Tetrahedron. Vol 24, pp. 1765 to 1776. Pergamon Press 1968 Printed in Great Britain

STUDIES ON JULIMYCINS—I

THE STRUCTURE OF JULIMYCIN B-II

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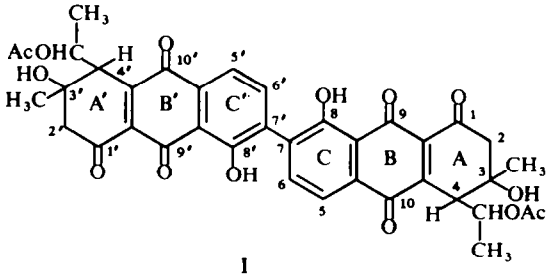
(*Received in Japan* 12 *June* 1967; *accepted for publication* 14 *August* 1967)

Abstract—The structure of a new anti-viral antibiotic, julimycin B-II, has been established as a derivative of β,β′-bianthraquinonyl by spectroscopic and degradative experiments.

THE isolation of a new anti-viral antibiotic, julimycin B-II, from the metabolites of *Streptomyces shiodaensis* nov. sp., and its molecular formula, $C_{38}H_{34-36}O_{14}$ as well as the peri-hydroxy quinonoid chromophore, based on preliminary experiments, has been reported.[1]

This substance shows interesting biological activities,[2] and in this paper are outlined the results of spectroscopic and degradative experiments which establish the structure of julimycin B-II (I) as a derivative of β,β′-bianthraquinonyl.



I

Julimycin B-II forms reddish orange plates which decompose slowly above 150° and result in tarry mass at 215–220°. The dimeric structure was based on its mol wt determination (about 700*) and the proton-countings on NMR spectra† of its derivatives. In each spectrum, assuming that one Me signal should contain three protons, the integration of the total signals is in proportion to one half of the proton number required for the mol wt (Fig. 3). Therefore, it was concluded that this antibiotic must have a symmetrical structure $(C_{19}H_{17}O_7)_2$.

The proposed peri-hydroxy quinonoid chromophore based upon UV and IR spectra (1634 cm^{-1}; chelated carbonyl and 1665 cm^{-1}; non-chelated carbonyl) as well as colour reactions especially with magnesium acetate[3] was confirmed by the

* 700 by Barger–Akiya's method in dioxan,[1] 730 by ebulliometry in acetone[1] and 749 by osmometry in acetone.

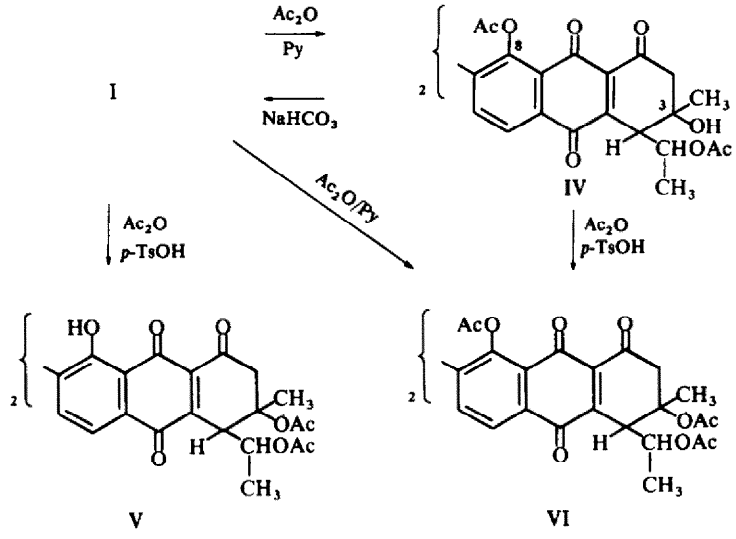
† NMR spectra were taken with a Varian A-60 spectrometer in $CDCl_3$ solution unless otherwise stated. Chemical shifts are expressed in δ (ppm) from TMS used as internal reference.

following experiments: The deep blue colour of an alkaline solution of I was decolourized immediately by the addition of sodium dithionite. On catalytic hydrogenation, I absorbs two moles of hydrogen to give yellow *leuco* compound which is oxidized to the original compound by air. The NMR spectrum of I in dioxan solution shows a chelated OH signal at 12·57 ppm. The IR spectra of 8,8′-O-dimethyl ether (II) and 8,8′-O-bis(*p*-nitrobenzoyl) derivative III show only non-chelated carbonyl bands in the quinone region.

The oxygen-containing groups, other than peri-hydroxy quinonoid parts, in accordance with spectroscopic results consist of OH groups (3445 cm^{-1}), CO groups (1704 cm^{-1}) and acetoxyl groups (1740 cm^{-1}; 1·77 ppm, 6H, in CD_3COCD_3). The OH band is also present in the IR spectrum of II.

In order to account for the OH groups, acetylation products of I were examined.

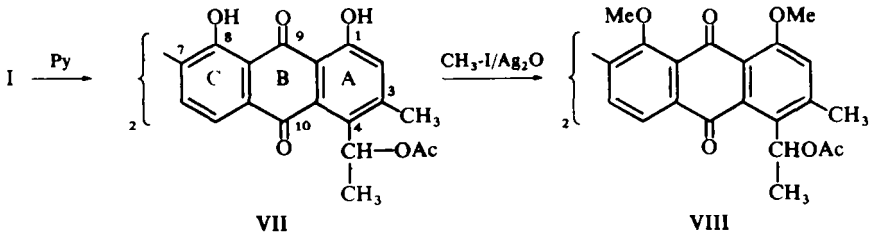
CHART 1



Julimycin B-II is easily acetylated with acetic anhydride-pyridine to the diacetate-I (IV)[1] which hydrolyzes to the original compound on treatment with sodium bicarbonate.[1] On the other hand, I gives the diacetate-II (V) on the acetylation with acetic anhydride–*p*-toluenesulfonic acid. Further, IV is easily converted to the tetraacetate VI, which is identical with the tetraacetate[1] prepared directly from I. In IV the two phenolic peri-hydroxyl groups have been acetylated because of the presence of phenolic acetoxyl groups (1780 cm^{-1}, 2·22 ppm, 6H) and the absence of peri-hydroxy quinonoid characters. Since V shows peri-hydroxy quinonoid characters (peri-hydroxy protons: 12·54 ppm, 2H; chelated carbonyl: 1633 cm^{-1}; magnesium acetate colour reaction: positive) and new acetoxyl functions (1740 cm^{-1}; 2·12 ppm, 6H), two alcoholic OH groups must be acetylated. Further, since VI has no OH group, the presence of four OH groups in the julimycin B-II molecule is corroborative. Accordingly, the number of the unknown carbonyl groups must be two.

In pyridine solution, I is converted to the bisanhydro derivative VII, $(C_{19}H_{15}O_6)_2$, *via* a labile monoanhydro derivative.*

CHART 2



VII VIII

In the IR spectrum of VII the alcoholic OH band (3445 cm^{-1}) and the CO band (1704 cm^{-1}) present in I have disappeared and the UV spectrum, as shown in Fig. 1, exhibits characters[4] similar to the anthraquinone derivatives which have two peri-hydroxy groups. These facts suggest that the treatment of I with pyridine results

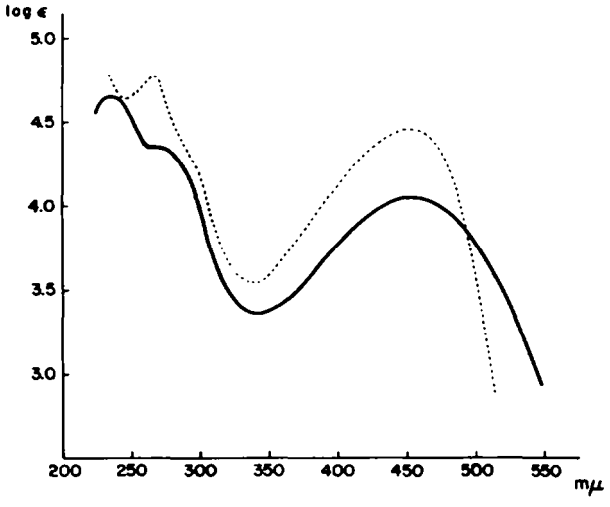


FIG. 1 UV spectra of I (———) in MeOH and VII (– – – –) in dioxan.

not only in the base-catalyzed dehydration of the alcoholic OH groups but also in aromatization which converts the CO groups into phenolic peri-hydroxyl groups. Actually, the NMR spectrum of VII shows two peri-hydroxyl signals (12·38 ppm, 2H, singlet and 12·25 ppm, 2H, singlet) and new aromatic protons (7·02 ppm, 2H, singlet). Moreover, the methylation of VII produces tetramethyl ether VIII.

* The isolation of monoanhydro derivative is possible under mild condition. The result will be reported later.

The position of the new OH groups was concluded from its IR spectrum in which the non-chelated quinone band is still observed at 1667 cm^{-1}. Therefore, the OH functions should bond with the same quinone CO groups which have been chelated by the original peri-hydroxyl groups. Thus, it is clear that VII is a derivative of 1,1′,8,8′-tetrahydroxybianthraquinonyl.

The unknown substituents, $[C_3H_{10}\text{-O-CO-}CH_3]_2$, of VII were easily resolved into

$$\left[\phi\begin{matrix}\underline{H}_{(f)}\\ \\ \underline{H}_{(f)}\end{matrix}\ (ortho);\ \phi\text{-}\underline{H}_{(e)};\ \phi\text{-}\underline{CH}_{3(c)}\ \text{and}\ \phi\text{-}\underline{CH}_{(d)}\text{-}(\underline{O}\cdot\underline{COCH}_{3(b)})\text{-}\underline{CH}_{3(a)}\right]_2$$

by the assignment of the NMR spectrum of VIII as shown in Fig. 2. Among these groups, the *ortho* hydrogen atoms ($H_{(f)}$) should be located on the original aromatic rings (Rings C, C′), because the corresponding signals ($H_{(g)}$ in Fig. 3) are also present in the spectra of julimycin B-II acetates (IV, V, VI) which are lacking the second aromatic rings. The remaining groups, the signals ($H_{(a)-(e)}$) of which are not observed in the spectra of IV, V and VI, must be placed on the new aromatic rings (Rings A, A′). Accordingly, the two anthraquinone moieties must be coupled, whether at the *ortho*- or *para*-positions to the phenolic OH groups of the original aromatic rings (Rings C, C′).

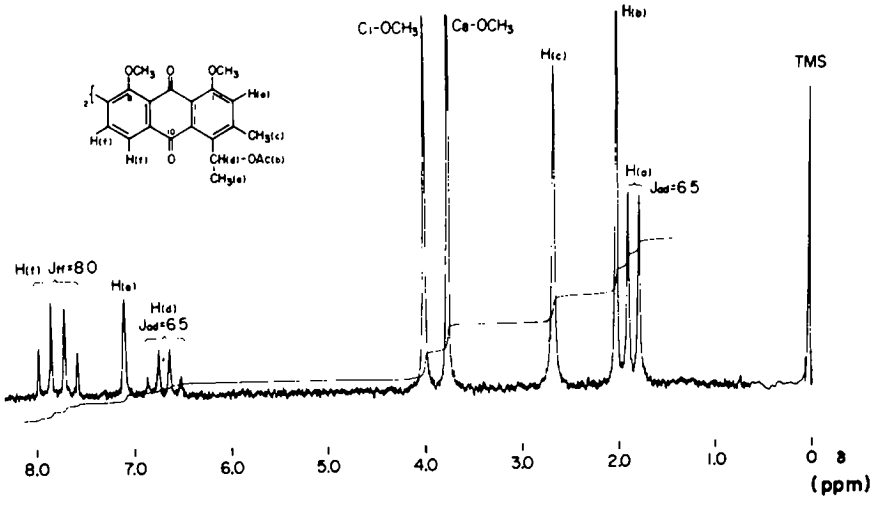


FIG. 2 NMR spectrum of VIII.

Since the conditions of dehydration of I were mild, the same number of rings must be present in the original compound and I is therefore, a derivative of 8,8′-dihydroxy-1,1′-dioxo-1,1′,2,2′,3,3′,4,4′-octahydro-5,5′- or 7,7′-bianthraquinonyl which differs from VII only in Ring A (A′). In this structure, because of the abnormal high frequency shift, it seems improper to attribute the absorption band at 1704 cm^{-1}

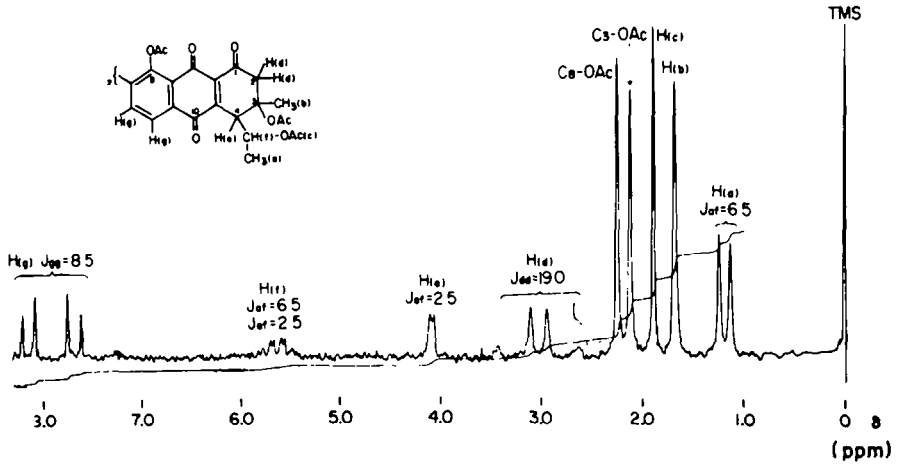
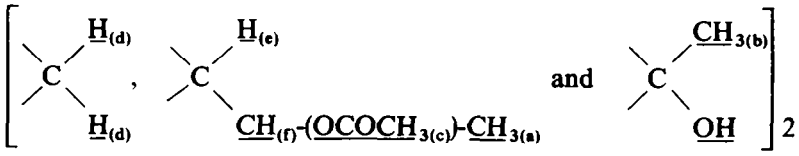


FIG. 3 NMR spectrum of VI.

to the CO group(s) at C_1 ($C_{1'}$). However, the wave number is comparable to that of rubroskyrin (1703 cm^{-1}).[5]

The substituents of the hydroaromatic rings of I were estimated as



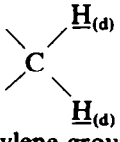
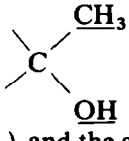
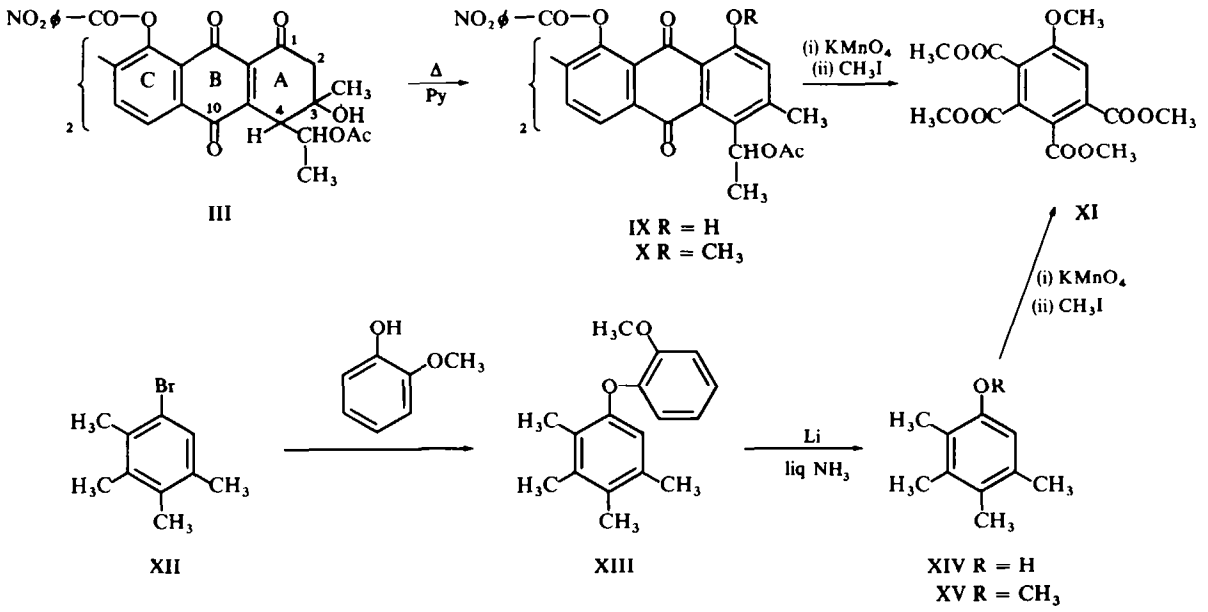
by the assignment of the NMR spectrum of VI (Fig. 3) referring to the structure of the bisanhydro derivative. Since the geminal methylene protons  exhibit a simple AB-type quartet, the carbon atoms adjacent to this methylene group(s) have no proton. Accordingly, the  groups could only be located at the β-position to the CO group(s) at C_1($C_{1'}$), and the assignment of this position is compatible with the easy base-catalyzed elimination of the OH function(s). Regarding the position of the methylene group(s), the large geminal coupling constant (J = 19·0 c/s) of the methylene protons suggests the presence of adjacent CO group.[6] This supports the locations of the methylene group(s) at C_2($C_{2'}$) and the side chain(s) at C_4($C_{4'}$). This was confirmed by the following experiments.

CHART 3

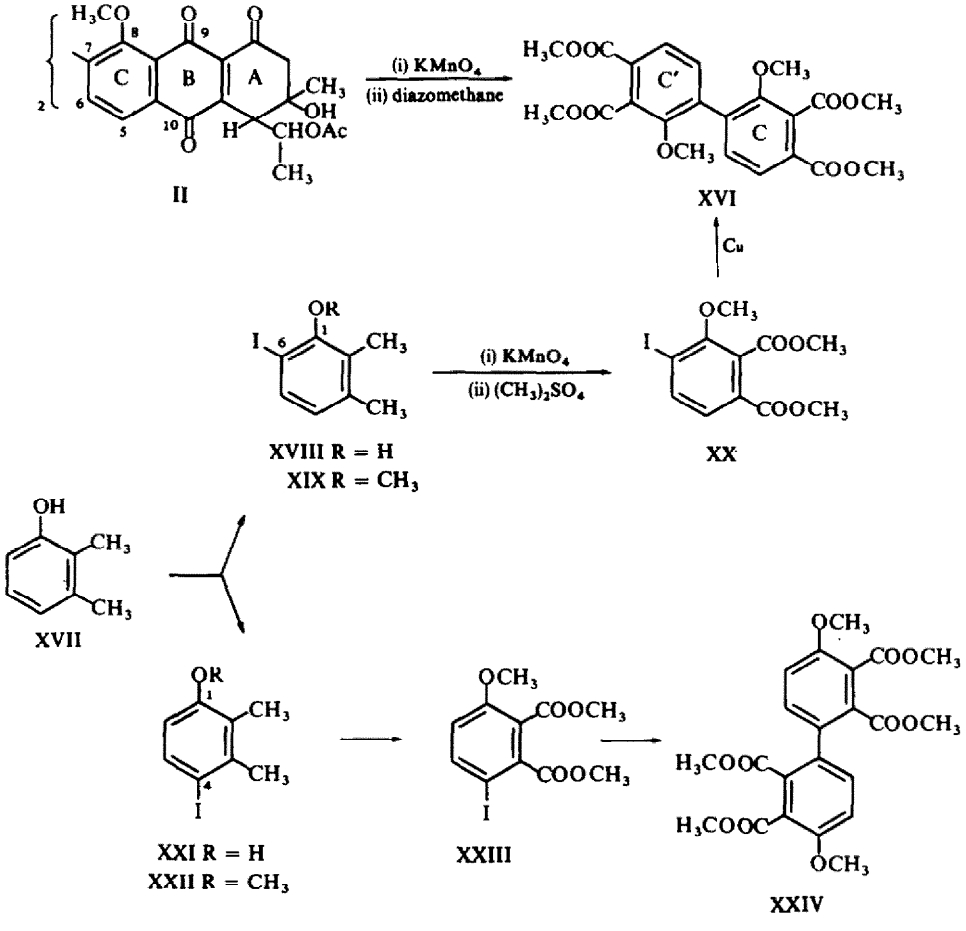

NO2φ—CO—O
C
B
A
CH3
OH
H
CHOAc
CH3
III
Δ
Py
OR
IX R = H
X R = CH3
(i) KMnO4
(ii) CH3I
H3COOC
COOCH3
XI
OH
OCH3
Br
H3C
CH3
XII
H3CO
O
XIII
Li
liq NH3
XIV R = H
XV R = CH3

In order to prepare the oxidation product which retains Ring A (A'), III was converted to the 1,1'-dimethoxyanthraquinone derivative X, which was oxidized with potassium permanganate. The oxidation product was isolated as the methyl ester XI. The synthesis of XI was carried out according to the scheme indicated in Chart 3.

By comparison with an authentic specimen the degradation product was proved to be tetramethyl 5-methoxybenzene-1,2,3,4-tetracarboxylate (XI). This result agrees with the conclusion from the NMR spectrum and establishes the structure of hydroaromatic ring(s).

In order to determine the positions at which the two quinonoid moieties are coupled, II was degraded by permanganate oxidation. Since the oxidation product (XVI) should include the biphenyl part (Rings C, C') of the original compound, the two possible esters were synthesized as shown in Chart 4.

CHART 4



Iodination of 2,3-dimethylphenol (XVII) yielded two monoiodides (XVIII and XXI). Both compounds exhibit the presence of *ortho* protons in the NMR spectra and XVIII, as distinct from XXI, shows intramolecular hydrogen bonding in its IR spectrum. Consequently, XVIII and XXI are 6- and 4-iodocompounds, respectively.

This assignment was confirmed by methylation of authentic 3-hydroxy-6-iodophthalic acid[7] to XXIII. From the iodo compounds the biphenyl compounds XVI and XXIV were obtained *via* a similar pathway.

The degradation product, therefore, was confirmed to be tetramethyl 2,2'-dimethoxybiphenyl-3,3',4,4'-tetracarboxylate (XVI) and the two quinonoid moieties are coupled at 7-7' giving julimycin B-II the structure as shown in formula I.

Though several α,α'-bianthraquinonyls have been isolated from fungal metabolites,[8] julimycin B-II is the first β,β'-bianthraquinonyl derivative isolated from microbial metabolites.*

EXPERIMENTAL†

Catalytic hydrogenation of julimycin B-II (I)

A soln of 100 mg I in 40 ml EtOH was hydrogenated over 5% Pd-C. The up-take of H_2 was 7·1 ml, which corresponded to two moles equivs for a molecule (theoretically 6·8 ml at 23°). The colour of the hydrogenated soln soon changed from yellow to the original red in the air and removal of the solvent afforded I.

Julimycin B-II 8,8'-O-*dimethyl ether* (II)

To a soln of 70 mg I in 20 ml acetone, 5 ml MeI and Ag_2O (prepared from 0·85 g $AgNO_3$ and 2·2 ml 10% NaOH) were added, and the mixture was refluxed for 1 hr under stirring. The reaction mixture was filtered and the filtrate was condensed to nearly dryness. Addition of MeOH gave 70 mg crude II as yellow fine prisms, which were recrystallized from a large amount of MeOH, m.p. 255–265° (dec). (Found: C, 64·76; H, 5·23; MeO, 8·46. $C_{40}H_{38}O_{14}$ requires: C, 64·68; H, 5·16; MeO, 8·36%); IR ν_{max}^{Nujol} cm^{-1}: 3460, 1720, 1670. UV λ_{max}^{dioxan} mμ (log ε): 232 (4·68), 380 (3·97).

8,8'-O-*bis*(p-*nitrobenzoyl*) *julimycin* B-II (III)

To a soln of 400 mg I in 15 ml pyridine, 1·0 g crystalline *p*-nitrobenzoyl chloride was added with stirring during a period of 5 min at room temp. After 15 min, the mixture was poured into 100 ml water, and the ppts were washed with water and then with MeOH. Recrystallization from acetone–MeOH gave 470 mg III as pale brown powder, m.p. 234° (dec). (Found: C, 59·29; H, 4·38; N, 2·62; H_2O, 3·85. $C_{52}H_{40}O_{20}N_2 \cdot 2H_2O$ requires: C, 59·54; H, 4·23; N, 2·67; H_2O, 3·44%). A dried sample is hygroscopic. (Found: C, 61·37; H, 4·08; N, 2·60. $C_{52}H_{40}O_{20}N_2$ requires: C, 61·66; H, 3·98; N, 2·77%); IR ν_{max}^{Nujol} cm^{-1}: 3514, 1755 (shoulder), 1745, 1713, 1670.

Diacetate-II (3,3'-O-*diacetyl julimycin* B-II) (V)

To a suspension of 100 mg I in 4 ml Ac_2O, 20 mg *p*-toluenesulfonic acid was added and the mixture stirred at room temp. After 2 hr the soln was poured into 40 ml water to give a crystalline acetate. Recrystallization from MeOH gave 68 mg pure V as dark red prisms, m.p. 231–235° (dec). (Found: C, 62·94; H, 4·87. $C_{42}H_{38}O_{16}$ requires: C, 63·15; H, 4·80%); UV λ_{max}^{MeOH} mμ (log ε): 233 (4·70), 462 (4·12). $Mg(OAc)_2$ colour reaction: blue.

Tetraacetate (VI) *from diacetate*-I (IV)

To a soln of 110 mg IV (prepared according to Katagiri *et al.*[1]) in one ml Ac_2O, 10 mg *p*-toluenesulfonic acid was added and the mixture allowed to stand for 3 hr at room temp. The soln was poured into 30 ml water to decompose Ac_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed 3 times with water, dried over $MgSO_4$ and evaporated. The residue was recrystallized from MeOH to give 60 mg VI as yellow plates, m.p. 225·5° (dec), whose IR spectrum was identical with that of julimycin B-II acetate-II.[1] (Found: C, 62·19; H, 4·85. $C_{46}H_{42}O_{18}$ requires: C, 62·58; H, 4·85%).

Bisanhydrojulimycin B-II (VII)

A soln of 30 mg I in one ml pyridine was heated at 90° for 2 hr. The pyridine was distilled off *in vacuo*, and the residue was recrystallized from acetone to give 17 mg VII as orange needles, m.p. 252° (dec).

* Cassiamin, a β,β'-bianthraquinonyl derivative has been isolated from a plant, *cassia siamae* Lam. (N. L. Dutta, A. C. Ghosh, P. M. Nair and K. Venkataraman, *Tetrahedron Letters* No. 40, 3023 (1964).

† All m.ps and b.ps are uncorrected.

(Found: C, 67·03; H, 4·49; M.W. (osmometry), 675. $C_{38}H_{30}O_{12}$ requires: C, 67·25; H, 4·46%; M.W., 678·62); $Mg(OAc)_2$ colour reaction: red.

Bisanhydro julimycin B-II 1,1′, 8,8′-O-*tetramethyl ether* (VIII)

To a soln of 100 mg VII in 20 ml acetone, Ag_2O (prepared from 0·85 g $AgNO_3$) and 5 ml MeI were added with stirring and the mixture refluxed for 1 hr. The inorganic compounds were filtered off and washed with $CHCl_3$. The combined solns were evaporated and the residue recrystallized from $CHCl_3$–n–hexane to afford 40 mg VIII as yellow powder, m.p. >315°. (Found: C, 68·92; H, 4·86. $C_{42}H_{38}O_{12}$ requires: C, 68·65; H, 5·21%); IR ν_{max}^{Nujol} cm^{-1}: 1727, 1672; UV λ_{max}^{dioxan} mμ (log ε): 227 (4·66), 265 (4·73), 372 (4·18).

Oxidative degradation of III

(i) *Dehydration of* III. A soln of 1·32 g III in 45 ml pyridine was heated on a steam bath for 2 hr. The reaction mixture was poured into 200 ml water, neutralized with 10% H_2SO_4 and extracted with $CHCl_3$. The $CHCl_3$ layer was dried over $MgSO_4$, chromatographed on silica gel* and eluted with $CHCl_3$–MeOH (100: 0·4–0·5). Removal of the solvent and recrystallization from $CHCl_3$–MeOH gave 1·02 g of IX as yellowish orange powder, which decomposed slowly above 195°. (Found: C, 63·85; H, 3·88; N, 2·89. $C_{52}H_{36}O_{18}N_2$ requires: C, 63·93; H, 3·71; N, 2·87%).

(ii) *Methylation of* IX. A mixture of 1·0 g IX, Ag_2O (prepared from 5·1 g $AgNO_3$), 30 ml MeI and 70 ml acetone was refluxed under stirring for 80 min. After working up as usual, the crude dimethyl ether was chromatographed on silica gel and eluted with $CHCl_3$–MeOH (100: 0·5). Removal of the solvent and purification from acetone–MeOH gave 654 mg X as yellowish green amorphous powder. (Found: C, 64·54; H, 4·21; N, 2·85. $C_{54}H_{40}O_{18}N_2$ requires: C, 64·54; H, 4·03; N, 2·79%).

(iii) *Permanganate oxidation of* X. A soln of 650 mg X in 10 ml 10% KOH–MeOH was heated on a steam bath for 10 min. After removal of the solvent the residue was dissolved in 20 ml water and treated with CO_2. The dark green ppt was collected and washed with water.

The ppt was oxidized in a soln of 20 ml 5% NaOH, 10 ml water and 5 ml pyridine at 60° with $KMnO_4$ until the colour of permanganate persisted for 1 hr. After removal of the pyridine 20 ml water was added to the residue and the mixture was reoxidized at 85–90° until the colour of permanganate was retained for 1 hr. MnO_2 was filtered off and washed with water, and the combined soln was acidified with HCl. Keeping the soln slightly acid, additional $KMnO_4$ was added to the soln at room temp till the generation of CO_2 ceased. The mixture was made alkaline with NaOH and MnO_2 was removed by filtration. The filtrate was extracted with EtOAc to remove 52 mg coloured material. The alkaline layer was neutralized with HCl and chromatographed on 100 ml Dowex 50 (× 12 proton type) and eluted with water. The water soln was evaporated to dryness *in vacuo* and the residue was extracted with a small amount of MeOH to give 50 mg crude acid, which was recrystallized from EtOAc to yield 16 mg pale yellow acid.

The acid was dissolved in 1·5 ml MeOH and the soln was refluxed with Ag_2O (prepared from 120 mg $AgNO_3$) and 1·5 ml MeI for 2 hr to afford 15 mg methyl ester as an oil, which was chromatographed on silica gel and eluted with $CHCl_3$–MeOH (100: 2·5). Removal of the solvent and recrystallization from ether with Norite gave 8 mg pure XI as colourless prisms, m.p. 128–128·5° (sintered at 103°), (m.p. 103–104° on hot stage). (Found: C, 53·18; H, 5·02. $C_{15}H_{16}O_9$ requires: C, 52·94; H, 4·74%); UV λ_{max}^{MeOH} mμ (log ε): 218 (4·51), 307 (3·53).

This ester was identical with tetramethyl 5-methoxybenzene-1,2,3,4-tetracarboxylate (XI) as mentioned below.

The synthesis of XI

(i) 1-(o-*Methoxyphenoxy*)-2,3,4,5-*tetramethylbenzene* (XIII). Compound XII was prepared from 1,2,4,5-tetramethylbenzene *via* 1,2,3,4-tetramethylbenzene according to the directions of Smith *et al.*,[9,10] and in each step, the product was purified successfully by preparative gas chromatography.

With vigorous stirring, a mixture of 2·13 g XII, 3·72 g *o*-methoxyphenol, 6·25 g finely powdered K_2CO_3,[11] 0·95 g Cu-powder and 25 ml pyridine was refluxed for 16 hr. During the reaction period, the condensed pyridine was forced to pass through an anhyd silica gel column to remove the water generated by the reaction. The reaction mixture was filtered and washed with pyridine. The residue from the pyridine soln was dissolved in ether and the soln was washed with dil HCl, then 3 times with dil NaOH aq, dried

* For the chromatography of such colouring matters, Kiesel gel (Merck) was purified to metal free according to the directions of Katagiri.[1]

over K_2CO_3, chromatographed on 12 g alumina and eluted with ether. Removal of the ether and recrystallization of the residue from MeOH gave 2·41 g (93·0%) XIII as colourless prisms, m.p. 67°. (Found: C, 79·77; H, 8·07. $C_{17}H_{20}O_2$ requires: C, 79·65; H, 7·86%).

(ii) 2,3,4,5-*Tetramethylphenol* (XIV). To a soln of metallic Li in 100 ml liquid ammonia was added slowly with stirring a soln of 1·65 g XIII in 35 ml ether at −50°. Metallic Li was added until the blue colour of the soln persisted for 1 hr. After evaporation of the solvents the residue was acidified with dil HCl and extracted with ether. The ether soln was dried over $MgSO_4$ and evaporated. The crude product was chromatographed on silica gel with $CHCl_3$ and then recrystallized from n-hexane to afford 784 mg (81%) pure XIV as colourless needles, m.p. 83·5–84°. (Found: C, 79·82; H, 9·17. $C_{10}H_{14}O$ requires: C, 79·95; H, 9·39%).

(iii) 2,3,4,5-*Tetramethylanisole* (XV). A soln of 300 mg XIV in 15 ml acetone was refluxed with 1·2 g K_2CO_3 and 1·2 ml Me_2SO_4 under stirring. After removal of the solvent, the residue was treated with 28% NH_4OH to decompose the excess Me_2SO_4 and extracted with ether. The distillation of the extract under diminished pressure gave 311 mg (95%) of XV as colourless prisms, $b.p._{11}$ 120–123° (bath temp), m.p. 33–34°. (Found: C, 80·58; H, 9·99. Calc. for $C_{11}H_{16}O$: C, 80·44; H, 9·83%); NMR δ: 6·55 (C_6-H, 1H, singlet), 3·75 (O-CH_3, 3H, singlet), 2·27 (φ-CH_3, 3H, singlet), 2·18 (φ-CH_3, 3H, singlet), 2·13 (φ-CH_3, 6H, singlet).

(iv) *Tetramethyl 5-methoxybenzene*-1,2,3,4-*tetracarboxylate* (XI). A mixture of 750 mg XV, 4 ml 5% NaOH, 30 ml water and 20 ml pyridine was gently refluxed under stirring and 4·35 g $KMnO_4$ was added portionswise. After consumption of $KMnO_4$, MnO_2 was filtered off and the filtrate was evaporated. The residue was acidified with dil HCl and extracted with ether to give 1·094 g intermediary crude tricarboxylic acid, which was dissolved in 10 ml 5% NaOH and 40 ml water and oxidized under reflux with 5·0 g $KMnO_4$ during a period of 24 hr.* MnO_2 was filtered off and the filtrate was neutralized with 10% HCl, diluted with water to about 120 ml, chromatographed on 100 ml Dowex 50 (× 12 proton type) and eluted with water. Evaporation of the soln gave 930 mg crude acid which was recrystallized from water to give two crops. Elementary analyses and IR spectra suggested that the less soluble crop (153 mg) was essentially tricarboxylic acid, while the more soluble crop (702 mg) was tetracarboxylic acid.

Methylation of the former crop and recrystallization from MeOH afforded trimethyl tricarboxylate, m.p. 108–109°. (Found: C, 56·78; H, 5·56. $C_{14}H_{16}O_7$ requires: C, 56·75; H, 5·44%).

The crude tetracarboxylic acid (370 mg) was methylated with 10 ml MeI and Ag_2O (prepared from 1·77 g $AgNO_3$) in MeOH under reflux for 1 hr. Working up as usual gave 334 mg crude ester, which showed the presence of a small amount of trimethyl tricarboxylate in the gas chromatogram. Recrystallization from MeOH and then from ether gave 170 mg pure XI as colourless prisms, m.p. 128–128·5° (sintered at 105°), (m.p. 103–104° on hot stage). (Found: C, 53·20; H, 4·74; MeO, 45·27. $C_{15}H_{16}O_9$ requires: C, 52·94; H, 4·74; MeO, 45·60%); UV λ_{max}^{MeOH} mμ (log ε): 218 (4·51), 307 (3·53); NMR δ: 7·57 (C_6-H, 1H, singlet), 3·93, 3·92, 3·89 and 3·85 (C_5-OCH_3 and 4-$COOCH_3$, 15H).

The mixed m.p. determination, gas chromatography and the comparison of IR spectra showed that this product was identical with XI derived from the natural product.

Oxidative degradation of II

To a soln of 760 mg II in 20 ml pyridine and 10 ml 10% NaOH, 6% $KMnO_4$ aq was added slowly on a steam bath until the colour of permanganate persisted for 1 hr; 72 ml of $KMnO_4$ aq was added during a period of 6 hr. After decomposition of excess $KMnO_4$ with HCHO aq, MnO_2 was filtered off, and washed with water. The combined soln was evaporated to nearly dryness *in vacuo*, acidified with dil HCl and evaporated completely *in vacuo*. The residue was extracted with acetone to give 600 mg crude acid as a brown syrup, which was refluxed with 3 ml Ac_2O for 15 min. After removal of Ac_2O the residue was chromatographed on 15 g silica gel with EtOAc. The crude anhydride (160 mg) was hydrolyzed with 10% NaOH and the soln was acidified with 10% HCl and extracted 4 times with ether. The ether extract was treated with excess ethereal diazomethane to afford an oily ester (13 mg) which was crystallized by the addition of MeOH. Recrystallization from MeOH gave 6 mg pure XVI as colourless prisms, m.p. 163–164° (on hot stage). (Found: C, 59·26; H, 4·67; MeO, 41·82. $C_{22}H_{22}O_{10}$ requires: C, 59·19; H, 4·97; MeO, 41·71%); UV λ_{max}^{MeOH} mμ (log ε): 209·5 (4·66), 268 (4·23).

* In this oxidation, it did not give a permanent colour with $KMnO_4$ presumably due to the further decomposition of the product.

This product was identical with synthetic tetramethyl 2,2'-dimethoxybiphenyl-3,3'4,4'-tetracarboxylate (XVI) as described below.

The synthesis of XVI *and* XXIV

(i) *Iodination of* 2,3-*dimethylphenol* (XVII). To a soln of 3·6 g XVII in 600 ml 5% KOH, a soln of 8·25 g I_2 and 19·5 g KI in 600 ml water at −0·5–0·5° was added dropwise with vigorous stirring. After the addition was complete, the stirring was continued for 20 min and the soln was acidified with 150 ml 10% HCl to separate a white ppt, which was extracted with ether. The ether soln was dried over $MgSO_4$ and evaporated to give 7·64 g of an oily product, which was chromatographed on 130 g silica gel and eluted with benzene and then with $CHCl_3$. The benzene eluate was distilled under diminished press to afford 1·38 g XVIII as a colourless oil, b.p_3 85°. (Found: C, 38·99; H, 3·75; I, 51·34. C_8H_9OI requires: C, 38·73; H, 3·66; I, 51·16%); NMR δ: 7·33 (C_5-H, 1H, doublet, J = 8·0 c/s), 6·48 (C_4-H, 1H, doublet, J = 8·0 c/s), 5·18 (ϕ-OH, 1H, singlet), 2·20 (ϕ-CH_3, 6H, singlet); IR $\nu_{max}^{CCl_4}$ in 20 mm cell: 3503 cm^{-1} (H-bonded OH).

The residue of this distillation was recrystallized from n-hexane to give 0·95 g 4,6-diiodo-2,3-dimethylphenol as nearly colourless needles, m.p. 84–84·5°. (Found: C, 25·97; H, 2·29; I, 67·66. $C_8H_8OI_2$ requires: C, 25·69; H, 2·16; I, 67·88%); IR $\nu_{max}^{CCl_4}$ in 20 mm cell: 3503 cm^{-1}.

The $CHCl_3$ eluate was evaporated and the recrystallization of the residue from n-hexane gave 4·12 g XXI as colourless needles, m.p. 82–83°. (Found: C, 38·84; H, 3·83; I, 51·01. C_8H_9OI requires: C, 38·73; H, 3·66; I, 51·16%); NMR δ: 7·50 (C_5-H, 1H, doublet J = 8·5 c/s), 6·37 (C_6-H, 1H, doublet J = 8·5 c/s), 4·70 (ϕ-OH, 1H, singlet), 2·40 (C_3-CH_3, 3H, singlet), 2·21 (C_2-CH_3, 3H singlet); IR $\nu_{max}^{CCl_4}$ in 20 mm cell: 3613 cm^{-1} (free OH).

(ii) 6-*Iodo*-2,3-*dimethylanisole* (XIX). To a soln of 2·07 g XVIII in 4 ml 10% NaOH and 4 ml water, 1·4 ml Me_2SO_4 was added and the mixture stirred at room temp for 2 hr. During the reaction period the mixture was kept alkaline by the addition of 10% NaOH. The excess Me_2SO_4 was decomposed with 28% NH_4OH and the mixture extracted with ether to give 1·93 g of XIX as pale yellow oil, which showed no OH band in IR spectrum and one spot in TLC. This product was used for the next step without further purification.

(iii) 4-*Iodo*-2,3-*dimethylanisole* (XXII). A soln of 2·48 g XXI in 5 ml 10% NaOH and 2·5 ml water was treated with 1·41 ml Me_2SO_4 as described above. Chromatography on alumina gave 2·49 g of XXII as a colourless oil. A small sample was crystallized from MeOH to afford colourless plates, m.p. 32–33°. (Found: C, 41·34; H, 4·25. $C_9H_{11}OI$ requires: C, 41·24; H, 4·23%).

(iv) *Dimethyl* 4-*iodo*-3-*methoxyphthalate* (XX). A mixture of 1·93 g XIX, 10 ml pyridine, one ml 10% NaOH and 35 ml water was gently refluxed and to the mixture was added 7·95 g $KMnO_4$ in 10 portions during a period of 6 hr. The mixture was acidified with 10% H_2SO_4, treated with $NaHSO_3$ to dissolve MnO_2 and extracted 3 times with ether. Evaporation of the ether soln gave 2·20 g of a mixture of mono- and dicarboxylic acid as a pale yellow solid. The acid mixture was dissolved in 8 ml 10% NaOH and 20 ml water and gently refluxed. While stirring, 2·50 g $KMnO_4$ was added to the soln in 5 portions during 3 hr. After acidification with 10% H_2SO_4, dissolution of MnO_2 with $NaHSO_3$ and saturation with NaCl, the reaction mixture was extracted with ether. Removal of the ether and recrystallization of the residue from ether–n-hexane gave 1·56 g 4-iodo-3-methoxyphthalic acid as colourless fine powder, m.p. 168–170° (dec). (Found: C, 33·73; H, 2·21; I, 39·46. $C_9H_7O_5I$ requires: C, 33·56; H, 2·19; I, 39·41%).

The acid (1·45 g) was esterified with ethereal diazomethane to yield 1·31 g of XX as a colourless oil, b.$p_{\cdot 0\cdot 02}$ 150–160° (bath temp). (Found: C, 37·86; H, 3·36; I, 36·32; MeO; 26·59. $C_{11}H_{11}O_5I$ requires: C, 37·73; H, 3·17; I, 36·25; MeO, 26·29%); NMR δ 7·93 (C_6-H, 1H, doublet J = 8·0 c/s), 7·51 (C_5-H, 1H, doublet J = 8·0 c/s), 3·97 (C_3-OCH_3, 3H, singlet), 3·88 (2-$COOCH_3$, 6H, singlet).

(v) *Dimethyl* 6-*iodo*-3-*methoxyphthalate* (XXIII). A mixture of 1·41 g XXII, 6 ml 5% NaOH, 8 ml pyridine and 10 ml water was gently refluxed and to the mixture 3·40 g $KMnO_4$ was added in 4 portions during a period of 140 min. The pyridine was removed by distillation and to the residue was added 10 ml water. The mixture was refluxed and reoxidized with additional 2·05 g $KMnO_4$ in 4 portions during 80 min.*[7] The mixture was filtered and the filtrate was acidified with 10% HCl and extracted with ether to afford 1·15 g crude acid. The acid was dissolved in 30 ml acetone and methylated with 3 g K_2CO_3 and 3 ml Me_2SO_4 by reflux for 3 hr. The mixture was filtered and the filtrate was distilled to remove the solvent and excess Me_2SO_4. The residue (750 mg) was chromatographed on 40 g silica gel and eluted with benzene and then with $CHCl_3$.

The benzene fraction gave about 200 mg colourless oil, b.p_2 160° (bath temp). The analytical data were

* See footnote * on page 1774.

in accord with the methyl ether of monocarboxylic acid. (Found: C, 39·35; H, 3·90; I, 41·54; MeO, 20·27. $C_{10}H_{11}O_3$ requires: C, 39·24; H, 3·62; I, 41·46; MeO, 20·28%).

The $CHCl_3$ fraction gave 450 mg dimethyl ester, which was recrystallized from ether–n-hexane to yield 425 mg of XXIII as colourless prisms, m.p. 80·5–81°. (Found: C, 37·85; H, 3·32; I, 35·95; MeO, 26·23. $C_{11}H_{11}O_5I$ requires: C, 37·73; H, 3·17; I, 36·25; MeO, 26·59%); NMR δ: 7·83 (C_5-H, 1H, doublet J = 9·0 c/s), 6·75 (C_4-H, 1H, doublet J = 9·0 c/s), 3·88 (C_3-OCH_3, 3H, singlet), 3·83 (2-$COOCH_3$, 6H, singlet).

(vi) *Tetramethyl 2,2'-dimethoxybiphenyl-3,3',4,4'-tetracarboxylate* (XVI). A mixture of 1·30 g XX and 1·30 g Cu-bronze[12] was heated on an oil bath and the temp kept at 220–250° for 30 min. The cooled reaction mixture was extracted with $CHCl_3$ and the extract was chromatographed on alumina with $CHCl_3$. Removal of the solvent and recrystallization from MeOH gave 778 mg of XVI as colourless plates, m.p. 165–166° (m.p. 165° on hot stage). (Found: C, 59·39; H, 5·14; MeO, 41·80. $C_{22}H_{22}O_{10}$ requires: C, 59·19; H, 4·97; MeO, 41·71%); UV λ_{max}^{MeOH} mμ (log ε): 209·5 (4·64), 268 (4·24); NMR δ: 7·83 ($C_{5,5'}$-H, 2H, doublet J = 8·0 c/s), 7·58 ($C_{6,6'}$-H, 2H, doublet J = 8·0 c/s), 3·96, 3·90 (4-$COOCH_3$, each 6H, singlet), 3·48 $C_{2,2'}$-OCH_3, 6H, singlet).

The mixed m.p. and the comparison of the IR spectra showed that this ester is identical with XVI obtained from III.

(vii) *Tetramethyl 4,4'-dimethoxybiphenyl-2,2',3,3'-tetracarboxylate* (XXIV). A mixture of 380 mg XXIII and 380 mg Cu-bronze was treated as described for XVI. Recrystallization from MeOH gave 136 mg of XXIV as colourless plates, m.p. 183–184·5° (m.p. 183–184·5° on hot stage). (Found: C, 59·36; H, 5·03; MeO, 41·66. $C_{22}H_{22}O_{10}$ requires: C, 59·19; H, 4·97; MeO, 41·71%); UV λ_{max}^{MeOH} mμ (log ε): 214 (4·64), 305 (3·83)); NMR δ: 7·23 ($C_{6,6'}$-H, 2H, doublet J = 9·0 c/s), 7·03 ($C_{5,5'}$-H, 2H, doublet J = 9·0 c/s), 3·87, 3·83 (4-$COOCH_3$, each 6H, singlet), 3·53 ($C_{4,4'}$-OCH_3, 6H, singlet).

Acknowledgements—The author is indebted to Prof. emeritus Ochiai of Tokyo University and Drs. K. Takeda and Y. K. Sawa of this Laboratory for their valuable advice. Thanks are also due to Dr. K. Tori for helpful suggestions on NMR spectra, to Dr. T. Kimura and Mr. H. Kyotani for supplying julimycin B-II and to Mr. K. Nagashima for technical assistance.

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