Metabolites of *Nectria coccinea*

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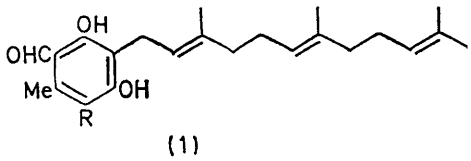
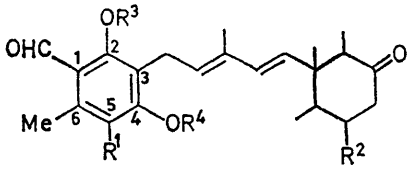
Nectria coccinea produces antibiotics LL-Z1272-β (1b), -γ (ascochlorin) (2a), -δ (3a), and -ε (3b) together with a new metabolite for which the name chloronectrin and structure (3c) {based on 3-[5-(3-oxocyclohexyl)pent-2-enyl]-5-chloro-orsellinaldehyde} are proposed. In the presence of bromide in place of chloride, *N. coccinea* produces an analogous 3-(penta-2,4-dienyl)-5-bromo-compound (2c). Several new derivatives in the series are reported.

ANTIBIOTICS LL-Z1272 [α (1a), β (1b), γ (2a), δ (3a), ε (3b), and ζ (2b)] have been isolated from an unidentified *Fusarium* sp.[1] Antibiotic LL-Z1272 γ (2a) (ascochlorin)



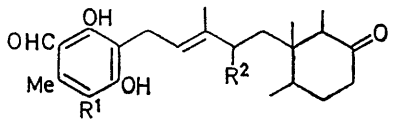
a; R = Cl
b; R = H

(1)



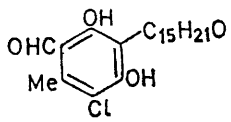
(2)

	R^1	R^2	R^3	R^4
a;	Cl	H	H	H
b;	Cl	OAc	H	H
c;	Br	H	H	H
d;	H	H	H	H
e;	Cl	H	H	Me
f;	Cl	H	Me	Me
g;	Cl	H	H	Ac
h;	Cl	H	Me	Ac
i;	Cl	H	Me	H

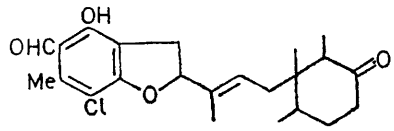


	R^1	R^2
a;	Cl	H
b;	H	H
c;	Cl	OAc
d;	Cl	OH

(3)



(4)



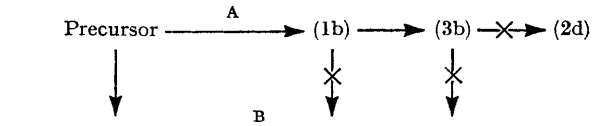
(5)

has been isolated independently from *Ascochyta viciae*,[2] and cylindrochlorin, a metabolite of a *Cylindrocladium* sp., has been assigned the related part-structure (4).[3] More recently, the isolation of ilicicolins A—H from *Cylindrocladium ilicicola* has been reported.[4] From the published data it seems likely that ilicicolin A is identical with antibiotic LL-Z1272-α, B with -β, C with -δ, D with -γ, and F with -ζ, though no reference was made in ref. 4 to the work of Ellestad *et al.*[1]

We report here the isolation from *Nectria coccinea* of compounds (1b), (2a), (3a), and (3b), together with a new member of the series for which we propose the name chloronectrin and the structure (3c). We also describe some new derivatives which we have prepared in the series.

Production of the Metabolites.—Whenever the culture medium contains chloride ions, the major metabolite of *N. coccinea* is ascochlorin (antibiotic LL-Z1272γ) (2a); under our optimum conditions the compound can be isolated by crystallization of the crude product from methanol. Ascochlorin (2a) is always accompanied by smaller amounts of the other metabolites, especially its dechloro-dihydro-derivative (antibiotic LL-Z1272ε) (3b), which are separated by chromatography on silica gel columns, sometimes supplemented by preparative t.l.c. The separation of ascochlorin from its dihydro-derivative (antibiotic LL-Z1272δ) (3a) is efficiently accomplished by crystallization from methanol, but the isolation of compound (3a) free from ascochlorin is more difficult and is best achieved by t.l.c. on silica gel impregnated with silver nitrate.[5]

In the absence of chloride ions no ascochlorin is produced (obviously), but the yield of compound (3b) remains essentially unchanged, so that compound (3b) becomes the major metabolite, and we have been unable to detect dechloroascochlorin (2d) under any conditions. This result suggests that the metabolites are related as shown in the Scheme, which incorporates two features



SCHEME

previously encountered in fungal biosynthesis: introduction of chlorine early in the biosynthetic sequence and prenylation of a polyketide-derived intermediate, probably at the aromatic level. The fact that in the

[1] G. A. Ellestad, R. H. Evans, jun., and M. P. Kunstmann, *Tetrahedron*, 1969, **25**, 1323.
[2] G. Tamura, S. Suzuki, A. Takatsuki, K. Ando, and K. Arima, *J. Antibiotics*, 1968, **21**, 539; Y. Nawata, K. Ando, G. Tamura, K. Arima, and Y. Iitaka, *ibid.*, 1969, **22**, 511.
[3] A. Kato, K. Ando, G. Tamura, and K. Arima, *J. Antibiotics*, 1970, **23**, 168.
[4] S. Hayakawa, H. Minato, and K. Katagiri, *J. Antibiotics*, 1971, **24**, 653.
[5] C. B. Barrett, M. S. J. Dallas, and F. B. Padley, *Chem. and Ind.*, 1962, 1050.

absence of chloride ions there is no increase in the yields of the non-chlorinated metabolites suggests that prenylation of chlorinated and non-chlorinated intermediates might be mediated by two different enzyme systems (indicated by A and B in the Scheme) and that production of the non-chlorinated metabolites in the absence of chloride ions is limited by the availability of enzyme A.

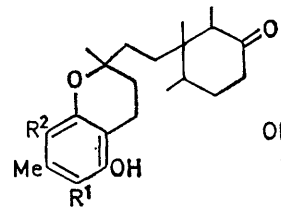
Addition of bromide to the chloride-free medium leads to production of the bromo-analogue (2c) of ascochlorin, the yield of bromo-compound being lower than that of ascochlorin [antibiotic LL-Z1272ε (3b) is the major product]. In an experiment in which chloride and bromide were present, in a molar ratio of 3 : 1, ascochlorin and the bromo-analogue (2c) were produced in the ratio 1 : 2, indicating the inhibitory effect [6] of bromide ions on the incorporation of chloride. Addition of iodide, fluoride, or isothiocyanate in the presence or absence of chloride did not significantly affect the products.

Structure of Chloronectrin (3c).—The n.m.r. spectrum of chloronectrin, $C_{25}H_{33}ClO_6$, is similar to that of compound (3a), with the addition of signals attributable to an acetyl group (τ 7·99) and a proton α to an acetoxy-group (τ 4·64, the X part of an ABX system). Hydrolysis of chloronectrin with sodium hydroxide yielded the alcohol (3d), which has the expected properties (notably the absence of an acetyl signal in its n.m.r. spectrum and an upfield shift of the one-proton signal from τ 4·65 to 5·84), together with the dihydrobenzofuran (5). The structure of the dihydrobenzofuran, $C_{23}H_{29}ClO_4$, follows from its n.m.r. spectrum, which shows an ABX system at τ 4·64, 6·65, and 7·04 (dihydrobenzofuran system), a broad triplet at τ 4·42 (olefinic proton), a broad singlet at τ 8·35 (allylic Me), a doublet at τ 7·92 (allylic CH_2), and a singlet at τ −2·43 (hydrogen-bonded phenol). The dihydrobenzofuran system presumably results from allylic displacement of the acetoxy-group of chloronectrin, which therefore has structure (3c).

It seemed that treatment of the alcohol (3d) with acid would provide an alternative route to the dihydrobenzofuran (5). However, the product, obtained under a variety of conditions, proved to be an inseparable mixture of compounds epimeric at the newly formed asymmetric centre. The presence of a mixture was revealed by the wide melting range and by doubling of the n.m.r. signals due to the two secondary methyl groups; the other n.m.r. signals remain sharp, as is the case in the chroman derivatives (see later). Since the base-catalysed reaction is stereospecific it must be synchronous, or nearly so, and the loss of stereochemistry in the acid-catalysed reaction presumably results from formation of an intermediate carbonium ion. Whereas the acid-catalysed cyclization of compounds (2a) and (3b) gives chromans derived from both phenolic groups, both the acid-catalysed and the base-catalysed formation of the dihydrobenzofuran give only the product derived from the non-bonded phenol.

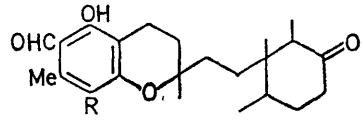
[6] M. A. Petty, *Bact. Rev.*, 1961, **25**, 111.

Miscellaneous Derivatives.—We have prepared several new derivatives of ascochlorin for biological testing. Methylation with methyl iodide in the presence of



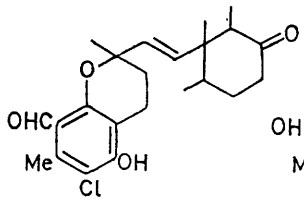
(6)

a; $R^1 = H$, $R^2 = CHO$
b; $R^1 = Cl$, $R^2 = CHO$
c; $R^1 = R^2 = Cl$



(7)

a; R = H
b; R = Cl



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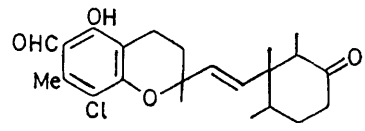

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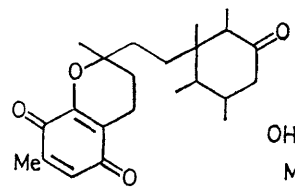

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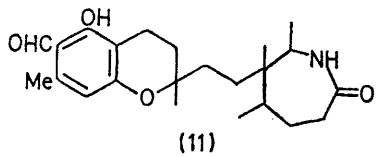

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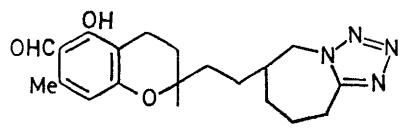

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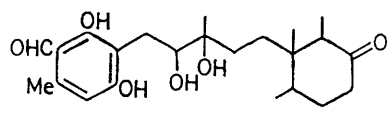

(13)

potassium carbonate gave first the monomethyl ether (2e), which retains the hydrogen-bonded phenol group, and then the bismethyl ether (2f). Acetylation of ascochlorin gave a diacetate, detected by n.m.r. spectroscopy, which decomposed to the monoacetate (2g) on attempted purification. The monoacetate (2g) formed a methyl ether (2h), hydrolysis of which yielded the monomethyl ether (2i), which has lost the hydrogen-bonded system.

Treatment of antibiotic LL-Z1272ε (3b) with concentrated sulphuric acid is reported to give the isomeric

chromans (6a) and (7a).[1] We have repeated this reaction and have obtained the chroman (7a), previously described as a gum,[1] in crystalline form. Doubling of the n.m.r. signals due to the secondary methyl groups of the chromans (6a) and (7a) shows that, as expected, each is a mixture of epimers, a point not previously commented upon. As in the case of the dihydrobenzofuran formed under acidic conditions, the remaining signals in the n.m.r. spectra are sharp; this doubling of the secondary methyl signals in the presence of sharp signals due to the other protons is a feature common to all the chroman derivatives. Treatment of ascochlorin (2a) with concentrated sulphuric acid gave the chromans (8) and (9). Under mild acidic conditions, the chroman (8) was converted into the chroman (9); under the same conditions the chromans (6a), (7a), and (9) are stable.

Chlorination of the chromans with t-butyl hypochlorite yielded the corresponding chloro-compounds (6b) and (7b). The reaction of the chroman (6a) with t-butyl hypochlorite also yielded the dichloro-compound (6c), presumably after oxidation of the aldehyde function to a carboxylic acid and decarboxylation. Oxidation of the chroman (6a) with Jones reagent yielded the quinone (10), a reaction analogous to that observed in the oxidation of orsellinic acid derivatives.[7] Treatment of either of the chromans (6a) and (7a) with sodium azide in concentrated sulphuric acid (Schmidt reaction) yielded the same mixture of products, owing to equilibration of the chroman system under the strongly acidic conditions. The caprolactam (11) and the tetrazole (12), both derived from the chroman (7a), were isolated from a Schmidt reaction on the chroman (6a). Treatment of antibiotic LL-Z1272ε (3b) with osmium tetroxide yielded the diol (13); this compound, too, showed doubling of the secondary methyl signals due to the presence of a mixture of epimers.

EXPERIMENTAL

I.r. spectra of solids were determined for Nujol mulls and of gums for solutions in chloroform. N.m.r. spectra were determined at 100 MHz for solutions in deuteriochloroform with tetramethylsilane as internal standard. Silica gel for chromatography was Hopkin and Williams M.F.C., and t.l.c. was performed on Merck GF silica gel. Preparative t.l.c. was carried out on layers 1 mm thick. Light petroleum had b.p. 60—80°.

Production of the Metabolites.—(a) *Ascochlorin* (*Antibiotic LL-Z1272γ*) (2a). *Nectria coccinea* (CMI 120337C, no. 2981 in our collection) was grown under stirred aerated conditions on a medium containing (g l^{-1}) Cerelose (50), sodium nitrate (1·0), potassium dihydrogen phosphate (0·5), magnesium sulphate heptahydrate (0·25), potassium chloride (0·25), iron(II) sulphate heptahydrate (0·005), yeast extract (Oxoid) (0·5), and 0·05% (v/v) minor element concentrate.[8] After 44 h, 36 l of culture was used to inoculate each of two tanks containing 675 l of medium containing Cerelose (50 g l^{-1}) and the other above ingredients at double concentration, and the mixture was stirred and aerated for 99 h, with the sugar level maintained at 10—20 g l^{-1} by addition of a solution of Cerelose. The contents of the fermenters were combined and filtered and the mycelium (320 kg wet wt.) was slurried twice with ethyl acetate. The combined ethyl acetate extract (1600 l) was evaporated *in vacuo* at 30° to give a dark brown gum (4·3 kg), which was stirred with light petroleum (25 l). The insoluble residue was collected and triturated with methanol to give a pale yellow solid (550 g), which crystallized from methanol to give ascochlorin (2a) (300 g), m.p. 164—167° (lit.,[1] 172—173°). The mother liquor from this crystallization contained (t.l.c.) a small amount of the dihydro-derivative, antibiotic LL-Z1272δ (3a).

(b) *Ascochlorin* (2a), *Antibiotic LL-Z*1272β (1b), *Antibiotic LL-Z*1272ε (3b), *and Chloronectrin* (3c). *N. coccinea* was grown as surface culture for 13 days in Thompson bottles each containing 1 l of the 'production' medium already described. The mycelium from 156 bottles was collected, dried, and extracted with hot chloroform. The chloroform was evaporated to give a mixture of oil and solid (434 g), which was washed with light petroleum leaving a solid (90 g). This was dissolved in benzene and chromatographed on a column of silica gel (2·86 l) that had been deactivated by equilibration with water (86 ml). Elution of the column with benzene and with benzene–chloroform (9 : 1) gave small quantities of gum. Elution with benzene–chloroform (3 : 1) gave first a fraction (100 mg) which crystallized from aqueous methanol to give antibiotic LL-Z1272β (1b), m.p. 98—100° (lit.,[1] 97·5°), and then fractions (34·1 g) which were combined and crystallized from benzene–light petroleum to give ascochlorin (2a) (24·4 g); the mother liquor from this crystallization contained a small amount of the dihydro-derivative, antibiotic LL-Z1272δ (3a) (see later). Elution of the column with benzene–chloroform (1 : 1) gave first an intermediate fraction (A) (4·9 g) and then fractions (26 g) which were combined and triturated with ether to give antibiotic LL-Z1272ε (3b) (9 g). Antibiotic LL-Z1272ε forms two crystalline modifications with markedly different solid state i.r. spectra: (*a*) m.p. 170—173°, $\nu_{max.}$ 3400, 1701, and 1640 cm^{-1}; (*b*) m.p. 179—182°, $\nu_{max.}$ 3250, 1700, 1640, and 1600 cm^{-1} (lit.,[1] m.p. 171·5—172·5°).

The ether washings from compound (3b) were combined with fraction (A) and subjected to repeated t.l.c. in chloroform (the plates being developed twice on each occasion) to yield *chloronectrin* (3c) (*ca.* 3 g) as a gum (Found: C, 65·1; H, 7·2%; *m/e*, 464·1979. $C_{25}H_{33}ClO_6$ requires C, 64·6; H, 7·2%; *M*, 464·1965); $\nu_{max.}$ 3515, 1728, 1712, and 1633 cm^{-1}; τ −2·44 (bonded OH), −0·11 (CHO), *ca.* 3·5br (OH), 4·44 (t, *J* 8 Hz, CH_2·C*H*=), 4·64 (dd, *J* 4 and 8 Hz, C*H*·OAc), 6·62 (d, *J* 8 Hz, CH_2·CH=), 7·43 (ArCH_3), 7·99 (O·CO·CH_3), 8·21 (d, *J* 2 Hz, CH=C·CH_3), 9·06 (d, *J* 7 Hz, CH·CH_3), 9·22 (d, *J* 7 Hz, CH·CH_3), and 9·48 (≥C·CH_3).

(c) *Antibiotic LL-Z*1272δ (3a). A mixture (160 mg) of ascochlorin (*ca.* 20%) and antibiotic LL-Z1272δ (*ca.* 80%) was chromatographed in chloroform on a layer of silica gel impregnated with silver nitrate.[5] Recovery of the lower R_F band [detected by spraying the edge of the plate with iron(III) chloride solution] gave a solid (147 mg), which was dissolved in chloroform and washed with dilute hydrochloric acid. The recovered product crystallized from acetone–light petroleum to give antibiotic LL-Z1272δ as prisms, m.p. 130—131° (lit.,[1] 129·5—130·5°).

[7] D. C. Aldridge, S. Galt, D. Giles, and W. B. Turner, *J. Chem. Soc.* (*C*), 1971, 1623.

[8] P. W. Brian, P. J. Curtis, and H. G. Hemming, *Trans. Brit. Mycol. Soc.*, 1946, **29**, 173.

(d) *The Bromo-compound* (2c). *N. coccinea* was grown under stirred aerated conditions in a medium containing the following analytical grade reagents (g l^{-1}): glucose (50), sodium nitrate (2), potassium dihydrogen phosphate (1), magnesium sulphate heptahydrate (0·5), iron(II) sulphate heptahydrate (0·01), and a minor element concentrate made up with sulphuric acid in place of hydrochloric acid. After 56 h potassium bromide (6·35 g) in water (4·5 l) was added and the fermentation was harvested after 262 h. The whole broth (22 l) was acidified to pH 2 and extracted with butyl acetate (2 × 5 l) to give an oily solid (69 g) which was triturated with ether to give antibiotic LL-Z1272ε (3b) (5·8 g). The mother liquor was evaporated to dryness and the residue was washed with light petroleum leaving a solid (10·1 g), which was adsorbed on silica gel (60 ml) by evaporation of a solution in acetone. The silica gel was placed on a column of deactivated silica gel (see before) (440 ml) made up in benzene. Elution of the column yielded first a mixture (1·9 g) containing antibiotic LL-Z1272β (1b) and a small proportion of the bromo-compound (see later) and then a fraction (1·6 g) which was purified by t.l.c. in chloroform. The main band gave a solid (698 mg) which was crystallized from benzene–light petroleum and from methanol to give the *bromo-compound* (2c) as needles, m.p. 164—167° [Found: C, 61·8; H, 6·6%; *m/e* (^{79}Br) 448. $C_{23}H_{29}BrO_4$ requires C, 61·5; H, 6·5%; *M* (^{79}Br) 448]; $\nu_{max.}$ 3382m, 1708s, and 1630s cm^{-1}; τ −2·73 (bonded OH), −0·13 (CHO), 3·61 (OH), 4·10 (d, *J* 16 Hz, C*H*=CH), 4·48 (t, *J* 7 Hz, CH_2·C*H*=), 4·64 (d, *J* 16 Hz, C*H*=CH), 6·46 (d, *J* 7 Hz, CH_2·CH=), 7·38 (ArCH_3), 8·09 (=C·CH_3), 9·17 (d, *J* 6 Hz, CH·CH_3), 9·20 (d, *J* 6 Hz, CH·CH_3), and 9·31 (≥C·CH_3).

Treatment of Chloronectrin (3c) *with Sodium Hydroxide.*—A solution of chloronectrin (63 mg) in 0·24N-sodium hydroxide (20 ml) was heated under nitrogen on a water-bath for 2 h. The cooled solution was acidified and extracted with ethyl acetate to give a gum (61 mg) which was fractionated by t.l.c. in chloroform. Recovery of the highest R_F band and crystallization of the product from ethyl acetate–light petroleum gave the *dihydrobenzofuran* (5) (4·6 mg) as prisms, m.p. 180—184° (Found: C, 68·0; H, 7·4%; *m/e*, 404. $C_{23}H_{29}ClO_4$ requires C, 68·2; H, 7·2; *M*, 404); $\nu_{max.}$ 1713, 1635sh, and 1620 cm^{-1}; τ −2·43 (bonded OH), −0·12 (CHO), 4·42 (t, *J* 8 Hz, CH_2·C*H*=) 4·64 (dd, *J* 4 and 5 Hz, ·O·C*H*·CH_2), 6·65 (dd, *J* 5 and 16 Hz, O·CH·CH_aH_b), 7·04 (dd, *J* 4 and 16 Hz, O·CH·CH_aH_b), 7·46 (ArCH_3), 8·35 (=CH·CH_3), 9·08 (d, *J* 7 Hz, CH·CH_3), 9·13 (d, *J* 7 Hz, CH·CH_3), and 9·39 (≥C·CH_3).

Recovery of the second highest R_F band and crystallization of the product from ethyl acetate–light petroleum gave the alcohol (3d) as needles, m.p. 133—134° and 145—148°, or prisms, m.p. 147—149° (Found: C, 65·4; H, 7·4. $C_{23}H_{31}ClO_5$ requires C, 65·3; H, 7·3%); $\nu_{max.}$ (needles) 3480, 3250, 1698, and 1631 cm^{-1}; $\nu_{max.}$ (prisms) 3534, 3320, 1706, and 1620 cm^{-1}; τ −2·22 (bonded OH), −0·05 (CHO), 4·57 (t, *J* 7 Hz, CH_2·C*H*=), 5·85 (dd, *J* 4 and 7 Hz, C*H*·OH), 6·66 (d, *J* 7 Hz, CH_2·CH=), 8·47 (ArCH_3), 8·22 (=C·CH_3), 9·05 (d, *J* 6 Hz, CH·CH_3), 9·21 (d, *J* 6 Hz, CH·CH_3), and 9·47 (≥C·CH_3).

Treatment of the Alcohol (3d) *with Acid.*—A solution of the alcohol (43·5 mg) in a mixture of glacial acetic and concentrated sulphuric acids (95 : 5; 3 ml) was set aside at room temperature for 15 min. The solution was poured into water and extracted with ethyl acetate to give a gum (40 mg), which was filtered in benzene through a short column of silica gel to give a gum (21 mg) which crystallized slowly. Recrystallization from methanol gave a mixture of prisms and rods, m.p. 130—175°, shown by the doubling of the secondary methyl signals in its n.m.r. spectrum to be a mixture of epimers of the dihydrobenzofuran (5). The mass spectrum of the epimeric mixture was identical with that of the dihydrobenzofuran (5) prepared before.

Derivatives of Ascochlorin (2a).—(a) 4-O-*Acetylascochlorin* (2g). Acetyl chloride (2 ml) was added dropwise to a rapidly stirred ice-cold solution of ascochlorin (400 mg) in 1 : 1 pyridine–benzene (4 ml) and stirring was continued for a further 10 min. The mixture was diluted with water and extracted with ether to give a brown gum (442 mg), which was purified by t.l.c. in chloroform. Recovery of the main band (390 mg) and crystallization from ethyl acetate–light petroleum gave 4-O-*acetylascochlorin* (2g), m.p. 140—142° (Found: C, 66·9; H, 7·2; Cl, 8·1%; *m/e*, 446. $C_{25}H_{31}ClO_5$ requires C, 67·2; H, 7·0; Cl, 8·0%; *M*, 446); $\nu_{max.}$ 1777, 1714, and 1644 cm^{-1}; τ −2·49 (bonded OH), −0·21 (CHO), 4·20 (d, *J* 16 Hz, C*H*=CH), 4·61 (d, *J* 16 Hz, C*H*=CH), 4·69 (t, *J* 7 Hz, CH_2·C*H*=), 6·63 (d, *J* 7 Hz, CH_2·CH=), 7·42 (ArCH_3), 7·70 (CO·CH_3), 8·16 (=C·CH_3), 9·19 (d, *J* 7 Hz, CH·CH_3), 9·22 (d, *J* 7 Hz, CH·CH_3), and 9·33 (≥C·CH_3).

(b) 4-O-*Acetyl*-2-O-*methylascochlorin* (2h). A solution of 4-*O*-acetylascochlorin (1·17 g) in acetone (40 ml) and methyl iodide (12 ml) was heated under reflux with potassium carbonate (6 g) for 2 h. The mixture was cooled and filtered, the filtrate was evaporated, and the residue was recrystallized from ethanol to give 4-O-*acetyl*-2-O-*methylascochlorin* (2h) as prisms, m.p. 114—116° (Found: C, 67·4; H, 7·2; Cl, 7·9%; *m/e*, 460. $C_{26}H_{33}ClO_5$ requires C, 67·7; H, 7·1; Cl, 7·7%; *M*, 460); $\nu_{max.}$ 1783, 1715, and 1693 cm^{-1}; τ −0·41 (CHO), 4·14 (d, *J* 16 Hz, C*H*=CH), 4·58 (d, *J* 16 Hz, C*H*=CH), 4·65 (t, *J* 7 Hz, CH_2·C*H*=), 6·19 (O·CH_3), 6·54 (d, *J* 7 Hz, CH_2·CH=), 7·39 (ArCH_3), 7·69 (CO·CH_3), 8·11 (=C·CH_3), 9·18 (d, *J* 7 Hz, CH·CH_3), 9·21 (d, *J* 7 Hz, CH·CH_3), and 9·32 (≥C·CH_3).

(c) 2-O-*Methylascochlorin* (2i). 4-*O*-Acetyl-2-*O*-methylascochlorin (257 mg) was heated under reflux with aqueous 10% sodium hydroxide (50 ml) for 2 h, the cooled solution was acidified with dilute hydrochloric acid and extracted with ethyl acetate, and the product was purified by t.l.c. in chloroform. Recovery of the major band (255 mg) and crystallization of the product from ether–light petroleum gave 2-O-*methylascochlorin* (2i) as prisms, m.p. 126° (Found: C, 68·7; H, 7·4; Cl, 8·1%; *m/e*, 418. $C_{24}H_{31}ClO_4$ requires C, 68·8; H, 7·4; Cl, 8·4%; *M*, 418); $\nu_{max.}$ 3170, 1712, and 1666 cm^{-1}; τ −0·31 (CHO), 3·58 (O*H*), 4·14 (d, *J* 16 Hz, C*H*=CH), 4·52 (t, *J* 7 Hz, CH_2·C*H*=), 4·63 (d, *J* 16 Hz, C*H*=CH), 6·20 (O·CH_3), 6·46 (d, *J* 7 Hz, CH_2·CH=), 7·37 (ArCH_3), 8·08 (=C·CH_3), 9·17 (d, *J* 7 Hz, CH·CH_3), 9·20 (d, *J* 7 Hz, CH·CH_3), and 9·30 (≥C·CH_3).

(d) 4-O-*Methylascochlorin* (2e). A solution of ascochlorin (2a) (500 mg) in acetone (20 ml) and methyl iodide (5 ml) was heated under reflux with potassium carbonate (2 g) for 10 min. The cooled mixture was filtered and evaporated and the residue was dissolved in ether and washed with aqueous 5% sodium hydroxide. Preparative t.l.c. in chloroform of the product from the ether yielded 4-O-*methylascochlorin* (2e) as a gum (253 mg) (Found: *m/e*, 418·1908. $C_{24}H_{31}ClO_4$ requires *M*, 418·1910); $\nu_{max.}$ 1715, 1650, and 1625 cm^{-1}; τ −2·54 (bonded OH), −0·25 (CHO), 4·14 (d, *J* 16 Hz, C*H*=CH), 4·52 (t, *J* 7 Hz, CH_2·C*H*=), 4·60 (d, *J* 16 Hz, C*H*=CH), 6·13 (O·CH_3), 6·48 (d, *J* 7 Hz,

CH_2·CH=), 7·38 (ArCH_3), 8·08 (=C·CH_3), 9·16 (d, *J* 7 Hz, CH·CH_3), 9·20 (d, *J* 7 Hz, CH·CH_3), and 9·30 (>C·CH_3).

(e) *2,4-Di-O-methylascochlorin* (2f). Ascochlorin (850 mg) was methylated as in (d) for 2 h; the product was chromatographed on silica gel in chloroform then distilled to give *2,4-di-O-methylascochlorin* (2f) as a gum (432 mg), b.p. 160—170° (bath temp.) at 0·03 mmHg (Found: C, 69·3; H, 7·7; *m/e*, 432·2059. $C_{25}H_{33}ClO_4$ requires C, 69·4; H, 7·6%; *M*, 432·2066); $\nu_{max.}$ 1705 and 1695 cm^{-1}; τ −0·34 (CHO), 4·15 (d, *J* 16 Hz, C*H*=CH), 4·58 (t, *J* 7 Hz, CH_2·C*H*=), 4·66 (d, *J* 16 Hz, C*H*=CH), 6·18 (O·CH_3), 6·23 (O·CH_3), 6·50 (d, *J* 7 Hz, CH_2·CH=), 7·42 (ArCH_3), 8·10 (=C·CH_3), 9·20 (d, *J* 7 Hz, CH·CH_3), 9·23 (d, *J* 7 Hz, CH·CH_3), and 9·32 (>C·CH_3).

Formation of Chromans.—(a) *From ascochlorin.* A solution of ascochlorin (2a) (1·4 g) in concentrated sulphuric acid (60 ml) was set aside at room temperature for 45 min, poured onto ice, and extracted with ethyl acetate to give a gum (1·3 g), which was fractionated by t.l.c. in benzene–acetone (19 : 1). Elution of the higher R_F product gave a gum (700 mg) which was crystallized from ether–light petroleum to give the *chroman* (9), m.p. 97—99° (Found: C, 68·5; H, 7·1; Cl, 8·2%; *m/e*, 404. $C_{23}H_{29}ClO_4$ requires C, 68·2; H, 7·2; Cl, 8·8%; *M*, 404); $\nu_{max.}$ 1720, 1710, 1640, and 1610 cm^{-1}; τ * −2·71 (bonded OH), −0·09 (CHO), 4·76 (C*H*=C*H*), 7·47 (ArCH_3), 8·54 (O·C·CH_3), and 9·39 (>C·CH_3).

Elution of the lower R_F product gave the *chroman* (8) (300 mg), m.p. 64—67° (Found: C, 68·3; H, 7·4; Cl, 8·4%; *m/e*, 404. $C_{23}H_{29}ClO_4$ requires C, 68·2; H, 7·2; Cl, 8·8%; *M*, 404); $\nu_{max.}$ 3350, 1710, and 1670 cm^{-1}; τ * −0·51 (CHO), 3·78 (OH), 4·66 (C*H*=C*H*), 7·35 (ArCH_3), 8·52 (O·C·CH_3), and 9·35 (>C·CH_3).

T.l.c. of the chroman (9) in benzene–ethyl acetate (96 : 4) (two developments) partially resolved the mixture of epimers.

(b) *From Antibiotic LL-Z1272ε* (3b).[1] Antibiotic LL-Z1272ε (10 g) was treated with concentrated sulphuric acid as described in (a) and the product was chromatographed on a column of silica gel. Elution with benzene–ethyl acetate (98 : 2) yielded a gum (3 g) which crystallized from ether–light petroleum to give the chroman (7a), m.p. 97—99° (Found: C, 74·2; H, 8·5. $C_{23}H_{32}O_4$ requires C, 74·2; H, 8·6%); the chroman (7a) had previously been obtained as a gum.[1] Subsequent elution of the column with benzene–ethyl acetate (9 : 1) yielded a gum (6·5 g) which crystallized from acetone–light petroleum to give the chroman (6a), m.p. 192—194° (Found: C, 74·5; H, 8·5%) (lit.,[1] m.p. 192—194°).

(c) *Conversion of the Chroman* (8) *into the Chroman* (9). A solution of the chroman (8) (102 mg) in ethyl acetate (25 ml) containing sulphuric acid (50%; 1 ml) was set aside at room temperature for 7 days. T.l.c. in benzene–ethyl acetate (96 : 4) and subsequent isolation of the product showed that complete conversion into the chroman (9) had occurred. Under the same conditions chromans (6a) and (7a) were unchanged.

Chlorination of the Chroman (6a).—A solution of t-butyl hypochlorite (300 mg) in acetic acid (5 ml) was added to a solution of the chroman (6a) (1 g) in acetic acid (10 ml). The mixture was set aside at room temperature for 10 min, poured onto ice, and extracted with ethyl acetate to give a gum (950 mg) which was fractionated by t.l.c. in chloroform–benzene (1 : 1). Three bands were recovered: (*a*) R_F 0·1, starting material (380 mg); (*b*) R_F 0·2, a gum (280 mg); and (*c*) R_F 0·3, a gum (190 mg). Crystallization of fraction (*b*) from aqueous acetic acid gave the *chlorochroman* (6b), m.p. 128—135° (Found: C, 67·6; H, 7·6; Cl, 8·6%; *m/e*, 406. $C_{23}H_{31}ClO_4$ requires C, 67·9; H, 7·6; Cl, 8·7%; *M*, 406); $\nu_{max.}$ 3320, 1710, and 1670 cm^{-1}; τ * −0·42 (CHO), 3·80 (OH), 7·41 (ArCH_3), 8·70 (O·C·CH_3), and 9·42 (>C·CH_3). Crystallization of fraction (*c*) from aqueous methanol gave the *dichlorochroman* (6c), m.p. 139—140° (Found: C, 64·2; H, 7·5; Cl, 17·2%; *m/e*, 412. $C_{22}H_{30}Cl_2O_3$ requires C, 63·9; H, 7·3; Cl, 17·2%; *M*, 412); $\nu_{max.}$ 3300, 1700, 1601, and 1570 cm^{-1}; τ * 4·45 (OH), 7·36 (t, ArCH_2), 7·66 (ArCH_3), 8·75 (O·C·CH_3), and 9·44 (>C·CH_3).

Chlorination of the Chroman (7a).—The chroman (7a) (1 g) was similarly chlorinated and the product (750 mg) was purified by t.l.c. in chloroform–benzene (1 : 1) to give the *chlorochroman* (7b) (580 mg), m.p. 139—146° (Found: C, 67·9; H, 7·5; Cl, 9·0%; *m/e*, 406. $C_{23}H_{31}ClO_4$ requires C, 67·9; H, 7·6; Cl, 8·7%; *M*, 406); $\nu_{max.}$ 1710 and 1625 cm^{-1}; τ * −2·83 (bonded OH), −0·11 (CHO), 7·47 (ArCH_3), 8·69 (O·C·CH_3), and 9·40 (>C·CH_3).

Oxidation of the Chroman (6a).—A solution of chromium trioxide (200 mg) in 20% sulphuric acid (2 ml) was added to a stirred solution of the chroman (6a) (372 mg) in acetone (5 ml). The mixture was stirred for a further 30 min, diluted with water, and extracted with ethyl acetate to give a yellow gum (340 mg), which was purified by t.l.c. in chloroform to give the *quinone* (10) (Found: *m/e*, 358·2148. $C_{22}H_{30}O_4$ requires *M*, 358·2143); $\nu_{max.}$ 1705, 1675, 1655, 1635, and 1609 cm^{-1}; τ * 3·62 (q, *J* 2 Hz, C*H*=C·CH_3), 8·05 (d, *J* 2 Hz, CH=C·CH_3), 8·72 (O·C·CH_3), and 9·44 (>C·CH_3).

Schmidt Reaction on the Chroman (6a).—Concentrated sulphuric acid (2 ml) and sodium azide (70 mg) were added to a stirred solution of the chroman (6a) (372 mg) in chloroform (5 ml) at 0°. The mixture was stirred at 0° for 2 h, poured onto ice, and extracted with chloroform to give a gum (400 mg). T.l.c. of the gum showed it to be similar to the product from a Schmidt reaction with the chroman (7a), two pairs of spots being present. The high R_F pair of compounds were isolated by preparative t.l.c. in chloroform–methanol (19 : 1): (*a*) R_F 0·45 (115 mg) and (*b*) R_F 0·65 (40 mg). Fraction (*a*) solidified at room temperature but could not be recrystallized; it was characterized as the *caprolactam* (11) by its spectroscopic properties (Found: *m/e*, 387·2405. $C_{23}H_{33}NO_4$ requires *M*, 387·2409); $\nu_{max.}$ 1670, 1630, and 1580 cm^{-1}; τ * −2·77 (bonded OH), −0·03 (CHO), 3·88 (ArH), 4·65 (d, NH), 6·51 (m, C*H*·NH), 7·53 (ArCH_3), 8·72 (O·C·CH_3), and 9·26 (>C·CH_3). Fraction (*b*) crystallized from benzene–light petroleum to give the *tetrazole* (12), m.p. 123—145° (Found: C, 67·2; H, 7·6; N, 13·9%; *m/e*, 412. $C_{23}H_{32}N_4O_3$ requires C, 67·0; H, 7·8; N, 13·6%; *M*, 412); $\nu_{max.}$ 1630br and 1580 cm^{-1}; τ * −2·76 (bonded OH), −0·02 (CHO), 3·87 (ArH), 5·55 (m, N·C*H*·CH$_3$), 7·55 (ArCH_3), 8·40 (O·C·CH_3), and 8·73 (>C·CH_3).

The Diol (13).—A solution of osmium tetroxide (1 g) in anhydrous ether (50 ml) was added to a solution of antibiotic LL-Z1272ε (3b) (1·45 g) in anhydrous ether (150 ml) and the mixture was set aside at room temperature for 48 h. The precipitate was collected and dissolved in ethanol, and the solution was saturated with hydrogen sulphide, filtered, and evaporated to a gum (900 mg),

* All the chromans and their derivatives are mixtures of epimers and the signals due to the secondary methyl groups appear as complex patterns of up to eight lines in the region τ 9·0—9·2.

which was chromatographed on a column of silica gel. Elution with chloroform gave a solid (680 mg) which crystallized from aqueous methanol to give the *diol* (13), m.p. 171—172° (Found: C, 67·7; H, 8·5. $C_{23}H_{34}O_6$ requires C, 67·9; H, 8·4%); $\nu_{max.}$ 3500, 3450, 3100, 1710, and 1620 cm^{-1}; τ −2·83 (bonded OH), −0·01 (CHO), 3·77 (ArH), 6·37 (m, C*H*·OH), 6·95 (d, *J* 14 Hz, ArCH_2), 7·58 (ArCH_3), 8·80 (O·C·CH_3), and 9·44 (>C·CH_3). Like the chroman derivatives (see footnote on p. 2140), the diol (13) shows a complex pattern in the τ 9·0—9·2 region.

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