BIOSYNTHESIS OF BUTIROSINS. II

BIOSYNTHETIC PATHWAY OF BUTIROSINS ELUCIDATED FROM COSYNTHESIS AND FEEDING EXPERIMENTS

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By cosynthetic studies, nine butirosin-non-producing blocked mutants of *Bacillus circulans* were classified into 7 groups (A to F and exceptional groups), based on their complementation patterns. Except for two strains of group A, mutant strains were all neamine-requiring idiotrophs. Mutants of group A produced xylostasin and ribostamycin and a mutant of group B accumulated 2-deoxystreptamine (DOS) in the culture broth. By feeding tests with a compound assumed to be an intermediate in butirosin biosynthesis, the following information was obtained: *myo*-inositol, conduritol B, 1-deoxy-*scyllo*-inositol and its per-acetate were not incorporated into the antibiotic by any mutants. 2-Deoxy-*scyllo*-inosose was converted to butirosins only by mutants of groups E and F. These mutants converted *scyllo*-inosose to 2-hydroxybutirosins, as well. 2-Deoxy-*scyllo*-inosamine and DOS, but not *N*-acetyl-DOS, were converted to butirosins by mutants of groups C to F. These mutants also converted *scyllo*-inosamine-2 and streptamine to 2-hydroxybutirosins.

Paromamine, neamine, ribostamycin and xylostasin were readily converted to butirosins by all mutants except those of group A. Mutants of group A could not convert any substances tested. By the above information, the biosynthetic pathway previous proposed for butirosins was extended as shown in Fig. 4, which indicated the blocked sites in the respective mutants.

In the previous paper of this series[1], we proposed the biosynthetic pathway of butirosins and 6′-deamino-6′-hydroxybutirosins from 2-deoxystreptamine (DOS) by experiments utilizing two neamine-requiring idiotrophs of *Bacillus circulans*.

We have further investigated the biosynthetic pathway of butirosins by cosynthesis and feeding experiments using additional seven butirosin-non-producing mutants of *B. circulans*. In the present paper, we describe the evidence which reconfirms the biosynthetic pathway of butirosins from DOS as previously proposed and show that DOS was formed from 2-deoxy-*scyllo*-inosose *via* 2-deoxy-*scyllo*-inosamine as in the case of actinomycetes aminoglycoside antibiotics[2].

In addition to the cofermentation technique in a liquid medium[3,4], cosynthesis on an agar plate devised by DELIC *et al.*[5]—hereafter called DELIC's technique—was advantageously applied in the present cosynthetic experiments. DELIC's technique is more useful than the cofermentation technique in a liquid medium for determining the relative role of these mutants in cosynthesis. However, the application of this technique has been hitherto limited to a streptomyces producing tetracycline[5] or platenomycins[6].

Encouraged by the successful application of this technique to the elucidation of the pathway of platenomycins biosynthesis, the present authors attempted to apply DELIC's technique to blocked mutants of butirosin-producing bacteria. This application first made it possible to classify seven

neamine-requiring idiotrophs and two butirosin-non-producing mutants into seven groups (A to F and exceptional groups), based on their complementation patterns. Furthermore it was possible to consider rationally the results obtained in the conversion tests of an assumable intermediate to butirosins by these mutants.

Materials and Methods

Microorganisms:

Nine butirosin-non-producing blocked mutants used in the present study were derived from *B. circulans* MCRL 5001 as described previously[4] and purified by repeated segregation. In addition to strains MCRL 5003, 5004, 5602 and 5605 already reported[4], strains MCRL 5380, 5670 and 5673 newly obtained were neamine-requiring idiotrophs. The remaining two strains MCRL 5011 and 5017, however, could not produce butirosins from DOS or neamine.

Pseudomonas aeruginosa No. 12 and *Bacillus subtilis* ATCC 6633 were used for detection of cosynthesized antibiotics and for bioassay of butirosins or 2-hydroxybutirosins.

Media:

The medium for cosynthesis by DELIC's technique was composed of 20 g of glycerol, 1 g of $MgSO_4 \cdot 7H_2O$, 30 mg of $ZnSO_4 \cdot 7H_2O$, 17 g of agar and 1,000 ml of soy bean meal extract prepared as follows: Soy bean meal (20 g) was extracted with 1,000 ml of water in an autoclave for 15 minutes at 120°C, and then centrifuged at 8,000 r.p.m. for 15 minutes. The supernatant was filled up to 1,000 ml with water, adjusted to pH 7.5 and used for preparation of the above medium.

The media for seed culture and for liquid culture were the same as described previously[4]. In conversion tests, a compound to be tested was supplemented to a liquid medium before inoculation.

The medium for the assay and detection of cosynthesized antibiotics (butirosins) using *P. aeruginosa* No. 12 consisted of 5 g of peptone, 15 g of agar and 1,000 ml of water. The pH was adjusted to 8.0 before autoclaving. As the assay medium for 2-hydroxybutirosin using *B. subtilis* ATCC 6633, Penassay agar (Kyoei Pharmaceutical Co., Ltd.) was used at pH 8.0.

Materials:

2-Deoxy-*scyllo*-inosose [DL-2,4/3,5-tetrahydroxycyclohexanone]*, conduritol B [DL-5-cyclohexene-1,3/2,4-tetrol], *scyllo*-inosose [2,4,6/3,5-pentahydroxycyclohexanone] and *scyllo*-inosamine-2 [1-amino-1-deoxy-*scyllo*-inositol] were gifts of Dr. S. J. DAUM, Sterling-Winthrop Research Institute. 1-Deoxy-*scyllo*-inositol [1,3,5/2,4-cyclohexanepentol] and 2-deoxy-*scyllo*-inosamine [DL-(1,3,5/2,4)-5-amino-1,2,3,4-cyclohexanetetrol] as the fully acetylated derivatives were gifts of Prof. T. SUAMI, Keio University. *myo*-Inositol was obtained from E. Merk.

The other precursors used in this study were prepared in our laboratory.

Cofermentation between blocked mutants in liquid culture:

Each of the blocked mutants was harvested in the medium for seed culture at 32°C for 48 hours. The seed culture (each 5 ml) of two blocked mutants to be paired was inoculated together into 250-ml flask containing 30 ml of the production medium and cultivated at 32°C on a rotary shaker. After 4～5 days, the broth was assayed for the production of butirosins by the cylinder-plate method.

Cosynthesis by DELIC's technique:

Simultaneous cultivation of a pair of mutants on an agar plate and recognition of one of paired strains as a converter or as a secretor were carried out by an analogous procedure to that of previously reported[6]. Different from streptomyces, bacteria tend to spread over the surface of plate, so that it was necessary to use an agar plate previously dried in an incubator. Two mutants to be tested were streaked with a glass spreader respectively on a half of the surface of plate, separated by 2 mm from each other, and cultivated for 4～8 days at 30°C. Since strains MCRL 5011 and 5017 produced xylostasin and ribostamycin, these strains were streaked 1～2 days after previous cultiva-

* For convenience, some of the tentative nomenclature systems for cyclitols were used in this paper, however, the names based on IUPAC-IUB 1973 recommendation for cyclitol were shown in parenthesis.

tion of the partner strain.

Production of butirosins or 2-hydroxybutirosins from the cyclitols and aminocyclitols:

A 2% inoculum of strain MCRL 5605 or 5673 growing in the seed medium was transferred to 30 ml of the production medium, in a 250-ml flask, containing 200 μg/ml of 2-deoxy-*scyllo*-inosose or 2-deoxy-*scyllo*-inosamine and incubated for 6 days at 32°C. Antibiotic component in each experiment was identified as butirosins by the procedure reported already[4]. When *scyllo*-inosose or *scyllo*-inosamine-2 was supplemented as a precursor, 2-hydroxy butirosins was produced and isolated by the similar procedures. This antibiotic was also produced from streptamine by mutants of groups C, D, E and F.

Production and isolation of xylostasin and ribostamycin from the mutants of group A:

In general, isolation and purification of the metabolites accumulated by a mutant of group A was performed as shown in Fig. 3. A typical example on a mutant MCRL 5017 was as follows:

Fermentation broths on a mutant MCRL 5017 obtained by 6-day incubation in a 500-ml flask, each containing 40 ml of the production medium, were combined (3.9 liters), diluted with three volumes of water and stirred for 1 hour with Amberlite IRC-50 (NH_4^+, 200 ml). After being washed with water, the resin was packed into a column and eluted with 1 N NH_4OH. The eluate was concentrated *in vacuo* to 60 ml. The concentrate was adjusted to pH 7 with 2 N H_2SO_4 and passed through a column of Amberlite CG-50 (NH_4^+, 40 ml). After being washed with water and 0.1 N NH_4OH (800 ml) succeedingly, the column was eluted with 0.15 N NH_4OH (800 ml) and then with 0.2 N NH_4OH (800 ml). Eluate was collected in portions of 80 ml. Tubes No. 11~28 gave 965 mg of crude powder, of which 785 mg was dissolved in 2 ml of water and passed through a column of CM-Sephadex C-25 (NH_4^+, 40 ml). After being washed with water, the column was eluted with 0.1 N NH_4OH and fractionized into portions of 40 ml. Tubes No. 2~6 gave 733 mg of white amorphous powder which was determined as a mixture of xylostasin and ribostamycin in 9~8: 1~2 ratio by TLC on alumina sheet (Merck Art. 5550, Type E) using the solvent system B[4].

m.p.: 190~200°C

Anal. Calcd. for $C_{17}H_{34}N_4O_{10}\cdot\frac{1}{2}H_2CO_3$: C 43.30, H 7.27, N 11.54

Found: C 43.55, H 7.31, N 11.55

Acid hydrolysis (0.4 N HCl for 3 hours at 65°C) products: xylose (major) and ribose

The pure xylostasin (93 mg) was obtained from the above mixture (115 mg) by preparative TLC on alumina plates, followed by CM-Sephadex C-25 (NH_4^+) chromatography.

m.p.: 193~200°C

Anal. Found: C 42.92, H 7.27, N 11.55

Acid hydrolysis product: xylose.

These antibiotics were finally identified as xylostasin and ribostamycin by direct comparison with authentic samples.

From the fermentation broth (1 liter) obtained by cultivating another mutant (MCRL 5011) for 5 days under shaking—the procedures were the same as those applied to strain MCRL 5017—104 mg of a mixture of xylostasin (major) and ribostamycin was recovered. The TLC and acid hydrolysis of this mixture showed a good agreement with that produced by strain MCRL 5017.

Isolation of DOS from the mutant of group B:

Isolation and purification of the metabolites accumulated by a mutant of group B was carried out as shown in Fig. 3. The procedure was essentially similar to those described for DOS in the previous paper[1]. In the attempt to isolate some precursor(s) of DOS, however, the previous isolation procedure was slightly modified.

Results and Discussion

1. Cosynthesis

(1) Cofermentation (mixed culture)

Cofermentation in a liquid medium was carried out by cultivating simultaneously two mutants

Table 1. Production of butirosins by the cofermentation between blocked mutants in mixed culture.

Strain (Group)	5011 (A)	5017 (A)	5003 (B)	5602 (C)	5670 (C)	5004 (D)	5605 (E)	5673 (F)	5380 (Ex.)
5011 (A)	—	—	18 (5003**)	26 (5602)	20 (5670)	29 (5004)	24 (5605)	17 (5673)	6 (5380)
5017 (A)		—	14 (5003)	15 (5602)	13 (5670)	18 (5004)	13 (5605)	14 (5673)	4 (5380)
5003 (B)			—	81 (5602)	62 (5670)	76 (5004)	78 (5605)	19 (5673)	—
5602 (C)				—	—	—	69 (5605)	18 (5673)	7 (5380)
5670 (C)					—	—	90 (5605)	15 (5673)	5 (5380)
5004 (D)						—	48 (5605)	10 (5673)	—
5605 (E)							—	11 (5673)	—
5673 (F)								—	4 (5380)
5380 (Ex.*)									—

Ex.*: Exceptional.
**: A strain in paranthesis showed the strain acting as a converter in cosynthesis.
Production (μg/ml) was expressed as butirosin A.

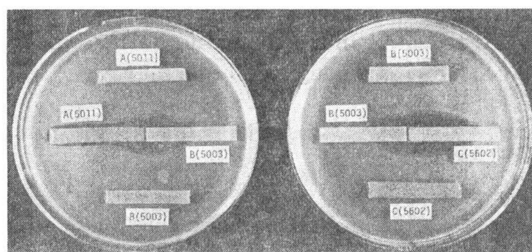
selected from nine blocked mutants. The results obtained on all combination of pairing are summarized in Table 1. Cosynthesis was not observed in combinations of strains MCRL 5011 with 5017, 5602 with 5670, 5602 with 5004, and 5670 with 5004. Behavior of strain MCRL 5380 was somewhat different from others. This mutant showed only a slight cosynthetic ability with strains MCRL 5011, 5017, 5602, 5670 and 5673, but not with others. In the case where strain MCRL 5380 was concerned, antibiotic production was too small to characterize the product. Except for this case, antibiotics coproduced were all identified as butirosins. In some pairs, antibiotic production was very excellent.

(2) Cosynthesis by DELIC's technique

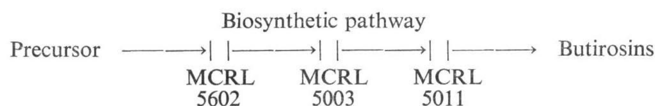
From the above results of cofermentation, no information was obtained as to the roles of the respective strains in cosynthesis or as to the relative blocked site in each mutant. To solve these problems, cosynthesis by DELIC's technique was attempted.

In Fig. 1, two examples of the experimental results are shown. In the experiment using strains MCRL 5003 and 5011, antibiotic production was observed on the side of MCRL 5003 (left photo.). Thus, it was concluded that MCRL 5011 acted as a secretor of some precursor which was converted to an antibiotic by MCRL 5003 acting as a converter. In the combination of strains MCRL 5003 with 5602, antibiotic production was demonstrated on the side of MCRL 5602 (right photo.), indicating the roles of MCRL 5003 as a secretor and MCRL 5602 as a converter. The above information further suggested the relative blocked site on the biosynthetic pathways of both mutants. Namely, strain

Fig. 1. Demonstration of antibiotic cosynthesis among mutants MCRL 5011 (group A), 5003 (group B) and 5602 (group C).



MCRL 5003 was blocked at a position nearer to butirosins than MCRL 5602, but far distant from that of MCRL 5011. Thus, the blocked sites of these three strains are shown by a mark "→| |→" as follows:



The results on cosynthesis by DELIC's technique were also described in Table 1. A strain in parenthesis showed the strain on whose side antibiotic activity was observed. In other words, a strain in parenthesis acted as a converter to a cooperative secretor strain.

It was to be noted that every couple gave the same cosynthetic result both in the present procedure and in the previous cofermentation.

(3) Reconfirmation of mutual cosynthetic ability

From the above experiments, the relative role of each mutant as a converter or a secretor was made clear. To confirm this relative role, cultivation of a converter strain in a culture filtrate of a secretor was carried out. These results shown in Table 2 supported the findings obtained in the cofermentation experiments.

(4) Classification of mutants and determination of their relative blocked sites

The information obtained in the above experiments, especially in the cosynthesis by DELIC's technique made it possible to classify the mutants based on their cosynthetic behavior and further to assume the relative blocked site where each mutant was hampered in a sequence of biosynthetic pathways.

Table 2. Cosynthesis of butirosins by a "converter" in the broth filtrate of a "secretor".

Filtrate of secretor (Group)	Converter (Group)	Production of butirosins			
		0 hr.	24 hrs.	48 hrs.	72 hrs.
5011 (A)	5003 (B)	—	44	52	48
	5602 (C)	—	28	36	45
	5004 (D)	—	19	25	23
	5605 (E)	—	26	27	32
	5673 (F)	—	2	7	7
5003 (B)	5602 (C)	—	35	53	75
	5004 (D)	—	37	60	70
	5605 (E)	—	40	46	55
	5673 (F)	—	20	25	24
5602 (C)	5004 (D)	—	—	—	—
	5605 (E)	—	19	21	36
	5673 (F)	—	10	16	17
5004 (D)	5605 (E)	—	23	27	34
	5673 (F)	—	2	7	8
5605 (E)	5673 (F)	—	4	8	10

Two ml of 72-hour growing culture of a converter strain was added to 30 ml of filtrated of 72-hour culture broth of a secretor strain and incubated for an additional 72 hours. Then, the amount of butirosins produced (μg/ml) was determined.

Strains 5011 and 5017 which acted similarly as a secretor to other strains but showed no cosynthesis between them were classified as group A. Strains in group A were blocked at nearer position to butirosins than others on the biosynthetic sequence. Contrary to mutants of group A, strain MCRL 5673 which acted as a converter to all others except for strain MCRL 5380 was classified into group F. Except for strain MCRL 5380 which was classified into the exceptional group by the reason mentioned below, the remaining strains behaved as a converter or as a secretor according to the strain to be coupled. Strain MCRL 5003, which played as a converter only to the mutants of group A, was classi-

fied into group B. This strain must be blocked at a site slightly further from a blocked site of the mutant of group A. Strains MCRL 5602 and 5670, behaving similarly to each other, acted as a converter to mutants of groups A and B and were classified into group C. Strain MCRL 5004 behaved similarly to the mutants of group C and showed no cosynthetic ability with the mutant of group C. However, the behavior of MCRL 5004 toward MCRL 5380 was different from that of the mutant of group C. Strain MCRL 5004 showed no cosynthetic phenomenon with strain MCRL 5380, but a strain of group C functioned as a secretor to strain MCRL 5380. A blocked site of the mutant belonging to group C or D must be located further from that of the mutant belonging to group B. The mutant of group C must have a blocked site in common with the mutant of group D. However, a mutant of group D must have another blocked site which existed also in strain MCRL 5380.

Strain MCRL 5605 which acted as a converter to other strains except MCRL 5673 (group F) was classified into group E and was thought to be blocked at a site somewhere between blocked sites of the mutants belonging to groups D and F. Behavior of strain MCRL 5380 was difficult to rationally understand. This strain must have at least two blocked sites respectively in common with the mutants of groups D and F. From this assumption, it was difficult to explain why strain MCRL 5380 did produce an antibiotic in cooperation with strain MCRL 5673 (group F). Moreover strain MCRL 5380, which is different from other mutants, produced only a small amount of butirosins very slowly from a precursor accumulated by a paired strain. Thus, strain MCRL 5380 was classified as the exceptional group at this moment.

The summary of the above results and the complementation pattern of the mutants, except for the exceptional group, is described in Fig. 2.

2. Isolation and Identification of Biosynthetic Intermediates Accumulated by Mutants of Groups A and B.

It was rational to think that blocked mutants of different groups would accumulate different types of biosynthetic intermediates. By the procedure shown in Fig. 3, DOS was isolated from the culture broth of MCRL 5003 (group B) and characterized. This fact was already described in the previous paper[1]. According to the complementation pattern elucidated above, mutants of group A must accumulate a substance derived from DOS and the mutants of groups C to F must produce some precursor(s) of DOS.

Fig. 2. Complementation pattern of the blocked mutants for the biosynthesis of butirosins.

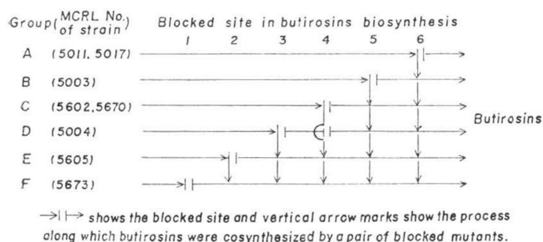
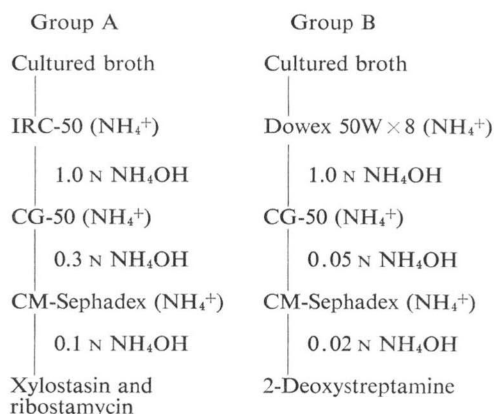


Fig. 3. Isolation steps for the metabolites produced by mutant of groups A and B.



Intermediate(s) accumulated by mutants MCRL 5011 and 5017 of group A were easily isolated and purified by the procedure shown in Fig. 3 and the recovered powder was identified as a mixture of ribostamycin (10~20%) and xylostasin (90~80%). Metabolite(s) produced by the other group mutants are now under study.

3. Bioconversion of the Plausible Intermediates to Butirosins and 2-Hydroxybutirosins

From the numerous reports on biosynthesis of aminoglycoside antibiotics, it was possible to assume the intermediates which might be concerned with the biosynthesis of butirosins. Thus, such plausible intermediates were examined whether or not they were incorporated by the present mutants

Table 3. Production of butirosins by blocked mutants of *Bacillus circulans*

Precursor	Inhibition diameter (mm)						
	5011 (A)	5003 (B)	5602 (C)	5670 (C)	5004 (D)	5605 (E)	5673 (F)
none	—	—	—	—	—	—	—
myo-Inositol	—	—	—	—	—	—	—
Conduritol B	—	—	—	—	—	—	—
1-Deoxy-*scyllo*-inositol	—	—	—	—	—	—	—
Pentaacetyl 1-deoxy-*scyllo*-inositol	—	—	—	—	—	—	—
2-Deoxy-*scyllo*-inosose	—	—	—	—	—	25.0	14.5
2-Deoxy-*scyllo*-inosamine	—	—	14.0	13.5	15.0	23.5	15.0
Pentaacetyl 2-deoxy-*scyllo*-inosamine	—	—	—	—	—	—	—
2-Deoxystreptamine	—	—	24.0	25.5	24.0	26.5	17.5
N-Acetyl 2-deoxy-streptamine	—	—	—	—	—	—	—
Paromamine	—	26.0	23.5	24.0	23.0	23.5	16.0
Neamine	—	21.5	18.0	18.5	22.0	21.0	16.0
Ribostamycin	—	18.5	17.5	16.0	19.0	19.0	15.0
Xylostasin	—	15.5	14.0	15.0	17.0	16.0	15.0

A blocked mutant was inoculated on an agar piece (6×7 mm) containing 100 μg/ml of a test compound, and incubated for 4 days at 30°C. Then, the piece was placed on an assay plate embedded with *Pseudomonas aeruginosa* No. 12 and assayed for the butirosins produced in a piece. The following dose-response relationship were observed between the amount of butirosin A in an agar piece and the inhibition diameter shown by this piece: concentration (inhibition diameter): 100 μg/ml (21.0 mm), 50 (19.5), 25 (18.0), 12.5 (16.5), 6.25 (15.0) and 3.12 (13.5).

Table 4. Production of 2-hydroxybutirosins by blocked mutants of *Bacillus circulans*.

	Inhibition diameter (mm)					
	5003 (B)	5602 (C)	5670 (C)	5004 (D)	5605 (E)	5673 (F)
none	—	—	—	—	—	—
scyllo-Inosose	—	—	—	—	19.0	13.0
scyllo-Inosamine-2	—	11.5	13.0	13.0	17.0	12.0
Streptamine	—	14.0	21.5	18.0	18.5	17.0

See footnote in Table 3.

Bacillus subtilis ATCC 6633 was used in place of *Pseudomonas aeruginosa* No. 12 to estimate the amount of 2-hydroxybutirosins produced in an agar piece.

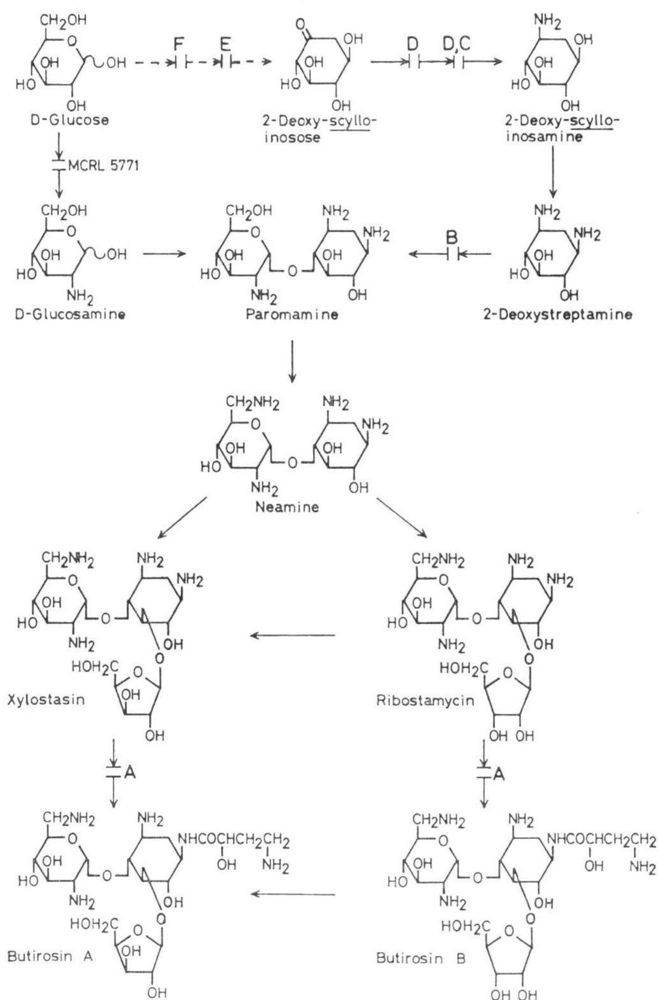
and, when incorporated, what kind of antibiotics were derived from them. Results are summarized in Tables 3 and 4. Table 3 concerns the biosynthesis of butirosins. *myo*-Inositol, conduritol B and 1-deoxy-*scyllo*-inositol were not incorporated to form the antibiotics. 2-Deoxy-*scyllo*-inosose was incorporated into butirosins by the mutants of groups E and F. 2-Deoxy-*scyllo*-inosamine and DOS were incorporated into butirosins by the mutants of groups C, D, E and F. Paromamine, neamine, ribostamycin and xylostasin were incorporated into butirosins by the mutants of groups B, C, D, E and F. However, strain MCRL 5011 of group A, a producer of ribostamycin and xylostasin, did not convert any intermediates tested into butirosins. It should be noticed that per-acetyl-1-deoxy-*scyllo*-inositol, per-acetyl-2-deoxy-*scyllo*-inosamine and *N*-acetyl-DOS did not form any antibiotic by mutants. Table 4 shows the results on the incorporation of *scyllo*-inosose, *scyllo*-inosamine-2 and streptamine. In these cases, antibiotics produced were confirmed to be 2-hydroxybutirosins reported by TAYLOR *et al.*[7], and the results were quite analogous to those observed on formation of butirosins.

4. Biosynthetic Pathway of Butirosins

Taking into consideration the present and previously reported experimental findings, we could demonstrate a more detailed biosynthetic pathway in butirosin formation as shown in Fig. 4. As to the pathways from DOS to butirosins, the present findings reconfirmed the pathways already elucidated.[1] The present findings added the information on DOS formation from 2-deoxy-*scyllo*-inosose *via* 2-deoxy-*scyllo*-inosamine. It was interesting that this route to DOS was the same as that demonstrated or postulated on aminoglycoside antibiotics produced by actinomycetes[2,8].

The blocked site where each mutant was hampered in its pathway was assumed from the complementation pattern and availability of the plausible intermediates. The blocked site of each mutant was also shown in Fig. 4 with the mark "→| |→". Mutants of group A could not acylate ribostamycin

Fig. 4. Proposed pathway for biosynthesis of butirosins.



→| |→ : The pathway (site) where mutants of a lettered group were blocked.

and xylostasin to butirosins by 4-amino-2-hydroxybutyrylation at the 1-NH_2 position. The mutant of group B was blocked on the pathway concerning pseudodisaccharide formation from DOS. The blocked site of mutants of group C was associated with the pathway from 2-deoxy-*scyllo*-inosose to 2-deoxy-*scyllo*-inosamine. The mutant of group D was blocked at least at two different sites on the pathway from 2-deoxy-*scyllo*-inosose to 2-deoxy-*scyllo*-inosamine, one being identical with the site of mutants of group C and the other existing slightly before the site of mutants of group C. If the products accumulated by mutants of group C and/or D were isolated and characterized, the pathway from 2-deoxy-*scyllo*-inosose to DOS would be more definitely elucidated. The blocked sites of mutants of groups E and F must be on the pathways which were concerned with 2-deoxy-*scyllo*-inosose formation. The blocked site of group E must be nearer to 2-deoxy-*scyllo*-inosose than that of group F, as the mutant of group F incorporated the metabolite produced by the mutant of group E. 1-Deoxy-*scyllo*-inositol, which was postulated as an intermediate in the biosynthetic pathway to 2-deoxy-*scyllo*-inosose from D-glucose by RINEHART *et al.*[9], was not utilized to form an antibiotic by any mutants. This result suggested that viboquercitol (1,2,4/3,5-cyclohexanepentol), which was demonstrated in gentamicin biosynthesis by DAUM *et al.*[2], may be an intermediate to 2-deoxy-*scyllo*-inosose or the blocked sites of the mutants of groups E and F may be on the pathway from 1-deoxy-*scyllo*-inositol to 2-deoxy-*scyllo*-inosose. It will be of great use, if the metabolites accumulated by such mutants are elucidated.

Acknowledgements

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