Three New Benzoic Acid Derivatives from the Glandular Excretion of *Eriodictyon sessilifolium* (Hydrophyllaceae)

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Eriodictyon sessilifolium, an endemic Hydrophyllaceae of Baja California (Mexico), has been studied for the natural products exuded by glandular trichomes covering its leaves and stems. Major components of this exudate were isolated and identified by spectroscopic methods to be 4-hydroxy-3-(2,3-dihydroxy-3-methylbutyl)-benzoic acid methyl ester (**1**), the natural methyl ester of anodendroic acid (**2**), and 2,2-dimethyl-3-hydroxychroman-6-carboxylic acid methyl ester (**3**). Possible biosynthetic relations between these new natural products are considered. In addition, 9 known flavonoid aglycones were identified as exudate constituents.

The genus *Eriodictyon* Benth. (Hydrophyllaceae) comprises "shrubs with resinous aromatic young branches and leaves, the latter being ... more or less glutinous-resinous" [1]. In species with high glandular activity, such as *E. angustifolium* Nutt. and *E. trichocalyx* Heller, the leaves and even the calyces are "sticky with a glandular secretion" [2]. These plants therefore attracted our interest and were investigated in our current research project "Exudate Flavonoids". Phytochemical studies on the constituents of *Eriodictyon* are scarce [3–5] and to our knowledge there is none that deals with *E. sessilifolium* Greene. This species is endemic to Baja California, where it grows in canyons, washes, burnt hillsides, ravines, and mesas [1]. The hirsute, glandular leaves of *E. sessilifolium* are not sticky with glandular exudate, *i.e.* the amount produced is much lower than in the species cited above. On rinsing the leaves with organic solvents, however, the glandular products are readily dissolved. A number of flavonoid aglycones are present in this leaf-wash, but the major constituents of the exudate were found to be benzoic acid derivatives. The structural elucidation of these new natural products will be reported here.

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Materials and Methods

Plant material

Aerial parts from flowering plants of *Eriodictyon sessilifolium* were collected on May 23, 1982, near San Telmo, Baja California Norte, Mexico. The plants were growing in an open sandy wash, with *Agave, Haplopappus,* and *Opuntia,* in a disturbed area at 70 m elevation. Aerial parts only were gathered and dried in a paper bag. Herbarium specimens (G. Yatskievych 82-177A) are kept at ARIZ and E.W.'s personal herbarium.

Isolation and characterization of compounds ***1-3***

On rinsing with acetone, the airdried plant material (76.3 g) yielded, after evaporation of the solvent, a dark brown resin (10.8 g = 16% d.w.) which was chromatographed by CC over silica, eluted with toluene and increasing quantities of methylethyl ketone and MeOH. Volumes and numbers of fractions were not recorded. Fractions were monitored by TLC on polyamide (for flavonoids) and on silica (for terpenoids) and similar fractions were combined. Two flavonoids were obtained in crystalline

form and seven further flavonoids were identified by direct comparisons with markers.

Two major non-flavonoid 'constituents' of the excretion were also isolated. Compound **1** forms fine colourless needles, m.p. 143–144 °C. $[\alpha]_D^{25}$ + 12.0° (EtOH, $c = 0.4$). MS: *m/z* (rel. int.) 254 (M^+, 19) 236 (M^+-H_2O, 7), 223 (M^+-OCH_3, 6), 196 (36), 181 (12), 178 (20), 165 (60), 152 (11), 147 (14), 134 (13), 107 (31), 91 (15), 77 (28), 71 (63), 59 (82), 43 (100), 41 (20). ^{1}H NMR ($CDCl_3$) δ ppm: 7.84 (H-6, dd, 8.5, 2), 7.72 (H-2, d, 2), 6.93 (H-5, d, 8.5), 3.87 (3H, s), 3.69 (H-2′, dd, 9.5, 2), 2.85 (H-1′, dd, 14.5, 9.5), 2.68 (H-1′, dd, 14.5, 2), 1.35 (3H, s), 1.29 (3H, s). For ^{13}C NMR data see Table I.

1 (19 mg) in dry acetone (5 ml) was treated with an excess of ethereal diazomethane at room temp. The solvent was evaporated and **1a** obtained as a yellowish oil (16 mg). MS: *m/z* (rel. int.) 268 (M^+, 7), 237 (M^+-OCH_3, 23), 221 (18), 210 (100), 179 (74), 166 (98), 149 (54), 135 (22), 121 (38), 105 (24), 91 (41), 77 (27), 59 (52). ^{1}H NMR ($CDCl_3$) δ ppm: 7.94 (H-6, dd, 8.5, 2), 7.88 (H-2, d, 2), 6.89 (H-5, d, 8.5), 3.89 (3H, s), 3.87 (3H, s), 3.64 (H-2′, dd, 10, 2), 2.96 (H-1′, dd, 14.2), 2.61 (H-1′, dd, 14, 10), 1.30 (3H, s), 1.27 (3H, s).

1a (16 mg) in Py/Ac_2O 1:1 (2 ml) kept at room temp., overnight, yielded **1b** (18 mg) as a colourless oil. MS *m/z* (rel. int.): 310 (M^+, 1), 292 (M^+-H_2O, 1), 279 (M^+-OM_3, 15), 263 (8), 250 (M^+-AcOH, 46), 235 (15), 218 (36), 203 (53), 179 (100), 177 (88), 166 (24), 149 (31), 105 (21), 91 (19), 77 (12), 59 (27). ^{1}H NMR ($CDCl_3$) δ ppm: 7.91 (H-6, dd, 8.5, 2), 7.79 (H-2, d, 2), 6.84 (H-5, d, 8.5), 5.08 (H-2′, dd, 10.5, 2.5), 3.90 (3H, s), 3.87 (3H, s), 3.19 (H-1′, dd, 13.5, 2.5), 2.66 (H-1′, dd, 13.5, 10.5), 1.86 (3H, s), 1.30 (3H, s), 1.27 (3H, s).

The mixture of compounds **2/3** was isolated as a light yellow resinous material. Since no acetates were observed in its ^{1}H NMR spectrum, 900 mg of this mixture were acetylated as above to yield 995 mg of crude product which was chromatographed on a "flash" Si-gel column using solvent E as eluent. Two pure products were isolated, **2** (200 mg) and **3a** (520 mg).

The MS data for compound **2** are *m/z* (rel. int.) 236 (M^+, 30), 221 (M^+-CH_3, 2), 218 (M^+-H_2O, 14), 205 (M^+-OCH_3, 16), 203 (M^+-H_2O-CH_3, 74), 178 (100), 165 (13), 147 (46), 119 (32), 105 (22), 91 (27), 77 (11), 59 (52). M.p. and ^{1}H NMR are in agreement with those reported for synthetic anodendroic acid methyl ester [6]. $[\alpha]_D^{25}$ 61.1° (EtOH, $c = 1.0$). For ^{13}C NMR data see Table I.

Compound **3a** forms a white powder, m.p. 98–100 °C. $[\alpha]_D^{25}$ −42.2° (EtOH, $c = 1.0$). MS: *m/z* (rel. int.) 278 (M^+, 5), 247 (M^+-OCH_3, 4), 218 (M^+-AcOH, 15), 203 (M^+-AcOH-CH_3, 100), 165 (13), 105 (5), 91 (4), 77 (6), 59 (4). ^{1}H NMR ($CDCl_3$) δ ppm: 7.81 (H-7, dd, 8, 2), 7.79 (H-5, d, 2), 6.85 (H-8, d, 8), 5.05 (H-3, t, 5), 3.87 (3H, s), 3.12 (H-, dd, 17.3, 5), 2.83 (H-4, dd, 17.3, 5), 2.06 (3H, s), 1.36 (3H, s), 1.33 (3H, s).

238 mg of the mixture **2/3** in MeOH (10 ml) and 8.5% KOH (6 ml) were refluxed for 8 h. After ether extraction and evaporation, 187 mg of crude hydrolysis mixture **2a/3b** were acetylated as above and the product (218 mg) was chromatographed on a 'flash' Si-gel column using solvent F as eluent to yield **2a** (40 mg) and **3c** (176 mg).

M.p. and ^{1}H NMR data of **2a** are identical with those reported for natural anodendroic acid [6], while m.p. and ^{1}H NMR data of **3c** agree with those reported for synthetic 2,2-dimethyl-3-acetoxy-chroman-6-carboxylic acid [6]. For **3b** $[\alpha]_D^{25}$ −24.8° (EtOH, $c = 1.0$).

TLC and general methods

The solvents used for TLC of fractions as well as for comparisons with authentic markers were A) toluene-petroleum ether (b.p. 100–140 °C)-methylethyl ketone-MeOH (12:6:2:1) and B) toluene-methylethyl ketone-MeOH (12:5:3) for TLC on Polyamid DC-11 [7]. Chromatograms were viewed at

Table I. ^{13}C NMR data of compounds **1**–**3**[a].

	1 (CD_3OD)	**2** ($CDCl_3$)	**3** ($CDCl_3$)
	122.1 (1)	122.0 (5)	119.0 (6)
	134.3 (2)	130.6 (4)	131.7 (5)
	128.2 (3)	127.4 (9)	121.5 (10)
	161.8 (4)	163.4 (8)	157.0 (9)
	116.0 (5)	108.4 (7)	116.6 (8)
	130.6 (6)	126.3 (6)	129.0 (7)
	33.9 (1′)	29.6 (3)	30.6 (4)
	79.5 (2′)	90.2 (2)	68.6 (3)
	73.8 (3′)	71.2 (1′)	77.6 (2)
2× CH_3	25.6, 25.0	25.3, 24.1	24.9, 21.1
$\underline{C}O_2CH_3$	168.9	166.6	166.8
$CO_2\underline{C}H_3$	52.2	51.4	51.4

[a] (δ in ppm, measured at 50.13 MHz; data are arranged in order to facilitate comparisons with structural formulae in Fig. 1).

366 nm before and after spraying with Naturstoffreagenz-A (NA; 0.5% in MeOH). For terpenoids and other compounds we used precoated silica plates (Polygram SIL-G) with solvents C) toluene-methyl-ethyl ketone (9:1), D) toluene-dioxane-HOAc (18:5:1), E) CH_2Cl_2-MeOH (99:1) and F) CH_2Cl_2-MeOH (95:5). Spots were visualized by spraying with $MnCl_2$-reagent (3 g of $MnCl_2$ dissolved in 150 ml H_2O, 750 ml MeOH, and 30 ml conc. H_2SO_4 carefully added), followed by heating to 130 °C [8].

All flavonoid markers were available in E. W.'s laboratory.

Mass spectra were recorded at 70 eV on a GC-MS Hewlett-Packard 5985. ^1H NMR spectra and ^{13}C NMR spectra were recorded on a Bruker WP 200 SY spectrometer at 200 and at 50.13 MHz, respectively, at the Departamento de Química Orgánica of the Universidad Autónoma de Madrid. Melting points are uncorrected. Adsorbents used for TLC and CC (Kieselgel N, Polyamid SC-6 and Polyamid DC-11) were from Macherey-Nagel/Düren. Naturstoffreagenz A (NA, β-aminoethyl ester of diphenyl boric acid) was from C. Roth, Karlsruhe).

Results

Nine flavonoid aglycones were identified from the relevant fractions of the glandular exudate of *Eriodictyon sessilifolium*. The two dominant flavonoids were obtained in crystalline form and were identified by their m.p., UV spectra, and mass spectra, as well as by direct TLC comparisons with markers, to be hispidulin (scutellarein 6-methyl ether) and pectolinarigenin (scutellarein 6,4′-dimethyl ether). In addition, the flavones chrysin, apigenin, genkwanin (apigenin 7-methyl ether), acacetin (apigenin 4′-methyl ether) and velutin (luteolin 7,3′-dimethyl ether) and the flavonols kaempferid (kaempferol 4′-methyl ether) and isorhamnetin (quercetin 3′-methyl ether) were identified by direct TLC with markers as well as by their UV spectra measured on small samples, isolated by preparative TLC.

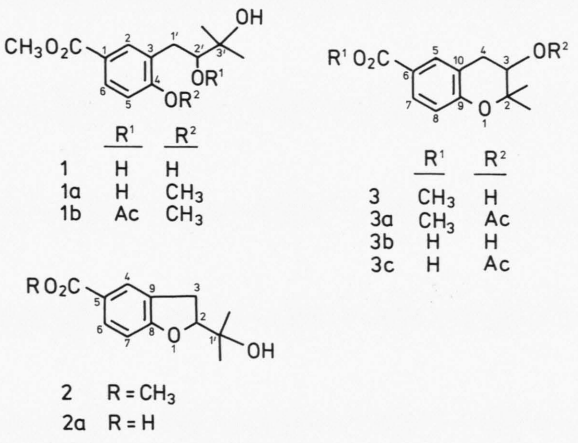

Fig. 1. Structural formulae of compounds **1**–**3** and their derivatives.

The two major 'components' of the leaf exudate, that were obtained in chromatographically pure form, were no flavonoids. Their color reactions with the $MnCl_2$-reagent [8] and also their molecular masses led us to assume that they were terpenoids, but detailed spectral analyses showed that this is not true.

Compound **1** was shown, by preparation of simple derivatives, to be a disubstituted methylbenzoate that contains three different hydroxyls. One of them is phenolic, as it reacts with diazomethane to yield **1a**. The second can, after methylation, be acetylated to yield **1b**, while the third is not affected by these reagents and is hence tertiary. The ^1H NMR data agree with a *p*-hydroxy-benzoate with a side-chain at C-3. Its chemical shifts and multiplicities are the same as observed for 2,3-dihydroxy-3-methylbutyl in elsholtzidiol, a substituted furane from *Elsholtzia densa* [9]. The MS and ^{13}C NMR data confirm this assignment. Compound **1** is, therefore, identified as 4-hydroxy-3-(2,3-dihydroxy-3-methylbutyl)-benzoic acid methyl ester.

The second 'component' appeared to be uniform chromatographically; the ^1H NMR spectrum revealed, however, that it was a mixture (ca. 1: 5) of two closely related compounds **2** and **3**. After acetylation, two spots were observed on TLC (solvent E; **2** at R_f 0.45 and **3a** at R_f 0.60) and the products were subsequently isolated by flash-CC over silica. Alkaline hydrolysis of the mixture **2/3** yielded a mixture of two free acids, **2a** and **3b**, that also appeared as a single spot on TLC. Again, these products could be separated after acetylation (solvent F; **2a** at R_f 0.30, **3b** at R_f 0.42). From the different behaviour on acetylation it became evident that **2** had a tertiary hydroxyl. The existence in the ^1H NMR of different chemical shifts and multiplicities of high-field protons indicated **2** to be dihydrobenzofurane and **3** to be a chroman. Detailed analysis of the total spectroscopic data led us to assign the structure of methyl-2-

(1-hydroxy-1-methylethyl)-2,3-dihydrobenzofuran-5-carboxylate to compound **2**, while compound **3** is identified as 2,2-dimethyl-3-hydroxychroman-6-carboxylic acid methyl ester.

Discussion

Although dried leaves of *Eriodictyon californicum* (Hook and Arn.) Torr. (Yerba Santa) and *E. glutinosum* Benth (gum bush, bearweed, mountain balm) have traditionally been used by American Indians and are still used today as a drug (Eriodictyonis Herba), phytochemical studies on this genus and even on the whole family Hydrophyllaceae are scarce [3]. The flavanones eriodictyol (5,7,3′,4′-tetrahydroxy flavanone) and homoeriodictyol (eriodictyol 3′-methyl ether) owe their trivial names to their first isolation from this plant source in 1907 [10]. Recent reports on *E. californicum* flavonoids [4, 5] are contradictory, probably due to the use of material from different populations. We have studied 6 different species of *Eriodictyon* for their exudate flavonoids. The results will be discussed in detail in a separate paper and compared with those for some species of *Wigandia,* another genus of the Hydrophyllaceae.

In the present paper, emphasis is on the isolation and structural elucidation of the new products, **1**–**3**, which are the major constituents excreted by the glandular trichomes on leaves and twigs of *Eriodictyon sessilifolium.* These were not detected in the 5 other species studied. Compound **1**, 4-hydroxy-3-(2,3-dihydroxy-3-methylbutyl)-benzoic acid methyl ester is a new natural product. A closely related compound, methyl-4-hydroxy-3-(2′-hydroxy-3′-methyl-but-3′-enyl)-benzoate has been reported recently from the bark of *Piper hostmannianum* (Piperaceae) [11]. Compound **2**, identified as methyl-2-(1-hydroxy-1-methylethyl)-2,3-dihydrobenzofuran-5-carboxylate, is the new natural methyl ester of anodendroic acid, previously reported from *Anodendron affine* (Apocynaceae) [6]. Compound **3**, 2,2-dimethyl-3-hydroxychromane-6-carboxylic acid methyl ester has thus far been reported only as a synthetic product [6]. 6-Carboxy-2,2-dimethyl chromanes are very rare in nature; a related 3-hydroxy chromane has been isolated from *Helichrysum stoechas* (Asteraceae) [12]. Interestingly enough, a similar compound was also reported from the bark of *Piper hostmannianum* [11]. Thus in *Piper* as well as in *Eriodictyon* the disubstituted benzoic acid derivative and the chromane occur jointly in the same species. This coincidence is probably not incidental. We would assume that compound **1**, the isoprenylated benzoate, is a precursor in the biosynthesis of compounds **2** and **3** by cyclization. That the newly reported compounds are indeed natural methyl esters follows from the observation that they are also obtained in leaf washes prepared with chloroform or with benzene, *i.e.* they have not been formed by reaction with MeOH, which was used as an eluent in chromatography.

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