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FLAVONOID AND CEROPTIN PIGMENTS FROM FROND EXUDATES OF *PITYROGRAMMA TRIANGULARIS*

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Abstract—Two chemically distinct golden–yellow flavonoid exudates occur on the underside of fronds of *Pityrogramma triangularis*: ceroptin and a newly described flavonol, 6-methyl-8-methoxy-3,5,7-trihydroxyflavone were detected in one of the exudates and two methylated kaempferol derivatives, 4′-methoxy-3,5,7-trihydroxyflavone and 3,5-dihydroxy-7,4′-dimethoxyflavone were isolated from the other.

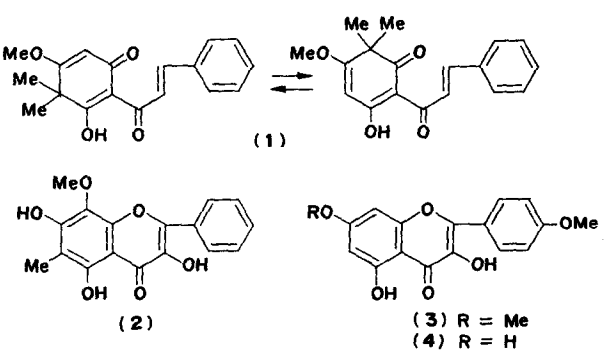
INTRODUCTION

The "golden-back" fern *Pityrogramma triangularis* (Polypondiaceae) derives its common name from a bright yellow farinaceous coating on the underside of its fronds. A novel chalcone-like compound, ceroptin (**1**), was previously reported from this exudate material [1]. (See ref. [2] for the use of the name *ceroptin*.) We have found that at least two chemically distinct golden farinaceous exudates occur naturally in morphologically homogeneous populations in the foothills of the Pacific Coastal ranges in Southern California. Initially, fronds from plants belonging to the two chemotypes were separated by examining them under UV light (366 nm): the ceroptin-synthesizing fronds fluoresce yellow while those which were later shown to produce kaempferol derivatives exhibit a deep burgundy color.

We report here the isolation and identification of four compounds, two from each chemotype. The previously investigated chemotype contains, in addition to ceroptin (the major component), a second exudate compound to which we assign the structure 6-methyl-8-methoxy-3,5,7-trihydroxyflavone (**2**) and for which we introduce the name "pityrogrammin". The two major components of chemotype-B are two kaempferol methyl ethers: 4′-methoxy-3,5,7-trihydroxyflavone (**4**), previously reported from the rhizomes of *Alpinia officinarum* (Zingiberaceae) [3] and 3,5-dihydroxy-7,4′-dimethoxyflavone (**3**), previously isolated from *Cheilanthes farinosa* [4] a fern in the same subfamily, Gymnogrammoideae [5], as is *Pityrogramma*, and from *Aesculus hippocastanum* buds [6].

RESULTS

Ceroptin (**1**), 3,5-dihydroxy-7,4′-dimethoxyflavone (**3**) and 4′-methoxy-3,5,7-trihydroxyflavone (**4**) were identified on the basis of UV spectra and



NMR analysis [7], co-chromatography with known compounds and mmps.

An ether extract of the dried fronds of chemotype-A yielded mostly ceroptin (**1**); however, a second minor component, pityrogrammin (**2**), was obtained as pale yellow crystals when the methanol-soluble material from the ether extract was chromatographed over polyamide [7]. The NMR spectrum for the di-trimethylsilyl ether of **2** indicated that it contained an aromatic *C*-methyl group (2·15δ), a methoxyl group (3·81δ), and 5 aromatic B-ring protons at 8·0–8·32δ (*m*) (H-2′,6′) and 7·32–7·69δ (*m*) (H-3′,4′,5′) and a 5-OH (13·37δ, *s*). The formation of a triacetate confirmed the presence of three aromatic hydroxyl groups (three singlets between 2·28 and 2·45δ) and that one hydroxyl group must be at *C*-5, i.e. the one which gave rise to the acetyl signal at 2·45δ [7]. On the basis of UV spectral data, one hydroxyl group could be assigned to position 3: MeOH absorption maximum at 380 nm and a 65 nm bathochromic shift of Band I in $AlCl_3$ [7]. In addition, in the mass spectrum of pityrogrammin an A-ring ion, $[A + H]^+$, at *m/e* 197 (33% relative intensity) corresponds to an A-ring containing one methoxyl, one *C*-methyl and two hydroxyl groups [8].

Therefore, since the NMR data established the presence of a *C*-5 hydroxyl group, the only question remaining with regard to the structure of pityrogrammin concerned the detailed substitution pattern at *C*-6, -7 and -8 utilizing three functional groups: one methoxyl, one *C*-methyl group and the third, unassigned hydroxyl group.

On the basis of general flavonoid biogenetic analogy, an oxygen function is assigned to position 7 and the remaining oxygen function and

Table 1. Comparison of UV spectra for 6- and 8-oxygenated flavones and flavonols

Compound	Band I (λ_{max}; nm)* for 6-oxygenated compounds	Band I (λ_{max}; nm)* for 8-oxygenated compounds
Flavones		
Baicalein		
(5,6,7-trihydroxyflavone)	323 [7]	
Norwogonin		
(5,7,8-trihydroxyflavone)		364 sh [7]
Scutellarin		
(6-hydroxyapigenin)	335 [9]	
6-Hydroxyluteolin	346 [9]	
Flavonols		
Alnusin		
(6-methoxy-3,5,7-trihydroxyflavone)	362 sh (EtOH†) [10]	
Pityrogrammin		
(8-methoxy-6-methyl-3,5,7-trihydroxyflavone)		380
6-Hydroxykaempferol	367 [9]	
Herbacetin		
(8-Hydroxykaempferol)		380 (95% EtOH) [11]
Herbacetin 3,7-dimethyl ether		372 [12]
Herbacetin 8,4′-dimethyl ether		374 [8]
Quercetagetin		
(6-Hydroxyquercetin)	365 (95% EtOH) [11]	
Quercetagetin 3′-methyl ether		
7-*O*-glucoside	364 (EtOH†) [13]	
Gossypetin		
(8-Hydroxyquercetin)		387 [7]
Gossypitrin		
(Gossypetin 7-*O*-glucoside)	386 (95% EtOH) [11]	385 [7]
Gossypin		
(gossypetin 8-*O*-glucoside)		380 [7]
Tambuletin		
(gossypetin 8- (or 7-) methyl ether		
7- (or 8-) glucoside		381 [14]
Gossypetin 3,7-dimethyl ether		378 [9]
Gossypetin 3,7,3′-trimethyl ether		365 sh [12]

* Spectrum recorded in MeOH unless otherwise stated.
† Presumably recorded in 95% EtOH.

the *C*-methyl group are assigned to the 8 and 6 positions, respectively, by comparing the UV spectral data for the parent compound with those observed for other 8- and 6-oxygenated flavones and flavonols. Comparison of the Band I (in methanol) observed for pityrogrammin with the Band I for other flavonols which contain either a 6- or an 8-oxygen function established that pityrogrammin contains an 8-oxygen substituent. 8-Hydroxy- (as well as 8-methoxy- and 8-*O*-glycosyl) flavones and flavonols have a 15–30 nm longer wavelength Band I (in MeOH or EtOH) than do the equivalent 6-oxygenated isomers (Table 1); thus, pityrogrammin must contain an 8-oxygen function in order to account for its Band I being at 380 nm.

That the 8-oxygen function is a methoxyl and not a hydroxyl group is supported by the mass spectral data. In the MS of pityrogrammin, the molecular ion appeared as the base peak at *m/e* 314 and the $[M-1]^+$ ion had only a 20% relative intensity and since it is known that *C*-8 hydroxyl groups (like *C*-6 hydroxyl groups) give an intense $[M-1]^+$ peak, pityrogrammin can not contain an 8-hydroxyl group [8]. For example, we have observed that the $[M-1]^+$ ion for 6-hydroxyflavonols is usually of approximately 60–80% relative intensity and the same ion for 8-hydroxyflavonols, although somewhat less intense, is nevertheless between 30–60% relative intensity. As expected, the M–Me peak for pityrogrammin appears at *m/e* 299 with a relative intensity of about 32% supporting the presence of a *C*-8 methoxyl function [8]. Also, we have observed that the chemical shift of δ3·81 is typical for an 8-methoxyl group in many (but no all) flavonols (7-methoxyl groups usually appear at around δ3·90). The assignment of the methoxyl group to the 8-position rather than the 7 is also supported by the benzene-induced shift (+0·2 ppm) for the methoxyl signal in the NMR spectrum; this value is in the range expected for a *C*-8 methoxyl function and considerably less than that expected for a *C*-7 methoxyl group (i.e. 0·5–0·6 ppm) [15]. All the data presented here support structure **2** for pityrogrammin.

In summary, the chemotype-A exudate of *Pityrogrammin triangularis* is distinguished by *C*-methyl compounds, i.e. the unusual ceroptin structure and the structurally-related flavonol pityrogrammin (**2**). In contrast, the exudate of chemotype-B contains two previously described methyl ethers of kaempferol.

EXPERIMENTAL

All fern material used in the present investigation was collected in foothills of San Ynez Mountains by D.M.S. Voucher specimens deposited in the Herbarium, University of California, Santa Barbara. NMR (data in ppm, δ scale) and UV spectra were obtained using standard procedures [7]. R_f values were determined on polyamide thin layer plates using a $CHCl_3$–MeOH–MeCOEt (12:2:1) (Egger's solvent system) and colors reported are those observed under UV light on these plates.

Chemotype-A. The isolation and properties of ceroptin (**1**) *and pityrogrammin* (**2**). Dried fronds (86 g) were washed with Et_2O. Et_2O was evapd, the gummy residue dissolved in hot MeOH, and the soln was 2× washed with *n*-hexane to remove the lipophilic material. Yellow crystals filtered off from each of the above solvents were determined to be ceroptin. *Ceroptin* (**1**), crystallized readily from Et_2O, MeOH and *n*-hexane, was isolated prior to column separation (2·027 g). It showed the following properties: R_f 1·00 (purple); yellow crystals; mp 135°; UV*, λ_{max} (MeOH): 230 (0·7), 295 sh (0·4), 365 (1·0) nm; λ_{max} (NaOMe): 292 (1·4), 345 (1·0) nm; λ_{max} ($AlCl_3$): 224 (0·5), 245 sh (0·3), 310 (0·3), 380 (1·0) nm; λ_{max} (NaOAc): 295 (1·4), 345 (1·0) nm; λ_{max} (NaOAc–H_3BO_3): 295 (1·0), 360 (1·0) nm; NMR spectrum† of the trimethylsilyl ether of **1** in $CDCl_3$: α and β protons, 8·38, 8·05 (*s*): H-2,3,4,5,6, 7·25–7·76 (*m*); H-3′ or 5′, 5·50 (*s*); 4′-methoxyl, 3·80 (*s*): two *C*-methyl groups, 1·40 (*s*, 6 protons). The MeOH extract was concentrated to 25 ml and applied to a polyclar polyamide column. Separation was effected using a $CHCl_3$–MeOH–MeCOEt (98:1·5:5) solvent system. Pityrogrammin was obtained as pale yellow crystals (8 mg): R_f 0·87 (purple); also purple on paper under UV and UV/NH_3; λ_{max} (MeOH): 280 (2·6), 300 sh (1·3), 380 (1·0) nm; λ_{max} (NaOMe): 265 sh (2·3), 370 (1·0) nm (some blue colour developed): λ_{max} ($AlCl_3$): 225 sh (2·0), 252 (2·9), 285 (2·4), 320 sh (0·9), 360 (1·7), 445 (1·0) nm; λ_{max} ($AlCl_3$–HCl): 225 (1·9), 225 (1·8), 285 (2·2), 310 sh (0·7), 360 (1·5), 445 (1·0) nm; λ_{max} (NaOAc): 278 (2·3), 325 sh (1·3), 375 (1·0) nm; λ_{max} (NaOAc–H_3BO_3): 275 (2·3), 325 sh (0·9), 373 (1·0) nm; UV spectra after de-*O*-methylation with pyridinium hydrobromide: [16] λ_{max} (MeOH): 280 (3·6), 325 sh (1·6), 380 (1·0) nm; λ_{max} (NaOMe): 250 (2·0), 290 (1·0), 355 (1·0) nm (dec.); λ_{max} ($AlCl_3$): 230 (5·5), 250 (5·0), 300 (4·0), 335 sh (2·3), 365 (2·3), 495 (1·0) nm; λ_{max} ($AlCl_3$/HCl): 260 (3·0), 280 (2·5), 320 sh (1·1), 360 (1·3), 440 (1·0) nm; λ_{max} (NaOAc): 300 (2·7), 365 (1·0) nm (dec.); λ_{max} (NaOAc–H_3BO_3): 295 (2·3), 345 sh (1·3), 370 (1·0) nm (dec.). NMR spectrum of the tri-acetate of **2** in $CDCl_3$: H-2′,3′,4′,5′,6′, 7·38–7·83 (*m*); 8-methoxyl, 3·95 (*s*); 6-*C*-methyl, 2·15 (*s*); three acetyl groups: 2·28, 2·38, 2·45.

Chemothype-B. Isolation and properties of the kaempferol derivatives **3** *and* **4**. Dried fronds (87 g) were washed with Et_2O.

* Numbers in parenthesis following values for λ_{max}'s for the UV data refer to relative intensities for the peaks assigning a value of 1·0 for the longest wavelength peak.

† Small signals adjacent to the methoxyl and *C*-methyl signals indicated that ceroptin exists in $CDCl_3$ as a mixture of two isomers (about 95:5), presumably the two shown in the structure presentation.

During evaporation of the ether, yellow crystals formed. Recrystallization from MeOH yielded bright yellow crystals (102 mg) of 3,5-dihydroxy-7,4'-dimethoxyflavone (**3**). Concentration of the mother liquor and recrystallization from $CHCl_3$ yielded deep yellow crystals (60 mg) of 4'-methoxy-3,5,7-trihydroxyflavone (**4**) (kaempferide). 3,5-Dihydroxy-7,4'-dimethoxyflavone (**3**) showed the following properties: R_f 0·96 (orange); mp 179°–181°; UV, λ_{max} (MeOH): 254 sh (0·9), 268 (1·0), 290 sh (0·5), 322 (0·6), 365 (1·0) nm; λ_{max} (NaOMe): 262 (1·2), 270 sh (1·1), 333 sh (0·3), 410 (1·0) nm; λ_{max} ($AlCl_3$): 239 (0·60), 245 sh (0·60), 272 (1·0), 308 sh (0·30), 350 (0·50), 422 sh (1·0) nm; λ_{max} ($AlCl_3$–HCl): 235 (0·8), 242 sh (0·8), 270 (1·1), 305 sh (0·4), 350 (0·6), 420 (1·0) nm; λ_{max} (NaOAc): 262 (1·4), 325 sh (0·5), 400 (1·0) nm; λ_{max} (NaOAc–H_3BO_3): 254 sh (1·0), 268 (1·1), 320 (0·6), 365 (1·0) nm; NMR spectrum of the trimethylsilyl ether of **3** in $CDCl_3$; H-2'6', 8·25 (*d*, *J* 9Hz); H-3'5', 7·08 (*d*, *J* 9Hz), H-8, 6·55 (*d*, *J* 2a5Hz); H-6, 6·43 (*d*, *J* 2·5Hz); 7,4'-methoxyls, 3·92 (*s*, 6 protons). 4'-Methoxy-3,5,7-trihydroxyflavone (**4**) showed the following properties: R_f 0·54 (orange); UV λ_{max} (MeOH): 270 (1·0), 330 (0·60), 370 (1·0) nm; λ_{max} (NaOMe): 280 (1·2), 325 sh (0·50), 415 (1·0) nm; λ_{max} ($AlCl_3$); 235 (0·69), 245 (0·60), 270 (0·90), 310 (0·30), 355 (0·40), 425 (1·0) nm; λ_{max} $AlCl_3$–HCl) 235 (0·70), 245 sh (0·60), 270 (0·90), 310 (0·30), 350 (0·50), 425 (1·0) nm; λ_{max} (NaOAc); 274 (1·2), 301 sh (0·8), 384 (1·0) nm; λ_{max} (NaOAc–H_3BO_3): 270 (1·0), 320 sh (0·60), 370 (1·0) nm. NMR spectrum of the trimethylsilyl ether of **4** in CCl_4: H-2'6', 8·05 (*d*, *J* 9·5Hz); H-3'5'; 6·88 (*d*, *J* 9·5Hz); H-8, 6·35 (*d*, *J* 2Hz); H-6, 6·12 (*d*, *J* 2Hz); 4'-methoxyl, 3·84 (*s*).

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