Total Synthesis of the Macrocyclic Antibiotic (±)-Pyrenophorin

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The total synthesis of the macrocyclic antibiotic pyrenophorin (8,16-dimethyl-1,9-dioxacyclohexadeca-3,11-diene-2,5,10,13-tetraone) (16) is described, involving the use of the 2-(*p*-tolylsulphonyl)ethyl ester as a selectively removable protecting group for the carboxy-function, and di-imidazol-1-yl ketone as a lactonising reagent.

A LARGE lactone ring is common to a wide variety of natural products with interesting biological properties,[1] but successful syntheses in this field have, until recently, been rare.[2] We now describe in full the total synthesis of the macrolide pyrenophorin. Since our preliminary communication,[3] work has intensified in this area and brief reports have appeared on the total synthesis of vermiculin,[4] methymycin,[5] and nonactin.[6]

Apart from the problems of constructing the necessary open-chain framework, the key aspects of synthetic strategy in this field comprise the choice of protecting groups for the terminal hydroxy- and carboxy-functions during this construction, and the development of a general high-yielding intramolecular lactonisation process for the resulting hydroxy-acid. For work on the polylactones (*e.g.* the nonactins) it is additionally desirable that the hydroxy- and carboxy-protecting groups should be such that each may be independently removable without affecting the other, or indeed any other sensitive part of the molecule. This imposes a particularly heavy demand on the nature of the carboxy-protecting group where ester functions are already present in the molecule. The use of benzyl and trichloroethyl esters goes some way towards solving the problem but the methods used for their removal cannot be employed when there are other reducible functionalities in the molecule.

We have found that a satisfactory carboxy-protecting grouping is the 2-(*p*-tolylsulphonyl)ethyl ester (1). The reported ready fragmentation [7] of these esters with strong inorganic bases would not of course result in the desired selectivity. However the use of 1,5-diazabicyclo[4.3.0]non-5-ene in benzene smoothly removes

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[1] W. Keller-Schierlein, *Fortschr. Chem. org. Naturstoffe*, 1970, **30**, 313; W. Keller-Schierlein and H. Gerlach, *ibid.*, 1968, **26**, 161; M. Binder and C. Tamm, *Angew. Chem. Internat. Edn.*, 1972, **12**, 370.

[2] D. Taub, N. N. Girotra, R. D. Hoffsommer, C. H. Kuo, H. L. Slates, S. Weber, and N. L. Wendler, *Tetrahedron*, 1968, **24**, 2443; I. Vlattas, I. T. Harrison, L. Tökes, J. H. Fried, and A. D. Cross, *J. Org. Chem.*, 1968, **33**, 4176.

[3] E. W. Colvin, T. A. Purcell, and R. A. Raphael, *J.C.S. Chem. Comm.*, 1972, 1031.

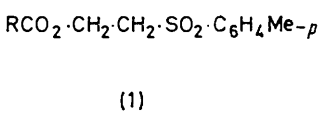
[4] E. J. Corey, K. C. Nicolaou, and T. Toru, *J. Amer. Chem. Soc.*, 1975, **97**, 2287.

[5] S. Masamune, H. Yamamoto, S. Kamata, and A. Fukuzawa, *J. Amer. Chem. Soc.*, 1975, **97**, 3513.

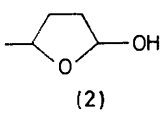
[6] J. Gombos, E. Haslinger, H. Zak, and U. Schmidt, *Tetrahedron Letters*, 1975, 3391; H. Gerlach, K. Oertle, A. Thalmann, and S. Servi, *Helv. Chim. Acta*, 1975, **58**, 2036.

[7] A. W. Miller and C. J. M. Stirling, *J. Chem. Soc.* (*C*), 1968, 2612.

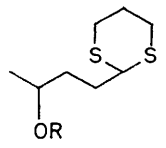
this protecting group at room temperature with no effect on other ester functions. Use of conventional



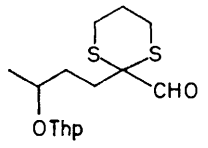
(1)



(2)



(3) R = H
(4) R = Thp



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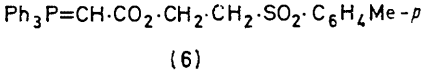


(6)

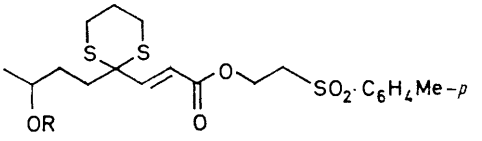
Thp = tetrahydropyran-2-yl

tertiary amines proved ineffective. For hydroxy-group protection in this context the tetrahydropyranyl group serves admirably. The conditions of its mild acid-catalysed removal do not affect the protective ester group.

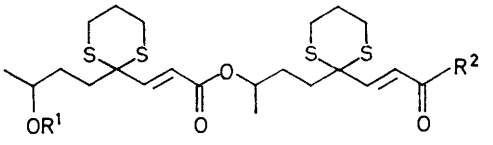
The macrolide pyrenophorin[8] (16) is a sixteen-membered dilactone antifungal and cytostatic metabolite of the plant pathogenic fungi *Pyrenophora avenae* and *Stemphylium radicinum*. The starting material for its synthesis, γ-valerolactone, was selectively reduced with sodium aluminium hydride[9] to the lactol (2), which gave the substituted dithian (3) on treatment with propane-1,3-dithiol and boron trifluoride–ether. The alcohol function of (3) was protected by formation of its 2-tetrahydropyranyl ether (4), and this was treated first with n-butyl-lithium and then with dimethylformamide[10] to produce the aldehyde (5). Reaction of (5) with the phosphonium ylide (6) derived from 2-(*p*-tolylsulphonyl)ethyl bromoacetate gave a homogeneous olefin (7), the required *E*-stereochemistry being defined by the 16 Hz coupling constant of the vinylic protons. Acid-catalysed removal of the tetrahydropyranyl group of (7) gave the parent alcohol (8), which was then converted into the bromoacetate (9). Treatment of (9) with triphenylphosphine and controlled treatment of the resulting salt with base gave the expected ylide (10). Condensation of (10) with the already elaborated aldehyde (5) gave the two diastereoisomers of the *E,E*-diene (11) as sole product. Separation was conveniently deferred until a later stage. After cleavage of the tetrahydropyranyl ether with acid the *p*-tolylsulphonylethyl group was quantitatively removed at room temperature with 1,5-diazabicyclo[4.3.0]non-5-ene in benzene to yield the two diastereoisomeric hydroxy-acids (13).

These were converted into the corresponding imidazolides (14) by the action of di-imidazol-1-yl ketone.[11] Spontaneous interaction between the hydroxy- and imidazolide groupings did not take place, but this was readily induced in benzene solution by a catalytic quantity of 1,5-diazabicyclo[4.3.0]non-5-ene to effect a smooth intramolecular cyclisation to yield a 1 : 1 mixture (as evinced by n.m.r.) of the diastereoisomeric dilactones (15) in a total yield of 60%. This comparatively high yield is in contrast with those obtained by older methods of lactonisation; this convenient method holds promise of generality in the macrocyclic lactone field and thus adds to the armoury of recently developed macrolactonisation techniques.[4-6,12]

Removal of the thioacetal groups of (15) with *N*-chlorosuccinimide in the presence of silver nitrate[13] gave a 1 : 1 mixture of two diastereoisomeric oxolactones (16), which were separable by preparative t.l.c. The less polar, m.p. 124—125°, was identical in all respects save rotation (n.m.r., i.r., and mass spectra; t.l.c. and g.l.c. behaviour) with the naturally occurring



(7) R = Thp
(8) R = H
(9) R = CO·CH$_2$Br
(10) R = CO·CH:PPh$_3$

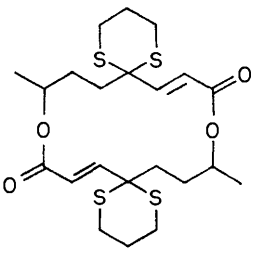


(11) R^1 = Thp, R^2 = O·CH$_2$·CH$_2$·SO$_2$·C$_6$H$_4$Me-*p*
(12) R^1 = H, R^2 = O·CH$_2$·CH$_2$·SO$_2$·C$_6$H$_4$Me-*p*
(13) R^1 = H, R^2 = OH
(14) R^1 = H, R^2 = 

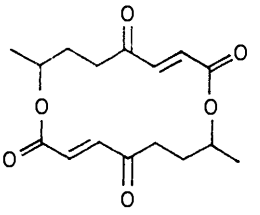
(−)-pyrenophorin (16). The more polar isomer, m.p. 118—119°, showed spectroscopic properties closely resembling those of the higher melting isomer, although it was chromatographically readily distinguishable. It

[8] S. Nozoe, K. Hitai, K. Tsuda, K. Ishibashi, M. Shirasaka, and J. F. Grove, *Tetrahedron Letters*, 1965, 4675.
[9] L. I. Zakharkin, V. V. Gavrilenko, D. N. Maslin, and I. M. Khorlina, *Tetrahedron Letters*, 1963, 2087.
[10] E. J. Corey and D. Seebach, *Angew. Chem. Internat. Edn.*, 1965, **4**, 1077.
[11] H. A. Staab and A. Mannschreck, *Chem. Ber.*, 1962, **95**, 1284.
[12] S. Masamune, S. Kamata, and W. Schilling, *J. Amer. Chem. Soc.*, 1975, **97**, 3515; E. J. Corey, K. C. Nicolaou, and L. S. Melvin, *ibid.*, p 653; E. J. Corey and K. C. Nicolaou, *ibid.*, 1974, **96**, 5614; H. Gerlach and A. Thalmann, *Helv. Chim. Acta*, 1974, **57**, 2661.
[13] E. J. Corey and D. Crouse, *J. Org. Chem.*, 1968, **33**, 298.

was therefore identified as the *meso*-diastereoisomer of (16).



(15)



(16)

EXPERIMENTAL

M.p.s were recorded with a Kofler hot-stage apparatus, i.r. spectra with a Pye–Unicam SP 1000 or a Perkin-Elmer 225 double-beam spectrophotometer (for liquid films, unless otherwise stated), u.v. spectra with a Unicam SP 800 instrument, ^{1}H n.m.r. spectra with a Varian T-60 (60 MHz) or a Varian HA100 (100 MHz) spectrometer (tetramethylsilane as internal reference), and mass spectra with an A.E.I.–G.E.C. MS12 or MS902S spectrometer. Analytical g.l.c. was performed with a Perkin-Elmer F11 gas chromatograph.

Kieselgel G (Merck) was used for analytical t.l.c., and Kieselgel HF_{254} or GF_{254} (Merck) for preparative t.l.c. All organic solutions were dried over anhydrous magnesium sulphate. Solvents were removed with a rotary evaporator.

5-Methyltetrahydrofuran-2-ol (2).—To a solution of γ-valerolactone (40 g, 400 mmol) in dry tetrahydrofuran (300 ml) at −78 °C in an atmosphere of nitrogen was added, with stirring, a suspension of sodium aluminium hydride (6 g, 111 mmol) in dry tetrahydrofuran (20 ml). After 5 h at −20 °C, the mixture was treated with water until precipitation ceased. The supernatant liquid was decanted, and the residue triturated with ether. The organic layers were combined, dried, and concentrated to yield the lactol–hydroxyaldehyde (2) as an oil (35 g), $\nu_{max.}$ 3 510 and 1 720 cm^{-1}, which was used without further purification.

4-(1,3-Dithian-2-yl)butan-2-ol (3).—To the crude lactol (2) were added, with ice-bath cooling, propane-1,3-dithiol (41 ml, 38 g, 351 mmol) and boron trifluoride–ether complex (37 ml, 32.7 g, 230 mmol), and the mixture was left for 10 min. Dry chloroform (300 ml) was added, and the solution left for 18 h at room temperature; it was then stirred vigorously with aqueous 5N-sodium hydroxide (300 ml). The organic layer was separated, washed with water, and brine, and dried. Removal of solvent gave the crude *dithian* (3) as an oil (26.1 g), which was used without further purification. Preparative t.l.c. (developing solvent 40% ethyl acetate–hexane) of a sample gave pure (3), b.p. 138° at 0.4 mmHg, $\nu_{max.}$ 3 430 cm^{-1}, δ($CDCl_3$) 1.2 (3 H, d, *J* 6 Hz), 1.74br (7 H; one H exchanges with D_2O), 2.88 (4 H, m), and 4.0 (2 H, m) (Found: C, 50.0; H, 8.65. $C_8H_{16}OS_2$ requires C, 49.95; H, 8.4%).

The Tetrahydropyranyl Ether (4) *of the Alcohol* (3).—To a solution of the crude alcohol (3) (26.1 g) in dry benzene (300 ml) were added dihydropyran (15 ml) and phosphoryl chloride (3 drops). After 18 h the solution was washed with dilute aqueous sodium hydrogen carbonate and brine, and dried. Removal of solvent and chromatography of the residue on alumina (grade II, neutral) yielded the pure *ether* (4) as an oil (27.6 g), b.p. 160° at 0.3 mmHg (no OH absorption in the i.r.), δ($CDCl_3$) 1.14 and 1.28 (3 H, 2 d, *J* 6 Hz), 1.7br (12 H, m), 2.9 (4 H, m), 3.9br (4 H, m), and 4.7 (1 H, m) (Found: C, 56.65; H, 9.0. $C_{13}H_{24}O_2S_2$ requires C, 56.5; H, 8.75%).

2-(3-Tetrahydropyran-2-yloxybutyl)-1,3-dithian-2-carbaldehyde (5).—To a stirred solution of the ether (4) (24.8 g, 90 mmol) in dry tetrahydrofuran (300 ml) at −78 °C in an atmosphere of nitrogen was added n-butyl-lithium (2.1M in hexane; 45.2 ml, 95 mmol), and the resulting solution was kept at −20 °C for 5 h. Dimethylformamide (15.1 g, 207 mmol) was then added, and stirring was continued at −20 °C for a further 3 h. The mixture was poured into water (300 ml) and ether (300 ml).

The organic extract was washed with water and brine, and dried. Removal of solvent, followed by chromatography on alumina (grade II, basic), afforded the thermally labile *aldehyde* (5) (18.5 g, 60.8 mmol), $\nu_{max.}$ 2 810 and 1 710 cm^{-1}, δ($CDCl_3$) 1.10 and 1.24 (3 H, 2 d, *J* 6 Hz), 1.62br (12 H, m), 2.2—4.0 (7 H, m), 4.62br (1 H, s), and 8.94 (1 H, two close singlets) [Found: *m/e*, 275.1132. $C_{13}H_{23}O_2S_2$ (*M* − CHO) requires 275.1139].

2-(p-Tolylsulphonyl)ethyl Bromoacetate (1; R = $BrCH_2$).—To a stirred suspension of 2-(*p*-tolylsulphonyl)ethanol (7.2 g, 36 mmol) [prepared [14] from 2-(*p*-tolylthio)ethanol] in dry benzene (250 ml) were added anhydrous sodium hydrogen carbonate (3.36 g, 40 mmol), molecular sieves (20 g; Linde type 4A), and bromoacetyl bromide (7.2 g, 36 mmol). After stirring for 18 h, the mixture was suction-filtered through Celite, and the filtrate was concentrated, to give the *ester* (1; R = $BrCH_2$) (10.3 g, 33 mmol), m.p. 47° (from ether), $\nu_{max.}$ (Nujol) 1 740, 1 320, 1 280, and 1 140 cm^{-1}, δ($CDCl_3$) 2.42 (3 H, s), 3.44 (2 H, t, *J* 6.5 Hz), 3.62 (2 H, s), 4.46 (2 H, t, *J* 6.5 Hz), and 7.34 and 7.76 (4 H, ABq, J_{AB} 9 Hz), $\lambda_{max.}$ (EtOH) 225 nm (ε 12 600) (Found: C, 41.0; H, 4.2. $C_{11}H_{13}BrO_4S$ requires C, 41.15; H, 4.1%).

(E)-*2-(p-Tolylsulphonyl)ethyl 3-[2-(3-Tetrahydropyran-2-yloxybutyl)-1,3-dithian-2-yl]prop-2-enoate* (7).—A solution of the bromoacetate (1; R = $BrCH_2$) (6.4 g, 20 mmol) and triphenylphosphine (5.7 g, 22 mmol) in benzene (100 ml) was stirred at room temperature for 24 h. The supernatant solution was decanted, and the gummy product washed with benzene. The phosphonium bromide so produced was dissolved in dichloromethane (100 ml) and cooled to 0 °C, and dilute aqueous sodium hydroxide (0.1N; 190 ml, 19 mmol) was added over 5 min with vigorous stirring. The layers were separated and the organic layer was dried and concentrated to give the crude ylide (6).

A solution of the crude ylide (6) and the aldehyde (5) (2.4 g, 7.9 mmol) in benzene (20 ml) was heated under reflux for 48 h. The mixture was concentrated, and the residue extracted with ether. The extracts were combined and concentrated. Preparative t.l.c. (developing solvent 40% ethyl acetate–hexane) gave the starting aldehyde (5) (1.09 g, 3.6 mmol) and the *olefin ester* (7) (1.48 g, 2.8 mmol), $\nu_{max.}$ 1 720, 1 643, 1 598, 1 320, 1 280, and 1 150 cm^{-1}, δ($CDCl_3$) 1.1 and 1.2 (3 H, 2 d, *J* 6 Hz), 1.7br (12 H, m), 2.54 (3 H, s), 2.72 (4 H, m), 3.56 (5 H, m and t, *J* 6 Hz), 4.56 (3 H, m and t, *J* 6 Hz), 6.0 (1 H, d, *J* 16 Hz), 6.9 (1 H, d, *J* 16 Hz), and 7.4 and 7.88 (4 H, ABq, J_{AB} 8 Hz), $\lambda_{max.}$ (EtOH) 222 nm (ε 19 340) (Found: M^+, 528.1665. $C_{25}H_{36}O_6S_3$ requires *M*, 528.1664).

(E)-*2-(p-Tolylsulphonyl)ethyl 3-[2-(3-Hydroxybutyl)-1,3-*

[14] H. S. Schultz, H. B. F. Freyermuth, and S. R. Buc, *J. Org. Chem.*, 1963, **28**, 1141.

dithian-2-yl]prop-2-enoate (8).—To a solution of the olefin (7) (1.47 g, 2.8 mmol) in methanol (20 ml) was added aqueous N-hydrochloric acid (10 drops), and the solution was stirred for 2 h. The mixture was poured into ethyl acetate, and washed successively with dilute aqueous sodium hydrogen carbonate, water, and brine, and dried. Removal of solvent gave the *hydroxy-ester* (8) as a gum (1.2 g, 2.7 mmol). A sample purified by preparative t.l.c. (developing solvent 50% ethyl acetate–hexane) showed $\nu_{max.}$ 3 610, 3 530, 1 720, 1 640, 1 595, 1 320, 1 280, and 1 150 cm^{-1}, $\delta(CDCl_3)$ 1.2 (3 H, d, *J* 6 Hz), 1.8 [7 H, m (one H exchanges with D_2O)], 2.48 (3 H, s), 2.78 (4 H, m), 3.52 (3 H, m and t, *J* 7 Hz), 4.54 (2 H, t, *J* 7 Hz), 6.0 (1 H, d, *J* 15 Hz), 6.90 (1 H, d, *J* 15 Hz), and 7.44 and 7.90 (4 H, ABq, J_{AB} 8 Hz) (Found: M^+, 444.1092. $C_{20}H_{28}O_5S_3$ requires *M*, 444.1098).

(E)-2-(p-*Tolylsulphonyl*)*ethyl* 3-[2-(3-*Triphenylphosphoranylideneacetoxybutyl*)-1,3-*dithian-2-yl*]*prop-2-enoate* (10).—A solution of the hydroxy-ester (8) (2 g, 4.5 mmol) and bromoacetyl bromide (0.5 ml, 1.1 g, 5 mmol) in benzene (30 ml) was stirred with anhydrous sodium hydrogen carbonate (1 g, 12 mmol) and molecular sieves (10 g; Linde type 4A) for 24 h. Suction-filtration through Celite, concentration, and preparative t.l.c. (developing solvent 60% ethyl acetate–hexane) gave the *bromoacetate* (9) (2.1 g, 3.7 mmol), $\nu_{max.}$ 1 740, 1 720, 1 643, 1 598, 1 320, 1 280, and 1 150 cm^{-1}, $\delta(CDCl_3)$ 1.2 (3 H, d, *J* 6 Hz), 1.8 (6 H, m), 2.48 (3 H, s), 2.78 (4 H, m), 3.52 (3 H, t, *J* 7 Hz), 3.84 (2 H, s), 4.54 (2 H, t, *J* 7 Hz), 4.9 (1 H, m), 6.0 (1 H, d, *J* 15 Hz), 6.9 (1 H, d, *J* 15 Hz), and 7.48 and 7.92 (4 H, ABq, J_{AB} 8 Hz) (Found: M^+, 556.0331. $C_{22}H_{29}BrO_6S_3$ requires *M*, 566.0288).

A solution of the bromoacetate (9) (1.84 g, 3.5 mmol) and triphenylphosphine (917 mg, 3.5 mmol) in benzene (10 ml) was heated under reflux for 4 h. After cooling, the supernatant solution was decanted, and the viscous product washed with benzene. The phosphonium bromide so produced was dissolved in dichloromethane (50 ml) and cooled to 0 °C, and aqueous sodium hydroxide (0.1N; 35 ml, 3.5 mmol) was added over 5 min, with vigorous stirring. The layers were separated and the organic layer was dried and concentrated, to give the crude ylide (10).

(E,E)-1-*Methyl*-3-{2-[2-(p-*tolylsulphonylethoxycarbonyl*)*vinyl*]1,3-*dithian-2-yl*}*propyl* 3-[2-(3-*Tetrahydropyran-2-yloxybutyl*)-1,3-*dithian-2-yl*]*prop-2-enoate* (11).—A solution of the crude ylide (10) and the aldehyde (5) (1.22 g, 4 mmol) in benzene (10 ml) was heated under reflux for 48 h. After cooling, the mixture was filtered and concentrated. Preparative t.l.c. (developing solvent 30% ethyl acetate–hexane) gave the *diene* (11) (1.13 g, 1.46 mmol), $\nu_{max.}$ 1 710, 1 638, 1 592, 1 315, 1 280, and 1 150 cm^{-1}, $\delta(CDCl_3)$ 1.15 (3 H, d, *J* 7 Hz), 1.28 (3 H, d, *J* 7 Hz), 1.72br (18 H), 2.44 (3 H, s), 2.72 (8 H, m), 3.44 (2 H, t, *J* 7 Hz), 3.8 (3 H, m), 4.44 (3 H, m and t, *J* 7 Hz), 4.86 (1 H, m), 5.95 (1 H, d, *J* 16 Hz), 6.15 (1 H, d, *J* 16 Hz), 6.84 (1 H, d, *J* 16 Hz), 6.94 (1 H, d, *J* 16 Hz), and 7.38 and 7.84 (4 H, ABq, J_{AB} 8 Hz), $\lambda_{max.}$ (EtOH) 215 nm (ε 28 300) [Found: *m/e* 688.1716. $C_{31}H_{44}O_7S_5$ (*M* − dihydropyran) requires 688.1691].

(E,E)-3-[2-(3-{3-[2-(3-*Hydroxybutyl*)-1,3-*dithian-2-yl*]*prop-2-enoyloxy*}*butyl*)-1,3-*dithian-2-yl*]*prop-2-enoic Acid* (13).—A solution of the diene (11) (1.1 g, 1.4 mmol) in ethyl acetate–methanol (20 ml; 1 : 1) and aqueous N-hydrochloric acid (4 drops) was stirred for 3 h at room temperature, then was poured into dilute aqueous sodium hydrogen carbonate and ethyl acetate. The organic layer was separated, washed with water, and brine, and dried. Removal of solvent and preparative t.l.c. (developing solvent 50% ethyl acetate–hexane) gave the *hydroxy-diene* (12) (900 mg, 1.3 mmol), $\nu_{max.}$ 3 500, 1 720, 1 650, 1 605, 1 310, 1 280, and 1 150 cm^{-1}, $\delta(CDCl_3)$ 1.2 (3 H, t, *J* 6 Hz), 1.24 (3 H, t, *J* 6 Hz), 1.87 (12 H, m), 2.33br (1 H, s, exchanges with D_2O), 2.5 (3 H, s), 2.77 (8 H, m), 3.53 (3 H, m and t, *J* 6.5 Hz), 4.6 (2 H, t, *J* 6.5 Hz), 5.0 (1 H, m), 6.03 (1 H, d, *J* 16 Hz), 6.42 (1 H, d, *J* 16 Hz), 6.98 (1 H, d, *J* 16 Hz), 7.07 (1 H, d, *J* 16 Hz), and 7.4 and 7.88 (4 H, ABq, J_{AB} 8 Hz) (Found: M^+, 688.1740. $C_{31}H_{44}O_7S_5$ requires *M*, 688.1687).

A solution of the hydroxy-diene (12) (300 mg, 0.44 mmol) in benzene (4 ml) was treated with 1,5-diazabicyclo[4.3.0]-non-5-ene (62 mg, 0.5 mmol) for 5 min at room temperature. The mixture was poured into ether (10 ml) and water (10 ml). The aqueous layer was acidified with dilute sulphuric acid and extracted with ethyl acetate, and the organic layer was washed with water and brine and dried. Removal of solvent and preparative t.l.c. (developing solvent 60% ethyl acetate–hexane) gave the *hydroxy-acid* (13) (210 mg, 0.42 mmol), $\nu_{max.}$ 3 500—2 500, 1 710, and 1 635 cm^{-1}, $\delta(CDCl_3)$ 1.2 (3 H, d, *J* 7 Hz), 1.3 (3 H, d, *J* 7 Hz), 1.84 (12 H, m), 2.74 (8 H, m), 3.8 (1 H, m), 5.0 (1 H, m), 6.1 (1 H, d, *J* 16 Hz), 6.14 (1 H, d, *J* 16 Hz), 6.45br (2 H, s, both exchange with D_2O), 6.88 (1 H, d, *J* 16 Hz), and 6.94 (1 H, d, *J* 16 Hz), $\lambda_{max.}$ (EtOH) 211 nm (ε 15 956) (Found: M^+, 506.1308. $C_{22}H_{34}O_5S_4$ requires *M*, 506.1287).

(E,E)-8′,16′-*Dimethyldispiro*-[1,3-*dithian*-2,5′-[1,9]*dioxacyclohexadeca*-[3,11]*diene*-13′,2″-[1,3]*dithian*]-2′,10′-*dione* (15).—A solution of the hydroxy-acid (13) (184 mg, 0.36 mmol) and di-imidazol-1-yl ketone (71 mg, 0.44 mmol) in dry tetrahydrofuran (1 ml) was stirred at room temperature for 18 h, then diluted with dry benzene (5 ml), and 1,5-diazabicyclo[4.3.0]non-5-ene (1 drop, *ca.* 20 mg, 0.16 mmol) was added. After a further 18 h at room temperature the solution was passed rapidly through a short column of alumina (grade II, neutral), with benzene as eluant. Evaporation afforded the *dilactone* (15) (107 mg, 0.22 mmol), a white solid, m.p. 190—194°, $\nu_{max.}$ 1 705 cm^{-1}, $\delta(CDCl_3)$ 1.2 (6 H, m), 1.9 (12 H, m), 2.7 (8 H, m), 5.0 (2 H, m), 6.1 (1 H, d, *J* 16 Hz), 6.12 (1 H, d, *J* 16 Hz), 6.73 (1 H, d, *J* 16 Hz), and 6.75 (1 H, d, *J* 16 Hz), $\lambda_{max.}$ (EtOH) 212 nm (ε 16 100) (Found: M^+, 488.1176. $C_{22}H_{32}O_4S_4$ requires *M*, 488.1182).

(E,E)-8,16-*Dimethyl*-1,9-*dioxacyclohexadeca*-3,11-*diene*-2,5,10,13-*tetraone* [(±)- *and* meso-*Pyrenophorin*] (16).—A mixture of silver nitrate (183 mg, 1.07 mmol) and freshly prepared *N*-chlorosuccinimide (128 mg, 0.96 mmol) in acetonitrile (5.2 ml) and water (2.1 ml) was cooled to 0 °C in an atmosphere of nitrogen. A solution of the lactone (15) (59 mg, 0.12 mmol) in acetonitrile (3 ml) was added, and the mixture stirred for 25 min, sodium hydrogen carbonate (80 mg, 0.96 mmol) being added in portions over the first 10 min. Dimethyl sulphoxide (0.52 ml) was added, and the mixture stirred for a further 30 min. The solution was then poured into water, and extracted twice with ethyl acetate. The organic extracts were combined, washed with water and brine, and dried. Removal of solvent and preparative t.l.c. (developing solvent 30% ethyl acetate–hexane) afforded two compounds. The faster moving, less polar substance was (±)-*pyrenophorin* (16) (5 mg), m.p. 124—125° (from ether–hexane), $\nu_{max.}$ (CCl_4) 1 729, 1 702, 1 682, and 1 650 cm^{-1}, $\delta(CDCl_3)$ 1.29 (3 H, d,

J 6 Hz), 2.1 (2 H, m), 2.6 (2 H, m), 5.05 (1 H, q, J 6 Hz), 6.5 (1 H, d, J 16 Hz), and 6.95 (1 H, d, J 16 Hz), $\lambda_{max.}$ (EtOH) 211 (ε 22 900) (Found: M^+, 308.1259. $C_{16}H_{20}O_6$ requires M^+, 308.1259), identical in i.r., n.m.r., and mass spectra with authentic (−)-pyrenophorin. Identity was also shown by t.l.c. and g.l.c.; coinjection with authentic material on 2% SE30 at 220° showed no peak separation. The more polar compound (5 mg), m.p. 118—119° (from ether–hexane), M^+ 308.1248, although chromatographically different, showed i.r., n.m.r., and mass spectra indistinguishable from those of the less polar compound. It is therefore identified as the *meso*-diastereoisomer of (16).

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