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THE CHARACTERIZATION OF 6-(PENT-1-ENYL)-α-PYRONE FROM *TRICHODERMA VIRIDE*

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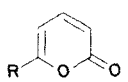
Key Word Index—*Trichoderma viride*, *Phytophthora cinnamomi*, volatile metabolites, 6-(pent-1-enyl)-α-pyrone

INTRODUCTION

Reeves and Jackson [1] demonstrated that oospore production by an A_2 compatibility-type isolate of the plant pathogenic fungus *Phytophthora cinnamomi* Rands in some soils, correlated with, and was due to, the presence of *Trichoderma viride* Pers. ex S F Gray in those same soils. It has been further shown that some isolates of *T viride* [1, 2], and of *T koningii* [3], produce volatile metabolites which induce oospore formation in pure cultures of the A_2 isolates of *P cinnamomi* growing on a variety of agar based media. During attempts to isolate and characterise the active constituent in the volatile metabolites of *T viride* it was observed that biological activity was associated with those strains possessing an aroma characteristic of coconuts The compound 6-pentyl-α-pyrone (**1**) has already been characterized as one of the constituents of the coconut-like aroma of *Trichoderma viride* [4] This metabolite has not been identified as a constituent of material extracted from culture filtrates of strains examined in these laboratories, although one compound has been shown to have the spectroscopic and chemical properties compatible with its being the related compound 6-(pent-1-enyl)-α-pyrone (**2**)



(**1**) R = Me—$(CH_2)_4$
(**2**) R = *t* Me—$(CH_2)_2$—CH = CH

RESULTS AND DISCUSSION

When it was found that ether extracts of *Trichoderma viride* culture filtrates had biological activity towards *Phytophthora cinnamomi*, the constituents were examined The residue remaining after removal of the solvent from extractions always represented a very low yield (10–30 mg/l.), in contrast to the yield of 180 mg/l of crude 6-pentyl-α-pyrone reported by Collins and Hallim [4]. The medium used by these authors was potato dextrose-based whereas the work reported here involved the use of modified Czapek Dox medium which had previously been shown to give good biological activity

GLC analysis showed a number of peaks with a single peak predominating. Initital experiments seemed to indicate that the size of this peak was correlated with biological activity; thus efforts were made to purify the compound responsible for the peak by using column chromatography. As the compound was purified it became more difficult to demonstrate the biological activity associated with the extract and it is concluded that this major constituent is probably not, on its own, responsible for the induction of oospore formation in *Phytophthora cinnamomi* After absorption onto silica gel and elution, first with *n*-pentane, then with *n*-pentane containing increasing concentrations of ether, a pale yellow oil, with the retention time on GLC of the major constituent of the crude mixture, was obtained

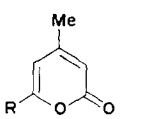
The MS gave a parent ion at *m/e* 164 and although insufficient material was available for elemental analysis, the pattern of (M + 1) and (M + 2) peaks was best explained by the molecular formula $C_{10}H_{12}O_2$ A predominant peak at *m/e* 95 indicated the possibility of a pyrone derivative The presence of an α-pyrone was

Table 1 NMR data of pyrone derivatives

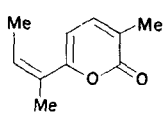
Compound	Proton chemical shifts (in ppm from TMS in $CDCl_3$) 3H	4H	5H	Ref
(1)	6·2 (*d*)	7·3 (*dd*)	5·95 (*d*)	
(2)	6·0 (*d*)	7·19 (*dd*)	5·83 (*d*)	4
(4)	5·66		5·66	5
(5)		6·94	5·9	5

further suggested by bands in the IR spectrum at 1740, 1650 and 1535 cm^{-1}. The UV spectrum showed a well defined maximum at 317 nm (ϵ *ca* 7000), indicating that the α-pyrone chromophore was further conjugated to a double bond. The compound (**3**) has been reported to have λ_{max} 295 nm (ϵ = 7000) whereas the corresponding unsaturated compound (**4**) has λ_{max} 330 nm (ϵ = 10200) [5].

The presence of peaks in the MS at *m/e* 149, 135 and 121 corresponding to $(M - Me)^+$, $(M - C_2H_5)^+$ and $(M - C_3H_7)^+$ indicate the presence of a simple side chain attached to the α-pyrone nucleus. These data are compatible with those expected of 6-(pent-1-enyl)-α-pyrone, and further confirmation for this structure comes from the NMR spectrum (see Table 1). As well as signals corresponding to the 3 protons of a 6-substituted α-pyrone the NMR spectrum of the new compound had 2 further one-proton signals at 6·00 ppm (*dt*; J_1 15; J_2 2) and 6·75 ppm (*dt*; J_1 15; J_2 7) corresponding to the olefinic protons of $-CH_2-CH=CH-$, the large coupling constant indicating a *trans* configuration.



(**3**) R = $Me_2CH=CH_2$
(**4**) R = $Me_2C=CH$



(**5**)

Further confirmation of structure (**2**) comes from the isolation of butanal and propan-2-one in the distillation products from heating the material with aqueous NaOH. These compounds could be formed by a retroaldol fragmentation of 5-keto-dec-2, 6-dienoic acid, itself formed by hydrolysis of the α-pyrone.

Finally structure (**2**) is of interest biogenetically representing a relatively simple pentaketide.

EXPERIMENTAL

Trichoderma viride (Strain T V 7) was isolated from a soil sample from Wädenswil, Switzerland and was maintained on potato dextrose agar (P D A)

Production of metabolite 20 × 250 ml flasks, each containing 100 ml of modified Czapek's soln ($NaNO_3$, 2·0, KH_2PO_4, 1·0 g; $MgSO_4$ $7H_2O$, 0·5, KCl, 0·5, $FeSO_4$ $7H_2O$, 0·1, Sucrose, 30 g/l), were each inoculated with 0·2 ml of a heavy conidial suspension Flasks were incubated at room temp on an orbital shaker (200 rpm, 25 mm throw) for 7 days Mycelium was removed by filtration and the culture filtrate (1400 ml) extracted with an equal vol of Et_2O using a continuous liquid/liquid extractor The extract was dried and evaporated to 50 ml A portion of the conc extract was retained for biological studies and 30 ml used for isolation and purification of metabolites

GLC This was carried out using a Perkin–Elmer F11 with a flame ionization detector and fitted with 2 m × 6 mm o d glass columns packed with 2½% silicone O V 1 on AW-DMCS Chromosorb G 80–100 mesh Carrier gas used was N_2 at a flow rate of 40 cm^3/min and analyses were run with the temp programmed from 100–250° at 10°/min Under these conditions the crude extract showed a number of peaks but a single component, with a retention time of 9·5 min after the appearance of Et_2O, accounted for over 90% of the material.

Column chromatography Concentrated Et_2O extract (30 ml) was further reduced to 2 ml and loaded onto a column of Si gel (30 × 2 cm) poured as a slurry in *n*-pentane The column was eluted with *n*-pentane (100 ml) followed by 100 ml each of the following mixtures of *n*-pentane–Et_2O 9·1, 5·5 and 1·9 The column was finally eluted with pure Et_2O (400 ml) Fractions (50 ml) were collected and monitored by GLC for the presence of the major metabolite, which appeared in fractions 7 and 8 contaminated only with material having a retention time of 8·6 min and accounting for about 4% of the material detected

Spectroscopic characterization Fractions 7 and 8 from the column chromatograph were bulked and the solvent removed yielding a very pale yellow oil (8 mg) This material was taken up in a few drops Et_2O, slurried with KBr powder (100 mg), Et_2O removed under vacuum and the material prepared as a KBr disc for IR (ν_{max} 1740 (broad), 1650 (with shoulders at 1640 and 1660), 1535 (with shoulder at 1545) cm^{-1}) KBr was broken up and extracted with Et_2O A portion of the extract (containing *ca* 3 mg of material) was taken up in EtOH (17 ml) and used to record a UV spectrum (λ_{max} 317 nm, ϵ *ca* 7000) The remainder was introduced into a capillary tube and used to record the MS after removal of Et_2O (164 M^+). Further material was prepared using the method outlined above and the NMR spectrum recorded at 90 MHz using $CDCl_3$ as solvent and trimethylsilane as internal standard The spectrum was complicated between δ 0–2 indicating contamination of this sample with silicon grease but the rest of the spectrum had the following characteristics δ 2·23 (2H, *qd*, J_1 7, J_2 2), δ 5·95 (1H *d*, *J* 7), 6·00 (1H, *dt*, J_1 15, J_2 2), δ 6·20 (1H, *d*, *J* 8), 6·75 (1H, *dt*, J_1 15 J_2 7), δ 7·3 (1H, *dd*, J_1 7, J_2 8)

Treatment with NaOH Material used for NMR was recovered and transferred to a 50-ml flask 1 M NaOH (20 ml) was added, the mixture brought to boiling point, and distilled over a period of 15 min to collect 10 ml of distillate to which was added 5 ml 2,4-dinitrophenylhydrazine sulphate (1%) Orange–yellow ppt formed was collected by filtration and dried (yield 4·9 mg) Material was taken up in $CHCl_3$, applied as a band to a plate of Si gel G (Merck), and resolved into

3 components using C_6H_6–EtOAc (19 1) Component I R_f 0 59 was bright yellow, co-chromatographed with propan-2-one DNP, MS 238 (M^+) MS was identical with that of pure propan-2-one DNP Component II R_f 0 72 bright yellow, co-chromatographed with butanal DNP, MS 252 (M^+) The MS was identical with that of pure butanal DNP Component III R_f 0 81, pale orange MS 306 (M^+) possibly the DNP of the unsaturated C_8 aldehyde formed by aldol condensation of 2 molecules of butanal

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LIGNANS AND SUGIOL FROM *LIBOCEDRUS BIDWILLII*

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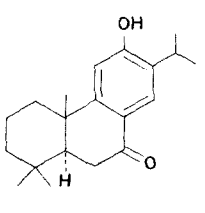
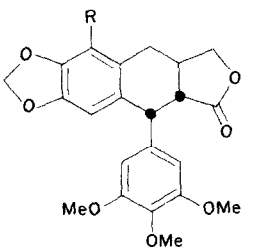
Key Word Index—*Libocedrus bidwillii*, Cupressaceae, sugiol, deoxypodophyllotoxin, β-peltatin-A methyl ether

Plant Libocedrus bidwillii Hook.f., common name, mountain ceder or pahautea *Source* Ruahine Ranges, State Forest 24, New Zealand. *Previous work* Terpenes of the essential oil [1-3]

Present work. Milled dried foliage (1·5 kg) was extracted with MeOH and the concentrate partitioned between light petroleum and MeOH–H_2O (4·1) The aqueous phase was extracted with Et_2O, after removal of the MeOH, and the Et_2O fraction was chromatographed on an alumina column with C_6H_6 followed by a silicic acid column with cyclohexane–EtOAc (4:1) This gave a series of fractions from which sugiol (**1**) mp 287°, deoxypodophyllotoxin [4,5] (**2**) mp 166–168°, $[\alpha]_D$ −114° ($CHCl_3$) and β-peltatin-*A* methyl ether [6,7] (**3**) mp 162–163° $[\alpha]_D$ −119° ($CHCl_3$) were obtained crystalline The identity of sugiol was established by direct comparison with authentic material (mmp IR, NMR, UV, MS) while the lignans were characterized from their IR, NMR, UV and MS [7,8]. Deoxypodophyllotoxin and β-peltatin-*A* methyl ether were refluxed in ethanolic sodium acetate for 18 hr to give their respective C-2 epimers Deoxypicropodophyllin mp 171–172°, $[\alpha]_D$ +31 ($CHCl_3$), and β-peltatin-*B* methyl ether mp 183–184°, $[\alpha]_D$ +9° ($CHCl_3$), were obtained in good yield



(**1**)



(**2**) R = H
(**3**) R = OMe

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