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PROTOGENKWANIN, A NEW FLAVONOID FROM *EQUISETUM ARVENSE* L.

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Abstract – From the fertile sprouts of *Equisetum arvense* L. have been isolated protogenkwanin-4′-glucoside and gossypitrin. The structure of protogenkwanin as 5-hydroxy-2-(1,4-dihydroxy-2,5-cyclohexadienyl)-7-methoxy-4-chromenon is proved on the basis of chemical and physical evidence. It is demonstrated that articulatin, a thiamine decomposing substance, from *E. arvense*, is identical with the known flavonol glycoside gossypitrin (= gossypetin-7-glucoside). The synthesis of 2,5-dihydroxy-2-(4-hydroxyphenyl)-7-methoxy-4-chromanon is reported.

In the course of our work on the flavonoids of various *Equisetum*-species[1-3] we became also interested in the fertile sprouts of the dimorphic species *Equisetum arvense* L. Previous work of Nakabayashi[4-6] on this part of *E. arvense*, which is used in Japan as a food, was aimed at the isolation of some thiamine decomposing substances; the probable identity of one of them will be discussed at the end of this paper. More recently Russian workers have isolated from the same source naringenin, dihydrokaempferol (= aromadendrin), dihydroquercetin (= taxifolin), apigenin and luteolin.[7]

As already reported in a preliminary note[8] we have isolated from the fertile sprouts of *E. arvense* by the procedure given in the experimental section, the glycoside of a very labile flavonoid whose mass-spectrum shows a molecular ion at 302 m/e^+, and which we named protogenkwanin (**2**) since on acid as well as thermal treatment it is dehydrated easily to genkwanin (**5**) (M^+ = 284 m/e). From these facts it was at first assumed that **2** was the 2-hydroxy-flavanone **6** (2-hydroxyflavanones are known to undergo dehydration to flavones under the conditions mentioned, and few of them have been found naturally occurring[9-13]), but synthesis of the 2-hydroxy-flavanone corresponding to genkwanin (**5**) (2,5-dihydroxy-2-(4-hydroxyphenyl)-7-methoxy-4-chromanon (**6**)) showed that it was not identical with protogenkwanin (**2**). By this synthesis the possibility that protogenkwanin was the diaroylmethane corresponding to **6** was ruled out as well, since both compounds are in a tautomeric equilibrium (at −60 in hexadeuteroacetone **6** contains about 8.5% of the diaroylmethane-tautomer (*cf.* PMR-data in the exptl. section)).

Hydrogenation of **2** led to a complex mixture, the main component of which (5-hydroxy-2-(4-hydroxy-cyclohexyl)-7-methoxy-4-chromenon (**3**)) could be isolated; its mass-spectrum revealed that two moles of hydrogen had been consumed to saturate two double bonds, and one to eliminate an OH group. The UV-spectra of **1**, **2** and **3** with and without added $AlCl_3$ are nearly identical with the published spectra of 5-hydroxy-7-methoxy-4-chromenon[14] (Table 1); this means that all three compounds contain the same chromenone-chromophor, which, in the case of **1** and **2** must not be conjugated with a double-bond. This proves that the elements of water in **1** and **2**, as compared with **4** and **5**, must be attached to the B-ring. Taking further into consideration that, because of the transformations **1** → **4** and **2** → **5**, the B-ring of protogenkwanin must bear a OH-group in the 4′ position, and that the second OH-group of that ring, since it is prone to hydrogenolysis, should be in a "benzylic" position, we deduced the structure **2** for protogenkwanin. This structure is compatible with all facts mentioned above and it has been proved by a careful analysis of the PMR-spectra of **2** and **3** (Table 2). The proton signals of the 5-hydroxy-7-methoxy-4-chromenon-moiety of **2** and **3** show the positions and multiplicity which are expected for this system.[14,15] The B-ring protons of **2** give rise to two two-proton signals (a doublet centered at δ 5.92 (J = 10.5 Hz) for H-2′,6′ coupled with H-3′,5′ and a quartet centered at δ 6.20 for H-3′,5′ coupled with H-2′,6′ (J = 10.5 Hz) and H-4′ (J = 4 Hz)) indicating that this ring possesses an axis of symmetry, which can be of course only in the 1′,4′-direction; further two OH-signals (a singlet at δ 6.26 for OH-1′ and a doublet centered at δ 5.20 for OH-4′ coupled with H-4′ (J = 7 Hz)), which disappear upon addition of D_2O, and finally a one-proton multiplet centered at δ 4.46 for H-4′, which becomes sharper after deuterium-exchange. Irradiation at δ 4.46 proves the correctness of the above assignments: the doublet of OH-4′ becomes a singlet and the quartet of H-3′,5′ becomes a doublet (J = 10.5 Hz). The B-ring signals of **3** are in agreement with the proposed structure: there is only one OH signal at δ 5.37 and an unresolved multiplet between δ 1.4 and 1.8.

The EI MS spectrum of **2** confirms the proposed structure: the strong peak at m/e 284 ($M-18$)$^+$ (35%) again proves the easy dehydration of the molecule, the

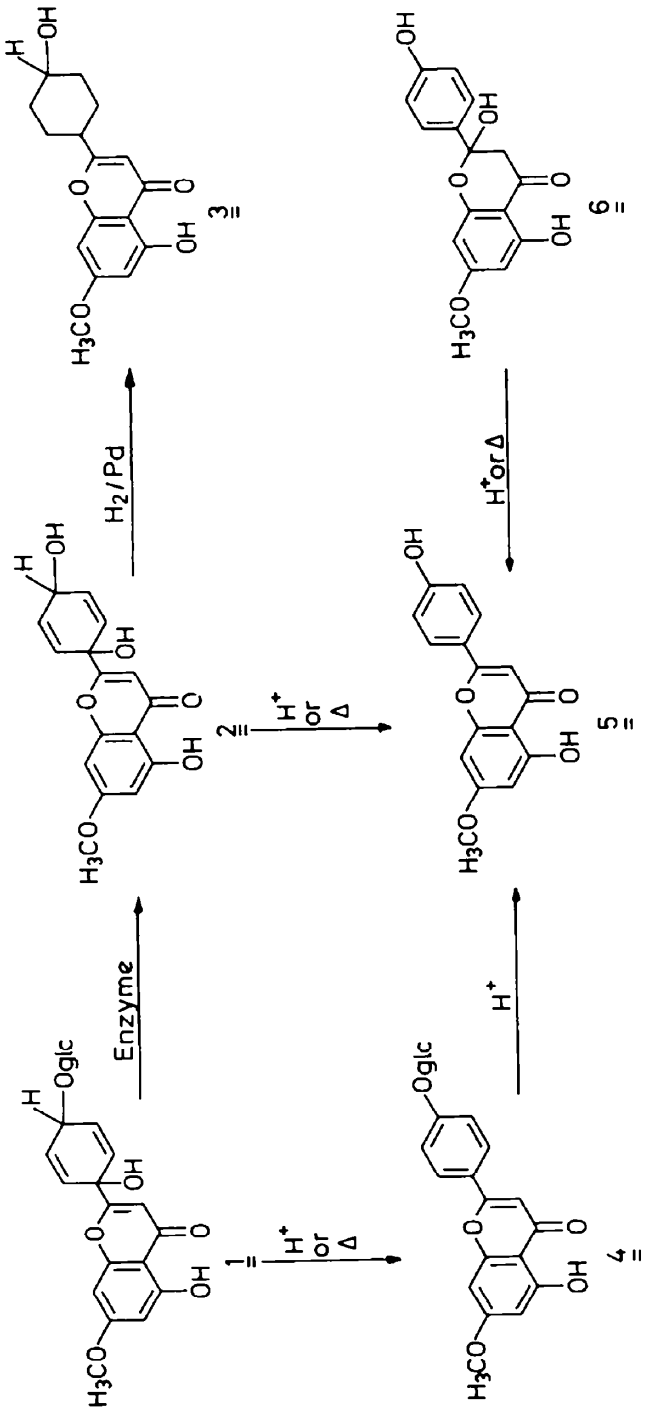
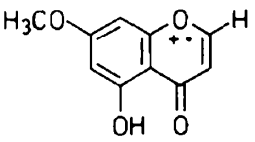

Enzyme
H+ or Δ
H2/Pd
H+ or Δ
H+
1
2
3
4
5
6
Oglc
OH
H3CO
O

Table 1. UV absorption maxima (nm) of **1**, **2**, **3** and 5-hydroxy-7-methoxy-4-chromenon. a = MeOH; b = MeOH + $AlCl_3$

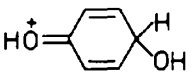
1	a	231		249	257	293	324
	b			258	263	308	363
2	a	231		249	257	293	324
	b			258	263	308	363
3	a	230		248	255	290	317
	b	225	234	256	263	304	363
5-hydroxy-7-methoxy-4-chromenon	a	227		252	257	293	310 } *
	b	223		253	266	310	320 }

†Taken from l.c.[14]

important peaks at m/e^- 167 and 121 derive from $M^{\cdot}$ or $(M-18)^{\cdot}$ by a retro Diels-Alder fragmentation, while fragmentation between B and C-rings gives peaks at m/e^- 192 and 111 which are characteristic of the new structure.



$m/e = 192$



$m/e = 111$

The above evidence proves unequivocally the constitution of **2**, but not its stereochemistry. This point will be clarified by an X-ray analysis, which shall be reported in an other context.

The attachment of the glucose moiety in **1** at the 4'-position is evident from the UV-spectra of **1** and **2**, and the transformation of **1** to **4**: the large $AlCl_3$-shift clearly demonstrates that in **1** OH-5 is free and the transformation **1** → **4** proves that OH-1'' is also free, leaving the 4'-glucosylation as the only possibility. The configuration of the glucose as β-D-glucoside can be deduced from the fact that **1** is hydrolysed by sweet almond emulsine; the PMR-data (Exptl. section) are also in agreement with the proposed structure of **1**.[15] **1** is to our knowledge the second example of a naturally occurring flavonoid with a non-aromatic B-ring, the other one is protofarrerol, which has been isolated from a fern.[16]

The second main flavonoid, which we isolated from *E. arvense* was identified as gossypitrin (**7**) by direct comparison with an authentical sample isolated from the flowers of *Chrysanthemum segetum* L.[17] **7** has been previously identified in the spores of the other dimorphic *Equisetum species*, *E. telmateja Ehrh.*[18] The properties of **7** and its aglycone gossypetin (**8**) are, as can be seen from Table 3, in good agreement with the published data of articulatin resp. articulatidin, its aglycone, which had been isolated by Japanese

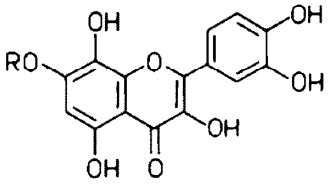
Table 2. PMR-signals (DMSO d_6) of **2** (100 MHz) and **3** (80 MHz)

	2		**3**	
	addn. of D_2O	irr. at H-4'		addn. of D_2O
OH-5	12.79 (s)	exchanged	OH-5 12.86 (s)	exchanged
H-8	6.63 (d) J=2.5		H-8 6.37 (d) J=2.5	
H-3	6.44 (s)		H-3 6.30 (s)	
H-6	6.42 (d) J=2.5		H-6 6.30 (d) J=2.5	
OCH_3-7	3.92 (s)		OCH_3-7 3.83 (s)	
OH-1''	6.26 (s)	exchanged		
H-3',5'	6.20 (dd) J=10.5 J=4	(d) J=10.5	H-2',3',5',6' 1.4-1.8 (m)	
H-2',6'	5.92 (d) J=10.5			
OH-4'	5.20 (d) J=7	exchanged	(s)	OH-4' 5.37 (broad) exchanged
H-4'	4.46 (broad)			

Table 3 Properties of articulatin and articulatidin[4,6] compared with 7 and 8

	m.p. (°C)	Colour reactions: $Zn(OH)(OAc)$	NaOH	$Mg(OAc)_2$	λ_{max} (mu)
Equisetrin (7)	240–242 (dec.)	reddish orange	orange yellow	orange yellow	
Articulatin	235	reddish orange	orange yellow	orange yellow	
Equisetin (8)	301–304 (dec.)	reddish orange	blue	blue	262, 278, 341, 386[17]
Articulatidin	293 (dec.)	not reported	blue	blue	263, 276, 340, 386

workers during a search for thiamine-decomposing substances in the sporestalks of *E. arvense*. The differences of the requirements of the molecular formula $C_{21}H_{22}O_{13}$ proposed for articulatin and that of 7 ($C_{21}H_{20}O_{13}$) are within the limits of error of an elemental analysis. The main heat-stable thiamin decomposing factor of *E. arvense* is therefore like that of *Pteridium aquilinum* (L.) Kuhn[19] a flavonol-glycoside.



7 : R = glc
8 : R = H

Other flavonoids detected in our material by tlc were apigenin, genkwanin and luteolin, which had been already isolated by other workers.[7]

EXPERIMENTAL

2-(4-β-D-Glucosyloxy-1-hydroxy-2,5-cyclohexadienyl)-5-hydroxy-7-methoxy-4-chromenon (= *protogenkwanin-4′-glucoside*) (**1**)

2.8 kg fresh, fertile sprouts of *Equisetum arvense* L. (collected in April 1975 near Oppenweiler, Rems-Murrkreis, Federal Republic Germany, and identified by H.G.) were extracted in an electric blender three times with 5 l of MeOH. The combined extracts were evaporated in a rotatory evaporator at a temp not exceeding 30° to a thin syrup. The latter was dissolved in 300 ml Me_2CO:H_2O (1:2), some waxy material was filtered off, and the filtrate mixed at first with 250 ml dry polyamide 6 and then with 250 ml H_2O. The resulting slurry was put on top of a column of polyamide 6 (8 × 100 cm, wet packed). The column was eluted with 2 l of each Me_2CO:H_2O 2:8, 3:7, 4:6, 5:5, 6:4 and 7:3. The fractions were monitored by tlc (cellulose:H_2O and cellulose:20% HOAc). After a forerun of 3.6 l, 1.3 l of an eluate containing **1**, 4.8 l containing some minor flavonoids, and finally 1.5 l containing **7** were obtained. Further purification of **1** was achieved by chromatography on Sephadex LH 20 with Me_2CO–MeOH–H_2O (2:1:1) as eluent (removal of some high molecular weight material), and by counter-current-distribution (100 elements, phase-volume 25 ml) between EtMeCO and H_2O (after 100 transfers **1** is found in the elements 15–45), and finally by crystallisation from water containing some Me_2CO. Colourless needles m.p. 126–128°, yield 1.27 g. (Found: C, 52.86; H, 5.50. $C_{22}H_{24}O_{13}.2H_2O$ (500.5) Calc: C, 52.80; H, 5.64%. Mr: 464 mu (FD MS); $[\alpha]_D^{25} - 31°$ (MeOH); PMR of TMS-ether (100 MHz, CCl_4): 3.7–4.4 (m, HGlc), 3.75 (s, 3H, OCH_3-7), 4.38 (d, 1H, H-1″Glc, J = 7), 4.56 (broad signal, H-4′), 5.84 (d, 2H, H-2′,6′, J = 10.5), 6.20 (m, 4H, H-6,8,3′,5′), 6.48 (s, 1H, H-3).

5-Hydroxy-2-(1,4-dihydroxy-2,5-cyclohexadienyl)-7-methoxy-4-chromenon (= *protogenkwanin*) (**2**):

(a) 200 mg **1** were dissolved in 100 ml 0.1 M acetate-buffer (pH 4.6) and incubated for 6 days at 40° with cellulase from *Aspergillus niger*[1] (activity from 1 g of the crude commercial preparation). At the end of this period **2** was extracted with EtMeCO and finally recrystallised from MeOH:H_2O, yield 92%.

(b) As above, but with 100 mg sweet almond emulsine (C. Roth, D-7500 Karlsruhe) and an incubation time of two weeks, yield 90%.

Flat yellowish needles m.p. 170–180° (dec). (Found: C, 63.52; H, 4.68; $C_{16}H_{14}O_6$ (302.4) Calc: C, 63.55; H, 4.67%. Mr: 302 mu (EI-MS); further spectral data see Tables 1 and 2.

Glucose was detected in the aqueous solutions after removal of the Na^+-ions by a strongly acidic ion-exchange resin by tlc.

5-Hydroxy-2-(4-hydroxycyclohexyl)-7-methoxy-4-chromenon (**3**)

12 mg **2** dissolved in 10 ml EtOH were hydrogenated at atmospheric pressure in the presence of 20 mg 10% palladised charcoal. Tlc (Silica gel; benzene:Me_2CO (9.5:5); bis-diazotized benzidine) revealed that a rather complex mixture of products had been formed. **3**, being the main product, was isolated by tlc using the above mentioned system, and recrystallised from EtOH, m.p. 158–159°, yield 22%. $C_{16}H_{18}O_5$ (290.3) Mr = 290 mu (EI MS); $\nu_{C=O}^{KBr}$ = 1660 cm^{-1}; further spectroscopic data see Tables 1 and 2.

4′-β-D-Glucosyloxy-5-hydroxy-7-methoxy-flavone (= *Phegopolin*) (**4**)

(a) 25 mg **1** were heated 5 hr with 5 ml 10% aqueous formic acid on a steam bath. After cooling, **4** was filtered off and recrystallised from MeOH:H_2O, yield 98%.

(b) **1** was heated without solvent 2 min. to 200–220°, yield almost quantitative yellowish needles m.p. 231–233° (Lit.[20]: 235–236°). $C_{22}H_{22}O_{10}$ (446.4) Mr = 446 mu (FD-MS).

5,4′-Dihydroxy-7-methoxy-flavone (= *genkwanin*) (**5**)

(a) 200 mg **2** were heated 4 hr with 50 ml 1% HCl on a steam bath whereby **5** separated in nearly quantitative yield.

(b) By heating **2** or **6** for 2 min. to 200°, yellow needles from MeOH m.p. 286–287° (Lit.[21]: 286°). $C_{16}H_{12}O_5$ (284.3) Mr = 284 mu (EI-MS).

2,5-Dihydroxy-2-(4-hydroxyphenyl)-7-methoxy-4-chromanon (**6**)

0.5 g of 2,6-dihydroxy-4-methoxy-acetophenone[22] dissolved in 15 ml pyridine and 2 g of *p*-benzyloxybenzoylchloride[23] were mixed and kept at ambient temp. After 24 hr the mixture was poured on ice, neutralised with 12 N HCl and extracted with EtOAc. The extract was washed thoroughly with $NaHCO_3$ aq and H_2O. Removal of the solvent left 2,6-dihydroxy-4-methoxy-acetophenone di-*p*-benzyloxybenzoylester as a slightly brownish oil, which was, without purification dissolved in 20 ml DMSO, and treated with 10 g NaOH finely ground under anhydrous Et_2O.[10,24] The resulting mixture was left 15 min at ambient temp, diluted with H_2O and after another 45 min neutralised with HOAc, whereby 2-(4-benzyloxyphenyl)-2,5-dihydroxy-7-methoxy-4-chromanon was precipitated. It was filtered off, dissolved in EtOAc, washed with aqueous $NaHCO_3$ and H_2O, and after removal of the solvent, recrystallised from benzene and benzene/MeOH (5:1), yield 55%, m.p. 140–142°.

λ_{max}^{EtOH} (log ε): 288 (4.36), 338 sh (3.68), 362 sh (3.78), 378 (3.85), 395 sh (3.75); $\lambda_{max}^{EtOH+AlCl_3}$: 307, 379, 395 sh; $\lambda_{max}^{EtOH+NaOH}$: 244, 284, 378. PMR (60 MHz, −60°C, $(CD_3)_2CO$): 12.53 (s, 1H, OH-5), 7.92 (d, 2H, H-3′, 5′, J = 9), 7.70 and 7.61 (2s, 5H, C_6H_5 benzyl), 7.32 (d, 2H, H-2′,6′, J = 9), 7.20 (d, 1H, OH-2, $J_{OH-2}^{H-2ax} = 2$), 6.30 (s, 2H, H-6,8), 5.33 (s, 2H, CH_2 benzyl), 4.83 (s, CH_2-β-diketoform, 3.5%), 3.98 (s, 3H, OCH_3-7), 3.45 (q, 1H, H-3ax, $J_H^{3eq}_{3ax} = 17$, $J_{H-3ax}^{OH-2} = 2$), 2.94 (d, 1H, H-3eq, $J_{H-3eq}^{H-3ax} = 17$).

600 mg of the above benzylether dissolved in 20 ml EtOH were hydrogenolysed in the presence of 10 mg Pd-C under atmospheric pressure. The catalyst was filtered off, the solvent removed, and **6** was crystallised from benzene/MeOH (5:1), white crystals m.p. ≈ 180°, at this temp **6** was dehydrated to **5** which finally melted at 286–287°, yield 61%. (Found: C, 63.24; H, 4.72 $C_{16}H_{14}O_6$ (302.4) Calc: C, 63.57; H, 4.67%).

λ_{max}^{EtOH} (log ε): 288 (4.33), 338 sh (3.70), 362 sh (3.80), 378 (3.87) 392 sh (3.81); $\lambda_{max}^{EtOH+AlCl_3}$: 306, 302, 392 sh; $\lambda_{max}^{EtOH+NaOH}$: 242, 298, 327. PMR (60 MHz, −60°C, $(CD_3)_2CO$): 12.50 (s, 1H, OH-5), 9.50 (broad signal, 1H, OH-4′), 7.81 (d, 2H, H-3′, 5′, J = 9), 7.11 (d, 2H, H-2′,6′, J = 9), 7.11 (d, 1H, OH-2, $J_{OH-2}^{H-3ax} = 2$), 6.26 (s, 2H, H-6,8), 4.7 (s, CH_2 β-diketoform, 8.5%), 3.95 (s, 3H, OCH_3-7), 3.40 (q, 1H, H-3ax, $J_{H-3ax}^{H-3eq} = 17$ $J_{H-3ax}^{OH-2} = 2$), 2.89 (d, 1H, H-3eq, $J_{H-3eq}^{H-3ax} = 2$).

7-Glucosyloxy-3,5,8,3′,4′-pentahydroxy-flavone (= *gossypitrin*) (**7**)

The crude **7**, which was eluted from the above mentioned polyamide column was further purified by chromatography on Sephadex LH 20 at first with Me_2CO/MeOH/H_2O (2:1:1) (mainly removal of some high molecular weight material), and then with MeOH/H_2O (7:3) (separation from some minor flavonoids), and finally crystallised from EtOH/H_2O, yellow needles, m.p. and mixed m.p. with an isolate from *Chrysanthemum segetum* L. 240–242°; MS, UV and R_f values (5 solvents) of both preparations are also identical, yield 160 mg.

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