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FLAVONOID 5-GLUCOSIDES FROM *PRUNUS CERASUS* BARK AND THEIR CHARACTERISTIC WEAK GLYCOSIDIC BONDING

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Key Word Index—*Prunus cerasus*; *Prunus avium*; Rosaceae; bark; pinostrobin 5-glucoside; flavonoid 5-glucosides; hydrolysis.

Abstract—Pinostrobin 5-glucoside, a novel flavanone glycoside, was isolated from the bark of *Prunus cerasus*. As it was not found in *P. avium*, the substance is useful to distinguish these two species. Apigenin 5-glucoside genkwanin 5-glucoside and neosakuranin were also isolated from the bark of *P. cerasus*. They occur in both species as minor components. These 5-glucosides together with genistein 5-glucoside, prunetin 5-glucoside, sakuranin, tectochrysin 5-glucoside and luteolin 5-glucoside were hydrolysed in malic acid. The isoflavone and flavone 5-glucosides were shown to be hydrolysed more rapidly than the flavanone 5-glucosides, whereas no hydrolysis was observed with the corresponding 7-glucosides under the same conditions. The chalcone 2′-glucoside neosakuranin was transformed at first to the corresponding flavanone 5-glucoside, which was hydrolysed thereafter.

In earlier work [1], two of the three flavonoids which distinguish *Prunus cerasus* from *P. avium* were identified as prunetin 5-glucoside (**8**) and tectochrysin 5-glucoside (**2**). The third is now identified as pinostrobin 5-β-D-glucoside (**1**) whose aglycone pinostrobin was reported earlier in *P. cerasus* by Nagarajan and Parmar [2]. The structure of **1** was proved by hydrolysis with β-D-glucosidase. It yielded the aglycon pinostrobin and glucose. Pinostrobin was identified by direct comparison with an authentic sample (HPLC, TLC and UV-spectra) (Table 1). The aglycone:glucose ratio was found enzymatically to be 1:1. As pinostrobin possesses only one hydroxyl the glucose must be linked to that group.

Apigenin 5-glucoside (**3**) and genkwanin 5-glucoside (**7**) were identified in the bark of *Prunus cerasus* by comparison with authentic samples [3] by HPLC, TLC and UV-spectroscopy. The chalcone neosakuranin (**5**) yielded sakuranin (**6**) by addition of acid and was additionally identified by UV-spectroscopy. Compounds **3**, **7** and **5** are minor components of the bark extracts of both *P. cerasus* and *P. avium*. Other authors have isolated from various *Prunus* species the following substances: genkwanin [4], genkwanin 4′-glucoside and **5** [5] from *P. puddum*, apigenin from *P. persica* [6] and apigenin 7-glucoside in trace amounts from *P. cerasus* and *P. avium* [7].

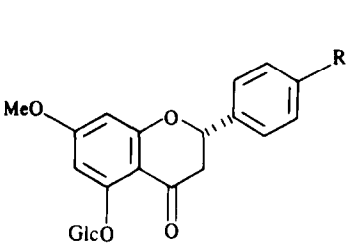
By using boiling ethanolic hydrochloric acid, Glennie and Harborne [8] showed that the hydrolysis of the 5-glucosides of luteolin, tricin and various flavonols occurred 60 times more rapidly than that of the 7-glucosides of quercetin and luteolin. A similar observation can now be made with the flavonoid 5-glucosides from *P. cerasus*. In addition, the hydrolysis was also observed using acids such as acetic acid, oxalic acid and malic acid at 20°. To determine the conditions necessary for the isolation of flavonoid 5-gucosides, several kinetic experiments were undertaken. The half-lives of all flavonoid 5-glucosides of *P. cerasus*, neosakuranin and additionally luteolin 5-glucoside (**9**) in 1 M malic acid at 60° are shown in Table 2. The flavone and isoflavone 5-glucosides were shown to hydrolyse more rapidly than the flavanone 5-glucosides. The isoflavone 5-glucosides hydrolysed first. The chalcone 2′-glucoside (**5**) was transformed at first to the corresponding flavanone 5-glucoside (**6**) which was hydrolysed thereafter. The chalcone aglycone could not be detected. Within each flavonoid group, there were no other significant differences. By contrast, related 7-glucosides and genkwanin 4′-glucoside were recovered unchanged after 24 hr treatment under the same conditions.

Aglycones are usually more toxic to microorganisms than glycosides [9–11]. This was also verified in the case of *Cytospora persoonii* [12], a fungus causing bark canker of *P. avium*. *Prunus cerasus* was described to be resistant to this fungus [13]. As *P. cerasus* has qualitatively and quantitatively more 5-glucosides than *P. avium*, the presence of readily hydrolysable 5-glucosides may contribute to the resistance of sour cherries to this fungus.

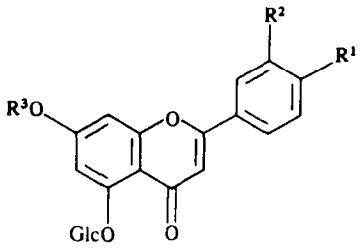
EXPERIMENTAL

General. Sample preparation [14], extraction and isolation [1] were described earlier. Authentic samples: **3**, **7**, **9**, and genkwanin 4′-glucoside from *Equisetum arvense* L. [3]; **2**, **4**, **8**, from *P. cerasus* [1], **5** [15] and dihydrowogonin 7-glucoside [16] from *P. avium* (but also occur in *P. cerasus*); aglycones and flavanone 7-glucosides from Roth (F.R.G.).

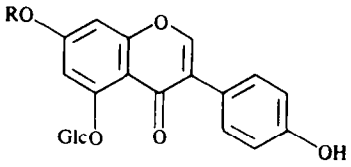
Identification. Compounds **1** and **5** were identified by UV spectroscopy [17]. Their aglycones and **3** as well as **7** were compared with authentic samples by analytical HPLC separation (conditions see [18], system 1) at a diode array detector, by UV spectroscopy, TLC [cellulose; solvent A (BAW): *n*-BuOH–HOAc–H_2O, 4:1:5, upper phase; solvent B: 10% HOAc]. Enzymatic hydrolysis by β-D-glucosidase showed that the sugar



1 R = H
6 R = OH



2 $R^1 = R^2 = H$, $R^3 = Me$
3 $R^1 = OH$, $R^2 = R^3 = H$
7 $R^1 = OH$, $R^2 = H$, $R^3 = Me$
9 $R^1 = R^2 = OH$, $R^3 = H$



4 R = H
8 R = Me

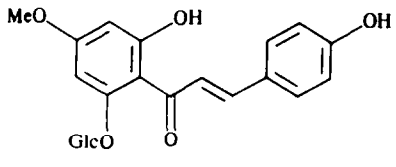


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Table 1. Spectroscopic and chromatographic behaviour of the flavonoid glucosides **1**, **3**, **5** and **7**

UV λ_{max}	**1**	**3**	**5**	**7**
MeOH	279, 305(sh)	258, 329	245(sh), 310(sh), 364	257, 326
$AlCl_3$*	279 decr., 308 incr.	273, 299, 345	255(sh), 326, 412	273, 296, 343, 375(sh)
$AlCl_3$–HCl*	279 decr., 308 incr.	273, 299, 345, 380(sh)	255(sh), 326, 412	273, 296, 343, 375(sh)
NaOMe	254(sh), 275(sh), 299	265, 318, 384	245, 317(sh), 394	260(sh), 277(sh), 379
NaOAc	279, 305 (sh)	265, 299, 360	245 (sh), 310 (sh), 364	257, 330, 375 (sh)
R_f				
BAW	0.84	0.59	0.72	0.66
10% HCO_2H	0.18	0.10	0.23	0.12
Fluorescence (350 nm)				
	Almost invisible	Light blue	Yellow	Light blue
Neu's reagent	Almost invisible	Light blue	Orange	Dark blue
HPLC-retention time	63.5 min	44.0 min	56.3 min	49.5 min

*Cf. text.

Table 2. Structures of the 5-glucosides tested and their half-lives in 1 M malic acid at 60°

Oxidation pattern						
OMe	OH	O-Glc	Chalcone	Flavanone	Flavone	Isoflavone
7		5		**1** 18.7 hr	**2** 3.1 hr	
—	7, 4′	5			**3** 2.8 hr	**4** 1.3 hr
7(4′)*	4′(4)*	5(2′)*	**5** 4.0 hr†	**6** 27.0 hr	**7** 2.1 hr	**8** 1.6 hr
—	7,3′,4′	5			**9** 2.5 hr	

*Chalcone numbering in parentheses.
†Transformation to sakuranin (**6**).

released was glucose. One mol glucose per mol aglycone was assayed enzymatically by hexokinase and glucose-6-phosphate dehydrogenase. Only glucose was detected on TLC (cellulose, BAW, as above, visualized with aniline hydrogen phthalate).

Hydrolysis. In addition to the 5-glucosides from Table 2, genkwanin 4'-glucoside and the following 7-glucosides were tested: of apigenin, genistein, luteolin, prunetin, eriodictoyl and dihydrowogonin. As the reaction of the 5-glucosides in HCl at 20° was very rapid, 1 M malic acid at 60° was used to demonstrate the differences in reaction time. Samples were taken at the beginning of the reaction and at 1, 2, 3, 4, 5, 7, 9, 12 and 24 hr and injected into the HPLC apparatus (solvents: 5% HOAc, MeOH, gradient 60% B in A in 10 min to 80% B in A, then 12 min isocratic, column 250 × 4 mm, Hypersil C-18, 3 μm). For each 5-glucoside a regression versus time was calculated ($0.89 < r^2 < 0.99$). From this the half lives were evaluated.

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REFERENCES

1. Geibel, M., Geiger, H. and Treutter, D. (1990) *Phytochemistry* **29**, 1351.
2. Nagarajan, G. R. and Parmar, V. S. (1977) *Planta Med.* **32**, 50.
3. Veit, M., Geiger, H., Cygan, F.-C. and Markham, K. R. (1990) *Phytochemistry* **28**, 2555.
4. Narasimhachari, N. and Seshadri, T. R. (1949) *Proc. Indian Acad Sci.* **30A**, 271.
5. Austin, P. W., Seshadri, T. R. and Sood, M. S. (1969) *Indian J. Chem.* **7**, 43.
6. Wildanger, W. and Herrmann, K. (1973) *Z. Lebensm. Unters. Forsch.* **151**, 103.
7. Treutter, D. and Feucht, W. (1985) *Bull. Liaison, Groupe Polyphenols* **13**, 45.
8. Glennie, C. W. and Harborne, J. B. (1971) *Phytochemistry* **10**, 1325.
9. Pridham, J. B. (1960) in *Phenolics in Plants in Health and Disease* (Pridham, J. B., ed.), pp. 9–15. Pergamon Press, Oxford.
10. Hildebrand, D. C. and Schroth, M. N. (1964) *Phytopathology* **54**, 640.
11. Müftüoglu, T. and Nienhaus, F. (1976) *Phytopath. Z.* **85**, 49.
12. Bayer, O. (1989) *Gartenbauwissenschaften* **54**, 138.
13. Kaltschmidt, B. (1984) *Arch. Gartenbau* **32**, 19.
14. Geibel, M., Treutter, D. and Meier, N. (1990) *Euphytica* **45**, 229.
15. Treutter, D., Feucht, W. and Schmid, P. P. S. (1987) *Sci. Hort.* **32**, 183.
16. Treutter, D., Galensa, R., Feucht, W. and Schmid, P. P. S. (1985) *Physiol. Plant.* **65**, 95.
17. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids.* Springer, Berlin.
18. Treutter, D. (1988) *J. Chromatogr.* **436**, 490.