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PROTOGENKWANIN 4'-GLUCOSIDE, A NEW TYPE OF NATURAL FLAVONOID WITH A NON AROMATIC B-RING

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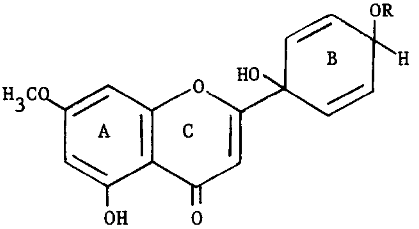
Abstract : From fertile sprouts of Equisetum arvense L. (Equisetaceae) a flavonoid has been isolated and called protogenkwanin 4'-glucoside (2-(4-β-D-glucosyloxy-1-hydroxy-2,5-cyclohexadienyl)-5-hydroxy-7-methoxy chromone). Its structure has been established on the basis of chemical and spectroscopical evidences.

Methanol extraction of fresh fertile stems of *Equisetum arvense* L. followed by chromatography on polyamide and Sephadex LH 20 as described in other cases[2] and counter current distribution between MeCOEt and H_2O, led to a new flavonoid : protogenkwanin 4'-glucoside <u>1a</u> {m.p. 128-129°C (EtOH) ; $[\alpha]_D^{25}$ - 31° (MeOH) ; λ_{max}^{EtOH} (log ε) 231 (4.27), 249 (4.31), 257sh (4.27), 293 (3.92), 324sh (3.62)}. Its hydrolysis by β-D-glucosidase (sweet almond emulsin) in acetate buffer pH 4.6 gave glucose and the corresponding aglycone, protogenkwanin <u>1b</u> {m.p. 175-176°C (EtOH) ; $\nu_{c=o}$ (KBr) 1680 cm^{-1} ; λ_{max}^{EtOH} (log ε) 231 (4.34), 250 (4.38), 257sh (4.35), 293 (3.99), 324sh (3.73) ; m/e (%) $M^{+\cdot}$ 302 (100), 284 (35), 192 (10), 167 (36), 121 (15), 111 (20)}.



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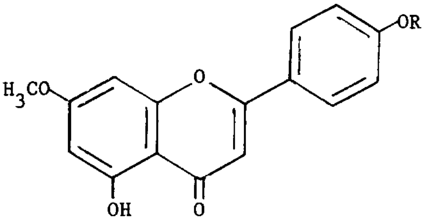

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a R = Glc

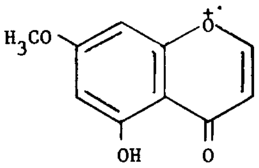
b R = H

1a and 1b are both easily dehydrated into the corresponding flavones, genkwanin 4'-glucoside[3] 2a ($M^{+\cdot}$ m/e 446) and genkwanin 2b ($M^{+\cdot}$ m/e 284) respectively, by heating to 200-220°C or by warming with 10 % HCOOH. Since the UV spectra of 1a and 1b, with and without added shift reagents, are identical with those of 5-hydroxy-7-methoxy chromone[4], the chromone ring of the flavone skeleton must be intact, and the elements of water attached to the B-ring so that no conjugation occurs with the chromone moiety or within the B-ring.

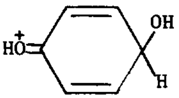
Analysis of the PMR spectrum (DMSO-d_6, 100 MHz) of 1b confirms the peculiar B-ring substitution. When D_2O is added the singlet of OH-1' (δ 6.26) disappears unmasking a group of signals between δ 5.9-7. These signals show that the B-ring has an axis of symmetry in the 1', 4' direction : the double doublet (2H, δ 6.20) for equivalent H-3'.5' coupled with H-2', 6' (J = 10.5 Hz) and with H-4' (J = 4 Hz), and the doublet (2H, δ 5.92) for equivalent H-2',6' only coupled with H-3', 5' (J = 10.5 Hz). D_2O also alters other parts of the spectrum : the doublet of OH-4' (δ 5.20) coupled with H-4' (J = 7 Hz) disappears and the broad signal of H-4' (δ 4.46) becomes sharper. The correctness of these assignments is proven by irradiation at δ 4.46 (H-4') : the doublet of OH-4' (δ 5.20) becomes a singlet and the double doublet of H-3', 5' (δ 6.20) a doublet.

The remaining signals from the chromone moiety, similar to those of flavones[5], occurred at δ 12.80 (s, 1H, OH-5), δ 6.62 (d, 1H, H-8, J_{meta} 2.5 Hz), δ 6.44 (s, 1H, H-3), δ 6.42 (d, 1H, H-6, J_{meta} 2.5 Hz) and δ 3.92 (s, 3H, OCH_3-7).

The EI-MS spectrum of 1b confirms the proposed structure. The strong peak at m/e 284 $(M-18)^{+\cdot}$ (35 %) again proves the easy dehydration of the molecule. The important peaks at m/e 167 and 121 derive from $M^{+\cdot}$ or $(M-18)^{+\cdot}$ by a retro Diels-Alder fragmentation[6], while fragmentation between B and C-rings gives peaks at m/e 192 and 111, which are characteristic of the new structure.

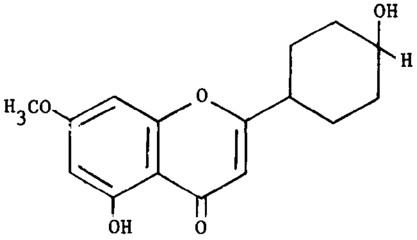


m/e 192



m/e 111

Additional evidence for the proposed structure was obtained by reduction of 1b with H_2 (EtOH, Pd/C) under normal conditions, giving a mixture from which the major product 3 could be isolated {m.p. 158-159°C (EtOH) ; λ_{max}^{EtOH} (log ε) 230 (4.09), 248 (4.16), 255sh (4.13) 290 (3.76), 320sh (3.51) ; $\nu_{C=O}$ (KBr) 1660 cm^{-1} ; m/e (%) $M^{+\cdot}$ 290 (100), 247 (13), 219 (27), 167 (14)}. Its UV spectrum shows that the chromone chromophore has not been affected.



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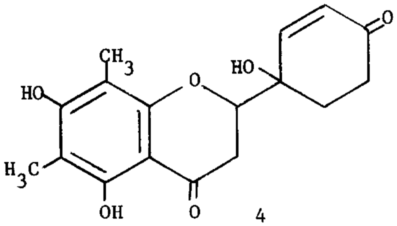
EI-MS indicates that saturation of the two double bonds of the B-ring and hydrogenolysis of the "benzylic" tertiary hydroxyl group at C-1' have occurred, in agreement with the PMR spectrum {$(CD_3)_2CO$, 100 MHz} which shows a broad multiplet system in the range of methylene protons between δ 1.6-2 and a singlet at δ 4.46 (OH-4') which disappears with D_2O or by irradiation at δ 3.25 (signal of H_2O). The signals of the protons of the chromone moiety are similar to those of 1b.

The only feature of the structure of protogenkwanin 1b and its 4'-glucoside 1a which remains undecided is the *cis* or *trans* configuration of the two substituents at C-1' and C-4'. This point will be examined by an X-ray study of the crystal structure.

To our knowledge, protogenkwanin 1b is the second example of a flavonoid bearing a 1'-OH group, the first one being protofarrerol 4 isolated from the fern *Leptorumohra* *miqueliana*[7] and easily dehydrated to the corresponding flavanone farrerol 5.



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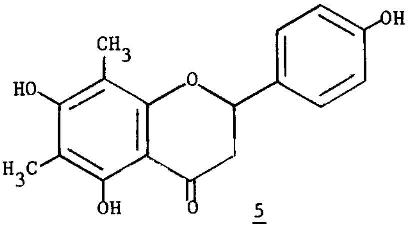

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The obvious similarity between the B-ring substitution patterns in 1b and 4 suggests a common biogenetic scheme through the oxidation of ring B of the flavone 2b and flavanone 5, respectively, to the corresponding p-hydroxycyclohexadienones, followed by reduction either of C = O or of C = C in the B-ring.

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