

213. *The Constitution and Synthesis of Fuscin.*

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The constitution (XI) has been deduced for the mould metabolite fuscin ; this formulation has been confirmed by total synthesis.

THE physiologically active mould metabolite fuscine, $C_{15}H_{16}O_5$, was first isolated by Michael,¹ from the culture filtrates of *Oidiodendron fuscum* Robak. Its chemistry was the subject of an elegant and detailed investigation by Birkinshaw, Bracken, Michael, and Raistrick.² At the very kind invitation of Professor H. Raistrick, F.R.S., we have correlated the extensive experimental work in terms of a constitutional formula (communicated to Professor Raistrick, with fully detailed argument, in October, 1954) and have then confirmed our deductions by a total synthesis.³

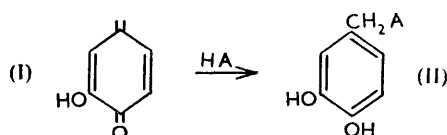
Fuscin contains one active hydrogen atom; it forms a monomethyl ether (methylhomofuscin; see below) with diazomethane, affords a highly coloured sodium salt, and gives a positive ferric chloride test. Fuscin contains, therefore, an enolic hydroxyl group. Pyrolysis of fuscine or dihydrofuscin alone or with copper chromite and quinoline gives 1 mol. of carbon dioxide. Since fuscine is clearly not a carboxylic acid, the ready formation of carbon dioxide is best explained by the presence of a lactone grouping. Of the remaining two oxygen atoms one is inert and is revealed by the degradations (see below) as ethereal; the other must be present as a carbonyl group in order to explain the presence of the enolic hydroxyl group. The lactone-carbonyl group cannot be responsible for the enolisation, for fuscine is clearly not an enolisable α - or β -keto-lactone.

¹ Michael, *Biochem. J.*, 1948, **43**, 528.

² Birkinshaw, Bracken, Michael, and Raistrick, *ibid.*, 1951, **48**, 67.

³ See also Barton and Hendrickson, *Chem. and Ind.*, 1955, 682, and Birch, *ibid.*, p. 682.

We consider next the quinonoid properties of fuscin. Fuscine is easily converted into the colourless dihydrofuscine, $C_{15}H_{18}O_5$, by reduction and this behaves like a dihydric phenol, affording a diacetate and a dimethyl ether (see below). The acetylation of fuscine is analogous, addition of 1 mol. of acetic acid to the quinonoid system affording a compound (not isolated) which is further acetylated to a triacetate. Fuscine also adds readily 1 mol. of mercaptoacetic acid, of methanol, of thiourea, and of hydrogen chloride, in every case affording a colourless product. The methanol addition compound gives a diacetate on acetylation. A methylene-hydroxyquinone structure, such as (I), would be compatible with all the addition reactions [(I) \rightarrow (II)] of fuscine but would not explain, without modification, the ready reversal of some of these processes (oxidation of dihydrofuscine in alkaline solution by air to furnish fuscine; elimination of hydrogen chloride from the "hydrochloride" to give back fuscine).



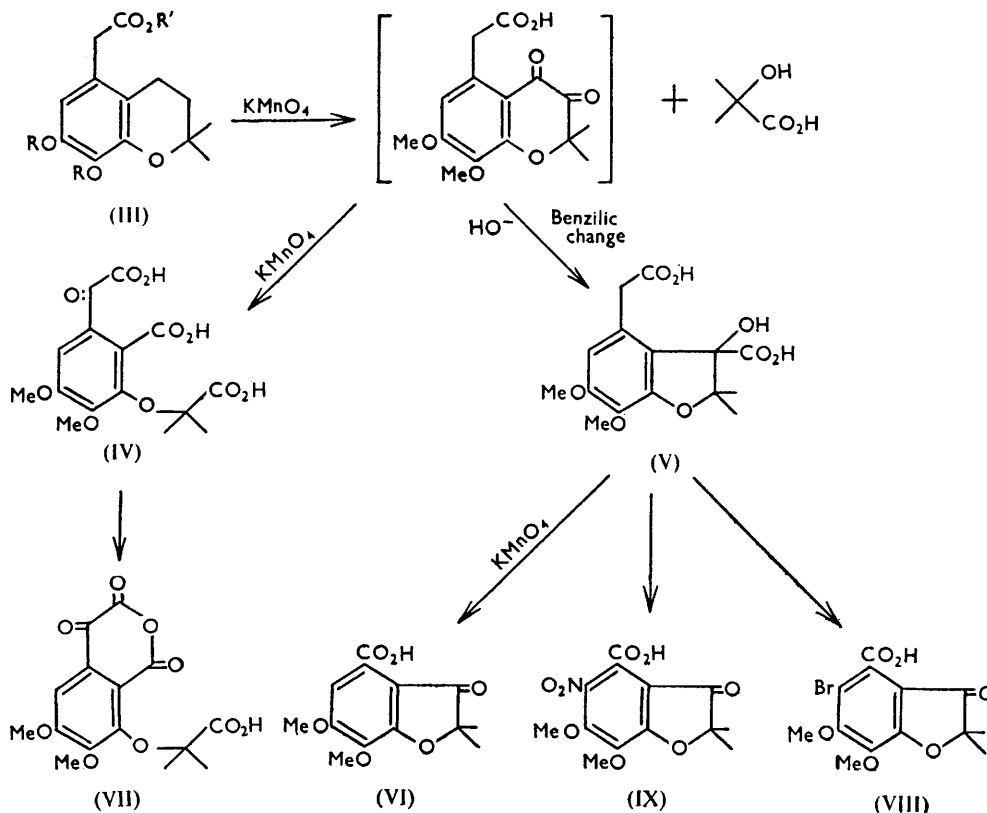
We turn now to the degradation products of dihydrofuscine obtained under alkaline conditions. Mild alkaline hydrolysis affords acetaldehyde and a phenolic carboxylic acid, $C_{13}H_{16}O_5$, conveniently designated fuscinic acid. The elimination of the acetaldehyde under such mild conditions must be a reversed aldol condensation, facilitated by a carbonyl (or potential carbonyl) group. The lactone-carbonyl group could not be responsible for this, for fuscine is obviously not a β -lactone or an $\alpha\beta$ -unsaturated δ -lactone. The more obvious explanation is that the group in dihydrofuscine leading to acetaldehyde is attached in the *ortho*-position to one of the phenolic (potentially carbonyl) hydroxyl groups as part

of the system $\text{Me}-\overset{\text{O}}{\underset{|}{\text{C}}}-\overset{|}{\text{C}}:\text{C}(\text{OH})-$.

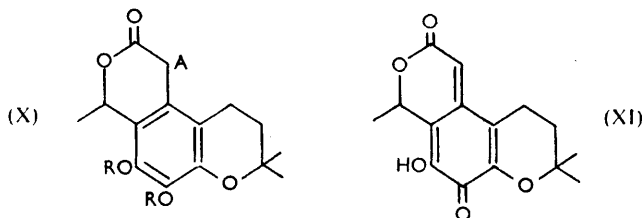
Fusion of dihydrofuscine with potassium hydroxide affords acetic, *isovaleric*, and 3:4:5-trihydroxyphenylacetic acid. The acetic acid must correspond to the acetaldehyde obtained under milder conditions. The *isovaleric* and arylacetic acid must represent degradation fragments from fuscinic acid; we note that all the carbon atoms are accounted for. Fuscinic acid is decarboxylated by heat, furnishing a dihydric phenol, $C_{12}H_{16}O_3$; the relatively easy decarboxylation is best explained if fuscinic acid itself is a phenylacetic acid.

We discuss next the constitution of fuscinic acid in the light of the evidence afforded by the products of oxidation of its dimethyl ether, $C_{15}H_{20}O_5$, with potassium permanganate. Four products were obtained: compound I, a monocarboxylic acid, $C_{13}H_{14}O_6$; compound II, a tricarboxylic acid, $C_{15}H_{16}O_{10}$; compound III, a dicarboxylic acid, $C_{15}H_{18}O_8$; and α -hydroxyisobutyric acid. Compound II retains all the carbon atoms and yet has three carboxyl groups. Its genesis is most simply explained by fission of the grouping $\cdot\text{CH}_2\cdot\text{CH}_2\cdot$ in a ring. Now fuscinic acid, $C_{13}H_{16}O_5$, contains twelve hydrogen atoms less than an aliphatic compound; of these, ten are taken up by the phenolic nucleus and the carboxyl group. The remaining pair must represent the extra ring required by the oxidation experiments; the residual uncharacterised ethereal oxygen atom must be part of this ring. The isolation of 3:4:5-trihydroxyphenylacetic acid (see above) clearly indicates the point of attachment of the ethereal oxygen to the phenolic nucleus. Remembering the isolation of *isovaleric* and α -hydroxyisobutyric acid, we are now in a position to advance complete structures for fuscinic acid (III; $R = R' = H$) and for Compound II (IV). Compound III is best regarded as (V), and Compound I as (VI). All oxidation products are formed according to the rational mechanisms indicated. In agreement with these formulations fuscinic acid dimethyl ether (III; $R = \text{Me}$, $R' = H$) showed bands (in CHCl_3) at 2500—3500 and at 1708 (CO_2H), at 1590 and 1605 (benzenoid) and at 1125 cm^{-1} (ether); compound II (IV) gave bands (in Nujol) at 2600—3200 and at 1722 (CO_2H), at 1690 ($\text{CO}\cdot\text{CO}_2\text{H}$), at 1580 and 1598 (benzenoid), and at 1114 and 1274 cm^{-1} (ether) [the anhydride (VII), formed from (IV) on melting, showed bands (in CHCl_3) at 1760 and 1840 cm^{-1} (anhydride)].

The decarboxylation and oxidation of Compound III [*i.e.*, (V)] by Br^+ and by NO_2^+ to give compounds $\text{C}_{13}\text{H}_{13}\text{O}_6\text{Br}$ (VIII) and $\text{C}_{13}\text{H}_{13}\text{O}_8\text{N}$ (IX) respectively is indicative of the α -hydroxy-acid structure $(\text{HO}\cdot\dot{\text{C}}\cdot\text{CO}_2\text{H} \rightarrow \text{X}-\text{O}-\dot{\text{C}}-\text{CO}_2\text{H} \rightarrow >\text{CO}; \text{X} = \text{Br or NO}_2)$ and the free aromatic position in this compound.



We are now in a position to deduce a constitution for dihydrofusicin. The group leading to acetaldehyde must (see above) be attached to the phenolic nucleus of fusicinic acid (III; $\text{R} = \text{R}' = \text{H}$) in the only vacant position and this then affords, on lactonisation, the structure (X; $\text{R} = \text{A} = \text{H}$) for dihydrofusicin. The obvious hydroxy-quinone structure for fusicin is (XI). The various addition compounds of fusicin mentioned above can be represented as (X; $\text{R} = \text{Ac}$, $\text{A} = \text{OAc}$) for the acetate derivative, as (X; $\text{R} = \text{H}$,

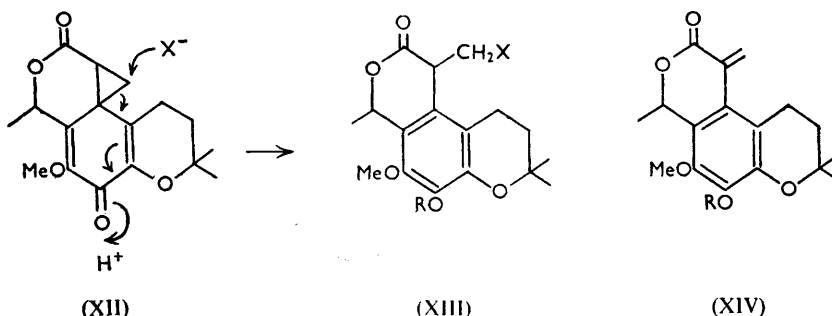


$\text{A} = \cdot\text{S}\cdot\text{CH}_2\cdot\text{CO}_2\text{H}$) for the mercaptoacetic acid adduct, as (X; $\text{R} = \text{H}$, $\text{A} = \text{OMe}$) and as (X; $\text{R} = \text{Ac}$, $\text{A} = \text{OMe}$) for the methanol adduct and its diacetate respectively, as (X; $\text{R} = \text{H}$, $\text{A} = \text{thiourea residue}$) for the thiourea adduct, and as (X; $\text{R} = \text{H}$, $\text{A} = \text{Cl}$) for the "hydrochloride."

In agreement with these formulations fusicin showed bands (in CHCl_3) at 3500 (OH), 1698 and 1668 ($\alpha\beta$ -unsaturated δ -lactone), 1638 ($\alpha\beta : \alpha'\beta'$ -diunsaturated *cyclohexadienone*), 1596 (C:C of this system), 1365 and 1380 (*gem*-dimethyl group) and 1112 (ether), and

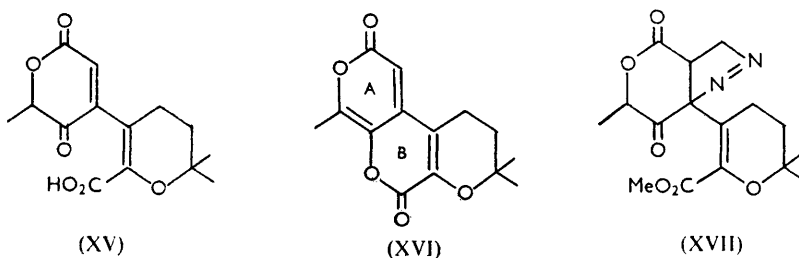
dihydrofuscine showed bands (in CHCl_3) at 3600 (OH), 1722 (δ -lactone), 1605 and 1638 (benzenoid), 1370 and 1380 (*gem*-dimethyl), and 1118 cm^{-1} (ether).

We next consider how structure (XI) explains the rest of the chemical evidence. Treatment of fuscine with diazomethane gives methylhomofuscine which may be represented as (XII). The interesting transformation products of this compound can be explained as follows. Hydrogenation gives a dihydro-derivative (XIII; $\text{R} = \text{X} = \text{H}$) characterised as the dimethyl ether (XIII; $\text{R} = \text{Me}$, $\text{X} = \text{H}$). Titration with alkali in the cold affords methylhomofuscine hydrate (XIII; $\text{R} = \text{H}$, $\text{X} = \text{OH}$), whilst reaction with hydrochloric acid furnishes the "hydrochloride" (XIII; $\text{R} = \text{H}$, $\text{X} = \text{Cl}$). Heating the last compound gives *isomethylhomofuscine* (XIV; $\text{R} = \text{H}$), also available by the more vigorous action of alkali on methylhomofuscine (XII) or by the action of iodine. Acetylation of methylhomo-



fuscine hydrate (XIII; $\text{R} = \text{H}$, $\text{X} = \text{OH}$) or of the *iso*-compound (XIV; $\text{R} = \text{H}$) gave *isomethylhomofuscine* acetate (XIV; $\text{R} = \text{Ac}$). In agreement with these formulations methylhomofuscine (XII) exhibited bands (in CHCl_3) at 1725 (δ -lactone), 1646 ($\alpha\beta : \alpha'\beta'$ -diunsaturated cyclohexadienone), 1610 (C:C of this system), 1360 and 1380 (*gem*-dimethyl), and 1113 and 1095 (ethereal). Likewise *isomethylhomofuscine* showed bands at 3550 (OH), 1720 (δ -lactone), 1605 and 1624 (benzenoid), 1118 (ether), and 895 cm^{-1} ($>\text{C}:\text{CH}_2$). The remarkable reactivity of the ether (XII) must be ascribed to the cyclohexadienone system blocked from aromaticity by the cyclopropane ring. There is thus an unusually powerful driving force [see (XII)] for the rupture of the cyclopropane ring.

On ozonolysis fuscine breaks down smoothly to a compound, $\text{C}_{14}\text{H}_{16}\text{O}_6$, and carbon dioxide. The ozonolysis product may be formulated as (XV). When heated alone or

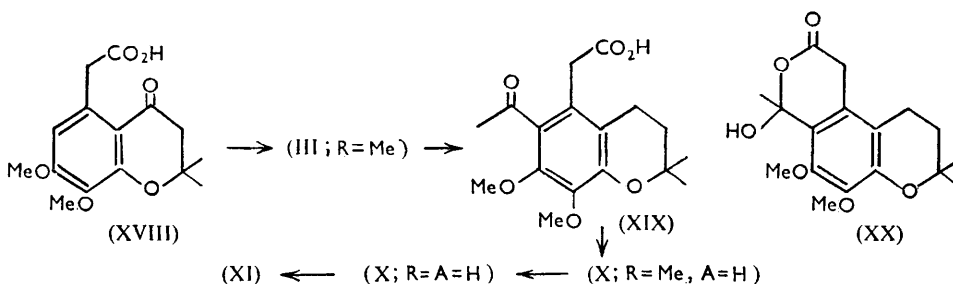


with sodium acetate-acetic anhydride, or when treated with cold alkali and then acidified, this product (XV) is transformed into a yellow product, $\text{C}_{14}\text{H}_{14}\text{O}_5$ (XVI). The methylation product (diazomethane) of the compound (XV) may be represented as (XVII), or an equivalent structure. In agreement with structure (XVI), the yellow compound showed bands (in CHCl_3) at 1742 (CO of ring A), 1718 (CO of ring B), 1642 and 1612 (conjugated C:C), 1360 and 1380 (*gem*-dimethyl) and at 1112 cm^{-1} (ether).

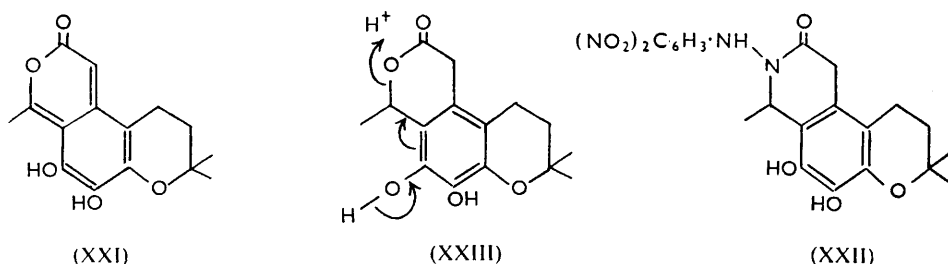
The constitution of fuscine (XI) has been placed beyond doubt by total synthesis. Treatment of methyl 3 : 4 : 5-trimethoxyphenylacetate with β -methylbut-2-enoyl chloride and aluminium chloride in ether⁴ gave the keto-acid (XVIII). This showed infrared

⁴ Bridge, Heyes, and Robertson, *J.*, 1937, 283.

maxima (Nujol) at 1712 (CO_2H), 1672 and 1575 ($\text{C}=\text{O}$ in a six-membered ring), 1598 and 1512 (benzenoid), 1372 and 1390 (*gem*-dimethyl), and 1300 cm^{-1} (ether) in agreement with the assigned constitution. Mild Clemmensen reduction furnished fuscinic acid dimethyl ether (III; $\text{R} = \text{Me}$, $\text{R}' = \text{H}$). Condensation of the methyl ester (III; $\text{R} = \text{R}' = \text{Me}$) with acetyl chloride and aluminium chloride as above gave the derived keto-acid (XIX), which probably existed in equilibrium with the lactol form (XX), since it showed (in CHCl_3) only one carbonyl band at 1754 cm^{-1} . Reduction of the acid (XIX) with potassium borohydride in methanol furnished dihydrofusicin dimethyl ether (X; $\text{R} = \text{Me}$, $\text{A} = \text{H}$), demethylated under controlled Zeisel conditions⁵ to dihydrofusicin (X; $\text{R} = \text{A} = \text{H}$), which was smoothly oxidised by air in alkaline solution to furnish fusicin (XI).



There remain for consideration those aspects of fusicin chemistry which are not at once explained by formula (XI). A possible objection is that structure (XI) has an asymmetric atom and yet fusicin and all its derivatives that we have examined are optically inactive. We believe that the asymmetric centre of fusicin must be readily racemised [see (XXI)]



and that this explains the lack of optical activity. A more serious objection is that dihydrofusicin forms a "2:4-dinitrophenylhydrazone." We found that the derived dimethyl ether (X; $\text{R} = \text{Me}$, $\text{A} = \text{H}$) did not react in this way and were therefore led to the view that the derivative was probably a 2:4-dinitrophenylhydrazone (XXII) formed as in (XXIII). The infrared spectrum of the compound (in CHCl_3) showed bands at 3550 (OH), 3400 (NH), 1678 ($\text{C}=\text{O}$), 1600 and 1620 (benzenoid), and 1110 cm^{-1} (ether) in agreement with formula (XXII). The observation that dihydrofusicin (X; $\text{R} = \text{A} = \text{H}$) forms two dimethyl ethers when alkylated with methyl sulphate also requires comment: the major product of m. p. 98° is clearly (X; $\text{R} = \text{Me}$, $\text{A} = \text{H}$) and was obtained in the synthesis reported above; the minor product of m. p. $238-242^\circ$ is probably to be formulated, in agreement with its analysis and high m. p., as (XXIV); a mechanism for the genesis of (XXIV)

can be devised without difficulty provided that the presence of a small amount of fusicin is admitted. We did not encounter this high-melting product in our own methylation studies.

⁵ Wilds and Close, *J. Amer. Chem. Soc.*, 1947, **69**, 3079.

EXPERIMENTAL

Infrared spectra were kindly determined by Messrs. Glaxo Laboratories Limited. Ultra-violet absorption spectra were taken in ethanol with the Unicam S.P. 500 Spectrophotometer. Unless stated to the contrary, the light petroleum used had b. p. 60–80°.

Fuscin and its Derivatives.—Fuscin and many of its derivatives were made available by Professor H. Raistrick, F.R.S., or were prepared by known methods.² Improvements in two preparations were secured as detailed below.

(a) *Fuscinic acid.* Dihydrofuscin (4.38 g.) in aqueous 2N-sodium hydroxide (80 ml.) was refluxed for 13 hr. in a stream of nitrogen. Extraction with ether and separation into acidic (aqueous sodium hydrogen carbonate) and neutral fractions gave acidic material (3.18 g.). All operations were carried out under sulphur dioxide. Treatment of the acid fraction with ether gave fuscine acid (1.52 g.). The mother-liquors were evaporated and the residue chromatographed over silica gel in 1 : 3 ether–benzene. Elution with 1 : 1 ether–benzene gave crystalline (from ether) fuscine acid (1.37 g.). The total yield of material of m. p. 182–186° was 2.49 g.

(b) *Dihydrofuscin dimethyl ether.* Dihydrofuscin (3.1 g.) in 2N-sodium hydroxide (100 ml.) was treated under nitrogen at room temperature with good stirring with methyl sulphate (25 ml.) and 2N-sodium hydroxide (100 ml.), both reagents being added portionwise and alternately during 1–2 hr. 6N-Sodium hydroxide (40 ml.) was added and stirring continued for 3 hr. The oily product was filtered through neutralised alumina (50 g.) in 1 : 9 benzene–light petroleum. Crystallisation from light petroleum containing a little benzene gave dihydrofuscin dimethyl ether (2.0 g.), m. p. 96.5–97.5°.

Condensation of Methyl 3 : 4 : 5-Trimethoxyphenylacetate with β -Methylbut-2-enoyl Chloride.—3 : 4 : 5-Trimethoxyphenylacetic acid (6.4 g.) was converted into the methyl ester with ethereal diazomethane. The excess of diazomethane and the ether were removed *in vacuo*. The residue was taken up in dry ether (150 ml.) with addition of β -methylbut-2-enoyl chloride (3.4 ml.) and aluminium chloride (25 g.; added portionwise under reflux). The red solution was left overnight at room temperature and then poured into excess of 2N-hydrochloric acid. Extraction with ether gave the organic product. This was separated in acidic and neutral fractions with N-sodium hydroxide. Acidification and re-extraction into ether gave the desired *keto-acid* (XVIII) (750 mg.), m. p. 187–188° (from benzene–ethanol), λ_{\max} , 222 and 285 m μ (ϵ 19,000 and 13,700 respectively), λ_{\min} , 252 m μ (ϵ 1500) (Found : C, 60.6; H, 6.0. $C_{15}H_{18}O_6$ requires C, 61.2; H, 6.15%). For analysis the *keto-acid* was chromatographed over silica gel, elution being with chloroform; it was characterised as the 2 : 4-dinitrophenylhydrazone. Prepared in the usual way and chromatographed in chloroform solution over kieselguhr–bentonite,⁶ this had m. p. 198–200° (from ethanol).

Conversion of the Keto-acid (XVIII) into Fuscine Acid Dimethyl Ether.—The *keto-acid* (730 mg.) in aqueous 17% hydrochloric acid (18 ml.) and warm ethanol (18 ml.) was treated with amalgamated zinc (20 g.) (prepared according to Organic Reactions, Vol. I, p. 162) under reflux for 6 hr. Extraction with ether gave the total organic product. This was separated into acid and neutral fractions with aqueous sodium hydrogen carbonate. Acidification and re-extraction into ether gave fuscine acid dimethyl ether (500 mg.), m. p. 164–168° (from ether). Purification by chromatography over silica gel and elution with 1 : 1 ether–benzene gave fuscine acid dimethyl ether, m. p. and mixed m. p. 166–168°, λ_{\max} , 230 and 282 m μ (ϵ 9400 and 1200), λ_{\min} , 258 m μ (ϵ 550), identical with that of an authentic specimen. The identity was confirmed by comparison of infrared spectra (identical in 1% $CHCl_3$ solution).

Condensation of Methyl Fuscinate Dimethyl Ether with Acetyl Chloride.—Fuscine acid dimethyl ether (920 mg.) was converted into the methyl ester with ethereal diazomethane in the usual way. The crude product was dissolved in dry ether (50 ml.) with addition of acetyl chloride (5 ml.) and aluminium chloride (4.0 g.; added portionwise under reflux). The dark solution was left for 3 hr. at room temperature, poured into excess of aqueous 2N-hydrochloric acid and extracted with ether. Shaking with aqueous sodium hydrogen carbonate extracted negligible amounts of acidic material from the ethereal layer. The ether was removed *in vacuo* and the residue was heated in ethanol (25 ml.) and aqueous 6N-sodium hydroxide (40 ml.) for 90 min. on the steam-bath. Separation of the product into neutral and acidic fractions gave crude acid (650 mg.). This was chromatographed on silica gel in benzene. Elution with

⁶ Elvidge and Whalley, *Chem. and Ind.*, 1955, 589.

3 : 1 benzene-ether (400 ml.) gave the desired *keto-acid* (XIX) (580 mg.), m. p. 147—148° (from benzene-light petroleum), λ_{max} . 225 and 273 m μ (ϵ 14,600 and 5100), λ_{min} . 255 m μ (ϵ 3900) (Found : C, 63.15; H, 6.5. $\text{C}_{17}\text{H}_{22}\text{O}_6$ requires C, 63.35; H, 6.85%).

Reduction of the Keto-acid (XIX) to Dihydrofusicin Dimethyl Ether.—The *keto-acid* (XIX) (125 mg.) in methanol (20 ml.) and water was refluxed with potassium borohydride (2.2 g.; added portionwise) for 4 hr. and then left overnight at room temperature. Dilution with water, acidification with dilute sulphuric acid, extraction with ether, washing with aqueous sodium hydrogen carbonate, and removal of the ether gave a neutral product (77 mg.). Crystallisation from ether-light petroleum gave dihydrofusicin dimethyl ether, identified by m. p., mixed m. p., ultraviolet absorption [λ_{max} . 285 m μ (ϵ 1500), λ_{inf} . 230 m μ (ϵ 11,500), λ_{min} . 258 m μ (ϵ 700); identical with that of an authentic specimen], and infrared absorption (identical with that of an authentic specimen in 1% CHCl_3 solution). The reduction was also effected, but less efficiently, by sodium borohydride.

Demethylation of Dihydrofusicin Dimethyl Ether.—(a) Dihydrofusicin dimethyl ether (560 mg.) was heated with hydriodic acid (10 ml.; d 1.7) and acetic acid (12 ml.) at 135—140° (reflux) in a stream of nitrogen for 5½ min. in a conventional Zeisel apparatus. A precipitate of silver iodide began to appear in the trap after 3½ min. The reaction was stopped by rapid cooling and the product, worked up in the usual way, was separated into acid (soluble in aqueous sodium hydroxide) and neutral (very small) fractions. The sodium hydroxide solution was acidified and extracted with chloroform. Removal of the chloroform *in vacuo* afforded crude dihydrofusicin (320 mg.), giving the characteristic grey-black ferric chloride reaction. Chromatography over silica gel in benzene and elution with 3 : 2 chloroform-benzene afforded crystalline dihydrofusicin (240 mg.). This was sublimed in a high vacuum to give, after one crystallisation from ether, pure dihydrofusicin, identified by m. p., mixed m. p., ultraviolet absorption [λ_{max} . 274 m μ (ϵ 900), λ_{min} . 266 m μ (ϵ 800), λ 210 m μ (ϵ 37,400); identical with that of an authentic specimen], and infrared absorption (identical with that of an authentic specimen in 1% CHCl_3 solution).

(b) Dihydrofusicin dimethyl ether (986 mg.) was heated with hydriodic acid (d 1.7) under the conditions specified above except that the heating was discontinued after 3½ min. The crude product, after filtration through silica gel in 1 : 1 ether-chloroform (460 mg.), was dissolved in ethanol (5 ml.) and aqueous 5% sodium hydrogen carbonate (30 ml.) with addition of aqueous 2N-sodium hydroxide (12 drops). Air was passed through this solution for 4 hr. at room temperature. Acidification of the resultant purple solution gave an orange precipitate of fusicin. Extraction with chloroform afforded crystalline fusicin (360 mg.), identified, after recrystallisation from ethanol, by crystal form (orange plates), m. p., mixed m. p., ultraviolet absorption [λ_{max} . 355 m μ (ϵ 27,600), λ_{min} . 283 m μ (ϵ 1000); identical with that of an authentic specimen], infrared absorption (1% solution in CHCl_3 ; identical with that of an authentic specimen), and colour reactions with ferric chloride and aqueous sodium hydrogen carbonate.

We thank the Government Grants Committee of the Royal Society, the Central Research Fund of London University, and Imperial Chemical Industries Limited for financial assistance. One of us (J. B. H.) is indebted to the National Research Council of the U.S. for Fellowship support. We express our deep appreciation to Professor H. Raistrick, F.R.S., for his interest and encouragement. Details for the preparation of 3 : 4 : 5-trimethoxyphenylacetic acid were kindly communicated by Professor R. A. Raphael (Queen's University, Belfast). Dr. W. D. Ollis (Bristol University) gave us valuable advice at various stages in this work.

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[Received, August 22nd, 1955.]