

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

Iris Djordjević<sup>a</sup>, Milka Jadranin<sup>b</sup>, Vlatka Vajs<sup>b</sup>, Nebojša Menković<sup>c</sup>, Vele Tešević<sup>d</sup>, Slobodan Macura<sup>e</sup>, and Slobodan Milosavljević<sup>d</sup>

<sup>a</sup> Faculty for Veterinary Medicine, University of Belgrade, Bulevar JA 18, 11000 Belgrade, Serbia

<sup>b</sup> Institute for Technology and Metallurgy, Njegoševa 12, 11000 Belgrade, Serbia

<sup>c</sup> Institute for Medicinal Plant Research, "Dr. Josif Pančić", Tadeuša Koščuška 1, 11000 Belgrade, Serbia

<sup>d</sup> Faculty of Chemistry, University of Belgrade, Studentski trg 16, P. O. Box 158, 11001 Belgrade, Serbia

<sup>e</sup> Department of Biochemistry, Mayo Clinic, College of Medicine, Rochester, MN 55905, USA

Reprint requests to PhD. S. Milosavljević. Fax: +381 11 636 061. E-mail: smilo@chem.bg.ac.yu

Z. Naturforsch. **61b**, 1437–1442 (2006); received April 4, 2006

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.

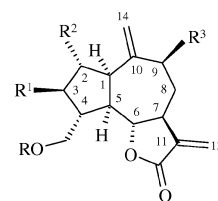
## Results and Discussion

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.

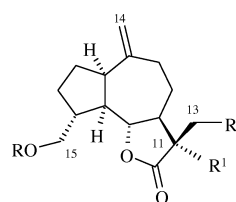
Lactone **5** showed an [ $\text{M} + \text{H}^+$ ] ion in the ESIMS at  $m/z = 323$ , corresponding to the molecular formula  $\text{C}_{17}\text{H}_{22}\text{O}_6$ .  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **5** were similar to those of **3** and **4** [3], thus indicating the same basic 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.

#### Acknowledgements

The authors from Serbia acknowledge their gratitude to the Ministry of Science and Ecology of Serbia (Project 142053) for financial support. We also wish to express our gratitude to Daniel Vincek, Botanic Garden, Dulovine, Kolašin (Montenegro) and Milutin Prašćević, Alpinetum of Prokletije, Plav (Montenegro) for the immense help in collecting the plant material.

- [1] V. Blečić, E. Mayer, *Phyton* (Austria) **12**, 150 (1967).  
[2] D.A. Webb, in T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters,

- D. A. Webb (eds): *Flora Europaea*, Vol. 4, p. 208, University Press, Cambridge (1972).  
[3] I. Djordjević, V. Vajs, V. Bulatović, N. Menković,

- V. Tešević, S. Macura, P. Janačković, S. Milosavljević, *Phytochemistry* **65**, 2337 (2004), [Erratum to this document: *Phytochemistry* **66**, 261 (2005)].
- [4] F. Bohlmann, C. Zdero, H. R. King, E. H. Robinson, *Phytochemistry* **23**, 1979 (1984).
- [5] K. C. Engvild, *Phytochemistry* **25**, 781 (1986).
- [6] M. Hamburger, J.-L. Wolfender, K. Hostettmann, *Nat. Toxins* **1**, 315 (1993).