

## Labdane and Tetranorlabdane Diterpenoids from *Botryosphaeria* sp. MHF, an Endophytic fungus of *Maytenus hookeri*

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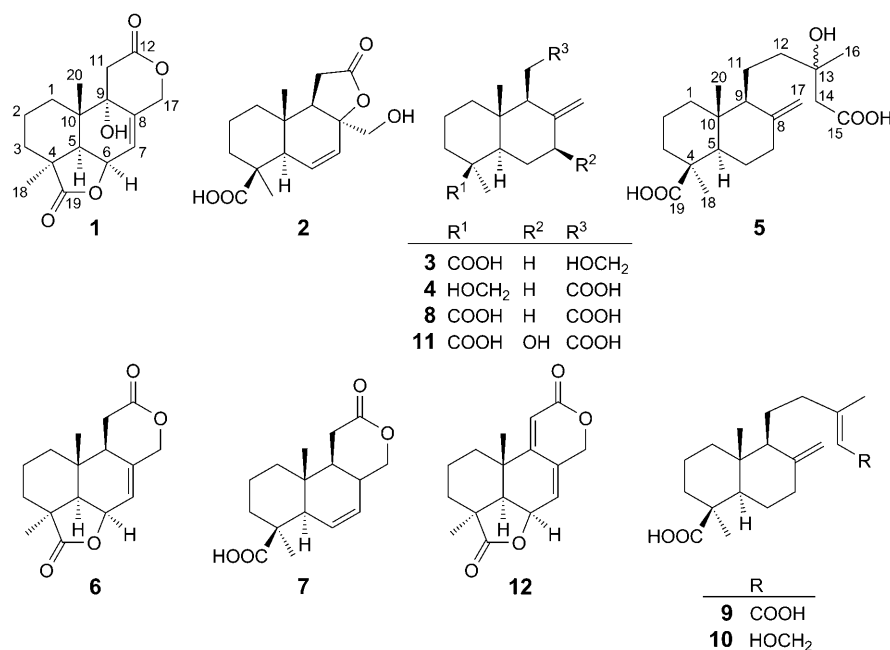
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Five new diterpenoids, named botryosphaerins A–E (**1–5**, resp.), including one labdane and four tetranorlabdane diterpenes, were isolated from the solid culture of the endophytic fungal strain *Botryosphaeria* sp. MHF of *Maytenus hookeri*, together with seven known diterpenoids, namely 13,14,15,16-tetranorlabd-7-ene-19,6 $\beta$ :12,17-diolide (**6**), acrostalidic acid (**7**), acrostalic acid (**8**), agathic acid (**9**), isocupressic acid (**10**), LL-Z1271 $\beta$  (**11**), and CJ-14445 (**12**). The structures of all compounds were established on the basis of comprehensive spectroscopic studies. The relative configurations of **1** and **2** were determined by single-crystal X-ray diffraction analyses. All compounds except **4** were evaluated for their inhibitory activities against several pathogenic bacterial and fungal strains. However, only compound **12** showed significant inhibitions against *Candida albicans*, *Saccharomyces cerevisiae*, and *Penicillium avellaneum* UC-4376 with nystatin as a positive control.

**Introduction.** – Endophytes are ubiquitous as a result of the balanced antagonism in the long co-evolution of the phytopathogens and their host plants. Endophytic microbes improve the resistance of the host plants to the outside intimidations by producing secondary metabolites with antimicrobial, insecticidal and many other bioactivities, which are becoming important resources of new pharmaceuticals and lead compounds [1]. During our research on the bioactive components of the endophytes from *Maytenus hookeri*, a medicinal plant containing the potent antitumor agent maytansine [2], various new compounds have been obtained [3][4]. In our continuing studies, we investigated the secondary metabolites produced by the solid cultured *Botryosphaeria* sp. MHF, an endophytic fungal strain isolated from the leave tissue of *M. hookeri*. The present work has resulted in the isolation of five new diterpenoids, named botryosphaerins A–E (**1–5**, resp.), including one labdane and four tetranorlabdane diterpenes, together with seven known diterpenoids (Fig. 1). Here, we report the isolation and structure elucidation of compounds **1–5**. The *in vitro* antimicrobial screenings of compounds **1–3** and **5–12** are also described.

**Results and Discussion.** – *Botryosphaeria* sp. MHF was cultured in Petri dishes with PDA medium and a total of 10 l for 10 d at 28°. The fermentation culture was extracted with AcOEt/MeOH/AcOH (80:15:5, v/v/v). The crude extract was partitioned between AcOEt and H<sub>2</sub>O. The organic layer was concentrated *in vacuo* to afford 5.8 g of a dark oil. The extract was purified by repeated column chromatography (RP-18, Sephadex LH-20, and silica gel) to afford five new and seven known diterpenoids.

Fig. 1. Structures of **1**–**12**<sup>1)</sup>

Botryosphaerin A (**1**) was obtained as colorless crystals. The molecular formula was determined as C<sub>16</sub>H<sub>20</sub>O<sub>5</sub> on the basis of HR-ESI-MS data ( $[M + Na]^+$  at  $m/z$  315.1211; calc. 315.1208). The IR spectrum pointed to the presence of OH (3424 cm<sup>-1</sup>) and CO groups (1763 and 1725 cm<sup>-1</sup>). The <sup>13</sup>C-NMR and DEPT spectra of **1** (Table 1) exhibited 16 C-signals, including those of two CO units ( $\delta(C)$  182.2 and 171.8), of three quaternary C-atoms ( $\delta(C)$  72.6, 42.8, and 38.2), a C=C bond ( $\delta(C)$  140.9 and 119.3), two CH ( $\delta(C)$  72.5 and 43.8), five CH<sub>2</sub> ( $\delta(C)$  69.2, 38.0, 28.3, 26.5, and 18.2), and two Me ( $\delta(C)$  24.3 and 22.1). The <sup>1</sup>H- and <sup>13</sup>C-NMR spectroscopic data (Tables 2 and 1, resp.) showed close similarity to those of 13,14,15,16-tetranorlabd-7-ene-19,6 $\beta$ :12,17-diolide (**6**) [5], which was also isolated in this study as a natural product for the first time, except that the CH C-atom at C(9) ( $\delta(C)$  44.7) in **6** was replaced by an O-bearing quaternary C-atom ( $\delta(C)$  72.6) in **1**. This contention was corroborated through HMBC correlations from H–C(11) ( $\delta(H)$  3.01, br. s), H–C(17) ( $\delta(H)$  5.04, s), and Me(20) ( $\delta(H)$  1.03, s) to C(9).

The relative configuration of **1** was deduced from a ROESY experiment and a single-crystal X-ray diffraction analysis. The ROESY cross-peaks of H–C(6)/H–C(5)<sup>1)</sup> and H–C(6)/Me(18) showed that the  $\gamma$ -lactone ring was  $\beta$ -oriented, and the correlations of Me(18)/H <sub>$\alpha$</sub> –C(3), Me(18)/H <sub>$\alpha$</sub> –C(2), Me(20)/H <sub>$\beta$</sub> –C(2), and Me(20)/H <sub>$\beta$</sub> –C(3) suggested the  $\beta$ -orientation of Me(20). Moreover, a single-crystal X-ray diffraction of **1** not only confirmed the assignment of the relative configuration

<sup>1)</sup> Arbitrary numbering, see Fig. 1.

Table 1.  $^{13}\text{C}$ -NMR Data of **1**–**5**, **8**, and **11**<sup>a)</sup>. At 100 MHz;  $\delta$  in ppm.

	<b>1</b> <sup>a)</sup>	<b>2</b> <sup>b)</sup>	<b>3</b> <sup>c)</sup>	<b>4</b> <sup>c)</sup>	<b>5</b> <sup>b)</sup>	<b>8</b> <sup>b)</sup>	<b>11</b> <sup>b)</sup>
C(1)	26.5 (t)	38.3 (t)	39.0 (t)	38.8 (t)	40.4 (t)	40.6 (t)	40.1 (t)
C(2)	18.2 (t)	19.9 (t)	19.8 (t)	18.8 (t)	21.1 (t)	21.0 (t)	21.0 (t)
C(3)	28.3 (t)	38.5 (t)	37.9 (t)	35.2 (t)	39.3 (t)	39.1 (t)	39.1 (t)
C(4)	42.8 (s)	44.0 (s)	44.1 (s)	38.7 (s)	45.1 (s)	45.0 (s)	44.8 (s)
C(5)	43.8 (d)	52.7 (d)	56.2 (d)	55.8 (d)	57.5 (d)	57.1 (d)	54.5 (d)
C(6)	72.5 (d)	135.9 (d)	25.9 (t)	23.8 (t)	27.5 (t)	27.0 (t)	36.0 (t)
C(7)	119.3 (d)	122.6 (d)	38.5 (t)	37.7 (t)	39.9 (t)	39.2 (t)	74.3 (d)
C(8)	140.9 (s)	86.4 (s)	148.1 (s)	148.2 (s)	149.6 (s)	150.3 (s)	152.3 (s)
C(9)	72.6 (s)	50.7 (d)	52.0 (d)	52.4 (d)	58.1 (d)	53.4 (d)	51.5 (d)
C(10)	38.2 (s)	37.3 (s)	40.1 (s)	38.7 (s)	41.7 (s)	40.6 (s)	40.1 (s)
C(11)	38.0 (t)	32.7 (t)	27.1 (t)	30.3 (t)	19.1 (t)	31.9 (t)	31.5 (t)
C(12)	171.8 (s)	179.9 (s)	62.3 (t)	176.9 (s)	42.0 (t)	178.0 (s)	177.4 (s)
C(13)	–	–	–	–	72.5 (s)	–	–
C(14)	–	–	–	–	46.2 (t)	–	–
C(15)	–	–	–	–	175.5 (s)	–	–
C(16)	–	–	–	–	27.2 (q)	–	–
C(17)	69.2 (t)	69.4 (t)	106.5 (t)	106.7 (t)	107.1 (t)	106.7 (t)	103.6 (t)
C(18)	24.3 (q)	28.6 (q)	28.9 (q)	27.0 (q)	29.5 (q)	29.5 (q)	29.3 (q)
C(19)	182.2 (s)	179.9 (s)	180.8 (s)	64.9 (t)	181.3 (s)	181.1 (s)	180.8 (s)
C(20)	22.1 (q)	12.6 (q)	12.7 (q)	15.2 (q)	13.3 (q)	13.4 (q)	13.3 (q)

<sup>a)</sup> Measured in  $\text{C}_5\text{D}_5\text{N}$ . <sup>b)</sup> Measured in  $\text{CD}_3\text{OD}$ . <sup>c)</sup> Measured in  $\text{CDCl}_3$ .

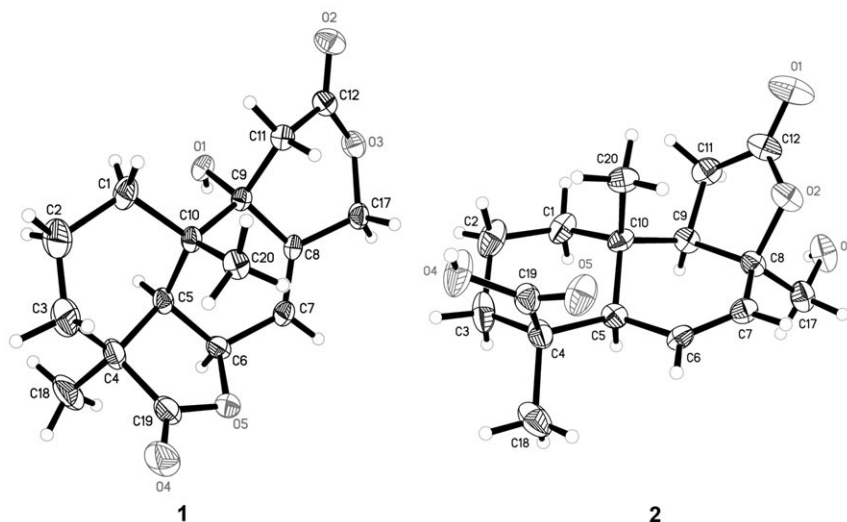
of **1** based on the ROESY data, but also determined that the OH substituent at C(9)<sup>1)</sup> was in  $\alpha$ -orientation (Fig. 2). Thus, from the above data, compound **1** was determined to be *rel*-(3a*S*,5a*R*,10a*S*,10b*S*,10c*R*)-1,2,3,3a,5a,7,10,10a,10b,10c-decahydro-10a-hydroxy-3a,10b-dimethyl-4*H*,9*H*-[2]benzofuro[7,1-*fg*]isochromene-4,9-dione.

Botryosphaerin B (**2**) was obtained as colorless crystals. The molecular formula was determined as  $\text{C}_{16}\text{H}_{22}\text{O}_5$  with five degrees of unsaturation according to the HR-ESI-MS data ( $[M + \text{Na}]^+$  at  $m/z$  317.1361; calc. 317.1364). The  $^{13}\text{C}$ -NMR spectrum (Table 1) showed that **2** possessed two tertiary Me, two olefinic C-atoms, an O-bearing  $\text{CH}_2$  group, an O-bearing quaternary C-atom, and two COO groups with overlapping resonances at  $\delta(\text{C})$  179.9, suggesting that it was structurally similar to isoacrostalidic acid [6], an isomer of acrostalidic acid (**7**) [6][7]. The key difference was that Me(17) ( $\delta(\text{H})$  1.40) in isoacrostalidic acid was oxygenated to a  $\text{CH}_2\text{OH}$  group in **2** ( $\delta(\text{H})$  3.60,  $d, J = 11.8$ ; 3.40,  $d, J = 11.8$ ), which was confirmed by the HMBC data from H–C(17) to C(7) ( $\delta(\text{C})$  122.6), C(8) ( $\delta(\text{C})$  86.4), and C(9) ( $\delta(\text{C})$  50.7).

The relative configuration of **2** could not be established unambiguously by ROESY experiments because the  $\text{H}_\alpha$ –C(1) and H–C(9)<sup>1)</sup> signals overlapped at  $\delta(\text{H})$  2.20–2.24, and  $\text{H}_\beta$ –C(1) and  $\text{H}_\alpha$ –C(3) overlapped at  $\delta(\text{H})$  1.03–1.13. Fortunately, crystals of **2** were obtained from  $^i\text{PrOH}$ . Consequently, the relative configuration of **2** was determined by single-crystal X-ray diffraction (Fig. 2), and compound **2** was identified as *rel*-(3a*S*,5a*R*,6*S*,9a*S*,9b*R*)-1,2,3a,5a,6,7,8,9a,9b-decahydro-3a-(hydroxymethyl)-6,9a-dimethyl-2-oxonaphtho[2,1-*b*]furan-6-carboxylic acid.

Table 2.  $^1\text{H}$ -NMR Data of **1–5**, **8**, and **11**<sup>1</sup>. At 400 MHz;  $\delta$  in ppm,  $J$  in Hz.

	<b>1</b> <sup>a</sup>	<b>2</b> <sup>b</sup>	<b>3</b> <sup>c</sup>	<b>4</b> <sup>c</sup>	<b>5</b> <sup>b</sup>	<b>8</b> <sup>b</sup>	<b>11</b> <sup>b</sup>
$\text{H}_\alpha\text{-C(1)}$	2.09–2.11 ( <i>m</i> )	2.20–2.24 (overlapped)	1.08–1.13 ( <i>m</i> )	1.17–1.21 ( <i>m</i> )	1.10–1.15 ( <i>m</i> )	1.18–1.23 (overlapped)	1.18 ( <i>t</i> , $J = 12.5$ )
$\text{H}_\beta\text{-C(1)}$	1.21–1.25 ( <i>m</i> )	1.03–1.13 (overlapped)	1.81–1.85 ( <i>m</i> )	1.60–1.64 ( <i>m</i> )	1.86–1.89 ( <i>m</i> )	1.71 ( <i>d</i> , $J = 12.6$ )	1.71 ( <i>d</i> , $J = 12.6$ )
$\text{H}_\alpha\text{-C(2)}$	1.50–1.53 ( <i>m</i> )	1.47–1.52 ( <i>m</i> )	1.50–1.54 ( <i>m</i> )	1.49–1.55 ( <i>m</i> )	1.47–1.51 ( <i>m</i> )	1.47–1.51 ( <i>m</i> )	1.47–1.52 ( <i>m</i> )
$\text{H}_\beta\text{-C(2)}$	1.60–1.64 ( <i>m</i> )	1.79–1.89 ( <i>m</i> )	1.82–1.87 ( <i>m</i> )	1.49–1.55 (overlapped)	1.87–1.92 ( <i>m</i> )	1.87–1.91 ( <i>m</i> )	1.86–1.91 ( <i>m</i> )
$\text{H}_\alpha\text{-C(3)}$	1.44–1.49 ( <i>m</i> )	1.03–1.13 (overlapped)	2.15 ( <i>d</i> , $J = 13.0$ )	1.83–1.87 ( <i>m</i> )	2.13 ( <i>d</i> , $J = 14.5$ )	2.12 ( <i>d</i> , $J = 13.0$ )	2.16 ( <i>d</i> , $J = 13.0$ )
$\text{H}_\beta\text{-C(3)}$	2.18–2.22 ( <i>m</i> )	1.71–1.75 ( <i>m</i> )	1.03–1.08 ( <i>m</i> )	0.96–1.01 (overlapped)	1.04–1.09 ( <i>m</i> )	1.07 ( <i>td</i> , $J = 13.4$ , 4.0)	1.09 ( <i>t</i> , $J = 13.2$ )
$\text{H-C(5)}$	2.90 ( <i>d</i> , $J = 5.1$ )	1.94–1.96 ( <i>m</i> )	1.36–1.38 ( <i>m</i> )	1.36–1.38 ( <i>m</i> )	1.33–1.36 ( <i>m</i> )	1.42 ( <i>dd</i> , $J = 12.5$ , 2.8)	1.47–1.50 ( <i>m</i> )
$\text{H}_\alpha\text{-C(6)}$	5.01–5.02 ( <i>m</i> )	6.67 ( <i>dd</i> , $J = 10.4$ , 1.8)	1.95–1.99 ( <i>m</i> )	1.80–1.84 ( <i>m</i> )	1.96–1.99 ( <i>m</i> )	1.98–2.01 ( <i>m</i> )	2.27 ( <i>d</i> , $J = 12.4$ )
$\text{H}_\beta\text{-C(6)}$			1.84–1.90 ( <i>m</i> )	1.32–1.35 ( <i>m</i> )	1.85–1.99 ( <i>m</i> )	1.85–1.88 ( <i>m</i> )	1.86–1.89 ( <i>m</i> )
$\text{H}_\alpha\text{-C(7)}$	6.05 ( <i>br. s</i> )	5.71 ( <i>dd</i> , $J = 10.4$ , 2.9)	1.89–1.93 ( <i>m</i> )	2.03–2.08 ( <i>m</i> )	1.89–1.92 ( <i>m</i> )	1.99–2.04 ( <i>m</i> )	3.94 ( <i>dd</i> , $J = 11.5$ , 4.9)
$\text{H}_\beta\text{-C(7)}$			2.41–2.43 ( <i>m</i> )	2.37–2.39 ( <i>m</i> )	2.35–2.39 ( <i>m</i> )	2.36–2.40 ( <i>m</i> )	
$\text{H-C(9)}$		2.20–2.24 (overlapped)	1.74 ( <i>d</i> , $J = 11.0$ )	2.32–2.35 ( <i>m</i> )	1.57 ( <i>d</i> , $J = 10.5$ )	2.29–2.33 ( <i>m</i> )	2.23 ( <i>d</i> , $J = 10.4$ )
$\text{CH}_2\text{(11)}$	3.01 ( <i>br. s</i> )	2.98 ( <i>dd</i> , $J = 18.2$ , 9.6, $\text{H}_\alpha$ ), 2.42 ( <i>d</i> , $J = 18.1$ , $\text{H}_\beta$ )	1.74–1.78 ( <i>m</i> ), 1.64–1.68 ( <i>m</i> )	2.52 ( <i>dd</i> , $J = 16.0$ , 3.5), 2.41 ( <i>d</i> , $J = 16.0$ )	1.62–1.67 ( <i>m</i> ), 1.38–1.42 ( <i>m</i> )	2.49 ( <i>dd</i> , $J = 21.2$ , 9.2), 2.31–2.34 ( <i>m</i> )	2.55 ( <i>d</i> , $J = 15.5$ ), 2.40–2.44 ( <i>m</i> )
$\text{CH}_2\text{(12)}$	–	–	3.71–3.75 ( <i>m</i> ), 3.49–3.54 ( <i>m</i> )	–	1.74–1.79 ( <i>m</i> ), 1.25–1.30 (overlapped)	–	–
$\text{CH}_2\text{(14)}$	–	–	–	–	2.43 ( <i>br. s</i> )	–	–
$\text{Me(16)}$	–	–	–	–	1.26 ( <i>s</i> )	–	–
$\text{CH}_2\text{(17)}$	5.04 ( <i>s</i> )	3.60 ( <i>d</i> , $J = 11.8$ ), 3.40 ( <i>d</i> , $J = 11.8$ )	4.85 ( <i>s</i> ), 4.54 ( <i>s</i> )	4.78 ( <i>s</i> ), 4.53 ( <i>s</i> )	4.83 ( <i>br. s</i> ), 4.57 ( <i>d</i> , $J = 8.4$ )	4.76 ( <i>s</i> ), 4.54 ( <i>s</i> )	5.14 ( <i>s</i> ), 4.70 ( <i>s</i> )
$\text{Me(18)}$	1.28 ( <i>s</i> )	1.28 ( <i>s</i> )	1.24 ( <i>s</i> )	0.99 ( <i>s</i> )	1.20 ( <i>s</i> )	1.20 ( <i>s</i> )	1.23 ( <i>s</i> )
$\text{CH}_2\text{(19)}$	–	–	–	3.75 ( <i>d</i> , $J = 10.8$ ), 3.41 ( <i>d</i> , $J = 10.9$ )	–	–	–
$\text{Me(20)}$	1.03 ( <i>s</i> )	0.72 ( <i>s</i> )	0.60 ( <i>s</i> )	0.67 ( <i>s</i> )	0.63 ( <i>s</i> )	0.64 ( <i>s</i> )	0.63 ( <i>s</i> )

Fig. 2. Crystallographic structures of **1** and **2**

Botryosphaerin C (**3**) was obtained as a colorless oil. The molecular formula was determined as  $C_{16}H_{26}O_3$  on the basis of HR-ESI-MS (positive-ion mode) data ( $[M + Na]^+$  at  $m/z$  289.1773; calc. 289.1779). The similarity between the  $^1H$ - and  $^{13}C$ -NMR spectra of **3** (Tables 2 and 1, resp.) and those of acrostalic acid (**8**) [6] suggested that **3** was a related tetranorlabdane derivative. The only difference between the two compounds was the replacement of one COOH group of **8** by a  $CH_2OH$  group in **3**. The HMBC data from the  $CH_2$  H-atoms of  $CH_2OH$  ( $\delta(H)$  3.71–3.75, *m*; 3.49–3.54, *m*) to  $C(9)^1$  ( $\delta(C)$  52.0) and  $C(11)$  ( $\delta(C)$  27.1) revealed that the  $C(12)OOH$  group of **8** was reduced to  $CH_2OH$  ( $\delta(C)$  62.3) in **3**. The relative configuration of **3** was determined by analysis of the ROESY spectrum. The NOE cross-peaks of  $H-C(5)/H-C(9)$  and  $H-C(5)/Me(18)$  indicated the  $\alpha$ -position for Me(18) and the  $\beta$ -position for  $C(19)OOH$ . The  $\beta$ -orientation of Me(20) was in agreement with the NOE correlations of  $Me(20)/H_\beta-C(6)$  and  $Me(20)/CH_2(11)$ . Therefore, compound **3** was established as *rel*-(1*S*,4*aR*,5*S*,8*aR*)-decahydro-5-(2-hydroxyethyl)-1,4*a*-dimethyl-6-methylidenenaphthalene-1-carboxylic acid.

Botryosphaerin D (**4**) was isolated as a colorless oil. The molecular formula was determined as  $C_{16}H_{26}O_3$ , identical to that of **3**, by the HR-ESI-MS (positive-ion mode) data ( $[M + Na]^+$  at  $m/z$  289.1776; calc. 289.1779). Moreover, **4** exhibited very similar spectroscopic data to those of **3**, indicating that they had the same skeleton (Tables 2 and 1, resp.). However, the position of the  $CH_2OH$  group in **4** was readily located to be  $C(19)^1$  by the HMBC correlations of  $CH_2OH$  ( $\delta(H)$  3.75, *d*,  $J = 10.8$ ; 3.41, *d*,  $J = 10.9$ ) with  $C(3)$  ( $\delta(C)$  35.2),  $C(4)$  ( $\delta(C)$  38.7), and Me(18) ( $\delta(C)$  27.0). The ROESY cross-peaks of  $H-C(5)/H-C(9)$  and  $H-C(5)/Me(18)$ ,  $Me(20)/CH_2(11)$  and  $Me(20)/CH_2(19)$  showed that **4** had the same relative configuration as **3**. Therefore, compound **4** was determined to be *rel*-(1*S*,4*aR*,5*S*,8*aR*)-decahydro-5-(hydroxymethyl)-5,8*a*-dimethyl-2-methylidenenaphthalen-1-yl]acetic acid.

Botryosphaerin E (**5**) was obtained as a colorless oil, and its molecular formula was indicated as  $C_{20}H_{32}O_5$  by HR-ESI-MS data ( $[M + Na]^+$  at  $m/z$  375.2147; calc. 375.2147). The NMR spectra of **5** (Tables 2 and 1, resp.) showed resonances characteristic of a labdane-type diterpene and high resemblance with those of agathic acid (**9**) [8], except for the side chain. Compound **5** was assumed to be a hydrate of **9**, as the  $^{13}C$ -NMR signals at  $\delta(C)$  114.8 and 164.0, assigned to a  $C=C$  bond in **9**, were shifted upfield to  $\delta(C)$  46.2, a  $CH_2$ , and  $\delta(C)$  72.5, an O-bearing tertiary C-atom, in **5**. The OH substituent was determined to be at C(13) rather than C(14) because of the *singlet* of Me(16) in the  $^1H$ -NMR spectrum and the HMBC between  $CH_2(11)$  ( $\delta(H)$  1.62–1.67) and C(13). The relative configuration of the bicyclic skeleton was the same as in botryosphaerin C and D, supported by ROESY correlations of H–C(5)/H–C(9), H–C(5)/Me(18), and Me(20)/ $CH_2(11)$ . Because the configuration of C(13) was difficult to determine by standard methods, we turned to the single-crystal X-ray diffraction analysis. After reaction with (trimethylsilyl)diazomethane (TMSCHN<sub>2</sub>) using a procedure published [9], the dimethyl ester of **5** was obtained, but no crystals were obtained, even after many attempts of recrystallization. Thus, compound **5** was identified as *rel*-(1*S*,4*aR*,5*S*,8*aR*)-5-(4-carboxy-3-hydroxy-3-methylbutyl)decahydro-1,4*a*-dimethyl-6-methylidenenaphthalene-1-carboxylic acid with as yet unknown configuration at C(13)<sup>1</sup>.

Compounds **6**–**12** were identified as 13,14,15,16-tetranorlabd-7-en-19,6 $\beta$ :12,17-diolide (**6**) [5], acrostalidic acid (**7**) [6][7], acrostalic acid (**8**) [6], agathic acid (**9**) [8], isocupressic acid (**10**) [10], LL-Z1271 $\beta$  (**11**) [11], and CJ-14445 (**12**) [12][13]. In addition, the NMR assignments for compounds **8** and **11** were completed, as they were only partially assigned in the literature [6][11].

By the disk diffusion assay on agar plates [14], all compounds **1**–**12**, except for **4** due to its limited amounts, were evaluated for their antibacterial and antifungal activities against *Staphylococcus aureus*, *Shigella dysenteriae*, *Candida albicans*, *Saccharomyces cerevisiae*, and *Penicillium avellaneum* UC-4376 with rifampicin and nystatin as positive controls, respectively. At a concentration of 50  $\mu$ g/disk, compound **12** showed antifungal activities against *C. albicans*, *S. cerevisiae*, and *P. avellaneum* UC-4376 with inhibitory zones of 2.3, 2.5, and 1.0 cm, respectively, but demonstrated no antibacterial activities, which was consistent with a report [12]. All other tested compounds were inactive against all organisms, whereas the positive control, nystatin, showed antifungal activities against *C. albicans*, *S. cerevisiae*, and *P. avellaneum* UC-4376 with inhibitory zones of 2.5, 2.1, and 3.1 cm, and rifampicin showed antibacterial activities against *S. aureus* and *S. dysenteriae* with inhibitory zones of 1.0 and 2.1 cm, respectively.

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### Experimental Part

*General.* Trimethylsilyldiazomethane (TMSCHN<sub>2</sub>) was from Aldrich. TLC: precoated SiO<sub>2</sub> GF<sub>254</sub> plates (Qingdao Marine Chemical Factory, P. R. China). Column chromatography (CC): SiO<sub>2</sub> H (200–

300 mesh or 10–40  $\mu\text{m}$ , *Qingdao*), *Sephadex LH-20* gel (*Amersham Pharmacia*, Sweden), and *RP-18* (reverse-phase  $\text{C}_{18}$ )  $\text{SiO}_2$  (40–63  $\mu\text{m}$ , *Merck*, Germany). Optical rotations: *Jasco DIP-370* digital polarimeter. UV Spectra: *Shimadzu 2401PC* spectrophotometer;  $\lambda_{\text{max}}$  in nm ( $\log \epsilon$ ). IR Spectra: *Bio-Rad FTS-135* spectrophotometer with KBr discs; in  $\text{cm}^{-1}$ . 1D- and 2D-NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometer instruments, resp.; chemical shift  $\delta$  in ppm rel. to  $\text{Me}_4\text{Si}$ ;  $J$  in Hz. ESI-MS and HR-ESI-MS: *Finnigan LCQ-Advantage* and *VG Auto-Spec-3000* mass spectrometers, resp.; in  $m/z$ . X-ray single crystal diffractometer: *Bruker Smart Apex*.

**Microbial Material and Fermentation.** The fungal strain was isolated from the surface-sterilized leaves of *M. hookeri* collected from Xishuangbanna, Yunnan Province, P. R. China, and deposited with the Kunming Institute of Botany, Chinese Academy of Sciences [4]. The strain was considered as non-sporulating fungus by traditional morphology and identified as one member of the genus *Botryosphaeria* by analysis of the internal transcribed spacer (ITS1 and ITS2) and 5.8S rDNA sequence (accession No. EU523117 in GenBank). Solid-state fermentation was carried out on 1.5% potato-dextrose-agar (PDA, 10 l), and the cultures were incubated at 28° for 10 d.

**Extraction and Isolation.** The cultures were extracted three times by  $\text{AcOEt}/\text{MeOH}/\text{AcOH}$  80 : 15 : 5 ( $v/v/v$ ). Subsequently, the crude extracts were participated between  $\text{H}_2\text{O}$  and  $\text{AcOEt}$  exhaustively. The org. solvent was evaporated to afford a residue (5.8 g), which was subjected to medium pressure liquid chromatography (MPLC) over *RP-18*  $\text{SiO}_2$  (145 g) and eluted with  $\text{H}_2\text{O}$ , 30, 50, 70, 85, and 100%  $\text{MeOH}$  (2 l for each gradient) to yield seven fractions, *Fr. 1–7*. After purification of *Fr. 2* (87 mg) by CC over *Sephadex LH-20* gel eluted with  $\text{MeOH}/\text{H}_2\text{O}$  (8 : 2,  $v/v$ ), crude crystals of **1** were obtained, which were recrystallized from  $\text{CHCl}_3/\text{MeOH}$  (1 : 1,  $v/v$ ) to afford **1** (20 mg). *Fr. 3* (552 mg) was subjected to CC over  $\text{SiO}_2$  *H* eluting with  $\text{CHCl}_3/\text{MeOH}$  (from 100 : 1 to 30 : 1 ( $v/v$ )) to yield **2** (10 mg), **6** (5 mg), **7** (3 mg), **11** (18 mg), and **12** (25 mg). *Fr. 4* (230 mg) was repeatedly chromatographed over  $\text{SiO}_2$  *H* and *Sephadex LH-20* gel to afford **3** (5 mg), **4** (2 mg), **5** (10 mg), and **8** (6 mg). Further purification of *Fr. 5* on  $\text{SiO}_2$  *H* and *Sephadex LH-20* gel yielded **9** (10 mg) and **10** (4 mg).

**Botryosphaerin A** (= rel-(3*a*S,5*a*R,10*a*S,10*b*S,10*c*R)-1,2,3,3*a*,5*a*,7,10,10*a*,10*b*,10*c*-Decahydro-10*a*-hydroxy-3*a*,10*b*-dimethyl-4*H*,9*H*-[2]benzofuro[7,1-*fg*]isochromene-4,9-dione; **1**). Colorless crystals ( $\text{CHCl}_3/\text{MeOH}$ , 1 : 1). M.p. 274–275°.  $[\alpha]_{\text{D}}^{25} = -23.5$  ( $c = 0.23$ ,  $\text{C}_5\text{H}_5\text{N}$ ). UV ( $\text{MeOH}$ ): 202 (3.74). IR: 3424, 2952, 1763, 1725, 1379, 1269, 1079, 922.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: *Tables 2 and I*, resp. HR-ESI-MS (pos.): 315.1211 ( $[\text{M} + \text{Na}]^+$ ,  $\text{C}_{16}\text{H}_{20}\text{NaO}_5^+$ ; 315.1208).

**X-Ray Crystallographic Data for 1<sup>2</sup>**.  $\text{C}_{16}\text{H}_{20}\text{O}_5$ ,  $M_r = 292.32$ , orthorhombic space group  $P2(1)2(1)2(1)$ ,  $a = 7.7054(10)$  Å,  $b = 9.6017(12)$  Å,  $c = 19.093(2)$  Å,  $V = 1412.6(3)$  Å<sup>3</sup>,  $Z = 4$ ,  $d = 1.375$  Mg/m<sup>3</sup>.  $F(000) = 624$ ,  $\mu = 0.102$  mm<sup>-1</sup>. A single crystal of dimensions  $0.50 \times 0.40 \times 0.23$  mm was used for X-ray measurements. The intensity data of all unique reflections within the  $\theta$  range 2.13–27.00° were collected at 292 K in a *Bruker Apex II* CCD area detector, using graphite monochromated  $\text{MoK}_\alpha$  ( $\lambda = 0.71073$  Å) radiation. A total of 8343 independent reflections was measured, and 1786 were considered to be observed ( $|F|^2 \geq 2\sigma|F|^2$ ). All calculations were performed using the Crystal-Structure crystallographic software package except for the refinement, which was performed using SHELXL-97 [15].

**Botryosphaerin B** (= rel-(3*a*S,5*a*R,6*S*,9*a*S,9*b*R)-1,2,3*a*,5*a*,6,7,8,9,9*a*,9*b*-Decahydro-3*a*-(hydroxymethyl)-6,9*a*-dimethyl-2-oxonaphtho[2,1-*b*]furan-6-carboxylic Acid; **2**). Colorless crystals ( $i\text{PrOH}$ ). M.p. 217–218°.  $[\alpha]_{\text{D}}^{18} = -32.6$  ( $c = 0.23$ ,  $\text{MeOH}$ ). UV ( $\text{MeOH}$ ): 202 (3.62). IR: 3432, 2926, 1757, 1627, 1202, 995.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: *Tables 2 and I*, resp. HR-ESI-MS (pos.): 317.1361 ( $[\text{M} + \text{Na}]^+$ ,  $\text{C}_{16}\text{H}_{22}\text{NaO}_5^+$ ; 317.1364).

**X-Ray Crystallographic Data for 2<sup>2</sup>**.  $\text{C}_{16}\text{H}_{22}\text{O}_5$ ,  $M_r = 294.34$ , orthorhombic space group  $P2(1)2(1)2(1)$ ,  $a = 7.5979(5)$  Å,  $b = 7.5979(15)$  Å,  $c = 53.725(5)$  Å,  $V = 3101.4(4)$  Å<sup>3</sup>,  $Z = 8$ ,  $d = 1.261$  Mg/m<sup>3</sup>.  $F(000) = 1264$ ,  $\mu = 0.093$  mm<sup>-1</sup>. A single crystal of dimensions  $0.26 \times 0.18 \times 0.09$  mm was used for X-ray measurements. The intensity data of all unique reflections within the  $\theta$  range 2.71–25.48° were collected at 293 K in a *Bruker Apex II* CCD area detector, using graphite monochromated  $\text{MoK}_\alpha$ .

<sup>2)</sup> CCDC-679522 and -681144 contain the supplementary crystallographic data of compounds **1** and **2**. These data can be obtained free of charge via [http://www.ccdc.cam.ac.uk/data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif) (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

( $\lambda = 0.71073 \text{ \AA}$ ) radiation. A total of 16372 independent reflections were measured, and 1830 were considered to be significant ( $|F|^2 \geq 2\sigma|F|^2$ ). All calculations were performed using the Crystal-Structure crystallographic software package except for refinement, which was performed using SHELXL-97 [15].

**Botryosphaerin C** (=rel-(1*S*,4*aR*,5*S*,8*aR*)-Decahydro-5-(2-hydroxyethyl)-1,4*a*-dimethyl-6-methylidenenaphthalene-1-carboxylic Acid; **3**). Colorless oil.  $[\alpha]_{\text{D}}^{15} = +44.0$  ( $c = 0.17$ ,  $\text{CHCl}_3$ ). UV ( $\text{CHCl}_3$ ): 239 (2.86). IR: 3424, 2959, 2932, 1693, 1644, 1468, 1384, 1045, 889.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: *Tables 2 and 1*, resp. HR-ESI-MS (pos.): 289.1773 ( $[M + \text{Na}]^+$ ,  $\text{C}_{16}\text{H}_{26}\text{NaO}_3^+$ ; 289.1779).

**Botryosphaerin D** (=rel-[(1*S*,4*aR*,5*S*,8*aR*)-Decahydro-5-(hydroxymethyl)-5,8*a*-dimethyl-2-methylidenenaphthalen-1-yl]acetic Acid; **4**). Colorless oil.  $[\alpha]_{\text{D}}^{15} = +25.0$  ( $c = 0.08$ ,  $\text{CHCl}_3$ ). UV ( $\text{CHCl}_3$ ): 212 (2.98). IR: 3425, 2925, 2852, 1710, 1646, 1460, 1382, 1022, 892.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: *Tables 2 and 1*, resp. HR-ESI-MS (pos.): 289.1776 ( $[M + \text{Na}]^+$ ,  $\text{C}_{16}\text{H}_{26}\text{NaO}_3^+$ ; 289.1779).

**Botryosphaerin E** (=rel-(1*S*,4*aR*,5*S*,8*aR*)-5-(4-Carboxy-3-hydroxy-3-methylbutyl)decahydro-1,4*a*-dimethyl-6-methylidenenaphthalene-1-carboxylic Acid; **5**). Colorless oil.  $[\alpha]_{\text{D}}^{18} = +72.8$  ( $c = 0.59$ ,  $\text{MeOH}$ ). UV ( $\text{MeOH}$ ): 202 (3.81). IR: 3422, 2966, 2939, 2846, 1697, 1645, 1449, 1385, 1030, 891.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: *Tables 2 and 1*, resp. HR-ESI-MS (pos.): 375.2147 ( $[M + \text{Na}]^+$ ,  $\text{C}_{20}\text{H}_{32}\text{NaO}_5^+$ ; 375.2147).

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