Study of volatile polyacetylenes and terpenoids of *Baccharis palustris* Heering through GC/MS, GC/HRMS-TOF and GCxGC/HRMS-TOF

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Introduction

Polyacetylenes are specialized bioactive metabolites disseminated in almost all the biological taxa, however, plants represent their main source especially Asteraceae and Apiaceae by the wide diversity of structures identified in their species [1,2]. Polyacetylenes are relevant compounds due to their pharmacological and therapeutic properties, including their promising cytotoxic and antitumoral activities, as is the case of the C_{17}-polyacetylenes falcarinol, panaxydol and panaxytriol from *Panax ginseng* C.A. Meyer (Apiaceae) [3].

In *Baccharis* L., as one of the largest taxa belonging to Asteraceae, a series of C_{10} and C_{17}-polyacetylenes have been identified in different organic extracts from at least 15 species, but their presence in essential oils is rare [4]. Previously, we identified polyacetylenes in *Baccharis palustris* Heering (Figure 1) essential oil (BPEO), and then fully elucidated them through a battery of spectroscopic techniques, which resulted in three new C_9- and two known C_{10}-polyacetylenes [5,6]. In this work, we present the results of the analysis of BPEO through GC/MS (ISO standard), GC/HRMS-TOF and GCxGC/HRMS-TOF.

Figure 1: ***Baccharis palustris*** **Heering. (Photo: H.A. González; National Museum of Natural History (NMNH, Montevideo)**

Materials and Methods

Aerial parts of *B. palustris* (Figure 1) were collected in Southern Uruguay region ("Paso Carrasco", Canelones) at vegetative stage in July 2021. Taxonomical identification was conducted by H.A. González (NMNH, Montevideo), and a sample was herborized. The extraction of the BPEO was conducted by hydrodistillation following our previous protocol [6]; yield: 0.5%.

Primary analyses of the BPEOs were performed on a GC/MS instrument equipped with two capillary columns of different stationary phases polarities (DB-5MS and DB-Wax). The injection of the samples and the chromatographic conditions were according to an ISO reference [7]. Mass spectra comparison with commercial libraries and with our previous results, and linear retention index (LRI) were employed to identify or tentatively identify the components [6]. GC/HRMS-TOF and GCxGC/HRMS-TOF analyses were conducted to confirm GC/MS results and to find out possible new components. The instrument was equipped with a DB-5MS capillary column (1st dimension) and a DB-17MS (2nd dimension). Experimental chromatographic conditions were the same as those for GC/MS analyses.

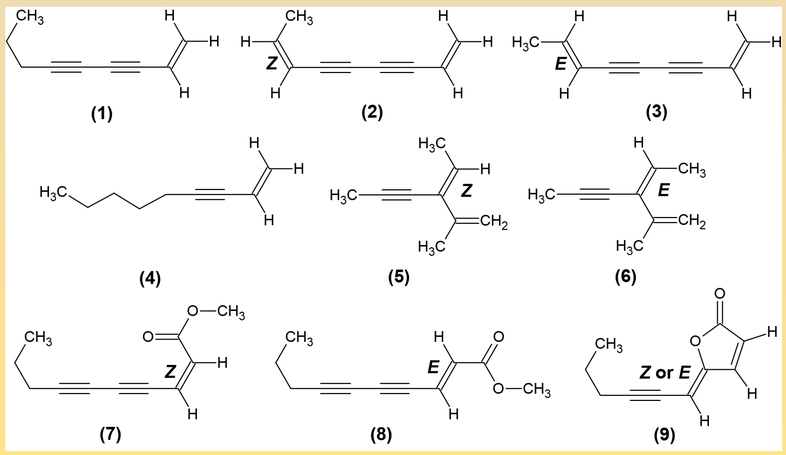

Figure 2: Polyacetylenes of BPEO: **1.** baccharisdyine; **2.** 7-*cis*-dehydrobaccharisdyine; **3.** 7-*trans*-dehydrobaccharisdyine; **4.** 1-nonen-3-yne; **5.** *cis*-3-ethylidene-2-methyl-1-hexen-4-yne; **6.** *trans*-3-ethylidene-2-methyl-1-hexen-4-yne; **7.** *cis*-lachnophyllum acid methyl ester; **8.** *trans*-lachnophyllum acid methyl ester; **9.** *cis*- or *trans*-lachnophyllum lactone.

Results and Discussion

Table 1 shows the main components of BPEO, where 42 components were identified or tentatively identified, most of them mono and sesquiterpenoids.

Compounds	Exp.	Lit.	LRI DB-5MS	LRI DB-Wax	% Abund. Pr. Inf.*
3-ethylidene-2-methyl-1-hexen-4-yne (5/6)£	1004	1070	1.1	0.7	No
(*trans*)-β-ocimene	1049	1044	4.2	3.0	Yes
baccharisdyine (1)	1089	na	56.3	63.1#	Yes
7-*cis*-dehydrobaccharisdyine (2)	1097	na	15.8	13.4	Yes
unidentified C_8H_8O	1110	na	0.7	0.6	No
7-*trans*-dehydrobaccharisdyine (3)	1121	na	2.1	1.6	Yes
unidentified $C_9H_{10}O$ + C_9H_{10}	1203	na	0.4	nd	No
α-copaene	1382	1374	0.3	0.2	Yes
(*trans*)-β-caryophyllene	1425	1417	0.3	0.3	Yes
germacrene D	1488	1484	0.6	0.5	Yes
bicyclogermacrene + *epi*-cubebol	1504	1500	0.7	0.1/0.3	Yes/No
(*cis*)-lachnophyllum acid methyl ester (7)	1519	1493	9.5	10.3	Yes
δ-cadineno + cubebol	1525	1522	0.6	0.3/0.3	Yes/No
trans-nerolidol	1573	1571	0.4	0.3	Yes
spathulenol + germacrene D-4-ol	1583	1577	2.0	1.5/0.2	Yes/No
caryophyllene oxide + unidentified $C_{15}H_{24}O$	1586	1582	0.6	0.2	Yes/No
α-cadinol	1650	1652	0.3	0.3	Yes

Table 1: Main BPEO components (≥0.3% of abundance) analyzed by GC/MS and GC/HRMS-TOF (bold). LRI Exp. and LRI Lit.: linear retention indexes obtained experimentally or from the literature respectively [6]. References: na (not available); nd (not detected); *(previously informed in [6]); # (co-elution with a compound C_9H_8); £ LRI from [8].

Ten BPEO minor oxygenated terpenoids (abundance <0.3%) not previously reported were identified in this work: α-pinene epoxide, *trans*-β-ocimene epoxide, *epi*-cubebol, cubebol, germacrene D-4-ol, junenol, *epi*-α-cadinol, *epi*-α-muurolol, germacra-4(15),5,10(14)-trien-1-β-ol and oplopanone. Several of these compounds co-eluted with other major components (Table 1) and, therefore, could not be identified by only one chromatographic dimension.

Confirming previous results (Table 1), the more abundant components of BPEO were the C_9-polyacetylenes [6]. GC/HRMS-TOF analyses established the molecular ions at m/z 118.0775 (Δ: 1.39 ppm) and 116.0619 (Δ: 1.08 ppm) corresponding to the formulas C_9H_{10} and C_9H_8 of **1** and **2**/**3,** respectively (Figure 2). Three other minor C_9-polyacetylenes were tentatively identified (**4** to **6**, Figure 2), including two geometric isomers **5**/**6** after GCxGC/HRMS-TOF analyses. Later compounds were previously reported to be major components of *Prangos* spp. (Apiaceae) essential oils [8]. To the best of our knowledge, **4** has not been previously reported in essential oils, but its structure is in line with the corresponding of **1** to **3** (Figure 2), suggesting a biogenetic pattern.

The putative C_{10} polyacetylene precursors of **1** to **4**, namely **6** and **7** (Figure 2) [6], were confirmed in BPEO after GC/HRMS-TOF analyses (molecular ions at m/z 176.0830; $C_{11}H_{12}O$; Δ: 0.82 ppm). A surprising result was obtained by GCxGC/HRMS-TOF analyses of the peak of **6**, which was resolved in two peaks in the second chromatographic dimension, the second one with the spectrum of **9** (undefined stereochemistry) [9] (Figures 2 and 3).

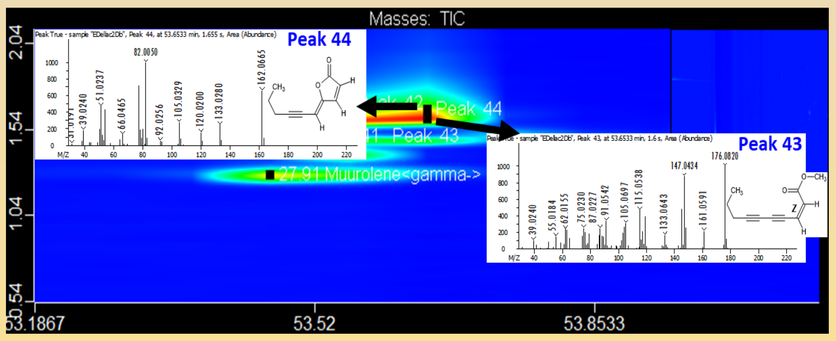

Figure 3: GCxGC/HRMSTOF counterplot and mass spectra of the co-elution of the peak #43 (*cis*-lachnophyllum acid methyl ester; **7**) and peak #44 (lachnophyllum lactone, unknown stereochemistry; **9**) from BPEO.

Compound **9** has been reported as a bioactive component of several essential oils, as allelopathic, fungitoxic and repellent [10]. Its presence in BPEO even at trace level, might contribute to its potential bioactivity [5], which need to be evaluated in further studies.

B. palustris is a highly endangered species in Uruguay and Southern Brazil. The presence of this uncommon volatile polyacetylenes (to an unprecedent level inside *Baccharis*, and inside Asteraceae) might contribute to its preservation as a valuable source of them.

Conclusion

Applying GC/MS (ISO standard), GC/HRMS-TOF and GCxGC/HRMS-TOF to BPEO was possible to confirm previous results and to add the identification of ten oxygenated terpenoids, three C_9-polyacetylenes and a lachnophyllum lactone (unknown stereochemistry).

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