167. *The Structures of Aurantio- and Rubro-gliocladin and Gliorosein.*

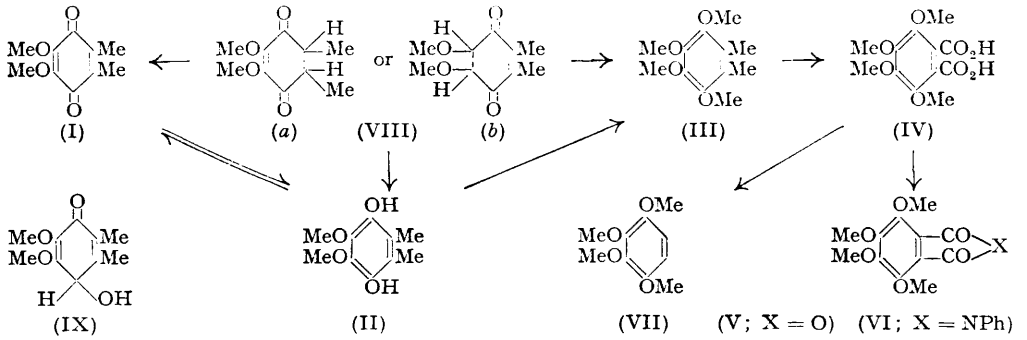
By E. B. Vischer.

The structures of the three antibiotics aurantio- and rubro-gliocladin and gliorosein, produced by a *Gliocladium* species (Brian *et al.*, *Experientia*, 1951, **7**, 266), have been determined as 2 : 3-dimethoxy-5 : 6-dimethyl-*p*-benzoquinone, the corresponding quinhydrone, and a dihydro-derivative of the former, respectively.

Brian, Curtis, Howland, Jefferys, and Raudnitz (*Experientia*, 1951, **7**, 266) isolated three new antibiotics, aurantiogliocladin, rubrogliocladin, and gliorosein, from a species of *Gliocladium*. The first named was shown to be a substituted *p*-benzoquinone $C_8H_6O_2(OMe)_2$. Rubrogliocladin was identified as the corresponding quinhydrone. Each was reduced by sulphur dioxide to the same quinol, and each gave the same dinitrophenylhydrazone. Gliorosein was isomeric with the quinol but showed a different ultraviolet absorption spectrum. This paper describes the continuation of this work and the exact structures of these antibiotics.

Aurantiogliocladin did not react with *o*-phenylenediamine and must therefore be a *p*-benzoquinone. Reduction with sulphurous acid, zinc and hydrochloric acid, hydrogen in the presence of Raney nickel, or, best, aqueous sodium dithionite (Baker, *J.*, 1941, 662) yielded the corresponding quinol (II), characterised as its bis-*p*-nitrobenzoate. The quinol reduced cold Fehling's solution and immediately decolorised 2 : 6-dichlorophenol-indophenol. On storage at room temperature, in the solid state or in solution, it gradually became red and formed the quinhydrone rubrogliocladin. This ready oxidation, which is characteristic of highly substituted *p*-benzoquinols (Erdtman, *Svensk Kem. Tidskr.*, 1932, **44**, 135; *Proc. Roy. Soc.*, 1934, *A*, **143**, 177), precluded its ready purification by recrystallisation. With methyl sulphate and alkali, under nitrogen or with addition of sodium dithionite (Baker, *loc. cit.*), the quinol yielded an oily tetramethyl ether (III), oxidised by permanganate at 90° to a tetramethoxy-dicarboxylic acid, $C_{12}H_{14}O_8$, as sole

product, m. p. 178—180°. At 180° this formed an anhydride, suggesting that the carboxyl groups were *ortho* and that the acid was thus tetramethoxyphthalic acid (IV) (which has not hitherto been described). Confirmatory evidence was: (1) The anhydride (V) gave a positive fluorescein reaction; (2) the acid formed a phthalanil (VI); (3) whereas the infra-red absorption spectrum of the acid showed an OH stretching band at 2600 and a carbonyl stretching frequency at 1704 cm.$^{-1}$, the anhydride showed only typical anhydride carbonyl bands at 1835 and 1771 cm.$^{-1}$; (4) decarboxylation of the acid by copper chromite



in quinoline at 175°, followed by re-methylation, yielded 1 : 2 : 3 : 4-tetramethoxybenzene, identified by comparison with an authentic specimen.

Accordingly, aurantiogliocladin must be 2 : 3-dimethoxy-5 : 6-dimethyl-*p*-benzoquinone (I) and rubrogliocladin the corresponding quinhydrone.

Spectrophotometric work confirmed this structure. Aurantiogliocladin shows an intense absorption band in the ultra-violet at 275 mμ (log ε 4·19; Fig. 1, curve I), whereas the absorption curve of the closely related 3 : 4-dimethoxy-2 : 5-toluquinone (Anslow, Ashley, and Raistrick, *J.*, 1938, 439) (Fig. 1, curve II) shows a maximum at 265 mμ (log ε 4·15). This shift of the maxima by about 10 mμ towards longer wave-length with increasing number of side chains was also observed by Braude (*J.*, 1945, 490) who found a maximum for toluquinone at 245 and for xyloquinone at 250—256 mμ.

The infra-red absorption spectrum shows no absorption attributable to hydroxyl groups in the region of 3 μ. It is very similar in the double-bond stretching region to that of the 3 : 4-dimethoxy-2 : 5-toluquinone. Thus aurantiogliocladin shows two strong bands at 1660 and 1615 cm.$^{-1}$ and the dimethoxytoluquinone corresponding bands at 1668 and 1615 cm.$^{-1}$. The quinol (II) shows no strong bands in the region of double-bond absorption, but has a strong hydroxyl absorption band at 3400 cm.$^{-1}$.

The final proof was given by Prof. W. Baker, who synthesised the 2 : 3-dimethoxy-5 : 6-dimethyl-*p*-benzoquinone (following paper) and found it to be identical with natural aurantiogliocladin.

Gliorosein usually formed about one-fifth of the total crystalline product obtained after extraction, but this yield varied considerably. In later experiments the organism had ceased to produce it and only aurantiogliocladin was present in the filtrates. For the last experiments the substance was therefore available only in minute quantities.

Gliorosein and the isomeric quinol (II) differed in melting point and ultra-violet absorption spectra, and gliorosein did not form the quinhydrone rubrogliocladin when recrystallised with an equal amount of aurantiogliocladin. It showed a positive reaction with tetranitromethane, indicating the presence of an olefinic double bond. It did not decolorise a solution of 2 : 6-dichlorophenolindophenol and was not oxidised by ferric chloride. It was optically inactive. On the other hand, gliorosein and the quinol (II) were closely related in that both reduced Fehling's solution instantaneously at room temperature and formed the same bis-*p*-nitrobenzoate in pyridine. Moreover, methylation of gliorosein with methyl sulphate and alkali yielded the oily tetramethyl ether (III), which was characterised by oxidation with potassium permanganate to tetramethoxyphthalic acid (IV). This relationship was explained when it was found that gliorosein rearranged

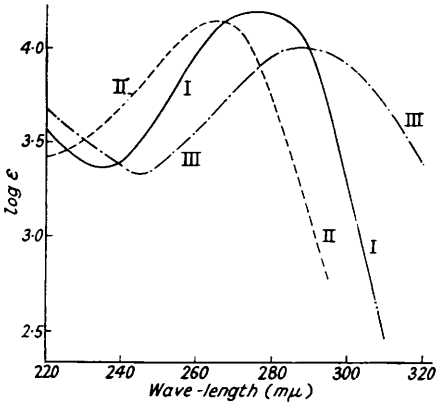
to the quinol (II) as soon as it came in contact with alkali or pyridine. Usually, owing to autoxidation, a mixture of the quinol and the quinhydrone (rubrogliocladin) was obtained, from which the pure quinone (aurantiogliocladin) and the quinol (II) could be obtained by oxidation with ferric chloride and reduction with sodium dithionite respectively.

Gliorosein and chromium trioxide in acetic acid at 100° (not at room temperature) yielded aurantiogliocladin quantitatively with consumption of one equivalent of oxygen.

The molecular weight confirmed the formula $C_{10}H_{14}O_4$. Formulæ (VIII*a* or *b*) and (IX) were considered for gliorosein. (IX) is less probable on chemical evidence, since all attempts to show the presence of a hydroxyl group in gliorosein, for instance by reaction with α-naphthyl *iso*cyanate, failed. Spectrophotometric work also favoured strongly formula (VIII). The ultra-violet spectra of aurantiogliocladin and gliorosein (Fig. 1, curves I and III) on one hand and of *p*-benzoquinone and *p*-benzoquinone dibromide (5 : 6-dibromo*cyclo*hex-2-ene-1 : 4-dione) (Fig. 2) on the other were compared. The disappearance of one double bond in *p*-benzoquinone causes a bathochromic shift of 12 mμ and a slight decrease in intensity. Similarly the change of aurantiogliocladin into

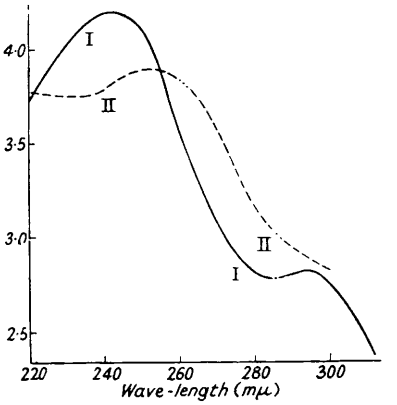
Ultra-violet absorption spectra, for ethanolic solutions.

FIG. 1.



I, Aurantiogliocladin. II, 3 : 4-*Dimethoxy*-2 : 5-*toluquinone. III, Gliorosein.*

FIG. 2.



I, p-*Benzoquinone. II,* 5 : 6-*Dibromo*-cyclo*hex-2-ene*-1 : 4-*dione.*

gliorosein caused a shift of 14 mμ accompanied by a decrease in intensity. In the infra-red spectrum of gliorosein there is a strong band at 1683 cm.$^{-1}$ indicating the presence of at least one carbonyl group in the molecule. The frequency of this band suggests that the group responsible for it is part of a conjugated unsaturated system, this conclusion being supported by the occurrence of a band at 1610 cm.$^{-1}$ which is probably due to a C=C bond conjugated to the carbonyl group. Here also formula (VIII) is favoured. For example, Flett (*J.*, 1948, 1447) has shown that oxanthrones have absorption bands near 3450 cm.$^{-1}$ in the solid state and near 3650 cm.$^{-1}$ in carbon tetrachloride solution due to their hydroxyl groups. Gliorosein has no band in this region. We regard this evidence as sufficient for proposing formula (VIII) for gliorosein.

p-Benzoquinones are well-known metabolic products of fungi (see Hoffmann-Osterhof, " Progress in the Chemistry of Organic Natural Products," Vienna, 1950, Vol. VI, p. 154). Most of them contain one methyl group and one or several hydroxyl or methoxyl groups, *e.g.*, spinulosin and fumigatin (Anslow and Raistrick, *Biochem. J.*, 1938, **32**, 687) to which aurantiogliocladin is very closely related. However, no *p*-benzoquinone with two methyl groups has hitherto been described, except for substances such as phoenicin (Posternak, *Helv. Chim. Acta*, 1938, **21**, 1326), oosporein (Kögl *et al.*, *Rec. Trav. chim.*, 1944, **63**, 5), and polyporic acid (Kögl, *Annalen*, 1926, **447**, 78), in which phenyl substituents can be regarded as " virtual " methyl groups. But in these cases the two virtual methyl groups are in the *p*-position to each other. It is not clear whether gliorosein is an artifact or a natural metabolic product; in the latter case, it might prove to be an interesting intermediate in

the formation of a benzene ring from glucose, especially in view of the easy rearrangement of this partially saturated *p*-benzoquinone to the normal quinol.

Experimental

All m. p.s were taken on a Kofler block and are corrected. Microanalyses are by Mr. W. Brown of these laboratories. The antibiotics were isolated as described by Brian *et al.* (*loc. cit.*).

2 : 3-*Dimethoxy*-5 : 6-*dimethylquinol* (II).—(*a*) Zinc dust was added in small portions to a mechanically stirred solution of aurantio- and rubro-gliocladin (6 g.) in dilute hydrochloric acid (5%; 500 ml.). Within 15 minutes the solution became colourless. It was extracted with ether. The washed and dried extract was evaporated to dryness. Crystallisation of the oily residue from light petroleum (b. p. 60—80°) gave the quinol (II) as colourless prisms, m. p. 84° (1·5 g.). The mother-liquor gradually became coloured and crystals (2·5 g.) of aurantio- and rubro-gliocladin separated out. These were again reduced in the same way to give more of the quinol.

(*b*) Rubrogliocladin (2·4 g.) and sodium dithionite (15 g.) were dissolved quickly in boiling water (50 ml.). On shaking, the orange solution became pale yellow within a few minutes. It was cooled quickly. An almost colourless solid (2·3 g.) separated out, which was filtered off and washed with a 0·5% solution of sodium dithionite. This crude quinol (II) was used for methylation without further purification. When recrystallised from light petroleum (b. p. 60—80°) it formed colourless prisms, m. p. 84°.

A solution of the quinol (17 mg.) and freshly distilled *p*-nitrobenzoyl chloride (34 mg.) in dry pyridine (0·5 ml.) was heated on a steam-bath for 1 hour with exclusion of moisture. 5% Sodium hydrogen carbonate solution (1 ml.) was added slowly to the cool reaction mixture. The pale yellow crystalline precipitate (35 mg.) crystallised from chloroform–ethanol (1 : 2) as almost colourless needles, m. p. 261—262°, of the *di*-p-*nitrobenzoate* (Found: C, 58·2; H, 4·25; N, 5·8. $C_{24}H_{20}O_{10}N_2$ requires C, 58·1; H, 4·1; N, 5·6%).

To the quinol (110 mg.) in methanol (3 ml.), ferric chloride (150 mg.) in N-hydrochloric acid (2·5 ml.) was added. The mixture became red instantaneously. It was kept at 35° for 5 minutes and the methanol removed *in vacuo*. The residue on crystallisation from light petroleum (b. p. 40—60°) gave aurantiogliocladin (86 mg.), m. p. and mixed m. p. 63—64°.

Tetramethoxy-o-*xylene* (III).—The crude quinol (II) (2·3 g.) and sodium dithionite (0·14 g.) in ethanol (5 ml.) were treated with methyl sulphate (7·4 ml.), under nitrogen, then with sodium hydroxide (2·8 g.) in water (7 ml.) during 2 hours, and heated on a steam-bath for 1½ hours. The mixture was cooled, diluted with water (20 ml.), and extracted with ether. The extract was washed with 2N-sodium hydroxide and water. After removal of the solvent a pale yellow oily *ether* (2·15 g.) was obtained which distilled almost quantitatively at 80°/0·5 mm. It was redistilled twice for analysis [Found: C, 64·0; H, 8·1; OMe, 48·8. $C_8H_6(OMe)_4$ requires C, 63·7; H, 8·0; OMe, 54·8%].

Tetramethoxyphthalic Acid (IV).—The tetramethyl ether (III) (5·8 g.), suspended in 2N-sodium hydroxide (60 ml.), was heated on a steam-bath in nitrogen and a 5% solution of potassium permanganate added slowly, with instantaneous decolorisation. The reaction became slow after uptake of 320 ml. and was complete after a total addition of 570 ml. Excess of permanganate was destroyed by a few drops of methanol. The manganese dioxide was filtered off and twice extracted with boiling water (50 ml.). The combined filtrates were neutralised and concentrated under reduced pressure to a small volume. The residue was made alkaline and extracted with ether; the extract contained only traces of an oil. The aqueous solution was acidified and extracted with ether (5 × 100 ml.). The extracts were washed until neutral, then dried, and the solvent was removed. *Tetramethoxyphthalic acid* was obtained as a colourless solid (3·5 g.) which crystallised from acetone–light petroleum (b. p. 40—60°) in prisms, m. p. 178—180° (after resetting at 135—136°) [Found: C, 50·2; H, 4·9; OMe, 41·8%; equiv., 142, 144. $C_6(OMe)_4(CO_2H)_2$ requires C, 50·35; H, 4·9; OMe, 43·4%; equiv., 143].

The acid (100 mg.) was sublimed at 170°/0·05 mm. The sublimate crystallised from light petroleum (b. p. 60—80°) in needles, m. p. 136—137° [Found: C, 53·8, 53·7; H, 4·5, 4·5; OMe, 46·1. $C_8O_3(OMe)_4$ requires C, 53·7; H, 4·5; OMe, 46·3%]. The *anhydride* (50 mg.) was suspended in 2N-sodium hydroxide (10 ml.) and heated at 50°, with gradual dissolution. The solution was acidified after 2 hours and extracted with ether. The extract was washed with sodium hydrogen carbonate solution and on evaporation left only a trace of colourless solid. The sodium hydrogen carbonate washings were acidified and extracted with ether. After evaporation of the washed extract, crystalline tetramethoxyphthalic acid was obtained [from acetone–light petroleum (b. p. 40—60°)], m. p. 178—180° (after resetting at 135—137°). The

mixed m. p. with the original acid (IV) was not depressed. The anhydride gave a positive fluorescein test.

Tetramethoxyphthalic acid (95 mg.) was boiled under reflux with aniline (0·6 ml.) for 15 minutes. Hot 50% ethanol (10 ml.) was added. On cooling, the *phthalanil* (VI) crystallised in needles, m. p. 124—125° (after two recrystallisations from 50% ethanol) (Found: C, 62·9; H, 4·9; N, 4·3. $C_{18}H_{17}O_6N$ requires C, 63·0; H, 5·0; N, 4·1%).

1 : 2 : 3 : 4-*Tetramethoxybenzene* (VII).—Copper chromite (0·5 g.) and tetramethoxyphthalic acid (1·5 g.) in quinoline (30 ml.) were heated under reflux at 175° (bath-temp.). Carbon dioxide was given off and was collected in a solution of barium hydroxide by means of a stream of nitrogen. After 50 minutes the hot reaction mixture was poured into 2N-hydrochloric acid (100 ml.). The brown solution was extracted with ether (4 × 100 ml.). The extract was washed with 2N-hydrochloric acid, 2N-sodium hydroxide (3 × 50 ml., see below), and water. After removal of solvent, the residual yellow oil (136 mg.) was distilled under reduced pressure at 110° (bath-temp.). It did not crystallise. The sodium hydroxide washings of the ether extract were acidified and extracted with ether. After evaporation a yellow phenolic solid (702 mg.) which gave a blue ferric chloride reaction was obtained. It was dissolved in ethanol (2 ml.) and methylated with methyl sulphate (2·5 ml.) and sodium hydroxide (1·5 g.) as previously described. A yellow oil (320 mg.) was obtained which was fractionally distilled under reduced pressure. The main fraction (b. p. 120°, bath-temp.) crystallised in colourless needles [from light petroleum (b. p. 40—60°)], m. p. 87—87·5° after three recrystallisations. The mixed m. p. with authentic 1 : 2 : 3 : 4-tetramethoxybenzene, m. p. 87—88°, was unchanged.

Gliorosein.—In addition to the microanalytical data supplied by Brian *et al.* (*loc. cit.*) the molecular weight of gliorosein was determined (Rast) (Found: 188. $C_{10}H_{14}O_4$ requires *M*, 198).

Di-p-*nitrobenzoate*. Gliorosein (350 mg.) and freshly distilled *p*-nitrobenzoyl chloride (400 mg.) in pyridine (1 ml.) were heated on a steam-bath for one hour. The reaction mixture was treated as described in a previous experiment. The ester formed pale yellow needles [from chloroform–ethanol (1 : 2)], m. p. 261—262° (Found: C, 58·0; H, 4·0; N, 5·7%). The mixed m. p. with the same derivative of the quinol (II) was unchanged.

0·1N-Sodium hydroxide was added to gliorosein (14 mg.) suspended in water (0·1 ml.). The solution instantaneously became yellow and after 3 minutes red, dissolution being complete. It was acidified with 2N-hydrochloric acid (0·05 ml.). On addition of 2% ferric chloride solution (0·1 ml.) orange needles of the quinone were formed (8 mg.), m. p. 62—63° after recrystallisation from light petroleum (b. p. 40—60°). The mixed m. p. with aurantiogliocladin was unchanged.

A solution of gliorosein (300 mg.) in pyridine (2 ml.) was heated on a steam-bath for 2 hours. The pale yellow solution became dark red. The solvent was removed *in vacuo* and the remaining dark oil extracted with light petroleum (b. p. 60—80°). Concentration of the extract afforded orange-red crystals (170 mg.), m. p. 45—66°. An aliquot (68 mg.) in methanol (1 ml.) on treatment with 10% ferric chloride solution (1·5 ml.) at 35° for 10 minutes yielded orange crystals, m. p. 62—63° after recrystallisation from light petroleum (b. p. 60—80°). A mixed m. p. determination with aurantiogliocladin showed no depression. The remainder (90 mg.) and sodium dithionite (300 mg.) were dissolved in hot water (1 ml.), shaken, and cooled. Colourless crystals (45 mg.) separated, of m. p. 81—82° after recrystallisation from light petroleum (b. p. 60—80°), mixed m. p. with the quinol (II), 81—83°.

A solution of gliorosein (100 mg.) in acetic acid (6 ml.) was treated gradually on a steam-bath with chromium trioxide (33 mg., 1 equiv. of oxygen) in 50% acetic acid (10 ml.). After 2 hours the mixture was concentrated under reduced pressure, diluted with water (10 ml.), and extracted with ether. The extract was washed, dried, and evaporated. The residual orange oil (91 mg.), on treatment with water, afforded aurantiogliocladin as needles, m. p. and mixed m. p. 63—64° after recrystallisation from light petroleum (b. p. 60—80°).

A solution of gliorosein (1·5 g.) in ethanol (3·5 ml.) was methylated with methyl sulphate (5·2 ml.) and sodium hydroxide (2·5 g.) as previously described. 1·6 G. of the tetramethyl ether (III), b. p. 78° (bath-temp.) were obtained. This was suspended in 2N-sodium hydroxide (16 ml.) and oxidised with potassium permanganate solution (170 ml. of 5%) as described earlier. 1·1 G. of tetramethoxyphthalic acid were obtained, m. p. 174—178° (after resetting at 135—136°). The mixed m. p. with the acid described earlier was 176—180° (after resetting at 134—135°).

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IMPERIAL CHEMICAL INDUSTRIES LIMITED, BUTTERWICK RESEARCH LABORATORIES,
THE FRYTHE, WELWYN, HERTS.

[Present address :
CIBA LTD., BASLE, SWITZERLAND.]

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