Illudalane Sesquiterpenoids from the Soft Coral *Alcyonium paessleri*: The First Natural Nitrate Esters

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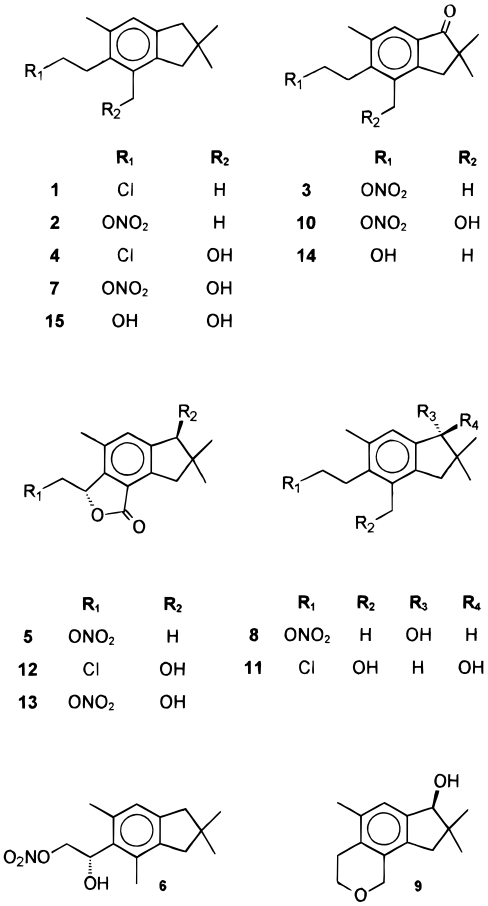
Fifteen illudalane sesquiterpenoids, alcyopterosins A−O (**1**−**15**) have been isolated from the subAntarctic soft coral *Alcyonium paessleri* which was collected at a depth of 200 m near the South Georgia Islands, and their structures were elucidated by spectroscopic techniques. Eight of these compounds (**2**, **3**, **5**−**8**, **10**, and **13**) are the first natural nitrate esters, while the other four (**1**, **4**, **11**, and **12**) are chlorinated. These compounds are as well the first illudalane sesquiterpenoids to be isolated from the marine environment. Compounds **1**, **3**, **5**, and **8** showed mild cytotoxicity toward human tumor cell lines.

Introduction

Although nitrates are common nutrients in seawater, a nitrate ester has never been found among marine natural products. This is indeed surprising since other common nutrients such as sulfate or phosphate are frequently found as esters in secondary metabolites from diverse marine phylla. In fact, to the best of our knowledge, nitrate esters have never been reported as natural products. As part of a program aimed at the discovery of biologically active metabolites from deep-water sub-Antarctic marine organisms, we studied the soft coral *Alcyonium paessleri* (May, 1899) from the South Georgia Islands which was a rich source of aromatic sesquiterpenoids of the rare illudalane class. Illudalane sesquiterpenoids are not widely distributed in nature, and up to date most of the compounds of this class have been isolated from ferns[1] and fungi.[2−10] In this paper we report the isolation and structure elucidation of 15 novel illudalane sesquiterpenoids, alcyopterosins A−O (**1**−**15**, see Chart 1). Eight of these compounds (**2**, **3**, **5**−**8**, **10**, and **13**) have a nitrate ester group while other four (**1**, **4**, **11,** and **12**) are chlorinated. These compounds are the first nitrate esters to be reported as natural products as well as the first examples of illudalane sesquiterpenoids from the marine environment.

Chart 1



	R_1	R_2
1	Cl	H
2	ONO_2	H
4	Cl	OH
7	ONO_2	OH
15	OH	OH

	R_1	R_2
3	ONO_2	H
10	ONO_2	OH
14	OH	H

	R_1	R_2
5	ONO_2	H
12	Cl	OH
13	ONO_2	OH

	R_1	R_2	R_3	R_4
8	ONO_2	H	OH	H
11	Cl	OH	H	OH

6 **9**

Results and Discussion

Alcyonium paessleri is a globular and spongy pink soft coral which was collected by netting (−100 to −200 m) near San Pedro Island (South Georgia Islands). The combined organic extracts of the frozen sample were worked-up using standard procedures (see Experimental Section), to yield 22 g of a viscous brown oil which was fractionated by vacuum flash chromatography on silica gel. Further fractionation by ODS vacuum flash chro-

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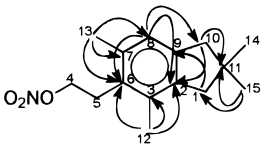

Figure 1. Long-range $^1H-^{13}C$ correlations observed for alcyopterosin B (**2**).

matography and final purification by reversed-phase HPLC allowed the isolation of compounds **1**−**15**.

Alcyopterosin A (**1**) had a molecular formula $C_{15}H_{21}Cl$ established by HRMS. The required five double bond equivalents suggested a structure of an aromatic bicyclic sesquiterpenoid. Although six aromatic carbons were present in the ^{13}C NMR spectrum, only a single aromatic proton was evident by ^1H NMR, which indicated a pentasubstituted aromatic ring. The nature of the remaining substituents was clear from the ^1H NMR spectrum: two aromatic methyls, a β-chlorinated C-2 side chain and two isolated benzylic methylenes. Additional NMR signals indicated the presence of two equivalent singlet methyls and a quaternary carbon. The carbon skeleton of **1** was established with the aid of 2D NMR spectra. Both benzylic methylenes correlated in a COLOC spectrum to the quaternary carbon at δ 39.2 which in turn correlated to both singlet methyls, thus forming a five-membered ring. The aromatic proton showed long-range coupling correlations in a COSY experiment to C-10 methylene and one of the aromatic methyls; on the other hand, the remaining aromatic methyl correlated to C-1. In a phase-sensitive NOESY experiment, H-8 showed NOE correlations to both H-10 and H-13 protons while H-12 showed NOEs to H-1 and H-5. These facts indicated that the C-2 side chain was located *ortho* to both aromatic methyls, thus defining an illudalane skeleton. Complete assignment of the NMR spectra of compounds **1**−**15** was achieved by HETCOR and COLOC experiments.

Alcyopterosin B (**2**) had a molecular formula $C_{15}H_{21}NO_3$ by HRMS and its ^1H NMR spectrum was almost identical to that of **1**, except for the downfield shift of C-4, which was the only functionalized position in **2**. In the ^{13}C NMR spectrum, C-4 was shifted downfield to δ 71.5, indicating oxygen substitution. Taking into account the molecular formula, the only possibility for this substituent was an ONO_2 group, namely a nitrate ester. The presence of this functional group was confirmed by two strong and sharp bands in the IR spectrum at 1640 and 1281 cm^{-1}. These bands are strongly characteristic of nitrates[11] and were present also in several other of the alcyopterosins. Long-range $^1H-^{13}C$ correlations observed for compound **2** are shown in Figure 1.

Once the structures of **1** and **2** were elucidated, characterization of compounds **3** and **4** was easily achieved. Alcyopterosin C (**3**) had a molecular formula $C_{15}H_{19}NO_4$ by HRMS. The chemical shift of C-4 methylene as well as the characteristic bands in the IR spectrum indicated again the presence of a nitrate ester. The ^{13}C NMR spectrum showed that the additional oxygen belonged to a cyclopentanone (δ 211.4), a fact supported by the absence in the ^1H NMR spectrum of one of the benzylic methylene signals. The large downfield shift of H-8 indicated that C-10 was the oxidized methylene. The ^1H NMR spectrum of alcyopterosin D (**4**) was very similar to that of **1**, except that one of the aromatic methyls was oxidized to a benzylic alcohol (δ ^1H: 4.69, s, 2H; δ ^{13}C: 60.5). Long-range COSY correlations as well as distinctive NOEs showed that C-12 methyl was oxidized.

The ^1H NMR spectrum of alcyopterosin E (**5**) showed no substitution in the five-membered ring, although some differences were observable at C-4 and C-5. The characteristic triplets of the ethyl side-chain were replaced by an AMX system, suggesting that an additional substituent was present at C-5. The molecular formula $C_{15}H_{17}NO_5$ indicated that compound **5** may have a nitrate ester plus two additional unsaturations. The presence of the nitrate ester was confirmed by IR, while signals at δ 169.6 and 76.9 in the ^{13}C NMR spectrum and a band at 1769 cm^{-1} in IR showed that the additional unsaturations were due to a five-membered lactone. A strong NOE correlation between H-8 and C-13 methyl indicated that C-12 was oxidized to the lactone carbonyl.

The ^1H NMR spectrum of alcyopterosin F (**6**), a minor metabolite, showed also an AMX system for the ethyl side chain, indicative of α,β disubstitution. However, the ^{13}C NMR spectrum did not show the presence of a lactone carbonyl. Moreover in the ^1H NMR spectrum, both aromatic methyls were present. The molecular formula of **6** ($C_{15}H_{21}NO_4$) was consistent with the presence of a nitrate ester and a hydroxyl group. The IR spectrum showed the characteristic bands of a nitrate ester, while the upfield shift (compared with compound **5**) of the ^{13}C signal of C-5 was attributed to the presence of a hydroxy group at C-5 instead of an ester. The absolute configuration at C-5 was established as 5*R* by the modified Mosher[12] method. A similar absolute configuration at C-5 for compound **5** was ascertained by comparison of the CD curves.

Alcyopterosins G and H (**7** and **8**, respectively) were isomeric to compound **6** as established by HRMS. Although both compounds showed the typical nitrate bands in the IR spectrum, together with a broad hydroxyl band, they showed significant differences in their ^1H NMR spectra. Analysis of the 1D and 2D NMR spectra, allowed the characterization of **7** as the nitrate ester analogue of **4.** In the ^1H NMR spectrum of **7**, it was evident that C-12 methyl was oxidized to an alcohol, while the downfield shift of the H-4 triplet was indicative of the presence of a nitrate group. In the ^1H NMR spectrum of **8**, signals of both aromatic methyls were present, but both CH_2 singlets of the cyclopentane ring were missing. One of them was replaced by a pair of one proton AB doublets at δ 2.72 and 2.56, which in a HETCOR experiment correlated to a methylene carbon at 44.2 ppm. The other methylene was replaced by a one proton singlet at δ 4.63, which indicated the presence of a hydroxylated methine. The fact that both methyl singlets were now nonequivalent (δ 1.17 and 1.05) was consistent with the presence of a chiral carbon at the cyclopentane ring. Analysis of the 2D NMR spectra proved that as in compound **3**, the oxidized methylene was C-10. The absolute configuration at C-10 was determined as 10*R* by the modified Mosher method.[12]

Compound **9** (alcyopterosin I) had a molecular formula $C_{15}H_{20}O_2$, which required six double bond equivalents.

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DEPT and ^{1}H NMR spectra of **9** gave a count of 19 protons bound to carbon, leaving only one interchangeable proton, which on the basis of comparison of the ^{1}H NMR spectra of **8** and **9** (δ H-10: 4.61 s, δ C-10: 83.3), was assigned to a hydroxyl group at C-10. No carbonyl groups were detected either in the ^{13}C NMR or IR spectra, indicating that the second oxygen had to belong to an ether and that the remaining unsaturation had to be an additional ring. Inspection of the ^{1}H NMR spectrum (two proton singlet at δ 4.64) showed that C-12 methyl was oxidized. As for the ethyl chain, both C-4 and C-5 methylenes appeared as two proton triplets in the ^{1}H NMR spectrum. The chemical shifts of C-4 and H-4 (δ ^{1}H: 3.98, t, 2H; δ ^{13}C: 65.2) indicated that C-4 was bound to oxygen. The only possible structure which could account for these features was that of a dihydropyran ring formed between C-4 and C-12. In the ^{1}H NMR spectrum of **9**, the triplet corresponding to the C-5 methylene showed a significant broadening, a fact consistent with the flexibility of a pyran ring. The absolute configuration of **9** was determined as 10*S* by the modified Mosher method.[12]

The ^{1}H NMR spectrum of alcyopterosin J (**10**) showed that this compound had structural similarities with **7**: a nitrate ester at C-4 and a hydroxyl group at C-12. A molecular formula $C_{15}H_{19}NO_5$, together with a carbonyl signal in the ^{13}C NMR spectrum (δ 211.1) and the absence of the C-10 protons, indicated that **10** was the 10-keto derivative of **7**. This structure was confirmed by the downfield shift of the aromatic proton (H-8). Interpretation of the NMR spectra allowed the identification of alcyopterosin K (**11**) as a 10-hydroxy derivative of **4**, while the absolute configuration was determined as 10*S* by the modified Mosher method.[12]

Alcyopterosins L and M (**12** and **13**, respectively) showed in their ^{1}H NMR spectra the characteristic AMX system of the five-membered lactone, and their molecular formulas were determined by HRMS as $C_{15}H_{17}ClO_3$ and $C_{15}H_{17}NO_6$ respectively. These formulas, together with the difference in the chemical shifts of the AMX systems indicated the presence of different substituents at C-4 in both compounds, namely a chlorine in **12** and a nitrate ester in **13**. Both compounds also showed ^{1}H NMR signals that indicated the presence of a hydroxyl group at C-10 (δ: 4.72 (s, 1H); 3.17 (d, J = 16.8 Hz); 2.92 (d, J = 16.8 Hz)). The remaining proton and carbon resonances were consistent with the proposed structures, and the presence of a nitrate ester in **13** was also confirmed by IR. The absolute configuration at C-5 was assumed as 5*R*, as was previously established for compounds **5** and **6**.

The ^{1}H NMR spectrum of alcyopterosin N (**14**) was similar to that of **3**, except for the different chemical shifts of H-4 and H-5 triplets. The missing H-10 protons, together with the pronounced downfield shift of H-8, suggested the presence of a ketone at C-10, which was confirmed by ^{13}C NMR (δ 211.1) and an IR band at 1692 cm^{-1}. The upfield shift of C-4 and H-4 signals (δ ^{13}C: 61.5; δ ^{1}H: 3.80 (t, 2H, J = 7 Hz)), together with the molecular formula $C_{15}H_{18}O_2$ showed the presence of a hydroxyl group at C-4 instead of a nitrate ester.

In the case of alcyopterosin O (**15**) the ^{1}H NMR spectrum showed the following features: C-10 was not oxidized, C-12 was oxidized to a benzylic alcohol (δ ^{13}C: 59.6; δ ^{1}H: 4.61 (s, 2H)), and a hydroxyl group was present at C-4 (δ ^{13}C: 61.3; δ ^{1}H 3.85 (t, 2H, J = 7 Hz)). A molecular formula $C_{15}H_{22}O_2$, obtained by HRMS and a complete set of 2D NMR spectra confirmed this structure and the assignment of all proton and carbon resonances.

An intriguing feature of the alcyopterosins is the difference observed in the absolute configuration of the compounds hydroxylated at C-10. Our attention was drawn by the fact that compound **8** was levorotatory while **9** and **13** were dextrorotatory. Analysis of these compounds by the modified Mosher method[12] showed that compounds **9**, **12**, and **13** had the absolute configuration 10*S* while compound **8** was 10*R*. Although more examples should be needed in order to put forward a conclusive theory, some observations can be drawn from the above-mentioned compounds. The most noticeable difference between the 10*R* and 10*S* compounds is that in the latter, C-12 methyl is oxidized, either to an alcohol, an ether, or an ester, and probably this group may influence the stereospecificity of a reductive hydroxylation process. However, a deeper knowledge on the biosynthesis of this family of sesquiterpenoids will be necessary in order to draw out significant conclusions.

Compound **5** showed mild cytotoxicity toward Hep-2 (human larynx carcinoma) cell line (IC_{50}: 13.5 μM), while compounds **1**, **3**, and **8** were cytotoxic toward HT-29 (human colon carcinoma) at 10 μg/mL.

Although several synthetic compounds having a nitrate ester are reported in the literature, ranging from the well-known trinitroglycerine to highly complex molecules, to the best of our knowledge a nitrate ester has never been found as a secondary metabolite. The reasons for this absence are not clear, but the ease of hydrolysis of these esters to the corresponding alcohols may play an important role. In fact, the 4-OH derivative of compound **5**, could be isolated and characterized (see Experimental Section) from the methanol/water mixture used as eluant for HPLC after 3 days storage at room temperature. The alcyopterosins should be considered the first natural nitrate esters, as well as the first marine illudalane sesquiterpenoids.

Experimental Section

General Experimental Procedures. Infrared spectra were recorded on a FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectral data and COSY, HETCOR, NOESY, DEPT, and COLOC 2D NMR experiments were measured on a 200 MHz FT-NMR spectrometer. ^{1}H NMR chemical shifts are relative to TMS (δ = 0), while ^{13}C NMR chemical shifts are relative to $CDCl_3$ (δ = 77). Vacuum flash chromatography was performed either on TLC grade silica gel or octadecyl functionalized silica gel (Aldrich Chemical Co.). HPLC separations were carried out using UV and RI detectors, HPLC grade solvents, and a YMC Rp-18 (20 × 250 mm) column. *R*- and *S*-α-Methoxy-α-(trifluoromethyl)phenylacetyl chlorides were purchased from Aldrich Chemical Co.

Collection, Extraction, and Isolation Procedures. Samples of the pink and globular soft coral *Alcyonium paessleri* (May, 1899) were collected by netting at a depth of 200 m near the South Georgia Islands, during the 1996 campaign of the research vessel "Dr. E. Holmberg" (INIDEP- Mar del Plata) and kept frozen until used. The soft coral was identified by Drs. Carlos D. Perez and Mauricio Zamponi [Laboratorio de Biología de Cnidarios (LABIC) Departamento de Ciencias Marinas, FCEyN, Universidad de Mar del Plata, Argentina]. A voucher specimen is preserved at the LABIC collection (Universidad de Mar del Plata). The frozen soft coral (3.5 kg) was triturated and extracted three times with EtOH (5 L) and then with EtOAc (5 L). The combined extracts were concentrated, and the resulting aqueous suspension was

Table 1. ^{1}H NMR Data ($CDCl_3$, 200 MHz)[a] for Compounds 1−5

H no.	1	2	3	4	5
H-1	2.66 (bs, 2H)	2.65 (bs, 2H)	2.87 (bs, 2H)	2.78 (bs, 2H)	3.04 (bs, 2H)
H-4	3.54 (t, 2H, J = 8)	4.48 (t, 2H, J = 8)	4.53 (t, 2H, J = 7.5)	3.64 (t, 2H, J = 8)	5.07 (dd, J = 12.6, 2.3) 4.58 (dd, J = 12.6, 6.6)
H-5	3.12 (t, 2H, J = 8)	3.06 (t, 2H, J = 8)	3.18 (t, 2H, J = 7.5)	3.21 (t, 2H, J = 8)	5.67 (dd, J = 6.6,2.3)
H-8	6.86 (bs)	6.87 (bs)	7.46 (bs)	6.87 (bs)	7.27 (bs)
H-10	2.70 (bs, 2H)	2.69 (bs, 2H)	−	2.70 (bs, 2H)	2.75 (bs, 2H)
H-12	2.22 (s, 3H)	2.21 (s, 3H)	2.32 (s, 3H)	4.69 (s, 2H)	−
H-13	2.32 (s, 3H)	2.32 (s, 3H)	2.42 (s, 3H)	2.33 (s, 3H)	2.41 (s, 3H)
H-14	1.16 (s, 3H)	1.15 (s, 3H)	1.23 (s, 3H)	1.15 (s, 3H)	1.19 (s, 3H)
H-15	1.16 (s, 3H)	1.15 (s, 3H)	1.23 (s, 3H)	1.15 (s, 3H)	1.16 (s, 3H)

[a] Spectra were referenced to residual solvent (δ 7.26).

Table 2. ^{1}H NMR Data ($CDCl_3$, 200 MHz)[a] for Compounds 6−10

H no.	6	7	8	9	10
H-1	2.65 (bs, 2H)	2.79 (s, 2H)	2.72 (d, J = 15) 2.56 (d, J = 15)	2.56 (d, J = 15) 2.39 (d, J = 15)	3.04 (bs, 2H)
H-4	4.92 (dd, J = 12,9.5) 4.52 (dd, J = 12,2.8)	4.60 (t, 2H, J = 7.5)	4.49 (t, 2H, J = 8)	3.98 (t, 2H, J = 5.8)	4.65 (t, 2H, J = 7.5)
H-5	5.49 (dd, J = 9.5,2.8)	3.17 (t, 2H, J = 7.5)	3.09 (t, 2H, J = 8)	2.70 (t, 2H, J = 5.8)	3.30 (t, 2H, J = 7.5)
H-8	6.86 (bs)	7.00 (bs)	7.07 (bs)	7.09 (bs)	7.58 (bs)
H-10	2.70 (bs, 2H)	2.70 (bs, 2H)	4.63 (s, 1H)	4.61 (s, 1H)	- - -
H-12	2.34 (s, 3H)	4.69 (s, 2H)	2.23 (s, 3H)	4.64 (s, 2H)	4.82 (s, 2H)
H-13	2.40 (s, 3H)	2.34 (s, 3H)	2.36 (s, 3H)	2.23 (s, 3H)	2.45 (s, 3H)
H-14	1.15 (s, 3H)	1.15 (s, 3H)	1.17 (s, 3H)	1.15 (s, 3H)	1.23 (s, 3H)
H-15	1.14 (s, 3H)	1.15 (s, 3H)	1.05 (s, 3H)	1.06 (s, 3H)	1.23 (s, 3H)

[a] Spectra were referenced to residual solvent (δ 7.26).

Table 3. ^{1}H NMR Data ($CDCl_3$, 200 MHz)[a] for Compounds 11−15

H no.	11	12	13	14	15
H-1	2.85 (d, J = 15.6) 2.68 (d, J = 15.6)	3.17 (d, J = 17) 2.92 (d, J = 17)	3.17 (d, J = 17.1) 2.92 (d, J = 17.1)	2.86 (s, 2H)	2.81 (s, 2H)
H-4	3.64 (t, 2H, J = 8)	4.18 (dd, J = 12.4; 2.2) 3.88 (dd, J = 12.4; 4.7)	5.08 (dd, J = 12.4; 2.2) 4.58 (dd, J = 12.4; 6.5)	3.81 (t, 2H, J = 7)	3.85 (t, 2H, J = 6)
H-5	3.23 (t, 2H, J = 8)	5.70 (dd, J = 4.7;2.2)	5.70 (dd, J = 6.5; 2.2)	3.06 (t, 2H, J = 7)	3.01 (t, 2H, J = 6)
H-8	7.18 (bs)	7.48 (bs)	7.50 (bs)	7.44 (bs)	6.98 (bs)
H-10	4.62 (s, 1H)	4.71 (s, 1H)	4.72 (bs, 1H)	−	2.70 (s, 2H)
H-12	4.68 (s, 2H)	−	−	2.31 (s, 3H)	4.61 (s,2H)
H-13	2.36 (s, 3H)	2.40 (s, 3H)	2.45 (s, 3H)	2.41 (s, 3H)	2.29 (s, 3H)
H-14	1.17 (s, 3H)	1.20 (s, 3H)	1.20 (s, 3H)	1.23 (s, 3H)	1.15 (s, 3H)
H-15	1.04 (s, 3H)	1.08 (s, 3H)	1.07 (s, 3H)	1.23 (s, 3H)	1.15 (s, 3H)

[a] Spectra were referenced to residual solvent (δ 7.26).

exhaustively extracted with EtOAc. The organic extract was taken to dryness to yield a brown oil that was vacuum flash-chromatographed on silica gel using a cyclohexane−CH_2Cl_2 gradient, and then CH_2Cl_2−EtOAc and EtOAc−MeOH gradients, giving 10 fractions, L1−L10. Fraction L2, eluted with cyclohexane−EtOAc (8:2), was further fractionated by reversed-phase vacuum flash chromatography using a MeOH−H_2O gradient, and the fraction eluted with MeOH−H_2O (7:3) was finally purified by column flash chromatography on silica (cyclohexane) to yield pure compounds **1** and **2**. Fractions L3−L8 from the original extract were worked-up in a similar way: the fractions were cleaned-up by reversed-phase flash chromatography using a MeOH−H_2O gradient, and the sesquiterpenoid containing fractions (usually eluted with MeOH−H_2O (8:2)) were finally purified by reversed phase HPLC using MeOH−H_2O (7:3) or (75:25) as eluants. Fraction L3 (cyclohexane−CH_2Cl_2 (1:1)) yielded compounds **3**−**5**; fraction L4 (cyclohexane−CH_2Cl_2 (1:3)) yielded **6**; fraction L5 (CH_2Cl_2) gave pure compounds **7** and **8**. Compounds **9**, **10**, **12**, and **13** were purified from fraction L6 (CH_2Cl_2−EtOAc (8:2)); fraction L7 (CH_2Cl_2−EtOAc (1:1)) yielded **11** and **14**, while fraction L8 (EtOAc) gave compound **15**.

Alcyopterosin A (1): colorless oil; HREIMS calcd for $C_{15}H_{21}Cl$ 236.1331, found 236.1332; EIMS *m/z* (%): 238 (8); 236 (25); 201(4); 187 (100); IR ν_{max} (KBr): 1471; 1382; 706 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ϵ): 232 (3.59); 272 (3.15); 282 (3.14); 358 (2.81) nm; ^{1}H and ^{13}C NMR see Tables 1 and 4, respectively.

Alcyopterosin B (2): colorless oil; HREIMS calcd for $C_{15}H_{21}NO_3$ 263.1521, found 263.1485; EIMS *m/z* (%): 263 (16); 217 (2); 201 (4); 187 (84); 85 (67); 83 (100); IR ν_{max} (KBr): 1640; 1281; 864 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ϵ): 234 (3.49); 274 (2.99); 283 (3.02) nm; ^{1}H and ^{13}C NMR see Tables 1 and 4, respectively.

Table 4. ^{13}C NMR ($CDCl_3$, 50 MHz)[a] Data for Compounds 1−8

C no.	1	2	3	4	5	6	7	8
1	47.1	47.1	42.3	46.4	44.7	47.1	46.3	44.2
2	140.8	140.9	149.7	141.5	147.6	142.1	141.5	139.2
3	130.6	132.8	134.6	134.8	122.2	133.0	135.0	133.2
4	42.5	71.5	70.6	43.7	76.9	74.7	72.6	71.3
5	33.4	27.1	27.8	33.0	71.6	68.6	26.8	27.2
6	131.9	129.3	139.4	133.0	140.5	131.4	130.6	131.7
7	134.5	134.8	136.7	135.3	130.1	134.7	135.5	135.5
8	124.3	124.5	123.5	127.2	132.1	125.5	127.2	124.2
9	141.8	142.1	134.0	142.8	141.7	143.6	143.0	143.1
10	47.9	47.4	211.4	47.8	46.9	47.9	47.7	83.7
11	39.2	39.3	45.5	39.7	40.9	39.3	39.7	43.8
12	15.9	15.9	14.8	60.5	169.6	16.9	60.4	15.6
13	20.1	20.1	20.2	20.0	17.9	20.9	19.9	20.2
14	29.3	29.2	25.4	29.0	28.7	29.2	29.0	27.1
15	29.3	29.2	25.4	29.0	28.7	29.2	29.0	21.8

[a] Spectra were referenced to $CDCl_3$ (δ 77.0).

Alcyopterosin C (3): recrystallized from MeOH as colorless needles, mp 83−84 °C; HREIMS calcd for $C_{15}H_{19}NO_4$ 277.1314, found 277.1315; EIMS *m/z* (%): 277 (56); 215 (22); 201 (100);

Table 5. ^{13}C NMR ($CDCl_3$, 50 MHz)[a] Data for Compounds 9−15

C no.	9	10	11	12	13	14	15
1	42.0	41.5	43.4	44.0	42.0	42.4	46.4
2	134.9	150.0	139.8	150.8	148.3	149.6	141.1
3	131.0	136.5	135.2	123.1	122.6	134.4	135.0
4	65.2	71.7	43.4	42.1	77.1	61.5	61.3
5	26.4	27.5	32.9	79.5	71.4	33.3	31.5
6	131.4	141.0	135.0	139.8	140.2	142.4	133.7
7	134.9	137.5	136.0	130.8	131.2	136.9	135.5
8	123.7	126.1	126.8	131.8	132.2	123.1	126.9
9	141.9	134.5	143.6	144.2	142.5	133.3	141.8
10	83.3	211.1	83.4	82.8	82.6	211.4	47.8
11	44.3	45.6	44.1	45.3	45.3	45.4	39.4
12	66.4	58.9	59.9	169.6	169.3	15.0	59.6
13	18.9	20.1	20.0	18.0	18.0	20.4	20.1
14	27.2	25.3	26.9	26.7	26.6	25.4	29.1
15	21.8	25.3	21.6	21.4	21.3	25.4	29.1

[a] Spectra were referenced to $CDCl_3$ (δ: 77.0).

IR ν_{max} (KBr): 1710; 1630; 1460; 1381; 1280; 857 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ϵ): 228 (3.68); 256 (4.22); 306 (3.54) nm; ^1H and ^{13}C NMR see Tables 1 and 4, respectively.

Alcyopterosin D (4): recrystallized from MeOH as colorless needles, mp 107−108 °C; HREIMS calcd for $C_{15}H_{21}ClO$ 252.1281, found 252.1288; EIMS *m/z* (%): 254 (6); 252 (17); 236 (17); 234 (45); 199 (100); IR ν_{max} (KBr): 3423; 978; 720 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ϵ): 214 (4.07); 232 (3.89); 286 (3.24) nm; ^1H and ^{13}C NMR see Tables 1 and 4, respectively.

Alcyopterosin E (5): colorless oil; $[\alpha]^{25}_D$ −31.28° (*c* 2.35 $CHCl_3$); HREIMS calcd for $C_{15}H_{17}NO_5$ 291.1106, found 291.1105; EIMS *m/z* (%): 291 (5); 215 (100); IR ν_{max} (KBr): 1769; 1640; 1277; 1053; 849 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ϵ): 242 (3.96); 302 (3.63) nm; ^1H and ^{13}C NMR see Tables 1 and 4, respectively.

Alcyopterosin F (6): colorless oil; $[\alpha]^{25}_D$ −29.13° (*c* 0.23 $CHCl_3$); HREIMS calcd for $C_{15}H_{21}NO_4$ 279.1470, found 279.1469; EIMS *m/z* (%): 279 (34); 261 (47); 215 (16); 203 (100); IR ν_{max} (KBr): 3420; 1630; 1281; 850 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ϵ): 220 (3.29); 234 (3.88); 274 (3.30); 282 (3.28) nm; ^1H and ^{13}C NMR see Tables 1 and 4, respectively.

Alcyopterosin G (7): colorless oil; HREIMS calcd for $C_{15}H_{21}NO_4$ 279.1470, found 279.1470; EIMS *m/z* (%): 279 (24); 261 (17); 215 (26); 203 (50); 83 (100) IR ν_{max} (KBr): 1640; 1278; 1029; 860 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ϵ): 212 (4.07); 232 (3.89); 286 (3.24) nm; ^1H and ^{13}C NMR see Tables 2 and 4, respectively.

Alcyopterosin H (8): colorless oil; $[\alpha]^{25}_D$ −13.90° (*c* 1.05 $CHCl_3$); HREIMS calcd for $C_{15}H_{21}NO_4$ 279.1470, found 279.1469; EIMS *m/z* (%): 279 (44); 215 (14); 203 (90); IR ν_{max} (KBr): 3406; 1632; 1465; 1279; 856 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ϵ): 232 (3.46); 272 (2.89); 306 (3.33) nm; ^1H and ^{13}C NMR see Tables 2 and 4, respectively.

Alcyopterosin I (9): recrystallized from cyclohexane as colorless needles, mp 97−99 °C; $[\alpha]^{25}_D$ 6.16° (*c* 2.59 $CHCl_3$); HREIMS calcd for $C_{15}H_{20}O_2$ 232.1463, found 232.1461; EIMS *m/z* (%): 232 (100); 199 (51); 187 (33); 83 (52); IR ν_{max} (KBr): 3413; 1121; 869 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ϵ): 212 (3.17); 230 (3.65); 280 (2.98) nm; ^1H and ^{13}C NMR see Tables 2 and 4, respectively.

Alcyopterosin J (10): recrystallized from MeOH as colorless needles, mp 118−121 °C; HREIMS calcd for $C_{15}H_{19}NO_5$ 293.1263, found 293.1261; EIMS *m/z* (%): 293 (5), 275 (14), 230 (90), 119 (100); IR ν_{max} (KBr): 3423; 1708; 1640; 1283; 857 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ϵ): 230 (3.87); 252 (4.21); 306 (3.67) nm; ^1H and ^{13}C NMR see Tables 2 and 5, respectively.

Alcyopterosin K (11): recrystallized from MeOH as colorless needles, mp 108−110 °C; $[\alpha]^{25}_D$ 10.0° (*c* 0.91 $CHCl_3$); HREIMS calcd for $C_{15}H_{21}ClO_2$ 268.1230, found 268.1227; EIMS *m/z* (%): 270 (7); 268 (22); 252 (10); 250 (34); 237 (22); 235 (43); 215 (100); IR ν_{max} (KBr): 3365; 1636; 859 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ϵ): 234 (3.41); 278 (3.04); 286 (3.08) nm; ^1H and ^{13}C NMR see Tables 2 and 5, respectively.

Alcyopterosin L (12): colorless oil; $[\alpha]^{25}_D$ −17.02° (*c* 0.58 $CHCl_3$); HREIMS calcd for $C_{15}H_{17}ClO_3$ 280.0866, found 280.0867; EIMS *m/z* (%): 282 (4); 280 (13); 264 (19); 262 (40); 231 (100); IR ν_{max} (KBr): 3415; 1753; 1099; 1046 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ϵ): 224 (3.19); 240 (3.70); 298 (3.42) nm; ^1H and ^{13}C NMR see Tables 3 and 5, respectively.

Alcyopterosin M (13): colorless oil; $[\alpha]^{25}_D$ −23.16° (*c* 0.79 $CHCl_3$); HREIMS calcd for $C_{15}H_{17}NO_6$ 307.1056, found 307.1057; EIMS *m/z* (%): 307 (6), 246 (100), 231 (40), 213 (23), IR ν_{max} (KBr): 3424; 1765; 1642; 1278; 1047; 850 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ϵ): 234 (3.65); 238 (3.64); 296 (3.35) nm; ^1H and ^{13}C NMR see Tables 3 and 5, respectively.

Alcyopterosin N (14): recrystallized from MeOH, mp 103−104 °C; HREIMS calcd for $C_{15}H_{20}O$ 232.1463, found 232.1461; EIMS *m/z* (%): 232 (12); 214 (32); 201(100); 186 (31); IR ν_{max} (KBr): 3422; 1712; 1692; 1604; 1049 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ϵ): 226 (3.72); 230 (3.58); 260 (4.18); 306 (3.52) nm; ^1H and ^{13}C NMR see Tables 3 and 5, respectively.

Alcyopterosin O (15): colorless oil, HREIMS calcd for $C_{15}H_{22}O$ 234.1619, found 234.1618; EIMS *m/z* (%): 234 (50); 216 (21); 201 (32); 186 (100); IR ν_{max} (KBr): 3418; 1740; 1226; 1034 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ϵ): 212 (4.01); 233 (3.89); 282 (3.28) nm; ^1H and ^{13}C NMR see Tables 3 and 5, respectively.

Preparation of MTPA Esters: 1 mg of compound was dissolved in 200 μL of anhydrous pyridine, and 5 μL of (*S*)- or (*R*)- MTPA chloride was added. The solution was stirred at room temperature for 1 h and then poured over crushed ice acidified with 20% aqueous HCl. The mixture was extracted several times with EtOAc and the organic layer taken to dryness under nitrogen. The MTPA esters were purified by preparative TLC on silica using an appropriate solvent mixture.

Hydrolisis of 5. HPLC fractions containing pure compound **5**, were left at room temperature for 3 days in the eluant mixture (methanol/water (7:3)), and an additional product was detected by TLC. This product was purified by prep TLC (CH_2Cl_2) and characterized as the 4-OH derivative of **5** (δ H-4: 4.23 (bd, J = 12.4 Hz), 3.80 (dd, J = 12.4, 5.9 Hz); δ C-4: 63.3, HREIMS calcd for $C_{15}H_{18}O_3$ 246.1256, found 246.1254).

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Supporting Information Available: ^1H and ^{13}C NMR spectra for alcyopterosins A−O (**1**−**15**). This material is available free of charge via the Internet at http://pubs.acs.org.

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