Tetrahedron. Vol 23, pp. 4449 to 4461. Pergamon Press Ltd. 1967 Printed in Great Britain

THE SYNTHESIS OF OCHRATOXINS A AND B

METABOLITES OF *ASPERGILLUS OCHRACEUS* WILH

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(*Received* 2 *February* 1967; *accepted for publication* 6 *March* 1967)

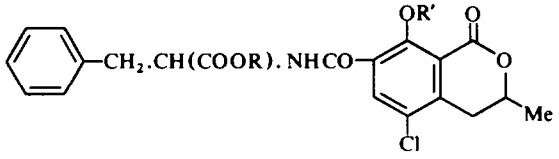
Abstract—Structure Ia was recently proposed for ochratoxin A, the main toxic metabolite of *Aspergillus ochraceus* Wilh. The structure of this compound is proved by an unambiguous synthesis. Modification of the synthetic sequence led to the related acid, ochratoxin B (II).

THE discovery of toxigenic strains of the fungus *Aspergillus ochraceus* Wilh.[1] led to the isolation of the two related acids called ochratoxin A (Ia) and B (II).[2] Recently the methyl and ethyl ester derivatives of these acids were isolated from the neutral fraction of cultures of this fungus.[3] Bio-assay revealed that the toxicity of the fungal cultures was due to the chlorine-containing compounds only.[3] The formulae suggested for the ochratoxins were based mainly on physical data and a direct synthesis was considered desirable to confirm the proposed structures.

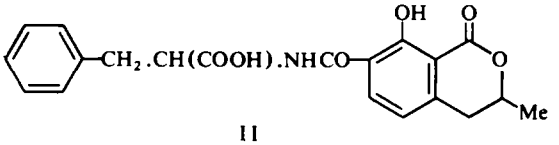
Acid hydrolysis of ochratoxins A and B gave L-β-phenylalanine and the lactone acids IIIa and IVa, respectively.[2] The first step towards the synthesis of ochratoxin A, therefore, was the preparation of 7-carboxy-5-chloro-3,4-dihydro-8-hydroxy-3-methylisocoumarin (IIIa), which could then be linked over its 7-carboxyl group to L-β-phenylalanine. The synthetic sequence furnishing this lactone IIIa was based on the fact that 3,4-dihydro-3-methylisocoumarins can be prepared by oxidation of the appropriate isochromans by means of a variety of agents.[4] The intermediate isochroman, 5-chloro-7-hydroxymethyl-8-methoxy-3-methylisochroman (XIIIb) was prepared according to Scheme I.

The starting material for the synthesis was 3-bromophenol (VIa), its corresponding tatrahydropyranyl derivative (VIb)[5] being converted into a Grignard reagent by reaction with magnesium in dry THF with ethyl bromide as catalyst. The Grignard reagent was treated in the cold with propylene oxide to furnish the 1-(3-tetrahydropyranyloxyphenyl)propan-2-ol (VIc). Without purification, this acid-labile intermediate was hydrolysed to 1-(3-hydroxyphenyl)propan-2-ol (VId). The IR, UV and high-resolution mass spectra of this compound were consistent with the structure. The NMR spectrum showed signals at τ 8·83 [three protons, doublet, J = 6·4 c/s, CH.Me]; 8·52 [one proton, singlet, CH.(OH).Me]; 7·40 [two protons, doublet, J = 6·3 c/s, $CH.CH_2.Ar$]; 6·05 [one proton, slightly broadened quartet, J = 6·33 c/s, $CH_2.CH.(OH).Me$]; 2·95 to 3·42 [four aromatic protons, complex pattern]; confirming the structure.

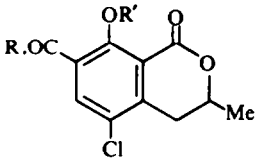
[1] De B. Scott, *Mycopathol. et Mycol. Appl.* **25**, 213 (1965).
[2] K. J. van der Merwe, P. S. Steyn, and L. Pourie, *J. Chem. Soc.* 7083 (1965).
[3] P. S. Steyn and C. W. Holzapfel. Unpublished results.
[4] R. D. Barry, *Chem. Revs.* 229 (1964).
[5] H. Gilman, L. Santucci, D. R. Swayampati and R. O. Ranck, *J. Am. Chem. Soc.* **79**, 3077 (1957).

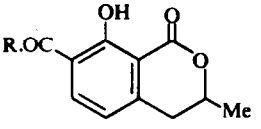


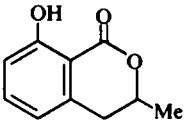
Ia; R = R′ = H
Ib; R = Me, R′ = H
Ic; R = R′ = Me



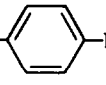
II







IIIa: R = OH, R′ = H
IIIb: R = Cl, R′ = H
IIIc: R = N_3, R′ = H
IIId: R = OMe, R′ = Me
IIIe: R = OH, R′ = Me

IIIf: R =  , R′ = Me

IVa: R = OH
IVb: R = N_3

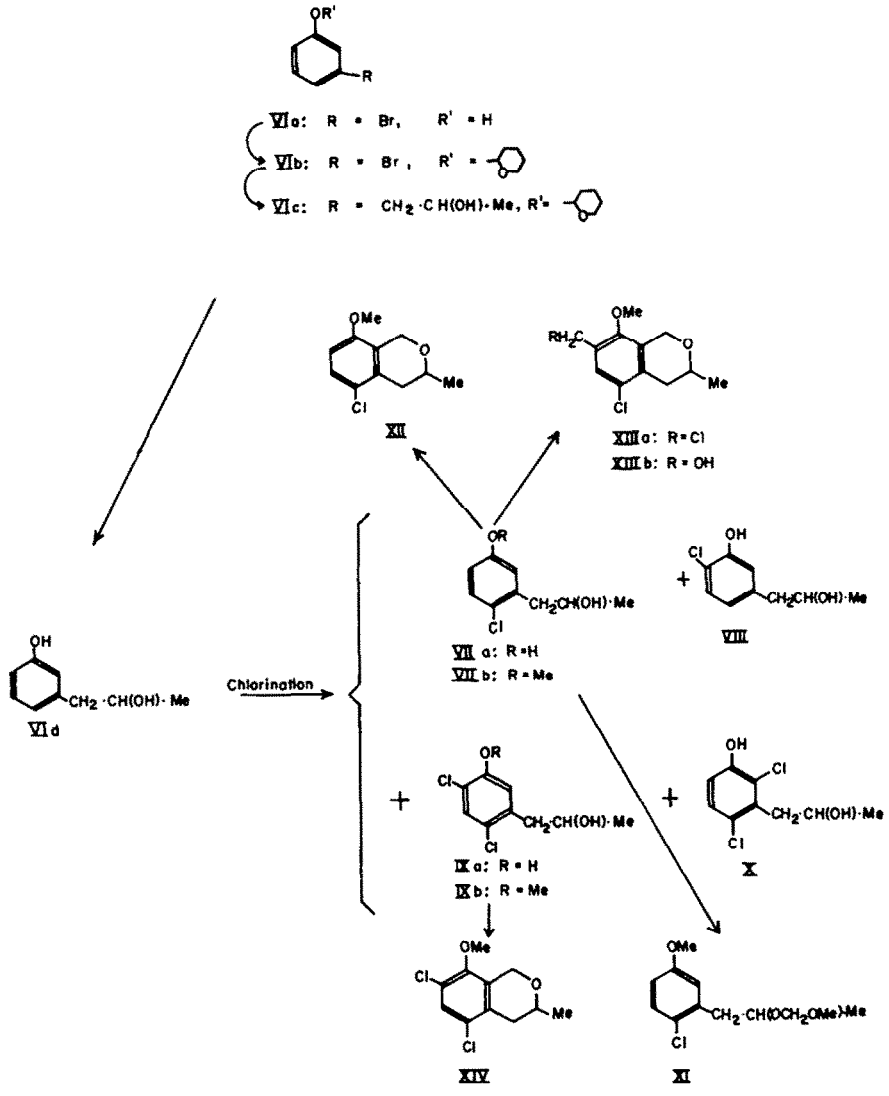
V

Relatively little is known about the chlorination of alkylphenols. It has been reported[6] that chlorination of 3-methylphenol in CCl_4 gives 4-chloro-3-methylphenol and 6-chloro-3-methylphenol in 29 and 49% yield, respectively; the 2-chloro-3-methylphenol has also been obtained. Sterically large groups (e.g. the t-Bu group) at the 3-position hinder chlorination in the 4-position. Thus, Kaeding[7] obtained the 4-chloro derivative in only 19% yield from the monochlorination of 3-t-butylphenol. From a survey of the influence of solvents on the chlorination of 2-methylphenol, Campbell[8] concluded that nitromethane gave a very favourable ratio of the 4-chloro- to the 6-chloro-isomer, *viz.* 6·6. The 1-(3-hydroxyphenyl)propan-2-ol was therefore chlorinated at 0° in nitromethane with one molar equivalent of chlorine gas. This procedure gave a mixture of chloroisomers which was separated on formamide-impregnated cellulose powder and silica chromatoplates. Mass spectrometry of the four major compounds showed that two of these contained one chlorine atom each, while the two others contained two chlorine atoms per molecule (molecular ions at

[6] G. P. Gibson, *J. Chem. Soc.* 1424 (1926).
[7] W. W. Kaeding, *J. Org. Chem.* 4851 (1961).
[8] A. Campbell and D. J. Shields, *Tetrahedron* **21**, 211 (1965).

m/e 186 and 220, respectively). The structures of these compounds were elucidated by UV and NMR spectroscopy. (See Tables 1 and 2).

Scheme 1



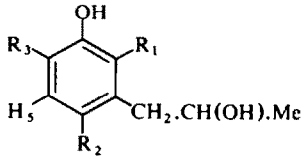
The UV spectra of the starting material VId and the two monochloro compounds were compared with those of phenol, 2-chlorophenol and 4-chlorophenol. The starting material resembled phenol, while, compared with the parent compound, the major and minor chloro compounds showed bathochromic shifts similar to those of 4- and 2-chlorophenol, respectively, due to the chlorine atom. The main mono chloro product was formulated as 1-(2-chloro-5-hydroxyphenyl)propan-2-ol

TABLE 1. UV ABSORPTION MAXIMA OF SUBSTITUTED PHENOLS

Compound	Wavelength (mμ) and molecular extinction coefficient (ε)	
Phenol[9]	210 (6,200)	270 (1,450)
2-Chlorophenol	216 (5,500)	276 (1,980)
4-Chlorophenol	227 (5,600)	284 (1,800)
VId	216 (7,200)	275 (2,000)
VIII	221 (8,100)	279 (2,700)
VIIa	229 (8,000)	284 (2,100)

(VIIa) and the minor one as 1-(4-chloro-3-hydroxyphenyl)propan-2-ol (VIII). The salient features of the NMR spectra of the two compounds as summarized (Table 2) confirm the correctness of the structural assignments.

TABLE 2. NMR SPECTRA OF SUBSTITUTED PHENOLS*



Compound	R_1	R_2	R_3	τ Values R_1	R_2	R_3	CH_2	H_5
VId	H	H	H	3·25	to	3·42m	7·40d	3·00m
VIIa	H	Cl	H	3·31d	—	3·44q	7·25m	2·88d
VIII	H	H	Cl	3·20d	3·35q	—	7·38d	2·82d
Aromatic Me-ether of IXa	H	Cl	Cl	3·17s		—	7·18m	2·65s
X	Cl	Cl	H	—	—	3·18d	6·86d	2·75d

s = singlet, d = doublet, q = quartet, and m = multiplet.

* In all these compounds the para-couplings were too small to be registered at 60 Mc/s.

The NMR spectrum of the aromatic methyl ether derivative IXb of the dichloro-compound IXa showed two signals characteristic of para-orientated aromatic protons (singlets at τ 2·65 and 3·17). This observation is compatable only with structure IXb. This conclusion was proved correct by converting the methyl ether derivative IXb into the isochroman XIV by heating under reflux in chloromethyl ether with $ZnCl_2$ as catalyst.

[9] A. I. Scott, *Interpretation of the Ultraviolet Spectra of Natural Products*, p. 93. Pergamon Press, London (1964).

The NMR spectrum of the other dichloro-compound contained the doublets of two ortho-aromatic protons, viz. τ 2·75 and 3·18 (J = 8·75 c/s). These data can be interpreted in terms of two possible structures, namely 1-(2,6-dichloro-3-hydroxyphenyl)propan-2-ol or 1-(2,4-dichloro-3-hydroxyphenyl)propan-2-ol. In order to distinguish between these possibilities, use was made of the fact that in chlorine-containing compounds the chlorine atom has a deshielding effect on nearby protons as shown by NMR spectroscopy.[10] This deshielding effect of the *ortho*-Cl atom on the methylene protons is clear from Table 2. The strong deshielding of the methylene protons (signal at τ 6·86), in the dichloro compound in question, subsequently led to a decision in favour of 1-(2,6-dichloro-3-hydroxyphenyl)propan-2-ol (X). Another interesting feature of these spectra was that an ortho-chlorine atom enhanced the magnetic non-equivalence of the methylene protons. The methylene protons in VId and VIII appear as doublets, while the corresponding protons in compounds VIIa, VIIb and IXb appear as the AB part of an ABX system.

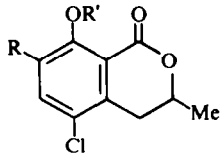
Treatment at room temperature of 1-(2-chloro-5-methoxyphenyl)-propan-2-ol (VIIb) in chloromethyl ether with $ZnCl_2$ gave an isochroman XII in quantitative yield. The NMR spectrum of XII showed signals centred at τ 8·63 [three protons, doublet, J = 6·5 c/s, CH.Me]; 7·38 [two protons, 8-line pattern of the AB part of an ABX system, J_{AB} = 17·9, J_{AX} = 9 and J_{BX} = 4 c/s, CH.CH_2.Ar]; 6·32 [one proton, complex pattern of the X part of an ABX system, CH_2.CH.(OR).Me]; 6·24 [three protons, singlet, Ar.OMe]; 5·30 [two protons, two doublets (τ 5·45 and 5·15, J = 16·0 c/s) of an AB system, Ar.CH_2.OR]; and 3·43 and 2·86 [two ortho-aromatic protons, J = 8·5 c/s]. Compound VIIb treated under reflux in chloromethyl ether in the absence of catalyst gave, apart from some unreacted starting material VIIb, also the isochroman XII and a new product formulated as XI on the basis of the following evidence: its mass spectrum which showed a molecular ion at *m/e* 244, its UV spectrum which was the same as that of the starting material, and in addition its IR spectrum showed no bands in the OH-stretching region in accordance with the view that etherification of the OH group had occurred. Cyclisation of the compound XI would yield the isochroman XII and it is therefore a likely intermediate in the $ZnCl_2$ catalysed reaction. Analogous electrophilic intramolecular aromatic substitutions were employed in the synthesis of dihydrocitrinin,[11] chroman,[12] and isochroman derivatives.[13]

At this stage an additional carbon atom had to be introduced into the aromatic nucleus at position 7. This was achieved by prolonged heating of compound VIIb under reflux in chloromethyl ether with $ZnCl_2$. The 5-chloro-7-chloromethyl-8-methoxy-3-methylisochroman (XIIIa) was obtained in quantitative yield. The NMR spectrum showed signals similar to those of the isochroman XII. The substitution at position 7 of an aromatic proton for a chloromethyl group was evident from the absorption of the chloromethyl group (τ 5·4, J = 1·5 c/s) and the single aromatic proton (τ 2·63).

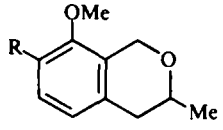
Oxidation of the isochroman XIIIa in AcOH with CrO_3 at room temperature gave the corresponding lactone XVa, showing IR absorption at 1723 and 1135 cm^{-1},

[10] R. C. Fort, G. W. Cheeseman and E. C. Taylor, *J. Org. Chem.* **29**, 2440 (1964).
[11] H. H. Warren, G. Dougherty and E. S. Wallis, *J. Am. Chem. Soc.* **71**, 3422 (1949).
[12] L. W. Deady, R. D. Topsom and J. Vaughan, *J. Chem. Soc.* 2094 (1963).
[13] J. Cologne and P. Boisde, *Bull. Soc. Chim. Fr* 1337 (1956).

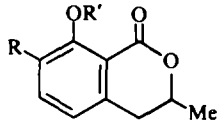
attributed to the lactone system. Compound XIIIa was subsequently hydrolysed to the hydroxyisochroman XIIIb, which was similarly oxidized to the 7-carboxy-5-chloro-3,4-dihydro-8-methoxy-3-methylisocoumarin (XVb). The methoxy acid XVb was demethylated by prolonged refluxing in 6N HCl. The compound obtained XVc was compared with the lactone acid IIIa, obtained by hydrolysis of natural ochratoxin A. These compounds possessed identical UV, IR (in Nujol), NMR (in DMS-d_6) and mass spectra [parent ion at *m/e* 256 and prominent fragments at *m/e* 212 (base peak) and 194] as well as identical chromatographic behaviour. After sublimation, the compound XVc had m.p. 229°, while that of IIIa was 238°, and that of a sublimate of the mixture, 233–234°. The physical data, furthermore, indicated that the compound XVc formed a racemic mixture on sublimation.[14]



XVa: R = CH_2Cl, R′ = Me
XVb: R = COOH, R′ = Me
XVc: R = COOH, R′ = H



XVIa: R = H
XVIb: R = CH_2OH



XVIIa: R = H, R′ = Me
XVIIb: R = COOH, R′ = Me
XVIIc: R = COOH, R′ = H

The above synthetic approach has been extended to the preparation of methoxymellein (XVIIa) and to 7-carboxy-3,4-dihydro-8-hydroxy-3-methylisocoumarin (IVa). Compounds XII and XIIIb were used as intermediates in the synthesis of the above compounds. Dehalogenation of the chloro-isochromans XII and XIIIb in MeOH over Raney nickel in the presence of alkali[15] furnished the isochromans XVIa and XVIb, respectively. The non-aromatic portions of the NMR spectra of these compounds were very similar; compound XVIb showed additional absorption at τ 5·39 owing to the 7-hydroxymethyl group, and compound XVIa showed a complex pattern of three ortho-aromatic protons, while compound XVIb contained the doublets of two ortho-aromatic protons (τ 2·89 and 3·2, J = 8·0 c/s).

Oxidation of compound XVIa in AcOH by CrO_3 furnished methoxymellein XVIIa, identified by its UV and IR (carbonyl absorption at 1711 cm^{-1}) spectra. Its NMR spectrum showed signals centred at τ 8·57 [three protons, doublet, J = 6·2 c/s, CH.Me]; 7·19 [two protons, the AB part of an ABX system, J_{AB} = 17·9, J_{AX} = 8·5,

E. L. Eliel, *Stereochemistry of Carbon Compounds*, p. 44. McGraw-Hill, New York (1962).

[15] H. Kämmerer, L. Horner and H. Beck, *Chem. Ber.* **91**, 1376 (1958).

J_{BX} = 5·4 c/s with $\nu_X \equiv 0$, ν_A = 180·3 and ν_B = 165·5 c/s, CH.$\underline{CH_2}$.Ar]; 6·13 [three protons, singlet Ar.$\underline{OMe}$]; 5·52 [one proton, complex pattern of the X part of an ABX system, $CH_2\underline{CH}(OR)$.Me]; 3·17 and 3·28 [two aromatic protons, broadened doublets, J = 7·5 c/s]; 2·63 [one proton, doublet of doublets, J = 7·5 c/s]. Similar NMR data were reported for the optical antipode of mellein,[16] and for 5-methylmellein.[17] Mellein can be obtained from methoxymellein by known methods.[18]

Oxidation of the hydroxyischroman XVIb furnished the corresponding methoxy lactone acid XVIIb, which was demethylated by acid hydrolysis. The resulting compound XVIIc was shown to be identical by direct comparison with the lactone acid IVa obtained from ochratoxin B.

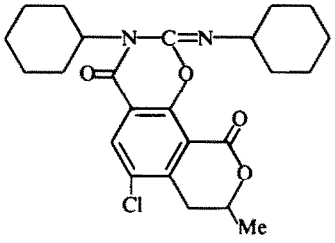
A study was undertaken of the coupling of L-β-phenylalanine with the acids IIIa and IVa obtained by hydrolysis of natural ochratoxins A and B. Various methods were investigated in order to find one that would ensure optical purity of the product. As criterion of the optical purity of the synthetic products, their optical rotatory dispersion (ORD) from 250–600 mμ was compared with that of natural material.

Treatment of the acid chloride IIIb of the lactone acid IIIa in dry pyridine with L-β-phenylalanine methyl ester[19] gave extensively racemized ochratoxin A methyl ester Ib (ORD evidence).

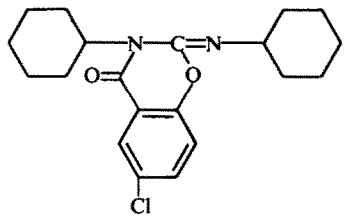
The acid chloride IIIb in DMF was subsequently treated for 1 hr at 0° with aqueous NaN_3. The acid azide IIIc in AcOEt was stirred for 60 hr at 5° with the triethylammonium salt of L-β-phenylalanine. The product obtained in 40% yield was shown to be identical with natural ochratoxin A. No racemization occurs in peptide synthesis on coupling of amino acid residues by the acid azide method.[20] The acid azide IVb was coupled in a similar fashion to L-β-phenylalanine giving rise to a product, identical to ochratoxin B in every detail.

The use of dicyclohexylcarbodiimide (DCC)[21] was studied as a direct coupling agent in the amide synthesis. Reaction of the lactone acid IIIa with L-β-phenylalanine methyl ester in the presence of DCC, however, yielded only 5% ochratoxin A methyl ester. The main product was a crystalline Dragendorff-positive adduct to which structure (XVIII) was as assigned on the basis of its IR, UV and mass spectra. A similar adduct (XIX) was obtained on treatment of 5-chlorosalicylic acid with DCC. It is of interest to note that this adduct is acid-labile and can be hydrolysed to a cyclic oxocarbamate derivative (XX). The above results can be compared with the finding of Zetsche *et al.*[22] that anthranilic acid reacts with diphenylcarbodiimide to give compound (XXIa) in high yield. It seems likely that this product originated from the hydrolysis of the adduct (XXIb). It has also recently been reported[23] that glycine ethyl ester or the p-nitrophenyl ester of glycine reacts with DCC to yield 1-cyclohexyl-2-cyclohexylimino-4,5-dihydro-5-imidazolone. These reactions are fast enough to be a serious limitation to the general applicability of DCC as an acylating agent.

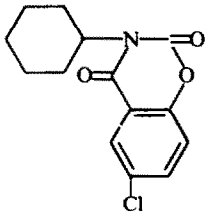
[16] E. L. Patterson, W. W. Andres and N. Bohonos, *Experientia* **4**, 209 (1966).
[17] A. Ballio, S. Barcellona and B. Santurbano, *Tetrahedron Letters* **31**, 3723 (1966).
[18] G. Bendz, *Arkiv. Kemi* **14**, 511 (1959).
[19] R. A. Boissonnas, St Guttmann, P.-A. Jaquenoud and J.-P. Waller, *Helv. Chim. Acta* **38**, 1491 (1955).
[20] F. Weygand, A. Prox and W. Kõnig, *Chem. Ber.* **99**, 1451 (1966).
[21] J. C. Sheenan and G. H. Hess, *J. Am. Chem. Soc.* **77**, 1067 (1955).
[22] F. Zetsche and G. Voigt, *Chem. Ber.* **74B**, 183 (1941).
[23] DeLos F. De Tar, R. Silverstein and F. F. Rogers, *J. Am. Chem. Soc.* **88**, 1024 (1966).



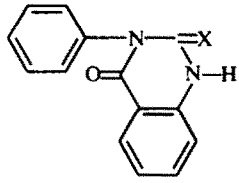
XVIII



XIX



XX



XXIa: X = O
XXIb: X = N—Ph

A derivative IIIe of the lactone acid IIIa in which the phenolic OH group was protected was obtained by alkaline hydrolysis of the O-methyl ester IIId. Reaction of this compound IIIe with L-β-phenylalanine methyl ester in the presence of DCC gave O-methyl ochratoxin A methyl ester in an unsatisfactory yield. This procedure was improved by esterifying the acid IIIe with 4-nitrophenol by means of DCC. The resulting ester IIIf was treated with L-β-phenylalanine methyl ester to give O-methyl ochratoxin A methyl ester Ic. Mild treatment with $AlCl_3$ in nitrobenzene[24] yielded ochratoxin A methyl ester Ib which was optically pure since it had the same ORD as the natural product.[3] Alkaline hydrolysis of Ib proceeded without any racemization to yield ochratoxin A (Ia).

The total synthesis of ochratoxin A was thus reduced to the separation of the optical antipodes of the synthetic lactone acid XVc. Considerable separation was achieved by fractional crystallization of the brucine salt of the synthetic material. The 3S-isomer crystallized preferentially from acetone. The lactone acid liberated from these crystals had an optical purity of 79·5%. This was shown by comparison of the amplitude of the Cotton effect (ORD extrema at 350 and 315 mμ) to that of the natural 3R-isomer. The required lactone acid (3R-isomer) was similarly isolated from the mother liquor and had an optical purity of 83%.

The results described above confirmed the correctness of the proposed formulae Ia and II for ochratoxins A and B, respectively.

EXPERIMENTAL

Unless specified to the contrary, UV absorption refers to EtOH and IR absorption to $CHCl_3$ solns. UV spectra (Cary 15 spectrometer) and IR spectra (Perkin-Elmer Model 237 spectrometer). NMR spectra (Varian A-60 or HA-100 spectrometer in $CDCl_3$ soln). The chemical shifts were measured on the τ-scale

[24] E. Hardegger, E. Widmer, K. Steiner and A. Pfiffner, *Helv. Chim. Acta* **47**, 2031 (1964).

relative to TMS as internal standard (τ 10·0). Mass spectra were taken on an MS-9 double focussing mass spectrometer. The ORD curves measured at 20° with a Jasco ORD/UV-5 spectrometer, concentration of solns given as mg/ml throughout. Silica for chromatography refers to a supply of E. Merck (0·05–0·20 mm). For preparative TLC chromatoplates were coated with Merck's Silica Gel G (thickness of silica gel layer ca 2 mm).

1-(3-*Hydroxyphenyl*)*propan*-2-*ol* (VId)

A Grignard reagent was prepared by slowly adding a mixture of VIb[7] (26 g), EtBr (2·0 g) and dry THF (75 ml) to Mg (5·0 g, activated by I_2) in a N_2 atm. The mixture was stirred and heated for 1 hr and then cooled on ice. Propylene oxide (20 ml) in THF (20 ml) was slowly added. After 16 hr 10% NH_4Cl soln was added and the aqueous phase extracted with ether. Evaporation of the solvent gave a residue which was separated on silica. $CHCl_3$ eluted the VIc (22 g) as a colourless oil. This compound VIc (21 g) in 1:1 MeOH—1N HCl (500 ml) was shaken overnight. The mixture was extracted exhaustively with Et_2O to afford the 1-(3-*hydroxyphenyl*)*propan*-2-*ol* (VId; 10·6 g) as an oil, λ_{max} 216 and 275 mμ (ε 7200 and 2000, respectively); ν_{max} 3590, 3320, 3005, 2970, 2930, 1603, 1590, 1160, 958, 929 and 880 cm^{-1}. (Accurate mass M^+ 152·084. $C_9H_{12}O_2$ requires: 152·084).

The Chlorination of 1-(3-*hydroxyphenyl*)*propan*-2-*ol* (VId)

Chlorine gas (3·76 g) in nitromethane (50 ml) was leaked in a stream of N_2 for 30 min into a soln of VId (8·0 g) and nitromethane (70 ml) at 0°. The solvent was removed after 15 min to furnish an oil (9·8 g) which was shown to consist of several compounds (descending paper chromatography on formamide-impregnated Whatman No. 1 filter paper with 1:1 C_6H_6–AcOEt as mobile phase, ethanolic $FeCl_3$ spray reagent).

The reaction product (9·8 g) was separated by column chromatography on formamide-impregnated cellulose powder (700 g) in 10:1 C_6H_6–AcOEt. Four main fractions were obtained.

Fraction 1. 1·5 g, eluted with 1·5 l. solvent, consisted of 4 minor compounds, not investigated further.

Fraction 2. 3·4 g, eluted with 4·5 l. solvent, consisted of 2 major compounds and one minor compound. Fractional crystallization from chloroform yielded 1-(2,4-*dichloro*-5-*hydroxyphenyl*)*propan*-2-*ol* (IXa; 1·8 g), m.p. 118°; λ_{max} 208, 224 and 289 mμ (ε 29,000, 8100 and 3000, respectively). (Found: C, 49·0; H, 4·5. $C_9H_{10}Cl_2O_2$ requires: C, 48·9; H, 4·6%.)

The mother liquor (1·55 g) from the above crystallization was separated on preparative chromatoplates (silica containing fluorescent indicator) with 98·5:1·5 $CHCl_3$–MeOH as mobile phase. Elution of the absorbing bands gave two compounds.

The major product, 1-(2,6-*dichloro*-3-*hydroxyphenyl*)*propan*-2-*ol* (X; 1·2 g), crystallized from 9:1 C_6H_6–n-pentane, and had m.p. 84°; λ_{max} 220sh, 277·5 and 283 mμ (ε 9500, 2500 and 2480, respectively). (Found: Cl, 31·55. $C_9H_{10}Cl_2O_2$ requires: Cl, 32·05%.)

The minor product (0·30 g), an oil was formulated as 1-(4-*chloro*-3-*hydroxyphenyl*)*propan*-2-*ol* (VIII), λ_{max} 221 and 279 mμ (ε 8100 and 2700, respectively). (Found: C, 57·7; H, 6·1. $C_9H_{11}ClO_2$ requires: C, 57·9; H, 5·9%.)

Fraction 3. 3·3 g, eluted with 3·8 l. solvent. Crystallization from $CHCl_3$ furnished the 1-(2-*chloro*-5-*hydroxyphenyl*)*propan*-2-*ol* (VIIa), m.p. 72°; λ_{max} 229 and 284 mμ (ε 8000 and 2100, respectively). (Found: C, 58·2; H, 5·7. $C_9H_{11}ClO_2$ requires: C, 57·9; H, 5·9%.)

Fraction 4. 1·9 g, eluted with 2·5 l. solvent. Unreacted starting material VId.

1-(2-*Chloro*-5-*methoxyphenyl*)*propan*-2-*ol* (VIIb)

The phenol VIIa (2 g) in dry acetone (180 ml) was heated under reflux for 20 hr with Me_2SO_4 (19 ml) and anhyd K_2CO_3 (35 g). Dilution of the reaction mixture with water and extraction into $CHCl_3$ gave a product which was separated on silica. $CHCl_3$ eluted the 1-(2-*chloro*-5-*methoxyphenyl*)*propan*-2-*ol* (VIIb; 1·93 g) as a colourless oil, which after distillation (60–70°/1 × 10^{-4} mm) had m.p. 45°; λ_{max} 229 and 281·5 mμ (ε 9800 and 2000, respectively). (Found: C, 59·9; H, 6·4. $C_{10}H_{13}ClO_2$ requires: C, 59·9; H, 6·5%.)

5-*Chloro*-8-*methoxy*-3-*methylisochroman* (XII)

$ZnCl_2$ (ca. 50 mg) was added to VIIb (125 mg) in chloromethyl ether (5 ml) and shaken at room temp (18°) for 1 min. Dilution of the reaction mixture with water and extraction into $CHCl_3$ gave material which was separated on silica. C_6H_6 eluted XII (130 mg), m.p. 47° (from n-pentane); λ_{max} 230, 278 and 285 mμ (ε 8300, 1550 and 1500, respectively). (Found: C, 62·6; H, 5·95. $C_{11}H_{13}ClO_2$ requires: C, 62·1; H, 6·15%.)

In a similar experiment VIIb (20 mg) in chloromethyl ether (10 ml) was heated under reflux for 30 min.

The reaction mixture was treated as above and the product separated on a preparative chromatoplate (silica containing fluorescent indicator) with $CHCl_3$ as mobile phase. The 3 main absorbing bands were eluted: band (1), R_F 0·55 being XII; band (2), R_F 0·30 being XI. (Found: M^+, 244 (MS). $C_{12}H_{17}ClO_3$ requires: M, 244). Band (3), R_F 0·18 was identified as unreacted starting material VIIb.

5-Chloro-7-chloromethyl-8-methoxy-3-methylisochroman (XIIIa)

$ZnCl_2$ (ca. 200 mg) was added to VIIb (1·0 g) in chloromethyl ether (20 ml) and heated under reflux for 45 min. The product was isolated as described for XII, and separated on silica. Elution with 1:1 $CHCl_3$–C_6H_6 gave the *5-chloro-7-chloromethyl-8-methoxy-3-methylisochroman* (XIIIa; 1·27 g), m.p. 85° (from hexane); λ_{max} 279 and 285 mμ (ε 860 and 865, respectively). (Found: C, 55·3; H, 5·4. $C_{12}H_{14}Cl_2O_2$ requires: C, 55·4; H, 5·4%.)

5-Chloro-7-hydroxymethyl-8-methoxy-3-methylisochroman (XIIIb)

The isochroman XIIIa (1·0 g) in water (200 ml) containing K_2CO_3 (12 g) was heated under reflux for 2 hr. Extraction into $CHCl_3$ gave a product which was separated on silica. $CHCl_3$ eluted the *5-chloro-7-hydroxymethyl-8-methoxy-3-methylisochroman* (XIIIb; 850 mg), m.p. 98° (from hexane); λ_{max} 273 and 281 mμ (ε 542 and 540, respectively). (Found: C, 59·3; H, 6·0. $C_{12}H_{15}ClO_3$ requires: C, 59·4; H, 6·2%.)

1-(2,4-*Dichloro-5-methoxyphenyl*)*propan-2-ol* (IXb)

The phenol IXa (530 mg) in dry acetone (50 ml) was heated under reflux for 20 hr with Me_2SO_4 (5·11 ml) and anhyd K_2CO_3 (9·0 g). The product was isolated as described for VIIb and separated on silica. $CHCl_3$ eluted the 1-(2,4-*dichloro-5-methoxyphenyl*)*propan-2-ol* IXb; 480 mg), m.p. 69° (from hexane); λ_{max} 227·5, 286 and 293 mμ (ε 8500, 2700 and 2600, respectively). (Found: C, 51·4; H, 5·1. $C_{10}H_{12}Cl_2O_2$ requires: C, 51·1; H, 5·1%.)

5,7-Dichloro-8-methoxy-3-methylisochroman (XIV)

$ZnCl_2$ (ca. 200 mg) was added to IXb (450 mg) in chloromethyl ether (10 ml) and heated under reflux for 5 min. The product was isolated as described for XII and separated on silica. C_6H_6 eluted the *5,7-dichloro-8-methoxy-3-methylisochroman* (XIV; 494 mg), m.p. 56° (from hexane), λ_{max} 224sh, 276 and 284·5 mμ (ε 11,200, 700 and 700, respectively). (Found: C, 53·5; H, 4·7. $C_{11}H_{12}Cl_2O_2$ requires: C, 53·5; H, 4·9%.)

5-Chloro-7-chloromethyl-3,4-dihydro-8-methoxy-3-methylisocoumarin (XVa)

The isochroman XIIIa (100 mg) in AcOH (3 ml) was treated at 0–5° over a period of 30 min with a soln of CrO_3 (228 mg) in water (0·19 ml) and AcOH (0·76 ml). The mixture was left at room temp for an additional hr and diluted with water. The aqueous phase was extracted with $CHCl_3$. The dried ($CaCl_2$) organic phase was concentrated *in vacuo* and the residue recrystallized from 1:1 C_6H_6–hexane to furnish the *5-chloro-7-chloromethyl-3,4-dihydro-8-methoxy-3-methylisochroman* (XVa; 72 mg), m.p. 112°; λ_{max} 214 and 310 mμ (ε 31,600 and 3100, respectively); ν_{max} in nujol 1723 and 1135 cm^{-1}. (Found: C, 52·5; H, 4·4. $C_{12}H_{12}Cl_2O_3$ requires: C, 52·4; H, 4·4%.)

7-Carboxy-5-chloro-3,4-dihydro-8-methoxy-3-methylisocoumarin (XVb)

The isochroman XIIIb (720 mg) in AcOH (45 ml) was treated at 0–5° over a period of 30 min with a soln of CrO_3 (3·42 g) in water (3·0 ml) and AcOH (8·40 ml). The reaction mixture was treated as described for XVa and gave the *dihydroisocoumarin* (XVb; 510 mg), m.p. 153° (after sublimation at 120°/5 * 10^{-4} mm); λ_{max} 214 and 311 mμ (ε 28,000 and 2800, respectively); ν_{max} (CO) in nujol 1727 and 1667 cm^{-1}. (Found: C, 53·2; H, 3·9. $C_{12}H_{11}ClO_5$ requires: C, 53·3; H, 4·1%.)

7-Carboxy-5-chloro-3,4-dihydro-8-hydroxy-3-methylisocoumarin (XVc)

Compound XVb (500 mg) was suspended in 6N HCl (150 ml) and heated under reflux for 20 hr. The reaction mixture was extracted with $CHCl_3$, dried ($CaCl_2$) and concentrated *in vacuo* to give XVc (425 mg). After sublimation at 140–150°/1 × 10^{-4} mm, it had m.p. 229°. Sublimate of a mixture of XVc and IIIa (m.p. 237–238°) had m.p. 233·234°. The synthetic product had λ_{max} 214 and 336 mμ (ε 28,000 and 5400, respectively); ν_{max} (CO) in nujol 1740, 1707 and 1670 cm^{-1}. (Found: C, 51·4; H, 3·5. $C_{11}H_9ClO_5$ requires: C, 51·5; H, 3·5%.)

The acid IIIa had λ_{max} 214 and 336·5 mμ (ε 30,000 and 5600, respectively) and ν_{max} (CO) in nujol 1740, 1707 and 1667 cm^{-1}.

Resolution of the racemic acid (XVc)

The acid XVc (120 mg) and brucine (184 mg) were heated in EtOH (25 ml) under reflux for 2 hr. Evaporation of the solvent gave a residue which was crystallized from acetone to furnish a solid (S_1) and a soln (L_1). The solid (S_1) (150 mg) was twice recrystallized from acetone to give a solid (S_3) (105 mg). The solid (S_3) was shaken with $CHCl_3$ and 2N HCl. The lactone acid component was isolated in the usual way and had a positive Cotton effect, ORD amplitude (a) 18·3 (extrema 350 and 315 mμ), optical purity 79·5%.

The mother liquor L_1 was similarly shaken with 2N HCl. The liberated acid component had a negative Cotton effect, a = 20·5 (extrema 350 and 315 mμ), optical purity 83%.

Corresponding ORD data for the optically active lactone acid IIIa were: negative Cotton effect. a = 31 (extrema 350 and 315 mμ).

8-*Methoxy*-3-*methylisochroman* (XVIa)

Raney Ni (100 mg) was added to XII (118 mg) in 0·25N methanolic KOH (4·0 ml), and the mixture stirred for 24 hr in a H_2 atm. The suspension was filtered and the filtrate diluted with water and extracted with $CHCl_3$. The organic phase was dried and concentrated under red press. The product was separated on silica; elution with 1:1 C_6H_6–hexane afforded the *isochroman* XVIa (85 mg) as an oil. (Found: C, 73·2; H, 7·6. $C_{11}H_{12}O_2$ requires: C, 73·0; H, 7·6%.)

7-*Hydroxymethyl*-8-*methoxy*-3-*methylisochroman* (XVIb)

Raney Ni (120 mg) was added to XIIIb (90 mg) in 0·25N methanolic KOH (3·0 ml) and the mixture stirred for 72 hr in a H_2 atm. The product was isolated as described for XVIa and separated on silica. Elution with 50:1 C_6H_6–acetone gave unreacted starting material XIIIb (14 mg), while 25:1 C_6H_6–acetone eluted the 7-*hydroxymethyl*-8-*methoxy*-3-*methylisochroman* (XVIb; 45 mg), m.p. 54° (from hexane); λ_{max} 215sh and 265 mμ (ε 9150 and 350, respectively). (Found: C, 69·30; H, 7·75. $C_{12}H_{16}O_3$ requires: C, 69·20; H, 7·75%.)

Methoxymellein (XVIIa)

The isochroman XVIa (60 mg) in AcOH (3·0 ml) was treated at 0–5° over a period of 30 min with a soln of CrO_3 (140 mg) in water (0·14 ml) and AcOH (0·50 ml). The reaction mixture was treated as described for XVa and XVIIa (25 mg) isolated. It had λ_{max} 210, 243 and 305 mμ (ε 28,400, 6550 and 4050, respectively [1]; ν_{max} (CO) 1711 cm^{-1}. (Accurate mass M^+ 192·078. Calc. for $C_{11}H_{12}O_3$ 192·078).

7-*Carboxy*-3,4-*dihydro*-8-*hydroxy*-3-*methylisochroman* (XVIIc)

Compound XVIb (25 mg) in AcOH (2·0 ml) was treated at 0° over a period of 30 min with a soln of CrO_3 (0·114 g) in water (0·05 ml) and AcOH (0·40 ml). The reaction mixture was treated as described for XVa, the product XVIIb suspended in 6N HCl (20 ml) and heated under reflux for 20 hr. The aqueous phase was extracted with $CHCl_3$, and concentrated *in vacuo* to give the *dihydroisocoumarin* (XVIIc; 11 mg), m.p. 223°, λ_{max} 218 and 322 mμ (ε 32,000 and 6300, respectively). (Accurate mass M^+ 222·052. $C_{11}H_{10}O_5$ requires: 222·052).

Corresponding data for the acid IV obtained by acid hydrolysis of ochratoxin B were: m.p. 227°, λ_{max} 219 and 322 mμ (ε 33,800 and 6800, respectively).

The preparation of ochratoxin A

(i) *By the acid chloride method.* The acid IIIa (27 mg) was heated under reflux (anhyd conditions) with $SOCl_2$ (8·0 ml) for 2 hr. The $SOCl_2$ was evaporated under red press and IIIb taken up in dry pyridine (1·0 ml) and cooled to 0°. L-β-phenylalanine methyl ester (50 mg) in dry pyridine (0·5 ml) was slowly added to the mixture and left at room temp for 3 hr. The mixture was poured on water and extracted with $CHCl_3$. The organic phase was washed with 2N HCl, 0·1N $NaHCO_3$, and with water. The organic layer was dried ($CaCl_2$) and concentrated *in vacuo* to furnish extensively racemized Ib (41 mg), $[\alpha]_{400}$ −187° (*c*, 0·75; MeOH); λ_{max} 213, 331 and 378 mμ (ε 34,800, 4100 and 2700, respectively), ν_{max} 3385, 1743, 1677, 1655, 1525, 1422 and 1134 cm^{-1}. (Accurate mass M^+ 417·097. $C_{21}H_{20}ClNO_6$ requires: 417·097).

In similar experiment DL-β-phenylalanine methyl ester was coupled to IIIa, $[\alpha]_{400}$ −180° (*c*, 0·75; MeOH).

Corresponding data for the natural product Ib were: $[\alpha]_{400}$ −250° (*c*, 0·75; MeOH), and ν_{max} 3385, 1743, 1677, 1655, 1525, 1422 and 1134 cm^{-1}.

(ii) *By the acid azide method.* The acid chloride IIIb (50 mg) prepared as described was dissolved in DMF

(0·50 ml). NaN_3 (14 mg) was slowly added to the soln at 0° and shaken for 30 min, water (0·20 ml) added and the mixture shaken for an additional 30 min. The soln was twice extracted with AcOEt (1·5 ml) and this organic phase added to a soln of L-β-phenylalanine (49·5 mg) in water (1·5 ml) containing Et_3N (0·05 ml). The soln was shaken at 5° for 55 hr and subsequently treated with 1N NaOH (1·5 ml). The mixture separated into 2 layers, the aqueous phase was extracted with AcOEt, acidified with AcOH, and extracted with $CHCl_3$. The $CHCl_3$ soln was concentrated under red press and the product separated by preparative TLC with 4:1 C_6H_6–AcOH as mobile phase. A green fluorescent band (R_F 0·50)[25] was extracted to yield Ia (20 mg). Crystallized from benzene it had m.p. 90–93°; λ_{max} 213 and 332 mμ (ε 37,650 and 6500, respectively); ν_{max} 3380, 1723, 1678, 1655, 1530, 1422 and 1140 cm^{-1}. ORD (*c*, 0·15; MeOH): $[\phi]_{600}$ −330°; $[\phi]_{589}$ −375°; $[\phi]_{345}$ −3600°; $[\phi]_{322}$ 0°; $[\phi]_{312}$ +1400°; $[\phi]_{295}$ 0°; $[\phi]_{269}$ −6600°.

The corresponding data for the natural product, Ia were: m.p. 90° (from C_6H_6); λ_{max} 213 and 332 mμ (ε 36,800 and 6400, respectively); ν_{max} 3380, 1723, 1678, 1655, 1530, 1422 and 1140 cm^{-1}. ORD (*c*, 0·18; MeOH): $[\phi]_{600}$ −385°; $[\phi]_{589}$ −400°; $[\phi]_{345}$ −4500°; $[\phi]_{322}$ 0°; $[\phi]_{312}$ +1600°; $[\phi]_{295}$ 0°; $[\phi]_{269}$ −6200.

(iii) *By the DCC method.* DCC (150 mg) was added at 0° to a soln of IIIa (100 mg) and L-β-phenylalanine methyl ester (106 mg) in DMF (2·0 ml) and left at room temp for 48 hr. The mixture was filtered, concentrated under red press and the product separated on silica. Benzene eluted the *adduct* (XVIII; 65 mg), m.p. 214° (from acetone); λ_{max} 218 and 333 mμ (ε 42,600 and 5600, respectively); ν_{max} 2925, 2858, 1728, 1668, 1435 and 1135 cm^{-1}. (Accurate mass M^+ 444·179. $C_{24}H_{29}ClN_2O_4$ requires: 444·181). Elution with 50:1 $CHCl_3$–MeOH gave Ib (8·1 mg).

(iv) *By the* p-*nitrophenyl ester method.* Compound IIIe (42 mg) was prepared by the saponification of IIId[2] (60 mg) under standard conditions. Compound IIIe was shown to be identical with XVb by direct comparison. DCC (30·5 mg) in AcOEt (0·30 ml) was added at 0° to a soln of IIIe (40 mg) and 4-nitrophenol (35 mg) in 1:1 DMF–AcOEt (0·50 ml). After 30 min the soln was allowed to come to room temp and held there for 12 hr. The N,N′-dicyclohexylurea which separated was filtered off, washed with $CHCl_3$, and the $CHCl_3$ phase concentrated *in vacuo* and the residue separated on silica. Elution with 2:1 $CHCl_3$–C_6H_6 gave IIIf (46 mg). The ester IIIf (45 mg) in AcOEt (0·5 ml) was treated with L-β-phenylalanine methyl ester (85 mg) in AcOEt (0·5 ml) and left at room temp for 24 hr. The mixture was diluted with AcOEt and washed with 1N NH_4OH, 1N HCl, and water. The organic phase was concentrated *in vacuo* to furnish Ic (45 mg); ν_{max} 3480, 1743sh, 1724, 1659, 1520 and 1133 cm^{-1}.

$AlCl_3$ (1·2 g) was added to Ic (43 mg) in nitrobenzene (10 ml) and stirred (anhyd conditions) at 35° for 2 hr. The mixture was diluted with $CHCl_3$ and extracted with water. The aqueous phase was acidified with 2N HCl and extracted with $CHCl_3$; the $CHCl_3$ extracts were combined, dried ($CaCl_2$), and concentrated *in vacuo*. The residue was separated on silica and elution with 50:1 $CHCl_3$–MeOH yielded Ib (28 mg), $[\alpha]_D$ −71·4° (*c*, 0·03; MeOH containing a trace of AcOH*), ORD amplitude, a = 33·5 (*c*, 0·03; MeOH containing a trace of AcOH) (extrema 334 and 310 mμ).

Corresponding ORD data for the natural product, ochratoxin A methyl ester[3] were: $[\alpha]_D$ −78° (*c*, 0·027; MeOH containing a trace of AcOH), a = 34 (*c*, 0·027; MeOH containing a trace of AcOH) (extrema 334 and 310 mμ).

Compound Ib (25 mg) in MeOH (1·5 ml) was treated with 1N NaOH (0·8 ml) and stirred at room temp for 1 hr. The mixture was acidified (2N HCl) and extracted with $CHCl_3$. The dried ($CaCl_2$) organic phase was concentrated *in vacuo* to yield Ia (19 mg). On crystallization from benzene it had m.p. 92°.

The preparation of ochratoxon B

The acid IVa (50 mg) was treated with $SOCl_2$ (20 ml) in the usual manner and the resulting acid chloride dissolved in DMF (1·0 ml). NaN_3 (22 mg) was slowly added to the soln at 0° and shaken for 30 min, water (0·30 ml) was added, and the mixture shaken for an additional 30 min. The soln was twice extracted with AcOEt (1·5 ml) and this organic phase added to a soln of L-β-phenylalanine (55·6 mg) in water (1·6 ml) containing Et_3N (0·05 ml). The mixture was shaken at 5° for 55 hr and worked up as described for ochratoxin A. The product was separated by preparative TLC in 4:1 C_6H_6–AcOH as mobile phase. A blue fluorescent band (R_F 0·35)[25] was extracted to yield *ochratoxin B* (II; 22 mg), m.p. 220° (from MeOH), $[\alpha]_D$ −62°

* AcOH was added to suppress ionization of the phenolic OH group.

[25] P. S. Steyn and K. J. van der Merwe, *Nature, Lond.* **211**, 5047 (1966).

(*c*, 0·02; MeOH); λ_{max} 218 and 318 mμ (ε 33,600 and 6500, respectively), ν_{max} in nujol 3460, 1728, 1666, 1658sh, 1532 and 1130 cm^{-1}; ORD amplitude a = 67·0 (*c*, 0·01; MeOH) (extrema 325 and 295 mμ).

Corresponding data for the natural product, II were: m.p. 220° (from MeOH), $[\alpha]_D$ −56° (*c*, 0·29; MeOH); λ_{max} 218 and 318 mμ (ε 34,300 and 6750, respectively); ν_{max} in nujol 3460, 1728, 1666, 1658sh, 1532 and 1130 cm^{-1}, ORD: a = 67·0 (*c*, 0·017; MeOH) (extrema 325 and 295 mμ).

Adduct of DCC and 5-chlorosalicylic acid (XIX)

DCC (298 mg) in AcOEt (1·0 ml) was added to 5-chlorosalicyclic acid (250 mg) in AcOEt (2·0 ml) at 0°. After 30 min the soln was allowed to come to room temp and held there for 1 hr. The mixture was filtered and the filtrate concentrated *in vacuo* to furnish a product which was separated on silica. C_6H_6 eluted the *adduct* (XIX; 120 mg), m.p. 133° (from MeOH); λ_{max} 217·5, 233sh, 264 and 314 mμ (ε 33,800, 19,900, 3400 and 2200, respectively); ν_{max} 2923, 2858, 1702, 1662, 1613, 1475, 1440 and 1330 cm^{-1}. (Found: C, 66·8; H, 6·9; N, 7·9. $C_{20}H_{25}ClN_2O_2$ requires: C, 66·9; H, 7·0; N, 7·8%.)

Hydrolysis of the adduct (XIX)

The adduct XIX (100 mg) in 1:1 MeOH–3N HCl (35 ml) was heated under reflux for 40 min. The soln was diluted with water and extraction into Et_2O yielded the *cyclic oxocarbamate* (XX; 70 mg). After crystallization from EtOH it had m.p. 163°; λ_{max} 214, 241sh, 301 and 310sh (ε 41,700, 7100, 2300 and 2000, respectively); ν_{max} 2940, 2860, 1766, 1702, 1620, 1480, 1440 and 1340 cm^{-1}. (Found: C, 60·2; H, 5·1; N, 5·3. $C_{14}H_{14}ClNO_3$ requires: C, 60·1; H, 5·1; N, 5·0%.)

Acknowledgements—The authors wish to express their thanks to Mrs. K. Barbara Norton of this Laboratory for ORD spectra, and the South African Oil Seeds Control Board, Pretoria, for financial support.