184. *The Chemistry of Fungi. Part V. The Constitution of Citrinin.*

By J. P. Brown, Alexander Robertson, W. B. Whalley, and (in part) N. J. Cartwright.

Hydrogenation of citrinin is accompanied by aromatisation of the nucleus, giving rise to dihydrocitrinin which on esterification and methylation yields methyl *O*-dimethyldihydrocitrinin. On oxidation the latter compound furnished a lactone, methyl *O*-dimethylcitrinone, and the acid obtained by the hydrolysis of the carbmethoxy-group of this lactone underwent simultaneous decarboxylation and demethylation when heated with glycerol, yielding a phenolic lactone, identical with the product formed by the action of hot hydriodic acid on methyl *O*-dimethyldihydrocitrinone. On being boiled with aqueous sodium hydroxide this lactone gave rise to phenol (A) by simultaneous hydration and loss of carbon dioxide. These reactions in conjunction with the conversion of dihydrocitrinin into (VIII) by means of hydriodic acid have made it possible to deduce rational structural formulæ for the phenolic lactone (VI), methyl *O*-dimethyldihydrocitrinone (V; R = Me), *O*-dimethyldihydrocitrinin (IV; R = H), and for dihydrocitrinin (III; R = H). The expression (II) appears best to represent the properties of citrinin.

Methylation of methyl citrinin gave a complex mixture from which a crystalline compound (M), $C_{14}H_{15}O_4(OMe)$, has been isolated in comparatively small yield. On alkaline hydrolysis this substance yielded acetaldehyde, diethyl ketone, and formic acid, together with a phenolic

alcohol which is represented by (XV; R = OH) since it can be reduced to the dihydric phenol (XV; R = H). When the crude methylation product is subjected to hydrolysis there is formed, in addition to the foregoing products, a considerable amount of an acid fraction. Esterification of this material followed by oxidation of the ester gave the lactone (V; R = Me). In consequence, it is concluded that the major component of the methylation mixture is formed by addition of the elements of methanol to citrinin and aromatisation with subsequent methylation.

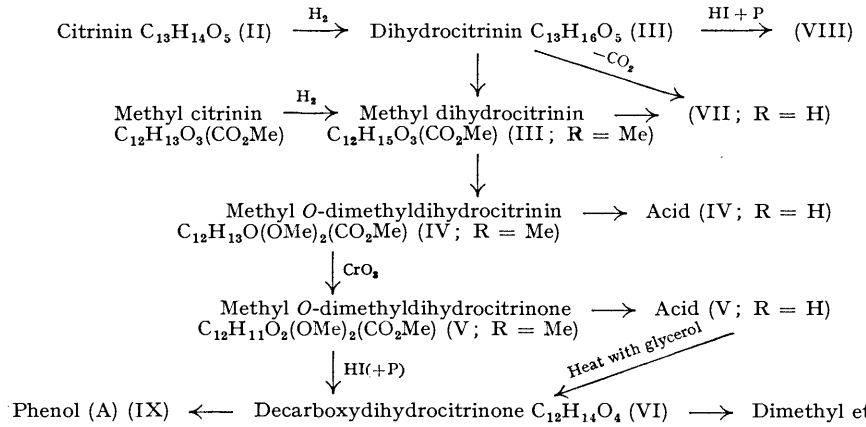
In Part IV of this series (previous paper) the phenolic hydrolysis product of citrinin, phenol (A), $C_{11}H_{16}O_3$, formed by the action of boiling dilute acids or alkalis was shown to be 3-(3 : 5-dihydroxy-2-methylphenyl)butan-2-ol (IX) and, as the only additional products in this decomposition are formic acid and carbon dioxide, the problem of developing a structure for citrinin consequently resolves itself into the question of inserting the ·CH· group eliminated as formic acid and of determining the position of the carboxyl group in the phenol (A) skeleton. In our view, leaving aside the erroneous orientation implied for phenol (A), a serious objection to the current structure for citrinin proposed by Coyne *et al.* (*Phil. Trans.*, 1931, *B*, **220**, 297) is that a compound having formula (I) would be expected to give oxalic acid and not formic acid on hydrolysis (or on ozonolysis) because the phenylglyoxylic acid, which would normally be a hydration product of (I) and thus constitute a stage in the hydrolytic fission, would not on general grounds be expected readily to undergo this kind of decomposition. In the course of attempts to obtain information contributing to a more exact knowledge of the structure of citrinin a study of the hydrogenation and methylation products of the compound was undertaken.

Although citrinin cannot be esterified under the usual conditions, the interaction of the sodium salt with methyl sulphate in aqueous sodium hydrogen carbonate has been found to give a satisfactory yield of a compound which appears to be a *methyl* ester.* The same substance is formed by treatment of citrinin for a short time with methyl iodide and potassium carbonate in boiling acetone or diethyl ketone, but with diazomethane a complex reaction appears to take place, depending to some extent on the amount of reagent employed and on the time of interaction. With less than one molecular proportion of diazomethane, however, a small yield of the methyl ester was obtained. Whilst this ester shows the expected properties and can be hydrogenated to give an almost quantitative yield of *methyl dihydrocitrinin*, the analytical results obtained with some of the specimens prepared indicate that the compound remains solvated after having been dried under the usual conditions; *e.g.*, material purified from benzene appears tenaciously to retain traces of this solvent, whilst the products from acetone have the composition approximating closely to that of a hemihydrate. On being heated at or above its melting point the ester decomposes, yielding a volatile fraction which contains methanol (isolated as the *p*-nitrobenzoate) and a complex residue from which citrinin can be separated by crystallisation. Unlike citrinin, the ester forms an unstable condensation product with acidic 2 : 4-dinitrophenylhydrazine hydrochloride which is regarded as the 2 : 4-*dinitrophenylhydrazone* of a hydrated form.

According to Hetherington and Raistrick (*Phil. Trans.*, 1931, *B*, **220**, 269), the reduction of citrinin with zinc dust and acetic acid furnished a highly unstable product from which they were able to prepare an acetyl derivative believed to be a diacetate. We have found that the hydrogenation of citrinin in methanol with hydrogen at atmospheric pressure or at 40 lb./sq. in. and a palladium–charcoal catalyst gives an excellent yield of a stable compound *dihydrocitrinin*. This substance, which is accompanied by traces of a phenol $C_{11}H_{14}O_2$ when the hydrogenation is effected at 40 lb./sq. in., and which is a stable dextrorotatory carboxylic acid giving a characteristic intense blue ferric reaction in alcohol, readily forms a *diacetate* and a methyl ester which is identical with the hydrogenation product obtained from methyl citrinin. On decarboxylation with hot glycerol, dihydrocitrinin gave rise to a dihydric phenol as an almost colourless glass which could not be induced to crystallise but was conveniently characterised by being converted into a well crystallised *di*-p-*nitrobenzoate*. This phenol, which gives a pale blue colouration with aqueous ferric chloride, was also formed by boiling dihydrocitrinin with dilute aqueous sodium hydroxide. Methylation of methyl dihydrocitrinin by the methyl

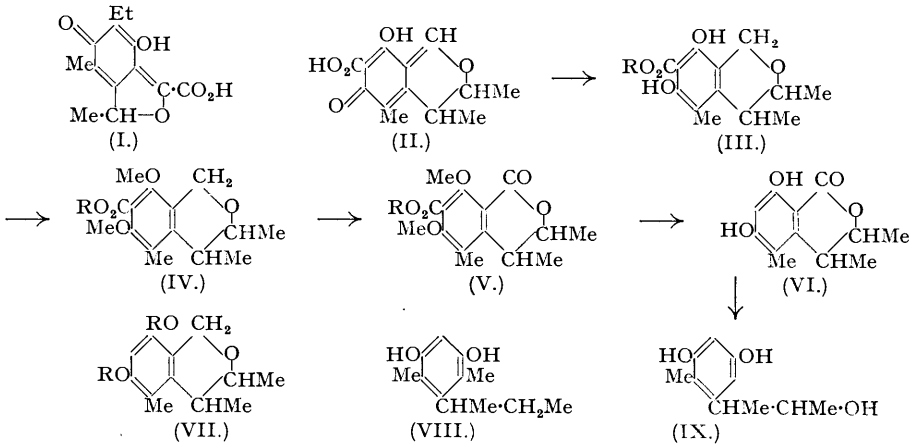
* As in the analogous case of the methyl ester of the carboxylic acid citromycetin (Part III, this vol., p. 848) we refer to the methyl ester of citrinin as methyl citrinin. The composition of trivial names for derivatives of these acids in so far as they are necessary and convenient would have been much easier and more systematic had the originators of the names followed the normal custom in naming acids, *e.g.*, citrinic and citromycetinic acid. This also applies to the more recently isolated unsaturated ketone, trichothecin (Freeman and Morrison, *Nature*, 1948, **162**, 30), for which a more appropriate name would have been, *e.g.*, trichothecenone.

iodide–potassium carbonate method gave rise to *methyl* O-*dimethyldihydrocitrinin* in good yield which on hydrolysis furnished the acid O-*dimethyldihydrocitrinin*. When methyl *O*-dimethyldihydrocitrinin, $C_{12}H_{13}O(OMe)_2(CO_2Me)$, is oxidised with chromic oxide in acetic acid it appears that a methylene group is converted into a carbonyl and a good yield of a product, $C_{12}H_{11}O_2(OMe)_2(CO_2Me)$, is obtained. The same compound is produced, but in considerably smaller yield, when aqueous potassium permanganate is employed as the oxidising agent. For convenience we have named this substance *methyl* O-*dimethyldihydrocitrinone*. Hydrolysis of the ester group in this compound yields the corresponding dextrorotatory *acid*, which, like the parent ester, resists reduction by the method of Clemmensen or by means of hydrogen and a palladium–charcoal catalyst and does not yield derivatives with carbonyl reagents. On being heated with glycerol at 250—260°, this acid underwent simultaneous decarboxylation and demethylation, yielding a dihydroxyphenolic lactone *decarboxydihydrocitrinone*, identical with the product formed by the action of boiling hydriodic acid on *O*-dimethylhydrocitrinone or its methyl ester. This compound was readily converted into a *dimethyl ether*, and the behaviour of this derivative with aqueous and alcoholic sodium hydroxide clearly indicated that the ether and consequently the parent substance contained a lactone group. In agreement with the lactone structure it was found that on being boiled with dilute aqueous sodium hydroxide the phenolic acid, formed as the sodium salt by the opening of the lactone ring in decarboxydihydrocitrinone, readily lost carbon dioxide, yielding the phenol (A) (IX). The foregoing degradations and transformations of citrinin may be conveniently summarised in the accompanying table :



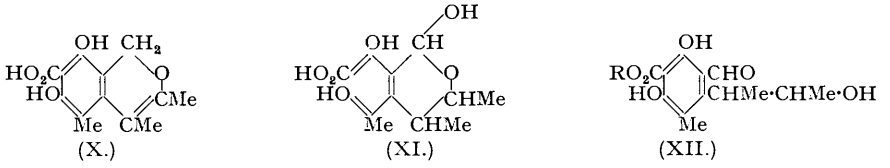
From its properties and ready conversion into phenol (A) (IX) by a simultaneous hydration and decarboxylation process in agreement with the expected behaviour of a β-resorcylic acid, it is clear that decarboxydihydrocitrinone has the structure (VI) and, since this substance is in turn derived from *O*-dimethyldihydrocitrinone by a simultaneous demethylation and decarboxylation process, it follows that the latter compound may be formulated as (V; R = H) and its methyl ester as (V; R = Me). Further, methyl *O*-dimethyldihydrocitrinone is formed by the oxidation of a methylene group in methyl *O*-dimethyldihydrocitrinin to a carbonyl group and, since the latter is lactonic and not ketonic and because of the fact that the parent dihydrocitrinin does not contain a free alcoholic hydroxyl group, it is clear that the oxidation involves the conversion of a $\cdot CH_2\cdot O\cdot C\vdots$ residue into $\cdot CO\cdot O\cdot C\vdots$, and therefore methyl *O*-dimethyldihydrocitrinin may be represented by formula (IV; R = Me) and the parent acid by (IV; R = H). Hence dihydrocitrinin and its methyl ester have formulæ (III; R = H) and (III; R = Me), respectively. The latter structure is in agreement with the important observation that on being boiled with hydriodic acid and red phosphorus dihydrocitrinin is converted, presumably by way of (VII; R = H), into the dihydric phenol 2-(3 : 5-*dihydroxy*-2 : 6-*dimethylphenyl*)*butane* (VIII), identified by comparison of the *di*-p-*nitrobenzoate* with a synthetical sample. With regard to the position of the carboxyl group in dihydrocitrinin (III; R = H), and consequently in (IV; R = H) and (V; R = H), it may be noted that (III; R = H) and (III; R = Me) give intense alcoholic ferric reactions, indicating that the carboxyl and the carbmethoxy-group are in the *o*-position to a phenolic hydroxyl group. The blue colouration of the acid (III; R = H) is in keeping with the compound being a γ-resorcylic derivative, *i.e.*, *p*-orsellinic acid type. On the other hand, the decarboxylation product (VII;

R = H) from dihydrocitrinin gives a faint blue ferric reaction with the aqueous reagent but, as would be expected in the absence of a carbonyl group in the *o*-position to a hydroxyl,



the substance gives a negative reaction with alcoholic ferric chloride. In contrast with the latter compound (VII; R = H) and in keeping with the structure allocated to it, the lactone (VI) gives a violet colouration with alcoholic ferric chloride.

On the basis of the structure (III; R = H) for dihydrocitrinin and the assumption that the aromatisation involves only a rearrangement of bonds simultaneously with the addition of hydrogen and not a deep-seated fundamental intramolecular change, a constitution for citrinin



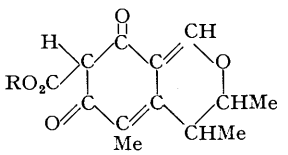
may be derived by the abstraction of two hydrogen atoms from (III; R = H). This may be done in two ways, giving rise to either (*a*) the *iso*chromen type (X) or a modification containing a vinyl group or (*b*) the hemiquinonoid type (II). Of these alternatives the former type is excluded for two main reasons—because the structure does not contain an asymmetric carbon atom (the presence of a vinyl group would allow this but would imply easy racemisation and give rise to formaldehyde on ozonolysis) and because the formation of formic acid both on the hydrolysis and on the ozonolysis of citrinin or its methyl ester cannot be satisfactorily explained on the basis of a formula type (X). On the other hand we regard the alternative expression (II), first advanced by us (*Nature*, 1948, **162**, 72), as best representing the chemical behaviour of citrinin because, *e.g.*, it allows for the optical activity of the compound which persists in dihydrocitrinin and its degradation products down to and including phenol (A) (IX). Further, formula (II) affords a rational explanation of the production of formic acid on ozonolysis unaccompanied by easily detectable amounts of formaldehyde. The hydrolysis with simultaneous aromatisation of the system together with the extrusion of the carboxyl group and of the potential formyl group as formic acid is obviously initiated by the addition of the elements of water at the 1 : 6-positions of the hemiquinonoid residue to give type (XI) comparable with the hydration or methosulphate addition in the case of an anhydronium base (compare Armit and Robinson, *J.*, 1925, **127**, 1604). The hemiacetal system of (XI) then presumably changes over to give the formyl system as in (XII; R = H). That this aromatisation is accompanied by the extrusion of the formyl and carboxyl groups is not unexpected in view of the fact that the potential benzenoid system is fully substituted and, further, the expulsion of the formyl group in warm acid media finds analogy in the case of other polyhydroxy-aldehydes. The final stages in the biological formation of citrinin may well be the reversal of this hydration process, passing from the aldehydo-form (XII; R = H) to (II) by way of (XI). A laboratory synthesis of citrinin along these lines is being explored.

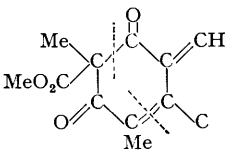
On the basis of formula (II) for citrinin, we regard the 2 : 4-dinitrophenylhydrazone formed

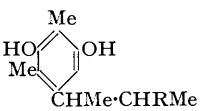
by methyl citrinin as being derived from the hydrated aromatic form (XII; R = Me) and believe that the decomposition of this compound in solution is associated with the tendency of the system (type XII) to revert to the citrinin system type (II).

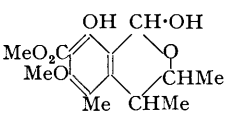
For the purpose of obtaining further evidence in support of the structure (II) advanced for citrinin, the methylation of methyl citrinin and the degradation of the product were studied. In the first instance, on account of the milder nature of the reaction involved, the methylation of the ester was carried out by the methyl iodide–potassium carbonate method in boiling acetone, but the time required for the completion of the methylation process as tested for by the ferric reaction was unexpectedly long and the final product proved to be a mixture containing two main components of which only one could be isolated in the pure state. From the viscous methylation product, which had been distilled in a high vacuum, a crystalline *compound* (M) was separated in comparatively poor yield by means of solvents, but the methoxyl content of this substance, which was completely insoluble in cold aqueous sodium hydroxide, appeared to be slightly lower than that of the initial ester. The substance (M), which has a negative ferric reaction, does not appear to contain a reactive carbonyl group and on hydrolysis with boiling 28% aqueous potassium hydroxide gave rise to a mixture of acetaldehyde, diethyl ketone, formic acid, and a crystalline lævorotatory *phenol*, $C_{12}H_{18}O_3$, together with a comparatively small amount of a liquid acid which was not further investigated. On methylation, the phenol furnished a *dimethyl ether*, $C_{12}H_{16}O(OMe)_2$, in which the third oxygen atom was shown to be present in an alcoholic group by the formation of a well-crystallised p-*nitrobenzoate* of the ether. Thus in its properties the phenolic alcohol, $C_{12}H_{18}O_3$, is strictly analogous to phenol (A), $C_{11}H_{16}O_3$, and like the latter it gave rise to a liquid dihydric phenol, $C_{12}H_{18}O_2$, on reduction with hydriodic acid and red phosphorus which was characterised by being converted into the *di*-p-*nitrobenzoate*. From the analogy with phenol (A) (IX) and the fact that the di-*p*-nitrobenzoate of phenol $C_{12}H_{18}O_2$ was isomeric and not identical with the *di*-p-*nitrobenzoate* of 2-(3 : 5-dihydroxy-2 : 6-dimethylphenyl)butane (VIII) from dihydrocitrinin, it seemed reasonably certain that the parent phenol, $C_{12}H_{18}O_3$, from (M) was 3-(3 : 5-dihydroxy-2 : 4-dimethylphenyl)butan-2-ol (XV; R = OH), a conclusion which was finally established by the synthesis of the di-*p*-nitrobenzoate of (XV; R = H), identical in all respects with the natural derivative.

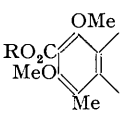
Although, owing to lack of sufficient crystalline material, we have not yet completed our investigations on the structure of (M), it seems reasonably certain from the methoxyl estimations that this compound is not an *O*-methyl ether of methyl citrinin but in all probability arises by the *C*-methylation of this ester. As a variation of the structure type (II), citrinin and its ester may exist in the ketonic forms (XIII; R = H) and (XIII; R = Me) and thus (M) may well be the *C*-methyl derivative (XIV) which could undergo hydrolytic fission with concentrated

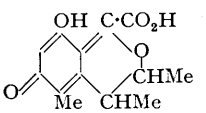

(XIII.)


(XIV.)


(XV.)


(XVI.)


(XVII.)

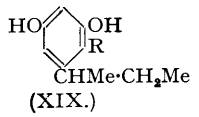

(XVIII.)

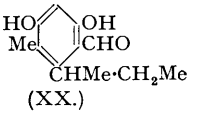
alkalis in several ways. In the formation of the chief hydrolytic product (XV; R = OH) the fission would seem to follow in the main the course taken by citrinin under similar conditions, in agreement with the fact that both compounds are in the same state of oxidation, *i.e.*, the extrusion of the potential formyl group, the hydrolysis of the carbomethoxy-group with the loss of carbon dioxide, and simultaneous aromatisation of the system. The only difference between the phenolic end products (IX) and (XV; R = OH) respectively from citrinin and from (M) is in the presence of the *C*-methyl group ultimately retained in the 4-position of (XV; R = OH). The minor products of hydrolysis, *viz.*, acetaldehyde and diethyl ketone, would seem to arise by the fission taking alternative courses; *e.g.*, the ketone in all probability arises by the initial fission of (M) proceeding as indicated by the dotted lines in formula (XIV), whilst a modification of this route could conceivably lead to acetaldehyde. Because of the difficulty at an earlier stage of

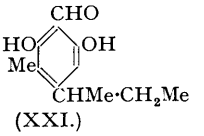
reconciling the analytical results with feasible structures for (M), careful consideration was given to the question of citrinin derivatives condensing with acetone in the presence of potassium carbonate during the methylation process, thus leading to an addendum responsible for acetaldehyde and diethyl ketone. Apart from other factors, however, the amounts of the latter products isolated as their 2 : 4-dinitrophenylhydrazones do not justify further consideration of this view, and, morover, it was subsequently found that when acetone was replaced by methanol as the solvent in the methylation procedure the same results were obtained.

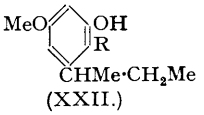
When the distilled mixed methylation product from methyl citrinin was hydrolysed under the conditions subsequently applied to substance (M), there was formed, in addition to the products obtained from (M), a comparatively large amount of an acid fraction which did not yield crystalline material and could not be distilled unchanged in a high vacuum. This acidic fraction was esterified by means of ethereal diazomethane but it was not possible to effect a satisfactory purification of the product by fractional distillation in a high vacuum. Oxidation of the distilled material with chromic oxide in acetic acid, however, gave rise to a comparatively good yield of a crystalline ester which was found to be identical in every way with methyl *O*-dimethyldihydrocitrinone (V; R = Me), giving *O*-dimethyldihydrocitrinone (V; R = H) on hydrolysis. Similar results were obtained when, before attempting to separate crystalline compound (M), the methylation product was distilled and subjected to oxidation with chromic oxide and the resulting mixture hydrolysed with boiling 28% aqueous potassium hydroxide. Under these conditions the products obtained were acetaldehyde, diethyl ketone, formic acid, the phenol (XV; R = OH), and *O*-dimethyldihydrocitrinone (V; R = H); the aldehyde and ketone were formed in comparatively small amounts as in the case of (M). It would thus appear that the second major product of the methylation process, which on oxidation gives rise to (V; R = Me), and on hydrolysis is not degraded like (M), contains methyl groups on the nuclear oxygen atoms, and consequently we may consider the first step in the formation of this methylation product to be the addition of the elements of methyl alcohol to methyl citrinin with simultaneous aromatisation to give substance (XVI). The latter intermediate is considered to undergo subsequent methylation, yielding the dimethyl ether (XVII; R = Me), and it is this substance which is oxidised, giving rise to methyl *O*-dimethyldihydrocitrinone (V; R = Me). A concentrate consisting largely of the ether believed to be (XVII; R = Me) has been obtained after the separation of crystalline (M), but this material is still contaminated with the latter because on hydrolysis it gives small amounts of the hydrolytic products characteristic of (M) as well as a high proportion of (XVII; R = H).

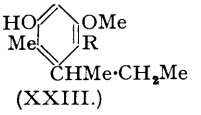
When the methylation of methyl citrinin was carried out in methyl alcohol instead of in acetone a similar mixed product was obtained, as shown by hydrolysis, but the amount of (M) present appears to be less than in the acetone product.

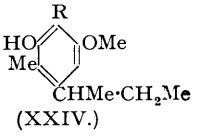

(XIX.)


(XX.)


(XXI.)


(XXII.)


(XXIII.)


(XXIV.)

In the synthesis of the phenol (VIII) obtained from dihydrocitrinin, the Gattermann reaction was applied to the resorcinol derivative (XIX; R = Me), but in place of the expected aldehyde (XX) as the sole product of the reaction, we obtained a mixture consisting mainly of the isomeric *γ-aldehyde* (XXI) accompanied by only small amounts of the required compound. The orientation of (XXI) was deduced in the first instance from its resemblance to *m*-xylorcylaldehyde (Robertson and Robinson, *J.*, 1927, 2196) in having a bright yellow colour and in giving an intense bottle-green colouration with alcoholic ferric chloride. On reduction by Clemmensen's method this aldehyde (XXI) gave rise to 2-(3 : 5-dihydroxy-2 : 4-dimethylphenyl)butane (XV; R = H), which was characterised by the formation of the di-*p*-nitrobenzoate, identical in every respect with the corresponding derivative of the phenol (XV; R = H) prepared by the degradation of compound (M) by way of (XV; R = OH). When, however, the mixed product from the Gattermann reaction, which had been distilled in a

vacuum once, was subjected to the Clemmensen reaction and the product acylated with *p*-nitrobenzoyl chloride and pyridine, it was possible to isolate a much less soluble di-*p*-nitrobenzoate, identical with the corresponding derivative of the phenol (VIII) from dihydrocitrinin. The absolute orientations of the isomeric aldehydes (XX) and (XXI) and consequently those of the phenols (VIII) and (XV; R = H) were established in the following way. The aldehyde (XIX; R = CHO) was converted into the monomethyl ether (XXII; R = CHO) which was characterised by the formation of the *semicarbazone*. The orientation of this ether (XXII; R = CHO) follows from the fact that it gives an intense ferric reaction in alcohol and reacts with acetophenone in the presence of hydrogen chloride in ethyl acetate to give a flavylium salt. On reduction by the Clemmensen method this monomethyl ether (XXII; R = CHO) yielded 2-(3-hydroxy-5-methoxy-2-methylphenyl)butane (XXII; R = Me), characterised by the formation of the p-*nitrobenzoate*. Application of the Gattermann reaction to the latter ether gave rise to the expected mixture of aldehydes (XXIII; R = CHO) and (XXIV; R = CHO), which were conveniently separated by taking advantage of the fact that 2-(3-*hydroxy*-5-*methoxy*-6-*formyl*-2-*methylphenylbutane* (XXIII; R = CHO), in agreement with its orientation, is readily extracted from an ethereal solution of the mixed reaction product by means of 0·5% aqueous sodium hydroxide, leaving the isomeride (XXIV; R = CHO) in the solvent. On reduction (XXIII; R = CHO), which did not give a ferric reaction, furnished the monomethyl ether (XXII; R = Me) and on demethylation this derivative yielded (VIII), characterised by being converted into the di-*p*-nitrobenzoate, identical with the product obtained from dihydrocitrinin, and by the reduction of the crude mixture of the aldehydes (XX) and (XXI). 2-(3-Hydroxy-5-methoxy-4-formyl-2-methylphenyl)butane (XXIV; R = CHO), which was characterised by the formation of the *semicarbazone*, gave the expected intense ferric reaction in alcohol, and a flavylium chloride on being condensed with acetophenone by means of hydrogen chloride. On reduction the aldehyde furnished the monomethyl ether (XXIV; R = Me). Demethylation of the latter compound gave rise to (XV; R = H), the di-*p*-nitrobenzoate of which was identical in every way with the derivative obtained from the compound (XV; R = OH) by way of (XV; R = H).

Addendum.—In view of the communication by Gore *et al.* (*J. Amer. Chem. Soc.*, 1948, **70**, 2287) proposing an alternative structure (XVIII) for citrinin, to which our attention was directed after the present memoir had been prepared, it seems desirable to summarise the available evidence which we believe proves that the carboxyl group is attached directly to the potential aromatic kernel as in (II) and not to a side chain of it as in (XVIII) :

(*a*) The formation of formic acid and not oxalic acid by citrinin, methyl citrinin, and its methylation product (M) on hydrolysis.

(*b*) The production of formic acid and not oxalic acid by the ozonolysis of citrinin, methyl citrinin, and mixed methylation product and the failure to obtain formic acid from dihydrocitrinin and its derivatives by this means.

(*c*) The oxidation of methyl *O*-dimethyldihydrocitrinin to a lactone without loss of the carbomethoxy-group together with the subsequent conversion of the product into the lactone (VI) by loss of this group.

(*d*) The failure to convert dihydrocitrinin by heating into a lactone (α-keto-dihydrofuran type) which would be expected on the basis of formula (XVIII).

(*e*) The intense alcoholic ferric reactions given by dihydrocitrinin and its methyl ester are in keeping with formulæ (III; R = H) and (III; R = Me) but not with a dihydro-derivative of (XVIII).

Experimental.

Methyl Citrinin.—The citrinin employed in this investigation was obtained from *Penicillium citrinum* [A.D. 23 (Baarn)] grown on the glucose medium employed by Hetherington and Raistrick, (*loc. cit.*). The acid, which can be sublimed unchanged in a high vacuum, has been found to have m. p. 178—179° (decomp.) after repeated crystallisation from methanol, $\lambda_{min.}$ ~235 mμ, $E^{1\%}_{1\,cm.}$ ~309; $\lambda_{max.}$ ~250 mμ, $E^{1\%}_{1\,cm.}$ ~333; $\lambda_{min.}$ ~285 mμ, $E^{1\%}_{1\,cm.}$ ~66; $\lambda_{max.}$ ~331 mμ, $E^{1\%}_{1\,cm.}$ ~266 with inflexions at λ ~371, ~390, ~481 mμ (Hetherington and Raistrick give m. p. 169°, decomp.). Repeated attempts under a variety of conditions to effect the decarboxylation of citrinin and obtain decarboxycitrinin have failed.

A well agitated solution of citrinin (5 g.) in saturated aqueous sodium hydrogen carbonate (100 ml.), maintained at 45—50°, was treated with methyl sulphate (25 ml.) with the further addition of sodium hydrogen carbonate as required to keep the reaction mixture neutral (Congo-red); 45 minutes later more methyl sulphate (12 ml.) and aqueous sodium hydrogen carbonate as required were added and, after a further 45 minutes, the solid ester (3·8 g.) was collected, washed, dried, and crystallised from benzene and then acetone, forming colourless, flat, stout, diamond-shaped prisms, m. p. 138° (decomp.), $[\alpha]_D^{20°}$ +96·9° (*c*, 1·878 in chloroform), $\lambda_{min.}$ ~240 mμ, $E^{1\%}_{1\,cm.}$ ~150; $\lambda_{max.}$ ~260 mμ, $E^{1\%}_{1\,cm.}$ ~450; $\lambda_{min.}$ ~285 mμ, $E^{1\%}_{1\,cm.}$ ~11; $\lambda_{max.}$ ~335 mμ, $E^{1\%}_{1\,cm.}$ ~140, giving an olive-green colouration with alcoholic

ferric chloride [Found, in material crystallised from acetone and dried over phosphoric oxide at room temperature: C, 61·9; H, 5·9. Found, in specimen dried in a high vacuum at 60° for 2 hours: C, 62·1; H, 6·0; OMe, 10·9. Found, in specimen crystallised from benzene: C, 63·1; H, 6·8. Found, in same material dried in a high vacuum at 80° for 2 hours: C, 63·2; H, 6·9. $C_{13}H_{13}O_4(OMe)$ requires C, 63·6; H, 6·1; OMe, 11·7%. $C_{13}H_{13}O_4(OMe),0{\cdot}5H_2O$ requires C, 61·5; H, 6·2; OMe, 11·4%]. Acidification of the aqueous sodium hydrogen carbonate liquor left on separation of the ester gave unchanged citrinin (approx. 0·7 g.).

The same ester mixed with much gummy material was formed when a solution of citrinin (2 g.) in chloroform (25 ml.) and methanol (25 ml.) was mixed with ethereal diazomethane (0·6 mol. of diazomethane), kept for 15 minutes, and evaporated. The residual product was extracted with ether, the extract was washed with aqueous sodium carbonate, dried, and evaporated, and the residual oil was dissolved in a little warm benzene. On being kept, the solution slowly deposited the methyl ester (0·2 g.), m. p. 138° (decomp.), identical with a specimen prepared by the methyl sulphate method.

A solution of citrinin (6 g.) in acetone (150 ml.) and methyl iodide (150 ml.) was boiled with potassium carbonate (12 g.) for 24 hours with the addition of more iodide (5 ml.) and carbonate (3 g.) after 12 hours. The voluminous yellow solid was collected, washed with acetone, dissolved in warm water (300 ml. at 50°), and decomposed with dilute hydrochloric acid, yielding the colourless ester. This compound was collected, dried, and crystallised from benzene, forming diamond-shaped prisms, m. p. and mixed m. p. 137° (decomp.) (Found, in material dried at 60° in a high vacuum: C, 63·2; H, 6·8; OMe, 10·3%). When the acetone was replaced by diethyl ketone the same ester, m. p. and mixed m. p. 137° (decomp.), was obtained. On distillation in a high vacuum the residue left on evaporation of the acetone filtrate from the sodium salt gave a colourless neutral oil, b. p. 130—140°/0·3 mm., which had a negative ferric reaction and did not react with carbonyl reagents. The analogous product obtained when diethyl ketone was employed in the methylation process was a pale yellow oil, b. p. 130—140°/0·2 mm., having a negative ferric reaction and forming a 2 : 4-dinitrophenylhydrazone, red needles from acetic acid (Found: C, 57·9; H, 5·1; N, 14·1%).

The methyl ester of citrinin is sparingly soluble in hot alcohol, moderately soluble in chloroform, and insoluble in light petroleum. When the ester (0·5 g.) was heated under reflux with 5N-sulphuric acid (25 ml.) for 1 hour the almost clear solution was found to contain formic acid, identified by distillation of a portion and the isolation of the acid as *NN'*-diphenylformamidine hydrochloride (Whalley, *J.*, 1948, 1015). From the remaining portion of the solution, phenol (A) was isolated with ether. Similarly, when the ester (0·5 g.) was heated under reflux with 2N-aqueous sodium hydroxide (25 ml.) for 1 hour, formic acid and phenol (A), m. p. and mixed m. p. 128°, were obtained from the acidified hydrolysate. When a specimen of the ester (1·8 g.), which had been dried in a desiccator at room temperature, was heated to 130—140° in a slow current of nitrogen for ½ hour and the effluent gas passed through a trap cooled with solid carbon dioxide, a small amount of benzene was obtained, originally present as solvent of crystallisation. The residue contained, *inter alia*, citrinin (0·6 g.) and a small amount of a product exhibiting ketonic properties. In another experiment the effluent gas was passed into a mixture of pyridine and *p*-nitrobenzoyl chloride, and from the latter methyl *p*-nitrobenzoate, m. p. 96°, was then isolated and identified by comparison with an authentic specimen.

A mixture of methyl citrinin (0·6 g.), prepared by either method, in methanol (50 ml.), containing 2 : 4-dinitrophenylhydrazine (1 g.) and concentrated sulphuric acid (2·5 ml.), was kept at room temperature for 24 hours. The addition of water (50 ml.) to the mixture gave a yellow precipitate (0·6 g.) which, on being chromatographed from chloroform on alumina, was found to be homogeneous, beling eluted with chloroform–methanol. Repeated crystallisation of this 2 : 4-*dinitrophenylhydrazone* from alcohol tended to cause decomposition. For analysis, the substance was purified from warm methanol, forming slender yellow needles, m. p. 165—166° (decomp.) [Found: C, 52·2; H, 4·8; N, 12·3; OMe, 6·3. $C_{19}H_{19}O_8N_4(OMe)$ requires C, 52·0; H, 4·8; N, 12·1; OMe, 6·7%]. On being bolled for a short time with alcohol, the 2 : 4-dinitrophenylhydrazone partly decomposed. Treatment of the alcoholic filtrate from the unchanged hydrazone with an excess of alcoholic 2 : 4-dinitrophenylhydrazine sulphate regenerated the derivative.

Dihydrocitrinin (III; R = H).—Citrinin (5 g.) was hydrogenated in methanol (200 ml.) with hydrogen at 40 lb./sq. in. and a palladium–charcoal catalyst (from 1 g. of charcoal and 0·2 g. of palladium chloride) in the course of 1½ hours. After the removal of the catalyst by filtration, the solution rapidly assumed a bluish tinge which changed to green when the liquor was kept for several days. The product left on evaporation of the solution under diminished pressure was triturated with benzene and crystallised from the same solvent and then from benzene–acetone, giving *dihydrocitrinin* in small colourless prisms (4·2 g.), m. p. 171°, $[\alpha]_D^{18°}$ +18·3° (*c*, 2·981 in chloroform), $\lambda_{min.}$ ~240 mμ, $E_{1\,cm.}^{1\%}$ ~250; $\lambda_{max.}$ ~260 mμ, $E_{1\,cm.}^{1\%}$ ~400; $\lambda_{min.}$ ~285 mμ, $E_{1\,cm.}^{1\%}$ ~10; $\lambda_{max.}$ ~330 mμ, $E_{1\,cm.}^{1\%}$ ~100 [Found: C, 62·0; H, 6·4; *M* (by titration), 251·7. $C_{13}H_{16}O_5$ requires C, 61·9; H, 6·4%; *M*, 252). This compound, which is also obtained when the hydrogenation is carried out at atmospheric pressure, gives an intense Prussian-blue colouration with ferric chloride in water or alcohol, and is sparingly soluble in benzene or light petroleum, and readily soluble in alcohol, acetone, or chloroform. Acetylation of dihydrocitrinin (0·8 g.) with acetic anhydride (3 ml.) and pyridine (1 ml.) at room temperature for 36 hours gave the *diacetate* (0·8 g.), which separated from benzene in colourless prisms, m. p. 144—145°, readily soluble in aqueous sodium hydrogen carbonate, alcohol, or acetone and having a negative ferric reaction (Found: C, 60·8; H, 5·9. $C_{17}H_{20}O_7$ requires C, 60·7; H, 6·0%).

From the benzene liquor left from the purification of about 50 g. of dihydrocitrinin a small amount of a second crystalline, non-acidic, phenolic product (0·5 g.) was obtained. Recrystallised from benzene–acetone, this substance formed colourless prisms, m. p. 188°, insoluble in water, chloroform, or light petroleum and soluble in acetone or alcohol, and giving a pale green ferric reaction in alcohol (Found: C, 74·1, 73·8; H, 7·9, 7·7. Calc. for $C_{11}H_{14}O_2$: C, 74·2; H, 7·9%).

A solution of dihydrocitrinin in ether was treated with slightly more than one molecular proportion of ethereal diazomethane, and 10 minutes later the unchanged reagent was destroyed with a little

hydrochloric acid. Slow evaporation of the solution in the course of 7 days gave the crystalline *methyl* ester (III; R = Me). Recrystallised from light petroleum (b. p. 40—60°), this derivative formed large colourless prisms, m. p. 60°, soluble in the usual organic solvents except light petroleum and giving a violet colouration with alcoholic ferric chloride and a weak blue with the aqueous reagent, in which the substance is but sparingly soluble [Found: C, 63·0; H, 6·5; OMe, 11·6. $C_{13}H_{15}O_4(OMe)$ requires C, 63·2; H, 6·8; OMe, 11·6%]. Acetylation of this ester (1·5 g.) with acetic anhydride (8 ml.) and pyridine (10 ml.) on the water-bath for ½ hour and subsequent dilution of the cooled mixture with water and dilute hydrochloric acid gave the *diacetate*, which formed colourless needles (1·7 g.), m. p. 114°, from aqueous methanol, having a negative ferric reaction (Found: C, 61·7; H, 6·5. $C_{18}H_{22}O_7$ requires C, 61·7; H, 6·3%). This compound (0·5 g.) was recovered unchanged after having been heated under reflux with acetic anhydride (8 ml.) and sodium acetate (0·3 g.) for 1 hour.

Hydrogenation of the methyl ester of citrinin (0·5 g.) in methanol (30 ml.) with hydrogen and a 20% palladium–charcoal catalyst (0·25 g.) for 1 hour gave the same dihydro-ester, m. p. and mixed m. p. 60°, forming the diacetate, m. p. 114°.

Decarboxydihydrocitrinin (VII; R = H).— A mixture of dihydrocitrinin (0·5 g.) and glycerol (5 ml.) was kept at 170° in an atmosphere of nitrogen for 10 minutes and then at 200° for one minute, and the product isolated from a solution of the cooled mixture in water (30 ml.) with ether. Evaporation of the extracts, which had been washed with aqueous sodium hydrogen carbonate and dried, left a very viscous liquid which did not crystallise and on distillation gave a phenol as a yellowish glass, b. p. 180°/0·1 mm., exhibiting a negative reaction with alcoholic and a pale blue-green colouration with aqueous ferric chloride and a yellow colouration with aqueous bleaching powder. On acylation with *p*-nitrobenzoyl chloride–pyridine, this product gave the *di-p-nitrobenzoate*, forming colourless needles, m. p. 160°, from acetic acid and then alcohol (Found: C, 61·8; H, 4·4; N, 5·5. $C_{26}H_{22}O_9N_2$ requires C, 61·7; H, 4·4; N, 5·5%).

Decarboxylation of dihydrocitrinin (1 g.) was also effected with boiling 2N-aqueous sodium hydroxide during 20 minutes, and the product isolated from the acidified liquor with ether, being obtained as a glass which gave the same di-*p*-nitrobenzoate, forming colourless needles, m. p. and mixed m. p. 160°, from alcohol (Found: C, 61·8; H, 4·4; N, 5·5%). When the dihydric phenol was methylated by the methyl iodide–potassium carbonate method the alkali-insoluble product did not form a *p*-nitrobenzoate.

O-*Dimethyldihydrocitrinin* (IV; R = H).—A solution of the foregoing methyl ester (2 g.) in acetone (150 ml.) and methyl iodide (4 ml.), containing potassium carbonate (8 g.), was heated under reflux for 18 hours, with the addition of more iodide (2 ml.) at intervals of 6 hours and 12 hours, until a test portion did not give a ferric reaction. On isolation, *methyl* O-*dimethyldihydrocitrinin* (IV; R = Me) was purified by distillation in a high vacuum and obtained as a pale yellow oil, b. p. 152—154°/0·3 mm., $[\alpha]_D^{20°}$ −19·5° (*c*, 4·692 in chloroform), which did not solidify and had a negative ferric reaction [Found: C, 65·3; H, 6·9; OMe, 25·4. $C_{13}H_{13}O_2(OMe)_3$ requires C, 65·3; H, 7·5; OMe, 31·4%]. This ester (4 g.) was hydrolysed with boiling 6% methanolic potassium hydroxide (80 ml.) for 2 hours, the greater part of the solvent was distilled off in a vacuum, the residue was treated with water (50 ml.), unchanged ester was removed by extraction with ether, and the residual alkaline solution was acidified with hydrochloric acid. The resulting O-*dimethyldihydrocitrinin* (IV; R = H) separated as an oil which solidified on trituration with water and then formed colourless prisms (3·5 g.), m. p. 124°, from ether–light petroleum (b. p. 40—60°). Recrystallised from aqueous methanol, the substance was obtained as a hydrate in large hexagonal plates, m. p. 66—68°, which on being dried in a vacuum over phosphoric oxide lost solvent of crystallisation and then had m. p. 124° [Found, in anhydrous specimen: C, 64·2, 64·4; H, 7·0, 7·1; OMe, 19·5. $C_{13}H_{14}O_3(OMe)_2$ requires C, 64·3; H, 7·1; OMe, 22·1%]. This compound has a negative ferric reaction and is readily soluble in aqueous sodium hydrogen carbonate and in the usual organic solvents except light petroleum.

Oxidation of Methyl O-*Dimethyldihydrocitrinin*.—A solution of the ester (1 g.) in acetic acid (20 ml.) was gradually treated with chromic oxide (1·5 g.) with occasional cooling to moderate the rather vigorous reaction. 20 Minutes later the solvent was distilled off in a vacuum, the residue was triturated with water (50 ml.), and the colourless solid was isolated with ether and freed from traces of acidic material by treatment with dilute aqueous sodium hydrogen carbonate followed by dilute aqueous sodium hydroxide. Crystallised from light petroleum (b. p. 60—80°), the resulting *methyl* O-*dimethyldihydrocitrinone* (V; R = Me) formed large needles (0·5 g.), m. p. 104°, $[\alpha]_D^{20°}$ +145° (*c*, 3·410 in chloroform), soluble in the usual organic solvents except cold light petroleum or water and having a negative ferric reaction [Found: C, 62·3, 62·5; H, 6·7, 6·7; OMe, 30·3. $C_{13}H_{11}O_3(OMe)_3$ requires C, 62·3; H, 6·5; OMe, 30·2%]. A small amount of an acidic by-product was sometimes obtained by acidification of the aqueous sodium hydrogen carbonate washings and on crystallisation from methanol formed colourless needles, m. p. 208°, but there was insufficient material for further purification and analysis.

Methyl *O*-dimethyldihydrocitrinin (1 g.), dissolved in acetone (70 ml.), was treated with a solution of potassium permanganate (3 g.) in water (140 ml.), added gradually (5 hours), the mixture was then cleared with sulphur dioxide, and the greater part of the acetone evaporated off in a vacuum. Isolated with ether, the product was separated from a trace of acidic material by means of sodium hydrogen carbonate and crystallised from light petroleum (b. p. 60—80°), giving methyl *O*-dimethyldihydrocitrinone in large needles (0·3 g.), m. p. 104°, identical with a specimen obtained with chromic oxide.

A mixture of the methyl *O*-dimethyldihydrocitrinone (2 g.) and methanol (30 ml.), containing potassium hydroxide (3 g.), was heated under reflux for 2 hours, the greater part of the solvent was distilled, and a solution of the residue in water was acidified with hydrochloric acid. Thus precipitated, the *acid* (V; R = H) (1·75 g.) was purified by crystallisation from benzene–acetone, forming colourless plates, m. p. 233—234° (decomp.) $[\alpha]_D^{20°}$ +141·9° (*c*, 1·691 in methanol), soluble in alcohol, chloroform, or ethyl acetate, sparingly soluble in water or benzene, and having a negative ferric reaction [Found: C, 61·2; H, 6·0; OMe, 20·5; *M* (by titration), 298, 288·4. $C_{13}H_{12}O_4(OMe)_2$ requires C, 61·2; H, 6·1; OMe, 21·1%; *M*, 294]. Esterification of this compound with diazomethane regenerated the parent ester, m. p. and mixed m. p. 104°.

Decarboxydihydrocitrinone (VI).—Simultaneous demethylation and decarboxylation of the foregoing acid was effected by heating the compound in portions of 0·5 g. with glycerol (10 ml.) at 250—260° for 10 minutes, and after the addition of water the product (from 4 g. of acid) was isolated with ether and separated into neutral, phenolic, and acidic fractions. The neutral fraction (1 g.) was an intractable gum and the acidic fraction (0·8 g.) consisted mainly of unchanged acid. The viscous phenolic material (2 g.) was dissolved in ether and on slow evaporation of the solvent a crystalline phenol, *decarboxydihydrocitrinone*, m. p. 210°, separated which on recrystallisation from benzene–acetone formed colourless prisms, m. p. 214°, readily soluble in the usual organic solvents except benzene or light petroleum and having a violet ferric reaction (Found: C, 64·7; H, 6·1. $C_{12}H_{14}O_4$ requires C, 64·9; H, 6·3%). When *O*-dimethyldihydrocitrinone was heated with quinoline containing copper-bronze at 230° a small amount of an acid, m. p. 160—163°, with a red ferric reaction in alcohol and a violet in water, was obtained along with an intractable phenolic fraction which gave a violet ferric reaction and may have contained the phenol, m. p. 214°.

O-Dimethyldihydrocitrinone (1 g.) was boiled with hydriodic acid (10 ml.; *d* 1·7) and red phosphorus (1 g.) for ½ hour, and the resulting pale yellow liquor was diluted with water (30 ml.) and extracted with ether. The combined ethereal extracts were washed with aqueous sodium hydrogen carbonate and then with 2N-aqueous sodium hydroxide, and on acidification with hydrochloric acid the latter washings gave decarboxydihydrocitrinone (0·2—0·5 g.; the variable yield did not appear to depend entirely on the time of refluxing) which on purification from benzene–acetone had m. p. and mixed m. p. 215°, $[\alpha]_D^{20°}$ +99·2° (*c*, 1·533 in methanol) (Found: C, 64·7; H, 6·2%).

Methylation of the phenolic lactone (VI) (0·5 g.), dissolved in acetone (30 ml.), with methyl iodide (4 ml.) and potassium carbonate (5 g.) in the course of 10 hours gave rise to a neutral *dimethyl ether* (0·4 g.), which separated from ether in large rectangular plates or from benzene–light petroleum (b. p. 60—80°) in hexagonal plates, m. p. 138°, soluble in benzene, chloroform, or alcohol and having a negative ferric reaction (Found: C, 67·4; H, 7·0. $C_{14}H_{18}O_4$ requires C, 67·2; H, 7·2%). This compound, which dissolved in warm aqueous sodium hydroxide and did not separate from the cooled solution, behaved as a lactone on titration. Addition of an excess of dilute aqueous sodium hydroxide to a solution of the lactone in the minimum quantity of alcohol did not give a precipitate until the solution had been acidified with hydrochloric acid.

On treatment with *p*-nitrobenzoyl chloride (0·6 g.) and pyridine (3 ml.), decarboxydihydrocitrinone (VI) (0·2 g.) gave an almost theoretical yield of the *di*-p-*nitrobenzoate*, which formed colourless prisms, m. p. 206°, from ethyl acetate (Found: C, 59·8; H, 3·9; N, 5·4. $C_{26}H_{20}O_{10}N_2$ requires C, 60·0; H, 3·8; N, 5·4%).

Conversion of Decarboxydihydrocitrinone into Phenol (*A*).—A solution of this lactone (0·5 g.) in 25% aqueous potassium hydroxide (20 ml.) was boiled in an atmosphere of nitrogen for 5 hours, the cooled solution was acidified with hydrochloric acid, and the product was isolated with ether. This gummy substance was triturated with chloroform and then on crystallisation from the same solvent gave phenol (A) in almost colourless prisms, m. p. 128°, after having been sublimed at 150°/0·01 mm. to remove solvent of crystallisation, identical with an authentic specimen (Found: C, 67·3; H, 8·3. Calc. for $C_{11}H_{16}O_3$: C, 67·4; H, 8·2%). A mixture of phenol (A) (0·5 g.), diazoaminobenzene (2 g.), and alcohol (20 ml.) was warmed on the steam-bath until a clear solution was formed. On being kept at room temperature the mixture deposited the *bisphenylazo*-derivative in the course of 4 days, which then separated from alcohol in deep red needles, m. p. 204—205° (decomp.), undepressed on admixture with an authentic specimen, m. p. 204—205° (decomp.) (Found: C, 68·4; H, 6·1; N, 13·8. $C_{23}H_{24}O_3N_4$ requires C, 68·3; H, 5·9; N, 13·8%).

Oxidation of Methyl O-Dimethyldihydrocitrinone.—To a mixture of the compound (0·5 g.) in water (15 ml.), maintained at 80°, a solution of potassium permanganate (2 g.) in water (120 ml.) was added gradually during 4½ hours. The reaction mixture was clarified with the minimum amount of sulphur dioxide and on acidification with sulphuric acid gave the *acid-ester* (0·2—0·3 g.), m. p. 204°, which separated from water in needles, m. p. 206°, $[\alpha]_D^{20°}$ +142·6° [Found: C, 56·7; H, 5·1; *M* (by titration), 343·5. $C_{16}H_{18}O_8$ requires C, 56·8; H, 5·3%; *M*, 338]. This compound (1 g.) was heated on the steam-bath with 2N-aqueous sodium hydroxide (20 ml.), and the solution cooled, acidified with hydrochloric acid, and allowed to evaporate at room temperature. Extraction of the residue with warm acetone gave a dibasic *acid* which, on recrystallisation from a small volume of acetone, formed colourless plates (0·7 g.), m. p. 200° [Found: C, 55·6; H, 5·0; equiv. (by titration), 175·9, 174·7. $C_{15}H_{16}O_8$ requires C, 55·6; H, 5·0%; equiv., 162].

Action of Hydriodic Acid and Red Phosphorus on Dihydrocitrinin.—A mixture of the dihydrocompound (2 g.), hydriodic acid (15 ml.; *d* 1·7), acetic acid (from 10 ml. of anhydride), and red phosphorus (1 g.) was gently heated under reflux for 20 minutes, cooled, diluted with water (100 ml.), and extracted several times with ether. The combined extracts were washed with aqueous sodium hydrogen sulphite and then with aqueous sodium hydrogen carbonate, dried, and evaporated. To remove traces of iodine-containing compounds a solution of the oil in acetic acid (10 ml.) was heated with zinc dust (3 g.) on the steam-bath for 3 hours, the acetic acid was evaporated, and the residue extracted with ether. Evaporation of the washed and dried extract left a viscous oil which was twice distilled in a high vacuum, giving 2-(3 : 5-*dihydroxy*-2 : 6-*dimethylphenyl*)*butane* (VIII) as a colourless oil, b. p. 120—121°/0·1 mm. (Found: C, 74·4; H, 9·4. $C_{12}H_{18}O_2$ requires C, 74·2; H, 9·3%). The yield of this phenol, which gave a pale blue aqueous ferric reaction, was 1 g. from 10 g. of dihydrocitrinin. Prepared in the usual way, the *di*-p-*nitrobenzoate*, which was difficult to purify, was eventually obtained in long colourless, slender, rhombic prisms, m. p. 204—205°, after repeated crystallisation from alcohol and aqueous acetic acid alternately (Found: C, 63·3; H, 4·7; N, 6·0. $C_{26}H_{24}O_8N_2$ requires C, 63·4; H, 4·9; N, 5·7%).

Methylation of Methyl Citrinin.—A mixture of the methyl ester (10 g.), acetone (250 ml.), methyl iodide (25 ml.), and potassium carbonate was heated under reflux for 90 hours with the addition of more iodide (5 ml.) and carbonate (5 g.) at 10-hourly intervals; after this period a test portion of the product had a negative ferric reaction and was insoluble in alkali. A solution of the product in ether was washed

with 2N-aqueous sodium hydroxide and then with water, dried, and evaporated. Distillation of the residue gave an almost colourless, viscous liquid (6·5 g.), b. p. 178°/0·1 mm., which partly crystallised. Of the numerous analyses carried out on different specimens of the distilled product the following examples may be quoted: [(*a*) Found: C, 65·7; H, 7·6; OMe, 16·2. (*b*) Found: C, 64·1; H, 6·3. Calc. for $C_{13}H_{13}O_3(OMe)_3$: C, 61·9; H, 7·1; OMe, 30·0%]. When a solution of this product in light petroleum containing 2% of methanol was allowed to evaporate spontaneously during several days a small amount of a *compound* (M) separated in stout needles which on recrystallisation from the same solvent had m. p. 94°, $[\alpha]_D^{20°}$ −25·2° (*c*, 1·192 in methanol), $\lambda_{max.}$ ~230 mμ, $E^{1\%}_{1\ cm.}$ ~66; $\lambda_{min.}$ ~250 mμ, $E^{1\%}_{1\ cm.}$ ~20; $\lambda_{max.}$ ~315 mμ, $E^{1\%}_{1\ cm.}$ ~550 [Found: C, 64·6, 65·0; H, 6·4, 6·6; OMe, 8·9. $C_{14}H_{16}O_4(OMe)$ requires C, 64·7; H, 6·5; OMe, 11·2%]. Another specimen gave higher results for carbon which may have been due to solvent (Found: C, 65·7, 65·8; H, 6·7, 6·6%). Repetition of the crystallisation process with liquor from the first crop of (M) gave further (diminishing) amounts of the same compound.

The distilled mixed methylation product (5 g.) was heated under reflux with 28% aqueous potassium hydroxide (125 ml.) for 2½ hours and simultaneously a slow stream of nitrogen was passed through the apparatus. The effluent gas was bubbled through two traps containing dilute sulphuric acid solutions of 2 : 4-dinitrophenylhydrazine sulphate, and an orange precipitate gradually separated. By a combination of chromatography from chloroform and fractional crystallisation this precipitate was resolved into the 2 : 4-dinitrophenylhydrazones of acetaldehyde and of diethyl ketone. Recrystallised from methanol–light petroleum, the former formed orange-yellow needles, m. p. 162°, identical with an authentic specimen (Found: C, 43·1; H, 3·1; N, 25·2. Calc. for $C_8H_8O_4N_4$: C, 42·9; H, 3·6; N, 25·1%). On being purified from methanol–light petroleum, the 2 : 4-dinitrophenylhydrazone of diethyl ketone was obtained in orange needles, m. p. 154°, unchanged on admixture with an authentic specimen (Found: C, 49·2; H, 5·1; N, 21·0. Calc. for $C_{11}H_{14}O_4N_4$: C, 49·6; H, 5·3; N, 21·0%). The experiment was repeated, and the effluent nitrogen passed through aqueous-alcoholic dimedone, giving the derivative of acetaldehyde, m. p. 140°, identical with an authentic specimen.

A portion of the alkaline hydrolysate (75% of total) was saturated with carbon dioxide and extracted with chloroform (75 ml. × 4). The clear yellow oil (2 g.) left on the evaporation of the combined, dried extracts was dissolved in a little chloroform and on slow evaporation of the solvent a small amount of crystalline 3-(3 : 5-*dihydroxy*-2 : 4-*dimethylphenyl*)*butan*-2-*ol* (XV; R = OH) (0·5 g.) separated. Recrystallised from chloroform, the compound was obtained in colourless, stout prisms, m. p. 131°, $[\alpha]_D^{20°}$ −35·1° (*c*, 1·568 in methanol) (Found: C, 68·4; H, 8·3. $C_{12}H_{18}O_3$ requires C, 68·6; H, 8·6%). Considerable difficulty was experienced in the purification of this compound, which tended to separate as a glassy product. Methylation of this phenol (2 g.) with potassium carbonate (5 g.) and methyl iodide (5 ml.) in boiling acetone (100 ml.) during 12 hours with the addition of more iodide (5 ml.) after 6 hours gave a product a solution of which in ether was washed with 2N-aqueous sodium hydroxide and then with water, dried, and evaporated, leaving a pale yellow oil. Distillation of this material gave the *dimethyl ether* (1·7 g.) as an almost colourless oil, b. p. 124°/0·5 mm., having a negative ferric reaction [Found: C, 71·0; H, 8·9; OMe, 19·0. $C_{12}H_{16}O(OMe)_2$ requires C, 70·6; H, 9·2; OMe, 26·0%]. Acylation of this ether with *p*-nitrobenzoyl chloride–pyridine gave rise to a quantitative yield of the p-*nitrobenzoate*, forming pale yellow, stout prisms, m. p. 118°, from alcohol [Found: C, 64·9; H, 6·3; N, 4·1; OMe, 12·5. $C_{19}H_{19}O_4N(OMe)_2$ requires C, 65·1; H, 6·5; N, 3·6; OMe, 16·0%].

A mixture of the phenol (XV; R = OH) (3 g.), hydriodic acid (25 ml.; *d* 1·7), red phosphorus (2 g.), and acetic acid (from 10 ml. of anhydride) was heated under reflux for 3 hours, cooled, diluted with water (150 ml.), and extracted with ether (100 ml. × 3). The combined ethereal extracts were washed with sodium hydrogen sulphite, and then with sodium hydrogen carbonate, dried, and evaporated. A solution of the residue in acetic acid (15 ml.), containing zinc dust (5 g.), was heated on the steam-bath for 3 hours, the solvent evaporated in a vacuum, the residue treated with water (100 ml.), and the product isolated with ether and distilled in a high vacuum, giving 2-(3 : 5-dihydroxy-2 : 4-dimethylphenyl)butane as a colourless oil (2 g.), b. p. 130—131°/0·01 mm., with a negative ferric reaction in alcohol. Prepared in the usual way, the *di*-p-*nitrobenzoate* of this phenol separated from dilute acetic acid or a large volume of alcohol in almost colourless prisms, m. p. 146° (Found: C, 63·6; H, 4·9; N, 5·4. $C_{26}H_{24}O_8N_2$ requires C, 63·4; H, 4·9; N, 5·7%).

After the separation of the phenolic fraction, the hydrolysate was acidified (Congo-red) with hydrochloric acid and extracted with chloroform (100 ml. × 4). Evaporation of the combined dried extracts left an acidic pale yellow oil (2 g.) which gave a faint green ferric reaction and could not be distilled without decomposition. The acidic oil was treated with an excess of ethereal diazomethane, and the alkali-insoluble product distilled in a high vacuum, giving a colourless oil, b. p. 185°/0·1 mm. (Found: OMe, 29·0%), which regenerated the liquid acid on hydrolysis. Oxidation of the esterified material (1 g.), dissolved in acetic acid (10 ml.), by means of chromic oxide (1 g.) proceeded vigorously and was complete in about 5 minutes. On isolation and crystallisation from ether, the resulting oily product (0·8 g.) gave *methyl* O-*dimethyldihydrocitrinone*, m. p. 104°, identical with a specimen obtained by the oxidation of methyl *O*-dimethyldihydrocitrinin [Found: C, 62·2; H, 6·3; OMe, 29·0. $C_{13}H_{11}O_3(OMe)_3$ requires C, 62·3; H, 6·6; OMe, 30·2%].

The remaining portion of the original alkaline hydrolysate (25% of total) of the methylation product was acidified with 2N-sulphuric acid and distilled, giving an aqueous distillate containing formic acid which was identified by conversion into the *NN'*-diphenylformamidine hydrochloride in the usual manner.

When the distilled methylation product from methyl citrinin was oxidised with chromic anhydride in acetic acid and the product (1·5 g.) was hydrolysed with 28% aqueous potassium hydroxide (36 ml.) in an atmosphere of nitrogen, the volatile products gave a precipitate (0·3 g.) with aqueous 2 : 4-dinitrophenylhydrazine sulphate which was resolved into the 2 : 4-dinitrophenylhydrazones of acetaldehyde and of diethyl ketone, respectively identified by comparison with authentic specimens. From the alkaline liquor phenolic (0·3 g.) and acidic (1·0 g.) fractions were isolated. The phenol (XV; R = OH) formed

colourless, stout prisms, m. p. and mixed m. p. 131°, from chloroform. The acidic material, which partly solidified, was crystallised from ether, giving *O*-dimethyldihydrocitrinone in thick prisms (0·8 g.), m. p. and mixed m. p. 234° (Found : C, 61·2; H, 6·2; OMe, 19·6%).

The compound (M) (1·5 g.) was boiled with 28% aqueous potassium hydroxide for 2½ hours, a slow stream of nitrogen was passed through the apparatus, and the effluent gas was washed with aqueous 2 : 4-dinitrophenylhydrazine sulphate. The precipitate (0·6 g.) which formed in the wash-bottles was collected and separated into the 2 : 4-dinitrophenylhydrazones of acetaldehyde and of diethyl ketone. A portion of the alkaline hydrolysate (one-third) was acidified with sulphuric acid and distilled, and the presence of formic acid in the distillate was proved by the isolation of *NN'*-diphenylformamidine hydrochloride. The remainder of the alkaline liquor was saturated with carbon dioxide and the phenolic fraction (0·3 g. from two-thirds of the hydrolysate) was isolated with chloroform and crystallised from the same solvent, giving 3-(3 : 5-dihydroxy-2 : 4-dimethylphenyl)butan-2-ol (XV; R = OH) in stout, colourless prisms, m. p. and mixed m. p. 131°. Acidification followed by extraction of the carbonate liquor with ether gave a small amount of acidic material which was not investigated.

Ozonolysis of Citrinin.—A slow stream of ozone and oxygen was passed into a solution of citrinin (1 g.) in chloroform (75 ml.) at 0° for ½ hour, the solvent was removed in a vacuum, the brownish residue treated with water (75 ml.), and next day the clear, acidic liquor was decanted from a trace of oily material. An excess of 2 : 4-dinitrophenylhydrazine sulphate in dilute sulphuric acid was added to the aqueous solution, next day the voluminous deep red-brown precipitate (1 g.) was collected, the filtrate distilled, and the formic acid in the distillate converted into *NN'*-diphenylformamidine hydrochloride (0·75 g.). By chromatography from benzene on aluminium oxide the main constituent (0·8 g.) of the mixture of 2 : 4-dinitrophenylhydrazones was isolated and crystallised from methanol, forming deep crimson needles, m. p. 212° (decomp.). (Found : C, 53·3; H, 4·0; N, 11·5%).

Oxidised in a similar manner, methyl citrinin and the mixed methylation product gave similar yields of formic acid.

2-(3 : 5-*Dihydroxy*-4-*formyl*-2-*methylphenyl*)*butane* (XXI).—A solution of synthetical 2-(3 : 5-dihydroxy-2-methylphenyl)butane (Part IV, *loc. cit.*) (1 g.) in ether (100 ml.), containing zinc cyanide (1·5 g.), was saturated at 0° with hydrogen chloride and kept at room temperature for 48 hours. The viscous oily product, which had been repeatedly washed with ether by decantation, was dissolved in water (100 ml.) and, after having been neutralised with sodium hydrogen carbonate, the solution was warmed on the steam-bath for ½ hour. The resulting semi-solid was isolated with ether and on distillation in a high vacuum gave an aldehydic pale yellow oil (0·7 g.), b. p. 190—200°/0·001 mm., which solidified. Crystallisation of this product from benzene–light petroleum (b. p. 60—80°) gave the *aldehyde* (XXI) in long, slender, lemon-yellow prisms, m. p. 122°, easily soluble in alcohol, acetone, or ethyl acetate and having an intense bottle-green ferric reaction in alcohol (Found : C, 69·0; H, 7·5. $C_{12}H_{16}O_3$ requires C, 69·2; H, 7·7%). The 2 : 4-*dinitrophenylhydrazone* formed bright orange-red needles, m. p. 271° (decomp.), from alcohol (Found : N, 14·7. $C_{18}H_{20}O_6N_4$ requires N, 14·4%). The same aldehyde was obtained from a specimen of 2-(3 : 5-dihydroxy-2-methylphenyl)butane prepared from citrinin (Part IV, *loc. cit.*).

The residue from the purification of the aldehyde (XXI), which gave a brownish ferric reaction in alcohol, did not solidify.

2-(3 : 5-*Dihydroxy*-2 : 4-*dimethylphenyl*)*butane* (XV; R = H).—The foregoing aldehyde (1 g.) was boiled with a mixture of concentrated hydrochloric acid (15 ml.) and alcohol (15 ml.) containing zinc amalgam (8 g.) for 1½ hours, and on isolation the product was distilled in a high vacuum, giving the butane as a colourless oil, b. p. 128—130°/0·01 mm., having a negative ferric reaction in alcohol and a pale blue colouration with the aqueous reagent. The di-*p*-nitrobenzoate separated from aqueous acetic acid and then alcohol in rosettes of colourless prisms, m. p. 146°, undepressed on admixture with a natural specimen (Found : N, 6·1%).

The once-distilled mixture of aldehydes (XX) and (XXI) was reduced by the same method, and the product distilled and converted into the mixed di-*p*-nitrobenzoates. On repeated crystallisation from alcohol and 75% acetic acid alternately a sparingly soluble ester was obtained in characteristic colourless, slender, rhombic prisms, m. p. 204—205°, identical with the di-*p*-nitrobenzoate of 2-(3 : 5-dihydroxy-2 : 6-dimethylphenyl)butane from dihydrocitrinin (Found : C, 63·4; H, 5·0; N, 5·9%).

2-(3-*Hydroxy*-5-*methoxy*-2-*methylphenyl*)*butane* (XXII; R = Me).—A mixture of 2-(3 : 5-dihydroxy-2-formylphenyl)butane (XIX; R = CHO) (7 g.), potassium carbonate (14 g.), methyl iodide (9 g.), and acetone (200 ml.) was heated under reflux for an hour, and a solution of the product in ether (200 ml.) was extracted with 2N-aqueous sodium hydroxide (50 ml. × 3). The combined alkaline liquors were acidified and extracted with ether (100 ml. × 5), the ethereal extracts were washed with 0·5% aqueous sodium hydroxide to remove unchanged phenol (1 g.), and then with water, dried, and evaporated, leaving the monomethyl ether, which on distillation was obtained as a colourless oil (4 g.), b. p. 108—110°/0·0001 mm. This compound, which gave a red-brown ferric reaction in alcohol, furnished a *semicarbazone*, forming very pale greenish needles, m. p. 195°, from aqueous alcohol (Found : C, 58·8; H, 7·3; N, 15·9. $C_{13}H_{19}O_3N_3$ requires C, 58·9; H, 7·2; N, 15·8%). Interaction of the aldehyde and acetophenone in ethyl acetate saturated with hydrogen chloride gave an orange-red flavylium chloride.

Evaporation of the original ethereal solution which had been exhausted with alkali left an oil (1·3 g.), having a negative ferric reaction, which appeared to be the dimethyl ether of (XIX; R = CHO).

Reduction of the monomethyl aldehyde (XXII; R = CHO) (3·5 g.) with zinc amalgam (25 g.) in boiling concentrated hydrochloric acid (25 ml.) and alcohol (25 ml.) for 1½ hours gave the butane monomethyl ether (XXII; R = Me), which on distillation was obtained as a colourless liquid, b. p. 110—114°/0·01 mm., having a negative ferric reaction in alcohol. The p-*nitrobenzoate* formed colourless prisms, m. p. 109°, from aqueous methanol (Found : N, 4·1. $C_{19}H_{21}O_5N$ requires N, 4·1%).

Gattermann Reaction with 2-(3-*Hydroxy*-5-*methoxy*-2-*methylphenyl*)*butane.*—A solution of the phenol (2·3 g.) in ether (100 ml.), containing hydrogen cyanide (5 ml.) and zinc cyanide (2·5 g.), was saturated with hydrogen chloride at 0°. 48 Hours later the crystalline product was collected, washed with ether, and hydrolysed with water (75 ml.) (neutralise the cold solution with sodium hydrogen carbonate) at

60—70° for 20 minutes. The resulting red oil was isolated with ether (75 ml. × 5) and the combined ethereal solutions were extracted with 0·5% aqueous sodium hydroxide (75 ml. × 3), washed with water, dried, and evaporated, leaving 2-(3-hydroxy-5-methoxy-4-formyl-2-methylphenyl)butane (XXIV; R = CHO) as a pale brown oil (1·5 g.) having an intense greenish-brown ferric reaction in alcohol. The *semicarbazone* formed colourless needles, m. p. 209°, from aqueous alcohol (Found: C, 60·2; H, 7·6; N, 14·8. $C_{14}H_{21}O_3N_3$ requires C, 60·2; H, 7·5; N, 15·1%). Reduction of this aldehyde (1·2 g.) with zinc amalgam (12 g.) in boiling concentrated hydrochloric acid (15 ml.) and alcohol (25 ml.) gave 2-(3-hydroxy-5-methoxy-2 : 4-dimethylphenyl)butane (XXIV; R = Me) as a colourless oil (1 g.), b. p. 102—103°/0·05 mm., which was demethylated with boiling hydriodic acid (5 ml.; *d* 1·7) and acetic acid (from 3 ml. of anhydride) during ½ hour. On isolation, the phenol (XXIV; R = H) was converted into the di-*p*-nitrobenzoate, which separated from alcohol in colourless prisms, m. p. 146°, identical in every respect with a specimen prepared by way of the aldehyde (XXI) (Found: N, 6·0%).

The combined 0·5% aqueous sodium hydroxide extracts from the crude Gattermann product were saturated with carbon dioxide and extracted with ether. Evaporation of the washed and dried extracts gave 2-(3-*hydroxy*-5-*methoxy*-6-*formyl*-2-*methylphenyl*)*butane* (XXIII; R = CHO), which crystallised from methanol in colourless prisms, m. p. 160° (0·4 g.), having a negative ferric reaction (Found: C, 69·9; H, 8·3. $C_{13}H_{18}O_3$ requires C, 70·3; H, 8·1%). Reduction of this aldehyde (0·45 g.) by the procedure used for (XXI) gave the monomethyl ether (XXIII; R = Me) (0·2 g.), b. p. 110—112°/0·01 mm., which on demethylation with boiling hydriodic acid and acetic acid furnished the phenol (VIII) (0·15 g.). The di-*p*-nitrobenzoate of this compound formed colourless, slender, rhombic prisms (0·2 g.), m. p. 204—205°, identical with the corresponding derivative of (VIII) prepared from dihydrocitrinin (Found: N, 6·0%).

The data recorded for the ultraviolet absorption spectra of citrinin, methyl citrinin (III; R = H), and (XIV) have been determined by Professor R. A. Morton of the Biochemistry Department of this University, to whom we express our thanks.

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