A Novel C-Methylated Dihydrochalcone from *Pityrogramma triangularis* var. *viscosa*

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Pityrogramma triangularis var. *viscosa* is one of the clearly defined varieties within the *Pityrogramma triangularis* complex. The flavonoid constituents of its frond exudate show a pattern characteristic for this taxon. The major component of the exudate could now be isolated. By spectroscopic methods it has been shown to be 2′,6′,4-trihydroxy,4′-methoxy,3′-methyl dihydrochalcone, a novel natural compound.

The fern genus *Pityrogramma* is represented in California and adjacent areas by a complex treated by Weatherby [1] as a single species, *P. triangularis* (Kaulf.) Maxon, consisting of several varieties. Among the "silver-backed" members of the complex, *P. triangularis* var. *viscosa* (D. C. Eaton) Weatherby is the most abundant, occuring especially in mainland maritime habitats in southern California (USA) and Baja California (Mexico) as well as on several of the off-shore islands [2]. Besides the whitish powdery exudate, the plants are further distinguishable from the rest of the complex by their more narrow, elongate pinnae and a copious viscid exudate that appears conspicuously on the adaxial frond surface. Such plants have also been considered to be a distinct species by Maxon [3] and later by Alt and Grant [2] as *P. viscosa* (D. C. Eaton) Maxon. Alt and Grant [2] suggest, however, that *P. viscosa* hybridizes with *P. triangularis* var. *triangularis* as well as with *P. triangularis* var. *maxonii* Weatherby, in southern California.

A previous survey of California *Pityrogramma* revealed the existence of unique exudate constituents that sometimes coincided with recognized taxa [4]. One such taxon is *P. triangularis* var. *viscosa*. In view of the possibility that this fern hybridizes with other *Pityrogramma* taxa, and that its chemical uniqueness might provide a key to use in analyzing instances of hybridization, it seemed desirable to identify the flavonoid components of the exudate. Thus, we report here the structure elucidation of the major farina component of *P. triangularis* var. *viscosa*.

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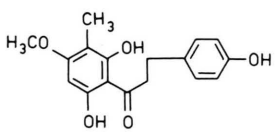
Materials and Methods

Dried fronds of *Pityrogramma triangularis* var. *viscosa*, collected in California (vouchers D. M. Smith 43352, 43383 in UCSB) in May, 1978, were rinsed with acetone to dissolve the exudate material. The solution was evaporated and the powdery remainder redissolved in a small volume of boiling MeOH. The major component crystallized on standing at room temperature. This product being not yet pure was dried onto polyamide SC-6 from acetonic solution, and chromatographed on a column of polyamide SC-6. Elution was with toluene and increasing quantities of methylethylketone and MeOH. Hereafter the compound was crystallized from boiling EtOH to yield light yellow crystals on cooling, m. p. 199 – 200 °C. λ_{max}^{EtOH}: (335), 291 nm, with no shift on addition of $AlCl_3$, NaOAc, H_3BO_3. Addition of NaOEt produced a bathochromic shift of band II to 300 nm; the shoulder became a small peak at 380 nm. On TLC (polyamide DC 11) the substance appears as a dark spot in UV_{366}, turning brownish-yellow after spraying with "Naturstoffreagenz A" (β-aminodiethyl ether of diphenyl boric acid). MS (70 eV) *m/e* (rel. int.): 302 (30), 270 (21), 193 (16), 181 (100), 167 (19), 166 (23), 154 (43), 138 (40), 120 (25), 107 (62). Acetylation yields a triacetate, MS (70 eV) *m/e* (rel. int.): 428 (16), 386 (20), 344 (82), 302 (31), 181 (100). – UV-spectra were recorded on a Beckman DB-GT, MS on a Varian MAT 311 A, PMR on a Brucker HFX 90 (DMSO, TMS). The melting point (Büchi SMP-20) is uncorrected.

Results and Discussion

The usual isolation procedure yielded the major component of the exudate as a crystalline substance. UV-spectrum and MS of the compound itself and of its acetyl derivative first led us to the assumption

that this compound would be a flavanone, with 3 OH-groups and 1 OCH_3-group (M^+ 302 would agree with the molecular formula $C_{16}H_{14}O_6$). PMR readily showed, however, the presence of a C-methyl group as a singlet at δ 1.86 ppm. Triplets at δ 2.77 and δ 3.26 ppm, integrating for 2 protons each (J 7 Hz), point to a $-CH_2-CH_2-$ bridge. Hence the substance is a dihydrochalcone, M^+ 302 corresponding to the molecular formula $C_{17}H_{18}O_5$. Since we already have 1 CH_3-group, there still must be 3 OH-groups and 1 OCH_3-group. Indeed the PMR spectrum shows 1 OCH_3-group as a singlet at δ 3.77. The AA'BB' spin system appearing at δ 6.66 (calc. 6.72) and δ 7.05 (calc. 7.03) (J 8 Hz), is typical for a *p*-substituted aromatic ring. Important peaks in the MS at *m/e* 107 and *m/e* 120 can be assigned to the fragments $-CH_2-C_6H_4-OH$ and $-CH_2-CH_2-C_6H_4-OH$, respectively, thus suggesting the presence of a OH-group at C-4. This fragmentation also explains the base peak at *m/e* 181. The PMR furthermore shows a single proton as a singlet at δ 6.08. Hydrogen-bonded OH-groups at C-2' or C-6', respectively, appear as singlets at δ 13.65 and δ 10.90, whereas the OH at C-4 appears as a broad signal at about δ 9.25. The C-methyl group can be assigned either to C-3' or to C-5'. The substance under investigation hence is 2',6',4-trihydroxy,4'-methoxy,3'-methyl dihydrochalcone.



This result is strongly supported by ^{13}C NMR spectral analysis of the natural product and by ^1H NMR analysis of the partially silylized compound [5], kindly performed by K. E. Malterud. Furthermore, identity of our compound with the most probable isomer 2',4',6'-trihydroxy,4-methoxy,3'-methyl dihydrochalcone could be excluded by direct comparison with a synthetic sample.

The major component of the farina of *P. triangularis* var. *viscosa* is a novel natural product. As yet only two C-methylated dihydrochalcones have been reported, namely 2',6'diOH,4'-OMe, 3',5'-diMe dihydrochalcone and 2'-OH,4',6'-diOMe,3'-Me dihydrochalcone from the fruits of *Myrica gale* [6, 7]. So this is the first time that a C-methylated dihydrochalcone is encountered as a farina constituent in *Pityrogramma* [8]. In its C-methylation of the A-ring, this new compound resembles ceroptin and triangularin, previously isolated from *P. triangularis* var. *triangularis* [9, 10]. Triangularin is the corresponding chalcone, but lacks the OH-group at the B-ring. From another variety of this species, namely var. *pallida* Weath., three C-methylated flavanones have been isolated recently as major farina constituents [11]. The capacity for biosynthesis of C-methylated flavonoids might be a typical feature of the species complex *P. triangularis*.

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