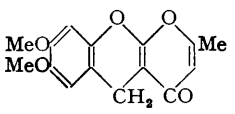
210. *The Chemistry of Fungi. Part XI. The Degradation of O-Dimethylcitromycinol and a Revised Structure for O-Dimethylcitromycin.*

By G. W. K. Cavill, Alexander Robertson, and W. B. Whalley.

By the hydrolysis of *O*-dimethylcitromycinol with alkalis the following ten products have been obtained: formic acid, acetic acid, acetone, (XVIII; R = Me), (XVIII; R = OH), (XVII), (XIX), (XV), (XIII), and (XII, R = H). The production of the more complex derivatives depends mainly on the conditions employed. From a study of their decomposition products the nature of the new compounds (XII; R = H), (XIII), and (XV) has been clarified and new formulæ (VIII) and (IX) for *O*-dimethylcitromycin and *O*-dimethylcitromycinol respectively have been developed (compare Part III, *J.*, 1949, 848).

It has been shown that *O*-dimethylcitromycin is oxidised with ozone, giving a mixture of *O*-dimethylcitromycinone and *O*-dimethylcitromycinol, of which the latter is the sole product when lead tetra-acetate is employed as the oxidising agent (Part III, *J.*, 1949, 848; Part VIII, *ibid.*, p. 1567). From a study of the general properties of *O*-dimethylcitromycin and of *O*-dimethylcitromycinone it was concluded in Part III (*loc. cit.*) that these compounds were best represented by either the linear structures (I) and (III) or the angular structures (VIII) and (X) respectively. Of these alternatives the linear type was preferred because in this way the easily oxidisable active methylene group appeared to be accommodated more satisfactorily, a conclusion which seemed to be substantially supported by our studies on the oxidation of xanthens with lead tetra-acetate and ozone (Part VIII, *loc. cit.*). It had been noted in Part III (*loc. cit.*), however, that the adoption of the linear structure implied that the production of 2-hydroxy-4 : 5-dimethoxyacetophenone and of 2-hydroxy-4 : 5-dimethoxybenzoic acid by the hydrolytic fission of *O*-dimethylcitromycin with alkalis was due to the simultaneous oxidation of the active methylene group in the 4-position of the chromen ring of (I), but from studies on 3 : 4-dihydrocoumarin derivatives (Part IX, *J.*, 1950, 895) it subsequently became clear that this



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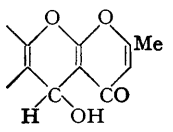

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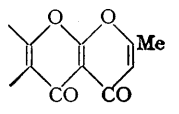

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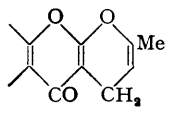

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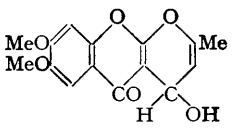

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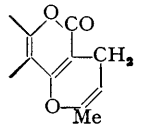

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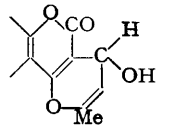


(VII.)

kind of oxidation was most unlikely in the case of a compound having formula (I) which may be envisaged as a derivative of a 3 : 4-dihydrocoumarin. In conjunction with the recognition of 4-hydroxy-6 : 7-dimethoxy-3-acetoacetylcoumarin as a primary hydrolytic product of *O*-dimethylcitromycinone, which it regenerates on cyclisation (Part X, *J.*, 1950, 903), these observations appeared to eliminate the possibility of *O*-dimethylcitromycin and its derivatives having the linear type of formula. This conclusion has now been substantiated by a study of the hydrolytic decomposition of *O*-dimethylcitromycinol.

As indicated in Part III (*loc. cit.*), in agreement with both the linear (II) and the angular formula (IX), *O*-dimethylcitromycinol behaves as a carbinol base of the pyrylium type and the corresponding *chloride* and *picrate* have now been prepared by standard methods. Although well-defined crystalline salts are also obtained with hydroferric chloride and with perchloric acid the analytical results given by these derivatives are not in agreement with their being the normal ferrichloride and perchlorate respectively. From the chloride the carbinol base is quantitatively re-formed by treatment with aqueous sodium hydrogen carbonate. On being boiled with methanol *O*-dimethylcitromycinyl chloride takes up another methyl group, giving O-*trimethylcitromycinol*, which is also formed, in almost theoretical yield, when the base is boiled with methanol containing small amounts of concentrated hydrochloric acid. When the methanol is replaced by alcohol the corresponding *ethyl ether* is obtained. The tendency of carbinol bases to form ethers in this manner is well recognised and in this respect the behaviour of *O*-dimethyl-

citromycinol finds an exceptionally close parallel in the case of 9-(2 : 2-diphenylvinyl)xanthhydrol (Zeigler, *Annalen*, **1923**, **434**, 34). Like other carbinol ethers *O*-trimethylcitromycinol ether is readily converted into the chloride by means of hydrogen chloride–acetic acid, whilst on prolonged boiling with solvents, *e.g.*, moist acetone or chloroform, the parent base is regenerated. On account of the low solubility of *O*-dimethylcitromycinol in the usual organic solvents compared with that of the trimethyl ether in methanol, the latter proved a convenient intermediate for the purification of *O*-dimethylcitromycinol in quantity. The carbinol base has also been characterised by the preparation of a *phenylurea* derivative.

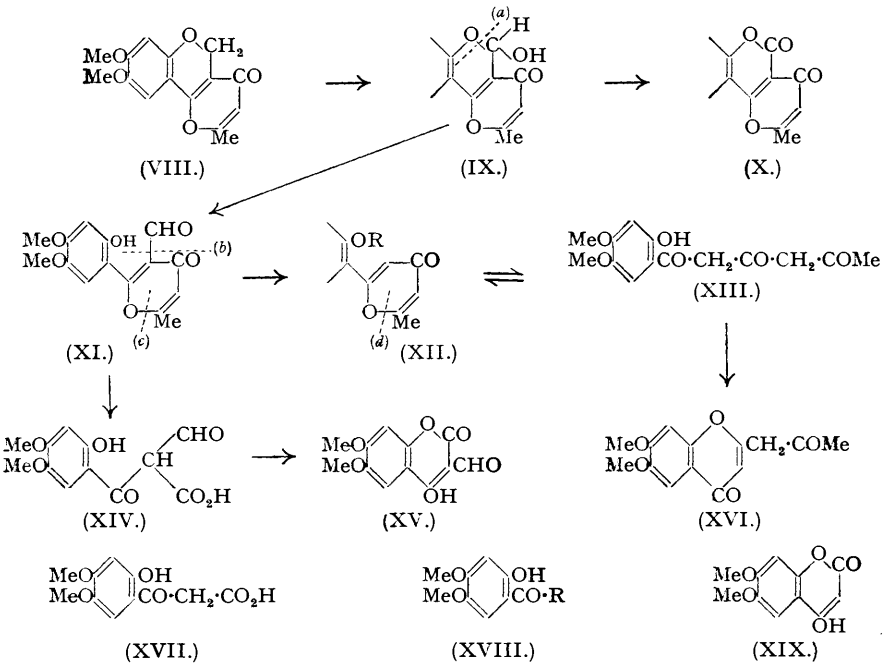
With a supply of *O*-dimethylcitromycinol (Part VIII, *loc. cit.*) available we have been able to carry out a study of the hydrolytic products formed by the action of alkaline reagents. In general, the use of the trimethyl ether in place of the parent base has been found to give identical results under similar conditions. The proportion of the various degradation products can be varied very considerably and in particular the more complex derivatives can be eliminated from the hydrolysate by alterations in the conditions of the hydrolysis. On being refluxed with dilute aqueous sodium hydroxide *O*-dimethylcitromycinol and the trimethyl ether gave rise to formic acid, acetic acid, acetone, 2-hydroxy-4 : 5-dimethoxybenzoic acid (XVIII; R = OH) and 2-hydroxy-4 : 5-dimethoxyacetophenone (XVIII; R = Me). When boiling barium hydroxide solution was employed in place of the aqueous sodium hydroxide there was isolated, in addition to acetone and (XVIII; R = Me), 2-*hydroxy*-4 : 5-*dimethoxybenzoylacetic acid* (XVII) the structure of which was established by the fact that it gave a ferric reaction, decomposed on being heated, giving carbon dioxide and (XVIII; R = Me), and on treatment with concentrated sulphuric acid furnished 4-hydroxy-6 : 7-dimethoxycoumarin (XIX). The main product formed by gently warming the carbinol base or its methyl ether with aqueous–methanolic sodium hydroxide was 4-*hydroxy*-6 : 7-*dimethoxy*-3-*formylcoumarin* (XV) which was accompanied by small amounts of the ketone (XVIII; R = Me). The structure of the formylcoumarin (XV) is conclusively established by the following reactions. The presence of the formyl group was demonstrated by the ready formation of a 2 : 4-*dinitrophenylhydrazone* and an *anil*, and by the fact that the compound readily condensed with resorcinol in hydrogen chloride–acetic acid to give a pyrylium salt which is clearly 7-*hydroxy*-6′ : 7′-*dimethoxycoumarino*(4′ : 3′-2 : 3)*benzpyrylium chloride* (XX). Further, the hydrolytic decomposition of (XV) with warm dilute aqueous sodium hydroxide gave rise to formic acid, (XVIII; R = Me), and (XVII), whilst with concentrated sulphuric acid in warm acetone the formyl group was eliminated with the production of 4-hydroxy-6 : 7-dimethoxycoumarin (XIX). Finally, the catalytic reduction of (XV) furnished an almost theoretical yield of 4-hydroxy-6 : 7-dimethoxy-3-methylcoumarin (Part II, *J.*, 1949, 562), thus proving conclusively that the formyl group is in the 3-position.

During attempts to obtain the precursor of the foregoing 3-formylcoumarin (XV) which arises from *O*-dimethylcitromycinol by the loss of three carbon atoms, we were able to isolate, from the complex mixture formed by the action of aqueous–methanolic sodium hydroxide on the base or its ether for 3—4 minutes, two closely related alkali-soluble compounds—(A) as a *hydrate* $C_{14}H_{16}O_6,H_2O$ and (B) $C_{14}H_{14}O_5$—in addition to (XV) and acetone. The compound (A) gave a ferric reaction and formed an iso*oxazole* derivative, indicating the presence of a 1 : 3-dicarbonyl system. On hydrolytic decomposition with alkali and subsequent treatment of the acidified hydrolysate with an excess of 2 : 4-dinitrophenylhydrazine sulphate in dilute sulphuric acid, (A) furnished the 2 : 4-dinitrophenylhydrazones of acetone and (XVIII; R = Me) along with 4-hydroxy-6 : 7-dimethoxycoumarin (XIX). The latter compound was considered to arise by the cyclisation of 2-hydroxy-4 : 5-dimethoxybenzoylacetic acid in the acidic medium, in keeping with the observed ready cyclisation of this acid. Thus it seemed that (A), which did not appear to be a carboxylic acid, contained the residue $C_6H_2(OMe)_2(OH)\cdot CO\cdot CH_2\cdot CO\cdot$, appearing in the hydrolysate as (XVII), and that the latter was derived by scission of (A) with the loss of a molecule of acetone. Hence (A) is a triketone which may be represented by formula (XIII) and in keeping with this view it was found that on treatment with concentrated sulphuric acid (A) undergoes cyclisation with the elimination of a molecule of water, giving 6 : 7-*dimethoxy*-2-*acetonylchromone* (XVI). Since it readily formed a 2 : 4-*dinitrophenylhydrazone*, (XVI) can be differentiated from (B) with which it is isomeric. In this connection it may be noted that the cyclisation of the triketone can take an alternative route leading to the formation of the γ-pyrone (XII; R = H) which would not be expected to form a 2 : 4-dinitrophenylhydrazone, thus clearly distinguishing it from the 2-acetonylchromone (XVI).

Unlike (A), the product (B) does not give a ferric reaction and appears not to contain a reactive carbonyl group, but on acetylation with pyridine–acetic anhydride it gave an alkali-insoluble *acetate* identical with that formed when the triketone (A) was subjected to the same

reaction. Obtained from either source, the acetyl derivative gave rise to compound (B) on treatment with warm hydrochloric acid. Thus the acetylation of (A) is accompanied by simultaneous dehydration and, in view of the foregoing cyclisation of (A) to give the chromone (XVI), the acetylation product must be the acetate (XII; R = Ac) of 2-methyl-6-(2-hydroxy-4 : 5-dimethoxyphenyl)-γ-pyrone (XII; R = H).

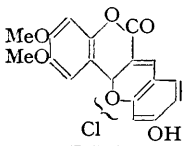
If the conclusions arrived at regarding the structure of *O*-dimethylcitromycin in Part IX (*loc. cit.*) and of *O*-dimethylcitromycinone in Part X (*loc. cit.*) are taken into account it is clear that the formation of formic acid, (XV), (XIII), and (XII) by the hydrolysis of *O*-dimethylcitromycinol excludes the possibility of this compound having the linear formula (II) originally proposed (Part III, *loc. cit.*). Of the remaining possible structures (V), (VII), and (IX) for



O-dimethylcitromycinol which follow from the alternative possible formulæ for *O*-dimethylcitromycin proposed in Part III, we consider, bearing in mind the general properties of the latter compound, that only formula (IX) affords a basis for a rational explanation of the formation of the inter-related series of decomposition products now obtained from the carbinol base. The first stage in the hydrolysis may be considered to be the opening of the ring containing the carbinol group along the broken line (*a*) yielding the intermediate (XI) which has not been isolated so far, but which by subsequent fission at (*b*) would give rise to formic acid and the 2-phenylpyrone (XII; R = H). Hydrolysis of the latter with the opening of the γ-pyrone ring at (*d*) accounts for the formation of the triketone (XIII) which by subsequent loss of acetone has been shown to furnish the benzoylacetic acid (XVII). On the other hand, scission of the γ-pyrone ring of (XI) at (*c*) would yield a formyl derivative of the triketone (XIII), giving by loss of acetone the formylbenzoylacetic acid (XIV) which we regard as the precursor of 4-hydroxy-6 : 7-dimethoxy-3-formylcoumarin (XV). The formation of the 4-hydroxycoumarin ring in preference to the pyrylium system on liberation of (XIV) from its sodium salt is in keeping with the relative ease of cyclisation of the respective systems.

It is evident that substances having these formulæ (V) or (VII) could give rise to the formylcoumarin (XV) by the opening of the pyranol ring with subsequent loss of an acetone residue on hydrolysis. On the basis of either formula the formation of the triketone from *O*-dimethylcitromycinol would involve the oxidation of the carbinol group with dilute alkalis, but under the conditions employed for the production of (XIII) this oxidation is regarded as highly improbable and in either case the formation of the γ-pyrone (XII; R = H) would be a secondary process. Whilst we have not observed the conversion of (XIII) into (XII; R = H) by acidification of an alkaline solution of (XIII) with mineral acid, a more cogent objection to formulæ (V) and (VII)

for *O*-dimethylcitromycinol is the fact that in the parent compounds (IV) and (VI) (*O*-dimethylcitromycin type) the methyl groups would not be expected to react with piperonal and the double bond would be expected to behave normally towards oxidising and hydrogenating agents (compare Part III, *loc. cit.*). Accordingly, therefore, we now consider that *O*-dimethylcitromycin and *O*-dimethylcitromycinol are represented by the structures (VIII) and (IX) respectively, in keeping with the revised formula (X) advanced for *O*-dimethylcitromycinone in Part X (*loc. cit.*). Further, the hydration product of the last-mentioned compound, which is soluble in aqueous sodium hydrogen carbonate (Part III), is now regarded as the *acid* (XXII) formed by the opening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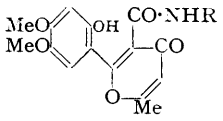
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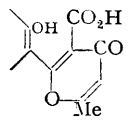
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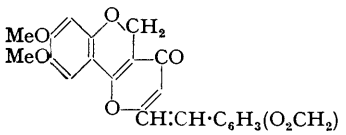
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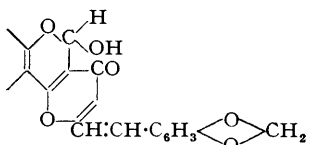
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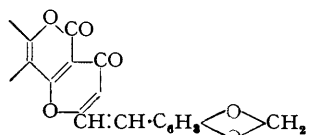
(XXV.)

of the potential 4-hydroxycoumarin ring. This acid (XXII), which can be readily cyclised by by acetic anhydride regenerating *O*-dimethylcitromycinone, is clearly the first stage in the hydrolytic decomposition of (X). Moreover, it now seems clear that the so-called anil and the phenylhydrazone of *O*-dimethylcitromycinone (Part III) are respectively the *anilide* (XXI; R = Ph) and the *phenylhydrazide* (XXI; R = NHPh) of the acid (XXII). We consider the oxime of *O*-dimethylcitromycinone to be the normal derivative involving the carbonyl group of the γ-pyrone system, but we are unable to account satisfactorily for the second oximation product.

The view expressed in Part III that the formation of the styryl derivative by condensation of *O*-dimethylcitromycin with piperonal does not involve the active methylene group which undergoes oxidation has now been substantiated. Oxidation of the styryl derivative (XXIII) with lead tetra-acetate gave the corresponding *derivative* of *O*-dimethylcitromycinol (XXIV) which on subsequent oxidation with chromic oxide gave the *compound* (XXV), identical with the product obtained directly from the piperonylidene derivative of *O*-dimethylcitromycin by the same oxidising agent.

Experimental.

Derivatives of O-*Dimethylcitromycinol.*—Treatment of a saturated solution of *O*-dimethylcitromycinol in acetic acid with hydrogen chloride at room temperature gave *O-dimethylcitromycinyl chloride* which separated from acetic acid containing a little hydrochloric acid in yellow prisms decomposing at 131° (Found : C, 58·4; H, 4·3; Cl, 12·9. $C_{15}H_{13}O_5Cl$ requires C, 58·4; H, 4·2; Cl, 11·5%). Prepared in the usual manner the *picrate* crystallised from warm alcohol in small orange needles, m. p. 179° (Found : C, 50·2; H, 3·1; N, 8·6. $C_{21}H_{15}O_{12}N_3$ requires C, 50·3; H, 3·0; N, 8·4%). With concentrated ferric chloride solution in hydrochloric acid the base gave a *derivative* which formed red prisms, m. p. 244° (decomp.), from acetic acid, analytical results of which did not agree with those required for a simple ferrichloride (Found : C, 46·3; H, 4·0; Cl, 20·8; Fe, 7·0. Calc. for $C_{15}H_{13}O_5Cl_4Fe$: C, 38·2; H, 2·8; Cl, 30·2; Fe, 11·9%). Similarly with perchloric acid a substance was formed which crystallised in lemon-yellow plates, m. p. 140—144° (decomp.), from acetic acid.

Methanol (10 ml.) containing a suspension of *O*-dimethylcitromycinol (1 g.) and 2 drops of concentrated hydrochloric acid was warmed on the steam-bath until the solid dissolved and on cooling, the yellow solution deposited O-*trimethylcitromycinol* which then separated from methanol in colourless needles (0·9 g.), m. p. 192° (Found : C, 63·2; H, 5·4. $C_{16}H_{16}O_6$ requires C, 63·2; H, 5·3%). Prepared in a similar manner O-*dimethyl*-O-*ethylcitromycinol* formed colourless elongated needles, m. p. 169°, from alcohol (Found : C, 64·0; H, 5·8. $C_{17}H_{18}O_6$ requires C, 64·1; H, 5·7%).

On being kept at room temperature the clear solution obtained by warming *O*-dimethylcitromycinol (0·5 g.), phenylurea (0·5 g.), and acetic acid (10 ml.) deposited the N-*phenyl*-N′-(O-*dimethylcitromycinyl*)-*urea* in almost colourless tiny needles, m. p. 275° (decomp.), which owing to its insolubility in most organic solvents was purified by extraction with much warm alcohol (Found : C, 64·8; H, 5·0; N, 6·8. $C_{22}H_{20}O_6N_2$ requires C, 64·7; H, 4·9; N, 6·9%).

Hydrolytic Degradation of O-*Trimethylcitromycinol.*—(*a*) A mixture of the methyl ether (4·0 g.) and 2N-aqueous sodium hydroxide (100 ml.) was refluxed for 1·5 hours and the resulting clear amber solution acidified with 2N-hydrochloric acid. The semi-solid precipitate (2 g.) was collected, washed, dried, and

dissolved in warm ethyl acetate. On cooling the solution deposited 4-hydroxy-6 : 7-dimethoxycoumarin in colourless plates, m. p. 278°, identified by comparison with an authentic specimen (Part II, *J.*, 1949, 562). The solid left by the evaporation of the ethyl acetate mother-liquor was dissolved in ether, and the solution extracted with aqueous sodium hydrogen carbonate, washed, dried, and evaporated, leaving 2-hydroxy-4 : 5-dimethoxyacetophenone in colourless prisms (0·8 g.), m. p. 111—112°, after recrystallisation. Acidification of the sodium hydrogen carbonate extracts gave 2-hydroxy-4 : 5-dimethoxybenzoic acid which formed colourless prisms (0·35 g.), m. p. and mixed m. p. 210°, from aqueous methanol.

Treatment of the filtered acidified hydrolysate, left after the separation of the semi-solid, with excess of aqueous 2 : 4-dinitrophenylhydrazine sulphate gave a red precipitate which was separated by chromatography on alumina from benzene as described in Part III (*loc. cit.*) into the 2 : 4-dinitrophenylhydrazone (0·6 g.) of acetone, m. p. and mixed m. p. 125—126°, after purification, and the 2 : 4-dinitrophenylhydrazone (0·3 g.) of 2-hydroxy-4 : 5-dimethoxyacetophenone, m. p. and mixed m. p. 227° (decomp.). Distillation of the filtrate from the mixture of the crude 2 : 4-dinitrophenylhydrazones furnished an aqueous distillate containing formic acid which was isolated as its sodium salt and identified by being converted in the usual manner into *NN'*-diphenylformamidine hydrochloride, forming colourless needles, m. p. 228—230° (decomp.), from dilute hydrochloric acid (Found : N, 12·2. Calc. for $C_{13}H_{13}N_2Cl$: N, 12·1%). The presence of formic acid in the distillate was also established by the conversion of the sodium formate into benziminazole picrate according to the method of Brown and Campbell (*J.*, 1937, 1699), which formed yellow needles, m. p. and mixed m. p. 227—229°, from alcohol (Found : N, 20·5. Calc. for $C_{13}H_9O_7N_5$: N, 20·2%). On several occasions the formic acid was accompanied by small amounts of acetic acid which was identified by conversion into acetanilide, m. p. and mixed m. p. 112°.

When *O*-dimethylcitromycinol (2 g.) was heated with 2N-aqueous sodium hydroxide (75 ml.) on the steam-bath for 15 minutes and the cooled reaction mixture acidified with 2N-sulphuric acid (100 ml.) the following products were isolated : 4-hydroxy-6 : 7-dimethoxycoumarin (0·55 g.), acetone 2 : 4-dinitrophenylhydrazone (0·5 g.), 2-hydroxy-4 : 5-dimethoxyacetophenone 2 : 4-dinitrophenylhydrazone (0·3 g.), formic acid as *NN'*-diphenylformamidine hydrochloride (0·1 g.), and acetic acid as the anilide.

(*b*) A mixture of *O*-trimethylcitromycinol (2 g.), 2N-aqueous sodium hydroxide (50 ml.), and methanol (50 ml.) was heated on the steam-bath for 2 minutes, the resulting clear solution was cooled and acidified with 2N-sulphuric acid (60 ml.), and the buff-coloured solid which separated immediately was collected, washed, and dried. Crystallised from chloroform this product gave *4-hydroxy-6 : 7-dimethoxy-3-formylcoumarin* in pale yellow prisms (0·9 g.), m. p. 242° (decomp.), readily soluble in aqueous sodium hydrogen carbonate and giving a red ferric reaction in alcohol (Found : C, 57·4; H, 4·3. $C_{12}H_{10}O_6$ requires C, 57·6; H, 4·0%). The 2 : 4-*dinitrophenylhydrazone* formed orange prisms, m. p. 308° (decomp.), from dioxan (Found : C, 50·3; H, 3·5; N, 13·3. $C_{18}H_{14}O_9N_4$ requires C, 50·2; H, 3·3; N, 13·0%). The *anil* separated from ethyl acetate in pale yellow prisms, m. p. 201° (Found : C, 64·5; H, 4·6; N, 4·1. $C_{18}H_{15}O_5N$ requires C, 64·5; H, 4·5; N, 4·2%).

The acidic filtrate left after the separation of the crude 3-formylcoumarin was treated with an excess of 2 : 4-dinitrophenylhydrazine sulphate, and the resulting precipitate separated by chromatography from benzene on aluminium oxide into acetone 2 : 4-dinitrophenylhydrazone (0·7 g.) and 2-hydroxy-4 : 5-dimethoxyacetophenone 2 : 4-dinitrophenylhydrazone (0·2 g.). 4-Hydroxy-6 : 7-dimethoxycoumarin was not detected in the hydrolysate.

When *O*-dimethylcitromycinol (2 g.) was heated with 2N-aqueous sodium hydroxide (25 ml.) and methanol (25 ml.) on the steam-bath for 3 minutes the same decomposition products were obtained.

(*c*) When heated under reflux with a saturated solution of aqueous barium hydroxide (100 ml.) for 15 minutes *O*-trimethylcitromycinol (1 g.) rapidly dissolved and was replaced by a buff-coloured precipitate which was isolated from the cooled reaction mixture and treated with dilute hydrochloric acid, giving *2-hydroxy-4 : 5-dimethoxybenzoylacetic acid* (0·4 g.). Crystallised from chloroform, this acid formed colourless octagonal plates, m. p. 140° (decomp.), readily soluble in aqueous sodium hydrogen carbonate and having a greenish-brown ferric reaction in alcohol (Found : C, 55·0; H, 5·1. $C_{11}H_{12}O_6$ requires C, 55·0; H, 5·0%). Treatment of 2-hydroxy-4 : 5-dimethoxybenzoylacetic acid with alcoholic 2 : 4-dinitrophenylhydrazine sulphate resulted in the formation of the 2-hydroxy-4 : 5-dimethoxyacetophenone 2 : 4-dinitrophenylhydrazone, m. p. 227° (decomp.), identified by comparison with an authentic sample. On being heated (oil-bath) at 150° for 15 minutes the acid (0·1 g.) decomposed, giving carbon dioxide and 2-hydroxy-4 : 5-dimethoxyacetophenone which formed colourless prisms (0·05 g.), m. p. 112°, from alcohol–light petroleum (b. p. 60—80°) and was identified by comparison with an authentic specimen. The acid (0·1 g.) and concentrated sulphuric acid (2 ml.) at room temperature for 24 hours gave 4-hydroxy-6 : 7-dimethoxycoumarin which separated from ethyl acetate in colourless plates (0·05 g.), m. p. 278° (decomp.), undepressed on admixture with an authentic specimen.

From the acidified hydrolysate left after the separation of barium 2-hydroxy-4 : 5-dimethoxybenzoate, the 2 : 4-dinitrophenylhydrazones of acetone and 2-hydroxy-4 : 5-dimethoxyacetophenone were isolated by the method employed in (*b*).

(*d*) *O*-Trimethylcitromycinol (4 g.) was added to a hot mixture of 2N-aqueous sodium hydroxide (100 ml.) and methanol (100 ml.), and the solution heated on the steam-bath for 3—4 minutes. Acidification of the cooled amber-coloured reaction mixture with 2N-sulphuric acid (150 ml.) gave a buff-coloured precipitate (1·85 g.) of almost pure 4-hydroxy-6 : 7-dimethoxy-3-formylcoumarin which had m. p. and mixed m. p. 238—240° (decomp.) after recrystallisation from chloroform. Extraction of the acidic liquor with chloroform (50 ml. × 3) gave a yellow extract exhibiting an intense green fluorescence, whilst treatment of the acidic aqueous liquor with aqueous 2 : 4-dinitrophenylhydrazine sulphate gave acetone 2 : 4-dinitrophenylhydrazone (0·05 g.), m. p. and mixed m. p. 124—6°. The yellow chloroform solution was extracted with 2N-aqueous sodium hydrogen carbonate and then with 2N-aqueous sodium hydroxide. From the acidified sodium hydrogen carbonate solution 4-hydroxy-6 : 7-dimethoxycoumarin was isolated by extraction with ethyl acetate–ether and purified from ethyl acetate, forming colourless plates (0·05 g.), m. p. and mixed m. p. 278° (decomp.). Concentration of the ethyl acetate mother-liquors from this purification gave 2-hydroxy-4 : 5-dimethoxybenzoylacetic acid (0·2 g.), m. p. 140° (decomp.).

Acidification of the foregoing aqueous sodium hydroxide extract with dilute hydrochloric acid, followed by repeated extraction with chloroform and evaporation of the combined extracts, gave an oily product which was dissolved in hot ethyl acetate. On cooling this solution deposited *2-methyl-6-(2-hydroxy-4 : 5-dimethoxyphenyl)-γ-pyrone* in colourless prisms (0·1 g.), m. p. 269° (decomp.), readily soluble in aqueous sodium hydroxide or in concentrated hydrochloric acid and having a negative ferric reaction in alcohol (Found : C, 63·8; H, 5·2. $C_{14}H_{14}O_5$ requires C, 64·1; H, 5·4%). Prepared by acetic anhydride–pyridine, the *acetate* of this compound formed colourless prisms, m. p. 152°, from ethyl acetate (Found : C, 63·3; H, 5·5. $C_{16}H_{16}O_6$ requires C, 63·1; H, 5·3%).

Concentration of the ethyl acetate liquors left after the purification of the γ-pyrone gave 1-(2-hydroxy-4 : 5-dimethoxyphenyl)hexane-1 : 3 : 5-trione as a *hydrate* which formed clusters of colourless prisms (0·7 g.), m. p. 100—102°, from the same solvent and gave a green ferric reaction in alcohol [Found : C, 56·1; H, 5·9; OMe, 20·4. $C_{12}H_{10}O_4(OMe)_2,H_2O$ requires C, 56·4; H, 6·0; OMe, 20·8%]. This triketone, which readily dissolved in aqueous sodium hydroxide, formed an *isooxazole* which separated from ethyl acetate in colourless needles, m. p. 172° (Found : C, 60·3; H, 5·7; N, 5·2. $C_{14}H_{15}O_5N$ requires C, 60·6; H, 5·5; N, 5·1%).

When the trimethyl ether was replaced by the parent *O*-dimethylcitromycinol (2 g.) in the foregoing hydrolysis, the following products were isolated and identified : 4-hydroxy-6 : 7-dimethoxycoumarin (0·02 g.), 4-hydroxy-6 : 7-dimethoxy-3-formylcoumarin (0·95 g.), 2-hydroxy-4 : 5-dimethoxybenzoylacetic acid (0·05 g.), 2-methyl-6-(2-hydroxy-4 : 5-dimethoxyphenyl)-γ-pyrone (0·05 g.), and 1-(2-hydroxy-4 : 5-dimethoxyphenyl)hexane-1 : 3 : 5-trione (0·2 g.).

Hydrolysis of 4-Hydroxy-6 : 7-dimethoxy-3-formylcoumarin—This compound (2 g.) was heated with 2N-aqueous sodium hydroxide (75 ml.) on the steam-bath for 20 minutes and the cooled amber-coloured solution acidified with 2N-sulphuric acid (125 ml.) giving a buff-coloured precipitate of the unchanged compound (0·25 g.), which was separated by filtration. On being kept the filtrate then gradually deposited a solid which was resolved by means of ether and aqueous sodium hydrogen carbonate into 2-hydroxy-4 : 5-dimethoxyacetophenone (0·2 g.), m. p. 112° (after purification), and 2-hydroxy-4 : 5-dimethoxybenzoylacetic acid (1 g.), m. p. 140° (decomp.). Treatment of the acidic filtrate from this mixture with 2 : 4-dinitrophenylhydrazine sulphate gave a small amount of a precipitate of the 2 : 4-dinitrophenylhydrazone of 2-hydroxy-4 : 5-dimethoxyacetophenone, which was removed; evaporation of the filtrate furnished an aqueous distillate containing formic acid which was isolated as the sodium formate. Part of this salt was converted into *NN'*-diphenylformamidine hydrochloride, forming colourless needles, m. p. 229—230° (decomp.). From the remaining portion of the sodium salt, benziminazole, m. p. 170°, was prepared.

On being heated under reflux with acetone (200 ml.) and sulphuric acid (5 g.) for 5 hours the 3-formylcoumarin (1 g.) slowly dissolved and after 3—4 hours was replaced by 4-hydroxy-6 : 7-dimethoxycoumarin which separated in plates. Isolated from the cooled mixture, this compound (0·7 g.) had m. p. 275—278° (decomp.) after purification.

Hydrogenation of 4-hydroxy-6 : 7-dimethoxy-3-formylcoumarin (0·5 g.) in ethyl acetate (200 ml.) at 100—110° with a palladium–charcoal catalyst and hydrogen at a pressure of 90 atmospheres during 8 hours gave 4-hydroxy-6 : 7-dimethoxy-3-methylcoumarin (0·4 g.) which formed colourless needles, m. p. 273° (from methanol), identical with a synthetical specimen (Part II, *loc. cit.*) (Found : C, 60·8; H, 5·3. Calc. for $C_{12}H_{12}O_5$: C, 61·0; H, 5·1%).

A solution of 4-hydroxy-6 : 7-dimethoxy-3-formylcoumarin (0·5 g.), and resorcinol (0·22 g.) in acetic acid (50 ml.) was saturated with hydrogen chloride and kept for several days. The red crystalline solid was collected, washed with ether, and crystallised from warm 2N-hydrochloric acid, giving *7-hydroxy-6' : 7'-dimethoxycoumarino(4' : 3'-2 : 3)benzpyrylium chloride* (XX) as red prisms (0·1 g.) which gave a wine-red colour with dilute aqueous sodium carbonate or sodium hydroxide (Found : C, 58·4; H, 4·0. $C_{17}H_{13}O_6Cl$ requires C, 58·5; H, 3·8%).

Hydrolysis of 1-(2-Hydroxy-4 : 5-dimethoxyphenyl)hexane-1 : 3 : 5-trione.—A solution of this triketone (0·5 g.) in 2N-aqueous sodium hydroxide (30 ml.) was heated on the steam-bath for 45 minutes, cooled, acidified with 2N-sulphuric acid, and treated with an excess of aqueous 2 : 4-dinitrophenylhydrazine sulphate. Next day the precipitate (0·3 g.) was collected, washed, dried, and extracted with benzene. Crystallisation of the insoluble residue from ethyl acetate gave 4-hydroxy-6 : 7-dimethoxycoumarin in colourless plates (0·05 g.), m. p. 278° (decomp.). After chromatography on alumina, acetone 2 : 4-dinitrophenylhydrazone (0·05 g.), m. p. 124—125°, and 2-hydroxy-4 : 5-dimethoxyacetophenone 2 : 4-dinitrophenylhydrazone (0·15 g.), m. p. 227° (decomp.), were isolated from the combined benzene extracts.

A solution of the triketone (0·05 g.) in concentrated sulphuric acid (2 ml.) was kept at room temperature for 12 hours, treated with ice-water (25 ml.), and extracted with chloroform. Evaporation of the combined, washed, and dried extracts left 6 : *7-dimethoxy-2-acetonylchromone* which formed colourless needles (0·03 g.), m. p. 144°, from ethyl acetate (Found : C, 63·9; H, 5·6. $C_{14}H_{14}O_5$ requires C, 64·1; H, 5·4%). This substance is insoluble in aqueous sodium carbonate, dissolves slowly in 2N-aqueous sodium hydroxide, and gives a 2 : 4-*dinitrophenylhydrazone* which separated from ethyl acetate in orange prisms, m. p. 210° (decomp.) (Found : C, 54·2; H, 4·3; N, 13·0. $C_{20}H_{18}O_8N_4$ requires C, 54·3; H, 4·1; N, 12·7%).

Acetylation of the triketone (0·2 g.) by means of acetic anhydride (1 ml.) and pyridine (3 ml.) at room temperature gave the acetate (XII; R = Ac) of 2-methyl-6-(2-hydroxy-4 : 5-dimethoxy)phenyl-γ-pyrone forming colourless prisms (0·1 g.), m. p. 151°, from ethyl acetate (Found : C, 63·3; H, 5·5%), undepressed on admixture with an authentic sample. On being heated with 4N-hydrochloric acid (5 ml.) on the steam-bath for 4 hours this acetate (0·05 g.) gave the parent γ-pyrone (XII; R = H), which separated from dioxan as colourless prisms (0·03 g.), m. p. 269° (decomp.).

Oxidation of the Piperonylidene Derivative of O-Dimethylcitromycin (XXIII).—(*a*) Lead tetra-acetate (0·6 g.) was added to the solution of this compound (0·5 g.) in acetic acid (50 ml.) during 30 minutes, at 35—40° and, after being maintained at this temperature for a further 15 minutes, the mixture, which then gave a negative test for lead tetra-acetate, was diluted with water (100 ml.). Crystallisation of the washed and dried yellow precipitate from dioxan gave the *piperonylidene* derivative (XXIV) of

O-dimethylcitromycinol in yellow prisms (0·25 g.), m. p. 258° (decomp.) (Found: C, 65·2; H, 4·4. $C_{23}H_{18}O_8$ requires C, 65·4; H, 4·3%). On being warmed with methanol (10 ml.) containing several drops of concentrated hydrochloric acid this base (0·1 g.) formed a deep-red solution which, when kept at room temperature for 24 hours, deposited the piperonylidene derivative of *O*-dimethylcitromycinyl chloride in dark red prisms (0·05 g.), decomposing at 223—225°.

(*b*) The compound (0·25 g.) was oxidised with chromic oxide (0·2 g.) in acetic acid at 30—40° for 30 minutes, the green solution was diluted with water (100 ml.) and extracted with chloroform (50 ml. × 6), and the chloroform extracts were washed, dried, and evaporated. Crystallised from acetic acid, the yellow residue gave the *piperonylidene* derivative (XXV) of *O*-dimethylcitromycinone in yellow prisms (0·1 g.), m. p. 290—292° (decomp.) (Found: C, 65·5; H, 4·0. $C_{23}H_{16}O_8$ requires C, 65·7; H, 3·8%). The same product (XXV) was obtained when the piperonylidene derivative (XXIV) (1 g.) was oxidised with chromic oxide (0·1 g.) in acetic acid under the same conditions.

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