Flavonoids and Terpenoids from the Leaf Resin of *Pluchea odorata*

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The chemical composition of the leaf resin of *Pluchea odorata* has been studied from material collected in Tamaulipas, Mexico. It mostly consists of triterpenes and phytosterols and an eudesmane type sesquiterpene. Very small amounts of ten different flavonol aglycones occur, dissolved in this resin; most of them are 6-methoxy compounds. A comparison with previous results on material from El Salvador shows that the latter contains a lesser number of triterpenes and especially of flavonoids, but more sesquiterpenes, while the n-alkane composition varies only quantitatively between both populations.

Introduction

The spur bush, *Pluchea odorata* (L.) Cass. (Compositae, tribe Inuleae) is a medicinal shrub known as "cuauhtematl," "hierba de Santa Maria," and "alinache" in Mexico, "siguapate" in El Salvador, "yerba del luceo" in Argentina, etc. It occurs from Florida through Mexico to South America and the West Indies. Leaf extracts are used in various household remedies against neuralgia and rheumatism, as stomachic, as general tonicum, and as a bath additive. Its leaves are pubsecent and exhibit a peculiar scent. They appear slightly sticky and thus attracted our interest in the scope of continuing studies on the distribution of flavonoid aglycones in plant excretions [1, 2]. For the present study we therefore dealt with the resin that was obtained from air-dried leaves of *P. odorata* by rinsing them with acetone. This solution exhibits neither chlorophyll degradation products nor carotenoids, and we are sure that it contains nothing but the external natural products. In the following we will report on the composition of the leaf resin of plant material collected in Tamaulipas, Mexico, as compared with material from El Salvador analyzed previously [3a, b].

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Materials and Methods

Leaves of *Pluchea odorata* (L.) Cass. were collected for this study on Highway 101 between Cd. Victoria and Juamave, Edo. Tamaulipas, Mexico on 3 May, 1983. The plants were growing on W-facing limestone roadside cliffs with grasses, *Cnidoscolus,* and shrubby legumes, at an elevation of some 800 m. The plant material was air-dried in a paper sack. Pressed vouchers (Yatskievych & Wollenweber 83–111) are deposited at ARIZ, IND, and the personal herbarium of E. Wollenweber.

The dry leaves (227 g) were rinsed with acetone to yield, after evaporation of the solvent, 3.3 g of resinous material (1.4% d.w.). The brownish resin was subjected to column chromatography on silica gel (Kieselgel N). Several fractions were then rechromatographed on polyamide (Polyamid SC-6). In both cases elution was done with toluene and increasing quantities of methylethyl ketone and methanol. Fractions containing flavonoids were finally purified, *i.e.* freed from terpenoids, by a passage over Sephadex LH-20, eluted with methanol. Only two non-polar flavonoids crystallized in minute amounts (PO-A/B and PO-C) and were characterized by their spectral data. The others were identified by direct comparisons with markers available from E. W.'s previous work on flavonoid aglycones [2]. – The very first fractions from the silica column yielded waxy material. This was purified by an additional passage over silica, eluted with petrol, and

then analyzed by gc (metal column, 2.5% SE-30 on Chromosorb-G-HP, 80–100 mesh; FID detection and injection port temp. 350 °C; He gas flow rate 60 ml/min; programmed temp. from 220 °C to 300 °C at 4 °/min). From the following, still relatively unpolar fractions of the silica column several portions of terpenoid material showed a tendency to crystallize. The major constituents of each portion could indeed be obtained by re-crystallization from ethanol: PO-D m.p. 185–190° (110 mg), PO-E m.p. 175–178° (60 mg), PO-F m.p. 138–140° (20 mg), PO-G 156–160° (190 mg). These terpenoids were identified on the basis of their spectroscopic data and by comparison with authentic samples.

The solvents used for chromatography of flavonoids on polyamide DC-11 were A) petrol ether (b.p. 100–140°)/toluene/methylethyl ketone/methanol 60:30:5:5, B) toluene/petrol ether (b.p. 100–140°)/methylethyl ketone/methanol 60:30:10:5, C) toluene/dioxane/methanol 80:10:10. The chromatograms were evaluated in UV_{366} before and after spraying with Naturstoffreagenz A (0.5% in MeOH). For TLC of terpenoids we used silica (Polygram SilG) with solvents D) petrol ether (b.p. 100–140°)/toluene/methylethyl ketone 18:1:1, E) toluene/methylethyl ketone 9:1, and F) toluene/dioxane/acetic acid 90:25:4. These chromatograms were visualized by spraying with $MnCl_2$ reagent (3 g $MnCl_2$ dissolved in 150 ml H_2O, 750 ml MeOH and 30 ml conc. H_2SO_4 added), followed by heating to 120 °C.

Mass spectra were recorded on a Varian MAT 311 at the Institute of Organic Chemistry of the TH Darmstadt. PMR spectra were measured on a Brukker WP-200-SY at the Department of Organic Chemistry of the Universidad Autónoma at Madrid (200 MHz; $CDCl_3$/TMS). A Perkin Elmer instrument 3920 was used for gas chromatography and peak areas were measured with a Minigrator Perkin Elmer M-2. IR spectra were recorded on a Pye Unicam SP 1100, UV spectra were recorded on a Beckman DB-GT. Melting points are uncorrected.

Results

A first test of the crude resin of *Pluchea odorata* had already shown faint traces of flavonoids and it was evident that most of the material consisted of terpenoids. After repeated column chromatography only two flavonoids were obtained in minute amounts as yellow crystalls. These two compounds were identified by their UV and mass spectra. PO-A/B has m.p. 170°. UV: λ_{max}(MeOH) 338, 275 nm; + $AlCl_3$ 364, 283 nm; + NaOH (321), 272 nm; no shift with NaOAc. MS: *m/z* (rel. int.) 358 (100, M^+), 343 (55, M-15), 339 (27, M-17), 315 (12, M-43).

M.p., UV and MS are in accordance with the data reported in the literature [4, 5] for 6-hydroxykaempferol 3,6,7,4′-tetramethyl ether (4′-methyl penduletin). PO-C has m.p. 160–162°. UV: λ_{max} (MeOH) 345, 272, 255 nm; + $AlCl_3$ 376, 265 nm; + NaOH 334, (273), 253 nm; no shift with NaOAc. MS: *m/z* (rel. int.) 388 (100, M^+), 373 (68, M-15), 369 (14), 358 (46, M-2×15), 343 (29, M-3×15). On the base of these data the compound was identified as quercetagetin 3,6,7,3′,4′-pentamethyl ether (artemetin) [4, 6]. In both cases identity was further corroborated by direct comparison with markers. Eight additional flavonoids could not be isolated in pure state, due to lack of material. However, they were identified unambiguously by direct comparisons with authentic samples in several solvents [7]. These flavonols are methyl derivatives of kaempferol, quercetin, 6-hydroxykaempferol and quercetagetin, as shown in Table I.

The terpenoid samples were elucidated by interpretation of their MS and PMR spectra and the identifications were confirmed by direct comparisons (PMR, TLC) with authentic samples. We deem it not necessary to report their spectral data here. PO-D is a mixture of α-amyrin acetate (72%) and β-amyrin acetate (28%). PO-E is a mixture of the corresponding triterpenes α-amyrin (80%) and β-amyrin (20%). PO-F consists of stigmasterol (55%) and β-sitosterol (45%). PO-G is the only sesquiterpene isolated from this material. It was identified (PMR, MS, IR, UV) to be the eudesmane 3-epoxyangeloyl-4-acetyl-cuauhtemone, previously described from three different *Pluchea* species [8, 10]. Alkaline hydrolysis (Na_2CO_3/MeOH, room temp.) of PO-G affords the parent compound cuauhtemone also isolated in this genus [8, 9, 18]. – GC analysis of the waxy fractions revealed the presence of n-alkanes C_{19}–C_{31}, with C_{25}, C_{27} and C_{29} as the major components (see Table I); *n*-heptacosane is dominating.

Discussion

The first phytochemical study of *Pluchea odorata* from Edo. Nuevo León, Mexico, reported β-amyrin acetate and campesterol from the petrol extract of

Table I. Natural products from *Pluchea odorata*.

	Plants from Mexico (Edo. Tamaulipas) (present work)	Plants from El Salvador [3a, b]
n-Alkanes	$C_{19}-C_{31}$ (C_{25} 16.4%, C_{27} 36.5%, C_{29} 18.8%)	$C_{23}-C_{34}$ (C_{27} 36.5%, C_{29} 24.7%, C_{31} 12.4%)
Triterpenes	α-amyrin, α-amyrin acetate β-amyrin, β-amyrin acetate	α-amyrin, taraxasteryl acetate
Phytosterols	stigmasterol, β-sitosterol	stigmasterol
Sesquiterpenes	1 eudesmane derivative	7 eudesmane derivatives
Flavonols	3-methyl kaempferol	
	3,7,4′-trimethyl kaempferol	
		6,7-dimethyl 6-hydroxykaempferol
	3,6,4′-trimethyl 6-hydroxykaempferol	
	3,6,7,4′-tetramethyl 6-hydroxykaempferol	
	3,3′-dimethyl quercetin	
	3,7,3′,4′-tetramethyl quercetin	
	3,6,3′-trimethyl quercetagetin	
	3,6,4′-trimethyl quercetagetin	
	3,6,7,4′-tetramethyl quercetagetin	
	3,6,7,3′,4′-pentamethyl quercetagetin	3,6,7,3′,4′-pentamethyl quercetagetin

ground aerial parts [11]. The sesquiterpene cuauhtemone was described from the non-saponifiable matter of aerial parts (presumably from the same locality) [8]. Later three sesquiterpenes of the cuauhtemone type were found along with two monoterpenes and caryophyllene in the ether extract of leaves and stems (Mexican material) [12]. Only recently a study on leaves of plants from El Salvador reporting 7 eudesmane sesquiterpenes [3] also mentioned the presence of two flavonol aglycones, namely artemetin (quercetagetin 3,6,7,3′,4′-pentamethyl ether) and herbacetin 3,7-dimethyl ether. Flavonol aglycones have also been found in *P. sagittalis* (Lam.) Cabrera (5,7,3′4′-tetrahydroxy 3,6,8-trimethoxy flavone) [13], in *P. sericea* (Nott.) Coville (quercetin 3,3′-dimethyl ether) [14], and especially in *P. chingoyo* DC. (7,4′-dihydroxy 3,6-dimethoxy flavone, 6-hydroxykaempferol 3,6-dimethyl ether, quercetagetin 3,6-dimethyl ether, and quercetagetin 3,6,4′-trimethyl ether) [15]. None of these previous publications took into account that the flavonoid aglycones encountered might well be localized externally, dissolved in a leaf resin of terpenoid nature. This fact has now been demonstrated unambiguously for our Mexican material of *P. odorata*. The leaf resin produced by this plant mainly consists of free and acetylated pentacyclic triterpenes and of phytosterols, a major sesquiterpene, and n-alkanes. Dissolved in this lipophilic matter we find ten different flavonols in minute or trace amounts. This confirms our previous observation that free flavonoid aglycones tend to be excreted along with lipophilic epicuticular products of mostly terpenoid nature and that this phenomenon is especially abundant in Asteraceae [1, 2].

It is obvious that so far all the flavonoids reported from *P. odorata* as well as from other species of the genus (see above) are flavonols. With few exceptions they all bear methoxy groups at C-6. It was therefore striking that the plants from El Salvador should produce the C-8 substituted flavonol, herbacetin 3,7-dimethyl ether [3a]. A reinvestigation of this compound revealed that the first identification was indeed incorrect. It was now shown to be identical with an authentic sample of eupalitin (6-hydroxykaempferol 6,7-dimethyl ether) by TLC, UV and MS comparisons. In addition, m.p. and spectral data of its acetyl derivative are in accordance with those reported in the literature [16]. The earlier statement [3a] thus needs to be revised.

It is of interest to compare the results of our investigation on *Pluchea odorata* collected in Tamaulipas, Mexico with those on the material collected in El Salvador. For this purpose the natural products identified in both studies are summarized in Table I. The observed differences in the leaf resin composition of

both populations indicate that they represent two different chemotypes. With respect to resin flavonoids we made similar observations recently in *Heterotheca grandiflora* and *H. psammophila* [17] and in *Dodonaea viscosa* (E. W., P. D., unpublished). The difference in the triterpene/phytosterol composition is also striking, while the deviation in n-alkane proportion seems not to be significant. One of us (F. J. A.) has also observed differences in terpenoid composition of Asteraceae species collected in different localities in South and Central America. With a plant like *P. odorata,* that invades disturbed habitats like roadsides and that is so widely distributed, one might indeed expect a greater amount of interpopulational variability than in more restricted species confined to a particular undisturbed habitat, but this would need to be checked in our case with comparison of samples from several geographic areas.

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