Table 1. ^{1}H NMR spectral data of 5 and 6 (400 MHz, $CDCl_3$, TMS as internal standard)

H	5	6
2	6.28 *d*	6.27 *d*
3	6.18 *dd*	6.16 *dd*
8	3.55 *dd*	3.20 *br d*
9	3.24 *dq*	3.23 *dq*
10	1.45 *d*	1.36 *d*
OMe	3.78 *s*	3.77 *s*

J (Hz): 2,3 = 11; 9,10 = 5; compound 5: 3,8 = 0.7; 8,9 = 4; compound 6: 3,8 ~ 0.5; 8,9 = 2.

the 400 MHz ^{1}H NMR spectra with those of authentic material.

Epoxides of matricaria ester (5 *and* 6). Colourless oil; UV $\lambda_{max}^{Et_2O}$ nm: 304, 287, 270, 210; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2240, 2160 (C≡C), 1720, 1610 (C=CCO$_2$R); MS *m/z* (rel. int.): 190.063 (5) [M]$^+$ (calc. for $C_{11}H_{10}O_3$: 190.063), 175 (6) [M − Me]$^+$, 159 (12) [M − OMe]$^+$, 146 (15) [M − C_2H_4O]$^+$, 131 (100) [159 − CO]$^+$. 20 mg *Z,Z*-matricaria ester were heated for 3 hr in 5 ml $CHCl_3$ with 40 mg *m*-chloroperbenzoic acid. TLC of the reaction products gave 8 mg 5, identical with the natural product (^{1}H NMR and TLC).

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(*E*)-9-CHLORO-8-NONENE-4,6-DIYNE-1,2,3-TRIOL, AN ARTEFACT FROM *PNEUMATOSPORA OBCORONATA*

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Key Word Index—*Pneumatospora obcoronata*; hyphomycete; polyacetylene; artefact.

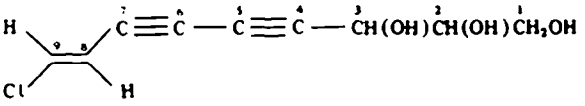
Abstract—A hyphomycete, Exeter Herbarium Number 23000, was isolated in Malaysia from green leaves of the fruit tree *Nephelium lappaceum* (rambutan) and from fallen leaves of *Hevea brasilienisis* (rubber). The fungus was grown for five days in a malt extract medium. From the mycelial filtrate, a novel compound, (*E*)-9-chloro-8-nonene-4,6-diyne-1,2,3-triol, was extracted.

INTRODUCTION

Pneumatospora obcoronata is a species of a newly-described genus found among the mycoflora of fruit tree leaves in Malaysia [1]. Extraction of cultures of the organism with ethyl acetate gave material which, although relatively stable in solution, rapidly polymerized on evaporation of the solvent to a dark-coloured, largely insoluble crystalline material. The portion of the evaporated material soluble in methanol was subjected to prep. TLC and HPLC to give a colourless compound for which structure 1 is proposed on the basis of the following evidence.

RESULTS AND DISCUSSION

An accurate mass determination indicated a molecular formula of $C_9H_9O_3Cl$. This was supported by the in-



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tensity ratio of the $[M]^+$ and $[M+2]^+$ peaks (2.96, calculated for $C_9H_9O_3Cl$, 2.99). The UV spectrum revealed a pattern of absorptions qualitatively and quantitatively similar to patterns reported for the enediyne system of naturally occurring polyacetylenes [2], but with a bathochromic shift of approximately 7 nm for each peak, attributable to the presence of the terminal chlorine atom.

The IR spectrum revealed strong absorption near 3340 cm^{-1} indicative of the presence of hydroxyl groups. No absorption was apparent in the C≡C bond stretching region (2200 cm^{-1}) and only a weak band was apparent in the olefinic region at 1584 cm^{-1}.

The 400 MHz ^1H NMR spectrum in deuteriomethanol (Table 1) revealed an AB quartet in the olefinic region with J = 13.6 Hz, which could be consistent with either a (*Z*)- or (*E*)-1,2-disubstituted alkene. X-ray crystallographic evidence described below revealed that the olefinic linkage had the (*E*)-configuration. The remaining part of the NMR spectrum was complex, consisting of multiplets, one centred at δ4.66 (1H) and the other in the range δ3.62–3.73 (2H). This left three protons unaccounted for and which were accordingly assumed to be hydroxylic protons. Irradiation of the signal attributable to C-8 caused collapse of the H-9 doublet to a singlet and also removed a 0.9 Hz coupling in the signal due to H-3. This seven-bond coupling was unusually large, but not unexpectedly so for such a system [3]. Irradiation at H-3 brought about the complementary simplification in the signal attributable to H-8. The multiplets at δ4.66 and δ3.62–3.73 were attributable to a 1,2,3-triol system at the chain terminus as indicated by the chemical shift and decoupling data given in Table 1. These inferences were supported by the NMR spectrum of the compound determined in deuteriochloroform solution (Table 1). In this spectrum the diastereotopic protons at C-1 gave well separated AB multiplets. In this spectrum also the hydroxyl protons gave distinct signals consisting of two doublets and a triplet, in agreement with the proposed terminal 1,2,3-triol system. Decoupling experiments (Table 1) gave results entirely in agreement with the proposed structure.

The only molecular constitution compatible with these data is that given in structure 1. This structure was confirmed and additional stereochemical features were clarified by an X-ray crystallographic analysis, which revealed the structure given in Fig. 1. This confirms the proposed constitution and further shows that the olefinic system has the (*E*)-configuration and that the C-2,3-diol system has the *erythro* (2*R*,3*S* or 2*S*,3*R*) configuration. The molecule is thus chiral, an observation confirmed by its low positive optical rotation and by its circular dichroism curve which showed positive bands associated with all of the principal UV transitions (see Experimental). The ^{13}C NMR spectrum, with and without proton noise decoupling (Table 2), was entirely consistent with the proposed structure.

The 1-chloroenediyne system of compound 1 has not been detected in any natural product and it is probably not present in any of the metabolites produced by *P. obcoronata* (see below). However, it has been generated synthetically [4] and the coupling constant for the (*E*)-1-chloro-1-ene system (14 Hz) in a synthetic analogue is in good agreement with the NMR data for compound 1 (Table 1).

Table 1. ^1H NMR data of compound 1 in deuteriomethanol and deuteriochloroform

H	CD_3OD	Signal(s) affected on irradiation	H	$CDCl_3$	Signal(s) affected on irradiation
9	6.94 *d* (13.6)*		9	6.70 *d* (13.7)	
8	6.15 *dd* (13.6, 0.9)	H-9 → singlet H-3 → simplification (disappearance of 0.9 Hz coupling).	8	5.99 *dd* (13.7, 0.9)	
3	4.66 *m*	H-8 (disappearance of 0.9 Hz coupling) H-1,2 (simplification)	3	4.62 m	H-8(→ doublet, with loss of 0.9 Hz coupling) H:2 (simplification) H-3 (OH) (→ singlet)
1,2	3.62–3.73 *m*	H-3 → simplification (disappearance of 5.2 Hz coupling).	1-*pro-R* or 1-*pro-S*	3.95 *m*	
			2,1-*pro-S* or 1-*pro-R*	3.81 *m*	
			OH-2	2.63† *d* (6.5)	
			OH-3	2.59† *d* (6.7)	
			OH-1	1.895† *dd*	

*J (Hz).

†Signals disappeared following D_2O exchange.

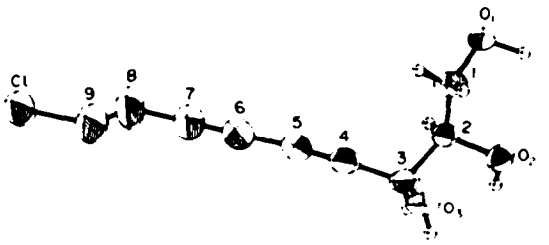

Fig. 1.

Table 2. ^{13}C NMR spectrum of compound 1 in methanol-d_4

C	
1	63.93 *t*
2	65.18 *d*
C≡C	70.07 *s*
C≡C	73.72 *s*
3	75.77 *d*
C≡C	76.90 *s*
7	84.28 *s*
8	113.75 *d*
9	135.56 *d*

Related C_9 polyacetylenes with a terminal 1,2,3-triol system have been reported in *Coprinus quadrifidus* [5] and in *Aleurodiscus roseus* [6]. A phenyldiynetriol has been isolated from a *Dahlia* hybrid [7]. The (2*S*,3*S*)-1,2,3-trihydroxy-4,6,8-triyne from *A. roseus* [6] was laevorotatory ($[\alpha]_D^{23} = -8°$) whereas the (2*S*,3*R*)-isomer from *C. quadrifidus* [5] was dextrorotatory ($[\alpha]_D = 6°$). The compound 1 from *P. obcoronata* had $[\alpha]_D^{20.5} = 5.3°$. It can therefore tentatively be assigned the 2*S*,3*R*-configuration.

Compound 1 is apparently not a natural constituent of *P. obcoronata*. Thus it cannot be detected by HPLC in fresh extracts of cultures of the fungus. It appears only as a product of the decomposition, by an unusual mechanism, of a major metabolite. The nature of its decomposition to compound 1 will be the subject of a forthcoming paper.

EXPERIMENTAL

Growth medium (malt extract L39 and yeast extract L21) were obtained from Oxoid Ltd., Basingstoke, Hants. Prep. TLC: silica gel plates 60F 254, 2 mm (E. Merck, Darmstadt, West Germany).

Growth of Pneumatospora obcoronata *and isolation of metabolites.* Three conical flasks (250 ml) containing 150 ml of malt extract broth (20 g/l) supplemented with yeast extract (0.5 g/l) were each inoculated with three 8 mm diameter agar plugs which had been taken from a 4-week-old plate culture of the fungus and fragmented in sterile distilled water in a MacCartney bottle fitted with a homogenizer rotating at 3000-4000 rpm. Flasks were incubated in an orbital shaker at 250 rpm for 5 days at 25° in the dark. The culture was filtered through Whatman No. 1 filter paper (Whatman Ltd., Maidstone, Kent) and the filtrate (pH 5.8) was extracted with EtOAc (2 × 250 ml). The combined extracts were evaporated to dryness on a rotary evaporator leaving a colourless deposit on the side of the flask which very rapidly darkened. The deposit was largely insoluble in MeOH, but the soluble portion was subjected to prep. TLC on 2 mm silica gel plates, with development in CH_2Cl_2–MeOH. The fluorescence-quenching band at R_f 0.37 was removed and extracted with EtOAc. The extract was filtered and evaporated to give a colourless crystalline residue that slowly turned pink. The material was purified by HPLC on a Spherisorb ODS column (10 μ, column 250 mm × 7 mm). The mixture was injected in MeOH and the eluting solvent was MeOH–H_2O (2:3) (flow rate 3 ml/min, detection at 225 nm). The fractions containing the compound with an R_t of 13.5 min were evaporated to give a crystalline compound (7 mg) that did not change colour on standing. The compound crystallized from H_2O as rectangular platelets, mp 123-124.5°, $[\alpha]_D^{20.5} = 5.3 \pm 1°$ (*c* 0.23; MeOH). The CD spectrum, determined in MeOH, showed the following maxima ($\Delta\varepsilon$): 221.4 (4.8), 249 (0.4), 259 (0.8), 272 (0.9), 290 (0.7). UV λ_{max}^{MeOH} nm (ε): 213 (29 100), 220 (38 500), 245 (5400), 258 (11 200), 273 (16 800), 289 (13 400); MS *m/z*: 200.0235, 202.0190 (calc. for $C_9H_9O_3Cl$: 200.0240, 202.0211), 182 $[M-H_2O]^+$, 139 $[M-CH(OH)CH_2OH]^+$; IR ν_{max}^{KBr} cm^{-1}: 3340 (OH), 1584 (C=C), 1097, 1052 (C–O), 914 (=CH), 700 (C–Cl).

X-ray crystal structure analysis. $C_9H_9O_3Cl$, M = 200.5, orthorhombic, space group $P2_12_12$, $a = 32.30(3)$, $b = 6.44$ (2), $c = 4.53(2)$Å, V = 942.3, $Z = 4$, $d_c = 1.413$ g/cm^3, d_f (flotation) = 1.40(1) g/cm^3. 1205 Independent reflections [699 with $I > 2\sigma(I)$] measured for layers HkO-4 and hOL with $\theta_{max} < 25°$ on a Stoe STADI-2 two-circle diffractometer (graphite monochromated MoKα^-, $\lambda = 0.7107$ Å). The structure was solved by direct methods and refined by full-matrix least squares analysis using SHELX-76 [8]. All H-atoms except those for the C=C bond (atoms C-1 and C-2 undergo considerable thermal motion) were located in difference syntheses. Weighted anisotropic refinement (H-atoms fixed, $U_{iso} = 0.06$Å^2) converged with $R = 0.067$, $R_w = 0.078$. Atomic coordinates have been deposited with the Cambridge Crystallographic Data Centre, Lensfield Road, Cambridge, U.K.

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