New Flavonoids from the Farina of *Pityrogramma* Species

Christiane Hitz, Karin Mann,
and Eckhard Wollenweber

Institut für Botanik der TH Darmstadt, Schnittspahnstraße 3, D-6100 Darmstadt

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2′,6′,4-OH, 4′-OMe-chalcone (neosakuranetin) and 2′,6′,4-OH, 4′-OMe-dihydrochalcone (asebogenin) were found as major constituents of the frond exudate on one individual plant of *Pityrogramma calomelanos*. Galangin-5,7-dimethyl ether was isolated from the farina on fronds of a certain population of *Pityrogramma triangularis* as a novel natural flavonol.

In a previous report on the composition of the flavonoid exudate on ferns of the genus *Pityrogramma* the occurrence of a new chalcone was mentioned [1]. This compound, 2′,6′,4-OH, 4′-OMe chalcone, had been detected only in the farina of two vouchers of *Pityrogramma tartarea* var. *aurata* (Moore) Tryon. These are J. A. Ewan 15672 from Bolivia (at US) and C. Vargas 22355 from Peru (at CR). Of course, only small fragments of these herbarium specimens were available and hence the chalcone could be identified only tentatively, by direct comparison on TLC. Meanwhile we found the same flavonoid in the farina of one plant of *Pityrogramma calomelanos* (L.) Link cultivated in a greenhouse [2]. Of this flavonoid a minute amount could now be isolated and identified unambiguously. From the same plant we could also isolate the corresponding dihydrochalcone, which had not been seen previously in any other *Pityrogramma* farina analysed. The third flavonoid we wish to report here is a novel natural methyl derivative of galangin, isolated from the farina of a distinct population of *Pityrogramma triangularis* Max.

Material and Methods

Pityrogramma calomelanos is cultivated in a greenhouse at the Botanical Garden of Darmstadt University. *Pityrogramma triangularis* was collected by E. W. at School Land Gulch at the Pardee Reservoir/California, in July, 1980. Vouchers are kept in the private herbarium of E. W. in Darmstadt (*P. calomelanos*, 'plant 11'; *P. triangularis*, EW-2). Fern fronds were rinsed with acetone to dissolve the exudate material. This was worked up by column chromatography on silica and on polyamide SC-6 in the usual manner. Preparative TLC and TLC comparisons were done on silica or on polyamide DC-11 (for details *cf.* [3]). – A sample of neosakuranetin was prepared by treatment of a solution of sakuranetin (naringenin-7-methyl ether) with alkali. The reaction product was, after slight acidification, rapidly extracted with diethyl ether. – Demethylation of comp. **III** was done with pyridine-HBr according to [4].

Reprint requests to Prof. Dr. E. Wollenweber.
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Results and Discussion

From the few leaves of *Pityrogramma calomelanos* 'plant 11' available a small amount of compound **I** could be isolated, but not crystallized, so we could not determine the m.p. The material was sufficient, however, to run a mass spectrum, which shows the following fragmentation. MS *m/z* (rel. int.): 286 (M^+, 53), 285 (23), 180 (18), 167 (78), 166 (32), 138 (37), 120 (84). These data indicate a chalcone with 3 OH-groups and 1 OMe-group 5 as well as a B-ring with 1 OH-group. The chromatographic identity of comp. **I** with the reaction product of alkali treatment of naringenin-7-methyl ether (sakuranetin), together with the mass spectral result shows that this compound is indeed 2′,6′,4-OH,4′-OMe-chalcone. Furthermore the UV spectra of both products are identical (±2 nm). UV λ_{max}^{MeOH} 365, 295 nm; $+AlCl_3$ 405, 312 nm; $+AlCl_3 + HCl$ 395, 310 nm; no shifts with NaOAc and H_3Bo_3.

This chalcone, as to our knowledge, has not been found in nature previously [2]. However, its 6′-O-glucoside was isolated as early as 1954 from the ethanolic extract of *Prunus puddum* stem bark [6]. As this glycoside is known under the name of neosakuranin, we proposed the trivial name neosakuranetin for the aglycone **I** [1, 2].

Compound **II** was isolated as light brownish crystals, m.p. 144 °C. Its chromatographic properties indicated a dihydrochalcone and this assumption is supported by the UV spectrum. UV λ_{max}^{MeOH} 268,

229 nm; + $AlCl_3$ 370, 312, 226 nm; + NaOH 364, 296, 242, 220 nm. MS *m/z* (rel. int.) 288 (52, M^+), 182 (13), 167 (83, $C_8H_7O_4^+$), 140 (19, $C_7H_8O_3^+$), 120 (31, $C_8H_8O^+$), 107 (29, $C_7H_7O^+$). The data show that this dihydrochalcone has also 3-OH-groups and 1 OMe-group. One OH- group is obviously located at ring B, which points to 2′,6′,4-OH, 4′-OMe-dihydrochalcone. This structure is supported by the PMR spectrum. ^{1}H-NMR (90 MHz, DMSO-d_6; ppm/TMS) 12.3 (1 H, s; OH at C-2′ or at C-6′; further OH-protons exchanged), 7.02 and 6.68 (2 H each, d; AA′BB′ spin system, B-ring p-substituted), 6.0 (2 H, s; H-3′/H-5′), 2.66 (3 H, s; OCH_3), ~3.2–2.6 (4 H, m; α- and β-methylens). Hence to compound **II** the structure of 2′,6′,4-OH,4′-OMe- dihydrochalcone is assigned. UV, MS, and PMR data are in agreement with those reported in literature [7]. The m.p. is 18° lower, but this certainly is due to impurity.

This dihydrochalcone has been found in nature only once before as an aglycone. In leaves of three *Rhododendron* species it occurs as such, jointly with its 2′-O-glucoside [7]. As this glycoside is named asebotin, we proposed the trivial name asebogenin for the aglycone (*cf.* 2).

Further compounds found in the farina of our distinct plant of *Pityrogramma calomelanos*, in trace amounts, are 2′,6′-OH, 4′-OMe-dihydrochalcone, 2′,6′-OH,4′,4-OMe-dihydrochalcone 2′,6′-OH,4′,4-OMe-chalcone, naringenin-7,4′dimethyl ether and naringenin-7-methyl ether (sakuranetin), and the complex flavonoid "D-1" (compound A in [8]).

Of *Pityrogramma triangularis* (EW-2) 56 g of air-dried fronds were available. The yellow farina was dissolved yielding 3.82 g of exudate material. From this we isolated, by column chromatography and finally by preparative TLC, a small amount of compound **III**, distinguished as a bright yellow fluorescent spot on polyamide TLC. From boiling ethanol if forms light yellow crystals, m.p. 174°. The bright yellow fluorescence on polyamide layer points to a flavonoid with either an OMe-group or no substituent at C-5. MS *m/z* (rel. int.) 298 (21, M^+), 297 (100, M-1), 279 (22), 267 (26), 252 (67), 181 (61, "Pic D" according to [9]), 105 (45, "Pic C"), 77 (29, "C-28"). M^+ 298 indicates a flavone or a flavonol with 1 OH and 2 OMe, and fragments *m/z* 181, 105 and 77 indicate an unsubstituted B-ring. Compound **III** could hence be a dimethyl ether of galangin, as this flavonol itself as well as its 7-methyl ether (izalpinin) are present in this fern farina, too. According to the fluorescence of the spot it should be the 5,7-dimethyl derivative. As a matter of fact, demethylation of compound **III** with pyridin-hydrobromide yields a mixture of galangin with gal-7-Me. This proves unambiguously that compound **III** is indeed galangin-5,7-dimethyl ether. Compound **III** exhibits the following UV spectrum. UV λ_{max}^{MeOH} (402), 352 (305), 265 nm; + $AlCl_3$ 406 (330), 273, 252 nm; + NaOH 395, 271, 250 nm. – This is the first report of this flavonol as a natural product (*cf.* [10]).

On *Pityrogramma calomelanos* the farina usually is either white, consisting mainly of 2′,6′-OH,4′-OMe-dihydrochalcone and/or 2′,6′-OH,4′,4-OMe-dihydrochalcone (*P. calomelanos* var. *calomelanos*), or it is yellow, then mainly consisting of 2′,6′-OH,4′-OMe-chalcone and/or 2′,6′-OH,4′,4-OMe-chalcone (*P. calomelanos* var. *aureoflava* (Hook.) Weath. = *P. austroamericana* Domin). Yellow farina can also consist of a mixture of the cited dihydrochalcone(s) and chalcone(s) [1]. The farina produced by 'plant 11' in our greenhouse is only slightly yellowish and it is produced in a rather low amount only. It is composed mainly of 2′,6′,4-OH,4′-OMe-dihydrochalcone (asebogenin) and the corresponding 2′,6′,4-OH,4′-OMe-chalcone (neosakuranetin), while the other chalcones and dihydrochalcones are only minor constituents. In the yellow farina of some other plants of *P. calomelanos* var. *aureoflava* cultivated in our greenhouse neosakuranetin can be detected as a trace constituent. All plants were originally grown from spores obtained from one plant cultivated at the Botanical Garden in Munich. They differ markedly in the shape of pinnae and pinnules as well as in the farina composition. This obviously is due to the well-known phenomenon of very easily occuring hybridization in *Pityrogramma* [11].

P. triangularis (EW-2), in its farina composition, resembles none of the previously described varieties or chemotypes of this species complex (*cf.* [12]). The novel flavonol, galangin-5,7-dimethyl ether, is present as a minor constituent only, but by its fluorescence it dominates the flavonoid pattern on TLC. A series of further flavonoids could be identified in this farina, which is still under investigation. The results will be reported and discussed elsewhere. The population from which this plant material has been collected represents either a new chemotype or a hybrid.

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[1] E. Wollenweber and V. H. Dietz, Biochem. Syst. Ecol. **8,** 21 (1980).
[2] E. Wollenweber and V. H. Dietz, Phytochemistry **20,** 869 (1981).
[3] E. Wollenweber, V. H. Dietz, D. Schillo, and G. Schilling, Z. Naturforsch. **35 c,** 685 (1980).
[4] G. Howard and T. J. Mabry, Phytochemistry **9,** 2413 (1970).
[5] E. Wollenweber and V. H. Dietz, Phytochem. Bull. **12,** 48 (1979).
[6] B. Puri and T. R. Seshadri, J. Sci. Industr. Res. **13 B,** 698 (1954).
[7] T. J. Mabry, M. Sakakibara, and B. King, Phytochemistry **14,** 1448 (1975).
[8] H. Wagner, O. Seligmann, M. V. Chari, E. Wollenweber, V. H. Dietz, D. M. X. Donnelly, M. J. Meegan, and B. O'Donnell, Tetrahedron Letters **1979,** 4269.
[9] H. Audier, Bull. Soc. Chim. France **1966,** 2892.
[10] E. Wollenweber, in: The Flavonoids – Advances in Research 1975–1980. Chapman & Hall, London 1982.
[11] K. Domin: Rozpr. České Akad. Třída II, **38,** 1 (1929).
[12] D. M. Smith: Bull. Torrey Bot. Club. **107,** 134 (1980).