334. *The Chemistry of Fungi. Part VII. Syntheses of Citrinin and Dihydrocitrinin.*

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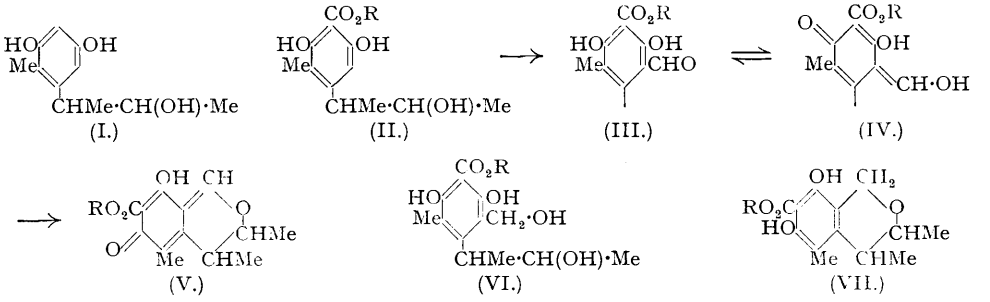
The carboxylation of the lævorotatory phenol (A) (I) furnished the acid (II; R = H) and on being subjected to the Gattermann reaction this acid and its methyl ester gave rise to products which are undoubtedly (III; R = H) and (III; R = Me) and which, on cyclisation with sulphuric acid, are converted into citrinin and its methyl ester respectively, identical in every way with natural specimens. By the same procedure optically inactive citrinin has been synthesised from phenol (B).

The interaction of the ester (II; R = Me) with formaldehyde in the presence of aqueous sodium hydroxide was accompanied by hydrolysis of the carbomethoxy-group, giving dihydrocitrinin (VII; R = H) in good yield, presumably by way of the intermediate type (VI).

We have been unable to obtain evidence that 2 : 4-dichlorobenzenediazonium sulphate couples with citrinin to form an azo-dye without the extrusion of any of the groups, and consequently we consider that the claim of Gore *et al.* (*Nature*, 1946, **157**, 333) cannot be maintained.

In deducing a revised structure (V; R = H) for citrinin (Part V, this vol., p. 867) we directed attention to the fact that the new formula was an anhydro-form of (III; R = H) and suggested that a compound having the latter structure might possibly be an intermediate in the phytochemical production of citrinin. Although the latter process is still obscure, we have now succeeded in effecting a synthesis of citrinin and its methyl ester by way of intermediates type (III), thus obtaining conclusive evidence in support of the new formula. Starting with the *lævo*-form of 3-(4 : 6-dihydroxy-*o*-tolyl)butan-2-ol (I), which is readily accessible from citrinin and which for the sake of brevity and convenience we shall continue to refer to as phenol (A) (compare Part IV, this vol., p. 859), we prepared the lævo-*acid* (II; R = H) by the carboxylation process employed for the synthesis of *p*-orsellinic acid (Robertson and Robinson, *J.*, 1927, 2196). The orientation of this acid (II; R = H) follows from its method of preparation, from its intense blue ferric reaction characteristic of γ-resorcylic acids, and from the ultimate conversion of the compound into citrinin. On being subjected to the Gattermann reaction, the acid (II; R = H) gave rise to a bright yellow oily product which was not further purified but which is essentially the aldehyde (III; R = H). The failure of this substance to react with carbonyl reagents is not unexpected and is considered to be due either to the steric effects of the groups adjacent to the formyl residue or to the existence of the compound in the hydroxymethylene form, type (IV), as in the case of *m*-xylorcylaldehyde (compare Robertson and Robinson, *loc. cit.*). On being dehydrated with concentrated sulphuric acid at room temperature the crude formyl derivative was smoothly converted into citrinin in good yield, identical in every way with the natural substance, and having almost the same specific lævorotation.



By the same procedure the *methyl* ester (II; R = Me) of the *lævo*-acid (II; R = H) was converted into methyl citrinin, identical with the natural derivative and showing the same characteristic tendency to retain solvent of crystallisation to which attention was directed in Part V (*loc. cit.*) when the compound was first described. We have also been able to record a new and higher value for the specific rotation (dextro-) of the ester. The lower rotation previously observed for the natural derivative would appear to have been due to the partial decomposition of the compound during the preparation of the required standard chloroform solution by warming, whereas in the present instance the solvent employed was acetone at room temperature, in which the ester is stable. We have found that methyl citrinin decom-

poses slowly in warm alcohol and more rapidly in cold impure chloroform containing traces of hydrogen chloride; when observed in a polarimeter the dextrorotatory solution of the ester in the latter solvent is seen to undergo a change in rotation as a result of hydrolysis and formation of the lævorotatory parent acid. On being boiled, a colourless solution of methyl citrinin in 95% alcohol, prepared at room temperature, becomes yellow owing to the decomposition of

FIG. 1.

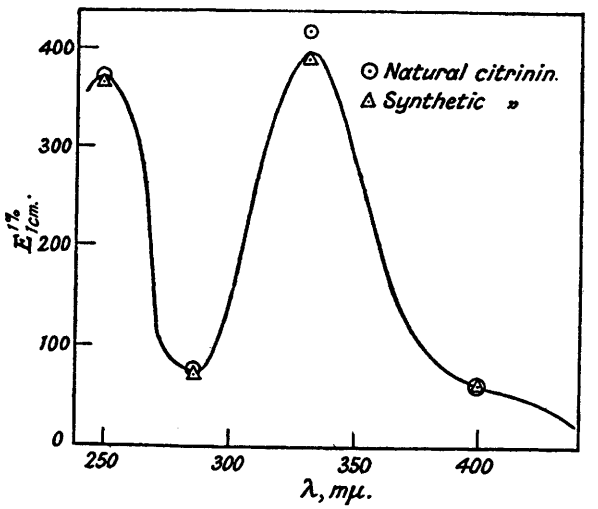
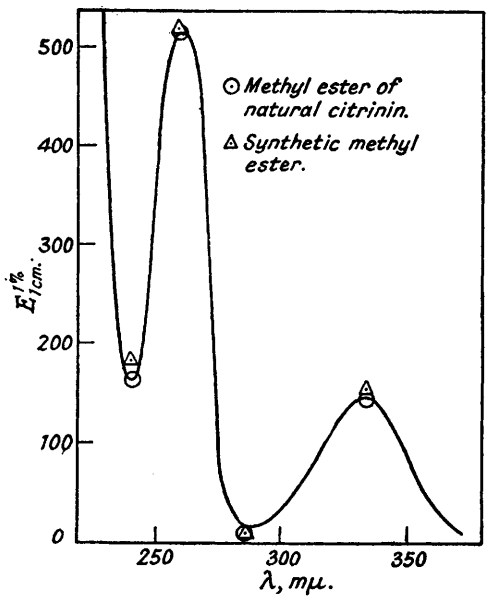


FIG. 2.



the ester; the considerable losses incurred in the purification of this ester from aqueous solvents arise mainly in this way.

From phenol (B), which is an optically inactive isomeride of phenol (A), an optically inactive citrinin has been prepared by the route employed for citrinin and its methyl ester and differs only from the natural compound in having a zero rotation. In this connection it may be noted that the synthesis of the *p*-nitrobenzoate of the dimethyl ether of phenol (B) has already been described together with the resolution of the acid phthalate of this ether to give the corresponding acyl derivative of the dimethyl ether of phenol (A) (Part IV, *loc. cit.*) which can be hydrolysed

without undergoing racemisation. We have now found that demethylation of the dimethyl ether of phenol (A) is accompanied by racemisation, yielding phenol (B), and consequently the main essential stage required to complete the synthesis of optically inactive citrinin has been completed. The remaining stage required to complete the synthesis of natural citrinin is either the preparation of phenol (A) by the resolution of phenol (B) or, alternatively, the resolution of synthetical inactive citrinin.

Further confirmation of the identity of natural and synthetical citrinin and their respective methyl esters was afforded by a comparison of the ultra-violet absorption spectra in alcoholic solution. The general shapes of the absorption curves for citrinin and the methyl ester are recorded in Figs. 1 and 2, respectively. The curves obtained for the natural and the synthetical substances were almost coincident, within the limits of experimental error, and to facilitate clarity of presentation only the curves for the natural compounds have been drawn. With the aid of a Beckman quartz spectrophotometer particular attention was paid to the regions near the maxima, minima, and points of inflexion.

The main data are summarised in the table, where a higher value of the extinction coefficient at 330 mμ is recorded for a rigorously purified specimen of natural citrinin (compare Part V, *loc. cit.*).

Substance.	λ, max., mμ.	λ, min., mμ.	λ, infl., mμ.	$E^{1\%}_{1\ cm.}$
Natural citrinin	250			370
	331			418
			400	63·2
		286		77
Synthetic citrinin	250			367
	331·5			390
			400	62·1
		286		72·9
Natural methyl ester	260			520
	334			151·6
		241		187·6
		285		11·3
Synthetic methyl ester	259·5			515
	336			141·2
		241		160
		285		10

The structure (VII; R = H), which was proposed in Part V (*loc. cit.*) for dihydrocitrinin and was based on the results of degradation experiments, has now been confirmed by a synthesis of the compound. The condensation of the optically active ester (II; R = Me) with formaldehyde in aqueous sodium hydroxide is accompanied by the hydrolysis of the carbomethoxy-group and simultaneous cyclisation of the intermediate alcohol type (VI; R = H or Me), giving a good yield of dihydrocitrinin, identical with the natural substance. The identity of the two compounds was confirmed by a comparison of the methyl esters (VII; R = Me) and their respective *O*-diacetyl derivatives. Less satisfactory yields of dihydrocitrinin were obtained when the ester (II; R = Me) was replaced by the parent acid.

In maintaining the inadequacy of the structure originally proposed for citrinin by Coyne *et al.* (*Phil. Trans.*, 1931, *B*, **220**, 301), Gore *et al.* (*Nature*, 1946, **157**, 333) based their criticisms on the claim that certain benzenediazonium salts reacted with citrinin to give monoazo-derivatives without the displacement of any group originally present in the citrinin molecule. More recently the latter authors (*J. Amer. Chem. Soc.*, 1948, **70**, 2287) have advanced an alternative constitution for citrinin which we have already shown in Part V (*loc. cit.*) to be untenable. As in the original formula of Coyne *et al.* (*loc. cit.*), the potential benzenoid system in the structure (V; R = H) is fully substituted, and consequently the criticisms of Gore *et al.* (*loc. cit.*) should apply equally to it. These authors have claimed that citrinin reacts readily under the usual conditions with benzenediazonium salts derived from *o*-chloroaniline, 2 : 5-dichloroaniline, and sulphanilamide, yielding monoazo-dyes, but they describe only the 2 : 5-dichloro-azo-derivative. They further state that with diazotised aniline the reaction is complex and that the azo-derivative of citrinin, m. p. 182°, was accompanied by the bisazo-derivative of phenol (A), m. p. 205° (decomp.). It is now clear that the claims of these workers cannot be sustained unless it is assumed that the methine group of the residue ·CH·O· of (V; R = H) is concerned in the coupling reaction, a possibility which we are unable to support. Further, with the somewhat scanty experimental details described by Gore *et al.* as a guide, we have investigated the action of 2 : 5-dichlorobenzenediazonium sulphate on citrinin under a variety of conditions and have completely failed to find any evidence of the formation of a compound corresponding

to the 2 : 5-dichlorobenzeneazo-derivative of citrinin, m. p. 201°. Instead, we obtained the *bisazo*-derivative of phenol (A), m. p. 280° (decomp.) [identical with a specimen prepared from authentic phenol (A)], along with a product, m. p. 245° (decomp.), the composition of which is not in agreement with that required by the corresponding monoazo-derivative of citrinin; it may be noted that the bis-2 : 5-dichlorobenzeneazo-derivative of phenol (B) has m. p. 289° (decomp.). When benzenediazonium sulphate was employed we were unable to isolate a homogeneous substance from the complex reaction mixture.

Experimental.

Demethylation of lævo-3-(4 : 6-*Dimethoxy*-o-*tolyl*)*butan*-2-*ol* [*Dimethyl Ether of Phenol* (*A*)].—This ether (2 g.) was gently boiled with a mixture of hydriodic acid (5 ml.) and acetic anhydride (10 ml.) containing red phosphorus (1 g.) for 5 minutes, and the cooled reaction mixture was diluted with water (100 ml.), neutralised with sodium hydrogen carbonate, and extracted with ether (100 ml. × 5). The combined ethereal solutions were then extracted with 2N-aqueous sodium hydroxide (50 ml. × 3), the alkaline extracts were acidified, and optically inactive 3-(4 : 6-dihydroxy-*o*-tolyl)butan-2-ol, phenol (B), was isolated with ether (wash with sodium hydrogen sulphite). Crystallised from chloroform, phenol (B) formed colourless prisms (200 mg.), m. p. 169—170°, undepressed on admixture with a specimen prepared from citrinin. Evaporation of the residual ethereal solutions which had been washed with alkali gave unchanged dimethyl ether (1·4 g.). When the time of heating of the demethylation mixture was increased from 5 to 15 minutes the product consisted of phenolic material from which only traces of phenol (B) could be separated. Demetbylation by means of pyridine hydrochloride at 150—170° for 3 hours likewise gave only traces of tbe required phenol.

lævo-3-(4 : 6-*Dihydroxy*-5-*carboxy*-o-*tolyl*)*butan*-2-*ol* (II; R = H).—An intimate mixture of phenol (A) (3 g.), potassium hydrogen carbonate (20 g.), and glycerol (30 ml.) was kept at 150—155° for 7 hours in an atmosphere of carbon dioxide, cooled, treated with water (150 ml.), saturated with ammonium sulphate, and extracted with ether (100 ml. × 6) to remove a small quantity of unchanged phenol. The aqueous liquors were then acidified with hydrochloric acid and the *acid* (2·25 g.) isolated by extraction with ether (100 ml. × 6) and crystallised from benzene, forming colourless squat prisms, m. p. 185° (decomp.) (Found, in a specimen dried in a vacuum at 60° for 2 hours: C, 60·1; H, 6·3. $C_{12}H_{16}O_5$ requires C, 60·0; H, 6·7%), $[\alpha]_D^{18°}$ −38·2° (*c*, 2·83 in alcohol), readily soluble in methanol or alcohol and sparingly soluble in benzene or light petroleum and giving an intense blue ferric reaction in alcohol.

A solution of this acid (2 g.) in a mixture of ether (75 ml.) and methanol (25 ml.) was treated with slightly more than one molecular proportion of ethereal diazomethane and the crystalline product was dissolved in 2N-aqueous sodium hydroxide (20 ml.) at 0° to remove diazomethane retained by the solid. Acidification of this solution with hydrochloric acid followed by extraction with ether gave the *methyl* ester, which was purified by distillation in a high vacuum and obtained as a colourless oil (1·8 g.), b. p. 164—166°/0·1 mm., $[\alpha]_D^{18°}$ −38·5° (*c*, 3·996 in methanol), having a greenish-blue ferric reaction in alcohol (Found: C, 61·1; H, 7·5. $C_{13}H_{18}O_5$ requires C, 61·4; H, 7·1%).

Carboxylation of phenol (B) by the procedure employed for the phenol (A) gave rise to the optically inactive *acid* (II; R = H), forming colourless stout prisms, m. p. 175—176° (decomp.), exhibiting the same ferric reaction (Found, in a specimen dried in a vacuum at 100°: C, 60·4; H, 6·3%).

Optically Inactive Citrinin (V; R = H).—A solution of the optically inactive acid (II; R = H) (1·1 g.) and hydrogen cyanide (5 ml.) in ether (125 ml.), containing zinc chloride (1 g.), was saturated at 0° with hydrogen chloride, and 48 hours later the crystalline product was collected, well washed with ether, and dissolved in cold water (50 ml.). This solution, which had been almost neutralised with ammonia, was heated at 80—90° for 10 minutes, cooled, and extracted with ether (50 ml. × 5). The acidic product was separated from the combined ethereal extracts by means of aqueous sodium hydrogen carbonate (50 ml. × 3), and after acidification of the latter solution this compound was isolated with ether and obtained as a pale yellow oil which did not solidify and had a blue-green ferric reaction in alcohol. A solution of this material in cold concentrated sulphuric acid (10 ml.) was kept at room temperature for 10 minutes and then poured on ice (50 g.). The resulting solid was collected, washed, dried, and crystallised from methanol, giving *inactive citrinin* in bright yellow prisms (0·4 g.), m. p. 175° (decomp.), which exhibited the iodine-brown ferric reaction characteristic of natural citrinin (Found: C, 62·2; H, 5·4. $C_{13}H_{14}O_5$ requires C, 62·4; H, 5·6%).

Citrinin (V; R = H).—The condensation of the *lævo*-acid (II; R = H) (1·2 g.) with hydrogen cyanide (2·5 ml.) by means of zinc chloride (2 g.) and excess of hydrogen chloride in ether (50 ml.), according to the procedure employed for the inactive isomeride, gave an oily condensation product, which on treatment with sulphuric acid (10 ml.) at room temperature for 15 minutes furnished citrinin (0·4 g.). Crystallised from alcohol, the synthetical compound formed characteristic yellow needles, m. p. and mixed m. p. 175° (decomp.), $[\alpha]_D^{18°}$ −37·4° (*c*, 1·15 in alcohol) (Found: C, 62·2; H, 5·6%). The m. p. of citrinin, which is a decomposition point, varies slightly with the rate of heating of the specimen. Rapid heating gives m. p. 178—179° (decomp.) and slower heating gives m. p. 175° (decomp.).

When the methyl ester (2·2 g.) of the (−)-acid (II; R = Me) was employed in place of the parent acid (II; R = H) in the Gattermann reaction the product was a yellow oil which on treatment with sulphuric acid gave rise to the methyl ester of citrinin. Recrystallised from benzene, this compound formed colourless diamond-shaped plates (1·5 g.), m. p. 139° (decomp.), $[\alpha]_D^{18°}$ +217·1° (*c*, 0·380 in acetone) (Found: C, 63·4; H, 6·8%). This product was identical in every way with a specimen of the natural ester, m. p. 139° (decomp.), $[\alpha]_D^{18°}$ +211·3° (*c*, 1·0894 in acetone), which had been recrystallised from benzene (Found: C, 63·0, 63·3, 63·1; H, 6·8, 7·0, 6·8. Calc. for $C_{14}H_{16}O_5$: C, 63·6; H, 6·1%).

Dihydrocitrinin (VII; R = H).—A solution of the methyl ester of the (−)-acid (II; R = Me) (1·5 g.) in cold 2N-aqueous sodium hydroxide (10 ml.) was treated with 40% formalin solution (10 ml.), and the orange mixture kept at room temperature for 24 hours, acidified with 2N-hydrochloric acid, and extracted

several times with ether. Evaporation of the combined washed and dried extracts left a residue which on crystallisation from benzene gave dihydrocitrinin (1 g.) contaminated with some of the acid (II; R = H). The product was extracted with chloroform in which this acid (II, R = H) is insoluble, and the residue left on evaporation of the chloroform extract was repeatedly crystallised from benzene, giving dihydrocitrinin in rosettes of colourless prisms (0·5 g.), m. p. 171° (decomp.), undepressed on admixture with a specimen of the natural derivative, $[\alpha]_D^{18°}$ −18·8° (*c*, 4·148 in chloroform) (Found : C, 62·0; H, 6·6. Calc. for $C_{13}H_{16}O_5$: C, 61·9; H, 6·4%). This compound gave the blue ferric reaction in alcohol characteristic of the natural derivative. Esterification of dihydrocitrinin with diazomethane furnished the methyl ester, forming characteristic, colourless, flat prisms, m. p. 60°, identical with a specimen from natural sources (Found : C, 62·9; H, 6·7. Calc. for $C_{14}H_{18}O_5$: C, 63·2; H, 6·8%). Acetylation of the synthetical methyl ester (100 mg.) with acetic anhydride (2 ml.) and pyridine (3 ml.) on a steam-bath for 35 minutes gave rise to the *diacetate* which separated from aqueous methanol in colourless needles (100 mg.), m. p. 114°, identical with a specimen prepared from the natural ester (Part V, *loc. cit.*) (Found : C, 61·9; H, 6·1. $C_{18}H_{22}O_7$ requires C, 61·7; H, 6·3%).

Reaction of Citrinin with 2 : 5-Dichlorobenzenediazonium Sulphate.—Prepared from dichloroaniline (1·0 g.), dissolved in concentrated sulphuric acid (15 ml.) and water (15 ml.) at −5°, a solution of the diazonium salt was added drop-wise to a well stirred solution of citrinin (2·0 g.) in 2N-aqueous sodium hydroxide (270 ml.) at 0°; 15 minutes later the reaction mixture was acidified, and the resulting precipitate washed free from acid, ground with aqueous sodium hydrogen carbonate to remove acidic material, washed, and dried. Acidification of the aqueous sodium hydrogen carbonate liquors gave only unchanged citrinin (0·3 g.). Extraction of the carbonate-insoluble product with alcohol left a fraction insoluble in the latter solvent which on crystallisation from chloroform gave the *bis*-2 : 5-*dichlorobenzeneazo*-derivative of phenol (A) in rust-coloured needles, m. p. 280° (decomp.), identical with an authentic specimen [Found : N, 10·4; Cl, 25·9. $C_{23}H_{20}O_3N_4Cl_4$ requires N, 10·3; Cl, 26·2%. Calc. for the monoazo-derivative of (A), $C_{17}H_{18}O_3N_2Cl_2$; N, 7·6; Cl, 19·3%. Calc. for the monoazo-derivative of citrinin, $C_{19}H_{16}O_5N_2Cl_2$: N, 6·6; Cl, 16·8%].

Evaporation of the alcoholic extract of the crude dye left only an intractable tar, but evaporation of the chloroform liquors from the purification of the bisazo-compound and subsequent addition of alcohol to the residue gave a *product*, m. p. 245—248° (decomp.), which on recrystallisation from chloroform–alcohol, formed glistening deep red prisms, m. p. 248° (decomp.) (Found : N, 10·2; Cl, 23·4%). In this experiment and in a number of similar experiments carried out under a variety of conditions we failed to isolate the compound, m. p. 200—201° (decomp.), described by Gore *et al.* (*loc. cit.*).

An authentic sample of the bis-2 : 5-dichlorobenzeneazo-derivative of phenol (A) was prepared by the interaction of this phenol and 2 : 5-dichlorobenzenediazonium sulphate and on purification from acetic acid or chloroform formed rust-coloured needles, m. p. 280° (decomp.) (Found : N, 10·5; Cl, 26·0%). The *bis*-2 : 5-*dichlorobenzeneazo*-derivative of phenol (B) was prepared in the same way, and on crystallisation from a comparatively large volume of acetic acid was obtained in rust-coloured needles, m. p. 289° (decomp.) (Found : N, 10·5; Cl, 26·5%).

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