Phytochemistry, Vol. 29, No. 5, pp. 1661–1665, 1990.
Printed in Great Britain.

0031-9422/90 $3.00 + 0.00
Pergamon Press plc

THREE PIPERAZINEDIONES AND A DRIMANE DITERPENOID FROM *PENICILLIUM BREVI-COMPACTUM*

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(*Received in revised form* 20 *July* 1989)

Key Word Index—*Penicillium brevi-compactum*; fungal metabolites; isolation; piperazinedione; drimane sesquiterpene.

Abstract—Three new piperazinedione metabolites isolated from cultures of *Penicillium brevi-compactum* are described. A drimane derivative similar to macrophorin A was isolated and characterized.

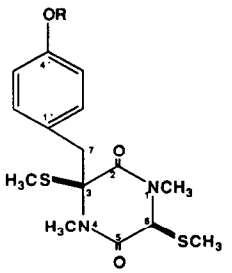
INTRODUCTION

The observation that a fungal contaminant,* later identified as *Penicillium brevi-compactum* (Dierckx), was strongly antagonistic to the Dutch elm disease fungus, *Ceratocystis ulmi* (Buisman) C. Moreau [1], prompted us to undertake an investigation of the secondary metabolites produced by this strain of *Penicillium*. Cultures of *P. brevi-compactum* provided extracts that contained several related piperazine-2,5-dione derivatives as well as mycophenolic acid [2], 1-deoxypebrolide [3], cerevisterol [4, 5], ergosterol [6], ergosterol peroxide [4], asperphenate [6], and *N*-benzoylphenylalaninol [7]. On one occasion, a drimane derivative, which showed strong antifungal activity against *C. ulmi* [8], was isolated. This report describes the structures of the piperazinedione metabolites, three of which have not been reported previously. In addition, the sesquiterpene derivative is characterized.

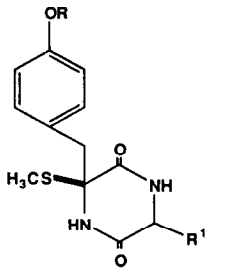
RESULTS AND DISCUSSION

The components of the toluene extract of the broth of a fermentation culture of *P. brevi-compactum* were separated by silica gel chromatography. The two most abundant metabolites in this extract co-eluted from the column and were isolated by preparative TLC. Comparison of their spectral properties revealed their similarity. The ^{1}H and ^{13}C NMR spectra of each compound has resonances associated with a 1,4-oxyphenylmethylene group, two methylthio groups, and two *N*-methyl groups. The IR and ^{13}C NMR spectra indicate the presence of two amide carbonyls. This information, together with other spectral properties revealed that these metabolites are the previously reported *cis*-bis(methylthio)silvatin (**1**) [9–11], and its parent phenol **2** [9, 11].

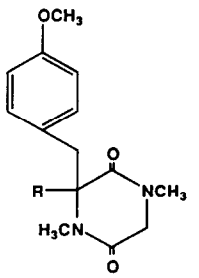
The most polar of the metabolites was crystalline (mp 205–207°) and is assigned structure **3** on the following basis. Its molecular formula, $C_{17}H_{22}O_3S$, was established by HRMS, and the presence of a 3-methylbut-2-enyl group in its ^{1}H NMR spectrum (δ4.43, *d*, 2H; 5.58, *br*, 1H; 1.70, *s*, 3H; 1.66, *s*, 3H), an *S*-methyl group (δ2.15, *s*, 3H) [12], and a 1,4-oxyphenylmethylene group (δ6.76, 2H, J = 9 Hz and 7.18, *d*, J = 9 Hz) suggested a relationship between compounds **3** and **1**. Comparison of the ^{1}H NMR spectrum of **3** with that of **1** revealed that compound **3** differs from **1** in the absence of two *N*-methyl groups and one *S*-methyl group. In addition, the C-6 methine group of **1** is replaced with a methylene group (δ3.30 and 3.79, AB) in **3**. The presence of a



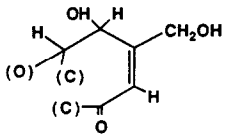
1 R=$CH_2CH=C(CH_3)_2$
2 R=H



3 R=$CH_2CH=C(CH_3)_2$, R^1=H
4 R=$CH_2CH=C(CH_3)_2$, R^1=OH



5 R=OH
6 R=H



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*We thank Dr. Y. Hiratsuka (Northern Forestry Centre, Canadian Forestry Service, Edmonton) for drawing our attention to this problem.

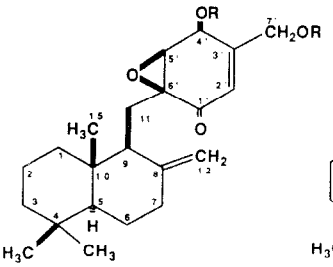
secondary amide [3180, 3070 (N-H), 1688 (–CONH) cm^{-1}] typical of a piperazinedione is indicated by the IR spectrum of **3**, while the HRMS fragmentation pattern confirms the presence of a 3-methylbut-2-enyl (*m/z* 266 [$C_{12}H_{14}N_2O_3S$]) and a thiomethyl group (*m/z* 219 [$C_{11}H_{11}N_2O_3$]) [13]. The chemical shifts of the carbon atoms corroborate the structural assignment of compound **3**.

A minor crystalline metabolite, compound **4**, was isolated from the very polar fractions of the crude extract. The molecular formula of this compound, $C_{17}H_{22}N_2O_4S$, was confirmed by CIMS and its HRMS spectrum displayed fragment ions corresponding to the loss of a 3-methylbut-2-enyl (*m/z* 282 [$C_{12}H_{14}N_2O_4S$]) and thiomethyl groups (*m/z* 235 [$C_{11}H_{11}N_2O_4$]). In addition to the strong absorption band at 1679 cm^{-1} typical of a pyrazinedione, the IR spectrum of **4** showed bands at 3325, 3200 and 3090 cm^{-1} suggesting the presence of an alcohol and secondary amides. The ^1H NMR spectrum of **4** confirmed the presence of the methylbutenyl, 1,4-oxyphenylmethylene, and thiomethyl groups. In addition, signals were observed which were assigned to an isolated secondary amide (δ8.82, *s*) and a secondary alcohol adjacent to an amide nitrogen (δ6.79, *d*, J = 6 Hz, OH; 4.44, *dd*, J = 4, 6 Hz, H-6; 8.61, *d*, J = 4 Hz, NH). The hydrogens on the hetero atoms exchanged with D_2O and couplings between hydrogens were confirmed by spin decoupling experiments.

One further piperazinedione metabolite, compound **5**, was isolated. Its IR spectrum showed the presence of hydroxyl (3360 cm^{-1}) and tertiary amide carbonyl (1670 cm^{-1}) bands. The ^1H NMR spectrum of **5** indicated a 1,4-oxyphenylmethylene group, two *N*-methyl groups (δ2.79, *s*, and 3.13 *s*, an isolated methylene group (δ2.34 coupled to 3.39, J = 17.5 Hz), and a methoxyl group (δ3.78). These data are consistent with a *cyclo*-glycyltyrosine structure with an oxygen substituent at C-3. The substituent at C-3 is assigned as the hydroxyl substituent based on a comparison of the chemical shift of C-3 (δ85.4) and C-4′ (δ159.7) in the ^{13}C NMR of compound **5** with that of model compound **6** (no OH at C-3: δ63.6; OMe at C-4′: δ159.4) (prepared in a two step sequence from L-tyrosine ethyl ester using the method of Kawai [14]) and co-metabolite **2** (SMe at C-3: δ75.7; OH at C-4′: δ156.4) [9–11].

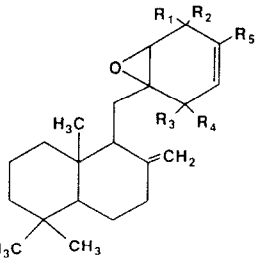
Isolation of this array of metabolites from a single organism suggests that they arise from a common biogenetic precursor such as a *cyclo*-glycyltyrosine. Conversion to a dithiol derivative [15] may precede the formation of thiomethylated or epidisulphide compounds [9–11], with *N*-methylation and *O*-prenylation occuring later in the biosynthetic sequence.

The bioactive fraction of the broth extract contained one major component. Purification by silica gel chromatography and fractional crystallization gave a crystalline compound (mp 124–126°) to which we assign structure **7**. The derivation of structure **7** is based on chemical and spectral analysis of the metabolite and some of its derivatives. Compound **7** ($C_{22}H_{32}O_4$, established by HRMS and CIMS) strongly inhibits the growth of *C. ulmi* when assayed by the disk diffusion method and this activity is comparable to that reported for other suggested control agents (e.g. hyalodendrin) of *C. ulmi* [16]. It contains exocyclic methylene (^{13}C NMR: 149, *s*, =C and 107, *t*, $=CH_2$), and a β,β-disubstituted α,β-unsaturated carbonyl functional groups (IR: 1680 cm^{-1}, ^{13}C NMR: δ194, *s*, C=O; 159, *s*, =C; 120, *d*, =CH; UV: λ_{max} 230 nm) [13]. The presence of these functionalities was verified in the following way. Treatment of **7** with lithium aluminium hydride gave a dihydro derivative **8** ($C_{22}H_{34}O_4$) whose IR spectrum shows hydroxyl (3380 cm^{-1}) but no carbonyl absorption bands whereas catalytic hydrogenation of **7** gave tetrahydro derivative **9** ($C_{22}H_{36}O_4$) whose IR spectrum shows hydroxyl and saturated carbonyl absorption bands (3400, 1730 (sh), 1710 cm^{-1}). Compound **7** contains two allylic hydroxyl groups (IR: 3400 cm^{-1}; ^1H NMR: δ4.57, 1H and 4.31, 2H) because acetylation (Ac_2O, pyridine) provided a diacetyl derivative **10** (IR: 1750, 1685, 1645 cm^{-1}). The primary and secondary nature of the hydroxyl groups was established on the basis of analysis of the acetoxyl-induced anisotropic shifts [12] of the hydrogens geminal to the hydroxyl groups of **7** and its diacetyl derivative **10** (Δ0.33 and 1.27, respectively). The allylic nature of the alcohols was verified by oxidation of **7** with manganate dioxide. Two products of molecular formula $C_{22}H_{30}O_4$ were obtained. One product, compound **11**, has enedione and primary hydroxyl groups (IR: 3440, 1710 cm^{-1}; ^1H NMR δ4.56, 4.36, each *dd*, CH_2OH) because acetylation gives a monoacetyl derivative **12** (IR 1750, 1710 cm^{-1}) whose ^1H NMR spectrum shows the characteristic acetoxyl shift for the methylene hydrogens (Δ=0.33). Compound **13**, the other oxidation product has hydroxyl, enone and aldehyde functional groups (IR: 3420, 2920, 1685 cm^{-1}; ^1H NMR δ9.71, *s*, CHO). These data indicate that the fourth oxygen of **7** must be present as an ether because its ^{13}C NMR spectrum shows signals corresponding to five carbons bearing oxygen; three previously assigned to the allylic alcohols and carbonyl groups. Extensive spin decoupling experiments [8] on **7** and its diacetyl derivative together with analysis the their ^{13}C NMR spectra allow the identification of partial structure **A** which includes all of the oxygen functionality of this compound. Detailed examination of the HRMS fragmentation of compound **7** led us to conclude that the



7 R=H

10 R=$COCH_3$

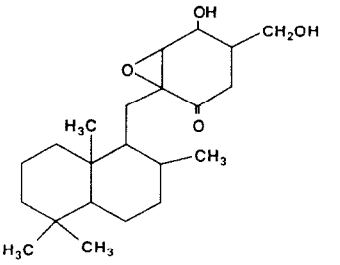


8 R_1=R_3=H
R_2=R_4=OH
R_5=CH_2OH

11 R_1,R_2=O R_3,R_4=O
R_5=CH_2OH

12 R_1,R_2=O R_3,R_4=O
R_5=CH_2OCOCH_3

13 R_1=H R_2=OH R_3,R_4=O
R_5=CHO



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molecule was divided into two distinct parts: one with 14–15 carbons and a smaller fragment carrying all the oxygen functionalities. This provides further evidence for partial structure **A**. The fragmentation pattern of the 15 carbon portion is reminiscent of the fragmentation pattern of a drimane sesquiterpene [17], and indeed the carbon shifts in the ^{13}C NMR of **7** are virtually identical with the corresponding carbon chemical shifts reported for several labdane derivatives including biformen [18], *epi*-manool, and methyl copalate [19]. Combination of the two structural fragments, a drimane and partial structure **A**, allowed us to assign the structure of the bioactive compound to be that shown in structure **7**. The stereochemistry of the drimane portion of compound **7** is assigned as shown since the epimeric form, if known, is extremely rare. The absolute stereochemistry of the cyclohexenone portion of **7** may be assigned as the same as that of epoxydon [20] because both compound **7** and (+)-epoxydon show positive Cotton effects in their CD spectra at 332 nm and 341 nm, respectively, and negative Cotton effects at 253 and 240 nm, respectively. The ^1H NMR spectrum of **7** shows H-5′ as a doublet ($J = 3$ Hz). In model cyclohexonene compounds the analogous hydrogen is a doublet with a 2.5–3 Hz coupling [20] and this suggests that the epoxide and H-4′ hydroxyl groups in **7** are *cis*. A structurally similar compound, macrophorin A [21], has been previously reported. It is possible that compound **7** is identical with macrophorin A, but we were unable to make a direct comparison since our supply of **7** was exhausted. Further work on the structure of compound **7** was not possible because of lack of material and the fact that subsequent cultures of *P. brevi-compactum* did not contain this compound.

EXPERIMENTAL

General. MS: data is reported as *m/z* (rel. int.). Unless diagnostically significant, peaks with intensities less than 20% of the base peak are omitted. ^1H NMR spectra were at 100, 200 or 400 MHz, and ^{13}C NMR were measured on a HFX-90 spectrometer interfaced to a Nicolet 1085 computer, a Varian HA-100 spectrometer interfaced to a Digilab FTS/NMR-3 data system or a Bruker WH-200 spectrometer. All NMR measurements employed TMS as int. standard and are reported in δ downfield from TMS. The multiplicity of C signals were determined with the off-resonance coherent proton-decoupling experiment or by a modified APT experiment [22]. Mps: uncorr.

TLC plates were 0.5 mm silica gel G (E. Merck, Darmstadt) containing 1% electronic phosphor (General Electric, Cleveland). Compounds were detected by viewing under UV light or by spraying with a solution of vanillin (1%) in conc H_2SO_4 and charring. Flash chromatography [23] was performed using silica gel 60 (Merck, 40–63, μm).

Plant materials. A culture of *Penicillium brevi-compactum* (isolated as an air contaminant on a culture of *Ceratocystis ulmi*) used in this study was obtained from Y. Hiratsuka (Northern Forestry Centre, Edmonton) and is on deposit in their culture collection under accession number C662. The fungus was maintained at 4° in slant tubes containing Czapek SM agar medium. An aq. suspension of mycelium was used to inoculate agar plates (10% mixed cereal, 2% agar in water). After 7–10 days at room temp., the mycelial growth from the agar plates was used to inoculate liquid shake cultures containing Czapek SM medium (2 × 250 ml). Inocula prepared in this way were used to inoculate still cultures grown in Fernbach flasks (10 × 1 l) for 4 weeks, or 10 l of medium in a microferm laboratory fermentor for 8 days (200 r.p.m., aeration at 2.5 l/min, 25°). The fungal culture was harvested in two different ways; either by exhaustive extraction of the total culture with EtOAc or by separately extracting the mycelium and the broth. The broth was concd to *ca* 1 l, filtered through celite, and extracted consecutively with toluene, Et_2O, and *n*-BuOH.

Isolation of metabolites. The toluene extract (170 mg) from a 10 l fermentation culture was chromatographed (silica gel, 20 g) providing impure compounds **1** (Me_2CO–toluene, 1:4), impure **5** (Me_2CO–toluene, 7:13), **2** (70 mg, Me_2CO–toluene, 1:1), and **3** (10 mg, MeOH). Compounds **1** and **5** were purified by prep. TLC (toluene–Me_2CO–HOAc, 69:30:1) to give 70 and 2 mg, respectively.

The EtOAc extract was chromatographed (silica gel). On one occasion only elution with EtOAc–CH_2Cl_2 (2:3) gave a fraction rich in one component from which compound **7** (60 mg) was isolated by fractional crystallization. Generally one of the more polar fractions from this chromatography (eluant $CHCl_3$) gave compound **4** (5 mg).

cis-*Bis(methylthio) silvatin* (**1**). Yellow solid from C_6H_6–petrol, mp 63–65°; $[\alpha]_D^{26} - 39.5°$ (*c* 1.36, $CHCl_3$) (reported 100–102°[10]). Identical by IR, UV, ^1H NMR with published spectral data.

cis-1,4-*Dimethyl*-3,6-*bis*(*methylthio*)-3-(4′-*hydroxyphenylmethyl*)-2,5-*piperazinedione* (**2**). Yellow crystals from C_6H_6–petrol, mp 154.5–156°; $[\alpha]_D^{26} - 27.6°$ ($CHCl_3$; *c* 0.642) (reported 68–69° [11]). Identical by IR, UV, ^1H NMR with published spectral data.

3-*Thiomethyl*-3-[4′-(3″-methyl-2″-*butenoyl*) *phenylmethyl*]-2,5-*piperazinedione* (**3**). Solid from CH_2Cl_2, mp 205–207°. $[\alpha]_D + 8.4°$ ($CHCl_3$; *c* 0.020); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3180, 3070, 2920, 2870, 1688, 1510, 1455, 1375, 1245. UV λ_{max}^{MeOH} nm (log ε): 207 (4.15), 229 (4.18), 277 (3.60). ^1H NMR (DMSO-d_6): δ 8.84 (1H, *s*, N-1H), 8.01 (1H, *br s*, $W_{1/2} = 5$ Hz, N-4H), 7.18 (2H, *d* $J = 9$ Hz, H-3′, H-5′), 6.76 (2H, *d*, $J = 9$ Hz, H-2′, H-6′), 5.58 (1H, *br t*, $J = 6$ Hz, H-2″), 4.43 (2H, *d*, $J = 6$ Hz, H-1″), 3.79 (1H, *d*, $J = 18$ Hz, H-6β), 3.41 (1H, *d*, $J = 13$ Hz, H-7α), 3.30 (1H, *dd*, $J = 3$, 18 Hz, H-6α), 2.81 (1H, *d*, $J = 13$ Hz, H-7β), 2.15 (3H, *s*, SMe), 1.70 (3H, *s*, H-4″), 1.66 (3H, *s*, H-5″). ^{13}C NMR (DMSO-d_6): δ 166.0 (C-2), 164.5 (C-3), 157.5 (C-4″), 136.7 (C-3″), 131.7 (C-3′, C-5′), 127.3 (C-1′), 120.1 (C-2″), 114.1 (C-2′, C-6′), 66.5 (C-3), 64.3 (C-1″), 44.3 (C-6), 41.6 (C-7), 25.3 (C-5″), 17.9 (C-4″), 12.6 (SMe). MS *m/z* (rel. int.): 334 $[M]^+$ (1), 266 $[M + H - C_5H_9]^+$ (13), 219 $[M + H - C_5H_9 - SMe]^+$ (62), 218 $[M - C_5H_9 - SMe]^+$ (57), 175 $[M - C_5H_7N_2O_2S]^+$ (23) 160 $[M + H - C_{12}H_{15}]^+$ (30), 159 $[M - C_{12}H_{15}O]^+$ (27), 107 $[M - 227]^+$ (100); exact mass calc for $C_{17}H_{22}N_2O_3S$: 334.1352; found (MS): 334.1371.

6-*Hydroxy*-3-*methylthio*-3-[4′-(3″-*methyl*-2″-*butenoxy*) *phenylmethyl*]-2,5-*piperazinedione* (**4**). Soft needles from $CHCl_3$, mp > 290° dec. IR ν_{max}^{film} cm^{-1}: 3325, 3200, 3090, 3055, 2975, 2880, 1679, 1510, 1450, 1060, 820. ^1H NMR (DMSO-d_6) δ 8.82 (1H, *s*, N-1H, D_2O exchangeable), 8.61 (1H, *d*, $J = 4$ Hz, N-4H D_2O exchangeable), 7.14 (2H, *d*, $J = 9$ Hz, H-3′, H-5′), 6.79 (1H, *d*, $J = 6$ Hz, OH, D_2O exchangeable, 6.76 (2H, *d*, $J = 9$ Hz, H-2′, H-6′), 5.39 (1H, *br*, $J = 8$ Hz, H-2″), 4.44 (2H, *d*, $J = 8$ Hz, H-1″), 4.44 (1H, *dd*, $J = 4,6$, Hz, H-6α), 3.33 (1H, *d*, $J = 14$ Hz, H-7α), 2.86 (1H, *d*, $J = 14$ Hz, H-7β), 2.18 (3H, *s*, SMe), 1.73 (3H, *s*, H-4″), 1.68 (3H, *s*, H-5″). MS *m/z* (rel. int.): 350 $[M]^+$ (1), 282 $[M + H - C_5H_9]^+$ (9), 264 $[M + H - C_5H_9 - H_2O]^+$ (7), 235 $[M + H - C_5H_9 - SMe]^+$ (19), 234 $[M - C_5H_9 - SMe]^+$ (17), 175 $[M - C_5H_7N_2O_3S]^+$ (14), 158 $[M + H - C_{12}H_{15}O - H_2O]^+$ (4), 107 $[M - 243]^+$ (100); exact mass calcd for $C_{17}H_{22}N_2O_4S$: 350.1301; Found (MS): 350.1300.

1,4-*Dimethyl*-3-*hydroxy*-3-(4′-*methoxyphenylmethyl*)-2,5-*piperazinedione* (**5**). Prisms from CH_2Cl_2, mp 166–167°.

IR ν_{max}^{film} cm^{-1}: 3360, 2935, 2840, 1670, 1605, 1515, 1455, 1395, 1300, 1240, 1180, 1155, 1120, 1095, 1025, 600, 570. UV λ_{max}^{MeOH} nm (log ε): 213 (3.82), 228 (3.94), 276 (3.08). ^{1}H NMR ($CDCl_3$): δ6.94 (2H, *d*, J = 8 Hz, H-3′, H-5′), 6.80 (2H, *d*, J = 8 Hz, H-2′, H-6′), 4.42 (1H, *br s*, $W_{1/2}$ = 13 Hz, OH), 3.78 (3H, *s*, OMe), 3.39 (1H, *d*, J = 17.5 Hz, H-6β), 3.13 (3H, *s*, NMe), 3.08 (1H, *d*, J = 8 Hz, H-7α), 2.95 (1H, *d*, J = 8 Hz, H-7β), 2.79 (3H, *s*, NMe), 2.34 (1H, *d*, J = 17.5 Hz, H-6α). ^{13}C NMR ($CDCl_3$): δ167.2 (C-2), 162.9 (C-5), 159.7 (C-4′), 131.1 (2, C-3′, C-5′), 125.6 (C-1′), 114.2 (2, C-2′, C-6′), 85.4 (C-3), 55.3 (OMe), 51.4 (C-6), 44.7 (C-7), 33.3 (NMe), 27.3 (NMe). MS *m/z* (rel. int.): 278 $[M]^+$ (1), 261 $[M+H-H_2O]^+$ (11), 260 $[M-H_2O]^+$ (8), 157 $[M-C_8H_9O]^+$ (23), 121 $[M-C_6H_9N_2O_3]^+$ (100); exact mass calcd for $C_{14}H_{18}N_2O_4$: 278.1267; found (MS): 278.1258.

(*s*)-1,4-*Dimethyl*-3-(4′-*methoxyphenylmethyl*)-2,5-*piperazinedione* (**6**). L-Tyrosine ethyl ester was reacted with $ClCH_2COCl$ and then satd. NH_3 based on Kawai's procedure [14]. The reaction product was dissolved in DMSO–THF, NaH was added followed by excess Me_2SO_4. Work-up in the usual way afforded compound **6** as needles, mp 133–135°. IR ν_{max}^{film} cm^{-1}: 2920, 1680, 1610, 1510, 1495, 1460, 1400, 1335, 1245, 1175. ^{1}H NMR ($CDCl_3$): δ6.97 (2H, *d*, J = 8 Hz, H-3′, H-5′), 6.82 (2H, *d*, J = 8 Hz, H-2′, H-6′), 4.15 (1H, *d*, J = 4 Hz, H-3), 3.79 (3H, *s*, OMe), 3.33 (1H, *d*, J = 17 Hz, H-6β), 3.22 (1H, *dd*, J = 3.5, 14 Hz, H-7α), 3.06 (3H, *s*, NMe), 3.04 (1H, *dd*, J = 4, 14 Hz, H-7β), 2.74 (3H, *s*, NMe), 2.38 (1H, *d*, J = 17 Hz, H-6α); ^{13}C NMR ($CDCl_3$): δ165.8 (C-2), 164.2 (C-5), 159.4 (C-4′), 130.8 (C-3′, C-5′), 126.4 (C-1′), 114.0 (C-2′, C-6′), 63.6 (C-3), 55.2 (OMe), 50.9 (C-6), 36.2 (C-7), 32.2 (NMe), 30.0 (NMe). Exact mass calcd for $C_{14}H_{18}N_2O_3$: 262.1317; found (MS): 262.1315.

11-(5′-*epoxy*-4′-*hydroxy*-3′-*hydroxymethylcyclo*-2′-hexenone)-$\Delta^{8(12)}$-*Drimene* (**7**). White crystals from Et_2O-pet Et_2O, mp 124–126°, $[\alpha]_D$ + 35° ($CHCl_3$; *c* 0.22), ORD (MeOH; *c* 0.43) $[\phi]_{530}$ 0°, $[\phi]_{360}$ + 4650°, $[\phi]_{340}$ 0°, $[\phi]_{300}$ − 9040°, $[\phi]_{250}$ − 13800, $[\phi]_{340}$ 0°; CD (MeOH; *c*, 0.43) $[\theta]_{381}$ 0, $[\theta]_{332}$ 9540 $[\theta]_{226}$ 0, $[\theta]_{253}$ − 5520, $[\theta]_{220}$ 0. IR ν_{max}^{film} cm^{-1}: 3400, 3080, 2940, 1680, 1460, 1440, 1385, 1275, 1200, 1100, 1030, 882, 665. UV λ_{max} nm (log ε): 221 (4650), 239 (8250). ^{1}H NMR ($CDCl_3$): δ5.96 (1H, *br s*, $W_{1/2}$ = 3 Hz, H-3′), 4.81 (1H, *br s*, $W_{1/2}$ = 3 Hz, H-12), 4.57 (2H, *br s*, H-12, H-5), 4.31 (2H, *br s*, $W_{1/2}$ = 3 Hz, H-4′), 3.71 (1H, *d*, J = 3 Hz, H-6′), 0.89 (3H, *s*, H-13), 0.82 (3H, *s*, H-14), 0.72 (3H, *s*, H-15). ^{13}C NMR ($CDCl_3$): δ 194.3 (*s*, C-1′), 158.9 (*s*, C-3′), 149.4 (*s*, C-8), 120.2 (*d*, C-2′), 106.8 (*t*, C-12), 65.3 (*d*, C-4′), 61.8 (*t*, C-7′), 61.2 (*d*, C-5′), 60.7 (*s*, C-6′), 55.7 (*d*, C-5), 51.8 (*d*, C-9), 42.3 (*t*, C-3), 39.9 (*s*, C-10), 39.0 (*t*, C-1), 38.3 (*t*, C-7), 33.7 (*s*, C-4), 33.6 (*q*, C-14), 24.7 (*t*, C-6), 21.8 (*q*, C-13), 21.3 (*t*, C-11), 19.5 (*t*, C-2), 14.7 (*q*, C-15). MS *m/z* (rel. int.): 360 $[M]^+$ (2), 342 (10), 327 (7), 313 (6), 206 (3), 205 (5), 191 (12), 177 (12), 167 (15), 152 (19), 137 (86), 123 (32), 109 (28), 107 (25), 105 (24), 95 (49), 93 (29), 91 (33), 81 (60), 79 (33), 77 (25), 69 (71), 67 (38), 66 (70); exact mass calcd for $C_{22}H_{32}O_4$ 360.2301, found 360.2290.

$LiAlH_4$ *reduction of compound* **7**. Compound **7** (8.5 mg) in dry Et_2O (10 ml) was cooled to 0° and excess $LiAlH_4$ was added. The stirred reaction mixture was warmed to room temp. for 1 hr, then H_2O (10 ml) added. The aq. fraction was extracted with Et_2O (3 × 10 ml), the Et_2O extracts combined, dried ($MgSO_4$), and concd. Separation by prep. TLC (silica gel, $CHCl_3$–MeOH–HOAc, 84:15:1) gave compound **8** (3 mg). IR ν_{max}^{film} cm^{-1}: 3380, 2920, 1640, 1455, 1435, 1380, 1360, 1205, 1020, 875, 750. ^{1}H NMR ($CDCl_3$): δ5.51 (1H, *m*, H-2′), 4.84 (1H, *m*, H-12), 4.81 (1H, *m*, H-4′), 4.64 (1H, *br s*, H-12), 4.43 (1H, *m*, H-1′), 4.22 (≅ 4H, *br s*, $W_{1/2}$ = 7 Hz, H-7′, OH), 3.53 (1H, *d*, J = 3.5 Hz, H-5′), 0.87 (3H, *s*, H-13), 0.80 (3H, *s*, H-14), 0.70 (3H, *s*, H-15); MS *m/z* (rel. int.): 344 $[M-H_2O]^+$ (6), 326 (6), 206 (5), 204 (8), 191 (42), 190 (20), 189 (22), 140 (15), 137 (100), 136 (24), 123 (42), 121 (31), 120 (20), 109 (37), 107 (33), 95 (60), 93 (38), 91 (34), 81 (72), 79 (38), 69 (79), 67 (44), 55 (66).

Catalytic reduction of compound **7**. Compound **7** (10 mg) in MeOH (15 ml) was hydrogenated over 5% Pd-C (30 mg) in a Parr hydrogenator at room temp. for 1 hr. The reaction mixture was filtered through celite and concd. The product was purified by prep. TLC (silica gel, $CHCl_3$–MeOH–HOAc, 89:10:1) to give tetrahydro derivative **9**. IR ν_{max}^{film} cm^{-1}: 3400, 2920, 1730, 1710, 1460, 1380, 1260, 1040. ^{1}H NMR ($CDCl_3$): δ4.18 (1H, *br m*, H-4′), 3.72 (2H, *br m*, H-7′), 3.18 (1H, *d*, J = 3 Hz, H-5′), 0.92 (3H, *d*, J = 3 Hz, H-12), 0.83 (6H, *s*, H-13, H-14), 0.81 (3H, *s*, H-15). MS *m/z* (rel. int.): 364 $[M]^+$, (1), 206 (83), 191 (46), 144 (40), 137 (25), 131 (37), 126 (37), 123 (100), 109 (44), 99 (45), 97 (20), 95 (54), 83 (25), 81 (68), 69 (85), 57 (27), 55 (82); exact mass calcd for $C_{22}H_{36}O_4$ 364.2593, found 364.2603.

Acetylation of compound **7**. The diacetyl derivative, compound **10**, was prepared using standard acetylation conditions. IR ν_{max}^{film} cm^{-1}: 2960, 1750, 1685, 1645, 1460, 1440, 1370, 1240, 1100, 1040, 885, 760. UV λ_{max}^{MeOH} nm (ε): 229 (10,400); ^{1}H NMR ($CDCl_3$): δ6.04 (1H, *m*, $W_{1/2}$ = 4 Hz, H-2′), 5.84 (1H, *br s*, $W_{1/2}$ = 6 Hz, H-4′), 4.82 (1H, *br s*, $W_{1/2}$ = 4 Hz, H-12), 4.77 (1H, *br d*, J = 15 Hz, H-7′), 4.56 (1H, *br d*, J = 15 Hz, H-7′), 4.48 (1H, *br s*, $W_{1/2}$ = 4 Hz, H-12), 3.75 (1H, *d*, J = 3 Hz, H-5′), 2.19 (3H, *s*, OAc), 2.08 (3H, *s*, OAc), 0.87 (3H, *s*, H-13), 0.80 (3H, *s*, H-14), 0.71 (3H, *s*, H-15). ^{13}C NMR ($CDCl_3$): δ 192.1 (*s*, C-1′), 169.9 (*s*, OCOMe), 169.7 (*s*, OCOMe), 148.7 (*s*, C-8), 147.3, 124.0 (*d*, C-2′), 106.6 (*t*, C-12), 66.3, 62.1, 59.7, 56.9, 55.2 (*d*, C-5), 51.0 (*d*, C-9), 41.8 (*t*, C-3), 39.4 (*s*, C-10), 38.7 (*t*, C-1), 37.8 (*t*, C-7), 33.3 (2, C-4, C-14), 24.1 (*t*, C-6), 21.5 (*q*, C-13), 20.5 (*t*, C-11), 20.3 (*q*, COMe), 20.0 (*q*, COMe), 19.1 (*t*, C-2), 14.3 (*q*, C-15); MS *m/z* (rel. int.): 444 $[M]^+$ (1), 429 (2), 384 (4), 342 (14), 324 (20), 309 (11), 295 (5), 233 (6), 205 (9), 203 (11), 195 (16), 191 (8), 189 (24), 137 (100), 136 (22), 123 (37), 121 (30), 119 (23), 109 (30), 107 (29), 105 (25), 95 (59), 93 (32), 91 (30), 81 (66), 79 (31), 69 (69), 55 (51); exact mass calcd for $C_{26}H_{36}O_6$ 444.2512; found 444.2511.

Oxidation of compound **7**. Compound **7** (10 mg) was stirred in dry Et_2O (15 ml) with activated MnO_2 (60 mg) for 2.5 hr at room temp. The reaction mixture was filtered through celite and the filtrate concd. The residue was purified by prep. TLC (silica gel, Et_2O–petrol, 7:3) to give enedione **11** (3 mg) and enone aldehyde **13** (3 mg) [8].

Compound **11**. IR ν_{max}^{film} cm^{-1}: 3440, 2960, 1710 (sh), 1687, 1460, 1440, 1385, 1200, 885. ^{1}H NMR ($CDCl_3$): δ6.65 (1H, *d*, J = 2 Hz, H-2′), 4.84 (1H, *d*, J = 1 Hz, H-12), 4.56 (1H, *dd*, J = 2, 16 Hz, H-7′), 4.53 (1H, *d*, J = 1 Hz, H-12), 4.36 (1H, *dd*, J = 2, 16 Hz, H-7′), 3.75 (1H, *s*, H-5′), 0.87 (3H, *s*, H-13), 0.80 (3H, *s*, H-14), 0.71 (3H, *s*, H-15); MS *m/z* (rel. int.): 358 $[M]^+$ (4), 343 (8), 340 (5), 273 (4), 204 (7), 203 (17), 189 (20), 137 (100), 123 (31), 109 (22), 95 (37), 91 (20), 81 (41), 69 (48), 67 (21), 55 (35); exact mass calcd for $C_{22}H_{30}O_4$ 358.2144; found 358.2146.

Compound **13**. IR ν_{max}^{film} cm^{-1}: 3420, 2920, 1685, 1455, 1440, 1380, 1090, 1035, 880, 755. ^{1}H NMR ($CDCl_3$): δ9.72 (1H, *s*, H-7′), 6.48 (1H, *d*, J = 1 Hz, H-2′), 4.98 (1H, *m*, $W_{1/2}$ = 8 Hz, H-4′), 4.84 (1H, *br s*, H-12), 4.55 (1H, *br s*, H-12), 3.61 (1H, *d*, J = 4 Hz, H-5′), 0.88 (3H, *s*, H-13), 0.81 (3H, *s*, H-14), 0.72 (3H, *s*, H-15). MS *m/z* (rel. int.): 358 $[M]^+$ (14), 343 (22), 340 (11), 311 (14), 273 (10), 204 (6), 203 (11), 191 (19), 189 (24), 137 (100), 123 (40), 121 (23), 119 (23), 109 (29), 107 (25), 105 (36), 95 (49), 93 (33), 91 (55), 81 (61), 79 (44), 77 (35), 69 (87), 67 (43), 55 (76), 53 (29); exact mass calcd for $C_{22}H_{30}O_4$ 358.2144; found 358.2155.

Acetylation of compound **11**. The acetyl derivative, compound **12**, was prepared using standard acetylation conditions. IR ν_{max}^{film} cm^{-1}: 2920, 1750, 1685, 1640, 1460, 1440, 1365, 1220, 880. ^{1}H NMR ($CDCl_3$): δ6.51 (1H, *d*, J = 2 Hz, H-2′), 4.96 (1H, *dd*, J = 2, 16 Hz, H-7′), 4.82 (1H, *br s*, H-12), 4.77 (1H, *dd*, J = 2, 16 Hz, H-7′), 4.52 (1H, *br s*, H-12), 3.76 (1H, *s*, H-5′), 2.13 (3H, *s*,

OAc), 0.88 (3H, *s*, H-13), 0.81 (3H, *s*, H-14), 0.71 (3H, *s*, H-15). MS *m/z* (rel. int.): 400 $[M]^+$ (2), 385 (6), 322 (10), 233 (3), 204 (4), 189 (14), 137 (62), 123 (31), 109 (29), 107 (20), 105 (21), 97 (30), 95 (45), 93 (23), 91 (28), 85 (35), 83 (37), 81 (51), 79 (27), 71 (52), 69 (100), 67 (33), 57 (89), 55 (83); exact mass calcd for $C_{24}H_{32}O_5$ 400.2250; found 400.2259.

Biological assay of P. brevi-compactum *metabolites. Ceratocystis ulmi* was grown on agar plates until it was established. Paper disks (6 mm, Schleicher and Schuell) were soaked in test soln (test compound dissolved in MeOH), air-dried, then placed at least 15 mm from the growing edge of the fungus. Compound **7** showed the following zone radii of inhibition (concentration in mg/ml of **7** in MeOH): 7 mm (0.3), 8 mm (0.6), 9 mm (1.2), 8 mm (2.4), 8 mm (4.8).

Acknowledgements—We gratefully acknowledge the financial support of the Natural Sciences and Engineering Research Council of Canada. We thank Drs Y. Hiratsuka and A. Tsuneda, Canadian Forestry Service, for bringing this problem to our attention and R. Andrade for helpful discussions.

REFERENCES

1. Stipes, R. J. and Campana, R. J. (1981) in *Compendium of Elm Disease*, p. 2. American Phytopathological Society, Department of Agriculture, U.S.
2. Harrison, W., Shearer, H. M. M. and Trotter, J. (1972) *J. Chem. Soc., Perkin Trans II* 1542.
3. McCorkindale, N. J., Calzadilla, C. H., Hutchinson, S. A., Kitson, D. H., Ferguson, G. and Campbell, I. M. (1981) *Tetrahedron* **37**, 649.
4. Sebryakov, E. P., Simolin, A. V., Kucherov, V. F. and Rosynov, B. V. (1970) *Tetrahedron* **26**, 5215.
5. Valisolalao, J. Luu, B. and Ourisson, G. (1983) *Tetrahedron* **39**, 2785.
6. Bird, B. A. and Campbell, I. M. (1982) *Appl. Environ. Microbiol.* **43**, 345.
7. Doefler, D. L., Bird, B. A. and Campbell, I. M. (1981) *Phytochemistry* **20**, 2303.
8. van Altena, I. A. (1982), Ph.D. Thesis, The University of Alberta, Edmonton, Alberta, Canada.
9. Kirby, G. W., Rao, G. V. and Robins, D. J. (1988) *J. Chem. Soc., Perkin Trans I* 301.
10. Kawahara, N., Nozawa, K., Nakajima, S. and Kawai, K. (1987) *J. Chem. Soc., Perkin Trans I* 2099.
11. Hanson, J. R. and O'Leary, M. A. (1981) *J. Chem. Soc., Perkin Trans I* 218.
12. Jackman, L. M. and Sternall, S. (1969) in *Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* 2nd Edn, p. 176. Pergamon Press, Oxford.
13. Silverstein, R. M., Bassler, G. C. and Morril, T. C. (1981) in *Spectrometric Identification of Organic Compounds.* John Wiley, New York.
14. Kawai, T. (1928) *Acta Schol. Med. Univ. Imp. Kyoto* **11**, 131. *Chem. Abs.* (19) 23: 2195.
15. Kirby, G. W., Robins, D. J. and Stark, W. M. (1983) *J. Chem. Soc. Chem. Commun.* 812.
16. Dunn, A. W., Johnstone, R. A. W., Sklarz, B. and King, T. J. (1976) *J. Chem. Soc., Chem. Commun.* 270.
17. Ayer, W. A. and Fung, S. (1977) *Tetrahedron* **33**, 2771.
18. Bohlmann, F. and Czerson, H. (1979) *Phytochemistry* **18**, 115.
19. Wehrli, F. W. and Nishida, T. (1979) in *Progress in the Chemistry of Organic Natural Products*, Vol. 36 (Herz, W., Grisebach, H. and Kirby, G. W., eds.), p. 55. Springer, New York.
20. Sekiguchi, J. and Gaucher, G. M. (1979) *Biochem. J.* **182**, 445.
21. Sassa, T. and Yoshikoshi, H. (1983) *Agric. Biol. Chem.* **47**, 187.
22. Brown, D. W., Nakashima, T. T. and Rabenstein, D. L. (1981) *J. Magn. Reson.* **45**, 302.
23. Still, W. C., Kahn, M. and Mitra, A. (1978) *J. Org. Chem.* **43**, 2923.