Structures and Absolute Configurations of Antibiotics of the Oligosporon Group from the Nematode-trapping Fungus *Arthrobotrys oligospora*

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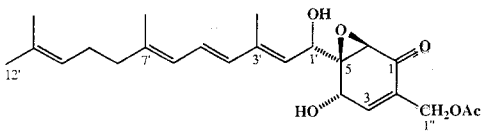
Spectroscopic data define the structures of three new antibiotics, 4′,5′-dihydro-oligosporon (**4**), hydroxyoligosporon (**5**) and 10′,11′-epoxyoligosporon (**6**) from the nematode-trapping fungus *Arthrobotrys oligospora*, and confirm the structures of the recently reported antibiotics oligosporon (**1**) and oligosporol B (**3**). The absolute configuration of the substituted 7-oxabicyclo[4.1.0]hept-3-ene nucleus of these metabolites is determined by circular dichroic spectroscopy. Oligosporon (**1**) and its dihydro-derivative (**4**) represent the second and most complex structural type of nematocidal metabolite to be characterised from cultures of nematophagous fungi.

The dramatic ability of a fungal species, *Arthrobotrys oligospora*, to capture nematodes was first reported by ZOPF in 1888.[1,2] This widely occurring fungus has since been extensively investigated as a model for nematode-fungus interactions,[3,4] and may have potential in the biological control of nematode parasites of plants[5] and animals.[6] STADLER, STERNER and ANKE have recently reported the isolation, structure elucidation and biological activities of oligosporon (**1**), oligosporol A (**2**) and oligosporol B (**3**), novel metabolites from cultures of a strain of *A. oligospora* obtained from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.[7] The compounds exhibited weak antibacterial, antiyeast, cytotoxic and haemolytic effects, but were not active against filamentous fungi or the nematode *Caenorhabditis elegans*. They represent a new class of natural products, and are the first antimicrobial antibiotics from predacious *Deuteromycetes* to be described.

In parallel to the German work, we have independently isolated oligosporon (**1**) and oligosporol B (**3**) from an Australian isolate of *A. oligospora* and established their structures as (**1**) and (**3**).[8] Furthermore, our strain yields a number of related antibiotics. We report here the structures of three of these, namely 4′,5′-dihydro-oligosporon (**4**), hydroxyoligosporon (**5**) and 10′,11′-epoxy-



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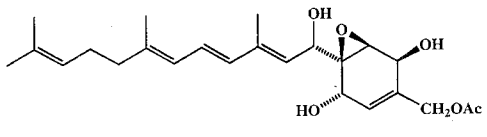

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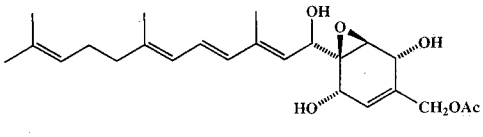

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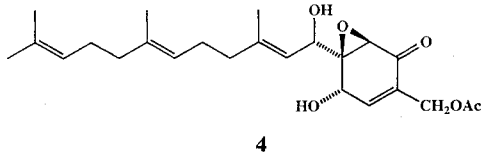

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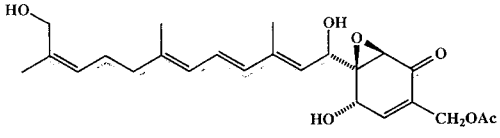

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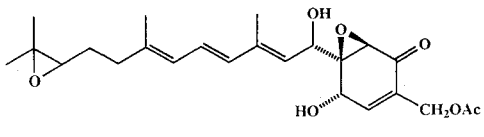

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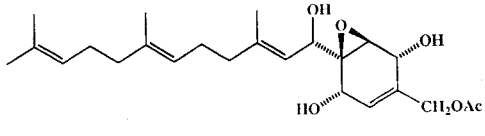

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oligosporon (**6**).[8] Oligosporol B (**3**) fractions contain a minor inseparable component which is probably 4′,5′-dihydro-oligosporol B (**7**). We present evidence for the absolute configuration of the substituted 5-hydroxy-7-oxabicyclo[4.1.0]hept-3-ene-2-one nucleus of 4′,5′-dihydro-oligosporon (**4**), and the extrapolation of this configuration to corresponding stereogenic centres in the other metabolites. Results from antimicrobial and nematocidal assays on these metabolites are also described.

Experimental

General

NMR spectra were recorded for acid-free $CDCl_3$ solutions on Varian Gemini-300 or VXR-500 instruments at 300 or 500 MHz for ^1H and 75.43 or 125.75 MHz for ^{13}C. The solvent ^1H and ^{13}C signals, 7.27 ppm for residual $CHCl_3$ and 77.0 ppm for $CDCl_3$, were used as internal references. Signal assignments are based on direct spectrum analysis, selective proton decoupling, H, H-COSY, APT, HMQC and HMBC data for compounds (**1**), (**4**) and (**5**), and on direct and comparative analysis and APT data for compounds (**3**) and (**6**). Small coupling constants were obtained by resolution enhancement of the spectra. EI, HR-EI and CI mass spectra were recorded on a VG Micromass 7070F spectrometer, FAB spectra on a VG ZAB2-SEQ spectrometer using 3-nitrobenzyl alcohol as the matrix. UV spectra were recorded on a Hewlett-Packard 8450A spectrophotometer, FTIR spectra on a Perkin-Elmer 1800 spectrophotometer, and CD spectra on a Carey 61 spectrophotometer.

Fermentation and Isolation of Antibiotics

Potato dextrose broth (4 liters, Difco Laboratories, Detroit, U.S.A.) adjusted to pH 5.6 with 2 M sodium hydroxide was inoculated with *A. oligospora* (strain M2, Dr. G. R. STIRLING, Queensland Department of Primary Industries, Indooroopilly, Queensland, Australia) grown on corn meal agar. Fermentation was carried out in 250-ml Erlenmeyer flasks, each containing 100 ml of medium, shaken at 200 rpm at 25°C for 7 days. Bioautography of EtOAc extracts of the culture liquor on silica gel TLC (Merck Kieselgel 60 F_{254}) in MeOH - $CHCl_3$ (1 : 9) showed two main zones of activity corresponding to quenching of UV fluorescence at Rf 0.56 and 0.40. Each zone inhibited the growth of *Streptomyces aureofaciens*, *Pythium ultimum* and *Phytophthora cinnamomi*, whilst the Rf 0.56 zone also inhibited *Rhizoctonia solani*. The mycelium was removed by filtration, and the filtrate extracted with EtOAc (3 × 1 liter). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo* on a rotary evaporator to a brown oil (400 mg). Extraction of the wet mycelium with stirred Me_2CO (1 liter), filtration, concentration *in vacuo* to an aqueous residue, and back extraction with EtOAc (3 × 100 ml) afforded after drying (Na_2SO_4) a darker oil (1.73 g).

The culture filtrate extract, after passage through a short silica gel column in EtOAc, was subjected to gradient elution in EtOAc - CH_2Cl_2 (3 : 7 to 9 : 1) on a silica gel Chromatotron plate (2 mm) to give a series of bioactive fractions. Combined fractions 4 and 5 were subjected to reverse phase HPLC on an octadecylsilane column in MeCN - H_2O (3 : 1) to yield oligosporon (**1**) (14 mg) and 4′,5′-dihydro-oligosporon (**4**) (4 mg). Further chromatography of combined Chromatotron fractions 6 and 7, and 9 and 10, on Sephadex LH-20 (Pharmacia LKB, Uppsala, Sweden) in MeOH - CH_2Cl_2 (3 : 97) gave 10′,11′-epoxyoligosporon (**6**) (4 mg) and hydroxyoligosporon (**5**) (14 mg), respectively. Metabolites (**1**) and (**5**) were primarily responsible for the zones of activity, observed at Rf 0.56 and 0.40 respectively, on TLC of culture extracts.

The mycelium extract was subjected to similar filtration through silica gel and gradient elution on Chromatotron plates. Combined fractions 7 and 8 were re-chromatographed in EtOAc - CH_2Cl_2 (3 : 7) on a silica gel Chromatotron plate (1 mm) to afford a mixture of oligosporon (**1**) and 4′,5′-dihydro-oligosporon (**4**) (8.9 mg). Similar re-chromatography of combined fractions 15 ~ 18 gave oligosporol B (**3**) (21 mg), containing a minor component tentatively identified as 4′,5′-dihydro-oligosporol B (**7**).

Bioassay of the Antibiotics

Antimicrobial assays were carried out by incorporating into warm agar solutions of the test compounds in Me_2CO, and examining the ability of the resulting agar to inhibit growth of the test organisms from aq. drop (bacteria) or agar plug (fungi) inocula over a period of 48 hours. Nutrient agar (Difco Laboratories, Detroit) was used for bacteria, potato dextrose agar (Oxoid, Basingstoke) for fungi. Nematode larval development assays were carried out by Dr. E. LACEY, McMaster Laboratory, Division of Animal Health, CSIRO, Sydney.[9]

Physico-chemical Properties of the Antibiotics

MS, UV, IR, CD, and ^1H and ^{13}C NMR data for oligosporon (**1**), 4′,5′-dihydro-oligosporon (**4**), hydroxyoligosporon (**5**), 10′,11′-epoxyoligosporon (**6**), and oligosporol B (**3**) are compared in Tables 1, 2 and 3. Data for oligosporon (**1**) and oligosporol B (**3**) were in agreement with reported data,[7] with the proviso that NMR spectra of the latter showed varying amounts (10 ~ 30%) of a second component. This component, provisionally identified as 4′,5′-dihydro-oligosporol B (**7**), showed a separate ^1H NMR signal at δ 5.30 (dq, J = 8.7, 1, 3 Hz, H-2′) and ^{13}C NMR signals at δ 121.0 (C-2′), 142.5 (C-3′), 16.1 (3′-Me), 39.7 (C-4′), 26.3 (C-5′), 123.4 (C-6′), 135.6 (C-7′), 16.0 (7′-Me), 39.7 (C-8′), 124.2 (C-10′).

Results and Discussion

Structures of the Antibiotics

All five metabolites were unstable, decomposing on storage even when refrigerated. Mass spectrometry in our hands gave relatively weak molecular ions under EI, CI and FAB ionisation conditions, in contrast to the results of STADLER and co-workers who report molecular ions with 30~40% of the base peak intensity in EI spectra of oligosporon (**1**) and oligosporols A (**2**) and B (**3**).[7] Attempts to prepare more volatile derivatives of oligosporon (**1**) for mass spectrometry failed, methylation with methanolic diazomethane, acetylation with acetic anhydride in pyridine, and trimethylsilylation with *bis*-(trimethylsilyl)-trifluoroacetamide in the presence of trimethylsilyl chloride all resulting in extensive decomposition. Molecular formulae could, however, be obtained by HR-MS measurements in EI spectra of molecular ions and daughter ions formed by water loss. These data (Table 1) indicated that compounds (**3**) and (**4**) were dihydro-derivatives of oligosporon (**1**), whilst compounds (**5**) and (**6**) were mono-oxygenated derivatives. Furthermore, mass measurement of the base peak at *m/z* 69 in EI-MS of oligosporon (**1**) and oligosporol B (**3**) established the composition $C_5H_9^+$, suggesting the presence of a terminal dimethylallyl unit in these compounds and in compound (**4**) which showed the same base peak. This fragment ion was absent in the more oxygenated metabolites (**5**) and (**6**).

UV spectra (Table 1) of oligosporon (**1**), oligosporol B (**3**), hydroxyoligosporon (**5**), and 10′,11′-epoxy-oligosporon (**6**) were all dominated by characteristic four-banded conjugated triene absorption around 280 nm,[10] which was absent in the dihydro-compound (**4**). IR spectra (Table 1) of all five compounds showed hydroxyl absorption between 3500~3700 cm^{-1} and a non-conjugated carbonyl stretching frequency near 1745 cm^{-1}.[11] In addition, compounds (**1**), (**4**), (**5**) and (**6**) showed conjugated carbonyl stretching frequencies near 1685 cm^{-1}[11] which were absent in oligosporol B (**3**). The close structural relationships between the five compounds were reinforced by the extensive similarities in their ^1H and ^{13}C NMR spectra (Tables 2 and 3).

The structure of oligosporon was determined by NMR analysis in conjunction with the data noted above. Since we concur with the structure (**1**) recently proposed by STADLER *et al.*,[7] which is also based on NMR data, we summarise here only the differences in our approach. The ^1H NMR spectrum with selective decoupling provided the proton multiplicities and coupling constants (Table 2), and in conjunction with H,H-COSY established the structural segments C-2~C-4, C-1′~C-3′, C-4′~C-7′, and C-9′~C-12′ with their substituents. An APT ^{13}C spectrum defined the extent of protonation

Table 1. Physico-chemical properties of *A. oligospora* metabolites.

	Oligosporon (1)	4′,5′-Dihydro-oligosporon (4)	Hydroxy-oligosporon (5)	10′,11′-Epoxy-oligosporon (6)	Oligosporol B (3)
Appearance	Colourless oil	Colourless oil	Colourless oil	Colourless oil	Colourless oil
Molecular formula	$C_{24}H_{32}O_6$	$C_{24}H_{34}O_6$	$C_{24}H_{32}O_7$	$C_{24}H_{32}O_7$	$C_{24}H_{34}O_6$
EI-MS	M^+	M^+	M^+	M^+	M^+
Found (*m/z*)	416.2198	418.2355	432.2145	432.2150	418.2354
Calcd (*m/z*)	416.2199	418.2355	432.2148	432.2148	418.2355
		M^+-H_2O	M^+-H_2O	M^+-H_2O	M^+-H_2O
Found (*m/z*)		400.2249	414.2055	414.2043	400.2249
Calcd (*m/z*)		400.2250	414.2042	414.2042	400.2250
	$C_5H_9^+$				$C_5H_9^+$
Found (*m/z*)	69.0704				69.0704
Calcd (*m/z*)	69.0704				69.0704
UV (EtOH)	261_{sh} (10260)	240_{end} (2146)	261_{sh} (10510)	260_{sh} (9500)	261_{sh}
λ nm (ε)	273_{sh} (15740)		273_{sh} (15550)	271_{sh} (14760)	270_{sh}
	280_{max} (18300)		280_{max} (18340)	280_{max} (17620)	280_{max}
	293_{sh} (14420)		291_{sh} (13820)	288_{sh} (14040)	290_{sh}
IR (CH_2Cl_2)	3579,	3685, 3593,	3685, 3593,	3579, 3056,	3589,
υ_{max}	1743, 1687	1744, 1685	1745, 1686	1743, 1687	1741
CD	(c. 0.03)	(c. 0.15)	(c. 0.03)	(c. 0.03)	(c. 0.03)
nm Δε	390 0	390 0	390 0	390 0	
(dioxane)	341 +2.40	342 +1.39	341 +1.91	338 +1.56	
	310 +0.83	294 +0.18	306 +0.45	308 +0.36	301 0
	290_{sh} +1.53		293_{sh} +0.95	292_{sh} +1.15	288_{sh} +0.45
	273_{sh} +2.38		281_{sh} +1.37	281_{sh} +1.60	280 +0.80
	250 + 9.11	252 +3.18	250 +6.35	252 +5.47	250_{sh} +0.45
	232 0	232 0	231 0	232 0	232 0
	218 - 8.90	222 -2.18	224 -3.62	219 -5.04	222 -0.54

Table 2. ^{1}H NMR data for *A. oligospora* metabolites[a].

Proton(s)	Oligosporon (1)	4',5'-Dihydro-oligosporon (4)	Hydroxy-oligosporon (5)	10',11'-Epoxy-oligosporon (6)	Oligosporol B (3)
1-H					4.33, m
3-H	6.71, dt, 4.7, 1.3	6.70, dt, 4.7, 1.3	6.71, dt, 4.8, 1.4	6.72, dt, 4.7, 1.5	5.77, dt, 5.5, 1.4
4-H	5.03, dm, 4.7	5.02, m	5.03, dm, 4.7	5.04, dm, 4.7	4.68, m
6-H	3.29, d, 1.4	3.27, d, 1.4	3.29, d, 1.3	3.28, d, 1.1	3.26, dd, 1.8, 1.8
1'-H	5.17, d, 8.9	5.06, m	5.16, d, 9.0	5.18, d, 8.9	4.80, d, 8.6
2'-H	5.40, d, 8.9	5.19, dq, 8.9, 1.3	5.42, d, 9.0	5.42, d, 8.8	5.51, dm, 8.6
3'-Me	1.89, d, 1.2	1.72, d, 1.3	1.89, d, 1.1	1.89, d, 1.1	1.87, d, 1.2
4'-H	6.16, d, 15.4		6.16, d, 15.3	6.17, d, 15.3	6.18, d, 15.2
4'-H_2		2.05, m			
5'-H	6.54, dd, 15.3, 10.9		6.53, dd, 15.3, 10.9	6.53, dd, 15.3, 10.8	6.50, dd, 15.2, 10.8
5'-H_2		2.11, m			
6'-H	5.90, dm, 10.9	5.1, m	5.89, d, 11.0	5.94, d, 10.8	5.89, dm, 10.8
7'-Me	1.82, d, 1.3	1.60, m	1.81, d, 1.1	1.83, d, 0.8	1.80, d, 1.2
8'-H_2	2.11, m	2.00, m	2.14, m	2.24, m	2.1, m
9'-H_2	2.13, m	2.05, m	2.21, m	1.69, m	2.1, m
10'-H	5.10, m	5.1, m	5.30, tm, 7.0	2.72, t, 6.3	5.09, m
11'-Me	1.62, d, 1.2	1.62, d, 1.3		1.27, s[b]	1.60, d, 1.3
11'-CH_2OH			4.12, s		
12'-H_3	1.69, d, 1.1	1.68, d, 1.3	1.80, d, 1.2	1.31, s[b]	1.68, d, 1.2
1"-H_a	4.76, dt, 14.1, 1.4	4.77, m	4.76, dt, 14.1, 1.3	4.77, dm, 14.2	4.64, m
1"-H_b	4.79, dt, 14.2, 1.2	4.77, m	4.80, dm, 14.2	4.79, dm, 14.2	4.64, m
1"-OAc	2.09, s	2.09, s	2.10, s	2.10, s	2.09, s

a Chemical shifts (δ) with $CHCl_3$ (7.27) as reference, multiplicities and coupling constants (Hz) for solutions in $CDCl_3$ at 300 MHz.

b May be interchanged.

Table 3. ^{13}C Chemical shifts for *A. oligospora* metabolites[a].

Carbon	Oligosporon (1)	4',5'-Dihydro-oligosporon (4)	Hydroxy-oligosporon (5)	10',11'-Epoxy-oligosporon (6)	Oligosporol B (3)
1	192.4	192.6	192.4	192.3	63.4
2	131.9	131.8	131.7	131.9	132.9
3	141.0	141.1	141.2	141.0	124.8
4	63.9	63.9	63.8	64.0	63.4
5	67.6	67.6	67.6	67.6	60.2
6	57.2	57.2	57.3	57.2	57.6
1'	67.5	67.5	67.6	67.6	69.7
2'	124.1	119.8	124.7	124.5	126.2
3'	140.6	144.0	140.3	140.4	139.3
3'-Me	13.3	17.0	13.2	13.3	13.2
4'	133.2	39.6	133.8	133.8	133.8
5'	127.0	26.1	126.6	126.7	126.1
6'	124.7	123.3	125.2	125.3	124.9
7'	141.3	135.9	140.2	139.9	140.5
7'-Me	17.0	16.1	17.0	16.9	16.9
8'	40.1	39.6	40.1	36.8	40.1
9'	26.5	26.6	26.0	27.3	26.6
10'	123.7	124.2	127.7	63.9	123.8
11'	131.9	131.4	134.8	58.5	131.8
11'-Me	17.7	17.7		18.8[b]	17.8
11'-CH_2OH			61.6		
12'	25.7	25.7	21.3	24.9[b]	25.7
1"	60.4	60.4	60.4	60.3	65.0
Ac-CO	170.5	170.5	170.5	170.4	171.4
Ac-Me	20.9	20.8	20.8	20.9	20.9

a Chemical shifts (δ) with $CDCl_3$ (77.0) as reference for solutions in $CDCl_3$ at 75 MHz.

b These assignments may be reversed.

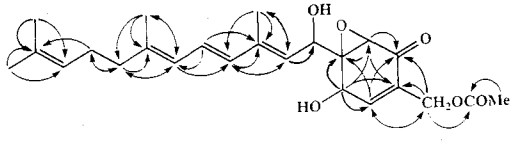
of each carbon (inherent in the assignments in Table 3), and the respective protons attached to each carbon (Table 2) were determined by HMQC spectroscopy. Extensive HMBC data summarised in Fig. 1 then provided the H, C connectivities necessary to establish the structure (**1**) for oligosporon. We note in particular that strong crosspeaks arising from 3-bond couplings between H-3 and C-5, and H-6 and C-2 of the cyclohexenone ring necessitate that the farnesyl substituent is attached to C-5, not C-6, of the ring. Inherent in this structure are the triene, hydroxyl, ester and enone functions observed in UV and IR spectra.

The olefinic stereochemistry of the oligosporon (**1**) side chain follows directly from the ^{13}C chemical shifts of the 3′-Me and 7′-Me groups in comparison with literature data for model terpenes and carotenoids.[12] The *trans* relative stereochemistry of the hydroxy and epoxy substituents on the cyclohexenone ring follows from comparison of the $^{3}J_{3,4}$ and $^{4}J_{4,6}$ values of 4.7 and 1.4 Hz with those for known model metabolites, assuming similar preferred conformations. Such *trans* systems show $^{3}J_{3,4}$ and $^{4}J_{4,6}$ values near 5 and 1 Hz, while *cis* systems show values near 2.5 and 0 Hz.[13,14] Like STADLER *et al.*,[7] we were unable to determine the relative configuration of the C-1′-hydroxyl group.

With the structure of oligosporon (**1**) established, the structures and depicted stereochemistry of the related metabolites (**3**), (**4**), (**5**) and (**6**) were deduced primarily from comparison of their spectroscopic data with those of oligosporon (Tables 1, 2 and 3). Structures (**4**) and (**5**) were also confirmed by extensive HMQC and HMBC NMR studies.

The dihydro-oligosporon (**4**) lacked the triene chromophore of oligosporon. Significant differences in its ^{1}H and ^{13}C NMR spectra relative to oligosporon were restricted to resonances arising from the C-2′～C-7′ segment of the side-chain (Tables 2 and 3), where two allylic methylene groups have replaced vicinal olefinic methine groups. It is clearly 4′,5′-dihydro-oligosporon (**4**), a probable biogenetic precursor of oligosporon in which desaturation of the farnesyl unit has yet to occur. The olefinic stereochemistry of its side chain is again defined by the ^{13}C chemical shifts of the allylic 3′- and 7′-methyl groups.[12,15]

Fig. 1. Long range couplings ($^{1}H \rightarrow ^{13}C$) observed in HMBC NMR spectra of oligosporon (**1**).



The ^{1}H and ^{13}C NMR data for the hydroxyoligosporon (**5**) closely resemble those for oligosporon (**1**) except for the C-10′～C-12′ region of the side-chain (Tables 2 and 3), where one of the terminal methyl groups is now hydroxylated. In agreement, the methylene protons of this hydroxymethyl group show strong HMBC crosspeaks to the neighbouring C-10′, C-11′ and C-12′ carbons. Literature data for such terpene systems indicate that hydroxylation of the methyl group *Z* or *E* to the chain leads to chemical shifts near 61.5 and 21.3 ppm, or 68.9 and 13.4 ppm, for the terminal hydroxymethyl and methyl carbons, respectively.[15] Comparison with the values for hydroxyoligosporon, 61.5 and 21.2 ppm, defines the structure (**5**) in which the *Z* 11′-methyl substituent carries the hydroxyl group.

The structure of 10′,11′-epoxyoligosporon (**6**) follows directly from its ^{1}H and ^{13}C NMR spectra, which differ significantly from those of oligosporon (**1**) only in the resonances from the terminal isoprene unit of the side-chain (Tables 2 and 3). We are unable to comment on the relative configuration at the remote C-10′ centre in this metabolite.

Oligosporol B (**3**) differed from oligosporon (**1**) and its other derivatives (**4**～**6**) in lacking conjugated carbonyl IR absorption (see above). Furthermore, the NMR signal of the oligosporon-type C-1 carbonyl group near δ 192 ppm was replaced by the ^{13}C and ^{1}H signals of a secondary carbinol group at δ 63.4 and 4.33 ppm, respectively, with concomitant changes in other resonances in the vicinity of C-1. These data suggested the triol structure (**3**) for oligosporol B, which is in agreement with that proposed by STADLER *et al.*[7] The coupling constants $^{3}J_{3,4}$ and $^{4}J_{4,6}$ of 5.5 and 1.8 Hz for oligosporol B show that the conformation of the cyclohexenone ring of oligosporon (**1**) is little changed on reduction of the C-1 carbonyl group. The observed $^{3}J_{1,6}$ value of 1.8 Hz indicates, but does not necessitate, a *trans* relationship between the 1-hydroxyl and the epoxide group of oligosporol B (**3**), as suggested by STADLER *et al.*[7] from NOESY correlations between 1-H and 1″-H_2. Literature data indicate *J* values of 1.5～2.3 Hz for such *trans* systems.[16]

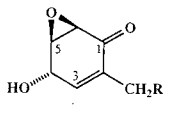
Oligosporol B (**3**) co-elutes from chromatograms with a minor contaminant, visible in NMR spectra, which is probably the related 4′,5′-dihydro-oligosporol B (**7**). APT ^{13}C NMR spectra show, together with the signals of oligosporol B, additional weak signals (*cf.* Experimental) which in chemical shift and extent of protonation closely resemble those from the C-2′～C-10′ region of the

side-chain of 4',5'-dihydro-oligosporon (**4**). Comparison of the ^{13}C data of oligosporon (**1**) and its dihydro-derivative (**4**) (Table 3) suggests that the undetected weak signals of the remaining carbons of 4',5'-dihydro-oligosporol B (**7**) could coincide with those of oligosporol B.

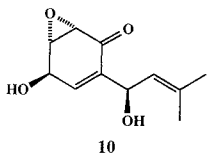
Absolute Configuration of the Antibiotics

With the relative configuration of the ring substituents of oligosporon (**1**) and its co-metabolites (**3**~**6**) established, attention was directed to the absolute configuration of this group of antibiotics. 4',5'-Dihydro-oligosporon (**4**) has a single chromophore absorbing in the accessible UV region, which gives rise to successive positive CD extrema at 342 and 252 nm associated with $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ transitions of the enone system (Table 1). These Cotton effects define respectively the 5*R*,6*R* and 4*S* configurations of the 5,6-epoxide and 4-hydroxy groups as depicted in structure (**4**). Thus the CD curve of 4',5'-dihydro-oligosporon (**4**) parallels those of the 4*S*,5*R*,6*R* systems of (+)-desoxyepiepoxydon (**8**)[17] and (+)-epiepoxydon {(+)-isoepoxydon} (**9**),[13,17] and is the mirror image of that of (−)-(4*R*,5*S*,6*S*)-panepoxydon (**10**).[13,18] In contrast, (+)-epoxydon (**11**) gives rise to successive positive and negative extrema at 341 and 245 nm arising from 5*R*,6*R* and 4*R* configurations of the epoxide and hydroxy groups, respectively.[13,19] This 4*S*,5*R*,6*R* absolute configuration of 4',5'-dihydro-oligosporon (**4**) is in accord with the corresponding relative configuration deduced from the 1.4 Hz long range coupling between 4-H and 6-H (Table 2) and discussed above for oligosporon (**1**) itself.

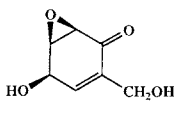
Analysis of the CD spectra of the triene-containing cyclohexenones (**1**), (**5**) and (**6**) is potentially complicated by the presence of overlapping chromophores and associated Cotton effects in the 250~300 nm region. Their CD spectra, however, resemble markedly that of 4',5'-dihydro-oligosporon (**4**), with strong positive extrema near 340 and 250 nm (Table 1). These spectra are clearly dominated by the enone chromophore, with weak additional structure between 250 and 300 nm reflecting the asymmetric environment of the triene. The long wavelength extrema originate solely from the $n\rightarrow\pi^*$ transition of the enone systems, and as with 4',5'-dihydro-oligosporon (**4**) define the chirality of the epoxide groups. The short wavelength extrema are associated primarily with the $\pi\rightarrow\pi^*$ transition of the enone systems, and define the configuration of the 4-hydroxy groups. The resulting 4*S*,5*R*,6*R* absolute configurations depicted for the oligosporons (**1**), (**5**) and (**6**) again accord with the corresponding relative configurations deduced from ring proton coupling constants (Table 2), as discussed above in the case of oligosporon (**1**).



8: R = H
9: R = OH



10



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Oligosporol B (**3**) lacks the cyclohexenone chromophore and consequent definitive CD Cotton effects (Table 1). Its $^3J_{3,4}$ and $^4J_{4,6}$ coupling constants (Table 2) are similar to those of the co-occurring cyclohexenones (**1**), (**4**), (**5**) and (**6**), however, suggesting the same relative and absolute configuration at C-4, C-5 and C-6. The absolute configuration of the C-1 centre in oligosporol B (**3**) and its 4',5'-dihydro derivative (**7**) then follows from the relative configuration at this centre, as does that depicted for oligosporol A (**2**).

Biological Activity of the Antibiotics

The biological activity of the *A. oligospora* antibiotics against various test organisms is summarised in Table 4. MIC values for oligosporon (**1**) and oligosporol B (**3**) against the Gram-positive bacteria *B. subtilis* and *S. aureofaciens* are in the range 25~100 μg/ml. These values from agar incorporation assays are comparable to those observed by STADLER *et al.*[7] for their compounds (**1**), (**2**) and (**3**) in agar diffusion assays against one Gram-negative and three Gram-positive bacteria. Our dihydro- and oxidized analogues (**4**), (**5**) and (**6**) are less active than (**1**) and (**3**) against the Gram-positive bacteria, and all are inactive at ≤100 μg/ml against the Gram-negative plant pathogenic bacterium *E. carotovora.* Only oligosporon (**1**) and 4',5'-dihydro-oligosporon (**4**) inhibit vegetative growth of the plant pathogenic fungus *P. cinnamomi* at ≤100 μg/ml, and none of the compounds inhibit the other plant pathogens *R. solani* and *P. ultimum* at this concentration. STADLER *et al.*[7] reported no inhibition of two different filamentous fungi at 100 μg/disc in agar diffusion assays.

Nematocidal activity was assessed in larval development assays[9] using the intestinal parasitic nematode *Haemonchus contortus.* Oligosporon (**1**) and 4',5'-dihydro-oligosporon (**4**) were the most active with LD_{50} values of 25 and 50~100 μg/ml, while the oxidised oligosporons (**5**) and (**6**) were inactive. STADLER *et al.*[7] observed no nematocidal effect for their compounds (**1**), (**2**) and (**3**) towards the bacterial-feeding nematode *Caenorhabditis elegans* at concentrations up to 100 μg/ml. They further concluded[20] that linoleic acid, obtained

Table 4. Biological activity of *A. oligospora* metabolites[a].

Organism	Oligosporon (1)	4',5'-Dihydro-oligosporon (4)	Hydroxy-oligosporon (5)	10',11'-Epoxy-oligosporon (6)	Oligosporol B (3)
Bacteria (MIC)					
Bacillus subtilis	50	100	50	100	25
Streptomyces aureofaciens	100	>100	>100	>100	50
Erwinia carotovora	>100	>100	>100	>100	>100
Fungi (MIC)					
Phytophthora cinnamomi	50	100	>100	>100	>100
Rhizoctonia solani	>100	>100	>100	>100	>100
Pythium ultimum	>100	>100	>100	>100	>100
Nematode (LD_{50})					
Haemonchus contortus	25	50-100	inactive	inactive	- b

a MIC and LD_{50} values in ug/ml.

b Not tested.

from mycelial extracts, was the only detectable nematocidal agent produced by cultures of various *Arthrobotrys* species, including *A. oligospora*. We attribute this discrepancy between their results and our own to the different nematocidal assays employed.

Conclusion

The present work defines the structures of a group of related cyclohexene oxide metabolites from the nematode-trapping fungus *A. oligospora*, and establishes the absolute configuration of their substituted 7-oxabicyclo[4.1.0]hept-3-ene nucleus. The work independently confirms and extends recent work by STADLER *et al.* on *A. oligospora* metabolites.[7] The metabolites are probably of mixed biosynthetic origin, with a carbon skeleton formed by alkylation of a polyketide-derived nucleus[13,21,22] with a terpenoid-derived farnesyl unit. Members of the group display various biological activities, from nematocidal to antibacterial and antifungal. Oligosporon (**1**) and 4',5'-dihydro-oligosporon (**4**) represent only the second and most complex structural type of nematocidal metabolite to be isolated and characterised from cultures of nematophagous fungi.[20,23] They may play a rôle in the interaction between the nematophagous fungus and its nematode prey,[1~4,23] and may also contribute to the potential ability of *A. oligospora* to protect crops and livestock from infestation by nematodes or microorganisms.[5,6]

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