Pergamon

Phytochemistry, Vol. 42, No. 6, pp. 1647–1652, 1996

0031-9422/96 $15.00 + 0.00

S0031-9422(96)00150-1

ZYTHIOSTROMIC ACIDS, DITERPENOIDS FROM AN ANTIFUNGAL *ZYTHIOSTROMA* SPECIES ASSOCIATED WITH ASPEN

WILLIAM A. AYER and ABDUL Q. KHAN

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

(*Received in revised form* 26 *January* 1996)

Key Word Index—Fungal metabolites; aspen; *Populus tremuloides*; Salicaceae; *Zythiostroma* sp.; zythiostromic acids; antifungal diterpenoids.

Abstract—Three new cleistanthane type diterpenes, zythiostromic acid A, zythiostromic acid B and zythiostromolid, have been isolated from liquid cultures of a *Zythiostroma* sp., a fungus associated with aspen. Their structures were determined both by spectroscopic methods and chemical correlation. This class of diterpenoids has previously been reported only from plant sources.

INTRODUCTION

During our investigation of the 'black gall effect' on aspen [1], an unidentified *Zythiostroma* species was isolated from aspen (*Populus tremuloides* Michx.) and found to be antagonistic to the blue stain fungus *Ophiostroma crassivaginatum* (Hutchinson, L., personal communication). We have investigated the metabolites produced when this fungus is grown in liquid culture and have isolated three new diterpenes of the relatively new cleistanthane skeleton [2, 3]. This is the first report of the isolation of this class of compounds from fungal sources.

RESULTS AND DISCUSSION

The fungus was grown in shake culture on malt extract–yeast extract broth. After four weeks the broth was separated from the mycelium and extracted with ethyl acetate. Flash chromatography of this extract led to the isolation of the three new diterpenes, zythiostromic acid A (**1**), zythiostromic acid B (**2**) and zythiostromolide (**3**).

Compound **1**, $[\alpha]_D$ −31.7°, shows no molecular ion in its high resolution electron impact (HREI) mass spectrum, but it shows a fragment peak corresponding to $[M-2H_2O]^+$ at *m/z* 314.1877 ($C_{20}H_{26}O_3$). The chemical ionization mass spectrum indicates a molecular weight of 350 (peak at 368 $[M+18]^+$, reagent gas: ammonia). The IR spectrum shows strong absorption at 3217 (OH), 1693 (COOH) and 1650 cm^{-1} (C=C). Treatment of **1** with acetic anhydride–pyridine yields diacetate **1a**, which also does not show an $[M]^+$ in the mass spectrum. The monomethyl ester **1b**, formed on treatment with diazomethane, does show a molecular ion in the HREI mass spectrum. Acetylation of **1b** (acetic anhydride–pyridine) provides the diacetate **1c**.

The ^{13}C NMR spectrum (Table 1) confirms the presence of 20 carbons. The spectrum shows signals for a carboxyl carbon (δ 184.2), two oxygenated methine carbons (δ 70.8 and 76.1) and an oxygenated tertiary carbon (δ 81.0). It further shows signals from an exocyclic methylene (δ 106.4 CH_2; 154.1 C) and a vinyl group (δ 139.4, CH; 115.8, CH_2). The presence of these groups is also apparent from the ^1H NMR spectrum (Table 1), which shows signals at δ 4.6 and 4.5 (1 H each *d*, $J = 2$ Hz) for the methylene and at δ 6.1 (1H, *ddd*, $J = 17$, 10 and 9 Hz), 5.0 (1H, *dd*, $J = 17$ and 2 Hz) and 4.9 (1H, *dd*, $J = 10$ and 2 Hz) for the vinyl group. The ^1H NMR spectrum of **1** shows apparent triplets at δ 4.3 ($J = 4.5$ Hz) and 4.05 ($J = 4.0$ Hz), indicative of protons geminal to hydroxyl groups. The spectrum also shows singlets at δ 1.4 and 1.0 for two tertiary methyl groups. Of the six degrees of unsaturation required by **1**, one is accounted for by the carboxyl group and two by the carbon–carbon double bonds. This functionality suggests that **1** might be a tricyclic diterpene of the cleistanthane type. The cleistanthanes are a relatively small group of diterpenes isolated from plants whose structures are based on the 14-ethyl-13-methylpodocarpane skeleton [2, 3].

The conclusion that the exocyclic methylene and the vinylic side chains are attached at C-13 and C-14 is consistent with a ^1H–^1H COSY experiment. The proton resonance at δ 6.1 (H-15) shows correlation with the C-16 protons at δ 4.9 and 5.0 and with the proton at δ 2.75 (H-14). The H-14 proton shows correlation with the protons at δ 6.1 (H-15), 5.0, 4.9 (H_2-16) and 1.9 (H-8). The COSY spectrum further shows that the protons at δ 4.6 and 4.5 (H-17) correlate with each other and with the protons at δ 2.2 (H_2-12). Analysis of the HMBC spectrum supports these structural assignments (Table 2). In particular, the long range ^1H–^{13}C correlation of H-14 (δ 2.75) with C-13 (δ 154.1), C-15

Table 1. ^{13}C NMR and ^{1}H NMR data for compounds **1**–**3***

	1		**2**		**3**	
	δ (^{13}C)	δ (^{1}H)	δ (^{13}C)	δ (^{1}H)	δ (^{13}C)	δ (^{1}H)
1	30.8	1.3, 1.8	29.4	1.26	31.4	1.36
2	27.8	1.6, 2.3	28.7	1.64, 2.3	26.9	1.8, 1.65
3	76.1	4.3	74.1	4.1	67.7	4.45
4	54.0		52.2		52.0	
5	81.0		80.8		80.3	
6	70.8	4.05	27.6	2.3	80.4	4.4
7	34.8	1.2, 1.95	26.3	1.7, 1.16	27.5	1.88, 1.95
8	36.3	1.9	41.4	1.54	38.3	1.76
9	42.8	1.93	42.0	1.9	38.9	2.06
10	42.5		42.9		38.4	
11	28.9	1.76, 1.16	28.2	1.76, 1.06	27.7	1.7, 1.16
12	32.8	2.2	32.6	2.2	32.3	2.2
13	154.1		153.9		153.0	
14	56.0	2.75	56.2	2.8	55.4	2.8
15	139.4	6.1	139.3	6.1	139.5	6.1
16	115.8	5.0, 4.9	115.9	5.0, 5.1	117.7	5.0, 5.1
17	106.4	4.6, 4.5	106.5	4.6, 4.5	107.5	4.65, 4.55
18	21.3	1.4	20.6	1.3	12.11	1.25
19	184.2		184.0		184.3	
20	16.7	1.0	15.5	0.85	20.1	0.95

*Carbon–hydrogen connectivity established by HMQC methods.

(δ 139.4), C-16 (δ 115.8), C-17 (δ 106.4), C-8 (δ 36.3) and C-7 (δ 32.8) supports the vicinal positioning of exocyclic methylene and vinyl groups at C-13 and C-14 in ring C.

The ^{1}H–^{1}H COSY experiment also allows us to position the secondary hydroxyl groups at C-6 and C-3. The signal at δ 1.9 (H-8) shows coupling with the protons at δ 2.75 (H-14), 1.95 (H-7) and 1.2 (H-7′). These latter methylene protons show coupling with the proton at δ 4.05 (H-6). The proton resonance at δ 4.3 (H-3) shows cross peaks with the methylene protons at δ 1.6 and 2.3 at C-2. These in turn are coupled with each other, with the resonance at δ 4.3 (H-3) and with the two methylene protons at C-1 (δ 1.3 and 1.8).

Key supporting evidence for the structure of **1** is provided by a 2D-INADEQUATE experiment [4]. The spectrum establishes that the methine carbon at δ 36.3 assigned as C-8 is coupled with two other methine carbons at δ 56.0 (C-14) and 42.8 (C-9) and to a methylene carbon at δ 34.8 (C-7). The methylene

Table 2. HMBC data for compounds **1**–**3**

1		**2**		**3**	
δ (^{1}H)	δ (^{13}C)	δ (^{1}H)	δ (^{13}C)	δ (^{1}H)	δ (^{13}C)
1.4 (H-18)	184.2, 81.0, 76.1, 54.0	1.3 (H-18)	184.0, 80.8, 74.1, 52.2	1.25 (H-18)	184.3, 80.3, 67.7, 52.0
1.0 (H-20)	81.0, 42.5, 30.8	0.85 (H-20)	80.8, 42.9, 42.0, 29.4	0.95 (H-20)	80.3, 38.4, 31.4
4.6, 4.5 (H-17)	32.8, 56.0, 154.0	4.6, 4.5 (H-17)	32.67, 56.2, 153.9	4.65, 4.5 (H-17)	55.4, 32.3
5.0, 4.9 (H-16)	139.4, 56.0	5.0, 5.1 (H-16)	139.3, 56.2	5.1, 5.05 (H-16)	139.5, 55.4
4.3 (H-3)	81.0, 30.3	4.1 (H-3)	80.8, 28.7	4.45 (H-3)	80.3, 38.4, 184.3
4.05 (H-6)	81.0, 42.5, 36.3			4.4 (H-6)	80.3, 38.3, 184.3
2.75 (H-14)	154.1, 139.4, 115.8, 106.4, 36.3, 34.8	2.8 (H-14)	153.9, 139.3, 115.9, 106.5, 41.4	2.8 (H-14)	153.0, 139.5, 117.7, 38.9, 32.3
2.2 (H-12)	154.1, 106.4, 56.0, 42.8, 28.9	2.2 (H-14)	153.9, 106.5, 28.2	2.2 (H-14)	153.0, 107.5, 27.76
		1.7 (H-7)	41.4	1.88 (H-7)	80.4, 38.3
		1.16 (H-7)	80.8, 41.4	1.95 (H-7)	55.4, 38.3
				1.8 (H-2)	67.7, 38.3
				1.76 (H-2)	67.7

carbon at δ 34.8 (C-7) shows connectivity with C-6 (δ 70.8) and C-8 (δ 36.3). The spectrum further shows that the quaternary carbon at δ 81.0 (C-5) is connected to two other quaternary carbons at δ 54.0 (C-4) and 42.5 (C-10) and as well as a methine carbon at δ 70.8 (C-6). Similarly, the carbon at δ 30.8 (C-1) shows coupling with a methylene carbon at δ 27.8 (C-2), while the carbon at δ 76.1 (C-3) is coupled with the carbon at δ 27.8 (C-2) and at δ 54.0 (C-4).

The structure of **1** is fully supported by INEPT and HMBC (Table 2) experiments. A series of selective INEPT experiments were consistent with the carbon assignments. Irradiation of H-18 (δ 1.4) enhanced the carbon signals at δ 184.2, 76.1, 54.0 and 81.0, thus confirming the assignments of C-19, C-3, C-4 and C-5. Irradiation of H-3 (δ 4.3) resulted in the enhancement of the C-5 (δ 81.0), C-4 (δ 54.0) and C-1 (δ 30.8) signals, while irradiation of H-6 (δ 4.05) shows enhancement of the C-5 (δ 81.0), C-4 (δ 54.0), C-10 (δ 42.5) and C-8 (δ 36.3) signals.

The stereochemistry at the different chiral centres in the molecule is established by the coupling constants in the ^{1}H NMR spectrum and by nOe difference spectra. In the ^{1}H NMR spectrum of **1**, H-14 (δ 2.75) shows a 9 Hz coupling with H-15 and a 4.5 Hz coupling with H-8, suggesting that H-14 is equatorial. This assignment is confirmed and extended by nOe enhancement experiments. Irradiation at δ 2.75 (H-14) shows 10% nOe at δ 4.6 (H-17) and 11% nOe at δ 1.9 (H-8), while irradiation at δ 6.1 (H-15) causes 7% nOe at δ 1.93 (H-9), showing the α-axial orientation of H-9. Irradiation at δ 1.0 (C-10 methyl) resulted in 5.5% enhancement at δ 1.9 (H-8) and 6% nOe at δ 1.76 (H_{ax}-11). Irradiation of the methyl at C-4 (δ 1.4) resulted in a 2% nOe enhancement at H-3 and 6.5% enhancement at H-6. The absence of nOe interaction between the axial 10- and 4-methyl groups indicates that the carboxylic group at C-4 is in the β-axial orientation. The structure of **1** is thus 3α,5α,6β-trihydroxycleistanth-13(17),15-dien-19-oic acid.

Compound **2**, $[\alpha]_D$ −15.5°; displays broad absorption at 3338 (hydroxyl) 1690, 1262, 1104 (carboxyl) and 1650 cm^{-1} (C=C). The molecular ion was not detected in the HREI mass spectrum of this compound, but a fragment ion appeared at *m/z* 316.2039 $[M - H_2O]^+$ corresponding to $C_{20}H_{28}O_3$. Compound **2** gave the monoacetate (**2a**) when treated with acetic anhydride–pyridine, indicating the presence of a secondary hydroxyl group. The HREI mass spectrum of **2a** also shows a fragment ion at *m/z* 316.2026 $[M - HOAc]^+$. The CI mass spectrum of the monoacetate confirmed its molecular weight as 376 (peak at 394 $[M + 18]^+$, reagent gas: ammonia).

The ^{13}C NMR spectrum of **2** (Table 1) confirms the presence of 20 carbons in the molecule. The ^{1}H NMR spectrum shows the presence of an exocyclic methylene (δ 4.6, 4.5, 1H each, *d*, *J* = 2 Hz) and a vinyl group (δ 6.1, 1H, *ddd*, *J* = 17, 10 and 9 Hz; 5.1, 1H, *dd*, *J* = 17 and 2.5 Hz; 5.0, 1H, *dd*, *J* = 10 and 2.5 Hz). In addition, it shows an apparent triplet at δ 4.1 (*J* = 4.5 Hz) for a proton geminal to a hydroxyl group and singlets at δ 1.3 and 0.85 for two quaternary methyl groups.

A comparison of the ^{1}H and ^{13}C NMR (Table 1) ^{1}H–^{1}H COSY and HMBC spectral data (Table 2) of **2** with those of **1** suggested that they were closely related, possessing the same cleistanthane skeleton and containing a secondary and a tertiary hydroxy and a carboxyl group. The partial structure of the segment from C-1 to C-3 is elucidated by a combination of ^{1}H–^{1}H COSY and HMBC experiments. In the COSY spectrum the proton at δ 4.1 (H-3) shows coupling interaction with methylene protons at δ 2.3 and 1.64 (H-2's). The C-2 protons correlate with each other and with H-3 (4.1) and with two methylene protons at δ 1.26 (H-1's). The position of secondary and tertiary hydroxyl groups at C-3, C-5 and the carboxyl group at δ C-4 are confirmed on the basis of HMBC long range correlations. The 18-methyl protons (δ 1.3) show long range correlations with C-3 (δ 74.1), C-5 (δ 80.8), C-19 (δ 184.0) and C-4 (δ 52.2). The C-20 protons (δ 0.85) show long range coupling interactions with C-5 (δ 80.8), C-10 (δ 42.9), C-9 (δ 42.0) and C-1 (δ 29.4). The other long range correlations are summarized in Table 2.

In the ^{1}H NMR spectrum of **2** the apparent triplet at δ 4.1 (*J* = 4.5 Hz) assigned to H-3 is indicative of an equatorial carbinyl proton interacting with adjacent axial and equatorial protons, showing the α-axial orientation of the hydroxyl group at C-3. The signal for H-14 at δ 2.8 appeared as a double doublet ($J_{14,15}$ = 9 Hz and $J_{14,8}$ = 4.5 Hz), suggesting a *cis* relationship between H-14 and H-8; thus, the vinyl group must be α-axial. The signal for H-9 at δ 1.9 shows two large ($J_{8_{ax},9_{ax}}$ = 12 Hz and $J_{9_{ax},11_{ax}}$ = 9.5 Hz) and a small coupling ($J_{9_{ax},11_{eq}}$ = 3 Hz), which reveals that the B/C ring fusion is *trans*. Assuming that the configuration at C-4 and C-5 are as in **1**, compound **2** is identified as 3α,5α-dihydroxycleistanth-13(17),15-dien-19-oic acid.

Compound **3**, $[\alpha]_D$ +4.6°, shows intense absorption for hydroxyl (3425 cm^{-1}), γ-lactone (1747 cm^{-1}) and carbon–carbon double bond(s) (1649 cm^{-1}) in its IR spectrum. The molecular formula for **3** was established as $C_{20}H_{28}O_4$ through HREI mass spectrometry (*m/z* 332.1981, calc. 332.1987) and is confirmed by CI mass spectrometry (*m/z* 350 $[M + 18]^+$, reagent gas: ammonia), indicating seven sites of unsaturation. The ^{13}C NMR spectrum (APT) of **3** indicates that the 20 carbons in the molecule are present as two methyls, seven methylenes, six methines and five fully substituted carbons. The ^{1}H and ^{13}C NMR assignments were established by HMQC experiments (Table 1). Other structural features are evident from the ^{1}H NMR spectrum of **3**. It displays five alkenic protons [exocyclic methylene (δ 4.65, 4.55, 1H each, *d*, *J* = 2 Hz: (vinyl group δ 6.1, 1H, *ddd*, *J* = 16, 10 and 9 Hz; 5.1, 1H, *dd*, *J* = 10 and 2 Hz; 5.0, 1H, *dd*, *J* = 16 and 2 Hz)] and two methine protons on oxygenated carbon appearing as overlapping double doublets at δ 4.45 (1H, *J* = 10 and 5 Hz) and 4.4 (1H, *J* = 6 and 1 Hz). In

addition, it shows the presence of quaternary methyl groups at δ 1.25 and 0.95 (3H each, *s*).

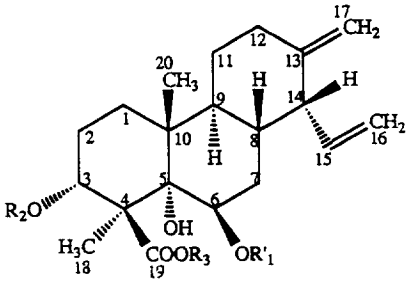
Treatment of **3** with acetic anhydride–pyridine yields the monoacetate **3a**, which shows $[M]^+$ at *m/z* 374.2077 ($C_{22}H_{30}O_5$, calc. 374.2093) in its HREI mass spectrum and this is confirmed by its CI mass spectrum (392 $[M + 18]^+$). The IR spectrum of **3a** shows strong absorption at 3461 (hydroxyl), 1776 (γ-lactone), 1743 (ester) and 1650 cm^{-1} (carbon–carbon double bonds). In the ^1H NMR spectrum of **3a**, the signals for the oxygenated methines appear as well separated double doublets at δ 5.6 ($J_{ax,ax}$ = 10 Hz and $J_{ax,eq}$ = 5 Hz) and 4.5 (J = 6.6 and 1 Hz). The downfield shift of one signal (δ 4.45 → 5.6) indicates that the compound has only one secondary hydroxyl group.

The ^1H–^1H COSY spectra of **3** and **1** show similarities which reveal that oxygen functionalities are attached to C-3 and C-6. The spectrum represents three spin systems, one initiated from the protons on C-3 (δ 4.45) to C-2 (δ 1.65 and 1.8) and C-1 (δ 1.36). The other correlation is initiated from the exocyclic methylene protons on C-17 (δ 4.55, 4.65) to C-12 (δ 2.2), C-11 (δ 1.7 and 1.16), C-9 (δ 2.06) and C-8 (δ 1.76). The third spin system shows the connection between the protons on C-16 (δ 5.1, 5.0) to C-15 (δ 6.1), C-14 (δ 2.8), C-8 (δ 1.76), C-7 (δ 1.95 and 1.88) and C-6 (δ 4.4).

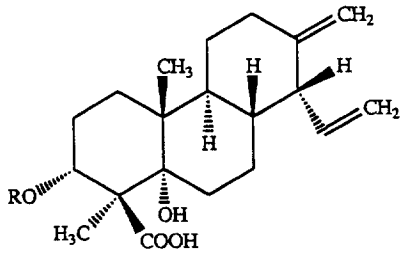
The long range (J^2_{CH}, J^3_{CH}) correlations in the HMBC spectrum of **3** establish the positions of the γ-lactone and secondary and tertiary hydroxyl groups. The proton signal at δ 1.25 (H-18) shows long range correlations with the carbon signals at δ 52.0 (C-4), 67.7 (C-3), 80.3 (C-5) and 184.3 (C-19). The spectrum also shows that the proton signal at δ 0.95 (H-20) is correlated with carbon signals at δ 38.4 (C-10), 31.4 (C-1) and 80.3 (C-5). Other CH long range correlations observed are shown in Table 2.

The stereochemistry of the molecule is confirmed by nOe difference measurements and chemical correlation between **3** and **1**. Compound **1** is easily lactonized to **3** by treatment with sulphuric acid (two drops) in acetone. The spectral data for the lactonized product are found to be identical with those for **1**, indicating the relationship between the two. In the ^1H NMR spectrum of both **3** and **3a** the proton at C-3 appears as a double doublet with $J_{ax,ax}$ = 10 Hz and $J_{ax,eq}$ = 5 Hz, indicating that in **3** the A ring adopts a twist conformation as shown in **4**. In agreement with this, irradiation of the C-10 methyl (δ 0.95) shows a 28% nOe enhancement at δ 4.45 (H-3) and 14% enhancement at δ 1.76 (H-8). This conformation also explains the differences in the γ-lactone frequencies in **3** and **3a**, since the hydroxyl group in conformation **4** is perfectly situated at the hydrogen bond [5] linked to the lactone carbonyl. In **3a** this hydroxyl is acetylated. Irradiation of the proton at C-15 (δ 6.1) shows nOe interaction to H-9 and vice versa, indicating the *syn*-relationship of the vinyl group and H-9. The structure of zythiostromolide is thus 3α,5α-dihydroxycleistanth-13(17),15-dien-6,19-olide (**3** = **4**).

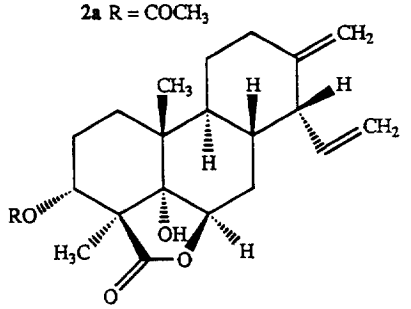
Compounds **1**, **2** or **3** showed no activity at 1000 ppm against *O. crassivaginatum* on agar culture plates, using 1 cm impregnated discs.



1 $R_1 = R_2 = R_3 = H$
1a $R_1 = R_2 = COCH_3$, $R_3 = H$
1b $R_1 = R_2 = H$, $R_3 = CH_3$
1c $R_1 = R_2 = COCH_3$, $R_3 = CH_3$



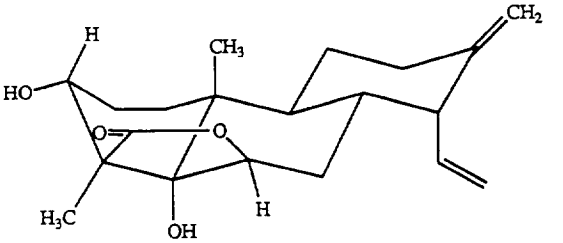
2 R = H
2a R = $COCH_3$



3 R = H
3a R = $COCH_3$

EXPERIMENTAL

General procedures. FTIR spectra were recorded on a Nicolet 7199 FTIR interferometer. Optical rotations were determined with a Perkin Elmer 241 polarimeter. HRMS were recorded on an AEIMS-50 mass spec-



4

trometer and CIMS were recorded on an AEIMS-12 mass spectrometer with NH_3 as reagent gas. NMR spectra (^1H and ^{13}C) were obtained on Bruker AM-300 or Varian Unity 500 multinuclear spectrometers and the solvent signal was used as standard. HMQC and HMBC experiments were recorded on a Varian Unity 500 spectrometer. Long range CH correlations were established using the HMBC experiment optimized for $^nJ_{CH} = 10$ Hz. Flash CC was performed on silica gel (230–400 mesh), General Intermediates of Canada. Analyt. TLC was carried out on E. Merck precoated aluminium sheets of silica gel 60F-254 (0.2 mm thickness). TLC plates were visualized using I_2 vapour or 5% phosphomolybdic acid in 5% H_2SO_4. All solvents were distilled prior to use. Skellysolve B (SKB) refers to Skelly Oil Co. petrol (bp 62–70°). All new compounds reported gave single spots on TLC in at least two different solvent systems and showed no substantial extraneous peaks in the ^1H and ^{13}C NMR spectra.

Isolation of metabolites. The culture of *Zythiostroma* sp. (strain NOF 1560) was obtained from Dr Y. Hiratsuka, Forestry Canada, Northern Forestry Centre, Edmonton. It is deposited in the University of Alberta Microfungus Herbarium as UAMH 7464. Five 4-l Fernbach flasks each containing 2 l 2% malt extract and 0.2% yeast extract were inoculated with *ca* 20 ml mycelial suspension of *Zythiostroma* sp. in H_2O and were shaken at room temp. for 4 weeks. The mycelium was sepd by filtration and the broth was concd to *ca* 3 l under red. pres. (water bath temp. 35–40°) and then extracted with EtOAc (3 × 1.5 liter). The organic extract was dried over Na_2SO_4 and the solvent was evapd under red. pres. to afford 2.6 g crude organic extract. The crude metabolites were subjected to flash CC over silica gel using gradient elution (0–100% EtOAc in SKB and then 0–5% MeOH in EtOAc). Frs of similar composition as determined by TLC and ^1H NMR were pooled. Further purification is described for individual components.

Zythiostromic acid A (**1**). The frs eluted with EtOAC–MeOH (49:1) were evapd and purified by prep. TLC (MeOH–EtOAc, 1:4] to afford **1** (150 mg) as a gum, which solidified to an amorphous solid when tritiated with Et_2O; $[\alpha]_D$ −31.7° (*c* 0.35, MeOH); IR ν_{max} (MeOH cast) 3217, 2934, 1693, 1650, 1202, 1076 cm^{-1}; ^{13}C NMR (75 MHz, CD_3OD): see Table 1; ^1H NMR (300 MHz, CD_3OD): δ 6.1 (1H, *ddd*, J = 17, 10, 9 Hz, H-15), 5.0 (1H, *dd*, J = 17, 2 Hz, H-16), 4.9 (1H, *dd*, J = 9, 2 Hz, H-16), 4.6, 4.5 (1H each, *d*, J = 2 Hz, H-17), 4.3 (1H, *t*, J = 4.5 Hz, H-3), 4.05 (1H, *t*, J = 4.0 Hz, H-6), 2.75 (1H, *dd*, J = 9, 4.5 Hz, H-14), 2.2 (2H, *m*, H-12), 1.4 (3H, *s*, H-18), 1.0 (3H, *s*, H-20); HREIMS: $[M - 2H_2O]^+$ m/z 314.1877 calc. for $C_{20}H_{26}O_3$ 314.1880; other fragment peaks appear at m/z 270.1980 ($C_{19}H_{26}O$) (23), 255.1742 ($C_{18}H_{23}O$) (16), 188.1196 ($C_{13}H_{16}O$) (22), 173.0962 ($C_{12}H_{13}O$) (25), 135.0807 ($C_9H_{11}O$) (44), 119.086 (C_9H_{11}) (43), 105.0702 (C_8H_9) (57), 91.0549 (C_7H_7) (100); CIMS 368 $[M + 18]^+$.

Zythiostromic acid A diacetate (**1a**). A mixt. of **1** (16.0 mg), Ac_2O (1.0 ml) and pyridine (10 drops) was kept overnight at room temp., then worked up in usual manner to yield **1a** as a gum (15.5 mg); $[\alpha]_D$ −30.7° (*c* 1.64, MeOH); IR ν_{max} (MeOH cast); 3561, 2932, 1737 (br), 1722, 1652, 1238, 1024 cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ 6.1 (1H, *ddd*, J = 16.5, 10, 9.5 Hz, H-15), 5.4 (1H, *m*, H-3), 5.15 (1H, *t*, J = 4.5 Hz, H-6), 5.01 (1 H, *dd*, J = 17, 2 Hz, H-16), 4.98 (1 H, *dd*, J = 10, 2 Hz, H-16), 4.6, 4.5 (1 H each, J = 2 Hz, H-17), 2.75 (1H, *dd*, J = 9, 4.5 Hz, H-14), 1.25 (3H, *s*, H-18), 1.2 (3H, *s*, H-20), 2.05, 1.95 (3H each, *s*, 2 × OCOC<u>H</u>$_3$); ^{13}C NMR (75 MHz, CD_3OD): δ 31.5 (C-1), 25.2 (C-2), 77.1 (C-3), 54.0 (C-4), 78.4 (C-5), 72.0 (C-6), 32.2 (C-7), 37.0 (C-8), 42.8 (C-9), 42.5 (C-10), 28.6 (C-11), 32.6 (C-12), 153.4 (C-13), 55.5 (C-14), 138.8 (C-15), 116.4 (C-16), 107.1 (C-17), 22.8 (C-18), 184.0 (C-19), 17.9 (C-20), 171.42, 172.3 (2 × O<u>C</u>OCH$_3$), 21.7, 21.1 (2 × OCO<u>C</u>H$_3$); HREIMS: $[M - HOAc]^+$ m/z 374.2082: calc. for $C_{22}H_{30}O_5$, 374.2093; fragments appear at m/z 330.2193 ($C_{21}H_{30}O_3$) (5), 312.2089 ($C_{21}H_{28}O_2$) (16), 296.1772 ($C_{20}H_{24}O_2$) (16), 270.1985 ($C_{19}H_{26}O$) (61), 237.1643 ($C_{18}H_{21}$) (40), 188.1195 ($C_{13}H_{16}O$) (13), 145.1012 ($C_{11}H_{13}$) (48), 124.0886 ($C_8H_{12}O$) (100), 91.0547 (C_7H_7) (92); CIMS: 392 $[M - HOAc + 18]^+$.

Methyl zythiostromate A (**1b**). Compound **1** (10 mg) was dissolved in Et_2O ether (5.0 ml) containing a few drops of Me_2CO and treated with excess of an ethereal soln of CH_2N_2. Removal of solvent gave **1b** (10.2 mg). $[\alpha]_D$ −71° (*c* 0.52, $CHCl_3$); IR ν_{max} ($CHCl_3$ cast): 3329, 2975, 1697, 1649, 1253, 1017 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 6.1 (1H, *ddd*, J = 17, 10, 9 Hz, H-15), 5.1 (1H, *dd*, J = 17, 2 Hz, H-16), 4.9 (1H, *dd*, J = 10, 2 Hz, H-16), 4.65, 4.55 (1H each, *d*, J = 2 Hz, H-17), 4.3 (1H, *t*, J = 4.5 Hz, H-3), 4.1 (1H, *t*, J = 4.5 Hz, H-6), 2.8 (1H, *dd*, J = 9, 4 Hz, H-14), 2.2 (2H, *m*, H-12), 1.5 (3H, *s*, H-18), 0.9 (3H, *s*, H-20), 3.75 (3H, *s*, OMe); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.8 (C-1), 27.0 (C-2), 73.1 (C-3), 51.9 (C-4), 79.9 (C-5), 69.9 (C-6), 33.7 (C-7), 34.7 (C-8), 41.7 (C-9), 41.3 (C-10), 27.7 (C-11), 31.6 (C-12), 152.1 (C-13), 54.2 (C-14), 139.7 (C-15), 116.1 (C-16), 106.6 (C-17), 20.7 (C-18), 179.5 (C-19), 15.8 (C-20), 53.3 (OMe); HREIMS: m/z 364.2243; calc. for $C_{21}H_{32}O_5$, 364.2249: fragment peaks appeared at m/z 346.2145 ($C_{21}H_{30}O_4$) (30), 314.1878 ($C_{20}H_{26}O_3$) (16), 296.1767 ($C_{20}H_{24}O_2$) (6), 256.1825 ($C_{18}H_{24}O$) (16), 220.1464 ($C_{14}H_{20}O_2$) (62), 149.1017 ($C_{11}H_{17}$) (16), 137.0604 ($C_8H_9O_2$) (23), 115.0409 ($C_5H_7O_3$) (100), 91.0547 (C_7H_7) (61); CIMS: 382 $[M + 18]^+$, 365 $[M + 1]^+$.

Methyl zythiostromate A diacetate (**1c**). Compound **1b** (7.0 mg) in Ac_2O (1.0 ml) and pyridine (6 drops) was warmed at 60° to produce a clear soln. The mixt. was left for 24 h at room temp. and then worked up in usual manner to yield **1c** as an oil (7 mg); $[\alpha]_D$ −18.5° (*c* 0.7, MeOH); IR ν_{max} ($CHCl_3$ cast): 3565, 1749, 1703, 1649, 1253, 1019 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 6.1 (1H, *ddd*, J = 17, 10, 9 Hz, H-15), 5.4 (1H, *m*, H-3), 5.1 (1H, *t*, J = 4.5 Hz, H-6) 5.05 (1H,

dd, $J = 17$, 2 Hz, H-16), 4.95 (1H, *dd*, $J = 10$, 2 Hz, H-16), 4.6, 4.5 (1H, *b rs*, H-17), 2.75 (1H, *dd*, $J = 9$, 4.5 Hz, H-14), 1.25 (3H, *s*, H-18), 1.1 (3H, *s*, H-20), 2.05, 1.95 (3H, each, *s*, $2 \times OCOCH_3$), 3.6 (3*H*, *s*, OMe); ^{13}C NMR (75 MHz, $CDCl_3$): δ 31.3 (C-1), 25.1 (C-2), 77.6 (C-3), 53.0 (C-4), 80.0 (C-5), 72.2 (C-6), 32.2 (C-7), 36.9 (C-8), 42.8 (C-9), 42.3 (C-10), 28.6 (C-11), 32.6 (C-12), 153.3 (C-13), 55.4 (C-14), 138.7 (C-15), 116.5 (C-16), 107.2 (C-17), 19.1 (C-18), 182.0 (C-19), 17.7 (C-20), 171.7, 172.4 ($2 \times OCOCH_3$), 21.8, 21.0 ($2 \times OCOCH_3$) 52.8 (OMe); HREIMS: *m/z* 448.2440: calc. for $C_{25}H_{36}O_7$448.2409; CIMS: 466 $[M + 18]^+$.

Zythiostromic acid B (**2**). The fr. eluted with SKB–EtOAc (3:2) was further purified by prep. TLC using EtOAc–CH_2Cl_2 (3:2) as eluent to give **2** as a gum, (2.0 mg); $[\alpha]_D$ −15.5° (*c* 0.18, MeOH); IR ν_{max} ($CHCl_3$ cast): 3338, 2929, 1690, 1650, 1262, 1104 cm^{-1}; ^{13}C NMR (75 MHz, CD_3OD): see Table 1; ^1H NMR (300 MHz, CD_3OD); δ 6.1 (1H, *ddd*, $J = 17$, 10, 9 Hz, H-15), 5.1 (1H, *dd*, $J = 17$, 2.5 Hz, H-16), 5.0 (1H, *dd*, $J = 9$, 2.5 Hz, H-16), 4.6, 4.5 (1H each, *d*, $J = 2$ Hz, H-17), 4.1 (1H, *t*, $J = 4.5$ Hz, H-3), 2.8 (1H, *dd*, $J = 9$, 4.5 Hz, H-14), 2.2 (2H, *m*, H-12), 1.9 (1H, *ddd*, $J = 12$, 9.5, 3 Hz, H-9), 1.3 (3H, *s*, H-18), 0.85 (3H, *s*, H-20); HREIMS: *m/z* $[M - H_2O]^+$ *m/z* 316.2039; calc. for $C_{20}H_{28}O_3$, 316.2038; fragment peaks appeared at *m/z* 298.1929 ($C_{20}H_{26}O_2$) (4), 272.2139 ($C_{19}H_{28}O$) (14), 257.1904 ($C_{18}H_{25}O$) (4), 204 ($C_{14}H_{20}O$) (100), 189.1280 ($C_{13}H_{17}O$) (13), 175.1120 ($C_{12}H_{15}O$) (10), 122.0729 ($C_8H_{10}O$) (23), 105.0704 (C_8H_9) (19), 91.0548 (C_7H_7) (30).

Zythiostromic acid B monoacetate (**2a**). Compound **2a** (1.8 mg) was prepd as described above for **1a**; $[\alpha]_D$ −27° (*c* 0.07, MeOH); IR ν_{max} ($CHCl_3$ cast) 3581, 2925, 1747, 1650, 1231, 1021 cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ 6.1 (1H, *ddd*, $J = 17$, 10, 9 Hz, H-15), 5.5 (1H, *t*, $J = 5$ Hz, H-3), 5.0 (1H, *dd*, $J = 17$, 2 Hz, H-16), 4.95 (1H, *dd*, $J = 10$, 2 Hz, H-16), 4.6, 4.5 (1H each, *d*, $J = 2$ Hz, H-17), 2.8 (1H, *dd*, $J = 9$, 4 Hz, H-14), 1.2 (3H, *s*, H-18), 0.9 (3H, *s*, H-20), 2.1 (3H, *s*, OAc); HREIMS: $[M - HOAc]^+$ *m/z* 316.2026; calc. for $C_{20}H_{28}O_3$, 316.2038; fragment peaks appeared at *m/z* 254.2031 ($C_{19}H_{26}$) (40), 239.1798 ($C_{18}H_{23}$) (51), 213.1661 ($C_{16}H_{21}$) (14), 204.1523 ($C_{14}H_{20}O$) (14), 160.1250 ($C_{12}H_{16}$) (31), 132.0949 ($C_{10}H_{12}$) (41), 120.0946 (C_9H_{12}) (34), 119.0860 (C_9H_{11}) (43), 107.0862 (C_8H_{11}) (41), 91.0538 (C_7H_7) (50); CIMS: 394 $[M + 18]^+$.

Zythiostromolide (**3**). Evapn of frs eluted with SKB–EtOAc (3:7) gave oily material which was purified by prep. TLC, using EtOAc–CH_2Cl_2 (3:2) to give **3** (8.0 mg) as a gum; $[\alpha]_D$ +4.6° (*c* 0.54, MeOH); IR ν_{max} ($CHCl_3$ cast): 3425, 2923, 1747, 1649, 1245, 1061 cm^{-1}; ^{13}C NMR (125 MHz, CD_3OD): see Table 1; ^1H NMR (300 MHz, CD_3OD): δ 6.1 (1H, *ddd*, $J = 16$, 10, 9 Hz, H-15), 5.1 (1H, *dd*, $J = 10$, 2 Hz, H-16), 5.0 (1H, *dd*, $J = 16$, 2 Hz, H-16), 4.65, 4.55 (1H each, $J = 2$ Hz, H-17's) 4.45 (1H, *dd*, $J = 10$, 5 Hz, H-3), 4.05 (1H, $J = 6.1$H, H-6), 2.8 (1H, *dd*, $J = 9$, 5 Hz, H-14), 2.2 (2H, *m*, H-12), 1.25 (3H, *s*, H-18), 0.95 (3H, *s*, H-20); HREIMS: *m/z* 332.1981; calc. for $C_{20}H_{28}O_4$, 332.1987; fragment ions appear at *m/z* 314.1884 ($C_{20}H_{26}O_3$) (38), 299.1644 ($C_{19}H_{23}O_3$) (17), 255.1744 $(C_{18}H_{23}O)^+$ (5), 221.1173 ($C_{13}H_{17}O_3$) (11), 159.1167 ($C_{12}H_{15}$) (17), 105.0702 (C_8H_9) (48), 91.0540 (C_7H_7) (65), 79.0548 (C_6H_7) (100); CIMS: 350 $[M + 18]^+$.

Zythiostromolide monoacetate (**3a**). Compound **3** (2.0 mg) was treated with a mixt. of Ac_2O (1.0 ml) and pyridine (5 drops) and the soln was kept overnight at room temp. Work-up in the usual way yielded **3a** as a glass (1.8 mg); $[\alpha]_D$ +2.3° (*c* 0.9, MeOH); IR ν_{max} ($CHCl_3$ cast); 3461, 2931, 1776, 1743, 1640, 1243, 1024 cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ 6.1 (1H, *ddd*, $J = 17$, 10, 9 Hz, H-15), 5.6 (1H, *dd*, $J = 10$, 5 Hz, H-3), 5.1 (1H, *dd*, $J = 10$, 1 Hz, H-16), 5.0 (1H, *dd*, $J = 17$, 1 Hz, H-16), 4.65, 4.55 (1H each, *d*, $J = 2$ Hz, H-17), 4.5 (1H, *dd*, $J = 6.6$, 1 Hz, H-6), 2.85 (1H, *dd*, $J = 9$, 4.5 Hz, H-14), 2.2 (2H, *m*, H-12), 1.25 (3H, *s*, H-18), 0.95 (3H, *s*, H-20), 2.04 (3H, *s*, $OCOCH_3$); ^{13}C NMR (75 MHz, CD_3OD): δ 31.1 (C-1), 23.9 (C-2), 70.7 (C-3), 52.0 (C-4), 80.4 (C-5), 80.2 (C-6), 27.4 (C-7), 38.3 (C-8), 38.9 (C-9), 38.4 (C-10), 27.9 (C-11), 32.2 (C-12), 152.9 (C-13), 55.3 (C-14), 138.4 (C-15), 117.8 (C-16), 107.6 (C-17), 12.8 (C-18), 184.3 (C-19), 21.0 (C-20), 20.0 ($OCOCH_3$), 171.8 (COMe); HREIMS: *m/z* 374.2077; calc. for $C_{22}H_{30}O_5$, 374.2093; CIMS: 392 $[M + 18]^+$, 375 $[M + 1]^+$.

Lactonization of zythiostromic acid A (**1**) *to zythiostromolide* (**3**). A soln of **1** (8.0 mg) in Me_2CO (15 ml) containing H_2SO_4 (2 drops) was stirred at room temp. for 24 hr. The major product **3** was purified by prep. TLC using EtOAc–CH_2Cl_2 (3:2) as eluent (6.0 mg), $[\alpha]_D$ +2.3° (*c* 0.51, MeOH). The IR, ^1H NMR and MS were identical with those reported above.

Acknowledgements—We gratefully acknowledge the financial support of the Natural Sciences and Engineering Research Council of Canada. We thank Dr Y. Hiratsuka, Northern Forestry Service, Forestry Canada, Edmonton, for his interest and for cultures of the *Zythiostroma* sp. We also thank Drs T. T. Nakashima, D. Muir and L. Jiminez for assistance with the NMR measurements and Dr P. Chakravarty for bioassays.

REFERENCES

1. Ayer, W. A. (1995) *Can. J. Chem.* **73**, 465.
2. McCorry, E. J., Pegel, K. H., Phillips, L. and Wright, E. S. (1969) *J. Chem. Soc., Chem. Commun.* 1074.
3. Hussaini, F. A., Roy, R., Shoeb, A., Pragya, G. M., Jacobsen, J. P. and Boll, P. M. (1993) *Nat. Prod. Letters* **3**, 71.
4. Mareci, T. H. and Freeman, R. (1982) *J. Magn. Reson.* **48**, 158.
5. Golfier, M. (1977) in *Stereochemistry: Fundamentals and Methods* (Kagan, H. B., ed.), Vol. 1, pp. 39–41. Georg Thieme, Stuttgart.