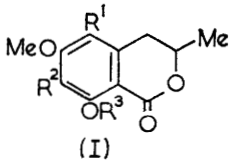
Chlorine-containing Metabolites of *Periconia macrospinosa*

By (the late) **D. Giles** and **W. B. Turner**, Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire

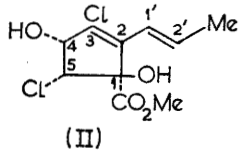
Two metabolites of the fungus *Periconia macrospinosa* are shown to be 5-chloro-3,4-dihydro-8-hydroxy-6-methoxy-3-methylisocoumarin (Ia) and methyl 2-allyl-3,5-dichloro-1,4-dihydroxycyclopent-2-enoate (II). Related compounds had previously been isolated from *Sporormia affinis*.

WE have isolated two chlorine-containing compounds from cultures of *Periconia macrospinosa* grown on Czapek-Dox medium and report here chemical and spectroscopic properties which lead us to assign to them structures (Ia) and (II). After this work was complete, two papers from the Lederle Laboratories described the isolation of the dihydro-isocoumarins (Ib), (Ic), and (Id),[1] and of the cyclopentenones (IIIa), (IIIb), and the 5-epimer of (IIIb) [2] from *Sporormia affinis*. The Lederle group did not isolate compounds (Ia) and (II) from *S. affinis*, but obtained the methyl ether (Ie) by chlorination of the methyl ether (If). On the other hand, we have not isolated any of the *Sporormia* metabolites from *P. macrospinosa* but have obtained the ketone (IIIa) by oxidation of the alcohol (II); since the Lederle publication [2] we have detected the ketone (IIIa) (by t.l.c.) in crude extracts from *P. macrospinosa*.

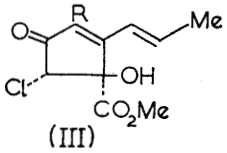


(I)

	R^1	R^2	R^3
a;	Cl	H	H
b;	H	H	H
c;	H	Cl	H
d;	Cl	Cl	H
e;	Cl	H	Me
f;	H	H	Me



(II)



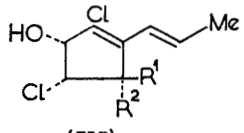
(III)

a; R = Cl
b; R = H

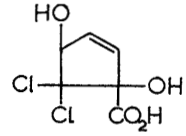
The n.m.r. spectrum of the dihydro-isocoumarin (Ia), $C_{11}H_{11}ClO_4$, shows signals attributable to an aromatic proton, a hydrogen-bonded phenolic proton, a methoxy-group, and the system $MeCH(OR)CH_2Ar$. These features, together with biosynthetic considerations, led us to conclude that 3,4-dihydro-8-hydroxy-6-methoxy-3-methylisocoumarin (Ib) was the basic structure for the metabolite; this was consistent with the u.v. ($\lambda_{max.}$ 219, 265, and 312 mμ) and i.r. ($\nu_{max.}$ 1650 and 1610 $cm.^{-1}$) spectra of the compound. The low carbonyl-frequency is characteristic of hydrogen-bonded lactones (I; $R^3 = H$) and is shifted to 1720 $cm.^{-1}$ in the methyl ether (Ie). Even without the evidence of hydrogen-bonding, the *O*-methyl group could be placed at position 6 with some confidence since all 30 isocoumarins and related compounds which have been obtained from fungi have a free hydroxyl-group at position 8. The chlorine atom was assigned to position 5 because the protons of the 4-methylene group are magnetically non-equivalent; similar considerations were used by the Lederle group in assigning the chlorine atom to position 7 in the *Sporormia* metabolite (Ic).

The n.m.r. spectrum of the second *P. macrospinosa* compound (II), $C_{19}H_{12}Cl_2O_4$, is complicated by the overlapping of signals and by virtual coupling; this is further discussed below. It did, however, show the presence of two olefinic protons, two protons on carbon atoms carrying electronegative substituents, two exchangeable protons, an *O*-methyl group, and an allylic methyl group. The presence of a methoxycarbonyl group is shown by a carbonyl band at 1710 $cm.^{-1}$, and by reduction of compound (II) with sodium borohydride to the triol (IVa) which shows no carbonyl absorption or methoxy-signal. The signals due to all the protons of the triol (IVa) are clearly resolved, and its n.m.r. spectrum shows the presence of the following groupings, each of which is isolated from the others: $-CH{=}\overset{t}{C}HMe$, CHCl·CHOH, CH_2OH, and t-OH.

Oxidation of the triol (IVa) to the ketone (IVb) with periodate shows that the primary and tertiary hydroxy-groups are vicinal and that the secondary and tertiary hydroxy-groups are not. The u.v. spectra of the ester (II) and the triol (IVa) show a maximum at *ca.* 245 mμ characteristic of a conjugated diene. The u.v. spectra of the ketone (IIIa) ($\lambda_{max.}$ 289 mμ) and of the ketone (IVb) ($\lambda_{max.}$ 219 and 272 mμ) are characteristic of conjugated and cross-conjugated dienones respectively.



(IV)



(V)

a; R^1 = OH, R^2 = CH_2OH
b; R^1, R^2 = O

Only structure (II) will accomodate the above data and this has now been confirmed by comparison of the ketone (IIIa) with a sample kindly provided by Mr. E. L. Patterson of The Lederle Laboratories. The Lederle group established the structure and absolute configuration of the ketone (IIIa) by *X*-ray crystallographic analysis [2] and the absolute stereochemistry of the alcohol

[1] W. J. McGahren and L. A. Mitscher, *J. Org. Chem.*, 1968, **33**, 1577.

[2] W. J. McGahren, J. H. van den Hende, and L. A. Mitscher, *J. Amer. Chem. Soc.*, 1969, **91**, 157.

(II) follows from this; the *cis*-configuration of the substituents at C(4) and C(5) is shown [3,4] by the coupling constant (6 c./sec.) of the protons in the triol (IVa) and the ketone (IVb).

The two olefinic protons of the metabolite (II) are magnetically equivalent leading to virtual coupling between H(1′) and the methyl group, the signal of which appears as a four-line pattern of relative intensities 13·6 : 7 : 6·9 : 13·2 with spacing of 1·5, 2, and 1·5 c./sec. Similarly, 4-H and 5-H are equivalent so that there is virtual coupling between 5-H and the hydroxy-proton which appears as a six-line signal. The patterns of the signals from the hydroxy-proton and from 4-H and 5-H are very similar to those calculated [5] for an ABX system with $\delta_X = 0$ and $\delta_A = \delta_B = 50$ c./sec. and J_{AX} 13, J_{BX} 0 and J_{AB} 10·5 c./sec.

The biogenetic significance of the co-occurence of the dihydro-isocoumarins and the cyclopentene derivatives has already been discussed.[2] Chemical analogy for the formation of chlorocyclopentenes from benzenes is provided by the conversion [4,6] of phenol into the cyclopentenol (V) with sodium hypochlorite.

EXPERIMENTAL

I.r. spectra were determined for Nujol mulls and u.v. spectra were measured for ethanol or methanol solutions. N.m.r. spectra were determined on a Varian A60 (for the isocoumarins) or a Varian HA100 spectrometer (for the cyclopentenes) with tetramethylsilane as internal standard and, unless otherwise stated, deuteriochloroform as solvent. The coupling constant (J) is in c./sec. Molecular weights were determined on an AEI MS9 spectrometer and the theoretical values are calculated for Cl = 35. Purity of compounds was checked by t.l.c. on Merck silica gel GF in the system chloroform–acetone (95 : 5). Silica gel for chromatography was Hopkin and Williams MFC.

Isolation of the Metabolities.—*Periconia macrospinosa* (CMI 24411; no. 1744 in our collection) was grown as surface culture in Thomson vessels each containing 1-l. of Czapek-Dox medium with 5% Cerelose and 0·1% yeast extract. After 27 days the medium (74 l.) was extracted at the natural pH (6·4) with ethyl acetate (1 × 7 l. and 2 × 3·5 l.) to give a brown gummy solid (40·5 g.). The extract was triturated with ether to give a solid which was recrystallized from ether–light petroleum to give the cyclopentenol (II) (12·5 g.). The mother liquors were combined and evaporated and the residue (28 g.) was dissolved in benzene, filtered free from a dark, insoluble solid (0·85 g.), and passed down a column of silica gel (750 g.) made up in benzene. Elution of the column with benzene, benzene–chloroform (9 : 1), and benzene–chloroform (4 : 1) gave small amounts of gum which were discarded. Elution with benzene–chloroform (1 : 1) gave, first, fractions containing the dihydro-isocoumarin (Ia) (900 mg.) which was crystallized from acetone–light petroleum as prisms (703 mg.), then fractions [containing mixtures (5·9 g.) of the dihydro-isocoumarin (Ia), the cyclopentenol (II), and a compound with the same R_F as the cyclopentenone (IIIa)] from which the cyclopentenol (II) (2·34 g.) was obtained by crystallization from ether–light petroleum, and, finally, fractions (17·0 g.) which yielded the cyclopentenol (II) (4·0 g.) on crystallization. Further elution of the column with chloroform and with mixtures of chloroform and ethyl acetate gave small amounts of mixtures of uncharacterized compounds.

Properties of the Metabolites.—(a) *5-Chloro-3,4-dihydro-8-hydroxy-6-methoxy-3-methylisocoumarin* (Ia). This compound forms prisms, m.p. 123—124° with a phase-change at *ca.* 120°, $[\alpha]_D^{25}$ −68° (*c* 0·51%, MeOH) (Found: C, 54·5; H, 4·7; Cl, 14·7%; *M*, 242. $C_{11}H_{11}ClO_4$ requires C, 54·4; H, 4·6; Cl, 14·6%; *M*, 242); $\nu_{max.}$ 1650s, 1610s, and 1580m cm.$^{-1}$; $\lambda_{max.}$ 219 (ε 23,000), 265 (ε 11,700), and 312 mμ (ε 5780); τ −1·4 (1H, s), 3·5 (1H, s), 5·3 (1H, m), 6·05 (3H, s), 6·7 (1H, dd, *J* 4, 17), 7·25 (1H, dd, *J* 11, 17), and 8·6 (3H, d, *J* 6).

Methylation of the dihydro-isocoumarin (Ia) with methyl iodide in the presence of potassium carbonate yields the *methyl ether* (Ie), m.p. 118—119°, $[\alpha]_D^{24}$ −173° (*c* 0·56%, MeOH) (Found: C, 56·2; H, 5·1%; *M*, 256; Calc. for $C_{12}H_{13}ClO_4$: C, 56·1; H, 5·1%; *M*, 256), $\nu_{max.}$ 1720s and 1593s cm.$^{-1}$; τ 3·53 (1H, s), 5·6 (1H, m), 6·05 (3H, s), 6·08 (3H, s), 6·8 (1H, dd, *J* 3, 17), 7·35 (1H, dd, *J* 11, 17), and 8·55 (3, d, *J* 6).

(b) *Methyl 2-allyl-3,5-dichloro-1,4-dihydroxycyclopent-2-enoate* (II). This compound forms prisms, m.p. 121—122°, $[\alpha]_D^{25}$ −90° (*c* 0·56%, MeOH) (Found: C, 44·9; H, 4·5; Cl, 26·2%; *M*, 266. $C_{10}H_{12}Cl_2O_4$ requires C, 44·9; H, 4·6; Cl, 26·5%; *M*, 266), $\nu_{max.}$ 3490s, 3450sh, 1710s, 1650w, and 1605w cm.$^{-1}$; $\lambda_{max.}$ 247 (ε 22,500), λ_{inf} 256 mμ (ε 16,000); τ 3·9 (2H, m), 5·6 (2H, m), 6·05 (1H, s), 6·17 (3H, s), 7·18 (1H, m), and 8·23 (3H, m) (on addition of D_2O, the signals at 6·05 and 7·18 disappear and the signal at 5·6 becomes a singlet).

Methyl 2-Allyl-3,5-dichloro-1-hydroxy-4-oxocyclopent-2-enoate (IIIa).—A solution of chromium trioxide (38·5 mg.) and concentrated sulphuric acid (0·03 ml.) in water (0·12 ml.) was added during 30 min. to a stirred solution of the cyclopentenol (II) (100 mg.) in acetone (1·0 ml.) at 0°. The mixture was stirred at 0° for a further 30 min., diluted with water (10 ml.), and extracted with ether (4 × 10 ml.) to give a solid (93 mg.) which was recrystallized from ether–cyclohexane to give the cyclopentenone (IIIa) (41 mg.) as needles, m.p. 134—136°, $[\alpha]_D^{24}$ +92·4° (*c* 0·6%, EtOAc) (Found: C, 45·5; H, 3·9; Cl, 26·7%; *M*, 264. Calc. for $C_{10}H_{10}Cl_2O_4$: C, 45·3; H, 3·6; Cl, 26·8%; *M*, 264), $\nu_{max.}$ 3420m, 1755s, 1730s, 1630m, and 1570m cm.$^{-1}$; $\lambda_{max.}$ 289 mμ (ε 22,300); τ 3·2 (1H, dq, *J* 6·5, 16), 3·55 (1H, dq, *J* 1·5, 16), 5·38 (1H, s), 5·6 (1H, br), 6·2 (3H, s), and 8·03 (3H, dd, *J* 1·5, 6·5) (on addition of D_2O the signal at 5·6 disappeared). The above product was identical (m.p., mixed m.p., and i.r. spectrum) with a sample of the cyclopentenone (IIIa) isolated from *Sporormia affinis*.

2-Allyl-1,4-dichloro-3,5-dihydroxy-3-hydroxymethylcyclopentene (IVa).—Sodium borohydride (185 mg.) was added to a solution of the ester (II) (523 mg.) in isopropyl alcohol (20 ml.) and the mixture was set aside overnight at room temperature. The solution was cautiously acidified with 3N-sulphuric acid, diluted with water, and extracted with ethyl acetate to give a gum (490 mg.) which was dissolved in chloroform and passed down a column of silica gel (25 ml.). Elution of the column with chloroform gave a solid which

[3] H. J. Jakobsen, *Tetrahedron Letters*, 1967, 1991.
[4] C. J. Moye and S. Sternhell, *Austral. J. Chem.*, 1966, **19**, 2107.
[5] J. I. Musher and E. J. Corey, *Tetrahedron*, 1962, **18**, 791.
[6] A. W. Burgstahler, T. B. Lewis, and M. O. Abdel-Rahman, *J. Org. Chem.*, 1966, **31**, 3516.

was recrystallized from ether–light petroleum to give the *triol* (IVa) as rosettes, m.p. 128—130° (decomp.) (Found: C, 45·3; H, 5·0; Cl, 30·0%; *M*, 238. $C_9H_{12}Cl_2O_3$ requires C, 45·2; H, 5·1; Cl, 29·7%; *M*, 238), $\nu_{max.}$ 3400s, 1650w, and 1600w cm.$^{-1}$; $\lambda_{max.}$ 245 mμ (ε 20,400); τ [$^2H_6$]Me_2SO 3·5 (1H, dq, *J* 6·5, 16), 4·0 (1H, dq, *J* 1·5, 16), 4·28 (1H, t, *J* = 4·5), 4·34 (1H, s), 5·23 (1H, d, *J* 11), 5·66 (1H, d, *J* 6), 5·88 (1H, dd, *J* 6, 11), 6·58 (2H, d, *J* 4·5), and 8·22 (3H, dd, *J* 6·5, 1·5) (on addition of D_2O the signals at 4·28, 4·5, and 5·66 disappear and the signals at 5·88 and 6·58 become a doublet and a singlet respectively).

2-*Allyl*-1,4-*dichloro*-3,5-*dihydroxy*-3-*hydroxymethylcyclopentene* (IVb).—Sodium periodate solution (0·1M; 6 ml.) was added to a solution of the triol (IVa) (78 mg.) in methanol (6 ml.) and the mixture was set aside at room temperature for 1 hr. The methanol was removed under reduced pressure and the aqueous residue was extracted with ethyl acetate to give a solid (71 mg.) which was recrystallized from ether–light petroleum to give the *cyclopentenone* (IVb) (49 mg.), needles, m.p. 111—112° (Found: C, 46·5; H, 3·8; Cl, 34·5%; *M*, 206. $C_5H_5Cl_2O_2$ requires C, 46·4; H, 3·8; Cl, 34·2%; *M*, 206); $\nu_{max.}$ 3420 br, 1700s, 1650m, and 1580m cm.$^{-1}$; $\lambda_{max.}$ 219 (ε 16,600) and 272 mμ (ε 8100); τ 3·0 (1H, dq, *J* 6·5, 16), 3·92 (1H, dq, *J* 1·5, 16), 5·22 (1H, dd, *J* 6, 8), 5·51 (1H, d, *J* 6), 7·32 (1H, d, *J* 8), and 8·16 (3H, dd, *J* 6·5, 1·5) (on addition of D_2O the signal at 7·32 disappears and the signal at 5·22 becomes a doublet).

We thank Mr. R. Bowling for the fermentations, Messrs. P. J. Suter and D. Greatbanks for the determination of n.m.r. spectra, and Dr. B. R. Webster for mass spectroscopy. Valuable technical assistance was provided by Mr. G. Halliday, and the interpretation of the n.m.r. spectrum of compound (II) is due to Dr. G. R. Bedford.

Note Added in Proof. Cryptosporiopsin, a metabolite of a *Cryptosporiopsis* sp., has been assigned a structure isomeric with (IIIa) (G. M. Strunz, A. S. Court, J. Komlossy, and M. A. Stillwell, *Canad. J. Chem.* 1969, **47**, 2087). Dr. Strunz informs us that cryptosporiopsin has now been shown to be identical with (IIIa).

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