Antimycobacterial Polyynes of Devil's Club (*Oplopanax horridus*), a North American Native Medicinal Plant

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Two new (**3** and **5**), as well as three known (**1**, **2**, and **4**), polyynes were isolated from Devil's Club (*Oplopanax horridus*; Araliaceae), a medicinal plant of North America. The structures were established by ^{1}H and ^{13}C NMR. The absolute configurations of **2** and **5** were determined by application of Mosher's method. All the polyynes exhibited significant anti-*Candida*, antibacterial, and antimycobacterial activity, with an ability to kill *Mycobacterium tuberculosis* and isoniazid-resistant *Mycobacterium avium* at 10 μg/disk in a disk diffusion assay.

During the course of investigations into the constituents of British Columbian medicinal plants, a MeOH extract of the inner bark of *Oplopanax horridus* (Smith) Miq. (Araliaceae) exhibited antibacterial and antifungal activity.[1] Extracts were also active against *Mycobacterium tuberculosis* and *Mycobacterium avium*.[2] *O. horridus*, commonly known as Devil's Club, is a well-known shrub of western North American forests. The inner bark and roots are used by First Nations peoples for a variety of ailments such as diabetes, rheumatism, tuberculosis, colds, headaches, and lung hemorrhages.[3,4] From *O. horridus*, we have identified two novel and three known polyynes, with antimycobacterial and antifungal activity.

A concentrated MeOH extract of finely ground fresh inner bark of *Oplopanax horridus* was successively partitioned into H_2O and CH_2Cl_2. Biological evaluation of the extract and fractions indicated that most of the activity was in the CH_2Cl_2 fraction. Further fractionation by vacuum liquid chromatography (VLC),[5,6] yielded five active fractions (F2−F6) as determined using a TLC-overlay bioassay.[7] Repeated VLC separation of fraction F2 afforded the known falcarinol (**1**) identified by UV, IR, and ^{1}H-NMR data identical to those previously described for falcarinol.[8−10] The absolute stereochemistry was assigned as 3*S*, based on optical rotation measurements and comparison with literature values.[11,12]

Fractions F3−F6 were subjected to successive C-18 reversed-phase chromatography to yield a series of diyne diols (**2**−**5**). Compound **2** was identified as falcarindiol, first isolated from *Falcaria vulgaris* Bernh,[10] and subsequently identified in species of Apiaceae[13−15] and Araliaceae.[11]

The third bioactive metabolite was an optically active oil, $C_{17}H_{26}O_2$ (HRMS). Its UV spectrum suggested a polyyne.[13] The ^{13}C-NMR spectrum (see Table 1) showed resonances of four nonprotonated acetylenic carbons (by HMQC), two olefinic carbons (δ 127.8 and δ 134.6), two oxygen-bearing sp^3 carbons at δ 58.63 and δ 64.1, and seven methylenes and two methyls at δ 9.25 and δ 14.23. The ^{1}H-NMR spectrum showed two methyl triplets (δ 0.86 and 0.99). The latter was coupled to a methine at δ 4.36 (by ^{1}H−^{1}H COSY). The COSY spectrum also showed another hydroxy methine at δ 5.2 coupled to olefinic protons at δ 5.5 and δ 5.6. The structure was then linked by an HMBC experiment (see Figure 1). On the basis of these structural determinations, the new metabolite was identified as 9-heptadecene-4,6-diyne-3,8-diol, which we have named oplopandiol (**3**). The 9,10 alkene bond was found to be *Z*, as evidenced by the small vicinal coupling constant ($J_{9,10}$ = 10.8 Hz). The *cis* geometry of this double bond was also supported by the chemical shift of C-11(δ 27.7), since the signals of carbons next to a *trans* double bond usually appear at δ 33.7.[16]

From the more polar chromatography fractions (F5−F6) we isolated a related optically active oil, $C_{20}H_{28}O_4$ (HRMS). This compound was identified as 9,17-octadecadiene-12,14-diyne-1,11,16-triol 1-acetate **4**. Although **4** has been reported from Apiaceae,[17,18] this is the first time it has been found in a member of the Araliaceae, and this is the first report of its activity. As limited NMR data of this compound have been previously described,[17,18] we report here the ^{1}H- and ^{13}C-NMR spectral data (see Table 1).

The fifth antibiotic metabolite was an optically active oil, $C_{20}H_{30}O_4$ (HRMS). Its IR spectrum displayed an intense carbonyl absorption, while the UV spectrum showed bands indicative of a polyyne. This finding was confirmed by ^{13}C NMR (see Table 1), which exhibited signals for the carbonyl group at δ 171.2, four nonprotonated acetylenic carbons, as well as two olefins (δ 134.3 and δ 127.9). The region between δ 4 and 6 in the ^{1}H-NMR spectrum, as well as the triplet at δ 0.99 and the multiplet at δ 1.72, were almost identical to that of **3**. This indicates that there is only a subtle difference at the end of the chain between **5** and **3**, namely the

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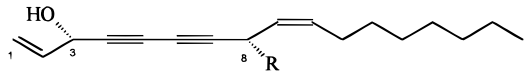
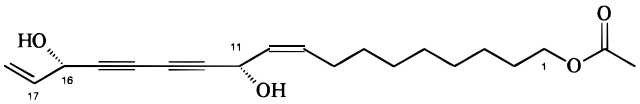
Table 1. ^{1}H-NMR and ^{13}C-NMR (500 MHz) Spectral Data for the Polyynes (**3**−**5**) in $CDCl_3$, from HMBC, HMQC, and APT

carbon no.	compound **3**		compound **4**		compound **5**	
	^{1}H m (*J*, Hz)	^{13}C	^{1}H m (*J*, Hz)	^{13}C	^{1}H m (*J*, Hz)	^{13}C
1	0.99, t (7.4)	9.25	4.05, t (6.7)	64.6	4.04, t (6.8)	64.7
2	1.72, m	30.6	1.6, m	25.7	1.6, m	25.8
3	4.36, t (6.6)	64.1	1.28, m	27.5[c]	1.25, m	29.1[e]
4		80.7	1.28, m	28.5[c]	1.25, m	28.9[e]
5		68.9[a]	1.28, m	28.5[c]	1.25, m	28.9[e]
6		69.01[a]	1.28, m	28.8[c]	1.25, m	28.5[e]
7		79.2	1.35, m	29.1[d]	1.37, m	29.2
8	5.18, br d (8.3)	58.6	2.1, dq (1.5, 7.3)	29.0[d]	2.1, dq (1.5, 7.3)	27.6
9	5.6, ddt (10.8, 7.3, 1.5)	134.6	5.59, ddt (10.7, 1.0, 7.2)	134.4	5.5, ddt (10.8, 8.3, 1.5)	127.9
10	5.5, ddt (10.8, 8.3, 1.5)	127.8	5.5, ddt (10.7, 8.1, 1.0)	127.7	5.6, ddt (10.8, 7.3, 1.5)	134.3
11	2.09, dq (1.5, 7.3)	27.7	5.18, d (8.3)	58.5	5.18, br d (8.3)	58.59
12	1.37, m	29.3		79.7		79.1
13	1.25, m	29.1[b]		68.6		68.9[f]
14	1.25, m	29.1[b]		70.1		68.9[f]
15	1.25, m	22.6		78.3		80.8
16	1.25, m	31.7	4.92, br d (5.3)	63.3	4.35, t (6.6)	64.1
17	0.86, t (7.1)	14.2	5.92, ddd (17.2, 10.2, 1.0)	135.7	1.72, m	30.6
18			5.24, dt (10.2, 1.0)	117.2	0.99 t (7.4)	9.3
			5.45, dt (17.2, 1.0)			
CO				171.4		171.2
CO*CH*$_3$			2.03, s	20.9	2.03, s	21.0, s

[a]−[f]Assignments may be interchanged.



(**1**) R = H
(**2**) R = OH
(**3**) R = OH; 1,2 dihydro



(**4**)

(**5**) 17,18 dihydro

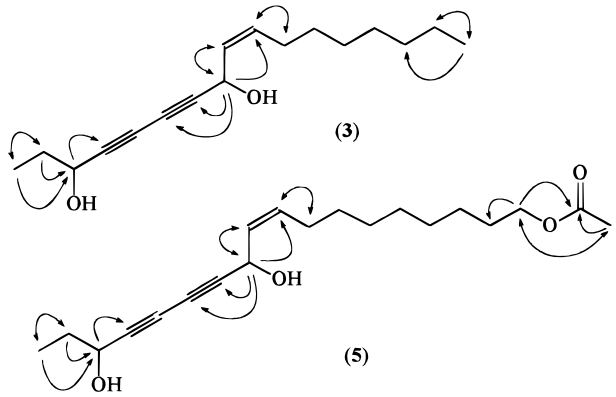


Figure 1. HMBC experiment for compounds **3** and **5**.

absence of a methyl resonance at δ 0.86 and the addition of a methyl singlet resonance at δ 2.03 and a methylene triplet at δ 4.04. From these data and the results of the useful HMBC correlations as shown in Figure l, the structure was established to be 9-octadecene-12,14-diyne-1,11,16-triol, 1-acetate, which we have named oplopandiol acetate **5**. The *Z* stereochemistry at C-9/C-10 was derived from the $J_{9,10}$ (10.8 Hz).

For the determination of absolute configuration of compounds (**2**−**5**), Mosher's ester method[19−21] for selected polyynes was applied. Thus, reactions of compound **2** with (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid chloride (MTPA-Cl) gave the (*S*)- and (*R*)-MTPA esters, respectively. The differences in ^{1}H chemical shifts between the (*S*)- and (*R*)-MTPA ($\Delta\delta_H = \delta_S - \delta_R$) of **2** were identical to that reported by Bernart et al.[11] suggesting the 3*S*,8*S* configuration for this compound. This result was confirmed by ^{19}F-chemical shift data (Table 2). The absolute configuration of the polyyne **5** was also determined by application of Mosher's method. The $\Delta\delta_F = \delta_S - \delta_R$ for the CF_3 resonance in the ^{19}F-NMR spectra were negative at C11 and C16. This result (Table 2) established the absolute stereochemistry for polyyne **5** as 11*S*,16*S*. Because **3** and **4** have similar optical rotations to those of **2** and **5**, the absolute configurations of **3** and **4** are assumed to be the same as in **2** and **5**, respectively.

Falcarinol (**1**) and falcarindiol (**2**) have been reported to be antibiotics.[15,22,23] Antimicrobial activity of the polyynes was evaluated by an overlay bioautography technique[7] and finally confirmed by minimum inhibitory concentration tests using a twofold serial microbroth dilution assay[24] (Table 3). The polyynes **1**−**5** showed antibiotic activity against two Gram-positive bacteria (*S. aureus and B. subtilis*), and two Gram-negative bacteria (*Escherichia coli* DC2 and *Pseudomonas aeruginosa* Z61) and the yeast *Candida albicans*. Falcarinol (**1**) was more active than the polyyne **3** and falcarindiol (**2**), and these three were substantially more active than **4** and **5**. The results are summarized in Table 3.

We tested the crude fractions and pure compounds for antimycobacterial activity against *M. tuberculosis* and isoniazid-resistant *M. avium* using a disk-diffusion assay.[2] We found that the MeOH extract, the CH_2Cl_2 extract, and all compounds isolated were active at a concentration of 10 μg/disk. Both **1** and **5** completely inhibited growth at 20 μg/disk. The results are summarized in Table 4. These preliminary results support the usage of *O. horridus* in traditional medicines in British Columbia.

Experimental Section

General Experimental Procedure. IR spectra were acquired with a Perkin-Elmer 1600 spectropho-

Table 2. Stereochemical Analysis ($\Delta\delta_F = \delta_S - \delta_R$; Hz) of the Polyynes **2** and **5** with the (*R*)- and (*S*)-MTPA Mosher Ester Derivatives

compound **2**					compound **5**				
CF_3	(*S*)-MTPA	(*R*)-MTPA	$\Delta\delta$	carbinol configuration	CF_3	(*S*)-MTPA	(*R*)-MTPA	$\Delta\delta$	carbinol configuration
3	4.19	4.36	negative	3S	11	4.01	4.2	negative	11S
8	4.27	4.48	negative	8S	16	3.89	4.14	negative	16S

Table 3. MIC Values (μg/mL) for Antibacterial Activity of the Extracts and Pure Compounds of *O. horridus*

	microorganisms used1,24							
	E. coli		*P. aeruginosa*		*S. aureus*		*B. subitilis*	*C. albicans*
compds	DC2	UB1005	K799	Z61	SAP00017	RN4220	UBC 221	UBC 54
H_2O extract	>500.0	>500.0	>500.0	>500.0	>500.0	>500.0	>500.0	>500.0
MeOH extract	125.0	>500.0	>500.0	>500.0	125.0	125.0	62.5	>500.0
CH_2Cl_2 extract	125.0	>500.0	>500.0	>500.0	125.0	62.5	62.5	>500.0
compound **1**	6.25	>50.0	>50.0	6.25	3.1	3.1	3.1	6.25
compound **2**	25.0	>50.0	>50.0	50.0	25.0	25.0	6.25	25.0
compound **3**	6.25	>50.0	>50.0	>50.0	6.25	6.25	6.25	12.5
compound **4**	>50.0	>50.0	>50.0	>50.0	>50.0	50.0	25.0	>50.0
compound **5**	>50.0	>50.0	>50.0	>50.0	>50.0	50.0	25.0	>50.0
gentamicin	0.60	1.25	1.25	0.6	>10.0	0.6	1.25	NT
methicillin	1.6	>100.0	>100.0	3.1	500.0	1.6	100.0	>100.0
nystatin	NT	NT	NT	NT	NT	NT	NT	1.60

[a] NT means not tested.

Table 4. Antituberculosis Activity of the Extracts and Pure Compounds of *O. horridus*

	microorganisms used[2,25]					
	Mycobacterium tuberculosis (μg/disk)			*Mycobacterium avium* (μg/disk)		
	100	20	10	100	20	10
MeOH extract	+++	+++	++	+++	+++	++
CH_2Cl_2 extract	+++	+++	++	+++	+++	++
water extract	+	−	−	+	−	−
compound **1**	+++	+++	++	+++	+++	++
compound **2**	+++	++	+	+++	+++	++
compound **3**	+++	++	+	+++	++	+
compound **4**	+++	++	+	+++	+++	++
compound **5**	+++	+++	++	+++	+++	++
control MeOH	−	−	−	−	−	−
control (isoniazid)	+++	+++	+++	−	−	−

[a] Key: −, no inhibition; +, zone of inhibition with a few less susceptible colonies within it or small zone of clearing (colonies too numerous to count); ++, large zone of clearing (less than 50 colonies present); +++, complete inhibition.

tometer, and UV spectra with a Philips PU 8720 UV/vis scanning spectrophotometer. Optical rotations were measured on a Perkin-Elmer 550-SE parameter. ^{1}H- and ^{13}C-NMR, HMBC, and HMQC were obtained with a Bruker AM-500 (500 MHz, $CDCl_3$). ^{19}F-NMR and APT spectra were determind on a Bruker WH-400 (400 MHz, $CDCl_3$). DCI and DCI-HRMS were recorded on a Kratos MS 50. Si gel (Kieselgel F_{254}) for column chromatography and μNOVAPAK C_{18} (RCM 25 × 10) for high performance liquid chromatography (HPLC) were supplied from Waters Co. Ltd.

Plant Material. *O. horridus* bark was collected from M. Knapp Research Forest, Maple Ridge, B.C., in June 1996. Voucher specimens are deposited in the herbarium of the Botany Department, UBC.

Antimicrobial Assays. Assays for activities against microorganism are as follows: *Bacillus subtilis* (UBC collection UBC 221), *Escherichia coli* UB1005, *Escherichia coli* DC2, *Pseudomonas aeruginosa* Z61, *Pseudomonas aeruginosa* K799, *Staphylococcus aureus* meth^S RN4220, *Staphylococcus aureus* meth^R SAP00017,[24] *Mycobacterium tuberculosis* (strain Erdmon, Trudeau Mycobacterial collection (T.M.C. 107), *Mycobacterium avium* (T.M.C. 724),[2,25] and *Candida albicans* (UBC Collection UBC 54).[1]

Extraction and Purification. Fresh ground inner bark (125 g) was extracted three times with MeOH, and combined extracts were concentrated *in vacuo*, followed by fractionation with CH_2Cl_2. The CH_2Cl_2 extract was concentrated under reduced pressure to give (7.93 g) of a brown oil. This was subjected to VLC on Si gel using eluents of increasing polarity from hexane to EtOAc. Ten fractions were collected; fractions 2−6 showed antibacterial activity. Fraction 2 was subjected to VLC {Si gel; hexane−EtOEt (9:2)} to yield falcarinol **1** as a minor component.

The presence of the dehydroderivatives did not permit the separation of the pair falcarindiol (**2**) and compound **3**, and the polyyne **4** from **5** by classical column chromatography or preparative TLC methods. Therefore HPLC was applied for the purification of compounds **2**−**5**. Fractions 3 and 4 afforded the polyynes **2** and **3** after purification by HPLC {μNOVAPAK C_{18} RCM 25 × 10, Waters Co. Ltd.; CH_3CN−MeOH−H_2O (45:5:50); 12.0 mL/min}. The fifth and sixth fractions were purified by HPLC {μNOVAPAK C_{18} RCM 25 × 10, Waters Co. Ltd.; CH_3CN−MeOH−H_2O (55:5:40); 12.0 mL/min} to afford compounds **4** and **5**.

Falcarinol 1: oil; $[\alpha]^{25}_D$ +29.4° (*c* 1.7, $CHCl_3$); spectral properties in accordance with published data.

Falcarindiol 2: oil (1.6 g, 1.3% dry wt); $[\alpha]^{25}_D$ +218.3° (*c* 6.9, $CHCl_3$); spectral properties in accordance with published data.

Oplopandiol 3: oil (1.4 g,1.12% dry wt); $[\alpha]^{25}_D$ +219.5° (*c* 10.7, $CHCl_3$), IR ν_{max} (film) 3342, 2928, 2856, 1456, 1452, and 1016 cm^{-1}; UV λ_{max} (EtOH) 206, 233, 246, 268 nm (ϵ 10 795, 2094, 2178, 1801); HRCIMS 262.19277 (calcd for $C_{17}H_{26}O_2$, 262.1932); ^{1}H- and ^{13}C-NMR data in Table 1.

9,17-Octadecadiene-12,14-diyne-1,11,16-triol, 1-acetate 4: oil (2.2 g, 1.6% dry wt); $[\alpha]^{25}_D$ +178.3° ($CHCl_3$, *c* 8.9); ^{1}H- and ^{13}C-NMR data in Table 1.

Oplopandiol acetate 5: oil (0.81 g, 0.65% dry wt), $[\alpha]^{25}_D$ +164.5° (*c* 5.7, $CHCl_3$) IR ν_{max} (film) 3392, 2918, 1720, 1612, 1510, 1461, 1252, 1156, and 1037 cm^{-1}; UV λ_{max} (EtOH) 206, 233, 245, 259 nm (ϵ 14 846, 1354, 1277, 808) nm; HRCIMS: 334.21442 (calcd for $C_{20}H_{30}O_4$, 334.21441); ^{1}H- and ^{13}C-NMR data in Table 1.

Determination of the Absolute Configuration of Compounds 2 and 5 by the Mosher Ester Method. To a dry round-bottom flask containing 5 mg of the polyyne were added sequentially dry pyridine (0.5 mL), (dimethylamino)-pyridine (1.0 mg), and 4 molar excess of MTPA-Cl. The mixture was stirred under N_2 at room temperature overnight, followed by removal of solvent *in vacuo*. The product was purified by VLC on Si gel. Both *S*- and *R*-esters were produced for each of the two natural products. The four derivatives were characterized by ^{1}H NMR, ^{19}F NMR, and HREIMS.

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