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# New Massarilactones, Massarigenin E, and Coniothyrenol, Isolated from the Endophytic Fungus *Coniothyrium* sp. from *Carpobrotus edulis*[‡]

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New massarilactones C (**3**) and D (**4**), massarigenin E (**5**), and coniothyrenol (**6**) were isolated from *Coniothyrium* sp. together with the known graphislactone A (**1**) and massarilactone A (**2**). The relative configuration of coniothyrenol (**6**) with an unprecedented benzo[*a*]xanthene skeleton and ten chirality centers was determined by 1D- and 2D-NMR spectroscopy.



## Introduction

Endophytic fungi are a rich source of new biologically active natural products. They represent a relatively unexplored ecological source and their secondary metabolism is particularly active due to their metabolic interactions with their hosts.[2–4] In our screening program for new biologically active fungal secondary metabolites, we investigated an endophytic *Coniothyrium* sp., which was isolated from the plant *Carpobrotus edulis*, a halotolerant succulent growing on the Canary island Gomera. From the ethyl acetate extracts of the fungal cultivation, four previously unknown polyketide-derived natural products, **3**–**6**, together with the known graphislactone A (**1**) and massarilactone A (**2**) were isolated. Two of these compounds, named massarilactone C (**3**) and D (**4**) are related to the massarilactones, first isolated by Oh et al. from the aquatic fungus *Massarina tunicata*.[5] One cyclohexene derivative, named massarigenin E (**5**), shows a substitution pattern similar to the one of massarigenins [e.g. massarigenin A (**5a**), Scheme 1], later isolated from the same fungus.[6] The formation of the stable hemilactol **2a** upon water addition to massarilactone A (**2**) was also observed. The absolute configurations of the new compounds **3**–**5** were assigned by comparison of the optical rotations with related natural products as shown in Scheme 1. Coniothyrenol (**6**), named after the producing strain, has an unprecedented structural hexadecahydro-1*H*-benzo[*a*]xanthene skeleton, to date unknown as a natural product. We now report on the details of the isolation, structure elucidation, and bioactivity tests of the new natural products.

## Results and Discussion

The optically inactive compound **1** crystallized from dichloromethane as colorless plates with m.p. 232–234 °C. The structure was identified as the known graphislactone A (**1**) (Scheme 1) by comparison of the published NMR spectroscopic data. This compound was isolated for the first time by Tanahashi et al. in 1997 from a lichen.[7]

Compound **2** is a yellow, optically active oil ($[a]_D^{20}$ = +15.5), whose structure was elucidated by detailed analysis of the 1D- and 2D-NMR spectra. The spectroscopic data were identical to those reported for massarilactone A (**2**), isolated by Ho et al. from *Massarina tunicata*.[5] The optical rotation is of the same sign and order of magnitude {$[a]_D^{20}$ = +15.5 (*c* = 0.35, $CH_2Cl_2$). $[a]_D^{20}$ = +8.7, *c* = 0.3, $CH_2Cl_2$[5]}, suggesting the identical absolute configuration (Scheme 1) as reported for massarilactone A (**2**).[5] Interestingly, the compound proved to be unstable in deuterated chloroform for NMR measurement. A freshly prepared and an "aged" $CDCl_3$ solution that was obtained after standing for two days at room temperature give rise to completely different NMR spectra. The signals for the exocyclic methylene group had disappeared, and instead a signal for a methyl group at $\delta$ = 1.57 ppm in the $^1H$ and a signal for a hydroxylated carbon atom at $\delta$ = 108.7 ppm in the $^{13}C$ NMR spectra were detected. Evidently, under the influence of a small amount of hydrochloric acid present in the chloroform solution, addition of water to the exocyclic double bond to form the hemiacetal **2a** had occurred. The NMR

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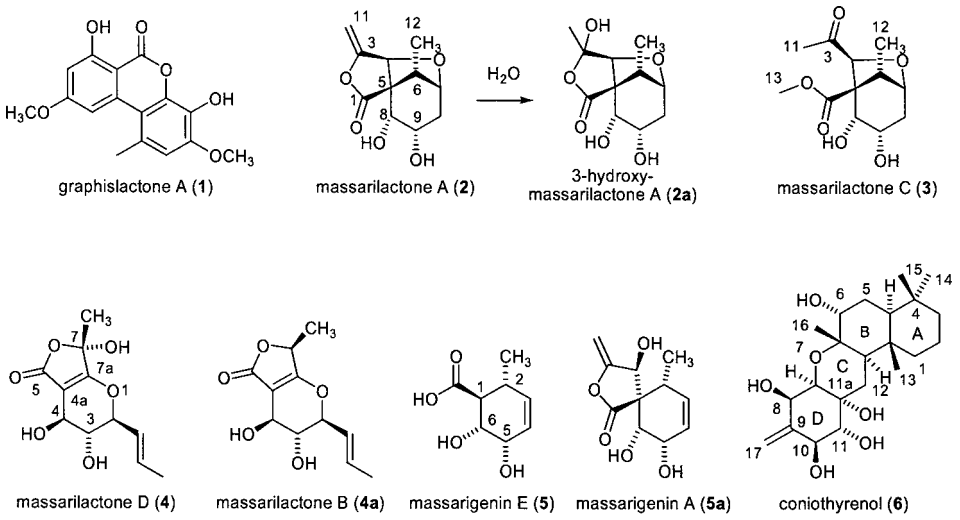


Scheme 1. Compounds **1**–**6** isolated from *Coniothyrium* sp. and related known structures massarilactone B (**4a**)[5] and massarigenin A (**5a**).[6]

spectroscopic data (see Exp. Sect.) proved that a complete transformation of massarilactone A (**2**) to the new 3-hydroxy-massarilactone A (**2a**) had occurred (Scheme 1). This was additionally confirmed by measurement of an INADEQUATE NMR spectrum of the new derivative **2a**. The structurally unique hemiacetal **2a**, which is at the same time part of a γ-lactone, is remarkably stable. To the best of our knowledge, only one structurally related natural product was reported by Khafagy et al.[8] in which the hemiacetal is, however, part of a *six*-membered lactone ring.

The third likewise optically active compound **3** ($[a]_D^{20}$ = +51.1) was also isolated as a yellow oil. The HRMS led to a molecular formula of $C_{12}H_{18}O_6$, accounting for four unsaturations. The $^{13}C$ NMR spectra had a great similarity with those of massarilactone A (**2**) as shown in Table 1. The assignment of all primary, secondary and tertiary carbon atoms was based on the analysis of the DEPT and HMQC spectra.

Table 1. $^{13}C$ NMR spectroscopic data of massarilactone A (**2**) and compound **3**.

| Atom number | Massarilactone A (**2**) | Compound **3** |
|---|---|---|
| 12 | 14.2 ($CH_3$) | 15.1 ($CH_3$) |
| 11 (**3**) | – | 28.7 ($CH_3$) |
| 8 | 38.5 ($CH_2$) | 38.2 ($CH_2$) |
| 6 | 46.0 (CH) | 45.7 (CH) |
| 13 | – | 52.3 ($CH_3$) |
| 5 | 61.5 ($C_q$) | 64.5 ($C_q$) |
| 9 | 66.3 (CH) | 66.4 (CH) |
| 10 | 69.4 (CH) | 73.6 (CH) |
| 7 | 76.8 (CH) | 81.1 (CH) |
| 4 | 83.8 (CH) | 81.5 (CH) |
| 11 (**2**) | 89.6 ($CH_2$) | – |
| 3 | 156.6 ($C_q$) | – |
| 1 | 171.8 ($C_q$) | 170.8 ($C_q$) |
| 3 (**3**) | – | 206.9 ($C_q$) |

As can be seen from the data in Table 1, the spectrum of compound **3** is lacking the signals at $\delta$ = 89.6 ppm due to a terminal double bond. In addition, signals for two methyl groups can be identified, one showing the typical chemical shift for a methoxy group at $\delta$ = 52.3 and one for an acetyl group at $\delta$ = 28.7 ppm. Furthermore, compound **3** shows a signal at $\delta$ = 206.9 ppm accounting for a keto carbonyl group, not present in massarilactone A (**2**). Analysis of the HMBC spectrum revealed the connections of the quaternary carbon atom to the remaining CH group at $\delta$ = 81.5 ppm and the connection of the methoxy group to the ester group. The coupling between the two CH groups resonating at $\delta$ = 81.5 and 81.1 ppm finally led to structure **3**, named massarilactone C, missing the lactone ring of compound **2** as shown in Scheme 1. The chemical relationship to massarilactone A (**2**) and the hemiacetal **2a** is obvious and it cannot be excluded that the keto methyl ester **3** is formed by methanolysis during chromatography of massarilactone **2**. Based on the likewise positive optical rotation found for **2**,[5] we tentatively assign the same absolute configuration to **3** as shown for **2** in Scheme 1.

Compound **4** forms colorless, optically active crystals ($[a]_D^{20}$ = –116.1) with the melting range 84–88 °C. Analysis of the NMR spectra reveals the presence of two double bonds with four and two substituents, respectively. This situation is typical of carbon atoms like C-4a and C-7a with oxygen-substituted α,β-unsaturated carbonyl compounds. Furthermore, three oxygenated CH groups, two methyl groups and one quaternary carbon atom can be identified from the $^1H$,$^1H$ COSY couplings. Correlation of the methyl group with the quaternary carbon atom at $\delta$ = 101.2 ppm, suggesting a twofold oxygenation, leads to structure **4**, which is supported by the molecular ion at *m*/*z* = 243 in the CIMS. Compound **4** is named massarilactone D in agreement with the related structure of massarilactone B (**4a**), which is missing the hydroxy group at C-3 and was isolated by Gloer et al. from *Massarina tunicata*.[5] Although showing some common structural elements, suggesting a common biosynthesis, the skeleton of massarilactones D (**4**) and B (**4a**) deviated notably from those of the massarilactones A (**2**), **2a**, and C (**3**) by incorporation of an oxygen atom

  

into the six-membered ring. The optical rotation of massarilactone B (**4a**) ($[\alpha]_D^{20}$ = –109) and massarilactone D ($[\alpha]_D^{20}$ = –116.1) had the same sign and order of magnitude, suggesting the same absolute configuration for both compounds, established by X-ray crystal structure analysis of the 4-bromobenzoate of massarilactone B (**4a**).[5]

Compound **5** was isolated as an optically active ($[\alpha]_D^{20}$ = +58.1) colorless oil. The $^1$H NMR spectrum shows signals for a *cis*-disubstituted double bond with a coupling constant of $J_{3,4}$ = 9.9 Hz, four CH groups, two of which are oxygenated, and one methyl group. The $^{13}$C NMR spectrum further reveals a low-field signal at $\delta$ = 176.9 ppm, typical of ester or acid carbonyl groups. Analysis of the $^1$H,$^1$H COSY spectrum leads to a tetrasubstituted cyclohexene ring with two hydroxy groups and one methyl group as the substituents. This information supports the assumption of the planar structure of **5** (Scheme 1). Finally, the relative configuration was determined by analysis of the coupling constants of the relevant ring protons. The large coupling constants between 1- and 2-H (10.9 Hz) as well as 1- and 6-H (11.5 Hz) showed the axial orientation of these protons and consequently the equatorial position of the carboxylic acid and methyl groups. In addition, the relatively small coupling constant of $J_{5,6}$ = 4.3 Hz shows the equatorial position of 5-H and thus the *cis*-orientation of the two hydroxy groups with 5-OH adopting an axial position.

Structure **5** is related to the fungal metabolites rosigenin[9] and massarigenin A (**5a**)[5] (Scheme 1), missing the C-3 side chain at the spiro center at C-5 of **5a**. Also, the close resemblance to massarilactone A (**2**) again suggests a common biosynthetic pathway and therefore the same absolute configuration is tentatively assigned to compound **5**, named massarigenin E (Scheme 1).

Compound **6** crystallizes as a white amorphous powder with m.p. 283–285 °C. The compound is optically active ($[\alpha]_D^{20}$ = +11.1) and the HRMS shows an M peak for matching $C_{22}H_{36}O_6$, including five double bond equivalents. From the NMR spectra four methyl groups, six $CH_2$ groups, seven CH groups and five quaternary carbon atoms can be deduced. The proton signals are assigned to the respective carbon signals by analysis of the HMQC spectrum as shown in Table 2. The low-field chemical shift supports the existence of not only a terminal double bond ($CH_2$ at $\delta$ = 103.6 ppm) but also five oxygenated CH groups.

Analysis of the $^1$H,$^1$H COSY and HMBC spectra, listed in Table 2, leads to fragments **A**–**C** as shown in Figure 1.

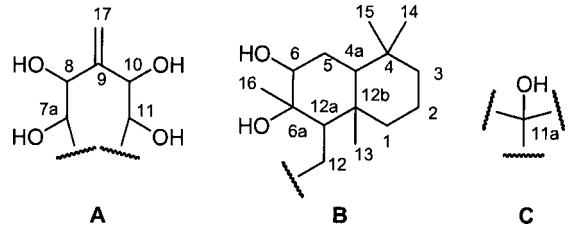


Figure 1. Fragments **A**–**C** as deduced from the $^1$H,$^1$H COSY and HMBC spectra of **6**.

Connection of the open valences in fragments **A**–**C**, e.g. carbon atom 11a of fragment **B** to the carbon atoms 7a, 11 of fragment **C** and C-12 of fragment **A** and inclusion of C-

Table 2. $^1$H and $^{13}$C NMR spectroscopic data of compound **6**.[a]

| Atom number | $^{13}$C NMR | $^1$H NMR (multiplicity, *J* in Hz) | $^1$H,$^1$H COSY | HMBC | NOESY |
|---|---|---|---|---|---|
| 1 | 39.0 | $1_{ax}$: 0.99 (t)<br>$1_{eq}$: 1.55 (dt, 13.9, 2.8) | 2 | 13 | $1_{eq}$, 4a, 12a |
| 2 | 18.2 | $2_{ax}$: 1.43 (m)<br>$2_{eq}$: 1.62 (m) | 1, 3 | – | $2_{eq}$, $3_{eq}$<br>$2_{ax}$ |
| 3 | 41.9 | $3_{ax}$: 1.17 (m)<br>$3_{eq}$: 1.35 (dt) | 2 | – | 4a, $3_{eq}$, $2_{eq}$<br>$2_{ax}$, $2_{eq}$ |
| 4 | 32.3 | – | – | – | – |
| 4a | 47.5 | 1.43 (dd, 14, 2.4) | 5 | 5 | $1_{ax}$, $3_{ax}$, $5_{eq}$, 12a, 14 |
| 5 | 26.1 | $5_{ax}$: 1.58 (td, 14, 2.4)<br>$5_{eq}$: 1.76 (dt, 14, 2.4) | 4a, 6 | 6a, 12b | $5_{eq}$, $6_{eq}$<br>$5_{ax}$, 4a, $6_{eq}$, 14 |
| 6 | 74.1 | 3.57 (t, 2.4) | 5 | 4a, 6a | – |
| 6a | 77.5 | – | – | – | – |
| 7a | 75.5 | 3.99 (d, 3.7) | 8 | 8, 9, 11, 11a | $8_{ax}$, 12a |
| 8 | 68.9 | 4.33 (br. d) | 7a, 17 | – | 7a, $10_{ax}$ |
| 9 | 147.9 | – | – | – | – |
| 10 | 71.3 | 3.99 (d, 9.6) | 11, 17 | – | |
| 11 | 78.8 | 3.27 (d, 9.6) | 10 | 10, 12 | 7a, $12_{ax}$, 16 |
| 11a | 71.6 | – | – | – | – |
| 12 | 30.0 | $12_{eq}$: 1.58 (dd)<br>$12_{ax}$: 1.99 (t, 14.3) | $12_{ax}$ | 7, 11 | $11_{ax}$, $12_{eq}$, 13, 16 |
| 12a | 42.1 | 2.27 (dd, 14.3, 4.3) | 12 | 1, 6a, 13, 16 | $1_{ax}$, 4a, 7a; $12_{eq}$ |
| 12b | 36.8 | – | – | – | – |
| 13 | 14.3 | 0.77 | – | 1, 4a, 12a, 12b | 12-$H_{ax}$ |
| 14 | 32.1 | 0.81 | – | 3, 4, 4a, 15 | $5_{eq}$, 4a |
| 15 | 20.3 | 0.76 | – | 3, 4, 4a | $5_{ax}$ |
| 16 | 23.1 | 1.00 | – | 6, 6a, 12a | $5_{ax}$, $6_{eq}$, $11_{ax}$, $12_{ax}$ |
| 17 | 103.6 | 5.06/5.16 | 8, 10 | 8, 9, 10 | – |

[a] For graphical representation of the NOESY correlations see the Supporting Information.

  

12 into a six-membered ring and an ether bridge between the oxygens on carbon atoms 6a and 7a leads to the planar structure of **6** (Scheme 1), named coniothyrenol after the producing fungal strain.

Coniothyrenol (**6**) has ten chiral centers and the relative stereochemistry was elucidated by a combination of the analysis of coupling constants and NOESY correlations (see Table 2 and Supporting Information) in combination with model considerations. The connection of rings A and B in a rigid *trans* decalin partial structure with two chair conformations of the rings could unambiguously be established by the strong NOESY correlation of all axial protons in 1,3-position: $1_{ax}$, $3_{ax}$, and 12a-H, placing these protons on the same α-side of the molecule. This is not possible in a *cis*-decalin configuration. The absence of any NOESY correlation between 4a-H and 13-Me, placed on the opposite face of the β-face of the decalin system, confirms this. The spin systems of the methylene groups are in part overlapping, but the clear coupling constants (dd, $J_{4a,5ax}$ = 14.0 and $J_{4a,5eq}$ = 2.4 Hz) deduced from the 4a-H signal at $\delta$ = 1.43 ppm confirm this assignment. The hydroxy group at C-6 must adopt an axial position due to the absence of any *trans*-diaxial coupling between 6-H and the C-5 protons. The equatorial position of 6-H is further confirmed by the absence of any NOESY correlation with the axial protons 4a-H and 12a-H. On the other hand, a NOESY correlation between $6_{eq}$-H and the 16-Me group places this neighboring methyl group in an axial position. This is independently confirmed by the strong correlation of 16-Me with $12_{ax}$ and $5_{ax}$-H.

The next open question was the mode of connection of the pyrane ring C to ring B. There are several strong indications of a *trans*-connection with ring B. First of all, the NOESY correlation between 12a-H and 7a-H places these protons into axial positions. The coupling constant of $J_{12a,12ax}$ = 14.3 Hz (Table 2) confirms the axial position of 12a-H. With 16-Me and 12a-H both in axial positions, rings C and B must be *trans* annulated. In addition, the strong NOESY correlation between the axial 7a-H and 12a-H, with the hydrogen atoms in 1–4 position of the tetrahydropyran ring, strongly supports the assumption of a boat conformation for ring C. This is confirmed by the correlation of $12_{ax}$-H with $11_{ax}$-H which again is only possible with a boat conformation of ring C. Fortunately, the comparatively rigid four-membered ring system also allows the assigments of the connection of ring D, adopting a chair conformation, as well as the configuration of the hydroxy groups. The simultaneous coupling of 8-H to 7a-H and 10-H establishes its axial position. This is in perfect agreement with the coupling constant of $J_{7a,8}$ = 3.7 Hz as 7a-H adopts a pseudo-axial position in the boat conformation of ring C and shows a ca. 45° dihedral angle to 8-H. By contrast, the coupling of $J_{10,11}$ = 9.6 Hz indicates a bis-diaxial position of these protons and the respective hydroxy groups 10-OH and 11-OH are thus equatorial. The remaining question is the orientation of the tertiary 11a-OH. Although no coupling constant can directly confirm the orientation of the tertiary 11a-hydroxy group, the strong NOESY correlation between $11_{ax}$-H and $12_{ax}$-H leaves no doubt about the axial α-position of 11a-OH. The opposite configuration at C-11a strictly excludes any such NOESY correlation. The axial β-position of 11-H is further confirmed by a strong NOESY correlation 16-Me. The other NOESY correlations and coupling constants, compiled in Table 2, are in harmony with the *relative* configuration of coniothyrenol (**6**) as shown in Scheme 1.

### Bioactivity

In spite of the fact that the crude extract showed antifungal, antibacterial and antialgal activities, the pure substances were inactive in tests for biological activity (0.05 mL of 5 mg/mL = 0.25 mg) against the gram positive bacterium, *Bacillus megaterium*, the fungus, *Microbotryum violaceum*, and the green alga, *Chlorella fusca.*

## Experimental Section

**General Experimental Procedures:** Melting points were determined in open capillaries on a melting point apparatus from Büchi and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter. The IR spectra were taken on a NICOLET–510P spectrometer. NMR spectra were run on a Bruker Avance 500 NMR spectrometer with TMS as internal standard. EIMS and CIMS (isobutane) were obtained on a MAT 8200 mass spectrometer. Silica gel (230–400 mesh) was used for column chromatography. Spots were detected on TLC under UV or by heating after spraying with a solution of 0.5 mL anisaldehyde in 50 mL of acetic acid and 1 mL of $H_2SO_4$.

**Extraction and Isolation:** The endophytic fungus *Coniothyrium* sp. (7067) was isolated from the succulent plant *Carpobrotus edulis* from Gomera. It was cultured for 28 d on 12 L of biomalt solid agar at room temperature. The agar plates were then frozen for 3 d at –10 °C and filtered after thawing. The liquid and solid portions were each extracted with ethyl acetate to give 27.8 g of combined crude extract. This was then separated into 7 fractions by column chromatography on silica gel using a gradient of dichloromethane/methanol (0–10% in 1%-steps of 500 mL each). Fraction 4 gave compound **1** (52.8 mg) after column chromatography on Sephadex LH 20 using dichloromethane/methanol (3:2). The compound was further purified by crystallization from dichloromethane. Fraction 5 contained compounds **2** (1.03 g) and **3** (32.7 mg), which were separated by column chromatography on Sephadex LH 20 (dichloromethane/methanol, 3:2) and silica gel using dichloromethane/methanol (9:1) as the eluent. Compound **3** was finally purified by TLC on silica gel (dichloromethane/methanol, 92:8). Fraction 7 was further separated into 8 fractions by column chromatography on silica gel (dichloromethane/methanol, 95:5). The 6$^{th}$ fraction contained compound **4** (134.5 mg), which was isolated by column chromatography on Sephadex LH 20 and silica gel using dichloromethane/methanol (98:2). The 8$^{th}$ fraction gave compound **6** (8.2 mg), which crystallized directly from dichloromethane/methanol (3:2) after column chromatography on Sephadex LH 20. The mother liquor underwent another column chromatography on silica gel which resulted in crude compound **5** (3.1 mg). This was finally purified by column chromatography on Sephadex LH 20.

**Graphislactone A (1):** Colorless crystals, m.p. 232–234 °C (ref.[7] 236–237 °C).

  

**Massarilactone A (2):** Yellow oil. $[\alpha]_D^{20}$ = +15.5 (*c* = 0.35, $CH_2Cl_2$); (ref.[5] $[\alpha]_D^{20}$ = +8.7, *c* = 0.3, $CH_2Cl_2$). $^1H$ NMR ($CDCl_3$, 500 MHz): $\delta$ = 1.07 (d, $J_{12,6}$ = 7.1 Hz, 3 H, 12-H), 1.78 (ddd, $J_{gem}$ = 15.6 Hz, $J_{8a,9}$ = 6.0 Hz, $J_{8a,7}$ = 1.9 Hz, 1 H, 8-H$^a$), 1.95 (q, $J_{6,12}$ = 7.1 Hz, 1 H, 6-H), 2.18 (dd, $J_{gem}$ = 15.6 Hz, $J_{8b,7}$ = 3.0 Hz, 1 H, 8-H$^b$), 4.17 (m, 3 H, 7-H, 9-H, 10-H), 4.52 (dd, $J_{gem}$ = 2.7 Hz, $J_{11a,4}$ = 2.2 Hz, 1 H, 11-H$^a$), 4.71 (d, $J_{gem}$ = 2.7 Hz, 1 H, 11-H$^b$), 5.63 (t, $J_{4,11a}$ = 2.2 Hz, 1 H, 4-H) ppm.

**Hydroxy-massarilactone (2a):** Yellow oil. $^1H$ NMR ($CDCl_3$, 500 MHz): $\delta$ = 0.89 (d, $J_{12,6}$ = 6.9 Hz, 3 H, 12-H), 1.57 (s, 3 H, 11-H), 1.62 (dd, $J_{gem}$ = 14.6 Hz, $J_{8a,9}$ = 5.7 Hz, 1 H, 8-H$^a$), 1.86 (m, 1 H, 6-H), 2.41 (dd, $J_{gem}$ = 14.6 Hz, $J_{8b,9}$ = 2.2 Hz, 1 H, 8-H$^b$), 4.21 (m 2 H, 2-H, 9-H), 4.56 (d, $J_{4,10}$ = 2.0 Hz, 1 H, 4-H), 4.71 (dd, $J_{10,9}$ = 4.4 Hz, $J_{10,4}$ = 2.0 Hz, 1 H, 10-H). $^{13}C$ NMR ($CDCl_3$, 125 MHz): $\delta$ = 14.5 ($CH_3$, C-12), 17.0 ($CH_3$, C-11), 35.6 ($CH_2$, C-8), 41.2 (CH, C-6), 67.8 ($C_q$, C-5), 73.4 (CH, C-9), 79.7 (CH, C-10), 82.0 (CH, C-7), 86.6 (CH, C-4), 108.7 ($C_q$, C-3), 174.1 ($C_q$, C-1) ppm. EIMS (70 eV, 200 °C): *m/z* (%) = 226 (6.5), 166 (94.9), 139 (21.2), 93 (28.4), 43 (100.0).

**Massarilactone C (3):** Yellow oil. $[\alpha]_D^{20}$ = +51.1 (*c* = 0.7, $CH_2Cl_2$). IR (film): $\tilde{\nu}$ = 3446, 2949, 1732, 1720, 1271, 1248, 1111, 1093, 1070, 1014 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 500 MHz): $\delta$ = 1.01 (d, $J_{12,6}$ = 7.0 Hz, 3 H, 12-H), 1.79 (ddd, $J_{gem}$ = 15.5 Hz, $J_{8a,9}$ = 5.7 Hz, $J_{8a,7}$ = 1.2 Hz, 1 H, 8-H$^a$), 1.86 (q, $J_{6,12}$ = 7.0 Hz, 1 H, 6-H), 2.16 (s, 3 H, 11-H), 2.20 (dd, $J_{gem}$ = 15.5 Hz, $J_{8b,7}$ = 3.8 Hz, 1 H, 8-H$^b$), 3.69 (s, 3 H, 13-H), 4.11 (d, $J_{10,9}$ = 5.5 Hz, 1 H, 10-H), 4.14 (m, 1 H, 9-H), 4.18 (m, 1 H, 7-H), 5.07 (s, 1 H, 4-H) ppm. $^{13}C$ NMR ($CDCl_3$, 125 MHz) see Table 1. EIMS (70 eV, 200 °C): *m/z* (%) = 258 (1.3) [$M^+$], 227 (6.0) [$M^+ - OCH_3$], 215 (47.1) [$M^+ - COCH_3$], 183 (100.0), 153 (27.8), 137 (26.0). HRMS (EI): calcd. for $C_{12}H_{18}O_6$: 258.11035; found 258.10992.

**(2*S*,3*R*,4*S*,7*S*,*E*)-3,4,7-Trihydroxy-7-methyl-2-(prop-1-enyl)-3,4-dihydro-2*H*-furo[3,4-*b*]pyran-5(7*H*)-one [Massarilactone D (4)]:** Colorless crystals, m.p. 84–88 °C. $[\alpha]_D^{20}$ = –116.1 (*c* = 0.56, MeOH). IR (Film): $\tilde{\nu}$ = 3404, 2918, 1747, 1678, 1441, 1308, 1209, 1140, 1051, 1024, 966, 930, 903 $cm^{-1}$. $^1H$ NMR ($CD_3OD$, 500 MHz): $\delta$ = 1.63 (s, 3 H, 11-H), 1.77 (dd, $J_{10,9}$ = 6.5 Hz, $J_{10,8}$ = 1.3 Hz, 3 H, 10-H), 3.82 (t, $J_{3,2}$ = $J_{3,4}$ = 4.2 Hz, 1 H, 3-H), 4.31 (d, $J_{4,3}$ = 4.2 Hz, 1 H, 4-H), 4.85 (dd, $J_{2,8}$ = 7.9 Hz, $J_{2,3}$ = 4.2 Hz, 1 H, 2-H), 5.79 (m, 1 H, 8-H), 5.95 (m, 1 H, 9-H) ppm. $^{13}C$ NMR ($CD_3OD$, 125 MHz): $\delta$ = 16.6 ($CH_3$, C-10), 21.8 ($CH_3$, C-11), 62.9 (CH, C-4), 71.2 (CH, C-3), 84.9 (CH, C-2), 99.9 ($C_q$, C-4a), 101.2 ($C_q$, C-7), 125.2 (CH, C-8), 131.7 (CH, C-9), 170.1 ($C_q$, C-5), 174.2 ($C_q$, C-7a) ppm. CIMS (isobutane, 150 °C): *m/z* (%) = 485 (73.1) [2M + $H^+$], 243 (100.0) [M + $H^+$], 225 (11.5) [M – $H_2O$ + $H^+$], 181 (18.3).

**(1*S*,2*R*,5*S*,6*R*)-5,6-Dihydroxy-2-methylcyclohex-3-enecarboxylic Acid [Massarigenin E (5)]:** Colorless oil. $[\alpha]_D^{20}$ = +58.1 (*c* = 0.16, MeOH). $^1H$ NMR ($CD_3OD$, 500 MHz): $\delta$ = 1.07 (d, $J_{8,2}$ = 7.0 Hz, 3 H, 8-H), 2.40 (ps. t, $J_{1,2} \approx J_{1,6} \approx$ 10.9 Hz, 1 H, 1-H), 2.49 (m, 1 H, 2-H), 3.78 (dd, $J_{6,1}$ = 11.3 Hz, $J_{6,5}$ = 4.3 Hz, 1 H, 6-H), 4.03 (ps. t, $J_{5,4} \approx J_{5,6} \approx$ 4.7 Hz, 1 H, 5-H), 5.69 (dd, $J_{3,4}$ = 9.9 Hz, $J_{3,2}$ = 1.9 Hz, 1 H, 3-H), 5.80 (ddd, $J_{4,3}$ = 9.9 Hz, $J_{4,5}$ = 5.3 Hz, $J_{4,2}$ = 2.6 Hz, 1 H, 4-H) ppm. $^{13}C$ NMR ($CD_3OD$, 125 MHz): $\delta$ = 18.4 ($CH_3$, C-8), 35.2 (CH, C-2), 50.5 (CH, C-1), 65.0 (CH, C-5), 70.8 (CH, C-6), 125.3 (CH, C-4), 135.0 (CH, C-3), 176.9 ($C_q$, C-7) ppm. CIMS (isobutane, 150 °C): *m/z* (%) = 173 (3.1) [M + $H^+$], 155 (4.6) [M + $H^+$ – $H_2O$], 137 (1.6) [M + $H^+$ – $2H_2O$].

**(4a*S**,6*R**,6a*S**,7a*R**,8*R**,10*S**,11*R**,11a*R**,12a*R**,12b*S**)-4,4,6a,12b-Tetramethyl-9-methylene-hexadecahydro-1*H*-benzo[*a*]-xanthene-6,8,10,11,11a-pentaol [Coniothyrenol (6)]:** White powder, m.p. 283–285 °C. $[\alpha]_D^{20}$ = +11.1 (*c* = 0.19, MeOH). NMR spectra ($CD_3OD$, 500 MHz) see Table 2: EIMS (70 eV, 200 °C): *m/z* (%) = 396 (4.1) [$M^+$], 378 (12.2) [$M^+ - H_2O$], 360 (38.3) [$M^+ - 2\ H_2O$], 331 (34.9), 293 (100.0), 189 (35.0), 177 (40.9), 123 (93.1). HRMS (EI): calcd. for $C_{22}H_{36}O_6$: 396.25119; found 396.25116.

**Supporting Information** (see also the footnote on the first page of this article): Graphical representation of the NOESY correlations.

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