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STUDIES ON JULIMYCINS—VIII

THE STRUCTURES OF JULICHROMES $Q_{1\cdot7}$, $Q_{8\cdot8}$, $Q_{3\cdot8}$, $Q_{3\cdot3}$ AND $Q_{1\cdot9}$

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Abstract—The structures of five new julichromes have been elucidated. The isolation of the pigments having a Q_8 unit, corresponding to the hydroquinone of the Q_1 unit, suggests the biogenesis of the anthraquinone system. The biogenetic relationships of the nine units found in the julichromes are also described.

IN THE preceeding papers, the isolation[1] and structural investigation[2-5] of fourteen julichromes have been reported. The present paper deals with the structures of the remaining four pigments and of julichrome $Q_{1\cdot9}$ lately isolated from julimycin B-complex.

Julichrome $Q_{1\cdot7}$ (I) is a pigment which turns violet with magnesium acetate as do julichrome $Q_{1\cdot3}$ (II)[2,3] and its C_9-epimer[3,*] (III) (cf. Chart 1). On acidic silica gel, I shows an R_f value very close to that of III but is distinguishable from III by its IR spectrum. The UV spectrum of I is also similar to those of II and III, therefore it was assumed that I may be an isomer of II, as was supported by the elementary analysis which coincided with the molecular formula, $C_{38}H_{36}O_{15}$.

The NMR spectrum† (cf Fig 1) showed that I is composed of the known Q_1 unit and an unknown unit which resembles the Q_3 unit. The unknown part, the Q_7 unit, has two aromatic protons (*ortho*), a secondary OH group at a benzylic position and the same substituents in the hydroaromatic ring as the Q_3 or $Q_{3'}$ unit (epimeric at C_9). However, some shifts of proton signals are observed. The C_3-Me signal, distinct from those of the Q_3 and $Q_{3'}$ units, appears at a higher field (1·27 ppm) than that of Q_1 unit (1·42 ppm). In the case of the Q_3 and $Q_{3'}$ units, as detailed in the previous paper,[3] the C_3-Me (axial) signal (1·67 ppm) shows a considerable downfield shift due to the β-epoxide at the A/B juncture. In addition, the proton signals of the methylene group at C_2 split into an AB-type quartet, whereas those of the Q_3 or $Q_{3'}$ unit appear as a singlet overlapping the signal of C_2-H_2 of the Q_1 unit. These observations suggest that the Q_7 unit is an isomer of the Q_3 or $Q_{3'}$ unit as regards the configuration of the epoxide or of the C_3-Me group.

In order to confirm the structure, I was oxidized with chromic acid. The IR spectrum of the product was similar to that of julichrome $Q_{1\cdot4}$ (IV)[2] but was not identical.

* This compound has not been found in the natural metabolites.

† NMR spectra were taken at 60 Mc in $CDCl_3$ solution unless otherwise stated. Chemical shifts are expressed in δ (ppm) downfield from TMS used as internal reference. For the measurement of poor samples a semi-micro cell was used.

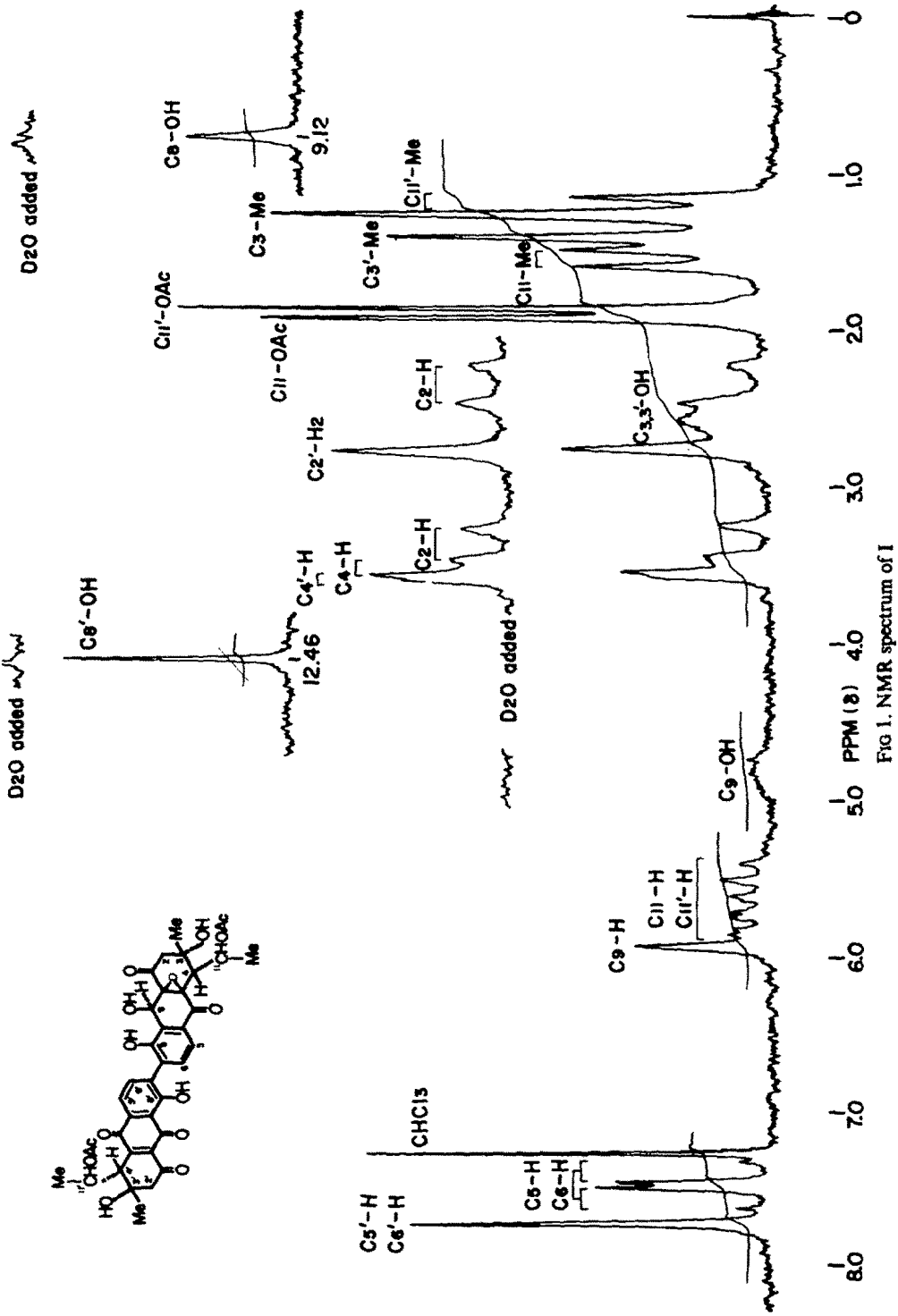


FIG 1. NMR spectrum of I

However, treating the product with potassium iodide in acetic acid gave julimycin B-II (V) in almost quantitative yield.

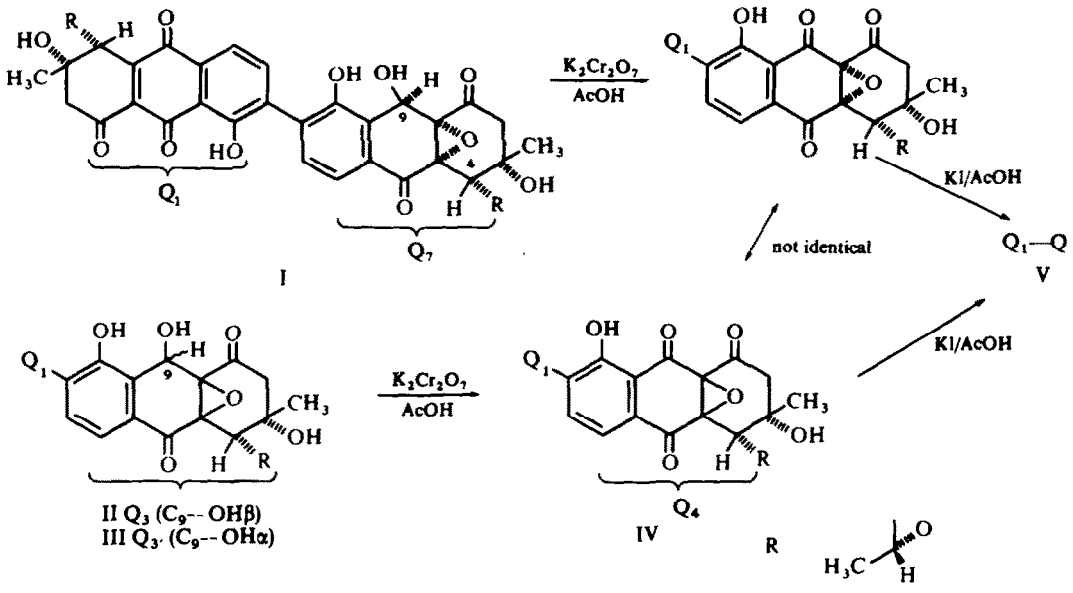
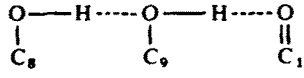


CHART 1

These experiments shown in Chart 1 clearly prove that the Q_7 unit is a diastereoisomer of the Q_3 or $Q_{3'}$ unit associated with the configuration of the epoxide, and since the configuration of the epoxide of the Q_3 unit has been determined as β, the epoxide of this unit must be α.

Now, the configuration at C_9 remains uncertain. The chemical shifts of C_8-OH (9·12 ppm) and $C_{8'}$-OH (12·46 ppm) are similar to those of the $Q_{3'}$ unit and suggest the existence of the intramolecular H-bonding system,[3]



and therefore a quasiequatorial conformation of the C_9-OH group. Accordingly, if the C_9-OH group is β, the structure of the Q_7 unit should be shown as Ia (cf Fig 2), and if the C_9-OH group is α then it must be indicated as Ib.

The structure Ib, however, would not allow for interpretation of the abnormally high shift of the C_3-Me signal, but in the case of Ia, the upfield shift due to the long-range shielding effect of the aromatic ring is predicted. Accordingly, the configuration of the OH group at C_9 is reasonably assigned as β, and in this structure the considerable downfield shift of the C_{11}-Me signal is properly explained by the anisotropic effect of CO group at C_{10}.

Julichrome $Q_{8\cdot8}$ (VI) is an unstable pigment isolated from fresh extract of the fermentation beer of *Streptomyces shiodaensis*. It crystallized from chloroform as a solvate but did not give constant analytical data. The IR spectrum was identical with that of julimycin B-III isolated by Katagiri *et al.*[6]

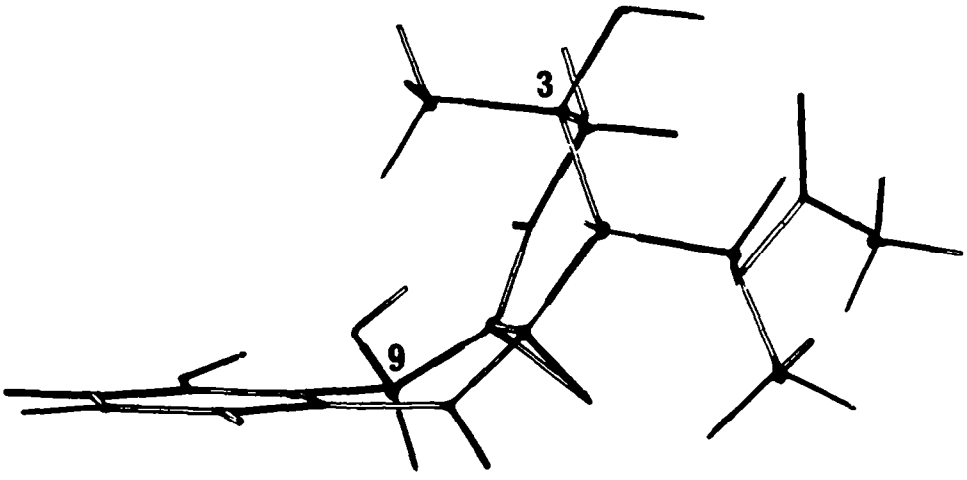


FIG 2a. Ia

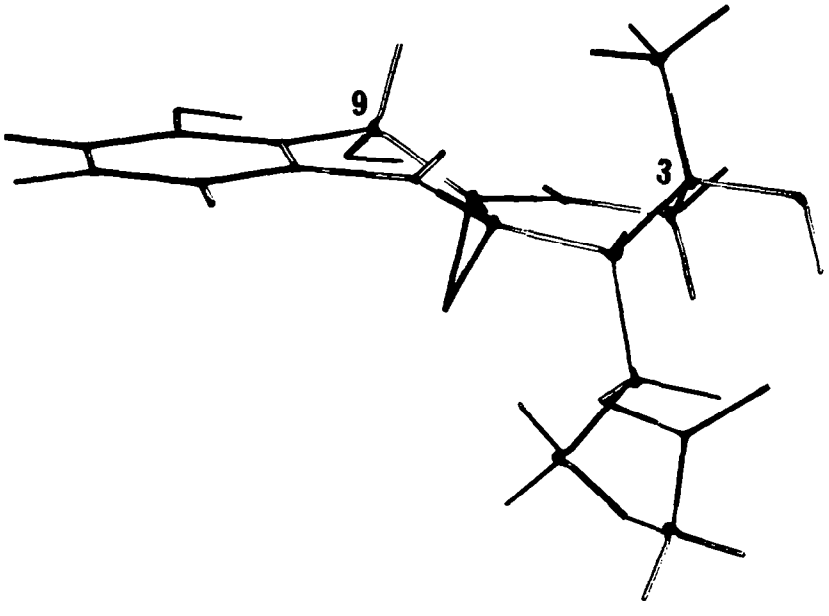


FIG 2b. Ib

On standing, VI was converted to V, even in the solid state, and the change was rapid in alcohol or acetone solution. Therefore, it was readily assumed that VI might be the hydroquinone of V. As expected, treatment of V with sodium dithionite gave VI. The NMR spectrum of VI (in CD_3COCD_3) shown in Fig 3 is in good agreement with the structure Q_8—Q_8 (cf Chart 2).

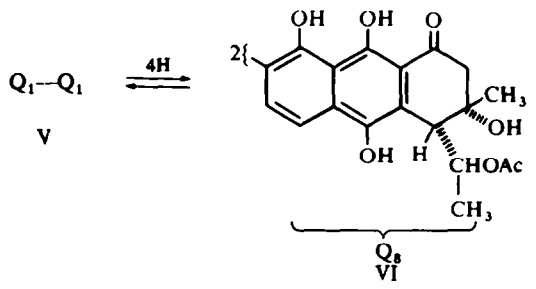


CHART 2

Julichrome $Q_{3\cdot8}$ (VII) is obtained as a yellow powder which shows a negative colour reaction with magnesium acetate. Though VII is more stable than VI, it gradually changed to II, suggesting the structure Q_3—Q_8.

The catalytic hydrogenation of II gave VII, and its NMR spectrum, which revealed the overlapping pattern of the signals attributable to Q_3 and Q_8 units, supported the structure.

Julichrome $Q_{3\cdot3}$ (VIII), distinct from other julichromes, is almost colourless. It is negative to the magnesium acetate reaction and its R_f value is as low as that of VII. Therefore, the structure of VIII was predicted as Q_3—Q_3.

The NMR spectrum (in CD_3OD) as shown in Fig 4 reveals only the signals assignable to a Q_3 unit and is in good agreement with its structure.

Further, the oxidation of VIII with potassium bichromate in acetic acid gave two products whose R_f values correspond to julichrome $Q_{3\cdot4}$ (Q_3—Q_4)[2] and julimycin B-II diepoxide[2] (Q_4—Q_4). On treating with potassium iodide in acetic acid, the

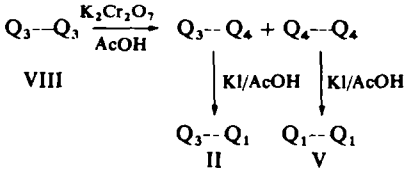


CHART 3

former product gave II and the latter afforded V. These results clearly prove the structure of VIII.

The last pigment, julichrome $Q_{1\cdot9}$ (IX), was recently isolated from the B-II fraction of julimycin B-complex. IX appears as a red spot between V and I on acidic silica gel plate, and changes its colour to violet with magnesium acetate. The IR spectrum of IX shows a characteristic absorption band at 1790 cm^{-1}, which has not been observed in the spectra of the other julichromes. Therefore, the presence of a new type of unit was predicted in this pigment, while the other part of the molecule was likely to be a Q_1 unit from its R_f value and colour reaction.

Unfortunately, the yield was too low for the measurement of a good NMR spectrum (cf Fig 5), but the structure of IX could be presumed from its assignment.

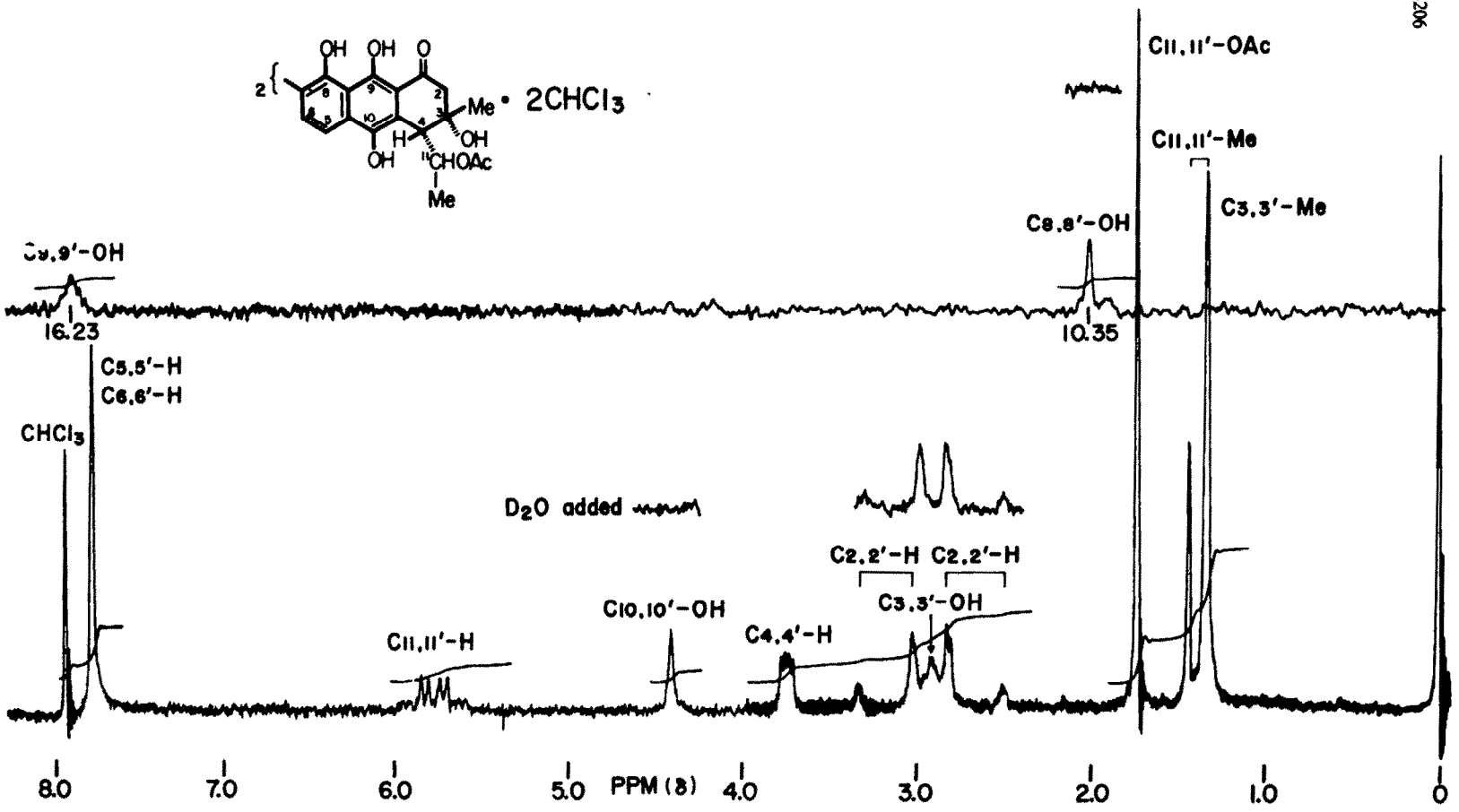


FIG 3. NMR spectrum of VI (CD_3COCD_3)

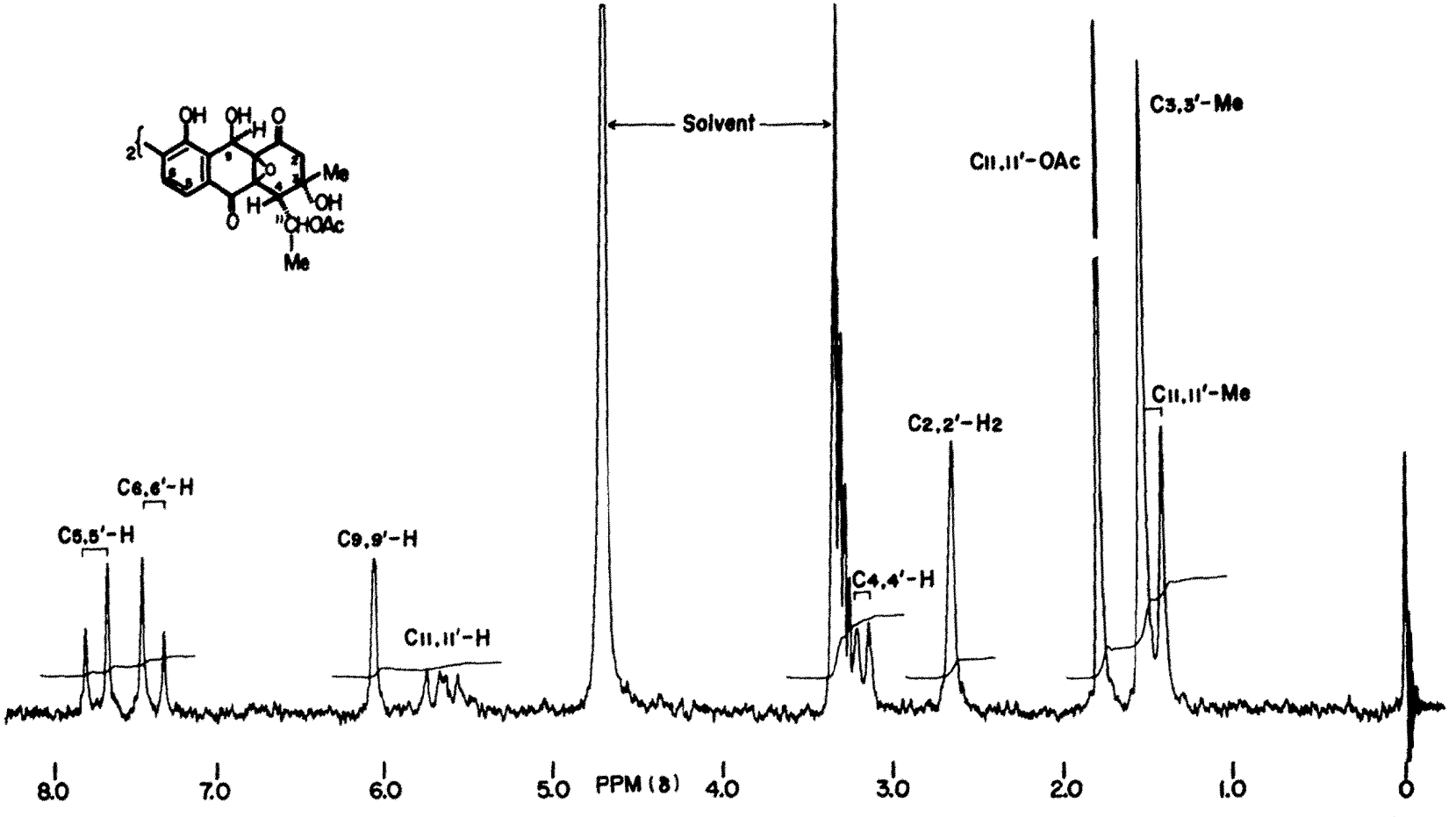


FIG 4. NMR spectrum of VIII (CD_3OD)

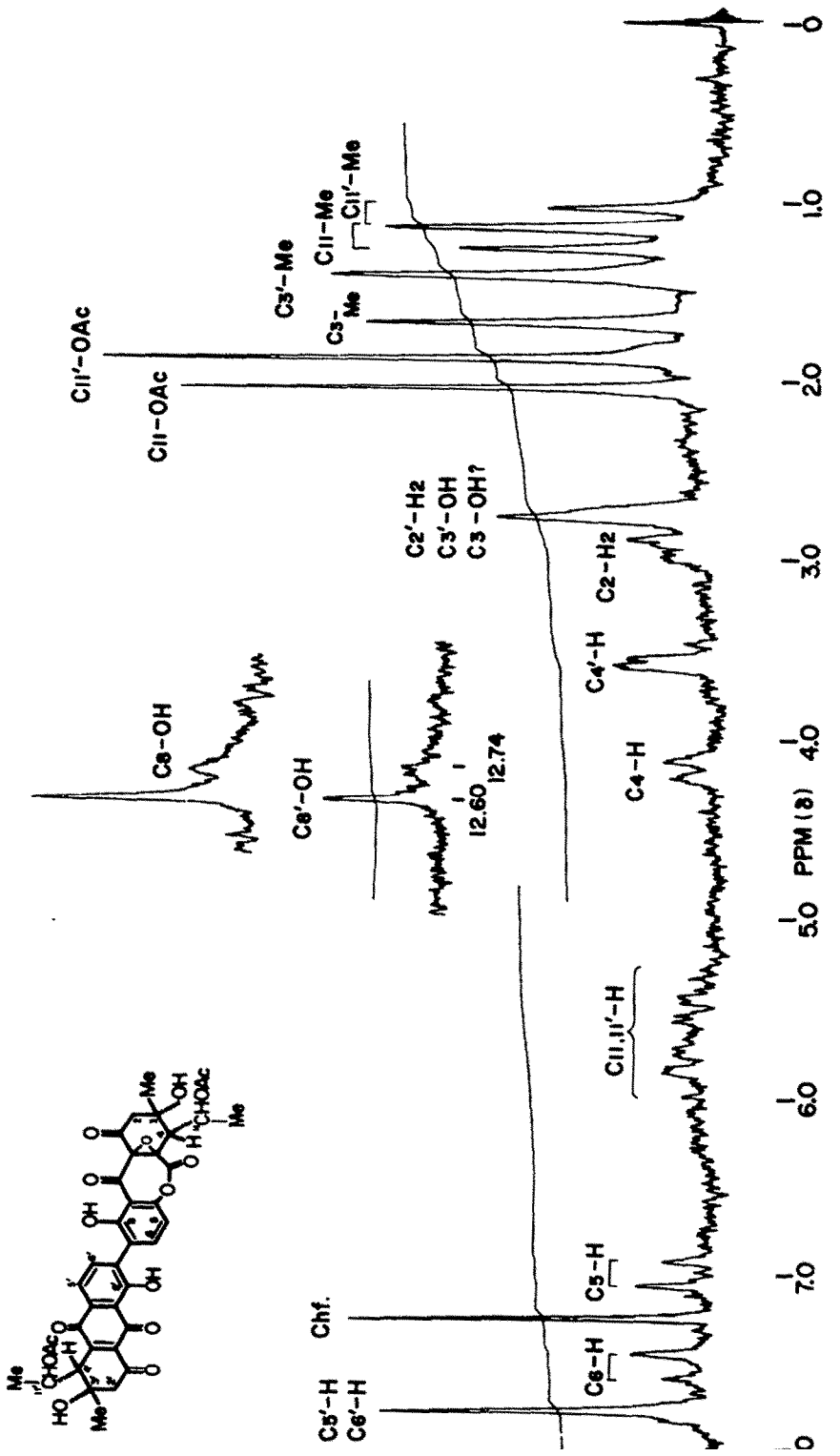
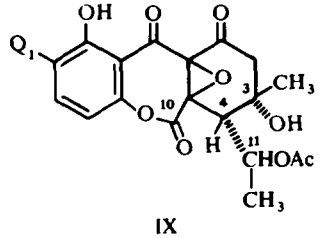


FIG 5 NMR spectrum of IX

The spectrum exhibits the signals attributable to a Q_1 unit, supporting the above assumption. The remaining signal pattern is not in accordance with any of the known units, but suggests that the unknown unit, the Q_9 unit, has a hydroaromatic ring substituted with groups common to other julichromes, and an aromatic ring which has two *ortho* protons as other units. Moreover, since the C_3-Me signal is the same as those of the Q_3 or $Q_{3'}$ unit, the presence of the β-oxide at the A/B juncture is likely. Regarding the aromatic ring the OH proton signal at 12·43 ppm together with the above-mentioned two aromatic proton signals suggests the following partial structure.

Since the CO band at 1790 cm^{-1} cannot be attributed to the CO functions indicated in this partial structure, an additional CO function must be present in the unindicated part of the molecule, presumably at C_{10a} or C_{4a}. The chemical shift of C_5-H (7·00 ppm) appears at an abnormally higher field in comparison with those of other known units. Accordingly, the location at C_{10a} of the OH or ether group, instead of the CO group, is reasonable. Again, the situation of the CO function at C_{4a} is favourable from the biogenetic point of view.

As for the CO group which shows the absorption band at 1800 cm^{-1} region, either the γ-lactone or the vinyl ester (phenolic ester) are conceivable, though the high frequency shift of about 20–30 cm^{-1} must be explained. In the case of IX, the γ-lactone system does not completely account for the partial structure, however, the depside-type linkage shown as IX nicely satisfies the requirements.



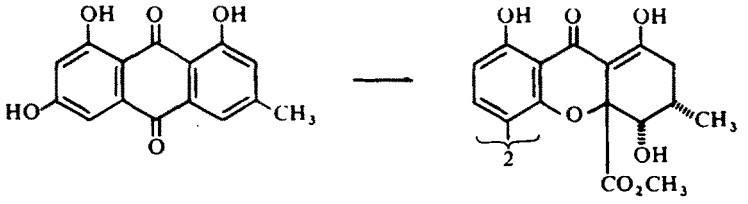
IX

In this structure, the upfield shift of the C_5-H signal is reasonable, and since the CO group at C_{10} is probably orientated upward due to non-bonding interaction with the C_{11}-CH_3 group, and its dipole is orientated in the same direction as the C_{4a}-O, the considerably high frequency shift of the CO absorption in the IR spectrum is predicted.[7] Moreover, the remarkable downfield shift of the C_4-H signal as well as the upfield shift of the C_{11}-CH_3 signal is possibly due to this orientation of the CO function.

It is to be regretted that insufficient pigment was available for further confirmation

of this novel ring system, but the assumed structure of the Q_9 unit is possible as an oxidation product of the Q_4 unit by a biological reaction of Baeyer-Villiger type.

Moreover, this structure bears a close relation to the ergochromes, the ergot pigments. Franck *et al.*[8] reported the biosynthesis of ergochromes from emodin, but they described that since the Baeyer–Villiger oxidation of the anthraquinones was



emodin ergochrome BB

chemically unsuccessful, this type reaction is unlikely for the formation of ergochromes, but that the easy oxidative cleavage of an anthrone would be conceivable. Nevertheless, the compound IX is suggestive of the convertibility of the anthraquinone derivatives to the hydroxanthone ring system.

TABLE 1. THE CONSTITUTION OF 20 PIGMENTS

Q_1--Q_1	Q_1--Q_6	Q_2--Q_5	Q_4--Q_5
Q_1—Q_2	Q_1—Q_7	Q_3--Q_3	Q_5--Q_5
Q_1—Q_3	Q_1--Q_9	Q_3--Q_4	Q_5—Q_6
Q_1—Q_4	Q_2--Q_2	Q_3--Q_5	Q_6--Q_6
Q_1—Q_5	Q_2--Q_3	Q_3--Q_8	Q_8--Q_8

The julichromes isolated hitherto are summarized in Table 1. Each of them consists of two of the nine component units (Q_1—Q_9), which have variety to a certain extent though all retain a structural correlation with the anthraquinone system (cf Chart 4). The presence of a close biogenetic relation between the pigments is evident, because all these compounds have been produced by the same strain in the same nutrient medium and most of them have asymmetric structures which are composed of two units differing from each other in biogenetic stage.

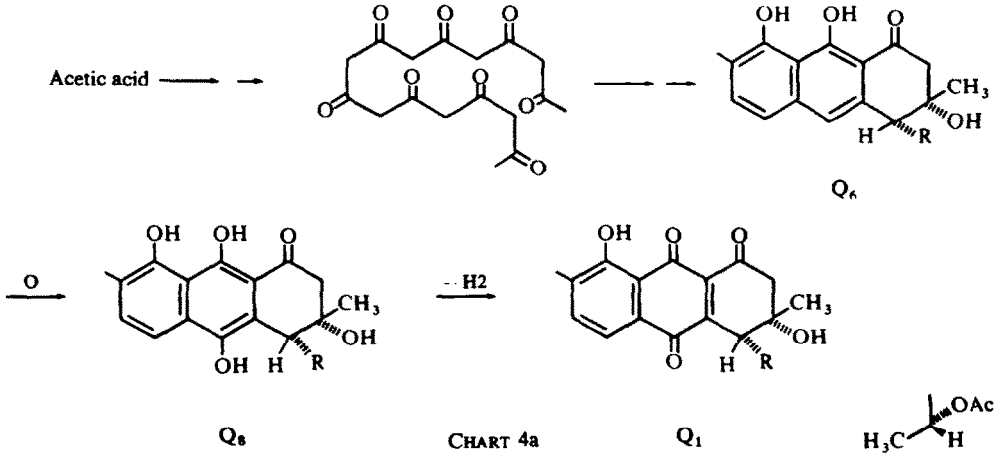


CHART 4a

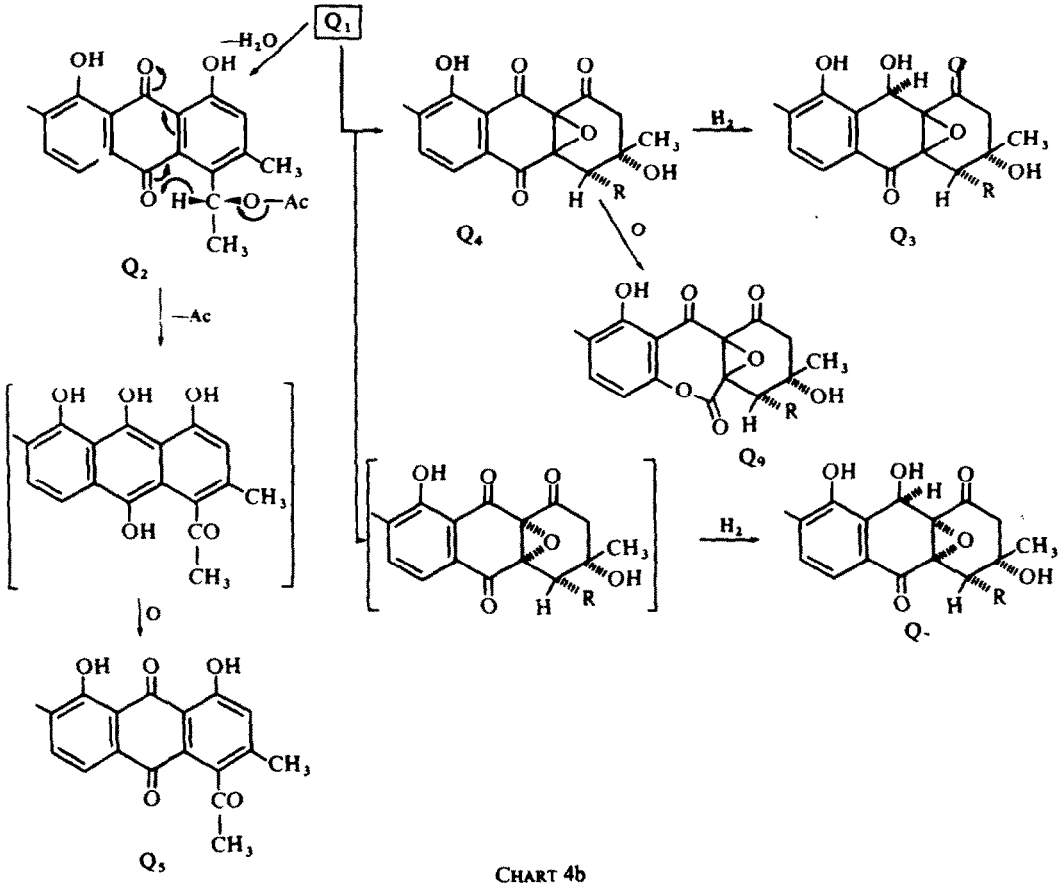


CHART 4b

The biogenesis of julichromes is possibly as shown in Chart 4. Despite the precise separations made, no monomeric pigment could be detected, further, all the pigments isolated have structures coupled at the β,β′-position and neither α,α′- nor α,β′-coupled compounds were found in the metabolites. Therefore, the biosynthesis of julichromes by the acetate–malonate route probably proceeds through a dimerization at some early step of the biosynthesis, and then the conversion may progress stepwise at each half of the molecule.

Among the nine units, the Q_6 unit, which lacks an O function at C_{10}, is conceivably the first to be produced. The Q_8 unit resulting from the hydroxylation of the Q_6 unit at C_{10}, is easily convertible to the Q_1 unit which composes the main product, julimycin B-II. However, the Q_8 unit seems considerably stable, or, at any rate, accumulative in the fermentation medium, considering that julichromes $Q_{8\cdot8}$ and $Q_{3\cdot8}$ were isolated in a fair yield from fresh extract of the fermentation beer, even though they are very unstable in the air.

The biosynthesis from the Q_1 unit possibly proceeds according to the pathways shown in Chart 4b, and the main metabolic pathway of the Q_1 unit may be the route $Q_1 \rightarrow Q_4 \rightarrow Q_3$ judging from the population of the units and the amount of each pigment. T. Kimura *et al.*[9] described that julimycin B-II was not accumulated in the

fermentation medium but that the amount of julichrome $Q_{1\cdot3}$ increased with the rapid degradation of julimycin B-II. The above-mentioned main pathway is supported by this observation.

With respect to the combination of the nine units more pigments should exist in the julimycin B-complex and, in fact, numerous minor zones were observed on TLC, though it was impossible to characterize them due to their negligible quantities, nor could other possible units, written in parentheses in Chart 4, be detected.

The further metabolic pathways are unknown, but it is certain that hardly any coloured materials were found in the extract of the fermentation beer of the ultimate stage.

Since compounds related to these nine units have been found in other microbial metabolites, the results obtained in this series of investigations not only give information on the formation and degradation of julimycin B-II but also serve the interest in the biogenesis of other anthraquinone systems.

EXPERIMENTAL*

Julichrome $Q_{1\cdot7}$ (I). This pigment was reported as an amorphous powder, but later it was recrystallized from benzene as fine red prisms, which fade gradually above 200° and do not melt below 290°. (Found: C, 62·61; H, 5·31. $C_{38}H_{36}O_{15}$ requires: C, 62·29; H, 4·95%); IR ν_{max} (Nujol) cm^{-1}: 3200–3500 (OH), 1690–1740 (CO, ester), 1665 (non-chelated quinone CO), 1630 (chelated quinone CO); UV λ_{max} (MeOH) mμ (log ε): 220 (4·51), 247 (sh) (4.32), 275 (sh) (4·22), 450 (3·76); ORD: $[\phi]_{440}$ 0, $[\phi]_{358}$ −14500, $[\phi]_{308}$ +37300, $[\phi]_{275-250}$ 0, $[\phi]_{238}$ −20700, $[\phi]_{230}$ −11900. CD: $[\theta]_{370}$ 0, $[\theta]_{330}$ −22800, $[\theta]_{307}$ 0, $[\theta]_{292}$ +15500, $[\theta]_{260}$ +7060, $[\theta]_{244}$ +18200, $[\theta]_{230}$ 0 (1·328 mg/5 ml MeOH).

Conversion of I *to julimycin* B-II (V). A hot soln of 2 mg I in 2 drops of AcOH was treated with 3 drops of a saturated soln of $K_2Cr_2O_7$ in hot AcOH, and the mixture was heated on a steam bath for 10 min. The mixture was poured into H_2O and extracted with $CHCl_3$. The extracted product was separated by continuous development TLC[1] on acidic silica gel ($CHCl_3$–MeOH, 97:3). The main orange zone gave 1·5 mg of the oxidation product as an orange amorphous powder, which shows a colour reaction with $Mg(OAc)_2$ (brown) similar to that of IV. The IR spectrum of this product is also very close to that of IV except for some absorption bands in finger-print region.

The oxidation product was dissolved in AcOH and treated with KI at room temperature for 30 min. Working up as usual gave 1 mg V, identical with an authentic specimen in comparison of IR spectra and TLC.

Julichrome $O_{8\cdot8}$ (VI). The pigment recrystallized from $CHCl_3$ possibly includes 2 moles of the solvent, as indicated by the NMR spectrum and analyses. (Found: C, 50·92; H, 4·13; Cl, †19·20. $C_{38}H_{38}O_{14}\cdot2CHCl_3$ requires: C, 50·20; H, 4·11. Cl, 22·23%); IR ν_{max} (Nujol) cm^{-1}: 3400 (OH), 1741 (w), 1715 (s) (OAc), 1615–1630 (chelated CO and aromatic).

Reduction of V *with* $Na_2S_2O_4$. To a soln of V (5 mg) in $CHCl_3$ (2 ml) and 3 drops of AcOH an excess soln of $Na_2S_2O_4$ in H_2O was added. After stirring for 2 min at room temp, the $CHCl_3$ layer became yellow and the reduction product crystallized. The mixture was filtered, and the crystals were dissolved in hot $CHCl_3$. The hot soln was dried over $MgSO_4$ and reduced to afford VI as yellow prisms, which were identified with the specimen isolated from the natural metabolites by comparison of IR spectra.

Julichrome $Q_{3\cdot8}$ (VII). The pigment recrystallized from $CHCl_3$ includes the solvent, as did VI. (Found: C, 54·05; H, 4·66. $C_{38}H_{38}O_{15}\cdot CHCl_3\cdot\frac{1}{2}H_2O$ requires: C, 54·27; H, 4·67%); IR ν_{max} (Nujol) cm^{-1}: 3340 (broad) (OH), 1700–1730 (OAc, CO), 1626 (chelated CO); $[\alpha]_D^{24}$ +47·2 (±4·5°) (*c* 0·178, MeOH).

Catalytic reduction of II. A soln of II (100 mg) in EtOH (18 ml) was hydrogenated over 5% Pd-C (20 mg). The uptake of H_2 was 3·7 ml (at 24·5°) during 35 min. The solvent was evaporated under N_2 nearly to dryness, and $CHCl_3$ was added to the mixture. The catalyst was filtered off and the filtrate was reduced to give 105 mg of V as yellow prisms, $[\alpha]_D^{24}$ + 54 (±5°) (*c* 0·177 MeOH). The IR spectrum of this product was identical with that of a natural specimen.

V soon reverted to II in a soln in MeOH or EtOH.

* Mps were determined on a hot plate and are uncorrected.

† The analysis of Cl was low, possibly due to discharge of the solvent.

Julichrome $Q_{3\cdot3}$ (VIII). The sample recrystallized from acetone gave the following analytical data. (Found: C, 58·29; H, 5·05; H_2O, 4·99. $C_{38}H_{38}O_{16}\cdot 2H_2O$ requires: C, 58·08; H, 5·33; H_2O, 4·58%); IR ν_{max} (Nujol) cm^{-1}: 3340 (broad) (OH), 1690–1750 (broad) (OAc and CO); UV λ_{max} (MeOH) mμ (log ε): 295 (3·85), 333 (3·75), 400 (3·37); ORD: $[\phi]_{430}$ 0, $[\phi]_{360}$ +21200, $[\phi]_{350}$ +13700, $[\phi]_{308}$ −48300, $[\phi]_{270}$ −8760, $[\phi]_{235}$ −51100, $[\phi]_{224}$ +20100. CD: $[\theta]_{368}$ 0, $[\theta]_{340}$ +29500, $[\theta]_{330}$ +27100, $[\theta]_{305}$ 0, $[\theta]_{290}$ − 23800, $[\theta]_{246}$ 0, $[\theta]_{226}$ − 61700, $[\theta]_{210}$ 0 (0·863 mg/2 ml MeOH).

Conversion of VIII *to* II *and* V. To a soln of VIII (9 mg) in AcOH (0·5 ml) was added a soln of $K_2Cr_2O_7$ (3 mg) in AcOH (0·5 ml). The mixture was heated on a steam bath for 12 min, poured into H_2O and extracted with $CHCl_3$. The material (7 mg) from the extract showed several zones by continuous development TLC on acidic silica gel. The second yellow zone corresponding to julimycin B-II diepoxide gave 1 mg yellow substance, which was treated with KI in AcOH to afford ~1 mg V, identical with an authentic sample in comparison of TLC and colour reaction. The 4th yellow zone of the oxidation product gave 1 mg of yellow pigment, the R_f value of which corresponded to that of julichrome $Q_{3\cdot4}$ used as reference. On treating with KI in AcOH, this pigment afforded ~1 mg II, identified with an authentic sample by continuous development TLC and colour reaction.

Julichrome $Q_{1\cdot9}$ (IX). This pigment was isolated from the accumulated materials between the B-II fraction and the SV fraction,[1] and finally separated by repeated continuous development TLC on acidic silica gel ($CHCl_3$–MeOH, 96:4). From about 20 g of julimycin B-complex 13 mg of IX was obtained as an amorphous solid, which was used for the measurement of NMR spectrum. The recovered sample was further purified from $CHCl_3$–light petroleum as a red powder, m.p. 172–177° (dec). (Found: C, 59·24; H, 4·94. $C_{38}H_{34}O_{16}\cdot H_2O$ requires: C, 59·68; H, 4·75%); UV λ_{max} (MeOH) mμ (log ε): 226 (4·50), 284 (4·28), 446 (3·74); IR ν_{max} ($CHCl_3$) cm^{-1}: 3592 (w), 3526 (m), *ca* 3480 (broad), 2700–3200 (chelate OH), 1790 (α.β-epoxy lactone), 1739 (OAc), 1707 (six-membered ring CO), 1666 (non-chelated quinone CO), 1633 (chelated quinone CO).

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