171. *Derivatives of Hydroxyquinol. Part III.*[1] *The Structure of Fulvic Acid: Syntheses and Reactions of Degradation Products of Fulvic Acid and of Citromycetin.*

By F. M. Dean and D. R. Randell.

Methyl 6,7-dimethoxy-2-methylchromone-5-carboxylate has been synthesised and shown to reproduce the spectroscopic properties of the appropriate derivatives of fulvic acid and to be converted by alkali in the predicted way into 2-acetyl-7-hydroxy-4,5-dimethoxyindane-1,3-dione. These facts taken with data from nuclear magnetic spectra confirm the structure for fulvic acid proposed earlier.

2-Acetyl-3-hydroxy-5,6-dimethoxybenzoic acid and 4-hydroxy-6,7-dimethoxycoumarin-5-carboxylic acid have been synthesised and identified with degradation products of citromycetin. 3-Acetyl-4-hydroxy-6,7-dimethoxycoumarin-5-carboxylic acid has also been synthesised and shown to differ from the product which had been obtained by degradation of citromycetin and allocated this structure.

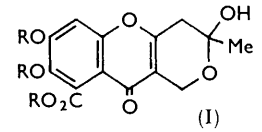
Although studies of fulvic acid were believed to indicate structure (I; R = H) for this compound, none of the simpler degradation products appeared to retain the chromone ring fundamental to this structure.[2] Thus alkaline hydrolysis of methyl anhydrodi-*O*-methylfulvate (II) was considered to involve opening of both heterocyclic rings, giving an intermediate (III) which then suffered both β-diketonic fission and retroaldol condensation leading to an *o*-acetoacetylbenzoic acid (IV; R = H) or its ester (IV; R = Me). A similar sequence had been invoked[3] to account for the alkaline degradation of methyl di-*O*-methylcitromycetinone (V; R = Me) which had led to a product regarded as the chromone-5-carboxylic acid (VI; R = H). This chromone was thought to be formed by cyclisation of the phenolic β-diketonic system in (IV; R = H or Me). In Part II (preceding paper), however, it was proved that this degradation product is not a chromone but the acetylindanedione (VII) formed by the alternative cyclisation of (IV; R = Me) in which the β-diketonic side-chain becomes linked, not to the hydroxyl, but to the ester grouping. The elusive chromone (VI; R = Me) has now been synthesised and converted into the indanedione (VII) in the conditions used for the degradation of methyl anhydrodi-*O*-methylfulvate (II) and methyl di-*O*-methylcitromycetinone (V; R = Me). Moreover, the close similarities in both the ultraviolet and the infrared spectra obtained from the chromone ester (VI; R = Me) and from the appropriate derivatives of fulvic acid (I; R = H) confirm that this compound is indeed a chromone; and this fact, taken with evidence from the nuclear magnetic resonance spectrum of methyl anhydrodi-*O*-methylfulvate (II), leaves no doubt that fulvic acid does have structure (I; R = H).

Unlike the corresponding trimethyl ether,[1,4] the benzyl ether (VIII) affords neither a stable iso-ozonide nor the desired aldehyde when treated with ozone: apparently the benzyl group is attacked at the same time as the propenyl substituent. Permanganate oxidation of the benzyl ether (VIII) led in moderate yield to an *o*-acetylbenzoic acid which behaved as the lactol (IX; R = CH_2Ph). Hydrogenolysis of this lactol was unsatisfactory when a palladium catalyst was used, but with Raney nickel the phenolic acid (X) was readily obtained and its properties suggested that chelation prevents its forming a lactol comparable to (IX). As mentioned in Part II, this acid is identical with one of the products obtained by alkaline degradation of methyl di-*O*-methylcitromycetinone (V; R = Me) and is presumably the substance which Hetherington and Raistrick believed to be a pyrone.

[1] Part II, preceding paper.
[2] Dean, Eade, Moubasher, and Robertson, *J.*, 1957, 3497.
[3] Robertson, Whalley, and Yates, *J.*, 1951, 2013.
[4] Dean, Randell, and Winfield, *J.*, 1959, 1071.

Although the phenolic acid (X) failed to undergo Claisen condensations with methyl acetate in a wide variety of conditions, the derived acetate [actually the lactol (IX; R = Ac)] rearranged in Baker–Venkataraman conditions. The expected β-diketone (IV; R = H) could not be obtained, however, since when liberated from its sodium salt it at once gave the chromone-5-carboxylic acid (VI; R = H): cyclisation to a chromone was expected at this point because it had been shown in Part II that indanedione formation would be restricted to the ester (IV; R = Me). In support of this view the chromone-5-carboxylate (VI; R = Me), obtained from the acid by means of diazomethane, rapidly changed into the 2-acetylindane-1,3-dione (VII) when treated with base, and the compound so made was identical with specimens obtained from degradation of fulvic acid or of citromycetin.

Of the transformation products of fulvic acid, two, methyl di-*O*-methylfulvate (I; R = Me) and methyl deoxydi-*O*-methylfulvate (XI), have been allocated structures [2] which should show much the same spectroscopic behaviour as the chromone-5-carboxylate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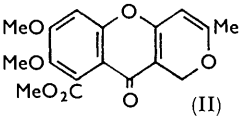
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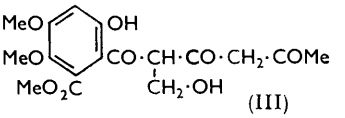
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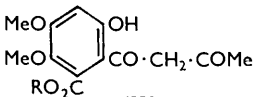
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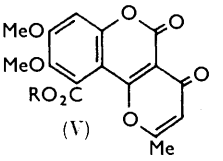
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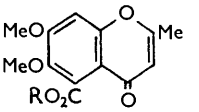
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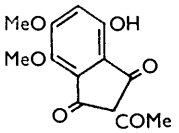
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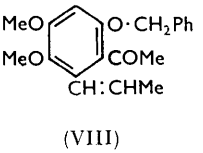
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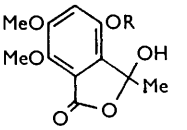
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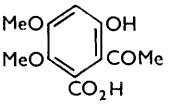
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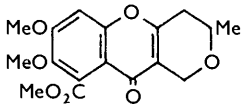
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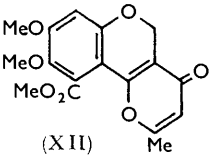
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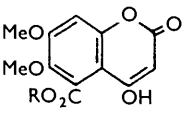
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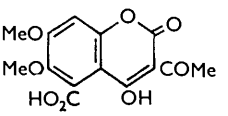
(XIV)

(VI; R = Me). With one exception (a band at 1623 cm.$^{-1}$ in the infrared spectrum of methyl deoxydi-*O*-methylfulvate for which we cannot account) the spectroscopic properties of all three compounds are so closely similar (Tables 1 and 2) that the formulation of fulvic as a chromone can hardly be doubted. Methyl anhydrodi-*O*-methylfulvate (II) and methyl di-*O*-methylcitromycetin (XII) are isomeric and have similar structural units. Their nuclear magnetic resonance spectra should therefore be comparable, Table 3 showing that this is so. That a band allocated to the methylene group in ·C·CH_2·O· should appear was particularly welcome, since this methylene group had defied chemical identification during degradative studies on fulvic acid.[2] Consequently, we are now confirmed in our belief that fulvic acid has structure (I; R = H).

A very minor hydrolysis product from methyl di-*O*-methylcitromycetinone (V; R = Me) has been formulated [3] as a 4-hydroxycoumarin-5-carboxylic acid (XIII; R = H), and the corresponding ester (XIII; R = Me) has been obtained [2] by permanganate oxidation of methyl anhydrodi-*O*-methylfulvate (II). While the phenolic acid (X) did not condense

directly with ethyl carbonate, its ethoxycarbonyl derivative cyclised to the 4-hydroxycoumarin (XIII; R = H) when treated with sodium methoxide. The synthetic product has the properties described for the citromycetin degradation product, but a direct identification could not be effected since the necessary specimen was not available and we failed to isolate the appropriate material by following the prescribed degradative route. Moreover, we failed to hydrolyse the coumarin-5-carboxylate (XIII; R = Me) to the corresponding acid using conditions designed to avoid collapse of the pyrone ring, and also failed to induce satisfactory esterification by treating the acid with diazomethane. Therefore we have been unable to identify either of the degradation products unequivocally,

TABLE 1. *Infrared frequencies* * (*cm.*$^{-1}$) *in the* 6μ *region.*

	Ester	Chromone carbonyl	Other absorption	
Me 6,7-dimethoxy-2-methylchromone-5-carboxylate ...	1735	1650	—	1600
Me di-*O*-methylfulvate	1742	1647	—	1592
Me deoxydi-*O*-methylfulvate	1740	1647	1623	1603

* In Nujol mulls. All strong bands.

TABLE 2. *Ultraviolet characteristics* (*in alcohol;* log ε *in parentheses*).

Me 6,7-dimethoxy-2-methylchromone-5-carboxylate	232 (4·34), 277 (4·03), 306 mμ (4·01)
Me di-*O*-methylfulvate	230 (4·41), 282 (4·08), 300 mμ (3·99)
Me deoxydi-*O*-methylfulvate	230 (4·40), 282 (4·08), 303 mμ (4·00)

TABLE 3. *Nuclear magnetic resonance spectra* [a] *of methyl di*-O-*methylcitromycetin* (XII) *and of methyl anhydrodi*-O-*methylfulvate* (II).

Band	Compound (XII)	Compound (II)	Relative intensity	Assignment
0–1	31·3 [a]	19·3 [a]	1	ArH
0–2	49·2	80·0	1	C:CH [b]
0–3	87·5	85·5	2	O·CH_2·C: [c]
0–4	133	136	3	OCH_3
0–5	135·5	139·5	3	OCH_3
0–6	138·5	143	3	OCH_3
[0–7]	180 [d]	213 [d]	$\ll$1	?
0–8	199	219	3	:C·CH_3 [b]

[a] H^1 Resonance at 40·00 Mc./sec. in chloroform as solvent and with chloroform as reference compound. Shifts in c./sec. [b] In ·CH:C·CH_3 but not in C:CH·CH_3. [c] In O·CH_2·C: but not in O·CH_2·CH:. [d] The source of these weak signals is not known.

though the spectroscopic and other data leave little doubt that these products have been correctly formulated.

Finally, the 4-hydroxycoumarin (XIII; R = H) was converted into its 3-acetyl derivative (XIV) by the boron fluoride–acetic anhydride technique.[5] The product was widely different from a substance which had been obtained [3] by hydrolysis of methyl di-*O*-methylcitromycetinone (V; R = Me) and shown to be di-*O*-methylcitromycetinone (V; R = H) in Part II. Like the parent compound [5] (XIV less the carboxyl group) this 3-acetyl-4-hydroxycoumarin could not be further acylated, so we could not synthesise di-*O*-methylcitromycetinone (V; R = H) from it.

EXPERIMENTAL

2-Acetyl-3-benzyloxy-5,6-dimethoxybenzoic Acid.—A hot solution of potassium permanganate (7·5 g.) and magnesium sulphate (7·5 g.) in water (150 ml.) was added in 5 min. to 6-benzyloxy-3,4-dimethoxy-2-propenylacetophenone [4] (3·0 g.) in boiling acetone (150 ml.), and the mixture was filtered at once to remove manganese dioxide. The filtrate was decolorised by means of sulphur dioxide and freed from acetone by evaporation. The product was isolated by extraction from the aqueous residue into chloroform, and thence into 2N-sodium carbonate. Precipitated by acidification, the *benzyloxybenzoic acid* formed plates (0·37 g.), m. p. 150—151°,

[5] Badcock, Dean, Robertson, and Whalley, *J.*, 1950, 903.

from aqueous methanol [Found: C, 65·1; H, 5·5; OMe, 19·2. $C_{16}H_{12}O_4(OMe)_2$ requires C, 65·4; H, 5·5; OMe, 18·8%]. In the solid state in Nujol mulls this acid had $\nu_{max.}$ 3472 (OH), 1748 (phthalide C:O), and 1608 cm.$^{-1}$, and therefore exists as the lactol (IX; R = CH_2Ph). When the oxidation was effected in more vigorous conditions the yield of the benzyloxybenzoic acid decreased and benzoic acid was also isolated.

2-*Acetyl*-3-*hydroxy*-5,6-*dimethoxybenzoic Acid* (X).—The foregoing benzyloxybenzoic acid (2·0 g.) in 2N-sodium hydroxide (60 ml.) was shaken with Raney nickel (~1 g.) and hydrogen. Absorption ceased after about 4 hr., and acidification of the filtered solution then gave 2-acetyl-3-hydroxy-5,6-dimethoxybenzoic acid which separated from aqueous alcohol in prisms (1·2 g.), m. p. 181° (decomp.) [Found: C, 55·2; H, 5·1; OMe, 25·5. Calc. for $C_9H_6O_4(OMe)_2$: C, 55·0; H, 5·0; OMe, 25·8%]. This acid dissolved readily in sodium hydrogen carbonate solution: the green colour it imparted to alcoholic ferric chloride became purplish-brown on the addition of water. In mulls it strongly absorbed infrared radiation at 3356, 3215 (bonded OH), 1718 (CO_2H), 1709sh, and 1613 cm.$^{-1}$ (chelated acetophenone C:O) and therefore showed no tendency to form a lactol. It was identified by the mixed-fusion and infrared techniques with one of the alkali-degradation products of methyl di-*O*-methylcitromycetinone (see Part II).

The *acetate* was prepared by shaking a solution of the acid (0·6 g.) in 2N-aqueous sodium hydroxide (15 ml.) with acetic anhydride (2 ml.) added dropwise during 15 min. at 0°, and it crystallised from benzene–light petroleum (b. p. 60—80°) in rectangular prisms (0·5 g.), m. p. 161° [Found: C, 55·3; H, 5·2; OMe, 22·1. $C_{11}H_8O_5(OMe)_2$ requires C, 55·3; H, 5·0; OMe, 22·0%]. This acetate appeared to be the lactol (IX; R = Ac) since it had $\nu_{max.}$ 3436, 3067, 1754, and 1600 cm.$^{-1}$ but no ketonic absorption.

6,7-*Dimethoxy*-2-*methylchromone*-5-*carboxylic Acid* (VI; R = H).—3-Acetoxy-2-acetyl-5,6-dimethoxybenzoic acid (0·70 g.) was treated with powdered sodium (0·4 g.) in tetrahydrofuran (40 ml.) at 90°. After 3 hr. the mixture was cooled and, after residual sodium had been destroyed by a little alcohol, diluted with ether (100 ml.). The orange sodium salts were then extracted into water. The extract was saturated with ammonium sulphate and acidified with hydrochloric acid. The yellow solid produced crystallised from 95% alcohol, giving the *chromone acid* in prisms (0·2 g.), m. p. 276° (decomp.) (Found, on specimen dried *in vacuo* at 140°: C, 58·9; H, 4·7. $C_{13}H_{12}O_6$ requires C, 59·1; H, 4·6%). This compound had $\nu_{max.}$ 3145 (CO_2H), 1733 (CO_2H), 1639 (chromone C:O), and 1595 cm.$^{-1}$: it gave no ferric reaction.

Similar results were obtained when the reaction was carried out in pyridine with sodium hydride or in tetrahydrofuran with sodium methoxide, but the yields were less good.

Diazomethane converted the acid in ether–methanol into the *methyl ester* which separated from benzene–light petroleum (b. p. 60—80°) in rectangular prisms, m. p. 135° (Found, on specimen dried *in vacuo* at 110°: C, 60·3; H, 5·1. $C_{14}H_{14}O_6$ requires C, 60·4; H, 5·1%).

2-*Acetyl*-7-*hydroxy*-4,5-*dimethoxyindan*-1,3-*dione* (VII).—When methyl 6,7-dimethoxy-2-methylchromone-5-carboxylate (0·02 g.) and 2N-aqueous sodium hydroxide (0·2 ml.) were heated together in methanol (2 ml.) on a steam-bath for 15 min., an orange solution was formed and then a yellow solid separated. This solid was collected, washed with methanol, and treated with dilute hydrochloric acid to give a buff-coloured product which, when purified from methanol, afforded 2-acetyl-7-hydroxy-4,5-dimethoxyindane-1,3-dione in pale yellow needles, m. p. 157° (Found: C, 58·9; H, 4·7. Calc. for $C_{13}H_{12}O_6$: C, 59·1; H, 4·6%). This compound was identical with specimens of " Compound B " prepared from methyl di-*O*-methylcitromycetinone or from methyl di-*O*-methylanhydrofulvate.

4-*Hydroxy*-6,7-*dimethoxycoumarin*-5-*carboxylic Acid* (XIII; R = H).—2-Acetyl-3-hydroxy-5,6-dimethoxybenzoic acid (0·6 g.) in 0·5N-aqueous sodium hydroxide (10 ml.) was allowed to react with methyl chloroformate (0·25 ml.) at 0° for 15 min. The precipitate formed when the resulting solution was acidified with dilute hydrochloric acid was collected, washed with water, and crystallised from aqueous methanol, giving 2-*acetyl*-3-*methoxycarbonyloxy*-5,6-*dimethoxybenzoic acid* as hexagonal plates (0·5 g.), m. p. 168° (Found, on specimen dried *in vacuo* at 100°: C, 52·5; H, 4·8. $C_{13}H_{14}O_8$ requires C, 52·4; H, 4·7%). This compound appeared to exist as the lactol (IX; R = CO_2Me) since it had $\nu_{max.}$ (in Nujol) 3401 (OH), 1770 ($ArO{\cdot}CO_2Me$), 1745 (phthalide C:O) and 1605 cm.$^{-1}$. It did not give a ferric reaction but was slowly soluble in aqueous sodium hydrogen carbonate.

After this 3-methoxycarbonyloxybenzoic acid (0·60 g.) had been subjected to the action of powdered sodium (0·2 g.) in boiling tetrahydrofuran (20 ml.) for 5 hr., the sodium salts formed were collected, dissolved in water, and decomposed by dilute hydrochloric acid. The solid

produced was fractionally crystallised from aqueous methanol. The less soluble fractions consisted of 2-acetyl-3-hydroxy-5,6-dimethoxybenzoic acid (0·05 g.), the more soluble of the desired product, which, when further purified from aqueous methanol, afforded *4-hydroxy-6,7-dimethoxycoumarin-5-carboxylic acid* as rhombs (0·11 g.), m. p. 256° (decomp.), soluble in aqueous sodium hydrogen carbonate, giving an amber ferric reaction, and having $\lambda_{max.}$ (in EtOH) 286, 316 mμ (log ε 4·37, 4·56) and $\nu_{max.}$ (in Nujol) 3534sh, 3436, 2770—2570 (ill-defined), 1733, 1704, 1669, and 1600 cm.$^{-1}$ (Found, on specimen dried *in vacuo* at 140°: C, 53·8; H, 4·0. $C_{12}H_{10}O_7$ requires C, 54·1; H, 3·8%).

3-Acetyl-4-hydroxy-6,7-dimethoxycoumarin-5-carboxylic Acid (XIV).—The foregoing 4-hydroxycoumarin (33 mg.), acetic anhydride (0·1 ml.), and a saturated solution (0·2 ml.) of boron fluoride in acetic acid were heated together at 90° for 15 min. A yellow boron complex (25 mg.) separated. This was crushed under ether (3 ml.), collected, and dissolved in 5% aqueous sodium acetate (1 ml.), giving a colourless solution which was then acidified. The precipitate separated from methanol, giving *3-acetyl-4-hydroxy-6,7-dimethoxycoumarin-7-carboxylic acid* in hexagonal prisms (17 mg.), m. p. 256° (decomp.), soluble in aqueous sodium hydrogen carbonate, giving an orange-red ferric reaction, and having $\lambda_{max.}$ (in EtOH) 343 mμ (log ε 4·25) and $\nu_{max.}$ (in Nujol) 1736sh, 1724 (coumarin C:O), 1701sh, and 1603 cm.$^{-1}$ (Found, on specimen dried *in vacuo* at 140°: C, 54·3; H, 4·1. $C_{14}H_{12}O_8$ requires C, 54·6; H, 3·9%).

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