Chemical Constituents of the Endophytic Fungal Strain *Phomopsis* sp. NXZ-05 of *Camptotheca acuminata*

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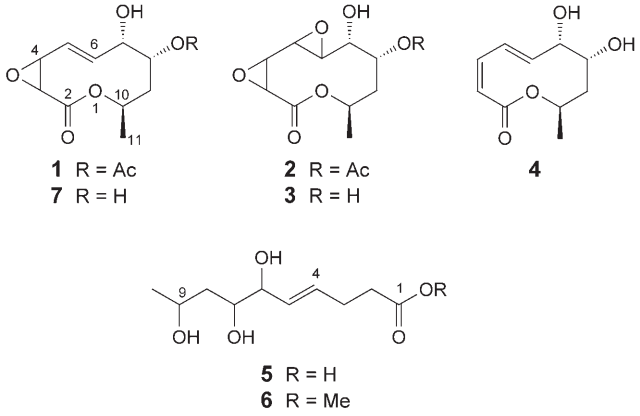
From the endophytic fungal strain *Phomopsis* sp. NXZ-05 of *Camptotheca acuminata* DECNE. (Nyssaceae), six new compounds were isolated, including the ten-membered macrolides **1**–**4** and their (known) parent compound multiplolide A (**7**), as well as a new unsaturated fatty acid, (4*E*)-6,7,9-trihydroxydec-4-enoic acid (**5**) and its methyl ester **6**. Their structures were elucidated by spectroscopic and mass-spectrometric analyses, including 1D- and 2D-NMR experiments and HR-ESI-MS, and their biological activities were elucidated.

Introduction. – Endophytes, a rich source of bioactive products, are one of the hot topics of research owing to their interesting biological activities [1]. *Camptotheca acuminata* (Nyssaceae), a medicinal plant specifically distributed in China, is rich in the anticancer compound camptothecin, the highest content (up to 0.4%) being found in the fresh leaves. It is conceivable that, in certain habitats, special endophytic fungi of *C. acuminata* exist that may take part in the synthesis or transformation of camptothecin. Therefore, it is important to study the bioactive components of the endophytic fungi isolated from this plant. So far, some 174 endophytic fungi have been isolated [2]. In this work, we report the isolation, structure elucidation, and bioactivities of the new ten-membered macrolides **1**–**4** and of the new unsaturated fatty acid **5** and its methyl ester **6**, which were obtained from the fermentation products of the endophytic fungal strain *Phomopsis* sp. NXZ-05. Also isolated was the known constituent multiplolide A (**7**).

Results and Discussion. – 1. *Structure Elucidation*. The morphological properties of the NXZ-05 strain were examined after incubation for 14 d at 28° on potato-dextrose agar medium. The organism was found to grow well, with white hyphal tips, and was identified as *Phomopsis* sp. NXZ-05 according to its ITS sequence of rDNA (ITS1-5.8S-ITS2) [2]. The fungal agar culture was extracted successively with AcOEt/MeOH/AcOH 80:15:5, and the crude extract was purified by repeated column chromatography (on *RP-18*, *Sephadex LH-20*, and silica gel) to afford compounds **1**–**7**.

Compound **1** was obtained as a colorless oil. Its HR-ESI-TOF mass spectrum showed the $[M+Na]^+$ peak at m/z 279.0844, establishing the molecular formula



$C_{12}H_{16}O_6$. The ^{1}H-NMR spectrum of **1** (*Table 1*)[1]) showed a Me signal [δ(H) 1.34 (J = 6.8 Hz)], a CH_2 moiety (δ(H) 1.26 (*dd*, J = 16.1, 2.9 Hz), 2.34 (*ddd*, J = 16.1, 8.0, 3.5 Hz)), five oxygenated CH groups (δ(H) 3.65, 3.79–3.80, 4.54, 5.06, 5.23), and two olefinic H-atoms (δ(H) 5.78, 5.90). The J(5,6) value of 15.4 Hz revealed the (*E*)-configuration for the olefin.

Table 1. *^{1}H-NMR Spectroscopic Data of* **1**–**4**. Recorded at 500 MHz in $CDCl_3$; δ in ppm, J in Hz. Arbitrary atom numbering.

Position	**1**	**2**	**3**	**4**
3	3.65 (*d*, *J* = 4.6)	3.63 (*d*, *J* = 5.0)	3.61 (*d*, *J* = 4.8)	5.75 (*d*, *J* = 10.5)
4	3.79–3.80 (*m*)	3.64–3.65 (*m*)	3.63–3.64 (*m*)	6.53 (*d*, *J* = 10.5)
5	5.78 (*d*, *J* = 15.4)	3.47 (*s*)	3.47 (*s*)	5.97 (*d*, *J* = 15.4)
6	5.90 (*d*, *J* = 15.4)	3.56 (*s*)	3.58 (*s*)	5.58 (*dd*, *J* = 15.4, 8.8)
7	4.54 (br. *s*)	4.18 (br. *s*)	4.22 (br. *d*, *J* = 9.4)	4.20 (*d*, *J* = 8.8)
8	5.06 (*dd*, *J* = 7.9, 1.8)	5.27–5.29 (*m*)	4.02 (br. *s*)	3.91–3.94 (*m*)
9	1.26 (*dd*, *J* = 16.1, 2.9), 2.34 (*ddd*, *J* = 16.1, 8.0, 3.5)	1.60 (*dd*, *J* = 15.5, 6.5), 2.43 (*ddd*, *J* = 15.6, 6.0, 3.0)	1.70 (*dd*, *J* = 15.5, 8.7), 2.22 (*ddd*, *J* = 16.0, 8.0, 3.0)	1.37 (br. *d*, *J* = 15.7), 2.27 (br. *d*, *J* = 7.4)
10	5.23 (*dq*, *J* = 6.4, 3.2)	5.27–5.29 (*m*)	5.48 (br. *t*, *J* = 6.2)	5.19–5.20 (*m*)
11	1.34 (*d*, *J* = 6.8)	1.35 (*d*, *J* = 6.0)	1.32 (*d*, *J* = 6.5)	1.20 (*d*, *J* = 6.6)
Ac	2.05 (*s*)	2.06 (*s*)	–	–

The ^{13}C-NMR spectrum of **1** (*Table 2*) showed twelve signals: two Me, one CH_2, and seven CH groups, and two quaternary C-atoms. The ^{1}H,^{1}H-COSY spectrum of **1** demonstrated the connectivity from H–C(3) to H–C(11). The epoxide at C(3) and C(4) was evident from the pertinent upfield chemical shifts. The downfield shift of H–C(8), from δ(H) 4.03 in multiplolide A (**7**) [3] (data not shown) to δ(H) 5.06 in **1**, indicated that the 8-OH group in **1** was esterified. Analyses of the HMQC and HMBC spectra revealed that **1** was the acetate of **7**. The HMBC spectra clearly demonstrated

[1]) Arbitrary atom numbering. For systematic names, see *Exper. Part.*

Table 2. *^{13}C-NMR Spectroscopic Data of* **1**–**4**. Recorded at 125 MHz in $CDCl_3$; δ in ppm, J in Hz. Arbitrary atom numbering.

Position	**1**	**2**	**3**	**4**
2	168.8 (*s*)	166.5 (*s*)	166.7 (*s*)	168.3 (*s*)
3	55.8 (*d*)	51.6 (*d*)	51.8 (*d*)	125.3 (*d*)
4	55.3 (*d*)	52.8 (*d*)	53.1 (*d*)	139.3 (*d*)
5	120.3 (*d*)	51.0 (*d*)	51.3 (*d*)	126.4 (*d*)
6	134.4 (*d*)	55.3 (*d*)	55.0 (*d*)	136.2 (*d*)
7	70.9 (*d*)	67.9 (*d*)	66.8 (*d*)	74.6 (*d*)
8	72.4 (*d*)	69.6 (*d*)	69.3 (*d*)	71.8 (*d*)
9	33.3 (*t*)	33.2 (*t*)	34.9 (*t*)	35.6 (*t*)
10	69.5 (*d*)	68.0 (*d*)	67.6 (*d*)	67.8 (*d*)
11	17.8 (*q*)	18.8 (*q*)	19.6 (*q*)	19.0 (*q*)
Ac	21.1 (*q*)	20.8 (*q*)	–	–
	172.0 (*s*)	170.1 (*s*)		

correlations of the C=O C-atom of the Ac group to both H–C(8) and the ester Me H-atoms, confirming the presence of an AcO moiety at C(8).

Comparison of the ^{1}H-NMR spectra of **1** and **7** with those of multiplolides A and B revealed that they not only had similar chemical shifts, but also identical coupling patterns, which indicated that **1** and **7** have the same configuration as the multiplolides. The relative configuration of **1** was further confirmed by NOE experiments. Thus, NOEs between H–C(4) and H–C(6), H–C(6) and H–C(7), H–C(6) and H–C(8), and H–C(8) and Me(11) were observed, as shown in the *Figure*. From these data, the structure of the macrolide **1** was, thus, elucidated as 8-*O*-acetylmultiplolide A.

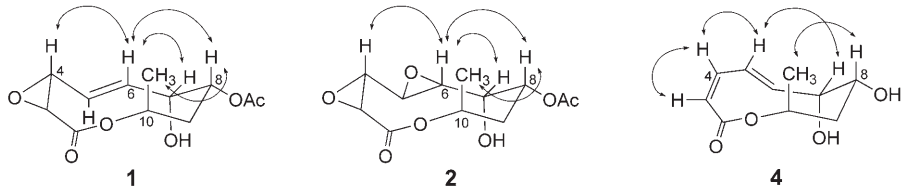


Figure. *Selected NOE correlations for compounds* **1**, **2**, *and* **4**

Compound **2** was obtained as colorless, cubic crystal upon crystallization from acetone/H_2O. The molecular formula was determined as $C_{12}H_{16}O_7$ by HR-ESI-TOF MS (m/z 295.0793 ($[M + Na]^+$)). The ^{13}C-NMR spectroscopic data (*Table 2*) disclosed the presence of two C=O groups (δ(C) 170.1, 166.5), seven oxygenated CH groups (δ(C) 51.6, 52.8, 51.0, 55.3, 67.9, 69.6, 68.0), and one Me group (δ(C) 18.8). The HMBC and ^{1}H,^{1}H-COSY spectra of **2** revealed the connectivities from C(2) to C(11). The long-range correlations from H–C(10) to C(2) and the lowfield-shifted resonance of H–C(10) (δ(H) 5.27–5.29 (m)) suggested that **2** was a ten-membered macrolide. The HMBC spectrum clearly demonstrated correlations of the C=O C-atom of an Ac group to both H–C(8) and the ester Me H-atoms, confirming the presence of an AcO moiety at C(8), just as in **1**. Further comparison of the NMR data of **1** and **2** indicated that **2** had an additional epoxide function, but no C=C bond. The relative configuration

of **2** was confirmed by NOE experiments. NOEs were observed between H–C(4) and H–C(6), H–C(6) and H–C(7), H–C(6) and H–C(8), and H–C(8) and Me(11), as shown in the *Figure*. Therefore, the structure of **2** was determined as 8-*O*-acetyl-5,6-dihydro-5,6-epoxymultiplolide A.

Compound **3** was obtained as colorless oil. Its molecular formula was determined as $C_{10}H_{14}O_6$ by HR-ESI-TOF-MS (*m/z* 253.0682 ($[M+Na]^+$)). Comparison of the ^{1}H- and ^{13}C-NMR data of **3** with those of **2** revealed that the two compounds had similar structures, except that **3** had no Ac group. This was confirmed by the upfield shift of H–C(8) from δ(H) 5.27–5.29 in **2** to δ(H) 4.02 in **3** (*Table 1*). Additionally, **3** showed similar NOE effects as **2** (data not shown), indicating identical relative configurations. Hence, the structure of **3** was determined as 5,6-dihydro-5,6-epoxymultiplolide A.

Compound **4** was obtained as needles (from $CHCl_3$). Its molecular formula was determined as $C_{10}H_{14}O_4$ by HR-ESI-TOF-MS (*m/z* 221.0785 ($[M+Na]^+$)). The ^{1}H-NMR spectrum (*Table 1*) showed a Me group (δ(H) 1.20 (*d*, *J* = 6.6 Hz)), a CH_2 group (δ(H) 1.37 (br. *d*, *J* = 15.7 Hz), 2.27 (br. *d*, *J* = 7.4 Hz)), three oxygenated CH groups [δ(H) 3.91–3.94, 4.20, 5.19–5.20], and four olefinic H-atoms (δ(H) 5.58 (*dd*, *J* = 15.4, 8.8 Hz); 5.75 (*d*, *J* = 10.5 Hz); 5.97 (*d*, *J* = 15.4 Hz); 6.53 (*d*, *J* = 10.5 Hz)). The *J*(3,4) and *J*(5,6) values of 10.5 and 15.4 Hz, respectively, indicated one (*Z*)- and one (*E*)-configured olefin. The ^{13}C-NMR spectrum of **4** (*Table 2*) showed ten signals: one Me, one CH_2, seven CH, and one quaternary C-atoms.

The ^{1}H,^{1}H-COSY spectrum of **4** demonstrated the connectivity from H–C(3) to H–C(11). The long-range correlations from H–C(10) to C(2) and the lowfield-shifted resonance of H–C(10) (δ(H) 5.19–5.20 (*m*)) suggested that **4** was a ten-membered macrolide as well. By comparing the ^{1}H- and ^{13}C-NMR data of **4** with those of multiplolide A (**7**), the structure of **4** was determined as 3,4-deoxy-3,4-didehydromultiplolide A. The configuration of **4** was determined by NOE experiments, key interactions being shown in the *Figure*.

Compound **5** was obtained as a colorless oil. Its molecular formula was determined as $C_{10}H_{18}O_5$ by HR-ESI-TOF-MS (*m/z* 241.1056 ($[M+Na]^+$)). The ^{13}C-NMR (DEPT) spectrum of **5** (*Table 3*) showed ten signals attributable to one Me, three CH_2, and five CH groups, as well as one quaternary C-atom [δ(C) 175.9 (C(1))], the latter being due to a carboxy function. The ^{1}H-NMR spectrum of **5** (*Table 3*) showed three hydroxylated methines (δ(H) 3.91–3.93 (*m*, H–C(6)); 3.74–3.77 (*m*, H–C(7)); 3.94–3.97 (*m*, H–C(9))) and two olefinic resonances (δ(H) 5.52 (*dd*, *J* = 15.4, 7.0 Hz); 5.69 (*dt*, *J* = 15.4, 6.6 Hz)). Two degrees of unsaturations were due to the COOH and C=C moieties. The ^{1}H,^{1}H-COSY and HMQC spectra revealed connectivities from H–C(2) to H–C(10), as confirmed by HMBC experiments. Therefore, from the above data, the structure of **5** was determined as (4*E*)-6,7,9-trihydroxydec-4-enoic acid.

Compound **6** was obtained as a colorless oil. HR-ESI-TOF-MS revealed the molecular formula $C_{11}H_{20}O_5$ (*m/z* 255.1209 ($[M+Na]^+$)). The ^{1}H- and ^{13}C-NMR spectra of **6** (*Table 3*) were similar to those of **5**, except for an additional signal [δ(H) 3.68 (*s*, MeO)] due to a Me ester, as confirmed by comparison of the ^{1}H- and ^{13}C-NMR spectra of **5** and **6**, and corroborated by an HMBC correlation between δ(H) 3.68 and δ(C) 173.5 (C=O). Thus, the structure of **6** was determined as methyl (4*E*)-6,7,9-trihydroxydec-4-enoate.

Table 3. *^{1}H- and ^{13}C-NMR Spectroscopic Data of* **5** *and* **6**. Recorded at 500/125 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz. Arbitrary atom numbering.

Position	**5**		**6**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	–	175.9 (*s*)	–	173.5 (*s*)
2	2.31–2.36 (*m*)	27.4 (*t*)	2.39–2.41 (*m*)	27.6 (*t*)
3	2.37–2.38 (*m*)	33.4 (*t*)	2.42–2.45 (*m*)	33.5 (*t*)
4	5.69 (*dt*, J = 15.4, 6.6)	131.9 (*d*)	5.78 (*dt*, J = 15.4, 6.5)	132.3 (*d*)
5	5.52 (*dd*, J = 15.4, 7.0)	129.1 (*d*)	5.57 (*dd*, J = 15.4, 6.8)	129.3 (*d*)
6	3.91–3.93 (*m*)	75.1 (*d*)	4.03–4.05 (*m*)	75.4 (*d*)
7	3.74–3.77 (*m*)	74.3 (*d*)	3.86–3.88 (*m*)	74.8 (*d*)
8	1.43–1.51 (*m*)	39.2 (*t*)	1.60 (*m*)	39.2 (*t*)
9	3.94–3.99 (*m*)	67.5 (*d*)	4.06–4.09 (*m*)	68.4 (*d*)
10	1.14 (*d*, J = 6.2)	23.1 (*q*)	1.23 (*d*, J = 6.1)	24.2 (*q*)
MeO	–	–	3.68 (*s*)	51.6 (*q*)

2. *Biological Studies.* Compounds **1**–**4** and **7** exhibited no evident antifungal activities against *Candida albicans* at 200 μg/ml, which is quite unexpected when considering related results [3]. The cytotoxicities of **1**–**3** and **7** towards human-tumor *Raji* cells were tested by the MTT method [4][5], but none of them was found to be cytotoxic at 100 μg/ml. The protease-inhibitory activities of **1**–**3** and **7** towards acetylcholinesterase (AChE) were determined according to the modified *Ellman* method [6]. Compound **1** exhibited significant inhibitory activity against AChE, with an IC_{50} value at 1.19 μg/ml, relative to the positive control galanthamine (with an inhibition of 97.8% at 2.5 μg/ml); compounds **2**, **3**, and **7**, however, exhibited no distinct inhibitory activities in this assay ($IC_{50} > 10$ μg/ml).

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 and 80–100 mesh; *Qingdao Marine Chemical Factory*, Qingdao, China), silica gel GF_{254} (*Merck*), *RP-18* gel (*Merck*), or *Sephadex LH-20* gel (*Amersham Biosciences*). TLC: precoated silica-gel GF_{254} plates (0.20–0.25 mm, *Qingdao*). UV Spectra: *Jasco V-530* spectrophotometer, in MeOH. IR Spectra: *Perkin-Elmer 552* spectrophotometer, with KBr cells; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker DRX-500* spectrometer, at 500/125 MHz, resp., in $CDCl_3$; δ in ppm rel. to Me_4Si, J in Hz. ESI-MS: *Thermo-Finnigan Advantage-LCQ* mass spectrometer; in *m/z*. HR-ESI-MS: *API QStar-Pulsar LC-Q-TOF* mass spectrometer; in *m/z*.

Fungal Material. The fungal strain *Phomopsis* sp. NXZ-05 was isolated from the twigs of *Camptotheca acuminata* DECNE. (Nyssaceae), which were collected in the 'Shaowu Jiangshi Nature Conservation Area', Fujian Province, P. R. China, from a plant that was approximately ten years old. Surface-sterilized samples from different parts of the twigs were cut into 1-cm fragments, with ten fragments per sample. The sterilized samples were placed onto the surface of melted potato dextrose agar (PDA; 15 ml) as medium in *Petri* dishes, and then cultured at 25°. During cultivation, the hyphal tips of the growing fungi were removed, inoculated onto fresh PDA medium, and incubated for at least two weeks at 25°. After being purified by the hyphal-tip method [7], the pure isolates were transferred to PDA slant tubes as deposit. The strain *Phomopsis* sp. NXZ-05 was inoculated on slope of PDA medium

in a test tube, and cultivated for 5 d at 25° to afford seed cultures. Solid-state fermentation was performed with PDA media for 14 d at 25°.

Extraction and Isolation. The fungal strain *Phomopsis* sp. NXZ-05 was fermented twice. Each of the cultures was extracted three times with an equal volume (see below) of AcOEt/MeOH/AcOH 80:15:5 (*v*/*v*/*v*) at r.t. The org. solns. were filtrated and concentrated *in vacuo* at 40° to yield a crude syrup (20.1 and 49.2 g, resp.).

a) For the first fermentation (5 l), the crude extract (20.1 g) was partitioned between H_2O and AcOEt. The org. layer was concentrated *in vacuo* to afford 12.5 g of extract, which was subjected to MPLC (130 g *RP-18*; H_2O, then MeOH/H_2O 1:3, 1:1, 2:1, 3:1, and 5:1) to afford six fractions. The fraction eluted with MeOH/H_2O 1:3 (1.4 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 10:1, 8:1, 5:1, 3:1, and 1:1) to yield two fractions: *Fr. C1* and *Fr. C2*. *Fr. C1* (159.2 mg) was purified by CC (SiO_2; petroleum ether (PE)/AcOEt 10:1, 8:1, 5:1, and 3:1) to yield **1** (5.9 mg) and **2** (79.6 mg). *Fr. C2* (334.1 mg) was further purified by repeated CC (1. 100 g *Sephadex LH-20*, MeOH; 2. SiO_2, $CHCl_3$/MeOH 50:1; 3. 100 g *Sephadex LH-20*, MeOH) to afford **7** (23.9 mg).

b) For the second fermentation (10 l), the crude extract (49.2 g) was successively extracted with PE, AcOEt, and MeOH. Half of the AcOEt-soluble extract (13.5 g in total) was subjected to MPLC (130 g *RP-18*), eluting with H_2O, and then H_2O/MeOH 70:30, 50:50, 30:70, and 0:100 (2 l each), to yield five fractions: *Fr. D1*–*Fr. D5*. *Fr. D2* (2.03 g) was subjected to CC (100 g *Sephadex LH-20*; MeOH). All fractions were analyzed by TLC ($CHCl_3$/MeOH 10:1), and pooled accordingly into three portions (*Fr. D2a*–*D2c*). *Fr. D2a* (1.22 g) was subjected to MPLC (80 g *RP-18*; MeOH/H_2O 25:75) to afford three portions (*Fr. D2a$_1$*–*D2a$_3$*). *Fr. D2a$_1$* (234.5 mg) was subjected to repeated CC (1. 30 g *RP-18*, MeOH/H_2O 15:85 and 23:77; 2. SiO_2, $CHCl_3$/MeOH 50:1) to yield **5** (38.1 mg) and **6** (12.1 mg). *Fr. D2a$_2$* (536 mg) was subjected to CC (SiO_2; $CHCl_3$/MeOH 200:1) to afford **4** (23.5 mg). *Fr. D2b* (708.2 mg) was purified by repeated CC (1. 100 g *Sephadex LH-20*, MeOH; 2. *RP-18*, MeOH/H_2O 20:80) to yield two portions (*Fr. D2b$_1$*–*D2b$_2$*). *Fr. D2b$_1$* (442.3 mg) was subjected to CC (SiO_2; $CHCl_3$/MeOH 400:1 and 200:1) to afford **2** (31.3 mg). *Fr. D2b$_2$* (229.4 mg) was subjected to repeated CC (SiO_2; $CHCl_3$/MeOH 350:1 and 250:1, cyclohexane/AcOEt 10:1 and 8:1) to afford **4** (10.8 mg). *Fr. D2c* (91.1 mg) was purified by CC (SiO_2; $CHCl_3$/MeOH 200:1) to yield **3** (19.3 mg).

*8-*O*-Acetylmultiplolide A* (=*(4*R**,6*R**,7*S**,8*E*)-7-Hydroxy-4-methyl-2-oxo-3,11-dioxabicyclo[8.1.0]undec-8-en-6-yl Acetate*; **1**). Colorless oil. UV (MeOH): end absorption. $[\alpha]_D^{20} = +41.5$ (c = 0.20, MeOH). IR (KBr): 3433,1632, 1392, 1241, 1113, 1051. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-TOF-MS: 279.0844 ($[M+Na]^+$, $C_{12}H_{16}NaO_6^+$; calc. 279.0845).

*8-*O*-Acetyl-5,6-dihydro-5,6-epoxymultiplolide A* (=*(7*R**,9*R**,10*R**)-10-Hydroxy-7-methyl-5-oxo-3,6,12-trioxatricyclo[9.1.0.0^{2,4}]dodec-9-yl Acetate*; **2**). Colorless, cubic crystal (acetone/H_2O). UV ($CHCl_3$): end absorption. $[\alpha]_D^{20} = -73.7$ (c = 0.68, $CHCl_3$). IR (KBr): 3473, 1728, 1372, 1289, 1239, 1053. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-TOF-MS: 295.0793 ($[M + Na]^+$, $C_{12}H_{16}NaO_7^+$; calc. 295.0794).

5,6-Dihydro-5,6-epoxymultiplolide A (=*(7*R**,9*R**,10*R**)-9,10-Dihydroxy-7-methyl-3,6,12-trioxatricyclo[9.1.0.0^{2,4}]dodecan-5-one*; **3**). Colorless oil. UV ($CHCl_3$): end absorption. $[\alpha]_D^{20} = -115.9$ (c = 0.77, $CHCl_3$). IR (KBr): 3429, 1725, 1369, 1292, 1057. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-TOF-MS: 253.0682 ($[M+Na]^+$, $C_{10}H_{14}NaO_6^+$; calc. 253.0688).

3,4-Deoxy-3,4-didehydromultiplolide A (=*(3Z,5*E*,7*S**,8*R**,10*R**)-7,8,9,10-Tetrahydro-7,8-dihydroxy-10-methyl-2*H*-oxecin-2-one*; **4**). Needles ($CHCl_3$). UV (MeOH): end absorption. $[\alpha]_D^{20} = -60$ (c = 0.18, MeOH). IR (KBr): 3444, 2926, 2365, 1706, 1385, 1054. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-TOF-MS: 221.0785 ($[M+Na]^+$), $C_{10}H_{14}NaO_4^+$; calc. 221.0790).

*(4*E*)-6,7,9-Trihydroxydec-4-enoic Acid* (**5**). Colorless oil. UV ($CHCl_3$): end absorption. $[\alpha]_D^{20} = -0.6$ (c = 2.0, $CHCl_3$). IR (KBr): 3429, 2926, 1718, 1389, 1025. ^{1}H- and ^{13}C-NMR: see *Table 3*. HR-ESI-TOF-MS: 241.1056 ($[M+Na]^+$, $C_{10}H_{18}NaO_5^+$; calc. 241.1052).

*Methyl (4*E*)-6,7,9-Trihydroxydec-4-enoate* (**6**). Colorless oil. UV ($CHCl_3$): end absorption. $[\alpha]_D^{20} = -0.7$ (c = 0.15, $CHCl_3$). IR (KBr): 3435, 1620, 1384, 1054. ^{1}H- and ^{13}C-NMR: see *Table 3*. HR-ESI-TOF-MS: 255.1209, ($[M+Na]^+$, $C_{11}H_{20}NaO_5^+$; calc. 255.1208).

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