Fungal Products. Part V.[1] The Absolute Stereochemistry of Colletodiol and the Structures of Related Metabolites of *Colletotrichum capsici*

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Colletoketol (2), colletol (3), and colletallol (4), three new co-metabolites of colletodiol (1) (10,11-dihydroxy-2,8-dimethyl-1,7-dioxacyclotetradeca-4,12-diene-6,14-dione), have been isolated from culture filtrates of *Colletotrichum capsici* and shown to be the 11-oxo-analogues of colletodiol, 11-deoxycolletodiol, and 10-deoxycolletodiol, respectively. The absolute stereochemistry for colletodiol [*i.e.* (2*R*,8*R*,10*R*,11*S*)] has been assigned from chemical and optical rotatory evidence, and that for colletoketol [*i.e.* (2*R*,8*R*,10*R*)] has been established by chemical correlation with colletodiol.

FROM the culture filtrates of the plant pathogen, *Colletotrichum capsici*, Grove *et al.*[2] isolated the metabolite colletodiol, which was subsequently shown to be the 14-membered cyclic dilactone (1) [3a] (*cf.* ref. 3*b*). The absolute stereochemistry (5) has now been assigned to this metabolite (see later). In the course of isolating colletodiol for this stereochemical investigation, three closely related dilactones, colletoketol (2), colletol (3), and colletallol (4), have been isolated from the culture filtrates of *C. capsici* together with 2-phenylethanol.

Structure (1) for colletodiol was deduced mainly from spectroscopic data [3a] which is not discussed in detail here. To facilitate comparison with the new dilactones, i.r. and n.m.r. data for colletodiol are included in Tables 1 and 2, respectively. In the mass spectrum of the bistrimethyl silyl (TMS) ether of colletodiol, it was suggested [3a] that the base peak at *m/e* 143 ($C_7H_{15}OSi$) arose by McLafferty rearrangement to the ion (6) followed by cleavage *a* to the ion (8). The presence of an intense *m/e* 143 ion in the mass spectrum of the TMS ethers of colletodiol and its analogues is therefore diagnostic of the partial structure (9) and this correlation proved useful (see later) in the determination of the structures of colletoketol (2), colletol (3), and colletallol (4).

[1] Part IV, R. I. Crane, P. Hedden, J. MacMillan, and W. B. Turner, *J.C.S. Perkin I*, 1973, 194.

[2] J. F. Grove, R. N. Speake, and G. Ward, *J. Chem. Soc.* (*C*), 1966, 230.

[3] (*a*) J. MacMillan and R. J. Pryce, *Tetrahedron Letters*, 1968, 5497; (*b*) J. W. Powell and W. B. Whalley, *J. Chem. Soc.* (*C*), 1969, 911.

Colletodiol, which formed a dibenzoate [2] and a diacetate, was hydrolysed by dilute aqueous sodium hydroxide to 5-hydroxyhex-2-enoic acid and, in low yield, to 4,5,7-trihydroxyoct-2-enoic acid. Hydrogenation of the former acid gave 5-hydroxyhexanoic acid, which was lactonised by heating in chloroform. The *R*-configuration of the resulting lactone (10) was established in three ways. First, by application of the Hudson–Klyne lactone rules [4,5] the positive $\Delta[\alpha]$ observed in going from the acid, $[\alpha]_D^{20}$ −7·6°, to the lactone, $[\alpha]_D^{20}$ +18·4°, indicated the *R*-configuration. Secondly, the specific rotation of the lactone (10) from colletodiol had the sign opposite to that, $[\alpha]_D$ −39°, reported for the lactone obtained [6] from sorbin oil and assigned the *S*-configuration. Thirdly, the c.d., $[\theta]_{207}$ +495°, of the lactone from colletodiol agrees with the positive Cotton effect predicted for the *R*-configuration from the lactone sector rule.[7] From these data, the 2*R*-configuration is assigned to colletodiol.

TABLE 1

I.r. absorption data for dilactones from *C. capsici*

$\nu_{max.}$ ($CHCl_3$)/cm^{-1}				Assignment
Colletodiol	Colletoketol	Colletol	Colletallol	
985	985	985	985	γ(C–H) of *trans*-HC:CH
1175	1175	1178	1180	ν(C–O)
1320	1320	1319	1318	δ(O–H)
1630	1630			
1657	1657	1655	1654	ν(C=C)
1710	1710			
1721	1721	1715	1715	ν(C=O)
3400	3300—3400	3400	3400	ν(OH)

Mild alkaline hydrolysis of the tetrahydro-derivative (11), obtained by hydrogenation of colletodiol, gave 5-hydroxyhexanoic acid and 5,7-dihydroxyoctan-4-olide (13). The structure of the lactone (13) was established by high resolution mass spectroscopy and by the i.r. (1768 cm^{-1}) and n.m.r. [τ 5·6 (1H, sextet, *J* 4, 7, and 7 Hz)] spectra. Like that of the lactone (10),[3b] the mass spectrum of the lactone (13) showed a strong $M^+ + 1$ ion. The lactone (13) had $[\alpha]_D^{20}$ −42° in ethanol and +1·4° in ethanol containing 2% potassium hydroxide. From application of the Hudson–Klyne lactone rules this $\Delta[\alpha]_D$ (−43·4°) indicated the *S*-configuration (13), which was supported by the positive c.d. curve, predicted from the lactone sector rule.[7] The magnitude (+528°) and sign of the molecular ellipticity of the lactone (13) agree with the data, $[\theta]_{210}$ +440°, reported [8] for the lactone (14), whereas the lactone (15), with the opposite configuration, gives a negative Cotton effect.[9] The 11*S*-configuration is therefore assigned to colletodiol.

To determine the C-8 stereochemistry the 4,5,7-trihydroxyoctenoic acid was oxidised by periodate to give a neutral product which, without characterisation, was oxidised with silver oxide to yield β-hydroxybutyric acid with $[\alpha]_D^{20}$ −21°. Since (*R*)-β-hydroxybutyric acid [10] has $[\alpha]_D^{20}$ −24°, colletodiol possesses the 8*R*-stereochemistry.

The *R*-configuration was assigned to the remaining asymmetric centre (position 10) in colletodiol in two ways. The first utilised the findings of Harada and Nakanishi [11] that the chirality of α-glycols can be determined from the c.d. of their dibenzoates. The interacting chromophores of the dibenzoates give rise to two strong Cotton effects of the same amplitude ($\Delta\varepsilon$ *ca.* 9—18); the first occurs at *ca.* 233 nm and its sign is determined by the chirality (right- or left-handed screw) of the dibenzoate. The second Cotton effect occurs at *ca.* 219 nm and is of opposite sign. The c.d. of colletodiol dibenzoate showed a negative first Cotton effect at 237 nm of the expected magnitude (Figure 1) but the second Cotton effect was also negative. However the c.d. of colletodiol and its diacetate also showed a strong negative Cotton effect, due to the αβ-unsaturated lactone chromophores, at the same wavelength as the second Cotton effect of the dibenzoate (Figure 1). Subtraction of the c.d. curve of the diacetate from that of the dibenzoate revealed the expected positive second Cotton effect of the interacting dibenzoate chromophores. Assuming the conformation [see (5)] for colletodiol which uniquely accounts for the distinct, non-averaged vicinal coupling constants observed [3a] for the metabolite, the negative sign of the first Cotton effect in the c.d. of the dibenzoate indicates a negative chirality, as defined by Harada and Nakanishi, and thus the 10*R*-configuration for colletodiol.

The 10*R*-configuration was also inferred from the reduction of colletoketol, shown later to be the ketol (2), to colletodiol. This reduction with metal hydrides was com-

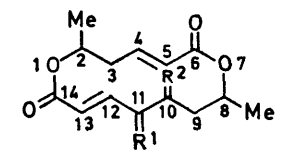
TABLE 2

Measured chemical shifts (τ) of protons in the 100 MHz n.m.r. spectra [a] of metabolites from *C. capsici*

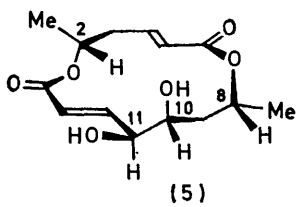
Compound	2-Me	2-H	3-H	4H	5-H	8-Me	8-H	9-H	10-H	11-H	12-H	13-H	OH
Colletodiol (1)	8·62	4·70	7·50, 7·79	3·30	4·28	8·62	4·84	8·00, 8·50	6·37	5·94	3·27	3·86	6·96
Colletoketol (2)	8·60	4·91	7·36, 7·66	3·29	4·16	8·64	4·65	7·85, 8·18	5·49	*b*	2·89	3·53	6·65
Colletol (3)	8·64	4·80	*c*	3·30	4·22	8·64	4·80	*c*	6·00	*c*	3·30	4·20	7·17
Colletallol (4)	8·64	4·78	*c*	3·32	4·22	8·81	4·78	*c*	*c*	5·42	3·19	4·12	7·25

[a] For $CDCl_3$ solutions. [b] Absent. [c] In overlapping multiplets τ 7·30—8·5.

[4] C. S. Hudson, *J. Amer. Chem. Soc.*, 1910, **32**, 338.
[5] W. Klyne, *Chem. and Ind.*, 1954, **32**, 1198.
[6] R. Kuhn and K. Kum, *Chem. Ber.*, 1962, **95**, 2009.
[7] W. Klyne and P. M. Scopes, 'O.R.D. and C.D. in Organic Chemistry,' ed. G. Snatzke, Heyden Press, London, 1967.
[8] S. Ito, K. Endo, S. Inoue, and T. Nozoe, *Tetrahedron Letters*, 1971, 4011.
[9] M. Ohno, M. Okamoto, N. Kawabe, H. Umezawa, T. Takeuchi, H. Iinuma, and S. Takahashi, *J. Amer. Chem. Soc.*, 1971, **93**, 1285.
[10] See J. A. Mills and W. Klyne, *Progr. Stereochem.*, 1954, **1**, 182.
[11] N. Harada and K. Nakanishi, *Accounts Chem. Res.*, 1972 **5**, 257.



	R^1	R^2
(1)	H,OH	H,OH
(2)	O	H,OH
(3)	H_2	H,OH
(4)	H,OH	H_2



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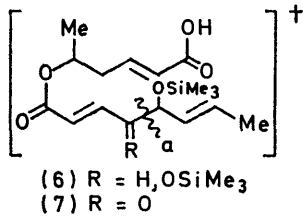
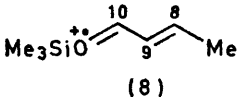


(6) R = H,OSiMe$_3$
(7) R = O



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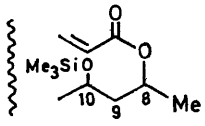

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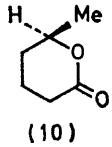

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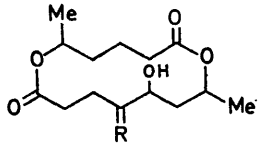
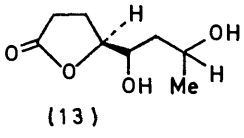


(11) R = H,OH
(12) R = O



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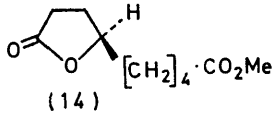

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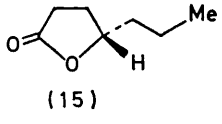


(15)

pletely stereospecific indicating the approach of hydride at C-10 *trans* to the 11-hydroxy-group, probably *via*

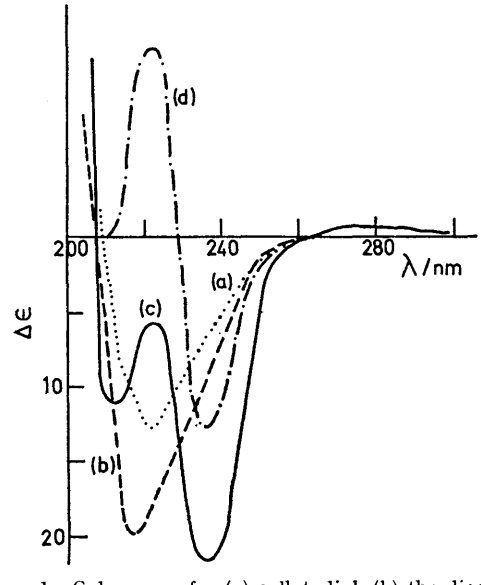
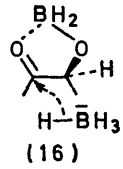


FIGURE 1 C.d. curves for (a) colletodiol, (b) the diacetate, and (c) the dibenzoate; curve (d) is curve (c) minus curve (b)

the complex (16).[12,13] The 10,11-*erythro*-configuration was also supported by the formation of an acetonide from colletodiol without conformational change as judged by



(16)

the similarity of the coupling constants in this derivative and in colletodiol; for example $J_{10,11}$ values for colletodiol and its acetonide were 9·0 and 10 Hz, respectively.

The foregoing data define the absolute stereochemistry (5) for colletodiol. The structures of the three dilactone metabolites of *C. capsici* were determined as follows.

Colletoketol (2) was the major metabolite (*ca.* 20 mg l^{-1}) from one fermentation; otherwise it was obtained in yields of *ca.* 5 mg l^{-1}. By g.l.c. monitoring of the fermentations it was shown that colletodiol (5) was formed initially, then reabsorbed from the medium to be replaced by colletoketol. The ketol structure (2) for colletoketol was indicated by the molecular formula, $C_{14}H_{18}O_6$, determined by high resolution mass spectrometry and by the spectroscopic properties. The i.r. spectrum (Table 1) was similar to that of colletodiol and the presence of a single hydroxy-group was shown by the formation of a monobenzoate and a mono-TMS ether. The mass spectrum of the TMS ether, like that [3a] of the bis-TMS ether of colletodiol, had a base peak at *m/e* 143 (8) indicative of the partial structure (9). The n.m.r.

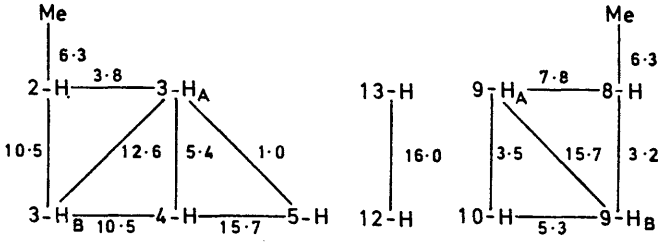


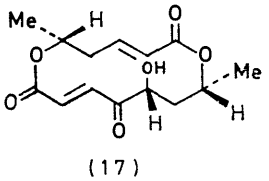
FIGURE 2 Observed coupling constants (Hz) for colletoketol (2)

spectrum of colletoketol was fully analysed by comprehensive decoupling experiments. The chemical shifts (Table 2) and coupling constants (Figure 2) are in complete agreement with structure (2); in particular the observed multiplicity of the 9- and 10-proton signals confirms the mass spectroscopic evidence that the hydroxy-group is positioned at C-10, excluding the alternative 11-hydroxy-10-oxo-structure. Thus the reduction of colletoketol, described earlier, to give colletodiol provided final proof of structure (2) for colletoketol. Oxidation of colletodiol (5) with either Jones reagent or silver carbonate on Celite [14] gave colletoketol (2). These chemical

[12] H. O. House, 'Modern Synthetic Reactions,' Benjamin, New York, 1965.
[13] J. H. Stocker, P. Sidisunthorn, B. M. Benjamin, and C. J. Collins, *J. Amer. Chem. Soc.*, 1960, **82**, 3913.
[14] M. Fetizon and M. Gulfier, *Compt. rend.*, 1968, 335.

correlations with colletodiol (5) establish the absolute stereochemistry (17) for colletoketol.

Like colletodiol (5), mild alkaline hydrolysis of colletoketol gave 5-hydroxyhex-2-enoic acid. Mild alkaline hydrolysis of the tetrahydro-derivative (12), obtained by hydrogenation of colletoketol, gave 5-hydroxyhexanoic acid and succinic acid but no 5,7-dihydroxy-4-oxo-octanoic acid. The succinic acid may be formed *via* the hydroperoxide as shown in (18) (*cf.* ref. 15).* Reduction of colletoketol (2) with sodium borohydride in methanol gave colletodiol (5) and, in one experiment only, a methanolysis product to which structure (19), rather than



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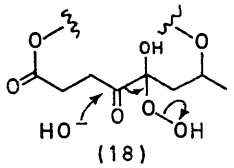

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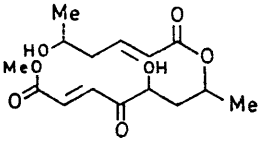

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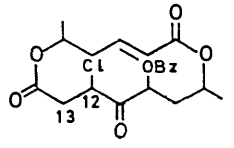


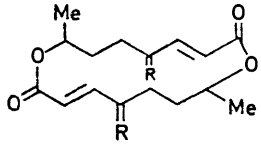
(20)

that of the alternative lactone cleavage product, was assigned from the base peak at *m/e* 143 (8) in the mass spectrum of the mono-TMS ether. An analogous methanolysis of colletodiol (5) was observed on treatment with methanolic sodium hydroxide. Formation of a monobenzoate by treatment of colletoketol (2) with benzoyl chloride and pyridine gave a minor product which was formulated as $C_{21}H_{23}ClO_7$ on the basis of a high resolution mass measurement on the parent ion; the presence of one chlorine atom was also indicated by the $^{35}Cl : ^{37}Cl$ isotopic ratio and by ions at *m/e* 35 and 37. The n.m.r. spectrum of this minor product showed that the vinylic protons at C-12 and C-13 of colletoketol (2) were not present and a new ABX system was present at τ 7·99, 7·73, and 5·22, respectively, with J_{AB} 20, J_{AX} 6, and J_{BX} 4 Hz. Structure (20) is therefore proposed for this product; this orientation for the addition of hydrochloric acid is deduced by assuming concerted attack of benzoylium ion and chloride from within the same solvent cage and therefore attack of chloride at C-12 rather than C-13.

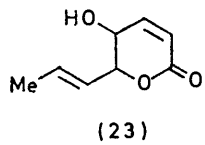
Colletol (3) was a minor metabolite (*ca.* 1 mg l^{-1}), the i.r. (Table 1) and n.m.r. (Table (2) spectra of which indicated a close relationship with colletodiol (1) and colletoketol (2). The molecular formula, $C_{14}H_{20}O_5$, and the formation of a monoacetate and of a mono-TMS ether suggested that colletol was a deoxycolletodiol. The hydroxy-group was assigned to C-10 on the basis of two pieces of evidence: (*a*) the mass spectrum of the mono-TMS ether had a base peak at *m/e* 143 indicative of the partial structure (9); and (*b*) the vinylic 13-protons in colletodiol (1) and colletoketol (2) are, respectively, deshielded by 0·42 and 0·63 p.p.m., as compared to the vinyl 5-protons (Table 2), whereas the 5- and 13-protons in colletol (3) have about the same chemical shift, indicating the absence of an 11-oxygen function.

Colletallol (4), isolated in low yield (<0·5 mg l^{-1}), showed i.r. (Table 1) absorption similar to the other dilactonic metabolites. Isomeric with colletol (3), it formed a mono-TMS ether; the absence of an ion at *m/e* 143 in the mass spectrum of this derivative indicated the absence of a 10-hydroxy-group in colletol (4). Structure (4), with an 11-hydroxy-group, was established for colletallol by the n.m.r. spectrum (Table 2), which showed the following features. First, spin-decoupling revealed an AMX system at τ 3·19, 4·12, and 4·78 with J_{AM} 16, J_{AX} 5, and J_{MX} 2 Hz, diagnostic of the 13-, 12-, and 11-protons of structure (4). Secondly, the 13-proton was deshielded by 9·93 p.p.m. with respect to the 5-proton by the 11-hydroxy-group. Thirdly, in the absence of the 10-hydroxy-group, the 8-methyl signal occurs at 0·17 p.p.m. to higher field than in colletol (3) (Table 2).

The macrocyclic dilactones from *C. capsici* are closely related to pyrenophorin (21), isolated [16] from both *Pyrenophora avenae* and *Stemphylium radicinum*, and to pyrenophorol (22), isolated [17] from *Byssochlamis nivea*. They are presumably derived from the hydroxy-C_8- and -C_6-acids of which the following C_8-derivatives are known fungal metabolites: the δ-lactone (23) and the γ-lactone (24), both from a *Nigrospora* species; [18] and the tetra-acetic acid lactone (25) from *Penicillium stipitatum*.[19]



(21) R = O
(22) R = H,OH



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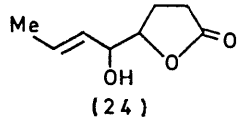

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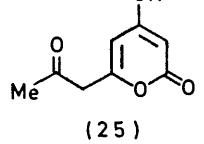

(25)

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus and are corrected. Silica gel M.F.C. (Hopkin and Williams) and Kieselgel G and HF (Merck) were used for column and thin-layer chromatography (t.l.c.), respectively. Analytical t.l.c. plates were developed by spraying with 2% ceric sulphate in 40% sulphuric acid and heating. Silica HF plates were viewed under u.v. light (254 and 366 nm). I.r.

* We thank a referee for this suggestion.

[15] M. F. Ansell, I. S. Shepherd, and B. C. L. Weedon, *J. Chem. Soc.* (*C*), 1971, 1857.

[16] S. Nozoe, K. Hirai, K. Tsuda, K. Ishibashi, M. Shirasaka, and J. F. Grove, *Tetrahedron Letters*, 1965, 4675.

[17] Z. Kis, P. Furger, and H. P. Sigg, *Experientia*, 1969, **25**, 123.

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spectra were obtained on a Perkin-Elmer 257 spectrometer for chloroform solutions unless otherwise stated. N.m.r. spectra (τ values; J in Hz) were obtained with a Varian HA100 spectrometer for deuteriochloroform solutions with tetramethylsilane as internal standard. Mass spectrometry was carried out with an A.E.I. MS9 spectrometer and the data were processed by an on-line Linc 8 computer for probe samples. Combined gas chromatography–mass spectrometry (g.l.c.–m.s.) was carried out with a G.E.C.–A.E.I. MS30 spectrometer. U.v. spectra were taken for solutions in methanol on a Unicam SP 800 spectrophotometer. Specific rotations were measured on a Perkin-Elmer 141 polarimeter. C.d. data were determined for methanolic solutions by Dr. P. M. Scopes, Westfield College, University of London. For gas chromatography (g.l.c.) a Pye 104 instrument was used with columns (5 ft × 0·25 in o.d.), packed with 2% SE33, 2% QF1, 2% OV1, or 2% OV210 on Gaschrom Q. Light petroleum had b.p. 60—80°.

Culture Methods.—Master cultures of *Colletotrichum capsici* Syd. Butler and Bisby were stored on slopes of 2% malt agar under liquid paraffin. Sub-cultures, grown for 7 days on slopes of the same medium in medicine bottles, were used to prepare a spore suspension for the inoculation of cultures on a preparative scale. For the latter the following liquid medium was used (% in parentheses): Dextrosol (5), potassium dihydrogen phosphate (1), ammonium nitrate (0·24), magnesium sulphate heptahydrate (0·2), trace elements concentrate (1 ml l^{-1}). This medium (pH 4·5) was heated in an autoclave at 15 lb in^{-2} for 20 min, and portions (200 ml), dispensed into Glaxo vessels, were inoculated with the spore suspension (1 ml). The culture flasks, kept at 25° under fluorescent light, were monitored by g.l.c. of the trimethylsilylated extract. Colletodiol production reached a maximum after 12 days and this was then rapidly converted into colletoketol. The culture filtrates were extracted twice with 1/3 vol. of chloroform. A typical fermentation consisted of 75 flasks (15 l culture medium).

Isolation and Characterisation of Metabolites.—The crude extract (0·5—3·0 g), obtained from the chloroform extract, was chromatographed on a column (20—25 × 3·5—4·0 cm) of silica gel and the following fractions were collected (eluant in parentheses): (i) 2-phenylethanol (diethyl ether containing 50% benzene); (ii) colletoketol (diethyl ether containing 20—10% benzene); (iii) colletol (diethyl ether containing 0—1% methanol); (iv) colletallol (diethyl ether containing 1% methanol); and (v) colletodiol (diethyl ether containing 2% methanol).

(*a*) Colletodiol, obtained as a waxy solid in fraction (i), crystallised from acetone–light petroleum as needles (yield *ca.* 10 mg l^{-1}), m.p. 162—164° (lit.,[2] 163—164°); $\Delta\varepsilon_{270}0$, $\Delta\varepsilon_{233}-13{\cdot}0$, $\Delta\varepsilon_{211}0$. The dibenzoate, prepared with benzoyl chloride and pyridine, crystallised from ethyl acetate–light petroleum as prisms, m.p. 102—104° (lit.,[2] 103—105°) (Found: M^+, 492·178. Calc. for $C_{28}H_{28}O_8$: M, 492·178); $\Delta\varepsilon_{300}0$, $\Delta\varepsilon_{282}+0{\cdot}53$, $\Delta\varepsilon_{280}+0{\cdot}48$, $\Delta\varepsilon_{276}+0{\cdot}53$, $\Delta\varepsilon_{265}0$, $\Delta\varepsilon_{237}-21{\cdot}6$, $\Delta\varepsilon_{220}-5{\cdot}55$, $\Delta\varepsilon_{213}-11{\cdot}3$, $\Delta\varepsilon_{209}0$, $\Delta\varepsilon_{207}+9{\cdot}3$!; $\lambda_{max.}$ 229 and 274 nm (ε 36,300 and 2040); $\nu_{max.}$ 980, 1115, 1172, 1215, 1450, 1585, 1601, 1654, and 1710 cm^{-1}; τ 2·10 (4H, m), 2·80 (6H, m), 3·10 (1H, dd, J 5 and 16), 3·27 (1H, dt, J 6 and 16), 3·79 (1H, d, J 16), 4·14 (1H, d, J 16), 4·20 (1H, m), 4·40 (1H, m), 4·73 (2H, m), 7·3—8·3 (4H, m), 8·69 (3H, d, J 6), and 8·74 (3H, d, J 6); *m/e* (%) 492(1), 387(0·1), 272(2), 183(3), 122(10), 113(2), 105(100), 77(37), and 68(3). The diacetate prepared from colletodiol (100 mg), acetic anhydride (2 ml), and pyridine (1 ml) was obtained as a gum which, after preparative t.l.c. [silica gel; ethyl acetate–light petroleum–acetic acid (45 : 50 : 5); R_F 0·62], crystallised from ethyl acetate–light petroleum as needles (68 mg), m.p. 132—133° (lit.,[2] 130—131°) (Found: M^+, 368·147. Calc. for $C_{18}H_{24}O_8$: M, 368·147); $\Delta\varepsilon_{265}0$, $\Delta\varepsilon_{219}-19{\cdot}55$, $\Delta\varepsilon_{206}0$, $\Delta\varepsilon_{204}+7{\cdot}5$!; $\lambda_{max.}$ 219 nm (ε 4780); $\nu_{max.}$ 980, 1173, 1327, 1373, 1450, 1658, 1720, and 1745 cm^{-1}; τ 3·28 (2H, m), 3·91 (1H, d, J 16), 4·22 (1H, d, J 16), 4·70 (2H, m), 4·81 (2H, m), 7·3—8·4 (4H, m), 7·95 (6H, s), 8·64 (3H, d, J 6), and 8·80 (3H, d, J 6); *m/e* (%) 368(0·1), 326(3), 282(10), 197(32), 183(19), 137(41), 126(24), 113(35), 95(29), 84(24), 68(18), and 43(100).

(*b*) *Colletoketol* (2) (*10-hydroxy-2,8-dimethyl-1,7-dioxacyclotetradeca-4,12-diene-6,11,14-trione*), obtained as a semi-crystalline solid from fraction (ii), crystallised from acetone–light petroleum as prisms (yield 5—20 mg l^{-1}), m.p. 138—139° (Found: M^+, 282·111. $C_{14}H_{18}O_6$ requires M, 282·110); $[\alpha]_D^{21}$ −33° (*c* 1·48 in $CHCl_3$); *m/e* (%) 282(3), 264(1), 254(5), 213(3), 183(8), 181(5), 170(6), 154(17), 152(14), 139(10), 125(12), 113(100), 97(15), 95(45), 824(42), 69(93), 68(85), 55(26), and 44(32). Impure fractions were purified by preparative t.l.c. on silica gel HF with ethyl acetate–light petroleum–acetic acid (45 : 50 : 5); colletoketol had R_F 0·45. Colletoketol mono-TMS ether, prepared with trimethylsilyl chloride–hexamethyldisilazane–pyridine (1 : 1 : 1) at 20°, showed one peak on g.l.c.–m.s.; *m/e* (%) 354(2), 339(2), 310(2), 227(4), 183(7), 171(5), 143(100), 113(4), 95(10), 81(12), 75(14), 73(50), and 68(19).

(*c*) *Colletol* (3) (*10-hydroxy-2,8-dimethyl-1,7-dioxacyclotetradeca-4,12-diene-6,11-dione*), obtained as an oil from fraction (iii), crystallised from ethyl acetate–light petroleum as needles (yield 1 mg l^{-1}), m.p. 101—104° (M^+, 268·131. $C_{14}H_{20}O_5$ requires M, 268·131); *m/e* (%) 268(0·1), 224(3), 183(6), 121(45), 113(32), 95(41), 71(21), 68(100), 55(15), and 44(11). The *mono-TMS ether*, prepared as in (*b*) showed a single peak on g.l.c.–m.s.; *m/e* (%) 340(0·2), 325(0·3), 269(3), 206(2), 183(1), 160(5), 143(100), 120(10), 95(8), 75(14), 73(42), and 68(23). The *mono-acetate*, prepared from colletol (15 mg), acetic anhydride (2 ml), and pyridine (5 drops), was obtained as an intractable gum (10 mg) (Found: M^+, 310·142. $C_{16}H_{22}O_6$ requires M, 310·142); $\nu_{max.}$ 980, 1101, 1170, 1314, 1655, and 1720 cm^{-1}; τ 3·32 (2H, m), 4·14 (1H, d, J 16), 4·21 (1H, d, J 16), 4·76 (1H, m), 4·88 (2H, m), 7·2—8·4 (4H, m), 7·95 (3H, s), 8·62 (3H, d, J 6), and 8·81 (3H, d, J 6); *m/e* (%) 310(1), 266(7), 334(46), 183(12), 121(35), 113(35), 95(42), 68(80), 58(26), and 43(100).

(*d*) *Colletallol* (4) (*11-hydroxy-2,8-dimethyl-1,7-dioxacyclotetradeca-4,12-diene-6,14-dione*) was obtained as an oil from fraction (iv), admixed with colletol (3). The mixture was separated on silica gel HF plates by multiple development with ethyl acetate–light petroleum–acetic acid (20 : 75 : 5). The less polar band (colletol) was separated from the slower moving colletallol (5), which was obtained as an intractable oil (yield <0·5 mg l^{-1}) (Found: M^+, 268·132. $C_{14}H_{20}O_5$ requires M, 268·131); *m/e* (%) 268(2), 244(12), 191(13), 157(24), 140(66), 122(100), and 69(50). The mono-TMS ether, prepared as in (*b*), showed one peak on g.l.c.–m.s.; *m/e* (%) 340(1), 325(0·3), 296(2), 211(14), 183(27), 169(33), 156(26), 143(5), 211(9), 113(10), 95(41), 83(11), 75(50), 73(100), 68(17), and 55(11).

Alkaline Hydrolysis of Colletodiol.—Colletodiol (100 mg) in 0·05N-sodium hydroxide (25 ml) was stirred at 20° for 24 h. Continuous extraction of the acidified solution with ether for 48 h and recovery from the ether gave a dark gum (95 mg) which was subjected to preparative t.l.c. in silica gel

HF plates. Development with ethyl acetate–light petroleum–acetic acid (45 : 50 : 5) revealed two products, R_F 0·35 and 0·12, which were collected to give: (*a*) the less polar 5-hydroxyhex-2-enoic acid as a gum (30 mg) (Found: C, 55·4; H, 7·5. Calc. for $C_6H_{10}O_3$: C, 55·4; H, 7·7%); $[\alpha]_D^{23}$ −10·0° (*c* 1·0 in EtOH); $\lambda_{max.}$ (EtOH) 212 nm (ε 10,650); $\nu_{max.}$ 3600—2100, 1704, 1660, and 986 cm^{-1}.

(*b*) The more polar 4,5,7-trihydroxyoct-2-enoic acid crystallised from acetone–light petroleum as prisms (21 mg), m.p. 110—112° (Found: M^+, 190·084. $C_8H_{14}O_5$ requires *M*, 190·084; $[\alpha]_D^{23}$ +63° (*c* 1·4 in EtOH); $\lambda_{max.}$ (EtOH) 211 nm (ε 9700); $\nu_{max.}$ (mull) 1080, 1655, 1720, and 3200—3600 cm^{-1}; *m/e* (%) 190(0·2), 157(2), 131(3), 115(1), 113(9), 111(3), 102(100), 89(31), 34(99), 73(34), 71(34), and 57(31).

Tetrahydrocolletodiol.—Colletodiol (100 mg) in ethyl acetate (15 ml) was hydrogenated for 2 h over 10% palladium–charcoal (25 mg) to give tetrahydrocolletodiol, crystallised from acetone–light petroleum as needles (85 mg), m.p. 129—132° (lit.,[2] 133—134°); $\nu_{max.}$ 1056, 1138, 1380, 1725, 3450br, and 3585 cm^{-1}; τ 4·88 (2H, m), 6·38 (2H, m), 7·04br (2H, exchanged with D_2O), 7·4—8·4 (12H, m), 8·72 (3H, d, *J* 6), and 8·78 (3H, d, *J* 6); *m/e* (%) 266(6), 244(2), 224(47), 185(14), 156(11), 139(49), 121(25), 115(100), 97(62), 89(19), 68(41), and 43(58).

The *dibenzoate*, prepared by similar hydrogenation of colletodiol dibenzoate, was obtained as an intractable gum (Found: M^+, 496·212. $C_{28}H_{32}O_3$ requires *M*, 496·210); $\Delta\varepsilon_{265}0$, $\Delta\varepsilon_{235}+7·99$, $\Delta\varepsilon_{204}+7·26$!; $\lambda_{max.}$ (EtOH) 229 and 273 nm (ε 19,500 and 1020); $\nu_{max.}$ 1110, 1176, 1452, 1596, 1605, and 1720 cm^{-1}; τ 2·90 (4H, m), 3·25 (6H, m), 4·38 (2H, m), 4·77 (1H, m), 4·98 (1H, m), 7·4—8·5 (12H, m), 8·69 (3H, d, *J* 6), and 8·75 (3H, d, *J* 6); *m/e* (%) 496(2), 383(2), 290(10), 271(7), 219(42), 185(18), 138(18), 115(26), 105(100), 77(22), and 69(4).

Alkaline Hydrolysis of Tetrahydrocolletodiol.—Tetrahydrocolletodiol (98 mg) in 0·05N-sodium hydroxide (25 ml) was stirred at room temperature for 24 h. Work-up as for the colletodiol hydrolysis gave a brown gum (85 mg), which was separated into two products, R_F 0·20 and 0·45, by preparative t.l.c. on silica gel HF plates with ethyl acetate–light petroleum–acetic acid (45 : 50 : 5). The more polar product crystallised from ethyl acetate–light petroleum to give 5,7-*dihydroxyoctan-4-olide* as needles, m.p. 90—91° (Found: M^+, 174·090. $C_8H_{14}O_4$ requires *M*, 174·089); $[\alpha]_D^{20}$ −42·7° (*c* 0·7 in EtOH); $\Delta\varepsilon_{240}0$, $\Delta\varepsilon_{213}+0·15$, $\Delta\varepsilon_{206}+0·16$; $[\alpha]_D^{20}$ +1·4° (*c* 0·3 in 2% ethanolic KOH); $\nu_{max.}$ 911, 1175, 1377, 1768, 3420br, and 3590 cm^{-1}; τ 5·56 (1H, sx, *J* 4 and 8), 5·91 (1H, m), 6·10 (1H, m), 6·50br (2H, exchanged with D_2O), 7·44 (2H, m), 7·75 (2H, m), 8·34 (2H, m), and 8·75 (3H, d, *J* 6); *m/e* (%) 175(0·6, M^+ + 1), 174(0·4), 156(0·2), 141(4), 130(11), 115(11), 112(13), 89(100), 86(80), 71(41), 57(23), and 45(75).

The more polar product, R_F 0·45, was obtained as a gum, $[\alpha]_D^{20}$ −7·6° (*c* 0·97 in MeOH), characterised as 5-hydroxyhexanoic acid by conversion into the corresponding lactone (see later) and by the following data: $\nu_{max.}$ 930, 1204, 1385, 1724, and 3300—2700 cm^{-1}; $\lambda_{max.}$ 211 nm (ε 209); τ 6·10 (1H, m), 7·65 (2H, m), 8·41 (4H, m), and 8·79 (3H, d, *J* 6).

(5R)-*Hexan-5-olide.*—5-Hydroxyhex-2-enoic acid (30 mg) was hydrogenated over 10% palladium–charcoal (20 mg) for 2 h to give a mixture of 5-hydroxyhexanoic acid and the corresponding lactone. This mixture was heated in chloroform to give the *lactone* (10), obtained as an intractable gum (20 mg) (Found: M^+, 114·068. $C_6H_{10}O_2$ requires *M*, 114·068); $\Delta\varepsilon_{235}0$, $\Delta\varepsilon_{214}+0·15$, $\Delta\varepsilon_{207}+0·15$; $[\alpha]_D^{20}$ +18·4 (*c* 1·7 in MeOH); $\lambda_{max.}$ 211 nm (ε 209); $\nu_{max.}$ 930, 1204, 1385, and 1724 cm^{-1}; τ 5·55 (1H, m), 7·49 (2H, m), 8·10 (4H, m), and 8·62 (3H, d, *J* 6).

Oxidation of Colletodiol.—(a) *Jones reagent.* Colletodiol (50 mg) in acetone (3 ml) was treated with Jones reagent (5 drops) at 0° for 5 min. After the addition of water the gummy product was recovered in chloroform. It gave only one peak on g.l.c., corresponding to colletoketol, and recrystallisation from ethyl acetate–light petroleum gave colletoketol as needles, m.p. 138—140°. Prolonged oxidation gave an intractable mixture of products.

(b) *Silver carbonate–Celite.* Colletodiol (20 mg) in chloroform (10 ml) was stirred for 24 h with silver carbonate–Celite reagent.[14] G.l.c. indicated 20% conversion into colletoketol.

Oxidation of 4,5,7-Trihydroxyoct-2-enoic Acid to (R)-β-*Hydroxybutyric Acid.*—The trihydroxy-acid (15 mg), sodium periodate (60 mg), and water (4 ml) were stirred at 20° overnight. After basification with N-sodium hydroxide, the neutral product was recovered in ether and stirred with silver oxide (200 mg) and water (3 ml) for 48 h. Continuous extraction of the filtered reaction mixture with ether gave (*R*)-β-hydroxybutyric acid (1 mg), $[\alpha]_D^{20}$ −21° (*c* 0·1 in MeOH), which ran concurrently with an authentic sample on a silica gel layer (developed with acetone, then sprayed with Methyl Orange).

Alkaline Hydrolysis of Colletoketol.—Colletoketol (100 mg) and 0·05N-sodium hydroxide (25 ml) were stirred at 20° for 48 h, then acidified with N-hydrochloric acid and continuously extracted for 48 h with ether. The gum (70 mg) recovered from the extract was subjected to preparative t.l.c. on silica gel HF with ethyl acetate–light petroleum–acetic acid (45 : 50 : 5). The band at R_F 0·36 was eluted with ethyl acetate to give 5-hydroxyhex-2-enoic acid (25 mg), identical with that obtained from alkaline hydrolysis of colletodiol. No other products were isolated from the band R_F 0·0—0·2.

Reduction of Colletoketol to Colletodiol.—(*a*) Sodium borohydride (108 mg) was added in portions to an ice-cold, stirred solution of colletoketol (48 mg) in methanol (50 ml). After 40 min, the mixture was worked up in the usual manner. The gummy product, recovered in ethyl acetate, was crystallised from ethyl acetate–light petroleum to give colletodiol as needles (38 mg), m.p. 161—163°.

(*b*) From an identical experiment, the gummy product (40 mg) showed a g.l.c. retention time of 4·95 min on an OV-1 column at 190°; *cf.* 4·25 min for colletodiol. This gum, which was intractable, showed no parent ion in the mass spectrum but was presumed to be the methyl ester (19); $\nu_{max.}$ 984, 1175, 1280, 1657, 1715, and 3410br cm^{-1}; τ 3·02 (2H, m), 3·98 (1H, d, *J* 16), 4·14 (1H, d, *J* 16), 4·83 (1H, m), 5·84 (1H, t, *J* 5), 6·02 (1H, q, *J* 6), 6·32 (1H, m), 6·25 (3H, s), 7·64 (2H, t, *J* 7), 8·17 (2H, m), 8·69 (3H, d, *J* 6), and 8·74 (3H, d, *J* 6). The tri-TMS ether, prepared as already described, showed a single peak on g.l.c.–m.s.; *m/e* (%) 532(0), 488(0·1), 485(0·4), 260(6), 241(5), 143(100), 117(65), 75(8), and 73(32).

Tetrahydrocolletoketol.—Colletoketol (48 mg) in ethyl acetate (10 ml) was hydrogenated for 3 h over 10% palladium–charcoal. The usual work-up gave the *tetrahydroderivative* (12), which crystallised from ethyl acetate–light petroleum as needles, m.p. 106—108° (Found: M^+, 286·142. $C_{14}H_{22}O_6$ requires *M*, 286·142); $\nu_{max.}$ 915, 1075, 1170, 1280, 1376, 1380, 1725, 1740, and 3490 cm^{-1}; τ 4·93 (2H, m), 5·94 (1H, t, *J* 4), 6·76 (1H, m), 7·22 (1H, t, *J* 4), 7·50 (3H, m), 7·80 (4H, m), 8·36 (4H, m), and 8·76 (6H, d, *J* 6);

m/e (%) 286(1), 271(3), 258(4), 185(23), 155(12), 155(100), 97(39), 15(24), and 69(27).

Tetrahydrocolletoketol Monobenzoate.—Colletoketol monobenzoate (9 mg) in ethyl acetate was hydrogenated over 10% palladium–charcoal in the usual way to give *tetrahydrocolletoketol monobenzoate*, needles (6 mg), m.p. 121—124° (from ethyl acetate–light petroleum) (Found: M^+, 390·142. $C_{21}H_{26}O_7$ requires M, 390·142); $[\alpha]_D^{20}$ −58·5° (*c* 0·53 in $CHCl_3$); τ 1·94 (2H, m), 2·48 (3H, m), 4·43 (1H, t, *J* 5), 6·74 (1H, m), 6·93 (1H, m), 6·25 (1H, m), 7·0—8·5 (11H, m), 8·67 (3H, d, *J* 6), and 8·76 (3H, d, *J* 6); *m/e* (%) 390(4), 362(8), 277(15), 219(100), 185(78), 134(23), 115(98), 105(100), 95(21), 77(100), 69(59), and 55(73).

Alkaline Hydrolysis of Tetrahydrocolletoketol.—Tetrahydrocolletoketol (100 mg) and 0·05N-sodium hydroxide (30 ml) were stirred at 20° for 21 h. The usual work-up gave a brown gum (73 mg) which was subjected to preparative t.l.c. on silica HF with ethyl acetate–light petroleum–acetic acid (45 : 50 : 5). Recovery from the bands at R_F 0·5 and 0·2 gave, respectively, 5-hydroxyhexanoic acid (25 mg), identical with that obtained from tetrahydrocolletodiol, and succinic acid (20 mg), identified by comparison (m.p., mixed m.p., and i.r.) with an authentic sample.

Benzoylation of Colletoketol.—Colletoketol (32 mg), benzoyl chloride (2 ml), and pyridine (1 ml) were stirred at 20° for 8 h. Work-up as for colletodiol dibenzoate gave a gum which was subjected to preparative t.l.c. on silica HF plates, which were successfully developed four times with ethyl acetate–light petroleum–acetic acid (25 : 75 : 2) to give two closely running bands. The upper band gave *colletoketol monobenzoate*, prisms (13 mg), m.p. 133—135° (from ethyl acetate–light petroleum) (Found: M^+, 386·138. $C_{21}H_{22}O_7$ requires M, 386·137); $[\alpha]_D^{20}$ −64° (*c* 0·94 in $CHCl_3$); $\nu_{max.}$ 987, 1100, 1600, 1650, and 1725 cm^{-1}; τ 1·90 (2H, m), 2·44 (3H, m), 2·94 (1H, d, *J* 16), 31·8 (1H, d, *J* 16), 3·24 (1H, dt, *J* 5 and 16), 4·30 (1H, d, *J* 16), 4·47 (1H, t, *J* 5), 4·72 (2H, m), 7·72 (4H, m), 8·59 (3H, d, *J* 6), and 8·71 (3H, d, *J* 6); *m/e* (%) 386(2), 342(3), 288(9), 183(75), 122(14), 113(13), 105(98), 95(17), 77(100), 68(55), and 44(23).

The lower band gave the *chloro-monobenzoate* (20), needles (10 mg), m.p. 122—124° (from ethyl acetate–light petroleum) (Found: M^+, 422·114. $C_{21}H_{23}{}^{35}ClO_7$ requires M^+, 422·113); $\nu_{max.}$ 990, 1120, 1603, 1655, and 1725 cm^{-1}; τ 1·92 (2H, m), 2·44 (3H, m), 3·20 (1H, dt, *J* 8 and 16), 419 (1H, d, *J* 16), 4·45 (1H, dd, *J* 1 and 7), 4·71 (1H, m), 5·04 (1H, m), 5·22 (1H, dd, *J* 5 and 6), 7·73 (1H, dd, *J* 4 and 20), 7·99 (1H, dd, *J* 6 and 20), 7·60 (4H, m), 8·60 (3H, d, *J* 6), and 8·71 (3H, d, *J* 6); *m/e* (%) 422(4), 394(6), 387(3), 359(38), 342(5), 289(10), 183(48), 167(10), 113(15), 105(100), 95(24), 82(20), 77(64), 68(76), and 55(23).

We thank the S.R.C. for a Research Studentship and for a Research Grant towards the purchase of a G.E.C.–A.E.I. MS30 mass spectrometer. We also thank I.C.I. Ltd. for a culture of *Colletotrichum capsici*; Dr. P. M. Scopes, Westfield College, University of London, for the c.d. measurements; and Mr. D. Morgan for technical assistance.

[3/170 *Received*, 24*th January*, 1973]