695. *The Chemistry of Fungi. Part XXVII.* The Structure of Fulvic Acid † from* Carpenteles brefeldianum.

By F. M. Dean, R. A. Eade, R. Moubasher, and Alexander Robertson.

Structure (I; R = H) is proposed for fulvic acid, the yellow acidic metabolite of *Carpenteles brefeldianum* Dodge. The chief degradation product was identical with a compound first obtained from citromycetin and now shown to be 2-acetyl-7-hydroxy-4 : 5-dimethoxyindane-1 : 3-dione and not 6 : 7-dimethoxy-2-methylchromone-5-carboxylic acid as originally proposed.[2] The nature of this product in combination with spectroscopic data provided evidence for the existence of the chromone ring in fulvic acid and the behaviour of the acid as a hemiketal together with the formation of a compound considered to be a pyridine oxide from a third ring completed the evidence on which structure (I; R = H) is based. Several other reactions and properties of fulvic acid are shown to be consistent with this formulation.

In a study of the metabolites of various species of *Penicillia*, Oxford, Raistrick, and Simonart [1] noted that, when grown on a Raulin–Thom medium, *P. griseofulvum* Dierckx produced a yellow substance which was named fulvic acid. *P. flexuosum* Dale and *P. brefeldianum* Dodge produced the same metabolite when incubated on the same medium; the latter organism, now known as *Carpenteles brefeldianum* Dodge (Shear), was used as a source of material for the present work which has resulted in the allocation of structure (I; R = H) to fulvic acid.

Oxford *et al.* established that the new metabolite had the empirical formula $C_{14}H_{12}O_8$ and, from the formation of phenolic methyl and ethyl esters by the Fischer–Speier method, concluded that a carboxyl group was present. More vigorous alkylation furnished a neutral dimethyl ether of the methyl ester, thus indicating the existence of two phenolic hydroxyl groups, in agreement with the formation of an acidic di-*O*-acetate from fulvic acid. This diacetate, however, was in reality derived from anhydrofulvic acid $C_{14}H_{10}O_7$, itself easily obtainable by sublimation of fulvic acid. These facts enabled Oxford *et al.* to perceive a close resemblance between fulvic acid and citromycetin, the yellow acidic metabolite of *P. glabrum* Wehmer, and to attach importance to the fact that anhydrofulvic acid and citromycetin were isomerides. Fulvic acid and citromycetin were observed to have the same characteristic ferric reaction and both compounds were devoid of methoxyl groups and optical activity, but citromycetin formed salts with hydrogen bromide and hydrogen iodide whereas it appeared that fulvic acid did not. In its earlier stages, our examination of fulvic acid was guided by reference to the chemistry of citromycetin, for which structure (II; R = H) has been established.[2, 3, 4]

Attempted crystallisation of methyl di-*O*-methylfulvate from acetic acid effected dehydration, producing methyl anhydrodi-*O*-methylfulvate identical with the ester resulting from alkylation of anhydrofulvic acid with diazomethane or methyl sulphate. Because methyl anhydrodi-*O*-methylfulvate was comparatively readily purified, it was the preferred starting point for further studies. Oxidation of this ester by permanganate under relatively stringent conditions terminated in the phthalic acid derivative (III), previously obtained by oxidation of methyl di-*O*-methylcitromycetin (II; R = Me); as before, the identity of this acid was confirmed by its conversion into the ethoxy-anhydride (IV) [4]

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* Part XXVI, *J.*, 1957, 542.

† The use of the name fulvic acid to designate a fraction obtained by the extraction of soil has priority, but because in that context the name refers to a heterogeneous material, we prefer to adhere to the nomenclature of Raistrick's school.[1]

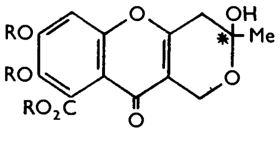
[1] Oxford, Raistrick, and Simonart, *Biochem. J.*, 1935, **29**, 1102.

[2] Robertson, Whalley, and Yates, *J.*, 1951, 2013.

[3] Mackenzie and Robertson, *J.*, 1949, 497.

[4] Robertson and Whalley, *J.*, 1949, 848; Cavill, Robertson, and Whalley, *J.*, 1950, 1031.

identical with a synthetic specimen. A second oxidation product was obtained in small amount, insufficient for further investigation, but a third is considered to be the 4-hydroxycoumarin (V; R = CO_2Me) because of its molecular formula $C_{10}H_3O_4(OMe)_3$, indifference to carbonyl reagents, acidity, pale brown ferric reaction, high decomposition point, and infrared absorption spectrum which possessed a band at 1735 cm.$^{-1}$ attributed to the methoxycarbonyl group since 4-hydroxy-6 : 7-dimethoxycoumarin (V; R = H) absorbed at, but not above, 1704 cm.$^{-1}$.



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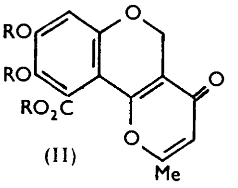

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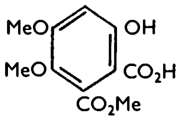

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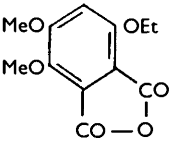

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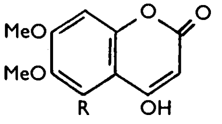

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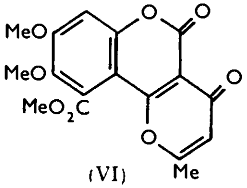

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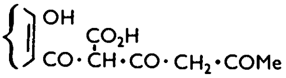

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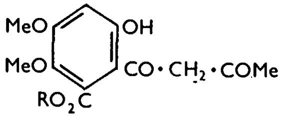

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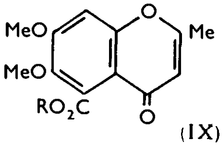

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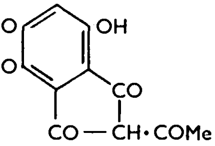

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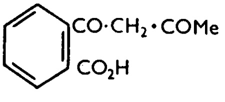

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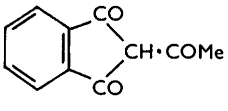

(XII)

The oxidation of methyl di-*O*-methylcitromycetin (II; R = Me) with chromic acid gave the lactone (VI), methyl di-*O*-methylcitromycetinone, hydrolysis of which furnished several important degradation products. It was evident that one mode of hydrolysis of (VI) included fission of both heterocyclic rings giving the polyketone (VII) as an unstable intermediate from which loss of carbon dioxide and the terminal acetyl group would yield a β-diketone (VIII; R = Me) able to cyclise to the chromone ester (IX; R = Me). Although the chromone ester (IX; R = Me) was not detected in the hydrolysate of (VI), there was present an acidic substance which was considered to be the corresponding acid (IX; R = H). The same compound has now been obtained by hydrolysis of methyl anhydrodi-*O*-methylfulvate without prior oxidation. Schwerin [5] has shown that, when liberated from its salts, *o*-acetoacetylbenzoic acid (XI) spontaneously generates acetylindanedione (XII), a type of cyclisation which, if it occurred in the case of the diketone (VIII; R = H), would result in the triketone (X) instead of the chromone (IX; R = H). Although β-triketones simulate carboxylic acids in many respects and are difficult to distinguish from them, a closer scrutiny of the acidic degradation product from methyl di-*O*-methylcitromycetin and methyl anhydrodi-*O*-methylfulvate has established that this must be the acetylindanedione derivative (X) and not the chromone (IX; R = H). In keeping with its character as an *o*-hydroxy-β-diketone, the degradation product gives

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[5] Schwerin, *Ber.*, 1894, **27**, 104.

(*a*) an intense purple ferric reaction distinct from the weak reddish colours given by carboxylic acids, (*b*) an oxime under conditions in which chromones are inert, and (*c*) a dimethyl ether instead of the monomethyl derivative expected from an acid having the structure (IX; R = H). Further, under conditions which destroy chromones this product, like acetylindanedione (XII), was remarkably resistant to alkaline hydrolysis. Moreover, the ultraviolet absorption spectrum differed widely from those of representative chromones, whereas the infrared absorption spectrum included bands at 1704, 1654, 1635, and 1605 cm.$^{-1}$ which tally with those shown by acetylindanedione at 1706, 1658, 1629, and 1601 cm.$^{-1}$.

Because the β-diketone (VIII; R = H) is the parent of both (IX; R = H) and (X), further revision of the chemistry of citromycetin was not implied in the adoption of structure (X) for the acidic degradation product, and the formation of acid (III) made clear the point from which elaboration of the carbon skeleton of fulvic acid was to be continued. On the other hand, the production of the indanedione (X) obscured the question as to whether fulvic acid and its derivatives contained a chromone ring, and, in fact, a simple degradation product containing a chromone ring has not been isolated. Nevertheless, we consider that system (XIII) must be present, because fulvic acid and its derivatives do not contain a reactive carbonyl group and have ultraviolet and infrared absorption spectra strongly reminiscent of simpler chromones. In detail, the fulvates have a band between 1740 and 1754 cm.$^{-1}$ because of the methoxycarbonyl group (this frequency is rather high for an aromatic ester but is not unexpected in view of the possibility of interaction with the rigidly held neighbouring carbonyl group[6] and the possibility that steric effects might be reducing the effective conjugation of the methoxycarbonyl group with the benzene ring), at 1647 (chromone carbonyl), 1592, 1555, and 1450 cm.$^{-1}$, a pattern which may be compared with that of 6 : 7-dimethoxy-2-methylchromone having bands at 1648, 1603, 1595 (shoulder), 1511, and 1486 cm.$^{-1}$. The ultraviolet absorption spectra are discussed below. In support of the formulation of fulvic acid as a chromone, it must also be pointed out that alternatives either lead to the structure (II) already ascribed to citromycetin or must be based on a structure of type (XIV; R = H or Me) which is extremely difficult to reconcile with the spectroscopic data and the reactions and properties now to be described.

With regard to the nature of the conversion of fulvic into anhydrofulvic acid, this change, which is paralleled by the transformation of methyl di-*O*-methylfulvate into methyl anhydrodi-*O*-methylfulvate, could be effected by sublimation or merely by dissolution in hot acetic acid, and was accompanied by disappearance of the hydroxyl absorption near 3450 cm.$^{-1}$ shown by fulvates (but not by anhydrofulvates which are transparent in this region). That the labile water was not water of crystallisation was evident from the readiness with which anhydrofulvates crystallised from aqueous solvents and from the ultraviolet absorption spectra of the anhydro-compounds which showed bathochromic shifts appropriate to the introduction of a new double bond in conjugation with a chromone ring. The hydration of anhydrofulvic acid and its derivatives to the corresponding fulvates was effected without difficulty by aqueous mineral acids and was extended to the acid-catalysed addition of alcohols with the production, for example, of methyl tri-*O*-methylfulvate, having an ultraviolet spectrum almost identical with that of methyl di-*O*-methylfulvate but devoid of hydroxylic absorption near 3400 cm.$^{-1}$. When it was also found that the elements of the appropriate alcohol could be removed from the new ethers by sublimation or by treatment with hot acetic acid, the resemblance of these reactions to those characteristic of dihydropyran and other vinyl ethers led to the view that anhydrofulvates contained the grouping $\cdot\overset{|}{C}{:}\overset{|}{C}\cdot O\cdot$, fulvates the grouping $\cdot\overset{|}{C}H\cdot\overset{|}{C}(OH)\cdot O\cdot$, and the new ethers the grouping $\cdot\overset{|}{C}H\cdot\overset{|}{C}(OR)\cdot O\cdot$. It was subsequently discovered that the

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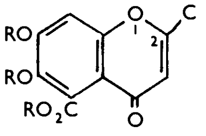
[6] Bellamy, " The Infra-red Spectra of Organic Molecules," Methuen, 1954, p. 158.

addition of alcohols to anhydrofulvates was also catalysed by bases. Since simple vinyl ethers are unresponsive to bases, it appeared necessary to develop a formulation permitting conjugation of the vinyl double bond with a carbonyl group as in the system $\cdot\mathrm{CO}\cdot\overset{|}{\mathrm{C}}:\overset{|}{\mathrm{C}}\cdot\mathrm{O}\cdot$. This interpretation was supported by hydrogenation of methyl anhydrodi-*O*-methylfulvate to the dihydro-derivative methyl deoxydi-*O*-methylfulvate, a compound that was inert to aqueous or alcoholic acids or bases and had an ultraviolet absorption spectrum very like that of methyl di-*O*-methylfulvate, facts which could readily be accommodated by the partial structure $\cdot\mathrm{CO}\cdot\overset{|}{\mathrm{C}}\mathrm{H}\cdot\overset{|}{\mathrm{C}}\mathrm{H}\cdot\mathrm{O}\cdot$. It was obvious that hydrogenation had not affected the carbonyl group because methyl deoxydi-*O*-methylfulvate could not be acetylated and did not have a hydroxyl band in its infrared absorption spectrum.

That methyl anhydrodi-*O*-methylfulvate contained one *C*-methyl group was evident from Kuhn–Roth determinations. This methyl group was clearly " active " since the ester gave a piperonylidene derivative devoid of *C*-methyl groups. Methyl deoxydi-*O*-methylfulvate did not condense with piperonaldehyde and therefore hydrogenation must have destroyed a double bond connecting the methyl group with a carbonyl group. This and the above facts were most neatly incorporated in partial structure (XV) for methyl anhydrodi-*O*-methylfulvate.



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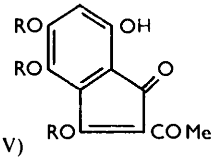

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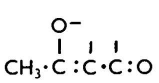
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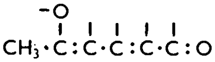
(XV)


$\mathrm{CH_3}\cdot\overset{\overset{\textstyle\mathrm{-O}}{|}}{\mathrm{C}}:\overset{|}{\mathrm{C}}\cdot\overset{|}{\mathrm{C}}:\overset{|}{\mathrm{C}}\cdot\overset{|}{\mathrm{C}}:\mathrm{O}$

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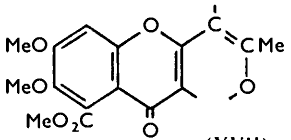

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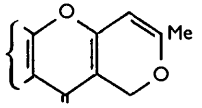
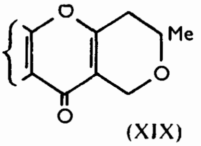


(XVIII)

In combination, the expressions (XIII; R = Me) and (XV) contain eight oxygen atoms whereas methyl anhydrodi-*O*-methylfulvate contains but seven. In the summation, at least one oxygen atom must therefore be common to both expressions, but identification of either oxygen atom of (XV) with the appropriate atom in (XIII) also necessitated identification of the double bond of (XV) with the 2 : 3-double bond of (XIII). This secondary identification was impossible because chromone double bonds are inert and do not have the properties attributed to that in (XV). It became clear that expression (XV) had to be expanded to the vinylogue (XVI), the combination of which with (XIII) presented no difficulty and gave the partial structure (XVII) for methyl anhydrodi-*O*-methylfulvate. This structure (XVII) differs from a complete structure by one methylene group which could be inserted in two ways, one giving the pyran (XVIII) and the other giving the oxeten (XXI). It has been possible to exclude the latter alternative, and therefore we represent methyl anhydrodi-*O*-methylfulvate by structure (XVIII), fulvic acid by (I; R = H), methyl tri-*O*-methylfulvate by (XX), and methyl deoxydi-*O*-methylfulvate by (XIX). The possibility of ring–chain tautomerism between fulvic acid (I; R = H) and the system (XXII) accounted for the absence of optical activity in spite of the presence of the asymmetric carbon atom starred in (I). The hydrolysis of (XXII) could have proceeded by way of (XXIII) from which the loss of the terminal acetyl group and of the hydroxymethyl group (by a reverse aldol condensation) would result in the triketone (X), but we have not been able to identify formaldehyde as a product of this reaction and have detected but small amounts of formic acid. The polyketone (XXIII) could also suffer hydrolysis to acetone and acetic acid; these products were easily detected. Oxidation of polyketone (XXIII) or an equivalent clearly accounted for the production of the 4-hydroxycoumarin (V; R = CO_2Me).



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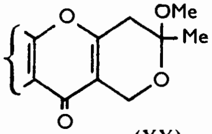

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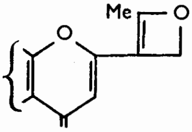
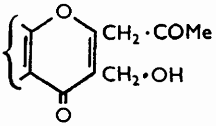
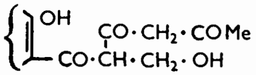


(XXI)

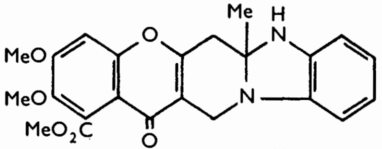
(XXII)





(XXIII)

As remarked earlier, derivatives of fulvic acid do not behave as ketones (methyl deoxydi-*O*-methylfulvate was completely inert to carbonyl reagents), but the relation of the fulvates to the system (XXII) would be expected to result in some kind of modified carbonyl activity in this series. This was observed in reactions with *o*-phenylenediamine and hydroxylamine.



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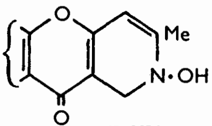

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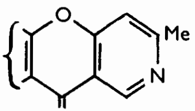

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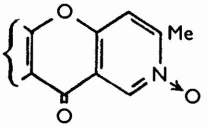

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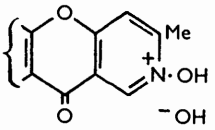

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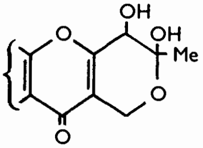

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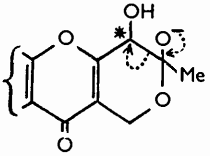

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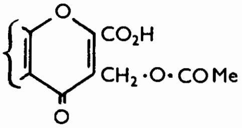

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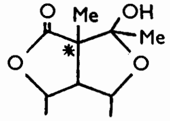

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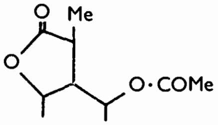

(XXXIII)

In contrast with the normal α-diketone reaction, interaction of methyl anhydrodi-*O*-methylfulvate (XVIII) and *o*-phenylenediamine was accompanied by elimination of only one mol. of water, but the product could not be diazotised and therefore did not contain a free primary amino-group. This reaction clearly involved the pyran ring only, because the product possessed infrared absorption bands at 1736 $cm.^{-1}$ (methoxycarbonyl), and at 1643, 1609, 1582, and 1479 $cm.^{-1}$ (chromone and benzene rings) and also because of the inertness of methyl deoxydi-*O*-methylfulvate (XIX) towards the same reagent. The anomaly can be explained in terms of structure (XVIII) for methyl anhydrodi-*O*-methylfulvate because replacement of the pyran oxygen atom by one nitrogen atom would permit the free amino-group to add to the active double bond after the fashion of water and alcohols. Consequently the product is considered to have structure (XXIV).

With hydroxylamine, methyl anhydrodi-*O*-methylfulvate (XVIII) underwent a complex reaction from which only a small quantity of a coherent product could be isolated. Much importance was attached to this compound because it seemed that the replacement of the pyran oxygen atom by a hydroxyamino-residue would give an intermediate (XXV) from which loss of the elements of water could yield the pyridine derivative (XXVI) by a simple variation of Knoevenagel's modification [7] of the Hantzsch pyridine synthesis, thus establishing the size of the terminal ring of fulvic acid. This pyridine $C_{17}H_{15}O_6N$ (XXVI), if formed, escaped isolation because the characterised product gave analytical data which accorded much more closely with the hydroxylamine derivative $C_{17}H_{17}O_7N$ (XXV). The data, however, fitted precisely a compound having the empirical composition $C_{17}H_{15}O_7N$, thus suggesting that the hydroxylamine derivative (XXV) has suffered oxidation to the pyridine oxide (XXVII), a formulation supported by the properties of the compound and by the discussions presented by Rogers,[8] which show that dialkylhydroxylamines readily undergo autoxidation to nitrones and other substances equivalent to the pyridine oxide (XXVII) under conditions similar to those obtaining in the preparation of this substance. In common with amine oxides,[9] the hydroxylamine condensation product liberated iodine from hydrogen iodide and was insoluble in alkaline media. It was, however, very weakly basic and dissolved only in relatively concentrated mineral acids, a behaviour which was not surprising because amine oxides are considerably weaker bases [9] than their parent amines, and, in addition, the present example was in effect derived from a 3-acylpyridine similar to 3-acetylpyridine which is a much weaker base [10] than pyridine. This low basicity also accounted for the apparent inability of the compound to form a hydrate (XXVIII) of the type usually formed by amine oxides. The infrared absorption spectrum of the compound was, as expected, devoid of hydroxyl or imino-stretching frequencies and showed a peak at 1752 $cm.^{-1}$ due to the methoxycarbonyl group; the chromone pattern was no longer evident. An important new band had appeared at 1664 $cm.^{-1}$, indicating that the pyran ring had aromatised, since frequencies in this region are typical of many of the more complex derivatives of benzophenone—in particular, 4-methoxyxanthone was found to absorb at 1661 $cm.^{-1}$. The ultraviolet and visible spectra of the condensation product were similar to those of the parent ester (XVIII) and to those of several xanthones [11] and were therefore at least not inconsistent with structure (XXVII). This interpretation automatically eliminated (XXI) as a possible structure for methyl anhydrodi-*O*-methylfulvate because the corresponding amine oxide would have had two double bonds in a four-membered ring and therefore be prohibitively strained; but the oxeten (XXI) might have reverted to a β-hydroxy-ketone from which condensation with hydroxylamine and loss of two hydrogen atoms could have produced an *iso*oxazole easily confused with a pyridine oxide of type (XXVII). Independent evidence against structure (XXI) for methyl anhydrodi-*O*-methylfulvate was sought.

Under mild conditions, permanganate oxidation of methyl anhydrodi-*O*-methylfulvate resulted in an acid devoid of carbonyl activity and formed simply by the addition of three oxygen atoms to the original ester, thus eliminating structure (XXI) which offered opportunity only for the formation of a neutral acetoxy-ketone by fission of the double bond in the four-membered ring. With diazomethane, this acid gave an ester containing four methoxyl groups and having the intense absorption near 1730 $cm.^{-1}$ expected from a polyester. The infrared absorption spectrum of the ester and the ultraviolet spectrum of the acid indicated that the chromone ring was intact, but peaks indicative of new carbonyl or hydroxyl groups were not present. Having regard to the course usually taken by permanganate oxidations, we considered the primary oxidation product to be the glycol

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[7] Knoevenagel, *Annalen*, 1894, **281**, 25.

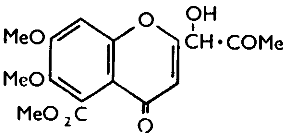
[8] Rogers, *J.*, 1956, 2784.

[9] Katritzky, *Quart. Rev.*, 1956, **10**, 395.

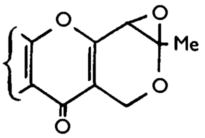
[10] Hall and Sprinkle, *J. Amer. Chem. Soc.*, 1932, **54**, 3469.

[11] Lund, Ph.D. Thesis, Liverpool, 1952.

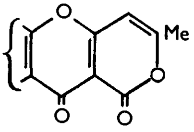
(XXIX), further oxidation of which appeared to be governed by the basicity of the reaction mixture, since this acid was never obtained when magnesium sulphate was included. Removal of a proton from the glycol (XXIX) to give an ion (XXX), in which electrons could move (as indicated) towards the starred carbon atom and be there stabilised by conjugation with the chromone carbonyl group, would result in a system that would subsequently be oxidised to the acid (XXXI). This mode of fission is virtually the same as that recently considered by Barton and de Mayo,[12] and by Braun, Herz, and Rabindran,[13] to account for the base-catalysed isomerisation of tenulin, partial structure (XXXII), to *iso*tenulin, partial structure (XXXIII). The structure (XXXI) agreed excellently with the properties of the acid given above and also with the isolation of approximately one mol. of volatile acid after acidic hydrolysis (chromone rings are generally stable in the conditions employed) as opposed to the neutralisation of three equivalents of base in alkaline hydrolysis. The absence of a 2-methylchromone system in the acid was clear from the negative Zimmermann reaction.[14]



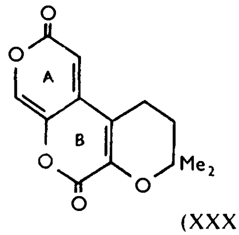
(XXXIV)



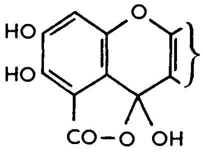
(XXXV)



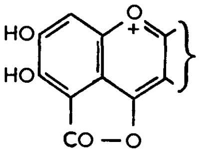
(XXXVI)



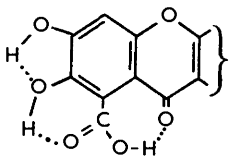
(XXXVII)



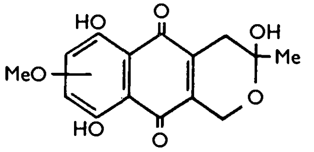
(XXXVIII)



(XXXIX)



(XL)



(XLI)

With osmium tetroxide, methyl anhydrodi-*O*-methylfulvate formed an intractable adduct. Although methyl deoxydi-*O*-methylfulvate (XIX) was inert, the anhydrofulvate (XVIII) rapidly destroyed one equivalent of perbenzoic acid and then suffered a further, slower oxidation. From the very variable reaction mixture, it was occasionally possible to isolate small quantities of an interesting crystalline product. The derivative which this compound rapidly formed with 2 : 4-dinitrophenylhydrazine unfortunately resinified, but the infrared absorption spectrum revealed a new unconjugated carbonyl group (peak at 1724 cm.$^{-1}$) and also a new hydroxyl group (peak near 3400 cm.$^{-1}$). Bands at 1745, 1647, and 1608 cm.$^{-1}$ attested the survival of the methoxycarbonyl and chromone systems. Taken in conjunction with the molecular formula $C_{16}H_{16}O_8$ and the stability of the substance to dilute acids, these facts enabled structure (XXXIV) to be allocated to the ketonic oxidation product. Whilst the ketone (XXXIV) was insoluble in aqueous sodium hydrogen carbonate, it dissolved in and was rapidly decomposed by aqueous sodium

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[12] Barton and de Mayo, *J.*, 1956, 142.
[13] Braun, Herz, and Rabindran, *J. Amer. Chem. Soc.*, 1956, **78**, 4423.
[14] Schönberg and Sidky, *J. Org. Chem.*, 1956, **21**, 476.

hydroxide, an observation which was not thought to invalidate structure (XXXIV) because the negative ferric reaction excluded the possibility that a phenolic or enolic hydroxyl group was present and also because a similar compound [(XXXIV) less the hydroxyl group] obtained from citromycetin behaved in the same way. On the other hand, there is no evidence as to the way in which the methylene group is lost during the formation of (XXXIV) in spite of the fact that the α-ketol system had an obvious origin in the epoxide (XXXV).

Ozonolysis of methyl anhydrodi-*O*-methylfulvate (XVIII) did not gave a tractable product and was of no interest except in that no volatile carbonyl compounds resulted. Oxidation of this ester with lead tetra-acetate or selenium dioxide also gave negative results. Chromic acid oxidation was not satisfactory but gave small quantities of a neutral product $C_{17}H_{14}O_8$ considered to have structure (XXVI) because of the analogy with the chromic acid oxidation of methyl di-*O*-methylcitromycetin (II; R = Me) to the corresponding citromycetinone (VI). In confirmation of this view, the new compound did not react with 2 : 4-dinitrophenylhydrazine and was transparent in the hydroxyl stretching region, whereas peaks at 1643, 1614, 1600, 1553, and 1468 cm.$^{-1}$ were consistent with the presence of a chromone ring. The absorption at 1754 cm.$^{-1}$ was noticeably intensified and clearly included that of the lactone ring in (XXXVI). On general grounds, this type of lactone was expected to absorb near 1745 cm.$^{-1}$ and this frequency has already been attributed to the somewhat similar lactone ring A in a degradation product (XXXVII) of fuscin.[15]

In conclusion, reference must be made to the decarboxylation and infrared spectrum of fulvic acid. The study of citromycetin (II; R = H) was greatly facilitated by the ease with which hot aqueous mineral acids eliminated the carboxyl group without affecting the remainder of the molecule but, in sharp contrast, fulvic acid was stable under these conditions. The chief difference in the environment of the carboxyl groups of structures (I; R = H) and (II; R = H) appeared to be the proximity of the pyrone-carbonyl group to the carboxyl group in (I; R = H). Although these groups would not normally interact with the formation of the lactol having the partial structure (XXXVIII), in strongly acidic media fulvic acid (I; R = H) could yield the oxonium ion (XXXIX) of which the lactol (XXXVIII) is the carbinol base; dilution of the solution would therefore regenerate fulvic acid of structure (I; R = H). As the positive charge on the ion is dispersed, its ability to promote extrusion of the carboxyl group would be much reduced. Although our investigations on these points are not yet complete we believe that the difficulty of isolating oxonium salts from fulvic acid may be at least partly due to disadvantageous solubility relations.

The infrared absorption spectrum of fulvic acid (and of anhydrofulvic acid) was curious in that carbonyl stretching frequencies did not occur above 1645 cm.$^{-1}$. Thus there was no band which could be specifically attributed to the carboxyl group and, in addition, the absorption due to the chromone-carbonyl group had been shifted to longer wavelengths. When fulvic acid was written as in (XL) it became obvious that the conditions for strong hydrogen-bonding were particularly favourable and would result in a spectrum of the observed type.

The structural connections between fulvic acid (I), citromycetin (II; R = H), and fusarubin (XLI) have been discussed elsewhere,[16] and related synthetical investigations will be published separately.

EXPERIMENTAL

Fulvic Acid (I; R = H).—(*a*) *Carpenteles brefeldianum* Dodge (Shear) (L.S.H.T.M. Catalogue No. P157) was incubated on Raulin–Thom medium in the manner described by Oxford, Raistrick,

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[15] Barton and Hendrickson, *J.*, 1956, 1028.

[16] Dean, Eade, Moubasher, and Robertson, *Nature*, 1957, **179**, 366.

and Simonart,[1] except that a somewhat higher temperature (26°) was used to allow the isolation of satisfactory yields of fulvic acid from the culture medium after an appreciably shorter period (28 days) of growth. Obtained by acidification of the culture fluid, the crude acidic pigment was completely soluble in aqueous sodium hydrogen carbonate and so the corresponding part of the original purification procedure was omitted in favour of extraction with ethyl acetate in which fulvic acid dissolved, leaving a residue which has not yet been examined closely. Further purification was effected by repeated crystallisation from anhydrous instead of aqueous dioxan and gave fulvic acid in pale yellow prisms, m. p. 244° (vigorous decomp.), which became brown and softened above 200° (Found, on a sample dried for 8 hr. at 110° *in vacuo* : C, 54·4; H, 4·1. Calc. for $C_{14}H_{12}O_8$: C, 54·6; H, 3·9%) [Oxford *et al.*[1] give m. p. 246° (decomp.)]. In alcohol, fulvic acid gave a deep green ferric reaction and had $\lambda_{max.}$ 225, 318, and 343 mμ (log ε 4·47, 4·07, 4·05). This acid had infrared bands at 3550 (sharp; free OH), 3540—2500 (broad; ill-defined; bonded OH), 1645, 1567, 1497, 1420, 1362, 1340, 1290, 1256, 1222, 1172 (broad), 1121, 1092, 1062, 975, 950, 926 (broad), 877, 855, 820, 807, 769, and 763 cm.$^{-1}$. Fulvic acid (0·15 g.) was recovered when its bright yellow solution in sulphuric acid monohydrate (15 ml.) was kept overnight and then poured on ice.

(*b*) Anhydrofulvic acid (0·2 g.) was heated under reflux with 2N-aqueous sulphuric acid (50 ml.). After 1 hr. the cooled solution was diluted to precipitate fulvic acid, which formed prisms (0·13 g.), m. p. and mixed m. p. 244° (decomp.), from dioxan; identification was effected spectroscopically. A similar experiment was conducted under nitrogen for 12 hr.; a little carbon dioxide was evolved and was detected by means of barium hydroxide but the only crystalline product was again fulvic acid.

Alkaline Degradation of Fulvic Acid.—(a) *Acetone and acetic acid.* A mixture of fulvic acid (1·5 g.) and 2N-aqueous sodium hydroxide was slowly distilled during ½ hr., with the addition of water to maintain the original volume. The distillate yielded acetone 2 : 4-dinitrophenylhydrazone which was purified by chromatography from light petroleum (b. p. 60—80°) on a column of aluminium oxide and then crystallised from aqueous alcohol, forming yellow needles, m. p. and mixed m. p. 124—125°.

Acidification of the residual alkaline liquor gave a yellow-brown amorphous precipitate which was removed. Distillation of the filtrate gave 0·94 equivalent of volatile acids including acetic acid, which was identified as 2-methylbenziminazole, m. p. and mixed m. p. 173—174°.

(b) *Carbon dioxide.* Fulvic acid (2·05 g.) was heated under reflux with 10% aqueous sodium hydroxide (30 ml.) in nitrogen for ½ hr. The cooled mixture was acidified carefully with 2N-sulphuric acid and then warmed gently during the passage of a slow stream of nitrogen from the mixture into standardised barium hydroxide solution. Titration of the unchanged alkali showed that 0·812 mol. of carbon dioxide had been produced. In similar experiments with anhydrofulvic acid, 0·850 mol. of carbon dioxide was produced, but methyl anhydrodi-*O*-methylfulvate gave negligible quantities of this gas.

*Methyl Di-*O*-methylfulvate* (I; R = Me).—(*a*) Methylation of fulvic acid with diazomethane or with methyl sulphate and sodium carbonate as described by Oxford *et al.*[1] gave methyl di-*O*-methylfulvate (I; R = Me), crystallising from aqueous dioxan in prisms, m. p. 186—187° (decomp.) (the previous authors quote m. p. 186—188°) (Found : C, 58·4, 58·4; H, 5·1, 5·0. Calc. for $C_{17}H_{18}O_8$: C, 58·3; H, 5·2%). This compound had $\lambda_{max.}$ 230, 282, and 300 mμ (log ε 4·41, 4·08, 3·99) with an inflection at 248 mμ (log ε 4·04). Infrared absorption occurred at 3448 (OH), 3050 (aromatic CH), 1742 (CO_2Me), 1647, 1592, 1550, 1420, 1400, 1312, 1274, 1258, 1235, 1205, 1171, 1153, 1111, 1039. 1022, 1010, 994, 953, 884, 866, 840, 813, 800, and 726 cm.$^{-1}$. This ester was insoluble in dilute sodium hydroxide and gave no ferric reaction.

(*b*) The solution obtained by heating methyl anhydrodi-*O*-methylfulvate (1·0 g.) for ¾ hr. with methanol (20 ml.) and 2N-sulphuric acid (20 ml.) was diluted with water (40 ml.) and concentrated under reduced pressure to 35 ml. in order to precipitate unchanged methyl anhydrodi-*O*-methylfulvate (0·1 g.). On being kept, the filtrate deposited methyl di-*O*-methylfulvate in prisms (0·85 g.), m. p. and mixed m. p. 191° (decomp.). This ester was not very soluble in dioxan or water but dissolved readily in mixtures of the two solvents.

*Methyl Tri-*O*-methylfulvate* (XX).—(*a*) Methanol (5 ml.) containing sodium methoxide (from 0·2 g. of sodium) was added to methyl anhydrodi-*O*-methylfulvate (1·0 g.) suspended in warm absolute methanol (10 ml.) which soon cleared, then became green, and finally brown. In a few days *methyl tri-*O*-methylfulvate* (XX) separated and was purified from benzene–light petroleum (b. p. 40—60°), forming cubes, m. p. 176—177° (decomp.), devoid of a ferric reaction

[Found, in specimen dried for 3 hr. at 80° *in vacuo*: C, 59·3; H, 5·8; OMe, 32·6. $C_{14}H_8O_4(OMe)_4$ requires C, 59·3; H, 5·5; OMe, 34·1%].

(*b*) From a 3% solution (50 ml.) of hydrogen chloride in absolute methanol containing methyl anhydrodi-*O*-methylfulvate (1·0 g.) a crystalline precipitate (0·8 g.) separated during 2 days. Purified from methanol, this solid gave methyl tri-*O*-methylfulvate (XX) in prisms, m. p. and mixed m. p. 175—176° (decomp.), readily soluble in chloroform and acetone, and having $\lambda_{max.}$ 230, 283, and 300 mμ (log ε 4·42, 4·09, 4·01) with an inflection at 248 mμ (log ε 4·06).

Methyl O-*Ethyldi*-O-*methylfulvate*.—This *ethyl ether* resulted when alcohol was substituted for methanol in the preceding preparations (*a*) and (*b*) and crystallised from alcohol in irregular plates, m. p. 208—209° (decomp.) (Found: C, 60·2, 60·3; H, 5·7, 5·8. $C_{19}H_{22}O_8$ requires C, 60·3; H, 5·9%).

Anhydrofulvic Acid (XVIII; OH for OMe).—When a solution of fulvic acid (8·0 g.) in 70% acetic acid (1 l.) was boiled for 10 min. and cooled, anhydrofulvic acid separated in long yellow crystals (6·4 g.) and on recrystallisation formed rods (from aqueous dioxan) or flat parallelepipeds (from the anhydrous solvent), m. p. 245—246° (after darkening near 235°) [Found, in sample dried to constant weight in a vacuum at 100°: C, 58·1; H, 3·6; *C*-Me (Kuhn–Roth), 5·4. Calc. for $C_{14}H_{10}O_7$: C, 57·9; H, 3·5; *C*-Me, 5·2%] (Oxford *et al.* give m. p. 242—243°). Anhydrofulvic acid (1·0 g.) was not hydrated when boiled for 4 hr. in dioxan (30 ml.) containing water (10 ml.). In alcohol it gave a deep green ferric reaction and had $\lambda_{max.}$ 233, 341, and 387 mμ (log ε 4·28, 4·03, 4·34). Because of the general similarity of this compound to fulvic acid and in particular the absence of noticeable m. p. depression in their mixtures, this compound was invariably characterised by its infrared spectrum: 3200 (bonded OH), 1639, 1623, 1587, 1562, 15·48, 1502, 1404, 1282, 1271, 1205, 1182, 1114, 1042, 990, 978, 914, 815, and 781 cm.$^{-1}$.

Methyl Anhydrodi-O-*methylfulvate* (XVIII).—(*a*) During the vigorous reaction between finely divided anhydrofulvic acid (10 g.), suspended in ether (200 ml.) containing methanol (20 ml.), and ethereal diazomethane [from nitrosomethylurea (30 g.)] the original yellow solid was replaced by buff-coloured solid which was collected next day and purified from methanol, giving *methyl anhydrodi*-O-*methylfulvate* in faintly yellow prisms (9·8 g.), m. p. 193—194° (decomp.), unchanged on sublimation but depressed to 170° on admixture with methyl di-*O*-methylfulvate [Found: C, 61·6, 61·5; H, 4·7, 5·0; OMe, 26·7%; *M* (micro-Rast), 324. $C_{14}H_7O_4(OMe)_3$ requires C, 61·4; H, 4·9; OMe, 28·0%; *M*, 332]. This compound was much more soluble in chloroform, hot benzene, or acetone than in methanol or ethyl acetate, and was unaffected by boiling dioxan (20 ml.) containing water (5 ml.) during 6 hr. It had $\lambda_{max.}$ 235, 257, 318, and 348 mμ (log ε 4·12, 4·09, 4·13, 4·20) and infrared bands at 3050 (aromatic and olefinic CH), 1751 (CO_2Me), 1647, 1597, 1562, 1425, 1403, 1314, 1277, 1258, 1133, 1203, 1190, 1163, 1153, 1110, 1042, 1024, 1012, 996, 990, 953, 878, 866, 839, 810, 799, 789, 751, and 727 cm.$^{-1}$.

(*b*) The mixture obtained by the addition of methyl sulphate (60 ml.) in methanol (30 ml.) to a stirred solution of anhydrofulvic acid (10 g.) in N-aqueous sodium carbonate (200 ml.) was treated gradually with 2N-aqueous sodium carbonate (400 ml.) so as to maintain slight alkalinity; the temperature was not allowed to rise above 40°. After 2 hr., the solid was collected, triturated with dilute aqueous sodium hydroxide, and purified from methanol, giving methyl anhydrodi-*O*-methylfulvate in prisms (9·3 g.), m. p. 193°, not depressed by admixture with a specimen from (*a*) (Found: C, 61·3; H, 4·7%). Acidification of the alkaline liquors gave a slight precipitate partly soluble in aqueous sodium hydrogen carbonate. Regained from this solution and crystallised from aqueous methanol, this acidic fraction supplied *anhydrodi*-O-*methylfulvic acid* (XVIII; CO_2H for CO_2Me) in pale yellow plates (0·1 g.), m. p. 219—220° (decomp.) (Found, on specimen dried *in vacuo* at 120° for 8 hr.: C, 60·0; H, 4·6%; equiv., 306. $C_{15}H_{13}O_5{\cdot}CO_2H$ requires C, 60·4; H, 4·4%; equiv., 318).

(*c*) When sublimed at 180°/0·005 mm., methyl di-*O*-methyl-, methyl tri-*O*-methyl-, or methyl *O*-ethyldi-*O*-methyl-fulvate gave methyl anhydrodi-*O*-methyl-fulvate, m. p. and mixed m. p. 193° (decomp.).

(*d*) Kept in boiling acetic acid (5 ml.) for 10 min., methyl di-*O*-methyl- (0·5 g.), methyl tri-*O*-methyl- (0·5 g.), or methyl *O*-ethyldi-*O*-methyl-fulvate (0·5 g.) gave methyl anhydrodi-*O*-methylfulvate which was precipitated with water and had m. p. and mixed m. p. 193° after purification from methanol (yield, 0·42 g.).

Interaction of methyl anhydrodi-*O*-methylfulvate (0·5 g.) and piperonaldehyde (0·5 g.) in warm methanol (20 ml.) containing sodium methoxide (from 0·2 g. of sodium) for 10 min. gave the *piperonylidene derivative* (0·4 g.) which separated from the mixture in two weeks and then formed long orange needles, m. p. 217—218° (decomp.), from methanol [Found, on specimen dried for 3 hr. in a vacuum at 80° : C, 64·8; H, 4·4; *C*-Me (Kuhn–Roth), 0. $C_{25}H_{20}O_9$ requires C, 64·7; H, 4·3%].

*Ethyl Anhydrodi-*O*-ethylfulvate* (XVIII; OEt for OMe).—Prepared from anhydrofulvic acid (1·0 g.) as in (*a*) above, but by using diazoethane instead of diazomethane, *ethyl anhydrodi-*O*-ethylfulvate* was freed from traces of phenolic material by means of cold dilute sodium hydroxide and then crystallised from alcohol, forming yellow plates (0·5 g.), m. p. 149—151° (decomp.) [Found, on specimen dried for 3 hr. at 80° *in vacuo* : C, 64·1, 64·0; H, 5·6, 6·0; OEt, 33·0. $C_{14}H_7O_4(OEt)_3$ requires C, 64·2; H, 5·9; OEt, 36·1%].

*Methyl Deoxydi-*O*-methylfulvate* (XIX).—Shaken with 10% palladium–charcoal (3 g.) a solution of methyl anhydrodi-*O*-methylfulvate (3 g.) in ethyl acetate (250 ml.) absorbed hydrogen (240—270 ml. Calc. for 1 mol. : 215 ml.). Concentration of the filtrate to 50 ml. induced the separation of *methyl deoxydi-*O*-methylfulvate* which formed prisms (2·3 g.), m. p. 199—200° (from ethyl acetate), with a negative ferric reaction [Found, on specimen dried for 3 hr. at 100° *in vacuo* : C, 61·1, 61·0, 61·3, 61·0; H, 5·4, 5·6, 5·3; OMe, 27·3, 27·3, 27·5, 27·2. $C_{14}H_9O_4(OMe)_3$ requires C, 61·1; H, 5·4; OMe, 27·8%]. This ester, which had $\lambda_{max.}$ 230, 282, and 303 mμ (log ε 4·40, 4·08, 4·00) and infrared absorption at 1740 (CO_2Me), 1647 (chromone C:O), 1623, 1603, and 1580 (shoulder) cm.$^{-1}$, was insoluble in dilute aqueous sodium hydroxide, and did not react under a wide variety of conditions with 2 : 4-dinitrophenylhydrazine, hydroxylamine, *o*-phenylenediamine, or piperonaldehyde. When kept in dioxan containing an equal volume of 2N-aqueous sulphuric acid at 100° for 1 hr. it was recovered unchanged, and it resisted the action of boiling acetic anhydride containing sodium acetate for 1 hr.

The residue from evaporation of the mother-liquors of methyl deoxydi-*O*-methylfulvate was purified by chromatography from benzene on aluminium oxide, giving a *compound* which separated from benzene–light petroleum (b. p. 60—80°) in irregular prisms (0·16 g.), m. p. 180—181° (Found : C, 60·8, 61·0; H, 5·8, 5·9. $C_{17}H_{10}O_7$ requires C, 60·7; H, 6·0%). Elution of the column with 1 : 9 acetone–benzene gave methyl anhydrodi-*O*-methylfulvate (0·14 g.), m. p. and mixed m. p. 197—198°.

*Condensation of Methyl Anhydrodi-*O*-methylfulvate with* o*-Phenylenediamine. Methyl 1 : 2 : 3 : 4 : 9 : 10-Hexahydro-6 : 7-dimethoxy-2-methyl-10-oxo-9-oxa-3 : 1′-diazaindeno(2′ : 3′-2 : 3)-anthracene-5-carboxylate* (XXIV).—A mixture of methyl anhydrodi-*O*-methylfulvate (0·5 g.) and *o*-phenylenediamine (0·5 g.) in alcohol (8 ml.) containing acetic acid (1 ml.) was heated under reflux for 1 hr. Addition of water (20 ml.) to the cold solution produced a turbidity that was cleared by charcoal, whereupon neutralisation of the filtrate gave a cream precipitate (0·6 g.) which, on crystallisation from alcohol, gave the *oxadiazaindenoanthracene* (XXIV) in prisms, m. p. 209—211° after being dried at 100° *in vacuo* [Found : C, 64·6, 64·1; H, 5·4, 4·9; N, 6·1; OMe, 22·8. $C_{20}H_{13}O_3N_2(OMe)_3$ requires C, 65·4; H, 5·3; N, 6·6; OMe, 22·0%]. This compound was readily soluble in dilute acids but gave only a yellow colour on treatment with nitrous acid followed by alkaline β-naphthol. The *picrate* separated from an alcoholic mixture and on purification from acetic acid formed plates, m. p. 263° (decomp.) (Found, on specimen dried for 2 hr. at 120° *in vacuo* : C, 53·9; H, 3·8; N, 10·5, 10·6. $C_{29}H_{25}O_{13}N_5$ requires C, 53·5; H, 3·9; N, 10·8%).

Methyl 6 : 7-Dimethoxy-6′-methylpyridino(4′ : 3′-2 : 3)chromone-5-carboxylate 1′-Oxide (XXVII).—Methyl anhydrodi-*O*-methylfulvate (1·0 g.) was heated with hydroxylamine hydrochloride (0·65 g.) and hydrated sodium acetate (1·4 g.) in boiling methanol (20 ml.) for 1 hr. and the dark yellow filtered mixture was concentrated to 10 ml., diluted with water (10 ml.), and kept in air for several days. The resulting semi-crystalline mass (0·15 g.) was repeatedly purified from alcohol, giving the *pyridine oxide* (XXVII) in small yellow prisms, m. p. 266° (decomp.), insoluble in dilute aqueous sodium hydroxide and 2N-hydrochloric acid, but soluble in 10N-hydrochloric acid (Found : C, 59·1, 59·1; H, 4·5, 4·1; N, 4·0. $C_{17}H_{15}O_7N$ requires C, 59·1; H, 4·4; N, 4·1%). This compound did not give a ferric reaction but under conditions in which methyl anhydrodi-*O*-methylfulvate was inert it liberated iodine from hydrogen iodide, identified on extraction into chloroform and subsequently into aqueous potassium iodide by the usual characteristic tests with starch and thiosulphate. The pyridine oxide had $\lambda_{max.}$ 242, 282, and 326 mμ (log ε 4·08, 4·49, 4·38) and infrared bands at 3040 (aromatic CH), 1752 (CO_2Me), 1664 (conjugated C:O), 1633, 1609, 1435, 1418, 1335, 1311, 1285, 1228, 1180, 1095, 1020, 1012, 982, 962, 935, 894, 856, 799, 788, and 758 cm.$^{-1}$.

Alkaline Degradation of Methyl Anhydrodi-O-*methylfulvate. 2-Acetyl-7-hydroxy-4 : 5-dimethoxyindane-1 : 3-dione* (X).—When a stream of nitrogen was led through a solution of methyl anhydrodi-*O*-methylfulvate (3·32 g.) in boiling dioxan or methanol (10 ml.) containing 2N-aqueous sodium hydroxide (80 ml.) for ¾ hr. the effluent gases gave negative tests for carbonyl compounds. On being kept at 0° for 8 hr. the brown solution deposited a yellow sodium salt (0·5 g.) and acidification of the filtrate from this gave a brown solid which with warm 2N-aqueous sodium hydroxide (20 ml.) yielded a further quantity (1·1 g.) of the yellow salt. Decomposed by acids, the sodium salts furnished the 2-acetyl-7-hydroxy-4 : 5-dimethoxyindane-1 : 3-dione (X) in pale yellow needles (1·2 g.), m. p. 157°, having a purple-brown ferric reaction in methanol [Found, on a specimen sublimed at 150°/0·01 mm. : C, 59·2; H, 4·5; OMe, 23·6. Calc. for $C_{11}H_6O_4(OMe)_2$: C, 59·1; H, 4·6; OMe, 23·5%]. Admixed with the so-called 6 : 7-dimethoxy-2-methylchromone-5-carboxylic acid prepared from citromycetin this had m. p. 157°; the two specimens had identical ultraviolet and infrared absorption spectra : $\lambda_{max.}$ 300 mμ (log ε 4·52); infrared bands at 3400 (phenolic OH), 1704, 1645, 1605, 1572 (shoulder), 1508, 1408, 1330, 1302, 1280, 1221, 1197, 1138, 1133, 1007, 945, 926, 907, 897, 845, 832, and 815 cm.$^{-1}$. The oxime separated from alcohol in small yellow needles, m. p. 213—214°, unchanged on admixture with the derivative from citromycetin (Found : C, 55·5, 55·5; H, 4·6, 4·8; N, 4·6. Calc. for $C_{13}H_{13}O_6N$: C, 55·9; H, 4·7; N, 5·0%). On methylation with methyl iodide (5 ml.) and potassium carbonate (1·5 g.) in boiling acetone (50 ml.) the indanedione gave a red gum which was purified from benzene with aluminium oxide and then extracted repeatedly with boiling light petroleum. The extract gradually crystallised and then on purification from the same solvent gave the *dimethyl ether* in needles, m. p. 77°, insoluble in aqueous sodium hydroxide and having a negative ferric reaction [Found : C, 61·2; H, 5·7; OMe, 43·0. $C_{13}H_{10}O_4(OMe)_4$ requires C, 61·6; H, 5·5; OMe, 42·5%].

After removal of the indanedione, the acidified hydrolysate was distilled almost to dryness to recover the volatile acids (1·07 mol. by titration). After treatment of the distillate with potassium permanganate and destruction of the excess of the oxidant by hydrogen peroxide and dilute sulphuric acid, re-distillation afforded volatile acid (1·03 ml.), identified as acetic acid by conversion into 2-methylbenziminazole, m. p. and mixed m. p. 175—176°, and into acetanilide, m. p. and mixed m. p. 112°.

Ozonolysis of Methyl Anhydrodi-O-*methylfulvate*.—A stream of ozone and oxygen was passed through a solution of methyl anhydrodi-*O*-methylfulvate (1·0 g.) in chloroform (40 ml.) cooled in ice-salt until ozone was no longer absorbed (½ hr.). The residue left on evaporation was treated with water (50 ml.) and, next day, the mixture was heated on the steam-bath for ½ hr., giving a cream solid which did not crystallise. With aqueous 2 : 4-dinitrophenylhydrazine sulphate the solution gave an amorphous precipitate which was still amorphous after purification from benzene on a column of aluminium oxide; a sample of the solution was distilled but the distillate did not give a precipitate with this reagent. In another experiment the distillate from the reaction mixture contained 0·3 equivalent of volatile acids, but the dried sodium salts of these failed to give a crystalline derivative with *o*-phenylenediamine hydrochloride.

Methyl 6 : 7-Dimethoxy-6′-methyl-α-pyrono(4′ : 3′-2 : 3)chromone-5-carboxylate (XXXVI).—Obtained by the dropwise addition of chromic oxide (1·4 g.) in 80% acetic acid (10 ml.) at 50° during 1 hr., the mixture was kept for an hour at the same temperature. Next day, the solvent was evaporated, the residue was stirred with water and extracted with chloroform (8 × 15 ml.), the extract was washed with N-aqueous sodium hydroxide, N-hydrochloric acid, and water, dried (Na_2SO_4), and evaporated, and the product extracted with boiling benzene to remove methyl anhydrodi-*O*-methylfulvate (0·8 g.). The residual solid was purified from alcohol, giving the *α-pyronochromone* (XXXVI) in pale cream plates (110 mg.), m. p. 250° (decomp.), inert towards ferric chloride and 2 : 4-dinitrophenylhydrazine sulphate [Found : C, 59·4; H, 4·1; OMe, 25·5. $C_{14}H_5O_5(OMe)_3$ requires C, 59·0; H, 4·1; OMe, 26·9%].

Oxidation of Methyl Anhydrodi-O-*methylfulvate with Potassium Permanganate*.—(a) *6-Hydroxy-3 : 4-dimethoxy-2-methoxycarbonylbenzoic acid* (III) *and methyl 4-hydroxy-6 : 7-dimethoxycoumarin-5-carboxylate* (V; R = CO_2Me). To methyl anhydrodi-*O*-methylfulvate (2·5 g.) in boiling acetone (200 ml.) potassium permanganate (9 g.) was added during 2 hr. and 15 min. later the mixture was cleared by means of water (100 ml.) and sulphur dioxide and concentrated under reduced pressure to *ca.* 120 ml. The products were extracted with ether (8 × 50 ml.) and washed with water, and the acids were separated with aqueous sodium hydrogen carbonate (2 × 25 ml.) from a neutral non-ketonic substance which formed prisms (30 mg.), m. p. 206°

(decomp.), from methanol (Found : C, 53·8; H, 4·2%). Liberated by dilute hydrochloric acid and isolated by ether, the acidic fraction formed a gum (1·2 g.) partly soluble in boiling benzene (2 × 10 ml.). Crystallised from aqueous dioxan the residue, insoluble in benzene, gave *methyl* 4-*hydroxy*-6 : 7-*dimethoxycoumarin*-5-*carboxylate* in long cream prisms (0·1 g.), m. p. 255—256° (decomp.), having a brownish ferric reaction but soluble in sodium hydrogen carbonate solutions and devoid of carbonyl activity [Found, in a specimen dried for 7 hr. at 110° *in vacuo* : C, 55·5; H, 4·3; OMe, 34·1, 32·9. $C_{10}H_3O_4(OMe)_3$ requires C, 55·7; H, 4·3; OMe, 33·2%].

When the concentrated benzene extract was kept 6-hydroxy-3 : 4-dimethoxy-2-methoxycarbonylbenzoic acid (III) separated and on recrystallisation from benzene formed prisms (0·7 g.), m. p. 147—148°, unaffected on admixture with a sample prepared from citromycetin [Found: C, 51·8; H, 4·6; OMe, 36·4. Calc. for $C_8H_3O_4(OMe)_3$: C, 51·6; H, 4·7; OMe, 36·3%]. This acid was characterised by its conversion [4] into 6-ethoxy-3 : 4-dimethoxyphthalic anhydride (IV), m. p. 195°, identical with a specimen prepared by the method of Mackenzie and Robertson [3] (Found : C, 57·5; H, 5·0. Calc. for $C_{12}H_{12}O_6$: C, 57·1; H, 4·8%).

(b) 3-*Acetoxymethyl*-6 : 7-*dimethoxy*-5-*methoxycarbonylchromone*-2-*carboxylic Acid* (XXXI).—Powdered potassium permanganate (2·0 g.) was added to a solution of methyl anhydrodi-*O*-methylfulvate (2·0 g.) in acetone (200 ml.) at 0° during 5 hr. and, after clarification with water (100 ml.) and sulphur dioxide, the mixture was concentrated under reduced pressure at below 40°, and extracted with ethyl acetate (6 × 25 ml.). The extracts were washed with water and then dilute sodium hydrogen carbonate (2 × 25 ml.), and evaporated, leaving methyl di-*O*-methylfulvate (0·5 g.), m. p. and mixed m. p. 192° (decomp.). Acidification of the alkaline extract furnished a buff solid (0·55 g.) which was leached with boiling benzene (2 × 10 ml.) and crystallised from aqueous methanol, giving the *acid* (XXXI) in needles, m. p. 233—234° (decomp.), devoid of a ferric reaction and of carbonyl activity towards 2 : 4-dinitrophenylhydrazine sulphate [Found: C, 53·3; H, 4·3; OAc, 10·0%; equiv. (hydrolysis by 0·04N-sodium hydroxide, at 100° for ½ hr., back-titration by 0·01N-hydrochloric acid), 123. $C_{15}H_{13}O_8{\cdot}OAc$ requires C, 53·7; H, 4·2; OAc, 11·3%; equiv. (3 acid functions), 127]. This acid had $\lambda_{max.}$ 221, 314 mμ (log ε 4·39, 3·97), inflections at 235, 292 mμ (log ε 4·35, 3·89), and was readily soluble in aqueous sodium hydrogen carbonate but gave a negative Zimmermann reaction.[14] Suspended in ether, the acid reacted rapidly with diazomethane, to furnish the *methyl ester* which separated from methanol in needles, m. p. 195° [Found : 54·5; H, 4·5; OMe, 29·9. $C_{14}H_6O_6(OMe)_4$ requires C, 54·8; H, 4·6; OMe, 31·4%]. The infrared spectrum of the methyl ester included peaks at 3050, 1730 (broad), 1641, 1605 (shoulder at 1582), and 1479 cm.$^{-1}$.

A careful search failed to reveal the presence of 6-hydroxy-3 : 4-dimethoxy-2-methoxycarbonylbenzoic acid in this oxidation mixture.

(c) Experiment (*b*) above was repeated, except that hydrated magnesium sulphate (0·55 g.) in water (60 ml.) was added before the oxidant. Methyl anhydrodi-*O*-methylfulvate (0·9), m. p. and mixed m. p. 192° (decomp.), was recovered, but the chromone-2-carboxylic acid (XXXI) was not detected, and the oily acidic fraction gave the intense ferric reaction characteristic of the phenolic acid (III) although this could not be obtained pure.

*Oxidation of Methyl Anhydrodi-*O*-methylfulvate with Perbenzoic Acid. Methyl* 2-1′-*Hydroxyacetonyl*-6 : 7-*dimethoxychromone*-5-*carboxylate* (XXXIV).—In preliminary experiments, methyl anhydrodi-*O*-methylfulvate destroyed 1·02 mol. of perbenzoic acid in ½ hr., but only 0·38 mol. in the next 3 days. Methyl deoxydi-*O*-methylfulvate was hardly attacked by this reagent.

Methyl anhydrodi-*O*-methylfulvate (0·95 g.) in chloroform (10 ml.) at 0° was allowed to react with perbenzoic acid (1·05 mol.) in the same solvent. After 1 hr., the solution was freed from acidic material with aqueous sodium hydrogen carbonate, washed with water, dried (Na_2SO_4), and evaporated at room temperature. The residue was fractionally crystallised from benzene, giving the *compound* (XXXIV) in faintly yellow feathery needles, m. p. 202—204° (decomp.), $\lambda_{max.}$ 314 mμ (log ε 3·99), with a negative ferric reaction (Found : C, 57·4; H, 4·5. $C_{16}H_{16}O_8$ requires C, 57·1; H, 4·8%). With 2 : 4-dinitrophenylhydrazine sulphate, this ketone gave an orange precipitate which resinified when heated in organic solvents. The ketone was insoluble in aqueous sodium carbonate but dissolved rapidly in dilute aqueous sodium hydroxide although it could not be recovered by acidification of the resulting solution; when heated for 1 hr. with N-sulphuric acid (1 ml.), water (20 ml.), and methanol (20 ml.), the ketone was recovered almost quantitatively by removal of the methanol and refrigeration at 0°.

From the fractionation, there was obtained a very small quantity of a substance which crystallised from ethyl acetate in small rods, m. p. 237—238° (decomp.) (Found : C, 54·1, 54·1; H, 4·2, 4·3%).

Spectra.—Ultraviolet spectra were determined in alcohol at concentrations near $\text{M} \times 10^{-3}$ on a Unicam S.P. 300 spectrophotometer. Infrared spectra were determined in paraffin mulls by means of a Grubb-Parsons double-beam spectrophotometer.

The analyses were performed by Mr. A. S. Inglis, M.Sc., and his associates of this Department.

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