Isolation of New Isocyanide Metabolites of *Trichoderma hamatum* as their (η^5-Pentamethylcyclopentadienyl)- or (η^5-Ethyltetramethylcyclopentadienyl)-di-μ-thiocyanato-rhodium Complexes

R. K. Boyd, A. J. McAlees, A. Taylor and J. A. Walter
National Research Council of Canada, Institute of Marine Biosciences, Halifax, Nova Scota B3H 3Z1, Canada

Many isolates of *Trichoderma spp.* have been shown to produce 10–20 unstable metabolites, characterized by the presence of an isocyano group, by a recently described analytical method. The purification of these metabolites can be followed by this method and in this way 4-hydroxy-8-isocyano-1-oxaspiro[4•4]cyclonon-8-en-2-one **9**,† 1-(2,3-epoxy-1-hydroxy-3-isocyanocyclopent-4-enyl)ethanol **13**, and 1-(1,4,5-trihydroxy-2-isocyanopent-2-enyl)ethanol **11** have been isolated and characterized. The presence of an isomer of methyl 3-(1,5-dihydroxy-3-isocyanocyclopent-3-enyl)prop-2-enoate **6**, previously reported has been confirmed.

We have shown[3,4] that unstable isocyano metabolites of *Trichoderma hamatum* can be converted into stable, usually crystalline complexes of the type **1** (R = Me, R^1 = CNR^2). These complexes are yellow or orange and quantities of 0.5 µg can be seen on thin layer chromatograms (TLC). Fig. 1 is a chromatogram of an ether extract[5] of a *T. hamatum* fermentation where the extract has been chromatographed along one edge of the plate in the usual manner, the plate rotated through 90°, the edge sprayed with a 0.1% solution of $[Rh\{(\eta^5\text{-}C_5Me_5)(SCN)_2\}_2]$, the reagent eluted into the first chromatogram and the complexes thus formed chromatographed. Among other metabolites, about 15 yellow Rh complexes could be seen on the chromatogram and these are marked in Fig. 1.

It has been shown[1] that mass spectroscopy of such spots provides details of the elemental composition of the isocyano ligand. It was, therefore, possible partly to characterize, the methyl esters of metabolites **4**,**5** and metabolite **3** in Fig. 1, and this partial characterization indicated them to be the known $[Rh(\eta^5\text{-}C_5Me_5)(SCN)_2]$ complexes of methyl 3-isocyanocyclopent-2-enylidenepropionate **1** (R = Me, R^1 = **4**), methyl 3-(3-isocyano-1,5-epoxycyclopentyl)prop-2-enoate **1** (R = Me, R^1 = **5**) and the unknown complex of 1-(2,3; 4,5-diepoxy-1-hydroxycyclopentyl)ethanol **1** (R = Me, R^1 = **3** = trichoviridin[6]). The components **6** and **9** (Fig. 1) were isolated by conventional chromatography. The elemental analysis and spectroscopic properties of component **6** suggested it to be the complex of a methyl 3-(1,5-dihydroxy-3-isocyanocyclopent-3-enyl)prop-2-enate *e.g.* **1** (R = Me, R^1 = **6**) one of the isomers of which has been characterized by Baldwin and his co-workers.[2]

Component **9** (Fig. 1) was purified as described in the Experimental section and the isocyano ligand was also characterized as its insertion complex formed with $[Pd_2(\mu\text{-}Ph_2PCH_2PPh_2)_2Cl_2]$.[8] Elemental analyses of these two complexes agreed with the molecular formula $C_9H_9NO_3$ for the isocyanato metabolite, which found support in the fragmentations $475^+ \rightarrow 296^+ + 179$ and $417^+ \rightarrow 238^+ + 179$ seen in the FAB mass spectrum of the rhodium complex. The IR spectrum of the metabolite had absorption maxima at ν/cm^{-1} 3400, 2125 and 1785 suggesting the presence of H-bonded hydroxy, an isocyanato group conjugated with an olefinic bond and a 5-membered lactone, in the molecule. The ^1H and ^{13}C NMR spectra of the rhodium complex confirmed the presence of a single trisubstituted double bond, and hence, from the molecular formula, the structure of the metabolite must contain two ring systems. The olefinic

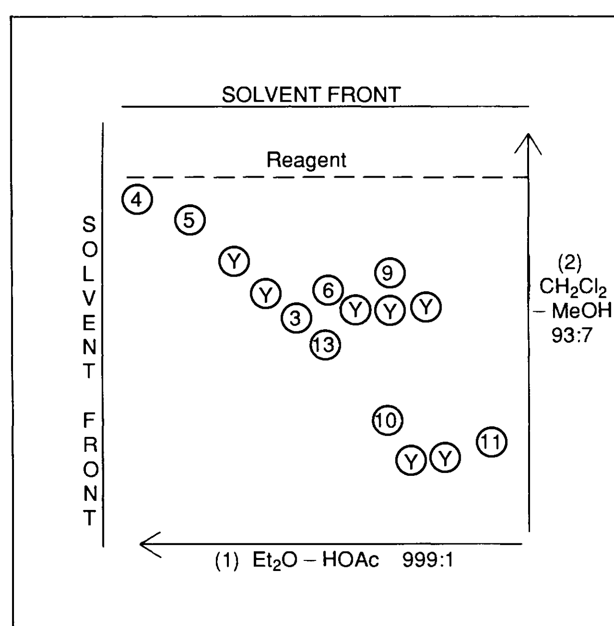
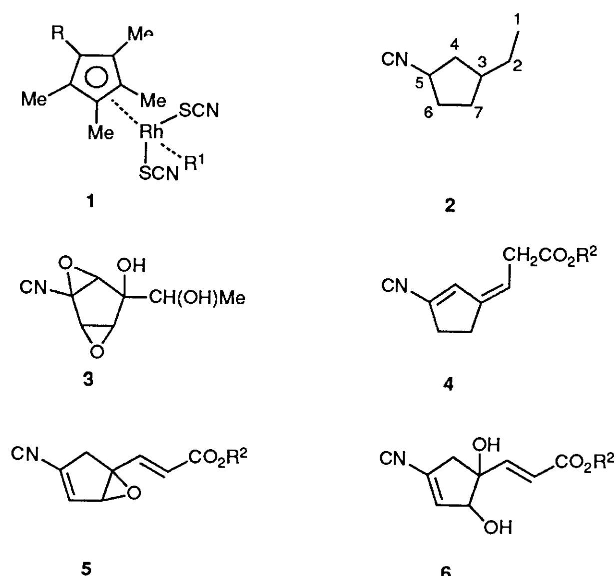


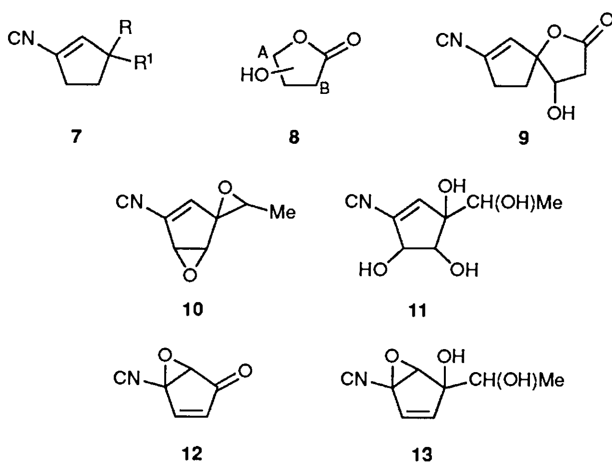
Fig. 1 Two dimensional chromatogram of crude ethyl acetate extract of a culture of *Trichoderma hamatum* (HLX 1379). Spots marked Y are unknown isocyanide complexes. Numbered spots refer to formulae.

proton was allylically coupled to a methylene group which, in turn, was coupled to a second CH_2 group; the coupling constants and chemical shifts were confirmed by spectral simulations. Thus the moiety **7** is present in the metabolite, where R and R^1 are not hydrogen atoms. Exchange of the hydroxy proton with deuterium revealed the presence of a three-spin system where a single proton substituent on a carbon bearing oxygen was coupled to a third methylene group whose geminal coupling (J_{obs} −17.8 Hz) indicated it to be α to a carbonyl group[9] the methylene group being located at position B **8** and the spiro junction at position A **8**. This lactone **8**, with the fragment **7** accounts for all the atoms in the molecule. The location of the hydroxy group and some aspects of the stereochemistry of the spiro junction

† There is some confusion in nomenclature in the literature with respect to these compounds *e.g.* trichoviridin **3**[6] is called isonitrin-C[7] and dermadin **5**,[5] isonitrinic acid-E.[7] Throughout the present report, systematic nomenclature is employed.



were also indicated by ^1H nuclear Overhauser effect (NOE) difference experiments with degassed samples in deuteriated chloroform or dichloromethane. Irradiation of the broad hydroxy resonance at δ_H 1.7 produced a NOE (*ca.* 4.5%) at the olefinic proton. This effect was not observed after exchange of the hydroxy proton with deuterium. Confirmation of this stereochemistry was obtained by demonstrating a NOE (*ca.* 3%) produced at the methylene protons (δ_H 2.19, 2.26) adjacent to the spiro junction by irradiation of the proton geminal to the hydroxy group. All of these data are consistent with formula **9**, whose dehydrated derivative has been reported as a metabolite of *T. hamatum.*[2]



It was possible to reduce the number of metabolites produced by *T. hamatum* by cultivation of the fungus on the synthetic medium previously reported.[5] Extracts of such fermentations from which the acidic metabolites had been mostly removed contained the neutral metabolites corresponding to complexes **3**, **13**, **10** and **11** (Fig. 1). The relatively polar nature of these compounds led us to attempt the purification of **13**, **10** and **11** as their $[Rh(\eta^5\text{-}C_5EtMe_4)(SCN)_2]$[2] complexes which have been shown to be more soluble and somewhat less polar than the corresponding pentamethyl derivatives **1** (R = Me).[4] The separations were complicated by the instability of some of the components of the mixture – bands recovered from PLC plates on rechromatography under the same conditions, providing a multitude of degradation products.

The most polar complex, **11** (Fig. 1) appeared, chromatographically to be homogeneous, but we were unable to induce it to crystallize. Its FAB mass spectrum had abundant ions at *m/z* 495.0825 ($C_{20}H_{28}N_2O_4RhS^+$ = M − SCN^+) and 437.1067 [$C_{19}H_{28}NO_4Rh^+$ = M − $(SCN)_2{}^+$], from which the molecular formula of the metabolite may be deduced as $C_8H_{11}NO_4$.[1] In the ^1H NMR spectrum of the complex, the olefinic H resonance at 6.24 ppm was coupled to the protons resonating at 4.74 and 4.54 ppm and the latter protons were also coupled to 2 exchangeable protons. In addition, the methyl doublet at 1.27 ppm was coupled to the proton observed at 3.80 ppm. In the proton noise decoupled ^{13}C NMR spectrum {ignoring signals due to the $[Rh(\eta^5\text{-}C_5EtMe_4)(SCN)_2]$ entity}, four signals at 134.7, 74.0, 72.7 and 70.6 ppm were shown to be CH groups by a DEPT experiment. The chemical shifts of the three latter carbon nuclei and that of the quaternary carbon atom at 80.9 ppm suggests that all of these carbon centres bear oxygen substituents. All of this spectroscopic evidence may be interpreted in terms of the formula **1** (R = Et, R^1 = **11**), tentatively proposed for this metabolite. Although we isolated component **10** (Fig. 1) as its beautifully crystalline rhodium complex on several occasions we were unable to obtain elemental analyses for C, H, N, O, Rh and S on these samples that accounted for 100% of the material analysed. Their FAB mass spectra were very similar to the mass spectrum of component **11** (Fig. 1), but their ^1H NMR spectra were similar to that of isonitrin A **10**.[7]

It was possible to separate an isocyanide corresponding to component **13** (Fig. 1) from 1-(2,3,4,5-diepoxy-1-hydroxycyclopentyl)ethanol **3** by careful chromatography on silica gel. The crystalline metabolite did not absorb in the UV region in the range λ/nm 210–360 and its IR spectrum had absorption bands at ν/cm^{-1} 3590 (OH), 2148 (unconjugated isocyanide) and 1630 (olefin). The metabolite readily formed a stable crystalline complex with $[Rh(\eta^5\text{-}C_5EtMe_4)(SCN)_2]$ whose analysis and FAB mass spectrum were consistent with the formula $C_8H_9NO_3$ for the metabolite. The exchangeable proton signals at 5.13 and 3.68 ppm indicated the presence of 2 hydroxy groups one of which was coupled to the proton at 3.41 ppm. This proton was also coupled to the protons of a methyl group (δ_H 1.42) and hence the system MeCH(OH) was present. The ^1H NMR spectrum of the complex also indicated the presence of a disubstituted olefin and a ^1H COSY experiment showed that these olefinic protons were coupled (*J*/Hz 1.5) to the proton found at 4.38 ppm whose chemical shift indicated it to be a substituent of an oxygenated carbon atom. These deductions were confirmed by analysis of the ^{13}C NMR spectrum of the complex; a DEPT experiment confirming the presence (in the metabolite) of 4 CH, 1 Me and 2 quaternary carbon atoms, one at 93.3 ppm confirmed the absence of conjugation between the olefin and the isocyano group. The foregoing data show that there are 2 ring systems present in the metabolite having the substituents OH, MeCH(OH) and NC. The metabolite in neutral solution readily consumed 1 mol of periodate and it was possible to extract from the reaction mixture material that had the typical UV absorption spectrum of an α,β-unsaturated ketone. The crude extract, on treatment with $[\{Rh(\eta^5\text{-}C_5EtMe_4)(SCN)_2\}_2]$ gave two rhodium complexes, the IR spectrum of the more polar having absorption bands characteristic of the groups (ν_{max}/cm^{-1}) SCN (2195), CN (2110), CO (1735) and C=C (1600). No absorption bands were observed in the 4000–3000 cm^{-1} region of the spectrum. This data is consistent with the formula **12** for the oxidation product and hence **13** for the metabolite.

Twelve isocyanide metabolites of tyrosine produced by *T. hamatum* have now been isolated[2,3,6,7] and eleven of these have been fully characterized. It is evident from Fig. 1 that several more isocyanides are produced by this fungus and it

seems probable that the utility of complexes of the type **1** will materially help studies of their chemistry.

Experimental

Melting points are not corrected. IR spectra were measured on a Perkin-Elmer 283 instrument. Mass spectra[1] were obtained using the instruments and techniques recently described in detail. NMR spectra (abbreviations: e = exchangeable, d = doublet, m = multiplet, q = quaternary, q' = quartet, t = triplet) were measured at 20 °C in chloroform ($CDCl_3$, unless stated otherwise) in 5 mm tubes on a Bruker MSL-300 instrument at 300 MHz for proton spectra, 75.5 MHz for ^{13}C and 121.5 MHz for ^{31}P. All chemical shifts (except ^{31}P) are given in ppm downfield from the signal of tetramethylsilane and unless stated otherwise are for single hydrogen, carbon or phosphorus nuclei. J Values are given in Hz. ^{13}C Resonances were assigned by DEPT experiments and coupling connectivities were determined as indicated by ^1H COSY experiments. ^1H NMR spectral simulations were obtained using the Bruker PANIC programme and these gave ^1H NMR chemical shifts and coupling constants and confirmed coupling patterns. Analytical thin layer chromatography (TLC) was carried out on commercially prepared glass plates (Merck, Kieselgel $60F_{254}$, 0.01 cm thick) and preparative layer chromatography (PLC) on silica gel (Merck, Kieselgel 60H, 0.05 × 20 × 20 cm) supported on glass plates. Tetrahydrofuran (THF) and dichloromethane were purified as described,[4] light petroleum was the fraction b.p. 30–60 °C; other reagents were used as received. Combustion analyses for C, H, N and S were obtained in the presence of vanadium pentoxide.

Isolation of Methyl 3-(1,5-*Dihydroxy*-3-*isocyanocyclopent*-3-*enyl)prop*-2-*enoate* **6** *and the* 1-*Oxaspiro*[4•4]*cyclonon*-6-*en*-2-*one* **9**.—An innoculum (2 dm^3) of *Trichoderma hamatum* (HLX 1379) was grown as reported by Brewer *et al.*[5] and was added to the glucose–malt extract–peptone medium (9 dm^3) as described.[5] The culture was stirred at 250 rev min^{-1} at 20 °C whilst air (2 dm^3 min^{-1}) was blown through it. After incubation for 70 h, the pH of the culture was adjusted to 8.0, the mycelium was filtered off and the filtrate was acidified to pH 4.5. The filtrate (4.5 dm^3) was treated with sodium chloride (750 g) and then extracted with ethyl acetate (4 × 1 dm^3). The bulked ethyl acetate emulsions were broken by treatment with sodium sulphate (500 g) and filtered. The filtrate was finally dried by storage at −15 °C for 18 h when the ice that had formed was filtered off. The filtrate was concentrated at 18 °C/15 mmHg and the concentrate (500 cm^3) treated with a solution of diazomethane (2 g) in ether (100 cm^3) until a small sample of the reaction mixture visibly reacted with benzoic acid. The reaction mixture was then concentrated and the concentrate (50 cm^3) applied to a silica gel column (5 × 20 cm, 100 g, Merck silica gel for TLC). The column was developed with diethyl ether–light petroleum (3:2; 250 cm^3), ether–light petroleum (4:1; 200 cm^3), ether (500 cm^3) and finally ethyl acetate (250 cm^3). Fractions were collected as follows: 1 (150 cm^3), 2 and 3 (100 cm^3), 4–15 (50 cm^3) and 16 (100 cm^3). Fraction 8 was concentrated and the resulting solution (0.5 cm^3) applied to two PLC plates. The chromatograms were developed at 3 °C with diethyl ether. Two bands, detected by reflectance UV light, had R_F 0.46 and 0.43, were clearly separated. The band of R_F 0.46 was eluted from the silica with ethyl acetate and the concentrated eluate (0.5 cm^3) applied to a PLC plate as before. The chromatogram was developed with dichloromethane–methanol (47:3) at 3 °C, when 4 bands, of R_F 0.06, 0.12, 0.16 and 0.26 were obtained. The first 3 polymerized on treatment with nickel(II) chloride.[10] The band of R_F 0.12 was eluted with ethyl acetate, the eluate evaporated and the residue [δ_H 7.17 (J 15.7), 6.18 (J 15.7), 6.04, 4.56, 3.77 (3 H), 2.94 (J 16.9), 2.59 (J 16.9)] taken up in dichloromethane (25 cm^3). This solution (20 cm^3) was treated with bis(η^5-pentamethylcyclopentadienyl)di-μ-thiocyanato-dithiocyanatodirhodium(III)[3] (75 mg) in dichloromethane (10 cm^3). After 3 h the solution was concentrated and the concentrate (0.5 cm^3) applied to a PLC plate. Three yellow–orange bands were obtained of lower R_F than the starting rhodium complex. The main band of intermediate R_F was eluted with acetone, the eluate evaporated and the residue (80 mg) was taken up in ethyl acetate (2 cm^3). The solution was diluted with ether (2 cm^3) and kept at 3 °C for 18 h. The supernatant liquid was then decanted and the residual gum and crystals washed with ethyl acetate–ether (1:4). The supernatant and washings were combined, kept in the dark at room temperature for 8 h and then for 18 h at 3 °C. The complex of 3-(1,5-dihydroxy-3-isocyanocyclopent-3-enyl)prop-2-enoate **1** (R = Me, R^1 = **6**) separated as orange–red needles, m.p. 176–178 °C (Found: C, 46.75; H, 4.7; N, 7.5; O, 12.0; Rh, 17.5; S, 11.4. $C_{22}H_{26}N_3O_4RhS_2$ requires C, 46.9; H, 4.65; N, 7.5; O, 11.4; Rh, 18.3; S, 11.4%); *m/z* 505 (M − CNS^+), 447 [M − $(SCN)_2{}^+$], 238 and 237; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3583, 3370, 2167, 2109, 1722, 1663 and 1656; δ_H 7.18 (J 15.7), 6.34 (J 1), 6.19 (J 15.7), 4.53 (^3J 1, $^3J_{H.OH}$ 10), 4.25 (e), 4.08 (e, $^3J_{H.OH}$ 10), 3.75 (3 H), 3.12 (J 16.5), 2.70 (J 16.5) and 1.88 (15 H).

Fraction 16 (ethyl acetate) was evaporated and the residue [51 mg; $\nu_{max}(CHCl_3)/cm^{-1}$ 3619, 3398, 2126, 1783 and 1643; δ_H 6.14 (J 1.8), 4.34 (J 5.8, 2.7), 2.89 (J 17.8, 5.8), 2.85 (m, 2 H), 2.60 (J 17.8, 2.7) and 2.27–2.13 (m, 2 H)] was dissolved in ether (50 cm^3). Part of this ethereal solution (= A, 40 cm^3) was added to a solution of the η^5-pentamethylcyclopentadienerhodium(III) complex (80 mg). After 4 h at 20 °C the reaction solution was concentrated and the concentrate (1 cm^3) applied to 2 PLC plates. Three yellow bands were obtained, the one of greatest R_F being the reagent and that of lowest R_F an artefact.[4] The band of R_F 0.41 was eluted with acetone, the eluate evaporated and the residue (76 mg) taken up in the minimum volume of warm acetone. This warm solution was treated with diethyl ether (5 cm^3) and the resulting solution allowed to cool slowly to room temperature, after which it was refrigerated for 18 h. The supernatant mother liquors were aspirated and the residual small yellow needles (65 mg) washed with ice cold acetone–ether (1:9, 3 × 1 cm^3). This *rhodium complex* of 4-hydroxy-7-isocyano-1-oxaspiro[4•4]cyclonon-6-en-2-one **1** (R = Me, R^1 = **9**) had m.p. 151–155 °C (Found: C, 47.3; H, 4.8; N, 7.95; O, 9.8; Rh, 18.4; S, 11.6. $C_{21}H_{24}N_3O_3RhS_2$ requires C, 47.3; H, 4.5; N, 7.9; O, 9.0; Rh, 19.3; S, 12.0%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3580 and 3375 (unchanged on dilution); $(CH_2Cl_2)/cm^{-1}$ 2176, 2116 and 1780; *m/z* 475 (M − SNC)$^+$, 417 [M − $(SNC)_2$]$^+$, 296, 237; $\delta_H(CD_2Cl_2$; subscripts refer to locants on **2**) 6.41 (H_3, $^4J_{3.6}$ 1.9, $^4J_{3.6'}$ 1.9), 2.92 (H_6 $^2J_{6.6'}$ −15.5, $^3J_{6.7}$ 3, $^3J_{6.7'}$ 8), 2.82 ($H_{6'}$ $^3J_{6'.7}$ 9, $^3J_{6'7'}$ 6), 2.26 (H_7 $^2J_{7,7'}$ −15.7), 2.19 ($H_{7'}$), 4.34 ($H_{2'}$ $^3J_{2,1}$ 5.9, $^3J_{2.1'}$ 2.5), 2.86 (H_1 $^2J_{1,1'}$ −17.8) and 2.53 ($H_{1'}$) δ_C 174.0 (q), 132.3 (CH), 122, 121.0 (CNS), 104.33, 104.27 ($J_{^{13}C,Rh}$ 4.7[11]), 97.7 (q), 74.0 (CH), 37.8 (CH_2), 33.5 (CH_2), 32.9 (CH_2) and 9.47 (Me_5). The ethereal solution (= A above, 10 cm^3) was evaporated and the residue (10 mg) in dichloromethane (5 cm^3) was added at 20 °C to a stirred solution of dichlorobis-μ-[methylenebis(diphenylphosphine)]-dipalladium(I)[9] (65 mg) in dichloromethane (7 cm^3). An orange–red precipitate separated immediately. After 10 min the precipitate was collected, washed with dichloromethane and dried (20 °C/0.01 mmHg). The *adduct* (56 mg) had m.p. 192–195 °C (Found: C, 56.1; H, 4.5; Cl, 6.3; N, 1.35; P, 9.8. $C_{59}H_{53}Cl_2NO_3P_4Pd_2{\cdot}2H_2O$ requires C, 55.9; H, 4.5; Cl, 5.6; N, 1.1; P, 9.8%); $\delta_P(CDCl_3$, relative to $PO_4{}^{-3}$) 25.2, 15.85, −2.8 and −53.8.

Isolation of the [Rh(η^5-C_5EtMe_4)$(SCN)_2$] *Complexes of* 1-(2,3-*Epoxy*-1-*hydroxy*-3-*isocyanocyclopent*-4-*enyl*)*ethanol* **1**

($R = Et$, $R^1 = 13$), *and* 1-(1,4,5-*Trihydroxy*-2-*isocyanopent*-2-*enyl*)*ethanol* **1** ($R = Et$, $R^1 = 11$).—A culture (25 dm^3) of *T. hamatum* (HLX 1379) was grown in shake flasks (250) on the glucose–potassium nitrate medium described by Brewer *et al.*[5] After 70 h cultivation at 25 °C the cultures were cooled to 3 °C, filtered through a bed of Hyflosupercel (1 cm thick) from which the fines had been removed by decantation. The filtrate was treated with sodium chloride (2.5 kg) and when this had dissolved the pH of the solution was adjusted to 8.5. This solution was extracted with ethyl acetate (25 dm^3, 25 dm^3 and 15 dm^3), the extracts were combined and kept at −15 °C for 24 h. The mixture was filtered (−15 °C) and the filtrate concentrated in a cyclone evaporator at 4 °C/25 mmHg to 2.5 dm^3. This concentrate was extracted with phosphate buffer ($\frac{1}{15}$ mol dm^{-3}, pH 7.5, 2 × 500 cm^3), the ethyl acetate raffinate freeze-dried as before and evaporated. The residue was taken up in ether (500 cm^3). This solution (25 cm^3) was diluted with diethyl ether–light petroleum (1:1, 25 cm^3) and the resulting cloudy solution applied to a silica gel column [Merck, Keiselgel 60H, 'for TLC', 150 g, column dia. 5 cm, packed with ether–light petroleum (1:1) and allowed to settle at 3 °C for 24 h]. The chromatogram was developed successively with ether–light petroleum mixtures as follows: 1:1 (200 cm^3), 3:2 (250 cm^3), 3:1 (250 cm^3), 4:1 (500 cm^3) and finally with ether (1 dm^3). The following fractions were collected: 1 (500 cm^3), 2–6 (100 cm^3 each), 7–18 (50 cm^3 each) and 19 (500 cm^3). Fractions 9 and 10 were combined and evaporated and the residue taken up in boiling ether (25 cm^3). The solution was evaporated to 2 cm^3, the concentrate kept 24 h at −15 °C and the crystals that separated added to fractions 7 and 8. The combined solution was concentrated to 5 cm^3, kept at −15 °C for 18 h and the 1-(2,3;4,5-diepoxy-1-hydroxycyclopentyl)ethanol (=trichoviridin) **3** [(308 mg) m.p. 95 °C; $[\alpha]_D^{25}$ −37° (*c* 1, $CHCl_3$)] collected. This material (15 mg) in diethyl ether (4 cm^3) was added to a solution of the η^5-pentamethylcyclopentadiene-rhodium(III) complex (25 mg) in dichloromethane (5 cm^3). After 2 h the reaction mixture was concentrated and applied to a PLC plate. The chromatogram was developed with methanol–dichloromethane (3:37) when two yellow zones were obtained. The least polar (7 mg) was the starting rhodium complex. The most polar was eluted from the silica with acetone, the eluate evaporated, the residue (24 mg) taken up in dichloromethane (2 cm^3) and an equal volume of ether carefully layered on the surface. The *isocyanide–rhodium complex* **1** ($R = Me$, $R^1 = 3$) separated as clumps of cone-shaped rods, m.p. 136–230 °C (decomp.) (Found: C, 44.2; H, 4.7; N, 8.0; O, 11.9; Rh, 18.1; S, 11.7. $C_{20}H_{24}N_3O_4RhS_2$ requires C, 44.7; H, 4.5; N, 7.8; O, 11.9; Rh, 19.1; S, 11.9%); *m/z* 479, 421, 296 and 237(8); λ_{max}(MeOH)/nm 251 and 330 (ε 18 560 and 7100); δ_H 4.32, 4.09 (e), 3.76, 3.69 (*J* 6), 3.64, 2.70 (e), 1.90 (15 H) and 1.32 (3 H, *J* 6). Fractions 11–16 were combined and rechromatographed on a PLC plate. The chromatogram was developed with ether to provide 4 bands of R_F 0.26, 0.22, 0.17 and 0.06. Only the band at R_F 0.06 absorbed UV light and the band at R_F 0.26 was shown to be identical to **3**. The main band at R_F 0.22 [ν_{max}($CHCl_3$)/cm^{-1} 3590, 3370 (relative intensity unchanged upon dilution), 2148, 1630, 1375, 1330, 1145, 1105 and 1075] was eluted from the silica with acetone, the eluate evaporated and the residue (24 mg) dissolved in dichloromethane (=B, 10 cm^3). This solution (4 cm^3) was treated with a solution of the [{Rh(η^5-C_5EtMe_4)(SCN)$_2$}$_2$] complex (50 mg) recently described.[4] The reaction mixture was kept at room temperature for 2 h, after which it was concentrated to 0.5 cm^3. This concentrate was applied to two PLC plates which were developed with methanol–dichloromethane (2:23). The major, orange bands (R_F 0.35) were eluted with acetone, the eluate evaporated and the residue (57 mg) was taken up in warm ethyl acetate (6 cm^3). The mixture was filtered and the filtrate concentrated to 3 cm^3 and light petroleum (1 cm^3) added. The *complex* **1** ($R = Et$, $R^1 = 13$) separated as clumps of yellow needles, m.p. 125–126 °C (Found: C, 47.3; H, 4.9; O, 8.5; Rh, 18.5; S, 11.7. $C_{21}H_{26}N_3O_3RhS_2$ requires C, 47.1; H, 4.9; O, 9.0; Rh, 19.2; S, 12.0%); *m/z* 477 (M − CNS)$^+$, 419 [M − (CNS)$_2$]$^+$, 310 and 251; δ_H 6.49 (^3J 6, ^4J 1.2), 6.24 (^3J 6, ^4J 0.9), 5.13 (e), 4.38, 3.68 (e), 3.41 (q′, ^3J 6.4), 2.35 (2 H, ^3J 7.7), 1.90 (12 H), 1.42 (d, 3 H, ^3J 6.4) and 1.16 (t, 3 H, ^3J 7.7); δ_C 141.5 (CH), 128.1 (CH), 123.1 (CNS), 122.6 (CNS), 106.8 ($J_{^{13}C,Rh}$ 6[11]), 104.2 ($J_{^{13}C,Rh}$ 6), 104.1 ($J_{^{13}C,Rh}$ 6), 103.6 ($J_{^{13}C,Rh}$ 6), 103.5 ($J_{^{13}C,Rh}$ 6), 93.3 (q), 87.5 (CH), 83.9 (q), 74.5 (CH), 17.4 (Me), 16.5 (CH_2), 13.0 (Me), 9.1 (2 Me) and 8.9 (2 Me).

Fraction 19 from the columm chromatography was evaporated and the residue (30 mg) taken up in dichloromethane (5 cm^3). The mixture was filtered and the filtrate treated with a solution (15 cm^3) of the [{Rh(η^5-C_4EtMe_4)(SCN)$_2$}$_2$] (48 mg) in dichloromethane. Analytical TLC of the reaction mixture showed the presence of at least six components but only two components were observed on reversed phase TLC (Whatman MKC_{18}F, methanol–water, 3:2). The component of R_F 0.64 (most polar) on reversed phase partition chromatography corresponded to the most polar component found on silica gel chromatography. The mixture of complexes (55 mg) was, therefore, subjected to PLC (methanol–dichloromethane, 2:23, 3 developments) and the most polar band (8 mg) was eluted with THF and rechromatographed under the same conditions. The major band was eluted from the silica with THF and the eluate evaporated to give an orange gum **1** ($R = Et$, $R^1 = 11$); *m/z* 495.0825 (M − CNS$^+$, $C_{20}H_{28}N_2O_4RhS$ requires 495.0825), 437.1067 [M − (CNS)$_2$]$^+$, $C_{19}H_{28}NO_4Rh$ requires 437.1073), 310 and 251; ν_{max}($CHCl_3$)/cm^{-1} 3660, 3390 vbr, 2920, 2850, 2180, 2112, 1605, 1375, 1300, 1110 and 1075; δ_H 6.24, 4.74, 4.58 (e, 2 H), 4.54, 3.80 (q′, ^3J 6), 2.36 (2 H, q′, ^3J 7.5), 1.91 (6 H), 1.89 (6 H), 1.27 (3 H, d, ^3J 6) and 1.15 (3 H, t, ^3J 7.5); δ_C 134.7 (CH), 129.4 (q), 123.5 (CNS), 122.1 (CNS), 106.95 ($J_{^{13}C,Rh}$ 7[11]), 104.4 ($J_{^{13}C,Rh}$ 7), 104.3, 101.1, 101.0 ($J_{^{13}C,Rh}$ 6), 80.9 (q), 74.0 [CH(OH)], 72.7 [CH(OH)], 70.6 [CH(OH)], 17.4 (2 C, Me, CH_2), 12.9 (Me), 9.2 (2 Me) and 9.0 (2 Me). The yellow band running in front of this complex was also stable on rechromatography on silica gel. This crude complex (53 mg) was applied to two PLC plates and the chromatograms developed twice with methanol–dichloromethane (2:23). Three compounds were separated on these chromatograms, that of lowest R_F corresponding with the polar complex described above **1** ($R = Et$, $R^1 = 11$). The main band of intermediate R_F was eluted from the silica with THF, the eluate evaporated and the residue (46 mg) taken up in warm ethyl acetate (6 cm^3, dissolved slowly). The warm solution was treated with light petroleum (1 cm^3) and the solution kept at 3 °C for 18 h. The red gum that separated slowly crystallized as clumps of yellow needles, m.p. 130–133 °C. The *complex* separated from tetrahydropyran or butyl acetate as yellow needles, m.p. 133–134 °C (first melting to an almost colourless liquid and then decomposing) [Found: C, 45.1; H, 4.8; N, 7.8; O, 11.0; Rh, 17.7; S, 11.1 (=97.5%). $C_{21}H_{28}N_3O_4RhS_2$ requires C, 45.6; H, 5.1; N, 7.6; O, 11.6; Rh, 18.6; S, 11.6%]; δ_H 6.35, 5.06, 4.56, 4.14 (^3J 6.5), 2.36 (2 H, ^3J 7.5), 1.92 (6 H), 1.90 (6 H), 1.28 (3 H, ^3J 6.5) and 1.17 (3 H, ^3J 7.5).

Periodate Oxidation of 1-(2,3-*Epoxy*-1-*hydroxy*-3-*isocyano*-*cyclopent*-4-*enyl*)*ethanol* **13**.—The dichloromethane solution (=B above, 2.5 cm^3) was evaporated and the residue (6 mg) taken up in methanol (2 cm^3). The solution was diluted with water (1 cm^3) and a solution (1 cm^3) of sodium metaperiodate (21 mg) added. The slightly opalescent reaction mixture was kept at room temperature for 1 h. The reaction mixture (0.5 cm^3) was titrated in the usual way with standard aqueous sodium arsenite and the titration indicated the presence of sodium periodate (12.3 mg, theory for 1 mol = 13.3 mg). The

remainder of the reaction mixture was concentrated (20 °C/9 mmHg) and the aqueous concentrate (0.2 cm^3) extracted with ether (3 × 1 cm^3). The ethereal solution (λ_{max}/nm 216 and 255) was evaporated, the residue taken up in dichloromethane (1 cm^3) and a solution (1 cm^3) of $[\{Rh(C_5EtMe_4)(SCN)_2\}_2]$ (10 mg) in dichloromethane added. The orange reaction mixture was kept in the dark at room temperature for 3 h and then concentrated. The concentrate was applied to a PLC plate to give three yellow bands on development with methanol–dichloromethane (1:19). That at R_F 0.47 was the starting complex. The band at R_F 0.22 was eluted with THF and had $\nu_{max}(CHCl_3)/cm^{-1}$ 2850, 2260, 2150, 2105, 1735 and 1605.

References

1 R. K. Boyd, D. J. Embree, A. J. McAlees and A. Taylor, *Can. J. Chem.*, 1991, 69, in the press.
2 J. E. Baldwin, R. M. Adlington, J. Chondrogianni, M. S. Edenborough, J. W. Keeping and C. B. Ziegler, *J. Chem. Soc., Chem. Commun.*, 1985, 816.
3 A. W. Hanson, A. J. McAlees and A. Taylor, *J. Chem. Soc., Perkin Trans. 1*, 1985, 441.
4 A. J. McAlees, K. Schwarzentruber and A. Taylor, *J. Chem. Soc., Perkin Trans. 1*, 1990, 2115.
5 D. Brewer, A. Feicht, A. Taylor, J. W. Keeping, A. A. Taha and V. Thaller, *Can. J. Microbiol.*, 1982, **28**, 1252.
6 M. Nobuhara, H. Tazima, K. Shudo, A. Itai, T. Okamoto and Y. Iitaka, *Chem. Pharm. Bull.*, 1976, **24**, 832.
7 A. Fujiwara, T. Okuda, S. Masuda, Y. Shiomi, C. Miyamoto, Y. Sekine, M. Tazoe and M. Fujiwara, *Agric. Biol. Chem.*, 1982, **46**, 1803.
8 A. L. Balch and L. S. Benner, *Inorg. Synth.*, 1982, **21**, **48**.
9 K. Mori, T. Takigawara and T. Matsuo, *Tetrahedron*, 1979, **35**, 933.
10 A. Feicht and A. Taylor, *Proc. Nova Scotian Inst. Sci.*, 1982, **32**, 313.
11 C. White, S. J. Thompson and P. M. Maitlis, *J. Chem. Soc., Dalton Trans.*, 1977, 1654.

Paper 0/05229I
Received 20*th November* 1990
Accepted 30*th November* 1990