Studies in the Biochemistry of Micro-organisms

83. FUSCIN, A METABOLIC PRODUCT OF *OIDIODENDRON FUSCUM* ROBAK. PART 2. DERIVATIVES AND DEGRADATION PRODUCTS

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Michael (1948) described the preparation, properties and antibacterial activity of fusicin, $C_{15}H_{16}O_5$, a mould metabolite which he isolated from culture filtrates of laboratory cultures of *Oidiodendron fuscum* Robak. A number of derivatives and degradation products of fusicin have been prepared and are described in the present communication. It is hoped to discuss their bearing on the molecular constitution of fusicin in a later paper.

EXPERIMENTAL

Derivatives of fusicin

Mono-sodium salt of fusicin. Fusicin (0.1 g.) was dissolved in saturated aqueous $NaHCO_3$ (50 ml.) and kept at room temperature for 1 week. The purple solution slowly deposited dark-purple cubes of the *monosodium salt* which were separated by filtration, washed quickly with a minimum of water and dried. (Found: Na, 6.3. $C_{15}H_{16}O_5Na \cdot 4H_2O$ requires Na, 6.2%.) It is much more soluble in ethanol than in water.

Addition compounds of fusicin

(a) **With hydrochloric acid.** Dry gaseous HCl was passed through a suspension, cooled in ice and salt, of fusicin (0.2 g.) in anhydrous ether (20 ml.). The fusicin slowly dissolved (0.5 hr.) to a pale cream-coloured solution from which colourless rods separated (1 hr.). They were collected, washed with anhydrous ether and dried *in vacuo* over KOH. The compound, 0.14 g., melted at 210° (decomp.) after darkening at 130 – 150° . (Found: C, 57.1; H, 5.4; Cl, 12.1. $C_{15}H_{16}O_5 \cdot HCl$ requires: C, 57.6; H, 5.5; Cl, 11.3%.) It is stable in contact with anhydrous ether or conc. HCl, but decomposes into fusicin and HCl in contact with ethanol or water. When 0.0352 g. in water (5 ml.) was heated at 100° for 1 hr. the solution became pale yellow and typical orange diamond-shaped crystals of fusicin separated. These were collected and the cold filtrate and washings were titrated with 0.1N-NaOH to phenolphthalein. (Found: 1.15 ml. 0.1N-NaOH. $C_{15}H_{16}O_5 \cdot HCl \rightarrow HCl$ requires 1.13 ml.) Dihydrofusicin, similarly treated with HCl in ether, did not give a hydrochloride.

(b) **With methanol.** Fusicin 'methanolate'. Methanolic KOH (15 ml. of a 25% (w/v) solution) was added to a suspension of fusicin (1 g.) in methanol (5 ml.). The resulting brownish solution was chilled, diluted with iced water (80 ml.) and rapidly acidified with ice-cold 5N-HCl (12–15 ml.). The buff-coloured precipitate (0.82 g.) which formed was purified by crystallization from ether or, with more loss,

from ethanol. Fusicin 'methanolate' was thus obtained as almost colourless short thick tablets. (Found: C, 62.4, 62.2; H, 6.6, 6.8; CH_3O , 11.0; active hydrogen, in pyridine 0.35, in anisole 0.23. $C_{15}H_{20}O_6$, i.e. $C_{15}H_{16}O_5 \cdot CH_3OH$, requires: C, 62.3; H, 6.5; $1CH_3O$, 10.1; 1 active H, 0.33%.) It forms a pure red solution in dil. NaOH, but is insoluble in aqueous $NaHCO_3$ (cf. fusicin). The substance has no sharp melting point. On heating, the colour changes to a pure orange at 160° and it then melts with decomposition at 210 – 225° , depending on the rate of heating. This is due to reversion to fusicin as was shown by the recovery of fusicin from the orange residue resulting from holding the substance at 200° for a short time. The substance gives a pure blue colour in ethanol with a trace of ethanolic $FeCl_3$, becoming green with a slight excess, and red and finally almost colourless on dilution with water. Fusicin itself gives a purplish red colour in ethanol. The substance appears to be a substituted quinol, since on shaking its colourless $CHCl_3$ solution with aqueous, slightly acid, $FeCl_3$ the $CHCl_3$ layer became intensely purple, and gave a dark-purple solid on removal of the solvent. The solid gave an intense orange-red colour with conc. H_2SO_4 , and was reduced in $CHCl_3$ solution with aqueous $Na_2S_2O_4$ with reformation of the parent 'methanolate'.

Diacyl derivative. Acetylation of the 'methanolate' with acetic anhydride and anhydrous sodium acetate in the usual way gave a good yield of a *diacetate* crystallizing from ethanol in colourless needles, m.p. 158 – 159° . (Found: C, 61.1; H, 6.3; CH_3O , 7.4. $C_{20}H_{24}O_8$ requires: C, 61.2; H, 6.2; $1CH_3O$, 7.9%.)

(c) **With thiourea.** Fusicin *thiuronium chloride*. Fusicin (0.2 g.) was added to a solution of thiourea (0.3 g.) in 2N-HCl (1.5 ml.). The initial orange colour disappeared after shaking for 0.5 hr. and a fine white precipitate was formed. This was collected after chilling for 3 hr., washed well with 2N-HCl and dried. The compound, *fusicin thiuronium chloride*, forms fine white needles, wt. 0.206 g., m.p. 205 – 208° . (Found: C, 46.1; H, 5.5; N, 6.95; Cl, 8.75; S, 7.9. $C_{15}H_{23}O_6N_2S \cdot HCl$, i.e. $C_{15}H_{16}O_5 + CS(NH_2)_2 + HCl + H_2O$ requires: C, 47.2; H, 5.7; N, 6.9; Cl, 8.7; S, 7.9%.) In contact with water it decomposes rapidly into its constituents. Its solution in NaOH rapidly develops the purple colour of the Na salt of fusicin. Its solution in ethanol, which is quite stable, gives a reddish brown colour with ethanolic $FeCl_3$.

Acetylation of fusicin

(a) **With acetic anhydride and conc. H_2SO_4 .** A suspension of fusicin (0.5 g.) in 6 ml. of a mixture of acetic anhydride (10 ml.) and conc. H_2SO_4 (0.2 ml.) was held at 50° for 2 min. The resulting pale-yellow solution, on treatment with ice and water, yielded a colourless solid (0.68 g.) which, on re-

crystallization from ethanol (15 ml.), gave the pure *acetyl derivative* as characteristic colourless prisms, 0.5 g., m.p. 193–194°. (Found: C, 59.8, 59.8; H, 5.8, 5.7; CH_3CO , 30.4. $\text{C}_{21}\text{H}_{24}\text{O}_9$, i.e. the triacetate of $\text{C}_{15}\text{H}_{16}\text{O}_5 \cdot \text{H}_2\text{O}$, requires: C, 60.0; H, 5.75; $3\text{CH}_3\text{CO}$, 30.7%.)

(b) *With acetic anhydride and anhydrous sodium acetate.* A mixture of acetic anhydride (1 ml.) and anhydrous sodium acetate (0.2 g.) was heated at 145–150° until complete solution was effected. After cooling to 100°, fuscine (0.1 g.) was added and the mixture was held at 100° for 2 min. The resulting yellow solution, on pouring into ice and water, gave 0.11 g. of a cream-coloured solid. On crystallization from glacial acetic acid (1 ml.) and water (2 ml.) characteristic colourless prisms of the acetyl derivative were obtained, wt. 66 mg., m.p. 193° alone or in admixture with the acetyl derivative of fuscine prepared by acetylation with acetic anhydride and conc. H_2SO_4 (see section *a* above).

Derivatives of dihydrofucsin

Dihydrofucsin, prepared from fuscine by reduction with $\text{Na}_2\text{S}_2\text{O}_4$ or catalytically with H_2 and Pd, has been described by Michael (1948).

(a) *Diacetyldihydrofucsin.* A warm solution of fuscine (0.2 g.) in glacial acetic acid (3 ml.) was reduced with Zn dust (0.2 g.). To the mixture, initially orange, but quickly becoming colourless, acetic anhydride (3 ml.) and anhydrous sodium acetate (0.5 g.) were added and the whole was boiled under reflux for 0.5 hr. The colourless product, wt. 0.33 g., m.p. 166–168°, formed on pouring the acetylation mixture into ice and water, was recrystallized, wt. 0.18 g., from ethanol (5 ml.). *Diacetyldihydrofucsin* was thus obtained as colourless prisms, m.p. 168–169°. (Found: C, 62.4; H, 6.2; CH_3CO , 23.0. $\text{C}_{19}\text{H}_{22}\text{O}_7$, requires: C, 63.0; H, 6.1; $2\text{CH}_3\text{CO}$, 23.75%.) It is insoluble in NaHCO_3 . It is also formed by the direct acetylation of dihydrofucsin.

(b) *Dimethyl derivatives: melting points 98 and 252°.* A solution of fuscine (2 g.) in CHCl_3 (100 ml.) was reduced by shaking with a solution of $\text{Na}_2\text{S}_2\text{O}_4$ (10 g.) in water (40 ml.). The colourless CHCl_3 solution was evaporated to dryness in a stream of N_2 . The residual dihydrofucsin was methylated at room temperature and under N_2 with dimethyl sulphate (20 ml.) and 2N-NaOH (125 ml.), added in portions. The residual alkaline liquid was acidified with HCl and extracted with ether. On removal of the solvent a partially crystalline residue remained. This was washed with a little dry ether and the residual crystals, 0.05 g., m.p. 238–242°, were recrystallized from ethanol. A *dimethyl derivative*, m.p. 252°, was thus obtained in colourless square plates. (Found: C, 66.1; H, 6.9; CH_3O , 20.5. $\text{C}_{17}\text{H}_{22}\text{O}_6$ requires: C, 66.6; H, 7.2; $2\text{CH}_3\text{O}$, 20.25%.)

The ethereal mother liquors from the crystals, m.p. 238–242°, were evaporated to a syrup which slowly crystallized on standing and was then drained and washed with ether. The solid, 0.4 g., m.p. 93–96°, on recrystallization from ether-light petroleum gave a second *dimethyl derivative*, m.p. 98°, in large colourless plates. (Found: C, 66.95; H, 7.2; CH_3O , 21.0. $\text{C}_{17}\text{H}_{22}\text{O}_6$ requires: C, 66.6; H, 7.2; $2\text{CH}_3\text{O}$, 20.25%.)

Both the dimethyl derivatives were insoluble in 2N-NaOH either in the cold or on heating. Neither of them gave a FeCl_3 colour reaction in ethanol or any precipitate with Brady's reagent.

(c) *Dihydrofucsin 2:4-dinitrophenylhydrazine.* Dihydrofucsin (0.05 g.) dissolved in ethanol (5 ml.) was treated with Brady's reagent (0.3% 2:4-dinitrophenylhydrazine in 2N-

HCl; 50 ml.). The precipitate which formed quickly was collected (0.07 g.) and crystallized from benzene (15–20 ml.). *Dihydrofucsin 2:4-dinitrophenylhydrazone* forms yellow rhombic plates, m.p. 274°, when heated quickly. (Found, on sample dried in a high vacuum at 150°: C, 55.0; H, 5.2; N, 12.3. $\text{C}_{21}\text{H}_{22}\text{O}_8\text{N}_4$ requires: C, 55.0; H, 4.8; N, 12.2%.) Fuscine, when treated similarly, does not form a dinitrophenylhydrazone.

Action of diazomethane on fuscine.

Formation of methylhomofucsin

A solution of fuscine (2.5 g.) in CHCl_3 (180 ml.) was treated with an excess of ethereal diazomethane. The reaction proceeded smoothly and quickly. Removal of the solvent left a gum which was crystallized from dry ether giving *methylhomofucsin* (2.49 g.) as colourless prisms, m.p. 153–154°, raised to a constant m.p. 155° by sublimation in a high vacuum at 140–150° and subsequent crystallization from ether. (Found: C, 67.0, 67.0; H, 6.7, 6.7; N, nil; CH_3O , 11.5, 11.6. $\text{C}_{17}\text{H}_{20}\text{O}_5$, i.e. $\text{C}_{15}\text{H}_{16}\text{O}_4 + \text{CH}_3 + \text{OCH}_3$, requires: C, 67.1; H, 6.6; $1\text{CH}_3\text{O}$, 10.2%.) The substance is readily soluble in CHCl_3 , ethanol or acetone, less so in ether or cold water (about 0.5%). It dissolves readily in 0.01N-NaOH in the cold to a colourless solution, but is insoluble in aqueous NaHCO_3 . It gives no colour with ethanolic FeCl_3 , but an intense blue colour with cold 50% (v/v) HNO_3 . Like fuscine, but unlike dihydrofucsin, it does not form a 2:4-dinitrophenylhydrazone. It does not appear to react with hydrazine hydrate in ethanol, and hence it is probably not a methyl ester. Unlike fuscine and dihydrofucsin (see p. 71) it does not give acetaldehyde on alkaline hydrolysis.

Acetylation product. Methylhomofucsin (0.1 g.) was treated in the usual way with acetic anhydride (2 ml.) and sodium acetate (0.2 g.). Since, on dilution with water, the acetylation mixture gave no precipitate, it was extracted with ether. The residue (0.07 g.) on removal of the ether consisted of methylhomofucsin hydrate, m.p. 180° (see next section).

Methylhomofucsin hydrate. Methylhomofucsin titrates fairly sharply in the cold to phenolphthalein as a monobasic acid. (Titration equivalent. Found: 289. $\text{C}_{17}\text{H}_{20}\text{O}_5$ requires 304.) A solution of methylhomofucsin (0.15 g.) in 0.1N-NaOH (5 ml.) was held for 1 hr. at room temperature and then neutralized by the addition of 0.1N- H_2SO_4 (5 ml.). A trace of white precipitate was filtered off. The filtrate was acidified with 0.1N- H_2SO_4 (5 ml.) and extracted with ether. On removal of the solvent the solid residue (0.11 g.) was crystallized from dry ether, and *methylhomofucsin hydrate* was obtained as large colourless bipyramidal crystals, m.p. 180°. (Found: C, 62.9; H, 6.9; CH_3O , 9.7. $\text{C}_{17}\text{H}_{22}\text{O}_6$ requires: C, 63.3; H, 6.9; $1\text{CH}_3\text{O}$, 9.6%.) It is readily soluble in N-NaOH but insoluble in NaHCO_3 . It gives no colour with FeCl_3 either in ethanol or in water.

Acetylation product. Acetylation of methylhomofucsin hydrate with acetic anhydride and anhydrous sodium acetate in the usual manner gave, on dilution with water, a precipitate, which on crystallization from 60% aqueous ethanol yielded the *acetate* in colourless prisms, m.p. 162°. (Found: C, 65.55; H, 6.5; CH_3O , 9.3. $\text{C}_{15}\text{H}_{22}\text{O}_6$, i.e. $\text{C}_{17}\text{H}_{22}\text{O}_6 + \text{C}_2\text{H}_5\text{O} - \text{H}_2\text{O}$, requires: C, 65.9; H, 6.4; $1\text{CH}_3\text{O}$, 9.0%.) The substance is neutral in reaction and is insoluble in NaHCO_3 or N-NaOH. It is a *monoacetate* and is identical with the monoacetate of isomethylhomofucsin (see p. 69).

Dihydromethylhomofuscin. A solution of methylhomofuscin (0.5 g.) in ethanol (50 ml.) was reduced by H_2 and a Pd-norite catalyst prepared from $PdCl_2$ (0.2 g.) and norite (0.5 g.). In 1 hr. 38.4 ml. H_2 were absorbed; absorption of 1 mol. H_2 requires 36.8 ml. The catalyst was removed by filtration and the ethanol by evaporation. The crystalline residue, on recrystallization from ethanol, gave *dihydromethylhomofuscin* (0.44 g.), m.p. 182°, as colourless short rods. (Found: C, 67.0; H, 7.4; CH_3O , 10.4; active H, in pyridine, 0.42, in anisole, 0.19. $C_{17}H_{22}O_5$ requires: C, 63.65; H, 7.2; $1CH_3O$, 10.1; 1 active H, 0.33%.) The substance gives no colour in ethanol with ethanolic $FeCl_3$, but in aqueous ethanol it gives a brownish-red colour with an excess of aqueous $FeCl_3$. It is insoluble in $NaHCO_3$, but dissolves in $N-NaOH$ to a colourless solution from which it is precipitated by CO_2 .

Dihydromethylhomofuscin monomethyl ether. Dihydromethylhomofuscin (0.2 g.) was treated with a large excess of ethereal diazomethane for several days. Removal of the solvent left an oil which slowly crystallized. Recrystallization from aqueous ethanol gave *dihydromethylhomofuscin monomethyl ether* as colourless fine plates, m.p. 107–108°. (Found: C, 67.6, 67.8; H, 7.6; 7.4; CH_3O , 19.5. $C_{18}H_{24}O_5$ requires: C, 67.5; H, 7.55; $2CH_3O$, 19.4%.) The substance gives no $FeCl_3$ colour. It is soluble in dil. $NaOH$ and is precipitated from this solution by CO_2 .

Compound, $C_{17}H_{21}O_5Cl$, of methylhomofuscin with HCl. Methylhomofuscin has the remarkable property of forming a stable compound with HCl even in aqueous solution. On the addition of 2N-HCl to a clear, saturated aqueous solution of methylhomofuscin (0.05 g. in 10 ml. water) an immediate white precipitate of the compound is formed. It is also formed by acidification with HCl of a solution of methylhomofuscin in the minimum excess of 0.1N- $NaOH$. It is most conveniently prepared as follows: conc. HCl (0.1 ml.) was added to a clear cold solution of methylhomofuscin (0.15 g.) in ethanol (2 ml.). The compound (0.19 g.) quickly crystallized in colourless broad needles which, on recrystallization from 50% (v/v) aqueous ethanol, melted at 191° with vigorous evolution of HCl. The melt then reset and remelted at 224° owing to the formation of *isomethylhomofuscin* (see below). (Found: C, 60.2; H, 6.3; Cl, 10.3; CH_3O , 8.7. $C_{17}H_{21}O_5Cl$ requires: C, 59.9; H, 6.2; Cl, 10.4; $1CH_3O$, 9.1%.) An aqueous ethanol solution of the compound, acidified with dil. HNO_3 , gives no precipitate with $AgNO_3$. The chlorine in the molecule is, however, converted into the ionized form by boiling the substance with aqueous $N-NaOH$.

isoMethylhomofuscin

This compound was prepared by three different methods, of which the first is the most convenient.

(a) *By heating the compound, $C_{17}H_{21}O_5Cl$ (see above).* The compound, $C_{17}H_{21}O_5Cl$ (0.33 g.) was heated in an oil bath to 200°. HCl was evolved. The resolidified melt (0.28 g.) was crystallized from a mixture of $CHCl_3$ (1 vol.) and ether (5 vol.) yielding *isomethylhomofuscin* (0.25 g.) as colourless glistening tablets, m.p. 226°. (Found: C, 66.9; H, 6.8; CH_3O , 10.0. $C_{17}H_{20}O_5$ requires: C, 67.1; H, 6.6; $1CH_3O$, 10.2%.) It is soluble in $N-NaOH$ but insoluble in $NaHCO_3$. It gives no colour with $FeCl_3$ either in ethanol or in water. Unlike methylhomofuscin, *isomethylhomofuscin* gives no marked colour with 50% aqueous HNO_3 .

(b) *By the action of iodine on methylhomofuscin.* 0.1N- I_2 (50 ml.) was added to a solution of methylhomofuscin

(0.30 g.) in ethanol (50 ml.). After 3 hr. the residual I_2 was titrated with 0.1N- $Na_2S_2O_3$. Only 2.87 ml. 0.1N- I_2 was absorbed. The colourless titrated solution was diluted with much water and the resulting precipitate (0.28 g.), m.p. 180–190°, was purified by crystallization, first from aqueous ethanol, and then from ether. The resulting *isomethylhomofuscin* melted at 226° alone or in admixture with material prepared by method (a).

(c) *By boiling methylhomofuscin with N-NaOH.* In an attempt to degrade methylhomofuscin by alkaline hydrolysis, a solution of 1.0 g. of it in $N-NaOH$ (100 ml.) was boiled for 5 hr. in a stream of N_2 . The issuing gases were scrubbed through Brady's reagent. Only a trace of precipitate was obtained. The cooled hydrolysate, on acidification and heating to 90°, gave only a trace of CO_2 and an amorphous precipitate (0.37 g.) from which, by fractionation first from ether-light petroleum and then ether, 0.03 g. of *isomethylhomofuscin*, m.p. 224–226°, was isolated. The solid material (0.66 g.), obtained by ether extraction of the filtrate from the amorphous precipitate (0.37 g.) referred to above, gave, on fractionation, only amorphous products which could not be purified.

Acetylation product. *isoMethylhomofuscin* (0.1 g.) was acetylated in the usual way with acetic anhydride (1 ml.) and anhydrous sodium acetate (0.2 g.). The resulting acetyl derivative, 0.10 g. of colourless prisms, melted at 160–162° alone or in admixture with the acetyl derivative, $C_{19}H_{23}O_6$, of methylhomofuscin hydrate (see p. 68).

Degradation products of fuscine and dihydrofuscine

A. By pyrolysis

In this series of experiments a weighed amount of fuscine or dihydrofuscine, contained in a small distillation flask, was heated in an oil bath. The side arm of the flask was connected through a small receiving flask to bubblers, the first containing Brady's reagent, in which no precipitate appeared either with fuscine or dihydrofuscine, the others a measured amount of 0.2N- $Ba(OH)_2$ solution. Before heating was commenced and also during heating, a stream of O_2 - and CO_2 -free N_2 was passed through the whole apparatus. At the end of the experiment the CO_2 absorbed in the $Ba(OH)_2$ bubblers was estimated by titration. The following results were obtained.

(1) *Fuscine*: 0.194 g. used. The substance melted at 230°, gas evolution commenced at 240–250°, and at 270–295° a colourless distillate appeared in the receiver. The heating was continued for 1 hr. at 295–320°. 0.0336 g. of CO_2 , equivalent to 1.09 mol. of CO_2 , was evolved. No pure solid product could be obtained from the oily distillate.

(2) *Dihydrofuscine*: 0.288 g. used. The substance melted at 204°, gas evolution commenced at 240°, and a colourless distillate collected in the side arm of the flask at 260–285°. Heating was continued for 0.5 hr. at 270–280°. 0.052 g. of CO_2 , equivalent to 1.15 mol. of CO_2 was evolved. (In a repeat experiment 1.09 mol. of CO_2 was evolved.) An undistilled brown residue (0.169 g.), which could not be crystallized, remained in the flask. The distillate in the side arm solidified overnight as an almost colourless solid (wt. 0.078 g., 27%). This was purified by extraction with hot light petroleum (100 ml.), sublimation in a high vacuum at 80–100° of the solvent-free extract and recrystallization of the sublimate from light petroleum. Colourless fern-shaped crystals, m.p. 139°, were thus obtained. (Found: C, 69.3, 69.5; H, 7.8, 7.8; $C-CH_3$, 11.8; active hydrogen, in anisole, 0.47, in pyridine,

0.34%. Mol. wt. (Rast) 202. $C_{12}H_{16}O_3$ requires: C, 69.2; H, 7.75; $2C-CH_3$, 14.4; 1 active H, 0.49%. Mol. wt. 208.) The substance is insoluble in $NaHCO_3$, but forms a yellow solution in $NaOH$. It gives a green colour in ethanol with ethanolic $FeCl_3$, changing to reddish-purple on the addition of water. It turns pink on exposure to air and is clearly a phenol although no crystalline acetate could be obtained from it. It was also obtained by decarboxylation, either by heating above the melting point or with quinoline-copper chromite, of the acid, $C_{12}H_{16}O_5$, which was the main hydrolysis product yielded by boiling dihydrofusicin with aqueous 2N-NaOH (see section E).

B. By heating with copper chromite and quinoline

In these experiments the same apparatus was used as is described in section A, the operation being carried out in an atmosphere of N_2 . No precipitate was obtained in the Brady's reagent with either fusicin or dihydrofusicin.

(1) *Fusicin*. Fusicin (0.9756 g.), quinoline (25 ml.) and copper chromite catalyst (Kinney & Langlois, 1931) (0.5 g.) used. CO_2 began to be evolved at 130° , and the mixture was held at $150-155^\circ$ for 4.5 hr. 0.141 g. of CO_2 , equivalent to 0.91 mol. of CO_2 , was evolved. The cooled reaction mixture was acidified with dil. HCl and extracted with ether. On removal of the solvent there remained 0.4 g. of a dark-brown oil which could not be obtained crystalline.

(2) *Dihydrofusicin*. Dihydrofusicin (0.4039 g.), quinoline (10 ml.) and copper chromite (0.2 g.) used. CO_2 began to be evolved at 155° , and the mixture was held at $170-180^\circ$ for 4 hr. 0.0600 g. of CO_2 , equivalent to 0.94 mol. of CO_2 , was evolved. The cooled reaction mixture was acidified with 2N-HCl (180 ml.) and extracted with six 100 ml. portions of ether. The ether residue, 0.5 g. of brown oil, was washed several times with water, dried *in vacuo*, and the brown glassy residue (0.306 g.) was extracted with hot light petroleum (2×50 ml.). Evaporation of the light petroleum extract to 20 ml. gave colourless crystals, m.p. $139-140^\circ$, not depressed on admixture with the substance $C_{12}H_{16}O_3$ obtained by pyrolysis of dihydrofusicin (see section A 2).

C. By zinc dust distillation

In these experiments fusicin and dihydrofusicin were heated to a dull red heat with Zn dust in a combustion tube in a stream of dry H_2 . The issuing H_2 was passed first through a small empty receiver, then bubbled through a second receiver containing saturated ethanolic picric acid solution (3 ml.). Both receivers were cooled in ice.

(1) *Fusicin*. Fusicin (1.0 g.) and Zn dust (15 g.) were used. A yellow oil (27 mg.) collected in the cool part of the combustion tube. It smelt strongly of tar, and its ether solution showed a strong blue fluorescence. It gave no colour reaction with $FeCl_3$. When mixed with saturated ethanolic picric acid solution (2 ml.) the colour changed to dark brown. The solution was boiled for 5 min., then evaporated slowly at room temperature. Brown needles formed and were washed with water to remove excess picric acid; wt. 65 mg., m.p. 95° after softening at 82° . (Found: C, 42.3, 41.9; H, 2.3, 2.2; N, 15.15, 14.95; $C-CH_3$, 1.8. $(C_4H_4)_n \cdot (C_6H_5O_7N_3)_n$ requires: C, 42.7; H, 2.5; N, 14.9; $1(C-CH_3)$ if $n=3$, 1.77, if $n=2$, 2.67%.) Hence the parent hydrocarbon is $(C_4H_4)_n$, and in view of the $C-CH_3$ estimation is probably $C_{12}H_{12}$. Its properties are different from any recorded tripicrates of known $C_{12}H_{12}$ hydrocarbons, e.g. ethylnaphthalenes or

dimethylnaphthalenes, etc., and also from known picrates of hydrocarbons C_8H_8 , e.g. styrene and cyclo-octatetraene.

(2) *Dihydrofusicin*. Dihydrofusicin (1 g.) and Zn dust (15 g.) were used. A completely liquid oil (17 mg.) collected at the cold end of the combustion tube. It gave the same picrate (orange needles, 7 mg., m.p. and mixed m.p. 95°) as was obtained from fusicin. A partially crystalline viscous oil (10 mg.), giving a blue fluorescent solution in ether, collected near the end of the Zn layer. It gave a picrate (15 mg.), m.p. 80° , in dark-brown needles. (Found: C, 50.55; H, 2.9; N, 12.4. $C_{14}H_{11}O_7N_3$, i.e. $C_8H_8 \cdot C_6H_5O_7N_3$, requires: C, 50.5; H, 3.3; N, 12.6%.)

No picrate could be isolated from the ethanolic picric acid bubbler either with fusicin or dihydrofusicin.

D. By potash fusion of dihydrofusicin

A nickel crucible containing dihydrofusicin (1 g.) and KOH (10.0 g.) was placed in a short wide glass tube closed with a rubber bung carrying inlet and outlet tubes, and a tap funnel. After displacing the air by O_2 -free N_2 through the inlet and outlet tubes, water (4 ml.) was added to the crucible through the tap funnel. The tube and its contents were heated slowly in a metal bath to 260° till most of the water had evaporated, and were then held at $280-290^\circ$ for 0.5 hr. after the melt had become quiescent. After cooling, water (10 ml.) and 13.5N- H_2SO_4 (14 ml.) were carefully added. The contents of the tube were washed into a Claisen flask with water (100 ml.) and evaporated *in vacuo* to low bulk three times, water (100 ml.) being added at the commencement of the second and third evaporations. The receiver was connected to a water trap.

Treatment of the distillate. The distillate was titrated with N-NaOH, requiring from 5 to 6 ml. in different experiments. The neutralized solution was evaporated to dryness *in vacuo*, and the dry Na salts (0.6 g.) were extracted with boiling acetone (400 ml.). The acetone-soluble and acetone-insoluble Na salts were separately converted into their *p*-bromophenacyl esters by boiling in 80% ethanol with the theoretical amount of *p*-bromophenacyl bromide, and were separately fractionated from light petroleum (b.p. $40-60^\circ$). The purified *p*-bromophenacyl ester from the acetone-soluble Na salt was obtained as colourless prisms, m.p. 68° alone or in admixture with authentic *p*-bromophenacyl isovalerate, m.p. 68° . (Found: C, 52.35; H, 5.1; Br, 27.9. Calc. for $C_{13}H_{15}O_3Br$: C, 52.2; H, 5.05; Br, 26.7%.) The purified *p*-bromophenacyl ester from the acetone-insoluble Na salt was obtained as long colourless glistening needles, m.p. 86° alone or in admixture with authentic *p*-bromophenacyl acetate, m.p. 86° .

Treatment of residue. The solution remaining after removal of the volatile acids was extracted with ethyl acetate. On removal of the solvent, the solid dark-brown residue (0.67 g.) was purified by crystallization from ethyl acetate-light petroleum, and was finally obtained by sublimation in a high vacuum as colourless crystals, m.p. 161° . (Found: C, 52.0, 52.4; H, 4.7, 4.6. $C_8H_8O_6$ requires: C, 52.2; H, 4.4%.) The substance, 3:4:5-trihydroxyphenylacetic acid, gives a steely blue colour in ethanol with ethanolic $FeCl_3$, the colour becoming a more intense blue with a tinge of purple on the addition of water.

Triacetate. 0.056 g. was acetylated with acetic anhydride (0.6 ml.) and sodium acetate (0.1 g.). 3:4:5-Triacetoxyphenylacetic acid was recovered by ether extraction of the acidified diluted acetylation mixture, and was purified from ether-light petroleum and finally ethanol, m.p. 196° . (Found C, 54.4; H, 5.0. $C_{14}H_{14}O_8$ requires: C, 54.2; H, 4.55%.)

Trimethyl ether. 0.2 g. was methylated for 3 days with ethereal diazomethane. The methylation residue was saponified and separated into acidic and neutral fractions. The acidic fraction (0.17 g.) was purified from ether and sublimed in a high vacuum giving colourless needles, m.p. 119°. (Found: C, 58.5; H, 6.2; CH_3O , 40.5; titration equivalent 230. Calc. for $\text{C}_{11}\text{H}_{14}\text{O}_5$: C, 58.4; H, 6.2; $3\text{CH}_3\text{O}$, 41.15%; equivalent, titrating as a monobasic acid, 226.) The substance was identified as 3:4:5-trimethoxyphenylacetic acid (Slota & Müller, 1936), by comparison with an authentic specimen kindly supplied by Dr A. J. Ewins, F.R.S. A mixture of the methylated fusion product (m.p. 119°) with the authentic specimen (m.p. 121°) melted at 120°. The identity of the trimethyl ether was confirmed by oxidation with alkaline KMnO_4 , when colourless needles, m.p. 167°, of 3:4:5-trimethoxybenzoic acid were obtained. The melting point was not depressed on admixture with an authentic specimen, m.p. 167°, obtained by methylation of gallic acid.

E. By alkaline hydrolysis

In these experiments fuscine and dihydrofucose were hydrolysed with boiling aqueous NaOH in an atmosphere of N_2 . The issuing N_2 was scrubbed during the hydrolysis directly through bubblers containing Brady's reagent. The cooled hydrolysis solution was acidified, heated to 90°, and CO_2 , swept out in a current of N_2 , was absorbed in baryta bubblers.

(1) *Fuscine.* Fuscine (1.0 g.) and N-NaOH (100 ml.) were used. The initial dark bluish-purple solution finally became pale yellow-brown in colour after 9.5 hr. hydrolysis. The Brady's reagent deposited a heavy bright-yellow precipitate, m.p. 148–150°, wt. 0.28 g., equivalent to 0.35 mol. of acetaldehyde 2:4-dinitrophenylhydrazone, which was identified by failure of a sample, recrystallized from light petroleum b.p. 60–80° in golden plates and yellow needles, m.p. 158–160°, to depress the m.p. of an authentic specimen, and by analysis. (Found: C, 43.1; H, 3.4; N, 24.1. Calc. for $\text{C}_8\text{H}_8\text{O}_4\text{N}_2$: C, 42.9; H, 3.6; N, 25.0%.) CO_2 equivalent to 0.39 mol. was evolved after acidification.

The acidified hydrolysate was filtered from some black amorphous material (0.22 g.) and exhaustively extracted with ethyl acetate. The extract was evaporated *in vacuo* to small bulk and fractionally precipitated by the gradual addition of light petroleum. The first fraction (0.13 g.) was mainly insoluble polymerized material; the third fraction (0.19 g.) was an intractable oil; the middle fraction (0.45 g.), on fractional crystallization from ether-light petroleum, gave finally 0.035 g. of pure colourless crystals, m.p. 186°, together with 0.065 g. of less pure crystals. (Found: C, 61.45; H, 6.5. Mol. wt. (Rast) 274, 273; titration equiv. 255. $\text{C}_{18}\text{H}_{16}\text{O}_5$ requires: C, 61.9; H, 6.4%. Mol. wt. 252; equiv., titrating as a monobasic acid, 252.) The substance forms colourless needles which are soluble in ether or water but not in light petroleum. Its aqueous solution is acid to litmus. Its ethanol solution gives with ethanolic FeCl_3 first a pure blue colour, then green, and changes to red on addition of a little water. It readily sublimates without decomposition in a high vacuum. It gives no reactions indicative of the presence of a carbonyl group.

(2) *Dihydrofucose.* Dihydrofucose (1.0 g.) and 2N- NaOH (20 ml.) were used. After 5 hr. hydrolysis 0.73 mol. of acetaldehyde 2:4-dinitrophenylhydrazone separated in the Brady bubbler, and 0.13 mol. of CO_2 was collected in the baryta bubbler. The acidified hydrolysate yielded an ethyl acetate extract, which on evaporation gave a dark gum con-

taining many colourless needles. This was purified by fractionation from ether-light petroleum, a process involving much loss. 0.18 g. of colourless needles was obtained, m.p. 184°, not depressed on admixture with the substance $\text{C}_{18}\text{H}_{16}\text{O}_5$, m.p. 186°, obtained by the alkaline hydrolysis of fuscine. It also gave the same colour reactions.

(3) *Derivatives and breakdown products of the hydrolysis acid, $\text{C}_{18}\text{H}_{16}\text{O}_5$.* (a) *Decarboxylation product*, m.p. 141°. The hydrolysis acid, $\text{C}_{18}\text{H}_{16}\text{O}_5$ (4.5 mg.), mixed with copper chromite (2 mg.) was placed in a small L-shaped tube closed at each end and fitted with a side arm. The other arm contained 1 ml. of 0.1N- $\text{Ba}(\text{OH})_2$. After evacuation, the side arm was closed and the pyrolysis mixture was heated at 150°. The baryta showed a film of carbonate and heating was continued at 160° for 2.5 hr. After cooling and allowing to stand for several hours, back titration of the baryta showed the absorption of 0.8 mol. of CO_2 . A sublimate of almost colourless needles which formed above the melt was collected. The melting point, 141°, was not depressed on admixture with the phenol $\text{C}_{12}\text{H}_{10}\text{O}_3$, m.p. 139–140°, obtained by pyrolysis or decarboxylation of dihydrofucose (see sections A2 and B2). It also gave the same colour reactions with aqueous and ethanolic FeCl_3 .

(b) *Diacetate.* The hydrolysis acid, $\text{C}_{18}\text{H}_{16}\text{O}_5$ (0.05 g.), was acetylated in the usual way with acetic anhydride (0.5 ml.) and anhydrous sodium acetate (0.05 g.). The recovered crude acetate (0.04 g.), m.p. 160–166°, was recrystallized from ether-light petroleum giving colourless sheaves of needles, m.p. 168°. (Found: C, 61.0; H, 6.35. Titration equiv. 326. $\text{C}_{17}\text{H}_{20}\text{O}_7$, i.e. $\text{C}_{18}\text{H}_{14}\text{O}_5(\text{O} \cdot \text{CO} \cdot \text{CH}_3)_2$, requires: C, 60.7; H, 6.0%. Equiv., titrating as a monobasic acid, 336.) The diacetate is readily soluble in ethanol or aqueous NaHCO_3 , and gives no colour with FeCl_3 .

(c) *Dimethyl ether.* The hydrolysis acid is somewhat unstable. It was therefore found preferable to isolate it for subsequent degradation experiments as a dimethyl ether. Methylation with ethereal diazomethane proved to be difficult and incomplete. The following procedure was satisfactory, the hydrolysis and methylation being carried out in an atmosphere of N_2 . A solution of dihydrofucose (15 g.) in 2N- NaOH (650 ml.) was boiled for 14 hr. Acetaldehyde 2:4-dinitrophenylhydrazone (6.7 g.) was precipitated in the Brady bubblers. The cooled reaction mixture was methylated with dimethyl sulphate (90 ml.) and aqueous NaOH (54 g. in 240 ml. water) added alternately in portions with vigorous stirring, first at room temperature and then at boiling point. The methylation was continued until a sample of the methylated product no longer gave a purple colour with FeCl_3 . A further 10 ml. of 10N- NaOH was then added and the solution was boiled for 2 hr. to complete saponification.

The cooled methylation mixture was acidified with HCl to congo red and extracted with ether (5 × 500 ml.). The ether extract, on evaporation to 30 ml., yielded the dimethyl ether in colourless plates, wt. 8.6 g., m.p. 167°, and a further 0.75 g. from the mother liquors evaporated to dryness and redissolved in dry ether. (Found: C, 64.1; H, 7.3; CH_3O , 21.9; titration equiv. 283.5. $\text{C}_{15}\text{H}_{20}\text{O}_5$, i.e. $\text{C}_{18}\text{H}_{14}\text{O}_5(\text{OCH}_3)_2$, requires: C, 64.3; H, 7.2; $2\text{CH}_3\text{O}$, 22.1%; equiv., titrating as a monobasic acid, 280.) The dimethyl ether may be crystallized from boiling water, or ethanol (1 vol.) + hot water (8 vols.). It gives no colour with FeCl_3 .

(d) *Oxidation of the dimethyl ether with alkaline KMnO_4 .* The dimethyl ether (14.2 g., m.p. 167°) was dissolved in N- NaOH (80 ml.) and water (100 ml.). Aqueous 10% (w/v) KMnO_4 solution was gradually added with constant stirring.

The first portions were readily decolorized in the cold. When approximately 300 ml. had been added, heating on the water bath was commenced. One litre, equivalent to 100 g. KMnO_4 , was added in all in the course of 2 working days. The hot, pale yellowish-green solution was separated by filtration from the MnO_2 which was thoroughly extracted with hot water. The combined filtrate and washings, acidified with HCl to congo red, were evaporated *in vacuo* to approximately 200 ml. On chilling overnight, colourless crystals, fraction A (4.9 g.), consisting of a mixture of large plates (oxalic acid) and needles, were separated by filtration from the mother liquor, B.

Fraction A was dissolved in *n*-NaOH (40 ml.) and acidified to litmus with 2*N*-HCl (5 ml.). Colourless needles of *oxidation product I* separated (wt. 0.21 g., m.p. 158°). The filtrate from these crystals, when made alkaline with NH_3 and treated with excess CaCl_2 , yielded a copious precipitate of calcium oxalate.

Mother liquor B, on similar treatment with NH_3 and CaCl_2 , yielded a further copious precipitate of calcium oxalate, the filtrate from which was strongly acidified with HCl and exhaustively extracted with ether. Removal of the solvent yielded 7.06 g. of yellow syrup which was dissolved in dry ether (30 ml.). Colourless crystals (0.65 g.) quickly separated and a further 0.65 g. of the same compound was obtained on concentrating and chilling the ether solution. This *oxidation product II*, wt. 1.30 g., melted at 187° with gas evolution, then reset while still hot and remelted on further heating at 224°. The ether mother liquors from *oxidation product II* were dried and the oily residue (4.66 g.) was dissolved in boiling benzene (150 ml.). On cooling, colourless silky needles separated as a suspension in the benzene together with a small amount of oil adhering to the surface of the flask. The crystals were filtered off without disturbing the oil, washed with benzene and dried. They constitute *oxidation product III*, wt. 2.6 g., m.p. 153–155°.

The benzene mother liquors from *oxidation product III* together with the oil were evaporated to dryness *in vacuo*. The residue (1.13 g.), oily at first, slowly partially crystallized. It was stirred with benzene (3 ml.) which dissolved the oil. The residual crystals constitute *oxidation product IV*, wt. 0.035 g., m.p. 79°. The benzene mother liquors from *oxidation product IV* were freed from solvent. The residue was dissolved in water and converted into the Zn salt by heating with ZnCO_3 . There was finally obtained by fractional crystallization from water 0.074 g. of the Zn salt of *oxidation product IV*.

Oxidation product I, m.p. 158°. This substance is readily purified by crystallization from ether or by acidification of an aqueous solution of its Na salt. It forms colourless needles, m.p. 158°. (Found: C, 58.4, 58.6; H, 5.4, 5.7; CH_3O , 23.0; active hydrogen in pyridine, 0.37; titration equiv. either hot or cold 264, 266. $\text{C}_{15}\text{H}_{14}\text{O}_6$ requires: C, 58.6; H, 5.3; $2\text{CH}_3\text{O}$, 23.3; 1 active hydrogen, 0.38%; equiv., titrating as a monobasic acid, 266.) The substance gives no colour with FeCl_3 either in water or ethanol, and no precipitate with Brady's reagent.

Oxidation product II, m.p. 187°, remelting at 224°. This substance was purified by crystallization from dry ether. It forms colourless crystals, m.p. 187°, with evolution of gas, resetting at once on further heating and remelting at 224°. On cooling the melt and reheating, the melting point remains unchanged. (Found: C, 51.3; H, 4.9; CH_3O , 17.4; $\text{C}-\text{CH}_3$, 11.4; active H in pyridine, 0.82; titration equiv. 113. $\text{C}_{15}\text{H}_{16}\text{O}_{10}$ requires: C, 50.6; H, 4.5; $2\text{CH}_3\text{O}$, 17.4; $3(\text{C}-\text{CH}_3)$,

12.5; 3 active H, 0.82%; equiv., titrating as a tribasic acid, 119.) The acid is readily soluble in water or ethanol and slightly soluble in dry ether. It gives no colour reaction with FeCl_3 , and no precipitate with Brady's reagent. The residue from a macro-Zeisel methoxyl estimation was diluted with water, extracted with ether, the ether extract shaken with $\text{Na}_2\text{S}_2\text{O}_3$ to remove I_2 and evaporated, yielding a small amount of crystals, m.p. 244°, an ethanolic solution of which gave a green FeCl_3 reaction, unchanged on the addition of water.

Silver salt. A quantity of oxidation product II dissolved in a minimum of water was made slightly alkaline with NH_3 and precipitated with a slight excess of 0.1*N*- AgNO_3 . The silver salt formed a colourless amorphous precipitate which was carefully washed and dried to constant weight. (Found: C, 26.4; H, 2.3; Ag, 48.8. $\text{C}_{15}\text{H}_{15}\text{O}_{10}\text{Ag}_3$ requires: C, 26.6; H, 1.9; Ag, 47.8%.)

Tri-p-bromophenacyl ester. Oxidation product II (0.107 g.) neutralized with *n*-NaOH (0.97 ml.) was mixed with ethanol (3 ml.), water (1 ml.) and *p*-bromophenacyl bromide (0.26 g.). The mixture was refluxed for 2 hr. The crude ester (0.20 g.) which separated was crystallized from acetone-light petroleum. The tri-*p*-bromophenacyl ester formed colourless fine needles, m.p. 96–97°. (Found: C, 49.45; H, 3.5; Br, 23.8. $\text{C}_{39}\text{H}_{31}\text{O}_{13}\text{Br}_3$, i.e. tri-ester of $\text{C}_{15}\text{H}_{16}\text{O}_{10}$, requires: C, 49.4; H, 3.3; Br, 25.3%.)

Anhydride. When oxidation product II (53.9 mg.) was heated in a current of dry N_2 at 195–200° for 0.5 hr. water (3.4 mg. = 6.3%) was evolved. There was no evolution of CO_2 or CO, which were tested for by bubbling the N_2 , after absorption of water in CaCl_2 , first through $\text{Ba}(\text{OH})_2$ and then dilute aqueous PdCl_2 . During the heating a lemon-yellow sublimate was formed. The whole of the heated material was suspended in dry ether (5 ml.) and the undissolved crystals (23 mg.) were separated and dried. The anhydride thus obtained formed colourless needles, m.p. 232°. The anhydride may also be prepared by subliming the acid at 160–170°/1 mm. It forms a colourless sublimate resembling snow, m.p. 232°. (Found: C, 53.0, 53.65; H, 3.8, 4.0; CH_3O , 17.7. $\text{C}_{15}\text{H}_{14}\text{O}_8$ requires: C, 53.25; H, 4.2; $2\text{CH}_3\text{O}$, 18.3%.) The anhydride (10.4 mg.) behaved in a characteristic fashion on titration with aqueous 0.1*N*-NaOH to phenolphthalein. NaOH (0.20 ml.) was neutralized rapidly in the cold and a further 0.68 ml. more slowly on heating before a permanent end point was reached. (Found: titration equiv. 118. $\text{C}_{15}\text{H}_{14}\text{O}_8$, titrating as a tribasic acid, requires 113.)

Oxidation product III, m.p. 167°. The crude product (2.6 g., m.p. 153–155°) was recrystallized from benzene (700 ml.) giving colourless needles with a constant m.p. of 167°. (Found: C, 55.6; H, 5.5; CH_3O , 18.2; $\text{C}-\text{CH}_3$, 5.66; active H in anisole, 0.64, in pyridine, 1.03; titration equiv. 168, 160. $\text{C}_{15}\text{H}_{16}\text{O}_8$ requires: C, 55.2; H, 5.6; $2\text{CH}_3\text{O}$, 19.0; $\text{C}-\text{CH}_3$, 1 group, 4.6, 2 groups 9.2; active H, 2H, 0.61, 3H, 0.91%; equiv., titrating as a dibasic acid, 163.) The acid is readily soluble in cold water, ethanol, acetone, ether and hot benzene; slightly soluble in cold benzene, and insoluble in light petroleum. Its aqueous solution gives a yellow colour with aqueous FeCl_3 ; no precipitate with Brady's reagent; no colour with azobenzenephénylhydrazinesulphonic acid or with ammonium molybdate; bromine water is rapidly decolorized with the formation of a white precipitate (see below). The acid completely resisted attempts to reduce it in aqueous ethanol either with Adams's PtO_2 catalyst or with a $\text{Pd}-\text{BaSO}_4$ catalyst, and was recovered unchanged. The only products, apart from oils, recovered by ozonization of the

acid in aqueous solution were oxalic acid and an at present uncharacterized 2:4-dinitrophenylhydrazone.

Di-p-bromophenacyl ester. This ester was prepared in a similar way to that of oxidation product II. It was recrystallized from hot ethanol giving colourless crystals, m.p. 170°. (Found: C, 51.2; H, 3.8; Br, 21.3. $C_{21}H_{18}O_{10}Br_2$, i.e. the di-ester of $C_{15}H_{18}O_8$, requires: C, 51.7; H, 3.9; Br, 22.2%.)

Demethylation product. This nor-derivative was prepared from the residues from a macro-Zeisel methoxyl estimation as described for oxidation product II. The ether residue, an oil, wt. 0.1 g., from 0.1011 g. of oxidation product III, was dissolved in ethanol (2 ml.) and mixed with light petroleum to give a faint turbidity. Clusters of colourless needles, wt. 29 mg., m.p. 258° (decomp.), were obtained. (Found: C, 52.8; H, 5.8; active H in anisole, 1.43; titration equiv. 148. $C_{15}H_{14}O_8$ requires: C, 52.4; H, 4.7; 4 active H, 1.34%; equiv., titrating as a dibasic acid, 149.) The nor-acid is readily soluble in water, ethanol, acetone and ether, but insoluble in light petroleum. Its aqueous solution gives a blue colour with a purple tinge with aqueous $FeCl_3$; no precipitate with Brady's reagent and no colour with azobenzene-phenylhydrazinesulphonic acid; an orange-yellow colour slowly fading to yellow with ammonium molybdate.

Action of bromine water. A solution of the acid (0.1 g.) in water (10 ml.) was treated with saturated bromine water (4.5 ml.) added in small portions in the course of 0.5 hr. Decolorization was almost immediate in the early stages. Colourless needles quickly separated, wt. 52 mg., m.p. 192° (resetting on cooling). The bromo compound may be purified by crystallization from hot water, or preferably by sublimation in a high vacuum at 125–145°. The melting point remains unchanged. (Found: C, 45.5; H, 3.8; Br, 23.2; CH_3O , 17.5. $C_{15}H_{13}O_8Br$ requires: C, 45.2; H, 3.8; Br, 23.2; $2CH_3O$, 18.0%.) 12.4 mg. in ethanol (2 ml.) required 0.38 ml. 0.1N-NaOH for neutralization to phenolphthalein in the cold. The end point was sharp and was unchanged on heating. $C_{15}H_{13}O_8Br$, titrating as a monobasic acid requires 0.36 ml. 0.1N-NaOH.

Action of conc. HNO_3 . The acid (0.1 g.) was mixed with cold conc. HNO_3 (2 ml.). It dissolved immediately and brown fumes were evolved. After holding the solution at room temperature for 3 days, it was mixed with water (4 ml.). Colourless needles (50 mg.) separated, m.p. 135°. The nitro compound is only slightly soluble in cold water. It dissolves more readily in hot water, but does not crystallize on cooling except on the addition of a little dil. HNO_3 . It is best recrystallized by dissolving in the theoretical amount of alkali and acidifying. The melting point remains unchanged. (Found: C, 49.3; H, 4.2; N, 4.7; titration equiv. 325. $C_{15}H_{13}O_8N$ requires: C, 50.2; H, 4.2; N, 4.5%; equiv., titrating as a monobasic acid, 311.)

Oxidation product IV, m.p. 79°. This substance was identified as α -hydroxyisobutyric acid. (Found: C, 46.2; H, 8.0; titration equiv. 106. Calc. for $C_4H_6O_3$: C, 46.2; H, 7.7%; equiv., titrating as a monobasic acid, 104.) Its melting point was not depressed on admixture with an authentic specimen of α -hydroxyisobutyric acid, m.p. 79°. The Zn salt of oxidation product IV was isolated as colourless shining plates from water, and was shown to be zinc α -hydroxyisobutyrate. (Found: on specimen dried in a high vacuum to constant weight at 100°: C, 35.7; H, 5.8; Zn, 23.9. Calc. for $(C_4H_5O_3)_2Zn$: C, 35.4; H, 5.2; Zn, 24.1%.) It had the same properties, crystal shape etc., as an authentic specimen of zinc α -hydroxyisobutyrate.

F. By ozonolysis of fuscine

Ozonized O_3 was bubbled through a solution of fuscine (1 g.) in $CHCl_3$ (100 ml.), cooled in ice, until the solution was almost colourless (2 hr.). The solution was mixed with water (100 ml.) and warmed with thorough shaking on the water bath. The separated cooled $CHCl_3$ solution was washed with water (100 ml.) and the $CHCl_3$ was removed by evaporation *in vacuo* leaving a crystalline residue (0.93 g.) which was purified by crystallization from 50% (v/v) aqueous ethanol, giving colourless platelets, m.p. 222–224° (decomp.), resetting on cooling and then remelting at 234°. (Found: C, 60.0; H, 5.8. $C_{14}H_{16}O_6$ requires: C, 60.0; H, 5.75%.) The substance is fairly soluble in $CHCl_3$ and readily soluble in ethanol. Its ethanolic solution gives no precipitate with Brady's reagent and no colour with $FeCl_3$, even after diluting with water.

In a repeat experiment carried out by Dr E. N. Morgan CO_2 -free ozonized O_3 was bubbled through a solution of fuscine (0.5 g.) in $CHCl_3$ (50 ml.), cooled in ice, for 2.5 hr., when the original orange colour had changed to pale yellow. The effluent gases were scrubbed first through distilled water and then through 0.2N-Ba(OH)₂. All connexions were made with plastic tubing which, unlike ordinary rubber tubing, is resistant to ozone. CO_2 , corrected for blanks, equivalent to 76.6% of 1 mol. was evolved. Water (50 ml.) was now added to the ozonized $CHCl_3$ solution and CO_2 -free O_3 was bubbled through for 2.5 hr., the mixture being held at room temperature. A further amount of CO_2 , equiv. to 23.4% of 1 mol., was absorbed in a fresh baryta bubbler. Hence 1 mol. of fuscine $C_{15}H_{16}O_8$, on ozonolysis, yields 1 mol. of CO_2 and 1 mol. of $C_{14}H_{16}O_6$ according to the equation

Prolonged ozonolysis of fuscine (0.5 g.) in $CHCl_3$ solution (40 ml.) led to extensive breakdown. The main product isolated was an uncrystallizable oil (0.38 g.) together with acetaldehyde (20 mg. of its 2:4-dinitrophenylhydrazone), acetic acid (about 80% of 1 equiv.) and considerable quantities of oxalic acid.

Methylation of the ozonolysis product $C_{14}H_{16}O_6$. The product (0.30 g.), suspended in dry ether (5 ml.) was treated with excess of ethereal diazomethane. The mixture was shaken and when solution was complete the solvent was removed. The residual dried methylated product (0.26 g.), m.p. 122° (decomp.), was recrystallized from ethanol. Colourless hexagonal prisms, m.p. 123° (decomp.). (Found: C, 57.1; H, 6.1; N, 8.4; CH_3O , 9.3. $C_{16}H_{20}O_6N_2$, i.e. $C_{14}H_{16}O_6 + CH_2N_2 + CH_2$, requires: C, 57.1; H, 6.0; N, 8.3; $1CH_3O$, 9.2%.) The substance, dissolved in 10N- H_2SO_4 containing a little $K_2Cr_2O_7$, gives no colour, indicating the probable absence of a pyrazoline ring (Knorr's test).

Conversion of the ozonolysis product $C_{14}H_{16}O_6$ into a yellow product, $C_{14}H_{14}O_6$. The colourless ozonolysis product, $C_{14}H_{16}O_6$, is readily and smoothly converted, with the loss of 1 mol. water, into a yellow product, $C_{14}H_{14}O_6$, by any of the following methods. (a) By heating above its m.p., 222–224°. (b) By heating with acetic anhydride and sodium acetate. (c) By treatment with excess cold aqueous NaOH or cold saturated aqueous $NaHCO_3$, and subsequent acidification in each case. The yellow product crystallizes beautifully from ethanol in long silky pure yellow needles, and may be readily sublimed in a high vacuum at 160–180°. M.p. 237° without decomposition, but with some sublimation. (Found: C, 64.15; H, 5.4; active H in pyridine, 0.0; Mol. wt. (Rast) 273.

$C_{14}H_{14}O_6$ requires: C, 64.1; H, 5.4%; mol. wt. 262.) The substance is almost insoluble in water, but dissolves to a yellow solution in hot NaOH, being recovered unchanged on acidification. It is insoluble in aqueous $NaHCO_3$. It is readily soluble in $CHCl_3$ from which it is precipitated in fine crystals by ether or light petroleum. Its ethanolic solution gives no marked colour with ethanolic $FeCl_3$ and no precipitate with Brady's reagent. It gives a clear orange-brown solution in cold conc. H_2SO_4 . It resists acetylation with sodium acetate and acetic anhydride, and on treatment with ethereal diazomethane a yellow oil, which could not be obtained crystalline, was formed. Similarly, ozonolysis in $CHCl_3$ afforded an uncrystallizable red gum. Catalytic reduction of its ethyl acetate solution (0.1 g. in 30 ml.) with Adams's PtO_2 catalyst (0.02 g.) resulted in the uptake in 3.5 hr. of 23 ml. H_2 . ($C_{14}H_{14}O_6$ absorbing 6H requires 25.7 ml. H_2 .) The isolated reduction product was an oil (0.12 g.). Similarly,

catalytic reduction of the ethanolic solution of the colourless ozonolysis product, $C_{14}H_{16}O_4$, with $PdCl_2$ -norite resulted in the slow uptake of almost 2H in 27 hr. and the formation of a stiff oil.

SUMMARY

An account is given of a number of derivatives and degradation products of fuscine, an antibacterial metabolite of the mould *Oidiodendron fuscum* Robak.

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Studies in Immunochemistry

9. THE OXIDATION OF THE HUMAN BLOOD-GROUP A SUBSTANCE WITH THE PERIODATE ION

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Apart from the methylation studies of Bray, Henry & Stacey (1946) few structural investigations have been carried out on the blood-group A substance. These workers isolated and identified 2:3:4-trimethyl- α -methyl-L-fucoside and 3:4:6-trimethyl- α -methylglucosaminide hydrochloride in the esterified products from acid hydrolysis of a fully methylated A substance derived from pepsin, thus indicating that both fucose and *N*-acetylglucosamine are present as non-reducing end groups in the alkali-stable residues examined. A methylated derivative of D-mannose was also identified, but according to other investigators (Bendich, Kabat & Bezer, 1947; Partridge, 1948b; Aminoff, Morgan & Watkins, 1950) mannose is not present in the A substance. The behaviour of the A substance during mild hydrolysis with alkali (Morgan, 1946) and with acid (Kabat, Baer, Bezer & Knaub, 1948; Baer, Dische & Kabat, 1948; Aminoff, Morgan & Watkins, 1948, 1950) has provided additional information regarding the *N*-acetylhexosamine and fucose components and the roles they play in the different serological activities associated with the blood-group substances.

Oxidation of the A substance with periodate offers an independent and more specific approach to the determination of the structure of this important biological material. Periodic acid is a selective oxidizing agent which breaks the C—C linkage in substances containing either two adjacent unsubstituted hydroxyl groups or an unsubstituted hydroxyl group adjacent to a primary or secondary amino group. The products of oxidation are aldehydes, acids and, with hydroxyamino derivatives, ammonia. The reactions, under suitable conditions, are practically quantitative. The application of oxidation with periodate to structural studies in the field of carbohydrate chemistry has been reviewed by Owen (1943), Jackson (1944), and Jones (1946), and further discussion here is unnecessary.

The investigation of the oxidation of the A substance with periodate was approached along the following lines: (1) studies on the lability of the serological properties during oxidation; (2) determination of the amount of periodate reduced and the rate of reduction by the A substance under different conditions; (3) qualitative and quantitative analyses of