes in bacteria where a streptomycin-mediated ion efflux can be demonstrated might yield interesting results.

REFERENCES

Asselineau, J. (1966). *The Bacterial Lipids*, p. 265. San Francisco: Holden–Day Inc.

Barber, G., Wolstenholme, W. A., Guinand, M., Michel, G. & Lederer, E. (1965). *Tetrahedron Lett.* no. 2, p. 1331.

Bermingham, M. A., Deol, B. S. & Still, J. L. (1970*a*). *Biochem. J.* **116**, 759.

Bermingham, M. A., Deol, B. S. & Still, J. L. (1970*b*). *Biochem. J.* **117**, 29P.

Brock, T. D. (1966). *Symp. Soc. gen. Microbiol.* no. 16, p. 131.

Bunting, M. I. (1946). *Cold Spring Harb. Symp. quant. Biol.* **11**, 25.

Cartwright, N. G. (1955). *Biochem. J.* **60**, 238.

Chandraseckhar, S., de Moute, A. J. H. & Subramanian, T. A. V. (1958). *Indian J. med. Res.* **46**, 643.

Chen, P. S., Toribara, T. Y. & Warner, H. (1956). *Analyt. Chem.* **28**, 1756.

Davies, J. & Davis, B. D. (1968). *J. biol. Chem.* **243**, 3312.

Dobler, M., Dunitz, J. D. & Krajewski, J. (1969). *J. molec. Biol.* **42**, 603.

Drown, D. & Galloway, R. (1969). *Arch. Mikrobiol.* **68**, 377.

Gevers, W., Kleinhauf, H. & Lipmann, F. (1969). *Proc. natn. Acad. Sci. U.S.A.* **63**, 1335.

Gorchein, A. (1968). *Proc. R. Soc.* B, **170**, 265.

Gorini, L. & Kataja, E. (1964). *Proc. natn. Acad. Sci. U.S.A.* **51**, 995.

Guinand, M., Michel, G. & Lederer, E. (1958). *C. r. hebd. Séanc. Acad. Sci., Paris*, **246**, 848.

Hassall, C. H., Martin, T. G., Schofield, J. A. & Thomas, J. O. (1967). *J. chem. Soc.* C, p. 977.

Hauser, G. & Karnovsky, M. L. (1954). *J. Bact.* **68**, 645.

Kahavcova, J. & Odavic, R. (1969). *J. Chromat.* **40**, 90.

Lennarz, W. J., Nesbitt, J. A. & Reiss, J. (1966). *Proc. natn. Acad. Sci. U.S.A.* **55**, 934.

Luzzatto, L., Apirion, D. & Schlessinger, D. (1968). *Proc. natn. Acad. Sci. U.S.A.* **60**, 873.

Luzzatto, L., Apirion, D. & Schlessinger, D. (1969). *J. molec. Biol.* **41**, 315.

Marshall, C. L. & Brown, A. D. (1968). *Biochem. J.* **110**, 441.

Modolell, J. & Davis, B. D. (1969). *Nature, Lond.*, **224**, 345.
Motomiya, M. (1960). *Scient. Rep. Res. Tohoku Univ.* **9**, 265.
Russell, D. W. (1966). *Q. Rev. chem. Soc.* **20**, 559.
Schlessinger, D. (1969). *Bact. Rev.* **33**, 445.
Sehgal, S. N. & Gibbons, N. E. (1960). *Can. J. Microbiol.* **6**, 165.
Shemyakin, M. M., Ovchinnikov, Yu. Y., Antonov, V. K., Kiryushkin, A. A., Ivanov, V. T., Shchelokov, V. I. & Shkrob, A. M. (1964). *Tetrahedron Lett.* no. 1, p. 47.
Siddiqui, M. A. Q. (1967). Ph.D. Thesis: University of Houston.
Vaczi, L. & Farkas, L. (1961). *Acta microbiol. hung.* **8**, 205.
Venis, M. (1969). *Nature, Lond.*, **221**, 5186.
Weisblum, B. & Davies, J. (1968). *Bact. Rev.* **32**, 493.
Williams, R. P. & Gott, C. L. (1964). *Biochem. biophys. Res. Commun.* **16**, 47.
Woolley, D. W., Schaffner, G. & Braun, A. C. (1955). *J. biol. Chem.* **215**, 485.
Wuthier, R. E. (1966). *J. Lipid Res.* **7**, 558.
Yamaguchi, J., Fukushi, K., Motomiya, M., Munakata, K. & Shinohara, C. (1960). *Jap. J. Tuberc.* **9**, 1.