189. *Production of Antibiotics by Fungi. Part III. Javanicin. An Antibacterial Pigment from* Fusarium javanicum.

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The isolation and characterisation of *javanicin* is described. It is regarded as a 5 : 8-dihydroxy-6-acetonyl-1 : 4-naphthaquinone carrying additional β-methyl and β-methoxyl groupings.

It was observed (Arnstein, Cook, and Lacey, *Nature*, 1946, **157**, 333; see also Part I, Cook and Lacey, *Brit. J. Exp. Path.*, 1945, **26**, 404) that certain metabolism solutions obtained from *Fusarium javanicum* inhibited the growth of *Staphyllococcus aureus* at a dilution of 1 : 100 and were also very active against the acid-fast *Mycobacterium phlei*. In view of the possibility of the active principle or principles having a useful inhibitory action against *M. tuberculosis* their nature

was studied more closely. Their production under various conditions and some biological properties are described elsewhere (Part II, Arnstein, Cook, and Lacey, *Brit. J. Exp. Path.*, 1946, **27**, 349) and the present communication records experiments on their chemical nature.

The materials of the present study were only produced under selected conditions; the most notable requirement was the presence of " Bactotryptone " in addition to mineral salts and glucose in the fermenting solution. Bactotryptone could be replaced by some but not all similar preparations, though the characteristic essential for the production of antibacterial activity could not be distinguished. Under the selected conditions the active solutions were heavily pigmented, resembling red ink in colour. The activity could be completely removed from slightly acid solutions by extraction with solvents such as ether, and the crude recovered pigment accounted for the whole of the activity of the original solutions. After preliminary experiments had shown that the antibacterial activity was insensitive to heat, acid, alkali, and solvents, attempts were made to purify the active principles, for example, by the ordinary methods of chromatography but without success. It was found that the active principle could be removed from the crude ethereal extract by shaking with sodium carbonate but not by sodium hydrogen carbonate, and that it could be returned from aqueous sodium carbonate to ether by neutralising the solution. The final solution was still strongly coloured (red-orange) and, as inactive pigment fractions were rejected at each point, it was apparent that several probably chemically similar pigments were initially present. The active solution on concentration gave a pigment which could be crystallised. The behaviour of the crystalline material varied from batch to batch. One compound, m. p. 207·5—208° (decomp.), was invariably obtained and sometimes another compound, m. p. 213—214° (decomp.), formed a second component. Occasionally a product, m. p. 178—179°, was obtained which was analytically intermediate between the two compounds, and as the m. p. behaviour was reproduced by an equimolecular mixture of the two it seems not unlikely that the third product was a molecular compound of the other two. Both compounds were very active antibacterial agents (see Arnstein, Cook, and Lacey, *loc. cit.*) and there was no reason to doubt that the whole of the activity of the original solutions was due to these two crystalline pigments. They were very similar in character but detailed attention could be given only to the compound, m. p. 207·5—208°, which, as it does not appear to have been isolated previously, is conveniently termed *javanicin*; the compound, m. p. 213—214°, contained one oxygen atom more than javanicin and is therefore termed *oxyjavanicin*.

The pigments produced by *Fusaria* are known to be very variable and delicately dependent on the conditions of growth (Brown and Horne, *Ann. Bot.*, 1924, **38**, 379; Brown, *ibid.*, 1925, **39**, 373; Sideris, *J. Agric. Res.*, 1925, **30**, 1011); probably for this reason there have been few occasions when *Fusarium* pigments have been isolated in pure condition and studied chemically. Ashley, Hobbs, and Raistrick (*Biochem. J.*, 1937, **31**, 388) isolated two pigments, rubrofusarin and aurofusarin, from *F. culmorum*. Aurofusarin is so different from the present pigments that it can hardly be confused with them, whilst the distinctive nature of rubrofusarin is made clear below. Javanicin and oxyjavanicin differ from the earlier pigments in diffusing into and being isolated from the medium instead of from the mycelium; the mycelium of *F. javanicum* although coloured did not yield any remarkable quantity of antibacterial pigment. Solid media on which *F. javanicum* was grown also became deeply pigmented; on the other hand, those on which *F. culmorum* was grown were scarcely coloured although the mycelia were very bright (Brown and Horne, *loc. cit.*). Mull and Nord (*Arch. Biochem.*, 1944, **4**, 422) mention diffusing *Fusaria* pigments and the experiments of Sideris (*loc. cit.*) particularly clearly demonstrate their formation, so the production of javanicin and oxyjavanicin is possibly not restricted to *F. javanicum*, and other species may produce nearly related pigments.

Javanicin contained only carbon, hydrogen, and oxygen. It apparently contained no easily hydrolysed groupings and could be heated in an autoclave without significant loss of activity, though very dilute solutions on prolonged exposure to light lost both colour and antibacterial activity, presumably as a result of photochemical oxidation.

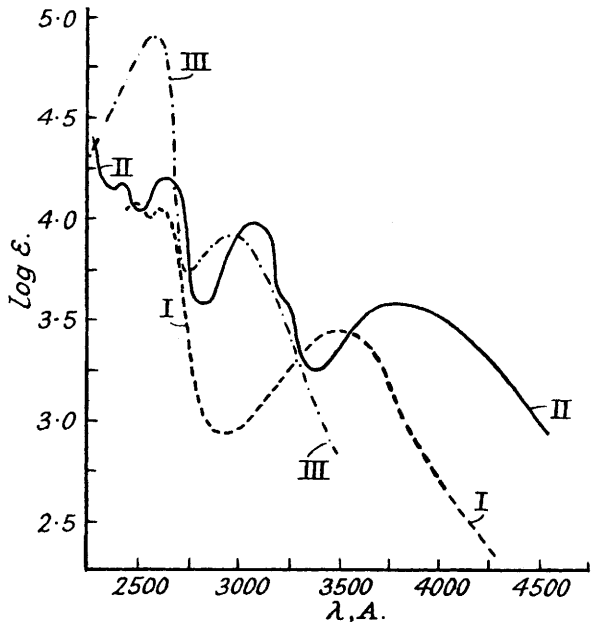
Analyses and determinations of molecular weight on different samples of javanicin from several batches indicated the formula $C_{15}H_{14}O_6$. Of the six oxygen atoms one was present as a methoxyl group. The molecule contained three active hydrogen atoms, but on acetylation in presence of concentrated sulphuric acid it yielded a yellow compound of the empirical and molecular formula, $C_{17}H_{14}O_6$, *i.e.*, a monoacetyl derivative of a hitherto unknown anhydrojavanicin. This *monoacetylanhydrojavanicin* contained one methoxyl group.

The reality of the dehydration of javanicin during acetylation was confirmed by the behaviour of the pigment itself. Javanicin showed signs of undergoing dehydration in sulphric acid solution and under other conditions, but reaction was most satisfactory with ethereal hydrogen

chloride containing zinc chloride. Under these conditions javanicin smoothly lost 1 mol. of water to give *anhydrojavanicin*, $C_{15}H_{12}O_5$, which on acetylation passed into the acetyl compound previously obtained directly. Anhydrojavanicin was chemically and physically quite distinct from the parent pigment, and contained only one active hydrogen atom in agreement with structure (III) (R = H) developed below. Two more oxygen atoms were revealed as a quinone grouping, for on reductive acetylation anhydrojavanicin was converted into a *leucoanhydrotriacetate*, $C_{21}H_{20}O_8$.

Javanicin anhydromonoacetate and leucoanhydrotriacetate showed spectra which indicated them to be α-naphthaquinone and naphthalene derivatives respectively (Fig. 1). It is known that if the hydroxyl groups of a hydroxynaphthaquinone be acylated, the spectrum loses any features peculiar to the tautomeric hydroxynaphthaquinone structure and reverts to that of a simple naphthaquinone (Macbeth, Price, and Winzor, *J.*, 1935, 325). Incidentally, consideration of the spectra of javanicin and its derivatives excluded the possibility of their being benzoquinone or anthraquinone derivatives; it was moreover impossible to accommodate a summation of other facts noted below on these formulations. Rubrofusarin, $C_{15}H_{12}O_5$, a pigment from

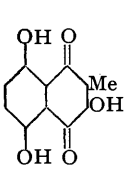
FIG. 1.



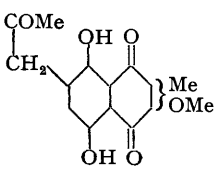
I. *Hydroxydroserone triacetate.* II. *Javanicin anhydromonoacetate.*
III. *Javanicin leucoanhydrotriacetate.*

F. culmorum (Ashley, Hobbs, and Raistrick, *loc. cit.*) appears formally closely related to javanicin, but rubofusarin behaves quite differently especially towards alkalis, with which it gives only a yellow colour, gives derivatives which are clearly distinct from those of javanicin, and is also spectrally quite distinct from javanicin (cf. Mull and Nord, *loc. cit.*). Javanicin gave a deep violet solution with 10% sodium hydroxide, purple solutions with concentrated ammonia or 2N-sodium carbonate, but no colour with sodium hydrogen carbonate. It was decolorised by bromine water or aqueous sodium hydrogen sulphite. The formulation of javanicin as a naphthaquinone derivative was supported by its colour reaction with lead acetate in methanol. Javanicin gave a violet solution; methylnaphthazarin also gave a violet solution, whilst hydroxydroserone (see below) gave a violet precipitate, and Kuhn and Wallenfels (*Ber.*, 1939, **72**, 1411) record that 5 : 8-dihydroxy- and 2 : 5 : 8-trihydroxy-naphthaquinone, echinochrome A, and its trimethyl ether all give violet solutions or precipitates. The formation of a coloured solution rather than a precipitate indicated the absence of any β-hydroxyl group (cf. Kuhn and Wallenfels, *loc. cit.*; see also below). Reaction of javanicin with diazomethane was complex, presumably because of reaction with the side chain, but anhydrojavanicin failed to react under normal conditions. As anhydrojavanicin is regarded as having the angular structure (III), and as only β-hydroxyl

groups in naphthaquinones are methylated under the normal conditions by diazomethane (Kuhn and Wallenfels, *loc. cit.*), this behaviour provides further though indirect evidence of the methoxyl group occupying a β-position. Comparison of the absorption spectrum of javanicin with that of representative hydroxynaphthaquinones revealed that its spectrum was practically



(I.)



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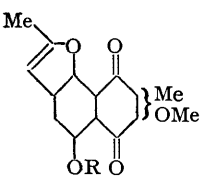
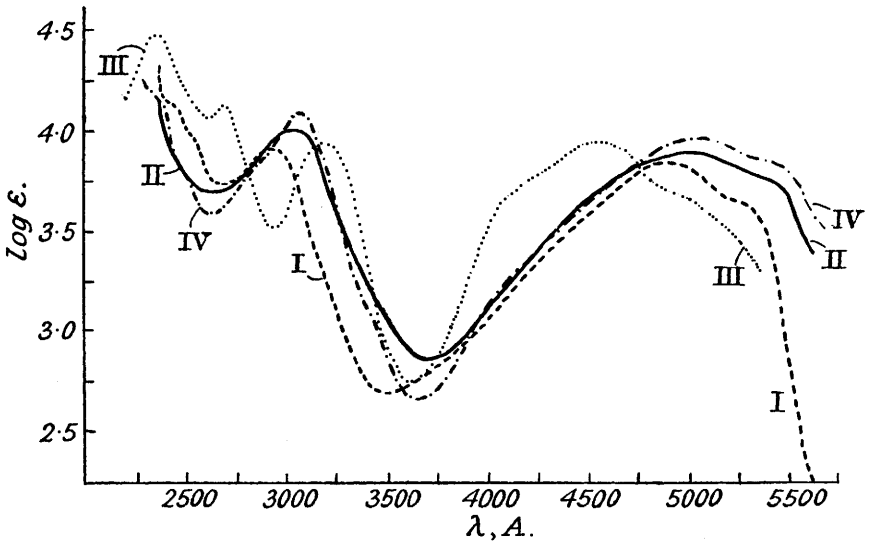


(III.)

coincident with that of hydroxydroserone (Macbeth, Price, and Winzor, *loc. cit.*) (Fig. 2) (3 : 5 : 8-trihydroxy-2-methyl-1 : 4-naphthaquinone, I) and the sequel shows that javanicin is indeed closely related structurally to hydroxydroserone, which also gives a magnificent violet colour with alkali (Rennie, *J.*, 1887, **51**, 374).

FIG. 2.



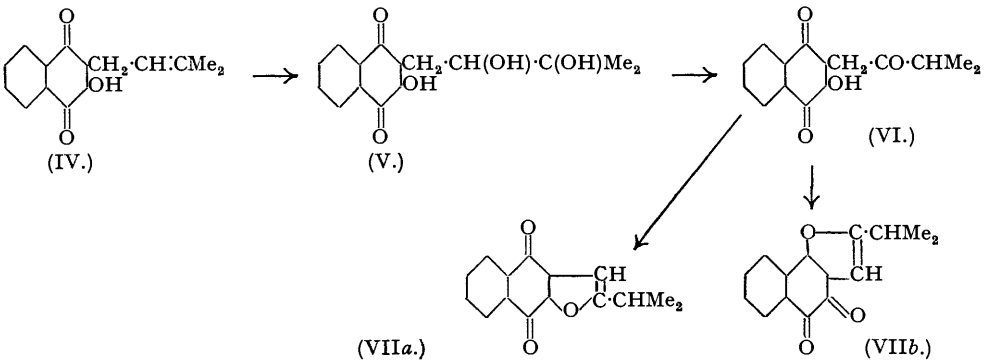
I. *Hydroxydroserone.* II. *Javanicin.* III. *Anhydrojavanicin.* IV. *Oxyjavanicin.*

These facts suggested then that javanicin was a dihydroxylated naphthaquinone carrying in addition to a β-methoxyl group a substituent which was able to undergo dehydration (cyclisation) by interaction with a neighbouring hydroxyl group. In view of the magnificent colour of javanicin in alkali and its acidity, the hydroxyls were believed to be in the *peri*-positions. Attempts were made to confirm this analytically by the formation of a boric acid complex (Macbeth and Winzor, *J.*, 1935, 334) but under conditions which precluded anhydride formation a stable complex proved unobtainable. That two hydroxyls were indeed present was shown by the behaviour of javanicin towards acylating agents under milder conditions than were used in the original experiments described above. When it was acetylated with acetic anhydride in pyridine *diacetyljavanicin*, $C_{19}H_{18}O_8$, was formed, and treatment with benzoyl chloride in pyridine afforded *dibenzoyljavanicin*, $C_{29}H_{22}O_8$. The acylation of a number of hydroxylated naphthaquinones has been described in the literature and no difficulty seems to have been encountered in fully acylating these compounds, so it was concluded that javanicin contained no more than two hydroxyl groups together with a β-methoxyl group.

On oxidation by the Kuhn–Roth method, javanicin gave two molecules of volatile fatty acid indicating two substituents, so the structure could be written in the form $C_{10}HO_2(OH)_2(OMe)RR'$, where $R + R' = C_4H_8O$. For the following reasons the four carbon atoms could only be distributed so that R contained C_1 and R′ contained C_3. The oxygen atom in the grouping C_4H_8O could not be placed on a terminal carbon atom for the side chain could not then have afforded a molecule of volatile fatty acid. If therefore there had been a

C_2,C_2 distribution the substituents would have been Et and COMe [or possibly CH(OH)Me], but these would not reasonably permit cyclisation. The remaining possibilities therefore for R, R′ are Me and CH_2·COMe, or less likely, in view of the analytical results, Me and CH_2·CH(OH)Me. When javanicin was oxidised with sodium hypoiodite or hypobromite, considerable yields of iodoform or bromoform were obtained. Moreover, when javanicin was treated with 2 : 4-dinitrophenylhydrazine a *mono*-2 : 4-*dinitrophenylhydrazone* was formed. Under the same conditions hydroxydroserone failed to react with dinitrophenylhydrazine and it was concluded that javanicin contained the substituents Me and CH_2·COMe. This conclusion was in agreement with the finding that javanicin had no observable optical activity, and that hydrogenation afforded no evidence of an ethylenic linkage. Catalytic hydrogenation resulted in rapid uptake of 1 mol. of hydrogen with disappearance of the colour, which was quickly regenerated in presence of air. From the recovered coloured solution the original pigment could be isolated, and obviously hydrogenation to this extent resulted only in formation of a leuco-compound. A second mol. of hydrogen was slowly absorbed, and further hydrogenation was very slow indeed; this stage corresponds supposedly to reduction of the ketone grouping followed by progressive reduction of the naphthalene nucleus, but no pure hydrocompounds were isolated. This suggested the partial structures (II) and (III) (R = COMe) for javanicin and its anhydromonoacetate respectively. These structures leave one unsubstituted position in the naphthalene nucleus; accordingly, after anhydrojavanicin was treated with bromine under very mild conditions a *monobromoanhydrojavanicin* was isolated. Furthermore, rather prolonged treatment of javanicin or anhydromonoacetyljavanicin with acetic anhydride gave an *acetoxy*-derivative of anhydroacetyljavanicin, $C_{19}H_{16}O_7$. Highly substituted naphthaquinones are known to undergo Thiele–Winter acetylation by this treatment, though with some difficulty.

The ready cyclodehydration of javanicin and its derivatives as pictured above is perhaps paralleled by the behaviour of lapachol (IV). Dihydroxylapachol (V) on dehydration gives, among other products, hydroxy*iso*lapachol (VI), which on further dehydration affords two products formulated by Hooker (*J.*, 1896, **69**, 1360) as (VII*a*) and (VII*b*).

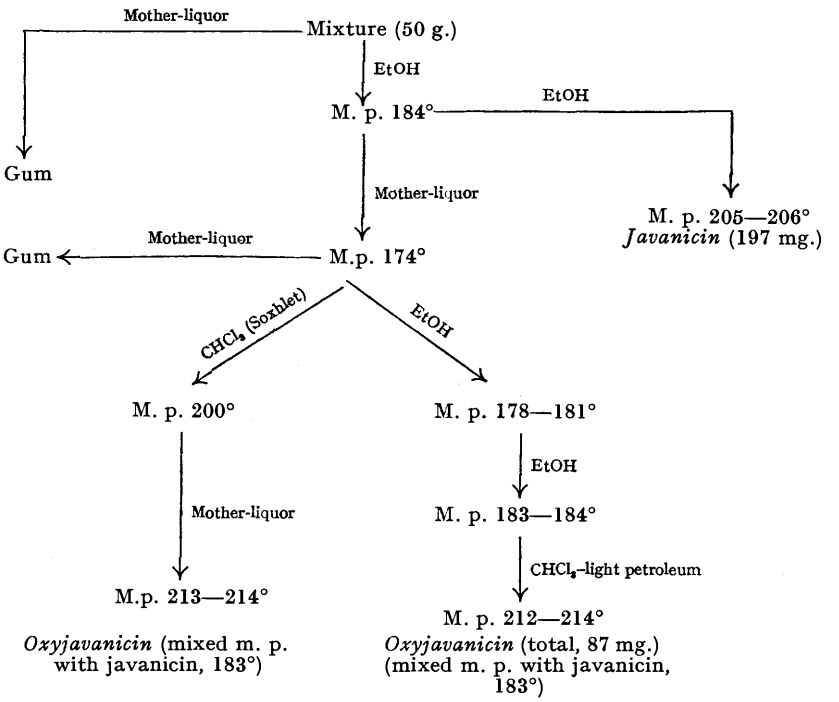


It may be pointed out that quinones of various kinds have been frequently observed to have strong antibacterial actions, and that the action of javanicin (and oxyjavanicin) is undoubtedly due in large measure to the quinone nature. Like that of other quinones, for example, its action is inhibited, though with some difficulty, by compounds containing thiol groupings. On the other hand, it seems equally certain that specific structural effects are exerted. Thus, comparison of javanicin with the nearly related hydroxydroserone showed that the activity of the latter especially towards *Myco. phlei* was surprisingly small (Arnstein, Cook, and Lacey, *Brit. J. Exp. Path.*, 1946, **27**, 349).

Experimental.

Production of Javanicin and Oxyjavanicin.—Stock slope cultures of *F. javanicum* were grown on a medium containing Kepler's malt extract (3%) and agar (2%) in 6″ × 1″ test-tubes. The mycelium of a single slope was pulped with sterile water (50 c.c.), and the suspension (2—3 c.c.) used to inoculate bottles of liquid medium. This technique was adopted to ensure even and prompt growth. The liquid medium contained: Sodium nitrate, 20 g.; potassium chloride, 5 g.; anhydrous magnesium sulphate, 2·5 g.; dipotassium hydrogen phosphate, 10 g.; ferrous sulphate heptahydrate, 0·1 g.; glucose, 400 g.; " Bactotryptone " (Difco) or " Eupepton " (No. 2) (Allen and Hanbury, Ltd.), 50 g.; distilled water to 10 l. The inorganic salts were dissolved separately in small portions of hot distilled water and added to distilled water (*ca.* 8 l.). Glucose in water (1 l.) and bactotryptone or eupepton in water (500 c.c.) were

added, and the volume made up. The medium, which was at pH 6·8—7·0 without further adjustment was distributed into quart-size milk bottles, each containing 400 c.c., furnished with cotton-wool plugs and sterilised at 15 lb. for 15 minutes. The inoculated bottles were incubated, stacked in an almost horizontal position at 25—28° for 10—14 days. The mycelium covered the surface only sparsely; it was highly pigmented (red) from the beginning and the medium became distinctly red after about 7 days. The antibiotic activity and colour of the medium increased to a maximum after about 10 days, the medium then having pH 3. All bio-assays were carried out by the serial dilution method, as plate assays were unreliable, especially on partly purified material. Before assay the solutions were adjusted to pH 7 and sterilised by heat; Seitz filtration resulted in the pigment and antibacterial principles being retained on the filter. (*a*) In a typical batch the filtered metabolism solution (11 l.), which inhibited the growth of *Staph. aureus* at a dilution of 1 : 100, was extracted twice with ether (2·5 l.) previously freed from peroxides. The ethereal solution was twice extracted with saturated aqueous sodium hydrogen carbonate, which removed a mixture of apparently at least several antibacterially inactive pigment components. The remaining ethereal solution was extracted with N-sodium carbonate (4 × 500 c.c.), which removed all the activity. By adjusting the aqueous solution to pH 7 and again extracting with ether (2 l.), the activity and pigment were returned to the organic solvent. The dried ethereal solution was evaporated to 50 c.c., whereupon most of the pigment separated; the mother-liquor yielded only a small quantity of gummy solid, which was rejected. The solid pigment (0·509 g.) had m. p. *ca.* 168° and inhibited the growth of *Staph. aureus* at a dilution of 1 : 200,000. The mixture was crystallised according to the following scheme :



(*b*) In another batch of metabolism medium (20 l.) the mycelium was filtered off, yielding 31 g. of dry material after drying at 100° for several hours. The finely powdered mycelium (20 g.) was extracted (Soxhlet) with light petroleum for 6 hours, no more red pigment then appearing to be extracted. On concentration to small volume (50 c.c.) and cooling, a small quantity of red solid (20 mg.), m. p. 200°, was obtained. One recrystallisation from hot acetone–ethanol (1 : 1) (5 c.c.) afforded pure crystalline material (laths), m. p. 207° (decomp.) alone or mixed with javanicin obtained from the filtrate (see below). (*c*) The filtrate from (*b*) (20 l.) was extracted with ether–benzene (1 : 1) (4 l.). After being extracted once with saturated aqueous sodium hydrogen carbonate solution (1 l.) and washed with water (1 l.), the organic solvent was shaken with 1% sodium hydroxide (1 l.). The deep violet-purple aqueous layer was separated, and the pigment re-extracted into benzene (1 l.) by acidifying with 10% phosphoric acid. The benzene layer was washed once with water (500 c.c.), dried, and evaporated to dryness under reduced pressure. The solid residue was extracted (Soxhlet) with acetone–ethanol (1 : 1) (50 c.c.). On cooling, almost pure javanicin, m. p. 200° (450 mg.), separated. One recrystallisation from hot acetone–ethanol (1 : 1) (50 c.c.) yielded pure javanicin, m. p. 207—208° (decomp.). Method (*c*) was more convenient than (*a*) and gave consistent yields provided spore suspensions had been used for inoculating the medium. When inoculation was carried out by using a platinum loop, the yields were very variable, as shown below :

Vol. of filtered metabolism solution (l.)		20	20	20	40
Yield of pure pigment {	Mg.	430	325	433	950
	Mg./l.	21·5	16·3	21·7	23·8

Javanicin when crystallised repeatedly from ethanol separated in red laths with a coppery lustre, m. p. 207·5—208° (decomp.). Analyses were carried out on different samples from several batches [Found: C, 62·1, 62·9, 61·8, 62·0, 62·4, 61·7; H, 4·95, 5·11, 4·92, 5·04, 5·11, 4·90. OMe, 10·4; side-chain Me (Kuhn–Roth), 9·2; active H, 0·98. $C_{15}H_{14}O_6$ requires C, 62·1; H, 4·86; 1 OMe, 10·7; 2 Me, 10·3; 3 H, 1·03%]. Light absorption in ethanol; $\lambda_{max.}$ 3030, 5050 A., log ε 3·97, 3·90; in chloroform, λ_{max} 3070, 5100 A., log ε 3·99, 3·86. Optical activity: A solution of javanicin (24·5 mg.) in acetone (50 ml.) in a 1-dm. tube showed no observable rotation.

Oxyjavanicin separated from chloroform or chloroform–light petroleum in clusters of red needles with a coppery lustre, m. p. 213—214° (decomp.; preheated bath) (Found: C, 58·6, 58·5; H, 4·65, 4·60; OMe, 9·9; side-chain Me, 5·3; active H, 0·79. $C_{15}H_{14}O_7$ requires C, 58·5; H, 4·61; OMe, 10·1; 1Me, 5·56; 2H, 0·66%). Light absorption: in chloroform, $\lambda_{max.}$ 3030, 5050 A., log ε 4·08, 3·96.

Monoacetylanhydrojavanicin.—(a) *From javanicin.* Javanicin (38 mg.) was dissolved in redistilled acetic anhydride (2·5 c.c.), and a trace of concentrated sulphuric acid added. There was an immediate darkening, and the mixture was heated at 100° for a few seconds till most of the red colour had disappeared, then poured into ice-cold distilled water (10 c.c.) and set aside for ½ hour; a yellow solid separated (yield, after drying in a vacuum, 26·3 mg.), m. p. 229—238° (decomp.). After two recrystallisations from ethanol the product formed long needles, m. p. 247—248° (decomp.) [Found, on different samples: C, 64·3, 65·05; H, 4·7, 4·6; OMe, 8·2, 9·3; *M* (cryoscopic in camphor), 317. $C_{16}H_{11}O_5(OMe)$ requires C, 64·9; H, 4·6, OMe, 9·85%; *M*, 314]. Light absorption: in ethanol, $\lambda_{max.}$ 2420, 2650, 3080, 3730 A., log ε 4·16, 4·20, 3·97, 3·59.

(b) *From anhydrojavanicin.* Anhydrojavanicin (29 mg.) was suspended in acetic anhydride (1 c.c.) containing a trace of concentrated sulphuric acid. On heating to 100° the colour changed to yellow. After 2 minutes the solution was poured on crushed ice (5 g.) and set aside for ½ hour, the yellow product separating in clusters of needles (28 mg.). After one crystallisation from ethanol, the m. p. was 249—250° (decomp.) alone and when mixed with javanicin anhydromonoacetate obtained above.

Anhydrojavanicin.—Javanicin (200 mg.) was suspended in dry ether (250 c.c.). Saturated ethereal hydrogen chloride (250 c.c.) was added, followed by anhydrous zinc chloride (0·5 g.). The mixture was shaken in a stoppered bottle for 3 hours, the colour having then changed to orange. The ethereal solution was washed several times with water till free from acid. The aqueous layer was washed with benzene (3 × 150 c.c.) to remove all the pigment. The benzene layer was combined with the ethereal solution, again washed with water (2 × 100 c.c.), and dried (Na_2SO_4). After removal of solvent, the solid residue was crystallised twice from acetone, from which it separated in clusters of needles, m. p. 244—245° (decomp.) (Found: C, 66·4; H, 4·50; active H, 0·53. $C_{15}H_{12}O_5$ requires C, 66·2; H, 4·42; one active H, 0·37%). Light absorption: in ethanol, $\lambda_{max.}$ 2300, 2640, 3160, 4500 A., log ε 4·49, 4·12, 3·95, 3·95. Javanicin (17 mg.) and concentrated sulphuric acid (1 c.c.) were heated together at 100° for 90 seconds. The solution was poured on crushed ice (10 g.), and a brown solid separated (20 mg.). The solid gave a violet coloration with 10% sodium hydroxide. It was dissolved in ethanol and reprecipitated by adding water, and then had m. p. 229—233° (decomp.). Repetition of this treatment yielded 1·3 mg. of a product which showed the following light absorption, in ethanol: $\lambda_{max.}$ 2350, 3160, 3240, 4560 A.; log ε (calculated for *M* = 284) 4·61, 3·92, 3·92, 3·91.

Javanicin Leucoanhydrotriacetate.—Anhydrojavanicin (250 mg.) in pyridine (20 c.c.) was warmed with acetic anhydride (2·5 c.c.). Zinc dust (1 g.) was added, and the reaction allowed to proceed for 5 minutes, the supernatant liquid then having become pale yellow. The solution was filtered and evaporated to small bulk. After a short time an almost colourless solid separated in rhombic prisms, m. p. 256—257° (decomp.) (321 mg., 87%). One recrystallisation from acetone–benzene afforded the pure *leucoanhydrotriacetate*, m. p. 258° (decomp.) (Found: C, 63·05; H, 5·10; CH_3·CO, 34·1. $C_{21}H_{20}O_8$ requires C, 63·0; H, 5·0; CH_3·CO, 32·25%). Light absorption in dioxan: $\lambda_{max.}$ 2610 A., log ε 4·92. Optical activity: A solution of 17·3 mg. in 2 ml. of chloroform in a 1-dm. tube showed no observable rotation.

Diacetyljavanicin.—Javanicin (50 mg.) was dissolved in pyridine (3 c.c.) and mixed with acetic anhydride (0·2 c.c.) in pyridine (2 c.c.). The solution was heated at 70° for 1 hour and kept at room temperature for a further 48 hours. After concentration to 1 c.c., a little ice was added which precipitated some gummy material. The supernatant liquid was decanted and further diluted with water (10 c.c.) and a yellow solid separated on cooling. The solid was crystallised from ethanol containing a little acetone. A second crystallisation from aqueous acetone yielded the pure *compound* (6·5 mg.) in clusters of needles, m. p. 207—208° (decomp.) (Found: C, 61·05; H, 4·95. $C_{19}H_{18}O_8$ requires C, 61·0; H, 4·81%). Light absorption, in ethanol: $\lambda_{max.}$ 2210, 2900, 4260 A., log ε 4·57, 4·17, 3·79.

Dibenzoyl Javanicin.—Javanicin (70 mg.), dissolved in pyridine (4 c.c.), was treated with benzoyl chloride (250 mg.). After about 10 minutes the red colour of the solution had changed to yellow. After a further 16 hours at room temperature, the solution was evaporated to dryness and the solid residue dissolved in ether–benzene (1 : 1) (25 c.c.). The solution was well washed with dilute hydrochloric acid (3 × 20 c.c.), followed by saturated aqueous sodium hydrogen carbonate (3 × 20 c.c.) and distilled water (20 c.c.). After drying (Na_2SO_4) the solvent was evaporated. The yellow residue was dissolved in ethanol (5 c.c.). On dilution with water (15 c.c.) some crystallisation took place, but most of the material was precipitated as a gum. On washing with cold ethanol, a pale yellow solid (47 mg.) was obtained. The *product* crystallised from hot ethanol–acetone (1 : 1) in bunches of needles, m. p. 216—217° (decomp.; preheated bath) (Found: C, 69·8; H, 4·41. $C_{29}H_{22}O_8$ requires C, 69·9; H, 4·42%). Light absorption in ethanol: $\lambda_{max.}$ 2290, 2600, 3650 A., log ε 4·63, 4·44, 3·52. Optical activity: A solution of 6·7 mg. in 2 ml. of chloroform in a 1-dm. tube showed no observable rotation.

Javanicin (29 mg.) was dissolved in N-sodium hydroxide (5 c.c.) and treated with sufficient iodine in potassium iodide to decolourise the solution completely. The solution had a strong smell of iodoform, and after standing at 0° overnight the precipitated iodoform was collected and dried (P_2O_5); yield 12·6 mg., m. p. 121—122° (decomp.) alone and when mixed with authentic iodoform.

Javanicin Mono-2 : 4-dinitrophenylhydrazone.—A hot solution of javanicin (25 mg.) in ethanol (3 c.c. and ethyl acetate (1 c.c.) was added to a cold solution of 2 : 4-dinitrophenylhydrazine, prepared by warming 2 : 4-dinitrophenylhydrazine (50 mg., 3 equivs.) with concentrated sulphuric acid (0·1 c.c.) till completely

dissolved, and diluting the cold solution with ethanol (1 c.c.). A red solid was immediately precipitated. The solution was warmed (100°) for a few seconds and cooled in ice. The solid was filtered off; yield 34 mg. (82%), m. p. 252—254° (decomp.; darkening at 249°). Recrystallisation from pyridine by adding ethanol yielded pure *javanicin mono-2 : 4-dinitrophenylhydrazone*, separating in needles, m. p. 255—256° (decomp.) (Found: C, 54·2, 53·4; H, 3·9, 3·7; N, 11·9. $C_{21}H_{18}O_9N_4$ requires C, 53·6; H, 3·83; N, 11·9%).

Monobromoanhydrojavanicin.—Anhydrojavanicin (50 mg.) was dissolved in chloroform (10 c.c.) and treated with excess of bromine in chloroform. The mixture was kept for 20 minutes at room temperature and evaporated to dryness. After being washed with a little ethanol, the residual solid was crystallised from benzene, from which it separated as tapered laths, m. p. 259—260° (decomp.) (yield 14 mg.). One recrystallisation from benzene–ethanol gave the pure *product*, m. p. 259—260° (decomp.) (Found: C, 51·35; H, 3·76. $C_{15}H_{11}O_5Br$ requires C, 51·3; H, 3·14%).

Acetoxyanhydroacetyljavanicin.—Javanicin (50 mg.) was refluxed for 15 minutes with acetic anhydride (5 c.c.) and anhydrous sodium acetate (50 mg.). The solution was poured on crushed ice, and the yellow solid filtered off after ½ hour. The solid was dissolved in acetone and reprecipitated with water; yield 14 mg. One recrystallisation from acetone–ethanol afforded the pure *compound*, m. p. 265° (extensive decomp.; darkening at 250°) (Found: C, 63·6; H, 4·8. $C_{19}H_{16}O_7$ requires C, 64·0; H, 4·5%).

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