Abibalsamins A and B, Two New Tetraterpenoids from *Abies balsamea* Oleoresin

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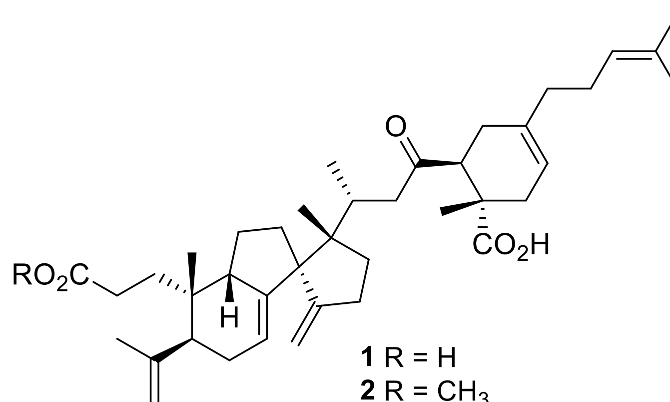
ABSTRACT



Abibalsamins A (1) and B (2), two unprecedented tetraterpenoids featuring a 3,4-seco-rearranged lanostane system fused with a *β*-myrcene lateral chain *via* a [4 + 2] Diels-Alder cycloaddition, were isolated from the oleoresin of *Abies balsamea*. Their structures were elucidated by means of extensive 2D NMR, IR and MS spectroscopy analyses. The absolute configuration of 1 was determined by single-crystal X-ray diffraction. Both compounds exhibited significant cytotoxic activity against cancer cell lines.

The genus *Abies* (Pinaceae) consists of 46 species, which are mainly distributed in temperate and boreal regions of North and Central America, Europe, Asia and North Africa.[1] Previous investigations on the chemical composition of firs (*Abies*) led to the identification of several secondary metabolites such as triterpenoids, flavonoids, stilbenes, chalcones and lignans, some of them exhibiting pharmaceutically relevant biological activities.[2-4]

Balsam fir-*Abies balsamea* (L.) Mill. is widely distributed in the eastern part of Canada. Traditionally, it has been used as an antiseptic, tuberculosis remedy and venereal aid.[5] Balsam fir, like other true firs, is characterized by the presence of blisters on the surface of young bark that contain an aromatic liquid called the cortical oleoresin. Oleoresin is known as a complex mixture of mono-, sesqui-, di- and triterpenoids.[6-9] Aboriginal people in Canada have employed fir oleoresin as a vulnerary, although nowadays its main field of utilization is as a cement for lenses and as mounting medium in microscopy.[10]

In the course of our research program aiming at the isolation and identification of bioactive substances from plant species of Quebec's boreal forest,[11-13] we have become interested in studying the constituents of *A. balsamea*.[12,14-16] This work led to the isolation of two new

tetraterpenoids, abibalsamins A (**1**) and B (**2**), which features an unusual 3,4-seco rearranged lanostane triterpene fused with a β-myrcene moiety. The closely related abiesonic acid (**3**)[17] was isolated as well. In this Letter, we report the isolation, structural elucidation and absolute configuration of the new compounds **1** and **2** (Figure 1) based on spectroscopic data, X-ray crystallographic analysis and comparison with literature data.

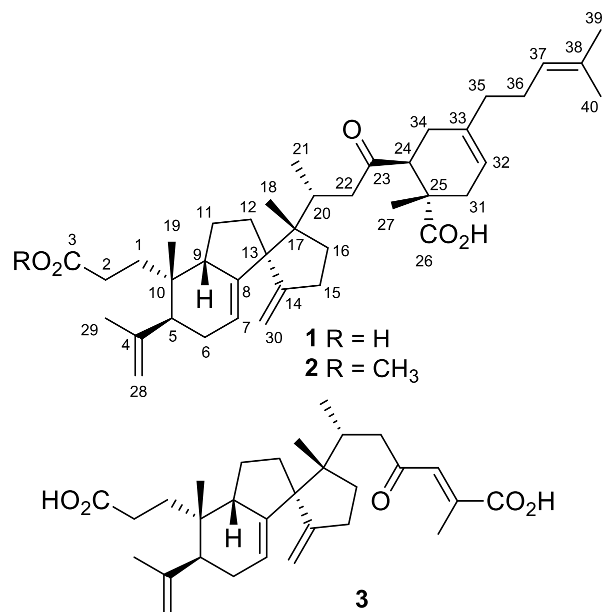


Figure 1. Structures of abibalsamins A (**1**) and B (**2**), and the related abiesonic acid (**3**).

The oleoresin of *A. balsamea* (500 g)[18] was directly subjected to silica gel column chromatography eluting with hexanes/EtOAc as a gradient (100:0 to 93:7), then MeOH. Both hexanes/EtOAc 93:7 and MeOH fractions were combined and concentrated under reduced pressure (75 g). A portion of the extract (60 g) was further fractionated on silica gel using a gradient of hexanes/EtOAc, affording three subfractions A-C. Subfraction B was purified by silica gel with hexanes/EtOAc (3:1), giving five other subfractions A'-E'. Repeated column chromatography of subfraction C' on C_{18} reversed-phase with H_2O/MeOH (1:4 to 0:1) followed by purification by preparative HPLC yielded compounds **1** (44.6 mg) and **2** (13 mg). Crystals of **1** were obtained by recrystallization with EtOH. Purification of subfraction E' on polyamide column followed by preparative HPLC separation yielded **3** (10 mg), which was identified as abiesonic acid after spectral caracterization (IR, NMR, MS).[17] This compound has been thoroughly described in literature in the form of a dimethyl ester.[8,19]

Compound **1**, $[\alpha]^{20}_D$ -24.8 (*c* 0.9, $CHCl_3$), showed a pseudomolecular ion peak at *m/z* 619.4348 $[M + H]^+$ in the HRESIMS suggesting the molecular formula $C_{40}H_{58}O_5$. IR absorptions band at 1702 (s) cm^{-1} implied the presence of carbonyl functionality. Analysis of ^{13}C NMR data and HSQC spectrum revealed the presence of seven methyls, twelve sp^3 methylenes, four sp^3 methines, four sp^3 quaternary carbons, two sp^2 methylenes, three sp^2 methines and eight sp^2 quaternary carbons. A close comparison of carbon chemical shift of **1** with abiesonic acid (**3**) suggested a 3,4-seco-rearranged lanostane system with a lateral chain constituted of an additional 6-membered ring. Analysis of the COSY spectrum revealed four spin systems (Figure 2) on the lateral chain which were connected by HMBC cross-peaks from H_3-21 to C-17, C-20 and C-22, from H_2-22 to C-23, from H-24 to C-23, from H_3-27 to C-24, C-25, C-26 and C-31, from H-32 to C-26 and C-35 and from H_3-39 and H_3-40 to C-38 and C-37.

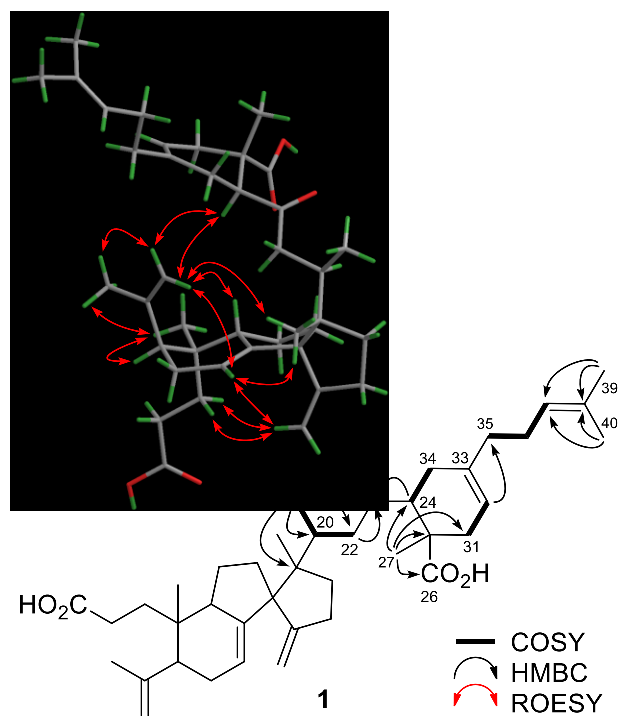


Figure 2. Selected ^1H-^1H COSY, HMBC and ROESY correlations for compound **1**.

The relative stereochemistry was established with the ROESY spectrum where cross-peaks from H-28*Z* to H-9 and from H_3-19 to H-9 and H_3-29 were observed (Figure 2). These facts indicated that H-5, Me-19 and H-9 are positioned α, β and β, respectively. Other correlations were observed between H_3-18/H-7 and H-30/H-1 suggesting that ring D is connected in such a way that C-17 is β-oriented and Me-18 is also β. The stereochemistry of the lateral chain could not be established unambiguously with the ROESY spectrum. Single-crystal X-ray diffraction analysis was carried out in order to confirm the structure of **1** (Figure 3). The absolute configuration was determined by Flack's method with

Flack's parameter determined as 0.0(2).[20] The X-ray structure demonstrated that the chiral centers in **1** were 5*S*, 9*S*, 10*S*, 13*R*, 17*S*, 20*R*, 24*S* and 25*S*. Furthermore, the ROESY correlation between H-28*Z* and H-24 could be better understood since the measured separation distance was 2.88 Å. On the basis of these spectroscopic evidences, the structure of **1** was assigned as (13*R*,17*S*,24*S*,25*S*)-24,25-[2-(4-methylpent-3-enyl)but-2-ene-1,4-diyl]-23-oxo-3,4-seco-17,13-friedo-8(14→13)-abeo-9*β*H-lanost-4(28),7,14(30)-triene-3,26-dioic acid and named abibalsamin A.[21]

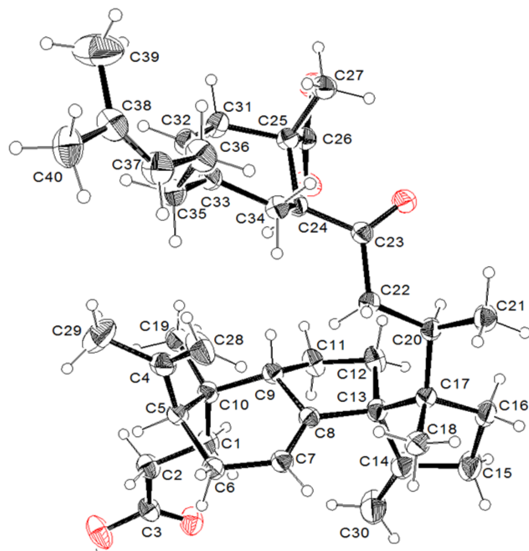


Figure 3. X-ray structure of abibalsamin A (**1**).

Compound **2**, $[\alpha]^{20}_{D}$ -44.5 (*c* 1.0, $CHCl_3$), showed a pseudomolecular ion peak at *m/z* 633.4514 $[M + H]^+$ in the HRESIMS suggesting the molecular formula $C_{41}H_{60}O_5$. IR absorptions band at 1737 and 1708 (s) cm^{-1} implied the presence of carbonyl functionalities. Comparison of the ^1H and ^{13}C NMR spectra with those of compound **1** revealed that they differ only by an additional methyl group linked at one of the carboxylic functionalities. An upfield shift at C-3 (6.4 ppm) for **2** along with a HMBC correlation between the new OCH_3 group and C-3 were observed and allowed us to assign the structure of **2**, named abibalsamin B,[22] as (13*R*,17*S*,24*S*,25*S*)-24,25-[2-(4-methylpent-3-enyl)but-2-ene-1,4-diyl]-23-oxo-3,4-seco-17,13-friedo-8(14→13)-abeo-9*β*H-lanost-4(28),7,14(30)-diene-3,26-dioic acid 3-methyl ester.

Table 1. NMR data of compounds **1** and **2** (δ in ppm, *J* in Hz)

no.	1		2	
	δ_H	δ_C	δ_H	δ_C
1	1.68, m (2H)	29.7	1.63, m	30.7
			1.76, m	
2	2.34, m (2H)	28.7	2.31, m (2H)	29.3
3	-	181.3	-	174.9
4	-	149.4	-	149.3
5	2.05, m	43.9	2.07, m	44.0
6	2.14, m	31.1	2.14, m	31.0
	2.34, m		2.39, m	
7	5.43, br s	122.2	5.45, br s	122.1
8	-	144.0	-	143.7
9	2.15, m	49.2	2.17, m	49.2
10	-	37.3	-	37.0
11	1.34, m	22.4	1.39, m	22.5
	1.67, m		1.64, m	
12	1.26, m	31.3	1.31, m	31.2
	1.66, m		1.75, m	
13	-	63.6	-	63.5
14	-	161.7	-	161.5
15	2.35, m	28.0	2.36, m	27.9
	2.47, m		2.47, m	
16	1.54, m (2H)	36.5	1.56, m (2H)	36.4
17	-	50.5	-	50.4
18	0.87, s	18.0	0.89, s	18.0
19	0.93, s	25.0	0.94, s	24.9
20	2.31, m	33.1	2.33, m	33.9
21	0.81, d (6.5)	17.0	0.80, d (6.4)	16.6
22	2.12, m	44.6	2.14, m	44.7
	2.36, m		2.30, m	
23	-	211.5	-	211.4
24	3.12, dd (12.4, 5.1)	50.4	3.08, dd (11.8, 5.4)	50.4
25	-	42.5	-	42.3
26	-	185.0	-	183.6
27	1.29, s	15.7	1.25, s	16.1
28	*Z* 4.92, br s	111.7	4.83, s (2H)	111.9
	E 4.82, s			
29	1.78, s	26.5	1.77, s	26.3
30	*E* 4.76, s	106.8	*E* 4.76, s	106.7
	Z 4.67, br s		*Z* 4.72, br s	
31	2.01, m	38.1	2.05, m	37.6
	2.28, m		2.33, m	
32	5.39, d (3.8)	118.8	5.39, br s	118.9
33	-	135.8	-	135.4
34	1.87, m	29.0	1.87, m	28.7
	2.22, m		2.27, m	
35	2.06, m (2H)	37.1	2.03, m	37.1
			2.08, m	
36	2.10, m (2H)	26.3	2.09, m (2H)	26.3
37	5.07, t (6.7)	123.7	5.06, t (5.9)	123.8
38	-	131.9	-	131.9
39	1.68, br s	25.7	1.68, br s	25.7
40	1.61, br s	17.8	1.60, br s	17.8
Me*	-	-	3.67, s	51.6

Although some marine species have been shown to contain polycyclic tetraterpenoids,[23-25] the isolation of non-carotenoid C_{40} compounds from plants is very scarce.[26] A plausible biosynthetic pathway of abibalsamins A (**1**) and B (**2**) is proposed in Scheme 1. In opposition to 'true' tetraterpenes such as the carotenoids, which are formed by a head-to-head condensation of two geranylgeranyl pyrophosphate (GGPP) units,[27] it is likely that abibalsamins **1** and **2** would come from a different biosynthetic route. Since abiesonic acid (**3**), a 3,4-seco-rearranged lanostane-type triterpenoid,[28] as well as the monoterpene *β*-myrcene have previously been isolated

from *A. balsamea*,[29] it seems reasonable to propose that abibalsamin A (**1**) would be formed by an enzyme-catalyzed [4 + 2] Diels-Alder-like cycloaddition between **3** and *β*-myrcene. Abibalsamin B (**2**) would then be obtained by the esterification of C-3 carboxylic acid function in **1**. Such a type of Diels-Alder-like biosynthetic reaction has already been suggested for dimeric triterpenoids[30] and tricyclic spirolactones,[31] to name a few recent examples.

Scheme 1. Plausible biosynthetic pathway of **1** and **2**

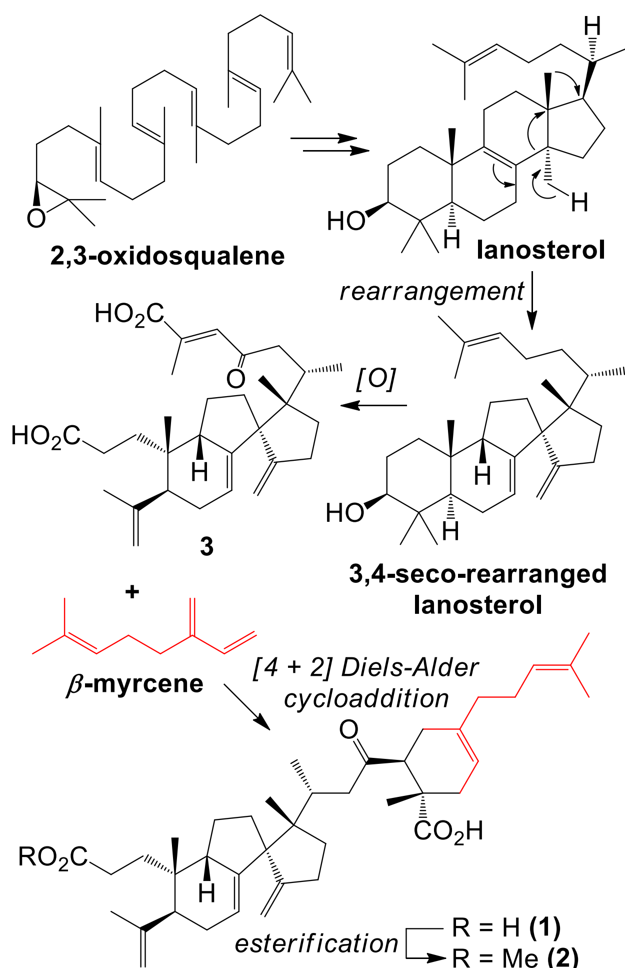


Table 2. Cytotoxic activity of compounds **1-3**[a]

Cancer cells lines	Compounds			
	1	**2**	**3**	**etoposide**
A549	22 ± 4	8.5 ± 0.7	22 ± 3	0.3 ± 0.1
DLD-1	>100	15 ± 1	>100	1.0 ± 0.4
WS1	>100	14.7 ± 0.2	30 ± 2	5 ± 1

[a] IC_{50} in μM.

The *in vitro* antiproliferative activities of compounds **1-3** against lung carcinoma (A549), colorectal adenocarcinoma (DLD-1) and normal skin fibroblasts (WS1) human cell lines were evaluated using the resazurin reduction test as previously described.[32] The cytotoxicity results presented in Table 2 are expressed as the concentration inhibiting 50% of the cell growth (IC_{50}). Etoposide was used as a positive control in this assay (IC_{50} 0.3-5 μM). The most cytotoxic compound was abibalsamin B (**2**) with IC_{50} values of 8.5 ± 0.7 and 15 ± 1 μM against A549 and DLD-1 cancer cell lines, respectively. Interestingly, abibalsamin A (**1**) selectively inhibit the growth of A549 cells (IC_{50} 22 ± 4 μM) compared to DLD-1 and WS1 (IC_{50} >100 μM).

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Supporting Information Available. Experimental section, NMR and IR spectra of new compounds (**1** and **2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Abibalsamin A (**1**): white crystals (EtOH), $[\alpha]^{20}_{D}$ -24.8 (c 0.9, $CHCl_3$); IR (film) ν_{max} 2960, 1742, 1702, 1460, 1376, 1295, 1217, 1160, 897, 756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 1; HR-ESI-MS m/z 619.4348 $[M + H]^+$ (calcd for $C_{40}H_{59}O_5$, 619.4357).
(22) Abibalsamin B (**2**): white amorphous solid, $[\alpha]^{20}_{D}$ -44.5 (c 1.0, $CHCl_3$); IR (film) ν_{max} 2958, 1737, 1708, 1435, 1375, 1294, 1193, 1172, 896, 756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 1; HR-ESI-MS m/z 633.4514 $[M + H]^+$ (calcd for $C_{41}H_{61}O_5$, 633.4514).

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