182. *The Chemistry of Fungi. Part III. The Degradation of O-Dimethylcitromycin and the Structure of Citromycetin.*

By ALEXANDER ROBERTSON and W. B. WHALLEY.

The degradation of *O*-dimethylcitromycin, shown to have a reactive methylene group and to retain the essential citromycetin structure, has been studied. Hydrolytic fission of this ether gave rise to a complex mixture from which acetone, acetic acid, the ketone (I; R = Me), the acid (I; R = OH), and a substance which may be 5 : 6-dimethoxy-3-acetyldihydrocoumarin (XIII) have been isolated. Oxidation of *O*-dimethylcitromycin with ozone furnished a ketone, *O*-dimethylcitromycinone, together with considerable amounts of a compound which appears to be the corresponding alcohol *O*-dimethylcitromycinol and gives the ketone on oxidation; the same ketone is formed by oxidation with chromic acid. On hydrolysis, *O*-dimethylcitromycinone gave rise to acetone, acetic acid, the ketone (I; R = Me), the acid (I; R = OH), the 4-hydroxycoumarin (II), and the 4-hydroxy-3-acetylcoumarin (III). Formulæ (IV), (V), (VIII), and (XV; R = CO_2H) have been deduced respectively for *O*-dimethylcitromycinone, *O*-dimethylcitromycinol, *O*-dimethylcitromycin, and citromycetin.

ON being boiled with dilute sulphuric acid citromycetin, a metabolic product of various species of *Citromyces*, is converted with loss of carbon dioxide into the dihydric phenol, citromycin,

which closely resembles the parent acid and like it readily forms a dimethyl ether (Hetherington and Raistrick, *Phil. Trans.*, 1931, *B*, **220**, 209). In the course of preliminary experimental studies on the degradation and constitution of citromycetin and its dimethyl ether in these laboratories (unpublished work by one of us, A. R., and M. Tunnicliffe carried out during the period 1937—39) it became clear that, in order to avoid the complications which arise as the result of the carboxyl group originally present in citromycetin obtaining in the various degradation products, the employment of citromycin would afford a simpler experimental approach to our objective. This compound is unaffected by boiling 40% sulphuric acid or by boiling concentrated hydriodic acid. From the latter it separates as the hydriodide which, like the hydrobromide (Hetherington and Raistrick, *loc. cit.*), is readily hydrolysed by water. Although it has been found that citromycin like citromycetin is sensitive to warm alkalis, the formation of the dimethyl ether of citromycin by the methyl sulphate–sodium hydroxide method is unaccompanied by molecular change, since the use of diazomethane or of the methyl iodide–potassium carbonate method gave rise to the same dimethyl ether. Accordingly we have employed this readily accessible derivative in the present series of degradation experiments.

The application of the Zerewitinoff reaction to *O*-dimethylcitromycin gave a positive result, indicating the presence of one active hydrogen atom, but the compound did not appear to contain a hydroxyl group since it could not be induced to form any of the usual diagnostic derivatives, and we concluded that the active hydrogen indicated by the Zerewitinoff reagent was in all probability due to a reactive methylene group. The failure of *O*-dimethylcitromycin to yield products with the usual carbonyl reagents clearly indicates the absence of a normal ketonic group, a conclusion which is supported by the fact that Clemmensen reduction gave intractable resins whilst reductive acetylation furnished only unchanged material. *O*-Dimethylcitromycin does not contain a lactone group, but, like methyl *O*-dimethylcitromycetin (Hetherington and Raistrick, *loc. cit.*), it has been found to be unstable in boiling alcoholic alkalis, and accordingly its hydrolytic fission has been studied under a variety of conditions. It has been established, *inter alia*, that when *O*-dimethylcitromycin is boiled with alcoholic potassium hydroxide a mixture of acetone, acetic acid, 2-hydroxy-4 : 5-dimethoxyacetophenone (I; R = Me), and 2-hydroxy-4 : 5-dimethoxybenzoic acid (I; R = OH) is obtained, whilst when boiling saturated aqueous barium hydroxide is employed the same products are obtained together with a more complex carbonyl derivative which we have so far been able to isolate only as its 2 : 4-*dinitrophenylhydrazone*, m. p. 282° (decomp.). The nature of this substance has not been completely established but, on the basis of the empirical formula of the 2 : 4-dinitrophenylhydrazone and the further degradations of *O*-dimethylcitromycin described in this paper, we are inclined to the view that the parent ketonic product is 6 : 7-dimethoxy-3-acetyldihydrocoumarin (XIII). In spite of a careful search in the course of a considerable number of experiments on the hydrolysis of *O*-dimethylcitromycin by means of alkalis under a variety of conditions, we failed to find any evidence of the formation of the compound $C_{10}H_6O_3(OMe)_2$, m. p. 177—180°, which was obtained (in poor yield) by the hydrolytic fission of methyl *O*-dimethylcitromycetin according to the procedure of Hetherington and Raistrick (*loc. cit.*), and for which these authors suggested possible pyrone structures. Although we have not a direct proof we are inclined to the view that this compound, which is invariably obtained only in small yield from methyl *O*-dimethylcitromycetin, is an artefact formed in the course of the alkaline degradation, and further, that the carbomethoxy-group is probably involved in its formation (cf. Part II, *loc. cit.*). Since *O*-dimethylcitromycin is not hydrogenated with hydrogen and an active palladium–charcoal catalyst and is not oxidised with perbenzoic acid under the usual conditions it seems reasonably certain that it does not contain a reactive double bond. Attempts to dehydrogenate *O*-dimethylcitromycin with a palladium–charcoal catalyst or with chloranil under a variety of conditions were unsuccessful, whilst oxidation in acetone with aqueous permanganate appeared to be difficult to control and gave only a small yield of a product, m. p. 246°, which has not been further investigated. On the other hand oxidation of *O*-dimethylcitromycin with chromic acid in acetic acid furnished a ketonic substance, $C_{13}H_6O_4(OMe)_2$, m. p. 316° (decomp.), in satisfactory yield, which we have named O-*dimethylcitromycinone* and which forms an *oxime*, m. p. 268° (decomp.), a *phenylhydrazone*, and a condensation product with aniline in warm alcohol or acetic acid. The last substance appears to have the composition of an *anil* of a hydrated *O*-dimethylcitromycinone, and on being gently warmed with concentrated sulphuric acid regenerates the parent ketone. In the preparation of the oxime, m. p. 268° (decomp.), by the sodium acetate method, there is formed a small amount of a second derivative, m. p. 212° (decomp.), which is the sole product when the oximation is carried out by the pyridine procedure. Since it was converted into the oxime, m. p. 268°

(decomp.), by solution in dilute aqueous sodium hydroxide followed by acidification with acid this compound at first seemed to be an isomeride (*cis–trans* type), but the analytical results, which clearly exclude this possibility, indicate that it may be a dioxime. In the course of experiments primarily designed to detect the presence of a double bond in *O*-dimethylcitromycin it was found that the action of ozone on this compound also gave rise to a good yield of *O*-dimethylcitromycinone together with a second oxidation product $C_{13}H_8O_4(OMe)_2$, m. p. 251—252°, which we have designated O-*dimethylcitromycinol*, and which on further oxidation with chromic acid in acetic acid furnished *O*-dimethylcitromycinone. The proportion of the two compounds in the crude ozonolysis product seems to vary somewhat according to the conditions employed, but in most experiments they appeared to be produced in almost equal quantities. Since *O*-dimethylcitromycinol and *O*-dimethylcitromycinone can be isolated directly from the ozonolysis mixture when the procedure is carried out in anhydrous solvents without treating the reaction mixture with water, it seems clear that these compounds are formed by direct oxidation without the formation of an intermediate ozonide, presumably according to the scheme, $\cdot CH_2 \longrightarrow \cdot CH(OH) \longrightarrow \cdot CO$. In addition to the two main oxidation products the crude ozonolysis mixture invariably contained small amounts of material which appeared to be an ozonide and was removed by triturating the crude reaction mixture with water. When the aqueous liquor was treated with 2 : 4-dinitrophenylhydrazine hydrochloride solution a complex mixture was obtained from which the 2 : 4-*dinitrophenylhydrazone*, m. p. 236° (decomp.), of a substance was isolated, identical with a 2 : 4-dinitrophenylhydrazone subsequently obtained from the mixed product formed by the hydrolysis of *O*-dimethylcitromycinone with alkalis.

In view of the abnormal results obtained with ozone and because of the detection of active hydrogen in *O*-dimethylcitromycin in the absence of a hydroxyl group, further experiments to test for the presence of a reactive methylene group were carried out. It was found that *O*-dimethylcitromycin reacts slowly with nitrous acid to give a substance which is not identical or isomeric with either of the oximation products. This result indicates that the reactive methylene group which is readily oxidised to a carbonyl is not in the α-position to a normal reactive ketonic group, *i.e.*, $\cdot CH_2 \cdot CO \cdot$ is not converted into $\cdot C(:NOH) \cdot CO \cdot$. Similarly, it was found that *O*-dimethylcitromycin condensed with piperonal in the presence of alcoholic sodium ethoxide, giving rise to a *styryl* derivative. From the fact that this compound does not appear to contain a *C*-methyl group whereas the parent ether as well as *O*-dimethylcitromycinone each contain one *C*-methyl group according to the Kuhn–Roth method of estimation, it would seem clear that a reactive methyl group and not the methylene group oxidisable to carbonyl is concerned in the formation of the styryl compound, *i.e.*, the system $CH_3 \cdot CO \cdot$ or, in view of the nitrosation results, more likely $CH_3 \cdot C(:) \cdot CO \cdot$ is present. Curiously enough, styryl derivatives were not obtained from *O*-dimethylcitromycin when piperonal was replaced by other aromatic aldehydes, *e.g.*, benzaldehyde or *p*-dimethylaminobenzaldehyde, but whether this is significant we are unable to say at present.

O-Dimethylcitromycinone has been found to be readily decomposed both by warm alkalis and (much less readily) by warm acids. With the former agents a complex mixture is obtained, the composition of which depends to a considerable extent on the conditions employed. Thus with warm aqueous sodium hydroxide, *O*-dimethylcitromycinone gives rise to a mixture of acetone, acetic acid, the 2-hydroxy-4 : 5-dimethoxyacetophenone (I; R = Me), 2-hydroxy-4 : 5-dimethoxybenzoic acid (I; R = OH), and small amounts of two products which have been isolated as their 2 : 4-dinitrophenylhydrazones, m. p. 236° (decomp.) and m. p. 220° (decomp.), respectively. The former 2 : 4-dinitrophenylhydrazone is identical with that obtained in small yield from the crude ozonolysis mixture. The second appears to be the 2 : 4-*dinitrophenylhydrazone* of a compound $C_{11}H_6O_4(OMe)_2$. When the hot aqueous sodium hydroxide is replaced by boiling aqueous barium hydroxide the main product of the reaction, apart from acetone and acetic acid, appeared to be 4-hydroxy-6 : 7-dimethoxycoumarin (II), which was invariably accompanied by a small amount of the ketone (I; R = Me).

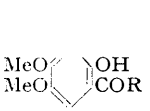
Decomposition of *O*-dimethylcitromycinone with warm concentrated hydrochloric acid gave rise to the product $C_{11}H_6O_4(OMe)_2$, m. p. 208°, which we consider to be 4-*hydroxy*-6 : 7-*dimethoxy*-3-*acetylcoumarin* (III) and which gives the 2 : 4-dinitrophenylhydrazone, m. p. 220° (decomp.), identical with that separated from the sodium hydroxide hydrolysate. Together with this compound there are formed carbon dioxide and small amounts of the substance giving the 2 : 4-dinitrophenylhydrazone, m. p. 236° (decomp.), but the presence of acetone in the hydrolysate has not been detected. When hydrochloric acid was replaced by 40% sulphuric acid, *O*-dimethylcitromycinone appeared to form a sparingly soluble sulphate which did not

undergo decomposition. A more detailed examination of the acidic decomposition of *O*-dimethylcitromycinone will be made when sufficient material is available. Although it has not yet been possible to synthesise 4-hydroxy-6 : 7-dimethoxy-3-acetylcoumarin (III) (cf. Part II, this vol., p. 562) in order to make a direct comparison with the natural degradation product, m. p. 208°, we consider that the structure of this compound follows from the fact that like 4-hydroxy-3-acetylcoumarin (Part II, *loc. cit.*) it forms a 2 : 4-dinitrophenylhydrazone, and on alkaline hydrolysis gives rise to the expected decomposition products, *viz.*, acetone, acetic acid, the ketone (I; R = Me), the acid (I; R = OH) and 4-hydroxy-6 : 7-dimethoxycoumarin (II). In support of the view that the coumarin, m. p. 208°, contains a *C*-acetyl group, it may be noted that in general the carbonyl group enolising to give the hydroxyl group in the 4-position of 4-hydroxycoumarins does not normally react with hydrazines. The degradation product (III) has been found to be isomeric and not identical with the synthetical substance believed to be 6 : 7-dimethoxy-2-methylchromone-3-carboxylic acid (Part II, *loc. cit.*), but in the course of experiments on the synthesis of the latter compound by the condensation of the ketone (I; R = CH_2·CO·CH_3) with ethyl carbonate by means of sodium, a method which can conceivably give rise to coumarin or chromone, it was found in two experiments that, together with the chromone, a small amount of a compound was formed which gave a 2 : 4-dinitrophenylhydrazone, m. p. 220° (decomp.), identical with that from *O*-dimethylcitromycinone.

If the assumption is made, which appears reasonable, that the hydrolysis of *O*-dimethylcitromycinone under the conditions discussed is not preceded by a deep-seated isomeric change in the parent molecular structure, then, from the formation of the various hydrolytic products—acetone, acetic acid (I; R = Me), (I; R = OH), (II), and (III), which have been defined, it seems fairly certain that *O*-dimethylcitromycinone is best represented by formula (IV). Formula (VII) is considered to be much less likely, whilst structures of the type (XII), which cannot give rise to 4-hydroxy-6 : 7-dimethoxycoumarin or its derivatives, can be excluded. The structure type (VI), or a variation of it, for *O*-dimethylcitromycinone, which could conceivably arise by the oxidation of an active methyl group, is excluded because the compound retains the *C*-methyl group originally present in *O*-dimethylcitromycin. Though a substance having a formula of the type (IV) might not on general grounds be expected readily to form ketonic derivatives, the instability of the chromono-γ-pyrone system in (IV) towards warm hydrochloric acid is more readily understood. The latter property is regarded as being due to the presence of the ether link involving the oxygen atom of the enolic form of the potential carboxyl group, *i.e.*, due to the tendency to regenerate the 4-hydroxycoumarin system by fission as indicated by the dotted line in formula (IV). The possibility that the degradation of *O*-dimethylcitromycinone with acids is initiated by hydration to give the intermediate (XIV) is considered unlikely. On the other hand, the main objection to formula (VII) for *O*-dimethylcitromycinone is that a substance having this structure, which could conceivably arise by the isomerisation of a compound having formula (IV), would hardly be expected to be sensitive to the hydrolytic effects of warm acids, and would certainly be less likely to form ketonic derivatives than (IV). The chemistry of *O*-dimethylcitromycinol, the oxidation product intermediate between *O*-dimethylcitromycin and *O*-dimethylcitromycinone, has not yet been fully investigated, but on the basis of the structure (IV) for the ketonic compound it seems clear that *O*-dimethylcitromycinol is best represented by formula (V). This implies that the compound is a 4-carbinol base comparable to the xanth-hydrols and consequently would be expected to react with acids, forming salts of the pyrylium type with the elimination of water. In agreement with this, a well-defined picrate, a perchlorate, and a ferrichloride have been prepared from the base (private communication from Mr. G. W. K. Cavill of this laboratory).

It is clear that *O*-dimethylcitromycin contains a reactive methylene group which is readily oxidised to give a reactive carbonyl group and further, that it contains a reactive methyl group which condenses with piperonal to give the styryl derivative. Thus on the basis of the two most probable formulæ, (IV) and (VII), for *O*-dimethylcitromycinone, *O*-dimethylcitromycin may be formulated as (VIII), type (IX), type (X), or type (XI), and of these the structures type (IX) and type (XI), which contain a γ-pyran nucleus (or the possible isomeric forms containing an α-pyran nucleus), may be excluded because in substances having this kind of residue the double bond reacts normally, undergoing hydrogenation and oxidation in the usual manner. Of the two remaining feasible structures (VIII) and (X) for *O*-dimethylcitromycin we prefer (VIII) in which the reactivity of the methylene group in the 4-position of the chromeno-residue is reminiscent of that in indene, fluorene, or xanthen, which is readily oxidised to a carbonyl group and responds to the Zerewitinoff reagent (one active hydrogen). Further we do not regard the angular structure, type (X), as being an attractive alternative to (VIII) because on

general grounds we would not expect the methylene group of the system ·O·CH_2·C⁞ to be reactive, and in support of this conclusion we have found in analogous chromeno(3′ : 4′ : 4 : 3)coumarins (Robertson, *et al.*, *J.*, 1936, 423) that the ·O·CH_2· group in the chromeno-system is not oxidised by ozone to ·O·CO·, *i.e.*, the chromen residue is not converted into a coumarin residue. On the basis of the formula suggested it would seem that the comparatively ready formation of the ketone (I; R = Me) and the acid (I; R = OH) in the course of the hydrolytic fission of *O*-dimethylcitromycin with aqueous alkalis is due to the oxidative action of these reagents on the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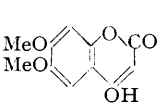
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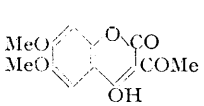
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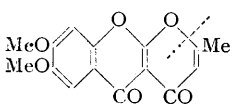
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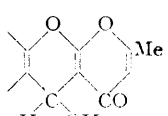
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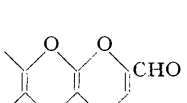
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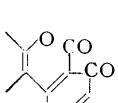
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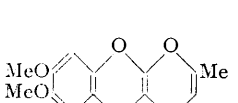
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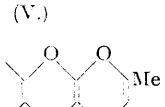
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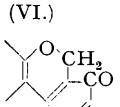
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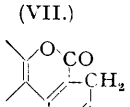
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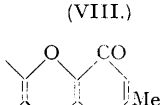
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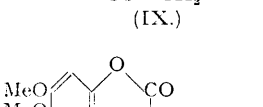
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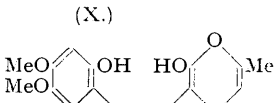
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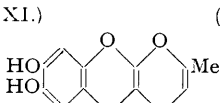
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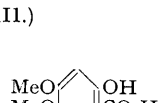

(XVI.)

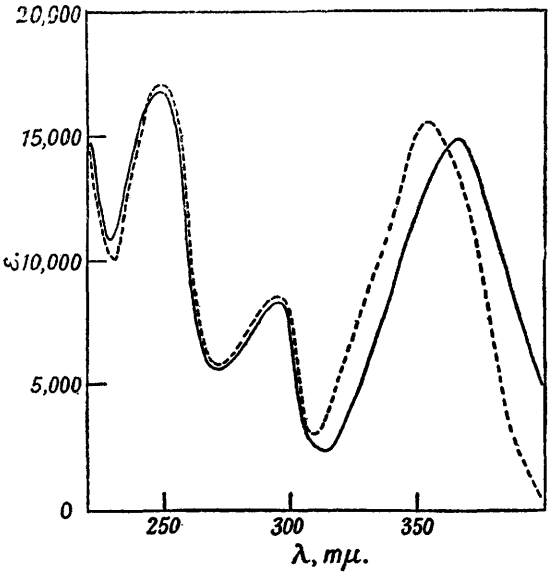
reactive methylene group. If the suggestion can be substantiated that the product obtained as its 2 : 4-dinitrophenylhydrazone, m. p. 282° (decomp.), from the hydrolysis of *O*-dimethylcitromycin by means of boiling aqueous barium hydroxide is the dihydrocoumarin derivative (XIII), then decisive evidence will be available in favour of formula (VIII) for *O*-dimethylcitromycin. Meanwhile, the spectroscopic evidence, as reported by Dr. Stubbs in the addendum, clearly supports the structure (VIII) for *O*-dimethylcitromycin, where the methylene group and the oxygen atom act as insulators between the chromophoric phenyl group and the γ-pyrone residue.

From the structure (VIII) allocated to its dimethyl ether citromycin is clearly represented by formula (XV; R = H). It has been assumed that the parent compound citromycetin is a carboxycitromycin (cf. Hetherington and Raistrick, *loc. cit.*), a conclusion which does not necessarily follow since the conversion of citromycetin into citromycin by means of warm dilute sulphuric acid may well involve a fundamental change in the original citromycetin structure simultaneous with the loss of carbon dioxide, and, further, the carboxyl group originally present may not be that ultimately eliminated. Accordingly, we have endeavoured to obtain evidence on this point. Although it was not found possible to decarboxylate *O*-dimethylcitromycetin by standard procedures, citromycetin is smoothly converted into citromycin in hot glycerol. Further, alkaline degradation of *O*-dimethylcitromycetin invariably gives rise, *inter alia*, to 3-hydroxy-5 : 6-dimethoxyphthalic acid, whereas from *O*-dimethylcitromycin the only phenolic acid obtained is 2-hydroxy-4 : 5-dimethoxybenzoic acid, indicating that the carboxyl group lost in the decarboxylation process is that appearing in the 1-position of the phthalic acid. Moreover, it has been found that the oxidation of methyl *O*-dimethylcitromycetin with aqueous potassium permanganate in acetone gives rise to an *acid-ester* (XVI) of 3-hydroxy-5 : 6-dimethoxyphthalic acid, a result which serves to prove that the carbomethoxy-group in methyl *O*-dimethylcitromycetin is present in the hydroxyquinol residue. These results, together with the spectroscopic evidence obtained by Dr. A. L. Stubbs (see addendum), confirm the view that the conversion of citromycetin into citromycin is a simple decarboxylation. Consequently citromycetin is represented by formula (XV; R = CO_2H).

In the course of attempts to hydrogenate *O*-dimethylcitromycinone dissolved in dioxan by

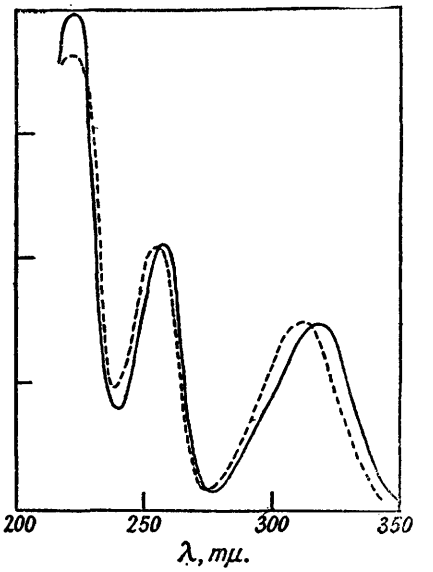
means of hydrogen and a palladium–charcoal catalyst, it was found that hydrogen was not absorbed but a product was obtained which appeared to be a *hydrate*, $C_{13}H_8O_5(OMe)_2$, of the original substance. Subsequently, it was discovered that the hydrate was formed by the catalyst in the absence of hydrogen and in the presence of a trace of hydrochloric acid. This hydrate, which gives a ferric reaction and is readily soluble in aqueous sodium hydroxide, regenerates *O*-dimethylcitromycinone on treatment with cold sulphuric acid or warm acetic anhydride, gives the same anil as *O*-dimethylcitromycinone, and on oximation furnishes the oxime of *O*-dimethylcitromycinone. On hydrolysis with warm 2N-sodium hydroxide the hydrate gave rise to acetone, the ketone (I; R = Me), and 4-hydroxy-6 : 7-dimethoxycoumarin, whilst on ozonolysis it gave rise to a complex mixture which has not been fully investigated but from which the 2 : 4-dinitrophenylhydrazone, m. p. 236°, has been obtained, identical with that formed by material from the alkaline hydrolysis of *O*-dimethylcitromycinone. In the meantime, we assign formula type (XIV) provisionally to this hydrate.

FIG. 1.



——— *O-Dimethylcitromycin.*
– – – – – *Methyl O-dimethylcitromycetin.*

FIG. 2.



——— *2-Hydroxy-4 : 5-dimethoxybenzoic acid.*
– – – – – *2-Hydroxy-6-carbomethoxy-4 : 5-dimethoxybenzoic acid.*

Addendum on Ultra-violet Absorption Spectra (A. L. STUBBS).—The ultra-violet absorption spectra of *O*-dimethylcitromycin (VIII) and methyl *O*-dimethylcitromycetin (XV; R = CO_2Me and OH groups replaced by OMe) have been examined in ethanol solution, using a Hilger Medium Quartz Spectrograph in conjuction with a rotating sector photometer. The absorption curves are shown in Fig. 1, and the positions and intensities of maximum absorption are tabulated below.

Compound.	$\lambda_{max.}$ (mμ).	ε, max.	Compound.	$\lambda_{max.}$ (mμ).	ε, max.
(I, R = OH)	318	7,390	(VIII)	365	15,100
	256·5	10,600		296	8,360
	222	19,800		249	16,900
(XVI)	311	7,420	Methyl *O*-dimethylcitromycetin	354	15,600
	255·5	10,400		293	8,570
	222	19,200		250	17,000

(Solvent, ethanol.)

In order to confirm that the sole difference between the citromycin and citromycetin derivatives is in the presence or absence of the carboxyl group, the spectra of these two compounds may be compared with those of the benzoic acid (I; R = OH) and its carbomethoxy-derivative (XVI), also measured in ethanol solution (Fig. 2 and Table). The elimination of the carbomethoxy-group in both pairs of compounds results in entirely analogous spectral changes,

which are in fact extremely small, and make it certain that no other structural change has taken place during the decarboxylation of the citromycetin compound.

It is interesting to discuss the spectra briefly in terms of chromophoric groups. The phenyl chromophore in (VIII) is " insulated " by the oxygen atom and the methylene group from the rest of the molecule. Consequently the characteristic absorption maxima of (I; R = OH) should appear with little change. It is therefore justifiable to associate the 318 mμ and 256·5 mμ maxima of (I; R = OH) with the 296 mμ and 249 mμ maxima of (VIII), the slight shift to shorter wave-lengths resulting from the replacements of carbonyl by methylene group. The 365 mμ maximum of (VIII) is, on this basis, to be ascribed to the remaining unsaturated linkages in the molecule, which chromophorically may be compared with phorone $CMe_2{:}CH{\cdot}CO{\cdot}CH{:}CMe_2$. Phorone shows $\lambda_{max.}$ 263 and 357 mμ with molecular extinction coefficient 23,500 and 82 respectively. The similarity in the positions of the 357 mμ maximum of phorone and the 365 mμ maximum of (VIII) justifies the analogy, but the ring closure and substitution have clearly given rise to considerable increase in extinction. Such an increase in extinction for a low intensity band, located in the carbonyl group, is not unusual (cf. Morton and Rosney, *J.*, 1926, 706).

Experimental.

Citromycin.—(*a*) A mixture of citromycetin (3 g.), water (180 ml.), and sulphuric acid (30 ml.) was boiled under reflux for 8 hours and then diluted with water (100 ml.). Next day the crystalline precipitate was collected, triturated with aqueous sodium hydrogen carbonate, and crystallised from dilute methanol, giving citromycin (2·2 g.) in pale greenish yellow needles, m. p. 296—298° (decomp.) (cf. Hetherington and Raistrick, *loc. cit.*, who give m. p. 285—290°).

(*b*) When a mixture of citromycetin (0·8 g.), glycerol (5 ml.), and copper bronze (0·3 g.) in an atmosphere of nitrogen was gradually heated (oil-bath) to 280° and then kept at this temperature for 10 minutes carbon dioxide was steadily evolved. The cooled mixture was diluted with aqueous sodium hydrogen carbonate, the precipitate was repeatedly extracted with boiling alcohol, and the combined extracts were concentrated. On cooling, the residue deposited citromycin in pale greenish yellow needles (0·1 g.), m. p. 298° (decomp.) after recrystallisation, identical with a specimen prepared by method (*a*) and giving *O*-dimethylcitromycin on methylation.

A mixture of citromycin (0·5 g.) and hydriodic acid (25 ml., of constant-boiling mixture) was boiled under reflux for 3 hours, and on cooling gave the hydriodide in golden needles. Treatment of this salt with aqueous sodium hydrogen carbonate regenerated citromycin, identified by comparison with authentic material. Similarly, citromycin was recovered unchanged when red phosphorus (0·2 g.) was added to the reaction mixture and the time of heating extended to 4 hours.

O-Dimethylcitromycin.—Citromycin (10 g.) was methylated by being heated with methyl iodide (20 ml.) and acetone (1000 ml.) containing potassium carbonate (40 g.) on the steam-bath for 6 hours until the ferric reaction was negative, with the addition of more iodide (10 ml.) after 3 hours. The hot solution was filtered, the potassium salts extracted with boiling acetone (2 × 100 ml.), the combined filtrates evaporated, and the residue crystallised from methyl or ethyl alcohol. After having been thoroughly extracted with water to remove traces of potassium salts, the dimethyl ether was recrystallised from methanol, forming colourless prisms (9 g.), m. p. 225—227°, identical with a specimen prepared by diazomethane (Hetherington and Raistrick, *loc. cit.*) [Found : C, 65·6; H, 5·3; *C*-methyl, 6·1. Calc. for $C_{12}H_5O_3(OMe)_2(Me)$: C, 65·7; H, 5·2; *C*-methyl, 5·2%].

When a mixture of *O*-dimethylcitromycin (0·2 g.), piperonal (0·2 g.), potassium ethoxide (from 0·05 g. of potassium), and alcohol (7 ml.) was heated on the water-bath for ½ hour and then kept at room temperature for 7 days, the *styryl* derivative gradually separated in clusters of pale greenish yellow needles (0·1 g.) which on isolation and recrystallisation from alcohol had m. p. 243° (Found : C, 67·5; H, 4·5. $C_{23}H_{18}O_7$ requires C, 68·0; H, 4·4%). When piperonal was replaced by benzaldehyde or by *p*-dimethylaminobenzaldehyde condensation did not take place.

A mixture of *O*-dimethylcitromycin (0·2 g.), alcohol (5 ml.), and concentrated hydrochloric acid (3 ml.) was cooled to 0°, treated with amyl nitrite (3 ml.), and then kept at room temperature for 4 days. On the addition of water to the clear solution an *iso*nitroso-derivative (0·2 g.) gradually separated, and on recrystallisation from aqueous alcohol formed almost colourless needles, m. p. 259—260° (Found : C, 61·9, 62·0; H, 5·3, 5·1. N, 4·4. Calc. for $C_{15}H_{13}O_6N$: C, 59·4; H, 4·3; N, 4·6%).

Hydrolysis of O-Dimethylcitromycin with Potassium Hydroxide.—(*a*) A mixture of *O*-dimethylcitromycin (2 g.), alcohol (16 ml.), and potassium hydroxide (4 g., dissolved in 7 ml. of water) was kept at 100° for 3½ hours, and simultaneously a slow stream of nitrogen was led through the containing vessel and the effluent gas was passed through a wash-bottle containing aqueous 2 : 4-dinitrophenylhydrazine sulphate. When the mixture had cooled, the yellow precipitate, which had formed in the wash-bottle, was separated, washed, and crystallised from methanol, giving the 2 : 4-dinitrophenylhydrazone of acetone in yellow needles, m. p. 125°, identified by comparison with an authentic specimen. The alkaline hydrolysate was diluted with water (50 ml.), the greater part of the alcohol was evaporated in a vacuum, the residual solution was saturated with carbon dioxide and extracted with chloroform (50 ml. × 4), and the dried chloroform extracts were distilled, leaving an intractable gum (0·6 g.). After acidification with 2N-sulphuric acid the residual aqueous liquor was extracted with ether (6 × 120 ml.), the combined extracts dried and evaporated, and the residue crystallised from aqueous acetone, giving 2-hydroxy-4 : 5-dimethoxybenzoic acid in colourless needles (0·35 g.), m. p. 213—214° (decomp.), which had a blue ferric reaction and were identified by comparison with an authentic specimen (Head and Robertson, *J.*, 1931, 2432) [Found : C, 54·6; H, 5·0; OMe, 31·2. Calc. for $C_7H_4O_3(OMe)_2$: C, 54·5; H, 5·1; OMe, 31·3%].

(*b*) The foregoing degradation was repeated with *O*-dimethylcitromycin (1 g.), and the cooled hydrolysate was acidified immediately with 2N-sulphuric acid, filtered to remove a gummy precipitate (X), and treated with excess of aqueous 2 : 4-dinitrophenylhydrazine sulphate solution. Next day the precipitate (0·4 g.) was collected, dried, dissolved in benzene, and chromatographed on a column of aluminium oxide, giving two main zones and with several minor ones. The lower main pale yellow zone (A) was washed through the column with benzene and then the main deep red zone (B) was washed through with chloroform. Evaporation of the benzene liquor containing material from zone (A) left the 2 : 4-dinitrophenylhydrazone of acetone, forming yellow needles, m. p. 125°, from methanol, undepressed on admixture with an authentic specimen. The residue obtained from the evaporation of the chloroform liquor containing the material from zone (B) was crystallised from benzene and then chloroform giving the 2 : 4-dinitrophenylhydrazone of 2-hydroxy-4 : 5-dimethoxyacetophenone, in red needles, m. p. 228° (decomp.), identified by comparison with an authentic specimen (Part II, *loc. cit.*) [Found : C, 51·5; H, 4·4; N, 13·5; OMe, 17·4. Calc. for $C_{14}H_{10}O_5N_4(OMe)_2$: C, 51·1; H, 4·3; N, 14·9; OMe, 16·5%]. Distillation of the acidic filtrate left after the removal of the 2 : 4-dinitrophenylhydrazones gave acetic acid which was isolated as the sodium salt and identified by conversion into the anilide, m. p. 112°, after purification, undepressed on admixture with an authentic sample (Found : N, 10·7. Calc. for C_8H_9ON : N, 10·4%).

On repeated crystallisation from methanol the precipitate (X) gave 2-hydroxy-4 : 5-dimethoxyacetophenone (10 mg.) in colourless stout prisms, m. p. 112°, identified by comparison with synthetical material (Part II, *loc. cit.*).

(*c*) *O*-Dimethylcitromycin (5 g.) was hydrolysed by being boiled with potassium hydroxide (10 g.), water (10 ml.), and alcohol (40 ml.) in an atmosphere of nitrogen during 3½ hours according to the procedure employed by Hetherington and Raistrick (*loc. cit.*) for *O*-dimethylcitromycetin. The effluent gas contained acetone which was isolated as the 2 : 4-dinitrophenylhydrazone, m. p. 125°, after purification. The dark alkaline hydrolysate was diluted with water (100 ml.), the greater part of the alcohol removed under reduced pressure, and the residual liquor saturated with carbon dioxide and extracted with chloroform (5 × 50 ml.). Crystallisation of the residue left on evaporation of the dried chloroform extracts from methanol gave 2-hydroxy-4 : 5-dimethoxyacetophenone (25 mg.), m. p. 112°, identical with an authentic sample.

After having been extracted with chloroform the aqueous liquor was acidified with dilute sulphuric acid and found to contain acetic acid in addition to small amounts of acetone and 2-hydroxy-4 : 5-dimethoxyacetophenone which were identified by treating a portion of the acidic liquor with 2 : 4-dinitrophenylhydrazine sulphate and separating the resulting mixed hydrazones by chromatography.

In spite of a careful search on the occasion of a number of experiments we failed to find any trace of the so-called pyrone, $C_{10}H_6O_3(OMe)_2$, which was isolated from *O*-dimethylcitromycetin by Hetherington and Raistrick (*loc. cit.*).

(*d*) A saturated solution of barium hydroxide (100 c.c.), containing a suspension of the *O*-dimethylcitromycin (1 g.), was refluxed in an atmosphere of nitrogen for 25 minutes, and half of the cooled solution was acidified with sulphuric acid, filtered, and distilled, giving an aqueous distillate which contained acetic acid, identified by conversion into the anilide. The remaining half of the barium hydroxide liquor was acidified with hydrochloric acid, and filtered to remove an intractable solid (0·5 g.) which constitutes the bulk of the product, and the filtrate was treated with aqueous 2 : 4-dinitrophenylhydrazine hydrochloride. A solution of the resulting precipitate (0·3 g.) in benzene was poured on a column of aluminium oxide giving (1) an orange yellow zone which was washed through the column with benzene, (2) a deep red zone which was washed through with chloroform, and (3) two smaller brownish zones which were eluted with chloroform and gave only resinous material. The product from zone (1) consisted of acetone 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 125°, and that from zone (2) was obtained as a bright crimson solid on evaporation of the chloroform and on crystallisation from ethyl acetate gave a *compound* in fluffy crimson needles, m. p. 282° (decomp.), which appeared to be a 2 : 4-dinitrophenylhydrazone of a compound $C_{11}H_8O_3(OMe)_2$ [Found : C, 53·0; H, 4·5; N, 13·1; OMe, 11·3. $C_{17}H_{12}O_6N_4(OMe)_2$ requires C, 53·0; H, 4·2; N, 13·0; OMe, 14·4%].

The effluent gas contained acetone which was isolated as the 2 : 4-dinitrophenylhydrazone (0·6 g.). In some experiments 2-hydroxy-4 : 5-dimethoxyacetophenone and 2-hydroxy-4 : 5-dimethoxybenzoic acid were also obtained.

Ozonolysis of O-*Dimethylcitromycin.*—Of many experiments carried out the following is typical. A moderate stream of dry ozone and oxygen was bubbled through a solution of the dimethyl ether (1 g.) in anhydrous chloroform (75 ml.) kept at 0° for 20 minutes, the chloroform was removed under reduced pressure, and the residue was triturated with water (150 ml.). Next day the solid was collected and refluxed with alcohol (125 ml.), the cooled solution kept for 24 hours, and the crystalline solid collected and recrystallised from much alcohol or dioxan or a small volume of glacial acetic acid, giving O-*dimethylcitromycinone* in very pale fawn needles (0·5 g.), m. p. 316° (decomp.) [Found : (mean of 4 carbon and hydrogen determinations) : C, 62·6; H, 4·4; OMe, 21·0; *C*-Me, 7·5. $C_{12}H_3O_4(OMe)_2(Me)$ requires C, 62·5; H, 4·2; OMe, 21·5; *C*-Me, 5·2%]. This compound has a negative ferric reaction, is insoluble in aqueous sodium hydroxide, sparingly soluble in the usual organic solvents, and forms a yellow solution in concentrated hydrochloric acid. A mixture of *O*-dimethylcitromycinone (0·25 g.), hydroxylamine hydrochloride (0·1 g.), sodium acetate (0·1 g.), and alcohol (25 ml.) was refluxed on the steam-bath for 6 hours; on cooling the clear solution deposited the *oxime* (0·2 g.) in pale greenish needles, which on recrystallisation from alcohol had m. p. 268° (decomp.) and had a negative ferric reaction (Found : C, 59·5; H, 4·1; N, 5·0. $C_{15}H_{13}O_6N$ requires C, 59·4; H, 4·3; N, 4·7%). In some experiments a second and more soluble product of oximation, m. p. 212° (decomp.), was obtained from the alcohol residues. This substance appeared to be the sole reaction product when the oximation was carried out in pyridine. A mixture of *O*-dimethylcitromycinone (0·5 g.), hydroxylamine hydrochloride (0·25 g.), and pyridine (5 ml.) was warmed on the steam-bath and the clear solution, which was obtained in the course of a few minutes, poured into excess of dilute hydrochloric acid. Crystallisation of the well washed precipitate from alcohol gave the *compound* in colourless needles (0·5 g.), m. p. 212° (decomp.),

having a negative ferric reaction (Found : C, 56·5; H, 4·0; N, 9·3. $C_{15}H_{14}O_6N_2$ requires C, 56·6; H, 4·4; N, 9·0%).

When this substance was dissolved in excess of 2N-sodium hydroxide and the solution acidified with hydrochloric acid, the oxime of *O*-dimethylcitromycinone, m. p. 268° (decomp.) after purification, was obtained in quantitative yield.

A mixture of *O*-dimethylcitromycinone (0·5 g.), phenylhydrazine hydrochloride (0·5 g.), sodium acetate (0·5 g.), and alcohol (25 ml.) was warmed on the steam-bath until a clear solution was obtained, and then a part of the solvent was evaporated. On cooling, the residual solution deposited the *phenylhydrazone* (0·5 g.) which, on recrystallisation from alcohol, formed colourless needles, m. p. 218° (Found : N, 6·8. $C_{21}H_{18}O_5N_2$ requires N, 7·4%).

When a mixture of *O*-dimethylcitromycinone (1 g.), aniline (3 ml.), and acetic acid (10 ml.) was warmed on the water-bath for 1 minute a bright yellow crystalline mass gradually separated. Recrystallisation of this from dioxan, chloroform, or acetic acid gave the *anil* of hydrated *O*-dimethylcitromycinone in bright yellow prisms, m. p. 252° (decomp.), sparingly soluble in alcohol, ethyl acetate, or chloroform (Found : C, 66·4; H, 5·0; N, 3·6. $C_{21}H_{19}O_6N$ requires C, 66·1; H, 5·0; N, 3·7%). The same compound was obtained when the reaction was carried out in boiling alcohol during 8 hours. When a solution of this product in a little concentrated sulphuric acid was warmed on the water-bath for 3 minutes, cooled, and poured into water, a precipitate of *O*-dimethylcitromycinone separated, m. p. 316° (decomp.), after purification.

Evaporation under reduced pressure of the alcoholic liquor left after the separation of the crude *O*-dimethylcitromycinone a viscous residue which was dissolved in hot dioxan (10 ml.). On cooling, the solution slowly deposited O-*dimethylcitromycinol* which then crystallised from acetone, alcohol, or dioxan, forming almost colourless needles, m. p. 251—252° (decomp.) [Found : C, 61·8; 62·3; H, 4·7, 5·0; OMe, 22·9; *C*-Me, 6·3. $C_{12}H_5O_4(OMe)_2(Me)$ requires C, 62·1; H, 4·8; OMe, 21·4; *C*-Me, 5·2%]. This compound dissolves in warm aqueous sodium hydroxide, has a negative ferric reaction, forms a bright yellow solution in concentrated hydrochloric acid, and does not form ketonic derivatives. A mixture of *O*-dimethylcitromycinol (0·3 g.), chromic anhydride (0·3 g.), and acetic acid (3 ml.) was boiled under reflux for 5 minutes and, on being cooled, deposited *O*-dimethylcitromycinone (0·2 g.) which had m. p. 316° (decomp.) after purification from acetic acid and was identical with an authentic specimen.

The aqueous extract of the crude ozonolysis product, which was only faintly acid and did not contain a detectable amount of volatile acid, gave a precipitate with aqueous 2 : 4-dinitrophenylhydrazine sulphate of which 0·8 g. was collected from 4 g. of *O*-dimethylcitromycetin. A solution of this material in chloroform was chromatographed on a column of aluminium oxide, giving four zones : (1) deep red, washed through the column with chloroform, (2) pale orange yellow, washed through with chloroform, (3) orange, washed through with a chloroform–methanol mixture (9 : 1), (4) deep red, washed through with methanol. Evaporation of the extracts from zones (1), (3), and (4) each gave resinous material which has not yet been further examined, whilst the elution of the material forming zone (2) with chloroform provided an orange crystalline solid (0·4 g.). Recrystallisation of this from chloroform or ethyl acetate gave a product in orange needles, m. p. 236° (decomp.), which appeared to be a 2 : 4-dinitrophenylhydrazone [Found : C, 53·4; H, 4·1; N, 13·3. OMe, 12·6. Calc. for $C_{17}H_{12}O_6N_4(OMe)_2$: C, 53·0; H, 4·2; N, 13·0; OMe, 14·4. Calc. for $C_{17}H_{10}O_6N_4(OMe)_2$: C, 53·3; H, 3·7; N, 13·1; OMe, 14·5%].

In several ozonolysis experiments *O*-dimethylcitromycinone partly separated from the chloroform solution during the passage of the gases, and it was subsequently discovered that this compound and its isomeride were most conveniently obtained by evaporating the chloroform solution and extracting the residue with warm alcohol (125 ml.). Evaporation of the alcoholic extract left *O*-dimethylcitromycinol (0·4—0·5 g.), whilst the alcohol-insoluble solid was almost pure *O*-dimethylcitromycinone.

Oxidation of O-*Dimethylcitromycin with Chromic Anhydride and with Potassium Permanganate.*—Chromic anhydride (0·8 g.), dissolved in acetic acid (25 ml.), was added gradually to a solution of the dimethyl ether (0·35 g.) in the same solvent (5 ml.), and the mixture boiled for 10 minutes. On cooling, the bright green solution gradually deposited *O*-dimethylcitromycinone in pale buff coloured needles (0·2 g.), m. p. 316° (decomp.) after recrystallisation (Found : C, 62·0; H, 4·9%). This material was found to be identical with an authentic specimen, and gave rise to the oxime, m. p. and mixed m. p. 268° (decomp.).

A solution of potassium permanganate (4 g.) in water (75 ml.) was added gradually during 6 hours to *O*-dimethylcitromycin (1 g.) dissolved in acetone (300 ml.), and next day the mixture was cleared with sulphur dioxide, the greater part of the acetone was removed under reduced pressure, and the residual solution was warmed with a little 2N-sulphuric acid and extracted with ether (6 × 100 ml.). Evaporation of the dried extracts left a residue which on repeated crystallisation from acetone gave a product in rosettes of plae yellow needles (10 mg.), m. p. 246° (decomp.), having a negative ferric reaction and exhibiting a green fluorescence in acetone (Found : C, 66·9; H, 5·7; OMe, 20·4%).

Hydrolysis of O-*Dimethylcitromycinone with Alkali.*—(*a*) A mixture of the compound (0·5 g.) in 2N-sodium hydroxide (20 ml.) was heated on the steam-bath for 3—4 minutes, and the resulting solution acidified with 2N-sulphuric acid, kept for 16 hours, filtered to remove a trace of amorphous solid, and treated with excess of 2 : 4-dinitrophenylhydrazine sulphate solution. Next day the precipitate (1·75 g., from four experiments) was collected, dried, and dissolved in benzene. When this solution was poured on a column of aluminium oxide and the chromatogram developed with the same solvent, four zones were obtained : (1) a pale orange-yellow zone which was washed through with benzene, giving acetone 2 : 4-dinitrophenylhydrazone (0·2 g.), m. p. and mixed m. p. 125° (Found : N, 23·8. Calc. for $C_9H_{10}O_4N_4$: N, 23·5%), (2) a pale orange zone which was washed through with chloroform and gave a 2 : 4-dinitrophenylhydrazone, forming orange needles (0·3 g.), m. p. 236° (decomp.), from ethyl acetate, identical with the 2 : 4-dinitrophenylhydrazone obtained from the aqueous washings of the ozonolysis mixture, (3) a greenish yellow zone which was washed through the column with a chloroform–methanol mixture (9 : 1) and the product crystallised from ethyl acetate, forming orange tablets, m. p. 220°, which we consider may be the 2 : 4-dinitrophenylhydrazone of 4-hydroxy-6 : 7-dimethoxy-3-acetylcoumarin,

(4) a deep red zone which was washed through the column with methanol. On evaporation of the solvent a substance was left which appeared to be a 2 : 4-dinitrophenylhydrazone (16 mg.), and crystallised from methanol in red needles, m. p. 296° (decomp.), but the amount available was insufficient for final purification and analysis.

Distillation of the aqueous liquors from the crude mixed 2 : 4-dinitrophenylhydrazones gave acetic acid which was identified by conversion into the anilide, m. p. and mixed m. p. 112° (Found : N, 10·7. Calc. for C_8H_9ON : N, 10·4%).

(*b*) *O*-Dimethylcitromycinone (1 g.) was heated with 2N-sodium hydroxide (65 ml.) for 15 minutes, and the cooled mixture was diluted with water (100 ml.), acidified with dilute sulphuric acid, filtered next day to remove a trace of solid, and divided into two equal portions. One portion of the solution was extracted with ether (6 × 100 ml.); the residue left on evaporation of the crude extracts was separated by means of aqueous sodium hydrogen carbonate into 2-hydroxy-4 : 5-dimethoxyacetophenone and 2-hydroxy-4 : 5-dimethoxybenzoic acid. The ketone was purified by repeated crystallisation from methanol, forming colourless prisms, m. p. and mixed m. p. 112°, identical with a synthetical specimen (Part II, *loc. cit.*). The acid separated from aqueous acetone in colourless needles, m. p. 213—214°, and was identified by comparison with synthetical material.

Addition of aqueous 2 : 4-dinitrophenylhydrazine sulphate to the second portion of the acidified hydrolysate gave an orange-red precipitate which was collected, washed, and dried. On being chromatographed from benzene on a column of aluminium oxide this was separated into the 2 : 4-dinitrophenylhydrazones of acetone and of 2-hydroxy-4 : 5-dimethoxyacetophenone which were identified by comparison with authentic specimens.

(*c*) *O*-Dimethylcitromycinone (1 g.) was boiled under reflux with saturated aqueous barium hydroxide (100 ml.) for ½ hour, and the filtered solution acidified with hydrochloric acid. After removal of a trace of solid the solution was treated with excess of aqueous 2 : 4-dinitrophenylhydrazine sulphate, and next day the product (0·7 g.) was collected, dried, and extracted with benzene, leaving an almost colourless residue (0·1 g.). Crystallisation of this from ethyl acetate gave 4-hydroxy-6 : 7-dimethoxycoumarin, m. p. 278° (decomp.), identified by comparison with a synthetical specimen (Part II, *loc. cit.*). Methylation of the coumarin (0·3 g.) with excess of potassium carbonate and methyl iodide in boiling acetone (20 ml.) during 2 hours gave the 4-methyl ether, forming colourless needles from methanol, m. p. 202°, identical with a synthetical specimen (Found : C, 61·0; H, 5·2. Calc. for $C_{12}H_{12}O_5$: C, 61·0; H, 5·1%). Acetylation of the coumarin (0·3 g.) with acetic anhydride (5 ml.) and pyridine (5 ml.) gave the acetate, which formed prisms, m. p. 242°, from alcohol, identical with an authentic specimen (Found : C, 59·3; H, 4·8. Calc. for $C_{13}H_{12}O_6$: C, 59·1; H, 4·6%) (Part II, *loc. cit.*). Chromatography of the yellow benzene extract of the crude product gave the 2 : 4-dinitrophenylhydrazone of acetone, m. p. and mixed m. p. 125°, and the 2 : 4-dinitrophenylhydrazone of 2-hydroxy-4 : 5-dimethoxyacetophenone, m. p. and mixed m. p. 228° (decomp.).

Hydrolysis of O-Dimethylcitromycinone with Acid.—(*a*) A solution of the dimethyl ether (1 g.) in concentrated hydrochloric acid (50 ml.) was kept at 100° for 15 minutes, cooled, diluted with water (80 ml.), almost neutralised with 2N-sodium hydroxide, and treated with excess of aqueous 2 : 4-dinitrophenylhydrazine sulphate. The copious precipitates (0·9 g.) from each of four such experiments were combined and extracted with a large volume of benzene, leaving a residue of unchanged material (2 g., m. p. 316°), and the benzene extract was run through a column of aluminium oxide, giving a yellow and an orange zone. The yellow zone was washed through the column with chloroform; the product left on evaporation of the solvent was an orange solid (1·3 g.), which on recrystallisation from ethyl acetate gave a hydrazone in small orange yellow needles, m. p. 236° (decomp.), identical with the 2 : 4-dinitrophenylhydrazone, m. p. 236° (decomp.), obtained from the aqueous extract of the crude ozonolysis product from *O*-dimethylcitromycin (Found : C, 52·9; H, 4·0; N, 12·1; OMe, 12·7%). The orange zone was washed through the column with a chloroform–methanol mixture (9 : 1), and on crystallisation from chloroform and then from ethyl acetate gave the 2 : 4-dinitrophenylhydrazone of 4-hydroxy-6 : 7-dimethoxy-3-acetylcoumarin (1 g.) in small orange tablets, m. p. 220° (decomp.) [Found : C, 51·6; H, 3·9; N, 12·4; OMe, 14·6. $C_{17}H_{10}O_7N_4(OMe)_2$ requires C, 51·3; H, 3·6; N, 12·6; OMe, 14·0%]. A synthetical specimen of this hydrazone was obtained as follows. The crude reaction product (0·3 g.) from the condensation of 2-hydroxy-4 : 5-dimethoxybenzoylacetone (1 g.) and ethyl carbonate (10 ml.), carried out according to the procedure described in Part II (*loc. cit.*), was dissolved in alcohol and treated with excess of alcoholic 2 : 4-dinitrophenylhydrazine sulphate. Next day the precipitate was collected, dried, dissolved in chloroform, and chromatographed on aluminium oxide, giving (1) a small zone washed through the column with chloroform, (2) an orange-yellow zone washed through with chloroform–methanol (9 : 1), and (3) a large dirty zone eluted with methanol. The residue left on the evaporation of the chloroform–methanol eluate containing zone (2) was crystallised from ethyl acetate, giving the 2 : 4-dinitrophenylhydrazone of 4-hydroxy-6 : 7-dimethoxy-3-acetylcoumarin in orange prisms, m. p. 220° (decomp.), identical with a specimen from natural sources (Found : C, 51·7; H, 3·7; N, 12·8; OMe, 14·9%).

The 2 : 4-dinitrophenylhydrazone of acetone could not be detected.

(*b*) Prepared according to method (*a*), the hydrolysate was extracted with ether (4 × 100 ml.) and the extract dried and evaporated, leaving a crystalline residue which on recrystallisation from ethyl acetate and then from methyl alcohol gave *4-hydroxy-6 : 7-dimethoxy-3-acetylcoumarin* in colourless needles (0·2—0·3 g.), m. p. 208°, readily soluble in aqueous sodium hydroxide or sodium carbonate, more slowly soluble in sodium hydrogen carbonate, and giving a faint pale brown ferric reaction in alcohol which gradually darkened when the solution was warmed (Found : C, 59·3; H, 5·0; *M*, 241·2. $C_{13}H_{12}O_6$ requires C, 59·1; H, 4·6%; *M*, 264). This compound gave the 2 : 4-dinitrophenylhydrazone, forming orange tablets, m. p. 220° (decomp.), from ethyl acetate, identical with the product obtained by method (*a*).

Continuous extraction of the aqueous liquors with ether in an extractor gave only a further small amount of the acetylcoumarin, together with unchanged *O*-dimethylcitromycinone (0·6 g.).

Degradation of 4-Hydroxy-6 : 7-dimethoxy-3-acetylcoumarin with Alkali.—A solution of the compound

(0·5 g.) in 2N-sodium hydroxide (25 ml.) was heated on the steam-bath for ½ hour, cooled, acidified with 2N-sulphuric acid, kept for 24 hours, filtered to remove a trace of solid, and treated with excess of aqueous 2 : 4-dinitrophenylhydrazine sulphate. From a benzene extract of the resulting precipitate (0·1 g.), the 2 : 4-dinitrophenylhydrazones of acetone, m. p. 125°, and of 2-hydroxy-4 : 5-dimethoxyacetophenone, m. p. 228°, were isolated by liquid chromatography with benzene as the solvent on a column of aluminium oxide, and identified by comparison with authentic specimens. Distillation of the aqueous liquor left on the separation of the crude hydrazones gave acetic acid which was identified by conversion into the anilide, m. p. and mixed m. p. 112°.

The residue, insoluble in benzene, was 4-hydroxy-6 : 7-dimethoxycoumarin, which separated from ethyl acetate in colourless prisms, m. p. 278°, identical with an authentic specimen (*loc. cit.*).

The degradation of the 3-acetylcoumarin was repeated, and the acidified liquors extracted with chloroform (3 × 25 ml.). From the chloroform extracts, 2-hydroxy-4 : 5-dimethoxybenzoic acid was isolated with the aid of aqueous sodium hydrogen carbonate and purified from aqueous acetone, forming colourless needles, m. p. 214°, identical with an authentic specimen.

Hydration of O-*Dimethylcitromycinone.*—A mixture of the dimethyl ether (0·5 g.), dioxan (50 ml.), water (5 ml.), palladium–charcoal (0·3 g., containing 10% of palladium), and 2N-hydrochloric acid (1 ml.) was boiled under reflux for 10 minutes, filtered (wash charcoal with dioxan), and the solvent evaporated under reduced pressure. Crystallisation of the residue from alcohol or aqueous alcohol gave the hydration *product* in very pale green needles (0·4 g.), m. p. 201°, having a reddish brown ferric reaction in alcohol [Found : C, 58·8; H, 4·6; OMe, 19·9. $C_{13}H_8O_5(OMe)_2$ requires C, 58·8; H, 4·6; OMe, 20·3%]. This compound dissolves very slowly in aqueous sodium hydrogen carbonate, but is readily soluble in aqueous sodium hydroxide. In attempts to hydrogenate dimethylcitromycinone (0·5 g.) dissolved in dioxan (60 ml.) with hydrogen and palladium–charcoal catalyst (from 0·5 g. of charcoal and 0·2 g. of palladium chloride), the same compound (0·4 g.), m. p. 201° after purification, was obtained. A mixture of this substance (0·25 g.), hydroxylamine hydrochloride (0·1 g.), sodium acetate (0·1 g.), and alcohol (25 ml.) was heated on the steam-bath for 6 hours; on cooling, the oxime of *O*-dimethylcitromycinone was deposited in pale greenish needles, m. p. 268° (decomp.), identical with an authentic specimen (Found : C, 59·6; H, 4·3. $C_{15}H_{13}O_5N$ requires C, 59·4; H, 4·3%). On being heated with aniline (0·2 g.) in acetic acid (2 ml.) at 100° for 5 minutes the hydration product gave rise to the anil of *O*-dimethylcitromycinone, m. p. 252°, identical with an authentic specimen, after purification from dioxan or acetic acid.

Attempts to acetylate the hydration product by the acetic anhydride–pyridine or the sodium acetate method regenerated *O*-dimethylcitromycinone, m. p. 316° (decomp.). Dimethylcitromycinone, m. p. 316° (decomp.), was almost quantitatively regenerated when the substance (0·1 g.) was dissolved in concentrated sulphuric acid (3 ml.), and the solution diluted immediately with water.

A solution of the hydration product (0·5 g.) in 2N-sodium hydroxide (20 ml.) was heated on the steam-bath for 10 minutes, diluted with water (25 ml.), acidified with sulphuric acid, filtered to remove a trace of solid, and treated with excess of aqueous 2 : 4-dinitrophenylhydrazine sulphate. Next day the solid (0·3 g.) was isolated, dried, and extracted with benzene, leaving an insoluble residue (0·1 g.) which, on crystallisation from ethyl acetate, gave 4-hydroxy-6 : 7-dimethoxycoumarin, m. p. and mixed m. p. 278° (decomp.), identical with a synthetical specimen. From the benzene extracts of the crude coumarin the 2 : 4-dinitrophenylhydrazones of acetone, m. p. 125°, and of 2-hydroxy-4 : 5-dimethoxyacetophenone, m. p. 228°, were isolated by chromatography on aluminium oxide and identified by comparison with authentic specimens.

A slow stream of ozone and oxygen was passed for 45 minutes into a solution of the hydrate (1 g.) in chloroform (75 ml.) kept at 0°, and, after the evaporation of the solvent, the residue was treated with water (100 ml.). Next day the almost clear solution was mixed with excess of aqueous 2 : 4-dinitrophenylhydrazine sulphate, and the product (0·6 g.) dissolved in chloroform and separated into 4 fractions by means of liquid chromatography on aluminium oxide. Of these, the main fraction consisted of the 2 : 4-dinitrophenylhydrazone, forming orange needles, m. p. 236°, identical with material obtained from alkaline hydrolysis of dimethylcitromycinone. Two of the remaining fractions consisted of small amounts of resinous materials, whilst the remaining fraction, forming red needles, m. p. above 300°, from methanol, was too small for investigation. Distillation of the filtrate from the mixed solid gave acetic acid which was identified by conversion into the anilide, m. p. and mixed m. p. 112°.

Oxidation of Methyl O-*Dimethylcitromycetin* (*with* J. B. D. MacKenzie).—A solution of potassium permanganate (4 g.) in water (75 ml.) was gradually added to the ester (1 g.) dissolved in acetone (100 ml.) during 6 hours. Next day the mixture was clarified with sulphur dioxide, the greater part of the acetone was removed under reduced pressure, the residue was extracted with ether (6 × 100 ml.), and the combined dried extracts were evaporated. Crystallisation of the residual solid from methanol gave 2-*hydroxy*-6-*carbomethoxy*-4 : 5-*dimethoxybenzoic acid* in colourless plates (0·2 g.), m. p. 145°, having a purple ferric reaction in alcohol [Found : C, 51·6; H, 4·8; OMe, 34·8. $C_8H_3O_4(OMe)_3$ requires C, 51·6; H, 4·7; OMe, 36·3%]. In some oxidation experiments only a trace of this acid was obtained, and another substance was encountered which formed colourless needles, m. p. 267—268° (decomp.), from methanol, and had a negative ferric reaction (Found : C, 61·7; H, 6·12; OMe, 30·5%). The half-ester of the phthalic acid (0·075 g.), dissolved in ether (20 ml.), was treated with excess of diazoethane (from 0·3 g. nitrosoethylurea) in ether (20 ml.), and next day the solvent was evaporated and the residue crystallised from light petroleum (b. p. 60—80°), giving the methyl ethyl ester of 5 : 6-dimethoxy-3-ethoxyphthalic acid (0·05 g.), m. p. 96—97°. To a solution of this compound (0·05 g.) in methanol (5 ml.), 4% methanolic potassium hydroxide (2 ml.) was added followed by water (2 ml.), and the mixture was boiled under reflux for ½ hour, cooled, neutralised (Congo-red) with dilute hydrochloric acid, and evaporated under reduced pressure. By means of ethyl acetate, 5 : 6-dimethoxy-3-ethoxyphthalic acid was isolated from the residue, and on crystallisation from ethyl acetate–light petroleum formed slender prisms (0·03 g.), m. p. 195° (with effervescence), identical with a synthetical specimen (Part I, this vol., p. 497). By being sublimed in a vacuum at 190°/14 mm., this acid was converted into the anhydride which on recrystallisation from ethyl acetate–light petroleum had m. p. 196°, undepressed on

admixture with a synthetical specimen (Part I, *loc. cit.*) (Found : C, 57·5; H, 5·0. Calc. for $C_{12}H_{12}O_6$: C, 57·2; H, 4·8%).

Very considerable difficulties have been encountered in obtaining consistent analytical results from the same samples of *O*-dimethylcitromycinone, *O*-dimethylcitromycinol, and their derivatives.

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UNIVERSITY OF LIVERPOOL. [*Received, May* 18*th*, 1948.]