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NOVEL DITERPENOID INSECT TOXINS FROM A CONIFER ENDOPHYTE

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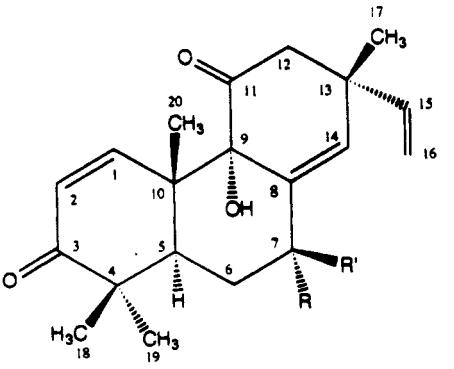
ABSTRACT.—The structures of two new insect toxins from an unidentified endophyte, originating from a needle of the balsam fir (*Abies balsamea*), have been determined by 1D and 2D nmr methods as 9α-hydroxy-1,8(14),15-isopimaratrien-3,7,11-trione [**1**] and 9α-hydroxy-1,8(14),15-isopimaratrien-3,11-dione [**2**]. Both compounds are toxic to spruce budworm (*Choristoneura fumiferana*) cells and larvae.

Earlier we reported the isolation and structure elucidation of several insect toxins from cultures of two endophytic fungi (*Hormononema dermatioides* and a *Phyllosticta* sp.) originating from needles of balsam fir [*Abies balsamea* (L.) Mill. (Pinaceae)] (1). This was the first report of the identification of insect toxins from fungal endophytes of woody plants. In a continuation of our search for naturally occurring insect toxins, we have isolated and identified two novel diterpenoid toxins [**1** and **2**] from the culture filtrates of an unidentified endophyte,[1] obtained from a needle of a balsam fir.

RESULTS AND DISCUSSION

Compound **1** was isolated as an optically active, white crystalline [mp 173–174°, $[\alpha]_D$ −213°] substance whose diterpenoid composition, $C_{20}H_{24}O_4$, was revealed by hreims. The ^{13}C-nmr spectrum and DEPT analysis showed the presence of nine sp^2 carbons, three of which were ketonic, while the remainder corresponded to mono-, di-, and tri-substituted double bonds. In addition to signals observed for four methyl singlets, two methylenes, one methine and three quaternary carbons, a signal at δ 78.43 ppm indicated the presence of a quaternary hydroxyl. These signals and their assignments are summarized in Table 1, together with the corresponding ^{1}H-nmr data.

The structure of **1** was deduced on the basis of 1D and 2D nmr experiments. Thus,



1 R,R^1=O
2 R=R^1=H

[1]The identification of this endophyte is currently being pursued.

TABLE 1. Nmr Data for Compounds **1** and **2**.

Position	Compound			
	1		**2**	
	δ_C	δ_H[b]	δ_C	δ_H
1	150.12	7.02	152.37	6.81
2	129.54	6.05	129.89	5.98
3	202.53		204.05	
4	44.06[a]		44.67	
5	39.76	3.15	43.90	2.63
6	36.99	2.62	22.58	1.74
		2.47		1.58
7	198.38		31.77	2.50
				2.32
8	137.72		137.62	
9	78.43		78.50	
10	43.94[a]		42.66	
11	210.26		211.89	
12	52.44	2.77	53.12	2.60
		2.60		2.70
13	42.74		44.38	
14	145.69	6.80	131.61	5.55
15	140.77	5.62	143.00	5.60
16	115.70	4.95	113.84	4.90
		5.04		4.90
17	27.48	1.32	28.47	1.24
18	21.62	1.10	22.24	1.10
19	25.39	1.16	27.45	1.20
20	18.63	1.29	20.40	1.16

[a]Signals may be interchanged.
[b]Assignments from HETCOR experiment.

a HETCOR spectrum afforded the carbon-hydrogen correlations which, together with the results of a COSY experiment, allowed the construction of partial structural moieties which were readily incorporated into a pimarane framework. Also important in this regard was an HMBC experiment whose major correlations are shown in Figure 1. The

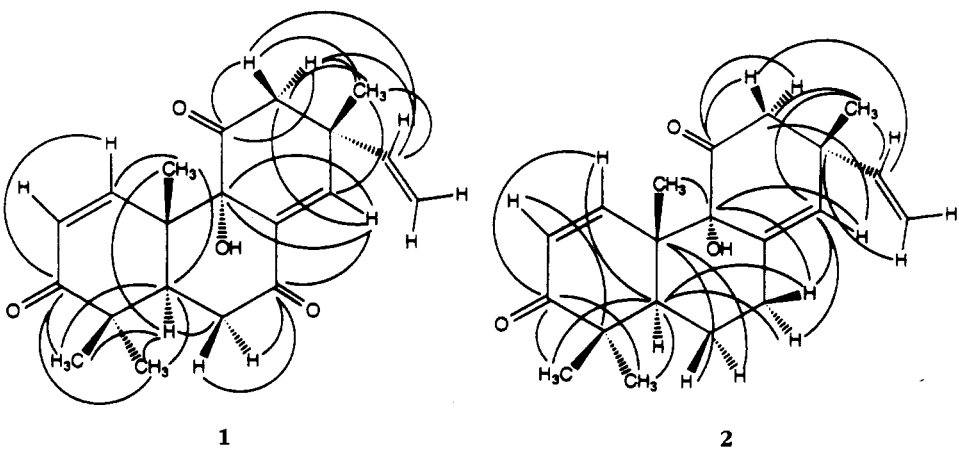


FIGURE 1. HMBC correlations for **1** and **2**.

stereochemistry of **1** is supported by nOe interactions summarized in Figure 2. The nOes between H_3-20 and H-6β and between H-6β and H_3-17 established the cis relationships of these groups and necessitates an α configuration for OH-9. Thus, we conclude that **1** is 9α-hydroxy-1,8(14),15-isopimaratriene-3,7,11-trione.

Compound **2**, $C_{20}H_{26}O_3$ (hreims), was obtained as a white crystalline solid [mp 105–107°, $[\alpha]_D$ −175°]. Its ^{13}C-nmr spectrum was similar to that of **1** but featured an additional methylene and one less carbonyl carbon.

Chemical shifts were determined from DEPT analysis and HETCOR experiments (see Table 1). The structure and stereochemistry of **2** were deduced from these data, together with information from COSY, HMBC, and nOe difference spectroscopy experiments. The pertinent HMBC correlations are depicted in Figure 1 and the nOe interactions are summarized in Figure 2. Thus, we conclude that **2** is 9α-hydroxy-1,8(14),15-isopimaratrien-3,11-dione. The absolute configurations of **1** and **2** are unknown.

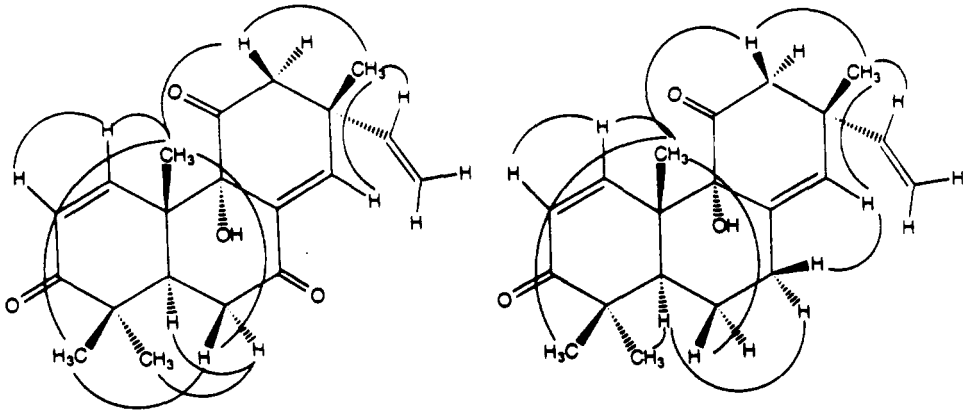


FIGURE 2. NOe correlations for **1** and **2**.

Both **1** and **2** displayed comparable toxicity to spruce budworm cells (2) and to larvae in a feeding bioassay (1) (Table 2). The toxicity to budworm larvae appears to be substantially lower than that of azadirachtin in view of the studies of Thomas *et al.* (3). These workers determined an LD_{50} value of 0.9–0.105 micrograms for ingestion of azadirachtin by 6th instar larvae.

Diterpenoid derivatives occur infrequently as fungal metabolites (4). Pimarane or

TABLE 2. Effects of Compounds **1** and **2** on Spruce Budworm Larvae.[a]

Treatment (quantity)[b]	Surviving larvae in instar (%)					Survival % (total insects)
	2	3	4	5	6	
Control diet	0	0	21	0	0	84 (25)
Solvent control	1	0	19	0	0	80 (25)
Compound **1** (6 μmol)	0	3	9	1	0	56 (25)
Compound **2** (6 μmol)	0	2	11	0	0	56 (25)

[a]For details of larval assay, see Calhoun *et al.* (1) and Clark *et al.* (2).
[b]Quantity of additive incorporated into diet and offered to total number (25) of insects.

isopimarane derivatives have been reported from *Cephalosporium aphidicola* (5), *Nigrospora sphaerica* (5), *Trichothecium roseum* (6), *Acremonium lazulae* (7), *Oospora virescens* (8), and *Armellaria mellea* (9), but in no case is the skeleton as highly functionalized as in **1** and **2**. While a number of plant-derived diterpenes (particularly kauren-19-oic acids) have been shown to possess insect antifeedant properties (10), we are not aware of any previous report of pimarane/isopimarane derivatives having insecticidal properties.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Melting points were taken with a Kofler hot-stage apparatus and are uncorrected. The ir spectra were recorded as films on a Bruker IF-S25 spectrometer. Prep. tlc was performed with precoated Si gel F254 (1 mm) plates.

Nmr data were recorded in $CDCl_3$ on a Varian Unity 400 spectrometer, using the solvent as reference. All 2D nmr spectra were recorded non-spinning at 25°. Hrms were recorded on a Kratos MS-50 instrument.

FUNGAL STRAIN.—The endophyte strain was isolated from a balsam fir needle. Attempts to induce the isolate to sporulate in culture were unsuccessful. In 2% malt extract agar incubated at 25° under fluorescent and uv light, the culture was slow-growing (1 cm/month), and had a dark mycelium. The isolate has been deposited in the Canadian Collection of Fungal Cultures (Centre for Land and Biological Resources Research, Agriculture Canada, Ottawa, Ontario K1A 0C6) as DAOM 208484. It is preserved in liquid nitrogen.

EXTRACTION AND ISOLATION.—Endophyte strain #7BF36H1 collected in New Brunswick in August 1991, was isolated and fermented (10 liter scale) according to our established protocols (1). The culture filtrate was extracted with EtOAc (3×ca. 3 liters) and the total extract evaporated to dryness at room temperature *in vacuo*, yielding a crude product which was toxic to budworm cells and larvae (1).

A portion of the crude extract (0.17 g) was purified by prep. tlc (ca. 40 mg/plate) using hexane-$CHCl_3$-EtOAc-MeOH (9:15:6:1) to yield crude **1** (R_f 0.48) and **2** (R_f 0.71), which were further purified by prep. tlc using hexane-EtOAc (3:2), giving pure **1** (70 mg, R_f 0.50) and **2** (16 mg, R_f 0.70).

9α-Hydroxy-1,8(14),15-isopimaratrien-3,7,11-trione [**1**].—White flat crystals, mp 173–174°; $[\alpha]_D$ −213° (c=0.0023, $CHCl_3$); ir ν max 3416 (br), 3020 (m), 2966 (s), 1714 (s), 1694 (s), 1678 (s), 1622 (m), 1460 (m), 1376 (m), 926 (m), 832 (m) cm^{-1}; ^1H- and ^{13}C-nmr data, see Table 1; eims *m/z* $[M]^+$ 328.1683 (14) (calcd for $C_{20}H_{24}O_4$, 328.1675), $[M-H_2O]^+$ 310 (14), 283 (26), 260 (27), 179 (82), 150 (33), 136 (33), 122 (24).

9α-Hydroxy-1,8(14),15-isopimaratrien-3,11-dione [**2**].—White feather-like crystals, mp 105–107°; $[\alpha]_D$ −175° (c=0.0040, $CHCl_3$); ir ν max 3448 (br), 3026 (m), 2962 (s), 1702 (s), 1672 (s), 1634 (m), 1456 (m), 1376 (m), 920 (m), 828 (m) cm^{-1}; ^1H- and ^{13}C-nmr data, see Table 1; eims *m/z* $[M]^+$ 314.1879 (9) (calcd for $C_{20}H_{26}O_3$, 314.1878), $[M-H_2O]^+$ 296 (6), 178 (12), 163 (24), 149 (100), 137 (27).

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