

# Further Guaianolides from *Amphoricarpos neumayeri* ssp. *murbeckii* from Montenegro

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Dedicated to Daniel Vinček, Botanic Garden Dulovine, Kolašin, Montenegro on the occasion of his 80<sup>th</sup> birthday

The aerial parts of *Amphoricarpos neumayeri* ssp. *murbeckii* afforded eleven guaianolides with the same relative (1 $\alpha$ H,4 $\beta$ H,5 $\alpha$ H,7 $\alpha$ H) configuration of the basic skeleton. All of them contained a CH<sub>2</sub>OX (X = H, acetyl or isovaleroyl) group in 4 $\alpha$ -position, typical for amphoricarpolides. Four compounds (**1**–**4**) were isolated before from the same species, originating from different localities. Guaianolides **5**–**11** are new compounds. Compounds **7** and **8** were epoxidized at the 10 $\alpha$ (14)-position. Instead of the  $\Delta^{11(13)}$ -double bond, observed in all previously isolated guaianolides from the same species, the four lactones contained 11 $\alpha$ ,13-diol (**8**–**10**) or 11 $\alpha$ -OH,13-chloro (**11**) moieties respectively.

**Key words:** *Amphoricarpos neumayeri* ssp. *murbeckii*, Sesquiterpene Lactones, Guaianolides

## Introduction

The classification of the genus *Amphoricarpos*, an endemic species of the western part of the Balkan Peninsula, is somewhat vague. Blečić and Mayer [1] reported two endemic species: *A. neumayeri* Vis. and *A. autariatus* Blečić et. Mayer, the latter comprising two subspecies, ssp. *autariatus* and ssp. *bertisceus* Blečić et. Mayer. On the other hand, Webb [2] recognized only a single species, *A. neumayeri* Vis., divided in two subspecies, ssp. *neumayeri* and ssp. *murbeckii* Bošnjak.

In our previous phytochemical study of the aerial parts of *A. neumayeri* ssp. *neumayeri* and ssp. *murbeckii* Bošnjak [3] originating from the Orjen and Visitor mountains, respectively, eleven sesquiterpene  $\gamma$ -lactones with the guaianolide skeleton (named amphoricarpolides) have been isolated. All of them exhibited a 11(13)-double bond and an  $\alpha$ -positioned C(15)H<sub>2</sub>OX group (X = H, acetyl or *i*-valeroyl). The majority of these lactones were oxygenated at the 3 $\beta$  position (OH or OAc) and most of them exhibited a

third oxygen functionality (OH or OAc, in one case) at the 2 $\alpha$ - or 9 $\beta$ -position. All of them were new compounds. Continuing our chemotaxonomic examination of the members of the *Amphoricarpos* complex, we now report the investigation of *A. neumayeri* ssp. *murbeckii* [2], collected at the Karanfili mountain (belonging to the mountain chains of Prokletije, North Albanian Alps). According to Blečić and Mayer [1] this taxon, mostly inhabiting Prokletije (covering the boundary areas of Montenegro, Kosovo and Albania) and the mountains of north Greece was denoted as *A. autariatus* ssp. *bertisceus*.

## Experimental Section

### General

Dry-column flash chromatography and column chromatography: silica gel 60 (Merck), under 0.063 mm. TLC: Kieselgel 60 G<sub>254</sub>, layer thickness 0.25 mm. IR: transparent dry films (Perkin-Elmer FT IR spectrometer 1725X). <sup>13</sup>C and <sup>1</sup>H NMR: at 50 and 200 MHz, respectively (Varian Gemini 2000) and 125/500 MHz (Bruker DMX 500). MS (EI and

DCI): double focusing mass spectrometer (Finnigan MAT 8230). MS (ESI): double focusing mass spectrometer + electro spray interface (Finnigan MAT 900). LC/ESI MS (positive mode): Agilent 1100 Series LC/MSD G 1946D, LiChrospher 100 RP-18 (250 × 4 mm, 5 μm), flow rate, 1 mL/min, mobile phase, A (H<sub>2</sub>O) + B (MeCN), elution, combination of gradient and isocratic modes: 90% A, 0–5 min, 90–65% A, 5–20 min, 65% A, 20–30 min, 65–50% A, 30–40 min, 50% A, 40–60 min. Optical rotations: Rudolph Research Analytical Autopol IV Automatic Polarimeter. Elemental analyses: Vario EL III C, H, N, S, O elemental analyzer (Elementar).

#### Plant material

Aerial parts of *A. neumayeri* ssp. *murbeckii* were collected during the flowering period (July) 2002 at the Karanfili mountain (Prokletije, Montenegro). A voucher specimen (BEOU AN 07072002) was deposited in the herbarium of the Botanical Garden “Jevremovac”, Faculty of Biology, University of Belgrade.

#### Extraction and isolation

Air dried aerial parts (380 g) were powdered and successively extracted twice with freshly distilled solvents (4.0 L): Et<sub>2</sub>O-petroleum ether-MeOH (1 : 1 : 1) at r. t., followed by MeOH treatment to remove long chain saturated hydrocarbons, according to the usual procedure [4].

The crude extract (10 g) was divided into 28 fractions (Frs. 1–28) by dry-column flash chromatography, starting elution with petroleum ether and gradually increasing the polarity of the solvent, first by addition of Et<sub>2</sub>O (up to 100%) and then MeOH (up to 30%).

From Fr. 13 (petroleum ether-Et<sub>2</sub>O, 3 : 7), after CC (CH<sub>2</sub>Cl<sub>2</sub>-MeOH, 9.8 : 0.2), 4 mg of the lactone **6** was isolated. Preparative TLC (toluene-EtOAc-MeOH, 7.5 : 2 : 0.5) of Fr. 14 (petroleum ether-Et<sub>2</sub>O, 2 : 8) yielded **11** (6 mg). The lactone **1** (24 mg) was isolated from Fr. 15 by silica gel CC (CH<sub>2</sub>Cl<sub>2</sub>-MeOH, 9.5 : 0.5). After preparative TLC (CH<sub>2</sub>Cl<sub>2</sub>-MeOH, 9.5 : 0.5) of Fr. 16 (petroleum ether-Et<sub>2</sub>O, 0.5 : 9.5), 3 mg of **10** were obtained. The lactones **2** (4 mg) and **7** (3 mg) were isolated from the combined Frs. 17 and 18 (Et<sub>2</sub>O-MeOH, 9 : 1), after preparative TLC (CH<sub>2</sub>Cl<sub>2</sub>-MeOH, 9.5 : 0.5). The lactones **5** (45 mg) and **9** (11 mg) were isolated from Fr. 25 (Et<sub>2</sub>O-MeOH, 8 : 2), after silica gel CC (elution started with CH<sub>2</sub>Cl<sub>2</sub>-MeOH, 9.5 : 0.5 and polarity was gradually increased by addition of MeOH). Fr. 26 (Et<sub>2</sub>O-MeOH, 8 : 2) was subjected to CC (CH<sub>2</sub>Cl<sub>2</sub>-MeOH, 9.5 : 0.5) yielding **3** (31 mg) and an additional fraction which, after further purification by two preparative TLCs (CH<sub>2</sub>Cl<sub>2</sub>-MeOH, 9.5 : 0.5 and 9.4 : 0.6, two developments in both cases) afforded 2 mg of **8**. The dominant lactone **4** (78 mg) was isolated from the combined Fr. 27 (Et<sub>2</sub>O-MeOH, 8 : 2) and 28 (Et<sub>2</sub>O-MeOH, 7 : 3).

#### 15-O-acetyl-9β-hydroxyamphoricarpolide (5)

Colourless gum. –  $[\alpha]_D^{25}$  – 7.1 (*c*, 0.38, CHCl<sub>3</sub>). – IR (film):  $\nu$  = 3457 (OH), 1670, 1650 (C=CH), 1768 (C=O, conjugated  $\gamma$ -lactone) cm<sup>-1</sup>. – <sup>1</sup>H and <sup>13</sup>C NMR: Tables 1 and 2, respectively. – MS (ESI, MeOH-H<sub>2</sub>O, 1 : 1 + 1% AcOH):  $m/z$  (%) = 667.3 (70), [2M+Na<sup>+</sup>], 645 (60) [2M+H<sup>+</sup>], 345 (100) [M+Na<sup>+</sup>], 323 (55) [M+H<sup>+</sup>], 305 (75) [M+H<sup>+</sup>-18]. – C<sub>17</sub>H<sub>22</sub>O<sub>6</sub> (322.36): calcd. C 63.34, H 6.88; found: C 63.50, H 6.72.

#### 15-O-acetyl-3-deoxyamphoricarpolide (6)

Colourless oil. – IR (film):  $\nu$  = 1768 (C=O, conjugated  $\gamma$ -lactone), 1736, 1245 (OAc) cm<sup>-1</sup>. – <sup>1</sup>H NMR: Table 1. – MS (ESI, MeOH-H<sub>2</sub>O, 1 : 1 + 1% NH<sub>4</sub>OAc):  $m/z$  (%) = 603 (2) [2M+Na<sup>+</sup>], 329 (38.5) [M+K<sup>+</sup>], 313 (34.5) [M+Na<sup>+</sup>], 308 (100), [M+NH<sub>4</sub><sup>+</sup>], 291 (7) [M+H<sup>+</sup>]. – HRMS (CI, 150 eV, *iso*-butane): [M+H<sup>+</sup>]  $m/z$  = 291.1586 (calcd. for C<sub>17</sub>H<sub>23</sub>O<sub>4</sub>: 291.1596).

#### 3-Deoxy-10α(14)-epoxyamphoricarpolide (7)

Colourless gum. – IR (film):  $\nu$  = 3428 (OH), 1761 (C=O, conjugated  $\gamma$ -lactone), 1666 (C=C) cm<sup>-1</sup>. – <sup>1</sup>H and <sup>13</sup>C NMR: Tables 1 and 2, respectively. – MS (ESI, MeOH-H<sub>2</sub>O, 1 : 1 + 1% NH<sub>4</sub>OAc):  $m/z$  (%) = 551 (2) [2M+Na<sup>+</sup>], 303 (24) [M+K<sup>+</sup>], 287 (100) [M+Na<sup>+</sup>], 282 (16) [M+NH<sub>4</sub><sup>+</sup>], 265 (16) [M+H<sup>+</sup>]. – HRMS (CI, 150 eV, *iso*-butane): [M+H<sup>+</sup>]  $m/z$  = 265.1437 (calcd. for C<sub>15</sub>H<sub>21</sub>O<sub>4</sub>: 265.1440).

#### 3-Deoxy-10α(14)-epoxy-11α,13-dihydroxy-11,13-dihydroamphoricarpolide (8)

Colourless oil. – IR (film):  $\nu$  = 3317 (OH), 1761 (C=O, lactone) cm<sup>-1</sup>. – <sup>1</sup>H and <sup>13</sup>C NMR: Tables 1 and 2, respectively. – MS (ESI, MeOH-H<sub>2</sub>O, 1 : 1 + 1% NH<sub>4</sub>OAc):  $m/z$  (%) = 635 (2) [2M+K<sup>+</sup>], 619 (13) [2M+Na<sup>+</sup>], 337 (100) [M+K<sup>+</sup>], 321 (49) [M+Na<sup>+</sup>], 316 (61) [M+NH<sub>4</sub><sup>+</sup>], 299 (9) [M+H<sup>+</sup>]. – HRMS (CI, 150 eV, *iso*-butane): [M+H<sup>+</sup>]  $m/z$  = 299.1499 (calcd. for C<sub>15</sub>H<sub>23</sub>O<sub>6</sub>: 299.1495).

#### 3-Deoxy-11α,13-dihydroxy-11,13-dihydroamphoricarpolide (9)

Colourless gum. – IR (film):  $\nu$  = 3433 (OH), 1766 (C=O,  $\gamma$ -lactone), 1638 (C=C) cm<sup>-1</sup>. – <sup>1</sup>H and <sup>13</sup>C NMR: Tables 1 and 2, respectively. – MS (EI, 70 eV):  $m/z$  (%) = 282 (20) [M<sup>+</sup>], 264 (8) [M<sup>+</sup>-18], 251 (6.5) [M<sup>+</sup>-31], 177 (100), 159 (58). – C<sub>15</sub>H<sub>22</sub>O<sub>5</sub> (282.34): calcd. C 63.81, H 7.85; found: C 63.70, H 7.93.

#### 15-O-acetyl-3-deoxy-11α,13-dihydroxy-11,13-dihydroamphoricarpolide (10)

Colourless oil. –  $[\alpha]_D^{25}$  + 5.3 (*c*, 0.4, CHCl<sub>3</sub>). – IR (film):  $\nu$  = 3393 (OH), 1768 (C=O, lactone), 1760, 1251 (OAc),

Table 1. <sup>1</sup>H NMR (CDCl<sub>3</sub>) chemical shifts, multiplicities and coupling constants (in parentheses) of compounds **5**–**11**.

H	<b>5</b> (500 MHz)	<b>6</b> (200 MHz)	<b>7</b> (200 MHz)	<b>8</b> (500 MHz)	<b>9</b> (500 MHz)	<b>10</b> (200 MHz)	<b>11</b> (500 MHz)
1	2.84 brq	2.83 brq	2.49 brq (~ 6)	2.68 brq (~ 9)	2.77 brq (~ 10)	2.79 brq (~ 10)	2.77 brq (~ 10)
2 $\alpha$	2.27 dt (7.5, 12.5)	~ 1.90 <sup>a</sup>	~ 1.91 m	1.73 dddd (2, 7, 7.5, 12.5)	1.93 m	~ 1.90 <sup>a</sup>	1.92 m
2 $\beta$	1.93 dt (9.5, 13)	~ 1.72 <sup>a</sup>	~ 1.71 m	1.22 m	1.66 dq (6.5, ~ 11.5)	1.66	1.64 dt (6, 11)
3	3.99 q (~ 8.5)	1.92 1.47	~ 1.96 ~ 1.48	1.37 m 1.84 dddd (2, 12.5, 6.5, 7)	1.96 m 1.41 dq (6.5, ~ 11.5)	~ 1.90 <sup>a</sup> 1.36 <sup>a</sup>	1.97 m 1.37 dt (6.5, 11.5)
4	2.16 <sup>a</sup>	2.25 m	~ 2.22 m	~ 2.1 <sup>a</sup>	2.22 m	2.39 <sup>a</sup>	2.39 <sup>a</sup>
5	2.18 <sup>a</sup>	2.17	2.15	~ 2.1 <sup>a</sup>	2.07 <sup>a</sup> m	2.13 <sup>a</sup>	2.09 <sup>a</sup>
6	3.94 t (~ 10)	3.89 dd (9.0, 10.5)	4.08 dd (9, 10)	4.22 t (~ 10)	4.06 t (10)	4.0 t (10)	4.06 t (11)
7	2.74 m	2.73 m	2.85 m	2.41 ddd (3, 10, 12.5)	2.28 ddd (3, 10, 13)	2.34 <sup>a</sup>	2.39 <sup>a</sup>
8 $\alpha$	2.59 dt (12.5, 3.5)	~ 2.30 <sup>a</sup>	2.29 m	~ 2.1 <sup>a</sup>	1.89 m	1.90 <sup>a</sup>	2.09 <sup>a</sup>
8 $\beta$	1.46 q (~ 12.5)	~ 1.36 <sup>a</sup>	~ 1.40	~ 1.5 <sup>a</sup>	1.50 dq (3.5, 12.5)	1.45	1.50 dt (6.5, 11.5)
9 $\alpha$	4.19 <sup>a</sup>	2.05 m	~ 2.40	1.97 ddd (1.5, 11, 14)	2.07 <sup>a</sup>	2.13 <sup>a</sup>	1.89
9 $\beta$		2.57 ddd (4.8, 4.8, 13.0)	~ 1.80	1.63 ddd (4.5, 4.5, 14)	2.61 dt (13, 4)	2.61 dt (4, 13)	2.62 dt (13, 4)
13	6.25 d (3.5) 5.54 d (3)	6.17 d (3.4) 5.46 d (3.0)	6.24 d (4.4) 5.53 d (3.2)	3.81 ABq (11) 3.77 ABq (11)	3.68 brs (2H)	~ 3.74 m <sup>b</sup> (2H)	3.56 s (2H)
14	5.57 brs 5.24 d (~ 1)	4.93 brs 4.91 brs	2.72 dd (1.4, 4.4) 2.58 d (4.4)	2.77 dd (1.5, 4) 2.56 d (4)	4.90 brs 4.86 brs	4.91 brs 4.88 brs	4.92 brs 4.88 brs
15	4.67 dd (3.5, 11.5) 4.19 <sup>a</sup>	4.28 dd (5.0, 11.0) 4.08 dd (7.2, 11.0)	3.75 dd (5.4, 11) 3.66 dd (5.4, 11)	3.75 dd (5, 11) 3.66 dd (5, 11)	3.70 dd (5, 11) 3.59 dd (6, 11)	4.26 dd (4.6, 11) 4.07 dd (6.8, 11)	4.22 dd (5, 11) 4.07 dd (7, 11)
OAc	2.13 s	2.07 s	–	–	–	2.07 s	2.06 s

<sup>a</sup> Overlapped (partly or completely); <sup>b</sup> partly resolved AB portion of an ABX spectrum, due to the additional coupling with OH.

C	<b>5</b> (125 MHz)	<b>7</b> (50 MHz)	<b>8</b> (125 MHz)	<b>9</b> (125 MHz)	<b>10</b> (50 MHz)	<b>11</b> (125 MHz)
1	39.9	48.8	45.1	47.6	47.4	47.5
2	37.1	26.7	26.3	30.4	29.7	29.6
3	73.5	29.1	29.0	29.5	30.3	30.3
4	50.8	47.8	48.7 <sup>a</sup>	47.1	43.8	44.0
5	45.2	46.1	47.8 <sup>a</sup>	48.9	48.7	48.8
6	84.8	85.5	84.5	84.4	83.8	83.8
7	44.2	45.9	52.2	54.1	53.4	54.1
8	38.6	26.6	22.3	27.2	27.2	27.0
9	74.3	34.5	36.5	36.7	36.4	36.0
10	152.8	58.1	58.9	150.1	149.6	149.2
11	138.5	139.4	~ 78.0 <sup>d</sup>	77.6	<sup>b</sup>	79.2
12	169.4	169.4	178.8	179.1	<sup>c</sup>	<sup>c</sup>
13	120.6	120.9	63.3	63.1	63.3	43.7
14	110.9	50.5	49.3	112.3	112.7	112.9
15	64.4	65.6	65.2	65.9	67.5	67.5
OAc	171.6 20.9			–	<sup>c</sup> 21.0	20.9

Table 2. <sup>13</sup>C NMR (CDCl<sub>3</sub>) chemical shifts of compounds **5**, **7**–**11**.

<sup>a</sup> The assignments can be interchanged; <sup>b</sup> overlapped with CDCl<sub>3</sub> signals; <sup>c</sup> not detected because of the small concentration; <sup>d</sup> detected in HMBC.

1638 (C=C)  $\text{cm}^{-1}$ . –  $^1\text{H}$  and  $^{13}\text{C}$  NMR: Tables 1 and 2, respectively. – MS (EI, 70 eV),  $m/z$  (%) = 282 (1) [ $\text{M}^+ - 42$ ], 264 (13) [ $\text{M}^+ - 60$ ], 246 (8) [ $\text{M}^+ - 60 - 18$ ], 159 (100), 43 (41). – HRMS (CI, 150 eV, *iso*-butane): [ $\text{M} + \text{H}^+$ ]  $m/z$  = 325.1645 (calcd. for  $\text{C}_{17}\text{H}_{25}\text{O}_6$ : 325.1651).

*15-O-acetyl-3-deoxy-11 $\alpha$ -hydroxy-13-chloro-11,13-di-hydroamphoricarpolide (11)*

Colourless gum. –  $[\alpha]_{\text{D}}^{25} + 2.3$  (c, 0.22,  $\text{CHCl}_3$ ). – IR (film):  $\nu = 3444$  (OH), 1781 (C=O, lactone), 1734, 1248 (OAc), 1639 (C=C)  $\text{cm}^{-1}$ . –  $^1\text{H}$  and  $^{13}\text{C}$  NMR: Tables 1 and 2, respectively. MS (CI, 150 eV, *iso*-butane),  $m/z$  (%) = 345 (34) [ $\text{M} + 2 + \text{H}^+$ ] 343 (100) [ $\text{M} + \text{H}^+$ ], 307 (90). –  $\text{C}_{17}\text{H}_{23}\text{ClO}_5$  (342.82): calcd. C 59.56, H 6.76; found: C 59.37, H 6.59.

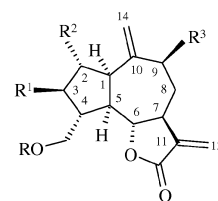
## Results and Discussion

Using the same extraction procedure as before [4], in combination with silica gel CC and preparative TLC, eleven guaianolides (**1**–**11**) have been isolated. 3-Deoxyamphoricarpolide (**1**), 15-*O*-acetyl-amphoricarpolide (**2**), 3,15-di-*O*-acetyl-9 $\beta$ -hydroxy-amphoricarpolide (**3**) and 15-*O*-isovaleroyl-3-*O*-acetyl-9 $\beta$ -hydroxyamphoricarpolide (**4**) were also obtained in our previous investigation of the *Amphoricarpos* complex [3].

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the new guaianolides, assigned by comparison with those of known closely related compounds, or using 2D NMR methods (COSY, NOESY, HSQC, HMBC), are listed in Tables 1 and 2.

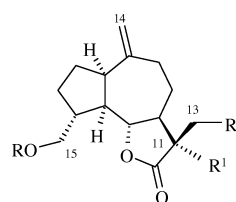
Lactone **5** showed an [ $\text{M} + \text{H}^+$ ] ion in the ESIMS at  $m/z = 323$ , corresponding to the molecular formula  $\text{C}_{17}\text{H}_{22}\text{O}_6$ .  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **5** were similar to those of **3** and **4** [3], thus indicating the same basic structure. The major difference was an upfield shift of H-3 ( $\Delta\delta = 1.06$  ppm) in **5** in comparison with H-3 in **3** and **4**, indicating 3 $\beta$ -OH substitution in **5**. This indicated the structure of 15-*O*-acetyl-9 $\beta$ -hydroxyamphoricarpolide for this lactone.

The  $^1\text{H}$  NMR data of **6** ( $\text{C}_{17}\text{H}_{22}\text{O}_4$ ) were similar to those of **1**, the only guaianolide isolated from the *Amphoricarpos* complex lacking a 3-oxygen functionality so far. The similarity of most of the NMR data of **6** (Table 1) to those of the co-occurring **1** indicated close structural and stereochemical relationships. Lactone **6** exhibited an acetoxy group ( $\nu_{\text{OAc}} = 1736, 1245$   $\text{cm}^{-1}$ ;  $\delta = 2.07$  s, 3H) attached to C-15, as evidenced from downfield shifts of H<sub>2</sub>-15 ( $\delta = 4.28$  and 4.05) in **6**,



	R	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>
<b>1</b>	H	H	H	H
<b>2</b>	Ac	OH	H	H
<b>3</b>	Ac	OAc	H	OH
<b>4</b>	<i>i</i> -Val	OAc	H	OH
<b>5</b>	Ac	OH	H	OH
<b>6</b>	Ac	H	H	H
<b>7*</b>	H	H	H	H

\* 10 $\alpha$ (14)-Epoxy.



	R	R <sup>1</sup>	R <sup>2</sup>
<b>8*</b>	H	OH	OH
<b>9</b>	H	OH	OH
<b>10</b>	Ac	OH	OH
<b>11</b>	Ac	OH	Cl

\* 10 $\alpha$ (14)-Epoxy.

compared with **1** ( $\delta = 3.74$  and 3.68). Accordingly, **6** was assigned as a 15-*O*-acetyl derivative of **1**.

The overall appearance of the  $^1\text{H}$  NMR spectrum of **7** ( $\text{C}_{15}\text{H}_{20}\text{O}_4$ ) was also rather close to that of **1**. The major difference was the occurrence of two mutually coupled one-proton signals ( $\delta = 2.72$  and 2.58,  $J = 4.4$  Hz) instead of broad one-proton singlets of the exocyclic  $\Delta^{10(14)}$  double bond in the olefinic region observed in most of the co-occurring compounds. The same pattern with the rather similar chemical shifts and couplings was also observed in the co-occurring lactone **8**, assigned as an 10 $\alpha$ (14)-epoxide, according to NOESY analysis (*vide infra*). This indicated the structure of a 3-deoxy-10 $\alpha$ (14)-epoxyamphoricarpolide for this lactone.

One of the common features of **8**–**11** was the lack of the characteristic  $^1\text{H}$  and  $^{13}\text{C}$  NMR resonances in the olefinic region ( $\delta_{\text{H}} > 5.5$  and  $\delta_{\text{C}} > 120$ ) of the exomethylene ( $\Delta^{11(13)}$ ) group (observed in **1**–**7**). Instead of this, two-proton signals (AB quartets or broad

singlets), typical for the isolated C(13)H<sub>2</sub>X (X = OH or Cl) group, were observed in the spectral region  $\delta = 3.56\text{--}3.81$  (Table 1). Whereas the chemical shifts of C-13 in **8–10** ( $\delta = 63.1\text{--}63.3$ ) indicated 13-OH substitution (X = OH), its chemical shift ( $\delta = 43.7$ ) in **11** was in accordance with the attachment of a chlorine atom (X = Cl, “heavy atom effect”). The presence of the chlorine atom was also deduced from the MS data of **11** (*vide infra*). The almost identical chemical shift of C-11 ( $\delta \sim 79\text{--}78$ ) in **8–11** indicated the same 11-OH pattern.

In addition to the above mentioned 11,13-diol group, the lactone **8** (C<sub>15</sub>H<sub>22</sub>O<sub>6</sub>) contained the 10(14)-epoxide unit identified according to a pair of mutually coupled one-proton doublets ( $\delta = 2.77$  and  $2.56$ ,  $J = 4$  Hz) occurring instead of  $\Delta^{10(14)}$  exocyclic vinyl protons. The occurrence of an AB quartet at  $\delta = 3.83$  (A) and  $\delta = 3.74$  (B) ( $J_{AB} = 11$  Hz), indicated an isolated C(13)H<sub>2</sub>OH group, which was confirmed in HSQC and HMBC spectra. The <sup>13</sup>C NMR signals of the epoxide ( $\delta = 58.9$  and  $49.3$ , C-10 and C-14, respectively) and a diol moiety ( $\delta = 63.3$  and  $\delta \sim 78$ , C-13 and C-11, respectively) also supported this assignment. A NOE between one of the H<sub>2</sub>-14 protons ( $\delta = 2.77$ ) and  $\beta$ -positioned H-6 revealed a 10 $\alpha$ (14)-epoxy configuration. Similarly, the NOE between H-6 and H<sub>2</sub>-13 indicated a  $\beta$ -orientation of the C(13)H<sub>2</sub>OH moiety. This, together with the remaining NOEs, such as H-6/H-4 and H-3 $\alpha$ /H<sub>2</sub>-15 were fully in accordance with the structure of 3-deoxy-10 $\alpha$ (14)-epoxy-11 $\alpha$ ,13-dihydroxy-11,13-dihydroamphoricarpolide for this compound.

Lactone **9** (C<sub>15</sub>H<sub>22</sub>O<sub>5</sub>) exhibited a broad two-proton singlet ( $\delta = 3.68$ ), typical for the isolated C(13)H<sub>2</sub>OH group (already observed in **8**). In addition, the occurrence of <sup>13</sup>C NMR resonances at  $\delta = 77.6$  and  $63.1$  of C-11 and C-13, assigned according to HMBC, indicated the 11,13-diol structure analogous to that in **8**. An additional common feature between **8** and **9** was the C(15)H<sub>2</sub>OH group exhibiting the same multiplicities and almost the same chemical shifts in these lactones (see Table 1). The NOEs between H-6 and H<sub>2</sub>-13, as well as with H-4

were in accordance with the same relative configurations at C-4 and C-11 (*i. e.* 4 $\beta$ H,11 $\alpha$ OH) in **8** and **9** and the structure of 3-deoxy-11 $\alpha$ ,13-dihydroxy-11,13-dihydroamphoricarpolide for **9**.

The lactone **10** (C<sub>17</sub>H<sub>24</sub>O<sub>6</sub>) exhibited rather similar spectral data to those of **9** (Tables 1 and 2). The main difference was the presence of an OAc group (3H s,  $\delta = 2.07$ ), as well as a downfield shift of H<sub>2</sub>-15 ( $\delta = 4.26$  and  $4.07$ ), compared with **9**, indicating C-15 as the acetylation site. This indicated that this compound was the 15-*O*-acetyl derivative of **9**.

According to [M+H<sup>+</sup>] and [M+2+H<sup>+</sup>] ions,  $m/z = 343$  and  $345$  (3:1), observed in DCIMS, lactone **11** exhibits the molecular formula C<sub>17</sub>H<sub>23</sub>ClO<sub>5</sub>. The attachment of the chlorine atom to C-13 as well as the hydroxyl group to C-11 was deduced by the above mentioned <sup>13</sup>C NMR chemical shifts of these carbon atoms. The chemical shift of H<sub>2</sub>-15, almost identical to that in **9**, and the occurrence of a singlet of an acetoxy methyl group ( $\delta = 2.06$ ) revealed acetylation of OH-15. The 4 $\beta$ H- (based on the NOE between H-4 and H-6) and 11 $\alpha$ OH-configuration (according to the NOEs between H-6 $\beta$  and H<sub>2</sub>-13, and also H-8 $\beta$ ), the same as in **8–11**, was also evident. 11 $\alpha$ ,13-Diol (**8–10**) and 11-chloro-13-hydroxy groups (**11**) were most probably formed by nucleophilic opening of the corresponding 11 $\alpha$ ,13-epoxide. Such chlorohydrins might be artefacts formed during the isolation procedure, where chlorinated solvents might serve as the source of Cl<sup>-</sup> [5, 6] as in the case of **11**, involving CH<sub>2</sub>Cl<sub>2</sub> for the extraction of the sample after TLC purification. However, HPLC/ESI MS analysis of the crude extract prepared using the usual procedure with exclusion of the chlorinated solvents [4], also revealed the presence of **11**, thus indicating that this compound was not an artefact.

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