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ARTICLE

Identification of Bisprenylated Benzoic Acid Derivatives from Yerba Santa (*Eriodictyon ssp.*) Using Sensory-Guided Fractionation

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Due to certain off-flavor problems and lacking bitter masking effects with Yerba Santa (*Eriodictyon angustifolium* and *E. californicum*) extracts, which are also described as bitter, herbal, medicinal, phenolic, or astringent, methanolic extracts were fractionated and evaluated for their taste properties using a high temperature liquid chromatography (HTLC)-based approach. The taste-guided fractionation led to the identification of a series of novel bisprenylated benzoic acids (erionic acids A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), and F (**6**) and eriolic acids A (**7**), B (**8**), C (**9**), and D (**10**), respectively), along with the known flavonoids eriodictyol, homoeriodictyol, hesperetin, and chrysoeriol. The new compounds were isolated in larger amounts for characterization from Narrow Leaf Yerba Santa (*E. angustifolium*) and California Yerba Santa (*E. californicum*), respectively, using fast centrifugal partition chromatography (FCPC) and HTLC. The structures were elucidated using one and two-dimensional NMR spectroscopy and high resolution mass spectrometry (HR-MS). For *E. californicum*, data regarding seasonal and climatic variation of the eriolic acid contents and of the flavonoids were collected. The flavor properties of some of the isolated new compounds were evaluated; they showed strong off-flavor characteristics, such as bitter, astringent, phenolic, or woody, and may contribute to the sensory effects observed for crude Yerba Santa extracts. Erionic acid C (**3**) was not only able to increase the absolute bitterness but also to extinguish the bitter masking effect of homoeriodictyol in a caffeine solution.

KEYWORDS: High temperature liquid chromatography (HTLC); Yerba Santa; Eriodictyon californicum; Eriodictyon angustifolium; taste dilution analysis; bisprenylated benzoic acids.

INTRODUCTION

Eriodictyon californicum, commonly called California Yerba Santa, and *E. angustifolium* (Narrow Leaf Yerba Santa) are evergreen shrubs with resinous branches and leaves from the family of *Hydrophyllacea* (*1*). This family consists of 25 genera and 300 different species, mainly found in Western America. Out of these 25 genera, 14 are endemic to California. California Yerba Santa is found in the southwest of the USA (California and Oregon) and Mexico, while Narrow Leaf Yerba Santa is widely distributed in Western America and can be found in California, Utah, and Arizona, among other states (*2*). Traditionally, Yerba Santa was used as a medicinal herb by Native Americans and was also very popular among the Spanish settlers in California, who named the plant "Yerba Santa", meaning "holy plant". *Eriodictyon* was used to treat different respiratory diseases, such as cough, colds, asthma, as well as influenza (*3*, *4*). Leaves were also smoked, chewed against gastrointestinal disorders, used as plasters, or used as a poultice to cure sores or wounds (*5*). Apart from these medicinal properties, *Eriodictyon* extracts are reported to act as a bitter masking agent to make bitter pharmaceuticals more pleasant for patients (*6*). Homoeriodictyol, eriodictyol, and, to some extent, sterubin were identified to be the active ingredients responsible for this effect (*7*). During investigations of the masking potential of crude Yerba Santa extracts, strong off-flavors suppressed the positive masking effects (*8*). A first taste-guided fractionation led to nonflavonoid fractions showing strong bitter, smoky off-flavors, but the responsible compounds could not yet be identified.

The present study reports the isolation and structural elucidation of ten new bisprenylated benzoic acid derivatives, isolated from *E. californicum* and *E. angustifolium*. To get more insight into the role of these new benzoic acid derivatives, their flavor profiles were evaluated. The seasonal variation of their occurrence in *E. californicum* was investigated in comparison to the flavonoid content, to find optimum harvesting periods.

MATERIALS AND METHODS

Solvents were purchased from Sigma Aldrich (Steinheim, Germany): methanol (puriss. p.a.), ethanol (absolute, puriss,

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99.8%), and acetonitrile Chromasolv (for HPLC, gradient grade min. 99.9%). CD_3OD and DMSO-D_6 were purchased from Deutero GmbH (Kastellaun, Germany).

Dried leaves of *E. angustifolium* and *E. californicum* were purchased from JPR Jenaer Pflanzenrohstoffe (Jena, Germany); additional plant material of *E. californicum* was collected by the same company as whole branches (leaves, stems, flowers) in Northern California from May to September of 2008. Each month, 3−5 branches were picked at the same growing place but not necessarily from the same individual plant. The plant material was directly dried in the sun. The leaves, flowers, and stems were separated prior to extractions.

NMR spectra were recorded in CD_3OD or DMSO-D_6 using Varian VXR400S (^{1}H: 400 MHz, ^{13}C: 100 MHz), INOVA-500 (^{13}C: 125 MHz, cold probe), as well as INOVA-600 (^{1}H: 600 MHz) spectrometers (Varian, Darmstadt, Germany) at 25 °C using tetramethylsilane as an internal standard.

LC/MS and HR-MS spectra were recorded using a mass spectrometer Bruker microTOF Q II (Bruker, Bremen, Germany, ESI electrospray ionization, pos/neg mode) in combination with a Waters Aquity UPLC system (Waters, Eschborn, Germany). Chromatographic separation was carried out on a C-18 column (BEH C-18, 1 mm × 50 mm; 1.7 μm particle size; Waters, Eschborn, Germany) at a flow rate of 0.2 mL min^{-1} using an acetonitrile/water gradient. The gradient started with 100% water containing 0.01% formic acid, increasing to 95% acetonitrile within 20 min. This concentration was kept for 5 min.

High temperature liquid chromatography was performed on a polymer-based PRP-1 column (semipreparative scale, 250 mm × 10 mm; 10 μm particle size; Hamilton, Bonaduz, Switzerland) at elevated temperatures (120 °C isotherm) on a HPLC system with detection on a DAD detector (SunChrom, Friedrichsdorf, Germany).

Fast centrifugal partition chromatography (FCPC) was carried out using a bench scale FCPC model, Version A (Kromaton Technologies, Angers, France), with a 200 mL semi-preparative rotor and detection on an ELSD detector.

Preparative HPLC was performed on a Kromasil 100 C-18-5 column (5 μm, 250 mm × 8 mm) at ambient temperature with detection with a DAD on a Merck-Hitachi preparative HPLC system (Merck-Hitachi, Darmstadt, Germany) and on a Dionex Ultimate 3000 system (Dionex, Idstein, Germany), employing the same column.

Extraction and Isolation. The material of *Eriodictyon californicum* (150 g) was infused with boiling water and stirred for 1 h to break up the plant material and prepare it for further extraction. The soaked plant materials were filtered, dried, and extracted twice with 1.5 L of methanol at room temperature for 1 h each while stirring continuously. The extract was filtered and dried *in vacuo* at 40 °C. The extraction resulted in 32.7 g of dried dark green solid.

The material of *Eriodictyon angustifolium* (500 g) was extracted as described for *E. californicum* using 2.0 L of methanol, resulting in 84.5 g of dried dark green solid.

FCPC. The FCPC experiment on *Eriodictyon angustifolium* extract was performed with a two-phase solvent system composed of *n*-heptane/ethyl acetate/methanol/water (5:4:4:5 v/v/v/v). After thoroughly equilibrating the solvent mixture in a separation funnel at room temperature, the two phases were separated and used for separation of the extract. In the ascending mode, the upper organic phase was used as stationary phase and the lower aqueous phase as a mobile phase. In the descending mode, the solvents were switched. A total of 1.5 g of the crude *E. angustifolium* extract was fractionated. Fractions were collected every minute in the ascending mode for 40 min (fractions A1 to A40) and for 30 min in the descending mode (fractions D1 to D30) at a flow rate of 8 mL min^{-1} and 10 mL min^{-1}, respectively. Homoeriodictyol, sterubin, hesperetin, and several minor flavonoids eluted in the ascending mode. Compound **1** eluted in fractions D2 and D3, and compound **2** eluted in fraction D4 in the descending mode. The corresponding fractions of 25 FCPC runs, using 60 mg of extract each, were combined, resulting in 57 mg of fractions D2 and D3, as well as 40 mg of fraction D4. Combined fractions D2 and D3 were further purified by preparative HPLC on the described Merck-Hitachi system using a water/acetonitrile gradient with a flow rate of 3.2 mL min^{-1}. The gradient started with a concentration of 10% acetonitrile, which was kept for 2.5 min. Within 15 min, the concentration was increased to 20% and held for 5 min. In a last step, the concentration was increased to 80% within 5 min and held for an additional 5 min. Compound **1** (2.6 mg) eluted between 8.6 and 14.6 min. Fraction D4 was also further purified by preparative HPLC on the same system using slightly modified conditions starting with 5% acetonitrile for 2.5 min. The concentration was increased to 20% within 15 min and held for 5 min, followed by an increase to 80% within 5 min. This concentration was kept for 5 min. Compound **2** (1.1 mg) eluted between 13.9 and 17.00 min.

High Temperature Liquid Chromatography (*HTLC*). 0.75 g of the crude *E. angustifolium* extract (300 mg mL^{-1} ethanol, injection volume 50 μL) was fractionated using a polymer-based RP column at 120 °C isotherm conditions using a water/ethanol gradient with a flow rate of 3 mL min^{-1}. The gradient started with 70% water and 30% ethanol, increasing the ethanol concentration to 100% within 50 min. This concentration was kept for an additional 10 min.

Erionic acid C (**3**) (MW 374; 32.7 mg) was obtained from a fraction collected from 15.0 to 15.5 min; erionic acid D (**4**), showing also a MW of 374, was collected between 15.5 and 16.0 min (5.9 mg), and erionic acid E (**5**) (MW 374; 7.2 mg) eluted between 17.0 and 17.7 min. Compound **6** (MW 358; 20.3 mg), erionic acid F, eluted between 23.0 and 24.5 min. Due to larger impurities, additional cleanup by preparative HPLC was necessary for compound C, using the Dionex system. Fractionation was carried out using water−acetonitrile gradients starting with 35% acetonitrile. The concentration of acetonitrile was increased to 77% within 8 min and to 100% after one additional minute. Cleanup resulted in 6 mg of compound **3**.

0.5 g of the crude *E. californicum* extract (300 mg mL^{-1} ethanol, injection volume 50 μL) were fractionated by HTLC under the same conditions as described for *E. angustifolium*. Eriolic acid A (**7**) (MW 388; 5.9 mg) was obtained from a fraction collected from 15.0 to 16.5 min; eriolic acid B (**8**), showing a MW of 358, was collected between 21.0 and 22.9 min (9.5 mg), and eriolic acid C (**9**) (MW 374; 23.0 mg) eluted between 12.5 and 14.0 min. Compound **10** (MW 372; 5.7 mg), eriolic D, was collected between 20.0 and 21.0 min.

Fractions obtained from the various preparation methods were evaporated using a parallel evaporator (Syncore, Buchi, Switzerland) at 40 °C followed by lyophilization.

Quantification. Quantification of flavonoids and selected benzoic acid derivatives (**8**, **9**) was carried out using a C18 column (Grom Saphier 110, 5 μm, 150 mm × 2.0 mm, Alltech Grom GmbH, Rottenburg-Hailfingen, Germany) with a gradient of water + 1% glacial acetic acid and acetonitrile, using standard samples for identification. The gradient started with 68% water and 32% acetonitrile, increasing to 50% acetonitrile within 10 min at a flow rate of 0.35 mL min^{-1}. Within an additional 5 min, the concentration of acetonitrile was increased to 100%. Detection was carried out using a UV detector at a wavelength of 285 nm; quantification was performed using external calibration. For the first two collections (May and July), quantifications were performed in duplicate; the observed deviations were below 5%. For the second two collections (August and September), only single determinations were carried out.

Sensory Analysis. Tasting sessions were carried out in a sensory room in the morning, 1−2 h after breakfast, during which time the testers were asked not to drink black or green tea or coffee. An average number of eight experienced testers (flavorists, expert panel members) participated in each session. Samples were tested by the sip and spit method. Extracts were tested at a concentration of 500 mg kg^{-1}, and isolates were tested at a concentration of 100 mg kg^{-1} on a testing solution. To profile the sample, the sample was added on sucrose (5%), salt solution (0.05%), and water (only compounds **2** and **3**). The testing solution (2−3 mL) was presented to the panelists in white plastic beakers; if only a limited amount of testing material was available, 2-mL-disposable pasteur pipettes were used. In the case of discoloration, the beakers were covered with aluminum foil. Flavor and taste attributes were determined by free discussion. No nose clips were used, as the overall off-flavor potential of the tested compounds was supposed to be evaluated. Flavor modifying effects for erionic acid C (**3**) were determined by duo comparison tests as described in ref 7 with 18 panelists.

Procedure for HTLC-Coupled Sensory Analysis. After removal of ethanol in an online evaporation chamber (*9*), fractions separated and

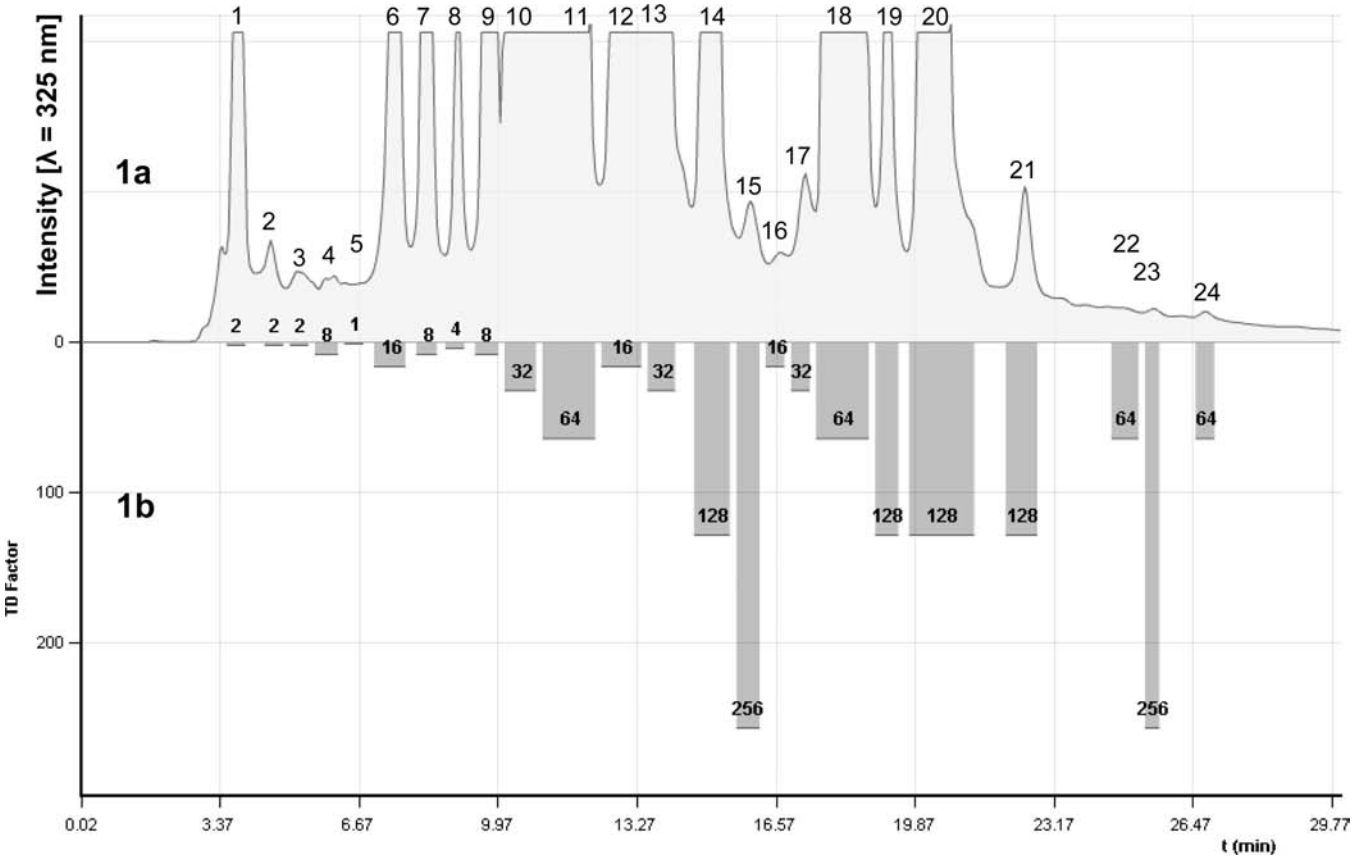

Figure 1. HTLC chromatogram of *E. angustifolium* methanolic extract (**a**) and TDA (**b**; effluent was evaluated in 24 separate fractions; fraction numbers are given on top of the peak). Starting concentration 400 mg mL^{-1} with 100 μL injection volume.

collected via HTLC-fractionation of *E. angustifolium* were evaluated sensorially by experienced testers. Taste dilution analysis (TDA) is used to estimate the contribution of a certain fraction to the overall flavor or taste of a mixture or an extract. In this study a modified version based on Frank et al. (*10*) was carried out by stepwise 1 + 1 diluting the extract with ethanol/water (1:1 v/v) and injecting each of the dilutions into the HTLC system. The sample was fractionated via HTLC using the conditions described before, and peak wise collected fractions were sensorially evaluated. The procedure was repeated, separating the samples of the different concentration steps in decreasing order and sensorially evaluating them, until no taste was perceivable any more by the assessor. As in TDA, the last dilution step, at which a taste could be perceived by the tester, was defined as the taste dilution (TD) factor.

RESULTS AND DISCUSSION

Separation and sensory evaluation of *Eriodicyton angustifolium* extract involving a previously reported high temperature liquid chromatography (HTLC)-based approach (*11*) indicated that a number of so far unknown compounds might be responsible for the lack of effectiveness of the crude extract, compared to the isolated taste modulating actives homoeriodictyol, sterubin, and hesperetin (*8*). *Eriodictyon* leaves accumulate a resin, which consists of approximately 80% of leaf waxes, mainly alkanes, and 20% of flavonoids, e.g. homoeriodictyol, hesperetin, sterubin, chrysoeriol, and luteolin (*3*, *7*, *12*). Only rare information can be found in the literature concerning other, especially nonflavonoid compounds in both *Eriodictyon* species. Gel permeation chromatography (GPC) on Sephadex LH-20 using methanol as eluent followed by HR-MS analysis indicated the presence of a number of nonflavonoid compounds (data not shown).

Sensory-guided fractionation of *E. angustifolium* extract was carried out using fractionation by HTLC and subsequent sensory evaluation of the single fractions by experienced assessors. Comparative LC/MS analyses of the crude extract and of the single fractions were carried out, to check for possible formation of artifacts; no obvious differences were observed. In addition, taste dilution analysis of the single HTLC fractions was carried out to estimate their contribution to the overall flavor of the extract and to find those fractions responsible for the off-notes in the crude extract (**Figure 1**). The sensory evaluation revealed a number of fractions (11, 12, 16, 17, 18, and 19) to have negative flavor attributes (e.g., bitter, medicinal, phenolic, or smoky). Some of them could not be assigned to known compounds after LC/MS analysis. The high TD factors determined in the dilution analysis suggested that these fractions might contribute to the off-flavor of the crude extract.

Isolation of these unknown compounds from the crude methanolic extract of *E. angustifolium* was carried out by fast centrifugal partition chromatography (FCPC) and in parallel by using HTLC for structure elucidation purposes, further analysis, and biological activity testing. Fractionation of the *E. californicum* extract was carried out by HTLC only. The structures of the compounds isolated from *E. angustifolium* are shown in **Figure 2**.

The molecular formula of compound **1**, which was isolated from the crude extract by FCPC, was established to be $C_{22}H_{30}O_6$ by HR-MS (MS data for compounds **1**−**6**; see **Table 1**). As can be seen in **Table 2**, the ^{1}H NMR spectrum of compound **1** reveals a singlet of two protons at 7.52 ppm. These were assigned to the two aromatic protons H-2 and H-6, indicating the presence of a benzene ring moiety. The proton spectrum also shows the presence of four methyl groups (1.07 ppm d, 1.22 ppm s, 1.23 ppm s, 1.65 ppm s) and of three olefinic protons (6.27 ppm, 6.93 ppm, 5.50 ppm). The ^{13}C NMR spectrum of **1** (see **Table 3**) shows the presence of four olefinic carbons and six aromatic atoms, as well as four oxygen-bearing carbons, indicating two hydroxyl groups

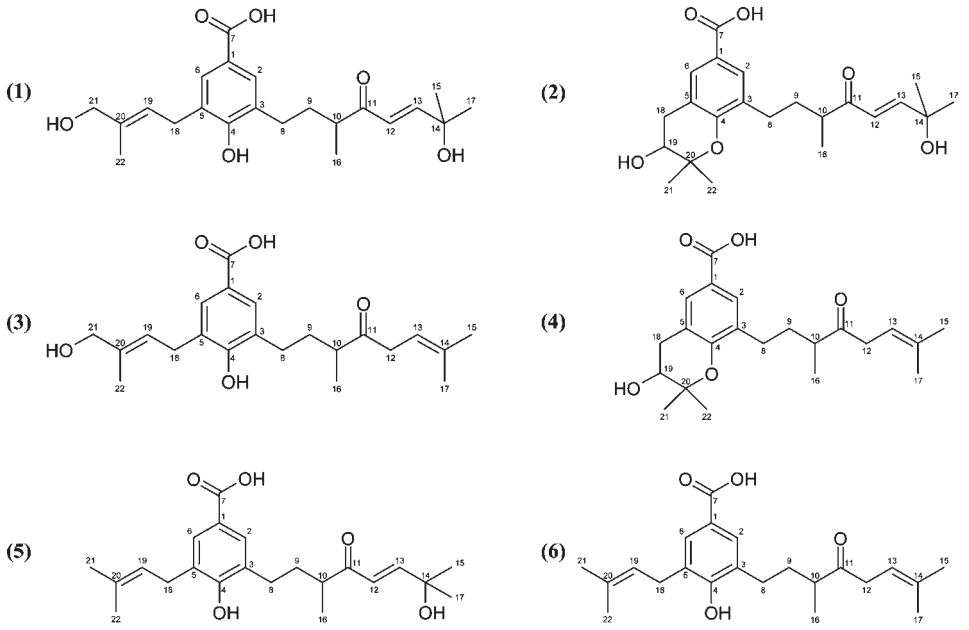

Figure 2. Structures of erionic acids A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), and F (**6**) isolated from *Eriodictyon angustifolium* by FCPC and HTLC.

Table 1. HR-MS and UV Data for Erionic Acids A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), and F (**6**)

compound	HR-MS	*m/z*	HR-MS (calc)	UV λ_{max} (nm)
1	389.194	371.187, 359.188, 345.203	389.196	210, 285
2	389.196	371.183, 359.181	389.196	215, 288
3	373.199	355.189, 343.189, 329.209	373.202	215, 260
4	373.201	355.191, 343.187, 329.209	373.202	218, 260
5	373.199	355.198, 329.213	373.201	225, 265
6	357.205	313.215	357.207	218, 260

(66.1 ppm and 69.1 ppm), one aromatic carboxyl (169.1 ppm), and one α,β-unsaturated keto group at 203.4 ppm (**Table 3**). The application of two-dimensional NMR techniques indicated that compound **1** is a tetra-substituted benzene, bearing the carboxyl group at position C-1, one hydroxyl group at C-4, and two prenyl moieties at C-3 and C-5. The ^{1}H,^{1}H-gCOSY spectrum reveals the correlations of proton−proton coupling of the two prenyl moieties, 3-methyl-2-butenyl, and 3,8-dimethyl-6-octenyl. Furthermore, the gHMBC and NOESY experiments confirmed the positions of the aliphatic hydroxyl groups as well as the keto group, leading to the structure of 4-hydroxy-3-((*E*)-7-hydroxy-3,7-dimethyl-4-oxo-oct-5-enyl)-5-((*E*)-4-hydroxy-3-methylbut-2-enyl)benzoic acid (**1**) (**Figure 2**). The key correlations determined by the 2D experiments are given in **Figure 3**. This compound was named erionic acid A, referring to its origin *Eriodictyon*.

The molecular formula of **2**, which was also isolated from *E. angustifolium* extract by FCPC, was determined to be $C_{22}H_{30}O_6$ by HR-MS. The ^{1}H and ^{13}C NMR spectra are in major parts similar to those of **1**. The proton NMR spectrum shows two signals at 7.57 ppm and 7.56 ppm for two aromatic protons which are close to those of **1**. In addition, the carboxyl group (170.2 ppm) could be confirmed in position C-1 (130.3 ppm). Furthermore, the NMR data indicate that both compounds **1** and **2** have the identical side chain at C-3. The ^{1}H NMR spectrum of **2** reveals the presence of five methyl signals at 1.30 ppm, 1.13 ppm, 1.29 ppm, 1.36 ppm, and 1.25 ppm, showing that no methyl group is attached to an olefinic double bond compared to the case of **1**. In addition to the described signals, **2** was suggested to have a second ring structure after gCOSY and gHMBC experiments, as it shows signals corresponding to a dihydrochromane ring with a hydroxyl group at C-19 (70.5 ppm) and two quaternary methyl groups at C-20 (78.4 ppm). The key correlations determined by the 2D experiments are given in **Figure 4**. Thus, the structure was assigned as 3-hydroxy-8-((*E*)-7-hydroxy-3,7-dimethyl-4-oxo-oct-5-enyl)-2,2-dimethylchroman-6-carbonic acid (**2**) and named erionic acid B.

HR-MS of compound **3**, which was isolated from *E. angustifolium* extract by HTLC, showed the molecular formula of $C_{22}H_{30}O_5$, reflecting the absence of one oxygen compared to compounds **1** and **2**. Again, the ^{1}H and ^{13}C NMR spectra of **3** display strong similarities with those of erionic acid A (**1**). The significant difference between this molecule and **1** can be found in the chemical shifts of C-14, indicating the absence of the tertiary hydroxyl group in the prenyl side chain at position C-3. Additional two-dimensional NMR experiments, including NOESY1D, gCOSY, and gHMBC (correlations analogous to **1**; see **Figure 3**), confirmed the structure of **3** to be 3-(3,7-dimethyl-4-oxo-oct-6-enyl)-4-hydroxy-5-((*E*)-4-hydroxy-3-methylbut-2-enyl)benzoic acid (**3**) (**Figure 2**), which was named erionic acid C.

A second compound (**4**), having the same molecular formula of $C_{22}H_{30}O_5$ as **3**, was also isolated from *E. angustifolium* extract by HTLC. The NMR spectra of this molecule are in some parts almost identical to those of **3**, indicating the carboxyl group at C-1 and the 3,7-dimethyl-(4-oxo)-oct-6-enyl side chain at C-3 as already detected in erionic acid C. The rest of the signals are strongly similar to the signals detected for the dihydrochromane ring of erionic acid B, generated by ring closure of the second prenylated side chain at C-5 with the hydroxyl group at C-4. After confirmation by 2D-NMR experiments (**Figure 4**), the final structure of the compound was therefore described as 8-((*E*)-3,7-dimethyl-4-oxo-oct-5-enyl)-3-hydroxy-2,2-dimethylchroman-6-carbonic acid (**4**) (**Figure 2**). The compound was given the trivial name erionic acid D.

According to HR-MS, compound **5**, isolated by HTLC as well, showed the same molecular mass as compounds **3** and **4** ($C_{22}H_{30}O_5$). The position of the carboxyl group at C-1, the octenyl side chain at C-3, as well as the hydroxyl group at C-4 are confirmed by extensive one- and two-dimensional NMR

Table 2. ^{1}H NMR Chemical Shifts for Compounds **1**−**6** Isolated from *E. angustifolium*[a]

pos.	compound **1**[b]	compound **2**[c]	compound **3**[c]	compound **4**[c]	compound **5**[c]	compound **6**[c]
2	7.52, s	7.57, d (2.1)	6.92, s	7.60, d (2.2)	7.60, d (2.2)	7.63, d (2.1)
6	7.52, s	7.56, d (2.1)	6.92, s	7.61, d (2.2)	7.63, d (2.2)	7.71, d (2.1)
8	2.55, m	2.58, m	2.59, m	2.56, m	2.62, m	2.61, m
9	1.83, m 1.51, m	1.96, m 1.61, m	1.95, m 1.61, m	1.94, m 1.60, m	1.97, m 1.63, m	1.95, m 1.61, m
10	2.85, ddq (6.8, 6.8, 6.8)	2.86, ddq (6.8, 6.8, 6.9)	2.66, m	2.63, m	2.89, ddq (6.9, 6.9, 6.9)	2.65, m
12	6.27, d (15.8)	6.29, d (15.8)	3.19, m	3.17, m	6.34, d (15.9)	3.18, m
13	6.83, d (15.8)	6.83, d (15.8)	5.24, m	5.23, m	6.88, d (15.9)	5.23, dd (7.1, 7.1)
15	1.22, s a	1.30, s a	1.72, s	1.72, s	1.31, s	1.77, s a
16	1.07, d (6.9)	1.13, d (6.9)	1.12, d (7.0)	1.11, d (6.9)	1.15, d (7.0)	1.12, d (7.0)
17	1.23, s a	1.29, s a	1.61, s	1.60, s	1.31, s	1.72, s a
18	3.33, d (7.3)	3.02, dd (5.4, 16.5) 2.74, dd (7.8, 16.5)	3.40, d (7.4)	3.05/3.06, dd/dd (16.7, 5.2) 2.76, dd (16.6, 7.2)	3.31 (covered by solvent)	3.33, d (7.3)
19	5.50, dd (7.3, 7.3)	3.75, dd (7.8, 5.4)	5.61, dd (7.4, 7.4)	3.78, m	5.33, dd (7.4, 7.4)	5.32, dd (7.3, 7.3)
21	3.83, s	1.36, s a	3.99, s	1.37/1.36 a	1.77, s	1.72, s b
22	1.65, s	1.25, s a	1.76, s	1.30/1.29 a	1.71, s	1.60, s b

[a] Letters a and b after entries indicate values that are not significantly different from one another, respectively. [b] DMSO. [c] CD_3OD.

Table 3. ^{13}C NMR Chemical Shifts for Compounds **1**−**6** Isolated from *E. angustifolium*[a]

pos.	compound **1**	compound **2**	compound **3**	compound **4**	compound **5**	compound **6**
1	127.7 a	130.3 a	n.d.	130.6 a	123.3	123.2
2	128.7 b	130.4 a	131.1 a	130.6 a	131.0a	130.8
3	128.3 a	129.6 a	130.3	130.6 a	130.3	129.4
4	156.5	155.0 b	158.4	156.2	158.1	158.4
5	121.0	131.4 a	125.6	120.9/120.8	128.9	129.4
6	128.9 b	130.4 a	130.8 a	131.3	130.8 a	130.5
7	169.1	170.2	171.1	171.0	171.5	170.8
8	27.4	28.9	29.2	28.9/28.8	29.3	29.0
9	32.6	34.6	33.9	34.0/34.0	34.4	33.8
10	42.7	44.4	46.5	46.3/46.3	44.5	46.2
11	203.4	207.0	217.4	215.6/215.5	207.1	216.1
12	123.8	125.6	42.2	41.9	125.8	44.5
13	154.3	154.9 b	117.5	117.3	155.5	117.2
14	69.1	71.2	136.8	136.6	71.2	136.7
15	29.0	29.3	25.9	25.9	29.3	25.9
16	16.2	16.8	16.9	16.7	17.2	16.9
17	29.0	29.3	18.3	18.3	29.3	17.9 a
18	27.6	32.4	29.2	32.2	29.7	29.4
19	120.8	70.5	124.2	70.0/70.0	123.4	123.0
20	136.2	78.4	137.8	79.1/79.1	134.5	134.2
21	66.1	24.3 c	69.1	26.1/26.0 b	26.2	26.0
22	13.1	21.0 c	13.9	21.6/21.3 b	18.0	18.1a

[a] Letters a, b, and c after entries indicate values that are not significantly different from one another, respectively.

experiments (correlations analgous to **1**; see **Figure 3**). The ^{13}C NMR spectrum shows significant shifts for the signals of C-21 and C-22, caused by the absence of the hydroxyl group, which is present in the terminal position of the butenyl side chain of erionic acid A (**1**). Therefore, the structure of **5** could be determined as 4-hydroxy-3-((*E*)-7-hydroxy-3,7-dimethyl-4-oxo-oct-5-enyl)-5-(3-methylbut-2-enyl)benzoic acid (erionic acid E) (**Figure 2**).

The sixth novel compound, which was isolated from *E. angustifolium* extract by HTLC and showed a molecular formula of $C_{22}H_{30}O_4$, again showed strong similarities to the above-described molecules in its NMR spectra (**Tables 1**−**3**). Two-dimensional NMR techniques (gHMBC and gCOSY, correlations analogous to **1**; see **Figure 3**) proved the carboxylic acid and the phenolic hydroxyl group in positions C-1 and C-4, respectively. Furthermore, interpretation led to the identification of the octenyl side chain at C-3 (see erionic acid C or D), as well as the butenyl side chain at C-5 (see erionic acid E). The final structure of erionic acid F is therefore 3-(3,7-dimethyl-4-oxo-oct-6-enyl)-4-hydroxy-5-(3-methylbut-2-enyl)benzoic acid (**6**) (**Figure 2**).

As described for *Eriodictyon angustifolium*, a number of structurally related novel compounds (**7**−**10**), named eriolic acids, could be isolated from *E. californicum* using HTLC. Although these compounds are closely related to erionic acids, eriolic acids show a characteristic difference in the octenyl side chain: the keto function is reduced to a secondary alcohol moiety.

Compound **7** was analyzed by HR-MS and shows the molecular formula of $C_{23}H_{32}O_5$ (MS data for compounds **7**−**10**; see **Table 4**). The ^{1}H NMR as well as the ^{13}C NMR spectra (**Tables 5** and **6**) show strong similarities to some of the erionic acids isolated from *E. angustifolium*, esp. erionic acid C.

Thus, the carboxyl group at C-1 and the prenyl side chain at C-5, similar to that of erionic acid C (**3**) at these positions, could be confirmed. However, this newly identified compound shows differences in the octenyl side chain, particularly at position C-11 (78.6 ppm). In addition, two more olefinic carbons were detected at 125.5 ppm and 139.6 ppm for positions C-9 and C-10. An additional methyl group (61.6 ppm) can be assigned to a methoxy group at position C-4 of the benzene ring instead of the hydroxyl group. The final structure was described as 3-((*E*)-4-hydroxy-3,

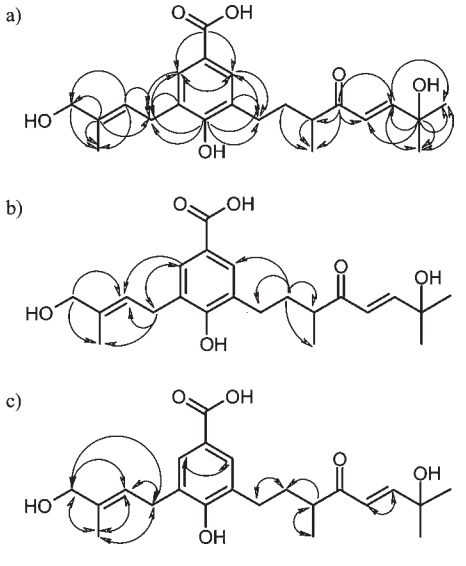

Figure 3. Key gHMBC (**a**), NOE (**b**), and gCOSY (**c**) correlations of erionic acid A (**1**); analogous correlations were found for **3**, **5**, and **6**.

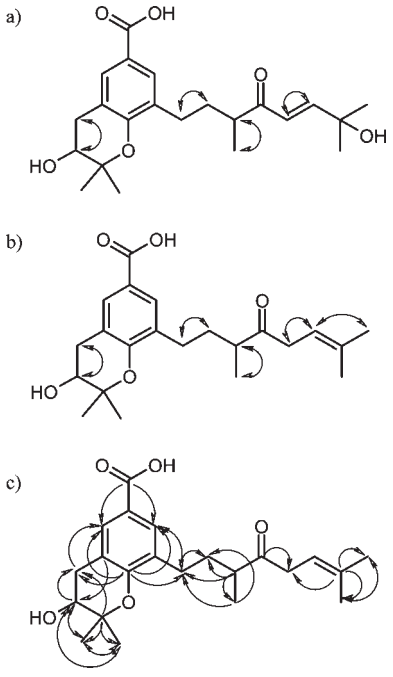

Figure 4. Key gCOSY correlations of (**a**) erionic acid B (**2**) and (**b**) erionic acid D (**4**), and gHMBC (**c**) correlations of erionic acid D (**4**).

Table 4. HR-MS and UV Data from Eriolic Acids A (**7**), B (**8**), C (**9**), and D (**10**)

compound	HR-MS	*m*/*z*	HR-MS (calc)	UV λ_{max} (nm)
7	387.215		387.217	215, 245
8	357.206	339.194, 313.213	357.207	218, 260
9	373.202	355.192, 343.188, 329.209	373.202	215, 260
10	371.222		371.222	215, 245

7-dimethylocta-2,6-dienyl)-5-((*E*)-4-hydroxy-3-methylbut-2-enyl)-4-methoxybenzoic acid (**7**) (**Figure 5**). In order to differentiate this new type of compound from erionic acids, which have been isolated from *E. angustifolium*, the molecule was named eriolic acid A, referring to the characteristic difference between both types of compounds.

Table 5. ^{1}H NMR Chemical Shifts for Compounds **7**−**10** Isolated from *E. californicum*

pos.	compound **7**[a]	compound **8**[a]	compound **9**[a]	compound **10**[a]
2	7.72, s	7.64, d (2.2)	7.65, s	7.71, d (2.2)
6	7.72, s	7.63, d (2.2)	7.65, s	7.70, d (2.2)
8	3.43, d (7.1)	3.38, d (7.4)	3.38, d (7.2)	3.43, d (7.3)
9	5.50, dd (7.2, 7.2)	5.50, dd (7.4, 7.4)	5.55, dd (7.4, 7.4)	5.50, dd (7.2, 7.2)
11	3.98, dd (7.0, 7.0)	3.99, dd (7.0, 7.0)	3.99, dd (7.1, 7.1)	3.98, dd (7.0, 7.0)
12	2.26, dd (7.0, 7.0)	2.26, dd (7.0, 7.0)	2.26, dd (7.1, 7.1)	2.26, dd (7.0, 7.0)
13	5.07, dd (7.0, 7.0)	5.08, dd (7.0, 7.0)	5.09, dd (7.1, 7.1)	5.06, dd (7.0, 7.0)
15	1.63, s	1.64, s	1.64, s	1.63, s
16	1.74, s	1.72, s	1.72, s	1.74, s
17	1.60, s	1.59, s	1.60, s	1.59, s
18	3.45, d (7.1)	3.33, d (7.1)	3.40, d (7.4)	3.38, d (7.3)
19	5.57, dd (7.3, 7.3)	5.32, dd (7.3, 7.3)	5.61, dd (7.4, 7.4)	5.28, dd (7.3, 7.3)
21	3.98, s	1.76, s	3.99, s	1.75, s
22	1.78, s	1.72, s	1.76, s	1.74, s
23	3.77, s			3.76, s

[a] Letters a and b after data points indicate values that are not significantly different from one another, respectively.

Table 6. ^{13}C NMR Chemical Shifts for Compounds **7**−**10** Isolated from *E. californicum*

pos.	compound **7**[a]	compound **8**[a]	compound **9**[a]	compound **10**[a]
1	129.6	123.1	123.4	128.4
2	131.0	130.3	130.4	130.8
3	135.7	128.8	128.8	135.5
4	161.4	158.2	158.1	161.3
5	135.7	129.2	128.8	136.1
6	130.9	130.3	130.4	130.8
7	170.6	170.9	171.1	170.7
8	28.8 b	29.0	29.1 a	28.8
9	125.5	124.9	124.7	125.5
10	139.6	139.6	139.6	139.5
11	78.6	78.7	78.6	78.6
12	34.8	34.8	34.8	34.8
13	121.7	121.7	121.6	121.6
14	134.0	134.0	134.0	133.9
15	26.0	26.0	26.0	26.0 a
16	11.8	11.6	11.6	11.7
17	18.0	17.9 a	18.0	18.0 b
18	28.9 a	29.4	28.9 a	29.2
19	124.5	123.0	124.1	123.7
20	137.5	134.1	137.4	133.9
21	68.7	26.0	68.8	25.9 a
22	13.9	18.0 a	13.8	17.9 b
23	61.6			61.5

[a] Letters a and b after data points indicate values that are not significantly different from one another, respectively.

Another compound, **8**, having the molecular formula $C_{22}H_{30}O_4$, shows strong similarities to eriolic acid A but also to erionic acid E and F according to the ^{1}H and ^{13}C NMR spectra. The similarities led to the conclusion that the molecule is a 4-hydroxybenzoic acid derivative bearing a 4-hydroxy-3,7-dimethylocta-2,6-dienyl side chain at C-3, as described for eriolic acid A, and a 3-methylbut-2-enyl side chain at C-5, as described for erionic acids E and F. Taking gCOSY, gHMBC, and NOESY1D (**Figure 6**) experiments into account, the structure was confirmed as 4-hydroxy-3-((*E*)-4-hydroxy-3,7-dimethylocta-2,6-dienyl)-5-(3-methylbut-2-enyl)benzoic acid (**8**), which was named eriolic acid B (**Figure 5**).

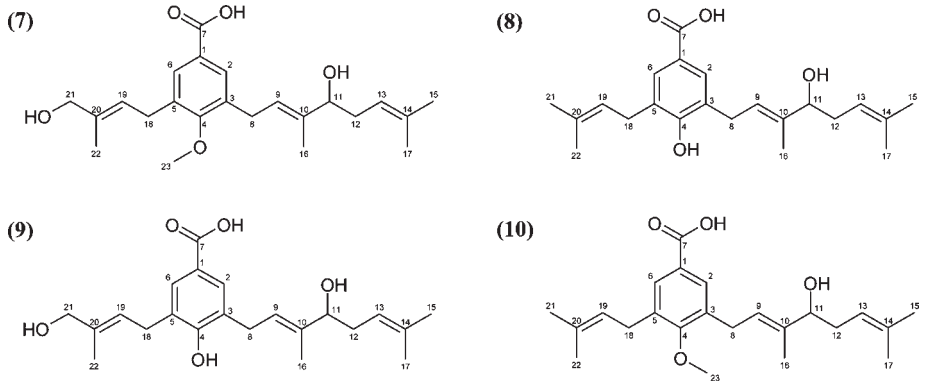

Figure 5. Structures of eriolic acids A (**7**), B (**8**), C (**9**), and D (**10**) isolated from *Eriodictyon californicum* by HTLC.

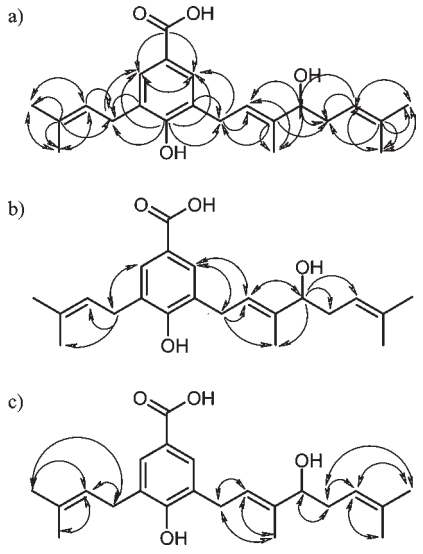

Figure 6. Key gHMBC (**a**), NOESY1D (**b**), and gCOSY (**c**) correlations of eriolic acid A (**8**); analogous correlations were found for **7**, **9**, and **10**.

Compound **9**, which was determined to have the molecular formula $C_{22}H_{30}O_5$ by HR-MS, was also analyzed by ^1H and ^{13}C NMR spectroscopy. The NMR data indicate that the complete structure of the molecule is almost identical to eriolic acid A (**7**). While five methyl groups were detected in eriolic acid A, only four could be found in compound **9** (1.64 ppm, 1.72 ppm, 1.60 ppm, and 1.76 ppm). The difference between both molecules can be found in the absence of the methoxy group referring to the phenolic hydroxyl group at C-4 (158.2 ppm). The compound was identified as 4-hydroxy-3-((*E*)-4-hydroxy-3,7-dimethylocta-2,6-dienyl)-5-((*E*)-4-hydroxy-3-methylbut-2-enyl)benzoic acid (**9**) (eriolic acid C) (**Figure 5**).

The fourth novel compound (**10**) isolated from *E. californicum* methanolic extract (molecular formula $C_{23}H_{32}O_4$ by HR-MS) shows the same characteristic signals in the ^1H NMR and ^{13}C NMR spectra as the molecules described before, especially eriolic acid B (**8**). The characteristic difference is the presence of a methoxy group instead of the hydroxyl group at C-4 (61.5 ppm, 161.3 ppm). The structure was assigned as 3-((*E*)-4-hydroxy-3,7-dimethylocta-2,6-dienyl)-4-methoxy-5-(3-methylbut-2-enyl)benzoic acid (**10**) and named eriolic acid D (**Figure 5**).

Structure elucidation by NMR shows that the basic structure of the newly identified open-chained compounds (**1**, **3**, **5**, **6**, **7**, **8**, **9**, and **10**) from FCPC and HTLC fractionation of *E. angustifolium* and *E. californicum* strongly resembles geranyl-4-hydroxy-5-(3′-methyl-2′-butenyl)benzoic acid, also referred to as myrsinoic acid A (**11**, **Figure 7**), which was isolated from the Asian plant *Myrsine seguinii* (*13*, *14*) and scoparal (**12**), which was recently described in *Artemisia scoparia*, originating from Pakistan (*15*). Additionally, the presence of two monoprenylated benzoic acid derivatives (**13** and **14**) was already reported for the related species *Eriodictyon sessilifolium* (**Figure 7** (*16*)). Prenylated benzoic acid derivatives are secondary metabolites, which are not widely distributed in nature (*17*). The biological activities of this class of compounds include anti-inflammatory (*18*), antifungal (*19*), antimicrobial (*20*), and antioxidative properties (*21*, *22*). No information is available in the literature concerning the flavor properties of this type of compounds.

It is still not fully clear whether erionic and eriolic acids occur exclusively in either of the two species, but by comparing the LC/MS analyses, it can definitely be seen that eriolic acids form the majority of benzoic acid derivatives in *E. californicum*, while erionic acids are the main representatives in *E. angustifolium*. In both species, however, erionic and eriolic acids seem to contribute significantly to the phytochemical profile, as they seem to be contained in the plant material in larger amounts. The distribution of both types of compounds to either of the two species might be used as a phytochemical marker together with the characteristic flavanones and may help to authenticate plant material of unclear origin. To complete this picture, additional *Eriodictyon* species should be investigated for their benzoic acid profile in further studies.

The isolated new compounds **2**−**10** were tasted at 100 mg L^{-1} on 5% sucrose and on 0.5% sodium chloride solution, respectively (compound **1** could not be isolated in sufficient amounts). The intention of this testing regime was not to find potential sweet or salt taste modulating effects but to determine possible contributions to the off-flavors described earlier for the Yerba Santa extracts. Slightly sweet and salty solutions are regularly used as test matrices to determine the flavor profiles of known or new compounds, because the potential flavor direction and, in particular, off-flavor notes are much easier to be detected than by simple tasting in aqueous solution. Nevertheless, compounds **2** and **3** were also tested in water, but the descriptors did not show significant differences. With the exeption of **2**, the compounds were bitter, medicinal, and phenolic, but also astringency-related flavor properties, such as scratchy, dry-dusty, or woody, were exhibited. The results of the sensory evaluation were compared with the descriptions gained from the HTLC-coupled sensory analysis for *E. angustifolium* of the corresponding fractions (**Table 7**). It is noteworthy that, except for **2** and **9**, all tested compounds were described as bitter by the experienced testers.

Unlike the case for *E. angustifolium*, no HTLC-coupled sensory evaluation was carried out for *E. californicum*. The descriptions of the isolated eriolic acids **7**−**10** (**Table 7**), however, strongly resemble the descriptions of erionic acids **3**−**6**. In a flavor

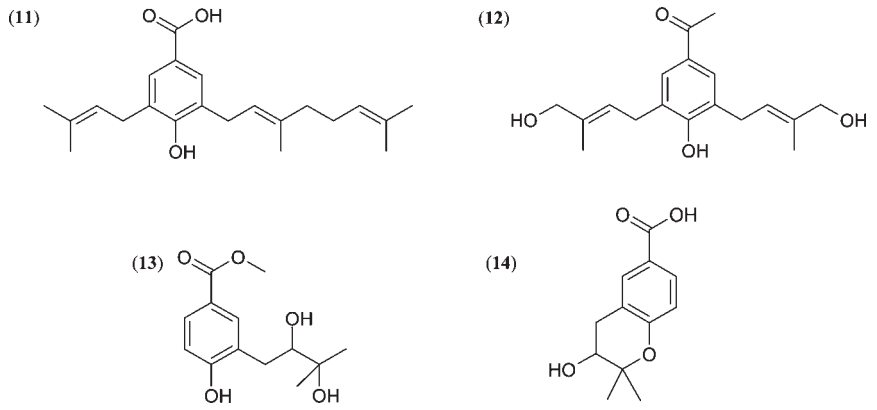

Figure 7. Structures of myrsinoic acid A (**11**) from *Myrsine seguinii*, scoparal (**12**) from *Artemisia scoparia*, and 4-hydroxy-3-(2,3-dihydroxy-3-methylbutyl)benzoic acid (**13**) and 2,2-dimethyl-3-hydroxychromane-6-carboxylic acid (**14**) from *E. sessilifolium*.

Table 7. Flavor Descriptions of HTLC Fractions and of the Corresponding Novel Compounds Isolated from *E. angustifolium* (**1**−**6**) and *E. californicum* (**7**−**10**), Respectively

fraction	flavor description of HTLC fraction	compound	flavor description of corresponding isolated compound at 100 mg L^{-1} [a]
E. angustifolium			
6	tealike, rum, phenolic, woody	**1**	n.d.
9	sweetish, weak	**2**	nearly neutral, weakly card board[b]
14	bitter, unpleasant, medicinal, phenolic, smoky	**3**	herbal, bitter, astringent[b]
13	estery, fishy, cod liver, bitter	**4**	bitter, dry-dusty, pencil, woody
14	fishy, cod liver, bitter	**5**	bitter,[c] phenolic, shoe polish
18	herbal, bitter, typ. Yerba Santa, musty, waxy	**6**	sweet, slightly bitter, phenolic, woody
E. californicum			
	n.a.	**7**	woody, bitter, tobacco
	n.a.	**8**	very bitter, woody, pencil
	n.a.	**9**	neutral, balsamic
	n.a.	**10**	fatty, rancid, bitter

[a] The descriptors for the evaluation of the neat compounds were determined in 5% sucrose and 0.5% salt solution on Vittel water and were identical for both matrices unless otherwise stated (n = 8, free discussion). [b] Compound tested on water and sucrose solution. [c] Perceived particularly in salty solution.

Table 8. Evaluation of Flavor Modifying Effects of Erionic Acid C (**3**), Homoeriodictyol Monosodium Salt (HED), and a Mixture of **3** and HED in an Aqueous 500 mg kg^{-1} Caffeine Solution[a]

compound	rating without compound(s)	rating with compound(s)	panelists all/ modification[b]	modification of bitter rating[d]
3	4.5 ± 1.6[c]	5.6 ± 1.8	18/11	+31% ($p < 0.1$)
HED	4.5 ± 1.5	4.0 ± 1.3	18/11	−14% (n.s.)
HED + 3	4.4 ± 1.5	3.1 ± 1.1	18/14	−38% ($p < 0.01$)

[a] Test concentration, 100 mg kg^{-1}; scale, 1 (no effect) to 10 (strong bitter); calculation of significance according to Student's t-test (n.s. not significant). [b] Ratio of number of all panelists against number of panelists who rated the bitterness of the test solution lower/higher than the standard solution. [c] Standard deviation. [d] Modification [%] = 100 × ($I_{test} - I_{blind}$)/I_{blind}; I = bitter rating − 1.

modifying experiment with a 500 mg L^{-1} caffeine solution (see **Table 8**), a test solution containing additionally 100 mg L^{-1} erionic acid C (**3**) was about 31% more bitter compared to the neat caffeine probe. Much more important, the known bitter masking effect of homoeriodictyol monosodium salt (7) was more or less inhibited by a combination of **3** with the flavanone. The small amount of isolated compounds did not allow additional tests to be carried out. Further isolation or synthesis is required to obtain more information about threshold values, dose-overthreshold (DoT), or the absolute contribution to the total flavor profile.

The preliminary results obtained support the conclusion that several of the ten novel compounds exhibit bitter and/or unpleasant taste and therefore seem to contribute to the taste profile of crude *Eridictyon* extracts. They might also explain why methanolic *E. angustifolium* or *E. californicum* extracts do not show the same bitter masking activity compared to the isolated bitter masking molecules homoeriodictyol and sterubin, which are contained in high amounts in these extracts. The preliminary data for erionic acid C (**3**) may lead to the conclusion that the newly identified bisprenylated benzoic acid can neutralize the bitter masking activity of homoeriodictyol and other flavanones due to their intrinsic bitter taste together with their high concentration. However, further recombination and omission experiments are necessary to determine the relative and absolute contributions of the remaining bisprenylated benzoic acids to the observed off-flavors.

Based on the described results, field trials were carried out, in order to find the optimum harvesting conditions for *Eriodictyon*. The aim of these experiments was to observe the formation of

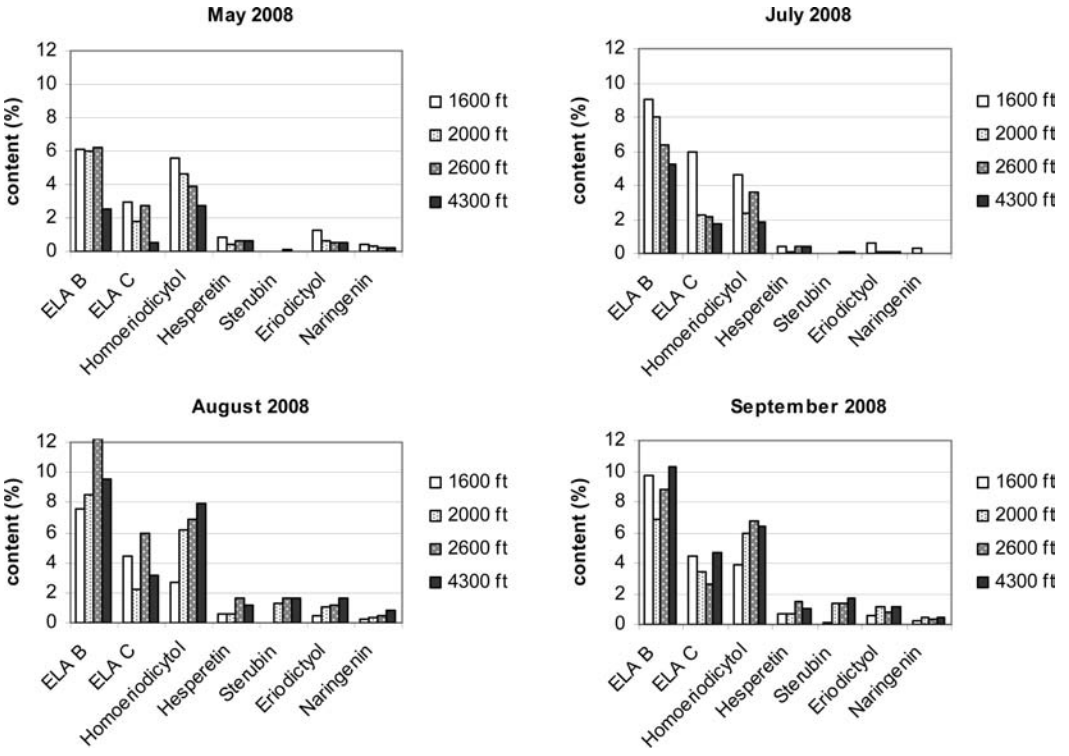

Figure 8. Seasonal variation of flavonoid and eriolic acid (ELA) content depending on altitude of origin (g 100 g^{-1} dried leaves).

both flavonoids and benzoic acid derivatives to find out if careful selection of plant material might lead to more favorable flavonoid/benzoic acid ratios, i.e. high levels of taste modulating flavonoids but low amount of benzoic acids. It is known that the phytochemical profile of plants changes under varying climatic conditions such as temperature, soil conditions, or altitude of growth, but also during different periods of growth depending on the seasons. Therefore, plant material (whole branches) of *E. californicum* was collected in four different locations in Northern California at altitudes of 1600 ft (approximately 500 m), 2000 ft (approximately 600 m), 2600 ft (approximately 800 m), and 4300 ft (approximately 1300 m); collection was carried out in May, July, August, and September 2008. Homoeriodictyol, hesperetin, eriodictyol, and naringenin were quantified from the dried leaves to observe their variation and formation depending on altitude and season. In addition, the two main compounds from the eriolic acid fraction of the extract, eriolic acid B (**8**) and C (**9**), were quantified and the data compared to the flavonoid content (**Figure 8**).

Surprisingly, the contents of eriolic acids and flavonoids are very high, regardless of the individual plant or seasonal variability and the impact of the altitude. Even taking into account that eriolic acids A (**7**) and D (**10**) were not quantified due to the lack of sufficient reference compounds, the amounts of eriolic acids B (**8**) and C (**9**) seem to outnumber the amount of total quantified flavonoids in the same period of time. When the concentrations of both types of compounds are compared over the different months, certain correlations can be found between the concentrations of eriolic acids and flavonoids in the leaves of *E. californicum*. In the first two months of the collection period, in May and July 2008, higher levels of flavonoids were detected in plant material originating from the lower areas. The leaves collected from higher altitudes, especially at 4300 ft, contain only half the amount of flavonoids than leaves from 1600 ft in the same period of time. This might be due to climatic reasons, as the temperature conditions are considered to be harsher at higher altitudes in early summer compared to low elevation collection sites, so that there is an advance in vegetation at the lower areas. Similar results can be found for eriolic acids, although the differences for the altitudes between 1600 and 2600 ft for the collection in May are marginal. In the July 2008 sample, clear correlations between the concentration of eriolic acid B and altitude can be found: the higher the site of collection, the lower was the detected amount of eriolic acid B (**8**). For eriolic acid C (**9**), the highest concentrations were detected. In midsummer and early fall, these findings seem to reverse, as higher concentrations of flavonoids and eriolic acids can be detected in the mountainous regions compared to the lower areas. This might be due to climatic variations between lower and higher elevation, so that the optimum conditions, leading to the formation of flavonoids and eriolic acids in the leaves of *Eriodictyon californicum*, are reached later during the year. The results indicate that there are certain correlations between the formation of flavonoids and benzoic acid derivatives in the plant, so that no optimum harvesting conditions, i.e. high flavonoid with concurrent low benzoic acid level, could be identified.

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LITERATURE CITED

(1) Howard, J. L. *Eriodictyon californicum*. In *Fire Effects Information System*; U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer), **1992**; available online: http://www.fs.fed.us/database/feis/plants/shrub/erical/all.html [2008, Oct. 17].

(2) Munz, P. A. *A California flora and supplement*. University of California Press: Berkeley, CA, 1973; p 547.

(3) Hadley, W. J.; Gisvold, O. A phytochemical study of Eriodictyon angustifolium, Nuttley. *J. Am. Pharm. Assoc. Sci. Educ.* **1944**, *33*, 275–7.
(4) Meyer, G. Sirupus Eriodictyonis, ein neues Geschmackskorrigens. *Pharm. Z.* **1905**, *82*, 870.
(5) Barrett, S. A.; Gifford, E. W. Miwok material culture: Indian life of the Yosemite region. *Bulletin of the Milwaukee Public Museum*; Yosemite Association: Yosemite National Park, CA, 1933; Vol. 2, Issue 4, 388 pp.
(6) Lewin, L. Ueber die Geschmacksverbesserung von Medicamenten und über Saturationen. *Berl. Klin. Wochenschr.* **1894**, *28*, 644–645.
(7) Ley, J. P.; Krammer, G. E.; Reinders, G.; Gatfield, I. L.; Bertram, H.-J. Evaluation of Bitter Masking Flavanones from Herba Santa (*Eriodictyon californicum* (H & A) Torr.; Hydrophyllaceae). *J. Agric. Food Chem.* **2005**, *53* (15), 6061–6066.
(8) Reichelt, K. V.; Peter, R.; Roloff, M.; Paetz, S.; Ley, J. P.; Krammer, G. E.; Engel, K. H. Characterization of Flavor Modulating Effects in Complex Mixtures via High Temperature Liquid Chromatography. *J. Agric. Food Chem.*, published online November 19, 2009.
(9) Roloff, M.; Peter, R.; Lütkenhaus, M.; Reichelt, K. Vorrichtung und Verfahren zur Reduzierung von Ethanol in einer flüssigen Zubereitung, **2009**, EP 2113770.
(10) Frank, O.; Ottinger, H.; Hofmann, T. Characterization of an Intense Bitter-Tasting 1*H*,4*H*-Quinolizinium-7-olate by Application of the Taste Dilution Analysis, a Novel Bioassay for the Screening and Identification of Taste-Active Compounds in Foods. *J. Agric. Food Chem.* **2001**, *49*, 231–238.
(11) Roloff, M.; Erfurt, H.; Kindel, G.; Schmidt, C. O.; Krammer, G. Process for the separation and sensory evaluation of flavors. 2006, WO2006/111476.
(12) Johnson, N. D. Flavonoid aglycons from *Eriodictyon californicum* resin and their implications for herbivory and UV screening. *Biochem. Syst. Ecol.* **1983**, *3*, 211–215.
(13) Dong, M.; Nagaoka, M.; Miyazaki, S.; Iriye, R.; Hirota, M. 3-Geranyl-4-hydroxy-5-(3′-methyl-2′-butenyl) benzoic Acid as an Anti-inflammatory Compound from *Myrsine* seguinii. *Biosci. Biotechnol. Biochem.* **1999**, *63* (9), 1650–1653.
(14) Hirota, M.; Miyazaki, S.; Minakuchi, T.; Takagi, T.; Shibata, H. Myrsinoic Acids B, C and F, Anti-inflammatory Compounds from Myrsine seguinii. *Biosci. Biotechnol. Biochem.* **2002**, *66* (3), 655–659.
(15) Ali, M. S.; Jahangir, M. Scoparal: A new Aromatic Constituent from *Artemisia scoparia* Waldst. *J. Chem. Soc. Pak.* **2008**, *30* (4), 609–611.
(16) Arriaga-Giner, F. J.; Wollenweber, E.; Schober, I.; Yatskievych, G. Three new Benzoic Acid Acid Derivatives from the Glandular Excretion of *Eriodictyon sessilifolium*. *Z. Naturforsch.* **1988**, *43c*, 337–340.
(17) Amaro-Luis, J. M.; Koteich-Khatib, S.; Carrillo-Rodriguez, F.; Bahsas, A. Myrsicorianol, A New Prenylated Benzoic Acid Derivative from *Myrsine coriacea*. *Nat. Prod. Commun.* **2008**, *3* (3), 323–327.
(18) Makabe, H.; Miyazaki, S.; Kamo, T.; Hirota, M. Myrsinoic Acid E, Anti-inflammatory Compound from *Myrsine seguinii*. *Biosci. Biotechnol. Biochem.* **2003**, *67* (9), 2038–2041.
(19) Terreaux, C.; Gupta, M. P.; Hostettmann, K. Antifungal Benzoic Acid Derivatives from *Piper dilatatum*. *Phytochemistry* **1998**, *49* (22), 461–464.
(20) Orjala, J.; Erdelmeier, C. A. J.; Wright, A. D.; Rali, T.; Sticher, O. Five New Prenylated p-Hydroxybenzoic Acid Derivative with Antimicrobial and Molluscicidal Activity from *Piper aduncum* Leaves. *Planta Medica* **1993**, *59*, 546–551.
(21) Masuoka, C.; Ono, M.; Ito, Y.; Nohara, T. Antioxidative constituents from the Aerial Part of *Piper elongatum* Vahl. *Food Sci. Technol. Int. Tokyo* **1997**, *3* (3), 285–289.
(22) Masuoka, C.; Ono, M.; Ito, Y.; Nohara, T. Antioxidative, Anti-hyaluronase and Antityrosinase Activities of Some Constituents from the Aerial Part of *Piper elongatum* Vahl. *Food Sci. Technol. Res.* **2003**, *9* (2), 197–201.

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