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STUDIES ON JULIMYCINS—III

THE ISOLATION AND PURIFICATION OF MINOR COMPONENTS

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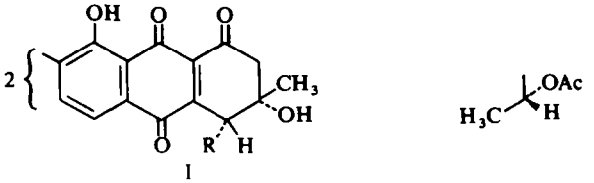
Abstract—In addition to julimycin B—II, a number of minor components, julichromes, were separated from the metabolites of *Streptomyces shiodaensis*. The isolation of new pigments, especially the preparative TLC techniques for the pigments having close R_f values, are described.

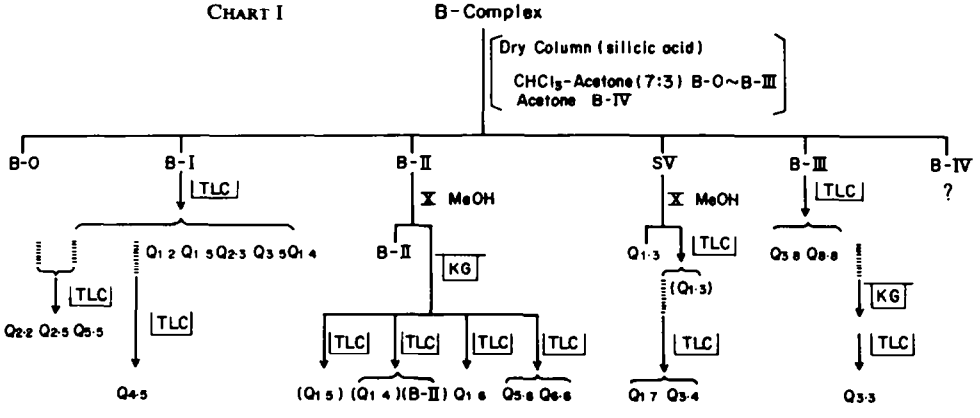
IT IS known that *Streptomyces shiodaensis* nov. sp. produces many other coloured materials besides julimycin B-II (I).[1,2] Katagiri *et al.*[3] briefly described the coloured metabolites (julimycin B-complex) as giving six spots (B-O, B-I, B-II, SV, B-III and B-IV) on TLC, and that julimycins B-I, B-II, SV and B-III had been isolated from the complex by the column chromatography on metal free silica gel.



I

Since it appeared of interest to isolate these minor components and to investigate the relationship between their structures, B-complex was carefully separated into nearly twenty coloured compounds. In order to avoid the disorder in nomenclature,

CHART I



these new pigments (including julimycins B-I, B-III and SV) other than julimycin B-II have been named julichromes, and individual pigments are indicated on the basis of constitution. This paper deals with the general isolation methods of julichromes.

A typical isolation scheme is shown in Chart 1, but modifications were required for the practical isolation since the composition of the B-complex depended not only on the fermentation conditions but also on the period of storage of the complex. For example, the yield of $Q_{8\cdot8}$ (cf. Chart 1) decreased on storing, and in extreme case could not be isolated at all.*

Firstly the B-complex was chromatographed on silicic acid using the dry column method[4] which gave results similar to the liquid column method but with fewer fractions.

The fractions were examined by TLC on metal free silica gel and combined according to the R_f values. The resultant six major fractions according to R_f values seemed to correspond to B-O, B-I, B-II, SV, B-III and B-IV.[3] But examination of the contents of each major fraction with the usual silica gel G plate showed a very different pattern on TLC, in which the R_f values and colours of pigments did not always agree with those on the metal free adsorbant (cf. Fig. 3). This behaviour was used for the detection of minor components and for the examination of purity.

Fig. 1 shows the elution diagram of the B-complex according to TLC and further separation experiments described below. The diagram indicates that the major six

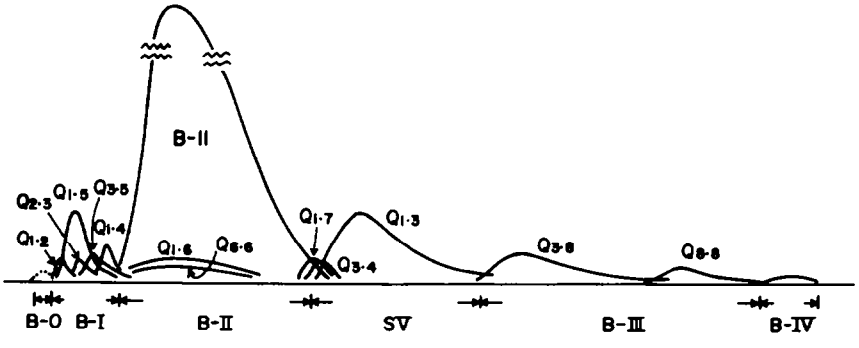


FIG. 1 Elution diagram of B-complex.

fractions were not sufficiently pure and differed considerably in quantity. Since the B-O fraction was too poor for further treatment, it was reserved until more was available. The plentiful B-II fraction was recrystallized to remove considerable amounts of julimycin B-II, and the mother liquor was subjected to separation into further fractions. The B-IV fraction showed only a dark brown tailing spot on both adsorbants, and no marked coloured material was obtained from this fraction.

Because of the close R_f values the definite separation of each major fraction by the ordinary preparative TLC as well as further column chromatography was unsuccessful. Therefore, the special TLC technique,[5] continuous development method, was applied.

* The reason will be reported later.

As shown in Fig. 2, the mixture (20–50 mg) on a preparative TLC plate (20 × 20 cm)* was developed allowing the solvent to evaporate from the top of the plate. The polarity of the developing solvent was adjusted so that the main zones could be developed to about 40–60% height of the plate during 2–16 hr (usually 2–3 hr). The minute

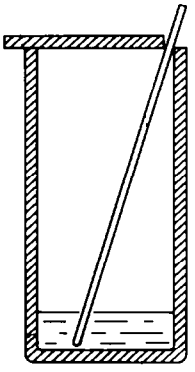


FIG. 2

components having large R_f values accumulated in narrow zones at the top of the plate, and could be purified by further treatment with a suitable solvent system. This technique is particularly useful for the separation of coloured materials such as julichromes and their derivatives, because the separation can easily be observed during the operation.

As shown in Chart 1 and Fig. 4 (cf. Fig. 3), most of the components were successfully fractionated, although some could not be distinctly separated even with this technique. In these cases, the preparative TLC on silica gel G sometimes gave efficient results. For example, $Q_{1\cdot6}$ and $Q_{6\cdot6}$ were separated from julimycin B-II by this method, although these pigments were apt to degenerate on this adsorbant. Therefore, to avoid continuous development, quick removal from the adsorbant, treatment of the extract with acid solution and further TLC of each zone on metal free silica gel were usually required.

A combination of the above isolation techniques resulted in the separation of the B-complex although many components were too poor to characterize.† In Figs 3 and 4 the TLC of the julichromes which were purified and characterized are shown in elution order on metal free adsorbant. The order reveals a close relationship with the constitutions as will be reported in following papers.

EXPERIMENTAL

All m.ps were determined on hot stage and are not corrected. For column chromatography, 100 mesh silicic acid (Mallinckrodt) was used without any further treatment. The adsorbant for TLC was metal free silica gel, Yamani-layer PG (Yamani Chem. Co. Ltd., Osaka, Japan), unless otherwise stated. As usual silica gel G, Kieselgel G (Merck) was used.

* For the plentiful sample, a large plate (20 = 100 cm) was used.

† About 20 g of B-complex was used for this study.

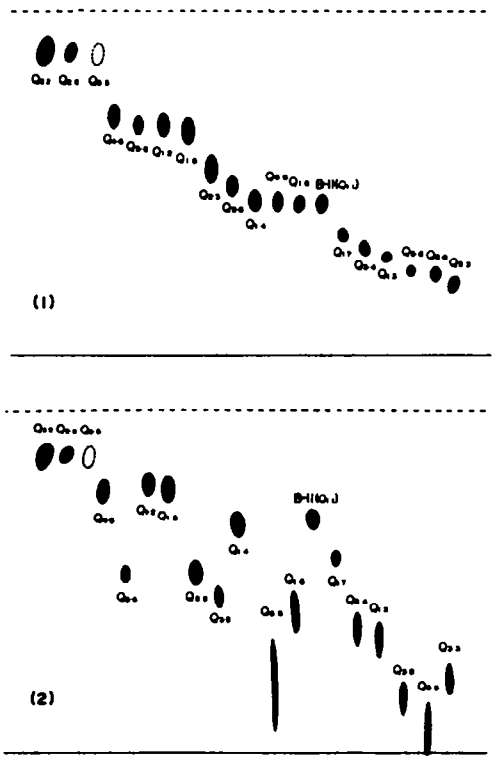


FIG. 3 (1) Ordinary TLC on metal free silica gel ($CHCl_3$–MeOH 9:1). (2) Ordinary TLC on silica gel G ($CHCl_3$–MeOH 8:2).

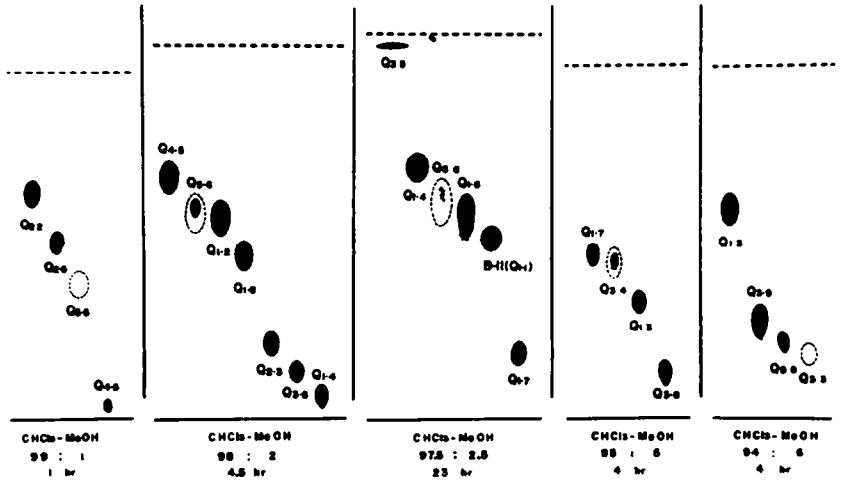


FIG. 4 Continuous development TLC on metal free silica gel.

Dry column chromatography of julimycin B-*complex*

To a soln of 2·0 g B-complex in 20 ml acetone, 10 g silicic acid was added, and the mixture was dried at room temp. The dried mixture was added to the top of the dry column (diam: 40 mm) tightly packed with 200 g silicic acid, and the sample was covered with additional 10 g silicic acid and then with cotton. The B-complex was eluted with $CHCl_3$-acetone (7:3), and the eluate was fractionated to each 15 g soln on a fraction collector. At the end of separation with this solvent system, elution with acetone gave B-IV fraction, which contained no marked components.

According to the R_f values, the fractions (about 300) were combined and evaporated. The components from each main fraction (except B-IV) were further separated as described below.

fraction	R_f($CHCl_3$—MeOH (9:1))	yield
B-O	0·88	ca. 1 mg
B-I	0·5–0·6	63 mg
B-II	0·4	1160 mg
SV	0·3	527 mg
B-III	0·20–0·25	303 mg
B-IV	0–	58 mg

B-O *fraction*

Since the yield of the components from this fraction was very poor, the crude materials were reserved. Later, 12 mg of the mixture was fractionated by continuous development TLC using $CHCl_3$–MeOH (99:1) into three major yellow zones. The material from each zone was recovered by extraction with $CHCl_3$–MeOH (9:1).

The first zone gave 2 mg $Q_{2\cdot2}$, which was crystallized from acetone to afford yellowish orange prisms, m.p. 250–251° (dec); $Mg(OAc)_2$ reaction: red.

The pigment (4 mg) from the second zone was crystallized from $CHCl_3$–MeOH to give $Q_{2\cdot5}$ as red prisms, m.p. >300°; $Mg(OAc)_2$ reaction: red.

The last zone afforded 6 mg $Q_{5\cdot5}$, which was recrystallized from $CHCl_3$ to give yellow prisms, m.p. >300°.

This compound is almost insoluble in most organic solvents and the yellow colour changed to red with $Mg(OAc)_2$, but soon reverted to original yellow.

B-I *fraction*

This fraction (63 mg) gave numerous bands on continuous development TLC using $CHCl_3$-MeOH (98:2).

(i) The pigments from the upper zones, with R_f values similar to those of B-O fraction, were combined and treated later together with the B-O fraction.

(ii) The second group was refractionated after several runs by the same method using $CHCl_3$–MeOH (98·5:1·5). The TLC showed two main bands. The first yellow band afforded $Q_{4\cdot5}$, which was crystallized from MeOH to give orange prisms, m.p. 241–246°. Its yield was estimated 1 mg or less from 2·0 g B-complex; $Mg(OAc)_2$ reaction: deep carmine. The second red zone gave $Q_{1\cdot2}$ as red powder, m.p. 166–170°. From 2·0 g B-complex, approximately 3 mg $Q_{1\cdot2}$ was isolated; $Mg(OAc)_2$ reaction: purple.

(iii) The main red zone of B-I fraction gave 18 mg $Q_{1\cdot5}$, which was recrystallized from acetone to yield red prisms, m.p. >300°; $Mg(OAc)_2$ reaction: purple. This pigment was identified as julimycin B-I and compared with the sample isolated by Katagiri *et al.*

(iv) From the yellow zone below $Q_{1\cdot5}$ 4 mg $Q_{2\cdot3}$ was obtained. Recrystallization from benzene gave a yellow powder, m.p. 165–190° (dec); $Mg(OAc)_2$ reaction: red.

(v) The next major yellow band afforded 6 mg $Q_{3\cdot5}$ which was recrystallized from MeOH to give orange needles, m.p. 228° (dec); $Mg(OAc)_2$ reaction: red.

(vi) The pigment (10 mg) from the bottom major orange zone was recrystallized from MeOH to give $Q_{1\cdot4}$ as red prisms, m.p. 200–220° (dec); $Mg(OAc)_2$ reaction: brown.

B-II *fraction*

The material from B-II fraction was recrystallized from MeOH to give 533 mg julimycin B-II as dark

red prisms. The residue from the mother liquor was separated into four major bands by the ordinary preparative TLC on silica gel G plate using $CHCl_3$–MeOH (9:1).

(i) The material from the first orange zone (turned to purple in the air) was washed with 1% acetone soln of oxalic acid until the colour changed to red, and then with $CHCl_3$–acetone (6:4). The combined solns were washed with H_2O, and the $CHCl_3$ layer was dried over $MgSO_4$ and evaporated. The pigment (20 mg) was identified as $Q_{1\cdot5}$ obtained from B-I fraction.

(ii) The material from the second brown zone (turned to blue in the air) was treated as above to give additional 350 mg julimycin B-II, which was purified by continuous development TLC with $CHCl_3$–MeOH (96:4). The main orange zone gave 335 mg pure julimycin B-II. The minor orange zone slightly above the main zone gave 8 mg $Q_{1\ 4}$, which was identical with the specimen isolated from B-I fraction.

(iii) The third dark green band afforded 57 mg deep red material, which was refractionated by continuous development TLC using $CHCl_3$–MeOH (96:4). The pigment (40 mg) from the main dark red zone was recrystallized from EtOAc to afford $Q_{1\cdot6}$ as deep red leaflets, m.p. 191–197°; $Mg(OAc)_2$ reaction: green.

The minor zones, which have R_f values similar to B-I fraction, were combined and the mixture was further separated by continuous development TLC using $CHCl_3$–MeOH (98·5:1·5). The main orange zone gave 4 mg $Q_{5\cdot6}$ as amorphous powder; $Mg(OAc)_2$ reaction: magenta. $Q_{5\ 6}$ was also isolated from B-I fraction, but the yield was very poor.

(iv) The bottom yellow zone of the TLC on silica gel G was treated as above. The extract (27 mg) was refractionated by continuous development TLC with $CHCl_3$–MeOH (96:4). The main yellow zone gave 10 mg $Q_{6\ 6}$, which was recrystallized from benzene to afford yellow prisms, m.p. 240–243°; $Mg(OAc)_2$ reaction: negative.

SV *fraction*

The material from SV fraction was recrystallized from MeOH to give 300 mg $Q_{1\cdot3}$ as red prisms, m.p. 190–210°. This pigment was identical with julimycin SV isolated by Katagiri *et al.*; $Mg(OAc)_2$ reaction: violet. The mother liquor was evaporated, and the residue was separated by the continuous development TLC using $CHCl_3$–MeOH (93:7).

(i) The pigments (20 mg) from the zones slightly above $Q_{1\ 3}$ were combined and fractionated by the repeated continuous development TLC with $CHCl_3$–MeOH (95:5). The above orange zone gave 6 mg $Q_{1\cdot7}$ as red amorphous powder; $Mg(OAc)_2$ reaction: violet. The lower pale yellow band afforded 1 mg $Q_{3\cdot4}$ as a pale yellow amorphous powder. This compound was negative to $Mg(OAc)_2$ colour reaction though its colour was somewhat deepened.

B-III *fraction*

The material (about 300 mg) was separated by the continuous development TLC using $CHCl_3$–MeOH (92:8). The main yellow zone gave 148 mg $Q_{3\cdot8}$, which was crystallized from $CHCl_3$ to afford yellow prisms, m.p. 195–200°; $Mg(OAc)_2$ reaction: negative. The zones below $Q_{3\cdot8}$ were combined and further separated on silica gel G plate using $CHCl_3$–MeOH (8:2). The main yellow zone was extracted with a soln of oxalic acid in MeOH. After addition of H_2O, the mixture was extracted with EtOAc to give 20 mg of almost colourless material, which was reseparated by continuous development TLC using $CHCl_3$–MeOH (92:8). The substance from the faint pale yellow zone was recrystallized from acetone to give 6 mg $Q_{3\cdot3}$ as almost colourless prisms, m.p. >300°. With $Mg(OAc)_2$, it deepened yellow.

$Q_{8\ 8}$ was not obtained from the B-complex used for this run presumably due to its instability on storage. In earlier experiments, however, $Q_{8\cdot8}$ was isolated in a fairly yield. On the continuous development TLC $Q_{8\cdot8}$ showed its band below $Q_{3\cdot8}$ as yellow zone, and recrystallization from $CHCl_3$ gave yellow prisms, m.p. 178–180°; $Mg(OAc)_2$ reaction: negative.

This compound was identical with julimycin B-III obtained by Katagiri *et al.* by comparison of IR spectrum.

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