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C_{15} Acetogenins from the red alga *Laurencia obtusa*

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Abstract

Four C_{15} acetogenins, 13-epilaurencienyne (3*Z*) (**1**), 13-epipinnatifidenyne (3*E*) (**2**), (3*E*, 6*S**, 7*R**, 9*S**, 10*S**, 12*R**)-9-chloro-13-bromo-6:12-epoxy-7, 10-diacetoxypentadec-3-en-1-yne (**3**), (3*Z*, 6*S**, 7*R**, 9*S**, 10*S**, 12*R**)-9-chloro-13-bromo-6:12-epoxy-7, 10-diacetoxypentadec-3-en-1-yne (**4**), along with the known 13-epilaurencienyne (3*E*) (**5**), have been isolated from the organic extract of the red alga *Laurencia obtusa*, collected in the Aegean Sea, Greece. The structures of the new natural products, as well as their relative stereochemistry, were established by means of spectral data analysis, including 2D NMR spectroscopic experiments. Some of the new metabolites exhibited significant insecticidal activity.

Keywords: *Laurencia obtusa*; Rhodomelaceae; Rhodophyta; Acetogenin; Vinyl acetylene; Cyclic ether; Insecticidal activity

1. Introduction

Red algae of the genus *Laurencia* elaborate a wide variety of structurally unusual secondary metabolites (Faulkner, 1999) and seem to be an endless source of new chemical constituents. The vast majority of these are halogenated diterpenes (Rochfort and Capon, 1996), sesquiterpenes (Amico et al., 1991; Wright et al., 1991) and C_{15} non-terpenoids containing ether rings of different sizes with terminal acetylenic, vinyl acetylenic or allenic side chains (Norte et al., 1991; Wright et al., 1993; König and Wright, 1994). Some of these metabolites have exhibited significant insecticidal properties (Dev and Koul, 1997; El Sayed et al., 1997). It is noteworthy that several *Laurencia* metabolites have also been isolated either from predators that feed upon this alga (Matsuda et al., 1967; Yamamura and Terada, 1977), or from symbiotic organisms (Imre et al., 1995).

Crop protection chemicals continue to be the major tool for protecting food and fiber crops from damaging pests. To date, research focused on isolating insecticidal prototype leads from marine origin has resulted in the report of about 40 active compounds (El Sayed et al., 1997). In the framework of our continuing investigations towards the isolation of biologically active compounds from marine organisms of the Greek seas (Mihopoulos et al., 1999, 2001; Iliopoulou et al., 2001), we examined a species of *Laurencia* collected off the coast of Southeastern Greece. In this paper we describe the isolation and structure elucidation of four novel non-terpenoid cyclic ethers **1**–**4** which were obtained from the non polar fractions of the organic extract of the alga, along with the known compound **5** (Imre and Aydogmus, 1997). The insecticidal activity of the extract and the metabolites was evaluated according to a previously established protocol (Tsoukatou et al., 2001) on the *Pheidole pallidula* ants.

2. Results and discussion

Compound **1** was purified along with its *trans* isomer **5**, following chromatographic separations and was isolated in minute quantities as a colorless oil. Compound **5** was identified by comparison of its NMR spectra with previously reported data (Imre and Aydogmus, 1997). The composition of metabolite **1** was deduced to be $C_{17}H_{23}O_3BrCl_2$ from the HRFAB mass spectrum and NMR spectral data. The HRFAB-MS showed $[M+1]^+$ peaks at *m*/*z* 425, 427, 429, 431 with intensities of 9.8/13.5/6.5/1.2 indicating the presence of one bromine and two chlorine atoms. The IR spectrum of the metabolite displayed the appropriate absorptions for a terminal alkyne (3295, 2100 cm^{-1}), an ether group (1066 cm^{-1})

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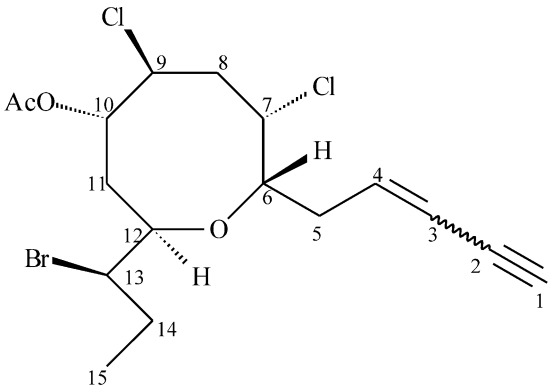
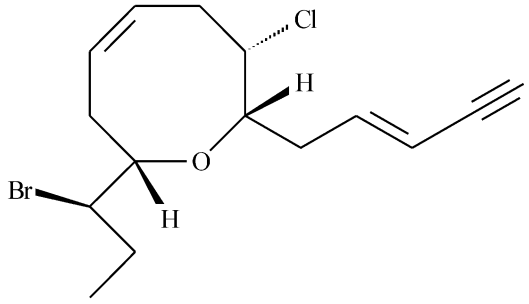


1 *Z*-isomer
5 *E*-isomer



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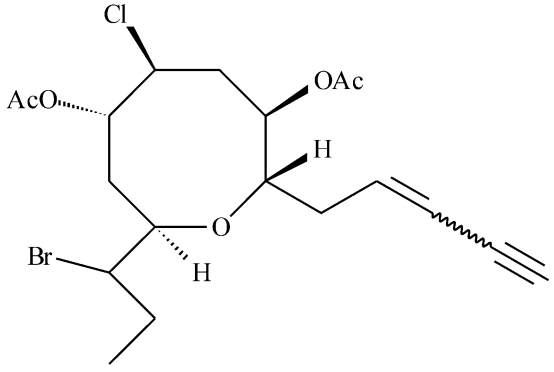

3 *E*-isomer
4 *Z*-isomer

and an ester carbonyl (1740 cm^{-1}). The stucture of compound **1** was elucidated by comparison of the ^{1}H and ^{13}C NMR spectral data with those of known metabolite **5**, as well as by 2D NMR techniques (HMQC, COSY, HMBC and NOESY). The most significant differences between metabolites **1** and **5** were observed in the ^{1}H NMR shielding value for the alkyne proton H-1 which in metabolite **1** was shifted to lower fields (δ 3.14, *d*, $J=2.0$ Hz), as well as at the ^{13}C NMR shielding values of methylene C-5 carbon which in metabolite **1** was shifted to higher fields (δ 35.2). Additionally the coupling constant of 10.8 Hz between H-3 and H-4 revealed the *cis* geometry of the C3–C4 double bond. From these differences, **1** was deduced to be 13-epilaurencienyne (3*Z*).

Compound **2** was purified by means of HPLC separations and isolated as a white powder. Both ^{13}C NMR data and EI–MS measurements supported the molecular formula $C_{15}H_{20}OBrCl$. The EI mass spectrum revealed a parent ion $[M]^{+}$ at *m*/*z* 332 together with isotopic peaks at *m*/*z* 330 and 334. The isotopic pattern as well as the fragment ion masses indicated the presence of one bromine and one chlorine atom. The presence of a conjugated terminal enyne group similar to that of compound **1**, was indicated by IR absorptions at 3280 and 2140 cm^{-1}. The ^{1}H NMR spectrum of **2** showed signals, which could be assigned to a *trans* enyne function, two non-conjugated olefinic protons, two protons on oxygenated carbons and two protons on halogenated carbons. The ^{13}C NMR spectrum of **2** revealed that, in addition to the acetylene carbons, two disubstituted double bonds were also present in the molecule. Furthermore, comparison of the ^{1}H NMR and the ^{13}C NMR spectroscopic data with those of related molecules, indicated a striking structural similarity with 13-epipinnatifidenyne (3*Z*) (San Martin et al., 1997). The most significant differences were observed in the ^{1}H NMR shielding value for the alkyne proton H-1 which in metabolite **2** was shifted to higher fields (δ 2.82, *br s*), as well as at the ^{13}C NMR chemical shift of methylene C-5 carbon which in metabolite **2** was shifted to lower fields (δ 37.9). Additionally the coupling constant of 15.9 Hz between H-3 and H-4 revealed the *trans* geometry of the C3-C4 double bond. According to the above observations and an extensive analysis of the 1D and 2D NMR spectra the structure of metabolite **2** was established as 13-epipinnatifidenyne (3*E*).

Compound **3**, after purification by HPLC, was isolated as a colorless oil. The composition of **3** was deduced to be $C_{19}H_{26}O_5BrCl$ from the HRFAB mass spectrum and ^{13}C NMR spectroscopic data. The HRFAB-MS showed $[M+1]^{+}$ peaks at *m*/*z* 449, 451, 453 with intensities of 7.9/8.1/3.1 indicating the presence of one bromine and one chlorine atom. The IR spectrum of the metabolite displayed absorptions for a terminal alkyne (3305, 2100 cm^{-1}), an ether group (1066 cm^{-1}) and two ester carbonyls (1742, 1739 cm^{-1}). The ^{13}C NMR spectrum along with the DEPT experiments showed the presence of 19 carbons corresponding to three quaternary, nine methine, four methylene and three methyl carbon atoms. From these carbons, two were carbonyls resonating at δ 169.9 (*s*), 169.2 (*s*), four were bonded to

oxygens, resonating at δ 71.9 (*d*), 71.1 (*d*), 76.0 (*d*), 73.7 (*d*) and two were halogenated at δ 61.4 (*d*), 57.2 (*d*). The presence of a terminal enyne group conjugated with a double bond was confirmed by the methine signals at δ 76.6, 111.5, 140.6 and the quaternary carbon at δ 81.9 ppm. With an unsaturation degree of 6 the structure was suggested to contain an ether linkage indicating the presence of an oxocane ring in the molecule. Based on the correlations between the carbonyl carbons (169.2 and 169.9 ppm) and H-7 (δ 4.75) and H-10 (δ 5.12) observed in the heteronuclear experiments (HMBC) the two acetoxyl groups were placed on C-7 and C-10. The ether bridge was placed on carbons C-6 and C-12 because of the long range correlation between H-12 (δ 4.12) and C-6 (71.9 ppm). Moreover, the correlation between the proton signal at δ 4.45 and the carbon signal at δ 57.2 placed the chlorine atom at C-9, while the correlation between H-13 (δ 3.80) and the carbon signal centered at δ 61.4 suggested the bromine atom to be at C-13.

The relative stereochemical configuration of the asymmetric centers was determined based on the nOe enhancements along with the coupling constants of the NMR spectrum. The strong NOE effect between H-9 and H-12 suggested a *cis* orientation between H-9 and H-12. The nOe interactions between H-9 and H-7 revealed a *cis* relationship of H-9 and H-7. Other significant correlations were between H-6 (δ 4.19) and H-8 (δ 2.09), H-6 (δ 4.19) and H-11 (δ 2.47), H-6 (δ 4.19) and H-13 (δ 3.80). Moreover, the coupling constants between H-6 and H-7 (10 Hz), H-9 and H-10 (9.5 Hz), H-11 and H-12 (10.8 Hz) indicated axial-axial orientations. Accordingly, the relative configuration of **3** was determined to be (3*E*, 6*S**, 7*R**, 9*S**, 10*S**, 12*R**)-9-chloro-13-bromo-6:12-epoxy-7, 10-diacetoxypentadec-3-en-1-yne.

To the best of our knowledge this is the first example of this class of C_{15} acetogenins with 6*S**, 7*R** configuration and only the second time that diacetylated oxocanols have been isolated from *Laurencia* species (Atta-ur-Rahman, 1989).

Compound **4**, after purification by HPLC, was isolated as a colorless oil. Both ^{13}C NMR data and HRFAB–MS measurements supported the molecular formula $C_{19}H_{26}O_5BrCl$. Comparison of the spectral data of **4** with those of **3** revealed a great similarity in the structures of the two compounds. The HRFAB–MS showed $[M+1]^+$ peaks at *m*/*z* 449, 451, 453 with intensities of 8.3/9.1/5.3 indicating the presence of one bromine and one chlorine atom. The IR spectrum of the metabolite displayed the appropriate absorptions for a terminal alkyne (3305, 2100 cm^{-1}), an ether group (1066 cm^{-1}) and two ester carbonyls (1742, 1739 cm^{-1}). The stucture of **4** was elucidated by comparison of the NMR spectral data with those of **3**, as well as by 2D NMR techniques. The most significant differences were observed in the ^1H NMR shielding value for the alkyne proton H-1 which in metabolite **4** was shifted to lower fields (δ 3.04, *d*, $J=2.0$ Hz), as well as at the ^{13}C NMR shielding values of methylene C-5 carbon which in metabolite **4** was shifted to higher fields (δ 33.3). Additionally the coupling constant of 11.0 Hz between H-3 and H-4 revealed the *cis* geometry of the C3-C4 double bond. According to the above observations the structure of **4** was deduced to be (3*Z*, 6*S**, 7*R**, 9*S**, 10*S**, 12*R**)-9-chloro-13-bromo-6:12-epoxy-7, 10-diacetoxypentadec-3-en-1-yne.

The biological activity of the isolated metabolites was evaluated by the mortality exerted to the *Pheidole pallidula* ants. Paper disks impregnated with the metabolites (0.5 mg/disk) and soaked with sugar water were given as food source to a statistically significant number of ants (Tsoukatou et al., 2001).

The results of the assays showed that the *cis* isomers (metabolites **1**, **4**) had a strong ant toxicity with a noticeable knockdown effect on the first day of application. All *trans* isomers (metabolites **5**, **3**, **2**) showed a gradual toxicity that was escalated at the 4th day of the experiments (mortality $>70\%$).

3. Experimental

3.1. General

Optical rotations were measured using a Perkin-Elmer model 341 polarimeter and a 10 cm cell. UV spectra were determined in spectroscopic grade C_6H_{14} on a Shimadzu UV-160A spectrophotometer. IR spectra were obtained using a Paragon 500 Perkin-Elmer spectrophotometer. NMR spectra were recorded using a Bruker AC 200 and a Brüker DRX 400 spectrometers. Chemical shifts are given on a δ (ppm) scale using TMS as internal standard (*s*, singlet; *d*, doublet; *t*, triplet; *m*, multiplet). The 2D experiments (^1H–^1H COSY, HMQC, HMBC, NOESY) were performed using standard Bruker microprograms. High Resolution Mass Spectra data were provided by University of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame, Indiana. EIMS data were recorded on a Hewlett Packard 5973 Mass Selective Detector. Column chromatography was performed with Kieselgel 60 (Merck), HPLC was conducted using a Pharmacia LKB 2248 model and an GBC LC-1240 refractive index detector, with Spherisorb S10W and Supelco CC1593 25 cm×10 mm, 5 um, columns. TLC were performed with Kieselgel 60 F_{254} (Merck aluminum support plates).

3.2. Plant material

The alga was collected by hand at Symi Island in the Aegean Sea, Greece, at a depth of 0.5–1 m in the August of 1999. A voucher specimen is kept at the Herbarium of the Pharmacognosy Laboratory, University of Athens (ATPH/MO/57).

3.3. Extraction and isolation

The alga was initially freeze-dried (137.4 g dry weight) and then exhaustively extracted at room temperature with mixtures of CH_2Cl_2/MeOH (2/1). The organic extract after evaporation of the solvents afforded a dark green oily residue (3.0 g). The crude extract was subjected to vacuum column chromatography (VCC) on Si gel using cyclohexane with increasing amounts (10%) of EtOAc and finally MeOH. The V fraction (40% EtOAc in cyclohexane) (210 mg) was further purified by VCC on Si gel using cyclohexane with increasing amounts (2%) of EtOAc. Fractions IV (6% EtOAc) (30.8 mg), VIII (14% EtOAc) (39.1 mg) and IX (16% EtOAc) (20 mg) were subjected to normal phase HPLC chromatography, using as mobile phase cyclohexane/EtOAc (90/10), to yield pure compounds **1** (4 mg), **2** (3 mg), **3** (6.3 mg), **4** (3.5 mg) and **5** (10.9 mg).

3.4. Compound *1*

Colorless oil; $[\alpha]_D^{20}$ +37.5 ($CHCl_3$; *c* 0.11); UV λ_{max} (*n*-hexane) nm (log ε): 225 (4.01); IR ν_{max} (thin film) cm^{-1}: 3295, 2980, 2100, 1740, 1455, 1375, 1240, 1100, 1066; ^1H and ^{13}C NMR ($CDCl_3$): see Table 1; HRFAB–MS (*m*/*z*): 425.0288 $[M+1]^+$ (calc. for $C_{17}H_{24}O_3{}^{79}Br^{35}Cl_2$ 425.0286); EIMS 70 eV, *m*/*z* (rel. int. %): 365, 363, 361, 359 $[M-C_5H_5]^+$ (3:20:45:28), 349, 347, 345 $[M-Br]^+$ (0.3:1:2), 332 (5), 303 (3), 285 (8), 251 (20), 213 (5), 183 (8), 165 (9), 143 (16), 129 (31), 105 (22), 91 (23), 65 (30), 43 (100).

3.5. Compound *2*

White powder; $[\alpha]_D^{20}$ +16 ($CHCl_3$; *c* 0.10); UV λ_{max} (*n*-hexane) nm (log ε): 225.6 (4.07); IR ν_{max} (thin film) cm^{-1}: 3280, 3020, 2943, 2140, 1450, 1380, 1250, 1090, 1020, 850; ^1H and ^{13}C NMR ($CDCl_3$): see Table 1; EIMS 70 eV, *m*/*z* (rel. int. %): 334, 332, 330 $[M]^+$ (1), 297, 295 $[M-Cl]^+$ (2:2), 269, 267, 265 $[M-C_5H_5]^+$ (8:32:24), 253, 251 $[M-Br]^+$ (2:6), 229 (5), 209 (14), 185 (19), 167 (14), 149 (32), 131 (52), 121 (42), 107 (55), 91 (100), 79 (95), 65 (68), 41 (46).

3.6. Compound *3*

Colorless oil; $[\alpha]_D^{20}$ +11.87° ($CHCl_3$; *c* 0.16); UV λ_{max} (*n*-hexane) nm (log ε): 225.3 (3.70); IR ν_{max} (thin film) cm^{-1}: 3305, 2930, 2100, 1742, 1739, 1374, 1243, 1106, 1066, 1029, 963; ^1H and ^{13}C NMR ($CDCl_3$): see Table 2; HRFAB–MS (*m*/*z*): 451.0664 $[M+1]^+$ (calc. for $C_{19}H_{27}O_5{}^{81}Br^{35}Cl$ 451.0710); EIMS 70 eV, *m*/*z* (rel. int.%): 387, 385, 383 $[M-C_5H_5]^+$ (6:22:16), 371, 369 $[M-Br]^+$ (1:3), 347 (5), 327 (3), 283 (8), 249 (14), 213 (19), 201 (6), 183 (8), 147 (11), 133 (12), 105 (16), 81 (23), 65 (15), 43 (100).

Table 1
NMR spectral data for compounds **1** and **2**[a]

Position	**1**		**2**	
	δ ^1H	δ ^{13}C	δ ^1H	δ ^{13}C
1	3.14 (*d*, 2.0)	82.8 *d*	2.82 (*br s*)	76.7 *d*
2		79.8 *s*		81.9 *s*
3	5.57 (*dd*, 10.8, 2.0)	111.8 *d*	5.60 (*br d*, 15.9)	112.1 *d*
4	5.92 (*dt*, 10.8, 7.7)	139.2 *d*	6.15 (*dt*, 15.9, 7.8)	141.4 *d*
5	2.85 (*dd*, 7.4, 7.4)	35.2 *t*	2.67 (*m*)	37.9 *t*
		2.55 (*m*)		
6	4.28 (*br dd*, 7.2, 7.2)	72.7 *d*	4.32 (*dd*, 9.1, 5.2)	73.7 *d*
7	4.07 (*dd*, 5.0, 1.0)	61.3 *d*	4.05 (*dd*, 5.9, 1.5)	63.8 *d*
8	2.63 (*dd*, 16.2, 5.0)	42.3 *t*	2.73 (*m*)	33.6 *t*
	2.39 (*ddd*, 16.2, 8.4, 2.4)		2.58 (*m*)	
9	4.90 (*br dd*, 9.1, 9.1)	56.8 *d*	5.76 (*br dd*, 14.2, 7.3)	127.2 *d*
10	5.17 (*ddd*, 9.8, 4.0, 3.2)	75.6 *d*	5.83 (*br dd*, 14.2, 7.0)	129.8 *d*
11	2.43 (*m*)	31.9 *t*	2.62 (*m*)	31.3 *t*
	1.86 (*ddd*, 16.5, 4.0, 1.0)		2.19 (*ddd*, 15.1, 6.3, 1.7)	
12	4.19 (*ddd*, 11.3, 3.6, 1.0)	73.4 *d*	4.03 (*m*)	78.7 *d*
13	3.83 (*ddd*, 8.1, 4.9, 3.8)	62.5 *d*	3.94 (*ddd*, 8.8, 4.4, 4.4)	62.0 *d*
14	1.96 (*m*)	29.2 *t*	1.96 (*m*)	29.3 *t*
15	1.04 (*t*, 7.2)	12.2 *q*	1.07 (*t*, 7.2)	12.4 *q*
16		170.0 *s*		
17	2.09 (*s*)	21.0 *q*		

[a] All spectra were recorded in $CDCl_3$. Chemical shifts are expressed in ppm. *J* values in parentheses are in Hz.

Table 2
NMR spectral data for compounds **3** and **4**[a]

Position	**3**					**4**	
	δ ^{1}H	δ ^{13}C	^{1}H–^{1}H COSY	HMBC	NOESY	δ ^{1}H	δ ^{13}C
1	2.80 (*d, 2.0*)	76.6 *d*	H-3	H-3		3.04 (*d, 2.0*)	81.7 *d*
2		81.9 *s*		H-4			n/o[b]
3	5.45 (*dd*, 16.0, 2.0)	111.5 *d*	H-1, H-4	H-1, H-5	H-5	5.57 (*dd*, 11.0, 2.0)	110.7 *d*
4	6.25 (*ddd*, 16.0, 8.0, 7.3)	140.6 *d*	H-3, H-5a, H-5b	H-3, H-5	H-5, H-6, H-7	6.14 (*ddd*, 11.0, 9.5, 5.7)	139.6 *d*
5	2.57 (*ddd*, 14.9, 7.4, 5.8)	36.4 *t*	H-4, H-5b, H-6	H-3, H-4	H-3, H-4, H-6	2.83 (*m*)	33.3 *t*
	2.42 (*m*)		H-4, H-5a, H-6			2.68 (*m*)	
6	4.19 (*ddd*, 10.0, 6.1, 4.0)	71.9 *d*	H-5a, H-5b, H-7	H-12	H-4, H-5, H-7, H-8b, H-11a, H-13	4.27 (*ddd*, 10.0, 4.4, 3.9)	71.9 *d*
7	4.75 (*ddd*, 10.5, 10.5, 4.4)	71.1 *d*	H-6, H-8a, H-8b	H-9, Ac-Me, H-8	H-4, H-6, H-8a, H-9	4.68 (*ddd*, 10.5, 10.5, 3.9)	70.8 *d*
8	2.45 (*m*)	39.2 *t*	H-7, H-8b	H-9	H-6, H-7, H-9	2.47 (*m*)	39.1 *t*
	2.09 (*m*)		H-7, H-8a, H-9		H-6, H-9, H-10	2.05 (*m*)	
9	4.45 (*br dd*, 9.3, 9.3)	57.2 *d*	H-8b, H-10	H-8, H-10	H-7, H-10, H-12	4.45 (*dd*, 9.5, 9.3)	57.4 *d*
10	5.12 (*ddd*, 9.5, 3.9, 2.7)	76.0 *d*	H-9, H-11a, H-11b	H-9, H-8, Ac-Me	H-8b, H-9, H-11a, H-11b	5.13 (*br dd*, 9.5, 2.2)	76.1 *d*
11	2.47 (*m*)	31.6 *t*	H-10, H-11b, H-12		H-10	2.48 (*m*)	31.7 *t*
	1.84 (*ddd*, 16.0, 4.1, 1.2)		H-10, H-11a, H-12		H-10, H-12, H-13	1.80 (*ddd*, 16.1, 3.6, 0.7)	
12	4.12 (*ddd*, 10.8, 4.0, 1.2)	73.7 *d*	H-11a, H-11b, H-13	H-10, H-11	H-9, H-11b, H-13, H-14, H-15	4.14 (*ddd*, 11.0, 3.4, 0.7)	73.7 *d*
13	3.80 (*ddd*, 8.8, 4.4, 4.4)	61.4 *d*	H-12, H-14	H-14, H-15	H-6, H-11b, H-12, H-14, H-15	3.83 (*ddd*, 7.8, 4.4, 3.4)	61.6 *d*
14	1.92 (*m*)	29.2 *t*	H-13, H-15	H-15	H-12, H-13, H-15	1.92 (*m*)	29.1 *t*
15	1.04 (*t*, 7.2)	12.0 *q*	H-14	H-14	H-12, H-13, H-14	1.03 (*t*, 7.5)	12.0 *q*
Ac-CO		169.9 *s*		Ac-Me, H-10			169.9 *s*
Ac-CO		169.2 *s*		Ac-Me, H-7			169.3 *s*
Ac-Me	2.02 (*s*)	21.0 *q*				2.02 (*s*)	21.2 *q*
Ac-Me	2.08 (*s*)	21.0 *q*				2.08 (*s*)	20.9 *q*

[a] All spectra were recorded in $CDCl_3$. Chemical shifts are expressed in ppm. *J* values in parentheses are in Hz.
[b] n/o, Not observed.

3.7. Compound 4

Colorless oil; $[\alpha]_D^{20}$ +48° ($CHCl_3$; *c* 0.10); UV λ_{max} (*n*-hexane) nm (log ε): 223.8 (3.91); IR ν_{max} (thin film) cm^{-1}: 3305, 2930, 2100, 1742, 1739, 1374, 1243, 1106, 1066, 1029, 963; ^1H and ^{13}C NMR ($CDCl_3$): see Table 2; HRFAB–MS (*m*/*z*): 451.0672 $[M+1]^+$ (calc. for $C_{19}H_{27}O_5{}^{81}Br^{35}Cl$ 451.0710); EIMS 70 eV, *m*/*z* (rel. int.%): 387, 385, 383 $[M-C_5H_5]^+$ (3:12:10), 371, 369 $[M-Br]^+$ (1:3), 347 (2), 327 (1), 283 (4), 249 (5), 213 (3), 183 (3), 165 (4), 147 (5), 133 (5), 105 (13), 81 (12), 65 (6), 43 (100).

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