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Phytochemistry, Vol. 20, No. 5, pp. 1181–1182, 1981.
Printed in Great Britain.

0031-9422/81/051181-02 $02.00/0

TWO FLAVONOIDS FROM THE FROND EXUDATE OF *PITYROGRAMMA TRIANGULARIS* VAR. *TRIANGULARIS*

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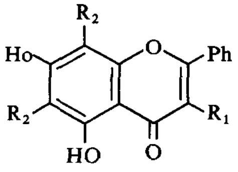
(*Received* 29 *August* 1980)

Key Word Index—*Pityrogramma triangularis* var. *triangularis*; Gymnogrammoideae; Pteridophyta; frond exudate; trace constituents; novel flavonoids; 5,7-dihydroxy-3-methoxy-6,8-dimethylflavone; 5,7-dihydroxy-6,8-dimethoxyflavone.

From a recently published review on flavonoid patterns in the farinose exudate of *Pityrogramma* species [1], it is apparent that *P. triangularis* (Kaulf.) Maxon is outstanding in its farina chemistry. Only in the members of this species-complex are *C*-methylated flavonoids found. The following representatives have been reported so far: ceroptin, pityogrammin [2] and triangularin [3] from var. *triangularis*; a *C*-methylated dihydrochalcone from var. *viscosa* D. C. Eaton [4] and three *C*-methylated flavanones from var. *pallida* Weath. [5]. The farinose frond exudate of the 'ceroptin-type' of var. *triangularis* [6] contains a series of trace constituents, as yet unidentified. Two of these have now been isolated in small amounts [7]. One of them is a novel *C*-methylated flavonol, the other a novel flavone.



1 $R_1 = OMe, R_2 = Me$
2 $R_1 = H, R_2 = OMe$

Compound 1 appears as a dark spot on polyamide (UV 366), which turns reddish-brown after spraying with 'Naturstoffreagenz A' (β-aminodiethyl ether of diphenyl boric acid). It therefore appears to be a flavonol methylated at C-3. According to its molecular peak at *m/z* 312, it could be either a flavone or a 3-methylflavonol [8], but this assumption does not agree with its polarity (R_f 0.56 in solvent A). However, the ^{1}H NMR data reveal the existence of one OMe group (δ 3.87 ppm) and two Me groups (δ 2.11, *s*; 2.29, *s*). Thus, in addition, there are two OH groups, one of which can be readily placed at C-5 (δ 12.80, broad signal). Since the ^{1}H NMR signals, as well as characteristic MS-fragments at *m/z* 235 (M − 77) and 105 ('Pic C' accord. to ref. [9]), prove the presence of an unsubstituted B-ring, the second OH group must also be located on the A-ring and at C-7, although in the UV spectrum no shift is observed on addition of NaOAc. The adjacent methyl groups at C-6 and C-8 may reduce the acidity of the 7-OH. The weak reaction with $AlCl_3$ probably is due to steric hindrance (cf. refs. [5] and [10]). The spot behaviour and the UV spectra are consistent with a methoxyl group at C-3. Compound **1** is thus 5,7-dihydroxy-3-methoxy-6,8-dimethylflavone.

Compound **2** appears as a dark spot on polyamide, which remains dark on spraying with 'Naturstoffreagenz A'. This behaviour again points either to a flavone or to a flavonol methylated at C-3, with an additional methoxyl at C-6. The molecular peak at *m/z* 314 indicates a flavonoid with two OH groups and two OMe groups [8]. These are corroborated by the relevant ^1H NMR data as well as by the formation of a diacetate (M^+ *m/z* 398). The ^1H NMR signals at δ 8.13 ppm (*m*, H-2′, 6′) and 7.62 (*m*, H-3′,5′ and H-4′) show the presence of an unsubstituted B-ring; hence the four substituents must be located on the A-ring. A proton signal at δ 12.08 can be assigned to OH at C-5, as shown by the UV shift on addition of $AlCl_3$. The second OH group can be placed at C-7. Ionization of the 7-hydroxyl appears to be hindered by the adjacent methoxyl groups at C-6 and at C-8. The presence of the latter is also shown by the UV spectrum in EtOH (375, 280 nm) [11]. Compound **2** is thus 5,7-dihydroxy-6,8-dimethoxyflavone.

Both substances are new natural flavonoids. The A-ring substitution pattern of **1** is known only for a 4′-OH flavonol, recently isolated from three Didieraceae [12] and for the flavanone desmethoxymatteucinol. The latter occurs in the frond exudate of *P. triangularis* var. *pallida* [5]. The same positions of the flavonoid skeleton are substituted in pityrogrammin [2]. A total of eight *C*-methylated flavonoids have now been reported from the farina of the varieties of *P. triangularis*. In addition, a *C*-methylated chalcone was isolated from the farina of var. *pallida*; its structural determination will be reported separately [13].

The parent compound of **2**, namely 5,6,7,8-tetrahydroxyflavone, has not yet been found in nature. Its 6,7-dimethyl, 5,6,7-trimethyl and 5,6,7,8-tetramethyl ethers have been isolated from the aerial parts of *Helichrysum herbaceum* [14]. The 6,7,8-trimethyl ether was found in the exudate of male flowers of *Alnus sieboldiana* [15]. The tetramethyl ether has also been described from leaves of *Zeyhera tuberculosa* [16] and roots of *Lindera lucida* [17]. The 6,8-dimethyl ether, however, is reported here for the first time.

EXPERIMENTAL

Fronds of *P. triangularis* var. *triangularis* (ceroptin-type) were collected at various locations in California (D. M. Smith). Vouchers are kept at Santa Barbara (UCSB). The fronds were rinsed with Me_2CO to dissolve the exudate. Dried fronds (109 g) yielded 3.78 g of crude material (4.1 % of dry wt). Most of the ceroptin, which is by far the major constituent of the farina, could be removed by crystallization from EtOH. The remainder was chromatographed on columns of Si gel, then on polyamide. Elution was with toluene and increasing quantities of MeCOEt and MeOH. The fractions thus obtained were still mixtures. From one of them, a small amount of the chalcone triangularin was isolated as red crystals. This was identified by direct comparison with an authentic sample. Another fraction, still with ceroptin as the major component, was dissolved in boiling C_6H_6 and the conc soln kept in a refrigerator. After 2 days, 10 mg light yellow crystals (**1**), mp 202°, separated. R_f on polyamide (solvent A) 0.56, on Si gel (solvent B) 0.44. UV λ_{max}^{EtOH} nm: 358 sh, 322, 280, 246 sh; $+AlCl_3$: 327, 285, 250 sh; +NaOEt: 388, 285, 268; +NaOAc: 368, 320, 279; +NaOAc + H_3BO_3: 368, 320, 279. MS *m/z* (rel. int.): 312 (M^+, 100 %; $C_{18}H_{16}O_5$, calc. 312.0998, found 312.1002), 311 (95), 294 (M − H_2O, 31), 293 (33), 283 (M − 29, 24), 281 (M − 31, 11), 269 (M − 43, 12; $C_{16}H_{13}O_4$, calc. 269.0814, found 269.0815), 115 (28), 105 ('Pic C' 9.48 %), 83 (21 %), 77 ('Pic C − 28', 33 %). ^1H NMR (δ in ppm/TMS): 12.80 (broad signal, OH-5), 9.87 (broad signal, OH-7), 8.25–8.00 (*m*, H-2′,6′), 7.80–7.55 (*m*, H-3′,4′,5′), 3.87 (*s*, 3 H, 1 OMe), 2.92 (*s*, 3 H, 1 Me), 2.11 (*s*, 3 H, 1 Me).

Another fraction crystallized from EtOH as a mixture of two components which were separated by prep. TLC on Si gel plates with concentrating zone (solvent B). **2** crystallized from EtOH as orange-yellow needles, mp 222–223° (17.5 mg). R_f on polyamide (solvent A) 0.7, on Si gel (solvent B) 0.4. UV λ_{max}^{EtOH} nm: 372, 280; $+AlCl_3$: 420, 295; +NaOEt: 300, 263; +NaOAc: 375, 280; +NaOAc + H_3BO_3: 375, 280. MS *m/z* (rel. int.): 314 (M^+, 100), 313 (M − 1, 57), 296 (5), 284 (3), 271 (14), 181 (3). ^1H NMR (δ in ppm/TMS): 12.08 (*s*, OH-5), 8.90 (*s*, OH-7), 8.13 (*m*, H-2′,6′), 7.62 (*m*, H-3′,4′,5′), 6.57 (*s*, H-3), 3.90 (*s*, 3 H, 1 OMe), 3.81 (*s*, 3 H, 1 OMe).

Solvent A for polyamide was toluene–petrol (bp 100–140°) MeCOEt–MeOH (6:3:1:1), solvent B for Si gel was toluene–MeCOEt (9:1). Mass spectra were recorded on a Varian MAT 311A and on a AEI MS 902, respectively. ^1H NMR spectra were recorded in DMSO.

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