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Secondary metabolites of *Peucedanum tauricum* fruits

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Abstract

From the essential oil of fruits of *Peucedanum tauricum* Bieb., two guaiane type sesquiterpene hydrocarbons guaia-1(10),11-diene (**1**) and guaia-9,11-diene (**2**) were identified. The structures of **1** and **2** were assigned by 1D and 2D NMR analysis. The relative configurations of the compounds were established by 2D-NOESY experiments while the absolute configurations were deduced through chemical correlations with (+)-γ-gurjunene (**9**) and capillary GC analysis using modified cyclodextrins as the stationary phases. From the dichloromethane extract of the less volatile fraction of the fruits, coumarins, viz. peucedanin (**3**), oxypeucedanin hydrate (**4**) and officinalin isobutyrate (**5**) were isolated. Compound **5** was confirmed to be 6-carbomethoxy-7-isobutyroxycoumarin by its 1D and 2D NMR data as well as by conversion into officinalin (**7**) by alkaline hydrolysis. Peuruthenicin, a positional isomer of officinalin, is assigned structure **8** on spectral basis. Bergapten (**6**) was identified by its mass spectrum. This is the first report on the isolation of compounds **4** and **5** from *P. tauricum*.

Keywords: *Peucedanum tauricum* Bieb.; Apiaceae; Essential oil; Guaia-1(10),11-diene; Guaia-9,11-diene; Coumarins; Peucedanin; Officinalin isobutyrate; Oxypeucedanin hydrate; Peuruthenicin; Bergapten

1. Introduction

Peucedanum tauricum Bieb. is an endemic perennial plant of the Apiaceae family, growing in nature at dry hillsides and pinewoods in Crimea, Caucasus, and in Romania (Tutin et al., 1968; Grosgiejm, 1967; Sziszkin, 1955). Previous chemical studies of the plant concerned the identification of phenolic acids in the foliage and fruits (Bartnik et al., 2003), GC/MS analysis of the essential oil of the fruits in which a number of sesquiterpene hydrocarbons were identified (Bartnik et al., 2002), isolation of coumarins from the fruits (Glowniak et al., 2002), isolation of an analog of chlorogenic acid and a chromone from the roots (Baranauskaite, 1970), determination of saponins in roots and fruits (Baranauskaite, 1968) as well as isolation of peucedanin from a combined extract of *P. tauricum* and *P. calcareum* (Baranauskaite and Nikonov, 1965). Here, we report the isolation and structural elucidation of two new guaiane type sesquiterpene hydrocarbons from the essential oil of the fruits of the title plant and isolation of coumarins from the high boiling dichloromethane fraction. In addition, revision of the structures of officinalin isobutyrate, officinalin as well as peuruthenicin is reported.

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† W.A. König passed away on 19th November 2004; his scientific achievements will keep him among us.

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2. Results and discussion

The essential oil obtained from the fruits of *P. tauricum* was analysed by coupled gas chromatography and mass spectrometry (GC/MS). Mass spectra and retention indices of the oil constituents were compared with a library of mass spectra of authentic compounds established under identical experimental conditions (Joulain and König, 1998; Hochmuth et al., 2003). Monoterpene hydrocarbons consisting of tricyclene, myrcene, limonene, (*Z*)-β-ocimene, 2,4(8)-*p*-menthadiene, the oxygenated monoterpene linalool and sesquiterpene hydrocarbons comprising α-ylangene, α-copaen, β-bourbonene, guaia-6,9-diene, selina-5,11-diene, valerena-4,7(11)-diene, γ-amorphene, γ-humulene, α-bulnesene, β-elemene, (*E*)-β-caryophyllene, α-guaiene, α-humulene, and γ-gurjunene were identified. The latter five sesquiterpenes have been earlier identified along with α- and β-selinene and γ-cadinene (Bartnik et al., 2002). Two unknown components were isolated by preparative gas chromatography. Their structures were elucidated by 1D and 2D NMR techniques to be guaia-1(10),11-diene (**1**) and guaia-9,11-diene (**2**), (Fig. 1). The relative configurations of the new compounds were established through 2D NOESY experiments. Their absolute configurations were assigned according to chemical correlations and capillary GC analysis using modified cyclodextrins as stationary phases (König, 1992; König et al., 1999).

In addition, from the less volatile dichloromethane fraction, known coumarins, peucedanin (**3**), oxypeucedanin hydrate (**4**), and officinalin isobutyrate (**5**) were isolated. (Fig. 1). Their structures were established by MS, 1D and 2D NMR data as well as comparison with the literature data (Perel'son et al., 1971; Gonzalez et al., 1976, 1978; Patra and Mitra, 1981; Sun et al., 1982). In addition, the structure of compound **5** was confirmed according to its 1D and 2D NMR data as well as by converting it into officinalin (**7**) by alkaline hydrolysis, thereby clearing the confusion concerning its structure. The earlier reported structure of peuruthenicin (Soine et al., 1973), an isomer of officinalin, is amended to be **8** based on its ^{1}H NMR. Bergapten (**6**) previously reported from the fruits (Glowniak et al., 2002) was identified by its mass spectrum. This is the first report on the isolation of officinalin isobutyrate and oxypeucedanin hydrate from *P. tauricum*.

2.1. Guaia-1(10),11-diene (1)

The mass spectrum of **1** exhibited a molecular ion signal at *m/z* 204 indicating an elemental composition of $C_{15}H_{24}$, typical for a sesquiterpene hydrocarbon with four degrees of unsaturations. The ^{1}H NMR spectrum and 2D HMQC experiment indicated six methylene groups, three methine and three methyl groups confirming the presence of twenty four protons directly attached to carbon atoms. Signals at δ 1.61 (H_3-14) and 1.71 (H_3-

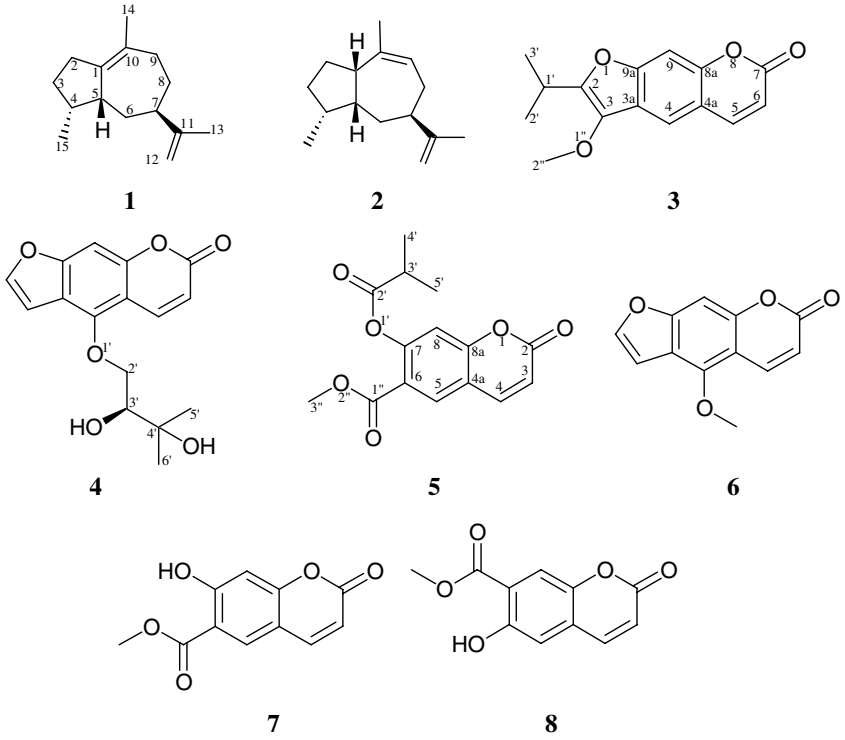

Fig. 1. Selected compounds from *P. tauricum*.

Table 1
Observed ^{1}H, ^{1}H COSY and HMBC correlations for compound **1**

^{1}H, ^{1}H COSY		HMBC	
Hydrogen	Correlated with	Carbon	Correlated with
H_2-2	H_2-3	C-1	H_2-3, H-5, H-4, H_3-14
H_2-3	H_2-2, H-4	C-2	H_2-3, H-4
H-4	H_3-15, H_2-3, H-5	C-3	H_3-15, H-4
H-5	H_2-6, H-4	C-4	H_3-15, H_2-3, H_2-6
H_2-6	H-5, H-7	C-5	H_3-15, H_2-3, H-4, H-7
H-7	H_2-6, H_2-8, H_2-12 (^3J)	C-6	H_2-8, H-7
H_2-8	H_2-9	C-7	H_2-12, H_2-8, H_3-13
H_3-13	H_2-12 (^3J)	C-8	H_2-6, H-7
		C-9	H_3-14, H_2-8
		C-10	H_3-14, H_2-8, H_2-9
		C-11	H_2-12, H-7, H_3-13
		C-12	H_3-13, H-7
		C-15	H_2-3, H-4

Table 2
Observed ^{1}H, ^{1}H COSY and HMBC correlations for compound **2**

^{1}H, ^{1}H COSY		HMBC	
Hydrogen	Correlated with	Carbon	Correlated with
H-1	H_2-2	C-1	H_2-3, H_2-2, H_3-14, H-5
H_2-2	H_2-3	C-2	H_2-3, H-5
H_2-3	H_2-2, H-4	C-3	H_3-15, H_2-2, H-5
H-4	H_3-15, H_2-3, H-5	C-4	H_2-3, H_2-2, H-5
H-5	H_2-6, H-4, H-1	C-5	H_3-15, H_2-3, H-7
H_2-6	H-5, H-7	C-6	H_2-8, H-5,
H-7	H_2-6, H_2-8, H_2-12 (^3J)	C-7	H_2-6, H_3-13, H_2-8, H-5
H_2-8	H-9	C-8	H_2-6, H-7
H_3-13	H_2-12 (^3J)	C-9	H_3-14
		C-10	H_3-14
		C-11	H_2-6, H_3-13, H-7
		C-12	H_3-13, H-7
		C-15	H_2-3, H-5

13) of two of the methyl groups were indicative of an allylic position while the signal at δ 4.84 (H_2-12) was indicative of an exocyclic methylene group. The ^{13}C NMR spectrum showed signals of a total of 15 carbon atoms comprising three primary, six secondary (including one exocyclic olefinic), three tertiary and three olefinic quaternary carbon atoms. Therefore, two of the four unsaturations were due to double bonds, suggesting a structure of a doubly unsaturated bicyclic sesquiterpene hydrocarbon for **1**.

In the ^{1}H, ^{1}H COSY spectrum of **1** (Table 1), the coupling between the protons of the allylic methyl singlet at δ 1.71 (H_3-13) and the exocyclic olefinic methylene protons at δ 4.84 (H_2-12) indicated the presence of an isopropenyl group. This was further substantiated by an HMBC experiment (Table 1), which exhibited couplings between protons of the allylic methyl singlet at δ 1.71 (H_3-13) with both the exocyclic olefinic methylene carbon at δ 108.92 (C-12) and an olefinic quaternary carbon at δ 150.32 (C-11). The presence of a methyl substituted five membered ring substructure was concluded from couplings observed in the ^{1}H, ^{1}H COSY spectrum between each of the two methylene protons centred at δ 2.15 (H_a-2) and 2.32 (H_b-2) and methylene protons centred at δ 1.36 (H_a-3) and 1.58 (H_b-3). The latter was also coupled to a methine multiplet at δ 1.99 (H-4) that in turn coupled to the secondary methyl doublet at δ 0.77 (H_3-15) and to a methine proton at δ 2.73 (H-5). These observations and examination of the remaining signals in the 2D ^{1}H, ^{1}H COSY and HMBC spectra indicated a guaiane skeleton with double bonds between C-1 and C-10 as well as between C-11 and C-12 as depicted in Fig. 1.

The relative configuration of **1** was determined by a 2D NOESY experiment. Correlations between H-4/H-5 indicated that the two methine protons were oriented in the same direction. Moreover, the absence of NOESY correlations between the methine protons H-5 and H-7 suggested a trans configuration. Considering the bulky isopropenyl group to keep a *pseudo* equatorial position and being β-oriented, H-7 has to be α (axial orientation). Consequently, H-5 and H-4 have to be β-oriented.

2.2. Guaia-9,11-diene (2)

Compound **2** exhibited similar spectral properties to **1** except that in the ^{13}C NMR spectrum instead of the olefinic quaternary resonance at δ 127.6 a methine resonance was displayed at δ 49.8 and instead of one of the ring methylene resonances at δ 33.8 an olefinic methine resonance at δ 122.0 appeared. Analysis of ^{1}H, ^{1}H COSY and HMBC NMR data (Table 2) revealed compound **2** to show the position of the ring double bond between C-9 and C-10. The depicted relative stereochemistry in **2** was established through a 2D NOESY experiment. Key NOESY correlations were seen between H-5/H-1, H-5/H-4 which led to the conclusion that these methine protons were on the same side of the ring. In addition, the fact that a NOESY correlation was exhibited between H-5/H-1 indicated that the ring was *cis*-fused.

2.3. Absolute configurations of 1 and 2

In order to determine the absolute configurations of the new guaiane sesquiterpenes, an authentic reference substance, (+)-γ-gurjunene (**9**) (Sigma–Aldrich), showing a guaiane skeleton of known absolute configuration, was hydrogenated. Each of the two new compounds was also hydrogenated separately. The hydrogenation products of **9** and the hydrogenation products of **1** and **2** were analysed by capillary GC using modified cyclodextrins as stationary phases which separated the diastereomeric products (Fig. 2) formed upon hydrogenation (König, 1992; König et al., 1999). At least one of the hydrogenation products of **1**, **2** and **9** (compound **10**,

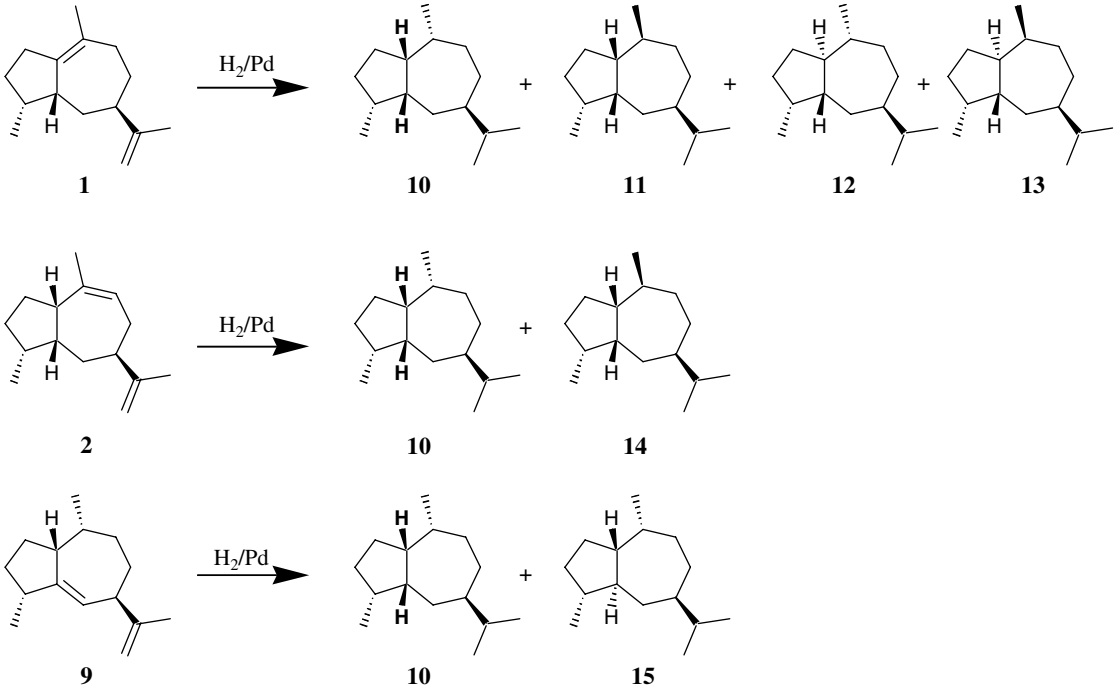

Fig. 2. Hydrogenation products of compounds **1**, **2** and **9**.

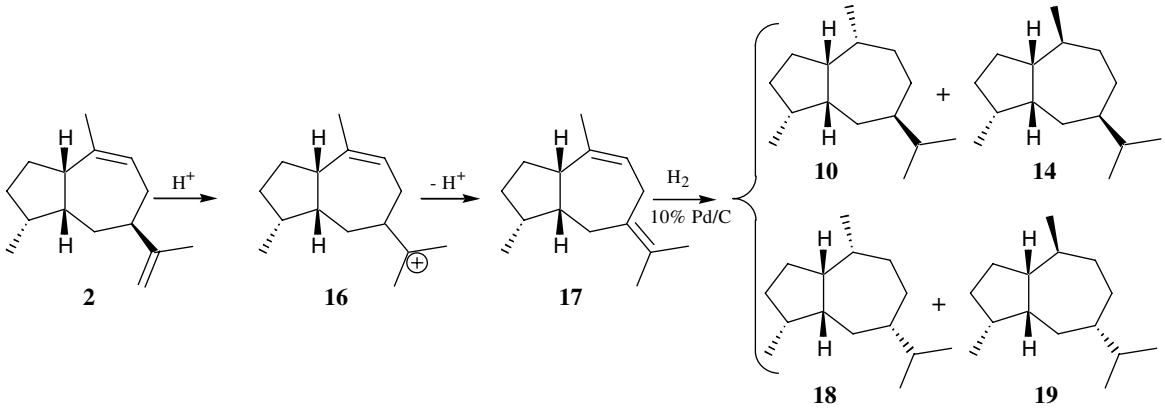

Fig. 3. Rearrangement of the C-11 double bond in **2** and its hydrogenation products.

in Fig. 2) should exhibit identical retention times, provided that the corresponding chiral centres of compounds **1** and **2** show the same absolute configuration as in compound **9**. This was found to be the caseon both 2,6-di-OMe-3-*O*-pentyl-γ-cyclodextrin and 6-*O*-TBDMS-2,3-di-OMe-β-cyclodextrin. As a result, the absolute configuration of **1** is (4*R*,5*R*,7*R*)-guaia-1(10),11-diene while **2** is (1*S*,4*R*,5*R*,7*R*)-guaia-9, 11-diene. In addition to the expected hydrogenation products shown in Fig. 2, formation of minor hydrogenation products were observed by enatioselective GC. The products and the possible pathway are shown for compound **2** (Fig. 3). The rearrangement of the double bond could have been induced by the acidity of the Pd/C catalyst, which arises from residual acids and/or palladium chloride in the production process of the catalyst (Ikawa et al., 2004).

2.4. *Officinalin isobutyrate (5)*

There is some confusion in the literature concerning the structures of the coumarins peuruthenicin (Soine et al., 1973) and officinalin (Gonzalez et al., 1976) as well as their corresponding acetates and isobutyrates. Officinalin isolated from *P. officinale* was reported to be 6-carbomethoxy-7-hydroxycoumarin (**7**) on the basis of its spectral data (Gonzalez et al., 1976). Peuruthenicin isolated from *P. ruthenicum* was independently assigned the same structure as officinalin on the basis of its spectral data as well as on chemical evidence

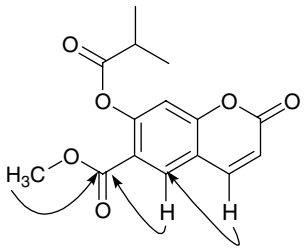

Fig. 4. Key HMBC correlations showing the position of the carbomethoxy group in compound **5**.

(Soine et al., 1973). Soon, it was noted (Ahluwalia and Prakash, 1977) that the two natural products that show the same molecular formula and very similar patterns of ^{1}H NMR spectra exhibit different m.p.s., 197–198 °C for the former and 162–164 °C for the latter. The observed discrepancy led to the re-evaluation of the structures of peuruthenicin as well as its acetate and isobutyrate by comparison of mainly m.ps. with synthetic samples. As a result, the proposed structure of peuruthenicin was concluded to be correct while that of officinalin, its acetate, and isobutyrate were reported to be untenable and had to be revised (Ahluwalia and Prakash, 1977). The confusion essentially concerns the positions of the carbomethoxy group as well as the hydroxyl group. This could be clarified as described below.

Sample of compound **5** obtained as white crystals exhibited a molecular ion peak at *m*/*z* 290. NMR spectral data revealed the presence of 15 carbon atoms indicating an elemental composition of $C_{15}H_{14}O_6$. In addition, it was observed that its ^{1}H NMR data as well as the m.p. (see experimental) were in good agreement with data reported for officinalin isobutyrate (Gonzalez et al., 1976). In the HMBC spectrum of **5**, both the methoxy proton signal at δ 3.9 (3H, *s*) as well as the aromatic methine signal at δ 8.18 (1H, *s*, H-5) correlated strongly to the carbomethoxycarbonyl carbon at δ 164.3 (Fig. 4). In addition, one of the AB system methine doublets at δ 7.71 (H-4, 1H, *d*, $J = 10$) was correlated to C-5 (δ 131.9). This observation clearly showed the carbomethoxy substituent to be linked to C-6 leading to 6-carbomethoxy-7-isobutyroxycoumarin as the structure of **5**, exactly the same as that reported for officinalin isobutyrate (Gonzalez et al., 1976). Furthermore **5** could be hydrolysed to yield **7,** that still kept the carbomethoxy moiety. The ^{1}H NMR of **7** was recorded (see experimental) and was found to be in agreement with that of officinalin (Gonzalez et al., 1976) rather than that of peuruthenicin (Soine et al., 1973). While this evidence confirms the structures of officinalin isobutyrate as well as officinalin, it also shows that the structure of peuruthenicin (Soine et al., 1973) could be 7-carbomethoxy-6-hydroxycoumarin (**8**). This is also supported by its ^{1}H NMR that displayed signals of a pair of AB system aromatic doublets, two aromatic singlets, a methoxy singlet as well as a hydroxy signal (Soine et al., 1973).

3. Experimental

3.1. General

The plant material used in this study was collected in September 2002 and 2003 in the Botanical Garden of Maria Curie-Sklodowska University (UMCS) in Lublin, Poland. The identity of plant material was established by K. Dbrowska, a botanical specialist from UMCS. Voucher specimen are deposited in the herbarium of the Medicinal Plant Laboratory, Department of Pharmacognosy (Skubiszewski Medical University in Lublin). The NMR spectra were recorded on Bruker WM 400 or 500 MHz spectrometers in either deuterated benzene (C_6D_6) or deuterated chloroform ($CDCl_3$). The chemical shift values are reported with reference to TMS, and the coupling constants are given in Hz. Optical rotations were measured as solutions in benzene on a Perkin–Elmer 341 polarimeter at 589 nm and 20 °C.

3.2. Hydrodistillation, extraction and isolation procedure

Cleaned, air dried, and pulverized fruits of *P. tauricum* were powdered under liquid nitrogen, homogenized and hydrodistilled in a Clevenger type apparatus for 2.5 h (Peyron, 1992), and a slightly greenish oil was collected in HPLC grade hexane. The oil was preliminarily analysed on an Orion capillary GC with FID detector, containing 2 columns, a 25 m × 0.25 mm i.d. non-polar CPSil-5 and a more polar CPSil-19 (chrompack) column of identical dimensions. The oven temperature was programmed from 50 to 230 °C at a rate of 3 °C/min. The injector and detector temperatures were kept at 200 and 250 °C, respectively. The oil was then further analysed using GC/MS on a HP 5890 GC coupled to a VG Analytical 70-250S mass spectrometer with electron impact (70 eV) ionisation. The crude oil was fractionated on a modified Varian 1400 preparative gas chromatograph, equipped with a stainless steel column (1.85 m × 4.3 mm) packed with Chromosorb W-HP coated with 10% polydimethylsiloxane SE 30. Subsequently, **1** and **2** were isolated from the fifth fraction using the same preparative GC equipped with a column (2 m × 5.3 mm) packed with chromosorb G-HD coated with 2.5% 2,6-OMe-3-*O*-pentyl-γ-cyclodextrin in OV 1701 (1:1, w/w).

The aqueous portion of the residue from the hydrodistillation was separated from the solid through filtration and was extracted with dichloromethane in order to obtain the high boiling fractions. The dichloromethane was removed under vacuum to give a dark oily residue. Part of the extract was subjected to column chromatography

on a silica gel column using dichloromethane and a methanol gradient. Fractionation was monitored by thin layer chromatography and UV detection. Fractions with similar compositions were combined. Further separations were carried out by HPLC (MERK-HITACHI) using an RP-18 column (Prodigy 5u ODS(3), 250 × 4.6 mm, Phenomenex) and acetonitrile as the mobile phase at a flow of 1 ml/min.

3.3. Hydrogenation

Hydrogenation of sesquiterpene hydrocarbons was performed by bubbling hydrogen gas through stirred solutions of ca. 0.5 mg samples in 1 ml hexane and 0.25 mg 10% Pd/C (Fluka) at room temperature for 1 h. The reaction mixture was filtered, and the products were analysed by GC and GC/MS.

3.4. Hydrolysis of officinalin isobutyrate

Officinalin isobutyrate (ca. 1 mg) was stirred overnight with 20% aqueous sodium hydroxide solution (5 ml). The solution was diluted to 20 ml with water, acidified, and extracted with chloroform. The chloroform was removed under vacuo, and the conversion was confirmed by MS and NMR.

3.5. Guaia-1(10),11-diene (**1**) (5β-isopropenyl-3α,8-dimethyl-1,2,3,3aβ,4,5,6,7-octahydroazulene)

$C_{15}H_{24}$, Colourless oil, RI_{CPSil5} = 1517, sense of optical rotation (benzene): (+) ^{1}H NMR (500 MHz, C_6D_6): δ 0.77 (3H, *d*, J = 7.0, H_3-15), 1.36 (1H, *m*, H_a-3), 1.58 (1H, *m*, H_b-3), 1.61 (3H, *s*, H_3-14), 1.68 (1H, *m*, H_a-8), 1.71 (3H, *s*, H_3-13), 1.74 (2H, *m*, H_2-6), 1.88 (1H, *m*, H_b-8), 1.99 (1H, *m*, H-4), 2.14 (2H, *m*, H_2 -9), 2.15 (1H, *m*, H_a-2), 2.28 (1H, *m*, H-7), 2.32 (1H, *m*, H_b-2), 2.73 (1H, *m*, H-5), 4.84 (2H, 2*s*, H_2-12); ^{13}C NMR (400 MHz, C_6D_6): δ 14.5 (C-15), 22.1 (C-13), 22.2 (C-14), 29.8 (C-8), 30.1 (C-2), 32.1 (C-6), 32.3 (C-3), 33.8 (C-9), 39.1 (C-4), 41.7 (C-5), 43.5 (C-7), 108.9 (C-12), 127.6 (C-10), 139.1 (C-1), 150.3 (C-11); MS (EI, 70 eV), *m*/*z* (rel. inten.): 204 [M^+] (30), 189 (70), 175 (25), 161 (82), 147 (38), 133 (41), 119 (62), 107 (87), 93 (100), 79 (87), 67 (40), 55 (58), 41 (39).

3.6. Guaia-9,11-diene (**2**) (5β-isopropenyl-3α,8-dimethyl-1,2,3,3aβ,4,5,6,8aβ-octahydroazulene)

$C_{15}H_{24}$, Colourless oil, RI_{CPSil5} = 1521, sense of optical rotation (benzene): (+) ^{1}H NMR (500 MHz, C_6D_6): δ 0.88 (3H, *d*, J = 7.0, H_3-15), 1.10 (1H, *m*, H_a-3), 1.50 (2H, *m*, H_2-6), 1.52 (1H, *m*, H_a-2), 1.55 (1H, *m*, H_b-3), 1.65 (3H, *s*, H_3-14), 1.68 (3H, *s*, H_3-13), 1.80 (1H, *m*, H_b-2), 1.86 (1H, *m*, H-4), 1.90 (1H, *m*, H_a-8), 2.18 (1H, *m*, H-5), 2.44 (1H, *m*, H-7), 2.45 (1H, *m*, H_b-8), 2.51 (1H, *m*, H-1), 4.83 (2H, 2*s*, H_2-12), 5.47 (1H, *m*, H-9); ^{13}C NMR (400 MHz, C_6D_6): δ 16.1 (C-15), 21.5 (C-14), 24.2 (C-13), 25.9 (C-6), 28.9 (C-2), 29.8 (C-8), 30.1 (C-3), 39.2 (C-4), 40.2 (C-5), 42.7 (C-7), 49.8 (C-1), 109.2 (C-12), 122.0 (C-9), 139.0 (C-10), 150.3 (C-11); MS (EI, 70 eV), *m*/*z* (rel. inten.): 204 [M^+] (30), 189 (70), 175 (25), 161 (42), 147 (38), 133 (41), 119 (62), 107 (87), 93 (100), 81 (87), 67 (40), 55 (58), 41 (39).

3.7. Peucedanin (**3**)

$C_{15}H_{14}O_4$, White solid, m.p. 108–109 °C; MS (EI, 70 eV), *m*/*z* (rel. inten.): 258 [M^+] (25), 243 (100), 228 (10), 200 (10), 189 (5), 171 (5), 160 (5), 144 (2), 115 (5), 108 (4), 88 (2), 76 (2), 69 (2), 51 (2), 39 (2). ^{1}H NMR, ($CDCl_3$, 500 MHz): 1.36 (6H, *d*, J = 7.0, H_3-2′, H_3-3′); 3.25 (1H, *sep*. J = 7.0, H-1′); 3.95 (3H, *s*, H3-2″); 6.37 (1H,*d*, J = 10.0, H-5); 7.33 (1H, *s*, H-9); 7.57 (1H, *s*, H-4); 7.79 (1H, *d*, J = 10.0, H-6).^{13}C NMR, ($CDCl_3$, 400 MHz): 21.6 (C-2′, C-3′); 26.8 (C-1′); 62.4 (C-2″); 100.1 (C-9); 114.6 (C-6); 115.0 (C-8a); 117.0 (C-4); 122.2 (C-9a); 136.7, (C-3); 144.2 (C-5);151.9 (C-4a); 152.9 (C-2); 154.3 (C-3a); 161.6 (C-7).

3.8. Oxypeucedanin hydrate (**4**)

$C_{16}H_{16}O_6$, ^{1}H NMR, ($CDCl_3$, 500 MHz): δ 1.31 (3H, *s*); 1.36 (3H, *s*); 3.91 (1H, *dd*, J = 10.0, 3.0, H-3′); 4.45 (1H, *dd*, J = 10.0, 3.0, H_a-2′); 4. 54 (1H, *dd*, J = 10.0, 3.0, H_b-2′); 6.31 (1H, *d*, J = 10.0, H-5); 6.99 (1H, *d*, J = 2.5, H-3); 7.20 (1H, *s*, H-9); 7.61 (1H, *d*, J = 2.5, H-2); 8.18 (1H, *d*, J = 10.0, H-6). ^{13}C NMR, ($CDCl_3$, 400 MHz): δ 25.6 (C-5′); 27.2(C-6′); 72.1 (C-3′); 74.9 (C-2′); 76.9 (C-4′); 95.4 (C-9); 105.1 (C-3); 107.8 (C-4a); 113.5 (C-3a); 114.7 (C-6) ; 138.6 (C-2); 146.4 (C-5);149.0 (C-8a); 153.0 (C-9a); 161.4 (C-7); 188.5 (C-4).

3.9. Officinalin isobutyrate (**5**)

White crystals; m.p. 118–120 °C; $C_{15}H_{14}O_6$; MS (EI, 70 eV), *m*/*z* (rel. inten.): 290 [M^+] (10), 259 (15), 220 (25), 188 (50), 160 (20), 71 (80), 43 (100). ^{1}H NMR, ($CDCl_3$, 500 MHz): 1.37 (6H,*d*, J = 7, H_3-4′, H_3-5′), 2.90, (1H, *septet* J = 7, H-3′); 3.9 (3H, *s*, H_3-3″); 6.44 (1H, *d*, J = 10, H-3); 7.04 (1H, *s*, H-8); 7.71, (1H, *d*, J = 10, H-4); 8.18 (1H, *s*, H-5). ^{13}C NMR, ($CDCl_3$, 400 MHz): δ 18.8 (C-4′, C-5′); 34.3 (C-3′); 52.5 (C-3″); 112.5 (C-8); 117.3 (C-3); 119.8 (C-4a); 120.4 (C-6); 131.9 (C-5); 142.5 (C-4); 153.7 (C-7); 157.3 (C-8a); 159.9, (C-2); 164.3, (C-1″); 175.3, (C-2′).

3.10. Bergapten (**6**)

$C_{12}H_8O_4$, MS (EI, 70 eV), *m*/*z* (rel. inten.): 216 [M^+] (100), 201 (25), 188 (10), 157 (2), 145 (35), 89 (20), 74 (10), 63 (15), 51 (23), 38 (10).

3.11. Officinalin (7)

$C_{11}H_8O_5$, MS (EI, 70 eV), *m/z* (rel. inten.): 220 [M^+] (60), 188 (100), 160 (48), 132 (18), 104 (12), 76 (15). ^{1}H NMR, ($CDCl_3$, 500 MHz): 3.99 (3H, *s*, –OMe); 6.29 (1H, *d*, *J* = 10, H-3); 6.88 (1H, *s*, H-8); 7.61, (1H, *d*, *J* = 10, H-4); 8.02 (1H, *s*, H-5); 11.20 (1H, *s*, –OH).

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