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Ligustolide A and B, two novel sesquiterpenes with rare skeletons and three 1,10-*seco*-guaianolide derivatives from *Achillea ligustica*

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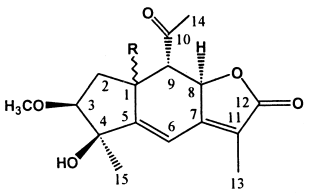
Abstract—*Achillea ligustica* afforded two novel sesquiterpene lactones with rare 5/6/5 skeletons, three 1,10-*seco*-guaianolides and a chlorine-containing sesquiterpene lactone as well as six known compounds, including two monoterpenes, two guaianolides, one eudesmane and one secocaryophyllene derivative. The structures of the compounds were elucidated by extensive application of one- and two-dimensional NMR spectroscopy. © 2003 Elsevier Science Ltd. All rights reserved.

1. Introduction

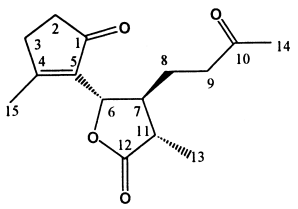
The genus *Achillea* (Asteraceae, Anthemideae) comprises over 100 species distributed worldwide. Aerial parts of different species of this genus are widely used in folk medicine for preparation of herbal teas with antiphlogostic and spasmolytic activity.[1] The extracts exhibit pharmacological activities such as, anti-inflammatory[2] and antiallergic[3] properties. Several species have been previously examined for flavonoids[4,5] and sesquiterpene lactones.[6–10] Recently, remarkable differences in the essential oils of several taxa of the genus have been found. It has been reported that C-glycosylflavones are an important chemotaxonomic character within the genus. In continuation of our chemical investigation of the chemical constituents of the member of the genus *Achillea*,[11–14] we studied the aerial parts of *Achillea ligustica*, which has been collected in Greece.

2. Results and discussion

Repetitive chromatographic steps of the methanol-dichloromethane (1:1) extract of the aerial parts of *A. ligustica* All yielded six novel sesquiterpene lactones (**1** –**6**) and six known compounds (**7**–**12**). The structural characterization of these compounds utilized spectroscopic techniques (high resolution 2D NMR measurements, HMQC, HMBC, COSY, NOESY and HRMS).



1 **R** = α OH
2 **R** = β OH



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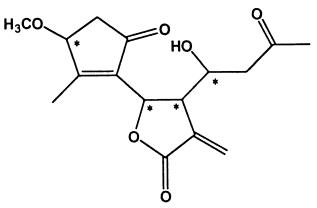

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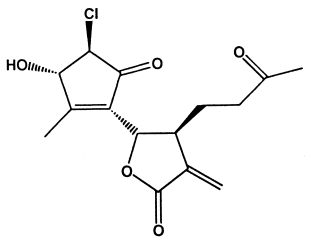

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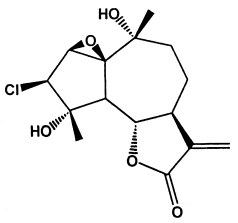

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Compound **1**, a rare 5/6/5 sesquiterpene skeleton, exhibited in the CIMS $[M+H]^+$ at *m/z* 309, corresponding to a

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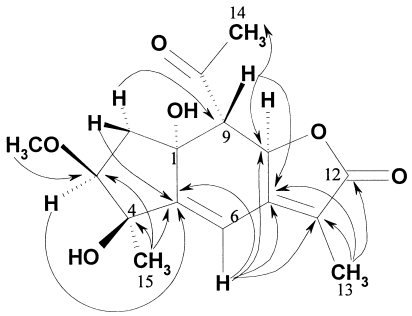


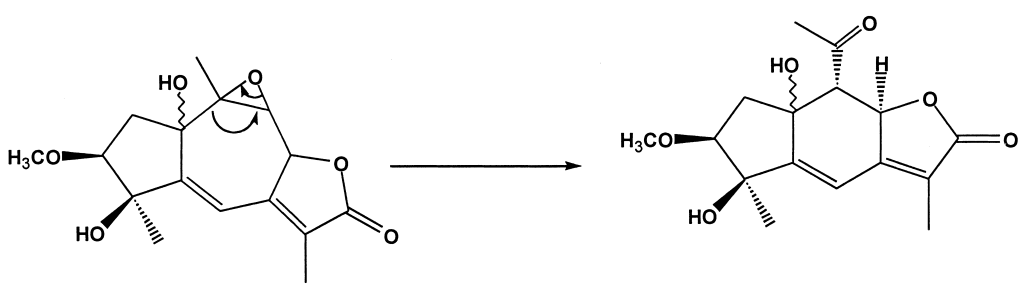
Figure 1. Arrows on formula **1** indicate characteristic HMBC connectivities obtained in chloroform-*d*.

molecular formula of $C_{16}H_{20}O_6$. The ^1H NMR spectrum showed one methyl singlet connected to an sp^3 carbon, and two methyls attached to sp^2 carbon atoms. Furthermore, one CH_3O (δ_H 3.42) and one =CH (δ_H 6.70) singlets were detected, in addition to five other protons attached to sp^3 carbon atoms. In the $^1H-^1H$ COSY experiment, the presence of an AMX-system (δ_H 3.74, t, J=5.7 Hz, H-3; 2.38, dd, J=5.7, 13.8 Hz, H_b-2; 1.79, dd, J=5.7, 13.8 Hz, H_a-2) and an AMX_3-system (δ_H 2.68, d, J=11.5 Hz, H-9; 5.36, dq, J=7.0, 11.5 Hz, H-8; 1.97, 3H, d, J=1.7 Hz, CH_3-13) were observed. The ^{13}C NMR spectrum indicated the presence of one ketone, one lactone, three quaternary sp^2 and one=CH, in addition to 10 sp^3 carbon atoms. To avoid the ambiguities resulting from the partial overlapping of the signals with the $CDCl_3$ signals, the ^{13}C NMR spectrum was also recorded in acetone-d_6. The assignment of the OCH_3 signal (3.42/57.9) was straightforward and can be utilized as a starting point for the determination of atom connectivities.

HMBC correlation leads from CH_3O to C-3 (δ_c 84.8). The connectivity of H-3 to H_a-2 was obvious from the $^1H-^1H$ COSY spectrum. HMBC cross-peaks (Fig. 1) proved the NMR signal assignments and the 5/6/5 membered tricyclic-ring-system. In the six membered ring, the presence of an acetyl substituent was straightforward (see H-9/C-14 connectivity). If the C=O group was incorporated in a ring system, seven member, the CH_3-14 signal should exhibit a doublet splitting in the ^1H spectrum. This was supported by the proposed biosynthetic route, which explain the formation of the methyl ketone, Scheme 1. The lactone should be in a five membered ring (γ-lactone) because H_3-13 protons correlate via $^3J_{CH}$ couplings with C-12 and C-7, and H-6 with C-11 and C-8, respectively. Due to the isochronous chemical shift of C-1 and C-4 atoms in chloroform-*d* the unambiguous assignment of the HMBC correlation to C-4 was not possible. This difficulty was overcome in acetone-d_6, where the C-1 and C-4 signals displayed different chemical shifts (δ_c 76.7 and 76.8, respectively). In this solvent, the H_b-2 proton gave $^3J_{CH}$ HMBC connectivities to C-5 atoms, which prove that these atoms are located in a five membered ring. The relative configuration of the five stereogenic centers of compound **1** and the preferred conformation was determined from the relevant $^3J_{H\text{-}8,H\text{-}9}$=11.5 Hz, $^3J_{Ha\text{-}2,H\text{-}3}$=$^3J_{Hb\text{-}2,H\text{-}3}$=5.7 Hz and from NOESY correlation. The value of $^3J_{H\text{-}8,H\text{-}9}$ suggested an antiperiplanar arrangement for these two protons with a calculated dihedral angle of 170°, whereas the $^3J_{Hb\text{-}2,H\text{-}3}$ coupling corresponds to 50° and $^3J_{Ha\text{-}2,H\text{-}3}$ to 140° dihedral angles, respectively.[15] The NOESY cross-peak between H-2β and H-9, H-2α and H-3, H-3 and H-15 supported the proposed stereochemistry of **1**.

Compound **2** exhibited ^1H and ^{13}C spectra rather similar to those for **1**. The same functional groups and spin-systems were identified, which suggested that compound **2** was a stereoisomer of **1**. Characteristic chemical shift differences $\Delta\delta=\delta(\mathbf{1})-\delta(\mathbf{2})$ were only observed for H_a-2, H-8 and H-9 protons (−0.39; −0.30; 0.40 ppm, respectively). In the ^{13}C NMR spectrum remarkable changes were found in the skeleton at C-1, C-2 and C-9 atoms (−3.2; 1.9; −1.9 ppm, respectively) as well as in the acetyl group at C-10 and C-14 (5.1 and 2.8 ppm, respectively). In compound **1**, the C-10 carbonyl signal had a 5.1 ppm deshielding comparable with the corresponding signal in **2**. This could be well explained by the strong intramolecular hydrogen bond between the *cis* arranged acetyl and HO-1 groups. This indicated that the relative configuration of C-1 was opposite in both compounds **1** and **2**. The steric proximity between H_a-2 and H-9 in structure **1** proved the antiperiplanar position of the hydroxyl group with respect to the two hydrogen atoms, whereas in compound **2**, due to the *cis* arrangement of the OH groups a H_a-2/H-9 interaction was not observed. Considering the NOESY cross-peak between CH_3-15 and H_b-2 protons, the two OH groups connected to the C-1 and C-4 carbons must be in a *cis* arrangement in **2** (Fig. 2).

Additionally, three 1,10-*seco*-guaianolide derivatives (**3**–**5**) were identified. The molecular formula of the first 1,10-*seco*-germacranolide (**3**) was determined to be $C_{15}H_{20}O_4$ by a high resolution FAB mass spectrum, $[M+H]^+$ at m/z 265.1447. The ^1H NMR spectrum of **3** showed three methyl signals at δ_H 2.18 (s), 2.11 (s), 1.35 (d, J=7.0 Hz) and a downfield signal at δ_H 4.82 (d, J=9.5 Hz). The ^{13}C NMR data (δ_c 207.3, 207.1, 178.4), together with IR absorption (1773, 1696, 1650 cm^{-1}), indicated the presence of three carbonyl carbons with one being a γ-lactone system. Two olefinic carbons were detected at δ_c 176.6 (s) and 135.7 (s) and one oxygen-bearing carbon at δ_c 78.3 (d). The lactone



Scheme 1. Proposed biosynthetic route of ligustolide A (R=αOH) and B (R=βOH).

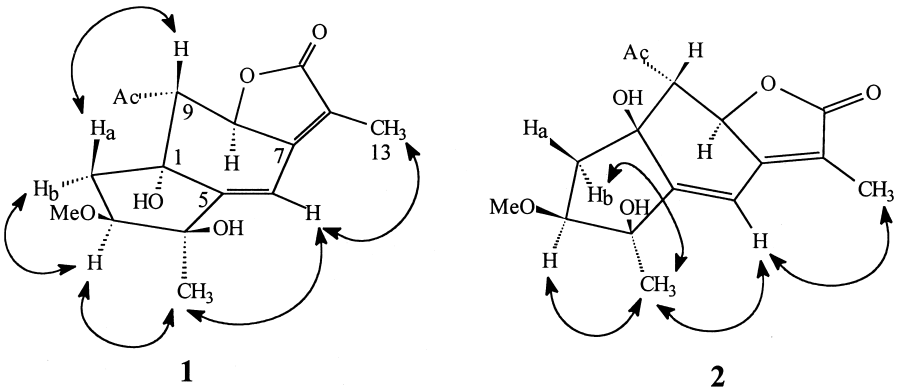


Figure 2. Stereo projections of **1** and **2**. Double arrows indicate significant steric proximities obtained by NOESY.

ring was substituted with three *trans* substituents, in position C-6 with a cyclopenten-il ring, in position C-7 with a butyl-on, and at C-11 with a methyl group. The sequences of these units was determined on the basis of $^{1}H-^{1}H$ COSY, TOCSY, HMQC and HMBC measurements. The respective correlation from the methyl doublet at δ 1.35 (H-13) to the carbons at δ 41.7 (C-11), 46.3 (C-7), 178.4 (C-12), from the doublet at δ_H 4.82 (H-6) to the carbons at δ_c 46.3 (C-7), 135.7 (C-5), 176.6 (C-4), 25.3 (C-8), 207.1 (C-1), allowed the sequences to be established. The *trans* arrangement of the substituents of the lactone ring was in accordance with the values of the $^{3}J_{(H\text{-}6,H\text{-}7)}$=9.5 and $^{3}J_{(H\text{-}7,H\text{-}11)}$=11.0 Hz couplings. Moreover, the relative stereochemistry of **3** was established by NOESY experiments. Therefore, compound **3** was identified as 3-deshydroxy-*iso*-*seco*-tanapartholide.[16]

The structure of **4** was established by comparison of its NMR data with those of **3**. The appearance of two sets of signals in both the ^{1}H and ^{13}C spectra indicated that **4** consists of two diastereoisomers in a ratio 1:1. The ^{1}H NMR exhibited an additional three proton singlet signal at δ_H 3.40/3.41 (δ_C 57.50/57.54) due to a methoxyl group. The placement of the methoxyl group at C-3 was achieved by $^{1}H,^{1}H$-COSY, HMQC and HMBC, measurements. In the HMBC spectrum, a correlation was observed between the methoxyl signal at δ_H 3.40/3.41 with C-3 at δ_C 79.7/79.9. Further correlation supported the location of the free hydroxyl group at C-8. Compound **4** was therefore the 8-OH derivative of 3-methoxy-3-deshydroxy-*iso*-*seco*-tanapartholide.[16]

The HR-CI-MS investigation of compound **5** showed a $[M+H]^{+}$ at *m/z* 313.08206, corresponding to a molecular formula $C_{15}H_{17}O_5Cl$. The presence of a chlorine atom in the skeleton was proved by an isotopic peak at *m/z* 315. The ^{1}H and ^{13}C NMR data for compound **5** differed from those for **3** by the presence of the following highly deshielded signals: δ_C 123.4 (t, δ_H 6.38, and 5.70), 169.5 (s), 80.0 (d, δ_H 4.72), and δ_C 62.5 (d, δ_H 4.22). The placement of an exomethylene at C-13, hydroxyl group at C-3 and the chlorine atom at C-2 were determined by $^{1}H-^{1}H$ COSY, HMQC and HMBC. In the HMBC spectrum, the proton resonance at δ_H 4.22 (d, H-2) was correlated to the carbons at δ_c 194.7 (C-1), 80.0 (C-3) and the proton resonance at δ_H 5.00 (d, H-6) was correlated to the carbons at δ_c 194.7 (C-1), 170.2 (C-4), 27.5 (C-8), 169.5 (C-12). Inspection of the nearly coplanar arrangement of the cyclo-penten-one ring proved that the vicinal protons could form a dihedral angle 0±30 for *cis* and 120±30 for *trans*. The measured $^{3}J_{H\text{-}2,H\text{-}3}$=2.8 Hz was in a good agreement with the *trans* orientation. Only, few seco-tanapartholides have been reported and most likely were obtained by the oxidative cleavage of the corresponding guainolide (Fig. 3).[17–21]

The $^{1}H-^{1}H$ COSY of compound **6** showed two vicinal protons at δ_H 3.70 and δ_H 4.07, and their small coupling ($^{3}J_{H\text{-}2,H\text{-}3}$<1 Hz) indicated a nearly orthogonal arrangement. The CH group δ_H 3.70 (δ 64.7) exhibited an extreme one-bond $^{1}J_{C,H}$=193 Hz coupling, which is characteristic for epoxides.[22] The other CH at δ_H 4.07 (δ_C 64.4) showed a $^{1}J_{(C,H)}$=160 Hz value. These coupling and chemical shifts suggested a methine group with a heteroatom substituent (Cl or O). The presence of a chlorine atom in the skeleton was supported by an isotopic peak at *m/z* 317 and HRCIMS at *m/z* 315.09994, corresponding to a molecular formula $C_{15}H_{19}O_5Cl$. The complete ^{1}H and ^{13}C NMR assignments were achieved with HMQC, HMBC and NOESY measurements. The observed NOE cross-peak between the protons CH_3-14/H-2 indicate that the epoxide was located at C-1/C-2. The β-configuration of CH_3-14 was supported by NOE cross-peak CH_3-14/H-6. A stereoisomer of **6** at C-1/C-2 was reported from *Chrysanthemum parthenium*.[23]

Compound **7** was found to be a 1:1 mixture of two diastereomers identical with (3*RS*, 6*RS*)-2,6-dimethyl-octa-1,7-dien-3,6-diol. The ^{1}H and ^{13}C NMR assignments were supported with HMQC and HMBC experiments, and the chemical shifts show a good agreement with data published by Knapp et al.[24] The next isolated component **8** was

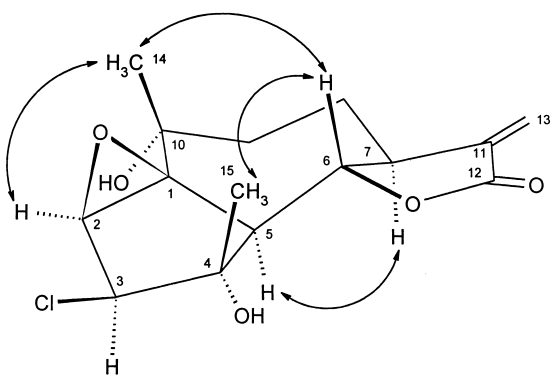
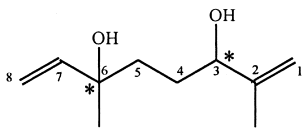


Figure 3. Double arrows indicate significant NOE contacts of **6**.

identified as monoterpene, namely 2,6-dimethyl-octa-1,7-diene-3,6-diol.[25]



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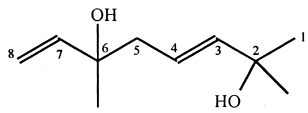

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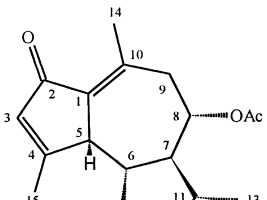

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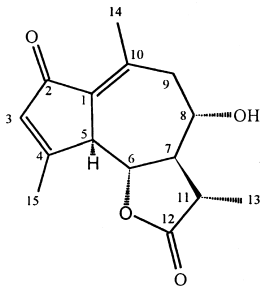

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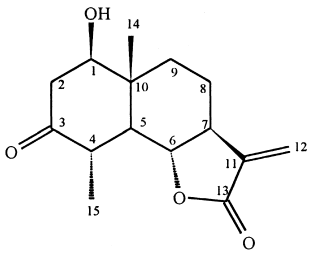

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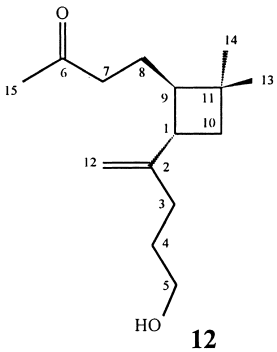

12

Compound **9** was identical with the sesquiterpene lactone matricarin.[26,27] The structure was supported with high resolution FAB-MS *m/z* 305.13875 $[M+H]^+$. The complete ^1H and ^{13}C NMR assignments were achieved with HMQC, HMBC and NOESY. Irradiation of the H-8 proton with different mixing times (τmix=25–90 ms), allowed the identification of the signals and coupling constants of H-5, H-6, H-7 and H-11 methine protons, as well as relative configuration with respect to H-8. The corresponding desacetyl matricarin (**10**) was previously reported.[28,29] A comparison of the ^1H chemical shifts of **9** and **10** showed the known acylation shifts and unambiguously proves the relation of these two compounds.

The next isolated component **11** was also a sesquiterpene with a eudesmane skeleton, and was identified as artecalin.[30] The high values of vicinal coupling constants (11 Hz) of the methine protons suggested that they all were in axial positions. Due to the axial orientation of the CH_3-14, the HMBC correlation map showed a strong response between the *trans* antiperiplanar located H-5/CH_3-14 atoms, whereas the gauche arrangement of the CH_3-15 and H-5 atoms, gave only a weak cross-peak.

Compound **12** showed a geminal dimethyl-substituted cyclo-butane ring with two further vicinal substituents, these latter were in the *trans* position, which was proven by the NOE responses between H-1/H_2-8 and H-9/H_Z-12 protons, respectively. The vicinal $J_{H\text{-}1,H\text{-}9}\sim 9$ Hz coupling was in accordance with a ca. 150° dihedral angle in the preferred conformation. Compound **12** was identical with 5-hydroxy-5,6-secocaryophyllene-6-on.[31]

In conclusion, the chemistry of *A. ligustica* was in agreement with other species of the genus *Achillea*. Only, one sesquiterpene lactone with 5/6/5 skeleton was reported from *A. crithmifolia*.[32] Additionally, sesquiterpene guaianolids are common in the genus,[12–14,19,20,25] in contract to chlorinated and seco-guaianolides which are rare.

3. Experimental

3.1. General

NMR spectra were recorded in chloroform-*d* at 300 K using a Bruker Avance DRX-500 spectrometer. Chemical shifts are given on the δ-scale. The pulse programs of the NMR experiments were taken from the Bruker software library. The gs-HMBC measurements were optimized to 7 Hz long-range couplings, whereas the NOESY experiments were run with 500 ms mixing time. MS measurements were recorded on Finnigan MAT 8430 and Micromass VG-ZAB-E instruments.

3.2. Isolation and structure elucidation

3.2.1. Plant material. The flowered aerial parts of *A. ligustica* All were collected June 1996 on Mt Parnitha (Attiki, Greece) at 1200 m. Plant material was identified by Dr T. Constandinidis, Department of Biotechnology, Institute of Systematic Botany, University of Athens. A voucher specimen of the collection (OT-6) has been deposited in the Herbarium of the University of Athens (ATHU).

3.2.2. Extraction and isolation. The aerial parts (1 kg) of *A. ligustica* were powdered and extracted with methanol-dichloromethane (1:1) at room temperature, and the solvent was evaporated under reduced pressure to give 35 g of a pale yellow extract. This extract was fractionated by dry-column chromatography on silica gel (Silica gel 60, 70–230 mesh, 1 kg, 6×80 cm) eluted with *n*-hexane-dichloromethane step-gradient. The first fraction (*n*-hexane-dichloromethane, 1:5, 20 mg) was purified on a Sephadex LH-20 column (*n*-hexane-dichloromethane-methanol, 7:3:0.5) to give *1,10-dioxo-1,10-secoguaia-4-en-6H-12,6-olide* (**3**) (5 mg), *3-methoxy-8-hydroxy-tanapartholide* (**5**) (4 mg) and *matricarin* (**9**) (15 mg). The second fraction (*n*-hexane-dichloromethane, 1:6, 28 mg) was separated by HPLC (methanol–water, 35:65) to afford ligustolide-A (1.5 mg), ligustolide-B (1.5 mg), 2,3-*trans*-dihydroxy-tanapartholide (**4**) (7 mg), desacetyl-matricarin (**10**) (11 mg) and 5-hydroxy-5,6-secocaryophyllen-6-on (**12**) (14 mg). The third fraction (*n*-hexane–dichloromethane, 1:7, 75 mg) was subjected to a Sephadex LH-20 column (*n*-hexane–dichloromethane-methanol, 7:3:1) to give *4α,10α-dihydroxy-1β,2β-epoxy-5α,7αH-guaia-11(13)-en-12,6α-olide*

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of **1** and **2** (in $CDCl_3$)

No.	**1**		**2**		
	δ_H[a]	δ_C[b]	δ_H	δ_C	HMBC
1		76.3 (s)		79.5	
2	2.38 (dd, 13.8, 5.7) 1.79 (dd, 13.8, 5.7)	42.4 (t)	2.25 (dd, 12.2, 5.8) 2.18 (dd, 15.2, 3.1)	40.5	C-5, 9
3	3.74 (t, 5.7)	84.8 (d)	3.54 (dd, 5.8, 3.1)	85.9	C-5
4		76.3 (d)		77.5	
5		158.4 (s)		156.8	
6	6.70 (s)	114.6 (d)	6.64	115.2	C-5, 7, 8
7		154.6 (s)		154.1	
8	5.36 (dq, 11.5, 1.7)	77.2 (d)	5.66 (dq, 11.8, 1.8)	77.1	
9	2.68 (d, 11.5)	60.0 (d)	2.28 (d, 11.8)	62.5	C-7, 8, 14
10		211.9 (s)		206.8	
11		121.2 (s)		122.0	
12		173.7 (s)		173.8	
13	1.97 (d, 1.7)	8.8 (q)	1.96 (d, 1.8)	8.8	C-7, 11, 12
14	2.43 (s)	33.4 (q)	2.48	30.6	
15	1.54 (s)	26.1 (q)	1.44 (s)	25.5	C-3, 4, 5
3-OCH_3	3.42 (s)	57.9 (q)	3.50	58.3	C-3

[a] Multiplicities and *J*-values in Hz are given in parentheses.
[b] Multiplicities were deduced from DEPT.

(**6**) (30 mg), *2,6-dimethyl-octa-1,7-dien-3,6-diol* (**7**) (17 mg), *2,6-dimethyl-octa-3,7-dien-2,6-diol* (**8**) (7 mg) and *artecalin* (**11**) (21 mg).

3.2.3. Ligustolide-A (1). Yellowish oil; IR ν_{max} (KBr film) cm^{-1}: 3490, 2922, 2845, 1748, 1707, 1370; CI-MS *m/z* (rel. int.) 309 (MH^+) (100), 291 (MH^+-H_2O); HR-CI-MS *m/z* 309.13382 (calcd for $C_{16}H_{20}O_6$, 309.13381); ^{1}H, ^{13}C NMR data see Table 1.

3.2.4. Ligustolide-B (1). Yellowish oil; IR and CIMS: similar to **1**; ^{1}H, ^{13}C NMR data see Table 1.

3.2.5. 3-Deshydroxy-*iso-seco*-tanapartholide (3). Colorless oil; IR ν_{max} (KBr film) cm^{-1}: 3427, 2922, 2845, 1773, 1696, 1650, 1461; FAB-MS *m/z* (rel. int.) 265 (MH^+)(22), 235 (MH^+-2CH_3) (12), 221 ($MH^+-2CH_3-CH_2$)(100); HR-FAB-MS *m/z* 265.14398 (calcd for $C_{15}H_{20}O_4$, 265.14470); ^{1}H NMR ($CDCl_3$, 500 MHz) 4.82 (1H, d, *J*=9.5 Hz, H-6), 2.61 (2H, m, H-3), 2.49 (1H, m, H-7), 2.47 (2H, m, H-9), 2.43 (2H, m, H-2), 2.35 (1H, dq, *J*=11.0, 7.0 Hz, H-11), 2.18 (3H, s, CH3-15), 2.11 (3H, s, CH_3-14), 1.81 (1H, m, H_X-8), 1.74 (1H, m, H_Y-8), 1.35 (3H, d, *J*=7.0 Hz, CH_3-13); ^{13}C NMR ($CDCl_3$, 125 MHz) 207.3 (s, C-10), 207.1 (s, C-1), 178.4 (s, C-12), 176.6 (s, C-4), 135.7 (s, C-5), 76.3 (d, C-6), 46.3 (d, C-7), 41.7 (d, C-11), 40.4 (t, C-9), 34.5 (t, C-2), 32.4 (t, C-3), 29.9 (q, CH_3-14), 25.3 (t, C-8), 17.8 (q, CH_3-15), 14.6 (q, CH_3-13).

3.2.6. 8-Hydroxy-3-methoxy-*iso-seco*-tanapartholide (4). Colorless oil; IR ν_{max} (KBr film) cm^{-1}: 3460, 2924, 1761, 1707, 1384; CI-MS *m/z* 309 (MH^+), 291 (MH^+-H_2O), 279 (MH^+-CH_2O); HR-CI-MS *m/z* 309.133154 (calcd for $C_{16}H_{20}O_6$, 309.133814); ^{1}H NMR ($CDCl_3$, 500 MHz) 6.43 (1H, d, *J*=2.7 Hz, H_Z-13), 5.73/5.74 (1H, d, *J*=2.7 Hz, H_E-13), 5.25/5.26 (1H, d, *J*=5.0 Hz, H-6), 4.31 (1H, d, *J*=2.8 Hz, H-3), 4.28 (1H, m, H-8), 3.40/3.41 (3H, s, H-3-OCH_3), 3.18/3.24 (1H, m, H-7), 2.66 (1H, d, *J*=2.8 Hz, H_Y-2), 2.65 (2H, m, H-9), 2.32 (1H, d, *J*=2.8 Hz, H_X-2), 2.19/2.21 (3H, s, CH_3-14), 2.15/2.16 (3H, s, CH_3-15); ^{13}C NMR ($CDCl_3$, 125 MHz) 209.0/209.1 (s, C-10), 202.8/203.1 (s, C-1), 170.9/171.3 (s, C-4), 169.8 (s, C-12), 134.7/134.8 (s, C-11), 138.5/138.8 (s, C-5), 124.3/124.6 (t, C-13), 79.7/79.9 (d, C-3), 72.4 (d, C-6), 68.9 (t, C-8), 57.50/57.54 (q, C-3-OCH_3), 48.1/48.3 (d, C-7), 44.9 (t, C-9), 40.7/40.8 (d, C-2), 30.8 (q, CH_3-14), 14.08/14.14 (q, CH_3-15).

3.2.7. 2α-Chloro-*iso-seco*-tanapartholide (5). White powder; IR ν_{max} (KBr film) cm^{-1}: 3474, 2923, 1768, 1648, 1466; CI-MS *m/z* 313 (MH^+), 295 (MH^+-H_2O); HR-CI-MS *m/z* 313.08206 (calcd for $C_{15}H_{17}O_5Cl$, 313.08427); ^{1}H NMR ($CDCl_3$, 500 MHz) 6.38 (1H, d, *J*=2.7 Hz, H_Z-13), 5.70 (1H, d, *J*=2.7 Hz, H_E-13), 5.00 (1H, d, *J*=5.0 Hz, H-6), 4.72 (1H, d, *J*=2.8 Hz, H-3), 4.22 (1H, d, *J*=2.8 Hz, H-2), 3.15 (1H, m, H-7), 2.59 (2H, m, H-9), 2.22 (3H, s, CH_3-15), 2.17 (3H, s, CH3-14), 2.02 (1H, m, H_X-8), 1.94 (1H, m, H_Y-8); ^{13}C NMR ($CDCl_3$, 125 MHz) 207.8 (s, C-10), 194.7 (s, C-1), 170.2 (s, C-4), 169.5 (s, C-12), 137.5 (s, C-11), 136.7 (s, C-5), 123.4 (t, C-13), 80.0 (d, C-3), 75.9 (d, C-6), 62.5 (d, C-2), 42.9 (d, C-7), 39.5 (t, C-9), 29.7 (q, CH_3-14), 27.5 (t, C-8), 13.9 (q, CH_3-15).

3.2.8. 4α,10α-Dihydroxy-1β,2β-epoxy-5α,7αH-guaia-11(13)-en-12,6α-olide (6). Greenish gummy material; IR ν_{max} (KBr film) cm^{-1}: 3474, 2923, 2851, 1768, 1379; CI-MS *m/z* (rel. int.) 315 (MH^+) (100), 297 (MH^+-H_2O) (23); HR-CI-MS *m/z* 315.099936 (calcd for $C_{15}H_{20}O_5Cl$, 315.099927); ^{1}H NMR ($CDCl_3$, 500 MHz) 6.20 (1H, d, *J*=3.5 Hz, HZ-13), 5.49 (1H, d, *J*=3.5 Hz, H_E-13), 4.34 (1H, dd, *J*=11.5, 9.7 Hz, H-6), 4.07 (1H, d, *J*=0.9 Hz, H-3), 3.70 (1H, d, *J*=0.9 Hz, H-2), 3.44 (1H, m, H-7), 2.63 (1H, d, *J*=11.5 Hz, H-5), 2.33 (1H, m, H-8), 2.05 (1H, m, H-9), 1.85 (1H, m, H-9), 1.56 (1H, m, H-8), 1.57 (3H, s, CH_3-15), 1.27 (3H, s, CH_3-14); ^{13}C NMR ($CDCl_3$, 125 MHz) 169.4 (s, C-12), 139.4 (s, C-11), 119.9 (t, C-13), 83.4 (s, C-4), 79.9 (d, C-6), 73.4 (s, C-1), 72.2 (s, C-10), 64.7 (d, C-2), 64.4 (d, C-3), 57.9 (d, C-5), 45.0 (d, C-7), 35.1 (t, C-9), 26.5 (q, CH_3-14), 23.5 (t, C-8), 22.1 (q, CH_3-15).

3.2.9. 2,6-Dimethyl-octa-1,7-dien-3,6-diol (7). Colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) 5.91/5.89 (1H, dd, *J*=17.3, 10.8 Hz, H-7), 5.23 (1H, dd, *J*=17.3, 1.3 Hz, H_a-8), 5.07

(1H, dd, J=10.8, 1.3 Hz, H_b-8), 4.96/4.95 (1H, d, J=1.5 Hz, H-1_a), 4.85/4.84 (1H, d, J=1.5 Hz, H-1_b), 4.07/4.06 (1H, t, J=5.7 Hz, H-3), 1.72 (3H, s, 2-CH_3), 1.63 (2H, m, H-4), 1.60 (2H, m, H-5), 1.30 (3H, s, 6-CH3); ^{13}C NMR ($CDCl_3$, 125 MHz) 147.5/147.3 (s, C-2), 144.9/144.8 (d, C-7), 112.0/111.9 (t, C-8), 110.9 (t, C-1), 76.2/75.7 (d, C-3), 72.9 (s, C-6), 38.2/37.6 (t, C-5), 29.3/29.1 (t, C-4), 28.3/28.0 (q, 6-CH_3), 17.9/17.7 (q, 2-CH_3).

3.2.10. 2,6-Dimethyl-octa-3,7-dien-2,6-diol (8). Colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) 5.93 (1H, dd, J=17.3, 10.7, Hz, H-7), 5.62 (1H, dt, J=15.6, 1.0 Hz, H-3), 5.60 (1H, ddd, J=15.6, 7.8, 6.6 Hz, H-4), 5.21 (1H, dd, J=17.3, 1.2 Hz, H_E-8), 5.06 (1H, dd, J=10.7, 1.2, Hz, H_Z-8), 2.29 (1H, ddd, J=13.8, 6.6, 1.0 Hz, Ha-5), 2.24 (1H, ddd, J=13.8, 7.8, 1.0 Hz, Hb-5), 1.32 (3H, s, H-1), 1.32 (3H, s, 2-CH_3), 1.28 (3H, s, 6-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) 144.7 (d, C-2), 142.6 (d, C-3), 121.7 (d, C-4), 112.0 (t, C-8), 72.7 (s, C-6), 70.8 (s, C-2), 45.0 (t, C-5), 29.9 (q, C-1), 29.9 (q, 2-CH_3), 27.4 (q, 6-CH_3).

3.2.11. Matricarin (9). Amouphous powder; IR (KBr) C=O lactone 1786, C=O ester 1742, C=O 1682, C=C 1637, 1618 cm−1; ^{1}H NMR ($CDCl_3$, 500 MHz) 6.20 (1H, s, H-3), 4.85 (1H, td, J=10.6, 2.0 Hz, H-8), 3.72 (1H, t, J=10.0 Hz, H-6), 3.41 (1H, d, J=10.0 Hz, H-5), 2.73 (1H, dd, J=13.6, 11.0 Hz, H-9), 2.50 (1H, dd, J=12.0, 7.0 Hz, H-11), 2.45 (3H, s, CH_3-14), 2.39 (1H, dd, J=13.6, 2.0 Hz, H-9), 2.33 (1H, ddd, J=12.0, 10.0, 2.0 Hz, H-7), 2.31 (3H, s, CH_3-15), 2.12 (3H, s, CH_3-17), 1.35 (3H, d, J=7.0 Hz, CH_3-13); ^{13}C NMR ($CDCl_3$, 125 MHz) 195.1 (s, C-2), 176.7 (s, C-12), 169.7 (s, C-16), 169.5 (s, C-4), 145.0 (s, C-10), 135.9 (d, C-3), 133.2 (s, C-1), 81.1 (d, C-6), 70.3 (d, C-8), 59.1 (d, C-7), 51.5 (d, C-5), 44.7 (t, C-9), 40.7 (d, C-11), 21.4 (q, C-14), 21.1 (q, C-17), 19.9 (q, C-15), 15.0 (q, C-13); HR-FAB-MS $[M+H]^+$ m/z 305.13875 (calcd for $C_{17}H_{21}O_5$, 305.13889). Characteristic NOE responses: H-3/H-15, H-5/H-7, H-5/H-9, H-6/H-8, H-6/H-11.

3.2.12. Desacetyl-matricarin (10). Colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) 6.17 (1H, s, H-3), 3.74 (1H, td, J=11, 2.1 Hz, H-8), 3.65 (1H, t, J=11 Hz, H-6), 3.39 (1H, d, J=11.0 Hz, H-5), 2.80 (1H, dd, J=13.8, 11.0 Hz, H_{ax}-9), 2.55 (1H, dq, J=11, 6.9 Hz, H-11), 2.43 (3H, s, CH_3-14), 2.36 (1H, dd, J=13.6, 2.0 Hz, H_{eq}-9), 2.31 (3H, s, CH_3-15), 2.13 (1H, t, J=11 Hz, H-7), 1.46 (3H, d, J=6.9 Hz, CH_3-13); ^{13}C NMR ($CDCl_3$, 125 MHz) 205.1 (s, C-2), 177.5 (s, C-12), 169.9 (s, C-4), 145.3 (s, C-10), 135.7 (d, C-3), 133.0 (s, C-1), 81.0 (d, C-6), 69.7 (d, C-8), 61.5 (d, C-7), 51.6 (d, C-5), 49.1 (t, C-9), 41.3 (d, C-11), 21.7 (q, CH3-14), 19.9 (q, CH_3-15), 15.5 (q, CH_3-13).

3.2.13. Artecalin (11). Colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) 6.12 (1H, d, J=3.3 Hz, H_a-12), 5.45 (1H, d, J=3.3 Hz, H_b-12), 3.99 (1H, t, J=11 Hz, H-6), 3.69 (1H, dd, J=11.7, 5.5 Hz, H-1), 2.74 (1H, dd, J=15.0, 5.5 Hz, H-2_a), 2.56 (1H, dd, J=15.0, 11.7 Hz, H-2_b), 2.54 (1H, t, J=11 Hz, H-7), 2.52 (1H, dq, 11, 6.9 Hz, H-4), 2.19 (1H, dm, J=12 Hz, H-9_a), 2.13 (1H, dm, J=12 Hz, H-8_a), 1.65 (1H, dddd, J=12, 12, 12, 3.8 Hz, H-8_b), 1.54 (1H, t, J=11 Hz, H-5), 1.36 (1H, td, J=12, 3.8 Hz, H-9_b), 1.29 (3H, d, 6.9 Hz, CH_3-15), 1.13 (3H, s, CH_3-14); ^{13}C NMR ($CDCl_3$, 125 MHz) 208.0 (s, C-3), 170.1 (s, C-13), 138.4 (s, C-11), 117.7 (t, C-12), 82.7 (d, C-6), 76.2 (d, C-1), 50.5 (d, C-7), 50.5 (d, C-5), 46.5 (t, C-2), 44.5 (d, C-4), 42.0 (s, C-10), 36.2 (t, C-9), 21.3 (t, C-8), 13.6 (q, CH_3-15), 11.8 (q, CH_3-14).

3.2.14. 5-Hydroxy-5,6-secocaryophyllen-6-on (12). Yellowish oil; ^{1}H NMR ($CDCl_3$, 500 MHz) 4.76 (1H, t, J=1.2 Hz, H_a-12), 4.74 (1H, t, J=1.2 Hz, H_b-12), 3.67 (2H, t, J=6.5 Hz, H-5), 2.41 (1H, ddd, J=10, 10, 9 Hz, H-1), 2.36 (2H, ddd, J=9.1, 6.7, 1.7 Hz, H-7), 2.12 (3H, s, CH_3-15), 2.06 (2H, t, J=7 Hz, H-3), 1.87 (1H, dt, J=9, 7.5, 7.5 Hz, H-9), 1.81 (1H, dd, J=10.5, 9.5 Hz, H-10_a), 1.70 (2H, tt, J=7, 6.5 Hz, H-4), 1.55 (2H, m, H-8), 1.45 (1H, t, J=10.5 Hz, H-10_b), 1.06 (3H, s, CH_3-14), 1.05 (3H, s, CH_3-13); ^{13}C NMR ($CDCl_3$, 125 MHz) 209.0 (s, C-6), 152.2 (s, C-2), 107.2 (t, C-12), 62.8 (t, C-5), 47.9 (d, C-9), 42.0 (t, C-7), 41.4 (d, C-1), 39.8 (t, C-10), 33.6 (s, C-11), 31.1 (q, CH_3-14), 30.8 (t, C-4), 30.8 (t, C-3), 29.9 (q, CH_3-15), 24.7 (t, C-8), 22.4 (q, C-13).

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References

[1] Wichtl, M. *Herbal Drug and Phytopharmaceuticals*. Scientific Publishers: Stuttgart, 1994.
[2] Goldberg, A. S.; Mueller, E. C.; Eigen, E.; De Salve, S. *J. Pharm. Sci.* **1969**, *58*, 883–941.
[3] Orkiszewska, A.; Lobarzewsky, R.; Jedrizejewska, M. Polish Patent, B1 119889, 1985.
[4] Valant-Vetschera, K. H.; Wollenweber, E. *Biochem. Syst. Ecol.* **1988**, *16*, 403–409.
[5] Ahmed, A. A.; El-Sayed, N. H.; Mabry, T. J. *Revista Latinoamericana de Quimica* **1989**, *20*, 5.
[6] Todorova, M. N.; Krasteva, M. I.; Markova, M. M.; Tsankova, E. T.; Taskova, R. M.; Peev, D. R. *Phytochemistry* **1998**, *49*, 2371–2374.
[7] Sabine, G.; Armin, P.; Disan, G.; Ingrid, W.; Samdan, N.; Ernest, H.; Johann, J.; Wolfgang, K. *Phytochemistry* **2001**, *58*, 1189–1194.
[8] Ulubelen, A.; Őksűz, S.; Schuster, A. *Phytochemistry* **1990**, *29*, 3948–3949.
[9] Rucker, G.; Manns, D.; Breuer, L. *Archive Pharmaceutical* **1990**, *32*, 678–679.
10. (a) Stefanovic, M.; Djermanovic, V.; Gorunvic, M.; Dermanovic, M.; Macura, S.; Miloslavlijevic, S. *Phytochemistry* **1989**, *28*, 1765–1767. (b) Ahmed, A. A.; Jakupovic, J.; Seif El-Dein, A. A.; Melek, F. R. *Phytochemistry* **1990**, *29*, 1322–1324.
[11] Balboul, B. A. A. A.; Ahmed, A. A.; Otsuka, H.; Bando, M.; Kido, M.; Takeda, Y. *Phytochemistry* **1997**, *46*, 1045–1049.
[12] Ahmed, A. A.; Mahmoud, A. A.; Ali, E. T.; Tzakou, O.;

Couladis, M.; Mabry, T. J.; Gáti, T.; Tóth, G. *Phytochemistry* **2002**, *59*, 851–856.
[13] Tzakou, O.; Couladis, M.; Verykokidou, E. *Biochem. Syst. Ecol.* **1995**, *23*, 569–570.
[14] Tzakou, O.; Skaltsa, H.; Harvala, C. *Sc. Pharma.* **1996**, *64*, 197–202.
[15] Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783–2792.
[16] Őksűz, S. *Phytochemistry* **1990**, *29*, 887–890.
[17] Huneck, S.; Zdero, C.; Bohlmann, F. *Phytochemistry* **1986**, *25*, 883–889.
[18] Tan, R. X.; Jia, Z. J.; Jakupovic, J.; Bohlmann, F.; Huneck, S. *Phytochemistry* **1991**, *30*, 3033–3035.
[19] Bohlmann, F.; Zdero, C. *Phytochemistry* **1982**, *21*, 2543–2549.
[20] Appendino, G.; Jakupovic, J.; Ozen, H. C.; Schuster, N. *Phytochemistry* **1993**, *34*, 1171–1172.
[21] Tan, R. X.; Jia, Z. J. *Phytochemistry* **1992**, *31*, 2158–2159.
22. Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*. VCH: Weinheim, 1987; pp 288.
[23] Wagner, H.; Fessler, B.; Lotter, H.; Wray, V. *Planta Medica* **1988**, *54*, 171–172.
[24] Knapp, H.; Straubimger, M.; Fornari, S.; Oka, N.; Watanabe, N.; Winterhalter, P. *J. Agric. Food Chem.* **1998**, *46*, 1966–1970.
[25] Bruno, M.; Herz, W. *Phytochemistry* **1988**, *27*, 1871–1872.
[26] Eekan, Z.; Procházka, V.; Herout, V.; Šorm, F. *Collect. Czech. Chem. Commun.* **1958**, *24*, 1554–1556.
[27] Romo, J.; Romo de Vivar, A.; Trevino, R.; Joseph-Nathan, P.; Díaz, E. *Phytochemistry* **1970**, *9*, 1615–1621.
[28] Shafizadeh, F.; Bhadane, N. bR.; Morris, M. S.; Kelsey, R. G.; Khanna, S. N. *Phytochemistry* **1971**, *10*, 2745–2754.
[29] Lee, K. H.; Simpson, R. F.; Geissman, T. A. *Phytochemistry* **1969**, *8*, 1515–1521.
[30] Yamakawa, K.; Nishitani, K.; Tominaga, T. *Tetrahedron Lett.* **1975**, *33*, 2829–2832.
[31] Bohlmann, F.; Zdero, C.; King, R. M.; Robinson, H. *Liebigs Ann. Chem.* **1984**, 503–511.
[32] Todorova, M. N.; Vogler, B.; Tsankova, E. T. N. *Prod. Lett.* **2000**, *14*, 463–468.